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PRODUCTS OF DIHYDROFOLATE AFTER OXIDATION IN AIR IN THE ABSENCE OF LIGHT

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(Received March 16, 1978)

Summary

Attempts have been made to identify the products of oxidation of dihydrofolate in the air.

Para-aminobenzoyl glutamic acid probably is the main product of the air oxidation of dihydrofolate, accompanied by small amounts of folic acid p-aminobenzoic acid. There some other oxidation products, which have not been identified.

Key words: Dihydrofolic acid, dihydrofolate reductase, folic acid, p-aminobenzoic acid, p-aminobenzoyglutamic acid.

Introduction

The use of reduced folate in the cytochemical technique for demonstration of dihydrofolate reductase (DHFR)[1,2] introduces some problems which are related to various considerations such as instability of the substrate, lack of knowledge about possible inhibitory effect of the products of non-enzymic degradation and uncertainty as to whether any of these derivatives act as substrate for the reductase. Accordingly the nature of the products of aerobic degradation of dihydrofolate (FH_2) was investigated. Investigators using FH_2 have been aware of the instability of this compound and many attempts have been made to characterize the products of this degradation.

O'Dell et al[3] and Zakrzewski[4] found that folic acid (FA) had been produced by air oxidation of FH_2 in alkaline pH.

Hillcoat et al[5] reported that phosphate-dependent decomposition of FH_2 produced a yellow product (λ max at 277 and 420 nm) and they suggested by analogy with the spectra of known compounds that this was identical to the 7,8-dihydroderivative of 2-amino-4-hydroxy-6-formyl pteridine. This compound had also been identified by Whiteley *et al.*[6]

It was therefore, necessary to obtain information about the products of degradation of FH_2 under conditions similar to those occuring during the preparation and use of this compound in the current study.

Materials and Methods

FH_2 was prepared by a modification of the standard technique as reported by Tzortzatou and Hayhoe.[1]

Thin layer chromatography (TLC) paper cellulose polygram cell 300 was obtained from Machery-Nagel and Co., 516 Duren, Werkstrasse 6-8 Post Fach 307 Germany.

The solvents for TLC were:

Solvent A: Ammonium hydroxide (s.g. 0.880): ter-Butan-l-OL: water 10:10:80 by volume.

Solvent B: Ascorbic acid 50 mM pH 6 (with KOH): ethanol 85:15 by volume. Completed chromatograms were examined in uv light (transmitted and reflected) at 254 and 366 nm. In some occasions sheets were sprayed with Ehrlich's reagent (2% p-dimethylaminobenzaldehyde in 5% HCl) to detect the presence of free aromatic amines. Absorption spectra were recorded using an SP 1800 B Pye Unicam recording spectrophotometer, with buffer as the blank. The remaining materials were from commercial sources.

Results

In these experiments FH_2 (10-30mg) was left in the air in the absence of light as a dry powder for periods from a few h to 20 days at room temperature. Within few h the colour changed from white-pale to dark-brown. This "oxidised FH_2" was dissolved in some tris-HCl buffer pH 7.5 or in ammonium hydroxide pH 10.

Columns (3 cm × 35 cm) of Whatman DE 52 DEAE-cellulose were prepared with 0,5m HCl acid, 0,5 m NaOH and 1M phosphate buffer pH 7,5 and were routinely eluted using a continuous linear gradient of tris-phosphate buffer pH 7.5 increasing in concentration from 0.0 to 0.7M.

Spectroscopic relationship between FH_2 and its oxidised form

The U.V. absorption spectrum of the "oxidised form" (48 h. exposure) of FH_2 when dissolved in phosphate buffer pH 7.2 was very similar to the spectrum of non-oxidised FH_2 taken under nitrogen (Figs. 1 and 2).

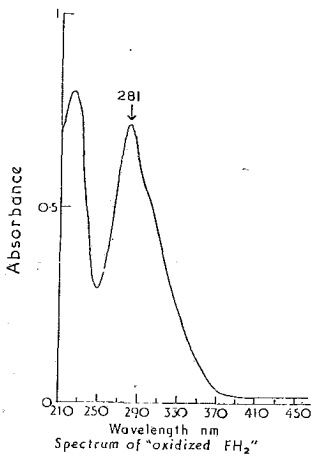

FIG. 1. *Absorption spectrum of "oxidised FH_2" in phosphate buffer pH 7.2.*

Hardly any difference can be discerned between the two spectra. The characteristic λ max. 282 for FH_2 has moved to 280/281 nm whilst the shoulder at 300 nm was preserved in the oxidised form. These spectra were repeated in 0.1N HCl and 0.1N NaOH, but again no useful distinction could be made. It is therefore impossible to assign a structure for the "oxidised FH_2" complex from these data. The "oxidised form" was found to be inactive in the cytochemical tests.

Stability of FH_2 as the cytochemical substrate

Since the U.V. absorption spectra did not provide a simple means of determining the stability of FH_2 a series of vials of FH_2 were exposed as described above for times up to 20 days. U.V. absorption spectra and cytochemical reactivity of each sample were determined.[1] As previously, there was little change in the absorption spectra. However, by three h exposure FH_2 has lost its cytochemical reactivity. This was not regained under conditions of prolonged exposure.

TLC analysis of "oxidised FH_2"

To understand whether a multiplicity of compounds contributed to the U.V. absoprtion spectra of "oxidised FH_2", samples were examined by TLC.

FA, FH_2 under N_2, and "oxidised FH_2" were examined, using solvent A and B. The results are shown in Table I.

The same colour spots with the same R_F as FH_2 were given by the "oxidised form of FH_2" in both solvents (Table I.). There was no evidence from these thin layer chromatograms that the oxidised form of FH_2 contained FA. The double spot of FH_2 which appeared in solvent A may be due to partial hydrolysis of the FH_2 by the solvent. Such hydrolysis would be likely to yield an amine, p-aminobenzoylglutamate and an unconjugated pteridine. The chromatograms (developed in both ascorbate and ammonium solvents) were treated by spraying for a few seconds with Ehrlich's reagent to detect aromatic amines as para-aminobenzoic acid (PABA) or para-aminobenzoylglutamic acid (PABG) and

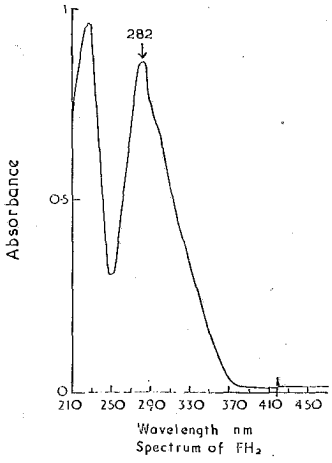

FIG. 2. Absorption spectrum of FH_2 in phosphate buffer at pH 7.2 under N_2

gave deep yellow spots immediately. After spraying the "oxidised FH_2" a large yellow spot which was not detectable previously appeared in both solvents close to the solvent front ($R_F = 0.96$).

FA and FH_2 (under N_2) gave in the same place, close to the solvent front, a very small and weak yellow spot after spraying with Ehrlich's reagent ($R_F = 0.96$). This indicates that under the circumstances of the TLC in either solvent A or B, both FA and FH_2 contained some free aromatic amines.

Pure PABA and PABG were examined in solvents A and B. A very weak fluorescent spot appeared at R_F 0.88 (PABA) and 0.96 (PABG) respectively for solvent A or R_F 0.85 (PABA) and R_F 0.92 (PABG) respectively for solvent B. At treatment with Ehrlich's reagent these spots assumed a deep yellow colour. These findings suggested that the "oxidised FH_2" was not degraded to FA as a terminal product but rather to PABA or PABG. Comparison of the R_F,s indicated that the component was probably PABG.

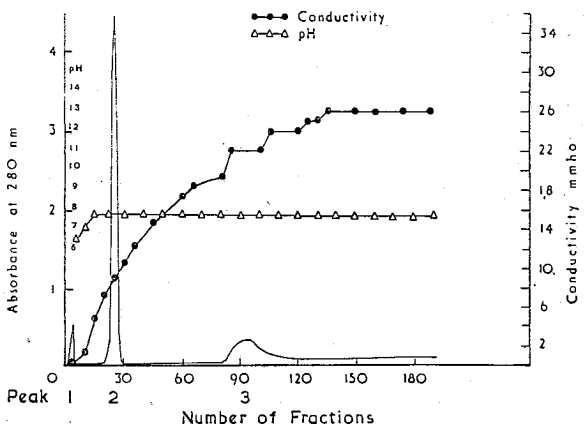

FIG. 3. *Elution profile of chromatography of "oxidised FH_2" dissolved at pH 7.5 on column of DE 52 cellulose.*

Analysis of "oxidised FH_2" by ion-exchange chromatography

By running "oxidised FH_2" through the ion-exchange column it was hoped to ascertain which other compounds of the decomposition of FH_2 were present. The tris-phosphate gradient eluted 3 main peaks, detected at 280 nm, when the "oxidised FH_2" was dissolved in tris-HCl buffer pH 7.5 and 4 main peaks when the "oxidised FH_2" was dissolved in ammonium hydroxide pH 10.

Profile of "oxidised FH_2" dissolved at pH 7.5

A typical profile is shown in Fig. 3 and consists of 3 peaks whose characteristics are summarized in Table II. Tentative identification of these peaks was made, though the identity of the first peak which eluted with water could not be established.

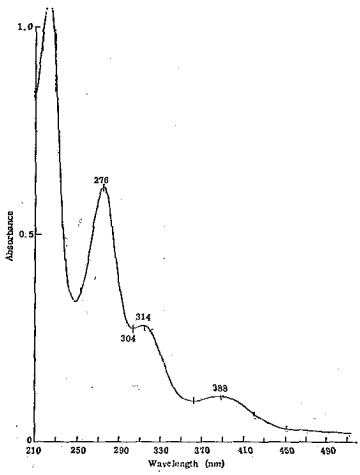

FIG. 4. Absorption spectrum of Peak 1. of "oxidised FH_2" dissolved at pH 7.5

Peak 1. The spectrum of material in this first peak (Fig. 4) has characteristics similar to those of pteridines, namely absorbance maxima at 276 and 388 nm. TLC (Table II) shows there to be at least two compounds present in this peak. This, together with the lack of retention by the exchanger, indicates that they could be pteridines without anionic charge at neutral pH. It is expected that 6-substituted pteridines would result from oxidative cleavage of FH_2 but this was not further investigated. The lack of charge implies that pteridine-6-carboxylic acid is not a degradation product.

Peak 2. This peak contains an aromatic amine, as it was strongly positive with Ehrlich's reagent and had a maximum of absorption at 272 nm (Fig. 5) showing spectrum similarities with PABG.

Peak 3. TLC resolved this peak into two compounds with R_F values 0.66 and 0.93 (solvent A) and 0.86 and 0.93 (solvent B) Table II. The U.V. absorption of peak 3 (containing both compounds) had absorption maxima at 284 nm and the ratio of the maxima was 3.80 (Fig. 6). The nature of the two components was not further investigated.

Profile of "oxidised FH_2" dissolved at pH 10

For these experiments FH_2 powder treated as described above was dissolved in ammonium hydroxide solution pH 10. The resulting solution was applied directly to the column without prior neutralization. In this way it was hoped that material retained by the column may be eluted by the pH-front passing through the column (Fig. 7). An additional peak appeared in the profile.

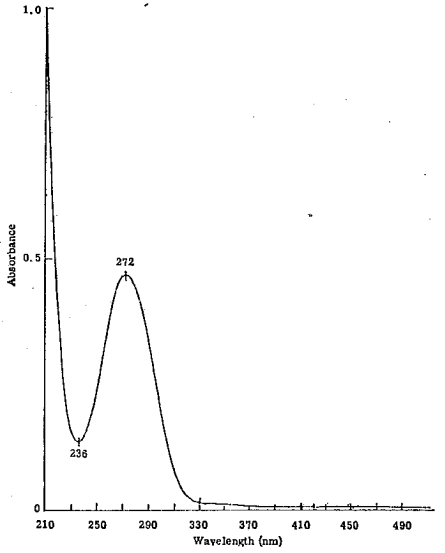

FIG. 5. *Absorption spectrum of Peak 2 of "oxidised FH_2" dissolved at pH 7.5.*

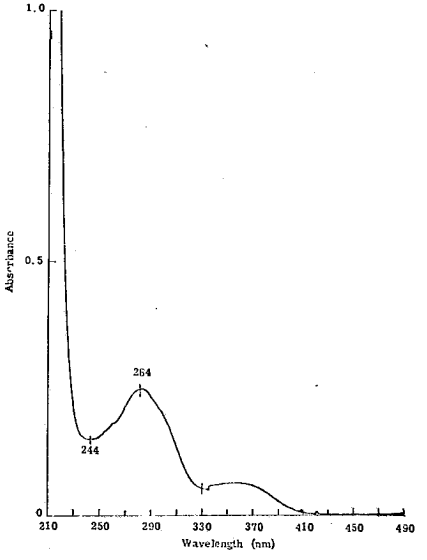

FIG. 6. *Absorption spectrum of Peak 3 of "oxidised FH_2" dissolved at pH 7.5.*

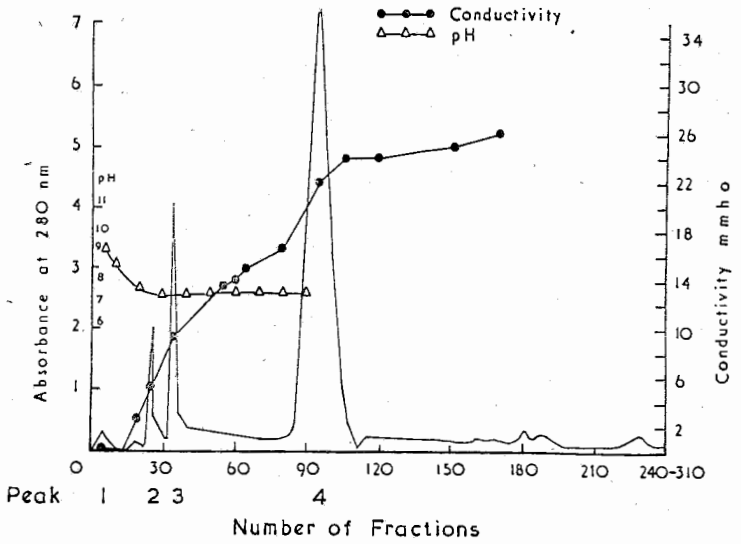

FIG. 7. *Elution profile of chromatography of "oxidised FH_2" dissolved at pH 10 on column of DE 52 cellulose.*

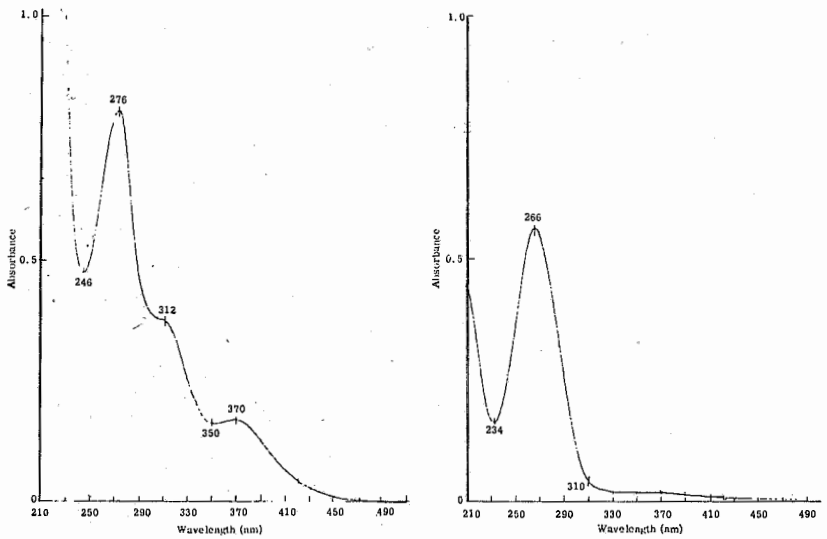

FIG. 8. *Absorption spectrum of Peak 2 of "oxidised FH_2" dissolved at pH 10.*

Peak 1. The absorption spectrum of peak 1 at pH 10 (not shown) was similar to that of peak 1 at pH 7.5 (Fig. 4). This identity was confirmed by TLC properties. Although the spectrum showed a general resemblance to that of pteridines, no identification could be made as the position of the absorption bands did not coincide with published data 7.

The fractions were negative to Schiff's reagent, indicating the absence of aldehyde groups, but the nature of the components was not further investigated.

Peak 2. This peak consisted of a poorly resolved complex. At the front end of the peak, fractions possessed absorption spectra similar in general form to those obtained from peak 1. (Fig. 8). Towards the end of the peak, the absorption spectrum of PABA became apparent (Fig. 8). The presence of PABA was confirmed by an Ehrlich's positive spot which cochromatographed with PABA.

Peak 3. The absorption spectrum of peak 3 at pH 10 (not shown) was similar to that of peak 2 at pH 7.5 (Fig. 5).

TABLE I. *Thin layer leyer chromatography of FA, FH_2, and "oxidised FH_2"*

Solvent	(Ammonium) A	B (Ascorbic acid)
	R_F	R_F
FA	0.85 (Abs^+)	0.51 (Abs^+)
FH_2	0.75 (PF^{*+x})	0.34 (BF^+)
"Oxidised FH_2"	0.75 ($P^{*+}F^x$)	0.34 (BF^+)

* yellow visible

\+ 254 nm UV; BF = blue fluorescence; PF = purple fluorescence

Abs = absorbing spot

x this spot contained two compounds: the front visible yellow and the tailing region colourless but blue fluorescence.

The R_F is taken from the centre of the visible yellow region.

TABLE II.: *Properties of compounds of "oxidised FH_2" resolved on DE 52 column chromatography.*

"Oxidised FH_2" dissolved at pH 7.5		Solvent A	Solvent B
Peak	Elution Molarity	R_F	R_F
1	water	0.72 (F) 0.68 (F)	0.37 (Y) 0.74 (F)
2	0.15 M	0.82 (F*)	0.82 (F*)
3	0.6 M	0.66 (F) 0.93	0.86 (F) 0.93
pH 10			
Peak			
1	water	0.72 (F) 0.68 (F)	0.37 (Y) 0.74 (F).
2	0.06 M	0.80 0.56 (F)	0.5 (F)
3	0.15 M	0.82 (F*)	0.82 (F*)
4	0.6 M	0.66 (F) 0.93	0.86 (F) 0.93

Y = yellow, F = fluorescent, F* = weak fluorescent

Peak 4. The compound in this peak had spectral characteristics similar to those of peak 3 at pH 7.5 TLC resolved this peak into two compounds with R_F values 0.66 and 0.93 (solvent A) and 0.86 and 0.93 (solvent B). Table 2. The U.V. absorption of peak 3 (containing both compounds) had absorption maxima at 284 nm and the ratio of the maxima was 3.80 (Fig. 6). The nature of the two compounds was not further investigated.

Discussion

Attempts were made to identify the products of oxidation of FH_2 by air in the absence of light. Several compounds were detected by ion exchange chromatography and TLC. However, the major product gave a yellow spot on TLC when sprayed with Ehrlich's reagent indicating a free amino group. The possibility was considered that this compound was either PABG, or PABA. The U.V. absorption spectra of this compound, with an absorption maximum at 272 nm, showed striking similarities to PABG ($PABG_{\lambda max}$ at 273 nm, $PABA_{\lambda max}$ at 266 nm). However there were discrepancies in the R_F values of the unknown compound, when concentrated from DEAE column, fractions, and authentic PABG on TLC, possibly due to the high salt load applied to the origin.

In relation to the use of FH_2 as cytochemical substrate, however, the study showed that since FA was probably not a major products of FH_2 oxidation, "oxidised FH_2" may also be inhibitory to DHFR. Kinetic studies, for example, of DHFR from L. Casei confirmed that PABG and PABA were competitive with FH_2. The DHFR activity in the presence of PABG 20 mM was less positive than the control without PABG. Hence in the cytochemical technique loss of FH_2 substrate activity may perhaps be ascribed to two mechanisms, although the precise details have yet to be elucidated:

1. Aerobic decay of FH_2 to complex mixtures of pteridine and PAB fragments.

2. Possible competitive inhibition of DHFR by either pteridine or PABG fragment.

In view of the complicated nature of the problem extreme precautions are necessary to prevent oxidation of FH_2 before or during its use.

Acknowledgments

The author wishes to thank Dr. N. Harding for advice and help throughout this work.

Περίληψις

Προϊόντα ὀξειδώσεως τοῦ διϋδροφολικοῦ ὀξέος μετὰ ἔκθεση εἰς τὸν ἀτμοσφαιρικὸν ἀέρα

Τὸ διϋδροφολικὸν ὀξὺ εἶναι ἕν ἀνοξείδωτον παράγωγον τοῦ φολικοῦ ὀξέος. Τοῦτο χρησιμοποιεῖται IN VITRO ὡς ὑπόστρωμα τοῦ ἐνζύμου διϋδροφολικῆς ρεδουκτάσης διὰ τὸν κυτταροχημικὸν προσδιορισμὸν τοῦ ἐνζύμου αὐτοῦ. Ἡ μέθοδος αὐτὴ ἀπέδειξεν τὴν ἀστάθειαν τοῦ διϋ δροφολικοῦ ὀξέος καὶ τὴν ταχεῖαν ὀξείδωσίν του. Μελέται ἐγένοντο διὰ τὴν ἀναγνώρισιν τῶν προϊόντων ὀξειδώσεώς του. Π-αμινο-βενζοϋλγλουταμινικὸν ὀξὺ πιθανὸν νὰ εἶναι τὸ κυριώτερον προϊὸν τῆς ὀξειδώσεως, συνοδευόμενον ἀπὸ μικρὸν ποσὸν φολικοῦ ὀξέος καὶ π-αμινοβενζοϊκοῦ ὀξέος. Ἐπιπροσθέτως ἀνευρέθησαν καὶ ἄλλα προϊόντα τὰ ὁποῖα εἶναι δύσκολον νὰ ἀναγνωρισθοῦν.

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COMPLEXES OF IRON (II), IRON (III), AND MANGANESE (II) WITH 2-BENZOYLPYRIDINE

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(Received April, 6, 1978)

Summary

The donor properties of the ligand 2-benzoyl-pyridine (L) towards iron (II), iron (III) and manganese (II) are further investigated and the complexes $FeBr_3L(H_2O)$, $FeCl_3L_2(H_2O)$, $FeCl_2L_2$ $Cl.6H_2O$, $FeCl_2L(H_2O)_2$, $FeCl_2L_2$ $.6H_2O$ and $MnCl_2L_2(H_2O)_2$ have been isolated and characterized by elemental analysis, conductivity and magnetic susceptibility measurements. Their UV, ir and mass spectra are also reported.

Key words: Iron (II), Iron (III), Manganese (II), 2-benzoylpyridine, Comlexes, Mass spectra.

Introduction

Molecules of the formula  -C-R, where R = H, CH_3,..., C_6H_5 etc., may act as monodentate or bidentate ligands by reacting either through the pyridin nitrogen or both pyridin nitrogen and carbonyl oxygen towards metal ions[1-5]. In a previous communication[5] we reported on complexes of the first row transition elements with 2-benzoyl pyridine and found that the binding sites are influenced by relatively minor factors such as the solvent and adduct formation between the complex and HCl or HBr. In this paper we report the preperation and characterization of several new complexes of Fe (II), Fe(III) and Mn (II) with the same ligand, together with the mass spectra of the complexes.

Results and Discussion

The analytical and physical data are summarized in Table I. The complexes $[FeLCl_3(H_2O)]$ and $[FeL_2Br_3(H_2O)]$ already reported[5] are also included for comparison.

The analytical data show that compositions agreed with calculated ratios. The magnetic moments indicate that all the complexes are of the high spin type having the maximum number of unpaired electrons (See Table I). The conductances in nitromethane show that only the complex $[FeL_2Cl_2]$ Cl is 1:1 electrolyte, while all others are non-electrolytes in this solvent. When the preparation is performed at elevated temperatures the non-chelate 1:2 complexes, $[FeL_2X_3(H_2O)]$ (where X is Cl or Br) are obtained. On the other hand, by carrying out the preparation at room temperature the chelate 1:1 complexes, $[FeLX_3(H_2O)]$ are obtained.

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

Compound		C%	H%	N%	M%	Melting point	Conductivity $ohm^{-1}.cm^2M$ 20°C in CH_3NO_2	$\mu_{eff.}$	Loss of Drying at 120°C
$FeLBr_3$ (H_2O)	Calc.	28.91	2.20	2.81	—	235°C D	15	5.80	
	Found	28.34	2.34	2.16					
$FeL_2Cl_3(H_2O)$	Calc.	52.60	3.65	5.11		113°C D	50*	5.70	
	Found	51.80	3.96	4.97					
$(FeL_2Cl_2)_2Cl.6H_2O$	Calc.	45.17	4.70	4.39	8.70	190°C D	66	5.77	
	Found	45.21	4.39	4.32	8.79				
$FeLCl_2(H_2O)_2$	Calc.	41.49	3.74	4.03	16.14	115°C D	32	5.10	Ø
	Found	41.60	3.52	4.66	15.78				Ø
$FeL_2Cl_2.6H_2O$	Calc.	47.84	4.98	4.65	9.31	148°C D	36	5.30	16.25
	Found	47.17	4.66	4.75	9.25				16.05
$MnL_2Cl_2(H_2O)_2$	Calc.	54.44	4.16	5.29	14.20	242°C D	Ø	5.30	Ø
	Found	55.14	4.77	5.10	14.38				Ø

where L= 2 -benzoylopyridine, D = Decomposition

* The conductivity of this compound was rapidly increasing, attaining a value of —200 where precipitation is taking place. The rather high imital value is probably due to the decomposition occured in the time between dissolution and measurement.

TABLE II.: *ir spectral assignments of the complexes*

Ligand	$FeLCl_3.H_2O$	$FeLBr_3H_2O$	$FeL_2Br_3H_2O$	$FeL_2Cl_3H_2O$	Assignments
—	3340w	340w	340w	—	O-H stretching
3050w	3050s	3040s	3050	3060s	C-H stretching
—	2500w	—	—	—	
1660s	1620s	1620s	1675m	1660m	C-O stretching
—	—	—	1610m	1620m	H_2O, O-H bending
157om	1570s	1588s	1580s	1595s	C=C, C=N stretch-
1560w	—	1565m	1560m	1556s	ing of the aro- matic rings
1320m	1340s	1340s	1340s	1330s	C-H in plane
—	—	—	—	—	deformation
—	—	—	—	—	
995s	1020s	1028s	1030s	1015m	Pyridine breathing motion
820s	850m	—	850s	842m	C-H out of
780s	825w	—	—	820m	plane vibration
—	785m	765m	—	—	
—	382s	—	263	370m	M-X stretching
—	362s	—	248	355m	
			228		

(FeL_2Cl_2) $Cl.6H_2O$	$FeLCl_2(H_2O)_2$	$(FeL_2Cl_2).6H_2O$	$MnL_2Cl_2(H_2O)_2$	Assignments
3400w	3400w	3400w	3400m	O-H stretching
3050w	3050w	3050w	3060m	C-H stretching
—	—	—	—	
1620s	1630s	1610s	1670s	C-O stretching
—	1610s	—	1630s	H_2O, O-H bending
1586s	1580s	1582s	1600m	C=C, C=N stretch-
1564s	—	—	—	ing of the aro- matic rings
1335s	1350s	1370s	1360s	C-H in plane
—	—	—	1372s	deformation
—	—	—	1395s	
1015s	1010w	1010m	1020m	Pyridine breathing motion
880m	855m	823w	830m	C-H out of
820m	828m	815w	—	plane vibration
—	—	—	—	
359s	385m		255s	M-X stretching
312s	360m		245s	

Generally the bromo-complexes are prepared easier and in better yields than the corresponding chloro analogs.

Structures are deduced mainly from the ir spectra. Tentative assignments are given in Table II.

The complex $[FeLBr_3(H_2O)]$ shows a strong band at 1620 cm^{-1} indicating coordinated carbonyl.[5,8,9,10] The same is also true for $[FeL_2Cl_2]$ $Cl.6H_2O$, $[FeLCL_2(H_2O)_2]$ and $[FeL_2Cl_2].6H_2O$, which show bands at 1620, 1630 and 1610 cm^{-1} respectively. On the other hand, the complexes $[FeL_2Cl_3(H_2O)]$ and $[MnL_2Cl_2(H_2O)_2]$ show bands at 1660 and 1670 cm^{-1} respectively, indicating that the carbonyl group is not a ligation site.[5,8,10] Coordination through pyridin nitrogen is indicated in all complexes by a positive shift of the pyridine breathing motion appearing at 995 cm^{-1} in the free and at 1010-1030 cm^{-1} in the complexed ligand[5].

Coordinated water is indicated by a band at ca. 1610-1620 cm^{-1} in the complexes,[5,8,9,10] while the bands at 1500-1600 cm^{-1} are attributed to the ring C=C and C=N motions[5]. The bands at ca. 1200 cm^{-1} are attributed to in plane C-H and the bands at ca. 750 cm^{-1} to out of plane C-H bending motions[6]. The M-X stretchings are assigned in the region 300-400 cm^{-1} for the iron complexes and at ca. 250 cm^{-1} for the manganese (II) complex. The presence of two bands in these regions for complexes $[FeL_2Cl_2]$ $Cl.6H_2O$, $[FeLCl_2(H_2O)_2]$ and $[MnL_2Cl_2(H_2O)_2]$ is indicative of cis configuration[5] (See Table II). Metal-nitrogen coordination[14,15] is also indicated by the UV spectra.[5,11,12,13] the 2636 A absorption of the free ligand in DMF solutions, attributed to the $\pi \rightarrow \pi^*$ transitions of the rings[5], is shifted to ca. 2655 Å in all complexes.

The mass spectrum of the complex $[FeL_2Cl_2]$ $Cl.6H_2O$ is shown in Fig. 1 and from this the possible fraction scheme is deduced and presented in Scheme I.

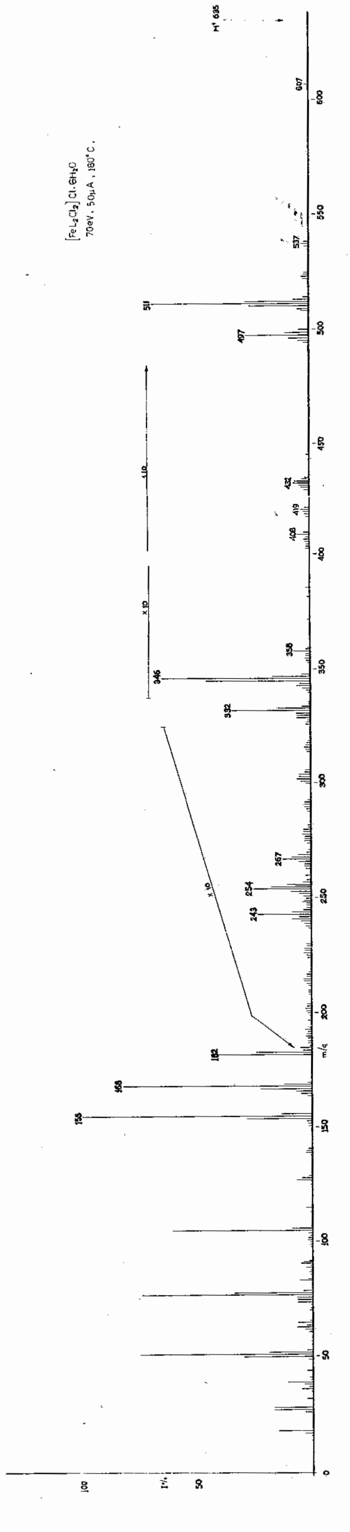
The fraction schemes of the complexes $[FeL_2Cl_2(H_2O)_2]Cl$, $[FeLBr_3(H_2O)]$ and $[FeL_2Cl_3(H_2O)]$ are also given in Schemes II, III and IV.

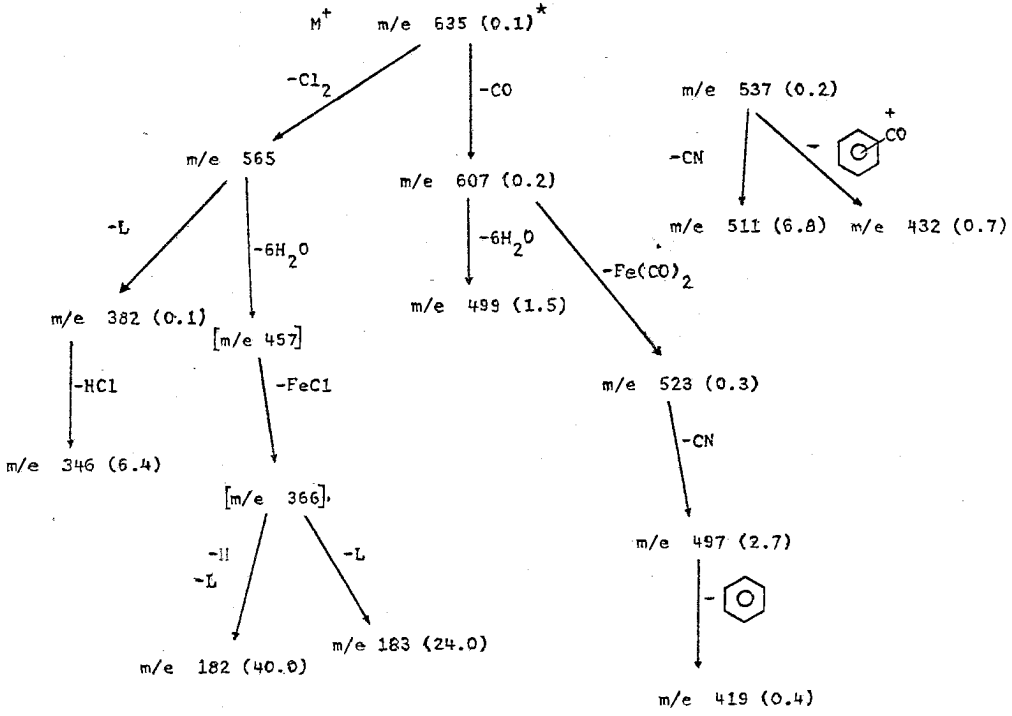
The general characteristic of the spectra is their low intensity due to very low vapor pressure of the respective complexes. The molecular ions have the lowest intensity and in some cases they did not appear at all. The usefull conclusion which is drawn from these spectra is that the respective complexes must be monomeric and this is in agreement with the proposed structures. The monomeric character is deduced from the fact that in none of the cases the observed peaks exceeded the mass number of the respective monomeric molecular ion.

Experimental

Materials: 2-benzoylpyridine was purchased from Fluka A.G. and used without further purification. All other chemicals were reagent grade.

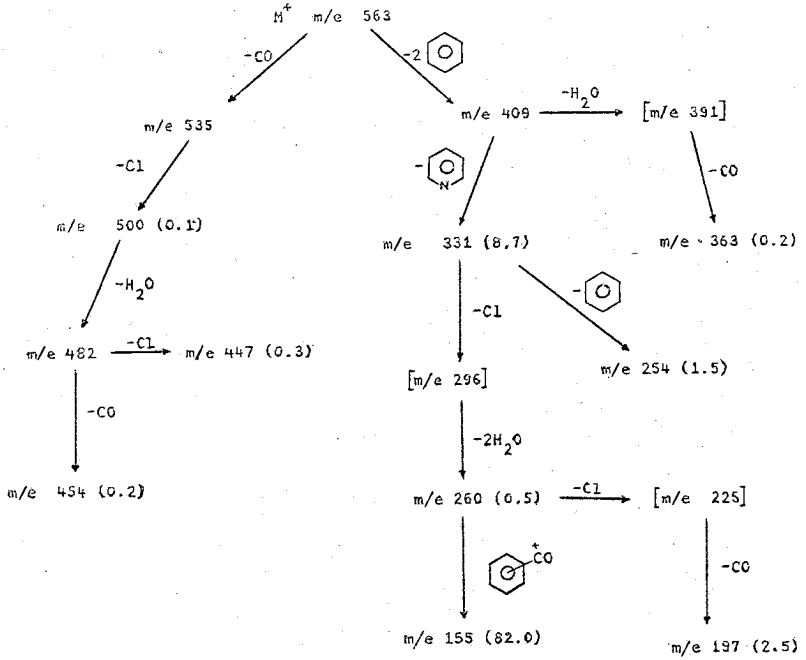
Methods: (a) The ir spectra were recorded in a Beckman 2050 model spectrophotometer in KBr pellets. The positions of the bands are given within ± 2 cm^{-1}. (b) The UV-Vis spectra were taken with a Cary model 17D spectrophotometer. (c) Conductivity measurements were performed using an E 365 B conductoscope, Metrohm Ltd., Herisau, Switzerland. (d) The melting points were determined on a W. Büchi melting point apparatus and are uncorrected. (e) The magnetic moments were determined by the Gouy method with diamagnetic corrections. (f) The mass spectra were recorded on a RMU-6M Hitachi-Perkin Elmer Mass Spectrometer with source operating at 50 mA, electron energy 70 eV and at temperatures 180-200°C.


[FeL2Cl2]Cl.6H2O
70eV, 50μA, 180°C.
M+ 635
607
537
511
497
432
419
408
358
346
332
267
254
243
182
168
155
x 10
m/e
I%

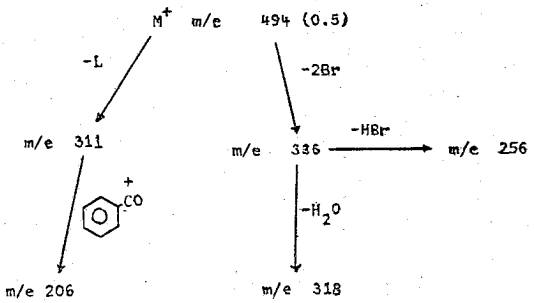


* The numbers in parentheses represent the % intensities of the different ions, as compared to the more intense ion.

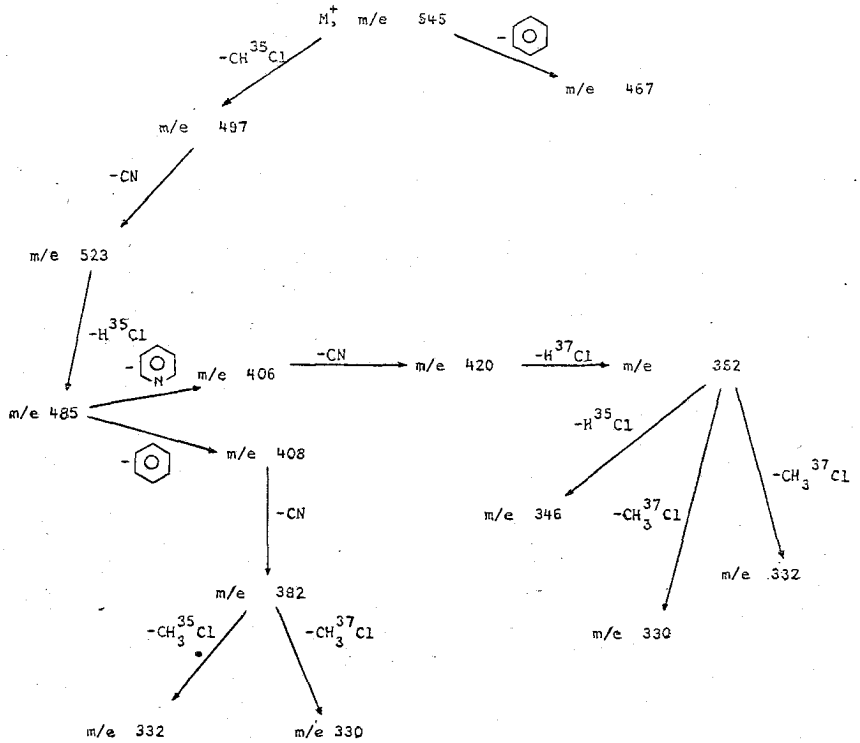
Scheme I. Decomposition of the complex $[FeL_2Cl_2]Cl.6H_2O$



Scheme II. Decomposition of the complex $[FeL_2Cl_2(H_2O)_2]Cl$



Scheme III. Decomposition of the complex $[FeBr_3L(H_2O)]$



Scheme IV. Decomposition of the complex $[FeL_2(H_2O)Cl_3]$

Microanalyses: C,H, N microanalyses were performed in the laboratories of the Hellenic National Research Foundation by Dr. Mantzos. The metal analyses were done according to literature methods[6] and the halogens were determined gravimetrically.

Preparation of the complexes

Aquo-tribromo (2-benzoylpyridine) iron (III).

$FeBr_3.6H_2O$ (1.5 mmole) were dissolved into 10 ml absolute ethanol and to the obtained solution was added dropwise 2-benzoylpyridine (1 mmole) dissolved into 5 ml absolute ethanol. The brown precipitate formed was filtered washed with ethanol and ether and dried at 50°C under vacuum. Yield 80%.

Aquo-trichloro-bis-(2-benzoylpyridine)Iron (III).

$FeCl_3.6H_2O$ (1 mmole) were dissolved into 10 ml absolute ethanol, and to that was added 2-benzoylpyridine (4mmole) dissolved into 20 ml absolute ethanol. The mixture was then heated on a steam bath for 1h. The green precipitate formed was filtered washed with ethanol and ether and dried at 50°C under vacuum. Yield 80%.

Dichloro-bis-(2-benzoylpyridine)iron (III) chloride hexahydrate.

$FeCl_3.6H_2O$ (1mmole) was dissolved into 10 ml absolute ethanol and to that was added 2-benzoylpyridine (7mmole) dissolved into 20 ml absolute ethanol. The mixture was refluxed for 2h and the yellow precipitate formed, filtered, washed with ethanol and dried at 50°C under vacuum. Yield 85%.

Bis-aquo-dichloro-(2-benzoylpyridine) iron (II).

$FeCl_2.4H_2O$ (1 mmole)was dissolved into 10 ml absolute ethanol and to that was added 2-benzoylpyridine (1 mmole)dissolved into 10 ml absolute ethanol. The mixture was stirred for 2h and roto-evaporated at 45°C. The blue residue was taken with a mixture hexane/ethanol, 3/1, filtered, washed with the same solvent and dried at 50°C under vacuum. Yield 90%.

Dichloro-bis-(2-benzoylpyridine) iron (II) hexahydrate.

$FeCl_2.4H_2O$ (1 mmole) was dissolved into 10 ml of a mixture of hexane/ethanol, 3/1, and filtered. The filtrate was added to 2-benzoylpyridine (4 mmole) dissolved into 10 ml of the same solvent. The deep blue precipitate formed was filtered, washed with the same solvent and dried at 40°C under vacuum. Yield 75%.

Bis-squo-dichloro-bis-(2-benzoylpyridine)manganese (II).

$MnCl_2.4H_2O$ (1 mmole) was dissolved into 10 ml absoulute ethanol and to the obtained solution was slowly added 2-benzoylpyridine (3 mmole) dissolved into 10 ml absolute ethanol. The mixture was refluxed for 3 h and the orange precipitate formed was filtered, washed with ethanol and ether and dried at 50°C under vacum. Yield 80%.

Περίληψη

Σύμπλοκες ἑνώσεις σιδήρου (II) καὶ (III) καὶ μαγγανίου (II) μὲ 2-βενζοϋλοπυριδίνη.

Ἐμελετήθησαν οἱ ἀντιδράσεις τῆς 2-βενζοϋλοπυριδίνης (L) μετὰ σιδήρου (II), σιδήρου (III) καὶ μαγγανίου (II) καὶ ἀπεμονώθησαν τὰ σύμπλοκα $FeBr_3L(H_2O)$, $FeCl_3L_2(H_2O)$, $[FeCl_2L_2]$ Cl. $6H_2O$, $FeCl_2L(H_2O)_2$, $FeCl_2L_2.6H_2O$ καὶ $MnCl_2L_2(H_2O)_2$. Τὰ σύμπλοκα αὐτὰ ἐχαρακτηρίσθησαν διὰ στοιχειακῆς ἀναλύσεως, ἀγωγιμομετρικῶν καὶ μαγνητικῶν μετρήσεων, καθὼς καὶ διὰ φασμάτων UV, ir καὶ μάζης.

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SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF NEW N-SUBSTITUTED DERIVATIVES OF 5,6 - DIHYDRODIBENZ [b, f] AZOCINE.

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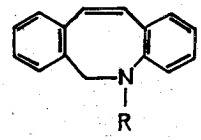
Summary

A new series of N-substituted derivatives of 5,6-Dihydrodibenz(b,f)azocine were synthesized. These derivatives were prepared by the reaction of 5H-dibenzo[a,d]cyclohepten-5-one (1) with hydroxylamine hydrochloride gave the corresponding oxime (2). Treatment of this oxime with polyphosphoric acid gave the dibenz[b,f]azocine-6(5H)-one (3) which by reduction with $LiALH_4$ yielded 5,6-dihydrodibenz[b,f]azocine (4). Reaction of 5,6-dihydrodibenz[b,f]azocine with phosgene leads to the corresponding chloride (5) which by treatment with diethanolamine gives the diole analog (6). To prepare the compounds of the general formula (I) the diole analog was treated with isocyanate esters or dimethyl carbamoylchloride.
Hence the present study deals with the synthesis of the N-Substituded derivatives of 5,6-dihydrodibenz[b,f]azocine and an initial investigation of their pharmacological properties.
Key words: N-Substituted Derivatives of 5,6-Dihydrodibenz[b,f]azocine.

Introduction

The pharmacological properties of the dibenzazocine derivatives of the following general formula have been described (6, 7, 8, 9, 10, 11, 12, 13, 14) in previous publications

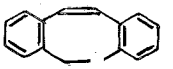


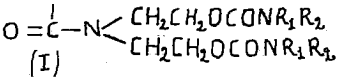
where:
R = alkylamino-acyl group

This seems to be a structural unit common to numerous pharmacologically active agents. (anticholinergic, psychotrope, analgesic) particularly those agents that affect the central nervous system.

Appropriate modification of group R can confer either stimulating or central inhibitory of the resulting molecule. This structural modification results in

N-substituted derivatives of 5,6-Dihydrodibenz[b,f]azocines of the following general formula (I).





(I)

where:
R_1 = H, CH_3
R_2 = CH_3, C_2H_5, nC_3H_7, nC_4H_9, C_6H_5

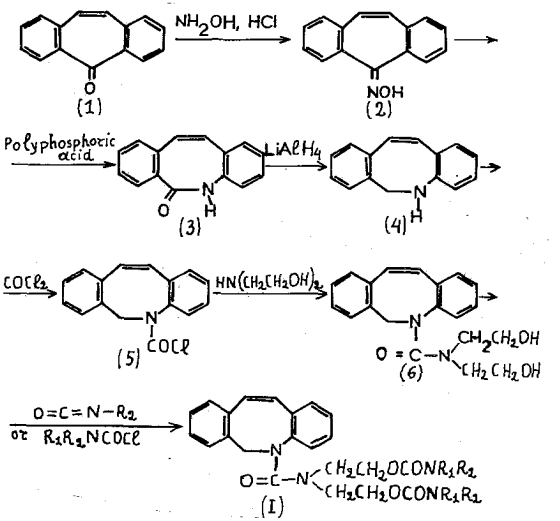
These compounds were therefore synthesized by the reaction of 5H-dibenzo[a,d]cyclohepten-5-onè (1) with hydroxylamine hydrochloride which gave the corresponding oxime[1] (2).

Treatment of (2) with polyphosphoric acid gave the dibenz[b,f]azocine-6(5H)-one[2] (3) and this by reduction with $LialH_4$ gave the 5,6-dihydrodibenz[b,f]azocine[3] (4).

Treatment of (4) with phosgene gave the chloride[4] (5) and reaction of (5) with diethanolamine provided the corresponding diole[5] (6).

To prepare the compounds of general formula (I) the reaction products (6) were treated with isocyanate esters or with dimethyl carbamoylchloride.[6]

The procedure followed was:



All the compounds prepared were studied for their pharmacological actions.

Experimental Section

Preparation of N′, N′-bis (2-hydroxyethyl)N-carbonylamide of 5,6-dihydro-dibenz[b,f]azocine (6). 3,38 g (0,1 mol) of the chloride (5) were diluted with 50 ml of chloroform and while stirred diethanolamine 2,05 ml (0,2mol) was added. The mixture was refluxed for 30 min, cooled and washed with water until neutral reaction. The solution was dried with anhydrous Na_2SO_4, the solvent was evaporated and the resulting residue was crystallized from acetone, to give 2,8 g of (6), (m.p 108°C, 68% yield).

Preparation of bicarbamate ester of N′, N′-bis (2-hydroxyethylamide) of 5,6 dihydro-dibenz[b,f]azocine (I, T3997).

0,7 g (0,02 mol) of the above diole (6) were diluted with 50 ml of anhydrous benzene and in this solution 0,5 ml ethyl isocyanate (=30% in excess) were added.

The mixture was left for 4 days at room temperature and the solvent was evaporated in vacuo. The resulting crude oily solid (0,98 g) was crystallized from ether to give 0,88 g of (I, T3997), m.p. 130°C; yield 88%. Recrystallization from acetone gave m.p. 137°C.

All the carbamate compounds of this series were prepared in the same way with the exception of (I, T4056) which was prepared by the addition of dimethylcatbamoylchloride instead of alkyl isocyanate.

Analytical data and physical constants of the prepared compounds are sited in Table I.

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

R_1	R_2	Molecular formula	N° Ref	Yield %	M.p°C	Analyses					
						Calculated %			Found %		
						C	H	N	C	H	N
H	CH_3	$C_{24}H_{28}N_4O_5$	T4100	34	133-4$^{(\alpha)}$	63,70	6,24	12,38	63,61	6,15	12,38
H	C_2H_5	$C_{26}H_{32}N_4O_5$	T3997	75	137$^{(\beta)}$	64,98	6,71	11,66	65,52	6,75	11,24
H	nC_3H_7	$C_{28}H_{36}N_4O_5$	T4099	88	104-5$^{(\beta)}$	66,12	7,13	11,02	66,44	7,29	10,84
H	nC_4H_9	$C_{30}H_{40}N_4O_5$	T4052	97	45$^{(\alpha)}$	67,14	7,51	10,44	67,37	7,21	10,60
H	C_6H_5	$C_{34}H_{32}N_4O_5$	T4053	96	176$^{(\alpha)}$	70,82	5,59	9,72	70,60	6,16	9,76
CH_3	CH_3	$C_{26}H_{32}N_4O_5$	T4056	42	114$^{(\beta)}$	64,98	6,71	11,66	65,33	6,72	11,80

α. Recrystallization from ethanol
β. Recrystallization from acetone

Pharmacology. Materials and methods

Compounds were tested as suspensions in 2% gum acacia solution and administered intraperitoneally. Initial general CNS screening was made according to previous described methods.[6]

Adult male Winstral albino rats of 180-220 g body weight and Swiss male albino mice of 20-25 g body weight were employed in the experiments. Purina laboratory chow and tap water were available adlibitum. The 24 hour preliminary toxicity and the results of the CNS screening are given in Table II.

From the first blank screeining a reduction of motor activity, of exploration and passivity as well as ataxia was observed. All the other observations for stereotypy, catalypsy, grooming, fearfulness, righting reflex, body tone, urination skin color and respiration rate were normal.

Acute Toxicity

Albino male mice weighing 20-25 g were administered the compounds i.p. Six mice were used per dose level and a minimum of 5 dose levels was employed for each toxicity determination. The dosed animals were observed 24 hours for mortality and LD_{50} was calculated according to the method of Miller and Tainter.[7].

Spontaneous Motor Activity (SMA)

Spontaneous motor activity was tested in an activity cage (Ugo Basile, Suisse) so adjusted that when a mouse breaks the beam of light, the cell activates a digital counter.[8]

Male albino mice were used for this test (a single subject per each experiment). There were three experimental subjects and 2 controls at each testing session, and each compound-dose group was compared with its appropriate control.

A minimum of three dose levels was employed for each compound. The activity counts were readed every five minutes for a half an hour.

Hypothermia

Male albino mice weighing between 20 and 25 g were used throughout. The animals were given access to food and water before and during the evaluation. Prior to the administration of any drug, the rectal remperature of each mouse was determined using a telethermometer with rectal probe. The rectal temperature was measured every half an hour for 5 hours, the same, every day for one week.[9] One week later, the same group of mice was given a dose of the synthesized compounds i.p and the temperature determined in the same way.

Antimetrazol-Anticonvulsant Activity

This technique was carried out according to the method of Bames et al[10] Adult albino mice are given the test compounds 30 minutes before the convulsant. The animals are allowed food and water up to time of injection. Groups of eight mice at a minimum of three dose levels were used and the ED_{50} was calculated as the dose which would prevent convulsions in 50% of the mice treated with 85 mg/kg of pentylenetetrazol by the i.p route.

Rotating Rod test

This test was used to measure the effects of compounds on muscle tone and/or muscular coordination particularly of muscle relaxants, sedatives and stimulants in mice. Five male albino mice, weighing 20-25 g were used dose level. The ED_{50} was the dose that caused 50% of the mice to fall of the rotating rod.[11]

Conditioned Avoidance

Subjects: Male Albino rats weighing 180-220 gr were employed.

Apparatus and procedure

Rats were placed in a plexi-glass two Way skinner type box(Ugo Basile, Suisse) with a grid floor through which electrical foot schock (0.6mA at 45V) could be delivered.

Optical sign (10″) prior to electrical schock (10″), interval time (50″). Each escape of the animal from one way to the other postponed the onest of the duration of electrical schock and the avoidance procedure was recorded on a counter (time in 1/12 sec). The duration of each avoidance session was one hour. In the control state a trained animal avoided more than 90% of the possible schocks. Each animal was given i.p the test compound immediatelly after the training and at hourly intervals thereafter for 3 hours as well as the next day, the response of each animal is determined by three succesive trials. The inhibition of the conditioned response is an indication of tranquillization.[13]

Inhibition of exploration

This test is done according to the methal of Nieschulz et al[14] The ED_{50} is the dose at which 50% of the animals slide down on an inclined plane. Male albino mice weighing 20-25 g used in this test.

Results

The CNS pharmacological profile has been studied of the six synthesized dibenzoazocines. As shown from table I the high LD_{50} indicates a relatively low toxicity for all the compounds tested. In mice some of the compounds (table I) inhibit the exploratory behavior and with higher doses they produce a hypothermia (35°C meanvalue). From the rotating rod test all of the compounds exert an effect on muscular coordination as it was also seen from the first blind screening. They also reduce the spontaneous motor activity byt they have no effect in the conditioned avoidance test. The lack of this effect and also the lack of antimetrazol test indicates the absence of major tranquilizer properties.

The very interesting finding of potentiation of pentylementrazol action and the high toxicity shown (synergy) as well as the inhibition of muscle coordination (table II) could indicate an indirect cholinergic mechanism. Such studies will be reported on next communications.

Table II: *Pharmacological profile of the synthesized derivatives*

		Test compounds					
Species	Test	T_{4099}	T_{4052}	T_{4100}	T_{3997}	T_{4053}	T_{4056}
Mouse	Acute Toxicity LD_{50} mg/kg	>500	>500	>500	>500	>500	>500
Mouse	SMA ED_{50} mg/kg	100	50	100	100	—	—
Rat	Inhibition of Cond Avoidance ED_{50} mg/kg	—	—	—	—	—	—
Mouse	Pentylenetetrazole antagonism ED_{50} mg/kg	Potentiation	Potentiation	Potentiation	Potentiation	—	—
Mouse	Hypothermia ED mg/kg	—	150	100	—	—	—
Mouse	Inhibition of exploration ED_{50} mg/kg	50	25	50	100	100	100
Mouse	Rotating Rod ED_{50} mg/kg	50	50	50	50	100	100

Περίληψις

Σύνθεσις καὶ φαρμακολογικὴ μελέτη νέων Ν-ὑποκατεστημένων παραγώγων τῆς 5,6-Διϋδροδιβενζο [b,f] αζοκίνης.

Εἰς τὴν παρούσαν ἐργασίαν παρασκευάζονται Ν-ὑποκατεστημένα παράγωγα τῆς 5,6-διϋδροδιβενζο[b,f]αζοκίνης τοῦ γενικοῦ τύπου (I).

Ἡ παρασκευὴ των ἐγένετο ἐκ τῆς 5Η-διβενζο[a,d]κυκλοεπτεν-5-ονης (1) ἡ ὁποία τῇ ἐπιδράσει ὑδροχλωρικῆς ὑδροξυλαμίνης μετατρέπεται εἰς τὴν ἀντίστοιχον ὀξίμην. Ἐκ ταύτης τῇ βοηθείᾳ πολυφωσφορικοῦ ὀξέος λαμβάνεται ἡ

διβενζο[b,f]αζοκιν-6(5H)-om (3) ἡ ὁποία δι' ἀναγωγῆς μὲ ὑδρίδιον τοῦ λιθίου-ἀργιλλίου μετατρέπεται εἰς 5,6-διϋδροδιβενζο[b,f]αζοκίνην (4). Ἐν συνεχείᾳ τῇ ἐπιδράσει φωσγενίου λαμβάνεται τὸ ἀντίστοιχον χλωρίδιον (5) καὶ ἐκ τούτου μὲ διαιθανολαμίνην ἡ ἀντίστοιχος διόλη (6). Ἐκ τῆς τελευταίας ταύτης τῇ ἐπιδράσει ἰσοκυανικῶν ἐστέρων ἢ διμεθυλοκαρβαμοϋλοχλωριδίου προκύπτουν τὰ προϊόντα τοῦ τύπου (I).

Τῶν παρασκευασθέντων προϊόντων ἐγένετο μία προκαταρκτικὴ μελέτη τῶν φαρμακολογικῶν των ἰδιοτήτων.

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COMPOSITIONAL ANALYSIS OF POLYESTER-POLYETHER RANDOM BLOCK COPOLYMERS BY N.M.R. SPECTROSCOPY

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Summary

Nuclear magnetic resonance spectroscopy has been used to evaluate the composition of a series of random block copolyesters derived from mixtures of dimethyl terephthalate, butane diol and poly (tetrahydrofuram). The method used provides a rapid assessment of composition and is based on the differences in the resonance position of the different types of hydrogen atoms present in these polymeric systems. Three methods of evaluating the composition from the n.m.r. data are described and critically examined.

Key words: Random block copolyesters, copolymer composition, nuclear magnetic resonance, chemical shift.

Abbreviations used: 4GT = Poly(tetramethylene terephthalate), PTHF = Poly(tetrahydrofuran), PTHFT = The tetrahydrofuran terephthalate repeating unit in the copolymers, DMT = Dimethyl terephthalate, 4GT/PTHF (MWt.) (X%) = A random block copolymer containing tetramethylene terephthalate and tetrahydrofuran terephthalate repeating units, derived from PTHF of the specified molecular weight (MWt.) and at X% incorporation by weight. Thus 4GT/PTHF 1000 (10%) refers to a block copolymer of 4GT and PTHFT derived from PTHF of molecular weight 1000 and having 10% by weight PTHFT units.

Introduction

Block copolymers containing tetramethylene terephthalate and tetrahydrofuran terephthalate repeating units have been prepared by transesterification. Butane diol and hydroxyl terminated PTHF of different molecular weights in admixture with DMT have been subjected to standard melt-phase polycondensation procedures.[1] This study forms part of a programme of work in the Department on structure-property relationships in copolyester systems.[2-7] The effect of both molecular weight and the percentage incorporation of PTHF (0 - 30%) on copolymer properties and dyeing behaviour has been investigated.[8,9]

Fifteen copolymers have been prepared and analysed using n.m.r. spectroscopy to evaluate copolymer composition. This proved necessary since the theoretical composition calculated from the weight of reactants using the Shivers equation[10] may not be the same as that obtained experimentally.

Such a situation may arise due to less of the more volatile components, namely, butane diol and DMT during polymerization or due to the degradation of a reactant. In the case of PTHF it has been reported that degradation can occur at the temperatures used in melt polycondensation.[1] Since structure-property relationships were under investigation a rapid and accurate assessment of the

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magnitude of these effects and hence the copolymer composition was required. The n.m.r. method of analysis has been previously used a variety of systems[12-16] and has been used by Still et al[7] for random copolymers containing tetramethylene terephthalate and tetramethylene sebacate repeating units. We now report studies on random block copolyester systems.

Experimental

N.m.r. spectra were obtained at room temperature from 20% solution of the copolymers in trifluoroacetic acid (B.D.H. spectroscopic grade) using a Varian HA-100 MHz instrument. The relative intensities of the resonance peaks were determined using an electronic integration device and for each spectrum the integral traces were recorded and used to calculate the mean value for a given set of protons.

Results and Discussion

The copolymers prepared consist of PTHFT units randomly distributed along 4GT chains as a result of their mode of preparation, namely, random transesterification. The repeating units of 4GT, PTHF and a typical 4GT/PTHF copolymer are shown in Figure 1 where different proton types are also designated. Such systems yield significantly different n.m.r. spectra as shown schematically in Figure 2. Thus 4GT yields a spectrum with three singlet absorptions at chemical shifts $\delta = 8.68$ aromatic protoms "a", $\delta = 5.10$ methylenic protons "b" adjacent to the electron withdrawing carboxyl grouping and at $\delta = 2.62$ methylenic protons "c". PTHF yields a spectrum with two singlet absorptions at $\delta = 4.32$ methylenic protons "d" adjacent to the ether oxygen atom and at $\delta = 2.26$ methylenic protons "e".

The copolymer spectrum is more complicated than either of the homopolymers and shows the combined features of both homopolymer spectra, Figure 2. The chemical shift data and the assignments made are shown in Table I and Figure 1.

TABLE *Chemical Shift Data and Assignments for the Copolymers*

Proton type		δ
aromatic	a	8.68
$-\overset{O}{\overset{\|}{C}}-O-CH_2-$	b+b*	5.10
$-O-CH_2-$	d	4.30
$-CH_2-CH_2-$	c	2.62
$-CH_2-CH_2-$	e	2.29
$-\overset{O}{\overset{\|}{C}}-O-CH_2-CH_2-$	c*	2.62

It can be seen by reference to Figures 1 and 2 and Table I that the absorptions at $\delta = 5.10$ and $\delta = 2.62$ in the copolymer spectrum are composite and arise from methylenic protons in both the PTHFT and the 4GT repeating units. Thus in any analysis utilizing these absorptions, due allowance must be made for the contribution of the different proton types.

Since the area under the absorption peak in n.m.r. spectrum is directly proportional to the number of hydrogen atoms producing the signal, this provides a suitable method for compositional analysis of these systems. In the previous study[7] the ratio of (total aromatic protons): (total aliphatic protons) was utilized for compositional analysis and this may be employed in this case leading to equation (1).

$$\frac{\text{Total aromatic protons}}{\text{Total aliphatic protons}} = \frac{1}{2(1 + nx - x)} \tag{1}$$

where x is the number of moles of PTHFT present in the copolymer and n is the degree of polymerization of the PTHF homopolymer used in preparation of the copolymer.

The value of x and hence the weight percentage incorporation of PTHFT may also be evaluated from the ratios of the methylenic protons adjacent to the carboxyl grouping in 4GT and PTHFT (b + b*) and the methylenic protons adjacent to the ether oxygen atom in the tatrahydrofuran unit "d". This leads to equation (2)

$$\frac{(b + b^*)}{d} = \frac{1}{x(n - 1)} \tag{2}$$

Alternatively the ratio of total aromatic protons to suitable methylenic protons in the tetrahydrofuran unit also be used as typified by protons of type "d" leading to equation (3)

$$\frac{a}{d} = \frac{1}{x(n - 1)} \tag{3}$$

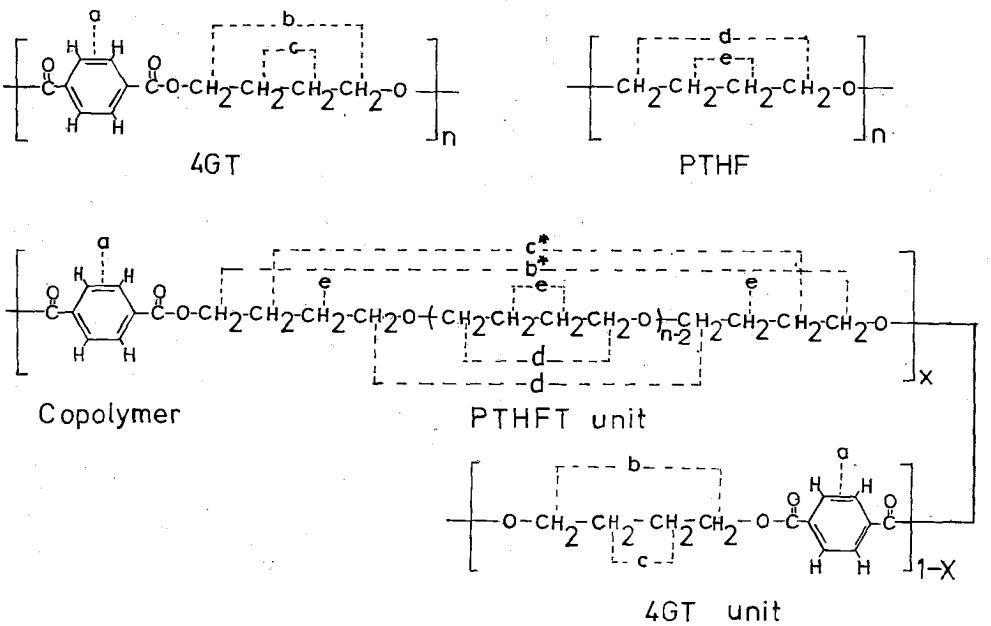

FIG. 1: *Repeating structures of 4GT, PTHF and a typical random block copolymer.*

The compositional data evaluated using the three methods are shown in Table II.

TABLE II. *Compositional Analysis of the Copolymers*

Copolymer	Weight Percentage PTHFT Units (a)	(b)	(c)
4GT/PTHF 1000(10%)	10.02	10.11	9.94
4GT/PTHF 1000(20%)	21.14	—	—
4GT/PTHF 1000(30%)	30.54	—	—
4GT/PTHF 2000(10%)	9.91	9.97	9.85
4GT/PTHF 2000(20%)	19.56	19.05	19.56
4GT/PTHF 2000(30%)	30.26	30.01	30.30
4GT/PTHF 3000(10%)	10.33	10.23	10.39
4GT/PTHF 3000(20%)	18.65	—	—
4GT/PTHF 3000(30%)	29.40	0	—
4GT/PTHF 4000(10%)	10.19	9,92	10.07
4GT/PTHF 4000(20%)	19.76	—	—
4GT/PTHF 4000(30%)	29.33	—	—
4GT/PTHF 5000(10%)	10.28	10.25	10.16
4GT/PTHF 5000(20%)	19.25	—	—
4GT/PTHF 5000(30%)	30.89	—	—

(a) calculated from equation (2)
(b) calculated from equation (1)
(c) calculated from equation (3)

The data obtained depends upon the accurate measurement of the integral step heights of the peaks. The data shown in Table II are self consistent and indicate that no significant changes in composition occur during polymerization. This indicates that PTHF does not degrade under the melt condensation conditions employed as was also found by Ghaffar *et al*[17] for the hexamethylene terephalate: PTHFT random block copolymer system.

The n.m.r. method used is more accurate when the ratio $\frac{b+b^*}{d}$ is employed. This arises because, as the PTHFT content and the molecular weight of the PTHF used is inereased, the integral step heights associated with the aromatic protons become progressively smaller leading to more significant errors in their measurement.

The method employed in this study gives a rapid estimation of copolymer composition in contrast to more laborious hydrolytic and degradative procedures.[18-21] The method has however the limitation that the degree of polymerization of the PTHF unit used in the preparation must be measured[22] in order that the composition may be calculated.

Περίληψις

Χρῆσις n.m.r. φασμάτων εἰς τὴν ἐξεύρεσιν τῆς συστάσεως στατιστικῶν κατὰ συστάδας συμπολυμερῶν πολυεστέρος-πολυαιθέρος.

Φάσματα Πυρηνικοῦ Μαγνητικοῦ Συντονισμοῦ (n.m.r.) ἐχρησιμοποιήθησαν διὰ τὴν ἐξεύρεσιν τῆς συστάσεως σειρᾶς συμπολυμερῶν ποὺ παράγονται

ἀπὸ μίγμα διμεθυλοτερεφθαλικοῦ ἐστέρος, 1,4-βουτανοδιόλης καὶ πολυτετραϋδροφουρανίου. Ἡ παροῦσα μέθοδος παρέχει τὴν δυνατότητα συντόμου προσδιορισμοῦ τῆς συστάσεως τῶν συμπολυμερῶν καὶ βασίζεται εἰς τὰς διαφορετικὰς θέσεις συντονισμοῦ τῶν διαφόρων τύπων ἀτόμων ὑδρογόνου ποὺ εὑρίσκονται εἰς τὰ ὑπὸ μελέτην συστήματα. Περιγράφονται καὶ ἐξετάζονται λεπτομερῶς τρεῖς μέθοδοι προσδιορισμοῦ τῆς συστάσεως τῶν συμπολυμερῶν ποὺ στηρίζονται εἰς τὰ δεδομένα τῶν φασμάτων n.m.r.

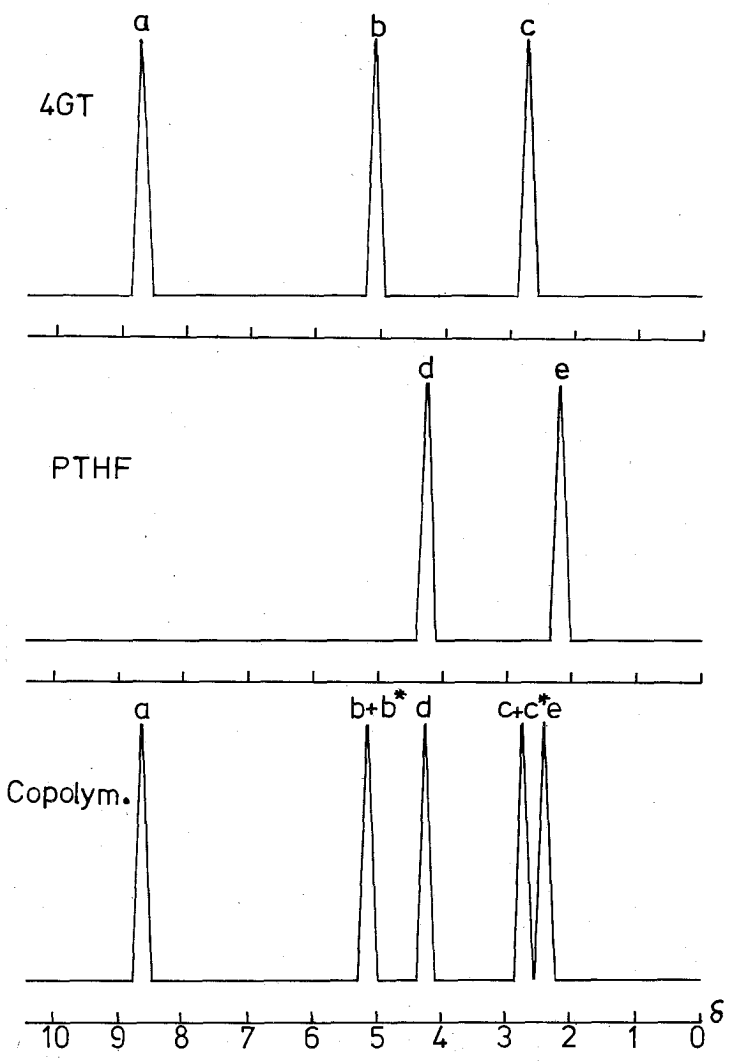

FIG. 2: *Schematic representation of 100 MHz n.m.r. spectra of 4GT, PTHF and a typical random block copolymer.*

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THE ANISOTROPY OF THE MAGNETIC SUSCEPTIBILITY OF BENZENE, 1,3,5-TRIFLUOROBENZENE AND HEXA FLUOROBENZENE

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Summary

The magnetic susceptibility of C_6H_6, 1,3,5-$C_6H_3F_3$ and C_6F_6 is obtained by using SCF perturbation theory. We propose a phenomenological explanation for the decrease of the magnetic susceptibility anisotropy, $\Delta\chi$, which results from the substitution of hydrogen by fluorine. It has also been found that the σ electrons make a significant contribution to $\Delta\chi$.

KEY WORDS: Magnetic anisotropy — SCF perturbation theory, benzene, 1,3,5-trifluorobenzene, hexafluorobenzene

Introduction

The magnetic anisotropy, $\Delta\chi$, or the anisotropy of the magnetic susceptibility, χ, has long been of interest.[1] A lot of people have attributed the large $\Delta\chi$ of benzene to its 6 π electrons.[2] However, this assumption has been questioned by a number of workers.[15,22] Our calculations have led us to reappraise the magnetic anisotropy problem since they suggest that the diamagnetic part of the magnetic anisotropy of the compounds examined can be explained in terms of the anisotropic contributions of the σ electrons.

We have also examined the reduction in $\Delta\chi$ with the substitution of hydrogen for fluorine in C_6H_6. A quantitative and accurate analysis would require computing time not readily available at present. However, by using our calculations and the experimental results from the literature[1] we propose an interpreation of the reduction in $\Delta\chi$.

Results and discussion

McWeeny's SCF perturbation theory[4] was applied to the calculation of the components of the susceptibility, $\chi_{\alpha\beta}$, which are given by

$$\chi_{\alpha\beta} = \mathrm{Tr}\ [f_{\alpha\beta}^{(2)}\ R^{(0)} + \tfrac{1}{2} f_{\alpha}^{(1)}\ R_{\beta}^{(1)}],$$

where α and β denote x, y or z directions of the applied magnetic field, $R^{(0)}$ is the zero order (non-perturbed) density matrix, $R_{\beta}^{(1)}$ is the first-order density matrix

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perturbed by a magnetic field along the β direction, $f_\alpha^{(1)}$ is the first-order framework Hamiltonian perturbed by a magnetic field along the α direction and $f_{\alpha\beta}^{(2)}$ is the second-order framework Hamiltonian perturbed by two perpendicular m agnetic fields along α and β directions. Detailed accounts concerning the application of the theory have been given elsewhere.[5,6]

The magnetic anisotropy, $\Delta\chi$, is given by

$$\Delta\chi = \chi_{zz} - \tfrac{1}{2}(\chi_{xx} + \chi_{yy}),$$

where χ_{xx}, χ_{yy} and χ_{zz} are the principal components of the susceptibility. Further we have

$$\chi_{\alpha\beta} = \chi_{\alpha\beta}^{d} + \chi_{\alpha\beta}^{p}$$

and

$$\Delta\chi = \Delta\chi^{d} + \Delta\chi^{p}$$

where d and p denote the diamagnetic and paramagnetic contributions, respectively.

For the calculation of $\chi_{\alpha\beta}$ we have used a minimal basis set, in which each STO (Slater type orbital) was simulated by 3 GTOs (Gaussian type orbitals). The exponents and the coefficients for this expansion were taken from Stewart[7] and the Slater exponents from Hehre *et al.*[8] The geometries of C_6H_6, 1,3,5-$C_6H_3F_3$ and C_6F_6 were taken from the literature.[9] The origin of the coordinate system was taken at the centre of mass of the molecule. The ATMOL program[10] as implemented on the Cambridge IBM 370/165 Computer was used for the calculation of the SCF integrals, eigenvalues and eigenvectors of the zero order Hamiltonian; together with a suite of programs developed at Cambridge[6] for computing molecular properties using the methods of SCF perturbation theory.

The decrease of the magnetic anisotropy

A reliable treatment of the magnetic anisotropy problem within our model would probably require a basis three times the minimal one.[3,11] However, for compounds of the size of C_6H_6 it is very difficult to perform ab-initio calculations with an extended basis since the computing time involved is enormous. Therefore, various methods have been used to circumvent this difficulty; e.g. some workers concentrated on the contribution of the π electrons, using empirical values for the σ contributions.[3,12,13] However, it is known that χ^d (and σ^d, the diamagnetic contribution to the magnetic shielding constant) can be calculated adequately with a minimal basis.[3,14,15] This can be seen if the results for C_6H_6 presented in this work (Table 1) are compared with Shoemaker's *et al.*[16] results (they extrapolated their results for C_6H_5F to C_6H_6) and with the extended basis calculations reported by Almlöf *et al.*[17]

In the present study of the anisotropy we use the calculated $\Delta\chi^d$, and since χ^p is almost certainly in error, a second approach to the evaluation of $\Delta\chi^p$ was adopted.[3] According to this method one estimates $\Delta\chi^p$ by subtracting the calculated $\Delta\chi^d$ from the experimental $\Delta\chi$. This method, of course, is not intended to replace either the calculation or the measurement of $\Delta\chi^p$; but in the absence of reliable values for both of them, offers a useful estimate of $\Delta\chi^p$ even though it concentrates all deficiencies and errors in the $\Delta\chi^p$ values. It is known that χ^d accounts for the simple diamagnetic circulation, induced by the applied magnetic field, and χ^p for the hindrance to this motion, e.g. by the massive nuclei.[18] In aromatic compounds both phenomena contribute anisotropically to χ^d and χ^p.

TABLE I. *Principal components of the susceptibility in units of* $4\pi \times 10^{-12} m^3\ mol^{-1}$[(a)]

Compound	χ_{xx}^d	χ_{zz}^d	χ_{xx}^p	χ_{zz}^p
C_6H_6	-285.4	-509.0	51.0	109.8
	-285.9[3]	-509.2[3]		
	-286±10[16]	-508±20[16]		
	-291.3[17]	-510.8[17]		
1,3,5-$C_6H_3F_3$	-682.8	-1288.0	52.5	115.8
C_6F_6	-1080.3	-2067.5	54.4	119.1

(a) The SI unit $4\pi \times 10^{-6} m^3\ mol^{-1}$ corresponds to the non SI $cm^3\ mol^{-1}$.

TABLE II. *Magnetic anisotropies in units of* $4\pi \times 10^{-12} m^3\ mol^{-1}$.

Compound	Exp. values (1)	Calculated values		
	$\Delta\chi$	$\Delta\chi^d$	$\Delta\chi^{p(a)}$	$\Delta\chi^{p(b)}$
C_6H_6	-53.4	-223.6	58.8	170.2
1,3,5-$C_6H_3F_3$	-39.0	-605.2	63.3	566.2
C_6F_6	-31.8	-987.2	64.7	955.4

(a) These values have been calculated.
(b) These values have been determined by: $\Delta\chi^p = \Delta\chi - \Delta\chi^d$. $\Delta\chi$ is the experimental value.

Interpreting the results of Table 2, we suggest that the substitution of H by F in C_6H_6 leads to increases in the contributions arising from both the diamagnetic circulation and its hindrance. But the rate of increase of the latter is faster than that of the former. So fluorination of C_6H_6 leads to a decrease of $\Delta\chi$.

We observe that the change in the diamagnetic anisotropy on going from C_6H_6 to $C_6H_3F_3$ (Table 2) is almost the same as that on going from $C_6H_3F_3$ to C_6F_6 ($= 382 \times 4\pi \times 10^{-12} m^3 mol^{-1}$). However, this does not mean that all fluorines contribute equally to $\Delta\chi$, since Hüttner et al[19] have found that the diamagnetic anisotropy changes from C_6H_6 to C_6H_5F by $107.3 \times 4\pi \times 10^{-12} m^3 mol^{-1}$ whereas if the contributions of all fluorines were the same this change would be $127.2 \times 4\pi \times 10^{-12} m^3 mol^{-1}$.

Resolution of the magnetic anisotropy

The π electrons of aromatic compounds have featured largely in the study of their characteristic properties. Elvidge and Jackman[20] define an aromatic compound as a compound which will sustain an induced ring current of π electrons. However, Lewis and Peters[21] maintain, that it is far from certain that it is the π electrons rather than the σ electrons which confer the aromaticity.

The present calculations show that the bulk of the anisotropy of the diamagnetic part comes from the σ electrons in all three examined compounds (Table 3). Further, the anisotropic contribution of the σ electrons disproves a popular assumption of the past[22] according to which the σ electrons contribute isotropically to the susceptibility. The calcualtions of the paramagnetic anisotropy, $\Delta\chi^p$, are much less reliable, but here also the σ electrons appear to contribute the main part of $\Delta\chi^p$ (Table 3). The present findings, therefore, appear to confirm the scepticism of Lewis and Peters.

TABLE III. *σ and π contributions to χ^d and χ^p in units of $4\pi \times 10^{-12} m^3 mol^{-1}$.*

Compound	χ_{xx}^d		χ_{zz}^d		$\Delta\chi^d$		χ_{xx}^p		χ_{zz}^p		$\Delta\chi^p$	
	σ	π	σ	π	σ	π	σ	π	σ	π	σ	π
C_6H_6	-246.2[3]	-39.2[3]	-453.8[3]	-55.2[3]	-207.6	-16.0	25.5	25.5	109.8	0.0	84.3	-25.5
	-246.2[3]	/39.7[3]	/453.8[3]	/55.4[3]								
1,3,5-$C_6H_3F_3$	-547.2	-133.6	-1050.5	-237.5	-503.3	-103.9	26.2	26.3	114.5	1.3	88.3	-25.0
C_6F_6	-847.8	-232.5	-1646.4	-421.1	-798.6	-188.6	27.2	27.2	117.7	1.4	90.5	-25.8

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Περίληψις

Ἡ Ἀνισοτροπία τῆς μαγνητικῆς ἐπιδεκτικότητας τῶν C_6H_6, 1,3,5-$C_6H_3F_3$ καὶ C_6F_6

Ἡ μαγνητικὴ ἐπιδεκτικότητα τῶν C_6H_6, 1,3,5-$C_6H_3F_3$ καὶ C_6F_6 ὑπολογίσθηκε χρησιμοποιῶντας τὴν SCF Θεωρία τῶν διαταραχῶν τοῦ Mc Weeny. Ἑρμηνεύτηκε ἡ ἐλάττωση τῆς μαγνητικῆς ἀνισοτροπίας ἡ ὁποία προκύπτει ἀπὸ τὴν ὑποκατάσταση τοῦ ὑδρογόνου μὲ φθόριο. Ἀπὸ τοὺς ὑπολογισμούς μας προκύπτει ὅτι ἡ συνεισφορὰ τῶν σ ἠλεκτρονίων στὴν μαγνητικὴ ἀνισοτροπία εἶναι ἰδιαίτερα σημαντική.

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Short Papers

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TRANS-UNSATURATION AND FATTY ACID COMPOSITION OF COOKING FATS AND MARGARINES IN GREECE.

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Summary

Samples of cooking fats and margarines manufactured in Greece were analyzed for fatty acid composition and *trans*-unsaturation. Marked differences were observed between summer and winter samples. The content of isolated *trans*-unsaturated fatty acids was quite variable for products with the same unsaturation. Fats prepared from olive oil were very poor in linoleic acid and had a small linoleic acid to *trans*-isomers ratio.

Key words: Greek cooking fats and margarines, fatty acids, *trans*-unsaturation.

Introduction

Sources of dietary fat have been changing in Greece in the last four decades. There has been a considerable shift from butter and animal fat to vegetable margarines and cooking fats. Recommendations by the medical profession to avoid cholesterol and saturated fatty acids gave this shift a significant impetus.

Although the relationship between dietary lipids and coronary heart disease are not clear and generalizations on the basis of unsaturation have often been criticized,[1,2,3] the concept that everyone is in a serious danger if he does not restrict the amount of saturated fat in the diet is often stated with positive assurance. This has often lead to unbalanced diets, due to misconceptions and serious misunderstanding.[1,3]

Acylglycerols are isomerized during hydrogenation both in positional and geometric configuration of their double bonds. Studies on the biological utilization of these modified fatty acids have been reviewed.[4-8] Kummerow presented a paper showing unfavourable data on pigs fed *trans* fatty acids in high proportions.[6]

It is undoubtedly difficult to make diet recommendations in the face of a problem as complicated as atherosclerosis. This task becomes far more difficult when the composition of dietary fats available to consumers is unknown. It is therefore imperative that more information on the composition of food fats becomes available. The present paper has been prompted by the lack of data on the fatty acid composition and *trans* isomers content of Greek cooking fats and margarines.

Experimental Procedures

Sampling

Samples of 250 g blocks of table margarines and 200 g plastic containers of cooking fats were purchased at regular intervals from three super-markets in

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Thessaloniki. Three samples of the same brand were obtained simultaneously from the three different super-markets every three months (winter, spring, summer and autumn). Thus, for seven brands used in this study, a total of 84 samples were collected and analyzed over a 1-year period (1976-1977). According to their label declaration the fats were prepared from hydrogenated olive oil, cotton seed oil and blends of different vegetable oils.

Cooking fats were transesterified immediately. Margarine samples were melted and the fat was filtered and dried with sodium sulphate. All the samples were stored in the refrigerator at -20°C until required for analysis.

Preparation of methylesters.

Methylesters for gas-liquid chromatography and infrared spectroscopy were prepared from the fats using A.O.C.S. method Ce 2-66.[9]

Determination of trans-unsaturation.

Isolated *trans*-unsaturated fatty acids were determined by IR spectroscopy of methylesters according to A.O.C.S. Method Cd 14-61. Secondary standards containing a known proportion of methyl elaidate were obtained from the Spectroscopy Committee of the American Oil Chemist' Society.

Gas-Liquid Chromatography.

Methylesters of fatty acids were separated isothermally at 180°C with a Hewlett-Packard, model 7620A, gas chromatograph equipped with a T.C. detector. The chromatograph was fitted with a 8'x1/8" column packed with ChromWAW, 80-100 mesh and coated with 20%polythyleneglycol succinate.

Results and Discussion.

The composition of cooking fats and margarines is given in Table 1. The table presents minimum, maximum and mean values obtained from the analysis of 12 samples for each brand.

As shown in the table, products of olive oil have a very small percentage of linoleic acid (approximately 5%). This ought to be expected since untreated olive oil contains very little linoleic acid (less than 10%). Values of oleic and palmitic acids differed very little from those of unhydrogenated olive oil, which suggests that only a small decrease of unsaturation results from hydrogenation. This also explains the presence of relatively high percentages of *trans*isomers. Apparently, with such a raw material, a non isosuppressive hydrogenation is necessary to obtain the required melting point of the base stock.[10]

Carola,[11] on the basis of existing knowledge, proposed the properties of an ideal balanced oil. This oil should contain at least 5% linoleic acid. As shown in the Table commercial samples of hydrogenated olive oil can hardly fulfill this requirement. It is also quite probable that part of the linoleic acid is in the form of nutritionally inferior isomers, which makes the actual amount of essential fatty acids smaller.[11,12]

Cooking fats and margarines prepared from vegetable oils have various proportions of linoleic acid and *trans* isomers. Differences were observed between summer and winter samples and between apparently similar products. Certain fats contained lauric and myristic acid. This suggests the addition of palm kernel or coconut oil.

Table I.: *Fatty Acid Composition of Cooking Fats and Margarines consumed in Greece. Results are given as mean values of 12 samples analyzed. The respective ranges (minimum / maximum) are also given in brackets.*

(Composition from labels)	Fatty acid (%)									
Cooking Fats	12:0		14:0		16:0		16:1		18:0	
	M.V	min. max.	M.V.	min. max.	M.V.	min. max.	M.V.	min. max.	M.V.	min. max.
1 Hydrogenated olive oil and liquid olive oil 95 %, butter fat 5%.	0.3	[0.2- 0.4]	0.5	[0.3-0.6]	12.8	[11.8-13.7]	1.0	[0.7-1.6]	7.7	[6.8-9.0]
2 Liquid and hydrogenated cotton seed oil 95%, butter fat 5%.	0.3	[0.1- 0.7]	1.2	[0.6-1.6]	22.3	[15.0-26.8]	0.7	[0.5-1.2]	4.4	[3.7-5.0]
3 Hydrogenated vegetable oils, liquid vegerable oils and peanut oil 95%, butter fat 5%.	1.7	[0.9- 4.3]	1.5	[1.2-2.0]	21.0	[18.2-24.2]	0.8	[0.6-0.9]	6.0	[4.4-7.5]
4 Hydrogenated vegetable oils and vegetable fats 95%, butter fat 5%.	2.1	[0.5-14.6]	1.4	[1.0-4.6]	18.2	[14.4-20.1)	0.8	[0.3-0.9]	5.5	[4.2-7.5]
Table margarines										
1 Olive oil	0.1	[- 0.2]	0.1	[- 0.2]	12.5	[11.8-14.1]	0.9	[0.7-1.6]	6.9	[4.4-8.0]
2 Hydrogenated and liquid cotton seed oil.	—	[- -]	0.7	[0.2-1.0]	19.6	[13.0-25.9]	0.8	[0.4-1.2]	5.2	[3.7-6.0]
3 Vegetable oils and peanut oil.	11.3	[10.1-12.7]	3.3	[2.6-3.6]	14.6	[12.7-16.3]	0.7	[0.4-0.8]	5.4	[4.5-7.5]

*							% isolated *trans* unsaturation by I.R. analysis.	
Cooking Fats (Composition brom labels)	18:1		18:2		Others			
*	M.V.	min. max.	M.V.	min. max.	M.V.	min. max.	M.V.	min. max.
1 Hydrogenated olive oil and liquid olive oil 95%, butter fat 5%.	71.9	[70.6-73.6]	5.3	[4.5- 6.7]	0.5	[0.3-1.1]	26.1	[19.2-32.5]
2 Liquid and hydrogenated cotton seed oil 95%, butter fat 5%.	54.7	[42.5-68.4]	16.1	[10.1-24.7]	0.3	[0.1-0.5]	48.8	[39.2-61.4]
3 Hydrogenated vegetable oil 95%, butter fat 5%.	51.6	[46.2-56.0]	16.9	[9.5-22.8]	0.5	[0.4-0.7]	29.4	[26.1-36.5]
4 Hydrogenated vegetable oils and vegetable fats 95%, butter fat 5%.	51.7	[42.5-59.1]	19.5	[12.8-25.9]	0.8	[0.3-3.3]	31.2	[24.0-37.7]
Table Margarines								
1 Olive oil	73.9	[72.0-75.9]	5.5	[3.8- 6.9]	0.1	[- 0.4]	22.2	[18.0-24.7]
2 Hydrogenated and liquid cottonseed oil.	51.2	[32.4-70.3]	22.3	[9.8-32.8]	0.2	[- 1.3]	35.3	[26.6-56,7]
3 Vegetable oils and peanut oil.	46.3	[42.5-52.3]	15.5	[12.1-19.5]	2.9	[1.7-4.2]	27.7	[18.1-39.6]

There are indications[6,7] that dietery *trans* fatty acids may elevate serum cholesterol levels. If this is the case, the content of *trans* isomers should be also taken into account (besides unsaturation) by those who consider cholesterol dangerous as postulated in the lipid theory of arteriosclerosis. It would, therefore,

seem proper that the approximate fatty acid composition and isolated *trans* unsaturated fatty acids content should be declared by the manufacturers. This information can be obtained readily by present day GLC and IR techniques. It would also seem judicious to establish and police adequate nutritional standards for cooking fats and margarines. These should include minimum amounts of essential fatty acids and maximum permissible concentrations of isomeric acids.

Περίληψη

Σύσταση σὲ λιπαρὰ ὀξέα καὶ περιεκτικότητα σὲ trans ἰσομερῆ μεγειρικῶν λιπῶν καὶ μαργαρινῶν παρασκευαζομένων στὴν Ἑλλάδα.

Σὲ μαγειρικὰ λίπη καὶ μαργαρίνες ποὺ παρασκευάζονται στὴν Ἑλλάδα προσδιορίσθηκαν ἡ σύσταση σὲ λιπαρὰ ὀξέα (ἀέριος χρωματογραφία) καὶ τὸ συνολικὸ ποσοστὸ τῶν ὀξέων μὲ μεμονωμένους *trans* διπλοὺς δεσμοὺς (φασματοσκοπία ὑπερύθρου). Τὰ δείγματα (84 συνολικὰ) συγκεντρώθηκαν ἀπὸ τὴν ἀγορὰ τῆς Θεσσαλονίκης σὲ χρονικὸ διάστημα ἑνὸς χρόνου (1966-67).

Διαφορὲς παρατηρήθηκαν ἀνάμεσα σὲ δείγματα ποὺ πάρθηκαν χειμώνα καὶ καλοκαίρι. Προϊόντα μὲ τὸν ἴδιο ἀκόρεστο χαρακτήρα εἶχαν διαφορετικὴ περιεκτικότητα σὲ ὀξέα *trans*. Σὲ ὁρισμένα δείγματα τὸ ποσοστὸ τῶν ἰσομερῶν αὐτῶν ὀξέων ἦταν σημαντικὰ ψηλὸ (μέχρι 61%). Λίπη παρασκευαζόμενα ἀπὸ ἐλαιόλαδο εἶχαν μικρὴ περιεκτικότητα στὸ ἀπαραίτητο βιολογικὰ λινελαϊκὸ ὀξὺ καὶ σχετικὰ ψηλὴ συγκέντρωση ὀξέων *trans* (πίν. 1).

Ἐπειδὴ ὁ ἀκόρεστος χαρακτήρας ἑνὸς ὑδρογονωμένου ἐλαίου μπορεῖ νὰ ὀφείλεται σὲ γεωμετρικὰ ἢ ἄλλα ἰσομερῆ, δηλ. συστατικὰ ποὺ θεωροῦνται μειωμένης βιολογικῆς ἀξίας καὶ πιθανὸν βλαβερὰ[6-8,11,12], συνιστᾶται νὰ δηλώνεται ἀπὸ τοὺς παρασκευαστὲς τῶν προϊόντων ἡ σύσταση σὲ λιπαρὰ ὀξέα καὶ ἡ περιεκτικότητα σὲ *trans* ἰσομερῆ. Τονίζεται ἐπίσης ἡ ἀνάγκη νὰ καθιερωθοῦν διατροφικὰ πρότυπα (standards) γιὰ τὶς μαργαρίνες καὶ τὰ λίπη. Αὐτὰ θὰ πρέπει νὰ περιλαμβάνουν τὰ ἐλάχιστα ποσοστὰ τῶν ἀπαραιτήτων ὀξέων καὶ τὶς ἀνώτατες ἐπιτρεπτὲς συγκεντρώσεις τῶν ἰσομερῶν.

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BASES DE SCHIFF ET THIOSÉMICARBAZONES DÉRIVÉS DE O-VANILLINE.

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Resumé

Synthèse des N-(dialcoxy-2,3 benzylidène)isonicotinhydrazice (II) et dialcoxy-2,3 benzaldéhyde thiosémicarbazones (III) par condensation des éthers de l'ortho-vanilline avec l'isoniazide et la thiosémicarbazone respectivement.

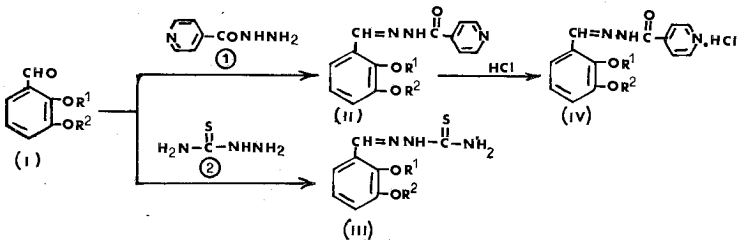
Terminologie: -Bases de Schiff et thiosémicarbazones dérivés de l'ortho-vanilline.

Introduction

L'activité tuberculostatique est reconnue chez certaines bases de Schiff qui sont préparées par condensation de l'isoniazide[1] avec une varieté de benzaldéhydes. La même activité accompagnée par une action foungicide[2] caractérise in vitro certaines thiosémicarbazones issues des cétones et aldéhydes de série aromatique.[3]

C'est donc la raison pour laquelle nous avons sythétisé les composés ci-dessus des formules générales (II) et (II) (schèma I) en condensant les éthers de l'ortho-vanilline avec l'isoniazide et le thiosémicarbazide. Dans le but d'étudier leurs propriétés phramacologiques nous avons préparé les chlorhydrates des composes (IV) (voir tableau II).

L'étude pharmacologique in vitro des produits en question est en cours, mais déjà certains resultats confirment notre supposition. La préparation des produits a été réalisée selon la schéma suivant:



Les matières premières que nous avons utilisé, mise à part de l'ortho-vanilline, sont les dialcoxy-2,3, benzyloxy-2 méthoxy-3 et dibenzyloxy-2,3 benzaldéhyde.

Celles-ci par condensation avec de quantités equimoléculaires d'isoniazide[4] 1 et de thiosémicarbazide[5] 2 respectivement ont donné les produits correspondants (II) et (III).

Parmi les aldéhydes que nous avons utilisé les methoxy-2,3 et éthoxy-2 méthoxy-3 ont été obtenues en faisant réagir le diméthyle sulfate et diéthyle sulfate avec l'ortho-vanilline.[6-9]

Les n-propoxy-2 méthoxy-3 et n-butoxy-2 méthoxy-3 benzaldéhydes ont été obtenues en faisant réagir le dérivé halogéné correspondant avec le sel de sodium de l'ortho-vanilline.[2,9]

Le benzyloxy-2 méthoxy-3 benzaldéhyde a été obtenu par action de chlorure de benzyle sur l'ortho-vanilline[10] et enfin le dibenzyloxy-2,3 benzaldéhyde par action de chlorure de benzyle sur le dihydroxy-2,3 benzaldéhyde; ce dernier a été préparée par deméthylation,[11,12] de l'ortho-vanilline à l'aide de HBr à 48% dans l'acide acetique glaciale.

Partie expérimentale

Les points de fusion ont été pris dans un appareil de Büchi, et ne sont pas corrigés. Les analyses ont été effectuées au Service Central de Microanalyse du S.N.R.S. et au Laboratoires Ciba que nous remercions vivement. Les résultats des microanalyses se trouvent dans les limites±0,4%des valeurs calculées. Les spectres IR ont été enregistrés à l'aide d'un appareil de Backman IR-4.

Benzaldéhydes Substitués (I)

Les benzaldéhydes utilisés sont décrits dans la litterature: dimethoxy-2,3 benzaldéhyde [I,R^1= CH_3,R^2= CH_3),[6,7] éthoxy-2 méthoxy-3 benzaldéhyde (I,R^1= C_2H_5,R^2= CH_3);[8,9] n-propoxy-2 méthoxy-3 benzaldéhyde (I,R^1= n-C_3H_7,R^2= CH_3);[9] n-butoxy-2 méthoxy-3 benzaldéhyde (I,R^1= n-C_4H_9,R^2= CH_3);[2] benzyloxy-2 méthoxy-3 benzaldéhyde (I,R^1= $CH_2C_6H_5$, R^2= CH_3);[10] dibenzyloxy-2,3 benzaldéhyde (I,R^1= R^2= $CH_2C_6H_5$).[11,12]

Préparation des bases de Schiff (II)

Méthode générale de préparation: En utilisant cette méthode nous avons préparé tous les produits du type général II. Pour cela, 0,05 mole d'isoniazide sont mis en solution dans l'éthanol (150 ml) puis on y ajoute 0,05 mole d'aldéhyde correspondant, en solution éthanolique (100 ml).

Le mélange est chauffé à reflux pendant 2h. Le produit qui cristallise déjà à chaud, précipite par refroidisement ou addition d'une petite quantité d'eau.

La plupart de produits sont d'une couleur jaune pâle et sont recristallisés dans l'éthanol.

L'absorption à l'IR donne les bandes suivantes: 3185 cm^{-1} (-NH-), 1655 cm^{-1} (C=O), 1575-1600 cm^{-1} (-C=N- et >c=c<), 1550 - 1565 cm^{-1} (pyridine).

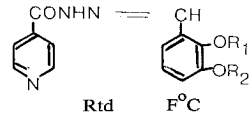
Préparation des thiosémicarbazones (III).

Méthode générale de préparation: Dans une solution éthanolique (50 ml) de 0.01 mole de thiosémicarbazide contenant 1 ml de AcOH on ajoute lentement et à chaud une solution de l'aldéhyde correspondant (0,01 mole) dans 50 ml d'éthanol.

Le mélange est chauffé doucement pendant 1h, puis on ajoute de l'eau. Le produit qui se forme est filtré, lavé avec une solution H_2O-EtOH (1:1) et séché. Les composes ainsi obtenus sont recristallisés dans une mélange H_2O-EtOH.

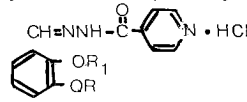
Les bandes d'absorption caractéristiques à l'IR des produits obtenus sont: 3440-3480 cm^{-1} (-NH_2), 3140-3280 cm^{-1} (-NH-), 1570-1600 cm^{-1} (>c=N- et >c=c<), 800-835 cm^{-1} (>c=s).

TABLEAU I: *N-(Dialcoxy-2,3 benzylidène) - isonicotinoyl hydrazides.*



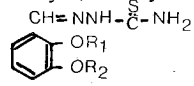
No	R_1	R_2	Rtd %	F°C	FORMULE MOLECULAIRE	Analyse C% Calc.	C% Tr.	H% Calc.	H% Tr.	N% Calc.	N% Tr.
1	H	CH_3	96	230-232	$C_{14}H_{13}N_3O_3$	61,98	61,89	4,83	5,10	15,49	15,17
2	CH_3	CH_3	86	164-165	$C_{15}H_{15}N_3O_3$	63,15	63,34	5,30	5,41	14,73	14,52
3	C_2H_5	CH_3	97	170-172	$C_{16}H_{17}N_3O_3$	64,20	63,98	5,72	5,81	14,04	19,38
4	C_3H_7-η	CH_3	90	144-146	$C_{17}H_{19}N_3O_3$	65,17	65,06	6,11	6,38	13,41	13,20
5	C_4H_9-η	CH_3	97	158-160	$C_{18}H_{21}N_3O_3$	66,04	65,94	6,46	6,62	12,83	12,64
6	$CH_2C_6H_5$	CH_3	99	172-174	$C_{21}H_{19}N_3O_3$	69,78	69,76	5,30	5,77	11,63	11,70
7	$CH_2C_6H_5$	$CH_2C_6H_5$	97	152-154	$C_{27}H_{23}N_3O_3$	74,12	74,24	5,30	5,51	9,61	9,71

TABLEAU II: *Chlorhydrates des N-(dialcoxy-2,3 benzylidène) - isonicotinoyl hydrazides.*



No	R_1	R_2	F°C	FORMULE MOLECULAIRE	Analyse C% Calc.	C% Tr.	H% Calc.	H% Tr.	N% Calc.	N% Tr.	Cl% Calc.	Cl% Tr.
1	H	CH_3	236-238 dec	$C_{14}H_{14}ClN_3O_3$	54,64	54,70	4,59	4,70	13,65	13,48	11,52	11,60
2	CH_3	CH_3	228-230 dec	$C_{15}H_{16}ClN_3O_3$	56,00	56,24	5,01	5,00	13,06	13,20	11,01	11,05
3	C_2H_5	CH_3	224-226 dec	$C_{16}H_{18}ClN_3O_3$	57,22	57,49	5,41	5,40	12,51	12,60	10,56	10,60
4	C_3H_7-η	CH_3	240-242 dec	$C_{17}H_{20}ClN_3O_3$	58,36	58,30	5,77	5,80	12,01	12,16	10,14	10,20
5	C_4H_9-η	CH_3	248-250 dec	$C_{18}H_{22}ClN_3O_3$	59,42	59,48	6,09	6,15	11,55	11,55	9,74	9,80
6	$CH_2C_6H_5$	CH_3	198-200 dec	$C_{21}H_{20}ClN_3O_3$	63,40	63,30	5,07	5,19	10,56	10,25	8,91	9,00
7	$CH_2C_6H_5$	$CH_2C_6H_5$	212-214 dec	$C_{27}H_{24}ClN_3O_3$	68,42	68,30	5,10	5,22	8,87	8,90	7,49	7,60

TABLEAU III: *N-(Dialcoxy-2,3 benzylidène) - thiosémicarbazones.*



No	R_1	R_2	Rtd %	F°C	FORMULE MOLECULAIRE	Analyse C% Calc.	C% Tr.	H% Calc.	H% Tr.	N% Calc.	N% Tr.
1	H	CH_3	92	238-240	$C_9H_{11}N_3O_2S$	47,98	47,87	4,92	4,97	18,65	18,08
2	CH_3	CH_3	91	232-234	$C_{10}H_{13}N_3O_2S$	50,19	50,31	5,47	5,53	17,56	17,39
3	C_2H_5	CH_3	95	182-184	$C_{11}H_{15}N_3O_2S$	52,16	51,24	5,97	5,83	16,59	16,70
4	C_3H_7-η	CH_3	94	176-178	$C_{12}H_{17}N_3O_2S$	53,92	53,82	6,41	6,39	15,72	15,55
5	C_4H_9-η	CH_3	68	165-167	$C_{13}H_{19}N_3O_2S$	55,48	55,46	6,81	6,81	14,94	15,02
6	$CH_2C_6H_5$	CH_3	89	166-168	$C_{16}H_{17}N_3O_2S$	60,92	60,86	5,43	5,41	13,32	13,22
7	$CH_2C_6H_5$	$CH_2C_6H_5$	69	128-130	$C_{22}H_{21}N_3O_2S$	67,49	67,22	5,41	5,42	10,74	10,64

Abstract

Schiff's bases and Thiosemicarbazone derivatives of o-vanillin.

A series of N-(2,3-dialcoxy benzyliden)-isonicotynhydrazide (II) and thiosemicarbazones (III) were prepared by condensation of isoniazide and thiosemicarbazide with various ethers of o-vanillin.

The products are currently being tested against antituberculosis and as antifungus agents. The results will be published in the near future.

Περίληψις

Βάσεις Schiff's καὶ θειοσεμικαρβαζόναι, παράγωγα τῆς ο-βανιλλίνης.

Εἰς τὴν ἐργασίαν αὐτὴν περιγράφεται ἡ παρασκευὴ N-(2,3-διαλκοξυ. βενζυλιδενο) - ἰσονικοτινοϋλοϋδραζιδίων (II) καὶ 2,3 -διαλκοξυ-βενζαλδεϋδοθειοσεμικαρβαζονῶν (III).

Τὰ προϊόντα λαμβάνονται εἰς καλὰς ἀποδόσεις διὰ συμπυκνώσεως ἰσονιαζιδίου καὶ θειοσεμικαρβαζιδίου ἀντιστοίχως, μετὰ αἰθέρων τῆς ο-βανιλλίνης, εἰς ἰσομοριακὰς ποσότητας.

Τὰ προϊόντα ταῦτα δοκιμάζονται ἤδη φαρμακολογικῶς διὰ φυματιοστατικὴν καὶ μυκητοκτόνον δρᾶσιν. Τὸ φαρμακολογικὸν μέρος θὰ δημοσιευθῇ προσεχῶς.

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PREPARATION AND CONFIGURATION OF 3-PHENYL (OR METHYL)-5α-PREGNAN-3-ols and 2,3-EPOXY-3-PHENYL (OR METHYL)-5α-PREGNANES

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Summary

The steroids 3-phenyl (or methyl)-5α-pregnan-3-ols and 2,3-epoxy-3-phenyl (or methyl)-5α-pregnanes, were synthesized from 5α-pregnan-3-one. The epimers of the above steroids were isolated by column chromatography and their structures were determined.

Key words: 5α-Pregnan-3-ols, preparation-configuration. 5α-Pregnanes-2,3-epoxy, preparation-configuration.

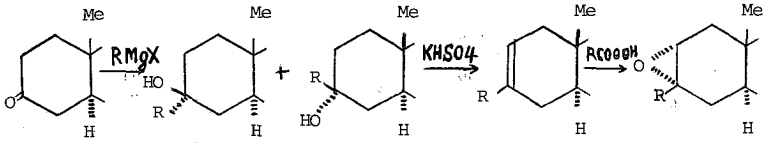
Theoretical

The synthesis of the title compounds is a part of a general program of steroidal preparations with pharmacological interest. 3β-Hydroxy-5α-pregnan-20-one-3β-acetate was transformed into 5α-pregnan-3β-ol by a modification of Wolff-Kshner reaction[1] and further to 5α-pregnan-3-one[2] I with potassium chromate in good yield.[2,3]

Reaction of phenylmagnesium bromide with the Ketone I gave a mixture of the epimeric alcohols: 3α-phenyl-5α-pregnan-3β-ol IIa and 3β-phenyl-5α-pregnan-3α-ol IIIa in a ratio 53:47.

The reaction of methylmagnesium iodide with the ketone I gave the epimeric alcohols IIb and IIIb in a ratio 57:43. In both reactions the a-epimers were formed in a slightly higher proportion than the β-epimers, in accordance with stereochemical requirements of the A-ring of the steroids, that is, the approach of Grignard reagent is slightly favorable from the α-side.[4,5]

Although there is a considerable difference in the size of the two Grignards reagents used, there is not any notable effect in the ratio of the obtained epimers. The above epimers were separated by column chromatography on alumina[3]. The configurations of the epimers alcohols were studied by ^{1}HNMR and I.R. spectroscopy. In the ^{1}HNMR spectra the signals arising from the methy hydrogens of IIa and IIb epimers (hydroxy-group at 3β-position) are shifted 2-4Hz downfield than the epimers IIIa and IIIb (hydroxy-group at 3α-position).[6,7,8] On the other hand the streching vibration absorptions of C-O group at 938 and 945 cm^{-1} for the epimers IIa and IIb (equatorial hydroxy-group) and at 887 and 899 cm^{-1} for the epimers IIIa and IIIb (axial hydroxy- group), are in agreement to the suggested structures.[4]



I IIa=R:Ph IIb=R:Me IIIa=R:Ph IIIb=R:Me IVa=R:Ph IVb=R:Me Va=R:Ph Vb=R:Me

Dephydration of epimers IIa or IIIa and IIb or IIIb yielded the Δ^2-olefins IVa nd IVb respectively. The double bond position in the steroid IVa was as expected[9,10] and this was confirmed by the HNMR spectroscopy. The olefin IVa shows a broad peak at 7.23 ppm arising from the benzene ring protons and a peak at 5.98 ppm ($\Delta\nu/2=11$Hz) from the vinyl proton, and the compound IVb shows the signal of the vinyl proton at 5.17 ppm ($\Delta\nu/2=9$Hz) and a singlet from the methyl protons at 1.63 ppm.

Epoxidation of the olefinic double bond with perbenzoic acid and/or m-chlorperbenzoic acid gave the corresponding epoxides in high yield. During chromatography of the mother liquor from the epoxidation with the m-chlorperbenzoic acid, a 6% yield of the 3β-phenyl-5α-pregnane-2β, 3α-diol was isolated. The ^{1}HNMR spectrum of this diol showed a signal at 3.73 ppm indicative of the C-2 methine proton ($\Delta\nu/2=6$Hz). this signal demostrates that the proton at C-2 position is equatorial and therefore couples with both C-2 position protons (Jea=Jee ~ 2-4Hz).

Reduction of the epoxide Va with $LiAlH_4$ led to a single product, which was identified as the alcohol IIIa (C, H analysis, ^{1}HNMR and IR). Similarly, reduction of the epoxide Vb led to the corresponding alcohol IIIb. The axial configuration of the alcohols IIIa and IIIb is in accordance to the mechanism of diaxal cleavage of the epoxides.[4,5,11,12]

Experimental

Melting points were taken in cappilary tubes with a Büchi apparatus and are uncorrected. Infra red Spectra were obtained in KBr pellets with a Perkin-Elmer 521 Spectrophotometer, ^{1}HNMR spectra were obtained in $CDCl_3$-TMS as an internal standard with a Varian A-60 spectrometer.

3β-Phenyl-5α-pregnan-3α-ol (IIIa) and 3α-phenyl-5α-pregnan-3β-ol (IIα):

To a well stirred ethereal solution of phenylmagnesium bromide, prepared in the usual manner from 2g (0.08 Gramtoms) magnesium turnings and 12g (0.08 mol) of bromobenzene in 200 ml of anhydrous ether, 4.5g (0.015 mol) of ketone (I) in 75 ml of anhyrdous ether was added dropwise. The reaction mixture was gently boiled for 2 hrs and allowed to stand overnight. It was decomposed with water and 10% HCl and the aqueous layer was extracted 3 times with ether. The combined ether extracts were dried ($MgSO_4$) and evaporated with a rotary evaporator to dryness. The crude product was 5.4g (96%). A part of the above mixture (2.5g) was chromatographed on 50g of neutral alumina. Compound (IIIa) was eluted first with hexane and (IIa) with benzene.

(IIIa). 1.7g (47%); p.m. 145-147° (acetone); I.R., ν (C-O) ≈ 887 cm^{-1}. ^{1}HNMR ($CDCl_3$) δO.57 ppm (s, 3H, 18-CH_3) 0,87 ppm (s, 3H, 19-CH_3), 1.70 ppm (s, 1H, 3-OH, exchangable with D_2O) and 7.13-7.59 ppm (m, 5H, aromatic protons).

anal. $C_{27}H_{40}O$:Calcd %: C:85.20, H:10.59
Found %: C:85.25, H:10.59

(IIa): 1.3g (53%); m.p. 127-130° (MeOH): IR (C-O) ≈ 938 cm^{-1}; ^{1}HNMR ($CDCl_3$) δ 0.53 ppm (s,3H, 18-CH_3), 0,91 ppm (s, 3H, 19-CH_3), 1.78 ppm (s, 1H, 3-OH) exchangable with D_2O) and 7.19-7.65 ppm (m, 5H, aromatic protons).
Anal. $C_{27}H_{40}O$:Calcd %: C:85.20, H:10.59
Found %: C:85.23, H:10.60

3β-Methyl-5α-pregnan-3α-ol (IIIb) and 3α-methyl-5α-pregnan-3β-ol (IIb): Experimental conditions and proportions were identical with the above experiment. The crude product was 4,45 gr (94%). A part of the crude product (2.5g) was chromatographed on 50g of neutral Alumina. Compound (IIIb) was eluted first with n-hexane and (IIb) with a mixture of n-hexane-benzene 1:1.

(IIIb): 1.08g (43%) m.p. 130-132° (acetone); IR: (C-O) ≈ 899 cm^{-1}: ^{1}HNMR ($CDCl_3$) δ 0.56 ppm (s, 3H, 18-CH_3) 0.74 ppm (s, 3H, 19-CH_3), 1.19 ppm (s, 3H, 3-CH_3) and 1.60 ppm (sl, 3-OH, exchangable with D_2O).
Anal. $C_{22}H_{38}O$Calcd %: C:82.95, H:12.03
Found %: C:83.11, H:11.99

(IIb): 1,4g (57%) m.p. 155-156° (MeOH). IR., ν(C-O) ≈ 945 cm^{-1} ^{1}HNMR ($CDCl_3$) δ0,53 ppm (s, 3H, 18-CH_3) 0.81 ppm (s, 3H, 19-CH_3) 1,23 ppm (s, 3H, 3-CH_3) and 1.62 ppm (sl, 1H, 3-OH, exchangable with D_2O).
Anal. $C_{22}H_{38}O$Calcd.%: C:82.95, H:12.03
Found. %: C:83.04, H:12.00

3-Phenyl-5α-pregn-2-ene (IVa). A mixture of 3g (0.08 mol) of the alcohol (IIa) or (IIIa) and 0,4g of $KHSO_4$, in 100ml of anhydrous toluene was refluxed for 10 hrs using a Dean-Stark water trap condenser. After cooling, the insoluble meterial was filtered off and the filtrate was evaporated in vacuo giving 2.5g (86%) of the crude olefin (IVa) m.p. 154-156° (MeOH-$CHCl_3$); ^{1}HNMR ($CDCl_3$) δO.58 ppm (s,3H, 18-CH_3), 0.88 ppm (s, 3H, 19-CH_3), 5.98 ppm (sl, 1H, vinylic proton at 2-c, Δν/2 ≈ 11Hz) and 7.23 ppm (sl, 5H, aromatic protons).
Anal. $C_{27}H_{38}$ Calcd %: C:89.43, H:10.57
Found %: C:89,32 H:10,43

3-Methyl-5α-pregn-2-ene (IVb). This compound was prepared in 88% yield following the procedure of the (IVa) m.p. 72-74° (MeOH); ^{1}HNMR ($CDCl_3$) δ0.55 ppm (s, 3H, 18-CH_3), 0.85 ppm (s, 3H, 19-CH_3), 1,63 ppm (s, 3H, 3-CH_3) and 5.17 ppm (sl, 1H, vinylic proton at 2-C, Δν/2 9Hz).
Anal. $C_{22}H_{36}$ Calcd %: C:87.92, H:12.08
Found %: C:88.13, H:12.01

2α, 3α-Epoxy-3β-phenyl-5α-pregnane (Va).

1. Epoxidation with perbenzoic acid: A solution of 3.35g (0.09 mol) of the olefin (IVa) is 15m of benzene was treated with 11ml 1.1N perbenzoic acid solution in benzene. The reaction mixture was allowed to stand at room temperature for 20 min and then at the refrigerator for 16 hours. The organic layer was washed with water, Na_2CO_3 10% and again with water and dried ($MgSO_4$). Evaporation of the solvent in vacuo gave 3.3g (yield 87%) of the crude epoxide (Va) which was crystallized from MeOH, m.p. 166-168°; ^{1}HNMR ($CDCl_3$) δ0.56 ppm (s, 3H, 18-CH_3), 0.87 ppm (s, 3H, 19-CH_3) 3.07 ppm (d, J ~ 4Hz, proton at 2-C) and 7.28 ppm (s, 5H, aromatic protons).
Anal. $C_{27}H_{38}O$:Calcd %; C:85.66, H:10.12
Found %: C:85.62, H:10./7

II. Epoxidation with m-chloroperbenzoic acid: To a solution of 3g (0.008 mol) of the olefin (IVa) in 40 ml of chloroform was added in small portion 1.5 g (0.08 mole) of m-chloroperbenzoic acid under stirring and cooling in ice-water bath. Stirring was continued for 30 min and at room temperature for 18hrs. After standing in the refrigerator for 48 hrs the mixture was filtered and the filtrate was washed with water, $NaHCO_3$ 5%, and again with water. After drying ($MgSO_4$) the solvent was removed in vacuo and the residue was recrystallized from methanol giving 2.4 g (80%) of the epoxide (Va). Evaporation of the mother liquor, gave a residue which was chromatographed on alumina (eluant solvent: mixture of petroleum ether-benzene 8:2) to furnish an additional amount of 0.3 g of the epoxide (Va). (Total yield 86%). Further elution of the column with benzene gave the 3β-phenyl-5α-pregnane-2β, 3α-diol in yield 6%; m.p. 209° (MeOH); ^{1}HNMR ($CDCl_3$) δ0.59 ppm (s, 3H 10-CH_3)σ, 0,96 ppm (s, 19-CH_3), 1.72 ppm (sl, 2H, 2-OH and 3-OH, exchangable with D_2O), 3.72 ppm (sl, 1H, proton at 2-C,$\Delta\nu/2 \approx 6$Hz). amd 7.23-7.72 ppm (m, 5H, aromatic protons).

Anal. $C_{22}H_{40}O$ Calcd %: C:81.76, H10.17, 0:8.07
Found %: C:82.01, H:9.79, 0:8.10

2α, 3α-Epoxy-3β-methyl-5α-pregnane (Vb), This epoxide was prepared in a 82% yield from the olefin (IVb) following the above described precedure. M.p. 100-102°, ^{1}HNMR ($CDCl_3$), δ0.55 ppm (s, 3H, 18-CH_3), 0.73 ppm (s, 3H, 19-CH_3), 1.22 ppm (s, 3H, 3-CH_3) and 2.93 ppm (d, 1H, J ~ 5Hz, proton at 2-C).

Anal. $C_{22}H_{36}O$ Calcd %: C:83.48, H:11.40
Found %: C:83.47, H:11.37

Reduction of the epoxide (Va). To a solution of 0.5g (0.0127 mol) of the epoxide (Va) in 60 ml of THF was added 1g (0.024 mol) of $LiAlH_4$, and the reaction mixture was refluxed for 12hrs. After cooling it at room temperature it was decomposed with a saturated solution of sodium sulfate, filtered and the fltrate was evaporated to dryness. The residue was chromatographed on 20 g. alumina using n-hexane to give 0.4g (80%) of a product having melting point 145-147°, (acetone). A mixed melting point of this product with the alcohol (IIIa) gave no depression. Also the I.R. and ^{1}HNMR spectra were identical with those of (IIIa). (See above).

Reduction of the epoxide (Vb).

Following the above described the epoxide Vb) gave a product in 75% yield (p.m. 130-132°), indentical to the alcohol (IIIb), (See above).

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Περίληψις

Σύνθεσις καὶ καθορισμὸς τῆς δομῆς τῶν ἰσομερῶν 3-φαινυλο- (ἤ μεθυλο)-5α-πρεγναν-3-ολῶν καὶ 2,3-εποξυ-3-φαινυλο (ἤ μεθυλο)-5-α-πρεγνανίων.

Ὡς πρώτη ὕλη ἐχρησιμοποιήθη ἡ 3β-ἀκετοξυ-5α-πρεγναν-20-ονη, ἡ ὁποία δι' ἀναγωγῆς μετατρέπται εἰς 5α-πρεγναν-3β-όλη[1] καὶ ἀκολούθως διὰ χρωμικῆς ὀξειδώσεως εἰς 5α-πρεγναν-3-όνη[2,3] I. Δι' ἐπιδράσεως φαινυλομαγνησιοβρωμιδίου ἐπὶ τῆς I λαμβάνεται μῖγμα τῶν ἐπιμερῶν 3α-φαινυλο-5α-πρεγναν-3β-όλης IIα καὶ 3β-φαινυλο-5α-πρεγναν-3α-όλης IIIα. Δι' ἀναλόγου ἐπιδράσεως

μεθυλομαγνησιοιωδιδίου ἐπὶ τῆς κετόνης I λαμβάνεται μῖγμα τῶν 3α καὶ 3β-μεθυλο ἐπιμερῶν ἀλκοολῶν IIb, IIIb. Εἰς ἀμφότερες τὶς ἀντιδράσεις τὰ α-ἐπιμερῆ λαμβάνονται σὲ ἐλαφρῶς μεγαλύτερη ἀναλογία.[4,5] Ὁ διαχωρισμὸς τῶν ἀνωτέρω ἐπιμερῶν ἀλκοολῶν ἀμφοτέρων τῶν παραγώγων ἔγινε διὰ χρωματογραφίας ἐπὶ στήλης[3]

Ἀφυδάτωσις τῶν καθαρῶν ἰσομερῶν IIα IIIα καὶ IIb, IIIb ὁδηγεῖ στὶς Δ^2-ὀλεφίνες: 3-φαινυλο-5α-πρεγνα-2-ένιον IVα καὶ 3-μεθυλο-5α-πρεγνα-2-ένιον IVb ἀντιστοίχως.[9,10] Ἐποξείδωσις τῶν ὀλεφινῶν αὐτῶν IVα καὶ IVb μὲ ὑπεροξέα: ὑπερβενζοϊκὸ καὶ m-χλωροϋπερβενζοϊκό, ὁδηγεῖ εἰς τὰ ἀντίστοιχα ἐποξείδια 2α, 3α-ἐποξυ-3β-φαινυλο-5α-πρεγνάνιο Va καὶ 2α, 3α-ἐποξυ-3β-μεθυλο-5α-πρεγνάνιο Vb εἰς ὑψηλὰς ἀποδόσεις.

Ἀναγωγὴ τῶν ἐποξειδίων Vα καὶ Vb μὲ $LiAlH_4$ ὁδηγεῖ ἀποκλειστικὰ εἰς τὶς ἀλκοόλες 3β-φαινυλο-5α-πρεγναν-3α-όλη καὶ 3β-μεθυλο-5α-πρεγναν-3α-όλη IIIb ἀντιστοίχως.[4,5,11,12]

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