CHIMIKA CHRONIKA

NEW SERIES

AN INTERNATIONAL EDITION
OF THE ASSOCIATION OF GREEK CHEMISTS

CMCRCZ 21(1-4), 3-96(1992)

ISSN 0366-693X

1-4/92

Volume 21, No 1-4 p.p. 3-96 March-December 1992

CHIMIKA CHRONIKA

NEW SERIES

AN INTERNATIONAL EDITION

Published by the Association of Greek Chemists (A.G.C.)
27 Kaningos str. Athens 106 82 Greece

Correspondence, submission of papers, subscriptions, renewals and changes of address should be sent to Chimika Chronika-New Series, 27 Kaningos street, Athens 106 82, Greece. The Guide to Authors is published in the first issue of each volume, or sent by request. Subscriptions are taken by volume at 2000 drachmas for Corporations in Greece and 50 U.S. dollars to all other countries except Cyprus, where subscriptions are made on request.

Phototypesetted and Printed in Greece by EPTALOFOS S.A.
12, Ardittou Str. 116 36 ATHENS Tel. 9217.513

Υπεύθυνος σύμφωνα με το νόμο: Π. Ξυθάλης, Κάνιγγος 27, Αθήνα 106 82.

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Chimika Chronika, New Series, 21, 3-10 (1992)

SOME APPROACHES TO THE OXIDATION OF WHITE WINE

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SUMMARY

Experimental white wines were made with the addition in the must casein hydrolysate (C.H.), cysteine (Cys), sodium diethyldithiocarbamate (DDC), sodium diethyldithiocarbamate plus casein hydrolysate (DDC + C.H.), and sodium diethyldithiocarbamate plus cysteine (DDC + Cys).

Color evaluations and chemical analyses (the absorbance at 420 nm, total phenolics, Cu content and UV spectrum) were made with the experimental wines in relation to control wines.

The DDC experimental wines (DDC, DDC + CH, DDC + Cys) had initially retained a non-oxidized color, but after four-six months they developed a red-brown color. Control and casein hydrolysate experimental wines had always a brown color while the cysteine experimental wines developed a more complicated behavior.

Color evaluation along with analytical results indicated that the three DDC experimental wines developed both browning and pinking phenomena while control and casein hydrolysate experimental wines only the first one.

The control wines, after four-six months developed better color in relation to experimental wines.

Key words: White wine, oxidation.

INTRODUCTION

The oxidation of white wines includes mainly the browning but also the pinking phenomena, especially in the case of well prepared and preserved white wines [1,2,3].

Browning is caused by oxidative polymerization of flavonoid polyphenols which can react either enzymically (polyphenoloxidase activity) or chemically with oxygen to produce quinones, which can further react to form brown polymeric pigments[3]. The main difference between enzymic and nonenzymic browing lie in the much faster rate of quinone production by the former at acidic pH[1].

Polyphenoloxidase which accurs naturally in the must, and laccase, which is produced by *Botrytis cinerea* (rotten grapes), appear to have oxidative activity on polyphenols.

Pinking is believed to be caused by the fast conversion of flavenes to red flavylium salts in the presence of oxygen. These flavenes can be formed by the slow dehydration of leucoanthocyanidins[3].

In this study, sensory and chemical properties of white wines, produced from must in which reagents for the inhibition of polyphenoloxidase activity or for the enhancement of the browing phenomenon were added, are examined.

EXPERIMENTAL

Must preparation:

Grapes used were Debina variety. Debina is a late ripening easily oxidizable variety which is cultivated at Zitsa (Epirus, Greece).

Handmade preparations (destemming, chrushing and juice extraction) were applied and so the free-van juice was used.

This, as determined by a digital refractometer, contained 181 g/l reducing sugars. The total acidity was 7.5 g/l as tartaric acid and the pH 3.1.

Must vinification:

Must was divided into six portions: the first was used as control [a] and in the rest casein hydrolysate (C.H.) 0.1 g/l [b], cysteine (Cys) 0.1 g/l [c], sodium diethyldithiocarbamate (DDC) 1.0 g/l [d], sodium diethyldithiocarbamate 1.0 g/l plus casein hydrolysate 0.1 g/l (DDC + CH) [e] and sodium diethyldithiocarbamate 1.0 g/l plus cysteine 0.1 g/l (DDC + Cys) [f] were added. But SO_2 was not used.

The fermentation was carried out into 2 lit green glass bottles at 18-20°C. It was not applied initial racking and yeast culture was not used except in the cases of DDC addition, where 3% must being in vigorously

fermentation by industrial selected yeast (*Saccharomyces cerevisiae*) was added because of the delay of fermentation start.

After fermentation was accomplished (variable duration) the wines were cooled for the required period and the lees was separated by sunction. After clarification the bottles stored in a dark place of about 15°C.

The produced wines had 5.5-6.5 g/l total acidity as tartaric acid. The fermentation leads to dry wines (<1 g/l reducing sugars) except in the cases of cysteine containing wines which retained reducing sugars (DDC + Cys wine 2.27 g/l and Cys wine 7.0 g/l).

Color evaluation and chemical analyses:

The analyses and color evaluation were made: a) after the clarification, b) four months after the starting of fermentation, and c) six months after the starting of fermentation.

The absorbance values at 420 nm were taken using a Milton and Roy comp. spectroning 1001 spectrophotometer. Total phenolics were determined by Folin-Ciocalteu method[4]. The UV spectra were taken using a Perkin-Elmer Lambda 15 UV/Vis spectrophotometer with a Perkin-Elmer FX-85 printer. Copper was determined by atomic absorption spectrophotometry using a Perkin-Elmer model 560 A.A.S..

All experiments were run in duplicate and results reported here are the mean values of two runs.

RESULTS AND DISCUSSION

Color evaluation and analytical results after clarification:

The a and b lees developed brown color whereas c, d, e, and f lees had a light grey-white color. Similary only the a and b wines developed brown color while the others had a light yellow nonoxidized color.

The wine adsorbances at 420 nm are given in Table I. It is shown that the absorbance values of a and b wines were higher than those of the rests. As it is known the absorbance at 420 nm is an index of browning development in white wines[1].

Subsequently from all the above is concluded that the browning phenomenon was developed into the control and casein hydrolysate experimental wines but not into the others, which can be attributed to polyphenoloxidase inhibition.

TABLE I. Absorbances at 420 nm of experimental wines (a,b,c,d,e,f) after clarification (I), four (II) and six (III) months after the starting of fermentation.

Experimental wine	A 420 nm		
	I	II	III
a	0.149±0.006*	0.191±0.008	0.205±0.007
b	0.150±0.007	0.28 ±0.01	0.33 ±0.02
c	0.089±0.007	0.259±0.008	0.40 ±0.01
d	0.108±0.007	0.214±0.009	0.188±0.009
e	0.100±0.008	0.25 ±0.01	0.245±0.007
f	0.078±0.008	0.14 ±0.01	0.12 ±0.02

* Errors given are the standard deviation of the mean for n=2.

Color evaluation and analytical results four months after the starting of fermentation:

A, b, and c wines appeared to be brown while d, e and f had a red color with brown hue.

The control wines had a better color in relation to b and c experimental wines which is also supported by the absorbances at 420 nm (Table I). It is noticeable that the oxidized red-brown color of d, e and f experimental wines can not be claimed by the absorbance at 420 nm.

TABLE II. Total phenolics of experimental wines (a,b,c,d,e,f) four (II) and six (III) months after the starting of fermentation.

Experimental wine	Total phenolics, mg/l gallic acid	
	II	III
a	113±13*	118±11
b	95±7	95±10
c	110±12	107±10
d	190±16	210±23
e	170±14	182±12
f	188±11	191± 6

* Errors given are the standard deviation of the mean for n=2.

Total phenolic values of a, b and c wines were lower than those of the others (Table II). This may indicates that the browning phenomenon was developed in a, b, and c wines in a greater extent than in the other wines, since part of the browning products removed as precipitate[1,5]. Similarly a, b and c wines gave lower peaks than d, e and f experimental wines at ~280 nm and ~320 nm (Figure 1), where it is known that the phenolic compounds (benzene ring) absorb[2,6].

Color evaluation, total phenolic values and profiles of UV spectra seem to suggest that a, b and c wines developed the browning phenomenon whereas d, e and f both browning and pinking phenomena.

Color evaluations and analytical results six months after the starting of fermentation:

Observations and measurements of six months wines were complying with those of four months wines.

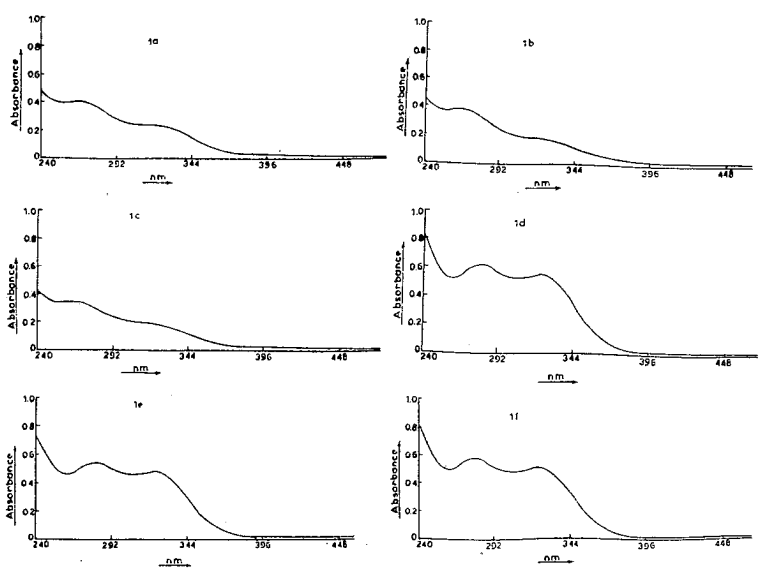

FIG. 1. UV spectra of experimental wines four months after the srarting of fermentation.
(a) control wine, (b) CH wine, (c) Cys wine, (d) DDC wine, (e) DDC+CH wine, (f) DDC + Cys wine.
The samples were diluted 12 times with distilled H_20.

A, b and c wines appeared brown color whereas d, e and f wines a redish brown color. Also A, b and c wines also contained lower total pheolics than the others (Table 2, Figure 2).

Consequently it seems that a, b and c six months wines also developed the browning phenomenon whereas d, e and f both browning and pinking phenomena.

After clarifications a, b and c wines contained 0.60, 0.35 and 0.25 ppm Cu respectively, whereas the Cu content of DDC (d, e, f) wines was approximately zero. The Cu content progressively decreased and six months after the starting of fermentation the a, b and c wines contained approximately zero copper. The above can be explained by the precipitation of copper complexes[7]. The approximate zero copper content in the DDC experimental wines probably indicated that the redish-brown color of these wines can't be attributed to a copper casse haziness.

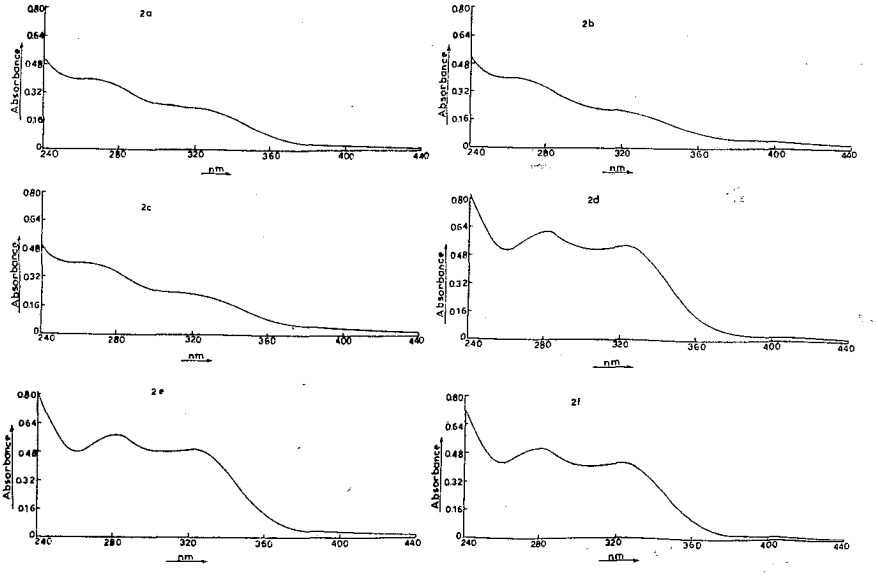

FIG. 2. UV spectra of experimental wines six months after the srarting of fermentation.
(a) control wine, (b) CH wine, (c) Cys wine, (d) DDC wine, (e) DDC+CH wine, (f) DDC + Cys wine.
The samples were diluted 12 times with distilled H_20.

It is remarkable noticing the behavior of cysteine experimental wines. The retardation of the oxidation can be explained by the inhibition of polyphenoloxidase activity, for the -SH group formes stable complexes with Cu[8,9]. The following enhancement of the browning development, in relation to the control wines , may be due to the reaction of the $-NH_2$ of cysteine with quinones (schiff base)[1]. On the contrary it is known that the -SH group reduces the quinones[8].

The casein hydrolysate experimental wines had always a deeper brown color than the control wines. It can be attributed to the formation of compounds, by the reaction of amino acids and peptides ($-NH_2$ group) with quinones , whose molar extinction coefficients are much higher than those of quinones or their polymerization products alone[1].

The addition of DDC in the three DDC experimental wines retarded the development of browning by the inhibition of the polyphenoloxidase activity [10,11,12].

Consequently the high amount of remained unpolymerized phenolics in the DDC wines may be related with the development of pink color.

It is obvious, comparing the control with DDC experimental wines, that the inhibition of polyphenoloxidase activity although initially had a better color effect in follow led to worse color in wines. This can be attributed to a higher browning capacity of DDC wines (higher phenolic content) and to the development of pinking phenomenon.

CONCLUSIONS

The inhibition of the polyphenoloxidase activity by the addition of diethyldithiocarbamate leads to the development of a red-brown color. This probably indicates that nonenzymic browning and pinking may coexist and that the usual brown color of oxidized wines is due to the fast action of polyphenoloxidases.

The better color of control in relation of DDC wines leads to the suggestion that the initial accelaration of the browning by must oxygenation may results, via the removal of phenolic compounds, in white wines of a better color and a low browning capacity.

ΠΕΡΙΛΗΨΗ

ΔΙΕΡΕΥΝΗΣΗ ΟΞΕΙΔΩΣΗΣ ΛΕΥΚΩΝ ΚΡΑΣΙΩΝ

Παρασκευάστηκαν πειραματικά λευκά κρασιά με προσθήκη στο μούστο υδρολύματος καζεΐνης (CH), κυστεΐνης (Cys), διαιθυλοδιθειοκαρβομιδικού νατρίου (DDC), DDC μαζί με CH, και DDC μαζί με Cys.

Τα DDC πειραματικά κρασιά (DDC, DDC+CH, DDC+Cys) αρχικά διατηρούσαν ένα μη οξειδωμένο χρώμα, όμως μετά από 4-6 μήνες εμφάνιζαν ένα κοκκινο-καφετί χρώμα. Τα κρασιά μάρτυρας και CH εμφάνιζαν συνεχώς ένα καφετί χρώμα, ενώ τα Cys κρασιά παρουσίαζαν περίπλοκη συμπεριφορά.

Οι παρατηρήσεις του χρώματος και οι χημικές αναλύσεις υπέδειξαν ότι στα τρία DDC πειραματικά κρασιά ελάμβαναν χώρα τα φαινόμενα browning και pinking ενώ στα κρασιά μάρτυρας και CH μόνο το browning.

Τα κρασιά μάρτυρες εμφάνιζαν, μετά 4-6 μήνες, καλύτερο χρώμα σε σχέση με όλα τα πειραματικά κρασιά.

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Chimika Chronika, New Series, 21, 11-30 (1992)

TERNARY COMPLEXES OF Pt(II) AND OTHER METALS WITH NUCLEOSIDES -NUCLEOTIDES AND AMINO ACID-PEPTIDES.

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(Received January 10,1992)

ABSTRACT

This article is a brief review of mixed complexes of metals with aminoacids or peptides and nucleobases-nucleosides or nucleotides as ligands. The bonding sites of these ligands with biological importance in the mixed complexes are described. Particular emphasis is also given to the interactions between the side chains of the aminoacids (peptides) and the aromatic rings or the phosphate groups of the nucleobases,nucleosides or nucleotides both coordinated to the same metal ion,as studied with various spectroscopic and physicochemical techniques. The stabilities of the mixed complexes are due to various forces and are greater than those of the simple complexes of the metal with one of the two groups of ligands. Finally the crystal structures of the mixed complexes in the solid state confirm the existence of inter- or intra-molecular interactions between the side chains of the aminoacids (peptides) and the aromatic rings of the nucleosides (nucleotides), as well as hydrogen bondings between these ligands.

Key words: Mixed complexes,nucleotides,nucleosides,aminoacids,peptides, stability constants,ligand-ligand interactions.

INTRODUCTION

The interaction between nucleic acids and proteins usually lead to the formation of stable complexes. Their stability is due to various forces exercised between the side chain of the aminoacids and the peptide bonds of the proteins on the one hand, and the aromatic rings or the phosphate groups of the nucleic acid chain, on the other. Such are electrostatic

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forces,hydrophobic interactions,hydrogen bonds etc.

It is also known, that such nucleic acid-protein interactions may be created in biological systems, with intervention of metal ions to form ternary complexes[1-3]. The ternary systems of the type metal-nucleoside(-tide)-amino acid (peptide) therefore, constitute the simplest models for the study of the more complex metal-DNA-protein interactions.

The use of the anticancer drug *cis*-DDP is known to be followed by various toxic side effects. These are believed to be due to the interaction of the drug with various other biologically important molecules, especially sulfur containing proteins. The possible formation on the other hand of crosslinks of the type DNA-Pt-proteins of *cis*-DDP in the body, were proposed to be the cause of the toxicological side effects of the drug, since they constitute the 0.15% of its total action[4]. The *trans*-DDP on the other hand, not possessing antitumor properties, forms DNA-Pt-protein crosslinks in a much larger proportion and has greater toxicity than the *cis*-analog[5].It is highly unlike that the antitumor action of *cis*-DDP is due to such a crosslink, although it was proposed as a possible such model earlier[6-8].

The present paper is a brief review of studies *in vitro* of ternary complexes of the type metal ion-nucleoside(-tide)-amino acid(-peptide) that were studied as models for the more general DNA-protein interactions mediated by metals with more emphasis to these of Pt(II) and Pd(II) analogs.

COORDINATION SITES.

The postulated coordination sites of Pt(II), in the DNA-Pt-protein crosslinks, are most propably the sulfur atoms of cysteine or methionine in the proteins, since the Pt-S bonds are more stable and favored[8-9].The coordination sites of DNA on the other hand, are solely the purine and pyrimidine bases.

Studies on the simple ternary systems of the type Pt(II)-nucleoside(--tide)-amino acid (peptide) are very limited. Pd(II) has been used instead as a possible model, because it aquates and reacts 10^5 times faster than Pt(II), though it has generally a similar chemistry, forming square planar complexes.

In this respect, the reaction of the complex Pd(Gly-L-Asp acid) with ATP was studied with ^{1}Hnmr spectroscopy. The purine base was found to

coordinate through either the N_1 or N_7 atoms of its ring[10]. These coordination sites of adenine were also found in the study of the reactions of a complex of Pd(II) with the dipeptide (Gly-L-Tyr) and ATP or ADP. An interaction of the purine ring with the aromatic ring of tyrosine was also observed in this system[11].

Hadjiliadis and Pneumatikakis[12] studied the interaction of a binuclear complex of Pt(II) with the O-Me ester of cysteine of the type $[Pt(O\text{-}MeCys)Cl]_2$ with various nucleosides and found that the purine bases coordinate with Pt(II) through N_7 or N_1 whenever both sites were available (e. g. adenine and guanine at high pH values), while cytidine coordinates through N_3. In all cases, the Pt-N bond was weak, due to the high *trans* influence of the opposite to them Pt-S bonds. The reactions of the complex $PtLCl_2$, where L is S-EtCys or S-MeCys, with nucl=inosine and guanosine produsd complexes of the type $[Pt(L)(nucl)_2]Cl_2$ in which the bases coordinate again through their N_7 atoms[13].

In reactions on the other hand, of *cis*-DDP with nucleobases and aminoacids the ternary complexes *cis*-$[(NH_3)_2Pt(nucleobases)(amacH)](NO_3)_2$ were isolated with the aminoacids coordinated through the $-NH_2$ group in strongly acidic aqueous solutions and the $-COO^-$ group in weakly acidic ones (Nucleobase:1-MeC or 9-MeG, amacH:Gly, L-Ala)[14].

Vestues and Martin[9] studied a series of ternary complexes of Pd(II) with the dipeptides Gly-L-Phe,Gly-L-Tyr,Gly-L-Ala and ATP or ADP. Pd(II) was reacting with both N_7 and N_1 of the purine rings of ATP and ADP with a ratio of 1:3 at the pH range 3 to 8. The H_8 of ATP was shifted more downfield in the complex of the base with Pd(Gly-L-Ala) than in its complex with Pd(Gly-L-Phe). This is due to the short distances between the aromatic rings of the Phe and purine especially when the latter coordinates through N_7 with the metal.

In the ternary complexes between Pd(II), Gly-GlyOEt,Gly-L-Tyr-L-Asp and cytidine, cytidine coordinates through N_3 and the oligopeptides are tridentate ($-NH_2$ terminal, -NH peptide, $-COO^-$ terminal)[15].

The reaction of the dimeric complex of Pd(II) with proline $[Pd(L\text{-}Pro)Cl]_2$ with guanosine and inosine yielded ternary complexes in which the purines coordinate again through N_7 with Pd(II)[16].

Ternary complexes of Pt(II) containing guanine, adenine, pyrimidine and various amino acids studied by Khan *et al*[17], showed again the N_7 atom of the purines and the N_3 of the pyrimidine to coordinate with the metal.

The amino acids were bound through their terminal $-NH_2$ group with the metal.

On the other hand the solid adducts of Ni(II), Cu(II), Co(II) and Zn(II) with glycine and uracil or thiouracil, were characterized with elemental analysis, conductivity and magnetic susceptibily measurements and ir spectra[18]. Thus the 1:1:1 complexes of Ni(II) and Zn(II) with glycine and thiouracil, as well as the ones of the same stoichiometry of Cu(II), Co(II) and Zn(II) with glycine and uracil had octahedral geometry. The corresponding Ni(II) complex of glycine and uracil however was distorted tetrahedral and the one of Co(II) with glysine and thiouracil square planar. In all these complexes[18], glycine was bidentate ($-NH_2$, $-COO^-$), uracil monodentate coordinated through N_3 except the case of Cu(II) where it coordinates through both N_3 and the carbonyl group. Thiouracil in its complexes of Cu(II) and Ni(II) coordinates through the carbonyl group and N_3 again, in its complex with Co(II), through the thiocarbonyl group and N_3 and in its complex with Zn(II), through the thiocarbonyl and carbonyl groups as well as N_3 , most propably thiouracil being tridentate.

In a series of papers, the synthesis of Cr(III)-nucleotide-amino acid complexes were recently reported[19-22]. These were including 5'-AMP, 5'-CMP, 5'-IMP, 5'-GMP, 5'-UMP/cysteine,L-His,L-Glu,L-Ser,L-Met and Gly. The coordination sites of the aminoacids included the $-NH_2$ and $-COO^-$ groups and the ones of the nucleotides the phosphate oxygens.

Phosphate coordination of metals was also found in the cases of Mn^{2+}, Cu^{2+} and Zn^{2+} in the M-ATP-Trp system[23]. Cu^{2+} and Zn^{2+} coordinate to the β- and γ- phosphate group of ATP, while Mn^{2+} coordinates to all three phosphate groups. Also phosphate coordination of Mg^{2+} was found in the case Mg-ATP-Trp[24].

LIGAND-LIGAND INTERACTIONS IN THE METAL-NUCLEOSIDE (-TIDE)-AMINO ACID (-PEPTIDE) SYSTEM.

According to Sigel[25], the stability of ternary complexes of Cu(II) with nucleotides and amino acids depend upon the following factors: (i) neutralization of the charge in the mixed complexes,(ii) steric factors due to either, the presence of bulky substituents or to large chelate rings,(iii) statistical reasons,(iv) formation of π back bonds between the filled d orbitals of the metal and the empty π* orbitals or empty d orbitals of the ligands; this being the most important factor, and (v) the stability in such systems is increased in the case of a strong inte-

raction between the two ligands,both coordinated to the same metal.

Ternary systems of Zn(II), Cu(II) or Mn(II) with ATP and tryptophane were more stable than the ones containing alanine alone, by 0.2 to 0.6 logarithmic units, due to a stacking interaction between the aromatic rings[24]. Also the ternary systems containing ATP and tryptophan were more stable than the binary ones containing only tryptophan, since the $\log K = [\log K_{M(ATP)(L-Trp)} - \log K_{M(L-Trp)}]$ had always positive values, due to the same type of stacking in the former, not existing in the latter[24]. A new band in the UV spectra of the ternary systems not found in the binary ones, may be due to such ligand-ligand interactions[24].

Also the stability constants in the case of AMP, CMP/Ni(II)/L-Ala, L-Trp systems were measured by Orenberg *et al*[26]. In the 1:2 complex of the Ni-AMP binary system, the stability constant was higher than in the Ni-CMP one. This was also true in the 1:2 Ni-(L-Trp) system, compared to Ni-(L-Ala),due to the autostacking existing in the former purely aromatic systems[26]. In the ternary systems, the stability constants followed the order: Ni/AMP/L-Trp>Ni/AMP/L-Ala>Ni/CMP/L-Trp>Ni/CMP/L-Ala.

Davidenko and Maronik[27] measured potentiometrically, the formation constants of ternary complexes of Co(II) with histidine, ATP,ADP and AMP, as well as purine and pyrimidine bases. The stability of these ternary systems increases in the sequence, adenine>uracil>cytosine>guanine for the bases and in the sequence, nucleotide triphosphate>nucleotide diphosphate>nucleotide monophosphate>nucleoside. The same authors[28] also calculated the formation constants of ternary complexes of Cu(II) and Ni(II) with ATP and Gly and compared them with the ones the literature for the corresponding ATP-(L-Ala) and ATP-(L-Try) systems. It was found that they were increasing in the order Gly<L-Ala<L-Try. They also calculated the formation constants of the ternary complexes of histidine, ATP, ADP and AMP, by varying the metal ion, Mn(II), Ni(II), Cu(II), Co(II) and Zn(II)[29-30]. The positive values of $\log K = \log K_{M(L-His)(A)} - \log K_{M(A)}$* show the greater stability of the ternary than the binary systems of the metal and adenosine alone. The stability follows the order Mn(II)<Co(II)<Zn(II)<Ni(II)<Cu(II) for the metals and ATP>ADP>AMP for the nucleotides.

Glycine, not possessing a side chain, is not ecpected to show intera-

*K is the equilibrium constant of the reaction

$$M^{2+} + \text{L-His} + A \longrightarrow M(\text{L-His})A$$

ctions with a nucleotide. It was therefore used for comparison purposes in ternary systems with Mn(II), Co(II), Zn(II) and ATP, ADP and AMP to the corresponding ones with histidine. The latter were 2 to 3 times more stable than the former, due to the existing ligand-ligand stacking interactions[31].

Complexes of Cu(II) with a series of dipeptides with systematically increased aliphatic side chains, were used to study their hydrophobic interactions with ATP in aqueous solutions[32]. It was found that the stability of the ternary systems increases with increasing the aliphatic side chain of the amino acids. Also, the interaction of ATP with the system $Cu(dipeptide)^{+}$, to form the ternary system, decreases the pK_a value of the protonation of the amide hydrogens of the peptides. Deprotonation of the latter modifies the structure of the complex (FIG. 1).

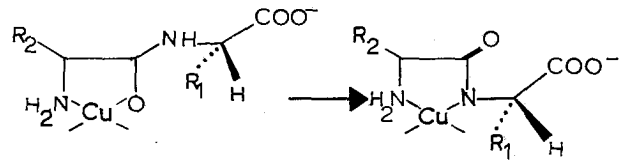

FIG. 1:*Rearrangement of the $Cu(dipeptide)^{+}$ after deprotonation of the amide nitrogen of the dipeptide*[32].

UV-Vis spectroscopy was used to calculate the formation constants of complexes of Zn(II) and Co(II) with aspartic acid, glutamic acid, histidine and ATP. The constants followed the order L-His>L-Glu>L-Asp[33],e.g., were larger in the case of a stronger ligand-ligand interaction.

Vlasova and Davidenko[34] studied the mixed Cu(II) complexes of the type Cu(OR)(Aa), with OR= orotic acid and Aa the anions of the aminoacids Gly, L-Ala, L-Ser, L-n-Val, L-Val, L-n-Leu, L-Pro, L-Phe, L-Tyr and L-Trp and found that the logK of the formation constants of the ternary minus the binary ones, are linear functions of their hydrophobic character (FIG. 2).

Reddy and Reddy[35] calculated the formation constants of the mixed complexes of CMP/Cu(II), Ni(II), Zn(II), Co(II), Mn(II), Mg(II), Ca(II)/ Gly, His, oxalic acid and histamine in aqueous solutions. They found that the formation constants of 1:1:1 mixed complexes were greater than the

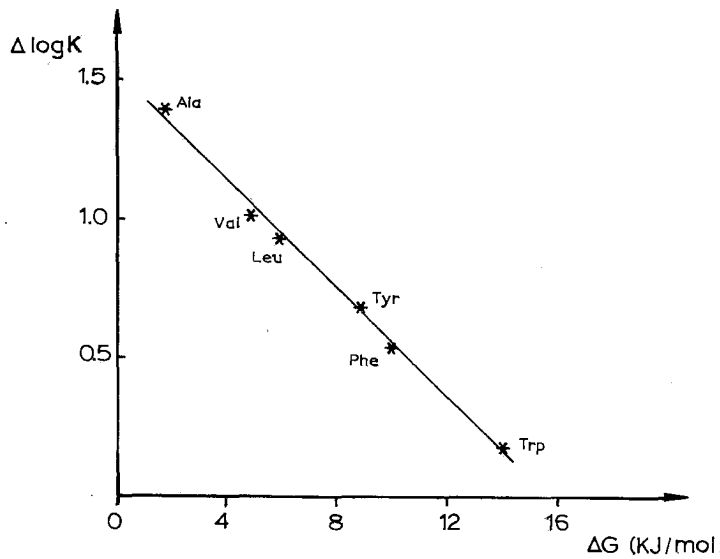

FIG. 2:*Plot of ΔlogK versus ΔG of the mixed complexes of Cu*[34].

binary ones, whenever the second ligand possessed an aromatic ring, due to the stacking interaction between the aromatic rings of the two ligands. Similar results were also found by the same authors[36-37] in ternary systems containing Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Mn^{2+}, Mg^{2+} and Ca^{2+} and xanthosine, inosine as well as histidine, histamine, catechol, glycine and alanine, phenylalanine and tryptophan.

Friedman *et al*[38] studied finally, with pHmetric titrations, the stability constants of mixed complexes of Cu(II), with thymine and glycine, alanine, serine, aspartic acid, phenylalanine, tryptophan and histidine, and of Co(II) and Ni(II) with thymine and glycine alone. They found that the stability of the mixed complexes of Cu(II) with thymine and various amino acids had almost the same order of magnitude (logβ=12.16-13.31).

The equilibrium constants of a "closed" and an "opened" form (FIG. 3) due to stacking interactions, in the ternary complexes of ATP, ITP, UTP/ Zn(II), Mg(II) and L-Tryptophane, bipyridyl and 1,10-phenanthroline, were calculated from the ^{1}Hnmr spectra by Mitcell *et al*[24]. The percentage of the "closed" form decreases as follows:Zn(phen)$(ATP)^{2-}$(>95%)>Zn(bipy)$(ATP)^{2-}$

^{1}Hnmr[40]. Intramolecular hydrophobic interactions between the methylenic groups of the aliphatic side chains of the amino acids and the purine rings, were detected in the "closed" form of the complex, being in equilibrium with the "opened" form (FIG. 4). In the "closed" form, the closed approach of the aliphatic side chain of the amino acids and the aromatic ring of purine is the consequence of a strong interaction between them, while the "opposite" is true in the "opened" form[40]. The strength of these interactions was increasing with increasing the aliphatic side chain[40], which also favor the "closed" form in the equilibrium of FIG. 4.

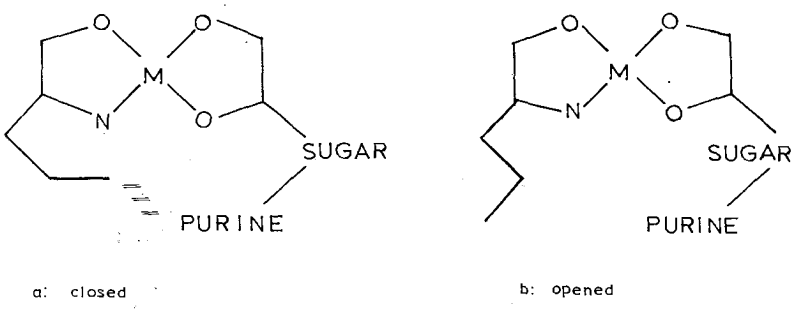

FIG. 4: *(a) The "closed" form of ternary complexes of metals with nucleotides and aminoacids, precenting a ligand-ligand interaction.(b) The corresponding "opened" form with no ligand-ligand interaction.*

Adenine-tryptophan stacking interactions were found to exist in the ternary system ATP/Mn(II)/L-Trp with e.s.r. spectroscopy[41].

Intramolecular interactions between the indol ring of tryptophan and ATP were also observed by Naumann and Sigel[42], when both coordinate with Zn(II) to form ternary systems, with ^{1}Hnmr spectroscopy.

Krattiger *et al*[43] investigated the relative position of the side chains, in a series of dipeptides like Gly-L-Asp, Gly-L-Val, Gly-L-Ileu, Gly-L-Tyr and Gly-L-Phe, in ternary square planar complexes of Pd(II), inosine, IMP and GMP, with ^{1}Hnmr. It was found that the percentage of the h rotamer was increasing in the complexes of the peptides with aliphatic side chain compared with t and g rotamers (FIG. 5). In the h rotamer, the aliphatic chain of the peptide is directed towards the metal ion, above the plane of the square planar complex. In the two other rotamers, this chain is far from the metal ion. It should be noted that in the free dipeptides, the h rotamer was found in smaller percentages[44].

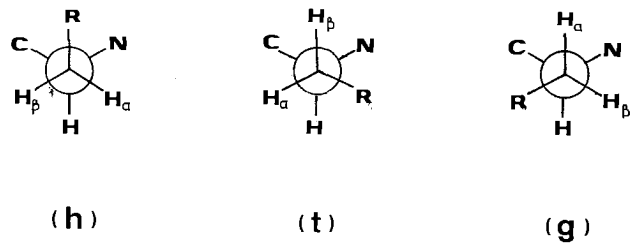

FIG. 5:*The (h), (t) and (g) rotamers around the* $C_{(\alpha)}$-$C_{(\beta)}$ *bonds of the aminoacids.*

Studies of aqueous solutions of the ternary system of Pd(Gly-L-His) and ATP with ^{1}Hnmr and ^{13}Cnmr, showed that a stacking interaction between the purine ring and the imidazole ring of histidine existed[45]. The same was also true for the system ATP/Pd/Gly-L-Phe, investigated by Vestues and Martin[9], where the two aromatic rings of the dipeptide and the nucleoside are almost perpendicular to the square plane of the metal.

Monodentate complexes of glycine and L-alanine with Pt(II), (coordinated through $-NH_2$) and guanosine, inosine (through N_7) or cytidine (through N_3) of the type *cis*-[Pt(amac)(nucl)]Cl_2 were isolated and characterized as solid adducts[46]. The α-protons of glycine were shifted downfield in the ^{1}Hnmr spectra of the Pt-glycine system (0.22 ppm) but did not change significantly when guo was added (0.04 ppm), as compared to the free ligand, showing a guo-glycine interaction.

The systems *cis*-[$(guo)_2$Pd(amac)]Cl, *cis*-[$(guo)_2$Pd(amacH)Cl]Cl and *trans*-[$(nucl)_2$Pd(dipeptide)$_2$]Cl_2, with amacH: Gly, L-Ala, L-Val, L-Ileu, L-Pro and L-Phe, dipeptide: Gly-Gly, Gly-L-Ala, Gly-L-Val and Gly-L-Leu and nucl: ino, guo, were studied as models for the DNA-Pt-protein cross-links, formed *in vitro*, in the presence of both *cis*- and *trans*-DDP[47-49]. The aminoacids were $-NH_2$, $-COO^-$ coordinated in the first series and only $-NH_2$ coordinated in the second series of complexes. The dipeptides were also $-NH_2$ coordinated, and the nucleosides through N_7.

Two main isomers with strong and weak ligand-ligand interactions called "closed" and "opened" forms respectively, were observed with ^{1}Hnmr in D_2O solutions[47-49]. In DMSO-d_6 solutions where diminishing hydrogen bonding and stacking interactions occured, the "opened" form was the only or the

major isomer.The *anti* conformation of the sugar moiety of the nucleosides was found to increase in the ternary systems than the binary Pd-nucleoside ones and even more in the *trans* analog[47]. It was thus concluded that the toxicity of the platinum drugs might be due to DNA-Pt-protein crosslinks[47].

The analogous complexes *cis*-[(ino)$_2$Pd(amac)]Cl, *cis*-[(ino)$_2$Pt(amacH)Cl]Cl and *trans*-[(guo)$_2$Pt(amacH)$_2$]Cl$_2$, with amacH: the same aminoacids as above, coordinated in the same way, were studied with the same aim[49-51]. Only one isomer was found in the *cis* series of complexes at room temperature, while in the *trans* analog, two forms of non equivalent aminoacids coordinated to Pt(II) were observed. A higher percentage of the *anti* conformation of the sugar moiety of the nucleoside was also found in the *trans* ternary systems, compared to the *cis* ones or the binary systems, in accordance with the higher toxicity of the *trans*-DDP, compared to the *cis*-analog[49-51].

Ligand-ligand interactions between the aliphatic part of the aminoacids and the aromatic rings of the guo molecules were observed in all the *cis*- and *trans*-Pd(II) and Pt(II) systems[47-51]. These however were stronger near the bonding sites in the *trans* analogs, and far from the coordination sites in the *cis* ones[47-51].

In a systematic study of the interactions of *cis* and *trans*-DDP with the aminoacids Gly, L-Ala, L-Val, L-Aba, L-Leu and the nucleobases 1-MeC (1-Methylcytosine) and 9-MeG (9-Methylguanine) of the type *cis*-, *trans*-[(NH$_3$)$_2$Pt(amacH)(nucleobase)](NO$_3$)$_2$ (with the aminoacids NH$_2$ coordinated and 1-MeC through N$_3$ and 9-MeG through N$_7$) hydrophobic ligand-ligand interactions of the two ligands were again detected in solution [52], though weaker than in the previous ternary systems of Pt(II) and Pd(II) described above. No indications however, of such interactions could be observed in the crystal structures of *cis*-[Pt(NH$_3$)$_2$(Gly)(1-MeC)](NO$_3$)[52] and *trans*-[(CH$_3$NH$_2$)$_2$Pt(1-MeC)(Gly)](NO$_3$)[53]. This was expected with Gly as ligand. However since the -COO$^-$ group of Gly was found to point out the pyrimidine plane, the situation might be the same if Gly was replaced in this Pt ternary complex, by other aminoacids, which would refain the same conformation. This remains to be seen in future crystal structure determinations of similar complexes.(See FIG. 6).

In the above system a hindered rotation around the Pt-N(3) bond was found by ^{1}Hnmr in the series of the complexes with 1-MeC persisting up to 90°C, but not in the series with 9-MeG, because the -NH$_2$ and -C=O groups

(55%)>Zn(bipy)(ITP)$^{2-}$(48%)>Zn(bipy)(UTP)$^{2-}$(40%). This sequence coincides with that of the binary adducts without the metal ion, where only an aromatic stacking interaction exists. In the case of the Mg(II) complexes, this stacking interaction is the only factor for the observed sequence of the equilibrium constants, whilst other factors may become also important in the case of Zn(II).

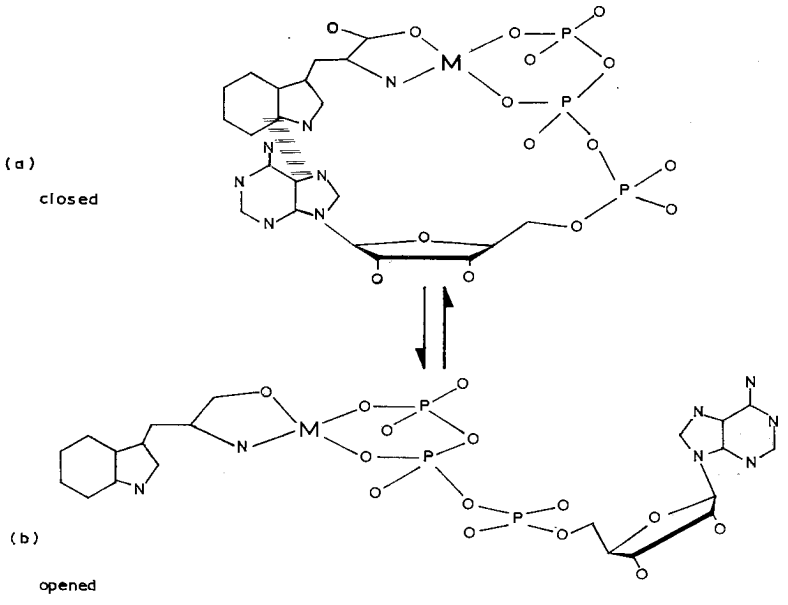

FIG. 3:*(a) The "closed" form of the complex of metals (Mg^{2+},Zn^{2+}) with tryptophan and ATP. (b) The corresponding "opened" form.*

Arena *et al*[39] calculated the thermodynamic parameters of binary complexes of Zn^{2+} and Cu^{2+} with ATP and of ternary complexes of these systems with L-AlaO$^-$ and L-TrpO$^-$. They found larger positive $\Delta H°$ and less positive $\Delta S°$ accompanying formation of [M(ATP)(L-TrpO$^-$)]$^{3-}$ compared with [M(ATP)(L-AlaO$^-$)]$^{3-}$ which they attributed to the presence of ligand-ligand interactions in the former complex.

The influence of the increasing aliphatic side chain in a series of amino acids in their complexes with ATP and Mn(II), Cu(II), Zn(II), Cd(II) and Pb(II) was also studied with various spectroscopic techniques and

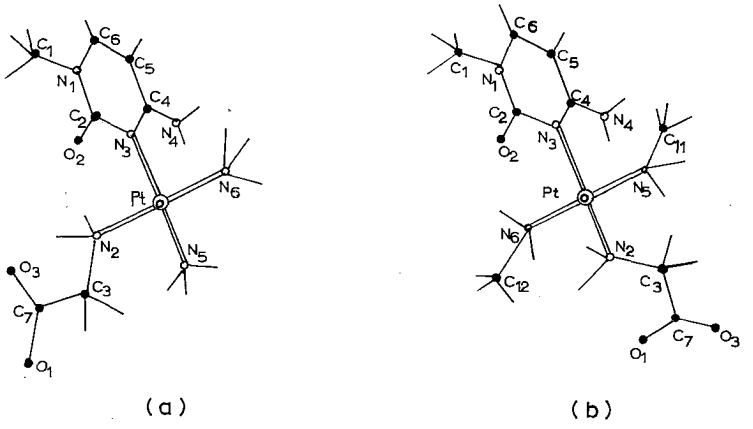

FIG. 6:*The crystal structures of the complexes: (a) cis-*$[Pt(NH_3)_2(Gly)(1\text{-}MeC)](NO_3)$[52] *(b) trans-*$[(CH_3NH_2)_2Pt(1\text{-}MeC)(Gly)](NO_3)$[53].

of the former are ortho to the metal position[52].A slight *cis*⇌*trans* isomerization (2.8-13.5%) was also observed in these systems increasing with temperature[52].

The comparison of the *cis-* and *trans-*$[Pt(NH_3)_2(amac)(nucleobase)](NO_3)$ complexes showed the existence of a much weaker ligand-ligand interaction in the *trans* than the *cis* isomers[53].

In attempts to prepare ternary complexes according to the scheme[54],

cis-$[(NH_3)_2Pt(amac)](NO_3)$ + *nucleobase* → *cis-*$[(NH_3)_2Pt(nucleobase)(amac)](NO_3)$

where, nucleobase=9-MeG, 9-MeA, amac=$-NH_2$,$-COO^-$ chelated aminoacids it was found that the chelated aminoacid could be replaced in many cases, resulting to the isolation of the 1:2 complexes of the type *cis-*$[(NH_3)_2Pt(nucleobase)_2](NO_3)_2$. Thus, the complex *cis-*$[(NH_3)_2Pt(9\text{-}MeA)_2](NO_3)_2$ with both adenine rings coordinated through N_7 was isolated for the first time and its crystal structure solved[55].

It should be noted here that in the vibrational spectra[56] (IR-Raman) of the series of complexes of the type *cis-*$[(NH_3)_2Pt(amac)](NO_3)$, it was found that the $\nu^a_{COO^-}-\nu^s_{COO^-}$ (difference of the asymmetric and symmetric $-COO^-$ stretching) of the aminoacids was increased gradually in the series Gly<

L-Ala<2-L-Aba<L-Val<L-n-Val. This propably reflect the stability of the corresponding complexes. If this were true, then the hydrophobic ligand-ligand interactions existing in the ATP-metal-aminoacids systems should not be the only reason for the larger stability constants observed with increasing aliphatic side chains of the amino acids[24,40], but also the bulkiness of the side chain itself.

Very strong stacking interactions between the purine rings and the imidazole ring of histidine were observed ih the ternary system GHL(Gly-L-His-L-Lys) with Pd(II) and the nucleotides 5'-IMP and 5'-GMP[57]. The C_2-H proton of imidazole shifts upfield by about 1 ppm in the ternary systems, as compared to the Pd-GHL binary system [57,58]. The tripeptide was bound to Pd(II) through the α-amino group, the N_1 of imidazole of histidine and the imino nitrogen atom of the peptide and the nucleotides through N_7[57,58].

Yamauchi and Odani studied the ligand-ligand interactions with ^1H and ^{13}Cnmr spectroscopies and circular dichroism in mixed complexes of Pd(II) with various amino acids[59,60]. Hydrophobic interactions of the aromatic rings and the aliphatic chains of the amino acids were detected and resulted at the greater stabilities of the ternary systems as compared to the binary ones.

Electrostatic interactions between the oppositely charged groups on the side chains of coordinated amino acids, as well as hydrogen bonds between the $-COO^-$ group of histidine and the -OH or $-CONH_2$ groups of asparagine, threonine etc., were detected with ^1Hnmr and circular dichroism, in complexes of Cu(II) and Pd(II), containing these amino acids[61].

Also in complexes of the type Cu(A)(L-B), where A=L, D-His, L-Tyr and B=L-Lys, L-Tyr, L-Trp, L-Phe, L-Ala, L-Val, L-Arg, L-Glu, L-Asn, L-Gln, L-Ser, L-Thr, ligand-ligand interactions were studied. These were detected in the case A=L-His, L-Tyr and B=L-Tyr, L-Trp. Thus, the equilibrium constant of the reaction,

$$Cu(A)(L\text{-}Ala) + Cu(en)(L\text{-}B) \rightleftharpoons Cu(A)(L\text{-}B) + Cu(en)(L\text{-}Ala)$$

had positive values, due to such ligand-ligand interactions. The same was true for the complex Cu(D-His)(L-Phe)[62].

TERNARY SYSTEMS OF METALS/AMINO ACIDS-PEPTIDES/NUCLEOBASES-NUCLEOSIDES, IN THE SOLID STATE.

There are not many crystal structures known of mixed complexes of me-

tals with amino acids-peptides and nucleobases-nucleosides. Gly-Gly was the peptide in most of them. Thus, the structure of the complex (Gly-Gly) $Cu(Cyt).2H_2O$ was elucitated with X-ray diffraction[63,64]. The dipeptide was a tridentate ligand ($-NH_2$, -NH, $-COO^-$) and cytosine was coordinated to the metal through N_3. Two weak interactions of the oxygen atoms at C_2 of cytosine and Cu(II) were found, one intramolecular and one intermolecular. Various hydrogen bonds between the dipeptide and cytosine, nitrogen and oxygen atoms were also found[63,64](FIG. 7).

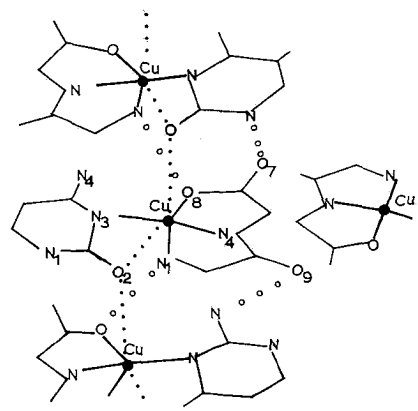

FIG. 7: *Crystal structure of the ternary system Cu(GlyGly)(Cyt)*[64].

Tomita *et al*[65] elucidated also the structure of the ternary complex (Gly-Gly)Cu(Ado), with the adenine coordinated through N_8 and the dipeptide acting again as a tridentate ligand. A strong intermolecular stacking interaction was found between the adenine rings of adjacent molecules. The distance between such rings was 3.8 Å (FIG. 8).

Similarly the structures of the complexes (Gly-Gly)Cu(9-MeAdo)(H_2O).$4H_2O$[66] and (Gly-Gly)Cu(Cyt).$2H_2O$[67] were also elucidated with X-ray diffraction. In both, GlyGly acted as a tridentate ligand, coordinated to Cu(II) via the terminal amino group, the deprotonated nitrogen of the peptide bond and the deprotonated carboxylate group. The C_6-NH_2 group was participating in a strong hydrogen bonding in the crystal structure of the 9-MeAdo derivative[66].

The crystal structure of a ternary complex of Pd(II) with Gly-L-Tyr and

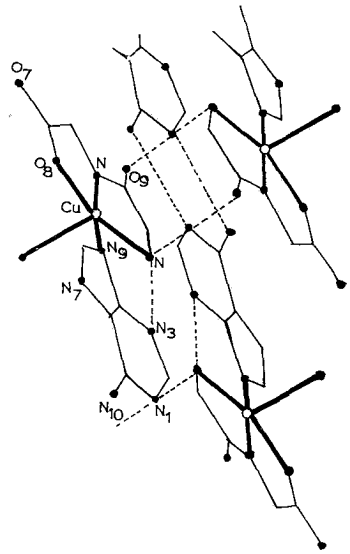

FIG. 8: *Crystal structure of the complex Cu(GlyGly)(Ado)*[65].

Cyd was also elucidated. The dipeptide was again coordinated through $-NH_2$, $-COO^-$ and the deprotonated peptide nitrogen and cytidine through N_3. Inter- and intramolecular stacking interactions were also observed between the aromatic rings of cytidine and tyrosine.

The structures of the ternary complexes *cis*-$[(NH_3)_2Pt(1\text{-MeC})(Gly)](NO_3)$[52], *trans*-$[(CH_3NH_2)Pt(1\text{-MeC})(Gly)](NO_3)$[53] were also recently reported. (See page 11).

The isolation of a Cu(II) complex with GlyGly and 5-fluorouracil was also reported[69]. The dipeptide was again tridentate, bonded through its usual sites, while 5-fluorouracil was bridging two Cu(II) units through its carbonyl groups, as esr, electronic spectra and other techniques revealed[69].

Finally the complexes of Cu(II) with GlyGly and uracil and 6-methyluracil were reported in the solid state, with the N_1 of the uracils and the $-NH_2$, $-COO^-$ and peptide nitrogen of Gly as the suggested coordination sites[70].

CONCLUDING REMARKS.

All the simple models ternary complexes of the type aminoacids(peptide)-M-nucleobase(,-side,-tide), can only roughly represent the more general DNA-M-Protein interactions. However conclusions such as the binding sites and the detection of ligand-ligand interactions even in these very simple systems can easily be extended to the more complex systems and distribute towards their understanding. The binding sites they are surely the same while the ligand-ligand interactions exist.

Thus the limited studies performed on ternary metal complexes of amino acids or peptides and nucleobases, nucleosides or nucleotides, allow by far the following conclusions to be made: (i) The bonding sites of both series of ligands (e.g. aminoacids etc and nucleobases etc) are the usual ones, found also in binary systems. Sulfur is a potential binding site in the sulfur containing amino acids, especially with heavy metals (Pt^{2+}, Pd^{2+} etc) besides the $-NH_2$ and $-COO^-$ terminal groups. The peptides act in a tridentate fashion, using the -NH peptide nitrogen (deprotonated or not) also as a binding site together with the $-NH_2$ and $-COO^-$ groups. The nucleobases on the other hand, use the N_7 site in guanine derivatives, the N_1 and N_7 in adenine derivatives and the N_3 in the pyrimidines. Oxygen carbonyls of pyrimidines can also be used as ligation sites. Finally hard Lewis acid e.g. Mg(II), Zn(II) prefer the phosphate groups of the nucleotides as binding sites. (ii) The simoultaneous coordination of an amino acid etc. or a nucleobase etc. with a metal ion allows strong interactions between them to take place. These were detected mainly with ^{1}Hnmr spectra and stability constant determinations. These interactions are; stacking, hydrophobic or electrostatic (hydrogen bonds etc.), with stronger the former. These exist when the ternary systems contain an aromatic amino acid and manifested with an upfield shift of the protons of the interacting rings, in their ^{1}Hnmr spectra and with the greater stability constants of the complexes, measured. The stability constants are generally greater in the case of the ternary systems with the ligand-ligand interactions present, than in the binary ones. The hydrophobic interactions of the aliphatic chains of the amino acids and the aromatic rings of the nucleobases were stronger near the coordination sites in the *trans-* ternary complexes of Pd(II) and Pt(II) with aminoacids and nucleobases and far from the coordination sites in the *cis-* analogs. Linear relationships were found in

the strength of these interactions and the number of the $-CH_2-$methylenic groups of the aliphatic side chains of the aminoacids. The presence of the amino acids and peptides increases the percentage of the *anti* conformations of the sugar moieties in the ternary *cis*- and *trans*- systems of Pd(II) and Pt(II) with amino acids-peptides and nucleosides and more in the case of the *trans* analogs, thus propably accounting for the greater toxicity of *trans*-DDP than its congener *cis*-DDP (anticancer drug), forming DNA-protein crosslinks to a larger extent.

ΠΕΡΙΛΗΨΗ

Μικτά Σύμπλοκα του Pt(II) και Άλλων Μετάλλων με Νουκλεοζίτες-Νουκλεοτίδια και Αμινοξέα-Πεπτίδια.

Το άρθρο αυτό αποτελεί μία σύντομη ανασκόπιση μικτών συμπλόκων μετάλλων, με υποκαταστάτες αμινοξέα ή πεπτίδια και νουκλεοβάσεις-νουκλεοζίτες ή νουκλεοτίδια. Περιγράφονται οι θέσεις δεσμού των υποκαταστατών αυτών με βιολογική σημασία, στα μικτά σύμπλοκα. Ιδιαίτερη έμφαση δίνεται στις αλληλεπιδράσεις μεταξύ της πλευρικής αλυσίδας των αμινοξέων (πεπτιδίων) και του αρωματικού δακτυλίου ή τις φωσφορικές ομάδες των νουκλεοβάσεων, νουκλεοζιτών ή νουκλεοτιδίων, και των δύο συνδεομένων με το ίδιο μεταλλικό ιόν, όπως μελετήθηκαν με διάφορες φασματοσκοπικές και φυσικοχημικές τεχνικές. Οι σταθερότητες των μικτών συμπλόκων οφείλονται σε ποικίλες δυνάμεις και είναι μεγαλύτερες εκείνων των απλών συμπλόκων του μετάλλου με μία από τις δύο ομάδες των υποκαταστατών. Κρυσταλλικές τέλος δομές των μικτών συμπλόκων στη στερεά φάση επιβεβαιώνουν την ύπαρξη δια- και ενδομοριακών αλληλεπιδράσεων μεταξύ των πλευρικών αλυσίδων των αμινοξέων (πεπτιδίων) και του αρωματικού δακτυλίου των νουκλεοζιτών (νουκλεοτιδίων) όπως επίσης και δεσμών υδρογόνου των υποκαταστατών αυτών μεταξύ τους.

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GLOSSARY

ino: inosine
ado: adenine
cyt: cytosine
9-MeG: 9-Methyl-guanine
amacH: aminoacid(zwiterion form)
AMP: Adenine monophosphate
ADP: Adenine diphosphate
IMP: Inosine monophosphate
GMP: Guanosine monophosphate
UTP: Uracil triphosphate
bipy: bipyridyl
L-Ala: L-alanine
L-Val: L-valine
L-Ileu: L-Isoleucine
L-Ser: L-serine
guo: guanosine
cyd: cytidine
1-MeC: 1-Methyl-cytosine
9-MeA: (-Methyl-adenine
amac: aminoacid (anionic form)
ATP: Adenine triphosphate
CMP: Cytidine monophosphate
ITP: Inosine triphosphate
UMP: Uracil monophosphate
phen: 1,10 phenanthroline
Gly: glycine
L-Pro: L-proline
L-n-Val: L-nor-valine
L-n-Leu: L-nor-leucine
L-His: L-histidine

L-Tyr: L-tyrosine
L-Arg: L-arginine
L-Asp: L-aspartic acid
L-Thr: L-threonine
L-Gln: L-glutamine
L-Aba: L-amino butyric acid
O-MeCys: O-Methyl-cysteine
S-EtCys: S-Ethyl-cysteine
Gly-L-Ala: glycyl-L-alanine
Gly-L-Tyr: glycyl-L-tyrosine
Gly-L-Val: glycyl-L-valine
Gly-L-Ileu: glycyl-L-isoleucine
K: stability constant
L-Lys: L-lysine
L-Glu: L-glutamic acid
L-Phe: L-phenylalanine
L-Met: L-methionine
L-Asn: L-asparagine
L-Trp: L-tryptophan
S-MeCys: S-Methyl-cysteine
Gly-Gly: glycyl-glycine
Gly-L-Phe: glycyl-L-phenylalanine
Gly-L-Asp: glycyl-L-aspartic acid
Gly-L-Leu: glycyl-L-leucine
Gly-L-His: glycyl-L-histidine
Gly-GlyOEt: glycyl-glycyl ethyl ester
Gly-L-Tyr-L-Asp: glycyl-L-tyrosyl-L-aspartic acid
GHL: Gly-L-His-L-Lys: glycyl-L-histidyl-L-lysine
cis or *trans* DDP: *cis* or *trans*-$(NH_3)_2PtCl_2$.

Chimika Chronika, New Series, 21, 31-40 (1992)

PHOTOCATALYTIC REACTION OF DOXYCYCLINE ON ZINC OXIDE POWDER

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SUMMARY

Photocatalytic reaction of doxycycline has been carried under various reaction conditions. The product was isolated and characterized. A tentative mechanism has been proposed for this reaction involving superoxide ion as an oxidant.

Key words : Photocatalytic oxidation, Photocatalytic reaction, Doxycycline, Zinc oxide.

INTRODUCTION

Doxycycline is an antibiotic drug and falls under the broad spectrum of tetracyclines. It is active against both gram-positive and gram-negative bacteria, mycobacteria, mycoplasma, treptonemas. It finds common use in the treatment of respiratory tract infections such as pneumonia and especially chronic bronchitis and mycoplasma pneumonia. It is also used in the treatment of urinary tract, minor staphylococcal, biliary tracts infections and peritonitis.

A great deal of literature survey reveals that photocatalytic oxidation of some organic compounds have been investigated in the presence of zinc oxide[1-5] but nil attention has been paid on photocatalytic reaction of pharmaceutical drugs in particular, doxycycline. However, recently

photo-oxygenation of doxycycline has been carried out by singlet oxygen[6]. Therefore, the present work has been undertaken.

EXPERIMENTAL

0.30 g of doxycycline was dissolved in distilled water (30 ml) in a beaker. 0.25 g photocatalyst was added to this solution. The solution was then irradiated with a tungsten lamp (200 watt) kept at a distance of 20 cm from the upper surface of the reaction vessel for visible light and a u.v. lamp (Toshniwal 366 nm, 125 W) was used for u.v. light. The progress of the reaction was checked with tlc, using the solvent system (n-Butanol : Formic acid : Water = 15:2:3 (v/v) as elute, at regular intervals. After three hours of irradiation, it was found that the tlc of the solution gave two spots; one corresponding to the original doxycycline (Rf = 0.29) and another corresponding to the product (Rf = 0.14). The reaction was allowed to proceed to completion. The light source was removed, when tlc plate showed only one spot corresponding to the product. The solution was then filtered and the filtrate was left for evaporation. A solid product was obtained and it was crystallized from water.

The effect of nature of the photocatalyst on photocatalytic reaction was studied by using different photocatalysts such as titanium dioxide, tungstic oxide, ferric oxide, stannic oxide etc. The results are reported in table-1.

Table 1 : Effect of nature of photocatalyst

Doxycycline = 0.30 g Time of irradiation = 180 min.
Photocatalyst = 0.25 g

Photocatalyst	Band gap (eV)	λ_{max} (nm)	Yield of photoproduct %	
			Visible	u.v.
Fe_2O_3	2.2	564	21.0	1.0
WO_3	2.6	477	14.0	3.0
TiO_2	3.1	400	8.0	8.0
ZnO	3.2	388	6.0	12.0
SnO_2	3.5	354	2.0	16.0

Keeping all other factors identical, the effect of amount of zinc oxide (photocatalyst) has also been observed. All the results are reported in table 2.

Table 2 : Effect of amount of photocatalyst

Doxycycline = 0.30 g Time of irradiation = 180 min.

Amount of ZnO (g)	Yield of photoproduct %	
	Visible	u.v.
0.10	2.0	3.0
0.15	4.0	5.0
0.20	5.0	9.0
0.25	6.0	11.0
0.30	7.0	13.0
0.35	6.0	13.0
0.40	6.0	13.0
0.45	6.0	13.0

The effect of variation of polarity of the solvent on the yield of the photoproduct has been observed and results are reported in table 3.

Table 3 : Effect of polarity of solvent

Doxycycline = 0.30 g Time of irradiation = 180 min.

ZnO = 0.25 g

Solvent	Dielectric constant (D)	Yield of photoproduct %	
		Visible	u.v.
Benzyl alcohol	12.74	0.0	2.0
Butanol	17.10	2.0	3.0
Isopropanol	18.30	3.0	4.0
Propanol	20.10	3.0	4.0
Ethanol	24.30	4.0	6.0
Methanol	32.63	4.0	7.0
Acetonitrile	37.50	1.0	3.0
Water	78.54	6.0	12.0

RESULTS AND DISCUSSION

The photoproduct obtained in the photocatalytic reaction of doxycycline was crystallised and characterised by its elemental analysis, physical, chemical and spectral data.

(i) m.p. = 215°C (decompose)

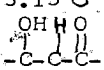
(ii) Colour = Orange

(iii) Elemental analysis = Found C = 61.58%, H = 4.45%, Calculated for $C_{19}H_{16}O_8$; C = 61.12%, H = 4.55%.

(iv) The product gave negative test for nitrogen and amido group but these groups were present in the original substrate.

(v) u.v. - The strong absorption band at 275 nm has been attributed to the presence of the substituted enone moiety in the product.

(vi) i.r. - The bands at 3458, 1314 and 1153 cm^{-1} have been attributed to the presence of the O-H group in the product. The bands at 2962-2853 and 1452 cm^{-1} are due to C-H stretching and bending vibrations of alkyl group, respectively. An intense band at 1631 cm^{-1} may be attributed to the stretching vibration of C=O in the enone moiety.  . The absorption bands at 1690, 3504 and 3403 cm^{-1}, which are due to C=O stretching and N-H stretching vibrations of amido group, have been disappeared in the original. This indicates that amido group has been lost during the photocatalytic oxidation of doxycycline. The presence of the strong bands at 1722 and 1634 cm^{-1} indicate the presence of the cyclic ketone and cyclic alkene, respectively. Whereas, the bands at 1990, 1605, 1567, 750 and 705 cm^{-1} have been assigned to the presence of the aromatic moiety in the product.

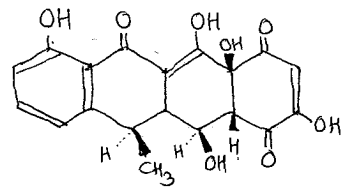
(vii) n.m.r. - The singlet and triplet at 0.88 and 1.48 δ are due to methyl and methylene protons, respectively whereas the signals centred at 1.98 and 2.28 δ are due to the methine protons. The doublet at 3.15 δ shows the presence of the methine proton in  skeleton. The singlet at 4.52, 5.19, 5.34 and 6.72 δ are due to the presence of the alcoholic

presence of TiO_2, ZnO and SnO_2. The reverse trend was observed in u.v. source.In this case, the yield was higher in the presence of TiO_2, ZnO and SnO_2 and relatively lower in the presence of the Fe_2O_3 and WO_3. This shows that the first two semiconductors are more efficient in the visible region, whereas, the last three photocatalysts work more efficiently in the u.v. region. It can explain on the basis that oxides having their $\lambda_{max} > 400$ nm absorb more effectively in visible range and oxide with $\lambda_{max} < 400$ nm absorb efficiently in u.v. region.

The effect of amount of photocatalyst has also been, studied on this reaction. It has been observed that as the amount of photocatalyst was increased, the yield of the photoproduct was also found to increase. However, this increase in the yield was observed only upto a certain amount of the photocatalyst. In the present investigation, the maximum yield was obtained for 0.25 g of the photocatalyst. Any further increase in the amount of photocatalyst showed no increase in the yield of the photoproduct. It indicates that there is a limiting value of photocatalyst above which, the increase in the amount of the photocatalyst will not affect the yield of photoproduct appreciably. This observation may be explained on the basis that in the initial stage, even a small addition of photocatalyst will increase the yield of photoproduct as the surface area of the photocatalyst increases, but after a certain amount (0.25 g), addition of photocatalyst do not affect the yield of the

protons in the product. The signals at 7.17, 7.28 and 7.36 δ have been assigned to the aromatic protons of the phenyl ring. A broad signal which was present in the n.m.r. spectrum of the reactant at 7.75 δ, disappeared in the n.m.r. spectrum of the product. This indicates that $-CONH_2$ group has been lost during the photocatalytic reaction of doxycycline. This has also been indicated by the appearance of a new signal at 5.70 δ, which is due to the olefinic proton in the enone ring. A broad singlet at 1.24 δ which is due to methyl protons of $-N(CH_3)_2$ group has disappeared in the n.m.r. spectrum of the product. This indicate the loss of these two methyl groups attached to the nitrogen atom.

On the basis of the above data, the following structure may be assigned to the product of the photocatalytic reaction of doxycycline.

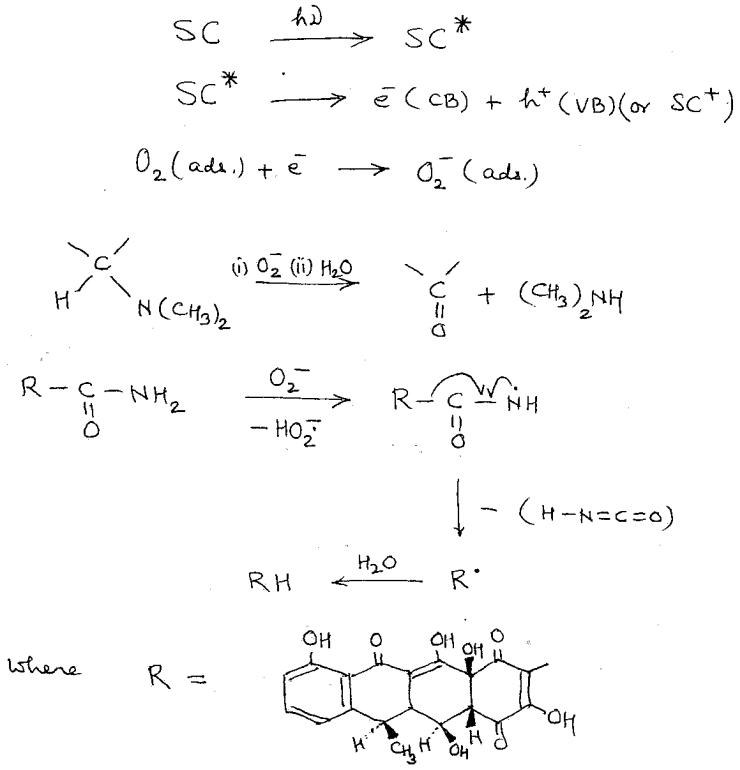


The effect of nature of photocatalyst on the yield of the photoproduct has been observed. It was observed that when the source of visible light was employed for irradiation, the yield of photoproduct increases with the increase in the band gap of semiconductor. The yield of photoproduct was higher in Fe_2O_3 and WO_3 as compared to the yield in

product, because of the fact that at this limiting amount, the surface at the bottom of the reaction vessel becomes completely covered with photocatalyst. Now increase in the amount of photocatalyst will only increase the thickness of the layer of the photocatalyst.

The effect of solvent on the rate of photocatalytic reaction was also observed and for this purpose various solvents of different dielectric constants were used. It was observed that the rate of the reaction increased with the increase in the polarity of the solvent. This suggests that some polar species is involved in this photocatalytic reaction as an intermediate. However, in presence of acetonitrile (inspite of its high polarity), the rate of the reaction was found to be much lower. This may be due to one of the following reasons : (i) Acetonitrile may be adsorbed on the photocatalyst, thereby preventing doxycycline from getting adsorbed on the surface of the photocatalyst and/or (ii) Acetonitrile may form a hydrogen bond with the substrate and this prevents it from occupying an active site on the photocatalyst in desired time limit.

On the basis of the above results and discussions, the following tentative mechanism has been proposed for the photocatalytic oxidation of doxycycline.



ΠΕΡΙΛΗΨΗ

Φωτοκαταλυτική αντίδραση της δοξυκυκλίνης με σκόνη οξειδίου του ψευδαργύρου

Η φωτοχημική αντίδραση της δοξυκυκλίνης έγινε σε διάφορες συνθήκες. Το προϊόν απομονώθηκε και χαρακτηρίστηκε. Προτείνεται προσορινά ένας μηχανισμός για την αντίδραση αυτή, που περιλαμβάνει ιόν υπεροξειδίου ως οξειδωτικό.

Acknowledgement

The authors are thankful to Head, Department of Chemistry, for providing laboratory facilities and to M/S Ranbaxy, India. Thanks are also due to Dr. Neeta Mangal, Miss Preeti Ameta for critical discussion.

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Chimika Chronika, New Series, 21, 41-52 (1992)

EFFECT OF *N*-NITROSATION ON THE ^{1}H AND ^{13}C NMR SPECTRA OF 2-, 3-, AND 4-METHYLAMINOPYRIDINE AND THEIR 1-OXIDE DERIVATIVES

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(Received: February 28, 1992)

SUMMARY

The ^{1}H and ^{13}C NMR spectra of the title compounds show that the presence of the methylamino group in the pyridine ring causes an upfield shift for all the aromatic protons especially in positions o- and p- to this group. However, it causes a downfield shift (10.5 - 23.9 ppm) for the aromatic carbons bearing the group and an upfield shift (11.4 - 20.4 ppm) for the carbons o- and p- to it. Carbons m- to this group are only slightly affected either way.

Upon nitrosation the aromatic protons are shifted downfield (0.4 - 1.4 ppm). The aromatic carbons bearing the nitrosated methylamino group are shifted upfield (3.4 - 10.7 ppm) whilst the carbons o- and p- to the nitrosated group show a downfield shift (3.3 - 18.1 ppm). Carbons m- to this group are only slightly affected. Nitrosation causes also a downfield shift (0.53 - 0.73 ppm) for the methyl protons but for the methyl carbons it causes a small shift either upfield or downfield.

Formation of the 1-oxide derivatives causes an upfield shift for the α- and γ- and a small downfield shift for the β- aromatic carbon atoms. These effects become more pronounced when the 1-oxide derivatives are nitrosated.

Key words: ^{1}H and ^{13}C NMR spectra, *N*-heteroaromatic amines, 1-oxide derivatives, *N*-nitroso derivatives.

INTRODUCTION

Aliphatic nitrosamines were shown, mainly by ^{1}H NMR studies, to have a restricted rotation about the *N-N* bond and to exist in two geometric isomers.[1] The simple aromatic analogues, eg. *N*-methyl-*N*-nitrosoaniline, were shown to exist mainly as the one isomer.[1-3] Similar conclusions were also reached from a study of the ^{15}N and ^{13}C NMR spectra of *N*-nitroso alipha-

tic and aromatic amines.[4]

In view of the interest in the carcinogenic properties of the secondary nitrosamines[5] and following our work on the formation and reactions of *N*-nitroso derivatives of heterocyclic amines,[6] the effect of the *N*-nitroso group on the ^{1}H and ^{13}C NMR spectra of 2-, 3- and 4-methylaminopyridine and their 1-oxide derivatives was examined.

RESULTS

^{1}H Spectra: The ^{1}H NMR spectra of the methylaminopyridines under study show one absorption peak (2.6 - 3.5 ppm) in the aliphatic region corresponding to the methyl protons. In the aromatic region they are of a higher order (6.4 - 8.9 ppm) due to coupling between the aromatic protons. In the case of the 2- and 3-isomers the spectra were analysed as ABCD spin systems whilst in the case of the 4-isomers as A_2B_2 spin systems.

Theoretical spectra were calculated by using the computer program LAOCOON III based on the work of Castellano and Bothner-by[7] as modified by Cooper[8]. However, some subroutines were introduced for the graphic representation of the results.

Chemical shifts and coupling constants of similar compounds[9-11] were taken as approximate values in order to calculate theoretical spectra, bearing in mind that, due to the -I effect and the magnetic anisotropy of the ring nitrogen,[10] the α-protons have chemical shifts at lower field[9] than the other ring protons. Moreover, in the 2-isomers the coupling constant between H-5 and H-6 is expected to be about 5 Hz whilst that between H-3 and H-4 is expected to be about 8.5 Hz.[9] In the case of the 3-isomers, the coupling constant between H-5 and H-6 is expected to be about 5 Hz whilst the coupling constants between H-2 and the other protons are expected not to exceed 3 Hz since no proton is present in position ortho to H-2.[11] Also, a coupling constant of about 8 Hz is expected betweeen H-4 and H-5.[11]

These considerations permitted the assignment of the chemical shifts and the calculation of the coupling constants for all protons in the aromatic region of the methylaminopyridines under study (Tables I, II and III). Chemical shifts of methyl protons have also been recorded.

TABLE I : ^1H Chemical shifts in ppm (δ) and coupling constants in Hz of 2-methylaminopyridine (2-NHMePy), 2-methylaminopyridine 1-oxide (2-NHMePy-Ox) and their *N*-nitroso derivatives (2-N(NO)MePy and 2-N(NO)MePy-Ox respectively).

Solvent	2-NHMePy a	2-NHMePy b	2-NHMePy-Ox a	2-NHMePy-Ox b	2-N(NO)MePy a	2-N(NO)MePy b	2-N(NO)MePy-Ox a	2-N(NO)MePy-Ox b
H-3	6.47	6.12	6.68	6.42	7.86	7.73	7.80	7.56
H-4	7.35	7.13	7.19	7.06	7.83	7.56	7.61	7.43
H-5	6.48	6.29	6.57	6.40	7.27	7.00	7.55	7.41
H-6	8.07	7.87	8.08	7.96	8.43	8.23	8.50	8.36
CH_3	2.87	2.66	2.85	2.85	3.39	3.33	3.32	3.46
J_{34}	8.45	8.44	7.96	8.42	8.51	8.39	8.10	8.07
J_{35}	1.02	0.88	1.19	1.97	1.80	0.90	1.69	1.80
J_{36}	0.67	0.90	0.57	0.60	0.74	0.97	0.20	0.21
J_{45}	7.02	7.13	7.63	7.33	6.72	7.33	7.79	7.70
J_{46}	2.01	1.94	1.36	1.50	1.97	1.88	1.45	1.39
J_{56}	5.06	5.08	6.13	6.58	4.82	4.95	6.67	6.47

a: DMSO-d_6 b: $CDCl_3$

TABLE II : ^1H Chemical shifts in ppm (δ) of 4-methylaminopyridine (4-NHMePy), 4-methylaminopyridine 1-oxide (4-NHMePy-Ox) and their *N*-nitroso derivatives (4-N(NO)MePy and 4-N(NO)MePy-Ox respectively).

Solvent	4-NHMePy a	4-NHMePy b	4-NHMePy-Ox a	4-NHMePy-Ox b	4-N(NO)MePy a	4-N(NO)MePy b	4N(NO)MePy-Ox a	4N(NO)MePy-Ox b
H-2	8.02	8.16	7.95	7.86	8.70	8.67	8.34	8.28
H-3	6.44	6.40	6.60	6.46	7.73	7.53	7.74	7.53
CH3	2.68	2.84	2.74	2.83	3.44	3.40	3.41	3.40

a: DMSO-d_6 b: $CDCl_3$

TABLE III : ^1H Chemical shifts in ppm (δ) and coupling constants in Hz of 3-methylaminopyridine (3-NHMePy), 3-methylaminopyridine 1-oxide (3-NHMePy-Ox) and their *N*-nitroso derivatives (3-N(NO)MePy and 3-N(NO)MePy-Ox respectively).

Solvent	3-NHMePy a	3-NHMePy b	3-NHMePy-Ox a	3-N(NO)MePy a	3-N(NO)MePy b	3-N(NO)MePy-Ox a
H-2	8.20	7.99	7.65	8.93	8.83	8.56
H-4	6.32	6.79	6.58	8.10	7.92	7.36
H-5	7.15	7.02	6.98	7.60	7.44	7.57
H-6	7.96	7.88	7.54	8.64	8.59	8.17
CH_3	2.80	2.74	2.78	3.52	3.47	3.40
J_{24}	2.91	2.88	2.01	2.73	2.63	1.99
J_{25}	0.62	0.65	0.23	0.65	0.72	0.35
J_{26}	0.00	0.00	1.77	0.00	0.00	1.46
J_{45}	8.30	8.29	8.55	8.36	8.30	8.51
J_{46}	1.38	1.39	1.00	1.44	1.46	1.41
K_{56}	4.64	4.68	6.04	4.70	4.66	6.05

a: DMSO-d_6 b: $CDCl_3$

^{13}C Spectra: Assignment of the chemical shifts of the carbons of the methylamino pyridine nucleus was done using the fully coupled spectra since the magnitudes of the ^{13}C-^1H coupling constants in other pyridine systems are already known.[12] The assignment was based on the observation that whilst the coupling constants in the aromatic systems along two or four bonds ($^2J_{CCH}$ or $^4J_{CCCCH}$) are relatively small (1 - 2 Hz), the coupling constants along three bonds ($^3J_{CCCH}$) are much larger (7 - 12 Hz).[13] In the *N*-heteroaromatic systems, such as pyridine derivatives, the coupling constants along two bonds ($^2J_{CCH}$) can also become significant especially when they are near the ring nitrogen[12] (e.g. $^2J_{C\beta H\alpha}$). For example, in the case of 3-methylaminopyridine, apart from a direct coupling of C-2 with H-2, coupling of C-2 with H-4 ($^3J_{C2H4}$ = 4.4 Hz) and with H-6 ($^3J_{C2H6}$ = 11.2 Hz) was also observed in agreement with previous observations on similar compounds.[12] However, C-2 shows no coupling with H-5 (<1 Hz).

On the other hand, the more complex group of absoptions was attributed to C-6 which is expected to show an additional splitting because of coupling with H-5 ($^3J_{C6H5}$ = 4 Hz). Likewise C-5 is coupled with H-6 ($^3J_{C5H6}$ = 8.1 Hz) and shows no detectable coupling with H-4 or H-2 (<1 Hz) whilst C-4 is coupled with H-2 ($^3J_{C4H2}$ = 4.8 Hz) and with H-6 ($^3J_{C4H6}$ = 6 Hz). Thus the ^{13}C chemical shifts of 3-methylaminopyridine follow the order δ3>δ6>δ2>δ5>δ4 which is in agreement with that found for 3-aminopyridine.[14]

In some cases difficulties in assigning chemical shifts can arise when coupling with longer distance hydrogen can be stronger than expected. For example, in the case of 2-methylaminopyridine 1-oxide, although C-6 can be differentiated from C-4 because of its more complex appearance, C-5 cannot be easily differentiated from C-3 because they both show similar splittings. Therefore, assignment of the chemical shifts was based on the assumption that the order of absorptions for C-3 and C-5 (β-carbons) in the case of 2-methylaminopyridine 1-oxide is similar to that of 2-methylamino-pyridine, since the introduction of an oxygen on the ring nitrogen has very little effect on the chemical shifts of β-carbons of the pyridine nucleus.[15]

Assignment of the chemical shifts of the aromatic carbons of the methylaminopyridines under study (Tables IV, V and VI) was based on the above considerations. Chemical shifts of methyl carbons have also been recorded.

TABLE IV : ^{13}C Chemical shifts in ppm (δ) of 2-methylaminopyridine derivatives

	2-NHMe-Py	2-N(NO)Me-Py	2-NHMe-Py-Ox	2-N(NO)Me-Py-Ox
C-2	160.1	154.9	151.5	148.1
C-3	106.5	112.6	105.3	123.5
C-4	137.2	138.3	127.9	126.4
C-5	112.4	121.5	111.1	125.9
C-6	148.1	148.1	137.2	140.6
CH_3	28.8	27.8	28.6	32.6

TABLE V : ^{13}C Chemical shifts in ppm (δ) of 4-methylaminopyridine derivatives.

	4-NHMe-py	4-N(NO)Me-py	4-NHMe-py-Ox	4-N(NO)Me-py-Ox
C-2,6	149.6	151.1	138.5	140.0
C-3,5	107.2	111.5	107.8	114.2
C-4	154.7	148.5	150.0	139.3
CH_3	29.2	29.0	29.5	29.4

TABLE VI : ^{13}C Chemical shifts in ppm (δ) of 3-methylaminopyridine derivatives.

	3-NHMe-py	3-N(NO)Me-py	3-NHMe-py-Ox	3-N(NO)Me-py-OX
C-2	135.3	140.2	127.5	136.9
C-3	145.9	138.7	148.3	141.8
C-4	117.9	126.1	111.4	114.7
C-5	123.8	124.0	125.5	126.1
C-6	137.5	148.2	124.0	129.8
CH_3	29.9	30.9	29.9	29.9

DISCUSSION

The *N*-nitroso-*N*-methylaminopyridines, like the *N*-nitroso-*N*-methylaniline,[1,2,4b] are expected to exist in the form of one isomer only with the methyl group *cis*- to the nitroso oxygen. This is evidenced by the single line resonance of the methyl protons or the methyl carbons and by the observation that proton and carbon resonances in the aromatic region correspond to those of one isomer only.

The inductive and the mesomeric effects of the substituents are considered to be mainly responsible for the ^{13}C chemical shift differences observed between the aminopyridines under study. These differences can be discussed[16] in terms of equation (1) were δ_k is the ^{13}C chemical shift of carbon k,C_k is the ^{13}C chemical shift corresponding to the parent com-

pound (pyridine[15] or pyridine 1-oxide[15]), Δ_{ik} is the chemical shift increment to carbon k due to the substituent (i.e. methylamino- or *N*-nitrosomethylamino-) in position i.

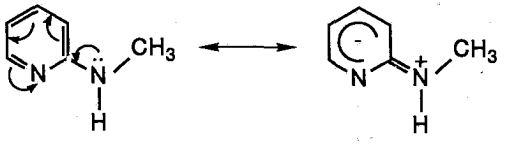
$$\delta_k = C_k + \Delta_{ik}(z_i) \qquad (1)$$

In the case of the methylaminopyridines the values of Δ_{ik} (Table VII) are strongly negative (strong shielding) for carbons in the o- and the p-position relative to the methylamino group (for which the -I and +M effects are operative) whilst for carbons in the m- position the values are slightly positive or negligible (slight deshielding). For example, in the case of 2-methylaminopyridine C-3 and C-5 are strongly shielded (Table VII).

TABLE VII : Empirical parameters (increments) for the calculation of ^{13}C chemical shifts for the substituted aminopyridines according to equation (1).

		2-NH_2-py	2-NHMe-py	2-N(NO)Me-py	2-NHMe-py-Ox	2-N(NO)Me-py-Ox
	Δ_{22}	+11.3	+10.5	+5.3	+12.4	+9.0
	Δ_{23}	-14.7	-17.0	-10.9	-20.4	-2.2
i=2	Δ_{24}	+2.3	+1.6	+2.7	+1.8	+2.4
	Δ_{25}	-10.6	-11.1	-2.0	-14.6	+0.2
	Δ_{26}	-0.9	-1.5	-1.5	-1.9	+2.8
		3-NH_2-py	3-NHMe-py	3-N(NO)Me-py	3-NHMe-py-Ox	3N(NO)Me-py-Ox
	Δ_{32}	-11.9	-14.3	-4.4	-11.6	-2.2
	Δ_{33}	+21.5	+22.4	+15.2	+22.7	+16.2
i=3	Δ_{34}	-14.2	-17.7	-9.5	-14.7	-11.4
	Δ_{35}	+0.9	+0.3	+0.6	-0.2	+0.5
	Δ_{36}	-10.8	-12.1	-1.4	-15.1	-9.3
		4-NH_2-py	4-NHMe-py	4-N(NO)Me-py	4-NHMe-py-Ox	4N(NO)Me-py-Ox
	Δ_{42}	+0.9	0.0	+1.5	-0.5	+0.9
	Δ_{43}	-13.8	-16.3	-12.0	-17.9	-11.5
i=4	Δ_{44}	+19.6	+19.1	+12.9	+23.9	+13.2
	Δ_{45}	-13.8	-16.3	-12.0	-17.9	-11.5
	Δ_{46}	+0.9	0.0	+1.5	-0.5	+0.9

For the carbons bearing the methylamino group, the values of Δ_{ik} (Table VII) are strongly positive (strong deshielding) because this carbon is depleted of its electrons since it is attached to the positively charged methylamino nitrogen (e.g. structures Ia - Ib). These results are similar to those for the primary aminopyridines[12] (Table VII), but in the case of the methylaminopyridines the methyl group causes through its +I effect, a small upfield shift which is more pronounced for the o-carbons to the group but less pronounced for the p- and the m- carbons (Table VII). For example, in the case of 3-methylaminopyridine C-2 and C-4 show respectively an upfield shift of 2.4 and 3.5 ppm, whilst C-5 and C-6 show an upfield shift of 0.6 and 1.3 ppm when compared to the corresponding chemical shifts of 3-aminopyridine (Table VII).

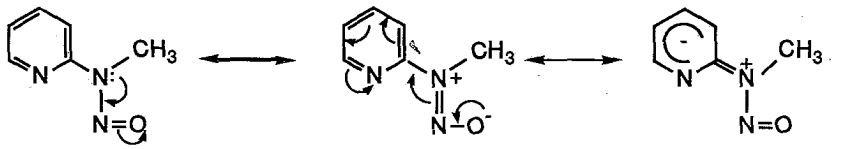


(Ia) (Ib)

In the case of the 2-isomers where the carbon bearing the methylamino group is next to the ring nitrogen (e.g. 2-methylaminopyridine) the deshielding of C-2 is less pronounced than in the case of the 3- and 4-isomers, presumably because it can withdraw electrons from the ring nitrogen. This is shown by the lower values of Δ_{ik} (Table VII) observed in the case of the 2-isomers (*cf.* structures Ia - Ib).

The introduction of the nitroso group causes a downfield shift for practically all aromatic carbons except for those bearing the nitrosomethylamino group for which an upfield shift of 5.2, 7.2 and 6.2 ppm was observed (Tables IV, V and VI) for 2-, 3- and 4-(*N*-nitroso)methylamino pyridine, respectively. This is attributed to additional resonance structures due to the presence of the nitroso group, which competes with the aromatic ring for the lone pair of electrons of the exocyclic amino nitrogen. For example, in

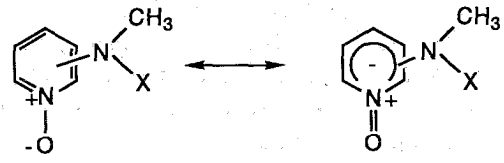
the case of 2-(*N*-nitroso)methylaminopyridine structures (IIa) and (IIb) cause a smaller shielding for C-3 and C-5 and a slightly greater deshielding of C-4 than in the case of 2-methylaminopyridine (Table IV). C-6 is practically unaffected by the presence of the nitroso group presumably because of the proximity of the ring nitrogen (*cf.* structures IIa - IIc).



(IIa) (IIb) (IIc)

The methylaminopyridine 1-oxide derivatives show an upfield shift for C-2, C-4 and C-6 and a small downfield shift for C-3 and C-5 (Tables IV, V and VI) when compared to the methylaminopyridines, presumably because of resonance structures (IIIa - IIIb). These structures show an increase in the electron density mainly in the α- and γ-positions of the pyridine nucleus, since the *N*-oxide moiety can act also as an electron donor group.[15,17] These effects are more pronounced upon nitrosation of the methylaminopyridine derivatives (Tables IV, V and VI).

The introduction of the nitroso group on the methylamino group causes a definite downfield shift of the methyl protons (0.56 - 0.73 ppm). However, it causes a small upfield or downfield shift of the methyl carbons depending on the position of the methylamino group in the pyridine ring (Table IV, V and VI).



(IIIa) (IIIb)

X = -H or -NO

EXPERIMENTAL

NMR Spectra were obtained with a Varian FT-80A spectrometer in deuterated chloroform and dimethyl sulphoxide-d_6 (Stohler Isotope Chemicals). Chemical shifts were measured from TMS. The effect of solvent on the ^{13}C chemical shifts was negligible.

Materials: 2-Methylamino-,[17] 3-methylamino-[18] and 4-methylamino-pyridine;[19] 2-methylamino-*N*-nitroso-,[20a] 3-methylamino-*N*-nitroso-[21b] and 4-methylamino-*N*-nitroso-pyridine;[20a] *N*-methyl-*N*-nitrosoaniline;[22] 2-methyl-amino,[23] 3-methylamino-,[24] 4-methylamino-pyridine 1-oxide;[25] 2-methylamino-*N*-nitroso-,[21a] 3-methylamino-*N*-nitroso-[21b] and 4-methylamino-*N*-nitroso-pyridine 1-oxide[21a] were prepared by known methods.[26] 2-Amino,[20b] 3-amino-[21b] and 4-amino-pyridine[20b] (Fluka; purum) were purified as described in the references cited.

ΠΕΡΙΛΗΨΗ

Επίδραση της *N*-Νιτρώδωσης στα φάσματα NMR ^1H και ^{13}C της 2-, 3- και 4-μεθυλαμινο-πυριδίνης και των αντίστοιχων 1-οξειδίων.

Τα φάσματα NMR ^1H και ^{13}C της 2-, 3- και 4-μεθυλαμινο-πυριδίνης και των αντίστοιχων 1-οξειδίων δείχνουν ότι η εισαγωγή της μεθυλαμινο

ομάδας στον πυριδινικό δακτύλιο προκαλεί μετατόπιση των απορροφήσεων των αρωματικών υδρογόνων προς υψηλότερες τιμές πεδίου, ιδιαίτερα σε θέσεις ο- και π- ως προς την εισαγόμενη ομάδα. Εν τούτοις, οι απορροφήσεις των αρωματικών ανθράκων, στους οποίους έχει γίνει η υποκατάσταση, παρουσιάζουν μετατόπιση προς χαμηλότερες τιμές πεδίου (10.5 - 23.9 ppm) ενώ οι απορροφήσεις των ο- και π- ανθράκων ως προς την εισαγόμενη ομάδα παρουσιάζουν μετατόπιση προς υψηλότερες τιμές πεδίου (11.4 - 20.4 ppm). Οι απορροφήσεις των μ- ανθράκων ως προς την εισαγόμενη ομάδα μετατοπίζονται ελάχιστα και προς τις δύο κατευθύνσεις.

Με την νιτρώδωση παρατηρείται μετατόπιση των αρωματικών υδρογόνων προς χαμηλότερες τιμές πεδίου (0.4 - 1.4 ppm). Ταυτόχρονα οι απορροφήσεις των υποκατεστημένων αρωματικών ανθράκων μετατοπίζονται προς υψηλότερες τιμές πεδίου (3.4 - 10.7 ppm) ενώ των ο- και π-ανθράκων ως προς την εισαγόμενη ομάδα προς χαμηλότερες τιμές πεδίου (3.3 - 18.1 ppm). Και σε αυτήν την περίπτωση οι απορροφήσεις των μ-αρωματικών ανθράκων ως προς την εισαγόμενη ομάδα μετατοπίζονται ελάχιστα. Με τη νιτρώδωση παρατηρείται επίσης μετατόπιση των υδρογόνων του μεθυλίου προς χαμηλότερες τιμές πεδίου (0.57 - 0.73 ppm) ενώ η μετατόπιση του άνθρακα του μεθυλίου που παρατηρείται είναι και πολύ μικρή και προς τις δύο κατευθύνσεις.

Η δημιουργία των 1-οξειδίων έχει σαν αποτέλεσμα την μετατόπιση προς υψηλότερα πεδία των απορροφήσεων των α- και γ- αρωματικών ανθράκων και μικρή μετατόπιση προς χαμηλότερες τιμές πεδίου των β-ανθράκων. Τα φαινόμενα αυτά γίνονται εμφανέστερα στα *N*-νιτρωδωμένα παράγωγα.

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EFFECT OF METHYL AND METHOXY GROUP ON THE IONISATION CONSTANTS OF 2-, 3- AND 4-AMINOPYRIDINIUM IONS.

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(Received: February 28, 1992)

SUMMARY

The pK_a values of 2-, 3-, 4-amino- and of the corresponding methylamino- and dimethylamino-pyridinium ions together with the pK_a values of their 1-methyl- or 1-methoxy- derivatives are reported. The results are discussed qualitatively in terms of steric hindrance to solvation, charge separation, steric inhibition of resonance and the inductive effect of the substituent groups.The pK_a values of (2-pyridyl)trimethylammonium and of 3-aminocollidinium ion are also reported and their implications are discussed. Where appropriate, the pK_{a1} values are shortly discussed in similar terms.

Key words: Ionisation constants, aminopyridinium ions, 1-methyl- or 1-methoxy-derivatives.

INTRODUCTION

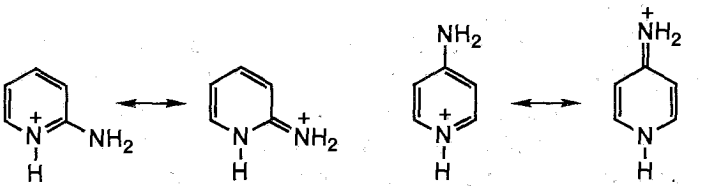
The present work reports the influence of methyl or methoxy group on the pK_a's of 2-, 3- and 4-aminopyridinium ions when born by the ring nitrogen. It reports also the effect of the methyl group when introduced on the exocyclic amino group. The results show that in most cases the methyl group decreases the basicity of the substituted aminopyridinium ions. Such a decrease is rather unexpected since in these cases the methyl substituent, which is attached to a more electronegative site, is expected to exert its +I effect.[1] On the other hand the diminishing effect of the methoxy group is consistent with its -I effect. Although all amino groups, especially in the 2- or 4-position of the pyridine ring, cause a substantial increase in the basicity of the ring nitrogen, the trimethylammonium group in position-2 causes a substantial decrease.

RESULTS AND DISCUSSION

(A). Protonation of the exocyclic amino group (pK_{a2}):

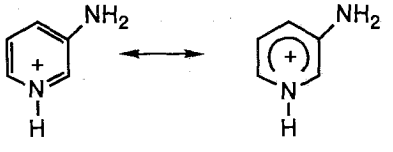
(1). Subsituent on the pyridine ring:

(a) Proton: The pK_{a2} values (Table) show that the 2- and the 4-aminopyridinium ions are much less basic (by 5 - 7 units) than the 3-aminopyridinium ions because in the case of the 2- and the 4-isomers, in which a resonance effect is mainly operative, the exocyclic amino group becomes positively charged and therefore more difficult to protonate than in the case of the 3-isomers in which an inductive effect is mainly operative (compare structures (Ia - Ib) and (IIa - IIb) with (IIIa - IIIb)). Also the 2-aminopyridinium ions (Ia - Ib) have lower basic strengths than the 4-isomers (IIa - IIb) since a greater repulsion of the positive charges in the dications



(Ia) (Ib) (IIa) (IIb)

of the 2-isomers is expected. For example, the pK_{a2} of 2-aminopyridinium ion is -8.39 whilst that of 4-aminopyridinium ion is -6.56 at 20°C (Table).



(IIIa) (IIIb)

TABLE : Ionisation constants of aminopyridine derivatives.

Pyridine derivative	t°/ C[a]	pK_{a1}	pK_{a2}	Spread[b] (±)	Conc. (10^5M)	A.w.l.[c] nm
2-NH_2-	2	-	-8.61	0.10	10.0	300
	20	-	-8.39	0.05	10.0	300
	20	6.86[d]	-	-	-	-
2-NH(Me)-	20	7.13[e]	-	0.04	11.1	328
	20	-	-8.56	0.06	10.0	306
2-$N(Me)_2$-	20	7.19	-	0.06	7.5	330
	20	-	-8.48	0.08	10.0	320
1-Me-2-NH_2-	2	-	-9.35	0.13	18.0	300
	20	-	-9.20	0.04	9.0	300
1-Me-2-NH(Me)-	20	-	-9.56	-	28.0	312
1-Me-2-$N(Me)_2$-	20	-	-6.24	0.07	10.0	328
1-MeO-2-NH_2-	2	-	-10.47	0.08	10.0	305
2-$N^+(Me)_3$-	20	-	-4.79	0.09	9.0	255
3-NH_2-	20	6.07[f]	-	0.05	-	-
	20	-	-1.38[g]	0.02	11.3	315
3-NH(Me)-	20	6.41[g]	-	0.03	5.22	267
	20	-	-1.63	0.04	39.1	340
3-$N(Me)_2$-	20	6.67[g]	-	0.03	21.6	300
	20	-	-1.91[g]	0.01	21.6	340
1-Me-3-NH_2-	2	-	-1.50	0.04	20.0	320
	20	-	-1.60	0.05	20.0	320
1-Me-3-NH(Me)-	20	-	-1.85	0.03	20.0	346
1-Me-3-$N(Me)_2$-	20	-	-2.25	0.10	20.0	360
3-NH_2-2,4,6-$(Me)_3$-	2	8.49	-	0.05	10.0	315
	2	-	-0.55	0.04	12.0	305
4-NH_2-	20	9.29[h]	-	0.02	-	-
	2	-	-6.84	0.05	5.0	260
	20	-	-6.56	0.06	5.0	260
4-NHMe-	20	9.65[h]	-	0.01	-	-
	20	-	-7.14	0.11	4.0	276
4-$N(Me)_2$-	20	9.70[h]	-	0.11	-	-
	20	-	-7.11	0.09	4.0	284
1-Me-4-NH_2-	2	-	-6.42[k]	0.04	5.0	269
	20	-	-6.14	0.11	5.0	269
1-Me-4-NHMe-	20	-	-6.77	0.13	4.0	278
1-Me-4-$N(Me)_2$-	20	-	-6.70	0.10	5.0	286
1-MeO-4-NH_2-	2	-	-7.34[k]	0.10	4.0	272

[a] The determination of some pK_a values at two temperatures, for comparison purposes, showed that the effect of a rise in temperature is unpredictable.

[b] The largest deviation between the negative logarithm of the average K_a value and any other value in the set.

[c] Analytical wavelength.

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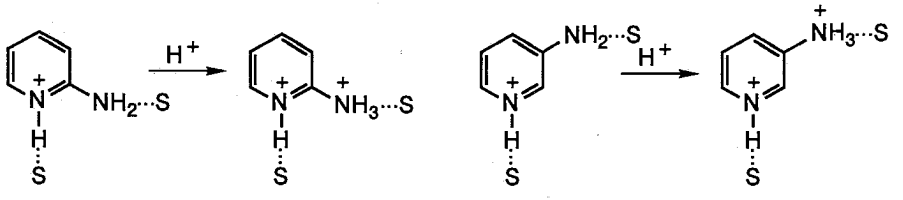
[h] Ref. 6a.

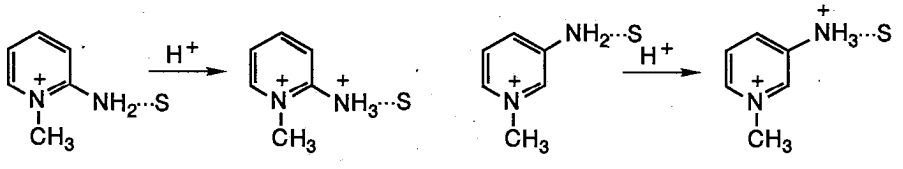
[k] Ref. 9 (temperature corrected)

(b) Methyl: The introduction of a methyl instead of a proton on the ring nitrogen causes a decrease in the basic strength of the 2- and, to a lesser extent, of the 3-aminopyridinium ions. In the relatively dilute solutions of perchloric acid in which the 3-aminopyridinium ions are protonated, solvation by water is predominant since water must be present in great excess, as in the case of solutions less than 50% w/w sulphuric acid.[2] However, in solutions in which the 2- and 4-aminopyridinium ions are protonated, i.e. solutions containing more than 70% w/w sulphuric acid, the amount of the bisulphate anion becomes much greater than the amount of water.[2] Solvation then may be effected by water and by the bisulphate anion, although as a nucleophile the bisulphate is about 100 times weaker than water.[3] In any case, solvation of the aminopyridinium ions by the solvent can be viewed as causing some deprotonation of the ring nitrogen,[4] i.e. causing attenuation of the positive charge. The introduction of a methyl on the ring nitrogen disrupts solvation and therefore increases the repulsion of the positive charges in the dications. This repulsion seems to outweigh the +I effect of the methyl more in the case the 2-isomers than in the case of the 3-isomers. This is probably due not only to the greater distance between the positive charges in the dications of the 3-isomers, but also to a greater reduction of their charge repulsion because of substantial solvation of the exocyclic amino group by water, which in these solutions must be in great excess[2] (compare structures (IVb) with (VIb) and (Vb) with (VIIb)). Thus a decrease of 0.81 and 1.00 units is observed when comparing the pK_{a2} values of 2-amino- and 2-methylamino-pyridinium ion with those of

the respective 1-methyl-2-substituted ions. Likewise, a decrease of 0.22, 0.22 and 0.34 units is observed when comparing the pK_{a2} values of 3-amino-, 3-methylamino- and 3-dimethylamino-pyridinium ion with the respective 1-methyl-3-substituted ions (Table).



(IVa) (IVb) (Va) (Vb)



(VIa) (VIb) (VIIa) (VIIb)

S = Solvent

When methyl groups are introduced on the ring carbons instead of the ring nitrogen, an increase in the value of the pK_{a2} is observed. Thus, the pK_{a2} of 3-aminocollidinium ion is greater than that of 3-aminopyridinium ion by about 0.8 units, indicating that the methyl groups attached to the ring exert mainly their inductive and field effect.

In the case of the 4-isomers the trend observed for the 2-isomers is reversed. Thus an increase of 0.42, 0.37 and 0.41 units is observed in the pK_{a2} values of 1-methyl-4-amino-, 1-methyl-4-methylamino- and 1-methyl-4-

dimethylamino-pyridinium ion when compared with those of 4-amino-, 4-methylamino- and 4-dimethylamino-pyridinium ion, respectively. It seems therefore that the +I effect of the methyl outweighs the charge repulsion of the dications of the 4-isomers because, in contrast to the case of the 2-isomers, the distance between the charges is greater. Although the disruption of solvation must be more pronounced for the 4-isomers, since protonation takes place in less acidic solutions in which more water is available for solvation,[2] the +I effect of the methyl is therefore still dominant.

(c) Methoxy: The introduction of a methoxy group on the ring nitrogen decreases the pK_{a2} values not only in the case of the 2- but also in the case of the 4-aminopyridinium ion by 1.86 and 0.50 units at 2°C, respectively (Table). The methoxy group, like the methyl, is expected to disrupt solvation of the protonated ring nitrogen but, unlike the methyl group which exerts a +I effect, the methoxy group exerts a -I effect. This should reinforce the charge repulsion in the dications. Therefore, a net decrease in the pK_{a2} values is observed which, because of the proximity of the positive charges, is much greater in the case of the 2-isomer than in the case of the 4-isomer (Table).

(2) Substituent on the amino group:

Methyl: A decrease in the pK_{a2} values is observed when a methyl is introduced on the exocyclic amino group. A decrease of 0.17 and 0.58 units is observed when comparing the pK_{a2} values of 2- and 4-amino-pyridinium ion with those of 2- and 4-methylamino-pyridinium ion, respectively (Table). This is presumably due to a decrease in solvation of the amino group upon methylation causing an increase in the charge repulsion in the dication which more than outweighs the +I effect of the methyl. The decrease in the pK_{a2} values is greater in the case of the 4-methylaminopyridinium ion presumably because the unsubstituted amino group in the case of the 4-isomers is more solvated and therefore more affected by methylation than in the case of the 2-isomers since less acidic solutions are required to protonate the 4-isomers. The decrease in the pK_{a2} values upon methylation of the exocyclic amino group is enchanced (from 0.17 to 0.36 units) in the case of the 1-methyl-substituted 2-isomers, but it remains almost the same (0.63 units) in the case of the 1-methyl-substitued 4-isomers (Table). This must be due to a greater increase in the charge repulsion in the case of the desolvated dications of the 1-methyl-substituted 2-isomers.

The introduction of a second methyl on the exocyclic amino group in

the case of the 2- and 4-isomers does not cause a further decrease in the basic strength of the dimethylamino-pyridinium ions. Thus the pK_{a2} values of 2- and 4-dimethylamino- and 1-methyl-4-dimethylamino-pyridinium ion are almost the same (slight increase) as those of the respective 2-and 4-methyl amino-derivatives (Table).

Protonation of the 3-aminopyridinium ions becomes more difficult upon progressive methylation of the exocyclic amino group (Table). Since the amount of free water in the acid solutions in which protonation takes place is in great excess and since there is no resonance between the exocyclic amino group and the ring nitrogen in the 3-aminopyridinium ions (structures (IIIa-b)) the disruption in the solvation of the amino group upon progressive methylation should increase the charge repulsion in the dications progressively. In all cases this repulsion appears to outweigh the +I effect of the methyl group. Thus, in contrast to the case of the 2- and 4-isomers, the introduction of a second methyl on the exocyclic amino group decreases further the pK_{a2} values of the 3-isomers (Table).

(B) Protonation of the ring nitrogen (pK_{a1}):

The pK_{a1} values (Table) show clearly that the methyl group exerts a +I effect in all cases. Thus an increase of 0.27, 0.34 and 0.36 units in the respective pK_{a1} values of 2-, 3- and 4-aminopyridine is observed upon methylation of the exocyclic amino group. An increase in the basicity is also observed when methyls are introduced on the pyridine carbons, as for example in the case of 3-aminocollidine which is more basic than 3-aminopyridine by about 2.4 units (Table).

Methylation of the exocyclic amino group must also increase the residual positive charge on the amino nitrogen of the mono-protonated molecules because of desolvation (*cf.* structures (Ib) and (IIb)). The increase therefore in the pK_{a1} values due to the +I effect of the methyl is somewhat smaller in the case of the 2-isomers than in the case of the other isomers because of the proximity of the positive charges (compare structures (Ia-b) with (IIa-b)). The introduction of a second methyl on the exocyclic amino group causes only a slight increase (~0.06 units) in the pK_{a1} values of the 2- and 4-isomers. However, it causes a greater increase (0.26 units) in the pK_{a1} values of the 3-isomers. Further methylation of the dimethylamino group produces the trimethylammonium group which when attached to the 2-position of the pyridine nucleus decreases the pK_a value (protonation of

the ring nitrogen) substantially. Compared to pyridine a decrease of about 10 units is observed, i.e. from a pK_a of 5.32 of pyridine[5] to a pKa of -4.79 of (2-pyridyl)trimethylammonium ion at 20°C. Since there can be no resonance between the trimethylammonium group and the ring nitrogen the pK_a value of (2-pyridyl)trimethylammonium ion may be taken as an indication

of the pK_{a2} values of the 2-aminopyridinium ions which would have been observed if only repulsive forces were predominant.

(C). Steric effects

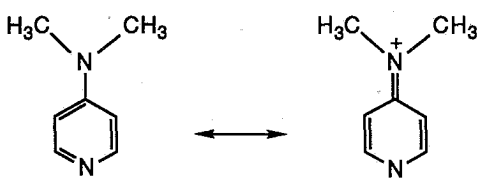
When a second methyl is introduced on the exocyclic amino group an increase in the pK_{a2} values of the 2- and 4-isomers is observed instead of the expected decrease, as in the case of the 3-isomers. The magnitude of the increase depends on the position of the amino group in the pyridine ring.

Although no steric hidrance has been proposed for the unsubstituted 4-dimethylaminopyridines[6] the small increase in the pK_{a2} values of 2- and 4-dimethylaminopyridinium ion and 1-methyl-4-dimethylaminopyridinium ion, as compared to those of the respective methylaminopyridinium ions, suggest that the introduction of a second methyl on the amino group renders effective some steric inhibition of resonance. This may be due to the lack of complete coplanarity of the dimethylamino group with the pyridine nucleus causing the positive charge of the protonated ring nitrogen to become less effectively transmitted to the exocyclic amino nitrogen in the monocations thus enhancing protonation of the amino group. Steric inhibition of resonance becomes very pronounced, however, in the case of 1-methyl-2-dimethylaminopyridinium ion, which is most probably sterically hindered and has a pK_{a2} value higher than that of 1-methyl-2-methylamino-pyridinium ion by 3.32 units (Table).

In contrast to the above the introduction of a second methyl on the exocyclic amino group of the 3-isomers lowers further the pK_{a2} values of the respective dimethylaminopyridinium ions. Since steric inhibition of resonance cannot become operative between the amino group and the ring nitrogen in the 3-isomers, because of lack of resonance between them, further desolvation of the amino group due to the introduction of the second methyl enhances the charge repulsion in the dications. Thus the pK_{a2} values of 3-dimethylamino- and of 1-methyl-3-dimethylamino-pyridinium

ion are lower by 0.28 and 0.40 units respectively than those of the respective methylamino-pyridinium ions (Table).

Steric inhibition of resonance becomes evident also when considering the pK_{a1} values of the 2- and 4-isomers. As mentioned above the introduction of a second methyl on the exocyclic amino group causes a slight increase in the pK_{a1} values of 2- and 4-dimethylamino-pyridinium ion as compared to those of 2- and 4-methylamino-pyridinium ion presumably because, due to steric inhibition of resonance, the transmission of electrons from the methyls to the ring nitrogen through resonances (structures (VIIIa) and (VIIIb)) is reduced. In the case of the 3-isomers, however, in which only inductive effects are operative the increase of the pKa_1 values from 3-methylamino- to 3-dimethylamino-pyridinium ion is more substantial.



(VIIIa) (VIIIb)

EXPERIMENTAL

Materials.

2-Amino- (Fluka, puriss), 4-amino- (Fluka, purum) and 4-dimethylamino-pyridine (Aldrich) were sublimed twice at 40°/0.1 Torr, 80°/0.2 Torr and 58°/0.03 Torr, respectively.

1-Methyl-3-methylamino-,[7] 1-methoxy-2-amino-,[8] 1-methyl-4-amino-,[9] 1-methyl-4-methylamino-,[10] 1-methyl-4-dimethylamino-[7] (δ_{ppm}: $-N(CH_3)_2$: 3.17, $^{+}N-CH_3$: 3.90 (DMSO-d_6) and 1-methoxy-4-amino-pyridinium perchlorate[9], as well as 3-methylaminopyridine[10] and 3-aminocollidine[11] were prepared as in the references cited.

2-Methylaminopyridinium perchlorate: Prepared by dissolving 2-methylaminopyridine (obtained as in ref. 12) in diethyl ether and then adding the required amount at 70% perchloric acid (Fluka) with stirring. The precipitate obtained by diluting with ether-chloroform (50 : 1) was recrystallised (charcoal) from methanol-chloroform (1 : 40) to give the perchlorate salt, m.p. 76 - 78°.

2-Dimethylaminopyridinium perchlorate: Prepared as described above from 2-dimethylaminopyridine (Aldrich). The precipitate was recrystallized (charcoal) from absolute ethanol to give the perchlorate salt, m.p. 98 - 100°.

4-Methylaminopyridinium perchlorate: Prepared as described above from 4-methylaminopyridine (obtained as in ref. 13). The precipitate was dissolved in absolute ethanol and the perchlorate salt was obtained by the addition of diethyl ether, m.p. 108 - 110°.

1-Methyl-2-amino-pyridinium perchlorate: Prepared by reacting 2-amino- and 2-methylaminopyridine respectively with methyl toluene-p-sulphonate. The product was treated as described in ref. 9 to give the perchlorate salt, m.p. 225 - 227°.

(2-Pyridyl)trimethylammonium perchlorate: Prepared by reacting 2-dimethylaminopyridine with methyl toluene-p-sulphonate. The product was treated as described in ref. 9 to give the perchlorate salt which was finally recrystallized from water, m.p. 211 - 212°, (δ_{ppm}: $^{+}N(CH_3)_3$: 3.59 (DMSO-d_6)).

1-Methyl-3-amino-, 1-methyl-2-methylamino- and *1-methyl-3-dimethylamino-pyridinium perchlorate:* Prepared by reacting 3-amino-, 2-methylamino- and 3-dimethylamino-pyridine with methyl iodide. The products were treated as described in ref. 7, m.p. respectively 88 - 89,° 117 - 118° and 127 - 128° (δ_{ppm}: $-N(CH_3)_2$: 3.06, $^{+}N-CH_3$: 4.25 (DMSO-d_6)).

1-Methyl-2-dimethylaminopyridinium perchlorate: Prepared by heating 2-bromopyridine (1.46 g) with methyl iodide (1.96 g) under reflux for 20h. 1-Methyl-2-bromopyridinium iodide (1.3 g, 47%), m.p. 201 - 203° (decomp.), (δ: $^{+}N-CH_3$: 4.42 (DMSO-d_6)) obtained was washed with chloroform and after drying under vacuum was boiled for 0.5h with a 33% ethanolic solution of dimethylamine (1.23 ml). Boiling was continued for another 4h after the addition of more dimethylamine solution (0.65 ml). After the addition of sodium carbonate (0.236 g) the reaction mixture was evaporated to dryness under vacuum, extracted with hot chloroform which was dried with calcium

chloride. The solid obtained after evaporating the solvent was dissolved in absolute ethanol, purified with charcoal and then precipitated by the addition of diethyl ether to give 1-methyl-2-dimethylaminopyridinium iodide (0.73 g, 64%), m.p. 95°. This product (0.7000 g) was dissolved in water (7.8 ml) and silver perchlorate (0.5495 g) was added. After filtration of the silver iodide the filtrate was condensed to a viscous liquid under vacuum. The solid obtained by the addition of ethyl acetate was purified by dissolution in a mixture of chloroform and absolute ethanol and treated with charcoal. Addition of ethyl acetate gave 1-methyl-2-dimethylaminopyridinium perchlorate as a white solid (0.45 g, 72%), m.p. 68-69° (δppm: $-N(CH_3)_2$: 3.09, $^{+}N-CH_3$: 4.01 (DMSO-d_6)).

The results of the elemetal analysis (C, H and N) of all compounds used in the present work were satisfactory.

pK_a Determinations.

These were carried out spectrophotometrically.[14] U.v. spectra were recorded on an SP 1800 Pye Unicam and a DMS-90 Varian Spectrophotometers fitted with a jacketed cell assembly to keep the temperature constant at 2° or at 20°C. Solutions of amines in aqueous sulphuric acid were used in all cases except for the following determinations: (a) pK_{a1} of 2-dimethylaminopyridine for which sodium hydroxide-sodium dihydrogen orthophosphate buffers were used, (b) pK_{a1} of 3-aminocollidine for which sodium hydroxide-potassium dihydrogen orthophosphate and tris(hydroxymethyl)aminomethane-perchloric acid buffers were used, and (c) pK_{a2} of 1-methyl-3-amino- and 1-methyl-3-methylamino-pyridinium ion as well as the pK_{a2} of 3-aminocollidinium ion for which perchloric acid solutions were used.[15] Weight per cent sulphuric acid and molarities of perchloric acid were determined by titration against standard alkali solutions. The H_0 values of sulphuric acid solutions at 2° or 20°C were estimated according to ref. 16.

Calculation of pK_{a1} and pK_{a2} values.

These were carried out by using equation (1)

$$pK_a = H_0 + \log [(d_M - d)/(d - d_I)] \quad (1)$$

were d_M and d_I are the optical densities of the aminopyridine or

aminopyridinium ion and of the corresponding fully protonated form, respectively, and d is the observed optical density of the amines in the various suphuric acid, perchloric acid or buffer solutions. In every case the appropriate acid or buffer solution was used as a reference. Attempts to determine the d_I value of the fully protonated 1-methyl-2-methylamino-pyridinium ion in solutions of suphuric acid mixed with oleum, failed because substantial changes were observed in the u.v. spectra after ca. 3mins. Therefore in this case the pK_a value was determined from equation (2) and from a plot of d against $(d_M\text{-}d) / h_o$.

$$d = K_a [(d_M - d) / h_o] + d_I \qquad (2)$$

Determination of pH.

pH values were determined by using a Philips 9414 pH meter.

ΠΕΡΙΛΗΨΗ

Προσδιορίσθηκαν οι pK_a των 2-, 3-, 4-αμινο- και των αντιστοίχων μεθυλαμινο- και διμεθυλαμινο- ιόντων του πυριδωνίου καθώς και των 1-μεθυλο- ή 1-μεθοξυ-παραγώγων. Η ποιοτική ερμηνεία των αποτελεσμάτων γίνεται με βάση την στερεοχημική παρεμπόδιση της επιδιαλύτωσης και της μεσομέρειας λόγω των μεθυλίων που φέρει η αμινομάδα, την ανάπτυξη απωστικών δυνάμεων μεταξύ ομώνυμων φορτίων καθώς και με βάση το επαγωγικό φαινόμενο που αναπτύσσεται στα μόρια αυτά. Επίσης προσδιορίσθηκαν οι pK_a του (2-πυριδυλ)τριμεθυλαμμωνίου και 3-αμινοκολλιδινίου και τα αποτελέσματα συσχετίζονται με τα προηγούμενα. Με ανάλογο τρόπο σχολιάζονται και οι τιμές των pK_{a1}.

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Chimika Chronika, New Series, 21, 67-73 (1992)

SHORT PAPER

TRACE ELEMENTS IN GUM MASTIC OF CHIOS

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ABSTRACT

The mastic gum is a natural resin product of Pistacia Lentiscus v. Chia. This product is well known because of its therapeutic properties.

It was found that this resin contains trace elements such as zinc, iron, cobalt, magnesium and manganese to which it is believed that the therapeutic properties may be ascribed.

Apart from the determination of the trace elements, the dissolution of these elements under gastric conditions, in vitro, is also studied.

Key words: Gum mastic of Chios, Trace elements.

INTRODUCTION

Gum mastic of the island Chios is a natural resin exudate obtained from the stem of the tree Pistacia Lentiscus v. Chia of the family Anacardiacae which has been cultivated in the southern part of the island for centuries[1].

The mastic is obtained after a nick on the stem of the trees. This product is named **"in lacrimis"** and mainly consists of resinous substances, terpenic acids[2] and essential oils[3]. After the separation of the essential oils, a non-smelling **"pulp"** is remained. The colour of this varies from yellow to dark brown.

The most important commercial use of mastic is for chewing either in the form "in lacrimis" or in the form of sugared pills.

Although gum mastic has been an important material of commerse for hundreds of years, its chemistry has received little attention.

In contrast with the limited number of the scientific works, there are many references in practical books on various uses of mastic. Its healing properties are known from the time of Dioscourides[4]. Today it is used for the preparation of plasters, bandages[5] and in solutions for healing. Mastic was also suggested in the therapy of gastric and duodenal ulcer[6].

Mastic is also investigated as an exepience for preparation of pills with slow release of active substance[7,8].

These facts prompted us to correlate its therapeutic properties etc, with the presence of trace elements. Because as it is well known, the deficiency of some fundamental trace elements causes serious disorders in humans[9-11]. These disorders are usually eradicated by supplements of trace elements preparations or with food rich in the deficient elements.

In this work we have determined the trace elements of mastic of Chios and we have studied the released quantity of them under gastric conditions, in vitro.

EXPERIMENTAL

Reagents: Zinc, iron, cobalt, magnesium and manganese solutions of a concentration of 1000 ppm were prepared by dissolving the appropriate amounts of salts (Aldrich Europe, gold label) in a small volume of acid, mainly HCl (Merck Pro Analysi) and then by diluting to one liter with 1% (v/v) HCl with deionized water. I.e. zinc standard stock solution was prepared by dissolving 1,0000 g of zinc metal in a minimum volume of (1+1) HCl and by diluting to one liter with 1% (v/v) HCl.

Apparatus: A Perkin-Elmer Model 403 atomic absorption spectrophotometer equipped with a deuterium background corrector was used for flame measurements.

A Metrohm 654 pH-meter was also used the pH measurements.

Operating parameters: The operating parameters are summarized in Table I.

Determination of zinc, iron, cobalt, magnesium and manganese in gum mastic samples.

The determination of these trace elements is performed either in whole mastic samples by using dry ashing or wet ashing decomposition procedures or in vitro conditions employing buffer systems.

A. In the first use a quantity of about 10 g accurately weighed of "in lacrimis" mastic or "pulp" mastic is ashed at 600°C or dissolved in HNO_3 1:1. The undissolved residue is separated by filtration and the filtrate is used for the analysis.

B. In vitro conditions stomach and bowel is followed the procedure.

i: Under stomach conditions: an amount of about 10 g of pulverised mastic sample

TABLE I. The operating parameters of atomic absorption spectrophotometer

Element	Zn	Fe	Co	Mg	Mn
Wave lengh	213.9 nm	248.3 nm	240.7 nm	285.2nm	279.5nm
Slit Setting	4	3	3	4	3
Light Source	EDL*	HCL**	HCL	HCL	HCL
Oxidant-Pressure	Air-75 psi	Air-80psi	Air-80psi	Air-75psi	Air-80psi
Fuel-Pressure	Acetylene-25psi	Acetylene-30psi	Acetylene-30psi	Acetylene-25psi	Acetylene-30psi

* EDL = Electrodelles Discharge Lamps

** HCL = Hollow Cathode Lamp

accurately weighed is mixed with 10.0 ml of 0.1 N HCl in a beaker. The beaker is placed on the water bath and thermostated at 37°C for 4 h. The pH of the mixture was 1.0-1.2. After that the mixture is filtered and the filtrate is used for the trace elements analysis.

ii: Under bowel conditions: we follow the same procedure but we adjust the pH at 7.2±0.1 using phosphate buffer (250 ml KH_2PO_4 0.2 M and 175 ml NaOH 0.2 M in 1000 ml H_2O).

iii: Under successive stomach and bowel conditions: a quantity ~10 g of pulverised sample is accurately weighed and thermostated at 37°C for 4 h. The pH of the mixture is adjusted at 1.0 - 1.2 using ~10 ml of 0.1 N HCl. Then the mixture is filtered and the filtrate is used for the trace elements analysis under stomach conditions.

The precipitate is then quantitavely transferred in a beaker and the pH is adjusted at 7.2±0.1 using the same as above phosphate buffer solution. The mixture is then thermostated at 37°C for 4 h. After that the mixture is filtered and the filtrate is used for the trace elements analysis under bowel conditions.

RESULTS AND DISCUSSION

The results of the qualitative and quantitative analyses and the mean values of mastic of Chios concenring the trace elements are shown in Tables II and III. The elements Zn, Fe, Co, Mg and Mn are all essential trace elements.

From these elements the dietary value and biochemical role of iron is very well known[12]. Although the content of iron in mastic is low it could be useful for the cases of long marginal deficiencies[13].

Zinc is also a well known essential trace element. It is a component in many enzymes,

TABLE II. Determination of trace elements Zn, Fe, Co, Mg, Mn in $\mu g\,g^{-1}$ with AAS.

Mastic "in lacrimis"					Mastic "pulp"		
Samples	Trace elements	Ashing (Mean value)[a]	Stomach condition (Mean value)[b]	Bowel condition (Mean value)[b]	Ashing (Mean value)[a]	Stomach condition (Mean value)[b]	Bowel condition (Mean value)[a]
I	Zn	3.07±0.18	3.20±0.24	-	2.41±0.13	3.38±0.21	1.81±0.11
	Fe	2.26±0.14	2.64±0.31	-	2.15±0.14	4.69±0.36	6.43±0.28
	Co	4.35±0.21	4.88±0.26	-	3.18±0.17	3.10±0.12	12.40±0.61
	Mg	2.68±0.17	2.25±0.12	1.53±0.14	1.59±0.14	1.53±0.14	3.18±0.23
	Mn	-	-	-	1.80±0.10	1.65±0.13	1.95±0.21
II	Zn	4.65±0.13	3.18±0.26	0.31±0.06	3.12±0.17	3.27±0.23	0.31±0.04
	Fe	1.40±0.11	2.66±0.19	1.98±0.10	3.64±0.14	1.52±0.10	4.00±0.23
	Co	3.88±0.17	4.88±0.26	0.60±0.08	1.91±0.10	1.21±0.09	9.86±0.43
	Mg	2.75±0.14	2.25±0.12	1.91±0.09	1.59±0.13	1.88±0.11	2.96±0.23
	Mn	0.79±0.04	-	0.49±0.07	4.39±0.32	1.76±0.15	-
III	Zn	4.80±0.21	3.08±0.19	1.72±0.10	1.47±0.14		
	Fe	1.47±0.14	3.55±0.17	1.28±0.09	1.78±0.10		
	Co	1.78±0.16	1.55±0.12	0.24±0.04	2.43±0.15		
	Mg	6.89±0.41	3.23±0.31	1.68±0.10	1.84±0.13		
	Mn	0.78±0.05	0.38±0.06	0.32±0.05	4.08±0.24		
IV	Zn	4.64±0.18	2.61±0.15	1.65±0.11	2.89±0.18		
	Fe	6.62±0.36	2.78±0.24	1.76±0.09	1.28±0.10		
	Co	5.89±0.43	4.54±0.39	0.76±0.04	2.35±0.16		
	Mg	5.84±0.31	4.20±0.26	2.13±0.12	1.73±0.13		
	Mn	0.73±0.05	0.21±0.04	0.26±0.03			

[a] eight determinations ± SD [b] six determinations ± SD

TABLE III. Mean values of the determination of Trace Elements in Mastic of Chios ($\mu g \cdot g^{-1}$)

Mactic "in lacrimis"				"Mactic pulp"
Trace elements	Ashing	Stomach condition	Bowel condition	Ashing
Zn	4.29	3.02	0.92	2.47
Fe	2.94	2.91	1.26	2.21
Co	3.98	3.96	0.40	2.47
Mg	4.54	2.98	1.81	1.69
Mn	0.58	0.15	0.27	2.57

in insulin etc.[14,15]. Zinc deficiency causes severe disorders in growth, taste, smell, healing, metabolism of carbohydrates etc. The recommended safe and adequated dietary intakes for adults per day is about 18 mg. This amount of zinc is usually taken by food but it is possible to become deficient in the case of pregnands, elderly, alkoolism or in people whose diet is very rich in fibres[15,16]. Also deficiencies have appeared in patients under special pharmaceutical treatments[17] and in the cases of long parenteral nutrition or malnutrition[18]. Zinc and copper deficiency have been also appeared in large groups of people whose every day consumed foods (e.g. milk) contain low quantity of zinc or copper[19]. In these cases mastic should be considered as a very useful natural supplement of nutrition.

The presence of zinc in mastic justifies its used healing ointments (prepared scientifically or not) as healing of gastric ulcer[6] and the decreasing the level of sugar in blood[20].

The magnesium in mastic is also interesting because its deficiency related to some heart disorders, spasm, tremor etc.[21]. The daily requirement of Mg for an adult per day is 300-350 mg. This requirement is increased in the cases of pregnancy, alcoolism, treatments with diouretics etc. Deficiencies also appeared in the areas where the drinking water is very soft.

Less important is the presence of cobalt. The amount of cobalt in an 1 g of mastic is much more than the daily requirements (10 μg). Cobalt is a constituent of vitamine B_{12}, (about 4,0% of the weight) and its functions are closely related to vitamine B_{12}. Slight amounts noted in some very strict vegetarious[10,11].

Experiments which have been carried out in our laboratory showed that after 30 min chewing the trace elements are released from the gum and are available for absorption. When

mastic is suspended in acid solution comparable to that of the stomach also all the trace elements are released from it.

These observations show that mastic could be a natural source of trace elements and could be used to provide them to humans in the case of very slight deficiencies of Zn, Mn, Co and even Fe and Mg.

This product is better than others commercially available, e.g. pills because of it's a natural product tested for ages.

ΠΕΡΙΛΗΨΗ

Ιχνοστοιχεία στη μαστίχη της Χίου

Η μαστίχη της Χίου είναι μία φυσική ρητίνη που προέρχεται από το φυτό Pistacia Lentiscus v. Chia, το οποίο καλλιεργείται αποκλειστικά στις νότιες περιοχές της νήσου Χίου. Είναι γνωστή από την αρχαιότητα για τις θεραπευτικές της ιδιότητες ενώ σήμερα έχει χρησιμοποιηθεί για την πρόληψη του έλκους στο γαστρεντερικό σωλήνα και στην παρασκευή δισκίων βραδείας αποδέσμευσης.

Η μαστίχη που λαμβάνεται αμέσως μετά από τη χάραξη του κορμού και των βλαστών του φυτού ονομάζεται "in lacrimis", "δάκρυ", ενώ μετά την απομάκρυνση του αιθερίου ελαίου λαμβάνεται μια άοσμη συμπαγής μάζα γνωστή ως "Pulp", "πούλπα".

Με τη χρησιμοποίηση φασματοφωτομέτρου ατομικής απορρόφησης (AAS) προσδιορίστηκαν τα ιχνοστοιχεία Zn, Co, Fe, Mg, Mn στη μαστίχη της Χίου. Μελετήθηκε επίσης το ποσοστό αποδέσμευσης αυτών in vitro, σε συνθήκες στομάχου (pH = 1,2±0,1) καθώς επίσης και σε συνθήκες εντέρου (pH=7,2±0,1).

Τα αποτελέσματα θα μπορούσαν να δικαιολογήσουν τις θεραπευτικές ιδιότητες της μαστίχης.

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Chimika Chronika, New Series, 21, 75-79 (1992)

SYNTHESIS AND CHARACTERIZATION OF NEW CATIONIC RHODIOUM(I) COMPLEXES WITH DIOLEFINS AND PHOSPHINE SELENIDES AS LIGANDS

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(Received November, 16,1990)

INTRODUCTION

The research activity in phosphine selenide coordination chemistry has not reached the extent of that of phosphine oxides and sulfides[1].

Relatively few phosphine selenide complexes have been[2-6] described until now. Although the rhodium(I) organometallic complexes with diolefins and phosphine and arsine oxides as ligands have been previously reported[7,8]. The corresponding chemistry of phosphine selenides is even more limited and is confined to two brief reports[9,10].

Our work on rhodium selenide compounds has recently[10] started by reporting the preparation and some physical data, concerning three complexes of triphenylphosphine selenide. In order to complete the existing work on this field, as well as to examine the ability of $[LRh\langle\rangle]^+$ (L=cycloocta-1,5-diene (COD), cyclooctatetraene (COT), norbornadiene (NBD)) species to act as Lewis acids towards different selenides we prepared several phosphine selenide complexes.

Key words: phosphine selenides, diolefins, $J(^{31}P—^{77}Se)$ Coupling Constants

EXPERIMENTAL

Physical measurements

Elemental analysis(C,H) were obtained with a Perkin Elmer 240 Analyser.

Infrared spectra were recorded on a Perkin Elmer 1430 ration recording spectrophotometer (over the range 4000-200 cm^{-1}) and samples prepared as KBr discs. Conductivities were measured on a WTW LF 530 conductivity bridge. The ^{31}P NMR spectra (in $CDCl_3$) were run in a Jeol FX-90 QFT spectrometer.

Preparation of the complexes

All operations were performed unter aerobic conditions. The starting complexes $[RhLCl]_2$ (L=COT, COD, NBD) were prepared as reported in the literature[14,15]. The phosphine selenides were prepared by known methods[13]. All the complexes of the general formulae $[RhLL_2]ClO_4$ and $[RhLP(Se)(CH_2)_2(Se)PPh_2]ClO_4$ were prepared according to the method described in our previous publication[10].

RESULTS AND DISCUSSION

In our preliminary report some data concerning complexes I-VI[9] were given. In the present work the title complexes were prepared by adding the respective selenide to an acetonic solution of $[RhL(Me_2CO)_x]ClO_4$ in the molar ratio 2:1.

The new compounds were formed, eqn(1), as coloured air-stable solids, slightly soluble in common organic solvents

$$(1)\quad \tfrac{1}{2}[RhClL]_2 \xrightarrow[Me_2CO]{AgClO_4} [RhL(Me_2CO)_x]ClO_4 \xrightarrow[Ph_2P(Se)(CH_2)_2(Se)PPh_2]{2SePAr_3 \text{ or}} [RhL(SePAr_3)_2]ClO_4 + xMe_2CO$$

Table 1 displays the analytical and other physical data of the prepared new compounds. The conductivity measurements of the complexes I-XII are suggesting the presence of 1:1 electrolytes in acetonic solution, which probably indicates non coordinated anions in this particular solvent[11].

All the complexes I-XII show at ca. 1100 (ν_3) and 620 (ν_4) cm^{-1} bands characteristic[12] of the uncoordinated anion ClO_4^- (Td) along with the absorptions of all the coordinated ligands. The band at ca. 520-550 is due to ν(P=Se) vibration shifted towards the lower wave numbers, than those of the free phosphine selenides[13]. The latter in fact suggests lowering of the bond order of the P-Se bond which in turn indicates coordination

of the ligands via the selenium atom. The capacity of the moiety $[LRh<]^+$ to act as Lewis acid is demonstrated either by the shift of the ν(Se=P)or by the variation of the $J(^{31}P-^{77}Se)$ coupling constant. However, the cor-

TABLE I. Analytical results, molar conductivities, yields and IR data for the new Rh(I) complexes.

	Complex	Found (calc.) % C	H	Λ_M (ohm^{-1}, cm^2mol^{-1})	Yield (%)	IR bands of P→Se (cm^{-1}) Ligand	Complex	Δν(P-Se)[c]
I	$[Rh(COD)(SeP(oCH_3C_6H_4)_3)_2]ClO_4$	55.4(55.7)[a]	5.4(5.0)	128[b]	72	560	545	-15
II	$[Rh(COD)(SeP(mCH_3C_6H_4)_3)_2]ClO_4$	54.4(55.7)	5.4(5.0)	120	70	574	540	-34
II'	$[Rh(COD)(SeP(pCH_3C_6H_4)_3)_2]ClO_4$	55.8(55.7)	5.0(5.0)	125	69	544	512	-32
IV	$[Rh(COT)(SeP(oCH_3C_6H_4)_3)_2]ClO_4$	55.0(55.7)	4.8(4.7)	112	78	560	550	-10
V	$[Rh(COT)(SeP(mCH_3C_6H_4)_3)_2]ClO_4$	56.0(55.7)	5.0(4.7)	118	76	574	550	-24
VI	$[Rh(COT)(SeP(pCH_3C_6H_4)_3)_2]ClO_4$	55.0(55.7)	5.0(4.7)	115	74	544	525	-19
VII	$[Rh(NBD)(SeP(oCH_3C_6H_4)_3)_2]ClO_4$	55.3(55.4)	4.6(4.7)	140	65	560	520	-40
VIII	$[Rh(NBD)(SeP(mCH_3C_6H_4)_3)_2]ClO_4$	55.0(55.4)	4.9(4.7)	138	68	574	550	-24
IX	$[Rh(NBD)(SeP(pCH_3C_6H_4)_3)_2]ClO_4$	56.0(55.4)	4.9(4.7)	142	64	544	545	+1
X	$[Rh(COD)(C_6H_5)_2PSeCH_2CH_2SeP(C_6H_5)_2]ClO_4$	47.0(47.1)	4.0(4.18)	120	70	539	520	-19
XI	$[Rh(COT)(C_6H_5)_2PSeCH_2CH_2SeP(C_6H_5)_2]ClO_4$	46.5(47.32)	3.0(3.73)	128	66	539	515	-24
XII	$[Rh(NBD)(C_6H_5)_2PSeCH_2CH_2SeP(C_6H_5)_2]ClO_4$	47.4(46.58)	3.1(3.79)	125	72	539	520	-19

[a] Theoretical values in parentheses

[b] Concentration 5×10^{-3}M in acetone

[c] Δν(P→Se) = ν(P→Se) (ligand) - ν(P→Se) (complex)

responding IR bands do not precisely correlate to the above argument in order to reach a safe conclusion.

Table II reports the ^{31}P NMR data of VI and VIII and of some other related species previously prepared[10]. Generally, the ^{31}P NMR spectra exhibit a sharp peak accompanied by a set of ^{77}Se satellites for which J(P-Se) is, in most cases, significantly lower than those of the free solenides; only ^1J(P-Se) of $[Rh(NBD)(SeP(m\text{-}MeC_6H_4)_3)_2]ClO_4$ is slightly lower than that of the free ligand. The observed decrease of J(P-Se) suggest weakening of the P-Se bond upon complexation. In the case of triphenylphosphine selenide complexes the magnitude of the decrease is greater for $[Rh(COD)(SePPh_3)_2]ClO_4$, indicating also stronger Lewis acidity for the $[(COD)Rh<]^+$ species.

Unfortunately, due either to solubility reasons in $CDCl_3$ or to decomposition problems, chloroform seems to attack some of them, we have been unable to record the ^{31}P NMR spectra for all complexes and to study more

accurately the variations of the coupling constants

TABLE II. ^{31}P NMR data for some cationic complexes

Complex	δ(P) (ppm)	$^1J(^{31}P-^{77}Se)$ (Hz)	$\Delta(^{31}P-^{77}Se)$ (Hz)
$[Rh(COT)(SePPh_3)_2]ClO_4$	34.2 (35.4)[b]	650 (730)	-80
$[Rh(NBD)(SePPh_3)_2]ClO_4$	31.0 (35.4)	640 (730)	-90
$[Rh(COD)(SePPh_3)_2]ClO_4$	34.4 (35.4)	632 (730)	-98
$[Rh(COT)\{SeP(p\text{-}MeC_6H_4)_3\}_2]ClO_4$	32.8 (33.8)	620 (720)	-100
$[Rh(NBD)\{SeP(m\text{-}MeC_6H_4)_3\}_2]ClO_4$	32.6 (35.4)	708 (723)	-15

[a] Measured in ($^2H_1$) chloroform

[b] Free-ligand values, Data taken from recorded by us spectra of pure ligands

SUMMARY

The preparation of cationic rhodium(I) complexes of the types $[RhLL'_2]ClO_4$ and $[RhLPh_2P(Se)(CH_2)_2(Se)PPh_2]ClO_4$ (L=COD, COT, NBD) L'=$(o\text{-}CH_3C_6H_4)_3PSe$, $(m\text{-}CH_3C_6H_4)_3PSe$, $(p\text{-}CH_3C_6H_4)_3PSe$ and some spectroscopic data (IR,NMR) are reported and discussed.

ΠΕΡΙΛΗΨΗ

"Σύνθεση και χαρακτηρισμός νέων κατιονικών συμπλόκων του Ροδίου(I) με διολεφίνες και ωωσφινοσεληνίδια ως ligands"

Παρασκευάσθηκαν και μελετήθηκαν νέες σύμπλοκες ενώσεις του Ροδίου(I) των γενικών τύπων $|RhLL'_2|ClO_4$ και $|RhLPh_2P(Se)(CH_2)_2(Se)PPh_2|ClO_4$ όπου (L=COD,COT,NBD) L' =$(o\text{-}CH_3C_6H_4)_3PSe$, $(m\text{-}CH_3C_6H_4)_3PSe$, $(p\text{-}CH_3C_6H_4)_3PSe$.

Η μελέτη τους έγινε με φάσματα IR και NMR (για μερικές εξ αυτών) με στοιχειακή ανάλυση και αγωγιμομετρία.

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Chimika Chronika, New Series, 21, 81-87 (1992)

DEOXYGENATION OF PYRAZOLE 1,2-DIOXIDES

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(Received May 28, 1991)

SUMMARY

The photochemical and triethyl phosphite deoxygenation of pyrazole 1,2-dioxides is reported. Irradiation of the latter, in methanol, yielded the products of partial and complete deoxygenation along with the mother diketones. A plausible mechanism is suggested. Furthermore, treatment of the above dioxides with triethyl phosphite effected their complete deoxygenation, and the corresponding pyrazoles were formed. Finally, some reported data about the deoxygenation of related systems are presented.

Key words : Pyrazole 1,2-dioxides, Deoxygenation.

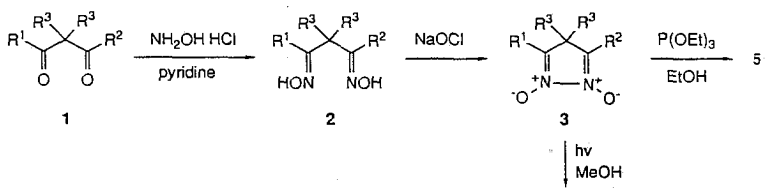
INTRODUCTION

Heterocyclic N-oxides have been the subject of extensive research over the past fifty years.[1] Important members of this class of molecules are the N-oxides of pyrazoles.[2] However, it was only in 1966 that Freeman proved[3,4] the pyrazole N-oxide structure and reported his work about 4-oxo-4*H*-pyrazole 1,2-dioxides. It was two decades later, in 1985, that the synthesis of 4,4-disubstituted pyrazole 1,2-dioxides **3** was reported[5-7] along with their electron impact mass spectra.[8]

The use of heterocyclic N-oxides as photochemical oxidizers[9] provided the interest to the present study of deoxygenation reactions of **3**. The N-oxides can be considered one of the best models of biological oxidation.[9]

RESULTS AND DISCUSSION

The pyrazole 1,-dioxides **3** were prepared according to the literature[7] by treatment of the corresponding 1,3-dioximes **2** with sodium hypochlorite (Scheme 1). Irradiation of the N-oxides **3** with a mercury lamp of medium pressure, in methanol, that generally favors deoxygenation, gave a mixture of products which was separated by column chromatography to afford the products of partial and complete deoxygenation, **4** and **5** respectively, as well as the parent diketones **1**. The yields of the products are shown in Table 1. The products **4**, **5** and **1** were identified by comparison of their data with those in the literature.[5-8, 10-13]



1-5	R^1	R^2	R^3
a	Me	Me	Me
b	Me	Me	Et
c	Me	Me	CH_2Ph
d	Me	Ph	Me
e	Ph	Ph	Me

Scheme 1

In the case of **3d** (R^1=R^3=Me, R^2=Ph) the partial deoxygenation occurs at the N-oxide group that is attached to the carbon with the methyl group. A plausible explanation is, that the stereochemical inhibition of the bulky phenyl group, along with the contribution of the exocyclic oxygen to the resonance of the aromatic nucleus and therefore its stabilization, favor the above deoxygenation of **3d** to **4d**. Furthermore, in the case of **3c** a very complicated mixture of products was formed. The only products isolated were the pyrazole **5c** and the diketone **1c**. It would be reasonable to assume that the presence of benzyl groups influences the re-

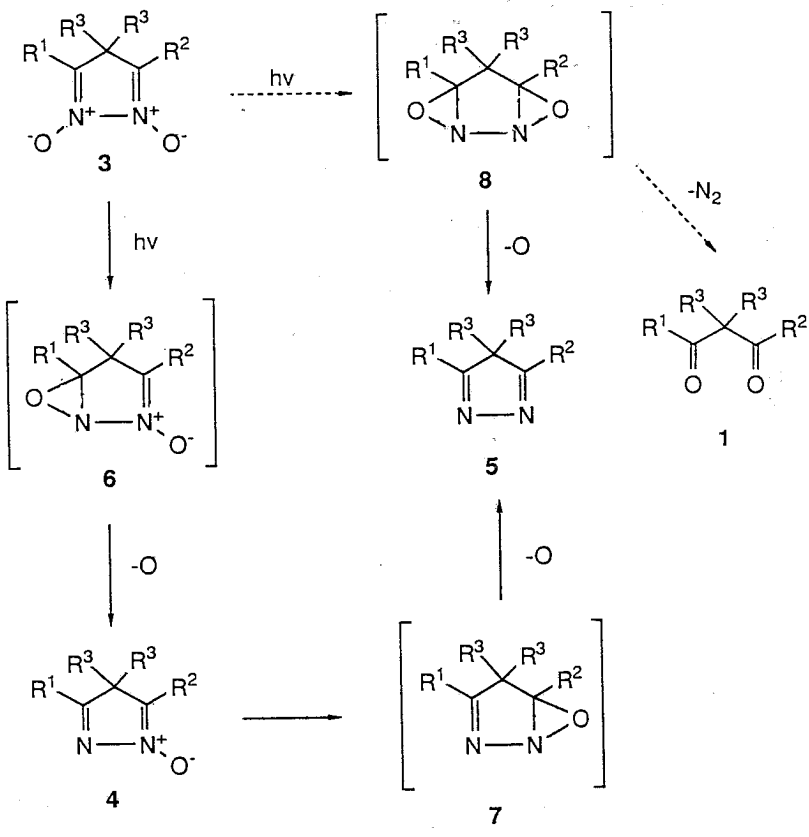
TABLE 1. Yields of the products **4**, **5** and **1** derived from A and B reactions (Scheme 1)

Compound	Yield %		Lit. ref.
	A	B	
4a	45	—	10, 13
4b	42	—	10, 13
4c	—	—	—
4d	54	—	13
4e	40	—	13
5a	21	80	10, 13
5b	20	85	10, 13
5c	14	81	11
5d	24	70	13
5e	18	64	13
1a	15	—	5, 7
1b	18	—	5, 7
1c	15	—	5, 7
1d	17	—	5, 7
1e	20	—	5, 7

activity of the molecule, giving alternative pathways.

According to the literature[9] it could be assumed that the mechanism that explains the formation of **4** and **5** involves as intermediates the oxaziridines 6 and 7 (Scheme 2). The formation of the N-oxides **4** could be thought of as an intermediate step in the formation of the complete deoxygenation products **5**. Alternatively, **4** and **5** could be formed by homolytic scission of the $N^{+}-O^{-}$ bond, analogously to the geometrical isomerization of nitrones.[9] Furthermore, the oxaziridine intermediate **8**, followed by abstraction of nitrogen could lead to the formation of **1**. However, no abstraction of N_2 was observed. Alternatively, **1** could be the result of the protonation and subsequent hydrolysis of **3**, by the protic solvent that was used.

Previously, it has been reported the photochemical deoxygenation of various heteroaromatic N-oxides.[1,9,14] Thus, pyridine,[15] 3-picoline,[1]



Scheme 2

benzo|c|cinnoline[14] as well as purine[1,9] N-oxides are smoothly deoxygenated photochemically. However, the N-oxides as quinoline,[9] isoquinoline,[9] phthalazine,[16] quinolizine,[14] quinoxaline,[17] and benzotriazine[18] undergo practically little or no deoxygenation. No deoxygenation study has been reported for non-aromatic N-oxides. However, it has been reported[19] that 4,4-dimethyl-3,5-diphenyl-4*H*-pyrazole 1-oxide 4e gives a photochemical rearrangement to 5,5-dimethyl-3,4-diphenyl-5*H*-pyrazole 1-oxide, when it is

irradiated in dichloromethane. We attempted some photochemical reactions in different solvents. However, in dichloromethane and chloroform the reactions lead to very complicated mixtures of products, whereas in benzene most of the unreacted starting material was recovered.

Furthermore, we attempted deoxygenation of **3** by treatment with triethylphosphite. This effected complete deoxygenation of **3** to give the pyrazoles **5**. However, the conditions required were vigorous (45 h reflux in ethanol with eightfold excess of the reagent). Milder conditions (heating in waterbath for 30 min in chloroform) that are efficient[10] to deoxygenate pyrazole N-oxides **4** were ineffective. Triethyl phosphite has been used extensively for the deoxygenation of a great variety of heteroaromatic N-oxides such as, pyridine and quinoline N-oxides, benzo|c|quinoline 1,2-dioxide, oxazole 3-oxide, furoxan and benzofuroxan.[1] Furthermore, benzo-|c|quinoline 5-oxide is reduced by triethyl phosphite under forcing conditions (5 h at 160 ^{0}C). This compound is not reduced by phosphorous trichloride under normal conditions.[20]

Finally, the relation of the reactivity of molecules that accept energy by electron ionization (mass spectra) to those that are irradiated, is well known[14] as both cases involve excited states. It should be noted that in the electron impact mass spectra of **3**, fragments at *m/z* $|M-16|^{+}$ that are due to the elimination of an oxygen from the molecular ion and correspond to pyrazole 1-oxides **4** are characteristic.[8] However, no fragment that corresponds to the complete deoxygenated pyrazoles **5** has been observed and the same is true for fragments corresponding to diketone **1**.[8]

EXPERIMENTAL

The preparation of diketones **1**, dioximes **2** and pyrazole 1,2-dioxides **3** was as described earlier.[7] Spectral and analytical procedures were also as earlier stated.[5-7] Compounds **4** and **5** are all known and they were identified by comparison of their data with those of the literature.[10-13]

Photochemical Reaction of Pyrazole 1,2-Dioxides **3**.

The N-oxides **3** (1 mmol) in methanol (100 ml) were irradiated in a quartz tube by a mercury lamp (125 W, medium pressure) for 45 h. Evaporation of the solvent gave an oil which was submitted to column

chromatography (silica gel ASTM 70-230 mesh), eluted with a mixture of pet. ether/ethylacetate 10/1 to afford the products **4**, **5** and **1** (Table 1).

Reaction of Pyrazole 1,2-Dioxides ***3*** *with Triethyl Phosphite.*

The N-oxides **3** (1 mmol) in ethanol (25 ml) were refluxed with triethyl phosphite (8 mmol) for 45 h under nitrogen. The mixture was cooled and the solvent was evaporated to live an oily residue which was hydrolysed by dilute aqueous hydrochloric acid. The products were recovered by ether extraction, were submitted to column chromatography (silica gel ASTM 70-230 mesh) and eluted with a mixture of pet. ether/ethylacetate 10/1 to yield as main products the pyrazoles **5** (Table 1).

The authors thank Dimitra Gemenetzi for her assistance.

ΠΕΡΙΛΗΨΗ

Στην εργασία αυτή παρουσιάζεται η αποξυγόνωση των 4,4-διϋποκατεστημένων πυραζολο-1,2-διοξειδίων, η οποία επιτυγχάνεται είτε φωτοχημικά είτε με επίδραση φωσφορικού τριαιθυλεστέρα. Ακτινοβόληση των παραπάνω 1,2-διοξειδίων σε μεθανόλη οδήγησε στον σχηματισμό μίγματος των μερικώς και των πλήρως αποξυγονωμένων προϊόντων ενώ απομονώθηκε και ποσότητα της μητρικής δικαρβονυλικής ένωσης. Η αποξυγόνωση που επιχειρήθηκε με επίδραση φωσφορικού τριαιθυλεστέρα είχε ως αποτέλεσμα τον σχηματισμό των προϊόντων πλήρους αποξυγόνωσης, των αντίστοιχων πυραζολίων. Επί πλέον παρουσιάζονται αποτελέσματα αποξυγόνωσης αναλόγων συστημάτων.

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Chimika Chronika, New Series, 21, 89-95 (1992)

DETERMINATION OF NOx AND SOLID TAR PARTICLES IN THE ENVIRONMENTAL SMOKE TOBACCO DURING CIGARETTE BURNING IN THE PRESENCE AND ABSENCE OF CITRIC ACID IN THE FILTER

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(Received November 20, 1991)

SUMMARY

The concentrations of nitrogen oxides NOx and solid tar particles in the environmental smoke tobacco during cigarette burning have been determined in the absence and presence of citric acid in the cigarette filter. The presence of citric acid causes a decrease of NOx varying from 19 to 64% and a decrease of tar in the smoke varying from 8 to 80%, depending on the type of cigarettes under examination.

Key words: Cigarette filter, Evans filter, smoke suction, optical absorbance, NOx concentration, tar withheld, environmental smoke tobacco.

INTRODUCTION

A large number of chemical compounds are found in the environmental smoke tobacco during the burning of cigarettes, cigars or pipes. One can distinguish the 25 alkaloids[1] such as nicotine (800 - 3000 μg/cigarette), harmane, benzimidazole, methylamine (4 - 6μg/cigarette), 2 - naphthylamine, 3, 4 - benzopyrene etc. A quantity of 1g of 3,4 benzopyrene[2] was isolated in the aerosol of 100 cigarettes. Spectrophotometric and chromatographic measurements have shown that the most toxic products found in tar are 11, 12-benzofluorene, 1,2-benzopyrene, methylpyrene, menthylfluorene, chrysene[3] etc. These products can have neoplasmic effects both on test animals and humans.

Two very important gaseous constituents of smoke stream are nitrogen oxides (NOx)[4,5] (500 - 1020 μg/cigarette), and carbon monoxide (CO) which

constitues 3 - 5% of the gaseous phase of smoke aerosol.
Citric acid can withhold alkaloids, such as nicotine and harmane, which did not break down during cigarette burning. It should be mentioned here that citric acid is held together with nocitine in tobacco leaves at 2 - 6% proportion[6]. Citric acid maybe placed between the tobacco and the filter of the cigarette in the same way as carbon crystals. Empirical tests in the past have shown that the taste of the cigarette during smoking is improved when the above substance is used.

In the present work an attempt is made to determine (i) the concentration of NOx in the smoke aerosol during burning of cigarettes belonging to various makes in the absence and presence of citric acid in the cigarette filter and (ii) the amount of tar withheld by EVANS filters in the absence and presence of citric acid in the cigarette filter. The motive for such an investigation was given by the fact that nicotine and citric acid exist together in tobacco leaves.

Determination of NOx has also been the object of research work in the past[7,8,9], but without making use of citric acid.

METHODS OF MEASUREMENT

1. *Determination of NOx in the environmental smoke tobacco*

Nitrogen oxides are formed both during burning of nitrogen compounds in tobacco and during the thermal reactions of the constituents of atmospheric air inhaled during smoking. Two sets of measurements were performed-one in the absence of citric acid and the other in the presence of citric acid in the cigarette filter.

Procedure: The Saltzman method[10] was used. A 15 ml solution consisting of sulfanilic acid and N-(1-naphthyl) ethylenediamine was introduced in a flasket. A total of 15 flaskets containing the above solution were then ready to receive the smoke from 15 cigarettes, each belonging to a different make. Each cigarette is mounted on the inlet of a syringe using an EVANS filter. A volume of 105 ml of smoke is sucked and then blown into the flasket. After 90 minutes we observe changes in the colour of the solution. Using a SHIMADZU single beam UV-VISIBLE spectrophotometer we measure the optical absorbance (D) and consequently the concentration (C) of NOx at a maximum wavelength (λ_{max}) of 560 nm. Figure 1 shows the variation of optical absorbance versus NOx concentration. The blank value of optical density was zero. The procedure was repeated for all 15 flaskets. More than 500 cigarettes were used and mean values were taken. Suctions were all at the same speed, since different speeds may alter the burning temperature and consequently the amount of NOx in smoke.

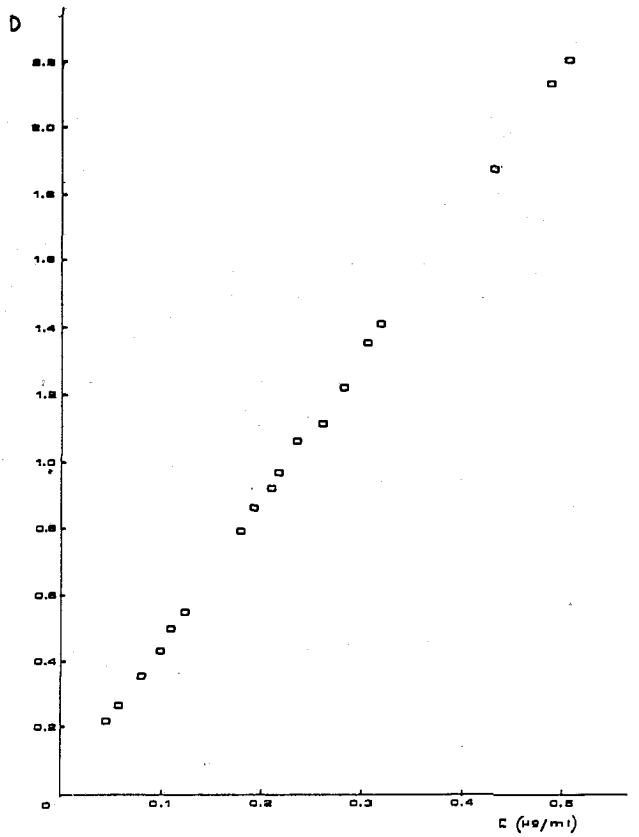

FIG. 1: *Optical Absorbance (D) versus NOx concentration (C)*

2. Determination of solid tar particles

Procedure:

Two sets of cigarettes belonging to 20 different makes were examined. The first set included cigarettes free of citric acid, while the second set included cigarettes with citric acid added in their filter. EVANS filters were mounted on all cigarettes. Each filter was used during the full burning (smoking) of each cigarette. The latter was simulated using 50 ml syringes to perform suctions. EVANS filters were weighed, before and after use, with an analytical balance (0.0001 g sensitivity). The difference between weighings gives the quantity of tar withheld by the EVANS filter, which is essentially the quantity of tar escaping from the cigarette filter.

RESULTS AND DISCUSSION

1. *Determination of NOx*

Saltzman method is 85% accurate for the NO/NO_x mixture. NOx concentrations with and without the addition of citric acid in the cigarette filter and % differences are given in Table I. It can be seen that concentration of NOx in smoke are increased in the case of absence of citric acid (1a), and decreased in the case of presence of citric acid in the cigarette filter (1b). It is concluded that cigarette filters containing citric acid can withhold more NOx.

% differences were calculated using the relation

% difference = 100x (NOx without citric acid-NOx with citric acid)/ NOx without citric acid. These varied form 19 to 64%.

TABLE I: Concentration of NOx in environmental smoke tobacco, in the presence and absence of citric acid in the cigarette filter.

Serial Number	Cigarette Code	NOx Concentration (μg/105ml±0.1μg)		Difference %
		1a. Filter without citric acid	1b. Filter with citric acid	
1	OSC	18.8	6.8	64
2	EO-LS	52.8	22.9	57
3	CPR	32.0	15.5	52
4	GSE	45.0	22.0	51
5	AS-FO	51.5	27.5	47
6	AS-AL	7.9	5.0	37
7	MIGS	15.5	10.3	34
8	SE	33.6	22.8	32
9	KLF	12.2	8.3	32
10	PA-FO	29.5	22.6	24
11	SG	7.0	5.4	23
12	KTG	11.5	9.1	21
13	ONE-R	7.7	6.1	21
14	KNS	12.8	10.2	20
15	ONY	24.7	20.0	19

2. *Determination of tar*

The quantity of tar withheld by EVANS filters during the full smoking of ci-

garettes is given in mg/cigarette in Table II. There are two cases - one in the absence of citric acid and the other in the presence of citric acid in the cigarette filter. It can be seen that in the first case, more tar is withheld by the EVANS filter than in the second case which means that more tar is withheld by the cigarette filter containing citric acid. % differences were calculated and varied between 7 and 83%.

TABLE II: Quantities of tar withheld by EVANS filter in the absence and presence of citric acid in the cigarette filter.

Serial Number	Cigarette Code	Quantity of tar withheld by Evans filter (mg/cigarette±0.1mg)		Difference %
		1a. Filter without citric acid	1b. Filter with citric acid	
1	FO-KA	26.9	4.5	83
2	AS-FO	17.7	3.5	80
3	GSE	12.4	6.4	48
4	CNA	10.2	5.8	43
5	DLF	19.9	12.1	39
6	MLR	23.7	15.4	35
7	ERG	26.1	17.1	34
8	SE	11.1	7.3	34
9	LT-KA	18.6	13.2	29
10	BGH	13.2	9.6	27
11	WTN	9.0	7.1	21
12	MLRS	10.6	9.1	14
13	KTG	12.1	10.5	13
14	CML	15.0	13.1	13
15	CPRS	11.7	10.3	12
16	OSC	22.2	19.6	12
17	EO-LS	12.6	11.3	10
18	ONY	19.4	17.6	9
19	ERG-S	12.9	11.9	8
20	PA-FO	19.1	17.8	7

According to the above results it may be concluded that the addition of citric acid in the cigarette filter results to a decrease of NOx concentration in smoke. The % decrease can even reach 50 - 60% for certain cigarette types.

Regarding tar in smoke, this can also be reduced by 8 - 80% in the presence of citric acid in the cigarette filter.

Cigarette manufacturers should investigate the possibility of producing cigarettes with citric acid filters, while manufacturers of filters of the Evans-type should investigate the addition of citric acid in these filters.

ΠΕΡΙΛΗΨΗ

ΠΡΟΣΔΙΟΡΙΣΜΟΣ NO_x ΚΑΙ ΠΙΣΣΑΣ ΣΤΟ ΑΕΡΟΛΥΜΑ ΤΟΥ ΚΑΠΝΟΥ ΚΑΤΑ ΤΗΝ ΚΑΥΣΗ ΤΟΥ ΤΣΙΓΑΡΟΥ ΠΑΡΟΥΣΙΑ ΚΙΤΡΙΚΟΥ ΟΞΕΟΣ ΣΤΟ ΦΙΛΤΡΟ

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Η συγκέντρωση των οξειδίων του αζώτου NO_x και των στερεών σωματιδίων πίσσας έχουν προσδιοριστεί στο αερόλυμα του καπνού κατά την καύση σιγαρέττων από διάφορες μάρκες, με ή χωρίς την παρουσία κιτρικού οξέος στο φίλτρο του τσιγάρου. Η παρουσία του τελευταίου προκαλεί ελάττωση των NO_x στον καπνό σε ποσοστό που κυμαίνεται από 19 εως 64% και μείωση της πίσσας στον καπνό σε ποσοστό από 8 εως 80%, αναλόγως του εξεταζόμενου τύπου σιγαρέττων.

Λέξεις κλειδιά:
Φίλτρο τσιγάρου, φίλτρο Evans, αναρρόφηση καπνού, οπτική απορροφητικότητα, συγκέντρωση NO_x , κατακράτηση πίσσας, αερόλυμα καπνού.

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Author Index (Volume 21, 1992)

Keyword index (Volume 21) 1992

CONTENTS

March - December 1992 Volume 21 No 1 - 4

This year visit Macedonia

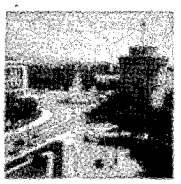
Macedonia

For 4,000 years* steeped in the history of Greece

Statue of Aristotle, Stagira.

Aristotle, the tutor of Alexander the Great, was born in Stagira in Macedonia in 384 BC. Together with Plato, he is regarded as one of the greatest philosophers the world has known. Aristotle was a true academic, concerned with Physics, Astronomy, Rhetoric, Literature, Political Science and History. His teachings laid the foundation for modern scientific thought.

The White Tower of Thessaloniki.

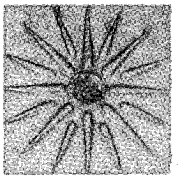


Thessaloniki, the heart of Macedonia, is a modern city with 1,000,000 inhabitants. It is strategically located at the crossroads of Europe with Asia. Having spread the Word at Philippi, the Apostle Paul continued his teachings in Thessaloniki. Its important monuments from antiquity and byzantium up to the present, provide testimony to the role that the city has played as the second capital of Hellenism.

The Bust of Alexander the Great. Acropolis Museum, Athens.

Alexander was born in 356 BC in Pella, Macedonia, established by his father Philip II, as the centre of Hellenism. Nurtured on the thoughts of his tutor, Aristotle, he rose to fame as a brilliant military leader. He influenced the course of history, rightfully earning his title as Alexander the Great. In 335 BC he became Commander in Chief of all the Greeks. By the time of his death in 323 BC he had created an enormous empire, stretching from the shores of the Adriatic to India, and from the Caucasus Mountains to Egypt. He spread the Greek spirit far and wide among nations who worshipped him as a god.

Symbol of the Greek Macedonian Dynasty from the tomb of Philip II. Archaeological Museum, Thessaloniki.



This 16 pointed star of Vergina was uncovered during the archaeological excavations at Vergina. This symbol of the Greek Macedonian Dynasty decorated the golden tomb of Philip II. The Star of Vergina, extracted from the soil of Macedonia, has since become the symbol of Hellenism.

The Olympian Aphrodite (3rd Century BC) Museum of Dion.

This statue of Aphrodite came to light during archaeological digs at the ancient sacred city of Dion. Dion, at the foot of Mt Olympus, was the most important spiritual site for the Northern Greeks, playing the same role in their lives as that of the oracle at Delphi.

4.000 years: Post-Mycenaean ceramic relics found in Assiros and Mycenaean swords found in Grevena date back 4.000 years, evidence of Macedonia's role at the vortex of Greek history. Even in mythology Macedon, mythical founder of the Macedonian race, is the son of Aeolos (god of the winds). Throughout the years Macedonia contributed to the fountain of knowledge of the Ancient Greeks. In the 5th century BC Demokritos, father of Atomic Theory, lived and worked in Avdira.*

St Dimitrios, detail of 7th Century Mosaic. Church of St. Dimitrios, Thessaloniki.

St Dimitrios, Protector of the city of Thessaloniki, was martyred in 305 AD defending Christianity. He is regarded as the Patron Saint of Thessaloniki and its saviour during difficult moments.

GREECE

Chosen by the Gods