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STIMULATION OF E. COLI POLY(A) POLYMERASES BY CHROMOSOMAL RNA

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(Received November 25, 1975)

Summary

Rat liver chromatin was found to be a potential stimulator of E. Coli poly(A) polymerase. Further study revealed that chromosomal RNA was the active constituent of chromatin. This RNA has been proved a much better primer, compared to other RNAs for poly(A) polymerase activity. The possible role of this fact in the formation of m-RNA is discussed.
Key words: Bacterial poly(A) polymerase, chromosomal RNA, primer specificity

Introduction

Poly(A) polymerase activity has been observed in bacteria by Gottesman *et al* since 1962[1] and in animal tissues by Edmonds and Abrams since 1960.[2] The function of this activity in bacteria is still uncertain because it is generally believed that bacterial mRNA does not contain poly(A) sequences. The synthesized poly (A) possibly serves only as a storage for adenylates, a hypothesis which is supported by the existence of specific polyadenylases in bacteria.[3,4] In eukaryotes, however, following the discovery that their mRNA contains poly(A) sequences,[5-9] the significance of poly(A) polymerases became evident.

Poly(A) polymerases need a nucleic acid in order to synthesize poly(A) clusters. Studies with labelled end groups showed that there is a primer and not a template activity of nucleic acid and that the synthesis of poly(A) starts from the 3′ end of the nucleic acid. The polyadenylation of RNA is generally considered to be a post transcriptional event. This fact, however, does not exclude the possibility that it takes place into or near the transcription machinery. In this study we present evidence that chromosomal RNA is a much better primer for poly(A) synthesis compared to other natural or synthetic polynucleotides.

Material and Methods

E. Coli bacteria of K_{12} strain, used throughout this work, were grown in minimal medium and collected at the end of logarithmic phase. The medium contained 1.0 gr NH_4Cl, 1.0 gr $(NH_4)_2SO_4$, 41mg $MgSO_4 \cdot 7H_2O$, 1.56mg $Fe(NH_4)_2(SO_4)_2$, 1.68mg citric acid and 10gr of glucose per liter of 0.1 M phosphate buffer pH 7.0. Male wistar rats 2-4 months old were used in the experiment. Rat liver chromatin was isolated according to Simpson *et al.*[10] The mixture of non histone protein and chromosomal RNA was obtained from this chromatin by the

method of Patel.[11] From this mixture, following incubation with pronase (3mg/ml), the chromosomal RNA was isolated by the SDS-phenol method, according to Getz *et al.*[12] The so obtained crude preparation of chromosomal RNA was then dissolved in 0.01 M Tris HCl buffer pH 8.0, containing urea 8M and 0.2M NaCl and loaded on to a 2×10cm DE 52 column pre-equilibrated with the same buffer. Two peaks of chromosomal RNA were obtained with the application of a 0.2-1.0 M NaCl gradient (50 ml each) (fig. 1). The first peak showed a A_{280}/A_{260} ratio of 0.6, while the second one of 0.7. Both peaks were moved to the same position (3-4S) after disc electrophoresis in 5% acrylamide and 0.1% agarose gels. We found that they do not contain poly(A) sequences, this being supported by the fact that they are not absorbed on poly(U) sepharose column. This is an evidence that they are not consistent of hnRNA fragments.

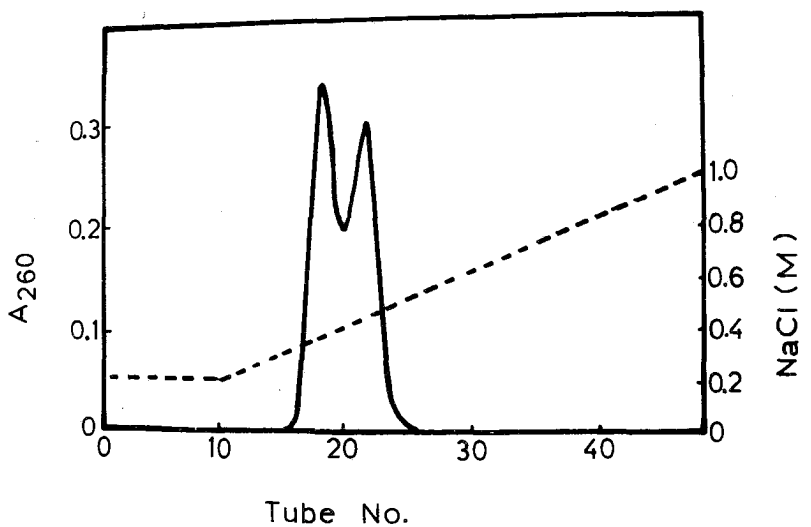

FIG. 1: *Anion exchange chromatography of chromosomal RNA.* A 0.2-1.0M NaCl gradient (50+50ml) was applied on a 1×10cm DE 52 column. Fractions of 3ml were collected and absorbance measured at 260nm.

E. Coli t-RNA was obtained according to Ehrenstein[13] using isopropanol for the final fractionation. Ribosomal RNA was obtained from E. Coli, essentially by the method of Takanami[14] with the only exception that the use of Sephadex G-200 step was omitted. The RNA of the two ribosomal subunits as well as the rat liver DNA were extracted by the SDS-phenol method.[15] Single stranded DNA was obtained by heating at 100° for 10 min. Histones and acid chromosomal proteins were prepared according to Bonner *et al.*[16] Bovine pancreatic RNase was a product of PL Biochemicals, while pancreatic DNase was purchased from Mann Research Lab. Sephadex G-100 and G-150 were purchased from Pharmacia, Uppsala. DEAE cellulose (0.85meq/g) was a product of Sigma, while preswollen DE 52 of Whatman. All radioactive compounds were labelled with tritium and purchased from Amersham, England.

Enzyme assay: the standard assay mixture (55μl) contained 2.5 μmoles Tris HCl buffer (pH 9.0), 1μmole $MgCl_2$, 40μmoles KCl, 0.1μmoles ATP (unlabelled), 0.25μCi ATP(^{3}H) (27Ci/mMole) and 2.5μg enzyme protein in 0.50 M Tris buffer pH 9.0 5% (v/v) glycerol, 0.1mM EDTA and 0.1mM dithiothreitol. Samples were

incubated for 40 min. at 37°C and 50μl were loaded on paper discs, washed three times with cold 5% TCA containing 1% Sodium Pyrophoshate, twice with ethanol, once with a mixture of ethanol and ether and finally with ether. After drying in the air, the paper discs were counted in toluene in a Packard liquid scintillator. One unit of enzyme is defined as the amount of enzyme that incorporates 1pMole of AMP in 40 min. under the conditions described above.

Results

Poly(A) polymerase was purified from E. Coli with ammonium sulfate fractionation, anion exchange chromatography and gell filtration, according to the following procedure: 15gr of wet bacteria were diluted in 10ml 0.05M Tris HCl buffer pH 7.5, containing 0.01M $MgCl_2$, 0.2M KCl. 0.1mM DTT, 0.1mM EDTA and 5% (v/v) glycerol. The bacteria were then disrupted with a Branson B-12 sonifier (4 bursts, 15 seconds each) at setting 8. After centrifugation of the homogenate for 10 min at 14.000g, the precipitate was sonicated two more times for 15 seconds, followed by centrifugation for 10 min at 14.000g. The two supernatants were combined and centrifuged once more at 16.000g for 20 min. The new supernatant was centrifuged at 105.000g for 2 hrs and the supernatant was treated with DNase (10μg/ml) and fractionated with ammonium sulfate. The 30-60% fraction was dissolved in 0.05M Tris buffer pH 9.0 containing 0.1mM EDTA, 0.1mM DTT and 5% glycerol, and dialyzed against the same buffer. The dialyzed solution was treated with pancreatic RNase (25μg/ml) at 37°C for 45 min and then was loaded on to a 1.5×30cm DEAE cellulose column previously equilibrated with the same buffer. After elution of the column with 2 volumes of buffer, a 0-0.3M $(NH_4)_2SO_4$ gradient was applied (fig. 2A). Two peaks of plymerase activity were obtained from the column, one not retained by the ion exchanger and the second eluted with 0.2M $(NH_4)_2SO_4$. Both enzymic preparations showed no RNase activity. The fraction of the enzyme eluted with the gradient, was used for this study. This fraction was further purified by filtration through Sephadex G-100 and G-150 (fig. 2C). The enzyme preparation obtained after the Sephadex G-150 column, was purified 200 times. This method, including gel filtration, was prefered to the earlier ones reported,[1,17] because it separates effectively poly(A) polymerase from RNA plymerase. This enzymic preparation was indeed free of DNA dependent RNA polymerase and incubation with different concentrations of AMP, ADP, UTP, and GTP in the presence of the corresponding labelled nucleotides, gave no incorporation at all. On the other hand, alkaline hydrolysis of the product obtained in the presence of ATP, gave exclusively AMP. The optimal conditions for the action of the enzyme are: pH 9.0, 0.4M KCl, 2mM ATP and 2mM $MgCl_2$. When $MnCl_2$ was used the optimal concentration was 2.4 mM at pH 8.0.

The poly(A) polymerase showed a characteristic lag period when incubated without the addition of exogenous RNA (fig. 3). The more purified the enzyme the more extensive this lag period was. The use of rat liver chromatin in the assay mixture restored the activity of the enzyme during this period, giving a time dependent linearity of poly(A) synthesis (fig. 3). Chromatin fractionation to its constituents revealed that chromosomal RNA was the active fraction which stimulated AMP incorporation (fig. 3). In fact, as it can be seen in fig. 4, DNA (double and single stranded), acid chromosomal proteins and histones were proved to be either simply not stimulatory or inhibitory. The hydrolysis of chromosomal RNA, on the other hand, in chromatin by $Zn(NO_3)_2$,[17] or the purified chromosomal RNA by RNase resulted in the elimination of their stimulatory activity (fig. 3).

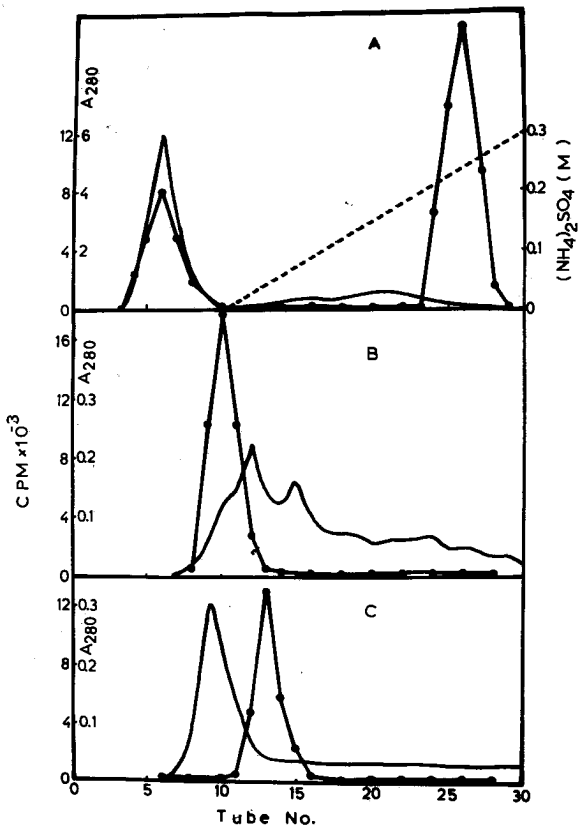

FIG. 2: *Purification of E. Coli poly(A) polymerase.*
A: DEAE cellulose chromatography. The enzymic preparation from 30-60% ammonium sulfate fractionation was loaded on to a 1.5×30cm column. After elution of the column with 2 volumes of buffer, a 0-03M ammonium sulfate gradient (50+50ml) was applied and 5ml fractions were collected.
B: Sephadex G-100 filtration of enzyme fraction obtained after DEAE cellulose chromatography. A 1.8×65cm column was used and 5ml fractions were collected.
C: Sephadex G-150 filtration of enzymic fraction obtained from Sephadex G-100 column. A 1.8×65 cm column was used and 5ml fractions were collected.
The enzymic activity was measured as described in the materials and methods. ——A_{280}, ●——● Enzymic activity.

Several other RNAs like t-RNA, r-RNA and RNA "in vitro" synthesized by E. Coli RNA polymerase showed no considerable activity (fig. 4) or were inhibitory. The effect of increased concentrations of different nucleic acids on the enzyme activity are presented in fig. 5.

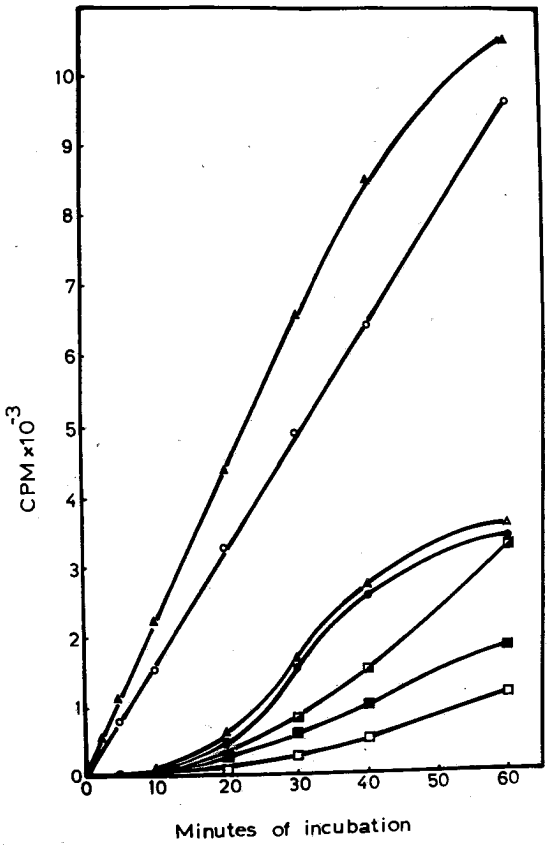

FIG. 3: *Poly(A) synthesis in the presence of chromatin and its constituents.* ●——● No chromatin constituent. o——o Chromatin. ▲——▲ Chromosomal RNA. Δ——Δ Chromatin after hydrolysis of its RNA. ■——■ Histones. □——□ Acid chromosomal proteins. ◪——◪ Native DNA. Final concentration of 0.4mg/ml in the case of chromosomal proteins and 0.1mg/ml in the case of nucleic acids, were used. The standard conditions for the enzyme assay, as described in Materials and Methods, were used.

Discussion

The considerable AMP incorporation which was observed when the purified poly(A) polymerase was incubated for more or less long periods of time, is a strong indication that in spite of RNase pretreatment, the enzyme preparation contained RNA traces of some kind. The nature of this RNA is not known and no attempt was made during this work to identify its origin. The dramatic stimulation of poly(A) synthesis, however, when exogenous chromosomal RNA was added to the reaction mixture, is an evidence that this RNA is a very good primer for the enzyme, in spite of their different origin, phylogenetically. The function of crRNA is not yet known. The fact, however, that it is highly heterogeneous concerning base composition and its absence from inactive nuclei, indicates a regulatory activity in chromatin and not a structural role. It is generally believed that crRNA plays a derepressor role

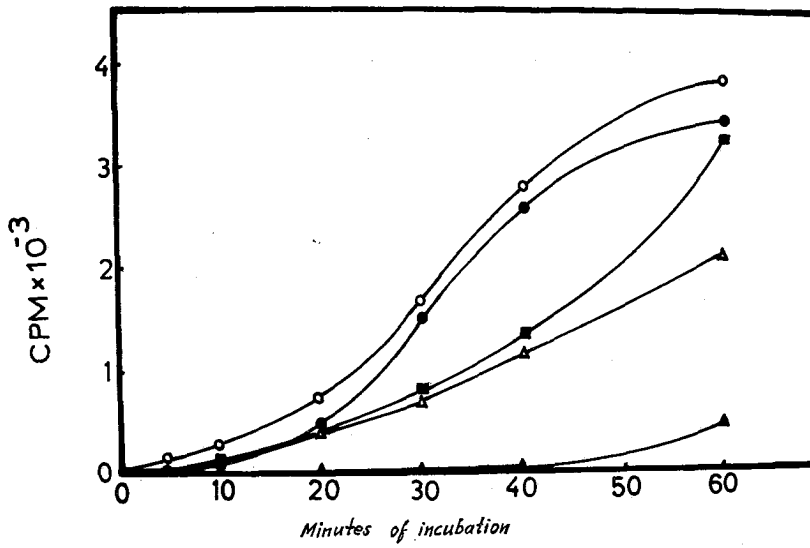

FIG. 4: *Poly(A) synthesis in the presence of different nucleic acids.* ●——● No additional RNA. o——o t-RNA. ■——■ Single stranded DNA. ▲——▲ "in vitro" synthesized RNA. Δ——Δ Ribosomal RNA. Final concentration of 0.1mg/ml of nucleic acids were used. The enzyme was assayed as described in the Materials and Methods.

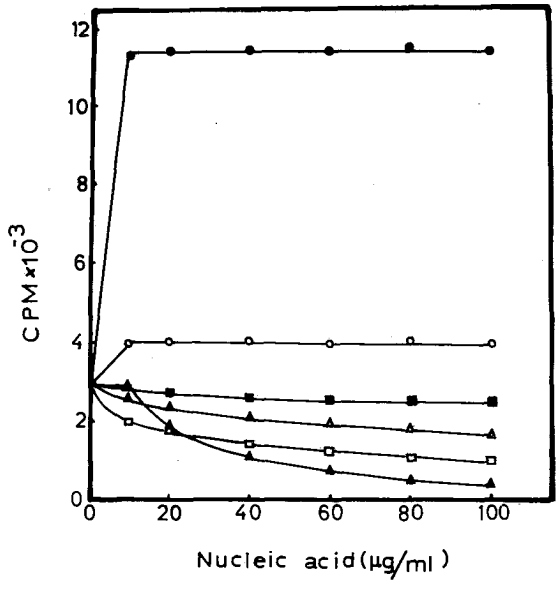

FIG. 5: *Effect of the concentration of different nucleic acids on the rate of poly(A) synthesis.* ●——● Chromosomal RNA. o——o t - RNA. ■——■ Double stranded DNA. Δ——Δ Single stranded DNA. □——□ "in vitro" synthesized RNA. ▲——▲ Ribosomal RNA. The enzyme was assayed as described in Materials and Methods.

recognizing specific sites in the genome and hybridizing to the middle repetitive sequences of the homologous genome DNA, this way destabilizing the template. Concerning its genesis, crRNA could be produced by sensor genes and in this case it is originated by the processing of the primary transcripts (HnRNA). Alternatively a small quantity of crRNA could be produced by integrator genes and then by derepression producing more crRNA.

In bacteria there are not known any polynucleotide species corresponding to crRNA which is considered a unique constituent of eukaryotic cell. The fact, however, that this RNA is a good primer for prokaryotic poly(A) polymerase in combination with the fact that this enzyme is highly specific towards different primers (compare the poor primer activity of rRNA, tRNA and various ribohomopolymers) suggests that there is probably some kind of RNA in E. Coli, similar to crRNA.

In any case, concerning eukaryotes, the localization of crRNA in chromatin, makes attractive the idea that it is the primer used by poly(A) polymerase for poly(A) synthesis. Following this hypothesis one could expect a regulatory role in the adenylation of crRNA. Possibly this adenylation is a first step for the derepression activity of this RNA.

A second assumption is, however, more probable. According to this, chromosomal RNA, homologous to one DNA strand, is hybridized to RNA synthesized on the other DNA strand, near the 5′ end of the latter. After that the 3′ end of crRNA is used for the synthesis of poly(A) the whole molecule representing the hnRNA. This assumption is consistent with the observation of Monckton *et al*[18] that there is double stranded RNA near the poly(A) cluster of hnRNA and that its size is 4-5S. This assumption could also explain the fact that histone mRNA does not contain poly(A) sequences if we adopt the hypothesis that histone genes are closely linked on chromosomal RNA.[19] Indeed in this case the RNA synthesized on the strand of DNA containing the histone genes can not be hybridized to an heterologous RNA as is the case of crRNA linked to these genes. The answer to these questions, of course, may come only from the study of the mode of action of nuclear poly(A) polymerase, and this is the direction which will be followed in this laboratory.

Περίληψη

Ἐνεργοποίηση τῆς Πολυ(Α) Πολυμεράσης τοῦ βακτηριδίου «Escherichia Coli» ἀπὸ χρωμοσωματιακὸ Ριβονουκλεϊκὸ ὀξύ.

Χρωματίνη ποὺ ἀπομονώθηκε ἀπὸ ἥπατα ἐπιμύων, βρέθηκε ὅτι ἀποτελεῖ ἕνα ἰσχυρὸ ἐνεργοποιητὴ τῆς πόλυ(Α) πολυμεράσης ἀπὸ Escherichia Coli. Περαιτέρω μελέτη ἀπέδειξε ὅτι τὸ χρωμοσωματιακὸ RNA εἶναι τὸ ἐνεργὸ συστατικὸ τῆς χρωματίνης. Συγκριτικὴ μελέτη ἀπέδειξε ὅτι τὸ RNA αὐτὸ εἶναι πολὺ καλύτερο "primer" ἀπὸ διάφορα RNAs ποὺ ἀπομονώθηκαν ἀπὸ ἄλλες πηγές. Ὁ πιθανὸς ρόλος τοῦ γεγονότος αὐτοῦ στὸν σχηματισμὸ τοῦ ἀγγελιοφόρου RNA συζητεῖται στὸ τέλος.

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PHOTOCHROMISM OF 2-NITROBENZYLIDENE-HYDRAZIDES

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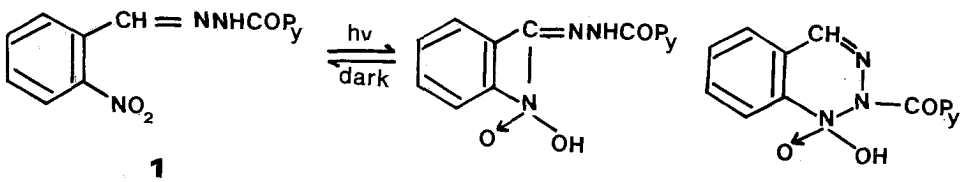
(Received June 7, 1976)

Summary

The photochromism of several 2-nitrobenzylidene-hydrazides is studied. The mechanism and the structure of the photoproducts is discussed on the basis of their spectral data.

Indroduction

It has been observed previously[1,2] that ultraviolet irradiation of 2-nitrobenzylidene-isonicotinic hydrazide 1, in the solid state, gives a red product, for which structure 2 was assigned. Although for this product structure 3 can be proposed as an alternative, it was excluded[3] on the basis of the different chromatographic behaviour of the normal products and those labeled with ^{14}C in α-position.



1 **2** **3**

Py =4-pyridyl

However, we think this evidence is not conclusive and this photochromic reaction is actually much more complicated. The purpose of this paper is to provide more evidence for the structure of the photoproduct(s) and to examine the mechanism of this photochromic reaction. For this reason we have prepared[4,5] and studied the photochromic behaviour of several 2-nitro-benzylidene-hydrazides, which are given in Table I.

Results and discussion

All the hydrazides studied are photochromic like compound 1. The irradiation was carried out in methanolic solution 0.02M using an immersion medium pressure uv lamp. The photoproducts are red to red brown, but in the

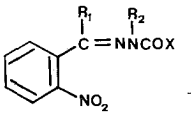


TABLE I: *Photochromic 2-nitrobenzylidene-hydrazides*

	Substituents				Hydrazide	Photoproduct
	R_1	R_2	X	m.p.	UV λ_{max} (nm)[b]	UV λ_{max} (nm)[b]
(1)	H	H	Py[a]	235-236°	277, 325s	220, 260s
(4)	H	H	C_6H_5	199-200°	278, 318s	223, 266s
(5)	H	H	$C_6H_4OCH_3$(-p)	186-187°	293, 320s	250, 280s
(6)	H	H	C_6H_4Cl(-p)	230-231°	285, 318s	229, 270s
(7)	H	$COCH_3$	C_6H_5	122-123°	275, 333s	220s, 262s
(8)	H	D	C_6H_5	198-200°	278, 315s	224
(9)	H	CH_3	C_6H_5	128-129°	276, 318s	221
(10)	CH_3	H	C_6H_5	140-142°	258	218s, 255
(11)	CH_3	H	Py[a]	163-165°	260	256

[a] 4-pyridyl

[b] In methanolic solution

s Shoulder

visible region of the spectrum there are only tails which, however, are found at higher level in respect to those of the starting materials (see Fig. 1). Analogous behaviour is shown by several other photochromic compounds.[6] The reactions are reversible and the colored products cannot be isolated, since they revert to the starting materials by heating for a short time or by leaving in the dark.

The uv spectra of the hydrazides studied show an absorption maximum at the region of 280 nm. The photoproducts of the hydrazides derived from 2-nitrobenzaldehyde show a new maximum or a shoulder at ∽230 nm, whereas those derived from 2-nitro-acetophenone continue to show a maximum or a shoulder in the same region, as the starting materials (Fig. 1). Assuming that the photoproducts have a structure like 2 a hypsochromic shift should be expected in the ultraviolet region because of interuption of the conjugation. On the other hand in structure 3 there is no significant change in the conjugation of the system, therefore in the case of photoproduct 10, where structure 3 seems most probable, there is a great similarity between the uv spectra of the photoproduct and its parent hydrazide (Fig. 1).

The substituents as well as the pH and the presence of oxygen do not influence remarkably the photo-reaction rate. However in the hydrazides of 2-nitroacetophenone the reaction rate is almost decreased by ten and in the N-substituted hydrazides the reaction rate is almost increased by ten in respect to that of 2-nitrobenzaldehyde.

The nmr spectrum of 4 in $CDCl_3$ showed a singlet at 1.15τ attributed to the =CH proton and two multiplets centered at 2τ and 2.4τ attributed to the aromatic protons. During the irradiation the intensity of the =CH peak was progressively reduced, with the appearance of a broad peak at 4.34 τ. The last peak disappeared

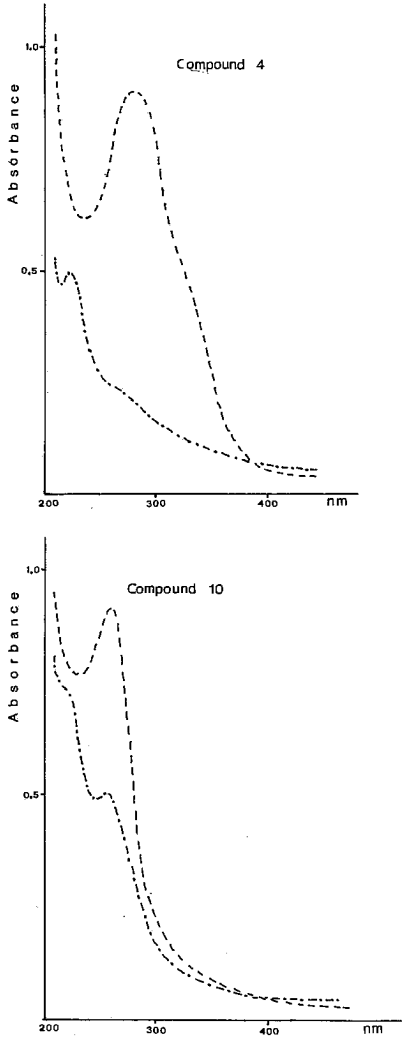
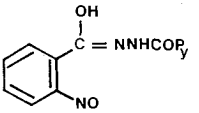


FIG. 1: *UV-Vis spectra of compounds 4,10 (– – –) and their photoproducts (—.—.), in methanolic solution (c = 5.10^{-5} mole/l).*

after addition of a small amount of D_2O. Furthermore the ir spectrum of the photoproduct showed a peak at 3500cm^{-1}, which is absent in the starting material and which is attributed to the N-OH group. It should be mentioned that similar hydrogen transfer has been observed in the photochromism of some o-nitrobenzyl derivatives.[7,8] Analogous is the behaviour of hydrazides 1, 5, 6 and 8 and especially of the N-substituted hydrazides 7 and 9, where the only possible structure for the photoproduct is structure 2. All these data are evidence in favour of structure 2 and

support the previous aspects.[1] On the other hand, it is of interest to note that hydrazides 10,11 derived from 2-nitroacetophenone are also photochromic and this is evidence in favour of structure 3. Both structures 2 and 3 are also found in agreement with the fact that nitronic derivatives of this type are unstable[9] and this is consistent with the mechanism of the photochromic process, which requires the photoproduction of an isomer thermodynamically unstable in its ground state[10] and explains the easy and fast transformation of them to the starting hydrazides.

It should be noticed that structure 12 or other structures similar to that, could be also proposed as alternatives, on the basis of the photochemical transformation of o-nitro-benzaldehyde[11,12] However, in this case the spectral data should be quite different[12] (NO absorption at ~670 nm) and mainly the reaction could not be reversible. On the other hand many "quinoide" structures like 13, which could be formed by a [1,7] hydrogen sigmatropic shift are excluded, mainly because the nmr spectra of the photoproducts should be quite different, from those of the parent hydrazides, a fact which is not observed. Furthermore, the product 13 should be expected to eliminate easily nitrogen.[13]



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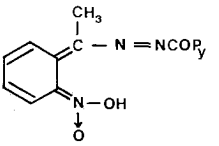

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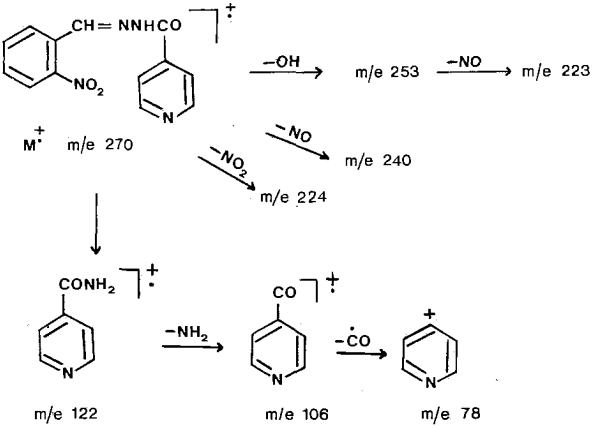
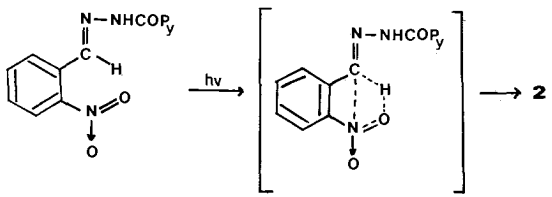
Of great importance are the mass spectra of 2-nitrobenzylidene-hydrazides. In a previous study[14] on the mass spectra of substituted aroylhydrazones it was found that only the 2-nitro derivative 4 gives a peak $[M-17]^+$ fact which suggests, in agreement with our observation, that an oxygen from the nitro-group should be eliminated with a =CH hydrogen as OH, through a four center transition state.

A typical example of fragmentation pattern of 2-nitrobenzylidene-hydrazides is given below for the compound 1.



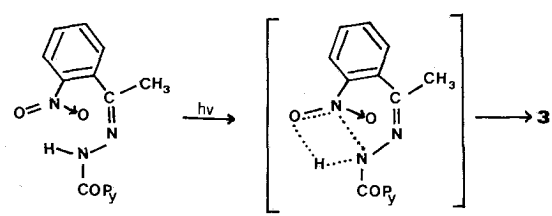
The same fragment $[M-17]^+$ has been also observed in compounds 5, 6, and 9 (Table 2). This transition, in the case of the N-substituted compound 9, is accompanied by a metastable peak at m/e 250. It is of interest to note that in the α-methyl substituted compounds 10 and 11 elimination of OH is observed only after the cleavage of the C-CH_3 bond, while the $[M-17]^+$ ion is not observed. In compound 8 (52% deuterated) a correlation of the peaks at m/e 270, with those at 253, 252, corresponding to elimination of OH, OD, shows that the main cleavage is that of the OH group, whereas the OD elimination is rare. These data are in agreement with the suggestion for the formation of a four membered ring (2).

In conclusion, the present data show that the photoproducts of 2-nitrobenzylidene-hydrazides 7 and 9 must probably have a structure analogous to 2, while the photoproducts of hydrazides 10 and 11 a structure analogous to 3. For the photoproducts of hydrazides 1, 4, 5, 6, and 8 an equilibrium mixture of 2 and 3 is also possible.



1 14

Concerning the mechanism of the formation of the photoproduct 2 it can be described as a [π2s + σ2s] or [π2a + σ2a] cycloaddition 14 which is photochemically allowed. In the case of the photoproduct 3 the conformation of the starting hydrazide must be like 15, which favors the [π2s + σ2s] cycloaddition between the nitro- and the N-H group.



15

Experimental

M.p'.s. are corrected and were determined with a Kofler hot-stage apparatus. Ir spectra were measured for Nujol mulls with a Beckman IR-4 spectrometer and uv spectra for solutions in methanol with a Perkin-Elmer 137 UV or a Beckman DU instrument. Nmr spectra were obtained with a Varian A-60A spectrometer with tetramethylsilane as internal standard. The mass spectra were obtained with a Hitachi Perkin-Elmer RMU-6L mass spectrometer; the ionization energy was maintained at 70 eV.

TABLE II: *Mass spectral data of 2-nitrobenzylidene-hydrazides. Mass numbers and relative intensities (values in parenthesis).*

Compound	Mass numbers (m/e) and relative intensities
1	270 (0,1), 253(0,3), 240(0,2) 224(0,2), 223(0,2), 122(42), 106(100), 78(66).
2	269(1), 252(2), 239(1), 223(0,5), 121(23), 105(100), 77(43).
5	299(3), 282(0,5), 269(0,5), 253(2), 252(2), 223(0,5), 151(21), 135(100), 133(6), 107(9), 105(7), 77(19).
6	303(1), 286(1), 273(0,5), 256(0,5), 252(0,5), 157(8), 155(24), 141(31), 139(100), 113(9), 111(27).
7	311(5), 269(8), 252(5), 222(2), 105(100), 77(27).
8	270(3), 269(2,5), 253(2,5), 252(2), 223(1), 222(1), 122(62), 121(46), 105(100), 77(82).
9	283(7), 266(6), 253(1), 135(11), 134(8), 105(100), 77(45).
10	283(0,1), 268(0,1), 251(3), 237(0,1), 222(0,1), 149(6), 105(100), 77(40).
11	285(0,1), 269(0,1), 252(3), 223(0,1), 150(13), 149(26), 106(100), 103(21), 78(72).

Preparation of 2-nitrobenzylidene-hydrazides.

All 2-nitrobenzylidene-hydrazides were prepared according to well known procedures.[4,5] Their m.p.'s and uv maxima are given in Table I.

Preparation of N-acetyl-2-nitrobenzylidene-benzoic acid hydrazide (7).

A mixture of 2-nitrobenzylidene-benzoic acid hydrazide (2 g) acetylchloride (2 ml) and a few drops of pyridine was stirred overnight at room temperature. By pouring the reaction mixture into crushed ice the product (100 mg, 4%) was separated m.p. 122-123°, from methanol; τ ($CDCl_3$) 2.10 (CH, singlet), 1.84-2.55 (aromatic protons, multiplet), 7.56 (CH_3, singlet); ν (Nujol) 1655 (CO) and 1530 and 1340 cm^{-1} (NO_2).

Preparation of N-deuterated-2-nitrobenzylidene-benzoic acid hydrazide (8).

A chloroform solution of 2-nitrobenzylidene-benzoic acid hydrazide was shaken with deuterium oxide (1 ml) for 12 hrs. The deuteration was 52% as shown by mass spectra.

Preparation of 2-nitroacetophenone-isonicotinic hydrazide (11).

To a solution of 2-nitroacetophenone (4 g) in ethanol (40 ml) a solution of isonicotinic acid hydrazide (3.3 g) in water (48 ml) was added. The mixture was refluxed (35 hrs) and after removal of the ethanol and filtration a white solid was left behind (37%), m.p. 163-165°, from methanol; τ ($CDCl_3$) 0.27 (NH, singlet), 1.14-2.55 (aromatic protons, multiplet), and 7.67 (CH_3, singlet); ν (Nujol) 3180 (NH), 1650 (CO), and 1520 and 1350 cm^{-1} (NO_2).

Analogous was the preparation of 2-nitroacetophenone-benzoic acid hydrazide (10). Yield 33%, m.p. 140-142°, τ ($CDCl_3$) 0.97 (NH, singlet), 1.40-2,63 (aromatic protons, multiplet), and 7.72 (CH_3, singlet); ν (Nujol) 3200 (NH), 1650 (CO), and 1520 and 1350 cm^{-1} (NO_2).

Irradiation of hydrazides.

A solution 0.02 M in methanol of the corresponding hydrazides was irradiated at room temperature with an immersion medium pressure uv lamp. The rate of photochromic transformation was examined with a uv spectrophotometer and an nmr spectrometer. Samples were examined every 30 mins until the uv spectrum remained unchanged. The photoproducts could not be isolated since they are very unstable giving the starting materials.

Acknowledgment

We wish to thank the National Science Foundation for financial support.

Περίληψις

Φωτοχρωμισμὸς 2-νιτροβενζυλιδενο-υδραζιδίων

Στὴν ἐργασία αὐτὴ γίνεται μὲ τὴν βοήθεια φασμάτων uv, nmr, καὶ μαζῶν μελέτη τοῦ φωτοχρωμισμοῦ ὡρισμένων ὑδραζιδίων τῆς 2-νιτρο-βενζαλδεΰδης καὶ τῆς 2-νιτροακετοφαινόνης, σὲ μεθανολικὰ διαλύματα.

Ὅλες οἱ ἑνώσεις ποὺ μελετήθηκαν ἔδωσαν μὴ ἀπομονώσιμα φωτοχρωμικὰ προϊόντα. Ἡ φωτοχρωμικὴ μεταβολὴ ὀφείλεται μᾶλλον στὴν μεταφορὰ τοῦ ὑδρογόνου τῆς =CH ἢ τῆς -NH ὁμάδος στὴν νιτρο-ομάδα τοῦ ἀρωματικοῦ δακτυλίου μὲ ἀποτέλεσμα τὸν σχηματισμὸ τετραμελοῦς (2) ἢ ἑξαμελοῦς (3) δακτυλίου ἀντιστοίχως. Ὁ μηχανισμὸς στὴν περίπτωση σχηματισμοῦ τετραμελοῦς δακτυλίου περιγράφεται σὰν [π2s + σ2s] ἢ [π2α + σ2α] φωτοχημικὰ ἐπιτρεπτὴ κυκλοπροσθήκη, ἐνῶ στὴν περίπτωση σχηματισμοῦ ἑξαμελοῦς δακτυλίου σὰν [π2s + σ2s] φωτοχημικὰ ἐπίσης ἐπιτρεπτὴ κυκλοπροσθήκη.

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Chimika Chronika, New Series, 6, 365-374 (1977)

Η ΔΟΜΗ ΤΩΝ ΚΗΡΩΝ ΤΗΣ ALKANNA TINCTORIA TAUSCH

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(Ἐλήφθη στὶς 26 Ἰουλίου 1976)

Περίληψη

Στὴν ἐργασία αὐτὴ περιγράφεται ἡ ἀπομόνωση καὶ ταυτοποίηση τῶν κηρωδῶν συστατικῶν τῆς φυτικῆς δρόγης Alkanna tinctoria Tausch,[1,2,3] σὲ μιὰ προσπάθεια ποὺ καταβάλλεται ἀπὸ τὸ ἐργαστήριό μας γιὰ τὴ χρησιμοποίηση φυτικῶν ἐκχυλισμάτων στὴ συντήρηση φρούτων καὶ τροφίμων.

Ἀπὸ τὴν ἔρευνα αὐτὴ προέκυψε ὅτι τὸ μίγμα τῶν κηρωδῶν συστατικῶν τοῦ φυτοῦ ἀποτελεῖται ἀπὸ ἕνδεκα ἐστέρες, ποὺ περιέχουν ἀπὸ τριάντα ὀκτὼ μέχρι πενήντα ἑκτὼ ἄτομα ἄνθρακος. Ἀπὸ τοὺς ἐστέρες αὐτοὺς σὲ μεγαλύτερη ἀναλογία βρίσκονται κατὰ σειρὰ ἐκεῖνοι ποὺ περιέχουν C_{52},C_{54},C_{50} καὶ C_{48}. Ὅλοι οἱ ἐστέρες τοῦ φυτοῦ ἔχουν προκύψει ἀπὸ τὴν ἐστεροποίηση ὀκτώ, συνολικά, ὀξέων καὶ ἕξι ἀλκοολῶν.

Ἀπὸ τὰ παραπάνω ὀξέα ταυτοποιήθηκαν τὰ ἕξι, εἶναι δὲ ὅλα μονοκαρβονικά, κορεσμένα, μὲ εὐθεία ἀνθρακικὴ ἁλυσίδα καὶ ἄρτιο ἀριθμὸ ἀτόμων ἄνθρακος, περιέχουν δὲ ἀπὸ C_{16} μέχρι C_{26}. Σὲ μεγαλύτερη ἀναλογία βρίσκονται τὰ ὀξέα μὲ C_{24} καὶ C_{26}.

Ὅλες οἱ ἀλκοόλες εἶναι εὐθείας ἀνθρακικῆς ἁλυσίδας μὲ ἄρτιο ἀριθμὸ ἀτόμων ἄνθρακος καὶ περιέχουν ἀπὸ C_{22} μέχρι C_{30}. Σὲ μεγαλύτερη ἀναλογία βρίσκεται ἡ ἀλκοόλη μὲ C_{28}, οἱ ἀλκοόλες δὲ μὲ C_{26},C_{28} καὶ C_{30} εἶναι ἀκόρεστες μὲ ἕνα διπλὸ δεσμό.

Συντμήσεις
UV-Vis = ultraviolet - visible, NMR = nuclear magnetic resonance, IR = infrared, GLC/MS = gas liquid chromatography / mass spectroscopy, m/e = μάζα / φορτίο ἰοντικοῦ θραύσματος, M^+ = μοριακὸ ἰόν, BSA = N,O-δὶς (τριμεθυλοσιλυλο) -ακεταμίδιο, TMS = τριμεθυλο-σιλύλιο, R_t = retention time, vs = very strong

Ἀποτελέσματα — Συζήτηση

Ὁλόκληρη ἡ δρόγη (ριζικὸ σύστημα, βλαστός, ἄνθη καὶ φύλλα) ξεραίνεται στὸν ἀέρα καὶ στὴ συνέχεια ἐκχυλίζεται μὲ πετρελαϊκὸ αἰθέρα. Τὸ προϊὸν τῆς ἐκχυλίσεως ὑποβάλλεται σὲ χρωματογραφικὴ ἀνάλυση στήλης (Kieselgel 60) καὶ κατὰ τὴν ἔκλουση μὲ μίγμα πετρελαϊκοῦ αἰθέρα-βενζολίου (50+50) ἀπομονώνεται λευκὸ κηρῶδες σῶμα περιοχῆς τήξεως 45-70°C.

I. Ἐστέρες

Τὰ φασματοσκοπικὰ χαρακτηριστικὰ τῶν κηρωδῶν συστατικῶν ποὺ ἀπομονώθηκαν περιγράφονται παρακάτω.

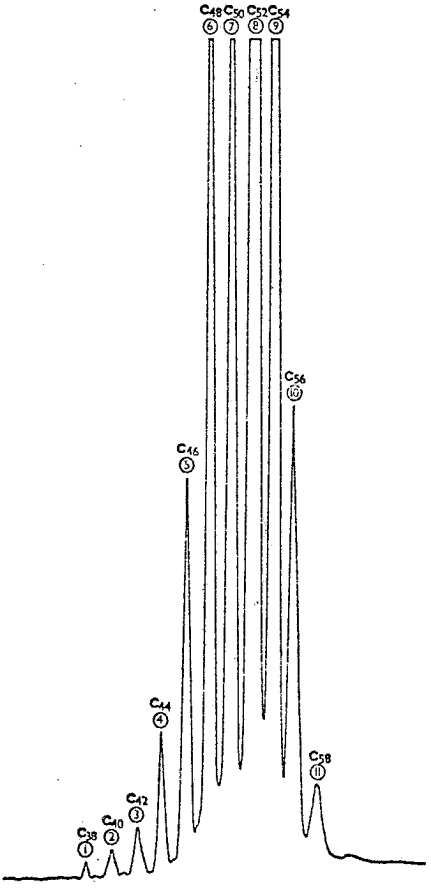
UV-Vis: Δὲν ἐμφανίζει λ_{max}, γεγονὸς ποὺ ὑποδηλώνει τὴν ἀπουσία συζυγίας.

IR: Ἐμφανίζει ἔντονες ἀπορροφήσεις στὰ 3000 (= CH-), 1735 (vs, -COOR), 1650 (>C = C<) καὶ 720 cm^{-1} [$-(CH_2)_n-$].

NMR: $\underset{\alpha}{H_3C}-\underset{\beta}{(CH_2)_x}-\underset{\varepsilon}{CH}=\underset{\varepsilon}{CH}-\underset{\beta}{(CH_2)_y}-\underset{\delta}{CH_2}-O-\underset{\underset{O}{\|}}{\overset{\gamma}{C}}-\underset{\beta}{CH_2}-\underset{\alpha}{(CH_2)_\omega}-CH_3$

δ (ppm)	Πολλαπλότητα	Πρωτόνια	
		Χαρακτηρισμὸς	ἀριθμὸς
0,9	τριπλὴ	α	6
1,3	πολλαπλὴ	β	2 (χ+ψ+ω)
2,1	τριπλὴ	γ	2
4,0	τριπλὴ	δ	2
5,3	πολλαπλὴ	ε	2

Ἀπὸ τὰ παραπάνω φασματοσκοπικὰ δεδομένα προκύπτει τὸ συμπέρασμα ὅτι τὸ κηρῶδες σῶμα ποὺ ἀπομονώθηκε εἶναι μίγμα ἐστέρων, ἀλειφατικῶν ὀξέων καὶ ἀλκοολῶν.



ΔΙΑΓΡΑΜΜΑ 1: *GLC/MS ἀνάλυση τοῦ μίγματος ἐστέρων τῶν κηρῶν σὲ στήλη 3% SE 30 (Chromosorb G-AW-DMCS), μήκους 0,37 m καὶ διαμέτρου 2 mm.* Προγραμματισμένη θερμοκρασία στήλης 200-350°C, μὲ ταχύτητα 10°/min. Φέρον ἀέριο He, παροχὴ 25 ml/min. Ἀνιχνευτὴς MS (Varian CH7). Θερμοκρασία χώρου ἐγχύσεως 380°C. Ταχύτητα χαρτιοῦ 0,1 inch/min.

ΠΙΝΑΚΑΣ Ι: *Κυριότεροι ἐστέρες τῶν κηρῶν τῆς Alkanna tinctoria*

Κορυφὲς χρωματογραφήματος διαγράμματος 1	MS m/e	Τύπος ἐστέρος
6	M^+ 704, 369, 351, 336	$C_{23}H_{47}COOC_{24}H_{49}$
7	M^+ 730, 369, 351, 362	$C_{23}H_{47}COOC_{26}H_{51}$
8	M^+ 758, 369, 351, 390	$C_{23}H_{47}COOC_{28}H_{55}$
9	M^+ 786, 397, 379, 390	$C_{25}H_{51}COOC_{28}H_{55}$
10	M^+ 814, 397, 379, 418	$C_{25}H_{51}COOC_{30}H_{59}$

Ὁ διαχωρισμὸς τοῦ φυσικοῦ μίγματος τῶν κηρωδῶν συστατικῶν τοῦ φυτοῦ ἔγινε μὲ GLC ἀνάλυση (διάγραμμα 1) καὶ μὲ ἐφαρμογὴ τῶν συνθηκῶν Eckert[4] γιὰ τριγλυκερίδια.

Ἡ ταυτοποίηση τῶν κορυφῶν τοῦ χρωματογραφήματος ἔγινε μὲ τὴ βοήθεια φασμάτων μάζης. Ἔτσι, ἡ ἐξέταση τῶν φασμάτων μάζης τῶν κορυφῶν τοῦ χρωματογραφήματος (διάγραμμα 1) ἔδωσε τὸ ἀκόλουθο ἀποτέλεσμα.

Ἀκολούθως, ὁ κηρὸς σαπωνοποιήθηκε[5] γιὰ νὰ ἐπιβεβαιωθῆ τὸ ἀποτέλεσμα τῆς GLC/MS ἀναλύσεως καὶ νὰ ταυτοποιηθοῦν τὰ συστατικὰ τῶν κηρῶν, δηλαδὴ οἱ ἀλκοόλες καὶ τὰ καρβοξυλικὰ ὀξέα. Ἡ σαπωνοποίηση τῶν κηρῶν παρουσίασε ὁρισμένες δυσκολίες, ποὺ ἀντιμετωπίστηκαν μὲ διαφοροποίηση τῶν συνθηκῶν τῆς σαπωνοποιήσεως (βουτανολικὸ διάλυμα 2N KOH).

Στὴ συνέχεια, μὲ ὄξινη ὑδρόλυση τῶν σαπώνων ποὺ σχηματίστηκαν καὶ ἐκχύλιση μὲ διαιθυλαιθέρα ἀπομονώθηκαν τὰ λιπαρὰ ὀξέα, τὰ ὁποῖα κατόπι μετασχηματίστηκαν στοὺς ἀντίστοιχους μεθυλεστέρες κατὰ τὴν κατεργασία μὲ διαζωμεθάνιο γιὰ νὰ διαχωριστοῦν καὶ νὰ ταυτοποιηθοῦν μὲ GLC/MS ἀνάλυση.

II. Λιπαρὰ ὀξέα

Τὸ μίγμα τῶν μεθυλεστέρων τῶν λιπαρῶν ὀξέων, ποὺ προέκυψε ἀπὸ τὴ σαπωνοποίηση τοῦ κηροῦ καὶ τὴν κατεργασία μὲ διαζωμεθάνιο, διαχωρίστηκε μὲ GLC/MS ἀνάλυση (διάγραμμα 2).

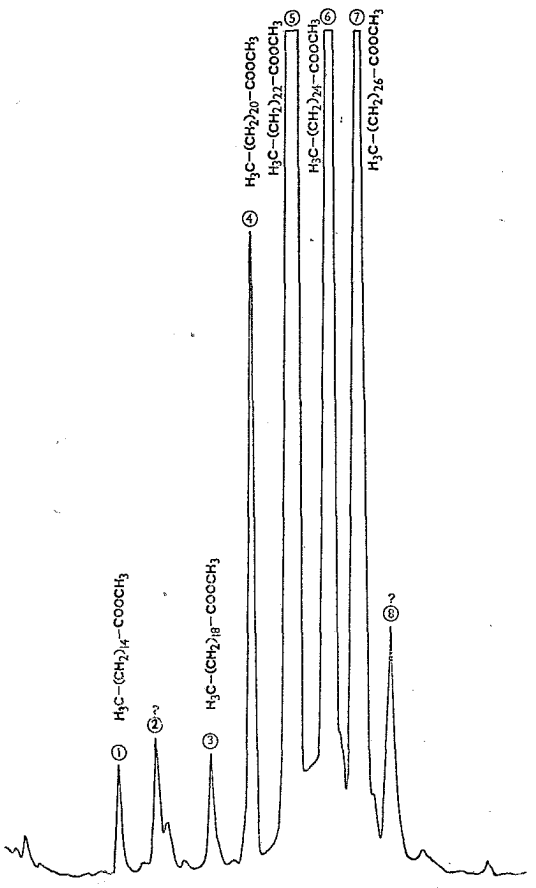
Ἡ ταυτοποίηση[6] τῶν κορυφῶν τοῦ διαγράμματος 2 ἔγινε μὲ τὴ βοήθεια φασμάτων μάζης, ποὺ πάρθηκαν γιὰ κάθε ἕνα μεθυλεστέρα τῶν λιπαρῶν ὀξέων. Στὸν πίνακα ΙΙ δίνονται τὰ ἀποτελέσματα αὐτῆς τῆς ταυτοποιήσεως.

III. Ἀλκοόλες

Τὸ μίγμα τῶν ἀλκοολῶν, ποὺ προέκυψε ἀπὸ τὴ σαπωνοποίηση τοῦ ἀρχικοῦ κηροῦ, κατεργάστηκε μὲ N, O-δὶς (τριμεθυλοσιλυλο) -ακεταμίδιο (BSA) γιὰ νὰ ὑποβληθῆ στὴ συνέχεια σὲ GLC/MS ἀνάλυση (διάγραμμα 3).

Ἡ ταυτοποίηση τῶν κορυφῶν τοῦ χρωματογραφήματος ἔγινε, ὅπως καὶ στὶς προηγούμενες περιπτώσεις, μὲ τὴ βοήθεια φασμάτων μάζης ποὺ πάρθηκαν ξεχωριστὰ γιὰ κάθε τριμεθυλο-σιλυλαιθέρα. Τὰ ἀποτελέσματα τῆς ταυτοποιήσεως αὐτῆς δίνονται στὸν πίνακα ΙΙΙ.

Ἡ ποσοτικὴ σχέση ἀνάμεσα στὴν ἀκόρεστη ἀλκοόλη n-$C_{26}H_{51}OH$ καὶ στὴν κορεσμένη n-$C_{26}H_{53}OH$ (πίνακας ΙΙΙ) βρέθηκε ὅτι εἶναι ∽1:1. Αὐτὸ κατορθώθηκε μὲ βρωμίωση τοῦ μίγματος τῶν ἀλκοολῶν καὶ στὴ συνέχεια μὲ GLC ἀνάλυση (διάγραμμα 4).



ΔΙΑΓΡΑΜΜΑ 2: *GLC/MS ἀνάλυση τοῦ μίγματος τῶν μεθυλεστέρων σὲ στήλη 3% SE 30 (Chromosorb G-AW-DMCS), μήκους 0,37 m καὶ διαμέτρου 2 mm.* Προγραμματισμένη θερμοκρασία στήλης 120-340°C, μὲ ταχύτητα 10°/min. Φέρον ἀέριο He, παροχὴ 30 ml/min. [3]Ανιχνευτὴς MS (Varian CH7). Θερμοκρασία χώρου ἐγχύσεως 350°C. Ταχύτητα χαρτιοῦ 0,2 inch/min.

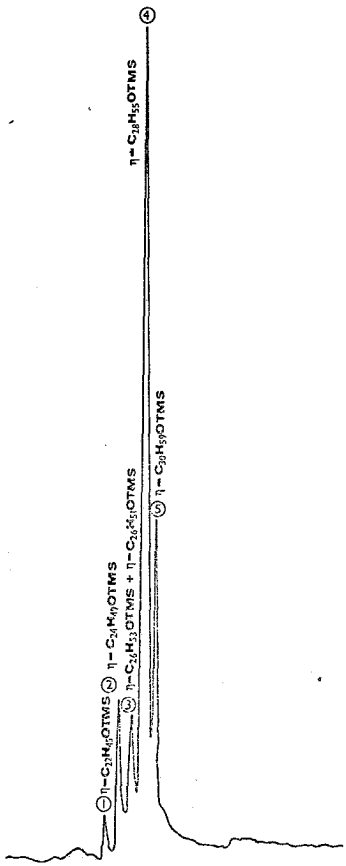
ΠΙΝΑΚΑΣ ΙΙ: *Λιπαρὰ ὀξέα τῶν κηρῶν ποὺ ἀπομονώθηκαν*

Κορυφὲς χρωματογραφήματος διαγράμματος 2	MS m/e*	Τύπος	%
1	M^+ 270, 241, 239, 227, 59, 74, 87, 101, ... 199, 213 κλπ.	$CH_3(CH_2)_{14}COOH$	0,7
2	M^+ 294 δὲν διευκρινίστηκε ἀκόμη		1,0
3	M^+ 326, 297, 295, 283, 59, 74, 87, 101, ..., 227 κλπ.	$CH_3\ (CH_2)_{18}COOH$	0,8
4	M^+ 354, 325, 323, 311, 59, 74, 87, 101, 115, ..., 227 κλπ.	$CH_3\ (CH_2)_{20}COOH$	4,1

Συνέχεια πίνακα II.

Κορυφὲς Χρωμογραφήματος διαγράμματος 2	MS m/e*	Τύπος	%
5	M^+ 382, 353, 351, 339, 59, 74, 87, 101, 115, ...	$CH_3\ (CH_2)_{22}COOH$	49,2
6	M^+ 410, 381, 379, 367, 59, 74, 87, 101, 115, ...	$CH_3\ (CH_2)_{24}COOH$	24,2
7	M^+ 438, 409, 407, 395, 59, 74, 101, ..., 199, 213 κλπ.	$CH_3\ (CH_2)_{26}COOH$	14,0
8	δὲν διευκρινίστηκε ἀκόμη		2,4

* Οἱ τιμὲς m/e ἀναφέρονται στοὺς μεθυλεστέρες τῶν ἀντίστοιχων λιπαρῶν ὀξέων.

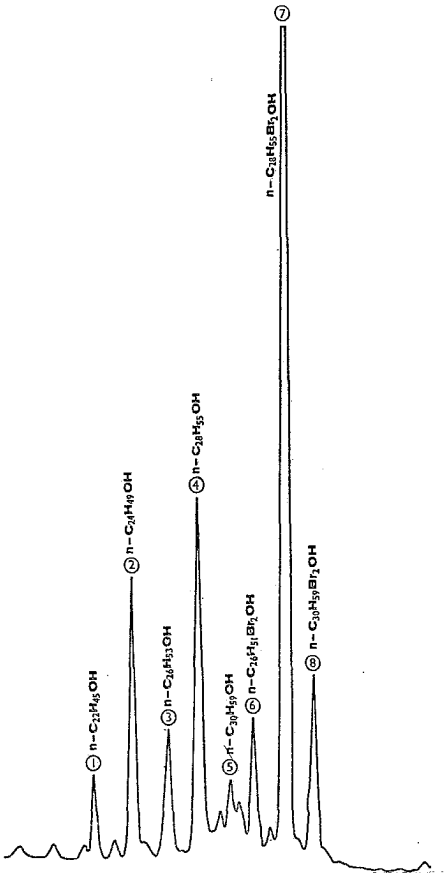


ΔΙΑΓΡΑΜΜΑ 3: *GLC/MS ἀνάλυση τοῦ μίγματος τῶν τριμεθυλοσιλυλαιθέρων σὲ στήλη 3% SE 30 (Chromosorb G-AW-DMCS), μήκους 0,37 m καὶ διαμέτρου 2 mm.* Προγραμματισμένη θερμοκρασία στήλης 150-350°C, μὲ ταχύτητα 10°/min. Φέρον ἀέριο He, παροχὴ 20 ml/min. Ἀνιχνευτὴς MS (Varian CH7). Θερμοκρασία χώρου ἐγχύσεως 350°C. Ταχύτητα χαρτιοῦ 0,1 inch/min.

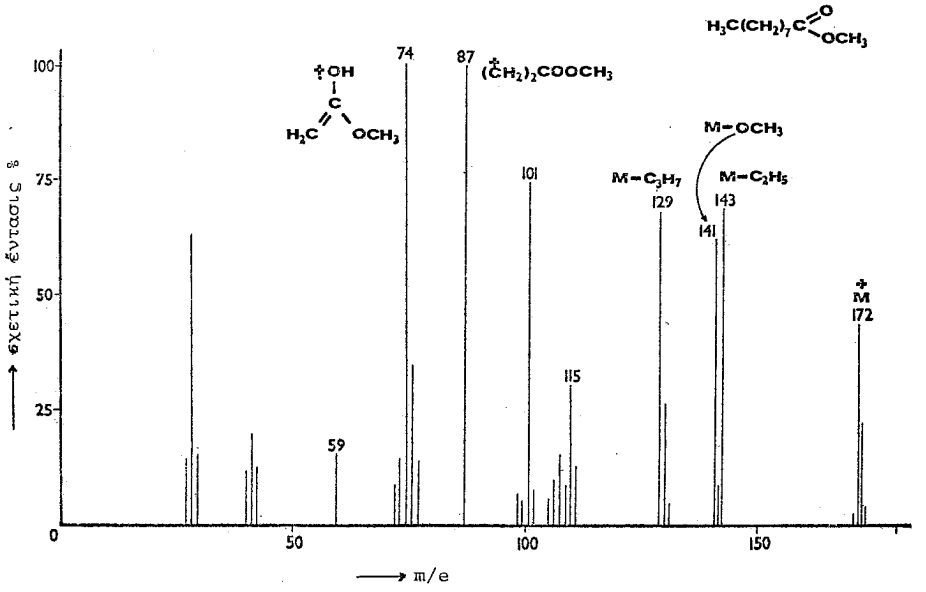
ΠΙΝΑΚΑΣ ΙΙΙ: *Ἀλκοόλες τῶν κηρῶν ποὺ ἀπομονώθηκαν*

Κορυφὲς χρωματογραφήματος διαγράμματος 3	MS m/e*	Τύπος	%
1	M^+ 398, 73, 75, 89, 103, 383 κλπ.	n-$C_{22}H_{45}OH$	3,0
2	M^+ 426, 73, 75, 89, 103, 411 κλπ.	n-$C_{24}H_{49}OH$	10,5
3	M^+ 452, 73, 75, 89, 103 κλπ. M^+ 454, 73, 75, 89, 103 κλπ.	n-$C_{26}H_{51}OH$ n-$C_{26}H_{53}OH$	11,1
4	M^+ 480, 73, 75, 89, 103, 390, 395 κλπ.	n-$C_{28}H_{55}OH$	56,7
5	M^+ 508, 73, 75, 89, 103, 418, 423 κλπ.	n-$C_{30}H_{59}OH$	11,6

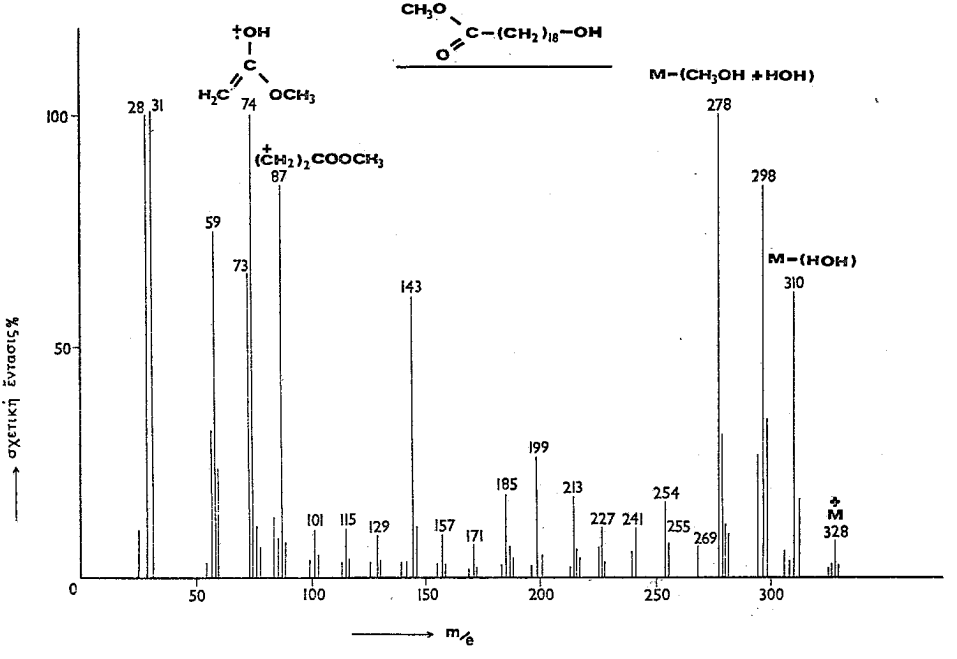
* Οἱ τιμὲς m/e ἀναφέρονται στοὺς τριμεθυλο-σιλυλαιθέρες τῶν ἀντίστοιχων ἀλκοολῶν.



ΔΙΑΓΡΑΜΜΑ 4: *GLC ἀνάλυση τοῦ μίγματος τῶν βρωμιωμένων καὶ μὴ σιλυλαιθέρων, τῶν ἀλκοολῶν τοῦ κηροῦ, σὲ στήλη 2% SE 30, μήκους 0,3 m καὶ διαμέτρου 3 mm.* Προγραμματισμένη θερμοκρασία στήλης 100-340°C, μὲ ταχύτητα 8°/min. Φέρον ἀέριο He, παροχὴ 25 ml/min. Ἀνιχνευτὴς ἰονισμοῦ φλογὸς (FID). Θερμοκρασία ἀνιχνευτῆ 350°C. Θερμοκρασία χώρου ἐγχύσεως 350°C. H_2/ἀέρας 40/240 ml/min. Ταχύτητα χαρτιοῦ 0,25 inch/min.



ΔΙΑΓΡΑΜΜΑ 5: *Φάσμα μάζης τοῦ ἐννεανικοῦ ὀξέος, ἑνὸς ἀπὸ τὰ προϊόντα τῆς ὀξειδωτικῆς διασπάσεως τοῦ διπλοῦ δεσμοῦ τῆς ἀλκοόλης n-$C_{28}H_{55}OH$.*



ΔΙΑΓΡΑΜΜΑ 6: *Φάσμα μάζης τοῦ ω-ὑδροξυ-δεκαεννεανικοῦ ὀξέος, τοῦ ἄλλου προϊόντος τῆς ὀξειδωτικῆς διασπάσεως τοῦ διπλοῦ δεσμοῦ τῆς ἀλκοόλης n-$C_{28}H_{55}OH$.*

Ἡ θέση τοῦ διπλοῦ δεσμοῦ τῶν ἀκόρεστων ἀλκοολῶν προσδιορίστηκε μὲ ὀξειδωτικὴ διάσπαση μὲ ὑπερμαγγανικὸ κάλιο (τροποποιημένη μέθοδος Kuemmel) καὶ μὲ τέτοιες συνθῆκες ὥστε νὰ ἐμποδιστῆ ἡ δημιουργία παραπροϊόντων ὀξειδώσεως.

Τὰ προϊόντα τῆς ὀξειδωτικῆς διασπάσεως τοῦ διπλοῦ δεσμοῦ τῶν ἀκόρεστων ἀλκοολῶν ταυτοποιήθηκαν μὲ GLC/MS ἀνάλυση. Σὰν κύρια προϊόντα ταυτοποιήθηκαν τὸ ἐννεανικὸ ὀξὺ καὶ τὸ ω-ὑδροξυ-δεκαεννεανικὸ ὀξὺ (διαγράμματα 5 καὶ 6), ποὺ προέκυψαν ἀπὸ τὴν ὀξειδωτικὴ διάσπαση τῆς C_{28}-ἀλκοόλης:

$$CH_3\ (CH_2)_7—CH=CH—(CH_2)_{17}CH_2OH$$

Δ^{19} - Εἰκοσιοκτενόλη

$\downarrow KMnO_4/O^-H^-$

$$CH_3(CH_2)_7\text{-}COOH + HOOC\text{-}(CH_2)_{17}CH_2OH$$

Ἐννεανικὸ ὀξὺ ω-Ὑδροξυ-δεκαεννεανικὸ ὀξὺ

Ἔτσι προέκυψε ὅτι ἡ ἀλκοόλη $n\text{-}C_{28}H_{55}OH$ εἶναι ἡ Δ^{19} - εἰκοσιοκτενόλη.

Πειραματικὸ μέρος

Ἀπομόνωση τῶν κηρῶν

200 g δρόγης, ἀφοῦ ξεραθῆ στὸν ἀέρα καὶ κονιοποιηθῆ καλά, ἐκχυλίζεται σὲ συσκευὴ Soxhlet μὲ πετρελαϊκὸ αἰθέρα (σ.ζ. 40-60°C) ἐπὶ 40 ὧρες.

Μετὰ τὴν ἀπομάκρυνση τοῦ πετρελαϊκοῦ αἰθέρα παραμένει στερεό, βάρους 8,18g , μὲ βαθὺ ἐρυθρὸ χρῶμα.

Στὴ συνέχεια τὸ στερεὸ αὐτὸ ὑποβάλλεται σὲ χρωματογραφικὴ ἀνάλυση στήλης (Kieselgel 60, 70-230 mesh), μὲ ὕψος στήλης 45 cm καὶ διάμετρο στήλης 3 cm. Ὡς ἐκλουστικὸ μέσο χρησιμοποιεῖται μίγμα πετρελαϊκοῦ αἰθέρα-βενζολίου (50+50). Ἔτσι, ἀπομονώνεται λευκὸ κηρῶδες σῶμα, βάρους 2 g καὶ περιοχῆς τήξεως 45-70° C.

Σαπωνοποίηση τῶν κηρῶν

α) Μὲ θέρμανση

0,626 g κηρῶν σαπωνοποιοῦνται μὲ θέρμανση ἐπὶ 2 ὧρες μὲ 25 ml βουτανολικοῦ διαλύματος 2N KOH, σὲ θερμοκρασία ὑψηλότερη ἀπὸ 60°C καὶ μέχρι τοῦ σ.ζ. τῆς βουτανόλης.

Τὸ προϊὸν τῆς σαπωνοποιήσεως ἐκχυλίζεται μὲ διαιθυλαιθέρα, ὁπότε παίρνεται τὸ μίγμα τῶν ἀλκοολῶν (55,8%).

Τὸ ὑδατικὸ διάλυμα τῶν σαπώνων ἐξουδετερώνεται μὲ θειικὸ ὀξὺ (50%) μέχρι ὄξινης ἀντιδράσεως. Τὰ λιπαρὰ ὀξέα ποὺ ἐλευθερώνονται ἐκχυλίζονται μὲ διαιθυλαιθέρα.

Στὸ αἰθερικὸ διάλυμα διαβιβάζεται διαζωμεθάνιο μέχρι κορεσμοῦ γιὰ τὸ σχηματισμὸ τῶν μεθυλεστέρων τῶν λιπαρῶν ὀξέων.

β) Χωρὶς θέρμανση

1,044 g κηρῶν σαπωνοποιοῦνται μὲ 50 ml βουτανολικοῦ διαλύματος 1N KOH, σὲ θερμοκρασία δωματίου, ἐπὶ 6 ἡμέρες. Τὸ μίγμα ἀναδεύεται σὲ ἀραιὰ χρονικὰ διαστήματα.

Στὴ συνέχεια ἀποστάζεται ἡ βουτανόλη καὶ τὸ προϊὸν τῆς σαπωνοποιήσεως ἐκχυλίζεται μὲ διαιθυλαιθέρα, ὁπότε παίρνονται 0,526 g ἀλκοολῶν.

Ἡ ὑδατικὴ στιβάδα κατεργάζεται μὲ θειικὸ ὀξὺ (50%) μέχρι ὄξινης ἀντιδράσεως. Τὰ λιπαρὰ ὀξέα ποὺ ἐλευθερώνονται ἐκχυλίζονται ὅπως καὶ προηγουμένως μὲ διαιθυλαιθέρα.

Ἀκολουθεῖ ἐστεροποίηση μὲ διαζωμεθάνιο, ὁπότε προκύπτουν 0,51 g μεθυλεστέρων.

Ὀξείδωση τῶν ἀκόρεστων ἀλκοολῶν

Ἡ μέθοδος ποὺ χρησιμοποιήθηκε ἔχει προταθῆ ἀπὸ τὸν Rudloff[7] καὶ ἔχει τροποποιηθῆ ἀπὸ τοὺς Kuemmel[8] καὶ Σαγρέδο.[9]

Ἀρχικὰ προσδιορίστηκε ὁ ἀριθμὸς ἰωδίου τῆς ὀξειδούμενης οὐσίας μὲ τὴ μέθοδο Wijs καὶ βρέθηκε ἴσος πρὸς 70.

Στὴ συνέχεια, 0,1 g τῆς οὐσίας διαλύεται σὲ 25 ml tert-βουτανόλης καὶ στὸ διάλυμα αὐτὸ προσθέτονται 27,5 ml διαλύματος τῶν ὀξειδωτικῶν μέσων. Τὸ διάλυμα τῶν ὀξειδωτικῶν παρασκευάζεται μὲ διάλυση 20,8 g μετα-υπεριωδικοῦ νατρίου καὶ 0,4 g ὑπερμαγγανικοῦ καλίου σὲ 1 lit νεροῦ, στὸ ὁποῖο προσθέτονται 47,3 mg ἀνθρακικοῦ καλίου.

Ἀκολουθεῖ ἀνάδευση ἐπὶ 5 ὧρες καὶ στὴ συνέχεια προσθήκη 1,5 g ὑδροξειδίου τοῦ καλίου. Ὕστερα ἀπὸ παραμονὴ μισῆς ὥρας καθιζάνει ἴζημα μὲ βαθὺ καφὲ χρῶμα ποὺ διηθεῖται.

Ἀπὸ τὸ διήθημα ἀποστάζεται ἡ tert-βουτανόλη. Τὸ ὑπόλοιπο μέρος τοῦ διηθήματος ὀξινίζεται μὲ 10 ml διαλύματος θειικοῦ ὀξέος (10%). Τὸ ὀξινισμένο διήθημα ἐκχυλίζεται τρεῖς φορὲς μὲ διαιθυλαιθέρα γιὰ τὴν παραλαβὴ τῶν ὀξέων ποὺ σχηματίστηκαν.

Τέλος, στὸ αἰθερικὸ διάλυμα διαβιβάζεται περίσσεια διαζωμεθανίου, ὁπότε σχηματίζονται οἱ μεθυλεστέρες τῶν ὀξέων.

Εὐχαριστίες

Εὐχαριστῶ θερμὰ τὸν Καθηγητὴ κ. Α. Σαγρέδο γιὰ τὴν προσωπική του συμβολὴ στὴν ἐκπόνηση αὐτῆς τῆς ἐργασίας.

Ἐπίσης εὐχαριστῶ τὸν Dr. R. Moser γιὰ τὴ λήψη τῶν φασμάτων μάζης.

Abstract

Structure of the wax substances of the plant Alkanna tinctoria Tausch.

In the present work is described the isolation and identification of the wax substances of the plant Alkanna tinctoria Tausch.

From the investigation arises that the mixture of wax substances of this plant constisted of eleven esters which have from thirty eight to fifty eight carbon atoms. From the above esters in a major proportion are found in the folowing order the ones having C_{52},C_{54},C_{50} and C_{48}. These esters are constructed from eight acids and six alcohols.

From the acids the six were identified and all of them were found to be saturated monocarbonic of unbranched chain and even number of carbon atoms having C_{16}-C_{26}. In a major proportion were found the ones having C_{24} and C_{26}.

All the alcohols were found having unbranched chain with a double bond while in a major proportion are found the ones having C_{28}.

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SEMIAUTOMATIC CATALYTIC TITRATION OF AMINOPOLYCARBOXYLIC ACIDS AND METAL IONS

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Summary

A semiautomatic spectrophotometric method is described for the catalytic titrimetric determination of aminopolycarboxylic acids, based on their inhibitory effect on the manganese (II)-catalyzed periodate - diethylaniline reaction. Microamounts of EDTA and DCTA in the 1-7500 μg range ($1,2\times10^{-7}$ - 8×10^{-4}M) were determined with accuracy and precision of 1-2%. The method has also been used for the indirect catalytic titrimetric determination of several metal ions (Ga^{3+}, Pd^{2+}, Cu^{2+}, Cd^{2+}, Pb^{2+}, Zn^{2+}, Hg (II), Co^{2+}, Ni^{2+}), of the solubilities of a large number of their insoluble salts, and for the analysis of certain of their mixtures.

Key words: Catalytic, Titration, Aminopolycarboxylic acids.

Introduction

In recent years many kinetic methods of analysis based on catalytic rates modified by inhibition or activation have been developed.[1-15] Inhibition in systems involving catalysts other than enzymes is mainly achieved by precipitation or complexation, particularly by chelating agents that complex metal ion catalysts.[10] Catalytic rates modified by inhibition are used in catalytic titrations. The term "catalytic or catalymetric titration" was coined by Yatsimirskii and Fedorova,[1] who first pointed out the possibility of using catalytic reactions for endpoint indication. Catalytic titrations are ideally fitted for the determination of inhibitors.

In the present paper a semiautomatic method is presented for the catalytic titrimetric determination of aminopolycarboxylic acids, e.g., of ethylenediaminetetraacetic acid (EDTA) and 1,2-diaminocyclohexanetetraacetic acid (DCTA), based on their inhibitory effect on the periodate - diethylaniline reaction. The determination of microamounts of anions of aminopolycarboxylic acids has become important recently in connection with their use in biology, medicine, food chemistry etc. The catalytic titrimetric determination of EDTA and DCTA involves two consecutive reactions: a) the *titration reaction,* in which the manganese(II) titrant (catalyst) is added to the sample at a constant rate and reacts rapidly and stoichiometrically with EDTA or DCTA, and b) the *indicator reaction,* that is the periodate-diethylaniline reaction, which involves the monitored species and can only occur at a noticeable rate once an excess of manganese(II) is present in the system. Since the excess of titrant at the endpoint does not react stoichiometrically

*Part of a doctoral thesis, University of Athens, 1976.

but catalytically, a small excess of titrant results in large changes in the rate of the indicator reaction and therefore in sharp absorbance changes which can easily be detected spectrophotometrically, by monitoring the absorbance at 470 nm where the yellow reaction product absorbs.[16] Actually, the output voltage of a photoconductive circuit is monitored. The absolute value of the variable chosen to monitor the indicator reaction does not need to be known accurately because only the measurement of the relative change of this parameter (absorbance, voltage) with time (volume of titrant) is required. Since the manganese(II) is added at a constant rate and the change of potential with time is recorded, the resulting titration curve shows a *pseudo-induction period*[10] whose length is proportional to the amount of inhibitor (EDTA or DCTA) present. The endpoint is read from the plot after the titration is completed.

The semiautomatic method is very senstitive, simple, rapid, and accurate. Microamounts of EDTA and DCTA in the range 1-7500 μg (1.2×10^{-7} - 8×10^{-4} M) were determined with relative errors and relative standard deviation of 1-2%.

The method has also been used for the indirect catalytic titrimetric determination of several metal ions (Ga^{3+}, Pd^{2+}, Cu^{2+}, Cd^{2+}, Pb^{2+}, Zn^{2+}, Hg(II), Co^{2+}, Ni^{2+}). In this case, a known amount of standard EDTA or DCTA solution is added to the metal ion solution and the unconsumed excess of the inhibitor is back-titrated with a standard manganese(II) solution. In addition, the method has been used for the determination of the solubilities of a large number of insoluble salts of the aforementioned metal ions, and for the analysis of certain of their mixtures.

Experimental

Apparatus

The titration system described previously[16] was used, except that a 470-nm second -order interference filter was inserted into the auxiliary holder of the Spectro unit and a Heath recorded (Model EU 301VE) was used.

Reagents

All reagents were prepared with deionized double - distilled water and reagent - grade materials.

Phosphate buffer, pH 6,0: 10 ml 0.5M H_3PO_4 and 10 ml 0.5M NaH_2PO_4 are diluted to 100 ml and neutralized with 2M NaOH to a pH of 6.0

Phosphate (0.05M) - citrate (0.03M) buffer, pH 7.0: 10 ml 0.5M H_3PO_4, 10 ml 0.3M citric acid and about 50 ml water are mixed, neutralized with 2M NaOH to a pH of 7.0 and diluted to 100 ml.

Potassium periodate, saturated aqueous solution.

Diethylaniline hydrochloride (DEA), 0.100 M stock solution. 1.49 g of diethylaniline are dissolved in 10 ml of 1 M HCl and diluted to 100 ml.

EDTA, 0.01000 M, stock solution. Standardized against standard zinc solution,[17] which is prepared by dissolving reagent grade zinc (>99,9% Zn) in hydrochloric acid, Eriochrome Black T being used as indicator; stored in polyethylene bottles.

DCTA, 0.01000 M stock solution. Prepared with recrystallized from hot water reagent, neutralized with NaOH and standardized against standard zinc solution[17].

Manganese, 0.0600 M stock solution. Prepared from $MnSO_4 \cdot H_2O$ and standardized against standard EDTA solution.[17] Working composite standards for the working curves containing 2×10^{-3} moles DEA/l are prepared by mixing with the stock DEA solution and dilution.

Metal ion stock solutions (Cu, Pb, Hg(II), Co, Ni, Cd, Zn, Pd, Ga), 0.01000 M. Standardized against standard EDTA solution.

Calcein indicator solution, 0.1% in 0.001 M NaOH.

Dilute standard EDTA, DCTA and metal ion solutions were prepared from the stock solutions daily. The very dilute solutions ($<10^{-5}$M) were prepared just before the measurements.

Procedure.

Preparation of equipment. Fill the burette with the appropriate composite standard manganese(II) solution, depending on the EDTA or DCTA concentration of the sample. Switch the regulated power supply "ON" and the "Spectro-Electro" titrator to the "Spectro" position, throw the polarity switch to position 1 and insert a Bosch and Lomb 470-nm second-order interference filter in the auxiliary holder. Set the pegs in the base to position the 50-ml beakers and connect a stirrer (S-76669-A), a right - angled delivery tip (S-29704) and the burette. Turn the lower pH index switch to 250 mV.

Semiautomatic titration of EDTA or DCTA. Pipet into a 50-ml beaker a 25.00 ml aliquot of the sample, 2,00 ml of phosphate - citrate buffer pH 7.0 or phosphate buffer pH 6.0 respectively and 1.00 ml of saturated potassium periodate solution. Lock the burette "ON", start the stirrer and turn the function switch to "Record" to obtain the titration curve. For the best accuracy, calibrate the recorder for each titration, using the burette reading.

Semiautomatic titration of metal ions. Pipet into a 50-ml beaker a 10.00-ml aliquot of the sample, add an excess of standard EDTA (or DCTA) solution, dilute to 25 ml and continue as in the titration of EDTA from the point of buffer addition. To estimate the amount of standard EDTA or DCTA solution to be added, add to the metal ion solution a drop of calcein indicator and then slowly the standard EDTA (or DCTA) solution until the appearance of the green fluorescence of the free indicator[18] and 2.0 ml in excess. In the case of cobalt murexide is substituted for calcein and the color change is from yellow to red-violet. The excess of back - titrated EDTA (or DCTA) is calculated from working curves obtained by titrating standard EDTA (or DCTA) solutions with the same manganese titrant. This procedure is also used for the determination of the solubility of an insoluble salt by titrating a 10.00 ml aliquot of its saturated solution.

Determination of metal ions in mixture. Pipet into a 50-ml beaker a 10.00-ml aliquot of the sample, containing mercury(II) and another ion M^{n+} at a molar ratio of 0.1:1 till 5:1, add an excess X of standard EDTA (or DCTA) solution, dilute to 25 ml and continue as in the titration of EDTA from the point of buffer addition. Subsequently, pipet into a 50-ml beaker another 10.00-ml aliquot of the sample, add 3.00ml of 0.0010 M KCN solution and the same excess X of EDTA (or DCTA), dilute to 25 ml and continue as in the titration of EDTA from the point of buffer addition. In both titrations, calculate the excess X_1 and X_2 of EDTA (or DCTA) from working curves and from the values of X, X_1, and X_2 calculate the concentrations of mercury(II) and M^{n+} in the sample.

Results and Discussion

Ions which react with manganese to form precipitates or strong complexes, and strong oxidants which could oxidize manganese(II) should be absent.

A phosphate - citrate buffer of pH 7.0 and a phosphate buffer of pH 6.0 were chosen for the titration of EDTA and DCTA respectively, because with these buffers the blanks were smaller and the results were more accurate.

The indicator reaction can be followed spectrophotometrically by monitoring the increase in absorbance at 470 nm. During the titration reaction the manganese(II) reacts with the inhibitor (EDTA or DCTA) and the signal remains practically constant (it may change slowly because of the uncatalyzed reaction) until the equivalence point is reached, at which point a small excess of titrant results in large changes in the rate of the indicator reaction and therefore in sharp changes of the signal level which can be easily detected. Recorded curves for the titration of EDTA and DCTA with manganese(II) are shown in Figs. 1 and 2. The endpoint was obtained by extrapolating the linear segments of the titration curve.

There is always a blank which is a function of titrant concentration and experimental conditions, such as rate of addition of titrant, stirring, volume of titrated solution, etc. However, all these factors can be kept constant, so that reproducible working curves were obtained. To minimize the blank, the diethylaniline was included in the titrant.

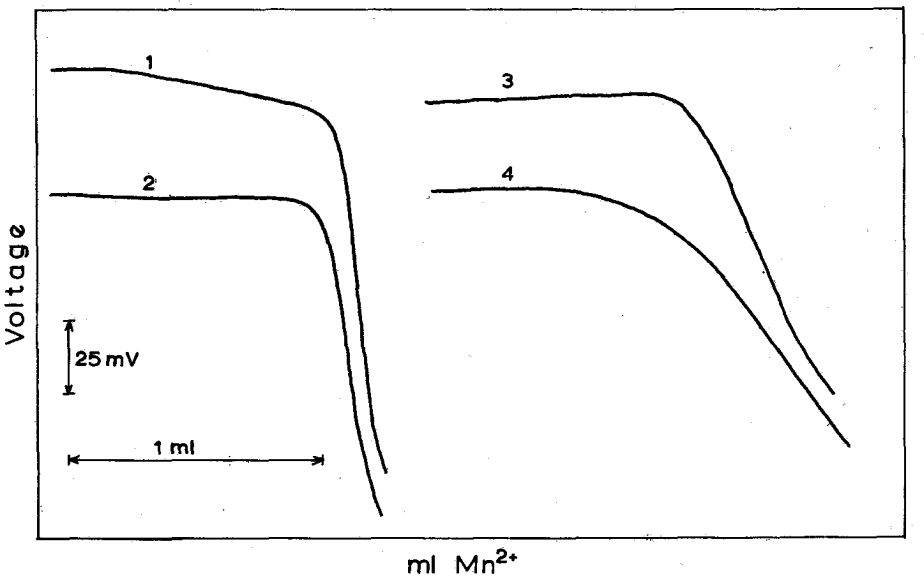

FIG. 1: *Recorded curves for the catalytic titration of EDTA with Mn (II).*
1 : 25 ml of 2×10^{-4} M EDTA titrated with 6×10^{-3} M Mn (II)
2 : 25 ml of 2×10^{-5} M EDTA titrated with 6×10^{-4} M Mn (II)
3 : 25 ml of 2×10^{-6} M EDTA titrated with 6×10^{-5} M Mn (II)
4 : 25 ml of 2×10^{-7} M EDTA titrated with 6×10^{-6} M Mn (II)

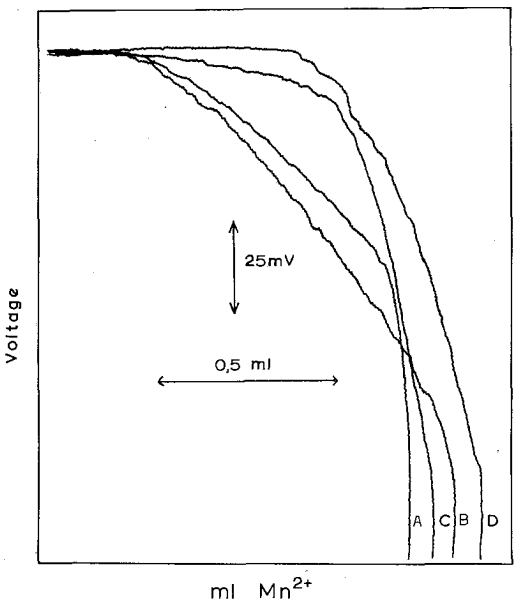

FIG. 2: *Recorded curves for the catalytic titration of DCTA with Mn (II).*
A. 25 ml of 2×10^{-4} M DCTA titrated with 6×10^{-3} M Mn (II)
B. 25 ml of 2×10^{-5} M DCTA titrated with 6×10^{-4} M Mn (II)
C. 25 ml of 2×10^{-6} M DCTA titrated with 6×10^{-5} M Mn (II)
D. 25 ml of 2×10^{-7} M DCTA titrated with 6×10^{-6} M Mn (II)

Working curves are obtained by plotting the endpoint volumes against the amount of EDTA (or DCTA). Working curves were made to read molar concentrations or micrograms of EDTA (or DCTA) in the titrated sample (25 ml). Working curves were prepared for each of four ranges (1×10^{-7} - 8×10^{-7}, 1×10^{-6} - 8×10^{-6}, 1×10^{-5} - 8×10^{-5}, and 1×10^{-4} - 8×10^{-4} M EDTA (or DCTA) with four manganese (II) standards (6×10^{-6}, 6×10^{-5}, 6×10^{-4} and 6×10^{-3} M respectively). Three standard EDTA (or DCTA) solutions were sufficient for each working curve. These plots were linear for all the concentration ranges tested.

The EDTA (or DCTA) concentration may also be found by the proportional method in which the result given by the sample is compared with that given by a standard EDTA (or DCTA) solution; conditions and especially the EDTA (or DCTA) concentration must then be as similar as possible in both titrations.

Results for aqueous EDTA and DCTA solutions of known concentrations are shown in Tables I and II respectively. In the proportional method, the unknown and the control had the same EDTA or DCTA concentration. The results point to the wide range of EDTA and DCTA concentrations that can be determined as well as to the good accuracy and precision afforded by semiautomatic titrations with catalytic end-point indication.

The conditional formation constants of the EDTA and DCTA complexes should be much larger for the titrated metal ion M^{n+} than for Mn^{2+}, so that during the back-titration of excess EDTA or DCTA with manganese (II) no substitution reaction takes place between Mn^{2+} and the EDTA or DCTA complex of M^{n+}. This was the case for all the ions titrated (Table III).

TABLE I. *Semiautomatic catalytic titration results for aqueous EDTA solutions*

Mn^{2+} titrant (M)	EDTA taken	% Error		% RSD
+ 2×10^{-3} M DEA	(μg) range	Working curve	Proportional method	(n=5)
6×10^{-6}	1.1- 7,4	9.1	2.6	1.9 (3.7 μg EDTA)
6×10^{-5}	11 - 74	1.6	1.7	1.0 (37 μg EDTA)
6×10^{-4}	110 - 740	0.7	1.5	1.0 (372 μg EDTA)
6×10^{-3}	1100 -7400	0.7	0.9	0.5 (3720 μg EDTA)
		Av. 3.0	1.7	1.1

TABLE II: *Semiautomatic catalytic titration results for aqueous DCTA solution*

Mn^{2+} titrant (M)	DCTA taken	% Error		% RSD
$+2\times10^{-3}$ M DEA	(μg) range	Working curve	Proportional method	(n=6)
6×10^{-6}	1.1 - 7.5	2.3	0.5	0.4 (3.7 μg DCTA)
6×10^{-5}	11 - 75	0.6	0.6	0.3 (37 μg DCTA)
6×10^{-4}	110 - 750	1.1	0.8	1.5 (373 μg DCTA)
6×10^{-3}	1100 -7500	2.5	0.9	1.1(3730 μg DCTA)
		Av. 1.6	0.7	0.8

For the titration of gallium the citrate-phosphate buffer was substituted by a 1% $NaHCO_3$ solution to avoid masking of gallium ions by phosphate ions.

For the determination of palladium and nickel with EDTA, it is necessary to let the solution stand for a few minutes before back-titrating the excess EDTA because the formation of their EDTA complexes is slow.

The detectability and the sensitivity are increased for the determination of lead (II) and mercury (II) in the presence of aminotriacetic acid (NTA) which enhances the catalytic effect of Mn (II) on the indicator reaction. A similar use of NTA to enhance the catalytic effect of manganese ions on the oxidation of Malachite Green cations has been reported.[19] Thus it was possible to extend the lower concentration limit to the 10^{-9} M level. The possibility of determining lead

TABLE III. *Semiautomatic indirect catalytic titration results for aqueous metal ion solutions*

Metal ion	Range, μg	Average error, %		% RSD
		Separate standard	Common standard	(n=5-8)
A. with EDTA				
Ga^{3+}	20 - 50	0.5		
Pd^{2+}	30 - 70	1.7		
Cu^{2+}	0.06 - 450	1.1	2.6	0.7 - 1.7[a]
Cd^{2+}	0.3 - 80	0.7	2.0	1.7 - 2.6[a]
Pb^{2+}	0.002-1500	2.0	2.0	0.8 - 2.1[a]
Zn^{2+}	0.07 - 50	1.4	1.7	0.5 - 1.2[a]
Hg (II)	0.006- 140	2.0	1.6	0.5 - 2.2[a]
Co^{2+}	0.006- 4	2.1	1.9	1.1 - 1.2[a]
Ni^{2+}	0.006- 4	3.1	3.0	0.4 - 2.7[a]
B with DCTA				
Cu^{2+}	0.6 - 5	0.6		1.1 (3.2 μg)
Cd^{2+}	1 - 8	0.6		0.5 (5.6 μg)
Pb^{2+}	2 - 15	0.8		2.1 (10.4 μg)
Zn^{2+}	0.7 - 5	0.6		0.3 (3.3 μg)
Hg (II)	2 - 14	0.2		0.8 (10.0 μg)
Co^{2+}	0.6 - 4	0.1		0.7 (3.0 μg)
Ni^{2+}	0.6 - 40	0.8		0.4 (2.9 μg)

[a] % RSD range

and mercury at the nanogram level makes the proposed method especially valuable in trace analysis. The possibility of determining microamounts of NTA on the basis of its promoting action on the manganese (II) - catalyzed periodate - diethylaniline reaction is under investigation.

In the indirect catalytic titration of a metal ion the proportional method is used. The volume of EDTA (or DCTA) found by subtracting the volume of unconsumed (back-titrated) EDTA (or DCTA) from the known volume of EDTA added to the sample is equivalent to the quantity of the metal ion in 10 ml of sample. To determine the blank either a separate metal ion standard of about the same concentration as the sample was titrated for each sample or a series of standard metal ion solutions covering a decade of concentration were titrated with each standard manganese solution, and the average blank was taken as common blank and was subtracted from the titrant volume for each sample. The first approach (separate standard) gave more accurate results but it took longer. Accuracy and precision were better with DCTA than with EDTA (Table III). The results indicate that microgram and in some cases nanogram amounts of metal ions can be determined with an accuracy and precision of about 1%.

The proposed method has been successfully applied to the determination of vitamin B_{12}. The method was also used for the determination of the solubilities of insoluble salts of many of the ions determined by the back - titrating procedure (Table IV). In most of the cases, there is satisfactory agreement between experimentally determined and reported values.

Results for the analysis of solution containing cobalt and nickel, and of binary mixtures containing mercury (II) and zinc or lead or copper or cadmium are shown in Table V. The analysis of the cobalt - nickel mixtures was made as follows: The sum of cobalt and nickel was determined in an aliquot of the sample by the proposed method by adding an excess of EDTA and back-titrating the excess of EDTA with standard manganese (II) solution. To an equal aliquot of the sample a borate buffer of pH 9.3 and dimethylglyoxime (DMG) were added, the Ni-DMG complex was extracted with chloroform, cobalt was determined in the aqueous phase by the proposed method and nickel was found by difference.

In the case of binary mixtures containing Hg (II) and a cation M^{n+}, first the sum of Hg (II) and M^{n+} is determined in an aliquot of the sample by the proposed procedure; then in another aliquot of the sample Hg (II) is masked by cyanide, ion M^{n+} is determined by the proposed method and Hg (II) is found by diference. The method for the analysis of binary mercury (II) mixtures is simple and fast in comparison with time - consuming procedures involving extraction and ion - exchange steps.

In conclusion, we have demonstrated the suitability of the manganese (II) - catalyzed periodate - diethylaniline reaction for the development of a rapid, simple, accurate and sensitive means of determining trace amounts of aminopolycarboxylic acids and of a variety of metal ions.

TABLE IV. *Determination of solubility of insoluble salts by catalytic titration*

Salt	Solubility, M		Salt	Solubility, M	
	Found	Reported		Found	Reported
$CuC_4H_4O_6$	3.7×10^{-4}	2.6×10^{-4}	$HgBr_2$	2.09×10^{-3}	1.84×10^{-3}
$CuCO_3$	1.63×10^{-5}	1.6×10^{-5}	$Hg(IO_3)_2$	1.00×10^{-4}	4.15×10^{-5}
$Cd_3[Fe)CN)_6]_2$	4.4×10^{-5}	4.6×10^{-5}	HgC_2O_4	4.7×10^{-5}	3.7×10^{-5}
$PbSO_4$	1.25×10^{-4}	1.26×10^{-4}	$HgCrO_4$	1.28×10^{-4}	4.5×10^{-5}
$Pb(IO_3)_2$	5.0×10^{-5}	$4,3\times10^{-5}$	Hg0	2.73×10^{-4}	2.39×10^{-4}
$PbBr_2$	4.7×10^{-3}	4.6×10^{-3}	CoC_2O_4	1.43×10^{-4}	$1,43\times10^{-4}$
$ZnCO_3$	2.05×10^{-5}	1.0×10^{-5}	$Ni(OH)_2$	1.74×10^{-5}[a]	1.41×10^{-5}
ZnC_2O_4	1.43×10^{-4}	1.16×10^{-4}	$NiCO_3$	3.4×10^{-5}[a]	8×10^{-5}

[a] DCTA was used

TABLE V. *Semiautomatic catalytic titration results for mixtures of metal ions*

Cobalt, μg		% Error	Nickel, μg		% Error
Taken	Found		Taken	Found	
1.178	1.174	— 0.3	1.174	1.178	+ 0.3
1.768	1.850	+ 4.6	1.761	1.674	— 4.9
2.357	1.472	+ 4.9	2.348	2.212	— 5.8
		Av. 3.3			Av. 3,7
Mercury, μg		% Error	Metal ion, μg		% Error
Taken	Found		Taken	Found	
			Zinc		
40.1	41.1	+ 2.5	13.07	12.75	— 2.4
100.3	100.1	— 0.2	6.54	6.28	— 4.0
20.06	17.45	— 13.0	32.7	33.4	+ 2.1
20.06	18.67	— 6.9	65.4	65.7	+ 0.5
		Av. 5.6			Av. 2.2
			Lead		
40.1	38.1	— 5.0	41.4	43.5	+ 5.1
100,3	98,7	— 1.6	20.7	20.7	—
20.06	20.06	—	103,6	103.6	—
20.06	20.46	+ 2.0	207.2	207.6	+ 0.2
		Av. 2.2			Av. 1.3
			Copper		
40.1	40.1	—	12.70	12.70	—
100.3	100.7	+ 0.4	6.36	6.04	— 5.0
20.06	18.46	— 8.0	31.8	31.8	—
20.06	21.27	+ 6.0	63.6	64.6	+ 1.6
		Av. 3.6			Av. 1.6
			Cadmium		
40.1	39.1	— 2.5	22.48	23.60	+ 5.0
100.3	99.1	— 1.2	11.24	12.36	+ 10.0
20.06	18.86	— 6.0	56.2	57.3	+ 2.0
20.06	20.06	—	112,4	116.9	+ 4.0
		Av. 2.4			Av. 5.2

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Περίληψις

Ἡμιαυτόματος καταλυτικὴ ὀγκομέτρησις ἀμινοπολυκαρβονικῶν ὀξέων καὶ μεταλλοϊόντων.

Περιγράφεται ἡμιαυτόματος φασματοφωτομετρικὴ μέθοδος διὰ τὸν καταλυτικὸν ὀγκομετρικὸν προσδιορισμὸν ἀμινοπολυκαρβονικῶν ὀξέων, βασιζομένη ἐπὶ τῆς παρεμποδιστικῆς δράσεως αὐτῶν ἐπὶ τῆς ὑπὸ τοῦ μαγγανίου (II) καταλυομένης ἀντιδράσεως ὑπεριωδικῶν - διαιθυλανιλίνης. Ἡ ἀντίδρασις

αὕτη ἐχρησιμοποιήθη ὡς ἐνδεικτικὴ διὰ τὴν καταλυτικὴν ὀγκομέτρησιν διὰ προτύπου διαλύματος μαγγανίου (II), οὕτω δὲ προσδιωρίσθησαν EDTA καὶ DCTA εἰς τὴν περιοχὴν 1 μg - 1,7 mg (1.2×10^{-7} - 8×10^{-4}M) μὲ σχετικὸν σφάλμα καὶ σχετικὴν τυπικὴν ἀπόκλισιν 1-2%.

Ἡ μέθοδος ἐχρησιμοποιήθη καὶ διὰ τὸν ἔμμεσον καταλυτικὸν ὀγκομετρικὸν προσδιορισμὸν κατιόντων διαφόρων μετάλλων, ἤτοι Ga, Pd, Cu, Pb, Zn, Hg (II), Co, Cd καὶ Ni, τὰ ὁποῖα οὐδεμίαν καταλυτικὴν ἐπίδρασιν ἔχουν ἐπὶ τῆς ἀνωτέρω ἐνδεικτικῆς ἀντιδράσεως. Πρὸς τοῦτο εἰς διάλυμα μεταλλοϊόντος M^{n+} προστίθεται γνωστὴ ποσότης EDTA ἢ DCTA, ἡ δὲ μὴ ἀντιδράσασα ποσότης EDTA ἢ DCTA ὀγκομετρεῖται διὰ προτύπου διαλύματος Mn (II), χρησιμοποιουμένης τῆς ἀντιδράσεως ὑπεριωδικῶν - διαιθυλανιλίνης ὡς ἐνδεικτικῆς. Μεγάλη εὐαισθησία, ταχύτης καὶ ἀκρίβεια εἶναι τὰ κύρια χαρακτηριστικὰ τῆς ἀναπτυχθείσης μεθόδου. Οὕτω προσδιωρίσθησαν τὰ ἀνωτέρω μεταλλοϊόντα εἰς ποσότητας τῆς τάξεως τοῦ μικρογραμμαρίου, ἐνίοτε δὲ καὶ τοῦ νανογραμμαρίου (1ng = 10^{-9}g), μὲ ἀκρίβειαν καὶ ἐπαναληπτικότητα περίπου 1%. Προσέτι ἐγένοντο ἀναλύσεις μιγμάτων τινῶν ἐκ τῶν ἀνωτέρω μεταλλοϊόντων διὰ καταλυτικῆς ὀγκομετρήσεως καὶ προσδιωρίσθησαν αἱ διαλυτότητες μεγάλου ἀριθμοῦ δυσδιαλύτων ἁλάτων τῶν ἐν λόγῳ μεταλλοϊόντων.

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DITHIOCARBAMATES OF MOLYBDENUM (V)

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Summary

Dithiocarbamate complexes of Molybdenum (V) having the molecular formulae $Mo(dtcb)Cl_4$, $Mo(dtcb)_2Cl_3$ and $Mo(dtcb)_3Cl_2$ have been prepared by the reaction of molybdenum pentachloride with the appropriate amount of dithiocarbamate ion. The complexes are non-electrolytes, monomeric in chloroform. The structure of the complexes has been formulated on the basis of the IR, NMR and electronic spectra.

Abbreviations:

$Mo(detc)Cl_4$, diethyldithiocarbamato molybdenum (V) tetrachloride, $[(C_2H_5)_2\ NCS_2]\ MoCl_4$.

$Mo(detc)_2Cl_3$, bis (diethyldithiocarbamato) molybdenum (V) trichloride, $[(C_2H_5)_2NCS_2]_2\ MoCl_3$.

$Mo(detc)_3Cl_2$, tris (diethyldithiocarbamato)molybdenum (V) dichloride, $[(C_2H_5)_2NCS_2]_3\ MoCl_2$.

$Mo(detc)_4Cl$, tetrakis (diethyldithiocarbamato)molybdenum (V) chloride, $[(C_2H_5)_2NCS_2]_4\ MoCl$.

$Mo(mdtc)Cl_4$, morpholinedithiocarbamato molybdenum (V) tetrachloride, [ $NCS_2]MoCl_4$.

$Mo(mdtc)_2Cl_3$, bis (morpholinedithiocarbamato)molybdenum (V) trichloride, [ $NCS_2]_2MoCl_3$.

$Mo(mdtc)_3Cl_2$, tris(morpholinedithiocarbamato)molybdenum (V) dichloride [ $NCS_2]_3MoCl_2$.

$Mo(pmtc)_4Cl$, pentamethylenedithiocarbamato molybdenum (V) tetrachloride, [ $NCS_2]MoCl_4$.

$Mo(pmtc)_2Cl_3$, bis(pentamethylenedithiocarbamato)molybdenum (V) trichloride, [ $NCS_2]_2MoCl_3$.

$Mo(pmtc)_3Cl_2$, tris(pentamethylenedithiocarbamato)molybdenum (V) dichloride, [ $NCS_2]_3MoCl_2$.

$Mo(tmtc)Cl_4$, tetramethylenedithiocarbamato molybdenum (V) tetrachloride, [ $NCS_2]MoCl_4$.

$Mo(tmtc)_2Cl_3$, bis(tetramethylenedithiocarbamato)molybdenum (V) trichloride, [$NCS_2]_2MoCl_3$.

$Mo(tmtc)_3Cl_2$, tris (tetramethlenedithiocarbamato)molybdenum (V) dichloride, [ $NCS_2]_3MoCl_2$.

Introduction

Heckley and co-workers have prepared the N,N-diethyldithiocarbamato complexes of Nb(V), Ta(V) and Pa(V) by reacting the pentahalides of the metals with sodium N,N-diethyldithiocarbamate.[1] Depending on the nonaqueous solvent used and more important the ratio of starting materials a variety of complexes can be isolated having the stoichiometries $M(dtcb)_2X_3$, $M(dtcb)_3X_2$ and $M(dtcb)_4X$. Based on their report and our continuing interest in the dithiocarbamates of the transition elements we undertook a study of similar complexes of Mo(V).

In a previous paper we reported our study of the complexes prepared by the reaction of molybdenum pentachloride with sodium morpholinodithiocarbamate.[2] We have continued our investigation to include the reaction of molybdenum pentachloride with diethyl-, pyrrolidino and piperidinodithiocarbamate ions. Reaction conditions are important in determining the kind of the products obtained. Because the desired products are part of a series of equilibrium reactions,[2] it is possible to prepare both the bis-dithiocarbamato and the tris-dithiocarbamato complexes by a proper selection of the stoichiometrie amounts of starting materials.

Experimental

Physical measurements

All physical measurements (IR, UV, visible, NMR, conductance, etc) were obtained using the same instruments and techniques used in the previous study.[2]

Molybdenum pentachloride was purchased from Alfa Inorganic. The sodium salts of the dithiocarbamates were prepared according to known procedure.[3] After crystallization the salts were dehydrated under vacuum at 40°C and were used without further purification.

Preparation of the complexes.

The complexes were obtained by the reaction of molybdenum pentachloride and sodium dithiocarbamate in different solvents. For example, to a suspension of 1,4 g (5,12 mmoles) of molybdenum pentachloride in methylene chloride (50 ml) the equivalent amount of anhydrous sodium dithiocarbamate was added rapidly with stirring. The mixture was stirred for one hour then filtered to remove sodium chloride and the product was obtained by evaporating the filtrate under vacuum at room temperature. The products were purified by dissolving them in either chlorofor or methylene chloride followed by precipitation with petroleum ether (60°-70°). Table I gives the analytical data for the compounds as well as colour melting points and molecular weights.

Results and Discussion

The solvent methylene chloride was used for all of the syntheses. Other solvents tried included carbon tetrachloride, chloroform, acetone and ethanol.

Alcohol proved to be unsuitable since only nonstoichiometric redbrown meterial formed. Carbon tetrachloride results in low yields and from chloroform the only isolable products are the $Mo(dtc)_3Cl_2$ and $Mo(dtc)_2Cl_3$ complexes. While acetone is the best choice for the preparation of the morpholino derivatives it is a poor one for all other since, the complexes appear to undergo side reactions and decomposition, as evidenced by many colour changes of the solution during the preparation.

The compounds are relatively stable when dry although, noticeable decomposition begins to take place after exposure to the atmosphere and light. In the absence of light and air the solutions are stable for at least a period of *ca.* 24 hours.

The complexes of the type $Mo(dtcb)Cl_4$ are extremely hygroscopic and undergo hydrolysis and they decompose rapidly. The other complexes of the general formulae $Mo(dtcb)_2Cl_3$ and $Mo(dtcb)_3Cl_2$, while they are air-sensitive and hydrolyze they are more stable in the solid state, compared to the $Mo(dtcb)Cl_4$ compounds.

Infrared spectra

The important band positions in the infrared spectra for the Mo(V) complexes are similar to those reported for other dithiocarbamate ones.[4,5]

Generally, a band appears at *ca.* 1000 cm^{-1} attributed to the dithiocarbamate group when both sulfur atoms are bound to the same central atom, i.e when acting as a bindentate chelating ligand. However, when both sulfur atoms are not bonded equivalently, a splitting results giving rise to another band[5] at *ca.* 950 cm^{-1} for the ν(C···S) stretching frequency. The data in Table II give the positions of the observed and show that for the majority of the complexes the ligands are not coordinated equivalently to molybdenum.

The ν(C···N) stretching mode is usually found in the range 1480-1560 cm^{-1}. The exact position of the band is effected by the presence of halogen bonded to the metal and the geometry of the complexes.[6] As an example the ν(C···N) band for the mono, bis and tris pyrrolydino complexes appears at 1545 cm^{-1}, 1535 and 1525 cm^{-1} respectively. There is a noticeable shift to higher frequencies as the number of chlorine atoms in the complexes increases. This trend is attributed to the inductive effect by halogen; thus, with increasing charge transfer from the ligand to the metal the multiple bond character of the C···N bond is increasing. This behaviour has been observed in the halo compounds of the dithiocarbamates of other elements.[7-9]

Electronic Spectra

Absorption spectra were obtained for the complexes in both methylene chloride and nitrobenzene solutions. The major band positions are the same in the two solvents. The log ε values were determined accurately only for the nitrobenzene solutions, because of the high solubility in this solvent.

The electronic spectra, Table III, are typical of those exhibited by the dithiocarbamate complexes in general. Assignments of the spectral frequencies to particular transitions were made on the basis of a comparison with known species and relative spectral intensities.

In the ultraviolet region, for solutions of the complexes in both solvents, sharp and intense bands are observed at about 37000 (270 nm) and 40000 cm^{-1} (250 nm). These bands are due to intraligand $\pi \rightarrow \pi^*$ transitions.[10] In addition to these, a third band is observed which is a shoulder at *ca.* 26000 cm^{-1} (385 nm). This weaker band is either an $n \rightarrow \pi^*$ transition or a charge transfer (ligand $\rightarrow$ orbital) band.[11,12]

The allowed band at about 23250 cm^{-1} (430 nm) is assigned to a charge transfer from the ligand to the metal. The absorption at 37000 cm^{-1} (270 nm) is more intense in methylene chloride solutions and a shoulder is present at about 23250 cm^{-1} (430 nm); however, the later, in nitrobenzene is a very sharp peak of greater intensity than the former.

In nitrobenzene solutions a broad band of low intensity is observed in the region 13000-15000 cm^{-1} (765-670 nm) and another broad band, that has no distinct maximum, appears in the region 18500 cm^{-1} (540 nm). These are assigned to d - d transitions corresponding to $^2B_{1g} \leftarrow {}^2B_{2g}$ and $^2A_{1g} \leftarrow {}^2B_{2g}$ respectively. The broad band occuring in the lowest frequencies indicates distortion of the symmetry from the octahedral configuration.[13] The high intensity of the second band is a consequence of appreciable covalency in the molybdenum-sulfur bonds.[14]

NMR Spectra

The NMR spectra were measured in chloroform and nitrobenzene solutions. The chemical shift values for the ligand protons of the alkyl or hetero ring of the complexes were found to shift substantially to lower frequencies from the

TABLE I: *Analytical and some physical data of the new complexes*

Compound	% Calcd						% Found						Mol. Weight		mp[b]	Colour
	C	H	N	S	Mo	Cl	C	H	N	S	Mo	Cl	Calcd.	Found	°C	
$Mo(detc)Cl^{a}_4$																green-blue
$Mo(pmtc)Cl_4$	18,10	2,51	3,52	16,11	24,11	35,63	18,20	3,06	4,09	16,20	24,20	34,7	398,00		98	green
$Mo(tmtc)Cl_4$	15,63	2,08	3,65	16,68	25,00	36,93	15,01	2,22	3,43	16,73	25,70	35,5	383,99	—	95	green
$Mo(mdtc)Cl^{c}_4$																
$Mo(detc)_2Cl_3$	24,07	4,01	5,61	25,71	19,24	21,36	23,82	3,98	5,20	26,50	19.00	19,65	498,80	520		pale-orange
$Mo(pmtc)_2Cl_3$	27,57	3,82	5,35	24,54	18,36	20,34	28,34	4,46	5,03	24,03	18,74	19,06	522,81	506	95	yellow
$Mo(tmtc)_2Cl_3$	24,28	3,23	5,66	25,92	19,40	21,50	25,08	3,32	5,32	25,08	19,70	20,02	494,75	511	126	orange
$Mo(mdtc)_2Cl_3^{c}$																
$Mo(detc)_3Cl_2$	29,45	4,90	6,87	31,42	15,69	11,60	28,96	4,84	6,55	29,41	15,01	11,80	611,60	593	75	brown
$Mo(pmtc)_3Cl_2$	33,38	4,63	6,49	29,71	14,81	10,95	33,39	5,00	6,06	24,60	14,52	10,31	647,6	608	75	brown
$Mo(tmtc)_3Cl_2$	29,75	3,96	6,94	31,75	15,84	11,71	28,77	3,85	6,49	30,56	15,82	11,50	608,57	578	133	red-brown
$Mo(mdtc)_3Cl_2^{c}$																
$Mo(detc)_4Cl$	33,17	5,52	7,74	35,38	13,26	4,90	33,30	5,52	8,00	34,38	12,40	5,00	724,39	—	—	violet

a = high hygroscopic

b = with decomposition in each case

c = see references

TABLE II: *Infrared Data for the Complexes*

Band Position cm^{-1}

Compound	v(C···N)	v(C···S)	Compound	v(C···N)	v(C···S)
$Mo(pmtc)Cl_4$	1545 vs	975 s	$Mo(tmtc)_2Cl_3$	1535 vs	950 vs
		1000 w	$Mo(mdtc)_2Cl_3$	1540 s	1025 vs
$Mo(tmtc)Cl_4$	1545 vs	970 m			945 s
		955 m	$Mo(detc)_3Cl_2$	1530 vs	950 s
$Mo(mdtc)Cl_4$	1550 s	975 s	$Mo(pmtc)_3Cl_2$	1525 s	1000 s
		1020 s			945 s
$Mo(detc)_2Cl_3$	1530 vs	950 vs	$Mo(tmtc)_3Cl_2$	1525 vs	995 m
$Mo(pmtc)_2Cl_3$	1525 s	1000 m	$Mo(mdtc)_3Cl_2$	1540 s	1040 s
		945 m	$Mo(detc)_4Cl$	1490 s	1000 s

corresponding proton signals of the free amines. The greater shift occurs for the methylene group which is bonded to the nitrogen atom.

The spectrum of the diethyl derivates consists of two signals. The region near $\delta = 1{,}40$ is complex and is probably the result of the overlap of two triplets. For the - CH_2 - group a multiplet signal is observed because of the overlap of two quartets. Generally a shift to higher values of δ is observed of both groups as a result of deshielding ($\Delta\delta = 1{,}22$ ppm for methylene and $\Delta\delta = 0{,}29$ ppm for methyl groups for diethyldithiocarbamate complexes. This behaviour is readily explained on the basis of restricted rotation about the C···N bond, which makes the two alkyl groups magnetically nonequivalent.[15]

The chemical shifts of the methylene protons of pentamethyleno and tetramethyleno dithiocarbamate complexes are similar to those for the diethyl complex but different from the morpholinoderivates.

TABLE III: *Electronic Spectral Data*
Wavelength, given in nm, for the principle absorption bands in the UV-visible region of solutions of the complexes in nitrobenzene and chloroform. The data in parentheses are log ε values.

	π^* π		π^* n or ch. tr.		ch. tr.		d - d
Compound	$CHCl_3$	$C_6H_5NO_2$	$CHCl_3$	$C_6H_5NO_2$	$CHCl_3$	$C_6H_5NO_2$	$C_6H_5NO_2$
$Mo(detc)Cl_4$[a]							
$Mo(pmdt)Cl_4$	235(4,36)	230(2,12)	370(3,36)	370(2,08)	430(—)	430(2,45)	740(2,29)
	265(4,40)	255(2,09)					
$Mo(tmtc)Cl_4$	230(4,34)	230(2,38)	350(3,48)			430(2,68)	735(2,75)
	250(4,42)	265(2,68)					
$Mo(mdtc)Cl_4$[b]		235(—)				430(—)	740(—)
		260(—)					
$Mo(detc)_4Cl$	230(4,32)	235(2,45)		390(3,21)	430(2,53)	430(2,97)	710(1,47)
	260(4,57)	260(2,45)					
$Mo(detc)_2Cl_3$	235(4,25)	240(2,67)	380(3,38)	390(2,02)	430(2,88)	430(2,90)	
	265(4,47)	265(2,77)					
$Mo(pmtc)_2Cl_3$	230(3,94)	235(2,70)	340(3,56)	345(2,11)		430(2,79)	530(1,62)
	270(4,38)	265(2,79)	390(3,17)				710(1,39)
$Mo(tmtc)_2Cl_3$	230(4,46)	245(2,50)	370(3,67)		445(3,84)	440(3,00)	
	265(4,59)	265(2,57)					
$Mo(mdtc)_2Cl_3$	240(4,29)	235(2,00)	380(3,30)	$370(2,15)_{sh}$		430(2,79)	535(1,55)
	265(4,40)	260(1,98)					760(0,70)
$Mo(detc)_3Cl_2$	248(4,46)	235(2,20)	360(3,51)	360(2,17)		430(2,95)	520(2,00)
	258(4,45)	257(2,19)					710(1,20)
$Mo(pmtc)_3Cl_2$	265(4,62)	235(2,79)	360(3,70)	$360(2,40)_{sh}$	440(3,16)	430(3,06)	540(2,04)
		265(2,87)					640(1,44)
$Mo(tmtc)_3Cl_2$	230(4,73)	230(2,43)	350(4,01)	310(2,65)		430(2,98)	660(1,50)
	261(4,79)	245(2,48)					
$Mo(mdtc)_3Cl_2$	246(4,06)	235(2,57)	380(3,12)			430(3,02)	535(2,45)
	265(4,17)						760(1,63)

a = high hygroscopic
b = little soluble

Περίληψις

Διθειοκαρβαμιδικὰ σύμπλοκα τοῦ πεντασθενοῦς μολυβδαινίου

Εἰς τὴν παροῦσαν ἐργασίαν μελετῶνται ὡρισμένα διθειοκαρβαμιδικὰ σύμπλοκα τοῦ πεντασθενοῦς μολυβδαινίου τὰ ὁποῖα προκύπτουν ἐκ τῆς ἀντιδράσεως τοῦ $MoCl_5$ μετά τοῦ διαιθυλοδιθειοκαρβαμιδικοῦ (detc), πυρολιδινοδιθειοκαρβαμιδικοῦ (pmtc) καὶ πιπεριδινοδιθειοκαρβαμιδικοῦ (pmtc) νατρίου. Τὰ μελετηθέντα σύμπλοκα ἦσαν δι- τρι- καὶ τετραχλωριωμένα σύμπλοκα τῶν γενικῶν τύπων $Mo(L)_3Cl_2$, $Mo(L)_2Cl_3$ καὶ $Mo(L)Cl_4$ ἀντιστοίχως, (ὅπου L διθειοκαρβαμιδικὴ ὁμάς). Περαιτέρω ἀντικατάστασις χλωρίων ὑπὸ διθειοκαρβαμιδικῶν ὁμάδων δὲν κατέστη γενικῶς ἐφικτή, πλὴν τοῦ σχηματισμοῦ τοῦ διαιθυλοκαρβαμιδικοῦ συμπλόκου τοῦ γενικοῦ τύπου $Mo(detc)_4Cl$.

Τὰ σύμπλοκα δὲν εἶναι ἠλεκτρολύται. Ὡς προκύπτει δὲ ἐκ τοῦ προσδιορισμοῦ τοῦ μοριακοῦ των βάρους, εἶναι ἐντὸς χλωροφορμίου μονομερῆ.

Ἡ ταυτοποίησις τῶν ἑνώσεων αὐτῶν ἐγένετο διὰ πλήρους στοιχειομετρικῆς ἀναλύσεως (πίναξ I) καὶ διὰ φασματοσκοπικῆς μελέτης. Συγκεκριμένως τὰ ὑπέρυθρα φάσματα αὐτῶν (πίναξ II) παρουσιάζουν τὰς χαρακτηριστικὰς ζώνας ἀπορροφήσεως εἰς τὰς περιοχὰς τῶν 1000 cm^{-1} καὶ 1500 cm^{-1} αἱ ὁποῖαι ἀποδίδονται εἰς τὰς δονήσεις C····S καὶ C····N ἀντιστοίχως.

Τὰ ἠλεκτρονικὰ φάσματα τῶν συμπλόκων (πίναξ III) παρουσιάζουν ἀπορροφήσεις εἰς τὰς περιοχὰς 37000-40000 cm^{-1} ($\pi - \pi^*$ διέγερσις), 23500 cm^{-1} (μεταφορὰ φορτίου) καὶ εἰς 13000-15000 cm^{-1} (d - d μετάπτωσις).

Τὰ φάσματα NMR γενικῶς δὲν διαφέρουν ἀπὸ ἀνάλογα διθειοκαρβαμιδικὰ σύμπλοκα ἄλλων στοιχείων.

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