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CHIMICA CHRONIKA, *NEW SERIES*

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Chimica Chronika, New Series, 8, 215-218 (1979)

A UNIFIED THEORY TO THE MECHANISMS OF ORGANIC REACTIONS IN SOLUTION

GEORGE A. GREGORIOU
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Introduction

Between 1958 and 1964 we developed and proposed a unified theory and approach to the mechanisms of organic reactions in solution which was applied to solvolytic reactions in particular.[1-6] This approach was radically different from the usual («classical») and a reinterpretation of the field in terms of this theory was proposed and initiated in some areas.

To test and apply this theory, a massive and ambitious project was needed and planned. This was to follow two mainstreams, one based on suitably designed experimental work and the other involving a reinterpretation of the literature data in the light of our theory.

Many developments in the field of solvolytic reactions since the time this theory was proposed[7] as well as our own experimental results,[8,9] have been in line with the positions and predictions of the above theory. Several of the presently known or accepted views[7] had been argued in our initial work[1-6] long before their development.

Since, except for an initial paper[1] and a recent rediscussion of some aspects of the theory,[10] this work has appeared primarily only in the form of four official reports of the Greek Atomic Energy Commission, the «DEMO» reports[3-6] published initially in 1963 and 1964*, it has not been widely available. This has created the problem of inaccessibility, as also expressed in several published[11,12] and private[13] communications. Characteristically, Lancelot, Cram, and Schleyer[11a] in their chapter on phenomium ions state that «Gregoriou has proposed a general unified theory of solvolysis mechanism... Some of the specific features of Gregoriou's unified theory (49)* forecast the more recent refinements which have contributed to ultimate resolution of the phenomium ion controversy. Unfortunately, the bulk of Gregoriou's published work was not widely available and only certain aspects of it have very recently

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* These reports were submitted in parallel to the Academy of Athens (Archives 46254, 46772 (1963), 48161, 18711 (1964).

* Ref. (8) *in this introduction.*

become readily accessible. Leading references to the rest of Gregoriou's work are found in Ref. 49.»

Statements like the above emphasize the inaccessibility of this work as well as its significance. The difficulty and interest regarding the above particular area of non-classical ions, which is but one of the many chapters and topics to which the proposed theory applies, is clearly illustrated by the statement that[11b] «probably few other topics in physical organic chemistry have been as widely and thoroughly studied. In spite of this, not many other theories in organic chemistry have been surrounded by such extended, often heated controversy as has been associated with the idea of participation by neighboring aryl groups in solvolysis reactions». It is added that regarding this controversy, which has raged through the entire decade of the sixties and has divided the researchers into two schools of thought, our theory had predicted in the very early stages[4,6] that both schools were partly incorrect and that the usual approach to solvolytic reactions was to blame for the situation[8]. This was finally partly recognised[11c] in accepting the importance of solvent assistance.

In view of reasons such as the above mentioned ones, and because of the additional need for referring to the initial work in our publications, its reappearance in a vehicle of wider circulation is necessary and long overdue. Thus, the four reports are reprinted in this and following issues of this journal under headings numbered I-IV (DEMO 1-4), in the very content in which they appeared initially, except for minor editorial corrections.* An exact reprinting is preferred if the right to refer to this as work of 1963-64 is to be maintained and also for reasons of expediency. Admittedly, this leaves the theory and work in its early shape which needs refinement, and particularly a more concise and precise presentation. However, the features and the essence of this initial approach are steadily being proven true during the fifteen years which have elapsed. Remarkably, many developments in the field, including most recent views such as the «S_N2-intermediate»[14] or «ion-sandwich»[15] mechanisms, can be recognized as implicitly covered by this theory or as steps towards its features.

In view of a host of greatly adverse conditions under which this project was called to develop and in view of its special features such as its size and radical nature, progress has not lived up to the initial plans. These involve several interrelated build-up steps, a brief outline of some of which is as follows.

Special experimental studies are conducted in many of the various chapters of the field of solvolysis such as isotope effects, solvent effects, salt effects, effects of nucleophiles, non-classical intermediates, etc. These studies are designed to test the predictions of the unified theory, in anticipation of answers different from those of the «classical» approach. They aim at the roots of each

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* The author thanks Dr. Anna Scordou-Matinopoulos for her contribution to these corrections.

chapter and their object is the reexamination and reinterpretation, if needed, of these chapters.

There are several reasons for dealing with several chapters simultaneously, one of them being the fact that they are interrelated and thus the particular reinterpretation of anyone of them stumbles on «opposite» literature «evidence» from other chapters as well which has to be faced also. Additionally, the merging of information from the simultaneous reexamination of more than one chapters should lead to a better and more valid reappraisal of each one and of the overall picture of them all as well.

These studies aim further at the common foundation of all these chapters which is the «classical approach» including the ionization hypothesis and at substituting this with the «unified approach» which starts from the non-ionization hypothesis. A still further scope of this project is the contribution to quantitative correlations and predictions, in addition to the qualitative ones, which should be unified within each chapter and possibly for all chapters as well.

Updated discussions of the theory presented here and its applications will be given in due course.

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Chimika Chronika, New Series, 8, 219-226 (1979)

I.* AN APPROACH TO THE MECHANISM OF ORGANIC REACTIONS. A UNIFIED THEORY

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(Demo 1, April 1963)*

Abstract

An approach to organic solution chemistry termed the «unified approach» or «unified theory» is briefly presented. The approach is based on the concept of the establishment of a gradient in the chemical properties causing reactions, principally charge and nucleophilicity, and leads to a «unified model» which is said to hold for all reactions in solution. The classical mechanistic schemes result as extreme cases of this model.

A crude schematic representation of the proposed mechanism is given and its application to a saturated carbon reaction center is examined in general terms. It is maintained that thinking in terms of this approach coupled with simple reasoning and experiments can lead with little effort to conclusions and predictions as well as to answers to many problems and controversies in the field of reaction mechanisms. These also offer, as claimed, a practical and effective basis for studies and a shortcut to knowledge in the field.

A small number among the many examples of the developments in the field in recent years which are in line with predictions of this approach are presented.

An approach to the mechanism of organic reactions. A unified theory

A unified theory,[1] or unified approach was introduced in 1958 as the unified mechanism for nucleophilic reactions involving saturated carbon[2] and was to be extended further. This theory, on the basis of which a reinterpretation of the field of reaction mechanisms was proposed but was carried out partly only recently,[1b] amounts to the study of organic solution chemistry in terms of the concept of the establishment of a *gradient* in the chemical properties causing reactions (*e.g.*, nucleophilicity, charge etc.), in all intra and intermolecular

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* This paper is a reprinting of DEMO 68/1 (April 1963, reprinted June 1968); *Atomindex*, **11**, No 5, 1486 (1969); Academy of Athens, *Archives*, No 46254, 46772 (1963), (see the introduction, p. 215, to the reprinting in this journal of the series DEMO 1-4 (papers I-IV), and ref 3 in this introduction).

directions, and also to focusing attention on the *entire range* of the gradient. This approach can also be referred to as the study of the field in terms of continuity.

One of the basic features of this approach becomes the fact that the picture of ionic *entities* in solution and the thinking in terms of them are abandoned.

One of the direct results of the concept of the gradient is, *e.g.*, that in a solvent containing a dissolved nucleophile the nucleophilicities are not just two, namely that of the nucleophile and that of the solvent, but there is instead a spectrum of nucleophilicities ranging from that of the nucleophile to that of the solvent. Another result is, *e.g.*, that a nucleophile attacking a substrate is studied as interacting not only with the reaction center but with several centers (either directly or through solvent molecules). Thus the attack is studied as involving forces acting on the molecule as a whole (nucleophilic pressure) instead of being looked at as a single force acting at the reaction center.

The concept leads to a unified model and mechanism for organic reactions in solution (free radical reactions* are also to be included) an approximate and symbolic representation of which is given by I (Fig. 1). The arrows symbolize the transmission of nucleophilic forces (the direction is an arbitrary choice). The transmission occurs by «interactions» (partial bond formations and ruptures whether covalent, induction of a dipole or other, although covalency is assigned a role greater than that generally accepted).

It should be noted that the term «unified model» is used instead of the «common intermediate» employed in the first paper.[2] This is done because the essential point is the *operation* of the *forces* indicated and not the question of whether an intermediate is formed or not. Intermediates formed in reactions are referred to as «unified intermediate complexes» and the model is said to apply all along the reaction coordinate. The term «unified» is thus emphasizing the action of the same type of forces as well as the application of the model and of the principles suggested by the theory to all reactions in solution. The term «unified intermediate», unless otherwise indicated, refers to the first formed intermediate(s). It should be kept in mind, however, that additional intermediates can also be formed.

According to this unified approach, prior to reaction, nucleophilic forces are considered to be transmitted from Y (nucleophile) to M (electrophile) mostly through solvent molecules EN (E and N being respectively the electrophilic and nucleophilic ends of the solvent molecules) as symbolized by path Y-EN-M, and only to a small extent through the organic substrate which is to undergo reaction. The number of EN molecules in the various paths shown in the model can vary between zero and a large number. It is obvious that Y is

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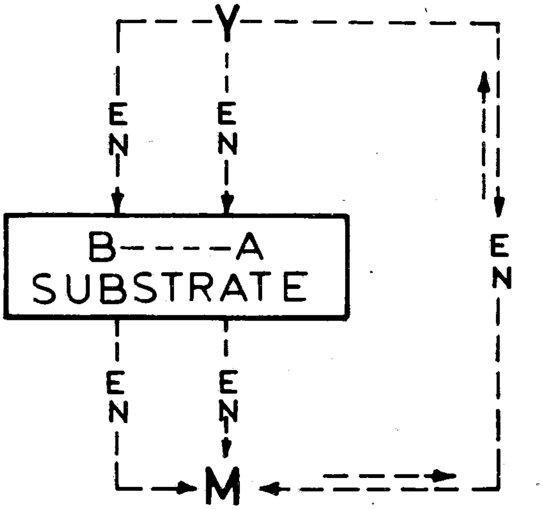
* Free radical reactions are said in terms of this theory to obey the unified model just as ionic ones are said to do, the difference being a quantitative one the interactions being probably weaker in radical reactions thus rendering the applicability and usefulness of the model less obvious.



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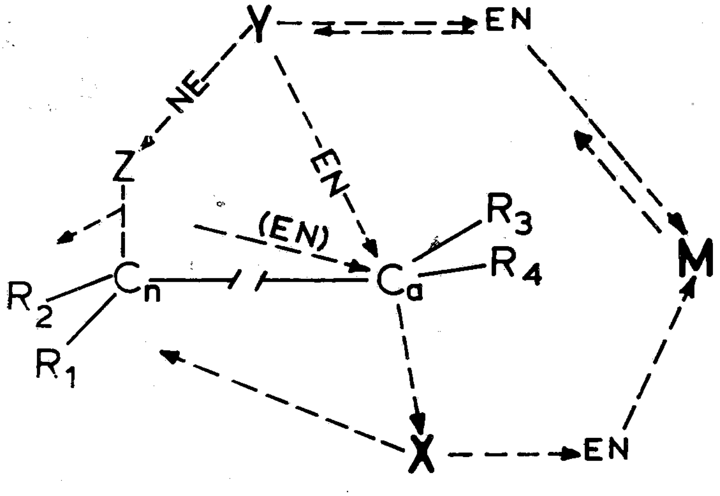

(II)

FIG. 1: *(I) A symbolic representation of the «unified model» for organic reactions. (II) A more detailed but still symbolic representation of the unified model, particularly as it applies to reactions at a saturated carbon* (illustrated best for nucleophilic reactions).

meant to be able to play also the role of a substrate as, *e.g.* in electrophilic displacements on carbon (*e.g.* in reactions of $R^- M^+$ where Y will stand for R).

During reaction, the transmission through the substrate increases at the expense of the path through the solvent. The decrease through the latter is symbolized by short arrows in the M-EN-Y direction. Reactions thus become *cyclic* processes and are studied as such. The cycle can be a diffuse one closing through the medium as a whole (cycle in an energy relation sense) but can also vary toward the other extreme becoming small and specific (cycle in a physical sense).

The attack on the entire substrate results in the transmission of the forces through the substrate by *several* paths operating *simultaneously*. Path Y-A-M («direct» path) stands for paths involving two atomic centers of the substrate, the second one being the leaving group (*e.g.* displacement reaction type). Path Y-B-A-M («indirect» path) stands for all those using more than two atomic centers but the last one being again the leaving group (*e.g.* elimination type path). Side paths, symbolized by Y-B-M or Y-A-B-M, cover those involving as an end in the substrate an atomic residue which is not a leaving group (*e.g.* addition reaction type).

The suggested simultaneous operation of a multitude of paths,* the fact that they should not reach their maximum contribution at the same point, and should also be reversed as the system proceeds further along the reaction coordinate, except for the path leading to the product, render most likely the formation of an intermediate (one or more) whether detectable or not, and not necessarily rate-determining, in most reactions, if not in all.

The general mechanistic models employed in the field result from the unified model as extreme cases by focusing attention on a certain path only. However, it is argued that actual reactions schould follow the unified model and that the use of simple models has resulted in a vast number of problems which are not real but created by the «classical» approach itself.

Application of the unified model to reactions involving saturated carbon (substitution, rearrangement, elimination) leads to the symbolic and approximate model II (Fig. 1). Analogous models are derived for other reaction classes, *e.g.* addition reactions etc. In the figure, Z stands for a variety of groups or paths of the substrate. A spectrum of possibilities is visualized. As already mentioned, the number of EN molecules in any part of the model may vary between zero and a large number. It goes without saying that as this number is increased, the specificity of interaction between the two groups

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* In this and subsequent papers on this theory, a *«path»* is defined as a series of elements and/or pairs of elements, belonging to a substrate and its surrounding medium, in sequence along which a smaller or larger change may occur in their covalency and, in general, in their properties involved in the formation of the gradient (charge, nucleophilicity, e.t.c.). On the other hand, *«operation or contribution of a given path»* to a process is defined as referring to the occurrence of the above mentioned change along this path during the process.

separated by EN decreases. The absence of Y (or M) does not alter the model in which the solvent EN molecules still play their role, a function which they supplement to a smaller or larger degree anyway even when Y or M are present. Y (M) symbolizes simply sources of relatively high nucleophilic (electrophilic) potential. The nucleophilic source, or one of them, may be part of the substrate. All of them are functioning by way of paths transmitted either through various residues (solvent, part of the substrate etc.) or directly. Y may even be on the same side of the substrate as X.

In the above figure, Y-C -X-M is the «direct» path. Indirect paths are symbolized by Y-Z-C_n-C_a-X-M (Z standing for any proper center).

Transmission from Z to C_a occurs mainly by partial double bond formation (hyperconjugation) for neighboring or conjugated C_n. However, for $C_n = C_\beta$, participation (Z = H, R etc), hyperconjugation, and attack on Z become interrelated and contribute simultaneously (to an extent governed by the gradient). This means that partial bonds are formed (directly or through solvent molecules) simultaneously between incoming groups (Y, solvent) and both the C_a-carbon and Z, and at the same time between Z and C_a (all these to degrees varying between extremes). The arrow from Z-C_n to C_a stands for both hyperconjugation and participation (when the latter is feasible) and for all other modes of transmission and partial bonds to C_a (*e.g.* nucleophilic contribution of Z to C_a by way of EN molecules). Side paths are symbolized by the arrow pointing to the left of Z-C_n. An illustration of a side path would be *e.g.*, a path Y-Z-C_n-R_1-M (it becomes more meaningfull as R is varied toward an electron attracting group).

The arrow pointing from the leaving group X toward the substrate symbolizes that this group is involved in interactions *qualitatively similar* to those of Y, their extent varying of course along the reaction coordinate in a manner opposite to that of Y.

The model leads to implications concerning the mechanism of all reaction classes, the molecularity, the geometry, energy and entropy relations, steric effects, ion pairs, the concept of nucleophilicity and a host of other features of reaction mechanisms, concepts, and phenomena in solution. The theory and the unified model are expected to apply to the inorganic fields as well but the subject has not been studied.

The unified theory reduces the problem from that of assigning specific mechanisms to that of evaluating the relative contributions of the various paths (including the one through the solvent Y-EN-M), namely to determining the effect of a number of factors on the contribution of these paths. The resulting «trends» lead to predictions many of which differ from a number of accepted views. Thus, the theory, although utilizing a more complex model and bringing in more variables, is nevertheless said to simplify the field by using the same variables for all problems. Furthermore, it provides a basis for quantitative studies and it allows one to predict «trends» in the chemical behavior of reacting systems as various factors are varied. It turns out that under this approach much of what was believed until 1958[2] and today (1963) and the great number of special mechanistic interpretations are modified and are proposed to be substituted by the unified interpretation suggested by the theory. The research efforts should concentrate instead mainly on the study of quantitative contributions along the different paths as well as on trends and correlations within this one approach and interpretation.

Evidence for the theory comes from the orderly and simple pattern into which it places the field, from its wide applicability in interpreting data, from the simple answers it provides to controversial and unresolved problems, (*e.g.* to the question of classical *vs.* non-classical carbonium ions) and from continued verification of a vast number of its predictions. In this interpretation, any aspect of the classical approach which did not agree with the predictions of the theory has been questioned regardless of how well established it has been. Several years will be required for conclusions to be written up. Since presentation of all the supporting evidence cannot be given in any one or few papers,[1a] only few pieces of evidence among the many which have appeared in the literature since our first publication[2] will be briefly mentioned below by way of example.

The unified theory leads to a study of reactions without making use of the concept and the term carbonium ions as reaction intermediates. Instead it makes use of the concept of the «intermediate complex» represented by the unified model. The ever increasing, since 1958, volume of publications where it is being realized that various reactions assumed to be carbonium ion reactions are actually ion-pair reactions[3] constitutes one of the most spectacular verifications of this aspect of the unified mechanism by bringing the field one step further away from carbonium ions, and also because the features of ion pairs are a step closer to those of the «unified model» (FIG. 1). It should be emphasized that even deamination reactions, some of the most notoriously carbonium ion reaction as accepted in 1958 and today as well, had been claimed[2] in terms of the unified mechanism to be non-carbonium ion reactions.

The solvent has been assigned by the unified mechanism a very important role (and the function of forming strong and specific bonds with the substrate (becoming only in extreme cases non-specific)[2] emphasized further by the concept of continuity. The sudden realization during the last few years of the importance of the solvent in chemical reactions[4,5] is one of the most inherent predictions of the unified approach.

The basic premise of a model and theory used recently to interpret bimolecular β-elimination reactions and the competition between Hofmann and Saytzeff type of elimination is that these reactions differ in the relative extents of C-X and C-H bond ruptures at the transition state.[5] This premise is the result of experimental findings. However, it is also one of the results of the unified mechanism. Use of this premise and of the *other features* of the unified model provide an interpretation to elimination reactions as will be discussed elsewhere. Furthermore the gradual transition between various elimination mechanisms,[5] and that between S_N1-S_N2 reactions[6] which are becoming more and more recognized are predicted by the unified mechanism as well as a gradual transition between elimination and substitution reactions which has not yet been recognized.

The experimental finding of cis-elimination accompanying substitution in the reaction of halide ions with alkyl toluenesulfonates, for which a merged mechanism[7] was proposed, are expected by the unified theory. A proper balance between the contribution of the various paths (direct, indirect, side paths) operating within the unified model results in the above experimental findings which thus do not require a special mechanism for their interpretation.

With respect to the arguments concerning classical vs. non-classical carbonium ions[8] and their distinctive difference in stereochemical behavior, the unified mechanism interprets experimental findings without differentiating sharply between these two types of ions and their interconversion. The «intermediate complex» of the mechanism has a representation (partial bond between Y and both C_a and Z and also partial bond between Z and C_a, all these to a degree varying between extremes, although geometry and entropy considerations may upset this continuity when approaching these extremes) different from that of either of these ions (classical or non-classical) and can give rise to displacement on C_a from either side although preferably from the side of X, the preference depending mainly on the relative contribution to this intermediate complex of the various partial bonds (Z-C_a, Y-C_a, X-C_a) or their relative ease of operation. Rearrangement leading to another intermediate with C_a and C_β having switched roles can also take place, the rate of this equilibration depending mainly on the relative extent of the partial bonds and the ease of operation of the respective paths.

The concept of steric effects as modified by the unified theory will be discussed elsewheve. The operation of paths and the concept of the gradient render the effect of a given group into a "variable" among other changes they bring about.

Detailed discussions of the above cases and of a large number of applications of the theory constitute the subject of forthcoming papers.

Περίληψη

Οἱ μηχανισμοὶ τῶν ὀργανικῶν ἀντιδράσεων ὑπὸ νέο πρίσμα. Μία ἑνιαία θεωρία

Εἰσάγεται περιληπτικὰ μία «ἑνιαία μέθοδος» ἢ «ἑνιαία θεωρία» γιὰ τὴ μελέτη ὀργανικῶν ἀντιδράσεων σὲ διαλύματα. Ἡ μέθοδος βασίζεται στὴν ἀρχὴ τῆς ἀναπτύξεως σταδιακῆς μεταβολῆς (gradient) στὶς χημικὲς ἰδιότητες ποὺ προκαλοῦν ἀντιδράσεις, κυρίως τὸ φορτίο καὶ τὴν πυρηνόφιλο ἱκανότητα. Ἡ ἀρχὴ αὐτὴ ὁδηγεῖ σὲ ἕνα «ἑνιαῖο πρότυπο» ποὺ θεωρεῖται ὅτι ἰσχύει γιὰ ὅλες τὶς ἀντιδράσεις σὲ διαλύματα. Ὅλα τὰ κλασσικὰ μηχανιστικὰ σχήματα προκύπτουν ὡς ἀκραῖες περιπτώσεις τοῦ προτύπου αὐτοῦ.

Δίδεται μία σχηματική παράσταση τοῦ προτεινόμενου μηχανισμοῦ καὶ ἐξετάζεται σὲ γενικὲς γραμμὲς ἡ ἐφαρμογή του στὴν περίπτωση ἀντιδράσεων σὲ κεκορεσμένο ἄνθρακα. Προβάλλεται ὁ ἰσχυρισμὸς ὅτι ἡ ἐξέτασις τῶν μηχανισμῶν τῶν ὀργανικῶν ἀντιδράσεων ὑπὸ τὸ πρῖσμα τῆς ἑνιαίας θεωρίας σὲ συνδυασμὸ μὲ ἁπλὴ λογικὴ καὶ πειράματα ὁδηγεῖ μὲ μικρὴ προσπάθεια σὲ συμπεράσματα καὶ προβλέψεις καθὼς καὶ λύσεις σὲ πολλὰ προβλήματα καὶ διαμφισβητήσεις στὸ πεδίο τῶν ὀργανικῶν ἀντιδράσεων. Ἐπίσης προβάλλεται ὁ ἰσχυρισμὸς ὅτι αὐτὰ προσφέρουν μία πρακτικὴ καὶ ἀποτελεσματικὴ βάση μελέτης καὶ ὁδηγοῦν ταχύτερα σὲ κατανόηση τοῦ πεδίου.

Παρουσιάζεται ἐπίσης ἕνας μικρὸς ἀριθμὸς παραδειγμάτων ἀπὸ τὶς πολλὲς ἐξελίξεις στὸ πεδίο κατὰ τὰ τελευταῖα χρόνια ποὺ συμφωνοῦν μὲ τὶς προβλέψεις τῆς προτεινόμενης θεωρίας.

References

1. (a) This theory, and a small part of the reinterpretation of the field of reaction mechanisms carried out in the light of this theory, have been covered in lectures delivered at the Weizmann Institute of Science, at the Hebrew University of Jerusalem, and at Brookhaven National Laboratory on July 24, 1962, July 25, 1962 and March 6, 1963 respectively.

   Research on the present paper was conducted in 1962 under the auspices of the Greek Atomic Energy Commission and at Brookhaven National Laboratory.

   The difficulty in publishing this and other papers on the theory and its application rests mainly with the lack of the author's own experimental work, and with the fact that all these papers are interrelated, and evidence for each one stems from all the others. In the meantime, the predictions of the theory discussed in these papers keep being verified in the literature at a fast rate, a fact which complicates and frustrates this entire undertaking.

   (b) Because of military and other obligations, the author has not had any contact with the field of chemistry from the time of the first publication in 1958 (ref 2) until 1962.
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II.* THE CONCEPT OF CARBONIUM IONS IN SOLUTION IN THE LIGHT OF THE UNIFIED THEORY

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Abstract

The purpose of this paper is to reemphasize and discuss further a suggestion made originally in 1958[1], that the concept of carbonium (and other) ions be abandoned in favor of the concept of the «unified intermediate complex» governed by the rules of the unified theory.[1-3] This suggestion is part of the more general proposal that solution chemistry be studied in terms of the unified theory which leads to a unified approach to reaction mechanisms and that the classical mechanistic models and the «thinking» associated with them be abandoned.

One of the claims deriving from the unified theory is that the concept of ionic *entities* in solution, the features of the mechanistic models employed, and the accepted approach to reaction mechanisms in general in addition to having contributed to the development of the field have nevertheless also created unnecessary problems and have hindered the solution of real problems.

Some among the differences between the carbonium ion and the suggested non-carbonium ion concept are pointed out.

The major sources of evidence which have established the concept of transient carbonium ion intermediates as chemical entities are examined in the light of the theory. It is concluded that most of these sources provide evidence only for the formation of «some intermediate» at the most but not necessarily for the formation of an *ionic entity*. It is maintained that the introduction of the carbonium ion concept into the everyday aspects of the field of reaction mechanisms has had deleterious consequences, even though this claim is obviously the reverse of one of the strongest convictions in the field, namely of the importance of the ionization hypothesis and the carbonium ion concept in the development of the field of reaction mechanisms.[23d]

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* This paper is a reprinting of DEMO 68/2 (November 1963, reprinted June 1968); *Atomindex*, No 5 1487 (1969); Academy of Athens, *Archives*, No 48161 (1964), (see the introduction to the present reprinting in this journal of the series DEMO 1-4, papers I-IV, p. 215 in the preceeding paper and ref 4 in this introduction).

By way of illustration, the theory is applied to two only systems (deamination reactions and the solvolysis of a secondary tosylate) among the numerous chemical systems for which it makes predictions different from those advanced in the literature in terms of the carbonium ion concept and the classical mechanisms.

Trends in the mechanistic thinking as they have appeared in the chemical literature during the last few years can be recognized as predictions of the unified theory but only few of these are brought up in this paper. It is pointed out that a large number of papers will be required in order for the predictions made on the basis of this approach to be discussed and for their verification to be illustrated.

Introduction

The concept of carbonium ions as intermediates in organic reactions and the distinction between carbonium and non-carbonium ion reactions, as well as the distinction between ionic and non-ionic reactions in general have dominated the thinking in the field of reaction mechanisms and have stimulated large research efforts along these lines.

On the basis of the unified approach or unified theory to reaction mechanisms introduced in 1958[1] and discussed further recently,[2,3] the concept of carbonium ion intermediates in solution (and of carbanions and other ions and to some extent of free radicals) as well as other features of the classical mechanisms have been questioned, particularly with respect to their usefulness. An interpretation of the field in terms of this approach has been suggested[1-3] and carried out. Part of this work has been reported.[2,3] In this approach, the concept of carbonium ion* and carbanion intermediates is substituted by that of a «unified intermediate», and the classical mechanistic models have been substituted by that of the «unified model»,[1-3] (FIG. 1).

It has been argued[1-3] that the significance of the unified approach draws support from a) the ease with which it interprets chemical data and provides answers to controversial problems, b) the *verification of a large number of the predictions* reached in terms of it, and c) the *orderly* pattern in which it places the field. It has also been argued that a large number of past and many present problems are not actual problems but have been *created* by the concept of carbonium ions and the classical mechanisms themselves.

The concept of carbonium ions has started its journey into the organic field on the basis of the picture of an organic species in which a carbon atom has only six electrons instead of an octet and thus carries a unit positive charge and has a covalency of three.[4,5] It is on the basis of this picture that the excep-

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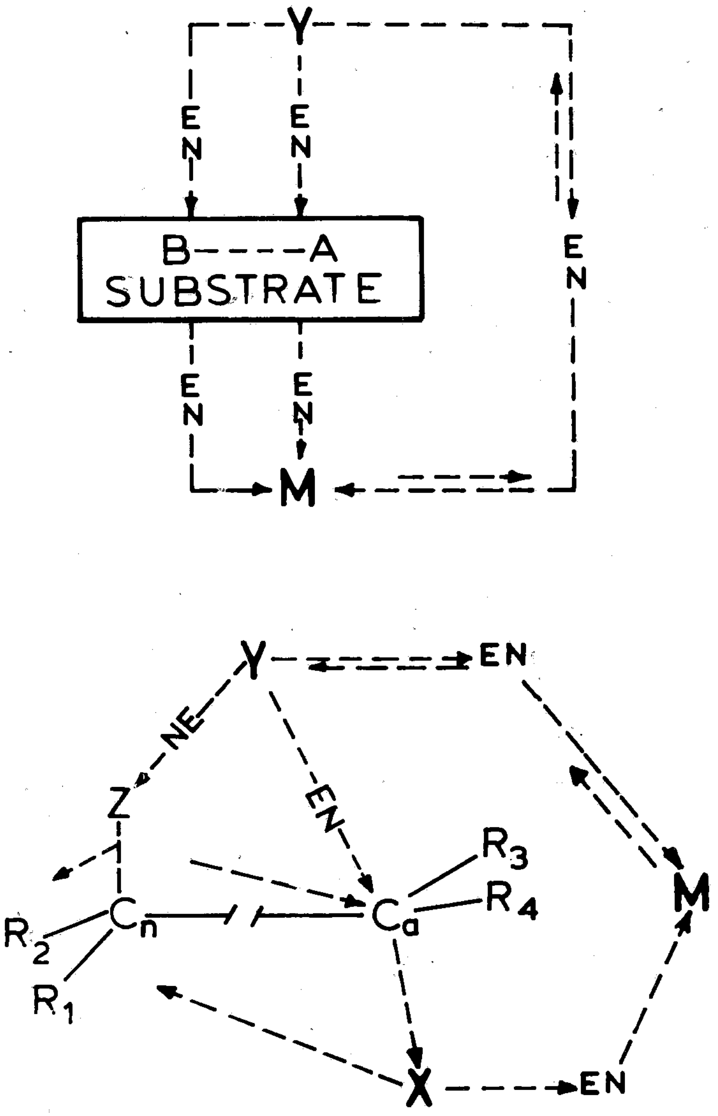
* A species involving bonds, (partially *covalent*) to a leaving group, to an incoming group, or to molecules of the medium through definite sites, such as the reaction center, or other centers (such as *e.g.* hydrogen residues) is not referred to in this discussion, and in studying its chemical behavior, as a carbonium ion (or ion in general).



(I)

(II)

FIG. 1: *(I) A symbolic representation of the «unified model» for organic reactions. (II) A more detailed but still symbolic representation of the unified model particularly as it applies to reactions at a saturated carbon (illustrated best for nucleophilic reactions). (For a discussion of this model see preceeding paper, ref. 3).*

tional reactivity and the geometry - planar due to sp^2-hybridization and an empty (or nearly so) p-orbital - have been assigned to reaction intermediates considered to be carbonium ions.[4-6] Extensive research efforts have been required before this picture could be modified to the present picture of a carbon residue bearing less carbonium ion character. The concept of resonance (π-, or s-bond) has provided a means for visualizing a *small* or large delocalization of this charge. The concept of ion-pairs introduced to organic reactions[7a] has provided also a means for stabilization of this charge. Still, however, the development of charge and of an empty p-orbital on a carbon center is discussed as a *flexible* feature which can even reach *large* values in a number of cases.[5,6,8,9,10] That large (and even extreme) carbonium ion character is allowed to develop on a carbon atom is obvious in a number of general discussions and in many individual cases such as for example the characterization of deamination reactions as «free» or «hot» carbonium ion reactions,[8,9] even in the case of primary systems or in discussions on bridgehead carbonium ions,[5,10]* as well as by the fact that the contribution of hyperconjugation to carbonium ion reactions has often been minimized.[11]

The application of the unified approach to the concept of carbonium ions leads to an alternative starting point for the study of reactions in solution which is the *reverse* of that from which the concept of carbonium ions has started. This starting point amounts to the suggestion that there are no carbonium ion intermediates formed in the usual media (water, alcohols, organic acids, ethers, ketones) by the usual chemical means, and more specifically that a carbon atom not only cannot reach but not even approach one having an empty or nearly so p-orbital (a total covalency of three) but maintains instead in the intermediate a total covalency *not very different from that in the starting material.* The development of charge and of an empty p-orbital on a carbon atom of such an intermediate becomes a feature having limited flexibility being able to reach only a small value beyond that in the initial state (small compared to one unit). Any substantial deviation from the concept of such an intermediate is said to be highly unfavorable and thus not attainable by the usual chemical means.

The idea of a carbonium ion intermediate* thus becomes an exception to

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* For a discussion of bridgehead carbonium ions under the prism of this approach, see ref. 29.

* The concept of carbonium ions as reaction intermediates has been substituted in the unified approach by that of a «unified intermediate complex[1-3], which is actually used to describe all reaction intermediates. The complex is governed by the concepts of «electrophilic and nucleophilic potential» and establishment of a «gradient in this potential» along the various paths[2,3] involved in the process. The nucleophilic or electrophilic potential of a residue is to be understood as a measure of the tendency of this residue to function, in the environmental conditions in which it is considered, as a nucleophile or electrophile as *e.g.* in the case of a differential extent of bond formation with some standard such as a solvent molecule. The potential depends on the charge on the residue, although it is not equivalent to it, and on other factors as well such as the immediate environment of the residue and particularly the *chemical element* in contact with it. The concept of the establishment of a gradient amounts to the requirement that the

be found, if possible, rather than being the rule to which exceptions are added gradually over decades of extensive research efforts.

Because of the suggested inability of a carbon atom to develop a substantially empty *p*-orbital, and because of the operation of the gradient,[2,3] reactions in solutions are treated by the unified approach as being the result of the operation of a multitude of paths along which nucleophilic forces are transmitted from high to low nucleophilic potentials. Thus the nucleophilic attack is considered to involve forces acting on the molecule as a whole, namely acting as «nucleophilic pressure» rather than as a single force at the reaction center, and is channeled through many paths. It should be pointed out however that the pressure is visualized as made up of specific forces (specific bonds)* acting on specific centers. The path starting from the medium (actually the nucleophilic end of the potential) and going through a carbon atom and the leaving group (displacement reaction type of path) on to the medium (actually to the electrophilic end of the potential) is termed «direct path». Those starting from the medium and extending through several centers of the substrate including at the end a carbon atom and the leaving group on to the medium (*e.g.*, elimination reaction type of path) are termed «indirect paths».

One of the results of the unified approach is that the classical mechanisms of organic reaction classes whether nucleophilic or electrophilic, on saturated or unsaturated carbon, in the aliphatic or aromatic series, result as extreme cases of the unified mechanism. To be more precise, the classical mechanistic models result as extreme cases of the unified model for chemical reactions in solution.[2,3] The need for the application of this model to free radical reactions is less obvious although these reactions as well must be studied under this prism.

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changes in the potential, along any path considered, be fairly gradual, thus establishing a gradient. Actually the drop between successive residues is expected to be larger between intermolecular than intramolecular ones. Charge is allowed to reside on the organic backbone of the complex (the substrate minus the leaving group) but this is visualized and treated as a rule as fractional, the difference between fractional and full charge being *exceedingly* important from a mechanistic standpoint. For a given substrate structure, the gradient along a path including a carbon atom or any ion in general and solvent molecules is said to depend to a great extent on, among other factors such as steric conditions, the nature of the nucleophilic end of the solvent molecule (and its bond to the rest of the solvent molecule) in immediate contact with the carbon atom in the intermediate complex (oxygen, which bears free electron pairs, for most of the usual solvents). On this basis, one cannot form an intermediate having a carbon atom bearing an empty or nearly so *p*-orbital in oxygen containing solvents (and thus in most of the usual media) by varying the leaving group, *e.g.* by the use of a deamination reaction, or by electrophilic action on the leaving group, *e.g.* by the use of Ag^+. This picture is a basic feature of the unified approach and has been applied to all phenomena and to all the species referred to as ions in solution, whether organic or inorganic,[2,3] the differences being only quantitative ones and not qualitative.

* On the basis of the unified theory hyperconjugative type contribution and bonding of the hydrogen (or to a much lesser extent of carbon) to the medium or to the entering or leaving nucleophile become factors of major importance in many reactions. As already mentioned, hyperconjugative type contribution, participation, and partial bonding of the group involved in hyperconjugation to the medium or nucleophiles are to be interrelated, by way of the concept of the gradient, conclusions some of which are in considerable agreement with Shiner's.[12]

With respect to S_N1 *vs* S_N2 reactions, the solvolysis of derivatives of the series methyl, ethyl, isopropyl, and *t*-butyl under the prism of the unified mechanism is studied under one mechanism with the various individual reactions representing quantitative trends within the frame of this one mechanism.[1-3] It should be noted that, with respect to S_N1 - S_N2 reactions, the conclusions reachedin terms of this approach resemble, but only in a limited way, to some among previously expressed views[13] which, it my be added, are hardly universally accepted.[14a] The trend is of an increase in the contribution of the indirect paths at the expense of the direct path.* In focussing attention to the intermediate, the relative contribution of the various paths is expected to be governed by their relative energetics. The energetics of the direct path relative to the indirect ones should increase in the series (from methyl to *t*-butyl) because of increased hindrance to its operation. This hindrance is not visualized as a simple geometric crowding of the incoming group but also, and very essentially, as one of hindrance to solvation (actually in terms of the unified approach it is hindrance to the development of the optimum gradient). On the other hand, the available indirect paths increase in number, and their energetics decrease from methyl to *t*-butyl. In solvolytic reactions, this is so because, among other reasons, the direct path creates an electrophilic potential in the solvent in contact with the substrate unfavorable for the operation of the indirect paths. The above render the decrease in the operation of the direct path not necessarily smooth. A substantial change could be visualized for example from the isopropyl to the *t*-butyl derivative.

Attention is thus focussed on the *absolute* and the *relative* extent of nucleophilic contributions along each path (direct and indirect ones in this system). Whether a carbonium ion is formed *does not enter into the picture* nor is there any interest in the molecularity (it is not definite anyway) which, on the basis of the suggested importance of covalency, has to be higher than unity anyway. The interest lies instead in the *extent* of bond formations and ruptures which are said to be *fractional* and not on whether a fixed number of species is or not involved in such interactions.

A pictorial illustration of the difference between the concept of carbonium ion intermediates and that of the unified intermediate complex is based on the same pictorial analogy to carbonium ions brought up by Whitmore[4] and termed «an excellent analogy». The ion was paralleled with a concave faced pile of sand that would be left if a log against which the pile has formed were to be removed quickly enough so that the sand could not start sliding. Such a pile, *once formed,* would *subsequently* undergo rapid changes *due to this instability.* This analogy is reversed by the present approach, according to which the sand slides along with the log *without first forming a concave face.* In fact the sand is said to behave almost as if it were glued onto the log, the only allowance made being that the glue may be visualized as elastic. On this basis, the in-

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* The definition of a «path» is given in DEMO No. 1, the first of the series.

crease in the instability and reactivity of so called carbonium ion intermediates in going to progressively more so «called unstable carbonium ions» becomes the result not so much of their «carbonium ion features» but mostly of the increase in their *non-carbonium ion* character namely their increased *similarity to the structure of an S_N2 reaction transition state.* The difference may also be illustrated by saying that this approach studies the reactions termed carbonium ion reactions (and carbanion and other ionic reactions) in terms of the *resistance* of the system to the formation of such an intermediate rather than in terms of the *tendency* of these systems to first form them. Thus, the application of the unified approach to the concept of carbonium ions leads to a concept which could be termed «non-carbonium ion», or, in general, «non-ionic».

The concept of carbonium ions has been carried over to the organic from the inorganic field on the basis of an analogy in the behavior between some organic and inorganic cations in conductivity measurements.[15] If the interest were in the external behavior of intermediates, such as the conductivity of the solution, namely if these species were viewed from a position somewhere in the bulk of the medium their characterization as ions could possibly be justified and this again only in a limited number of cases. However, in studying organic reactions, the internal behavior of the intermediate (that of the organic backbone) is of prime interest and even more so that of the reaction center, and thus the thinking in terms of ions is not justified.

Analogous considerations should apply to inorganic ions if their internal transformations are considered. Some large changes in the nucleophilic reactivities of anions (fluoride,[16] methoxide[17]) observed by changing the medium illustrate this point and instead of being surprising have been expected by this approach, which looks at the species called ions as being involved in extensive covalent bonding with the medium.[2,3] However, there is a difference in the consequences of this picture, rendering its use more required in the organic as compared to the inorganic field. This difference is that the usual inorganic cations or anions do not undergo intramolecular transformations and their reaction with the medium is reversible. Organic cations, however, can undergo intramolecular transformations (rearrangements, intramolecular partial bond formation etc.) and can react irreversibly with the medium, all these changes (and other behavior) being *greatly affected by the extent of bonding (especially covalent) to the medium or to other nucleophiles and by the centers involved in it.*

On the basis of what has been discussed it is expected that the similarities between the suggestions of this approach and those of the concept of carbonium ions are expected to increase as the structure of the substrate in a solvolysis is varied toward those believed to form a stable carbonium ion. On the other hand, the greater dissimilarities are expected in those cases where the intermediates called carbonium ions are formed not by a variation of the organic structure but by changing to a better leaving group (*e.g.* use of a deamination reaction) or by an increase in the electrophilic action on the leaving group (*e.g.* use of Ag^+ or better electrophilic action of the medium).

Some of the conclusions of the application of the unified theory to the concept of carbonium ions resemble to some extent views expressed before[13] and there are some respects in which the difference between the unified and the approach employed today is only subtle and often one of degree. Individual references to apparent similarities are not brought up here because a discussion of the differences would also be required with a resulting considerable deviation from the subject of this paper. Such detailed discussions will appear with the application of the unified approach to individual subjects. There are also some features of the approach which seem to be another way of expressing known facts. These too will be dicussed individually, since even in these cases there are several subtle differences. A general commet to all these similarities should be made however; this is that the numerous conclusions reached in terms of this approach, some of which resemble various suggestions and facts available in the literature, have been actually derived from one set of principles[2,3] which constitute the core of this approach, without knowledge or use of most of these other views or facts several of which have appeared during the last few years anyway. In fact such similarities, and thus the continued verification of predictions made in terms of this approach, constitute the best evidence for the usefulness of the approach.

Discussion

A. Sources of evidence for carbonium ion intermediates.

The present paper deals mainly with the evidence for transient carbonium ion intermediates. With respect to stable ions and several of the transient ones, the present approach leads to differences which will not be discussed here except for the general comment that the evidence for these intermediates (N.M.R., I.R., U.V., conductivity, freezing point depression) does not exclude fractional bonding (including partial covalency) of the intermediate, through carbon and hydrogen residues, to the environment *i.e.,* anionic leaving group, solvent molecules etc.

The general sources of evidence which have established the concept of transient carbonium ion intermediates are the following ones:

1. Evidence for stable carbonium ions.
2. The energetics of carbonium ion formation.
3. Kinetic data.
4. Product composition (stereochemical data, rearrangements, etc).

1. Evidence for stable carbonium ions.

The concept of transient carbonium ion intermediates, and one of the sources of evidence for such intermediates, stems from evidence for the existence of stable carbonium ions.[15] This line of evidence is strengthened by the recent interest in, and evidence for, stable aliphatic ions.[18] The inference for the formation of unstable carbonium ion intermediates from evidence for stable ions rests on the assumption that the two types of species differ *only in the degree of stability* and not in their intrinsic nature.[5,15] If, however, in progressing from the stable carbonium ions to the unstable ones, *e.g.* by a change in the reaction medium and in the structure of the substrate etc., there takes place a change not only in the stability of these intermediate species but also in their *nature, e.g.* increased bonding of the carbon center of the substrate to the environment (the term environment will refer to molecules of the medium, to the leaving group and to nucleophiles or electrophiles in general), as is expected by this approach, then there may be no justification for extending the picture of a carbonium ion to the unstable intermediates. In fact the studies on stable ions could prove to be of considerable value if emphasis were placed on their fine print, namely on possible trends, even small ones, in the physical properties of stable ions explainable in terms of increased covalent bonding to the environment as their stability is decreased, by a change in the medium or in their structure. If such trends were observed, their extrapolation to unstable ions could conceivably provide evidence *against* rather than *for* carbonium ion intermediates.

2. *Evidence from the energetics of carbonium ion formation.*

Another major source of evidence for short-lived carbonium ions comes from correlations of the energetics of carbonium ion formation in the gas phase with data from solvolysis studies.[19-21] The correlation has apparently provided support to the concept of carbonium ion intermediates in two ways. One is the agreement between the experimentally determined activation energies for the solvolysis of some alkyl halides and those calculated by subtracting from the heats of formation in the gas phase of the corresponding carbonium ions their calculated solvation energies in the reaction medium. In this argument the calculated activation energy for the sovolysis is obtained as a small difference between two large numbers, one of which (the calculated solvation energy) is obtained only as an approximation.[19,20] Furthermore, such an approximate agreement - a difference between calculated and experimentally obtained energy of activation smaller than 1 kcal/mole - has been obtained only for two compounds, *t*-butyl chloride and *t*-butyl bromide, in 80% ethanol. The discrepancy for *t*-butyl-iodide and the isopropyl halides varies between 1.2 and 4 kcal/mole. Such discrepancies however are not much smaller than the usual differences in the energy of activation between the so called unimolecular and bimolecular solvolyses. Furthermore, the lack of constancy in the agreement could very well suggest a possible cancelling out of effects which happens to be just right for two only of the halides.

A serious objection to inferences concerning the nature of the intermediate from such calculations is, in addition to the approximations involved which are recognized anyway,[19-20] the very nature of the method used. Thus, the ionic radii used to calculate the carbonium ion solvation energies were *estimated* from experimental values and further increased by an *empirical* constant (0.85 Å) amounting to between 40 percent and 30 percent of the radii in going from ethyl to the *t*-butyl ion. The approximations to the radius as well as the method used to estimate it may actually *question more rather than aid* the physical picture for the support of which they are used. Thus, they can very well cover up and include cancellations of the true effects in the actual physical picture of the intermediate which may be quite different from that used as a model, *i.e.* from visualizing the carbonium ion as an *entity* surrounded by solvent dipoles.

It may be argued that the use of the empirical constant (0.85 Å), by having been successful in the calculation of the heat and entropy of solvation of inorganic cations[20b] in terms of the Born model (and the ion-dipole model) which does not consider covalency in the ion-solvent interaction, is justified, and that its success in determining carbonium ion solvation energies supports a picture similar to the one in the inorganic field. However, what is valid for the inorganic field is not by necessity applicable to organic reactions. Furthermore, the unified theory involves the assumption of the existence of extensive covalencies between atomic residues ordinarily assumed to be carrying the charge of an ion and the solvent molecules (referring to the usual sovents) in the inorganic field as well,[2,3] a fact which is gradually being recognized.

The need to consider the possibility that models giving *approximate numerical* agreements may deviate substantially from the actual picture as well as the fact that many effects may be cancelled or masked in chemical reactions thus evading experimental observation *must be strongly emphasized and will often be repeated.* Even small deviations from perfect agreement may be a hint for the existence of substantial deviations from accepted models. The trouble with such approximations is that even if recognized as being only approximations, they nevertheless establish the image of a model (that used in the mathematical treatment) which with time and by being used out of context becomes established as a real model capable of influencing deeply the thinking in the field. That this can possibly have been the case with solvated carbonium ions will be discussed later in this paper and in future papers in particular.

It can be concluded that the available data on such energy relations do not necessarily distinguish between carbonium ions and intermediates involving partial bonds to the environment.

The second and most widely quoted argument[19-21] in favor of carbonium ion intermediates based on energetics is similar to the first one but employs differences rather than the absolute numbers themselves and should thus be more reliable. It is based on the existence of a parallelism between the trend in the rate of the unimolecular solvolyses of a series of substrates and the trend in the stabilities (in the gas phase) of the corresponding carbonium ions which leads to the conclusion that the intermediate is a carbonium ion. The example often quoted is the rate sequence in the *first-order*[19, 21,22] formic acid solvolysis of the ethyl, isopropyl, and *t*-butyl bromides which is said to parallel the gas phase stabilities of the corresponding carbonium ions. However, an examination of this argument from a quantitative standpoint is revealing.

This particular example is chosen for the application of the criterion because a) it is widely quoted, b) data are available for the solvation energies of the corresponding carbonium ions,[20] c) the system is quite representative of the usual systems on which the study of many reaction mechanisms has concentrated (secondary derivatives) and d) formic acid is the most limiting among the usual solvolytic media and thus the most likely one for the formation of carbonium ions if these are formed at all as believed.

By applying equation (1)[23] to the relative solvolysis rates of primary, secon-

$$\Delta F^{\neq} - \Delta F^{\neq}_{0} = 2.303 \text{ RT} \log k_0/k \qquad (1)$$

dary and tertiary halides, one can derive equation (2) in which the subscripts 1, 2, and 3 refer to the above halide order respectively.

$$\log k_3/k_2 - \log k_2/k_1 = [(\Delta F^{\neq}_{2} - \Delta F^{\neq}_{3}) - (\Delta F^{\neq}_{1} - \Delta F^{\neq}_{2})] / 2.303 \text{ RT} \qquad (2)$$

Equation (2) reduces to (3) if the concept of carbonium ion reactions is applied and if it is assumed that the solvation energies and entropies of the ions change by the same amount from ethyl to isopropyl and from isopropyl to *t*-butyl.*

$$\log k_3/k_2 - \log k_2/k_1 = (18\text{-}33) / 2.303 \text{ RT} \qquad (3)$$

The numbers in the parentheses are respectively the difference[23b] in the energy in the gas phase (in kcal/mole) between isopropyl and *t*-butyl and ethyl and isopropyl carbonium ions. They are obtained as the difference between the gas phase heterolytic bond dissociation energies of a series of alkyl bromides.

The experimental data for the rate of formolysis of the three bromides at 100°[23] give a value of 5.2 for the first half of equation (3). The second half is calculated to be —8.8. Thus the discrepancy between the experimental value for the ratio $k_3/k_2 : k_2/k_1$ and the one calculated in terms of the concept of a carbonium ion reaction for the members of this series is actually *enormous,* a factor of 10^{14}. Steric acceleration to any important extent of the solvolysis of the tertiary halide can be ruled out.[24]

With respect to the mechanism of these reactions, the above numbers suggest that one or more of the members of the series does not solvolyse in formic acid by a carbonium ion mechanism. This may not be considered unexpected. However the formolysis of these halides has been characteristically discussed as a unimolecular[19,21,22] carbonium ion reaction and thus no more than minor deviations from this picture could be allowed. In contrast, the data

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* This is justified in terms of the data given by Franklin[20a] in water, methanol, and 80% aqueous methanol. In fact some slight discrepancies are in a direction which would help the present argument. Also, since differences are employed, one can safely neglect some other quantities such as solvation of the initial state, possible differences in the energy of solvation of the same leaving group due to the blocking effect of the organic residue, and use of energy data for the intermediate rather than for the transition state.

suggest an enormous deviation from the formation of a carbonium ion intermediate at least for one member of the series.

The value of the difference log k_3/k_2 − log k_2/k_1 for the solvolysis of the same series of halides (ethyl, isopropyl, t-butyl bromide) in the solvents ethanol, 80% ethanol, 50% ethanol, water, formic acid varies in the sequence 3.18, 3.47, 3.77, 3.95, 5.16 respectively.* This trend indicates that in going from ethanol to a more ionizing solvent the change affects the rate of solvolysis of the tertiary system over that of the secondary more than it affects that of the secondary over the primary one by a factor which reaches a value of 100 for formic acid *vs* ethanol ($10^{5.16}/10^{3.18}$). This can also be put in other terms. Whereas the rate of solvolysis of isopropyl bromide over that of ethyl bromide changes from 0.725 in ethanol to 26.1 in formic acid, *i.e.* by a factor of 36 only, the corresponding change for *t*-butyl bromide over isopropyl bromide is a factor of 35×10^2 (1.1×10^3 to 3.8×10^6). These data suggest that the isopropyl derivative behaves very much more like the ethyl derivative than the *t*-butyl one. Since the formic acid solvolysis of at least one of these derivatives (the ethyl bromide, obviously) was argued above to deviate greatly from being a carbonium ion reaction, it follows that the solvolysis of the isopropyl compound must also deviate from being a carbonium ion reaction. Thus, at least two of the compounds, namely the primary and the secondary derivative, solvolyse in *formic* acid by way of a mechanism greatly different from involving a carbonium ion.

Similarly, the relative rates of solvolysis[23] of methyl, ethyl, and isopropyl bromides show only small variation** as the solvent is changed from ethanol to formic acid, suggesting that the change to the more ionizing among the usual solvolytic media *does not really change the mechanistic features of the reaction.*

The above analysis*** of long available data suggests that energetics not only

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* Calculated from data given in ref. 23.

** The fact that the variation is small is in agreement with the principle of this approach that the empty *p*-orbital character of a carbon atom is not a very flexible feature and that it can only reach a small value.[2-6] It is also in agreement with the concept of the gradient and its strong dependence on the nucleophilic residue of the medium (actually on the nucleophilic potential of this residue). The residue in these media is the same, namely an oxygen atom, and although its nucleophilic potential is expected to vary, due *e.g.* to variation in the hydrogen bonding ability of the medium, the resistance to the formation of a carbonium ion carbon atom is too large and thus the development of charge on carbon cannot be greatly influenced by these trends. Similar considerations apply to the effect of the increased electrophilic action of the medium on the leaving group being too weak when compared to the resistance of carbon to the development of an empty *p*-orbital.

*** NOTE added in this reprinting: It is apparent that the above predictions of the unified theory, and the method used above in demonstrating, and estimating the magnitude of solvent assistance (and this from already long available data) have since found later[a] their more recent verification and analogous treatment for estimating this assistance.
a. P.v.R. Schleyer, J.L. Fry, L.K.M. Lam, and C.J. Lancelot, *J. Amer. Chem. Soc.*, **92**, 2542 (1970).

do not provide evidence for carbonium ion intermediates in the most widely used systems of mechanistic interest (solvolysis of secondary derivatives) even in formic acid medium but suggest instead an intermediate with features substantially different from those of a carbonium ion. Still, even today the formation of a secondary (2-octyl) carbonium ion (solvated) even in aqueous dioxane is apparentyl accepted as a possibility.[14a]

3. Evidence from kinetic data

Kinetic criteria have provided some of the strongest arguments in favor of carbonium ion intermediates. Among these criteria the farthest reaching one, because of both its direct and indirect effects in developing the concept of carbonium ions, is whether the rate of a solvolysis reaction is accelerated or not, beyond that from a salt effect, by the lyate ion of the solvent or by a strong nucleophile in general. Lack of acceleration has been used as evidence for a carbonium ion reaction.

This criterion and thus the arguments derived from it rest apparently on the assumption that since lyate ions or strong nucleophiles in general behave as much better nucleophiles than solvent molecules they must do so to a more or less similar order of magnitude in the reaction under consideration as well.

Before discussing this criterion further a comment that must be made is that even if attention is focused away from the nucleophilic contribution of an incoming group, the criterion does not distinguish between a carbonium ion intermediate and an intermediate involving bonding to the leaving group, including covalency, a state different from that of a carbonium ion and even from an ion pair in some ways. What will be discussed below is that it does not prove either that fractional nucleophilic displacement is absent in the first step of the reaction, namely that the reaction does not proceed like a displacement reaction (involving nucleophilic attack on the reaction center and on hydrogen residues involved in a hyperconjugative sense) which somewhere along the reaction coordinate forms an intermediate.*

A postulate reached in terms of this approach is that the ratio of the displacing ability of a lyate ion (involving *direct* bonding of the ion with the residue considered, *e.g.* carbon or hydrogen) over that of its conjugate solvent molecule should be a sensitive function of, among other factors, the extent of bond formation in the transition state between the residue and the ion.

The extent of bond required for reaction with the lyate ion is of course different from that required for reaction with the solvent. However, the com-

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* NOTE added in this reprinting: As seen in this (DEMO 2) and earlier papers (DEMO 1 and ref. 1) the «unified mechanism» comprises as one of its very features also the recent views on S_N2 (intermediate) or ion-sandwich mechanisms[a-c] proposed fifteen years later.

a. T.W. Bentley and C.T. Bowen, *J.C.S. Perkin II,* 557 (1978).

b. T.W. Bentley and P.v.R. Schleyer, *J. Amer. Chem. Soc.,* **98,** 7658 (1976).

c. F.G. Bordwell, and T.G. Mecca *J. Amer. Chem. Soc.,* **97,** 123, 127, (1975); F.G. Bordwell, P.F. Wiley, and T.G. Mecca *ibid.,* **97,** 132 (1975).

parison is not between these two bonds, but with the corresponding bonds to another substrate as the substrate is changed. The ratio k_S/k_{SH}, where k denotes the rate constant of the reaction with lyate ion S and solvent SH respectively, should *decrease sharply* as the required bond formation decreases. In fact, beyond a certain point the *solvent could possibly become a better nucleophile than its lyate ion.*

Another argument for the above postulate* from the classical viewpoint can be given in terms of solvation effects. In order for a lyate ion to form a bond to a residue (whether to carbon or hydrogen) it has to become less solvated. This loss is two-fold. One part is due to the blocking effect of the organic substrate hindering solvation on one side of the ion. This is a steric effect. The other part is a loss associated with the bonding of the lyate ion to the residue considered. This bonding results in a decrease in the nucleophilic potential (approximated by the charge) of the anion with a consequent decrease in the solvation energy of the ion. The ratio of the change in solvation energy over the percent of bond formation required to reach the transition state in each case is expected to increase with a decrease in this percent. This conclusion is based on the second power dependence of the solvation energy on the charge of the ion.

In considering the ratio of the overall loss of solvation energy over the extent of bond formation, ratio should start from a large value and decrease sharply as the extent of bond required increases. Even for very weak bond formation there still is large loss due to steric hindrance to solvation. On the other hand, if a solvent molecule is considered to function as a nucleophile, the trend should be reversed, that is the ratio of the energy required because of the charge developed on the solvent molecule and the hindrance to solvation over the extent of bond formation should start from a low value (the solvent solvates the polar bond of the substrate in the initial state any way) and should increase sharply as the extent of the required bond increases. There is thus expected to be a region of extent of required bond formation where the solvent could be preferred over the lyate ion. What was said about lyate ions should apply to other ions as well but variations are expected** (because of solvation differences and other factors).

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* This postulate results from the application of the unified approach to the concept of nucleophilicity which has been discussed only briefly.[2] Since reactions are treated in this approach as multicyclic processes[2,4] a nucleophile cannot be assigned a fixed relative nucleophilicity because this is expected to be a function of a number of variables (suggested by the unified model) which vary greatly. One of these variables greatly affecting relative nucleophilicities is the *extent* of bond formation between the nucleophile and various centers of the substrate as well as that of bond rupture between the nucleophile and the medium (extent of desolvation) required to reach the transition state. Thus even reversals of relative nucleophilicities as well as of leaving group abilities are expected with a variation of these and other factors.

** The effect must be more pronounced with lyate ions, as *e.g.* those of water of alcohols, since these are expected to involve stronger partial bonds to the medium than many of the common nucleophiles.

It is difficult to cite experimental evidence for the above postulate because the reactions belonging to the region of the proposed reversal of relative nucleophilic reactivities are reactions which are termed carbonium ion reactions. However, some experimental support can be obtained by studying the trend in the ration $k_{\text{lyate ion}}/k_{\text{solvent}}$ as the extent of the required bond formation in the transition state decreases and assuming that this could be extrapolated further to the region of small extents of bonding to the nucleophile.

The k_{OH^-}/k_{H_2O} rate ratio for the hydrolysis of some esters and acid halides with hydroxide and water can serve for this purpose. These reactions have been shown to be bimolecular.[25] The ratio k_{OH^-}/k_{H_2O}* is equal to $10^{10.4}$, $10^{7.5}$, $10^{7.8}$, and $10^{6.0}$ respectively for ethyl acetate, acetyl fluoride, benzoyl fluoride, and benzoyl chloride respectively,[26] but only $10^{4.2}$ and $10^{3.0}$ for methyl bromide and ethyl *p*-toluensulfonate. It is therefore obvious that the ratio can vary enormously (a ten million fold change from $10^{10.4}$ to 10^3). It is also apparent that the ratio is much larger for acyl halides than for alkyl halides.

These data[27] suggest** but cannot prove on their own that the major cause for these large differences is the variation in the extent of the bond to the incoming nucleophile. The decrease of this ratio in the above sequence is what one might expect for a decreasing extent of bonding to the nucleophile. Thus, with respect to the leaving group, the ethoxy group for example is more difficult to remove than chloride as is fluoride compared to chloride, so that the extent of bond required to the incoming nucleophile should be larger in the former cases. The bond required is also expected to decrease in the order primary >secondary> tertiary as far as substrate structure is concerned.

If the drop in the k_{OH^-}/k_{H_2O} ratio in going from RCOX to CH_3X hydrolysis is attributed mainly to the difference in the extent of bond formation, the magnitude of this drop would make it reasonable to expect that further reduction in the extent of the bond could result in a substantial still further decrease of the ratio, even though the substrate may still require the formation of bonds (partially covalent) to nucleophiles in order for the reaction to take place. If furthermore the difference in the concentration of H_2O *vs* OH^- is also taken into account (study of the ration k_{OH^-}/k_W , $k_W = k_{H_2O}\,[H_2O]$) the decrease in the observed effect may be even more pronounced. Thus, the value of the ratio k_{OH^-}/k_W for the hydrolysis of ethyl benzenesulfonate[23a] is only 11.

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* Where $k_{H_2O} = k_W/[H_2O]$, k_W being the observed rate with water as solvent.

** It should be noted that under the prism of the unified theory,[27] the hydrolysis of esters or acid halides does not differ mechanistically in any qualitative way (it differs only quantitatively) from displacement reactions on saturated carbon. This allows the comparison of data between the reactions of the two types of substrates.

The large decrease in the ratio from the RCOX compounds to the RX ones cannot be interpreted in terms of a decreased selectivity of the substrate associated with a decrease in activation energy (although this factor plays its role also). Thus, e.g., the activation energy for the solvolysis of methyl bromide is not smaller but much larger than that for the solvolysis of benzoyl chloride.[26]

The use of isopropyl benzenesulfonate should result in a decrease of this value on the basis of the postulate. Hydrolysis of this substrate (in 36.5% aq. acetone) is actually unaffected by the addition of hydroxide[28] and has been concluded[28] to be a unimolecular reaction on the basis of this criterion. This constitutes an illustration of the effect this criterion has had on the thinking in reaction mechanisms. However, on the basis of the previous considerations this reaction does not have to be unimolecular in spite of these observations. In fact, according to the theory, the reaction center in the intermediate formed in this reaction (specifically the first formed intermediate) is expected to be involved in extensive total bonding (including covalency) to its environment as well as in intramolecular bonds. *The intermediate is expected to bear resemplance to what is called an S_N2 transition state.*

Emphasis has been placed above on the criterion of the effect of added nucleophiles on the rate of a solvolysis because other kinetic criteria supporting the concept of carbonium ions are also based on this one. This was also done in order to suggest that in nucleophilic displacements lyate ions are not necessarily better nucleophiles than solvent molecules, and that there may also be other cases in which this may be so, as discussed, *e.g.*, in the solvolysis of bridgehead halides.[29]

The mass-law effect and the more general observation of the trapping of carbonium ions by strong nucleophiles constitutes another general kinetic criterion. This criterion is based on the appearance of added strong nucleophiles in the product of a reaction and in their simultaneous lack of appearance in the rate expression (other than as a salt effect) for the reaction. This is taken to suggest that a rate determining step preceeds the attack by the added nucleophile and it is understood that this step is an ionization step *differentiated* from the displacement one (the second step) because if nucleophilic attack were involved in this step the added nucleophile by being a much better one would appear in the rate expression. Thus, this criterion depends indirectly on the first kinetic criterion discussed above, and specifically on the belief that if nucleophilic attack is involved the added anionic nucleophile has to be a better nucleophile than the solvent, which however was argued not to be valid. Thus, this second criterion as well does not necessarily prove that the first step, in all the cases in which the criterion applies, does not involve displacement proceeding to a limited extent and that it differs mechanistically from the second one, namely that the first step is an ionization whereas the second one is a displacement one. The fact that the strong nucleophile is in some cases preferred over the solvent in the second step but not in the first one can be understood without the use of a carbonium ion mechanism if it is realized that the transition state leading to the product by being further along the reaction coordinate than that leading to the intermediate should involve more extensive bonding to the incoming nucleophile. An enchanced difference in the reactivity between strong nucleophiles and solvent molecules in the second step over that in the first step is thus expected on the basis of the postulate discussed above. Below a certain extent of bond required in the first step the solvent may behave as a better nucleophile than the strong nucleophile. This, extent, however, may be considerable and need not be a mere solvation effect (it is suggested that the added nucleophile can displace the incoming solvent from the intermediate as has been suggested earlier[1]).

That this criterion provides evidence for the formation of some intermediate but not necessarily of a carbonium ion is substantiated by the fact that the criterion is positive when applied to the solvolysis of substituted benzyl tosylates,[30]* for which available evidence shows that it cannot proceed by a unimolecular or carbonium ion reaction,[31,32] a conclusion in agreement with the unified mechanism.

4. Evidence from product composition.

Several criteria for carbonium ion intermediates, both kinetic and stereochemical, are based on the expected geometry of a carbonium ion center, namely a planar one because of sp^2-hybridization due to a vacant or more or less so *p*-orbital. Experimental evidence for the planarity of such intermediates has thus provided support, by a reversal of the above reasoning, to the possibility of formation of intermediates having a carbon center with the above features. Evaluation of whether this evidence does support a carbonium ion intermediate in the cases in which the criterion has been used is not possible by means of a general discussion as the present one. However a general comment can be made. It results from the fact that the geometry at both an olefinic carbon atom and at a carbon atom in the transition state of an S_N2 displacement reaction is expected to be planar or nearly so. Thus even if the carbon center of an intermediate bears very little resemblance to a carbonium ion, namely even if it involves extensive bonding to leaving and incoming groups and/or to the rest of the molecule (*e.g.*, partial double bonds to adjacent carbon atoms), planarity or near planarity would still be the preferred geometry by the intermediate.

Some of the most direct evidence for the planarity of carbonium ions has come from kinetic data which have shown that carbonium ion reactions are greatly retarded if the reaction center is prevented from reaching planarity.[5,10] Thus bridgehead derivatives have been found to be very uncreactive to solvolysis reactions and this unreactivity has been attributed[5,10] to the fact that a carbon atom having six bonding electrons is more stable in a planar form (sp^2) than in a non planar one, the difference in energy between the former and a tetrahedral carbonium ion (sp^3) being estimated theoretically to be of the or-

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* The reactions of benzyl derivatives have been a subject of several questions as to their mechanistic interpretation and their correlation with the Hammett equation.[30-32] This system and its reactions is an example illustrating the creation of unnecessary problems by the concept of carbonium ions and the classical mechanisms.

der of 24 kcal. This interpretation is based on the picture of a bridgehead carbon atom bearing a unit or very large charge.

The interpretation offered by the present theory and discussed elsewhere[29] is that the observed uncreactivity and trends in these derivatives are to be interpreted instead as being due chiefly to an increase in the charge forced to develop on the bridgehead carbon because of unavailability of rearside bonding to the medium (even a small increase in charge is argued to be greatly resisted) and to the strain associated with the requirement for planarity, this planarity however being required to some extent in order to help intramolecular nucleophilic contribution to the bridgehead carbon rather than simply because of an electron deficient carbon atom. In short the classical approach assigns a large charge on the bridgehead carbon proper whereas the unified one assigns a small fraction of unit charge to that carbon.[29]

With respect to the evidence derived from the stereochemical outcome of reactions, little can be discussed in general terms except for the comment that many of the usual systems which have been used for mechanistic studies on carbonium ions such as saturated secondary systems or benzylic systems give on solvolysis mostly or essentially inversion of configuration a fact which speaks for itself against the concept of a carbonium ion intermediate. In general, stereochemical data do not support the concept of carbonium ions. Another general comment is that a number of reactions involving partial racemization may behave so by way of the formation of more than one intermediate differing substantially from a carbonium ion, as could be for example the case of some solvolyses in mixed solvents. Similar stereochemical results may be understood in some cases as a delicate balance between backside *vs.* front-side substitution again from an intermediate substantially different from a carbonium ion. This may be the case with, *e.g.*, deamination reactions as discussed at the end of this paper. The stereochemical outcome does not have to be necessarily associated with any one type of mechanism.

Rearrangements make up a group of reactions on the basis of which the concept of carbonium ions was put on a firm foundation originally[4]. However, as has been pointed out before[1-3] a rearrangement (on carbon), just as many other observations interpreted in terms of carbonium ions, can be interpreted as a step involving displacement on carbon thus breaking various partial bonds (that to an incoming group or outgoing group, or to the rest of the molecule) in which this carbon atom is involved, thus not requiring the concept of a carbonium ion for their interpretation. In general, a rearrangement, just as any other reaction process, is treated by the present approach as being the result of the operation of paths. In focusing attention to the section of the path containing the residue (generally carbon) bearing originally the migrating group, this residue has to be supplied with extensive total bonding from intra and intermolecular sources in order for migration to take place. The smaller the possiple intramolecular contribution the larger is expected to be the required intermolecular one. This offers also an interpretation of the ability of 1,3-

interactions (hydride shifts and cyclopropane formation) to compete with the more exothermic 1,2 rearrangements and proton eliminations in deoxidations and nitrous acid deaminations as compared to the lack of their occurrence in solvolyses.[8] Thus *e.g.* in addition to a possible effect of the energetics of these reactions and of the nature of their intermediate, the 1,2 rearrangement could be more difficult in deaminations as compared to solvolyses thus giving a chance to 1,3-rearrangements to take place. This happens because of the poor ability of the leaving group to contribute nucleophilically to the β-carbon atom in the case of deaminations, and also to the deleterious effect to such contribution by neighboring solvent molecules resulting from the positive charge on nitrogen. The difference of the effect of the leaving group in contribution (*e.g.* by the medium) to the γ-carbon atom could be much smaller and so should be its effect on 1,3 rearrangements.

These displacement - rearrangements are visualized as occuring in the unified intermediate complex and their rate may vary between extremes. The rate can be extremely large as concluded by the application of the mechanism to certain reactions such as those usually represented as proceeding by way of symmetrical non-classical intermediates.[33] Similarly, intermolecular displacements of these partially bonded groups are also normal reactions of the intermediate complex,[1,3] their rates varying between extremes.

B. The question of formation of intermediates.

The comments made above on the classical criteria for the formation of carbonium ion intermediates, several of which may be obvious or not new, lead to the conclusion that the evidence for the formation of carbonium ion intermediates constitutes actually evidence only for the formation of *some* intermediate at the most but does not require that this intermediate be a carbonium ion. However, according to the unified mechanism, all usual solvolysis reactions proceed as a rule by way of an intermediate *any way* and if this is correct, then the above criteria do not even offer any *mechanistic* information other than simply information on *e.g.*whether the intermediate can be trapped with the particular nucleophile in the particular solvolytic system employed. A spectrum of possible experimental observations is possible depending on the system and nucleophile used, but this does not have to be interpreted in terms of different mechanisms. Thus, several cases can be distinguished: a) The nucleophile may not appear at all in the product. This means that compared with the added nucleophile, the solvent is a powerful enough nucleophile to provide the nucleophilic contribution required by the particular substrate to reach the first and second, and more demanding, transition states which lead to the intermediate and the product respectively. This nucleophilic contribution will be to the most demanding of the many points of attack on the substrate. b) The nucleophile may appear in the product but not directly in the rate expression other than as a so-called salt effect. This means that compared to the added nucleophile the solvent is powerful enough for the nucleophilic requirements of the first transition state but not so for those of the second one c) The nucleophile may appear in both the product and directly in the rate expression. This means that the solvent is not powerful enough as compared to the added nucleophile to provide even the nucleophilic contribution required to reach the first transition state of the solvolysis reaction. In employing more and more demanding substrates '(in a nucleophilic sense) in a solvolysis reaction, the difference between the nucleophilic requirements of the first and the second transition states is expected to become smaller and for this and other reasons (*e.g.*, extent of the bond to the incoming solvent in the intermediate) the intermediate in the cases discussed above is expected to become progressively more difficult to be trapped until it may conceivably not be capable of being recognized experimentally.

In all the above cases, the mechanistic model has remained the same, the only thing that has changed are the species involved in the attacks and the relative contributions through the various paths.

That an intermediate is expected to form in the usual solvolysis reactions[2,3] follows from the principles of this approach and will be discussed in detail elsewhere[34] as the postulate of the «etiology for the formation of intermediates». The formation of an intermediate is considered to be a consequence of two factors, the one of interest in this discussion being that most chemical reactions are considered by this approach to proceed by the simultaneous operation of a multitude of paths of transmission of nucleophilic forces. Thus in solvolyses, «indirect paths» (and «side paths»[2,3] in certain substrates) are said to operate in addition to the «direct path» (displacement type) in order to decrease the energetics of the direct one. They are expected to reach their maximum contribution roughly around the point of the reaction coordinate of maximum energy requirement of the direct path (the transition state if the process were a pure S_N2 process). The superposition of the curve representing the decrease in the energy of the system due to the contribution of the indirect paths on that of the energetics of the direct displacement reaction could be anticipated to result in an energy minimum. It is understood of course that the larger the number of the indirect paths and the easier their energetics as compared to those of the direct path the larger the energy dip. Thus the formation of an intermediate in the usual solvolysis reactions is expected to be the rule whether it is detectable or not.

C. The Unified Mechanism for Cases for which the Carbonium Ion Concept is Applied.

Extensive evidence for carbonium ion intermediates seems to be deduced in the chemical literature by the successful, as is believed, application of the carbonium ion concept to a large number of problems of limited or general nature. These cases will be brought up individually in applying the unified mechanism to chemical data. This will allow for an evaluation of whether the concept of carbonium ions and the classical mechanisms represent a useful approach to the study of chemical reactions. A large number of extensive discussions will be required for this purpose and thus what is dealt with in the rest of this paper represents an extremely limited application to experimental data.

There is an ever increasing realization that various reactions assumed[23a] to be carbonium-ion reactions are actually ion-pair reactions.[23c] A recent example is the solvolysis in acetic acid of a secondary sulfonate (2-octyl tosylate) which was assumed to be a carbonium ion reaction in terms of the usual criteria but was shown recently to proceed by way of an ion-pair mechanism.[35]

Such trends, apparent throughout the recent chemical literature, amount essentially to a correction of the earlier conclusions reached in terms of the carbonium ion approach. Furthermore, these corrections represent a large volume of work since they deal with each chemical system individually. The problem, however, lies at the root, namely in the approach to solution chemistry in terms of the concept of carbonium ions and carbanions, and any ions for that matter. The above trends constitute also one step toward verification of the predictions of the unified theory which discards the use of the concept of ions in studying reaction mechanisms in solution and according to which even deamination reactions - some of the most notoriously carbonium ion reactions[8,9a] - had been argued as early as 1958[1] not to proceed by way of a carbonium ion intermediate but by way of the unified intermediate complex.* It has always been inconceivable, as far as this approach is concerned, that a secondary carbonium ion can be formed in acetic acid and that the first formed intermediate in acetic acid can have the tosylate group removed.

There has appeared recently a sudden precipitation of papers in which it is concluded that the chemical behavior of a carbonium ion may depend more than generally expected on the mode of its generation and the leaving group employed.[37,39] Such conclusions, however, are essential features of the unified approach,[1,3] and constitute one of the reasons for having argued strongly for the substitution of the carbonium ion concept by that of the intermediate complex.

The recent realization that the leaving group and the solvent can affect, in the so called carbonium ion reactions, the extent of elimination as compared to substitution,[39] as well as the type of olefin formed,[38] and that the leaving group can compete with the medium for the abstraction of a proton from the intermediate,[38] as well as the discussion of the factors affecting this behavior,[38,39] are also inherent in the unified mechanism and had been discussed in the

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* With respect to the function of the leaving group, the «intermediate complex»[2,3] resembles to some extent but only to some extent an ion pair. There are also objections to the term ion-pair. The term was introduced in the carbonium ion field to describe what it actually means, namely a pair of ions[36] not involving covalency and it is its use which has changed its meaning to cover such intermediates whether they involve or not some covalency. Thus the term helps emphasize further the carbonium ion image which has strongly influenced the thinking in reaction mechanisms.

original publication[1] and recently[2,3] and will appear in detail elsewhere. The recent conclusion[39] that elimination may be another general reaction which may proceed by way of undissociated ion-pairs has long ago been predicted[1] and has been inherent in the unified mechanism according to which the intermediate complex has been suggested to be capable of giving *directly* elimination products.[1-3] The factors controlling *cis* and *trans* elimination from the intermediate complex have also been discussed[1-3] in part and will appear in detail elswhere.

Some recent work[14] has led to the suggestion that some solvents such as dioxane and acetone called «inert», because they have been believed not to participate as reagents in solvolyses, may actually be functioning as nucleophiles. These conclusions, however, are inherent predictions of the present theory and have often been pointed out in various ways.[1-3] They follow from the «non-ionic» concept introduced by the theory. Chemical systems in solution are said to use all possible means in order not to form a carbonium ion-like carbon residue. To prevent this formation, covalent nucleophilic contribution to the carbon center from both intramolecular and intermolecular sources is anticipated to a relative degree governed by their energetics. On this basis even *saturated hydrocarbons* would be expected to function as nucleophiles if necessary (lack of other nucleophiles, forced formation of a species that could otherwise have a carbonium ion-like carbon atom etc). It is consequently incoceivable, as far as this approach is concerned, that an oxygen containing solvent could allow the formation of such a carbon center without functioning as a nucleophile (acetone and dioxane could not possibly be inert). If this were the case it would amount to a violation of the concept of the «gradient»[2,3] because it would allow the development of substantial charge (actually of electrophilic potential) on a carbon atom without the development of the proper charge (controlled by the gradient in this system and by other factors) on the oxygen residue in contact with it, by way of covalency between the carbon and the oxygen atom. This would amount to a large and abrupt decrease in the charge in going from the carbon atom toward the medium.

As was mentioned earlier in this paper the carbon center (and other residues of the reacting substrate such as hydrogen atoms) «sees» primarily, according to this theory, atomic residues of molecules of the medium, e.g. an oxygen atom of a solvent molecule, secondly the molecules to which they belong, and only thirdly the solvent as a whole namely as a medium having a dielectric constant. This picture is the reverse of that of an ionization process (formation of ions in a dielectric medium or of ions stabilized by dipoles).

In general, the boom in the realization in the last few years of the importance of the solvent in chemical reactions has been anticipated[1-3] in terms of the present theory. The importance of the solvent and its specific effects constitute a direct consequence of the non-ionic concept and of the basic principles of the theory as will be illustrated in detail elsewhere. The lack of their earlier realization is again claimed to have been the result of the classical approach itself.

Some recent data of the solvolysis of 2-octyl brosylate in aqueous dioxane and in methanolic acetone[14] are in agreement with the unified mechanism but there is basic disagreement between the interpretation offered by the above mechanism and the one advanced by the authors.[14] It was found that whereas the solvolysis of optically active brosylate in 75% aqueous dioxane gave the inverted alcohol of only 77% optical purity, the same solvolysis conducted in the presence of a proper concentration of sodium azide gave the inverted alcohol (formed now in competition with the azide) in 100% optical purity. These results were interpreted as suggesting that the solvolysis reaction follows two distinct mechanisms in the S_N1 - S_N2 sense. One of them was said to be a one-step S_N2 displacement by water giving the inverted alcohol and the other was assumed to involve the formation of an intermediate, believed to be either a solvated carbonium ion or an oxonium ion trapped when azide was added giving either racemic or inverted alcohol respectively.

As mentioned more than once, according to the unified approach, although various reactants may be involved the solvolysis is expected to proceed by way of one mechanism giving rise to an intermediate complex which reacts further. In examining the possibility of an S_N2 displacement by the medium, this approach suggests that, because of the tendency of the system to follow a course with an energy minimum and because of the energetics of the direct path which are influenced by both steric effects and the fact that charge is developed on the incoming group, the reaction is expected to derive assistance from the operation of indirect paths. This assistance is further facilitated by the nucleophilic action of the leaving group on the rest of the molecule. The fact that these paths do operate is supported by the observation of a secondary β-isotope effect in the hydrolysis of β-deuterated isopropyl tosylate ($\Delta\Delta F^* = 44$ cal/mole)[41]* Because of the operation of indirect paths the formation of an intermediate (the intermediate complex) becomes a reasonable expectation with the ease of its trapping expected to increase from ethyl to *t*-butyl if the series *n*-propyl, isopropyl, and t-butyl derivatives were under study. Thus, although attack by the medium is expected in the solvolysis the process cannot be a direct one but has to go through an intermediate.

In examining the second mechanism suggested by the authors, *i.e.* the possibility of formation of a solvated carbonium ion, the unified theory suggests that the large resistance to the emptying of a *p*-orbital on carbon, combined with the limited capacity for covalent nucleophilic contribution through the indirect paths (only two methyl groups are involved) renders necessary the supply of substantial total nucleophilic contribution (involving ex-

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* β-Hydrogen isotope effects have been interpreted[1,3,40] by this approach as being the result of the operation of indirect paths which is in fair agreement with the hyperconjugation picture as the cause of secondary β-isotope effects.[41]

tensive covalency) to the carbon atom from the leaving group and incoming solvent group. In short, the system does not have a choice to form or not a carbonium ion. This is so because of the requirement for extensive filling of the carbon *p*-orbital. Thus, if one wanted to imagine that the leaving group were completely broken off in the intermediate, the «non-carbonium ion concept» would require very extensive covalent bonding between the carbon atom and the solvent (since the supply by the two methyl groups can be only limited) a situation which would automatically have brought the system far along the formation of the product and the reaction could not stop there to render it an intermediate. There is another way of putting this argument: A reaction intermediate, according to this theory, and more specifically according to the resulting «postulate of the etiology of the formation of intermediates», has in principle nothing in common with an ion and vice versa an ion has nothing in common with a reaction intermediate. The actual intermediate is the species corresponding to the energy minimum along the reaction coordinate and this minimum is, according to the postulate, formed by the superposition of curves which are in principle such that the nature of the corresponding complex *has to be different* from that of an ion.

Thus, one mechanistic model, the unified one, has to be applied. This cannot be forced, as done by the classical approach, to be either a carbonium ion reaction or a direct S_N2 one for either the total or a fraction of the reaction.

On the basis of this theory, the simultaneous operation of an S_N2 and of a carbonium ion mechanism in borderline solvolyses is not a reasonable possibility, a suggestion which is in agreement with some views on the subject[13] and in disagreement with others.[14] Room for something resembling duality would exist not in the borderline but in the extreme region in which the intermediate complex formed, by having a very small energy dip, would resemble a transition state so much that some molecules could possess enough energy to give the product directly. But such statistical deviations cannot be referred to as a second mechanism. Besides, these would be systems for which the detection of the intermediate would not be easy anyway.

The above dicussion is also repeating the suggestion made by this approach concerning the usual solvolysis reactions according to which they all give an intermediate complex capable in principle of a number of further transformations to other intermediates or products.

In the solvolysis of the above brosylate in aqueous dioxane, the intermediate complex whether formed with water or dioxane functioning as the incoming nucleophile and acting on the carbon (or, one fraction with water and the other with dioxane), is expected when reacting further (either as such or by exchanging the incoming solvent molecule with another, water or dioxane, or by reacting with them directly) to give rise to the alcohol, possibly protonated, from its reaction with water or to an oxonium intermediate from its reaction with dioxane. And whereas the former is the product (or could readily give it by loss of a proton), the latter requires further reaction with a nucleophile (*e.g.*

solvent or the added azide ion) at the seat of the original reaction before giving a stable product. Furthermore, the azide could possibly intervene in the intermediate complex state in addition to reacting directly with the brosylate.

The more extensive the total bonding of the reaction center to its environment in the intermediate complex of a solvolysis (incoming and outgoing groups combined) the larger can be the expected stereospecificity of the reaction. The large specificity observed in the part of the reaction proceeding by reaction with water supports the existence of extensive bonding to the environment and thus supports the arguments against the development of a substantial charge on carbon, and the formation of a carbonium ion intermediate.

On the basis of the present considerations it is seen that the data do not necessarily support a dual mechanism* and that according to this interpretation the difference is *not one of mechanism but of reactants* (water *vs* dioxane). Analogous considerations are expected to apply in the case of the solvolysis in ethanolic acetone although here the difference in the behavior of the two nucleophiles (alcohol and acetone) may be more pronounced than in the water-dioxane medium since the nucleophilic end of acetone is a ketonic one. However, the reaction with acetone is not necessarily a direct displacement as suggested[14b] and the formation of an intermediate complex is considered to be a reasonable expectation as the first step of the reaction.

A class of reactions in which the concept of carbonium ions is manifested in its fullest extent are deamination reactions. Thus even «free»[8] (unsolvated) and «hot»[9] (non-resonating) primary, and essentially methyl[9a] carbonium ions are postulated as reaction intermediates.[8,9] Therefore the interpretation of deamination reactions should be a subject of large difference between the two approaches which should be most pronounced as the structure of the substrate is varied toward that suggested to form primary and methyl type carbonium ions in deaminations.

The present approach suggests for deaminations the same mechanistic principles proposed for all reactions in general. And, although certain reaction courses can be rendered very unlikely there are uncertainties as to the exact course of the reaction. These uncertainties arise mainly from the possible involvement of two reactants acting on the substrate (the diazomium intermediate RN_2X), namely the molecules of the medium and the anionic leaving group X. Some difficulty is also introduced by the presence of two leaving groups, the X and the N_2. At first the possible courses of the reaction will be

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* While this paper was being completed, another publication by the some authors appeared[42] in which some of the discussion presented in one of their earlier papers[14a] has been modified in the direction of what is proposed in this paper but not enough as to modify the mechanistic principles suggested previously.[14] Thus the S_N2 reaction has not been excluded and the suggestion of a dual mechanism apparently still remains in effect although it is not used in the discussion of their new data «for reasons of simplicity and symmetry». Furthermore a difference is obviously implied between the formation of the intermediate referred to as an ion-pair and the process involving nucleophilic attack on the starting material.

examined* when solvent and not the group X functions as a reactant.

The reaction course that has been argued against[1] on the basis of the unified mechanism is *that* involving the formation of an intermediate having a carbonium-like carbon atom («hot» and unsolvated or carrying a unit positive charge or anything approaching that kind of magnitude). This constitutes the main objective of the discussion on deaminations which follows. The suggestion of a possible definite course for the reaction is a means to this end.

It follows directly from the principles of this approach that such intermediates can not to be formed.*

Instead, the final intermediate responsible for the formation of products by the C-N bond rupture, is suggested to be, for the usual substrate structures, a species involving only partial C-N bond rupture (referring to the intermediate complex suggested[1] to accompany the C-N bond rupture reaction). This will be briefly discussed further. Attention is focussed on the course of the reaction involving rupture of the C-N bond. The part involving rupture of the N-X bond and whether this is partial or complete is not the focal point of this discussion on carbonium ions.

This subject has been discussed in 1958[1] and it was suggested that the reaction could be expected to proceed by way of an intermediate complex in which the carbon-nitrogen bond is only partially ruptured.* The rupture of the carbon-nitrogen bond is expected to be accompanied by nucleophilic contribution to the carbon atom by way of the direct and indirect paths.*

This operation of a multitude of indirect paths is reasonably expected to result in the formation of some intermediate complex on the basis of the postulate of the «etiology for the formation of intermediates»,[34]* and the postulate of the gradient which stands behind it.

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* The present discussion on deaminations refers to those which proceed as non-radical reactions. Although most deamination reactions are considered to be of this type[43] further study is needed especially since there are systems such as deaminations in pentane[44], where the data are not those expected by the application of the unified mechanism to a non-radical process.

* One reason for this is that a gradient in the nucleophilic or electrophilic potential which includes charge is said to be established as a rule in all directions. Thus, the occurence of a chemical phenomenon with such a large deviation from the optimum gradient for the particular medium and structure of the substrate employed, as would be the case if these «hot» and unsolvated carbonium ion intermediates were formed, is not an acceptable possibility, at least in principle.

* If this proves to be the case it will add further arguments against the thinking behind the concept of ion-pairs (see p. 26); it will also add support to the postulate of the etiology of intermediates[34] according to which an intermediate is not to be associated with the concept of ions or with that of preconceived fixed types of structures as done by the classical approach.

* If this were not so, the postulate of the gradient (occurence of the reaction so that the gradient in the nucleophilic or electrophilic potential along the paths, intra- and intermolecular, be as close as possible to the optimum for the particular system) would be violated.

* This postulate provides information on the probable structure of the intermediate and its position along the overall reaction coordinate as well as on which one of the two steps (the one to the intermediate or that to the product) is the probable rate determining one. Similar conclusions

Therefore, these postulates suggest that this intermediate complex is, not a carbonium ion and that in certain aspects (for example in the C-N bond elongation or in the *ratio* of the total intramolecular bonding of carbon, in excess of three, over its total bonding to its environment, outgoing and incoming group included, the suggested intermediate complex in deaminations may deviate from being or behaving as a carbonium ion more so than the intermediate from the corresponding solvolyses (of, *e.g.*, the tosylate). This possibility follows from the nature and partial positive charge of the leaving group and its effect as suggested by the postulate of the gradient. The above possibility would render deaminations more concerted (in some respects) and less carbonium ion reactions than the corresponding solvolyses, which turns out to be the reverse of what is believed.[8,9,37]

In the case in which the anionic X group may function as a reactant (while still associated with or partially broken off from the substrate) it could do so at the above discussed intermediate complex stage or at the diazonium intermediate stage (prior to the severance of the C-N bond). The former case is covered by the previous discussion (the same intermediate is formed). The latter possibility will not be detailed here, specifically whether the formation of an intermediate is expected or not. It will be discussed elsewhere in applying the unified mechanism to the reactions referred to as S_Ni. In either case, however, the intermediate is not allowed to be a carbonium ion.

Another possible special feature of deamination reactions to consider is that the nature and charge of the leaving group may render frontside nucleophilic contribution (by the leaving anion X acting directly or through solvent molecules) competitive with backside contribution. This possibility is suggested by the cyclic nature of reactions suggested by the unified mechanism.[2,3] On this basis, any lack of stereospecificity in the reaction could not necessarily mean, and be used as evidence for, the formation of a carbonium ion intermediate. In fact, a delicate balance between frontside and backside bond formation with a noncarbonium ion intermediate can explain* such results.

An evaluation of the predictions of the non-carbonium ion concept *vs* those of the carbonium ion concept with respect to deaminations can be obtained by an examination of available experimental data.

The stereochemistry of substitution in deaminations is said to support the carbonium ion mechanism for these reactions.[43] The stereochemical results however can also be understood in other ways, one of which is the delicate

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are arrived at by the application of the postulate of the gradient. These postulates suggest also that the greater the resemblance of the structure of R to that of a primary system the greater the deviation of the intermediate in these deaminations from that of a carbonium ion structure and the greater its similarity to the transition state leading to it. Thus the intermediate might even become of no practical significance in extreme cases and the system may behave as undergoing a direct displacement reaction.

* The leaving group X and the solvent, in addition to the structure of the substrate, should play an important role with respect to this «delicate balance».

balance between frontside and backside bond formation mentioned above. In fact an examination of the stereochemistry of the substitution product in the alicyclic series reveals a high degree of stereospecificity. Thus, in the cyclopentane series the results obtained vary from extensive inversion to predominant retention.[45] In the steroid series the reaction proceds with 100% retention of configuration (in 50% aqueous acetic acid) from either equatorial or axial amines.[46] In the decalin series the reaction proceeds with 100% retention from the equatorial epimer and with about 90% inversion from the axial epimer.[47] The above data are understandable in terms of a non-carbonium ion intermediate.* The change in the stereochemical outcome of the reaction with the system studied without loss of stereospecificity supports the view of the possible existence of a delicate balance and thus of this being the possible cause of the apparent racemizations in some systems in the acyclic series rather than the formation of a carbonium ion intermediate.

The formation of elimination product in deaminations has been assigned a carbonium ion mechanism and ground-state control from this ion is said to provide an adequate interpretation of the data.[43] However, the data in the rigid cyclohexane series (in the decalin and the steroid series) do not support this interpretation. Thus equatorial amines yield no elimination product whereas axial ones give a large amount of olefins (50% to 100%)[46,47] Here, as contrasted to the acyclic series, ground-state control from a carbonium ion could not explain the difference in the behavior of the two epimers since there is no question of rotation. The carbonium ion from either epimer would be expected to yield the same amount of olefin (assuming of course the formation of a planar carbonium ion). The above difference in behavior is however, anticipated[1] by the non-carbonium ion interpretation. The intermediate from the axial amine, due to the larger proximity to coplanarity** (smaller dihedral angle) between the

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* Whether the data are understood better in terms of the intermediate complex having a partially ruptured C-N bond or the diazonium intermediate itself will not be argued in this paper. The main interest here is the comparison with the carbonium ion intermediate.

** Elimination reactions have been argued[1-3] in terms of the unified mechanism to depend mainly on two factors. The first and very important one is the complanarity between the carbon-leaving group bond and that of the β-carbon-hydrogen bond in the intermediate complex or the unified model[2,3] in general, and *not necessarily on a trans arrangement* of these groups. This importance and its extent has recently been shown for bimolecular eliminations[48] (a measure of coplanarity is the dihedral angle between the two bonds, being larger the smaller the deviation of this angle from 0° or 180°). The second factor has been said to be the extent of interaction and affinity of the *leaving or incoming groups* with the corresponding β-hydrogen atoms in the intermediate complex.[1-3] This second factor could also be expected, as discussed earlier,[1] to work against elimination from the equatorial amine due to the N_2 leaving group which is a very poor base (cannot interact with the axial β-hydrogens) as compared to solvent molecules and has actually an electrophilic potential which would not facilitate the removal of a proton in its neighborhood. Although unlikely, the first leaving group X (from RN_2X), when it is still attached in the intermediate, could conceivably introduce uncertainties in this argument. Another complication could stem from the fact that both factors seem to hinder elimination from the equatorial amine rendering difficult an exact prediction as to which one, and to what extent, is the more important one in this system. The factor of coplanarity should generally be the more important one.

carbon-leaving group bond and the C-N bond as compared to that of the intermediate from the equatorial amine, is expected to yield more olefin (the transition states should resemble these intermediates).

Considerably different behavior* is observed in the solvolysis of the epimeric 4-*t*-butylcyclohexyl tosylates in either formic or acetic acid, or in 60% aqueous acetone.[49] Here there is small difference in the extent of elimination from the two epimers, the axial tosylate yielding about 85% of olefin and the equatorial one about 70%. This difference between the deamination and the sulfonate solvolysis supports the previous suggestion that the former may deviate from being a carbonium ion reaction even more than the solvolysis of sulfonates.

The deamination of *α*- and *γ*-methylallylamines in acetic acid[9a] is of interest. The two amines did not yield the same acetate product mixtures as for the silver ion catalyzed solvolysis of the corresponding halides in acetic acid, but yielded largely the unrearranged allylic acetate (80% from the primary amine and 67% from the secondary). To reconcile these data with the carbonium ion interpretation of deaminations, as much as 50% of the reaction was argued[9a] to proceed by way of a non-resonating or «hot» carbonium ion intermediate in which the p-orbital at the α-carbon forms an angle with the π-orbital of the double bond carbon atoms (this amounts to suggesting that an intermediate carrying a full positive charge on carbon is formed). Such a «hot» ion was considered to yield only non-rearranged allylic acetate, the rest of the reaction proceeding by way of a resonating carbonium ion intermediate. Extensive racemization in the deamination of optically active *α*-methylallylamine provided evidence for the carbonium ion mechanism.[9a] The limited rearrangement, however, can be understood by a non-carbonium ion nature of the intermediate in the usual deamination reactions. Also, a competition between formation of the bond to the nucleophile with retention or inversion can account for the observed extensive racemization. Thus the data are interpreted by this approach without the need to invoke carbonium ions.

Another source of evidence for the formation of carbonium ion intermediates in deaminations is argued[43] to be the multiplicity of products obtained, but this behavior, as argued earlier in this paper, does not necessitate the assumption of a carbonium ion intermediate. The ground state control of migration in deaminations[43] is also understood in terms of the suggested non-

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* The behavior of the sulfonates as compared to that of the amines can be understood in terms of a greater deviation in the former system of the structure of the non-carbonium ion intermediate from that of the starting material. These deviations result in such a product determining transition state that the dihedral angle between the axis of the leaving group and the C_β-H bond in the solvolysis of the equatorial sulfonate is decreased compared to the angle in the deamination reaction. In the case of the axial derivative, the deviations result in an increase of the same angle. Both these factors favor the observed difference in the behavior between deamination and the sulfonate solvolysis. The second factor affecting eliminations (interaction and affinity for the β-hydrogen atoms) would also favor a similar trend and thus one cannot argue convincingly that the behavior is due mainly to the first factor.

carbonium ion intermediate since the greater the resemblance of the intermediate (and thus of the transition state) to the starting material the greater the expected ground state control.

There also seem to be some difficulties within the «hot» and unsolvated carbonium ion interpretation of deaminations itself anyway. On one hand this ion is concluded to be capable of very rapid intramolecular rearrangement prior to its reaction with the medium even in the case of a primary,[8] thus unhindered system. On the other hand, such an ion formed without need for intramolecular contribution is postulated[9b] to explain the classical carbonium ion nature of the intermediate in deaminations[9b,37] and its behavior as such[9b,37b] (successful competition of substitution product formation with rearrangement). This successful competition of product formation compared with rearrangement in secondary[37] and relatively more hindered and rigid systems, such as the norbornyl system,[37a] cannot be reconciled with the unsuccessful competition of product formation with rearrangement in the case of primary and unhindered acyclic ions. In these acyclic substrates product formation would be expected to gain in importance over rearrangement as compared to the former systems, namely the reverse of what is observed, if these species were of the same type, that is, free and hot as suggested in the literature.

The highly exothermic nature of deamination reactions[37a,50] is apparently the basis for the acceptability of the formation of «hot», «free», and highly energetic carbonium ions in deaminations. However, according to the postulate of the «etiology for the formation of intermediates»,[34] the nature of an intermediate is not controlled so much by the overall energetics of the reaction but mainly by the relative contribution of the direct path as compared to the other paths involved. Thus, the important factors are the structure of the substrate, the nature of the leaving group and its effect on the gradient, and the nature of the medium, whereas the ease of breaking the carbon-leaving group bond results as playing the lesser role (it all follows from the postulate of the gradient as well).

The very exothermic nature of deamination reactions is substantiated in the literature by estimates of the energetics of the reaction $RN_2^+ \rightarrow R^+ + N_2$ which for R = *sec*-alkyl and in the gas phase gives $\Delta H = -3{,}6$ ev.[37a] This number would be expected to be considerably smaller if a «hot» carbonium ion (not involving internal resonance stabilization) or primary[8] or, even more, methyl type[9] carbonium ion were formed (the difference in the ionization potential between a *sec*-alkyl radical and a methyl radical is *ca* 2 *ev*). In considering deaminations in solution the substrate is not necessarily the RN_2^+ species but may have only a partially ruptured N-X bond or be an ion-pair $RN_2^+\,X^-$ especially in the usual deamination medium, acetic acid.[44] Even if the intermediate were to be the solvated RN_2^+, in view of the large values of solvation energies, solvation of the R^+ would also be necessary in order for the reaction to remain exothermic.

However, according to this approach, the *solvation model* of a charged atomic residue (carbon here) involves extensive covalency and thus the R^+ would have to be non-«hot». This example illustrates also the misleading influence of solvation models and of the pitfalls in the classical approach due to the approach itself.

The above lead to the conclusion that the formation of «hot», «free», and/or primary carbonium ion intermediates may deviate greatly from being a *highly* exothermic process.

One of the interesting areas of application illustrating the difference in the results obtained from the non-carbonium ion *vs* those from the carbonium ion concept is that of the field of non-classical carbonium ion intermediates. The description of the intermediate not as an entity (a carbonium ion or an ion pair in which the cationic part is generally treated as an entity interacting with the anionic counterpart by a geometric arrangement expected from a pair of ions, namely symmetrical) but as the unified intermediate complex[1-3] with its multitude of *fractional and specific* bonds and bond ruptures, renders the intermediate automatically unsymmetrical (as described[1-3]) and thus the representation of the intermediate system by a single mesomeric symmetrical species emerges in principle as unjustified. This has been suggested[2,3] to apply as a rule to intermediates formed in the usually employed systems and media whether the group forming the bridge is a *hydrogen* atom, a simple *alkyl* residue, or a *phenyl* group (some special considerations of a difference in degree have to be made here) and whether these are considered in the aliphatic or in the aromatic series. Another way of expressing this is by saying that the first formed intermediate, from these and other systems discussed elsewhere,[33] has to be, as a rule, unsymmetrical. On the basis of this approach, even if the chemical behavior of a system suggests the formation of a single symmetrical intermediate the system still has to be described by unsymmetrical ones. Whether in addition to these unsymmetrical ones the symmetrical structure represents an intermediate or a transition state as well as a detailed discussion of this subject as a whole and evidence for these conclusions appear elsewhere.[33]

One interesting correlation between the above subject and deaminations is the following. One of the reasons for arguing against the concept of a single mesomeric non-classical intermediate is the refusal of the present theory to accept the carbonium ion picture and study of intermediates. On this basis the larger the deviation of an intermediate obtained from a substrate having a given carbon skeleton (*e.g.* by a change in the leaving group) from the picture of a carbonium ion, the larger may be expected to be the deviation of the behavior and of the resemblance of this intermediate from that of a non-classical intermediate, and more specifically from that of a single mesomeric symmetrical intermediate. Actually more factors, such as the intramolecular cyclic interactions of the unified model enter into the picture.[33] On this basis and on the basis of the suggestion that deaminations may deviate from being carbonium ion reactions even more so than solvolyses, coupled with the poor function of the N_2 leaving group in intramolecular cyclic interations,[33] deaminations should deviate more than solvolyses from behaving as proceeding through a symmetrical non-classical intermediate. Experimental data on deaminations[37,9b] are in agreement with this prediction.

One of the most inner features of a carbonium ion and thus a minimum prerequisite for such a species, is that the relative and absolute charge distribution be governed and thus determinable by quantum-mechanical principles concerning the ion itself. On the other hand, the representation of actual reaction intermediates by a unified intermediate complex is expected, because of the features of this complex,* to lead to a distribution of charge (both relative and absolute) substantially different from that calculated for a carbonium ion. This remains to be shown although it seems to be rather obvious. If even this is the case, there is little left for the concept and the picture of a carbonium ion to stand on since it is difficult to find any feature of actual reaction intermediates that is really *characteristic* of a carbonium ion, and only of a carbonium ion, or any feature that suggests that these intermediates are entities (referring always to the usual reaction intermediates and not to some exceptional structures). The only feature that seems to be common to such reaction intermediates is that they carry *some fractional charge* on their organic skeleton but this is also a feature of the transition state of many apparently direct displacement reactions as well as starting materials.

The objective of the discussion presented in this paper was to overcome the obstacles to arguing against the intervention of carbonium ions imposed by the criteria which have established the carbonium ion concept. This was done only to some extent and further evidence will be needed. A strong emphasis is placed on the non-carbonium ion picture because it is one of the essential starting points, according to the theory presented, for reaching conclusions on reaction mechanisms. It will be shown in future papers** that the abandoning of the picture of ions and the use of the unified theory provide an answer to most past and present problems of reaction mechanisms as has been suggested before.[1-3]

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* Extensive covalencies and specific and fractional bonds.

** In the reinterpretation of organic reactions in the light of the unified theory suggested in 1958[1] and carried out recently[2,3] any aspect of the field which was not in agreement with the predictions of the theory has been questioned regardless of how well established it was. This had as a result the questioning of a large number of reaction mechanisms covering organic solution chemistry, of interpretations, and of concepts of the classical approach as they were established in 1958. It will require several years in order for these conclusions to appear in print.[51]

Περίληψη

Ἡ ἔννοια τῶν καρνοβιόντων σὲ διάλυμα ὑπὸ τὸ πρίσμα τῆς ἑνιαίας θεωρίας

Ὁ σκοπὸς τῆς παρούσης δημοσιεύσεως εἶναι νὰ τονισθῆ ξανὰ καὶ νὰ ἀναπτυχθῆ περισσότερο μία πρότασις ποὺ ἔγινε ἀρχικὰ τὸ 1958[1]. Ἡ πρότασις συνίσταται στὴν ἐγκατάλειψη τῆς ἐννοίας τῶν καρβονιόντων (καὶ ἄλλων ἰόντων) καὶ τὴν ἀντικατάσταση αὐτῆς μὲ τὴν ἔννοια τῶν «ἑνιαίων ἐνδιαμέσων συμπλόκων» ποὺ ἐλέγχονται ἀπὸ τοὺς κανόνες τῆς ἑνιαίας θεωρίας.[1-3] Εἶναι μέρος τῆς γενικώτερης προτάσεως τοῦ νὰ μελετηθῆ ἡ χημεία διαλυμάτων βάσει τῆς ἑνιαίας θεωρίας ποὺ ὁδηγεῖ σὲ μία ἑνιαία μέθοδο μελέτης τῶν μηχανισμῶν ἀντιδράσεων καὶ τῆς προτάσεως νὰ ἐγκαταλειφθοῦν τὰ κλασσικὰ μηχανιστικὰ πρότυπα καθὼς καὶ οἱ μηχανιστικοὶ συλλογισμοὶ τοῦ πεδίου ποὺ ἔχουν συνδυασθῆ μὲ αὐτά.

Ἕνας ἀπὸ τοὺς ἰσχυρισμοὺς ποὺ προέρχονται ἀπὸ τὴν ἑνιαία θεωρία εἶναι ὅτι ἡ ἔννοια τῶν ἰοντικῶν ὀντότητων σὲ διαλύματα καὶ τὰ χαρακτηριστικὰ τῶν μηχανιστικῶν προτύπων ποὺ χρησιμοποιοῦνται καθὼς καὶ ἡ συνήθης μέθοδος μελέτης τῶν μηχανισμῶν, ναὶ μὲν συνεισέφεραν στὴν ἀνάπτυξη τοῦ πεδίου ἀλλὰ ταυτόχρονα δημιούργησαν περιττὰ προβλήματα καὶ παρεμπόδισαν τὴ λύση πραγματικῶν προβλημάτων.

Ὑπογραμμίζονται μερικὲς ἀπὸ τὶς διαφορὲς μεταξὺ τῆς ἐννοίας τῶν καρβονιόντων καὶ τῆς προτεινομένης ἐννοίας τῶν μὴ-καρβονιόντων.

Ἐξετάζονται ὑπὸ τὸ πρῖσμα τῆς ἑνιαίας θεωρίας οἱ κύριες πηγὲς μαρτυριῶν ποὺ ἑδραίωσαν τὴν ἔννοια τῶν μεταβατικῶν καρβονιοντικῶν ἐνδιαμέσων ὡς χημικῶν ὀντότητων. Συμπεραίνεται ὅτι οἱ περισσότερες ἀπὸ τὶς πηγὲς αὐτὲς παρέχουν μαρτυρία μόνο γιὰ τὸν σχηματισμὸ τὸ πολὺ «κάποιου ἐνδιαμέσου» ἀλλὰ ὄχι ὑποχρεωτικὰ γιὰ τὸν σχηματισμὸ κάποιας ἰονικῆς ὀντότητας. Προβάλλεται ὁ ἰσχυρισμὸς ὅτι ἡ εἰσαγωγὴ τῆς ἐννοίας τῶν καρβονιόντων σὲ μεγάλη ἔκταση, εἶχε ἐπιβλαβεῖς ἐπιπτώσεις παρὰ τὸ γεγονὸς ὅτι ὁ ἰσχυρισμὸς εἶναι προφανῶς τὸ ἀντίθετο μιᾶς ἀπὸ τὶς πιὸ ἰσχυρὲς πεποιθήσεις τοῦ πεδίου καὶ συγκεκριμένα τῆς σημασίας τῆς ἐννοίας τῶν καρβονιόντων στὴν ἀνάπτυξη τοῦ πεδίου τῶν μηχανισμῶν.[23d]

Σὰν παράδειγμα, ἡ θεωρία ἐφαρμόζεται σὲ δύο μόνο συστήματα (ἀντιδράσεις ἀπαμινώσεως καὶ τὴ διαλυτόλυση ἑνὸς δευτεροταγοῦς σουλφονικοῦ ἐστέρος) μεταξὺ τῶν πολυαρίθμων χημικῶν συστημάτων γιὰ τὰ ὁποῖα οἱ προβλέψεις της εἶναι ἀντίθετες ἀπὸ τὶς ὑπάρχουσες στὴν βιβλιογραφία.

Οἱ μηχανιστικοὶ συλλογισμοὶ ὅπως αὐτοὶ ἐμφανίζονται στὴν βιβλιογραφία κατὰ τὰ τελευταῖα χρόνια ἀποτελοῦν βήματα πρὸς τὶς προβλέψεις τῆς ἑνιαίας θεωρίας, λίγα ὅμως μόνον παραδείγματα ἀναφέρονται εἰς τὸ παρὸν κείμενο. Σημειώνεται ὅτι ἀπαιτεῖται μεγάλος ἀριθμὸς ἄρθρων γιὰ νὰ ἀναπτυχθοῦν οἱ προβλέψεις τῆς θεωρίας καὶ ἡ ἐπαλήθευσις αὐτῶν.

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IN VITRO RADIOIODINATION OF NUCLEIC ACIDS WITH HIGH SPECIFIC ACTIVITY AND MINIMAL DEGRADATION

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Abstract

A modification of Commerford's iodination procedure is described where iodine is incorporated into nucleic acids through a stable covalent bond as 5-iodocytosine. According to this method solutions of polynucleotides containing $NaClO_4$ are heated at 60°C in the presence of thallic trichloride plus iodide at pH 4.7. Sodium perchlorate is used to maintain single strand denaturation during the iodination procedure, whereas $TlCl_3$ catalyzes the reaction. A second heating step at pH 7.5 follows the first one and this facilitates the removal of unstably bound iodine. Factors affecting the iodination reaction are discussed in detail. Several physiochemical studies using iodinated as well uncreacted DNA indicate that the iodination reaction does not induce significant alterations in the macromolecule.

Introduction

In the seven year period following the publication of Commerfod's procedure (1-2) for the *in vitro* iodination of nucleic acids, this method has been employed in a wide diversity of applications (3-5). Our interest for radiolabeled polynucleotides arose from preliminary studies showing that ^{131}I-DNA and ^{125}I-DNA accumulate in a variety of tumor tissues and have the potential for a useful tumor-imaging agent. In order to investigate further these results, it was necessary to have at hand a labeling procedure which could be applied to a wide variety of polynucleotides, would provide high specific activity, avoid degradation of the native nucleic acids and produce radioiodinated products with high *in vivo* and *in vitro* stability against dissociation of the label. Commerford's method has been reported to be associated with some not well understood factors resulting in undesirable properties for the purpose of our studies (6-7).

In this work a modification of Commerford's labeling procedure (1) is described which minimized the damage to native nucleic acids while maximizing the extent of iodination. A detailed discussion and experimental data are provided for several factors that affect the iodination efficiency. The physicochemical properties of iodinated and unreacted nucleic acids are compared.

Reagents and solutions

All reagents were analytical grade. The water used was doubly distilled.

Nucleic acid denaturing solution, 0.1 M acetate buffer, pH 4.7, containing 10 M sodium perchlorate ($NaClO_4$). The solution was made by dissolving the $NaClO_4$ in a mixture of equal parts of 0.1 M sodium acetate and 0.1 M acetic acid, and adjusting the pH to 4.7. This was stored at 4°C up to fifteen days and filtered through 0.22 µm Millipore filters before use.

Thallic trichloride ($TlCl_3$), reagent grade, was stored at 4°C in 0.1 M solutions. These were replaced monthly. Working solutions were made in 0.1 M acetate buffer, pH 4.7, and filtered through 0.22 µm Millipore filters before use.

Potassium iodide (KI) stock solution 1 M, was stored at 4°C. Working solutions were made in 0.1 M acetate buffer, pH 4.7.

Radioiodine, $Na^{125}I$, 350-600 mCi/ml, containing 1.8×10^{-4}M iodine at a concentration of 400 mCi/ml, and carrier free $Na^{131}I$, 600-950 mCi/ml, were obtained from New England Nuclear Co., Boston, Mass. Each shipment was tested for radionuclide purity, and in addition the buffering capacity per unit volume of solution was determined by titrating the amount of base in a small aliquot with bromcresyl green indicator solution (0.04%). Prior to use in radioiodination the pH of the radioiodine solutions was adjusted close to 4.7 by adding predetermined amounts of an acidification reagent made of a mixture of 3 ml 0.3 M HNO_3, 5 ml 0.1 M sodium acetate buffer (pH 4.7) and 4 ml water.

Dialysis buffer was made by mixing 0.4 M NaCl and 2×10^{-4}M ethylenediaminetetraacetic acid in 0.01 M phosphate buffer, pH 7.5.

Heat-denatured DNA was prepared by heating at 100°C for 5 minutes a calf thymus DNA water solution of 10 mg/ml and fast freezing at −20°C.

Methods

Purification of nucleic acids. A solution of nucleic acid (0.2 mg/ml) was shaken two times with an equal volume of freshly distilled phenol saturated with 0.01 M potassium phosphate buffer, pH 7 (approximately 35 g of buffer per 100 g phenol). The final aqueous nucleic acid layer was extracted 3 times with 4 volumes of diethyl ether followed by bubbling dry nitrogen through until it was free of ether fumes. After the nucleic acid was precipitated by adding 5 M lithium chloride, the gelatinous residue at concentration 10 mg/ml was suspended in 0.15 M sodium chloride – 0.015 M sodium citrate buffer, pH 7, and centrifuged at a cesium sulfate step-gradient at 35,000 g for 24 hours at room temperature (22°C) in a Spinco SW 65 rotor (8-11). Following centrifugation the nucleic acid band was recovered, dialysed overnight at 4°C against 0.1 M NaCl, lyophylized and stored at −20°C in the dark.

Concentrations of polynucleotides, expressed as molarities (1), were calculated from the measured ultraviolet absorption of the polynucleotide stock solutions at pH 7 using the values for morar extinction coefficients reported in the literature (12-15).

Modified radioiodination method. The concentrations of mucleic acids, KI, radioiodine, and $TlCl_3$, used are fiven in the text. Unless otherwise specified, a 1 ml reaction mixture (pH 4.7) containing 6M $NaClO_4$ and desired amounts of KI, $Na^{125}I$ or $Na^{131}I$, polynucleotides, and $TlCl_3$ was heated for 10 minutes at 60°C immediately after addition of $TlCl_3$. The reaction was cooled in ice; the mixture was chromatographed at 4°C on precalibrated Sephadex G-50 (fine) column 50×2cm; and eluted with dialysis buffer (pH 7.5). The iodinated polynucleotide- containing fractions were combined and concentrated overnight at 4°C by vacuum dialysis against 1 liter dialysis buffer solution. Then the buffer was replaced with preheated buffer at 60°C and both buffer and the mixture in the dialysis sac were heated for 1 hour at 60°C. Finally, the dialysis buffer was replaced with 0.9% NaCl and the nucleic acid was dialyzed for 12 hours at 4°C and stored at −20°C in the dark.

Commerford's radioiodination method. A mixture, 2 ml volume, containing 6-7 × 10^{-4} M (as nucleotides) polynucleotides, 2.5 × 10^{-4} M KI, 2 μCi of ^{125}I, and 1.5 × 10^{-3} M $TlCl_3$ in 0.1 M ammonium acetate – 0.04 M acetic acid (pH 5), was heated for 15 minutes at 60°C. Then the reaction mixture was cooled in ice, mixed with 0.1 ml of 0.1 M Na_2SO_3 and 0.4 ml of 1 M ammonium acetate – 0.5 M NH_4OH to raise the pH to 9, heated for 20 minutes at 60°C and chromatographed on Sephadex G-50 (fine), and eluted with 0.1 M ammonium acetate. The ultraviolet absorption at 260 nm and the net radioactivity was determined for each fraction. The iodinated polynucleotide - containing fractions were combined and the polynucleotides were concentrated by vacuum dialysis.

Specific activity and degree of polynucleotides. The specific activity of the radiolabeled products was calculated in dpm/μg. In cases where the concentration of the iodinated product was too small to be determined spectrophotometrically, the specific activity was approximated from the initial amounts of the polynucleotides added to the iodination reaction mixture assuming a complete recovery of the nucleic acid during the iodination steps (16).

The percent of radioiodine incorporated into polynucleotides was determined by measuring the activity of an aliquot of iodinated products and comparing it to the initial activity.

The counting efficiency was determined by means of a calibrated ^{129}I source and was generally 30-50%.

Density gradient sedimentation equilibrium (8-11). Studies were carried out using 3-5 μg of nucleic acids, and 4 ml of CsCl solution (1.71 g/ml) containing 0.01 M tris-HCl buffer, pH 7. The final density of the mixture was adjusted to 1.71 g/ml.

Melting temperature (Tm). DNA samples at a concentration of approximately 20 μg/ml were melted in stoppered 1 cm pathlength square cuvettes in a Gilford spectrophotometer with a temperature programmer to monitor the absorbance of four cells at 260 nm.

The Tm values corresponded to the midpoint of the absorbance rise obtained from a linear plot of absorbance versus temperature increase values (17).

DNase I - snake venom digest of iodinated DNA (18-19). Two ml ^{131}I-DNA solution, 7×10^{-4} M as nucleotides, were digested first for 90 minutes at 37°C in 5 mM $MgSO_4$ and 100 μg/ml DNase I, then overnight at 37°C with 0.5 mg/ml snake venom (crotalus adamanteus) at pH 9. The pH was raised using 1 M NH_4OH prior to snake venom addition. The nucleoside digest was chromatographed on a Sephadex G-10 column (90×1.5 cm) precalibrated with albumin, deoxycytidine, thymidine, deoxyadenosine, ^{125}I-deoxycytidine and deoxyguanosine (20) and was eluted at a flow rate of 30 ml/hr, controlled by a peristaltic pump, using 0.01 M citric acid – Na_2HPO_4 buffer, pH 3.5. Fractions of 3.0 ml were collected, and the absorbance at 260 nm as well as the radioactivity in cpm were determined.

Results and discussion

The iodination method described in this paper is a modification of that described by Commerford (1-2), where iodine was incorporated mainly into the position 5 of cytosine of polynucleotides, through the use of thallic chloride ($TlCl_3$) as an oxidizing agent to form a stable covalent bond (5-iodocytosine). Other iodination procedures such as those using ^{125}ICl (21) or N-iodosuccinimide (22) were found unsatisfactory for further study. The first method required conversion of the nucleic acid into a salt soluble in organic solvents and led to degradation. The second was inconvenient and provided low specific activity products.

In summary Commerford's method involved heating at 60°C single-stranded polynucleotides in an aqueous solution containing KI, Na^{125}I and $TlCl_3$ at pH 5.0, chilling the mixture to 0°C, and termination of the iodination reaction by reheating the mixture at 60°C with raised pH (~9.0) in the presence of the reducing agent Na_2SO_3. Although this method was simple to perform, it has been reported that it is associated with some unknown factors which sometimes may affect the specific activity, stability and uniform labeling of the iodinated products or in certain cases may introduce chain scission of nucleic acids as a consequence of the labeling procedure (1, 3-7, 16, 23-26).

In an attempt to overcome some of these difficulties, several changes were introduced to Commerford's method, which allowed iodination under milder conditions and produced increased specific activity and stability of radioiodinated products. a) The heat denaturation step required by Commerford's method for double-stranded nucleic acids prior to iodination was eliminated. Instead, the iodination reaction was performed in a concentrated sodium perchlorate ($NaClO_4$) solution, which allowed continuous denaturation of nucleic acids during iodination without interfering with the reaction. This change, in addition to simplifying the procedure, allowed homogeneous conditions of iodination for a large number of nucleic acids irrespective of melting temperature (Tm) values. b) The iodination reaction was terminated by cooling, raising the pH to neutral, instead of nine, and removing the unreacted iodide and other salts present by gel chromatography and dialysis. This step was not recommended in Commerford's procedure; however, it was introduced in order to eliminate unnecessary substances during the critical second heating step. c) The Na_2SO_3 reducing agent was eliminated in order to minimize risks of deamination and deiodination of the polynucleotides. d) Finally, the second heating step was done at pH 7.5, instead of 9, and the iodinated reagents were incubated in a dialysis bag in order to facilitate the removal of unstable iodine from the iodinated products.

Table I shows a comparison of the amount of radioiodine incorporated into polynucleotides following Commerford's and the modified iodination method described in this work. More than 20% increase in the specific activity was obtained in the majority of the cases.

Parameters Affecting the Iodination Reaction

Nucleic acid solutions. Contamination of nucleic acid preparations with substances capable of reacting with iodine, such as proteins, or with reducing substances which may inhibit labeling, such as sugars, is undesirable. As a consequence special precautions should be taken in the purification of polynucleotides when radioiodination is to be performed. However, purification steps should be performed with special concern for the fact that increasing the complexity of a procedure to purify nucleic acids also increases the tendency to introduce chain breaks (12).

Several methods of purification were investigated and led to buffer saturated phenol extraction followed by salt precipitation with lithium chloride and centrifugation in cesium sulfate gradients (11-14, 23). The phenol reagent should be freshly distilled in order to eliminate oxidation products and other contaminants. Two extractions removed about 60-80% of 100 µg highly purified iodinated human serum albumin (^{125}I-HSA) added to a control sample of RNA solution (0.2 mg/ml). Multiple extractions with phenol were avoided since the method is considered inadequate to completely remove proteins and, furthermore, may cause fragmentation of nucleic acids (27-28). The precipitation of nucleic acids with lithium chloride removed 30-50% the ^{125}I-HSA that remained after phenol extraction, but RNA collected in the gelatinous residue was iodinated more effectively, increasing iodine incorporation as much as 5-8 fold, indicating that some reducing agents may have been removed in this step (23). Additional purification by preparative sedimentation through cesium sulfate gradients eliminated more than 90% of the ^{125}I-HSA that remained after lithium chloride precipitation from the polynucleotide bands. Under these condi-

TABLE I: *Iodine Bound to Polynucleotides in TICl$_3$ Catalyzed Reaction**

Polynucleotides	^{125}I-Polynucleotides (dpm/μg)	
	Commerford's Method	Modified Method
Native calf thymus DNA	4957 (3115-6732)**	28470 (25861-32025)
Heat denatured calf thymus DNA	22017 (18195-24411)	28363 (26371-30515)
E. coli t-RNA	15176 (13268-17150)	20642 (18148-22972)
Chicken 28S r-RNA	14458 (12822-16604)	19653 (17814-22863)
Poly(A)	238 (125-361)	237 (115-311)
Poly(G)	227 (162-414)	287 (186-409)
Poly(C)	35922 (32378-38018)	42256 (40865-44376)
Poly(U)	1521 (1215-1705)	2102 (1904-2311)

* Polynucleotides were iodinated as described in the experimental section using 40-50 μg polynucleotides, 2.5 × 10 M KI, 2 μCi, ^{125}I^{4}; and 3×10 M TICl$_3$. $^{-3}$

** Mean (range) four experiments.

tions proteins remained near the surface of the gradient, whereas polynucleotides were concentrated in bands between $^1/_2$ and $^2/_3$ of the distance from the bottom of the tube. Comparative density gradient sedimentation equilibrium experiments showed no significant alterations of nucleic acids during the purification steps (10-11).

First heating step. Table I shows that native DNA solutions were poorly iodinated as compared to denatured DNA. Thus, initially double-standed nucleic acids were heat denatured at 100°C prior to iodination, as recommended by Commerford (1). Disadvantages of this approach to denaturation include the risk of chain scission (6, 7, 12), the different handling of single and double-standed polynucleotides in comparative studies, and most significantly heat denatured nucleic acids with low complexity may renature during the iodination resulting in heterogeneous products in terms of iodination (6-7). DNA complexity is defined as the number of base pairs present in a nonrepeating sequence (29).

In an effort to find an alternative method of denaturation, several denaturing solvents (7, 29) such as tetraethylammonium chloride, formamide, dimethyl sulfoxide, or sodium perchlorate in varying concentrations were added to the reaction mixture of native calf thymus DNA in Commerford's method. The efficiency of iodination was determined from the specific activity value of iodinated products. It was concluded that concentrated solutions of $NaClO_4$, 5-7 M, allowed iodination of native calf thymus DNA in specific activities comparable to those obtained using heat denatured DNA solutions. Other denaturing solvents inhibit iodination. Table II shows the effect of $NaClO_4$ concentration on the incorporation of iodine into calf thymus DNA. The presence of $NaClO_4$ did not affect basic iodination mechanisms since in an additional experiment it was found that denatured DNA was iodinated identically in the presence or absence of this agent. Probably $NaClO_4$ acted simply as a denaturing agent during the iodination without further participation in the reaction mechanisms. Nevertheless, heat denaturation of double-standed nucleic acids was not necessary in the presence of $NaClO_4$ and therefore this step was omitted in further experiments except when it is specifically indicated that heat denatured nucleic acids were used. Commercial samples of $NaClO_4$ yielded solutions which varied widely in pH (30). Thus, working solutions of $NaClO_4$ were buffered to pH 4.7. It should be emphasized that some pH meters equilibrated slowly (2-3 min) with concentrated $NaClO_4$ solutions. The buffer was filtered prior to its use because lack of filtering resulted in lower radioiodination yields.

The presence of $NaClO_4$ had no effect on the pH requirements of the reaction which lies between 4 and 5 (1, 5). It was decited to conduct the reaction at pH 4.7, which was close to the pK of acetate ion (pK = 4.76), in order to prevent the possibility of deamination, depurination or acid hydrolysis of the polynucleotides at low pH values (31). In this regard it should be noted that commercial radioiodine solutions included significant amounts of NaOH

TABLE II: *Effect of $NaClO_4$ Solutions on the Incorporation of Iodine into Calf Thymus DNA**

$NaClO_3$ (M)	^{125}I-DNA (dpm/μg)	$NaClO_4$ (M)	^{125}I-DNA (dpm/μg)
0	9,924	4.5	20,458
1	13,196	5.0	24,321
2	14,606	5.5	28,632
4	21,350	6.0	26,156
6	28,019	6.5	26,259
8	20,321	7.0	14,621

* Calf thymus DNA (51 μg) was iodinated as described in the modified method using 2.5 × 10 MKI, 2 μCi ^{125}I, 3 × 10^{-3} M $TlCl_3$ and appropriate concentrations of $NaClO_4$ denaturing agent.

which may change the final desired pH of the reaction mixture especially when the reaction is carried out in smaller than 0.5 ml volumes. To overcome this, the buffering capacity per unit volume of the radioiodine solutions was determined in each shipment and appropriate ammounts of acid were added to neutralize it.

The relative concentrations of KI, $TlCl_3$, and nucleic acids were found to influence critically the efficiency of the iodination reaction (24). Optimum KI concentrations were in the range of 2.5 × 10^{-4} – 2.5 × 10^{-5}M. Concentrations higher than 2.5 × 10^{-4}M decreased considerably the iodination yield. Optimum $TlCl_3$ concentrations were 3-12 times higher than the KI concentrations. Finally decreased nucleic acid concentrations were found to increase significantly the specific activity of the radiodinated products. This increase in specific activity was difficult to explain, but it was expected to be related to the mechanism of the reaction and the extent to which the intermediate product 5-iodo-6-hydroxy-dihydropyrimidine proceeds to 5-iodopyrimidine with varing nucleic acid concentrations (32).

Figure 1 shows that the iodination reaction was faster in concentrated $NaClO_4$ than in H_2O. Using Commerford's procedure, the percent of iodine incorporated into polynucleotides depended on the temperature and incubation time. Maximum iodination was obtained at 70-80°C and 60 min incubation time. On the contrary using the proposed procedure iodination was complete within 5 min at 60°C with comparable yield. The advantage of short incubation time at lower temperature was the minimized risk of degradation of the nucleic acids.

Reaction Termination. The iodination reaction termination step as described by Commerford (1) exmploys the addition of Na_2SO_3 to the chilled reaction mixture, raising the pH of the reaction to 8-9, and reheating at 60°C for 20 minutes. The Na_2SO_3 is considered as a safety step (3-5) which was mainly used to reduce volatile components of radioiodine (I_2) to radioiodide

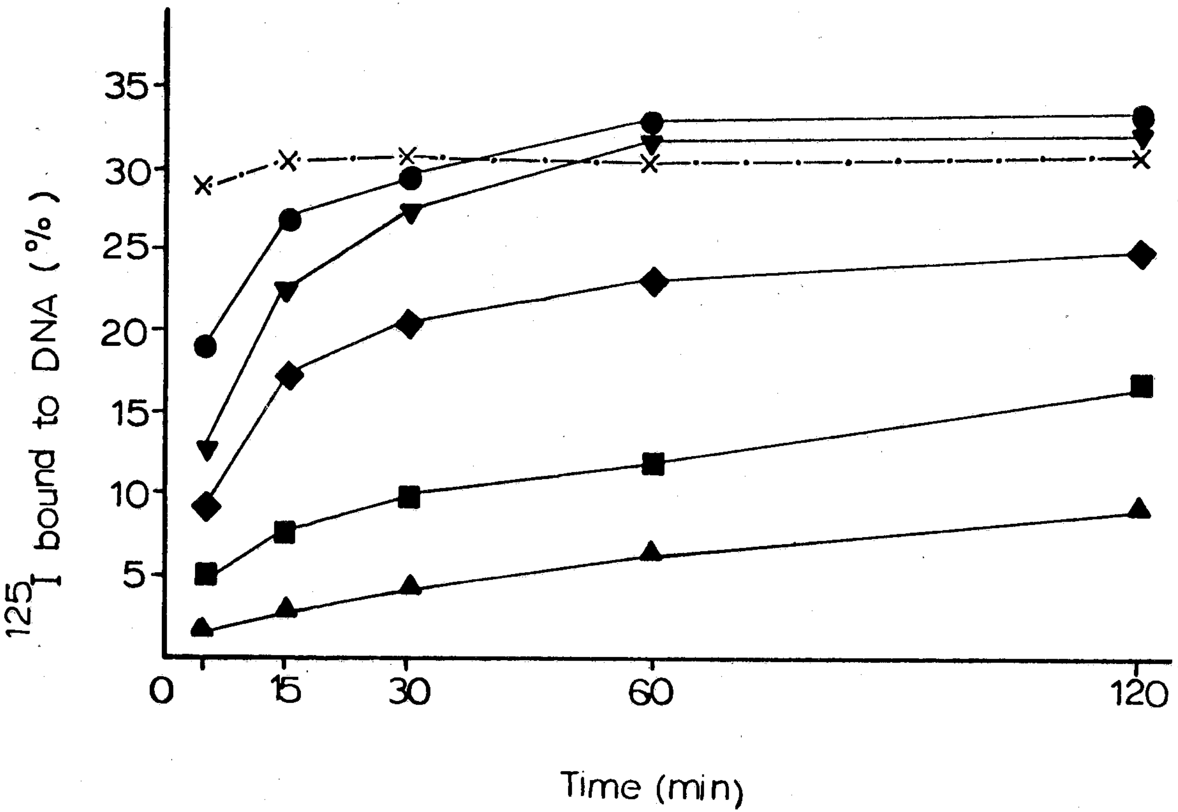

FIG. 1. *Effect of Incubation Temperature and Reaction Time on the Iodination of Calf Thymus DNA.*

Calf Thymus DNA (40 μg) was iodinated following Commerford's method at 40° (-), 50° (■ - ■), 60° (-), 70° (-), or 80° (● - ●), and following the modified method at 60° (X - X). The concentration of the reagents were as described in the legend of Table II.

(I). In the modified procedure, reported in this work, Na_2SO_3 was eliminated because of the disadvantages associated with its use. First under mild conditions Na_2SO_3 may convert cytosine to uracil by deamination (33), thus possibly altering the nucleic acid primary sequence. Second there is a marked instability of iodine atoms associated with uracil residues (1, 2, 24-26) and therefore Na_2SO_3 treatment may result in greatly enhanced deiodination of the nucleic acids. This was confirmed in the study shown in Table III where dissociation of iodine from iodinated poly(C) was observed when ^{125}I-poly(C) solutions were incubated for sixteen hours at 60°C in the presence of increasing concentrations of Na_2SO_3.

TABLE III: *Effect of Na_2SO_3 on the Liberation of Radiodine from ^{125}I-Poly(C) Solutions**.

Na_2SO_3 (M)	^{125}I-poly(C) (% radioactivity lost)
0.01	8.3
0.1	14.8
1.0	28.7
8.0	62.3

* One milliliter of Na_2SO_3 solution containing 10 μg of ^{125}I-poly(C) was incubated at 60°C for 16 hr and then chromatographed on Sephadex G-50 (fine) column.

The pH of the reaction mixture immediately following the first heating step was found to be very important factor influencing both the amount of iodine bound to polynucleotides and the stability of the iodinated products. Table IV shows the effect of pH on the amount of iodine bound to polynucleotides immediately after the first heating step. The study was conducted by observing the percent of the total radioactivity precipated with polynucleotides in the presence of trichloroacetic acid from solutions with pH 4 to 9 after the first heating step. Control solutions of ^{125}I and carrier polynucleotides had less than 0.1% radioactivity in the sediment, after precipitation with trichloroacetic acid, indicating that only iodine bound to polynucleotides was precipitated, whereas free iodine remained in the supernate solution. The results showed that in all cases the amount of iodine bound to polynucleotides was much higher in acidic versus neutral or basic solutions. At neutral or basic pH the percent of radioactivity bound to poly(C) or DNA was constant and independent of incubation time. In the case of poly(U) stable iodination products were obtained when the second heating step was performed at basic pH. Additional studies using dialysis and Sephadex chromatography after the first and second heating steps showed that about 60-70% of the radioactivity was eluted in association with DNA or poly(C) after the first heating step, but only 35-45% remained bound after the second heating step. This amount of radioactivity remained constantly bound during prolonged additional heating at 60°C. In a similar experiment using poly(U) it was found that 40-55% of radioactivity was bound to polynucleotide after the first heating step and this was reduced to less than 5% after the second heating at pH higher than 7. Thus, these experiments indicated that the steps immediately after the first heating one were the critical determinants for efficient incorporation of iodine into polynucleotides in a chemically stable form. In addition, unstable iodine bound to uracil bases required at least a weakly basic environment and 15-30 min incubation at 60°C to remove all unstable iodine. Therefore, in order to have similar iodination procedures for both DNA and RNA, the second heating step was done at pH 7.5 and 60 min incubation time.

TABLE IV: *Effect of pH on the Amount of Iodine Bound to Polunucleotides after the Second Heating Step**.

					Time (min)							
pH**	10	20	40	80	10	20	40	80	10	20	40	80
	% of Radioiodine Bound to Polynucleotides											
	^{125}I-poly(U)				^{125}I-poly(C)				^{125}I-DNA			
4	85	78	68	62	99	95	100	96	98	95	94	92
5	82	54	32	21	92	82	85	80	87	83	81	83
6	30	28	27	12	72	68	69	70	67	63	64	65
7	15	12	8	5	38	35	36	37	36	38	34	37
8	8	5	6	4	39	35	40	38	35	34	35	32
9	4	6	3	2	40	37	34	37	37	32	33	34

* Solutions of poly(U), poly(C) and heat denatured calf thymus DNA containing 40-50 μg were heated at 60°C for 30 min in the presence of 2.5×10^{-4} M KI, 25 μCi ^{125}I and 3×10^{-3} M TlCl$_3$. The mixtures were then cooled; 2.5×10^{-2} M KI was added[2] to stop further iodination and the pH of each solution was adjusted from 4 up to 9 using predetermined amounts of 0.5 N NaOH or HCl. Then the volumes of the solutions were brought to 4 ml by adding appropriate buffer and the mixtures were incubated at 60°C for up to 80 min. During heating 0.1 ml aliquots were removed at 10, 20, 40 and 80 min intervals, mixed with 5 μg carrier polycucleotide, counted and precipitated in 1 ml 10% (w/v) trichloroacetic acid at 4°C (15 min incubation). Thereafter, the precipitates were collected on Millipore RA nitrocellulose filter, washed with 5% trichloroacetic acid and counted. The radioactivity of the precipitate (radioiodine bound to polynucleotide) was compared to that of total radioactivity as follows:

$$\% \text{ radioiodine bound} = \frac{\text{radioactivity in precipitate}}{\text{total radioactivity of aliquot}} \times 100$$

** Buffer solutions with constant ionic strength (I = 0.1) and pH ranging from 4 up to 9 were: Acetate buffer 559 mM, pH 4, and 146 mM, pH 5. Phosphate buffer 81 mM, pH 6 and 66 mM, pH 7. Barbital buffer 23 mM, pH 8 containing 88 mM of NaCl. Basic glycine buffer 626 mM, pH 9.

In the modified method iodinated nucleic acids were chromatographed prior to reheating in order to remove unreacted iodine and other salts present in the first heating step. This was found to improve the physical properties of the iodinated products with respect to the melting temperature and sedimentation profile. Furthermore, during the second heating step iodinated polynucleotides were kept in dialysis bags in order to remove free iodide.

Physicochemical Properties of Radioiodinated Nucleic Acids

Ultraviolet-visible absorption spectra of iodinated DNA at pH 7 showed that the absorption profile was similar to that of unreacted DNA, but it was shifted toward longer wavelengths (10-20 nm) when more than 10% of the cytosine was iodinated. This shift was associated with a decrease in ultraviolet absorption a 260 nm and the appearance of ultraviolet absorption at 310 nm, an area where unreacted DNA did not absorb (34-36). This confirmed the previous similar observation by Commerford (1). Additional experiments indicated that the reduction of ultraviolet absorption at 260 nm was shown to be related to the stability of pyrimidine-iodine bonds. Thus, after the first heating step, where a good percent of the iodine was relatively unstably bound, there was as high as 15-20% reduction of the absorbance at 260 nm versus that of unreacted DNA. On the contrary iodinated DNA obtained after the second heating step showed only 2-4% decrease of absorbance. Both iodinated products had similar absorption at 310 nm which was 85-95% smaller than the absorbance at 260 nm, but it was distinct enough to be considered as a property of heavily iodinated DNA. Heavily iodinated t-RNA showed a similar absorption pattern.

In a comparative study testing the influence of the iodination reaction procedure on some physical characteristics of DNA, ^{131}I-DNA samples were reincubated under the iodination reaction conditions of Commerford's method and modified iodination method, without labeled iodine. After reaction completion the sedimentation pattern (8-11) as well as melting profile (17) of the iodinated compounds were determined. Figure 2 shows a comparison of sedimentation profiles of ^{131}I-DNA before and after reiodinations. Reiodination by the proposed method provided a sedimentation profile similar to that of unreacted ^{131}I-DNA, whereas Commerford's method provided a pattern indicating evidence of degradation (spread of radioactivity).

Table V shows the melting temperature (Tm) values (37) of the unreacted as well as reiodinated ^{125}I-DNA. These correspond to the midpoint of the absorbance rise when heated DNA solutions changed from double-standed structures (native) the denatured state (single-standed). Both unreacted and reiodinated ^{125}I-DNA showed Tm values similar to native DNA (37). In addition, they had close Tm values after renaturation and remelting processes. In the case of ^{125}I-DNA reiodinated using Commerford's procedure a 4-5% decrease of the Tm values versus native DNA was found.

Thus, the modified method provided higher iodination yields than Commer-

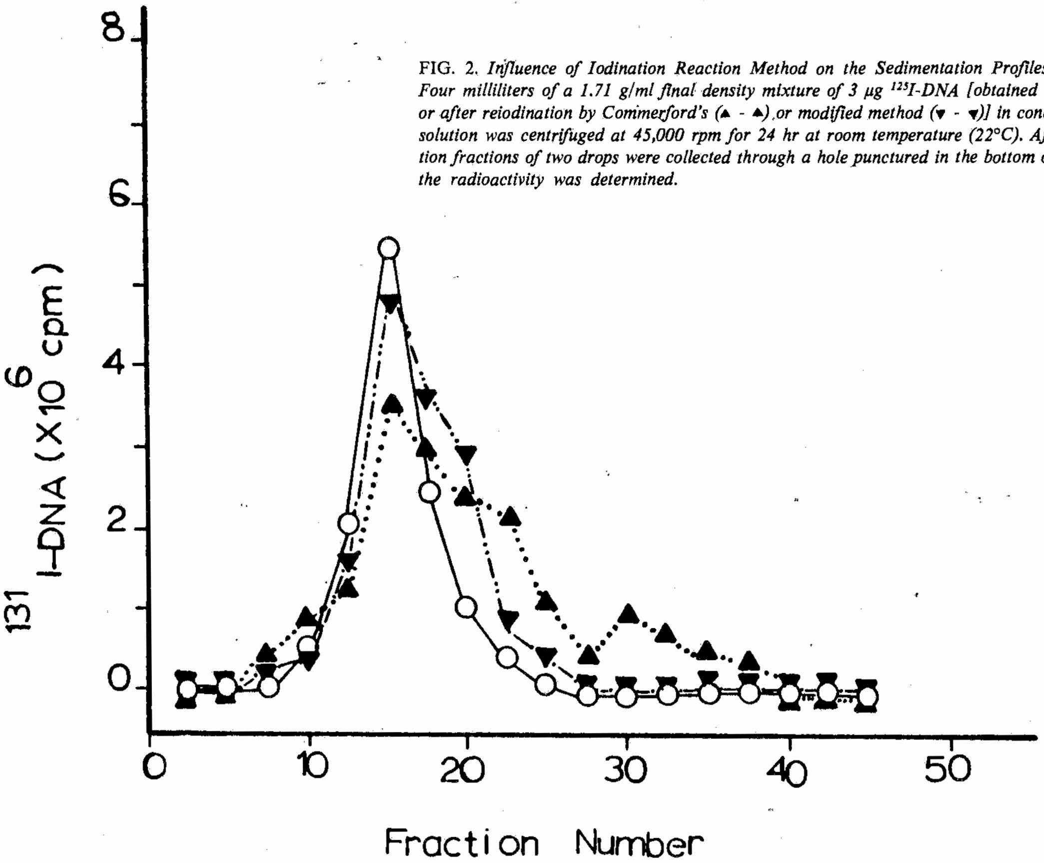

FIG. 2. *Influence of Iodination Reaction Method on the Sedimentation Profiles of ^{125}I-DNA. Four milliliters of a 1.71 g/ml final density mixture of 3 μg ^{125}I-DNA [obtained before (O - O) or after reiodination by Commerford's (▲ - ▲) or modified method (▼ - ▼)] in concentrated CsCl solution was centrifuged at 45,000 rpm for 24 hr at room temperature (22°C). After centrifugation fractions of two drops were collected through a hole punctured in the bottom of the tube and the radioactivity was determined.*

TABLE V: *Melting Temperature (Tm) Values of ^{131}I-DNA Before and After Reiodination**.

Method	1st denaturation (Tm)	2nd denaturation (Tm)
Uncreacted ^{131}I-DNA	86.1	83.9
Commerford's	82.8	80.4
Modified	85.8	83.1

* ^{131}I-DNA obtained before or after reiodination by Commerford's or proposed method was heat denatured as described in the experimental section and the Tm values were determined. Native DNA under the same conditions had Tm= 86.7° after first denaturation and Tm= 84.6° after the second denaturation.

ford's method (Table I). In addition iodinated products retained to a large extent similar physical characteristics compared to the unreacted parent compounds.

Chemistry and Specificity of the Iodination Reaction

As previously described nucleic acids may be labeled with iodine by heating in the presence of iodide and thallic trichloride. Not all polynucleotides react with iodide under the above conditions. As indicated in Table I only those having cytosine bases were iodinated with high yields. Poly(U), poly(A) and poly (G) had very low specific activities. Poly(T) was unavailable.

In another experiment ^{125}I-DNA was digested by pancreatic deoxyribonuclease (DNase I) followed by venom diesterase (18-19). The resulting nucleoside digest was chromatographed on precalibrated Sephadex G-10 column as previously described (20), and the elution profile was recorded by measuring the ultrabiolet absorption at 260 nm and the gamma radioactivity. The radioactivity was eluted as a single peak in the position where the iodinated deoxycytidine was eluted during the calibration. In this peak the ultrabiolet absorption was weak probably because of the small concentration of iodinated deoxycytidine.

Deoxycytidine (9.8×10^{-4} M) was iodinated by heating at pH 4.7 for 90 min at 60°C in the presence of 3.5×10^{-4} M KI, 2.2×10^{-3} M $TICl_3$, and 5 µCi of ^{125}I. The reaction products were chromatographed on a precalibrated Sephadex G-10 column. It was found that the main amount of radioactivity was associated with a peak which was eluted in the identical way as the 5-iododeoxycytidine standard. In a similar experiment deoxyuridine was also iodinated, but thymidine and other nucleosides substituted in the C-5 position of pyrimidines, such as 5-hydroxymethyldeoxycytidine, 5-methyldeoxycytidine or 5-hydroxymethyldeoxyuridine did not react with iodine.

Thus, these results confirmed Commerford's postulation that the unoccupied C-5 positions of pyrimidines and especially those of cytosine were the main sites of binding of iodine during iodination reactions of polynucleotides. Furthermore as previously indicated iodine was bound to pyrimidines in two forms, one heat stable and another heat unstable.

The mechanism of iodine incorporation into pyrimidines is unkown; however, it is expected to be qualitively similar to that proposed for pyrimidine bromination (32). Thus, the first heating step results in the covalent bonding of iodine mainly to pyrimidines with the formation of unstable 5-iodo-6-hydroxydihydropyrimidine derivatives. Reheating of the mixture at pH 6.0 or higher causes dehydration of the product and the formation of an iodinated derivative of the nucleic acid.

Conclusion

The method described in this work can be used to iodinate a variety of single and double standed polynucleotides irrespective of their melting temperature and conformation. The reaction proceeded at 60°C in a narrowly acidic pH range and required well adjusted reagent concentrations for optimum yields. The iodination proceeded only when the 5′ position of the pyrimidine base was unoccupied. Iodine was bound in two forms: one, stable and another unstable. Iodinated products showed a small U.V. absorption at 310 nm and a decreased absorption at 260 nm. Loosely bound iodine was eliminated by heating at 60°C at pH 7.5. More than 95% of the stably bound iodine was associated with cytosine. Comparative studies indicated that there was a considerable increase (>20%) of the specific activity of the iodinated products versus other previously reported procedures. The iodinated polynucleotides retained to a large extent their pre-reaction physicochemical characteristics.

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INTERACTION OF 2-ACETYLPYRIDINE WITH THE FIRST-ROW TRANSITION METALS

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Abstract

The donor properties of the ligand 2-acetylpyridine towards the first row transition metalions have been investigated and the following new complexes have been synthesized: CrL_2Cl_3, CrL_2Br_3, MnL_2Cl_2, MnL_2Br_2, FeL_2Cl_3. 2 H_2O, FeL_4Br_3. 2 H_2O, CoL_2Cl_2. 4 H_2O, CoL_2Br_2. 4 H_2O, NiL_2Cl_2, NiL_2Br_2, Cu (OH_2) L_3Cl_2, and Cu (OH_2) L_3Br_3 (L is 2-acetylpyridine). The structure of the complexes has been deduced from elemental analyses, conductivity and magnetic susceptibility measurements, and electronic and ir spectra. The properties of this ligand are finally compared with those of 2-benzoylpyridine.

Introduction

In our previous communications[1-3] we are concerned with the complexes of the first row transition metalions with 2-benzoylpyridine. As an extention of this work we studied the interaction of the same elements with 2-acetylpyridine. This investigation has been carried out in order to compare the donor properties of 2-acetylpyridine with those of 2-benzoylpyridine, as well as its ability to from N, O chelates and to satisfy the maximum coordination number of the used transistion elements. The only complexes of 2-acetylpyridine reported so far are those of Ti, Sn, Hf, and Zr[4].

This kind of studies are very important because as it was pointed out very recently by Hughes *et al*[5-7] that the reduction of the 2-pyridine carbonaldehyde (an analogous ligand) by N-benzyl 1,4-dihydronicotinamide (an analog of NADH) is metal ion dependent, and therefore the system studied may act as a biological model in reactions of the same type.

As an extention of this work we studied the interaction of the same elements with 2-acetylpyridine. This investigation has been carried out in order to compare the donor properties of 2-acetylpyridine with those of 2-benzoylpyridine as well as its ability to form N, O chelates and to satisfy the maximum coordination number of the ured transition elements.

Experimental

Materials. 2-acetylpyridine was purchased from Aldrich Chem. Co. and used without further purification. All the other chemicals used in this study were from Merck A.G.

Preparation of the Complexes

1. Dichloro-bis (2-acetylpyridine) Chromium (III) cloride, $[CrL_2Cl_2]Cl$. $CrCl_3$. $6H_2O$ (2mmoles) was dissolved in ethanol (30ml) and to the obtained solution 2-acetylpyridine (6mmoles) was added dissolved in ethanol (10ml). The solution was refluxed for 6hrs and the a brown compound was precipitated with excess ether. The precipitate was filtered, washed with ether: ethanol (4:1) and dried at 50°C under vacum. Yield 80%.

The bromo analog, dibromo-bis (2-acetylpyridine) Chromium (III) bromide $[CrL_2Br_2]$ Br, was prepared in a similar way starting with $CrBr_3$. $6H_2O$.

2. Dichloro-bis (2-acetylpyridine) Manganese (II). $[MnL_2Cl_2]$. $MnCl_2$. $4H_2O$ (2mmoles) was dissolved in ethanol (10ml) and to the obtained solution was 2-acetylpyridine (4mmoles) dissolved in ethanol added dropwise (10ml). The yellow precipitate formed was filtered washed with ethanol (20ml) and dried at 50°C under vacum. Yield 85%.

The bromo analog, dibromo-bis (2-acetylpyridine) Nickel (II) $[NiL_2Br]$, $Br_2]$ was prepared in a similar way starting with $MnBr_2$. 4 H_2O.

3. Dichloro-bis (2-acetylpyridine) Iron (III) chloride dihydrate, $[FeL_2Cl_2]$ Cl. 2 H_2O.

2-acetylpyridine (4mmoles) was dissolved in ethanol (10ml) and to the obtained solution was added dropwise $FeCl_3$. 6 H_2O (2mmoles) dissolved in ethanol (10ml). The yellow precipitate formed was filtered, washed with ethanol (20ml) and dried at 50°C under vacum. Yield 75%.

4. Dibromo-tetrakis (2-acetylpyridine) Iron (III) bromide dihydrate, $[FeL_4Br_2]$ Br. 2 H_2O.

$FeBr_3$. 6 H_2O (2mmoles) was dissolved in ethanol (30ml) and then was added 2-acetylpyridine (8mmoles) dissolved in ethanol (10ml). The solution was refluxed for 3hrs and the compound was precipitated with excess ether. The dark-red precipitate was filtered, washed with ether: ethanol (4:1) (30ml) and dried at 50°C under vacum. Yield 75%.

5. Dichloro-bis (2-acetylpyridine) Cobalt (II) tetrahydrate, $[CoL_2Cl_2]$. 4 H_2O.

2-acetylpyridine (4mmoles) was dissolved in ethanol (10ml) and then was added dropwise $CoCl_2$. 6 H_2O (2mmoles) dissolved in ethanol (10ml). The yellow precipitate formed was filtered, washed with ethanol (30ml) and dried at 50°C under vacum. Yield 80%.

The bromo analog, dibromo-bis (2-acetylpyridine) Cobalt (II) tetrahydrate $[CoL_2Br_2]$. 4 H_2O, was prepared in a similar way starting with $CoBr_2$. $6H_2O$.

6. Dichloro-bis (2-acetylpyridine) Nickel (II), $[NiL_2Cl_2]$.

2-acetylpyridine (6mmoles) was dissolved in ethanol (10ml) and then was added dropwise $NiCl_2 . 6\ H_2O$ (2mmoles) dissolved in ethanol (10ml). The yellow precipitate formed was filtered, washed with ethanol (25ml) and dried at 50°C under vacum. Yield 80%.

The bromo analog, dibromo bis (2-acetylpyridine) Nickel (II) $[NiL_2Br_2]$, was prepared in a similar way starting with $NiBr_2 . 6\ H_2O$.

7. Aquo-dichloro-tris (2-acetylpyridine) Copper (II), $[CuL_3Cl_2\ (H_2O)]$.
2-acetylpyridine (6mmoles) was dissolved in ethanol (10ml) and then was added dropwise $CuCl_2 . 6\ H_2O$ (2mmoles) dissolved in ethanol (10ml). The green precipitate formed was filtered, washed with ethanol (20ml) and dried at 50°C under vacum. Yield 80%.

The bromo analog, aquo-dibromo-tris (2-acetylpyridine) Copper (II) $[CuL_3Br_2\ (H_2O)]$, was prepared in a similar way starting with $CuBr_2 . 6\ H_2O$.

Physical measurements

(a) The ir spectra were recorded in a Beckman model 2050 spectrophotometer in KBr pellets and in Nujol mulls, the position of the bands are given within ± $2 cm^{-1}$. (b) The UV-VIS spectra were taken with a Cary model 17D spectrophotometer. (c) Conductivity measurements were performed using an E365B conductorscope Metrohm Ltd. (d) The melting points were determined on a W. Büchi apparatus and are uncorrected. (e) The magnetic moments were determined by the Goy method takin into account the diamagnetic corrections at 25°C.

Microanalyses: C, H, and N microanalyses were performed in the laboratories of the Hellenic National Research Foundation by Dr Mantzos. The halogens were determined gravimetrically.

Results and discussion

The analytical and physical data are given in Table 1 and show that compositions agreed well with calculated ratios.

The molecular conctivities in nitromethane and ethanol agree with the proposed formulae (See Table I). In water all complexes were found to be electrolytes and attain in a short time high conductivity values. In the case of the Cr (III) and Fe (III) complexes the increase in the conductivity is followed by a considerable decrease in the pH ($[CrL_2Cl_2]\ Cl$, after 24hrs, $\Lambda_m = 498$; pH= 3.13. $[FeL_2Cl_2]\ Cl. 2\ H_2O$, after 24hrs, $\Lambda_m = 730$; pH = 2.87). The increase in conductivity implies substitution of the coordinated halogens by water molecules and the decrease in pH by further hydrolysis of the aquo species according to the general scheme:

$$[ML_2Cl_2]\ Cl + 2\ H_2O \rightarrow [ML_2\ (OH_2)_2]\ Cl_3$$

$$H_2O$$

$$\cdot\ [ML_2\ (OH_2)_{2-\chi}\ (OH)_\chi]\ Cl_{3-\chi} + \chi H_3O^+ + \chi Cl^-$$

where M is Cr of Fe.

In the case of the Co(II), Ni (II) and Cu (II) complexes the only change observed was the increase in conductivities, which after 24hrs were stabilized in the following values:
$[CoL_2Cl_2]. 4 H_2O$, $\Lambda_m = 265$; $[NiL_2Cl_2]$, $\Lambda_m = 232$; $[CuL_3Cl_2 (OH_2)]$, $\Lambda_m = 235$. In these complexes, since the pH of their solutions remains neutral, the only change occured upon dissolution in water, is the substitution of the coordinated halogens by water.

The magnetic moments (see Table I) indicate that all the complexes are of the high spin type. (with the maximum number of unpaired electrons).
ir spectra: The tentative ir assignements are given in Table II. The complexes of Fe (III), Co (II) and Cu (II) showed a broad band at about 3400 - 3300 cm^{-1} which indicates the presence of water[8]. The weight lost by heating the Fe (III) and Co (II) complexes at 110°C corresponds to two and four molecules of

TABLE I: *Analytical and physical data of the compounds.*

Compound	%				M.P (°C)	Λ (Mho cm^2) ($MeNO_2$)	μ_{eff} (μB)
	C	H	N	X			
CrL_2Cl_3	37.22	2.95	6.31	26.76	260	76	3.80
	(37.06)*	(3.08)	(6.16)	(26.49)			
CrL_2Br_3	29.12	2.29	4.63	42.28	238		
	(29.42)	(2.45)	(4.90)	(42.03)			
MnL_2Cl_2	45.60	3.65	7.11	19.27	242D	10	5.50
	(45.90)	(3.80)	(7.60)	(19.30)			
MnL_2Br_2	36.46	2.98	6.01	34.88	228D		
	(36.84)	(3.07)	(6.14)	(34.99)			
$FeL_2Cl_3 . 2 H_2O$	38.38	4.28	6.08	24.50	208	83	5.75
	(38.18)	(4.09)	(6.36)	(24.18)			
$FeL_4Br_3 . 2H_2O$	41.26	3.72	6.66	29.54	215	76	
	(41.17)	(3.92)	(6.86)	(29.39)			
$CoL_2Cl_2 . 4H_2O$	38.65	4.65	6.23	16.05	218	14	4.15
	(37.80)	(4.95)	(6.30)	(15.99)			
$CoL_2Br_2 . 4 H_2O$	31.32	3.98	5.02	29.87	237		
	(31.51)	(4.12)	(5.52)	(30.02)			
NiL_2Cl_2	45.32	3.95	7.38	18.80	263	13	3.20
	(45.15)	(3.76)	(7.52)	(19.10)			
NiL_2Br_2	36.11	2.95	5.89	34.50	267		
	(36.44)	(3.04)	(6.08)	(34.70)			
$CuL_3Cl_2 (OH)$	49.06	4.29	8.47	12.64	172	14	1.92
	(48.93)	(4.46)	(8.15)	(13.79)			
$CuL_3Br_2 (OH_2)$	41.53	3.68	6.71	26.23	167		
	(41.72)	(3.80)	(6.95)	(26.49)			

L= 2-acetylpyridine
D= decomposition
* The numbers in parentheses correspond to the calculated amounts.

TABLE II: *Infrared spectra* (cm^{-1})

L	$CrCl_3L$	$MnCl_2L_2$	$MnBr_2L_2$	$FeCl_3L_22H_2O$	$FeBr_3L_32H_2O$	$CoCl_2L_24H_2O$	$CoBr_2L_24H_2O$	$NiCl_2L_2$	$NiBr_2$	$CuCl_2L_3H_2O$	$CuBr_2L_3H_2O$	Assignment
				3420 sh	3420 sh	3460 m	3460 m			3360 m	3350 m	O-H Stretching
						3530 m	3533 m					
3060 m	3066 m	3070 m	3060 m	3060 m	3060 m	3050 m	3060 m	3070 m	3070 m	3066 m	3066 m	C-H Stretching
				1610 m	1610 m	1630 m	1633 m			1630 m	1630 m	^{0}-H bending
1695 s	1615 s	1665 s	1665 s	1645 s	1700 s	1660 s	1660 s	1660 s	1650 s	1690 s	1695 s	C-O stretching
1570 s	1605 m	1590 s	1590 s	1590 s	1580 s	1590 s	1590 s	1605 s	1595 s	1600 s	1600 s	C=C and C=N
1550 s	1566 m	1566 m	1566 m	1570 m	1570 m	1560 m	1560 m	1570 s	1566 s	1570 s	1570 m	stretching of the
990 s	1030 s	1015 s	1015 s	1015 s	1025 s	1020 s						aromatic ring
615 m	660 s	670 m	670 m	650 m	640 m	640 m	1020 s	1030 s	1025 s	1030 s	1030 s	pyridine breathing
590 s	640 m	635 m	635 m	615 m	610 m	615 s						motion
	610 m	615 s	615 s	595 w	595 w	590 m	640 m	645 m	640 m	655 s	655 s	pyridine deformation
		590 m	590 m									motion
	560 w	505 m	505 m	500 w	500 w	595 w	615 s	630 s	625 s	615 s	615 s	
	530 w	580 w	480 w				590 m	605 m	595 m	590 m	590 m	
	480 s	425 m	425 m	425 w	420 w	425 m	495 w	485 w	485 w	485 w	—	M-N or M-O
	420 w	415 w	415 w	—	—	405 s						stretching
	410 w	400 w	400 w	—	—	—	425 m	450 s	435 s	450 s	450 s	ring deformation
	270 s	325 m	240 m	—	—	—	405 s	425 m	425 w	433 w	415 m	
								415 m	415 m	415 m	400 m	
										400 m		
							—	310 s	—	330 m	—	M-X stretching

water respectively and its easy removement suggests water of crystallization. In the Cu (II) complexes the water is retained after heating at 110°C, which indicates that it is coordinated to the metal. The presence of water in the above complexes is further confirmed by a band at 1630 - 1610 cm^{-1}. Small shift towards higher frequencies of the most stretchings of the pyridine ring was observed in all complexes prepared and this is common in the pyridine complexes[9].

The ligand exhibits a strong absorption at 1690 cm^{-1}, assingned to the carbonyl stretching and is expected to shift to lower frequencies when the oxygen is coordinated to the metal and to remain unchanged or to shift to slightly higher frequencies in the case of N-only coordination[1-3,8]. Thus, in the complexes of Cr (III), $FeCl_3$, Mn (II), Co (II) and Ni (II) the νC=O is lowered by 30 - 60cm^{-1}(see Table II), while in the other complexes it appears unchanged or at little higher frequencies (ca. 10cm^{-1}). The observed decrease in the ν C=O frequency upon complexation is explained by the synergic effect of simultaneous N, O coordination. This is a good indication of the formation of N, O chelate complexes. Where the band at 1690cm^{-1} remains unchanged or is shifted to higher values it is assumed that the ligand in the complexes is monodentate[1-3].

The out of plane bending motion of the carbonyl group is not observed in the free ligand and this is possibly due to the overlapping by the strong bending motion of the pyridine ring at 590 cm^{-1} 4,11.

The strong band at 900 cm^{-1} of the free ligand is assigned to the breathing motion of the pyridine ring and is shifted by 20 - 40 cm^{-1} towards higher frequencies in all the complexes (see Table II), consistent with N-coordination[1-4, 12, 14].

The band at 605 cm^{-1} in the free pyridine is assingned to the deformation motion of the ring and is shifted to higher frequencies in its complexes with the first row transition metals[14]. The shifting is characteristic of the stereochemistry of the complex[15] and of the element's group in the periodic table[16]. In the free 2-acetylpyridine the two bands at 615 and 590 cm^{-1} are assingned to the deformation motion of the pyridine ring[4]. In all the complexes prepared a new medium intensity band is appeared at ca. 640 cm^{-1} and this may correspond to those at 615 and 590cm^{-1} of the free ligand, which colapse to one and shift to higher frequencies due to coordination.

The free ligand does not show any bands in the region 550 - 400 cm^{-1}. The appearance of a weak band at ca. 500 cm^{-1} in the spectra of the complexes may be assingned to the M-N or M-O stretching motions[4].

The free picolinaldehyde and 2-benzoylpyridine shows bands at 440 and 445 cm^{-1} respectively assingned to the deformation modes of the ring, which are shifted to higher frequencies by ca. 10 - 20 cm^{-1} upon complexation[2]. In the spectra of the complexes of Cr (III), Ni (II) and Cu (II) a strong band appears at ca. 450cm^{-1} assigned to the deformation motion of the pyridine ring, which becomes active due to complexation. For the same reason the medium to strong intensity bands in the region 430 - 400 cm^{-1} are most possibly due to the deformations of the pyridine ring, because a band of this kind appears at 402 cm^{-1} in the spectrum of the free pyridine, which is shifted to higher frequencies by ca. 20 cm^{-1} upon complexation[14].

The tentative assingnement of the M-X stretchings in the far ir spectra is based on the comparison of the spectra of the chloro and bromo analogs of the same complex, where possible, and are included in Table II. In the complex MnL_2Cl_2 the strong band at 325 cm^{-1} appears at 240 cm^{-1} in the spectrum of the bromo analog and they are assingned to Mn-Cl and Mn-Br stretchings respectively. The complex NiL_2Cl_2 shows a strong band at 310 cm^{-1} which is absent from the spectrum of the bromo analog and is assingned to Mn-Cl stretching. The same is true for the complex CuL_3Cl_2 (OH_2), which shows the Cu-Cl stretching at 330 cm^{-1}

Electronic spectra

(a) UV region: The ligand exhibits an intence absorption at ca. 37590 cm^{-1}, which shifts to lower frequency (red shift) with increasing solvent polarity and is assingned to $\pi^* \leftarrow \pi$ transition of the ring[2]. A bathochromatic (red) shift is also expected for this transition when the N atom of the pyridine ring is metal coordinated[2]. As Table 3 shows this occurs in all the complexes investigated, implying nitrogen coordination in all of them.

A second weak band at ca. 30770 cm^{-1} which undergoes a hypsochromic (blue) shift with increasing solvent polarity is assingned to an $\pi^* \leftarrow n$ transition of the carbonyl group[2]. Coordination through the carbonyl group should cause a large hypsochromic shift of this transition, while non involvement of the carbonyl group in coordination must leave this transition unaffected[2]. Thus in the copper (II) complexes where the carbonyl group is supposed to be free (-from the ir data) this transition appears at the same frequency as in the free ligand. The iron (III) complexes exhibit strong charge trannsfer transitions in this region preventing the obserbation of the $\pi^* \leftarrow n$ band. In all other complexes a shift to higher frequencies by ca. 776 to 977 cm^{-1} is observed (see Table 3) indicating C=O involvement in coordination, as also was concluded from the ir spectra.

(b) Visible and near ir regions: The visible and near ir spectral data are given in Table III. In the Fe (III) complexes the strong absorption at ca. 29411cm^{-1} is assingned to charge transfer of the ligand - metal type. This band prevents the observation of the (d-d) transitions in the visible region, a common phenomenon for Fe (III) complexes[2, 17]. Similarly, the d-d absorption spectrum of the Mn (II) complex was not observed, due to the low intensity of the bands[2, 17].

The $[CuL_3Cl_2 (OH_2)]$ complex, in methanol, shows two bands at ca. 12739 and 6645 cm^{-1}. These two bands are usually found in trigonally distorted octahedral complexes of Cu (II) and are assingned to the $^2E_2 \leftarrow {}^2E$ and $^2A_1 \leftarrow {}^2E$ transitions respectively[17].

The complex $[CoL_2Cl_2]$, in methanol, exhibits two absorptions at ca. 19047

TABLE III: *Electronic spectral data* (cm^{-1})

Compound	$\pi^* \leftarrow \pi$	$\pi^* \leftarrow n$	$d \leftarrow d$	assingnement
L	37590	30769		
CrL_2Cl_3	37313	31746	38461	$^{4}T_{1g}$ (P) $\leftarrow$ $^{4}A_{2g}$
			27397	$^{4}T_{1g}$ (F) $\leftarrow$ $^{4}A_{2g}$
			12738	$^{4}T_{2g} \leftarrow {}^{4}A_{2g}$
$CoL_2Cl_2 \cdot 4H_2O$	36036	31545	19047	$^{4}T_{2g}$ (P) $\leftarrow$ $^{4}T_{1g}$
			7905	$^{4}T_{2g} \leftarrow {}^{4}T_{1g}$
NiL_2Cl_2	36036	31545	25316	$^{3}T_{1g}$ (P) $\leftarrow$ $^{3}A_{2g}$
			14598	$^{3}T_{1g} \leftarrow {}^{3}A_{2g}$
			7911	$^{3}T_{2f} \leftarrow {}^{3}A_{2g}$
$CuL_3Cl_2\ (OH_2)$	35971	30769	12739	$^{2}E_2 \leftarrow {}^{2}E$
			6645	$^{2}A_1 \leftarrow {}^{2}E$

and 7905 cm^{-1} assingned to the transitions $^{4}T_{1g}$ (P) $\leftarrow$ $^{4}T_{1g}$ and $^{4}T_{2g}$ respectively in an octahedral environment[2, 17, 18].

The complex [NiL_2Cl_2], in methanol, show three bands at ca. 25316, 14598 and 7911 cm^{-1}, assingned to the $^{3}T_{1g} \leftarrow {}^{3}A_{2g}$, $^{3}T_{1g} \leftarrow {}^{3}A_{2g}$ and $^{3}T_{2g} \leftarrow {}^{3}A_{2g}$ respectively in octahedral environment[17].

The octahedral Cr (III) complexes usually show three absorptions at ca. 14000, 25000 and 37000 cm^{-1} assingned to the transitions $^{4}T_{2g} \leftarrow {}^{4}A_{2g}$, $^{4}T_{1g}$(F) $\leftarrow$ $^{4}A_{2g}$ and $^{4}T_{1g}$(P) $\leftarrow$ $^{4}A_{2g}$ respectively[17]. In distorted complexes the first transistion is analyzed in two absorptions of lower intensity and the one of them in lower frequencies[17]. The third transition is characterized by very low intensity and in many cases it does not show up because it is obscured by the intense UV bands of the ligand. The complex [CrL_2Cl_2] Cl, in methanol, shows a strong band at 27397 cm^{-1} and two others of lower intensity at ca. 38461 and 12738 cm^{-1} assingned to the transitions $^{4}T_{1g}$ (F) $\leftarrow$ $^{4}T_{2g}$, $^{4}T_{1g}$ (P) $\leftarrow$ $^{4}A_{2g}$ and $^{4}T_{2g} \leftarrow {}^{4}A_{2g}$ respectively.

From the comparison of the 2-acetylpyridine complexes of the first row transition metals with those of 2-benzoylpyridine[1-3] the following two brief conclusions can be drawn:

(a) The carbonyl oxygen of 2-acetypyridine participates more easily in complex formation than the corresponding group of 2-benzoylpyridine, thus forming N, O chelates with the most elements of the first transition series. This, most probably, is due to the higher electron density which the methyl group induces on the carbonyl group. In the case of 2-benzolpyridine the oxygen atom of the carbonyl group is less basic due to electron withdrawing from the benzene ring.

(b) The ligand: metal ratio is higher in the most of the 2-acetylpyridine complexes than the corresponding complexes of the 2-benzoylpyridine and this, most probably, is due to the smaller molecular volume of the first, and thus more molecules of it can be accomodated around the central metal atom.

Περίληψις

Ἐμελετήθησαν αἱ ἰδιότητες ὡς δότου τοῦ ὑποκαταστάτου 2-ἀκετυλοπυριδίνη πρὸς τὰ ἰόντα τῆς πρώτης σειρᾶς τῶν στοιχείων μεταπτώσεως καὶ παρεσκευάσθησαν τὰ ἑξῆς νέα σύμπλοκα: CrL_2Cl_3, CrL_2Br_3, MnL_2Cl_2, MnL_2Br_2, FeL_3Cl_3. $2H_2O$, FeL_4Br_3. $2H_2O$, CoL_2Cl_2. $4H_2O$, CoL_2Br_2. $4\ H_2O$, $Cu\ (OH_2)\ L_3Br_3$ (L= 2-ἀκετυλοπυριδίνη).

Ἡ δομὴ τῶν παρασκευασθέντων συμπλόκων ἐξηγήθη κατόπιν στοιχειακῶν ἀναλύσεων, μετρήσεων ἀγωγιμότητος, τῆς μαγνητικῆς αὐτῶν ἐπιδεκτικότητας τῶν ἠλεκτρονιακῶν καὶ ὑπερύθρων φασμάτων. Τελικῶς συγκρίνονται αἱ ἰδιότητες τῆς 2-ἀκετυλοπυριδίνης μὲ ἐκείνας τῆς 2-βενζοϋλοπυριδίνης.

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MODEL STUDIES OF THE FIRST HYDRATION SHELL OF N, N-DIMETHYLACETAMIDE USING CNDO/2 VIEWED IN THE LIGHT OF NMR SPECTROSCOPIC AND OTHER EXPERIMENTAL EVIDENCE

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Abstract

CNDO/2 calculations on the hydration of N, N-dimethylacetamide involving up to three water molecules have been used to evaluate the most likely positions of the water molecules in the first hydration shell. Calculated electron densities and bond order parameters have been examined in a comparison with experimental data. The model proposed can be seen to be compatible with the barrier to rotation about the central C-N bond and viscosity measurements. Carbon-13 chemical shift data correlate in part only and additional intermolecular effects must be invoked to explain the results qualitatively.

Key words: *CNDO/2, N-dimethylacetamide, Hydration, First-Shell, NMR, proton, carbon 13 viscosity, energy barrier.*

r (C_1-O)	interatomic distance
<(OCN)	interbond angle
P (CN)	bond order calculed for C-N bond
D	deshielding
S	shielding
E_a	activation energy for rotation about C-N bond
E_t	total energy
Γ_e	equilibrium interatomic distance
ΔF_t	stabilisation energy

Introduction

The properties of mono- and di-substituted amides in aqueous solutions have been the subject of a number of interesting papers in which viscosities,[1,2] densities[1], volume changes[1,2], heats of mixing[2] phase diagrams[2] and ultrasonic absorption[3] have been measured as a function of the mole fraction of water. The use of amide-water mixtures as binary solvents or reaction media in chemical rate and equilibrium studies has been suggested as sufficient reasons to justify a critical look at the physical properties of such solutions.

Viscosity and volume changes show maxima at low and specific mole ratios which have led to suggestions that stoichiometric compounds or complexes are formed[1]. Low temperature phase diagram work has been presented to confirm this premise[2]. The emphasis in previous work has been on probing the extent of molecular association or hydrogen bonding and on possible changes in the water structure. It was not expected by these authors[1] that a clear molecular interaction mechanism could be inferred from viscosity, or similar, measurements alone.

The subject of this present paper has been to present some new data associated with, N, N-dimethylacetamide water mixtures in an attempt to provide a more detailed picture of the intermolecular interactions. Three approaches have been made in following changes in approximate barriers to rotation about the central C-N bond, considering the carbon-13 chemical shifts of the four DMA carbons and in calculating the total energy in a «united-molecule» approach using the CNDO/2 program.

Carbon-13 nmr has been used to examine the intermolecular interactions in N, N-dimethylacetamide water mixtures and the results show some compatibility with viscosity studies[3]. Barriers to internal rotation in simple amides have been determined using proton nmr but the quantitative nature of the results is in doubt especially where single parameter methods have been used because of the long-range coupling between protons which broadens the peaks[4]. However, the simplistic approach provides an indication in trends of results which, though lacking in accuracy, are distinctive and in keeping with the qualitative picture emerging from alternative approaches described above.

The CNDO/2 program has been applied in a critical way to specifically chosen configurations involving up to three water molecules for each DMA molecule.

Similar work has been described for formamide-water interactions[5], association between water and purine[6] bases and hydration of peptides[7].

Experimental

The amide was obtained commercially and purified by distillation at reduced pressure.

Activation Energy E_a.

The experimental activation energies, E_a, identified with the barrier height restricting rotation about the central C-N bond of the amide, have been obtained from measurements of the rate of internal rotation at a series of temperatures, for samples containing the amide and water in different mole ratios.

The spectra were obtained using a Varian A60A nmr spectrometer with a variable temperature controller. The effect of overlap of the components of N-methyl doublets was not taken into account and the approximate values for E_a

thus obtained came directly from the best straight-line through the seven points of each Arrhenius plot. The errors quoted are twice those given in the original paper and further undetermined errors arising from the approximate method employed would be expected to require exceedingly cautious use of these data in absolute terms. However, the changes observed between samples were outside the measured error, as shown in Table I, and the relative values are of more interest here than the absolute ones.

TABLE I: *The trends in activation energies associated with the restricted internal rotation about the central C-N amide bond in acet-N, N^1-dimethylamide and four mixtures including water.*

Composition	E_a (KJ mol^{-1})
DMA	51.0 ± 3.0[a]
DMA (H_2A)	74.0 ± 3.3
DMA $(H_2O)_2$	73.2 ± 3.7
DMA $(H_2O)_3$	72.7 ± 3.4
DMA $(H_2O)_4$	74.5 ± 4.0

[a] Errors were estimated from extreme gradients of the Arrhenius plots.

Approximate activation energies have been obtained from a single parameter analysis of variable temperature H *nmr* spectra[4].

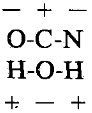
Carbon-13 chemical shifts.

The details of these results have been reported in a more general context in another paper[8].

Theoretical

Models for the DMA/water interaction.

Past evidence from viscosity and phase-diagram studies, at low temperatures, of amide-water mixtures has been interpreted in favour of the stability of particular compexes even at room temperature[2]. It has been suggested that two water molecules can associate strongly with the two unshared pairs of electrons on the carbonyl oxygen in N, N-disubstituted formamides and acetamides, and in N-substituted pyrrolidones. The viscosity data, however, distinguished between the acyclic and cyclic amides because the former show viscosity maxima for 3:1 water-amide mole ratio while the latter exhibit maxima for 2:1 mole ratio. This distinction may well hint at the spatial arrangement or the strength of attachment of the third water molecule for the N, N-disubstituted amides[1]. The location and orientation of the third water molecule remains a controversial issue. The evidence which has been obtained here suggests that a predominantly six-centred electrostatic interaction



$$\begin{array}{c} - \quad + \quad - \\ \text{O-C-N} \\ \text{H-O-H} \\ + \quad - \quad + \end{array}$$

is more likely than the weaker interaction between the water molecule and the sterically obstructed lone pair electrons on the disubstituted nitrogen[1]. The experimental viscosity curves are skewed towards higher mole ratios and this has been interpreted as suggesting that higher complexing ratios (>3:1) also occur[1]. Possible sites for solvation of the amides by water have been suggested which involve the alkyl groups[1]. The number of potential sites for water associating with amide is therefore likely to be at least six but it will be shown that these are not energetically equivalent. The models which have been investigated are shown in Figure 1.

Basis of the calculations.

CNDO/2 parameters. The CNDO/2 method (complete neglect of differential overlap) developed by Pople and Santry is adequately described in standard texts[9].

The main concern in this work has been with ground state energies of interacting «amide» and water molecules as a basis for prediction of the «first hydration shell» characteristics. The difference in energy between the calculated values when the molecules are well separated and at an equilibrium distance associated with a minimum energy has been taken as a measure of stabilisation of the complex (ΔE_t in Table II) and identified with an enthalpic dissociation energy[10]. In some cases no such minimum was observed (Table II). Illustration of three representative curves obtained by plotting E_t against an appropriate interatomic distance are given in Figure 2.

The calculated dipole moments provide a test of the molecular eigenvectors and hence of the electron density on each atom. This latter parameter is particularly relevant to the carbon-13 measurements since significant correlations have been observed in other systems which indicate that C-13 chemical shifts are linearly dependent on electron density[11]. The parameter recorded in Table II is nett atomic charge since we are primarily interested in the changes which take place. Further, it is possible to use the population matrix (generated from the eigenvectors) in the output from the program to obtain a measure of bond order[12], (Table III) and seek to correlate this with the *changes* in the activation energy E_a (discussed later).

The calculations were carried out by first choosing interatomic distances and inter-bond angles in accord with values listed in the literature[13]. These dimensions were kept fixed for DMA throughout the calculations since any attempt to take account of changes leads to a hopelessly complicated model. The parameters used were as follows: (planar skeleton).
r(C1-0) 0.121 nm, r (C1-N) 0.135 nm, r (C2-N) 0.145 nm, r (C3-N) 0.145 nm, r (C1-C4) 0.155 nm, r (C-H) 0.111 nm, <(OCN) 125.5°, <(0 C1 C4) 117°,

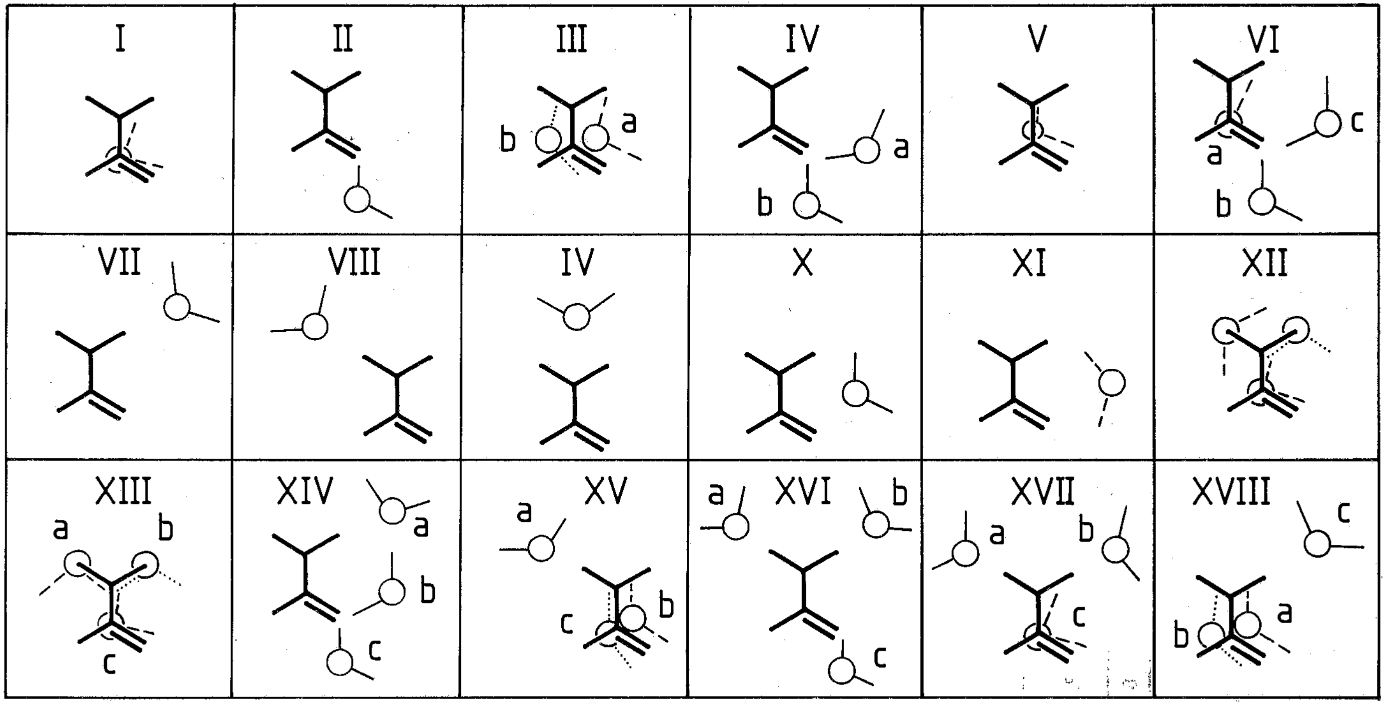


FIGURE 1: *Schematic diagrams representing the adopted models for DMA/water interactions: Dashed lines for the OH bonds O indicate the water molecule is above the plane of the DMA molecule. Dotted lines ...O indicate the water is below the DMA plane and full lines are used for coplanar molecules.*

<(C1 N C2) 120°, <(C1 N C3) 120°, <(HCH) 109°28′, ≧(CCH) 109°28′ H_2O r(OH) 0.10 nm, <(HOH) 104°.

TABLE II: *Total energies E_t in Hartress*$^+$ and nett atomic charges ρ calculed for the models in Figure 2 using the CNDO/2 program. Equilibrium distances r_e in nanometers and stabilisation energies in Hartrees$^+$ ΔE_t determined for the same models.

A	B	C	D	E
Model	r_e	E_t	ΔE_t	for $r = r_e$
Amide	—	—	—	1 0.3511 2 0.1009 3 0.0942 4 −0.0898
H_2O	—	—	—	0 −0.2708 H 0.1354 H 0.1354
I	0.18	−85.287	0.027	1 0.4305D 2 0.1019D± 3 0.0933S± 4 −0.0931S
II	0.25	−85.280	0.016	1 0.3654D 2 0.0933S± 3 0.0928S± 4 −0.0933S
III	a 0.18 b 0.18	−105.198	0.056	1 0.4980D 2 0.1020D 3 0.0920S 4 −0.0960S
IV	a 0.30 b 0.30	−105.149	0.037	1 0.3615D 2 0.0996S 3 0.0677S 4 −0.0918S
V	0.18	−85.284	0.021	1 0.4254D 2 0.1005S± 3 0.0928S 4 −0.0929S
VI	a 0.18 b 0.30 c 0.30	−125.061	—	1 0.4358D 2 0.1010− 3 0.0678S 4 −0.0950S

Table II (continued)

A	B	C	D		E
VII	0.225	−85.267	0.001	1	0.3522D±
				2	0.1017D±
				3	0.1059D
				4	−0.0900−
VIII	0.225	−85.266	0.001	1	0.3513D±
				2	0.1106D
				3	0.0950D±
				4	−0.0901−
IX	0.425	−85.266	0.001	1	0.3531D±
				2	0.0849S
				3	0.0948D±
				4	−0.0894−
X	r= 0.50	−85.261	No minimum	1	0.3519D±
				2	0.1011D±
				3	0.0945D±
				4	−0.0898−
XI	0.225	−85.268	0.003	1	0.3534D
				2	0.0999S
				3	0.0973D
				4	−0.0917S
XII	a 0.18	−125.048	−	1	0.4292D
	b 0.30			2	0.0960S
	c 0.30			3	0.0865S
				4	−0.0937S
XIII	a 0.300	−125.036	−	1	0.4251D
	b 0.300			2	0.0984S
	c 0.180			3	0.0870S
				4	−0.0939S
XIV	a 0.225	−125.040	−	1	0.3589D
	b 0.30			2	0.1007−
	c 0.30			3	0.0800S
				4	−0.0922S
XV	a 0.225	−125.088	−	1	0.4961D
	b 0.30			2	0.1129D
	c 0.30			3	0.0934S±
				4	−0.0964S
XVI	a 0.225	−125.060	−	1	0.3673D
	b 0.18			2	0.1099D
	c 0.18			3	0.1052D
				4	−0.0938S

Table II (continued)

A	B	C	D	E
XVII	a 0.225	−125.067	— 1	0.4310D
	b 0.225		2	0.1126D
	c 0.250		3	0.1060D
			4	−0.0937S
XVIII	a 0.180	−125.089	1	0.4964D
	b 0.180		2	0.1040D
	c 0.225		3	0.1046D
			4	−0.0964S

Footnote to table 2.

The lower case letters a, b, c, where appropriate, refer to the relevant water molecules in the schematic diagram of Figure 1.

* Nett atomic charge, ρ= 4-calculated electron density on the atom.

\+ 1 Hartree = 627.7 Kcal mol^{-1} = 2604 KJ mol^{-1}

± Small deviations from the corresponding neat amide value only. i.e. $\Delta q > 0.0010$

TABLE III: *Bond order parameters calculated for the C-N bond in Amide-water systems. Models I, II and III.*

Model I		Model II		Model III	
r	P (CN)	r	P (CN)	r	P (CN)
0.18 nm*	1.07	0.25 nm*	1.143	0.18 nm*	1.040
0.25 nm	1.19_5	0.35 nm	1.130	0.35 nm	1.1233
P(CN) for DMA = 1.12					

P (CN) = P_{ij}

P_{ij} are the elements of the population matrix generated by the CNDO/2 program

*r_E in each case.

Discussion

The choice of models shown in Figure 1 was constrained by computer time and by chemical intuition based on earlier arguments. Models I, III and V were suggested by the charge distribution both in DMA and in water, involving six-centre electrostatic interactions. Model V bears some relationship to the suggestion made that the water can hydrogen bond to the nitrogen through its lone pair and is justfied by the potential energy minimum observed at an equilibrium distance of 0.18 nm (compare model I in Figure 1). Models II and IV portray the conventional hydrogen bonding situation and it is significant to note that the stabilisation energy in these cases is less than in the corresponding models above although no emphasis is laid on *absolute* differences because of earlier qualifications.

Models VII to X represent situations which are less likely on energy grounds but which do have small stabilisation energies shown in Table 2 and represented schematically for model VII in Figure 2. Model XI showed no minimum probably because of unfavourable steric interactions.

The other models shown in table I represent combinations of the basic models which are likely. No attempt was made to extend the calculations to mole ratios greater than 3:1 mainly because of computing time but also because the experimental results suggest 3:1 to be a significant ratio.

The nett atomic charge is reported for the models studied in Table I and the effect of the change at a particular carbon compared with its environment in the isolated amide is recorded as a shielding, S, or deshielding, D, effect. The carbon nuclei C1, C2 and C3 were observed to be deshielded when water was added to the amide. The fourth carbon nucleus was not affected over the concentration range studied[8].

Earlier evidence accumulated by other workers has been interpreted in favour of stable amide-water complexes even at room temperature[2]. These conclusions are at variance with the results here since the more stable configurations chosen cannot explain alone the results observed. A dynamic system is more compatible with the chemical shift and energy barrier data. Hydrogen bonded systems exist on a time scale of the order of 10^{-5} seconds in the case of carboxylic acids (dimers)[14] falling to 10^{-10} seconds for weaker varieties[15]. The calculations suggest that model I is more stable than model II by some 30 KJ mol^{-1} although it should be emphasised that first this difference is probably exaggerated and second that it is an enthalpy difference. An additional and very important factor arises from entropy considerations. The closer approach and more restricted configuration of the water in model I must mean that the entropy change associated with that model (compared with the separate DMA and water molecules) is more negative than in model II (-10 to -20 ev)[16].

Consequently the free energy difference between models I and II will be less than the enthalpy difference (since $\Delta G = \Delta H - T\Delta S$) and so the ratio of the two configurations at equilibrium may well be nearer unity than suggested by enthalpy alone although it is not possible to estimate it quantitatively.

This is an important point because it may mean that no one configuration dominates the equilibrium. The free energy change for models such as VII therefore may well be small or even positive and thus reduce the probability that these models contribute at all through specific interactions. The entropy change for model VII would be expected to be less negative than for model II. Reference to Table II at this point indicates that model I does not explain the chemical shift data for C2, C3 or C4 through electron density changes alone. It is necessary, in the context of the chosen models, to invoke contributions from models such as VII and VIII to explain the direction of the chemical shifts of C2, C3 and C4 relative to DMA when water is added. Models XVII and XVIII predict the correct direction of chemical shifts for C1, C2 and C3 in the 3:1 mole ratio case. The C4 carbon chemical shift can be made to conform by including analogous models. These arguments omit contributions to the screening constant from Vander Waals interactions and anisotropy effects.

It should be noted that alternative sites for water molecules not involving DMA, for example $(H_2O)_2$ dimers, cannot be discounted and may well account for the small chemical shifts observed when water is added. The reasoning underlying this conclusion is based on the sensitivity of carbon-13 chemical shifts to electron density changes. The measured chemical shift change for a change in electron density by one unit in aromatic compounds has been reported to be 150 ppm per electron[11]. Preliminary calculations for a limited number of carbonyl containing compounds in this work suggest a higher sensitivity $\geqq 200$ ppm per electron.

Model I predicts a change in electron density for C1 compared with neat DMA of 0.08 electrons or a deshielding amounting to ~16 ppm. The appropriate figures for models II and VII are ~2.2 ppm and ~0.2 ppm respectively. Thus, either model II must be heavily weighted or if model I is included with comparable weighting there must be alternative sites for the water molecule which have little or no effect on the C1 chemical shifts. This is a highly complex problem because of the many contributions to the screening constant and no really satisfactory conclusions can be drawn from from C-13 data.

The barrier to rotation about the C-N bond.

The barrier to rotation about the central C-N bond in DMA has been shown to increase when water is added (Table 1) and that the increase is maximised for a mole ratio close to 1:1. The previous discussion led to a conclusion that model I is at least important in the dynamic model and it is tempting to suggest that such an association would sterically inhibit rotation about the CN bond. This would be even more likely if the water molecule moved to the position illustrated by model V.

A bond order parameter has been calculated (Table III) from the population matrix generated by the CNDO/2 program and normalised in the cases of ethane, ethylene and acetylene to the values 1, 2 and 3 respectively. It is clear that the approach of the water molecule in the configuration described by model I acts to decrease this bond order parameter for the CN bond and thus to reduce electronic contributions to the barrier to rotation. The same parameter determined for model II showed an increase compared with neat DMA and therefore restricts the intramolecular rotation. The nett result of these interactions could conceivably lead to an overall increase in the barrier to rotation but it could be argued that the barrier should continue increasing for mole ra-

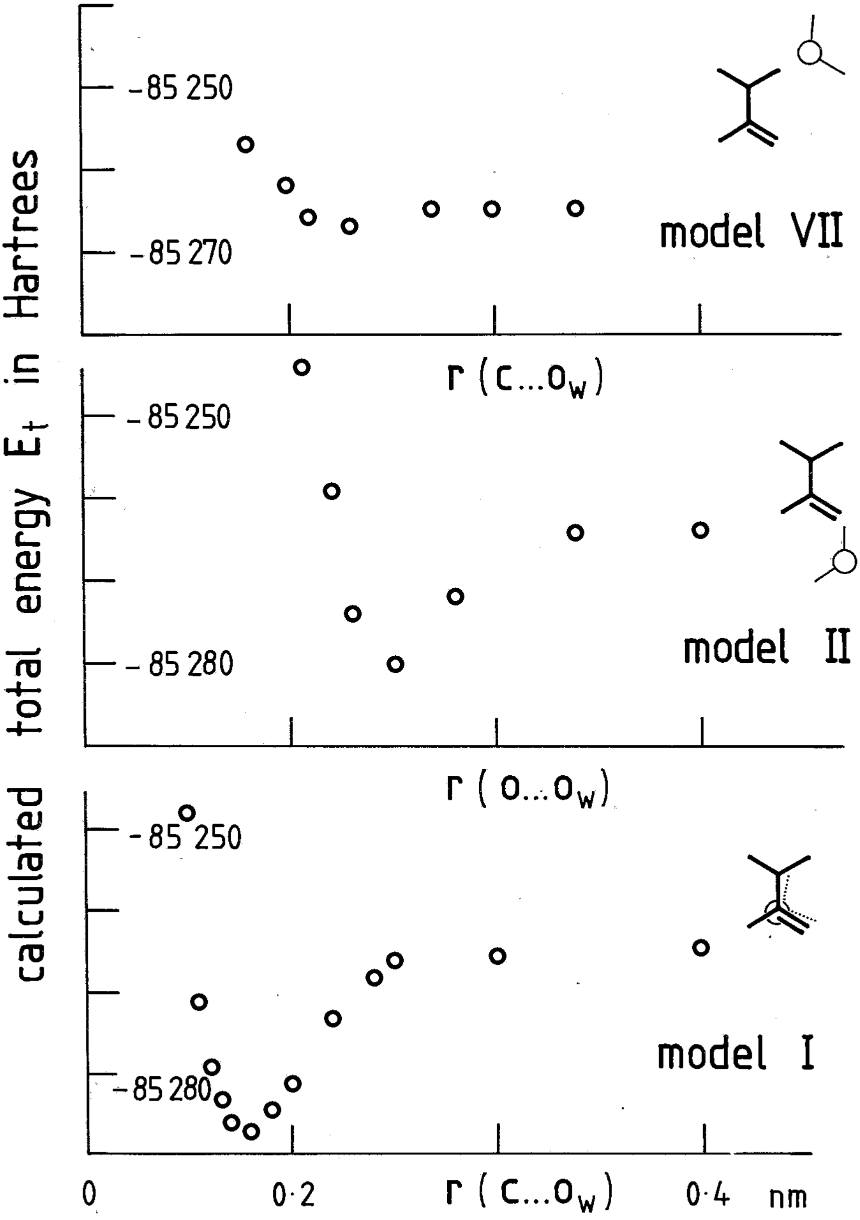


FIGURE 2: *Total Energy calculated using CNDO/2 for interacting Amide-Water molecules as a function of interatomic distance in some of the models where minima were observed.*

tios beyond 1:1 when water is added to DMA, unless the steric interactions in a «layered» structure of alternating amide and water molecules are dominant.

Conclusions

The models shown to be stable by the CNDO/2 calculations are as follows. The most stable involves the interaction between parallel amide and water molecules with a minimum energy when 0.8 nm apart. There are two possible sites for water on the amide according to this model but transient stacking or layering may lead to an apparent 1:1 ratio.

The next most stable involves the conventional hydrogen bonding situation between the water protons and the amide carbonyl oxygen atom. There are two sites per amide molecule again here, and each water molecule is capable of hydrogen bonding to two amide oxygens, or one amide and one water oxygen. The evidence from other work (carbon-13 chemical shifts viscosity) suggesting a first hydration shell of three water molecules is therefore compatible with these CNDO/2 results. The concept of electron density has been used to discover which changes are likely to occur in the change distribution of the N, N-dimethylacetamide when it interacts with water. The calculations show, in agreement with carbon-13 chemical shift data, the greater sensitivity of C1 in comparison to C2, C3 and C4. The correlation between electron density and chemical shift which was sought did not emerge, not surprisingly perhaps, on detailed examination. However, no account has been taken of other contributions to the screening constant which may involve anisotropy and Van der Waal's factors. The dominant factor in determining trends in activation energies can be interpreted to be steric in character and the picture where water and amide molecules alternate in parallel does explain the insensitivity of E_a beyond the mole ratio 1:1. The overall picture can best be thought of as a dynamic equilibrium between the different models and measurements made at the radiofrequency end of the electromagnetic spectrum reflect a time average of the environments of the amide molecule weighted according to their relative stabilities.

The use of liquid phase data in examining the trends of the CNDO/2 results can be justified because the importance of molecular interactions in the liquid phase requires some attempt at an explanation not wholly based on speculation. The systems most likely to prove of greatest intrinsic interest do not lend themselves to vapour phase study or study in other conditions which may be regarded as aesthetically desirable.

Περίληψη

Τὸ πρόγραμμα CNDO/2 χρησιμοποιήθηκε γιὰ τὴν μελέτη τῆς ἀλληλεπίδρασης τοῦ N, N-διμεθυλοακεταμιδίου (ΔΜΑ) μὲ 1 μέχρι 3 μόρια H_2O. Συνολικὰ μελετήθηκαν 18 μοντέλα ποὺ ἡ ἐπιλογή τους ἔγινε μὲ βάση φυσικὰ καὶ χημικὰ κριτήρια.

Οἱ ἠλεκτρονικὲς πυκνότητες καὶ ἡ τάξη τοῦ δεσμοῦ C-N ποὺ ὑπολογίστηκαν μὲ τὴν βοήθεια τοῦ CNDO/2, ἐξετάστηκαν σὲ σχέση μὲ τὰ πειραματικὰ δεδομένα.

Τὸ μοντέλο ποὺ προτείνεται ἐξηγεῖ τὰ πειράματα τῆς περιστροφῆς γύρω ἀπὸ τὸ δεσμὸ C-N, τοῦ ἰξῶδους καὶ μερικὲς ἀπὸ τὶς χημικὲς μετατοπίσεις τῶν 4 ^{13}C τοῦ ΔΜΑ.

Πιστεύουμε ὅτι περισσότερο πολύπλοκες ἀλληλεπιδράσεις τοῦ ΔΜΑ μὲ τὸ H_2O π.χ. ἀλληλεπίδραση τοῦ ΔΜΑ μὲ μεγαλύτερο ἀριθμὸ μορίων H_2O, θὰ ἔδιναν πληρέστερη ἀπεικόνιση καὶ ἑρμηνεία τῶν πειραματικῶν δεδομένων. Τέτοιοι ὅμως ὑπολογισμοί, πρὸς τὸ παρὸν τουλάχιστον, εἶναι ἀνέφικτοι.

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APPLICATION OF NMR SPECTROSCOPY TO STRUCTURE DETERMINATION OF SYNTHETIC DYES: PART I - CERTAIN CASES OF SPIN COUPLING OF N^+H PROTONS TO α-CARBON PROTONS

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Summary

Trphenylmethan, oxazine, thiazine and diazacarbocyanine dyes, do not show NMR N^+H absorption and spin-spin coupling of α-carbon protons of N-alkyl groups in trifluoroacetic acid. Such peaks and spin couplings, useful in structure elucidation of the dyes, was observed when the dye was in the reduced form.

Introduction

Cationic dyes of triphenylmethane, oxazine and thiazine type, that contain amino groups in their molecule, do not show any NMR, N^+H peaks and spin coupling of α-carbon protons to N^+H protons even in TFA.[1,2] It is obvious that there is no direct protonation of the amino group, as the positive charge is delocalized throughout the chromophoric system, and it is not localized on the nitrogen. It is apparent that such N^+H absorptions and spin-spin couplings of α-carbon protons, could be very informative in the structure determination of the dyes. Valuable information can be obtained concerning the structure of the chromophore and the substituents that belong to it, if the α-carbon protons could be recognized.

If instead of the dye, the reduced form is used, the charge is localized on the nitrogen and there is direct protonation of the amino group in TFA. In that case N^+H absorptions are observed, as like N^+H - α-carbon proton spin-spin couplings. The difficulty of the leucobase isolation, can be overcome by reducing the dye in the NMR tube by small amounts of zink powder and taking the NMR spectra, without previous isolation of the leucobase.

Experimental Part

Trifluoroacetic acid, Merck spectrophotometric grade Merck zink powder were used.

The spectra were recorded on a Varian T-60 spectrometer (measured probe temperature 35°C).

Commercial samples of dyes were purified by recrystallization and purity was checked by TLC chromatography.

TMS was used as reference.

Reduction Procedure:

Samples of 20 to 25 mg of the dye, were dissolved in the NMR tube in 0,3 ml TFA. Very small amount of zink powder was then added carefully into the tube till the samples were completely decolorized. The tube was then left for some time, till an excess of zink powder was settled, before the spectra were taken.

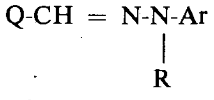
Results and Discussion

Proton NMR spectra of malachite green leucobase in TFA, shows a broad peak of N^+H at 575 Hz, a pattern of aromatics between 460 and 410 Hz (I), a singlet at 340 Hz, which belongs to the proton of the central carbon atom, and a doublet at 200 Hz (IIa). This doublet belongs to the protons of the four menthyl groups which are spin-spin coupled to the N^+H proton. If the N^+H peak is irradiated, spin-spin decoupling takes place, and the doublet collaplses to a singlet (IIb). If instead of the leucobase, the colour base of malachite green is dissolved in TFA, the proton NMR pectrum is quite different. There is no N^+H peak, and the four N-methyl groups give only a singlet. When the colourbase is reduced in the NMR tube with zink powder, the expected pattern of the leucobase is obtained.

In the case of oxazine dyes (III, X=N), and thiazine (III, X=S), when R_1, R_2, R_3 and R_4 are methyl groups, they appear as a sharp singlet when the dye is in the oxidized form. In the reduced form however, a broad N^+H peak appears at 536 Hz, while the N-methyl singlet, splits in a doublet. Irradiation of the N^+H peak, collapses the doublet to a singlet.

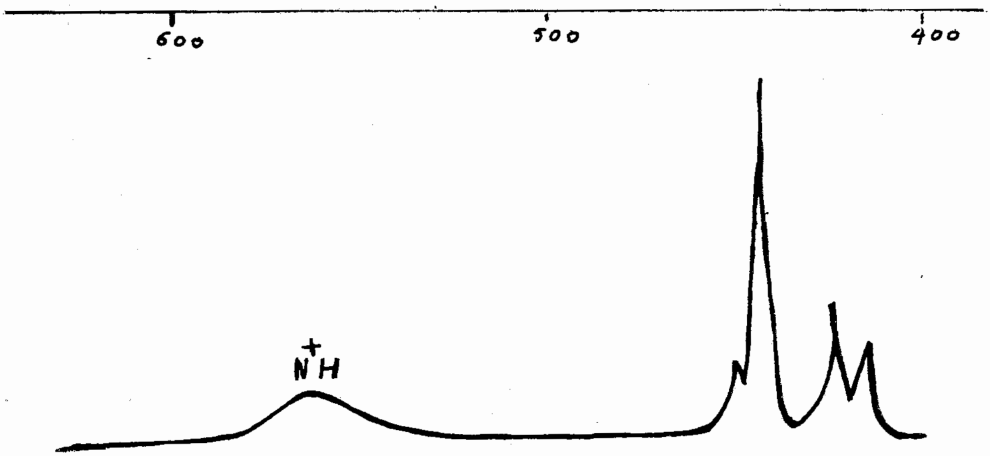
When R_1, R_2, R_3 and R_4 are ethyl groups, the α-carbon protons appear as a quartet (IVa) between 185 and 225 Hz, because of spin-spin coupling to the methyl protons of the ethyl group. In the reduced form, a broad N^+H peak appears at 475 Hz, which is far downfield from aromatics and can be easily integrated, and a second split appears in the quartet of the α-carbon protons (IVb), not so clear though as in the case of methyl substituents. This second splitting desappears, when the N^+H peak is irradiated (IVc).

Quite interesting is the case of another family of dyes for synthetic fibers. These dyes, known as diazacarbocyanine dyes, have a chromophore of the general structure:

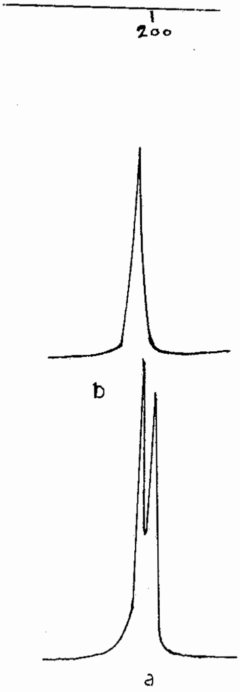


$$\text{Q-CH} = \underset{\displaystyle\overset{|}{\text{R}}}{\text{N}}\text{-N-Ar}$$

where Q is a heterocyclic or a heteroaromatic radical, and Ar is a substitued or unsubstitued aryl. R can be hydroze of alkyl group, and usually it is a methyl group.



I



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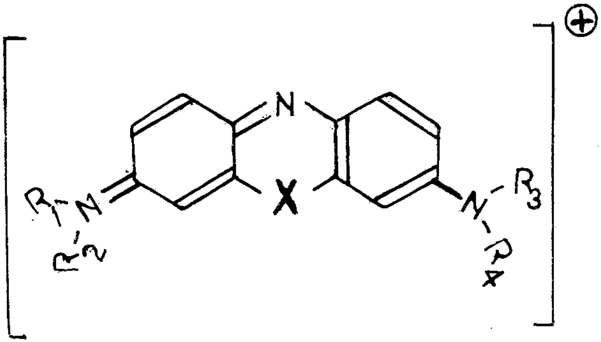
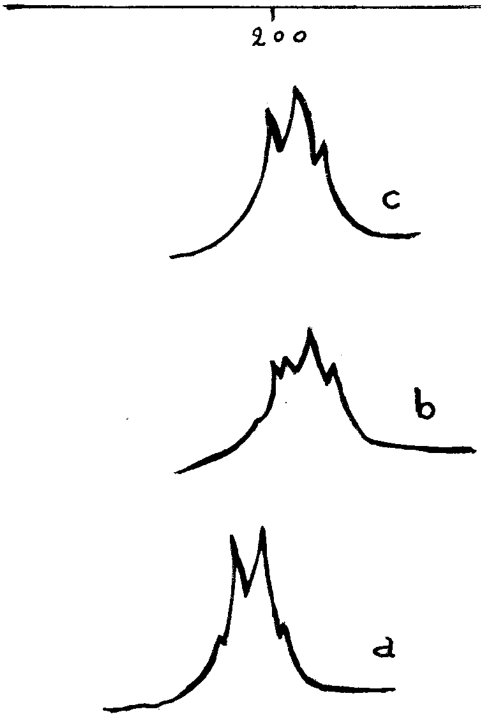
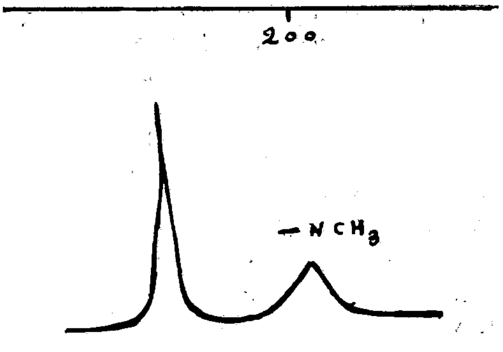


III



IV

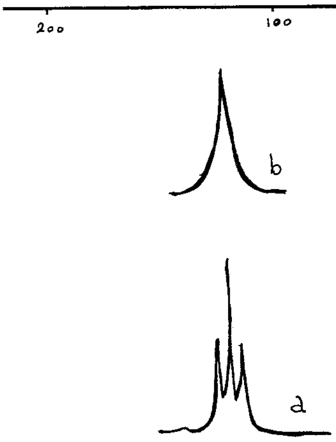
The proton NMR spectra or these dyes in TFA, besides of aromatic, heteroaromatic patterns, show a N-methyl peak at around 200 Hz, when R is a methyl group (V). When the dye is reduced in the NMR tube with zink powder, a broad N^+H_2 peak appears at 500 Hz, which is far downfield from aromatics and can be easily intergrated (VI).



V

The N-methyl peak splits in a very clear triplet (VIa), which collapses to a singlet (VIb), when the N^+H_2 peak is irradiated.

It is apparent that reduction splits the chromophore between the two nitrogen atoms, indication that there is double bond character between them. J values were always at the range of 5 Hz.



VI

Περίληψις

Ἐφαρμογὴ τῆς NMR φασματοφωτομετρίας στὸν προσδιορισμὸ τῆς συντάξεως τῶν συνθετικῶν χρωμάτων - Μέρος I, Μερικὲς περιπτώσεις σπὶν συζεύξεως τῶν N^+H *πρωτονίων μὲ τὰ πρωτόνια τοῦ α-ἄνθρακος.*

Χρώματα τῆς κατηγορίας τοῦ τριφαινυλομεθανίου, ὀξαζίνης, θειαζίνης καὶ διαζοκαρμποκυανίνης δὲν παρουσιάζουν N^+H ἀπορρόφηση στὸ NMR φάσμα τους σὲ τριφθοροοξικὸ ὀξὺ ἐπειδὴ τὸ πρωτόνιο δὲν εἶναι ἐντοπισμένο στὸ ἄζωτο, ἀλλὰ κατανεμημένο σὲ ὅλο τὸ μόριο. Στὴν ἀνηγμένη ὅμως μορφὴ ἐμφανίζεται N^+H ἀπορρόφηση καθὼς καὶ σύζευξη τῶν σπὶν τῶν N^+H μὲ τὰ πρωτόνια τοῦ α-ἄνθρακος τῶν ὑποκαταστατῶν τῶν ἀμινομάδων. Ἡ ἐντόπιση καὶ ὁλοκλήρωση τῶν N^+H πρωτονίων, ὅπως καὶ ἡ ἀναγνώριση τῶν πρωτονίων τοῦ α-ἄνθρακος εἶναι πολύτιμα στοιχεῖα γιὰ τὸν προσδιορισμὸ τῆς δομῆς τοῦ χρωμοφόρου. Γιὰ τὴ λήψη NMR φασμάτων λευκοενώσεων περιγράφεται μία ἁπλὴ τεχνικὴ ἀναγωγῆς τοῦ χρώματος στὸν σωλῆνα τοῦ NMR μὲ σκόνη ψευδαργύρου, χωρὶς νὰ χρειάζεται ἀπομόνωση τῆς λευκοενώσεως.

References and Notes

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Η ΕΠΙΔΡΑΣΗ ΤΟΥ ΔΙΑΛΥΤΗ ΚΑΙ ΤΗΣ ΣΥΓΚΕΝΤΡΩΣΗΣ ΣΤΙΣ ΠΑΡΑΜΕΤΡΟΥΣ ΠΥΡΗΝΙΚΟΥ ΜΑΓΝΗΤΙΚΟΥ ΣΥΝΤΟΝΙΣΜΟΥ ΠΡΩΤΟΝΙΟΥ (^{1}H-NMR) ΤΩΝ ΜΟΝΟΫΔΡΟΞΥΠΥΡΙΔΙΝΩΝ

ΧΑΡΙΚΛΕΙΑ ΙΩΑΝΝΙΔΟΥ-ΣΤΑΣΙΝΟΠΟΥΛΟΥ
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Περίληψη

Ἀναλύονται καὶ σχολιάζονται τὰ φάσματα NMR τῶν τριῶν μονοϋδροξυπυριδινῶν σὲ $CDCl_3$, DMSO-d_6 καὶ C_6D_6. Οἱ μεταβολὲς τῶν χημικῶν μετατοπίσεων ποὺ παρατηροῦνται κατὰ τὴν ἀλλαγὴ διαλύτη καὶ τὴν μεταβολὴ τῆς συγκέντρωσης ὀφείλονται σὲ ἀλληλεπιδράσεις διαλύτη-διαλυτοῦ ποὺ μετατοπίζουν τὶς ἰσορροπίες ταυτομέρειας ἤ/ καὶ αὐτοσύνδεσης. Ἡ ἐξάρτηση ἀπὸ τὴν συγκέντρωση καὶ τὸν διαλύτη ἀλλάζει τὴν σειρὰ ἐμφάνισης ὁρισμένων πρωτονίων, καί, ἐξ αἰτίας τῆς πολυπλοκότητας τῶν φασμάτων, μπορεῖ νὰ ὁδηγήσει σὲ λανθασμένη ἀπόδοση τῶν ἀπορροφήσεων.

Ἀπὸ τὴν μελέτη τῶν φασμάτων ^{1}H-NMR τῆς πυριδίνης καὶ τῶν παραγώγων της ἔχει προκύψει ὅτι ἡ φύση τοῦ διαλύτη ἐπηρεάζει ἔντονα τὴν χημικὴ μετατόπιση καὶ σὲ μικρότερο βαθμὸ τὶς σταθερὲς σύζευξης τῶν πρωτονίων τοῦ ἑτεροκυκλικοῦ δακτυλίου. Οἱ μεταβολὲς στὶς φασματικὲς παραμέτρους προκαλοῦνται ἀπὸ εἰδικὲς ἀλληλεπιδράσεις τῶν μορίων στὸ διάλυμα ποὺ εὐνοοῦν ὁρισμένες κατανομὲς τοῦ ἠλεκτρονιακοῦ φορτίου στὸ δακτύλιο.

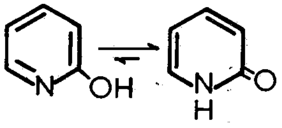
Οἱ μονοϋδροξυπυριδίνες εἶναι ὀξεοβασικοὶ καταλύτες[2]. Σὲ κατάσταση διαλύματος ὑφίστανται σὲ δύο ταυτομερεῖς μορφές, τὴν ἀμιδικὴ καὶ τὴν ἑτεροαρωματική. Οἱ ἰσορροπίες 2-ὑδροξυπυριδίνη **(1)** ⇄ 2-πυριδόνη **(2)** καὶ 4-ὑδρόξυπυριδίνη **(3)** ⇄ 4-πυριδόνη **(4)** εἶναι μετατοπισμένες πρὸς τὰ δεξιά[3], ἐνῶ ἡ ἰσορροπία 3-ὑδροξυπυριδίνη **(5)** ⇄ 3-πυριδόνη **(6)** εἶναι μετατοπισμένη πρὸς τὰ ἀριστερά[4]. Ἔτσι οἱ δομὲς ποὺ ἐπικρατοῦν στὸ διάλυμα εἶναι οἱ **2, 4** καὶ **5.**

Παρατηρήσαμε ὅτι τὰ φάσματα τῶν μονοϋδροξυπυριδινῶν ἐπηρεάζονται ἀπὸ τὴν φύση τοῦ διαλύτη καὶ ἀπὸ τὴν συγκέντρωση. Παρακάτω σχολιάζομε τὶς μεταβολὲς τῶν φασματικῶν παραμέτρων συσχετίζοντάς τις μὲ τὶς μοριακὲς ἀλληλεπιδράσεις μέσα στὸ διάλυμα.

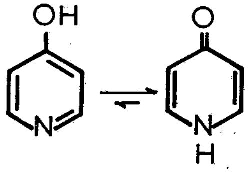
Ἀποτελέσματα καὶ Σχόλια

α. *Διαλύματα διμεθυλοσουλφοξειδίου καὶ χλωροφορμίου*

Σὲ μὴ πολικοὺς διαλύτες οἱ ὑδροξυπυριδίνες αὐτοσυνδέονται μὲ δεσμὸ ὑδρογόνου[2]:



1 2



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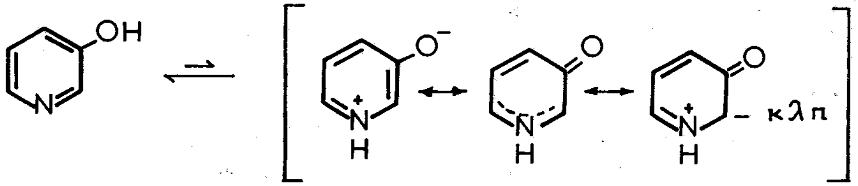
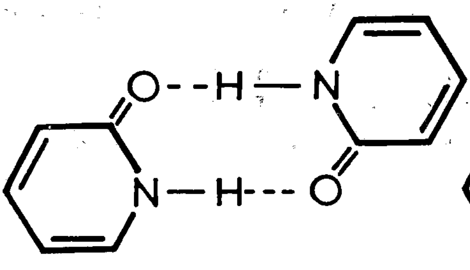


5 6

n Ὑδροξυπυριδίνη $\rightleftarrows$ (Ὑδροξυπυριδίνη)$_n$

Οἱ χαμηλὲς τιμὲς τῆς χημικῆς μετατόπισης τοῦ NH/OH ποὺ ἔχουν παρατηρηθεῖ στὶς 2- καὶ 4-ὑδροξυπυριδίνες ἑρμηνεύονται σὰν ἔνδειξη ἔντονης μετατόπισης τῆς ἰσορροπίας πρὸς τὰ ἀριστερά. Οἱ τιμὲς ποὺ μετρήσαμε σὲ χλωροφορμικὸ διάλυμα 0,5 M εἶναι 13,75 δ γιὰ τὴν 2- καὶ 13,13 δ γιὰ τὴν 4-ὑδροξυπυριδίνη, σὲ πολὺ καλὴ συμφωνία μὲ τὴν βιβλιογραφία[5,6]. Στὴν περίπτωση τῆς 2-ὑδροξυπυριδίνης, δεδομένα ἀπὸ IR, UV καὶ μετρήσεις ἐξασθένησης ὑπερήχων δείχνουν ὅτι τὸ n εἶναι 2[2,6,7]. Ὁ δακτύλιος στὸ διμερές, μὲ τὴν ταυτόχρονη (concerted) μεταφορὰ τῶν δύο πρωτονίων[8] μπορεῖ νὰ θεωρηθεῖ σὰν μεσομερὴς τῆς ἀμιδικῆς (7) καὶ τῆς ἑτεροαρωματικῆς δομῆς. Ἐξ αἰτίας τῆς ἐνέργειας μεσομέρειας ὁ ὑδρογονικὸς δεσμὸς εἶναι ἀπὸ τοὺς σταθερότερους γνωστούς.



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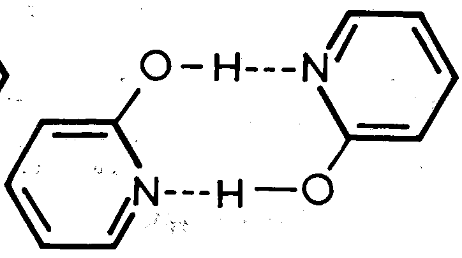

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Ἡ ἀπορρόφηση NH/OH τῆς 3-ὑδροξυπυριδίνης στὸ χλωροφόρμιο εἶναι στὰ 10,33 δ. Σὲ σύγκριση μὲ τὰ 2- καὶ 4-παράγωγα αὐτὸ ὑποδηλώνει μικρότερο βαθμὸ ὑδρογονικοῦ δεσμοῦ δηλαδὴ μικρότερης ἔκτασης αὐτοσύνδεση.

Τὸ DMSO-d_6 σχηματίζει ἰσχυρὸ ὑδρογονικὸ δεσμὸ μὲ τὶς μονοϋδροξυπυριδίνες καὶ παρεμποδίζει τὴν αὐτοσύνδεσή τους. Ἡ χημικὴ μετατόπιση τοῦ NH/OH στὰ διαλύματα διμεθυλοσουλφοξειδίου παραμένει σταθερὴ κατὰ τὴν ἀραίωση ἀπὸ 4 Μ ἕως 0,025 Μ (11.4 δ γιὰ τὴν 2-ὑδροξυπυριδίνη, 9,7 δ γιὰ τὴν 3-ὑδροπυριδίνη καὶ 10,4 δ γιὰ τὴν 4-ὑδροξυπυριδίνη). Ἡ παρατηρούμενη ἀπορρόφηση ὀφείλεται στὸ πρωτόνιο NH/OH τὸ δεσμευμένο ἀπὸ τὸν διαλύτη ποὺ ἤδη ἀπὸ τὴν συγκέντρωση 4 Μ εἶναι σὲ περίσσεια ὡς πρὸς τὸ διαλυτό.

Ἐξ ἄλλου τὸ διμεθυλοσουλφοξείδιο, ἐξ αἰτίας τῆς μεγάλης του διηλεκτρικῆς σταθερᾶς (ε= 45)[9], σταθεροποιεῖ τὴν πυροδονικὴ μορφὴ ὅπως προκύπτει[4] ἀπὸ τὴν σύγκριση τῶν φασμάτων σὲ DMSO-d_6 καὶ σὲ $CDCl_3$ (ε= 4,81): Τὸ πρόσημο τῆς συνολικῆς διαφορικῆς μετατόπισης Σ Δδ ($CDCl_3$ - DMSO-d_6) τῶν ὑδρογόνων τοῦ δακτυλίου εἶναι θετικό (Πίνακας I). Περιορισμοὶ διαλυτότητας δὲν ἐπιτρέπουν τὴν χρησιμοποίηση ἑνὸς τελείως μὴ πολικοῦ διαλύτη ὅπως ὁ τετραχλωράνθρακας (ε= 2,24) γιὰ σημεῖο ἀναφορᾶς. Οἱ ἐπιμέρους Δδ εἶναι ἐπίσης θετικὲς μὲ ἐξαίρεση τὴν Δδ τῶν πρωτονίων Η-2 καὶ Η-6 τῆς 4-ὑδροξυπυριδίνης ποὺ εἶναι ἀρνητική. Αὐτὸ θὰ μποροῦσε νὰ ἀποδοθεῖ σὲ μεταβολὲς ποὺ προκαλοῦνται στὴ μαγνητικὴ ἀνισοτροπία τοῦ ἀζώτου καθὼς ὁ δεσμὸς ὑδρογόνου μεταξὺ μορίων τοῦ διαλυτοῦ ἀντικαθίσταται, μερικά, ἀπὸ δεσμὸ διαλύτη-διαλυτοῦ.[10]

Μιὰ δεύτερη ἔνδειξη ὅτι τὸ DMSO-d_6 σταθεροποιεῖ τὴν πυριδονικὴ μορφὴ εἶναι ἡ γενικὴ αὔξηση τῆς προάσπισης μὲ τὴν ἀραίωση (Σχῆμα 1). Στὶς 2- καὶ 3-ὑδροξυπυριδίνες οἱ καμπύλες ἀραίωσης τῶν Η-4, Η-6 καὶ Η-4, Η-5, ἀντίστοιχα, διασταυρώνονται. Στὴν περίπτωση τῆς 3-ὑδροξυπυριδίνης ἡ ἀντιμετάθεση τῶν δύο ὑδρογόνων στὸ φάσμα γίνεται ἀντιληπτὴ ἀπὸ τὴν μορφὴ τῶν ἀσθενῶν ἀκραίων κορυφῶν (Σχῆμα 2). Γιὰ τὸν ὑπολογισμὸ τῶν χημικῶν μετατοπίσεων σὲ παράγωγα τῆς πυριδίνης ὑπάρχουν στὴν βιβλιογραφία τιμὲς προσθετικῶν παραμέτρων ποὺ ἔχουν προκύψει ἀπὸ πυκνὰ διαλύματα διμεθυλοσουλφοξειδίου. Σὲ ἀραιὰ διαλύματα τῆς ἴδιας συγκέντρωσης τὸ Η-4 εἶναι λιγότερο θωρακισμένο ἀπὸ τὸ Η-5 στὸ χλωροφόρμιο καὶ στὸ βενζόλιο σὲ ἀντίθεση μὲ ὅ,τι συμβαίνει στὸ διμεθυλοσουλφοξείδιο (Πίνακας I). Ὅπως εἶναι φανερὸ ἀπὸ τὰ δεδομένα μας, ὅταν ἡ ἐξάρτηση ἀπὸ τὴν συγκέντρωση καὶ τὸν διαλύτη δὲν λαμβάνονται ὑπόψη κατὰ τὸν ὑπολογισμὸ ἡ ἀποδοσὴ τῶν ἀπορροφήσεων εἶναι λανθασμένη[10]. Οἱ τιμὲς τῶν σταθερῶν σύζευξης στὸ DMSO-d_6 καὶ σὲ $CDCl_3$ (Πίνακας I) συμφωνοῦν ἱκανοποιητικὰ μὲ τὶς τιμὲς τῆς βιβλιογραφίας[1,4]. Ἡ ἀλλαγὴ τῆς συγκέντρωσης δὲν τὶς ἐπηρεάζει.

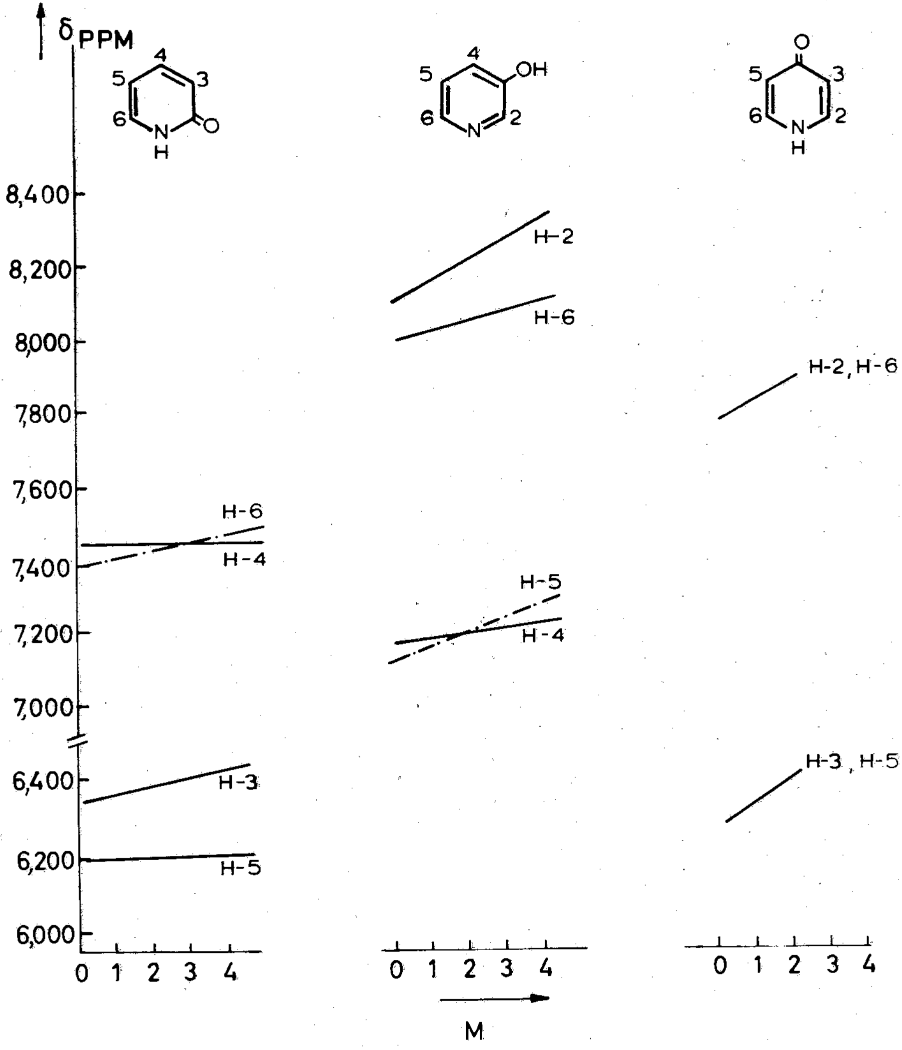
β. Βενζολικὰ διαλύματα

Φάσματα NMR μονοϋδροξυπυριδινῶν σὲ βενζόλιο δὲν ἀναφέρονται στὴ βιβλιογραφία πιθανὸν ἐξ αἰτίας τῆς περιορισμένης διαλυτότητας. Χρησιμο-

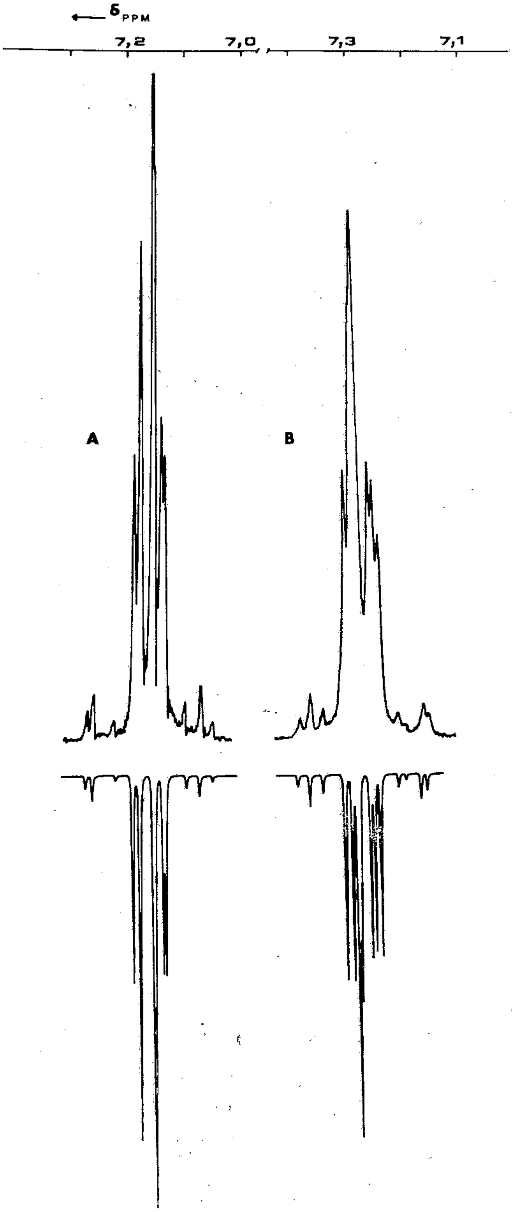
ΠΙΝΑΚΑΣ Ι: *Χημικὲς Μετατοπίσεις καὶ Σταθερὲς Σύζευξης* Διαλυμάτων Μονοϋδροξυπυριδινῶν 0,025 Μ.*

Οὐσία καὶ Διαλύτης			δ		ppm	ΣΔδ					J_{Hz}					
	H-2	H-3	H-4	H-5	H-6	ppm	2,3	2,4	2,5	2,6	3,4	3,5	3,6	4,5	4,6	5,6
2-ὑδροξυπυριδίνη																
$CDCl_3$	–	6,590	7,465	6,275	7,360	–	–	–	–		9,40	1,15	0,55	6,60	2,10	6,40
DMSO-d_6	–	6,290	7,390	6,130	7,325	0,555	–	–	–		9,40	1,10	0,60	6,40	2,00	6,40
C_6D_6	–	6,450	6,775	5,425	6,640	2,400	–	–	–		9,20	1,30	1,10	6,40	2,10	6,50
3-ὑδροξυπυριδίνη																
$CDCl_3$	8,295	–	7,294	7,247	8,100	–	–	2,80	0,45	0,30	–	–	–	8,50	1,25	4,75
DMSO-d_6	8,105	–	7,145	7,185	8,000	0,501	–	2,80	0,70	–0,15	–	–	–	8,35	1,75	4,65
C_6D_6	8,200	–	6,915	6,582	7,871	1,368	–	2,80	0,70	0,15	–	–	–	8,35	1,45	4,65
4-ὑδροξυπυριδίνη																
$CDCl_3$	7,590	6,440	–	6,440	7,590	–	7,00	–	–	–	–	–	–	–	–	7,00
DMSO-d_6	7,670	6,150	–	6,150	7,670	0,420	7,00	–	–	–	–	–	–	–	–	7,00

* Τὸ ἄζωτο συζεύγνυται μὲ τὰ πρωτόνια Η-2 καὶ Η-4. Ἡ λεπτὴ ὑφὴ ποὺ προκύπτει ἀπὸ τὴν σύζευξη αὐτὴ δὲν εἶναι ὁρατὴ ἐπειδὴ τὸ τετράπολο τοῦ ἀζώτου ἐπιταχύνει τὴν ἀποκατάσταση τῶν πυρηνικῶν του πληθυσμῶν. Ἔτσι, ὁ τριπλασιασμὸς ἀπὸ τὸ Ι $^3/_2$ τοῦ ^{14}N καταλήγει σὲ ἁπλὴ διεύρυνση τῶν κορυφῶν[18].



ΕΙΚ. 1: Ἐξάρτηση τῶν χημικῶν μετατοπίσεων τῶν πρωτονίων τοῦ δακτυλίου ἀπὸ τὴν συγκέντρωση σὲ διαλύματα DMSO-d_6.

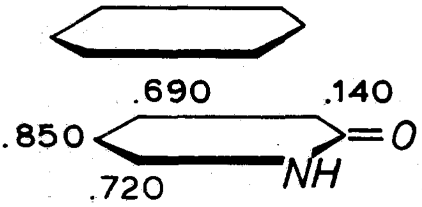


ΕΙΚ. 2: *Πειραματικά (ἄνω) καὶ ἐξομοιωμένα ἀπὸ τὸν ὑπολογιστὴ (κάτω) φάσματα τῆς ἀρωματικῆς περιοχῆς τῆς 3-ὑδροξυπυριδίνης σὲ διαλύματα* DMSO-d_6. *Εὖρος ἀπορρόφησης ἐξομοιωμένων φασμάτων 0,3 Hz. A: Συγκέντρωση 0,3 M. H-2: 8,150 δ, H-4: 7,165 δ, H-5: 7,205 δ, H-6: 8,035 δ. B: Συγκέντρωση 4,2 M. H-2: 8,350 δ, H-4: 7,295 δ, H-5: 7,242 δ, H-6: 8,135 δ.*

ποιῶντας τὴν παλμικὴ τεχνικὴ Fourier καταγράψαμε φάσματα συγκέντρωσης 10^{-2} M. Ἡ διαλυτότητα τῆς 4-ὑδροξυπυριδίνης εἶναι πολὺ χαμηλότερη ἀπὸ αὐτὸ τὸ ὅριο καὶ δὲν τὴν μελετήσαμε.

Τὸ βενζόλιο ἁπλοποιεῖ ἐντυπωσιακὰ τὸ φάσμα τῆς 3-ὑδροξυπυριδίνης (Σχῆμα 3). Στὸ χλωροφόρμιο καὶ τὸ διμεθυλοσουλφοξείδιο τὰ φάσματα εἶναι τοῦ τύπου ABCD. Ὁ λόγος (ν_A-ν_B) /J_{AB} εἶναι στὰ 100 MHz, τόσο μικρός (<0,5), ὥστε ἡ περιοχὴ AB νὰ μὴ μπορεῖ νὰ δώσει καμμιὰ πληροφορία χωρὶς εἰδικὴ ἐπεξεργασία. Ἀντίθετα στὸ βενζολικὸ διάλυμα τὸ φάσμα εἶναι σχεδὸν πρώτης τάξης. Γενικὰ παρατηροῦμε, ὅτι σὲ σχέση μὲ τοὺς ἄλλους διαλύτες, ὅλα τὰ πρωτόνια τοῦ πυριδινικοῦ δακτυλίου τῶν 2 καὶ 3-ὑδροξυπυριδινῶν παρουσιάζουν αὐξημένη θωράκιση.

Τὸ βενζόλιο ἀνήκει στοὺς λεγόμενους δισκοειδεῖς διαλύτες ποὺ τὰ μόρια τους παρεμβάλλονται σὰν δίσκοι ἀνάμεσα στὰ μόρια τῆς διαλυόμενης οὐσίας, ἰδίως ὅταν εἶναι καὶ αὐτὴ ἀρωματική, σὲ μία διάταξη κατὰ ἐπίπεδα[12]. Ἀποτέλεσμα τῆς διάταξης αὐτῆς εἶναι ἡ αὔξηση τῆς μαγνητικῆς θωράκισης ἐκείνων τῶν πρωτονίων ποὺ βρίσκονται στὴ ζώνη θετικῆς θωράκισης τοῦ βενζολικοῦ δακτυλίου (κοντὰ στὸν ἄξονα ἕκτης τάξης). Μὲ πολικὰ μόρια τὸ βενζόλιο παρουσιάζει καὶ μία ἀλληλεπίδραση ἄλλου τύπου[13]. Τὸ ἀρνητικὸ ἄκρο τοῦ διπόλου τοῦ διαλυτοῦ ἀπωθεῖται ἀπὸ π-νέφος τοῦ βενζολίου καὶ τείνει νὰ ἀποφύγει τὸν δακτύλιο. Ἐξ αἰτίας τῆς μεγάλης πολωσιμότητας στὸ ἐπίπεδο τοῦ δακτυλίου, ἐμφανίζεται μία ἐξ ἐπαγωγῆς διπολικὴ ροπὴ ποὺ ἀλληλεπιδρᾶ μὲ τὴν διπολικὴ ροπὴ τοῦ διαλυτοῦ. Ἡ ἀλληλεπίδραση διπόλου - ἐπαγομένου διπόλου μπορεῖ νὰ παρουσιαστεῖ στὶς μονοϋδροξυπυριδίνες. Σὲ διαλύματα διοξανίου ἡ διπολικὴ ροπὴ μ τῆς 2-ὑδροξυπυριδίνης εἶναι 1,95 D καὶ τῆς 3-ὑδροξυπυριδίνης 2,00 D[14]. Τὸ ἀρνητικὸ ἄκρο τοῦ διπόλου εἶναι τὸ ἄτομο τοῦ ὀξυγόνου. Οἱ διατάξεις 9 καὶ 10 ὅπου σημειώνονται οἱ Δδ ($CDCl_3$-C_6D_6) σὲ ppm, εἶναι σύμφωνες καὶ μὲ τοὺς δύο τρόπους ἀλληλεπίδρασης καὶ δικαιολογοῦν τὶς παρατηρούμενες μετατοπίσεις. Ἀνάλογα παραδείγματα ἀναφέρονται στὴν βιβλιογραφία γιὰ Ν-μεθυλαμίδια[15] καὶ Ν-μεθυλολακτάμες[16]. Οἱ σταθερὲς σύζευξης στὸ βενζόλιο δὲν παρουσιάζουν ἄξιες λόγου διαφορὲς ἀπὸ τὶς ἀντίστοιχες τιμὲς στὸ χλωροφόρμιο ἢ τὸ διμεθυλοσουλφοξείδιο δείχνοντας ὅτι οἱ ταυτομερεῖς δομὲς 2, 4 καὶ 5 ἐπικρατοῦν καὶ στοὺς τρεῖς διαλύτες.



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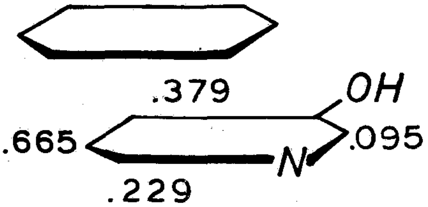

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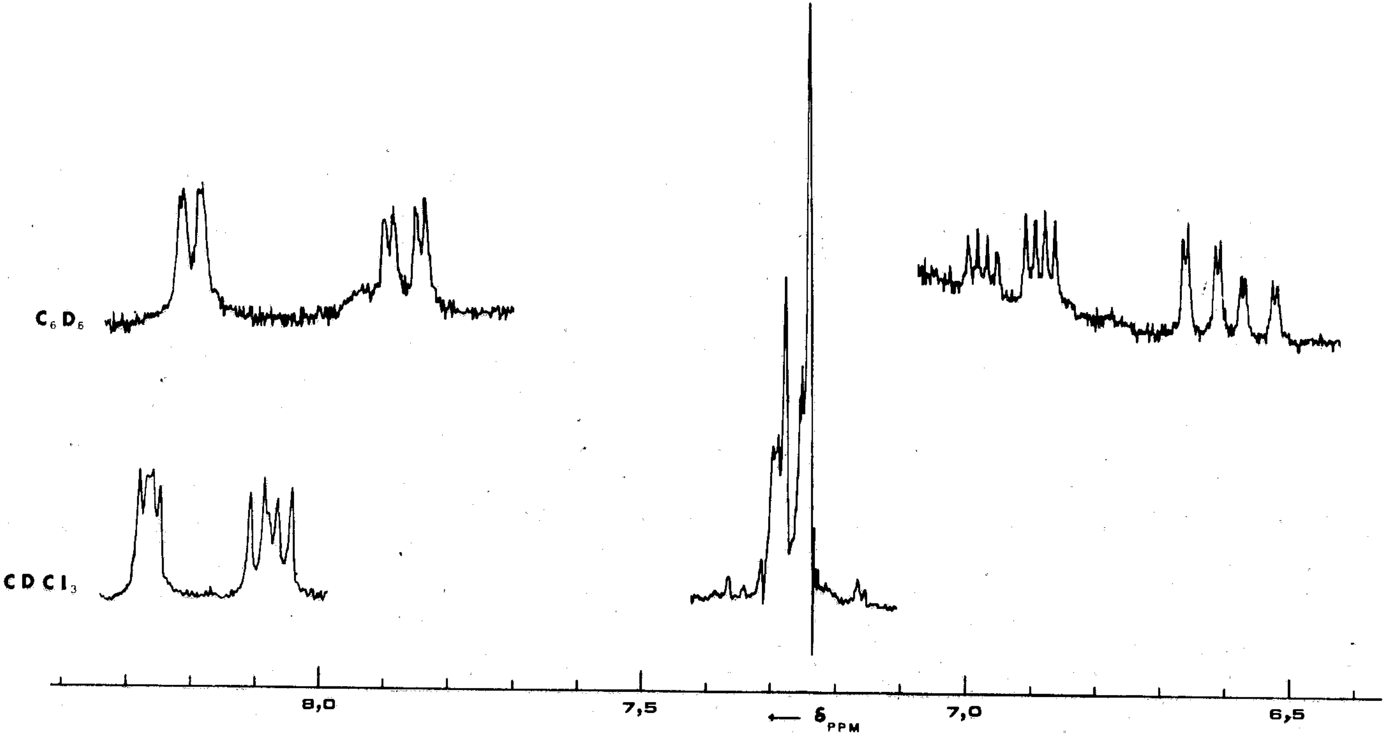

ΕΙΚ. 3: *Φάσματα ἀρωματικῆς περιοχῆς τῆς 3-ὑδροξυπυριδίνης. Ἄνω: Διαλύτης C_6H_6, συγκέντρωση 0,025 M, τεχνικὴ FT (211 transients). H-2: 8,200 δ, H-4: 6,915 δ, H-5: 6,582 δ, H-6: 7,871 δ. Ἀπὸ τὸ φάσμα παραλείπεται ἡ πολὺ ἰσχυρὴ ἀπορρόφηση τοῦ μερικὰ δευτεριωμένου διαλύτη (7,25 δ). Ἡ πλατειὰ ἀπορρόφηση στὰ 7,9 δ ποὺ συμπίπτει μερικὰ μὲ τὸ H-6 εἶναι δορυφόρος ἄνθρακα-13 τοῦ διαλύτη (μέση σταθερὰ σύζευξης $J_{13\,C\text{-}H}$ = 170 Hz). Κάτω: Διαλύτης $CDCl_3$, συγκέντρωση 0.1 M, τεχνικὴ CW. H-2= 8,262 δ, H-4: 7,294 δ, H-5: 7,247 δ, H_6: 8,072 δ. Ἡ ἀπορρόφηση τοῦ $CHCl_3$ στὰ 7,24 δ (κατάλοιπο μὴ δευτεριωμένου διαλύτη) συμπίπτει μὲ τὸ σύμπλεγμα κορυφῶν τῶν H-4 καὶ H-5.*

Πειραματικὸ μέρος

Οἱ ὑδροξυπυριδίνες ἀγοράστηκαν ἀπὸ τὴν Aldrich, ἀνακρυσταλλώθηκαν ἀπὸ χλωροφόρμιο καὶ ἡ καθαρότητά τους ἐλέγχθηκε ἀπὸ τὸ σημεῖο τήξεως καὶ τὸ φάσμα ^{1}H-NMR. Οἱ δευτεριωμένοι διαλύτες ἦταν τῆς Stohler. Ἡ ξηρότητα τοῦ δευτεριωμένου διμεθυλοσουλφοξειδίου (DMSO-d_6) μετὰ τὸ ἄνοιγμα τοῦ μπουκαλιοῦ ἐξασφαλίστηκε μὲ τὴν προσθήκη μοριακῶν κόσκινων καὶ τὴν φύλαξη σὲ ξηραντήρα. Ἐσωτερικὸ σημεῖο ἀναφορᾶς γιὰ τὴ μέτρηση τῶν χημικῶν μετατοπίσεων ἦταν τὸ τετραμεθυλοσιλάνιο (1% στὸ διάλυμα).

Γιὰ τὴ λήψη φασμάτων χρησιμοποιήθηκε τὸ φασματόμετρο XL-100-15 τῆς Varian μὲ συνεχῆ σάρωση (CW) γιὰ δείγματα πυκνότερα ἀπὸ 0,1 M καὶ μὲ παλμικὴ τεχνικὴ Fourier (FT) γιὰ ἀραιὰ δείγματα. Τυπικὲς τιμὲς σταθερῶν γιὰ τὴν παλμικὴ ἀκτινοβόληση ἦταν pulse width, PW=23 μs (παλμὸς 90°), pulse delay, PD=10 s καὶ data length, DL= 8092. Θερμοκρασία δείγματος κατὰ τὴν μέτρηση: 33°C.

Ἡ ἐξομοίωση τῶν φασμάτων ἔγινε ἀπὸ τὸν ὑπολογιστὴ τοῦ συστήματος XL-100-15. Χρησιμοποιήθηκε ἕνα πρόγραμμα ποὺ λογαριάζει τὶς πυρηνικὲς μεταβάσεις σὲ συστήματα ἀλληλεπιδρώντων πυρηνικῶν σπὶν (μέχρι 6 σπὶν μὲ $I=1/2$)[17]. Τὸ πρόγραμμα δὲν περιλαμβάνει ἐπαναληπτικὴ προσέγγιση. Οἱ τιμὲς τῶν ὑπολογισμένων παραμέτρων ἔχουν ἀκρίβεια χιλιοστοῦ τοῦ ppm γιὰ τὶς χημικὲς μετατοπίσεις καὶ δέκατου τοῦ Hertz γιὰ τὶς σταθερὲς σύζευξης.

Summary

The proton NMR spectra of the three monohydroxypyridines in $CDCl_3$, DMSO-d_6 and C_6D_6 are analyzed. The solvent and concentration effects on the chemical shifts are discussed in terms of solute-solvent interactions which perturb the tautomeric and/or self-association equilibria. The concentration and solvent dependence changes the order of appearance of some protons and, due to the complexity of the spectra, may lead to erroneous assignments.

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FLOUR ASH CONTENT OF TWO MEXICAN VARIETIES WHEAT: YECORA AND SIETTE GERROS

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The consequent works have been performed in one of the laboratories of the Ministry of Commerce.

Abstract

Greek legislation controls the wheat flours quality from its ash content, for fixed «extraction rate». Since the year 1977, two soft Mexican wheat varieties —Yecora and S. Gerros— have begun to be cultivated in Greece.

Certain wheat flour-mill owners expressed, during 1978, some complaints about higher ash content of flours, produced by the above mentioned Mexican varieties, in comparison with the Greek old ones.

Our laboratories have undertaken to research the problem in question and to suggest the proper solution.

In fact we have collected 55 samples, from about all Greek regions in which wheat is cultivated. These samples of wheat were milled at our laboratory flour-mill; its milling products and by-products were subjected to analyses in respect of the moisture, the ash and the starch.

From their results, it is clear seen that the complaints of the wheat flour-mill owners were exaggerated and the flour ash content must remain at the same limits, as previously.

Introduction

Two types of flours produced from Greek soft wheat and characterized as T55 and T70, must fulfill, among the other physical and chemical constants, the term of the ash content. It must amount to 0,45% and 0,50% correspondingly, for an «extraction rate» of 57% for the first type and 70% for the second one and on 13,5 - 14,5% moisture basis.

The Experimental of the work

For the performance of the work, in question, our laboratories have collected,

21 samples of Yecora wheat variety

20 samples of S. Gerros wheat variety
11 samples of Greek soft wheat variety and
4 samples of Greek durum wheat variety

The above samples are representative of about the whole area, of Greek country, cultivated with wheat.

Our laboratories are supplied with a laboratory flour-mill made by BUHLER BROTHERS UZWIL-SWITZERLAND, Model MLU-201, in which the milling process has taken place.

After preparatory work proceedings for cleaning the wheat samples, they were tempered in order to absorb the necessary moisture for milling, about 15%.

The duration of tempering amounted to 40-60 seconds. After tempering, each sample was left to rest about 24 hours, for the uniform distribution of the moisture. Afterwards, it was milled by the following conditions:

1. Distance adjustment between break rolls,

$$B1 = \frac{50}{100}\,\text{mm}, \quad B2 = \frac{10}{100}\,\text{mm} \quad \text{and } B3 = \frac{8}{100}\,\text{mm}$$

2. Distance adjustment between smooth rolls,

$$\text{On } C1 = \frac{7}{100}\,\text{mm till } C3 = \frac{3}{100}\,\text{mm}$$

3. Sieve coverings,
    I. Break rolls compartment
      upper part: wire sieves
      No 30 34 40
      lower part: silken sieves
      8χχ 9χχ 9χχ
    II. Smooth rolls compartment
      upper part from left: two wire sieves
      No 30 30
      directly down of them: three silken sieves
      9χχ 10χχ 10χχ
      and even down of them also three silken sieves
      9χχ 10χχ 10χχ

The mill was adjusted in such a way to yield 3 kilos per 80-90 minutes. After a number of tests, the above yield was found to be rather the optimum, for this variety of wheat.

Our laboratory flour-mill produced six flour qualities, the brans and the shorts. All these products and by-products were subjected to analyses as regard the moisture and the ash.

The brans, the shorts as well as the total flour, taken by mixing the above six qualities, were subjected to analyses of the starch content, in order to control the perfection grade of milling process.

The results are calculated on 14% moisture basis flour, produced from wheat on 15% moisture basis.

We also enumerated the percentage of vitreous kernels, at wheat in the initial condition and before tempering it.

The table I gives this percentage of the whole number of wheat samples.

The Fig. 1 gives the diagram of our laboratory flour-mill.

The results

The products of milling process, the results of their analyses and the corresponding calculations for three representative samples, are stated in tables II, III, and IV.

We have also drawn, in the Fig. 2, the relative plots which correspond to column 13 and 14 of the three tables.

The steps on the extraction rate cooridinate were fixed by the successives additions of the six milled flours parts in order to increase ash content.

TABLE 1: Number of vitreous kernels

Yecora sample No	Vitreous kernels %	S. Gerros sample No	Vitreous kernels %	Greek soft sample No	Vitreous kernels %
1	70	3	62	1α	48
2	78	5	88	2α	60
4	84	7	78	3α	20
6	86	10	88	4α	10
8	90	11	78	5α	42
9	90	14	74	6α	40
12	86	16	82	7α	48
13	76	19	24	8α	20
15	88	21	74	9α	38
17	78	22	62	10α	26
18	52	24	72	11α	24
20	56	25	58		
23	70	28	52		
25	74	31	76		
27	77	32	70		
29	94	34	90		
30	92	37	90		
33	98	38	84		
35	96	40	90		
36	94	41	76		
39	78				

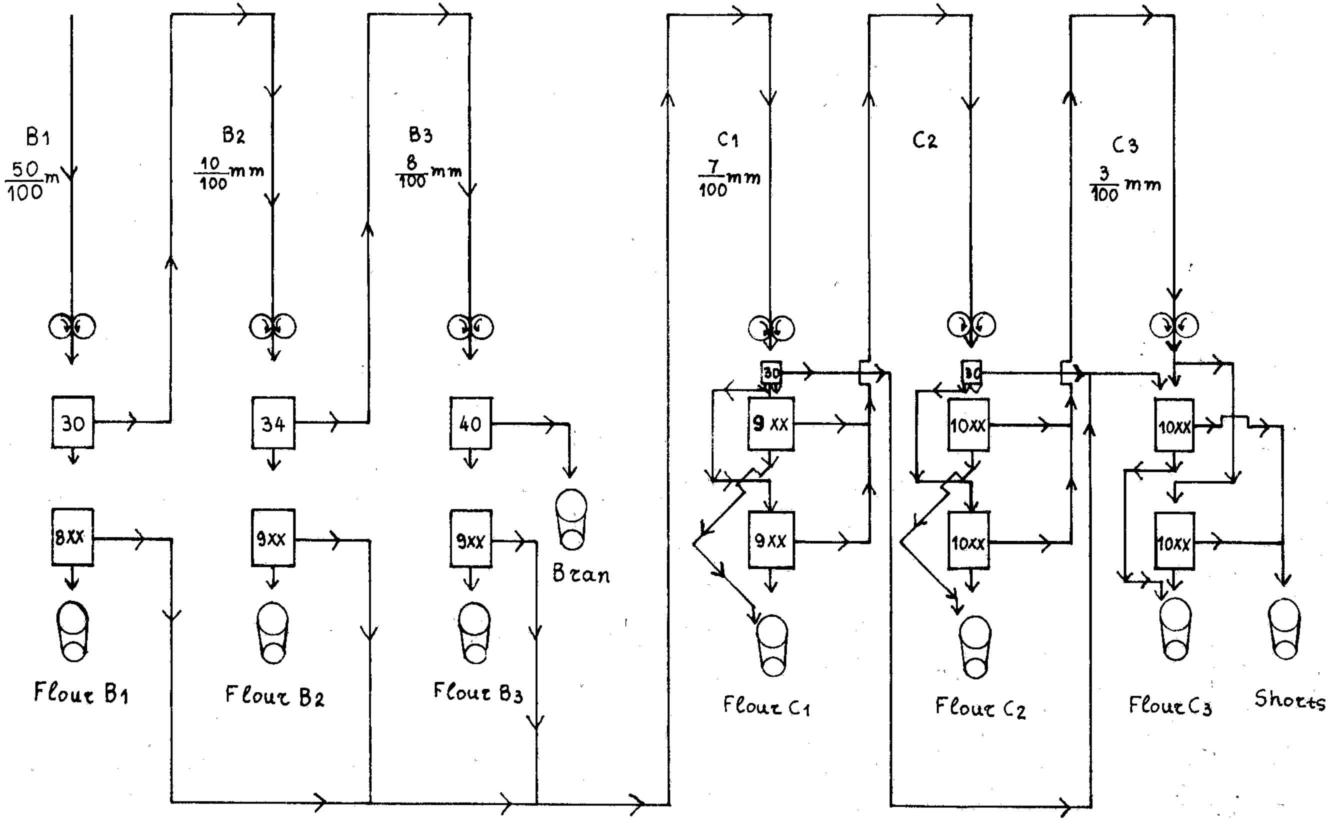

FIG. 1: *BUHLER laboratory flour-mill diagramm.*

TABLE II: *SAMPLE No 37 S. GERGOS*

1	2	3		4	5	6	7	8	9	10	11	12	13	14
	Weight as it is	Moisture %		Weight on dry basis	Ash after tempering %	Ash on dry basis %	Total ash	Starch %	Flour weight on 14% moisture basis	Column 9 from-to	Total ash from-to	Extraction* %	Extraction* % from-to column 12	Ash content % • column 10, 13
		Initial	After tempering											
WHEAT	3080	11,7	15,5	2602,6	1,11	1,31								
C1	570	14,3		488,5	0,35	0,41	2,00		568,03	568,03	2,00	18,55	18,55	0,35
C2	654	13,7		564,4	0,35	0,41	2,31		656,28	1224,31	4,31	21,43	39,98	0,35
B2	270	14,1		231,9	0,41	0,48	1,11	69,35	269,65	1493,96	5,42	8,81	48,79	0,36
B1	290	14,4		248,2	0,48	0,56	1,39		288,61	1782,57	6,81	9,43	58,22	0,38
B3	70	13,4		60,6	0,64	0,74	0,45		70,47	1853,04	7,26	2,30	60,52	0,39
C^3	366	12,3		321	0,63	0,72	2,31		373,26	2226,3	9,57	12,19	72,71	0,43
SHORTS	336	11,8		296,4	2,34	2,65	7,85	43,44	344,65	2570,95	17,42	11,26	83,97	0,68
BRAN	440	12,7		384,1	3,74	4,28	16,44	18,92	446,63	3017,58	33,86	14,59	98,56	1,13
TOTAL				2595,1			33,86							

% Ash content of Wheat from calculation = 1,31%
Wheat weight on 15% moisture basis = 3061,88
* From wheat on 15% moisture basis
To flour on 14% moisture basis
Losses = 0,29%

TABLE III: *Sample No 35 Yecora*

1	2	3		4	5	6	7	8	9	10	11	12	13	14
	Weight as it is	Moisture %		Weight on dry basis	Ash after tempering %	Ash on dry basis %	Total ash	Starch %	Flour weight on 14% moisture basis	Column 9 from-to	Total ash from-to	Extraction % *	Extraction % * from-to column 12	Ash content % * column 10, 13
		Initial	After tempering											
WHEAT	3360	10,3	15,4	2842,6	1,37	1,62	46,05							
C1	776	14,8		661,1	0,38	0,45	2,97		768,73	768,73	2,97	22,99	22,99	0,39
C2	740	14,6		632	0,41	0,48	3,03		734,89	1503,62	6	21,98	44,97	0,40
B1	355	15,1		301,4	0,53	0,62	1,87	66,39	350,47	1854,09	7,87	10,48	55,45	0,42
B2	250	15,1		212,2	0,54	0,64	1,36		246,75	2100,84	9,23	7,38	62,83	0,44
B3	62	14,3		53,1	0,75	0,88	0,47		61,74	2162,58	9,7	1,85	64,68	0,45
C3	307	13,9		264,3	0,86	1,00	2,64		307,33	2469,91	12,34	9,19	73,87	0,50
HORTS	311	13,2		269,9	3,21	3,70	9,99	34,25	313,84	2783,75	22,33	9,38	83,25	0,80
BRAN	479	14,3		410,5	4,91	5,73	23,52	18,98	477,33	3261,08	45,85	14,27	97,52	1,41
TOTAL				2804,5			45,85							

% Ash content of Wheat from calculation = 1,63%
Wheat weight on 15% moisture basis = 3344,2
* From wheat on 15% moisture basis
To flour on 14% moisture basis
Losses = 1,34%

TABLE IV: *SAMPLE No 5a SOFT*

1	2	3		4	5	6	7	8	9	10	11	12	13	14
	Weight as it is	Moisture %		Weight on dry basis	Ash after tempering %	Ash on dry basis %	Total ash	Starch %	Flour weight on 14% moisture basis	Column 9 from-to	Total ash from-to	Extraction % *	Extraction % * from-to column 12	Ash content % * column 10, 13
		Initial	After tempering											
WHEAT	2320	12,3	15,0	1972	1,52	1,79	35,30							
C1	609	14,0		523,7	0,31	0,36	1,88		609,0	609,0	1,88	26,2	26,2	0,31
B1	355	14,4		303,9	0,37	0,43	1,31		353,4	962,4	3,19	15,2	41,5	0,33
B2	214	14,1		183,8	0,44	0,51	0,94	68,56	213,7	1176,1	4,13	9,2	50,7	0,35
C2	382	13,6		330,0	0,51	0,59	1,95		383,7	1559,8	6,08	16,5	67,2	0,39
B3	53	13,3		45,9	0,62	0,71	0,33		53,4	1613,2	6,41	2,3	69,5	0,40
C3	107	12,4		93,7	1,53	1,75	1,64		108,9	1722,1	8,05	4,7	74,2	0,47
SHORTS	158	11,6		139,7	3,84	4,34	6,06	27,55	162,4	1884,5	14,11	7,0	81,2	0,75
BRAN	364	13,1		316,3	5,65	6,50	20,56	20,70	367,8	2252,3	34,67	15,8	97,1	1,54
TOTLA				1937			34,67							

% Ash content of Wheat from calculation = 1,79
Wheat weight on 15% moisture basis = 2320
* From wheat on 15% moisture basis
To flour on 14% moisture basis
Losses = 1,8%

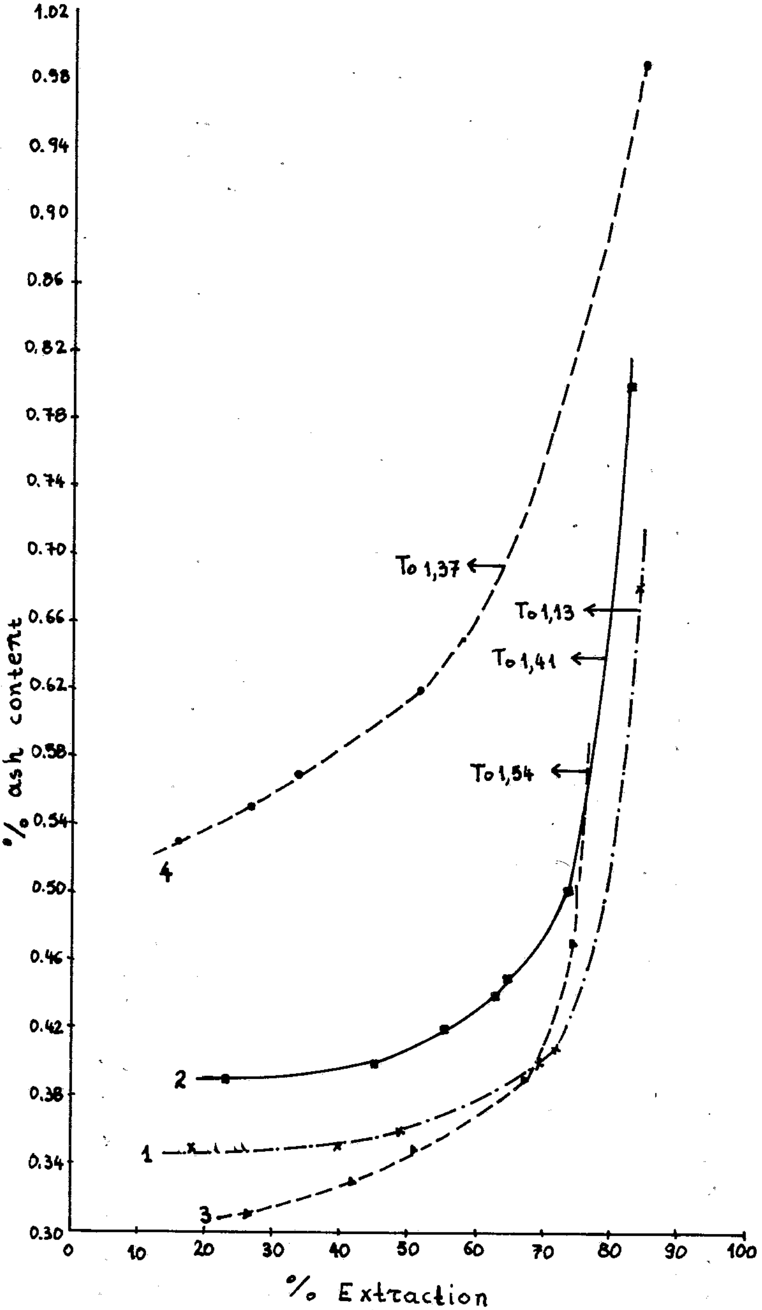

FIG. 2: *Ash change as a function of extraction (column 13 and 14 of tables 2, 3, 4)- 1. Siette Gerros, 2. Yecora, 3. Greek Soft, 4. Greek durum.*

Each sample wheat gives one flour curve, similar to the above, so we have: 21 plots of Yecora, 20 of S. Gerros, 11 of Greek soft and 4 of Greek durum.

From these plots we find the values for some fixed extraction rate, on the basis of which we draw up the tables V, VI and VII. The table VIII states the mean values, of ash for the marked extraction and for every variety, taken from the three aforesaid tables.

The four curves of the Fig 3 have been plotted from these mean values, which are the final ones to study the change of ash as a function of the extraction. Furthermore, we are forming, from the table VIII, the differences between ash values for the marked extractions of flours of the following pairs.

Yecora minus Greek soft

S. Gerros minus Greek soft

These values, for every one pair, are stated in Table IX, from which we are finaly constructing the two curves of the Fig. 4.

TABLE V: *% Ash content of flour wheat:* YECORA

Sample	Extraction %						
No	20	30	40	50	60	70	75
1	0,401	0,402	0,406	0,416	0,431	0,465	0,522
2	0,360	0,364	0,358	0,366	0,382	0,402	0,414
4	0,370	0,374	0,382	0,388	0,420	0,450	0,470
8	0,360	0,365	0,373	0,386	0,398	0,431	0,490
8	0,380	0,380	0,380	0,388	0,408	0,441	0,472
9	0,341	0,347	0,366	0,369	0,390	0,432	0,490
12	0,360	0,362	0,368	0,377	0,392	0,422	0,454
13	0,400	0,408	0,417	0,429	0,442	0,462	0,476
16	0,360	0,366	0,373	0,385	0,402	0,429	0,452
17	0,360	0,366	0,373	0,386	0,405	0,443	0,476
18	0,362	0,354	0,359	0,368	0,384	0,416	0,448
20	0,410	0,412	0,416	0,427	0,460	0,498	0,542
23	0,428	0,432	0,434	0,442	0,460	0,490	0,880
25	0,428	0,434	0,441	0,452	0,476	0,512	0,544
27	0,348	0,354	0,363	0,376	0,397	0,432	0,468
29	0,388	0,391	0,398	0,410	0,440	0,494	0,530
30	0,384	0,390	0,396	0,414	0,446	0,490	0,516
33	0,400	0,402	0,408	0,415	0,432	0,480	0,520
35	0,388	0,400	0,395	0,407	0,428	0,474	0,510
36	0,390	0,392	0,398	0,412	0,436	0,486	0,522
39	0,368	0,370	0,373	0,384	0,404	0,442	0,471
Total	7,966	8,063	8,163	8,396	8,823	9,589	10,315
M.V.	0,379	0,383	0,3887	0,3998	0,420	0,456	0,491

TABLE VI: *% Ash content of flour wheat:* S. GERROS

Sample	Extraction %						
No	20	30	40	50	60	70	75
3	0,371	0,373	0,375	0,385	0,403	0,450	0,520
5	0,406	0,421	0,439	0,461	0,490	0,540	0,570
7	0,379	0,384	0,397	0,426	0,471	0,510	0,544
10	0,391	0,399	0,412	0,431	0,454	0,516	0,546
11	0,410	0,415	0,424	0,441	0,470	0,513	0,550
14	0,371	0,373	0,380	0,394	0,423	0,476	0,520
16	0,429	0,439	0,452	0,471	0,495	0,531	0,560
19	0,376	0,382	0,393	0,403	0,425	0,472	0,516
21	0,379	0,382	0,407	0,431	0,468	0,524	0,560
22	0,360	0,366	0,375	0,389	0,414	0,466	0,500
24	0,390	0,392	0,398	0,412	0,434	0,490	0,550
26	0,383	0,390	0,398	0,414	0,442	0,498	0,550
28	0,321	0,330	0,342	0,358	0,382	0,424	0,452
31	0,340	0,343	0,352	0,370	0,402	0,450	0,490
32	0,292	0,296	0,304	0,320	0,360	0,413	0,466
34	0,296	0,306	0,320	0,338	0,360	0,398	0,422
37	0,350	0,350	0,350	0,364	0,384	0,418	0,442
38	0,421	0,424	0,430	0,440	0,462	0,500	0,540
40	0,436	0,440	0,446	0,464	0,494	0,534	0,570
Total	7,101	7,205	7,394	7,712	8,223	9,123	9,867
M.V.	0,373	0,379	0,389	0,406	0,433	0,480	0,519

TABLE VII: *% Ash content of flour wheat: greek soft*

Sample	Extraction %						
No	20	30	40	50	60	70	75
1α	0,328	0,334	0,340	0,348	0,358	0,410	0,516
2α	0,306	0,319	0,335	0,352	0,372	0,414	0,508
3α	0,322	0,327	0,335	0,344	0,365	0,440	0,450
4α	0,310	0,312	0,317	0,329	0,346	0,406	0,508
5α	0,310	0,312	0,327	0,344	0,366	0,410	0,470
6α	0,310	0,315	0,322	0,331	0,344	0,390	0,472
7α	0,309	0,316	0,326	0,340	0,360	0,422	0,496
8α	0,321	0,324	0,333	0,344	0,360	0,404	0,500
9α	0,330	0,336	0,345	0,360	0,386	0,445	0,560
10α	0,300	0,304	0,312	0,325	0,344	0,378	0,430
11α	0,360	0,361	0,364	0,374	0,393	0,430	0,496
Total	3,518	3,554	3,656	3,789	3,995	4,549	5,406
M.V.	0,320	0,323	0,332	0,344	0,363	0,413	0,491

TABLE VIII: *Ash mean values %, taken from tables 5, 6, 7*

Wheat variety	Extraction %						
	20	30	40	50	60	70	75
Greek soft	0,320	0,323	0,332	0,344	0,363	0,413	0,491
Yecora	0,379	0,384	0,388	0,399	0,420	0,456	0,491
Siette Gerros	0,373	0,379	0,389	0,406	0,433	0,480	0,519
Durum	0,548	0,581	0,618	0,661	0,720	0,805	0,861

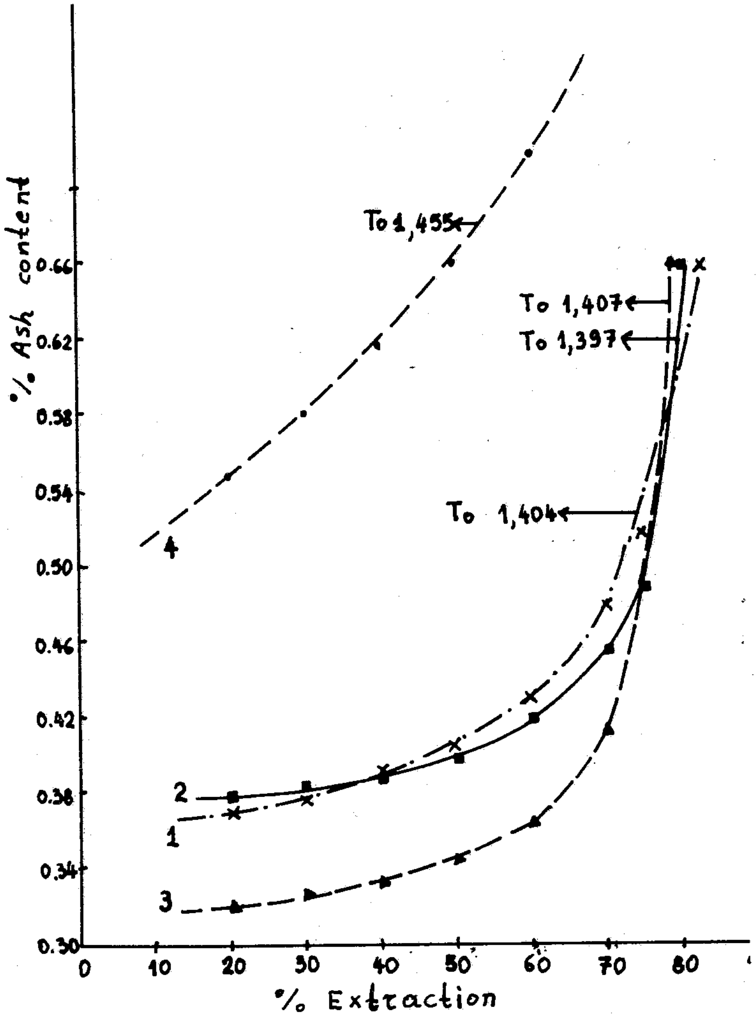

FIG. 3: *Ash change as a function of extraction 1. Siette Gerros, 2. Yecora, 3. Greek soft, 4. Greek durum.*

TABLE IX: *Ash difference values*

Varieties difference	Extraction %													
	20	30	40	50	60	70	71	72	73	74	75	76	77	78
Yecora minus Greek soft	0,059	0,061	0,056	0,055	0,057	0,043	0,032	0,018	0,017	0,009	0	–0,012	–0,025	–0,030
S. Gerros minus Greek soft	0,053	0,056	0,057	0,062	0,070	0,067	0,056	0,044	0,044	0,037	0,028	0,014	–0,004	–0,017

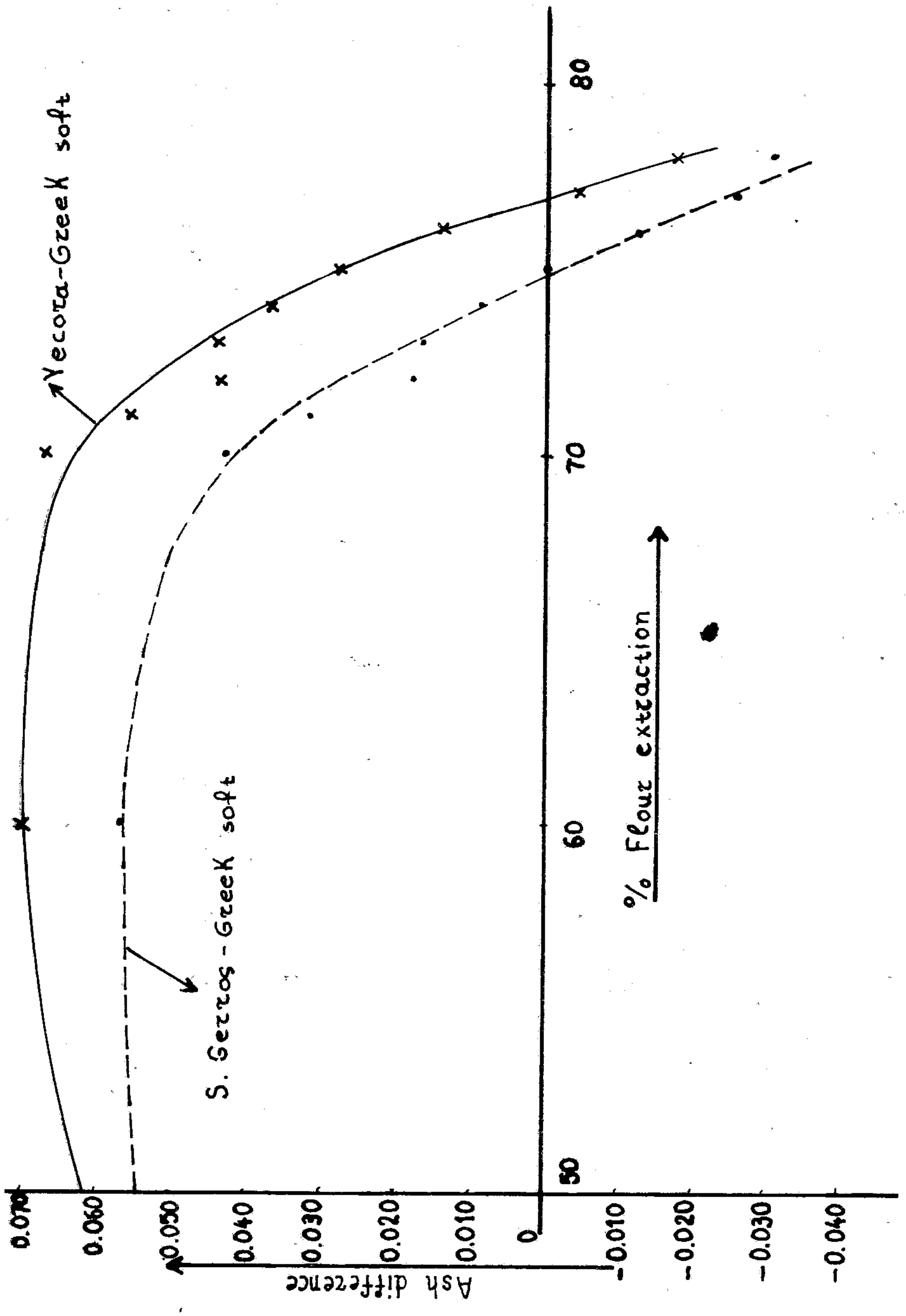

FIG. 4: *Showing ash difference values with respect to extraction.*

Discussion

The study of the curves of Fig. 3 gives the consequent results:

1. The content in metallic salts of the endosperm of the two Mexican varieties is, in fact, appeared rather a little increased, in comparison with the Greek soft.
2. The three curves, corresponding to the soft flours, are almost similar in shape, and show that the ash content increases little up to an extraction of 60% and a little more between 60% and 70%. Thereafter the curve of Greek soft rises much more steeply than the other two. This causes a markedly greater rise in mean ash content, to Greek soft flour, which has as result the sort meeting of this curve with the other two ones, and before 80% extraction.

This means further, that brans are mixed with clear flour much faster than in the case of the other two Mexican varieties.

So, in this short range of extraction between 70 and 80%, namely just before entering in the region of the clear bran, it compensates the initial differences of ash content.

3. All the three curves of soft flours lie much lower than the durun one.
4. So, for an 75% extraction we are taking a flour with a content of ash

for Yecora	0,491%
for S. Gerros	0,518%
for Greek soft	0,491% and
for Greek durum	0,860%

while for an extraction 70% the percentage of ash content is as following:

for Yecora	0,456%
for S. Gerros	0,480%
for Greek soft	0,413%
for Greek durum	0,805%

Thus the difference between the above values and the percentage 0,50% is more than clear. In addition it must be said that this percentage is the permitted limit of the Greek soft flours for the 70% extraction.

5. If we now pass to the other flour type T55, we are observing from the same diagram of Fig. 3 following:

The elevation of the ash values for the accepted extraction of 57% are formed as consequently

for Yecora flours	0,414%
for S. Gerros flours	0,423% and
for Gr. soft flours	0,358%

Inversely, upon any flour, including such a sum of mineral salts, that gives an ash content of 0,45%, we are taking the following extraction values

for Yecora	69%
for S. Gerros	64,5% and
for Gr. soft	73%

namely an average extraction value of about 68% for mixed grists of the above three varieties.

6. The above conclusions become more apparent, from the table 9 from which we notice at first that the difference of the ash value of the two mentioned pairs, remains almost constant, inside the errors experienced, up to 60% extraction.

There after Fig. 4 suggests the steep falling of the two curves from which the first arives to zero at a 75% extraction and the second one at about 77%.

Therefore, we recognize from all the above, that the same results and conclusions are also valid to the T55 flour type as to the T70 type.

Final Conclusions

The stated observations and remarks, inferred from the study of the curves of Fig. 3, and in general the whole range of our experiments, heve led us to the following decisions and results:

1. The complaints of the wheat flour-mill owners were not based on reliable observations and experiments, and for this reason, we can say they were exaggerated, and
2. Some modifications must take place either to the elevation of the ash for the admitted extraction, or to the elevation of the extraction, while the permitted values of the ash content must be kept to remain at the same limits.

All the above are valid for the two flour types.

Περίληψη

Περιεκτικότης εἰς τέφρα τῶν Μεξικανικῶν ποικιλιῶν σίτου Yecora καὶ Siette Gerros.

Ἡ Ἑλληνικὴ νομοθεσία ἐλέγχει τὴν ποιότητα τῶν ἀλεύρων ἐκ σίτου, βάσει τῆς περιεκτικότητός των εἰς τέφρα διὰ καθωρισμένο ὅριο ἀποδόσεως. Ἀπὸ τὸ ἔτος 1977 ἔχουν ἀρχίσει νὰ καλλιεργοῦνται εἰς τὴν Ἑλλάδα δύο ποικιλίες μαλακοῦ Μεξικανικοῦ σίτου - Yecora καὶ Siette Gerros.

Μερικοὶ ἀλευροβιομήχανοι ἐξέφρασαν παράπονα κατὰ τὸ ἔτος 1978 διὰ ὑψηλὴν περιεκτικότητα εἰς τέφρα τῶν δύο ἀνωτέρω ποικιλιῶν σίτου ἐν συγκρίσει μὲ τὶς ἀπὸ πολλῶν ἐτῶν καλλιεργούμενες ποικιλίες Ἑλληνικοῦ μαλακοῦ σίτου. Τὰ ἐργαστήριά μας ἔχουν ἀναλάβει τὴν διερεύνηση καὶ λύσιν τοῦ προβλήματος. Πράγματι, συγκεντρώθησαν 55 δείγματα ἀπὸ ὅλες τὶς σιτοπαραγωγὲς περιοχὲς τῆς Ἑλλάδος καὶ ἐκπονήσαμε τὴν παροῦσα μελέτη. Τὰ δείγματα σίτου ἀλέσθηκαν εἰς τὸν ἐργαστηριακό μας μῦλο εἰς δὲ τὰ προϊόντα καὶ ὑποπροϊόντα ἀλέσεως προσδιορίσθησαν ἡ τέφρα, τὸ ἄμυλον καὶ ἡ ὑγρασία.

Ἀπὸ τὰ ἀποτελέσματα εἶναι σαφὲς ὅτι οἱ διαμαρτυρίες τῶν ἀλευροβιομηχάνων ἦσαν ὑπερβολικὲς καὶ ἡ περιεκτικότης τῶν ἀλεύρων εἰς τέφρα συναρτήσει τῆς ἀποδόσεως θὰ πρέπει νὰ παραμείνει εἰς τὰ αὐτὰ ὅρια ὡς καὶ προηγουμένως.

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ALBUMIN MICROPARTICLE PHARMACEUTICAL COMPOSITION CONTAINING DNA-DAUNOMYCIN COMPLEX

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To the Editor:

I have attempted to develop a new pharmaceutical preparation for an anticancer drug consisting of a DNA-drug complex entrapped in an albumin microsphere matrix. Since the diameter of these particles can be made to vary from less than 1 μm up to 70 μm, the microparticles can be made to localize specifically in marrow, liver, spleen or lung (1). During biodegradation of the albumin particle the DNA-drug complex should be slowly released into the blood circulation. Because of the DNA carrier the complex should show higher affinity for tumor cells and other cells endowed with higher endocytic activity than the surrounding tissues. After intralysosomal digestion of the DNA the drug will become free to diffuse from the lysosomes and to reach other components or the extracellular space (2).

I have put this theoretical model to an experimental test using the antibiotic daunomycin (3) which has considerable clinical usefulness, particularly in the treatment of acute leukemia, but which is cytotoxic, as are all the main anticancer drugs, and in addition which produces severe cumulative dose-dependent cardiotoxicity (4). Here I report the results of the preliminary experiments carried out to investigate first the feasibility of preparation of such particles and second the retention of the antimicrobial and antitumor activity of daunomycin released from microparticles and dissociated from the carrier.

Human serum albumin microparticles containing DNA-daunomycin complex were prepared at room temperature by adding dropwise 2 ml DNA-daunomycin solution containing 5.08 mg human serum albumin into a rapidly stirred continuous phase composed of 200 ml of the dewatering agent n-butanol, 2.4 ml of the crosslinking agent formaldehyde (35% by weight in water) and 200 ml castor oil. The dispersion was mixed for two hours at room temperature (20-25°C) to crosslink the protein matrix. The DNA-daunomycin complex, with a ratio of 11.7 to 1 on a weight basis, was made by mixing one volume of the daunomycin solution, 20 mg/ml, with 100 times its volume of the DNA solution, 2.34 mg/ml, which was heat denatured by autoclaving at

120° C for 15 min and cooling rapidly. Both solutions were made in 0.15 M NaCl. Immediately after mixing a reduction of fluorescence at 580 nm indicated the formation of the complex (2). The resulting solid microparticles were collected by vacuum filtration. Residual oil was removed by washing repeatedly first with n-heptane and then with ether. Dried microparticles were sized. More than 75% had diameters of 15-65 μm and contained more than 65% of the original drug complex as estimated using radioiodinated DNA. For the *in vivo* and *in vitro* studies microparticles with diameters 10-25 μm were used.

The daunomycin complex released from albumin microparticle preparations did not inhibit bacterial growth of *Staphylococcus albus* compared to control cultures incubated without the complex, whereas 1.5 - 2.0 μg of the drug obtained after digestion of the carrier inhibited bacterial growth by 45-55%. This indicates that the activity of daunomycin is reduced by complexing which may be advantageous for reducing the toxic effects of the drug until its release at desired sites (2). The experiment was conducted by incubating at 37°C for six hours 0.5 ml of the supernate from a suspension, 1 mg/ml, of microparticles in deionized water with 4 ml bacterial suspension in BactoDextrose Broth (Difco). Bacterial growth was estimated by turbidimetry at 660 nm. The drug carrier was digested by pancreatic deoxyribonuclease prior to addition to the culture.

The cytostatic activity of albumin microparticles containing DNA-daunomycin complex was evaluated *in vitro* using human KB cells and *in vivo* using tumorous mice (3). In the first study increasing amounts, 5-200 μl, of a 1 mg/ml suspension of microparticles in deionized water were added to 5 ml of nutrient medium containing about 10^6 KB cells. The nutrient medium was made by mixing 80 volumes of a solution containing 0.5% hydrolysate of lactalbumin, 0.1% Difco yeast extract, and 0.5% glucose with 20 volumes of calf serum. The mixture was incubated at 37° C for 48 hours and the cell protein nitrogen was determined by the method of Oyama and Eagle (5). A 50% inhibition of cell growth, compared to controls incubated with corresponding amounts of microparticles containing DNA but no drug, was found using 50-100 μl of microparticle suspension.

In the second study suspensions of microparticles 10 and 80 mg/kg were randomly injected into the tail vein of R111/Rho female mice implanted with adenocarcinoma TM. The injections started immediately after implantation and the treatment was repeated every second day for five times. Implanted control mice received saline injections. Ten days after the last dosing animals were sacrificed (CO_2) and the solid tumors were removed and weighed. The percentage inhibition of the tumor weight was calculated from the equation:

$$\text{percent weight inhibition} = \frac{C - T}{T} \times 100 \text{ where}$$

C = average weight of control tumors
T = average weight of treated tumors.

A group of twelve animals per dosing was used. The results of the study showed that tumors in mice receiving the lower dose weighed 10-30% less than the controls and tumors in those receiving the higher dose weighed 29-55% less than the controls.

The experimental results reported here encourage further investigation of preparations of albumin microparticles containing DNA-drug complexes. It is expected that use of these preparations will decrease the number of dosings required for treatment; it will maintain better control of the amount of drug circulating in the blood; it will decrease the toxicity of the drug; and it will increase specificity of tissue localization.

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