CHIMIKA CHRONIKA

NEW SERIES

AN INTERNATIONAL EDITION
OF THE ASSOCIATION OF GREEK CHEMISTS

4/93

CMCRCZ 22(4), 189-254(1993)

ISSN 0366-693X

Volume 22, No 4 p.p. 189-254 October-December 1993

CHIMIKA CHRONIKA

NEW SERIES

AN INTERNATIONAL EDITION
OF THE ASSOCIATION OF GREEK CHEMISTS

4/93

CMCRCZ 22(4), 189-254(1993)

ISSN 0366-693X

Volume 22, No 4 p.p. 189-254 October-December 1993

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 drachms 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, Arditou Str. 116 36 ATHENS Tel. 9217.513

Υπεύθυνος σύμφωνα με το νόμο: Ν. Κατσαρός, Κάνιγγος 27, Αθήνα 106 82.

Responsible under law: N. Katsaros, 27 Kaningos St., Athens 106 82, Greece.

Chimica Chronica, New Series, 22, 189-204 (1993)

COORDINATION COMPLEXES OF CAFFEIC AND FERULIC ACIDS WITH Cu(II), Ni(II), Co(II) AND Fe(III)

A.L.PETROU[1*], M.V. KOROMANTZOU[1] and J. M. TSANGARIS[2]

1.Laboratory of Inorganic Chemistry, University of Athens, Panepistimiopolis, Kouponia, Athens, 15701, Greece.
2. Laboratory of Inorganic Chemistry, University of Ioannina,P.O.Box 1186, Ioannina, 45110, Greece.

(Received June 10, 1992)

SUMMARY

Complexes of caffeic and ferulic acids with Cu(II), Ni(II), Co(II) and Fe(III) were prepared in the solid state: $K_2[Cu_2(cafH)_2Cl_2].2CH_3OH$, $K_2[Co_2(cafH)_2Cl_2]$, $K_2[Ni_2(cafH)_2Cl_2].2CH_3OH$, $Fe_2(cafH)_2Cl_2.2H_2O$, $K_2[Cu_2(fer)_2Cl_2]$, $K_2[Co(fer)_2]$, $K_2[Ni_2(fer)_2Cl_2].2CH_3OH$, $Fe_2(fer)_2Cl_2$. Spectroscopic, magnetic and thermogravimetric results indicate bis(μ-chloro) tetrahedral binuclear structures with a catechol-type of coordination.

Key words: caffeic, ferulic acid complexes, catechol-type coordination.

INTRODUCTION

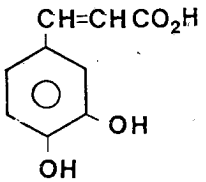
3-(3,4-dihydroxyphenyl)-propenoic acid (caffeic acid, abbr.$cafH_3$)1 and 3-(4-hydroxy-3-methoxyphenyl)-propenoic acid (ferulic acid, abbr. $ferH_2$)2 are early recognized as constituents of different plants and seeds[1].

Both acids can be found in the soil lignins as degradative products of vegetation, contributing by consequence to the availability of several biotrace metal cations from the soil to the plants[2].

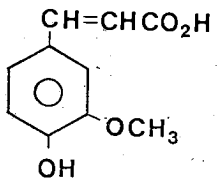
Ferulic acid is a precursor of aryl tetralin lignans related to anticancer active compounds as the podophyloto-

* Author to whom correspondence should be addressed.

xin and its methylated derivatives[3]. Also the antivirial activity of caffeic acid was lately investigated[4a].



(1)



(2)

Phenolic hydroxyl-containing ligands, which are known to be present in the root exudates, may participate in the transport processes of metal ions from the surrounding soil to the plant roots[4b]. Of special interest are trans - 3-(3,4-dihydroxyphenyl)propenoic acid (caffeic acid), which is believed to participate in the transport and in the reduction of iron(III) to iron(II) in the soil environment, and chlorogenic acid[1,3,4,5-tetrahydroxycyclohexanecarboxylic acid 3-(3,4-dihydroxycinnamate)], which is a precursor of caffeic acid. Complexes of 3,4-dihydroxyphenyl derivatives e.g. copper(II) complexes of chlorogenic acid and related compounds[4c] have been investigated by pH-metric and spectroscopic methods. Preparation of coordination complexes of 3,4-dihydroxyphenylpropionic acid with copper(II), nickel(II), cobalt(II) and iron(III) was achieved[5a]. Cupric complexes with 3,4-dihydroxybenzoic acid[5b] have been studied where the bidentate catechol part of the ligand studied predominates as the binding site for metal ion although the carboxyl group may also participate in the metal ion binding leading to creation of the dinuclear species. More con-

centrated solutions yield a trimeric complex molecule (Cu_3A_2) with one metal ion bound to four phenolate oxygens and two others bound to the respective carboxylates. Phenolic ligands are also important as the constituents of more complicated humic and fulvic acids, which are the main ligands taking part in the transport and accumulation of the nutrient ions[5c].

Considering the chemical and biological importance of catechol-like coordination complexes[5], the preparation in the solid state, the characterization and the study of the physicochemical properties of several complexes of the above ligands with Cu(II), Ni(II), Co(II) and Fe(III) was undertaken.

Earlier work on the caffeic acid coordination is covered by the potentiometric titrations of the caffeate-metal-proton system [6,7] and some spectroscopic (absorption, ESR) measurements on the complexes formed in solution[8].

It is not evident however, that the complexes formed are mononuclear involving only a catechol-type of coordination[6], or oligonuclear involving both catecholic and carboxylic coordination especially with Cu(II), playing the role of link between adjoining ligands[7,8].

Infering from analogous behaviour of several dihydroxy benzoic acids, the last type of coordination was proposed as more probable[9-11].

EXPERIMENTAL

The caffeic (dec.223-225°C) and ferulic (m.p.174°C) acids were obtained from Merck Co. and were used without further purification; mass spectra: m/e of the molecular ion for caffeic acid 180 (calc.180.163), for ferulic acid 194 (calc.

194.215). The metal salts MCl_2(M=Cu, Ni,Co) and $MCl_3.6H_2O$ (M=Fe) used as starting materials for the preparation, were pro analysi grade from Fluka.

Upon refluxing MCl_2 or $MCl_3.6H_2O$ with caffeic or ferulic acid in methanolic solution containing equimolecular amount of KOH with a final ratio metal ion:acid:KOH 1:2:2, solid coloured complexes (1-8, TABLE I) were obtained. Details for the preparation are given in a previous paper concerning hydrocaffeic acid[5a].The coloured precipitates were also washed with 80% MeOH. Elemental analysis, physicochemical and spectroscopic measurements were carried out by published methods[12-14]. The magnetic susceptibilities in the solid state were performed at 24°C. The $cafH_2Na.H_2O$ and $ferHNa.H_2O$ salts were prepared and characterized as previously[5a].

RESULTS AND DISCUSSION

Preparative and analytical data, colours and molar conductivity values are reported in TABLE I. The prepared complexes are either microcrystalline (2,3,4,8) or powder-like (1,5,6,7), relatively stable in atmospheric conditions (oxygen, humidity) except for the complexes 1, 5 which gave evidence of structural changes upon time (see TABLE II). All complexes have a limited solubility in DMSO, DMF and MeOH. The molar conductivities of the complexes 1,2,3,5,6,7 in both DMSO and MeOH are low for their formulation as 2:1 electrolytes. This probably arises from the existing large volume anions in the complexes[15]. The complexes 4 and 8 show a conductivity which increases with time and this can be attributed to the strong donor capacity of the solvent used, which leads to displacement of anionic ligands and changes the complexes to electrolytes[15].

TABLE I: Preparative data, colours, analytical results and conductivity measurements for the complexes

Compound number	Complex	Final pH in the preparation 1:2:2 [a]	Yield %	% M [b]	%Cl [b]	%C [b]	%H [b]	Λ_M [c] DMSO	Λ_M [c] CH_3OH	Colour
								$ohm^{-1}mol^{-1}cm^2$		
1	$K_2[Cu_2(cafH)_2Cl_2].2CH_3OH$	5.8	55	18.52 (18.25)	10.35 (10.20)	34.26 (34.48)	2.56 (2.87)	48	88	dark brown
2	$K_2[Co_2(cafH)_2Cl_2]$	5.5	35	19.03 (18.91)	11.54 (11.40)	34.92 (34.68)	1.86 (1.93)	80	92	dark green
3	$K_2[Ni_2(cafH)_2Cl_2].2CH_3OH$	6.0	62	17.33 (17.10)	10.18 (10.34)	34.52 (34.97)	2.87 (2.91)	77	82	dark green
4	$Fe_2(cafH)_2Cl_2.2H_2O$	2.0	17	19.65 (19.42)	12.41 (12.36)	37.81 (37.59)	2.54 (2.78)	57	60	black
5	$K_2[Cu_2(fer)_2Cl_2]$	4.5	25	19.56 (19.24)	10.66 (10.76)	36.12 (36.36)	2.39 (2.42)	95	94	brown
6	$K_2[Co(fer)_2]$	6.2	48	11.75 (11.31)	0.00 (0.00)	45.84 (46.07)	2.98 (3.07)	74	76	violet
7	$K_2[Ni_2(fer)_2Cl_2].2CH_3OH$	6.7	56	16.40 (16.43)	9.85 (9.94)	37.18 (36.95)	3.10 (3.36)	85	86	pale-green
8	$Fe_2(fer)_2Cl_2$	2.8	10	19.56 (19.70)	12.65 (12.53)	42.08 (42.36)	2.75 (2.82)	55	57	brownish

[a] ratio metal: acid:OH^- [b] theoretical values in parentheses

[c] molar conductivity for ca. 10^{-3} mol.l^{-1} solution in DMSO and CH_3OH respectively at 25 °C.

Thermogravimetric studies of the prepared complexes in the range 35-600°C show that the methanol present in 1,3,7 is completely lost between 60-70°C and is lattice-held. In 4, water is lost between 100-110°C and is also lattice-held. Complexes 1, 2, 3 decompose between 200-500°C with the same decomposition pattern possibly indicating analogous structures. Similarity in their structures is also indicated from the same thermal decomposition pattern of the complexes 5, 6, 7, which is effected in a relatively narrow temperature range between 240-320°C. Complexes 4, 8 are decomposed in higher temperatures. Complexes 1, 5 have a sudden and low temperature (200-220°C) decomposition, indicating bridged structures. All complexes were decomposed in a thermogravimetric experiment in the presence of atmospheric oxygen and gave as products metal oxides and KCl until a final temperature of 650-700°C.

The low magnetic moments of the complexes 1 and 5 are due to their binuclear structures and are typical for antiferromagnetic character. Their diffuse reflectance spectra clearly indicate distorted tetrahedral stereochemistry[16,17]. The $^1E \leftarrow {}^1T_1$ transition expected for a d^9 system like Cu(II) in a tetrahedral environment is split to more transitions, due to Jahn-Teller effect, known also to take place in such sy-stems[18]. More particularly the distortion of the tetrahedron as a flattening around the two-fold axis will result in D_{2d} symmetry, which retains the d_{yz}, d_{xz} degeneracy, splitting of both the ground and excited levels, so that either three or four transitions are expected[19] namely from the ground $^1B_1$ to ^1E, $^1B_1$ and $^1A_1$ states [20] as is in our case (TABLE II). The complexes 2 and 6 acquire diffuse reflectance spectra [18,21] and magnetic moments[22,23] close to tetrahedral structures, especially complex 6.

TABLE II: Electronic spectra and solid state magnetic moments of the complexes

Compound	Diffuse reflectance[a]						μ_{eff}^{b} BM
	CT			d-d			
1		22700	20800	18700	17700	15500	14200	1.30[c]
2		21050		18900 sh	16400 sh	14800		4.75
3		21500		17500	15800 sh	14800		3.88
4	26700	23300	21500	17900 sh	16700	15000		4.97
5	25600				16100	15000		1.41
6					16400 sh	15200		4.78
7		21500		19800		14285		4.26
8		21300			15600	14700		3.50

[a] in cm^{-1}. [b] at 298 K. [c] upon keeping the solids for 4-6 weeks at room temperature in a vacuum desiccator these values are increasing continuously to 2.10 BM.

A usual value for the magnetic moment of a tetrahedral weak field Ni(II) complex is 4.1 BM[23]. The values of magnetic moments for the complexes 3 and 7 suggest tetrahedral structures. The bands at 14800 and 14285 cm^{-1} in the diffuse reflectance spectra of 3 and 7 respectively, satisfactorily account for the $^3T_1$ (P)$\leftarrow$$^3T_1$(F) transition. In 3 there is a splitting due to low symmetry fields [4,25]. The observed μ_{eff} values for the complexes 4 and 8 at room temperature are too small for high spin S=5/2 complexes (μ_{eff} =5.9 BM) or too large for low spin S=1/2 complexes (μ_{eff} = 2.0 BM) assuming an octahedral d^5 system. An intermediate spin state (S=3/2) should have μ_{eff} = 4.0 BM. Both tetrahedral or octahedral complexes with A and E terms require higher values than those observed[23]. The measured values can be explained either by antiferromagnetic coupling of the iron(III) in dimeric complexes[26], or by a spin equilibrium between high and low spin states[27]. An alternative situation can also be proposed assuming a square pyramidal structure around iron (III) with $^6A_1$ electronic ground state, since a five coordinated structure is manifested by the stoichiometry of 4, or a spin-paired tetrahedral with $^6A_1$ ground term both with large orbital contribution[28].The last situation seems more probable for 4.

The bands in the diffuse reflectance spectra of the complexes 4 and 8 are weak, as it is expected since the electronic transitions of the iron(III) systems are spin forbidden. There is a considerable masking of the weak bands from the strong charge-transfer bands at 21300-26700 cm^{-1}. It is known that in spin equilibrium iron(III) systems the high spin form is characterized by a band at 18000-20000 cm^{-1} and the low spin by another one at 14000-16000 cm^{-1} [29-31]. Only in 4 the 17900 cm^{-1} shoulder demonstrates a high spin system,

but in both 4 and 8 the two low spin bands are present at 14700-15800 cm^{-1}. The presence of two low spin bands can be explained assuming low symmetry pseudo-tetrahedral structure in the complexes. No spectroscopic evidence has been noticed indicating presence of Fe(II) in complexes 4 and 8 owing to an oxidation-reduction mechanism sometimes found in analogous catecholic systems[32].

In TABLE III some diagnostic ir bands of the prepared complexes are reported. The broad band at 3500 cm^{-1} exhibited by the complexes 1, 3, 7 is due to lattice methanol. The lattice water in $cafH_2Na.H_2O$, $ferHNa.H_2O$ and in 4 is shown by the broad strong bands at 3600 and 3570 cm^{-1} respectively[33,34].

In the spectra 1-8 the following relation is observed :

$$\Delta_{complex} = \Delta_{LNa.H2O}$$

where L=$cafH_2$ or ferH and Δ is the separation between $\nu_{as(CO2)}^-$ and $\nu_{s(CO2)}^-$. This indicates that the carboxylate group of either of caffeic or ferulic acid is not coordinated to the metal ions in all prepared complexes[35,36]. Complexes 1, 2, 3, 4, 5, 7, 8 show a medium intensity ir band at 215-257 cm^{-1} assignable to the metal-halogen stretching mode associated with bridged structures[37].

The complexes 1-4 show medium intensity band at 470-485 cm^{-1} due to $\nu_{(M-OH)}$ vibration whereas the complexes 5-8 show a similar medium intensity band in higher frequencies at 580-590 cm^{-1} possibly assignable to the $\nu_{(M-OCH3)}$ stretch[38]. All complexes exhibit a strong band at 605-667 cm^{-1} assignable to the $\nu_{(M-OPh)}$ stretch[38].

In FIG 1 and FIG 2 the titration curves for deprotonation and coordination reaction in solution of MeOH:H_2O 9:1 for $cafH_3$ and $ferH_2$ respectively are reported. For the

TABLE III: Spectral IR (cm^{-1}) data for $cafH_3$, $cafH_2Na.H_2O$, $ferH_2$, $ferHNa.H_2O$ and the complexes 1-8

Assignments	$cafH_3$	$cafH_2Na.H_2O$	$ferH_2$	$ferHNa.H_2O$	1	2	3	4	5	6	7	8
$\nu(OH)_{alcoholic}$					3447sbr		3447sbr				3457sbr	
$\nu(OH)_{water}$		3600s		3600s				3567s				
$\nu(OH)_{phenolic}$	3421m	3420m	3440m	3435m	3368m	3395m		3377m	3368m	3335m	3386m	
$\nu(OH)_{acid}$	3243,2850		2920,2860m									
$\nu(C=O)_{acid}$	1663s		1690s									
ring stretching			1620,1600		1614m	1649,1596m	1649	1728,1623m		1658,1614m	1649,1614m	1640,1605m	1658,1605m
vibrations	1536s		1518			1500m	1518m	1535m		1526m	1518m	1518m	1570,1526m
$\nu_{as}(COO^-)$		1468s		1472s	1402s	1425s	1447s	1420s		1417s	1408s	1409s	1421s
$\nu_{s}(COO^-)$		1346s		1347s	1280s	1303s	1327s	1298s		1292s	1283s	1284s	1298s
$\delta(OH)_{acid}+\nu(C-O)_{acid}$		1293m		1325m,1290m									
$\tau(OH)_{acid}$	921m	902m	915m	900m	868s	860s	860s	860s		877s	877s	877s	877s
$\nu_{(M-OPh)}$					640s	640s	614s	614s		658s	614s	605s	667s
$\nu_{(M-OH)}$					480m	485m	480m	470m					
$\nu_{(M-OCH3)}$										550m	575m	590m	572m
$\nu_{b(M-Cl)}$					220m	218m	257m	220m		215m		220m	210m

s=strong, b=bridge, m=medium, ν_{as}= antisymmetric stretching, ν_{s}=symmetric stretching

coordination reactions the titration was effected in the presence of metal cation in the ratio metal to ligand 1:2. It can be seen from the potentiometric titration curves that the precipitation of the complexes occurs at relatively low pH's before the ionization of all carboxylic and phenolic protons of the acid ligands takes place. This behaviour of $cafH_3$ and $ferH_2$ resembles the complexing ability of 3,4-dihydroxybenzoic acid which coordinates to metal ions with a catecholic type of binding[19].

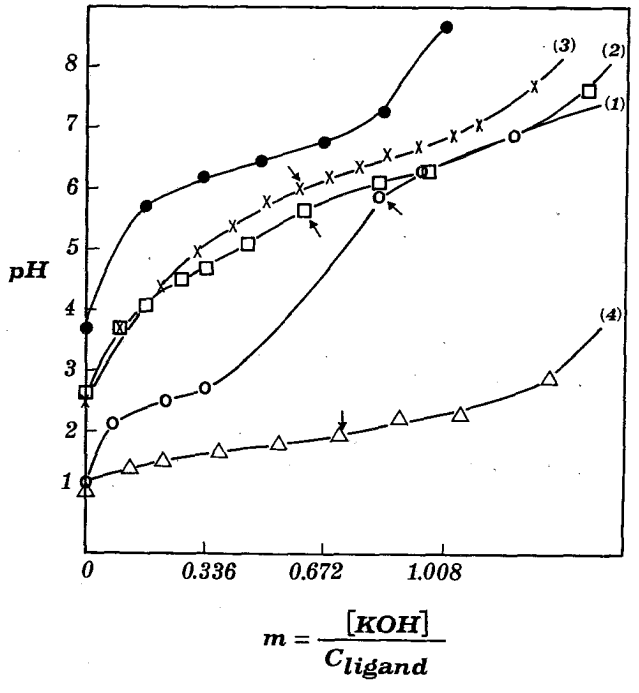


FIG.1.: *Potentiometric titration curves of the caffeic acid and the metal ions in a MeOH:H_2O solution (9:1).*

↓ *Complex precipitation*
L *$cafH_3$*
1 *$cafH_3$+ $CuCl_2$ 2:1*
2 *$cafH_3$+ $CoCl_2$ 2:1*
3 *$cafH_3$+ $NiCl_2$ 2:1*
4 *$cafH_3$+ $FeCl_3$ 2:1*

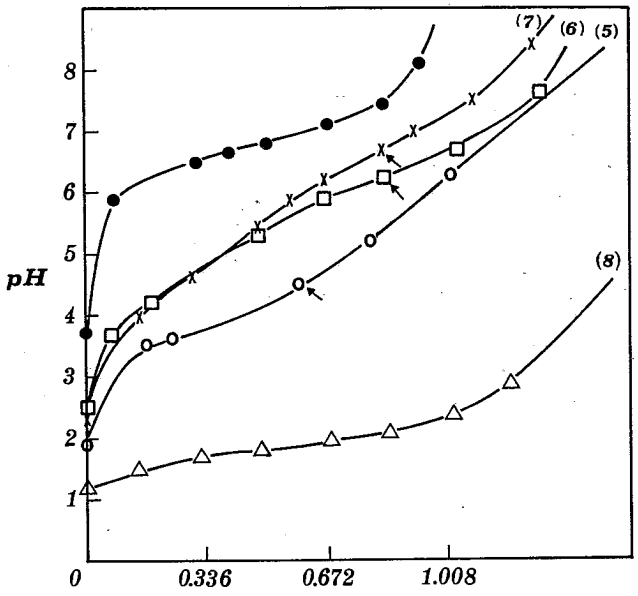


FIG.2: *Potentiometric titration curves of the ferulic acid and the metal ions in a MeOH:H_2O solution (9:1).*

↓ *Complex precipitation*

L *ferH$_2$*

5 *ferH$_2$ + CuCl$_2$* *2:1*

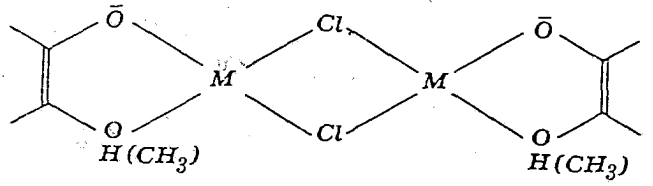
6 *ferH$_2$ + CoCl$_2$* *2:1*

7 *ferH$_2$ + NiCl$_2$* *2:1*

8 *ferH$_2$ + FeCl$_3$* *2:1*

Concluding remarks

From the overall study it is therefore concluded that caffeic and ferulic acids form 1:1 complexes with Cu(II), Co(II), Ni(II) and Fe(III) regardless of the ratio of the ligand to metal cation of the preparation mixture except for the case of the Co(II) complex with ferulic acid which is formed in the ratio 1:2. An analogous situation was met with the complex of hydrocaffeic acid with Co(II)[5]. In the prepared 1:1 complexes chlorine bridged structures are formed with a catechol type of coordination *3*.



3

A pseudotetrahedral or tetrahedral microsymmetry around the metal ion seems to be in prevalence. Owing to the difficulty in obtaining convenient monocrystals of the prepared complexes, their X-rays structural investigation is at present lacking.

ΠΕΡΙΛΗΨΗ

ΣΥΜΠΛΟΚΕΣ ΕΝΩΣΕΙΣ ΤΩΝ ΟΞΕΩΝ ΚΑΦΕΙΚΟΥ ΚΑΙ ΦΕΡΟΥΛΙΚΟΥ ΜΕ Cu(II), Ni(II), Co(II) ΚΑΙ Fe (III).

Παρασκευάσθηκαν και απομονώθηκαν στην στερεά κατάσταση τα σύμπλοκα των οξέων καφεϊκού και φερουλικού με Cu(II), Ni(II), Co(II) και Fe(III) : $K_2[Cu_2(cafH)_2Cl_2]$. $2CH_3OH$, $K_2[Co_2(cafH)_2Cl_2]$, $K_2[Ni_2(cafH)_2Cl_2]$. $2CH_3OH$, $Fe_2(cafH)_2Cl_2.2H_2O$, $K_2[Cu_2(fer)_2Cl_2]$, $K_2(Co(fer)_2]$, $K_2[Ni_2(fer)_2Cl_2]$. $2CH_3OH$, $Fe_2(fer)_2Cl_2$. Ανεξάρτητα από το λόγο μεταλλικού ιόντος: υποκαταστάτη στο μίγμα παρασκευής τα σύμπλοκα που σχηματίζονται είναι 1:1 εκτός από την περίπτωση του συμπλόκου του Co(II) με το φερουλικό οξύ που είναι 1:2. Ανάλογη συμπεριφορά αναφέρεται στην περίπτωση του συμπλόκου του υδροκαφεϊκού οξέος με το Co(II)[5]. Από τα φασματοσκοπικά, μαγνητικά και θερμοσταθμικά αποτελέσματα συμπεραίνεται ότι στα 1:1 σύμπλοκα οι δομές είναι τετραεδρικές διπυρηνικές με γέφυρες χλωρίου και με κατεχολικού τύπου σύμπλεξη.

ACKNOWLEDGEMENTS

We wish to thank the University of Athens for financial support of this work.

REFERENCES AND NOTES

1. Bate-Smith, E. C., *Chem. and Ind.*, 1457 (1954)
2. Stevenson, F., *Humus Chemistry, Genesis, Composition, Reactions.* Wiley-Interscience N. Y. 1982.
3. Jackson, D. E., and Dewick, D. M., *Phytochemistry,* 23(5), 1029 (1984)

4.a. Grodzinska-Zachiwieja, Z., Zgorniak-Nowosielska, I., Marcisjewska, M., and Gatkienicz, A., *Acta Biol. Cracov., (ser-Bot.)* 19(1), 29 (1976)

4.b. Olsen, R. A.,Brown, J. C., Bennett, J. H., and Bloom, D., *J. Plant Nutr.* 5,433, 1982.

4.c. Kiss, T., Nagy, G., Pecsi, M., Kozlowski, H., Micera, G., and Erre, L. S., *Polyhedron* 8, 2345(1989)

5.a. Petrou, A.L., Koromantzou, M. V., and Tsangaris, J. M.,*Trans. Met. Chem.*, 16, 48 (1991)

5.b. Gerega, K., Kozlowski, H., Kiss, T., Micera, G., Erre, L. S., *Inorganica Chimica Acta,* 138, 31-34 (1987)

5.c. Schnitzer, M. and Khan, S. U., *"Humic Substances in the Environment"*, Dekker, N. Y., 1972

6. Bizri, Y., Cromer, M., Lamy, I., and Scharff, J. P., *Analusis* 13, 128 (1985)
7. Linder, P. W., and Vaye, A. *Polyhedron,* 6,53 (1987)
8. Kiss, T., Nagy, G., Pecsi, M., Kozlowski, H., Micera, G., and Erre, L.S., *Polyhedron,*8, 2345 (1989)
9. Cariati, F., Erre, L., Micera, G., Panzanelli, A., Giani, G., and Sironi, A., *Inorg. Chim. Acta,* 80, 57 (1983)
10. Gerega, K., Kozlowski, H., Kiss, T., Micera, G., Erre, L.S., and Cariati, F., *Inorg. Chim. Acta,* 138, 31 (1987)
11. Kiss, T., Koslowski, H., Micera, G., and Erre, L.S., *Polyhedron* 8, 647 (1989)
12. Kabanos, T. A., and Tsangaris, J. M., *J. Coord. Chem.,* 13, 89 (1984)
13. Rahman, A.A., Nichols, D., and Tsangaris, J. M., *J. Coord. Chem.,* 14, 327 (1986)
14. Kovala-Demertzi, D., and Tsangaris, J. M., *Inorg. Chim. Acta,* 125, L31 (1986)
15. Geary, W. J., *Coord. Chem. Rev.,* 7, 81 (1971)
16. Sacconi, L., and Ciampolini, M., *J. Chem. Soc.*, 276 (1964)
17. Gaura, R. M., Stein, P., Willett, R. D., and West, D. X., *Inorg. Chim. Acta,* 60, 213 (1982)
18. Lever, A. B. P. *Inorganic Electronic Spectroscopy.* p. 203. Elsevier Scien. Publ. 1984.
19. Furlani, C. and Morpurgo, G. *Theor. Chim. Acta I,* 102 (1963)

20. Ferguson, J., *J. Chem. Phys.*, 40, 3406 (1964)
21. Figgis, B. N., *Introduction to Ligand Fields*. p. 29, Intern. Publ. N. Y., 1967
22. Earnshaw, A., *Introduction to Magnetochemistry*, Acad. Press. N. Y. 1968
23. Figgis, B. N., and Lewis, J. M. *Progr. Inorg. Chem.*, 6, 429 (1964)
24. Barefield, E. K., Bush, D. H., and Nelson, S. M., *Quart. Rev.*, 22, 457 (1969)
25. Rowley, D. A., and Drago, R. S., *Inorg. Chem.*, 7, 795 (1968)
26. Gerloch, M., Lewis, J., Mabbs, F. E., and Richards, A., *J. Chem. Soc., (A)*, 112 (1968)
27. Elizabathe, J. M., and Zacharias, P. S. *Polyhedron*. 6, 969 (1987)
28. Carlin, R. L., *Science*, 227, 4692 (1985)
29. Dose, E. V., Murphy, K. M. M., and Wilson, L. J., *Inorg. Chem.*, 15, 2622 (1976)
30. Tweedle, M. F., and Wilson, L. J., *J. Am. Chem. Soc.*, 98, 4824 (1976)
31. Maeda, Y., Tsutsumi, N., and Takashima, Y., *Inorg. Chem.*, 23, 2440 (1984)
32. Xu, J., and Jordan, R. B., *Inorg. Chem.*, 27, 4563 (1988)
33. Mikulski, C. M., Mattucci, L., Smith, Y., Tran, T. B., and Karayannis, N.M. *Inorg. Chim. Acta*, 80, 127 (1983)
34. Gelfand, L. S., Iaconianni, F. J., Pytlewski, L. L., Speca, A. N., Mikulski, C. M., and Karayannis, N. M., *J. Inorg. Nucl. Chem.*, 42, 377 (1980)
35. Deacon, G. B., and Phillips, R. J., *Coord. Chem. Rev.*, 33, 227 (1980)
36. Deacon, G. B., Huber, F., and Phillips, R. J., *Inorg. Chim. Acta*, 104, 41 (1985)
37. Nakamoto, K., *Infra Red Spectra of Inorganic and Coordination Compounds* , p. 216 Wiley-Intern. N. Y. 1970
38. Adams, D. M., *Metal- Ligand and Related Vibrations*. p. 248. St. Martin's Press. N.Y. 1968.
39. Kiss, T., Kozlowski, H., Micera, G., and Erre, L. S., *J. Coord. Chem.*, 20, 49 (1989)

Abbreviations

Caffeic Acid	abbr. $cafH_3$
Ferulic Acid	abbr. $FerH_2$

Chimica Chronica, New Series, 22, 205-212 (1993)

COMPARATIVE STUDY OF PARAQUAT TOXICITY ON MICE AND *TETRAHYMENA PYRIFORMIS*

ZUHAIR M. ABDEL-KADER

University of Athens, School of Natural Sciences, Laboratory of Food Chemistry, Athens, Greece

(Received October 30, 1993)

SUMMARY

Comparison of the acute toxicity of paraquat in mice and in *Tetrahymena pyriformis*, strain E resulted in almost identical values, i.e. LD_{50}=25 mg/kg after intramuscular injection to mice and EC_{50}=22 ppm for *Tetrahymena* cultures. The paraquat content of lung tissues of mice reached similar levels (16-22 mg/kg or ppm) 24-48 hours after injections. These data together with microscopical observations on alterations of motility and shapes of the *Tetrahymena* cells indicate that this system is a valuable tool in toxicological studies.

Key words: Paraquat toxicity, *Tetrahymena pyriformis*, mice

INTRODUCTION

Paraquat is the trade name of the dichloride salt of the radical 1,1'-dimethyl-4,4'-bipyridinium, a powerful herbicide used worldwide as a non-selective, contact weedkiller. It was originally synthesized in 1882[1], but its herbicidal properties were revealed in 1959[2]. While there is no evidence for deleterious side-effects in normal use of paraquat, its accidental ingestion can be lethal[2] and a large number of fatal intoxications have been reported during its wide use in farms, plantations and private gardens[3].

The biochemistry and clinical effects of paraquat poisoning have been extensively investigated. The toxicity of paraquat is due to the formation of superoxide free radicals[4] and singlet oxygen which attack the membrane lipids with the formation of lipid peroxides[4,5]. Cell membrane lipids polymerize as a result of the action of these agents and the integrity of cells is lost[5-8]. This action is profound in the epithelial cells of types I and II of lung alveoli, which selectively take up paraquat by energy dependent mechanisms[9-13]. The median lethal dose of oral paraquat shows considerable variation: 50mg/kg in mice, 30 mg/kg in cats, 200 mg/kg in chicken, 70 mg/kg in sheep and 36-54 mg/kg in the ox[14]. It seems that this variation is mainly due

to the variable absorption of paraquat from gastro-intestinal tract, ranging between 10% and 90%[15]. Therefore, paraquat toxicity by intramuscular injection should show much less variation between animal species and was chosen in the present comparative study.

In recent years, there is an internationally incrasing concern towards minimizing experimental animals' use in toxicological studies and there is much effort of research for the development of in vitro techniques able to substitute animal testing. The use of *Tetrahymena* as a toxicological tool has been well documented many years ago[16-23]. In these applications, apart from the lethal or sublethal effects reflected in the extent of inhibition of growth of *Tetrahymena* cultures, several other parameters such as motility and morphological changes of cell shapes or subcellular organelles may give valuable information about the mechanism of action or the side-effects of drugs[17,19,20]. Thus, especially when the mechanism of action of a drug or other toxic material is known or expected on the basis of toxicological data related to another substance of the same family, comparative evaluation of the aforementioned parameters may lead to conclusive evaluation of the toxicological properties of the new substance.

In this present study, the intramuscular paraquat toxicity in mice is compared with the toxic effects of paraquat on the cells of *Tetrahymena* cultures, sharing with the lung alveolar cells the property of phagocytosis.

MATERIALS AND METHODS

Tetrahymena pyriformis, strain E, was grown in 2% Difco proteose-peptone (stock cultures). Working cultures were prepared by axenic transfer of 0.5 ml stock culture into 200 ml of a medium containing 2% proteose-peptone, 0.5% dextrose, 0.2% yeast extract (Difco) and 2 ml of 900 mM Fe^{++}-EDTA complex[24]. The cultures were continuously aerated by magnetic stirring at room temperature (22-24°C). For the toxicity measurements, portion of working culture at the early stationary phase was axenically diluted with growth medium until its optical density (530 nm) was about 0.500. Then, 3 ml of this was added to each of 16 test tubes (1.5x18 cm) containing 0.5 ml of appropriate paraquat solutions in order to obtain final concnetrations of 0, 8, 12, 18, 27, 40, 60 and 90 ppm (in duplicate). The tubes were left tilted in a rack and their optical densities from that of the tubes without paraquat gave the respective inhibitions of growth.

As experimental animals, male mice were used, 15-20 days old and weighing 13-19 g. The animals were housed two per cage and maintained at room temperature (18-21°C) with controlled light-dark cycles. Groups of 8 mice were used for each dose of intramuscular paraquat injection, in the range of 0 to 90 mg/kg body weight..

For measuring the paraquat content in several mice tissues and organs (see below), the respective parts dissected from 4 animals were mixed, homogenized and paraquat was
assayed by the method of Calderbank and Yuen[25].

RESULTS AND DISCUSSION

In order to compare the acute toxicity of paraquat in mice and in *Tetrahymena*, both were intoxicated with equal doses of paraquat, ranging from 8 to 90 mg/kg (for mice) or ppm (for *Tetrahymena* cultures). All doses were given to the mice by intramuscular injection of 0.1 ml per 10 g body weight of appropriate solutions of paraquat in saline. Table I summarizes the mortality data 72 hours after paraquat injection. After this time, no further deaths occurred during the next 10 days. Statistical evaluation of these results by the probit analysis method of Litchfield-Wilcoxon[26] is illustrated in Fig. 1. From this, a value of LD_{50}=25 mg/kg body weight was calculated.

TABLE I: Toxicity data of paraquat on mice and *T.pyriformis*.

Paraquat (mg/kg or ppm)	Mice		*Tetrahymena*	
	Dead/treated	Mortality %*	Optical density	Inhibition %
0	0/8	-	0.417	
8	0/8	2.6	0.385	7.7
12	1/8	10.7	0.335	19.7
18	2/8	29.3	0.292	30.0
27	5/8	56.6	0.174	58.3
40	6/8	80.3	0.107	74.3
60	8/8	94.0	0.007	98.3
90	8/8	98.8	0.000	100.0

* Statistically corrected values according to Litchfield and Wilcoxon[26].

The data of the inhibitory action of paraquat on the growth of *Tetrahymena* cultures are also summarized in Table I and their statistical evaluation (as above) is illustrated in Fig. 2. From this a median effective concentration EC_{50}=22 ppm (or mg/kg) was found i.e. equal to the LD_{50} value of intramuscular paraquat in mice. As a matter of fact, this is a simple coincidence of numerical values, albeit useful to indicate how comparable may be the toxic effects of a substance to quite different living organisms.

On the other hand, a more justifiable comparison is possible on the basis of data on the paraquat content in tissues and organs of intoxicated mice. As shown in Table

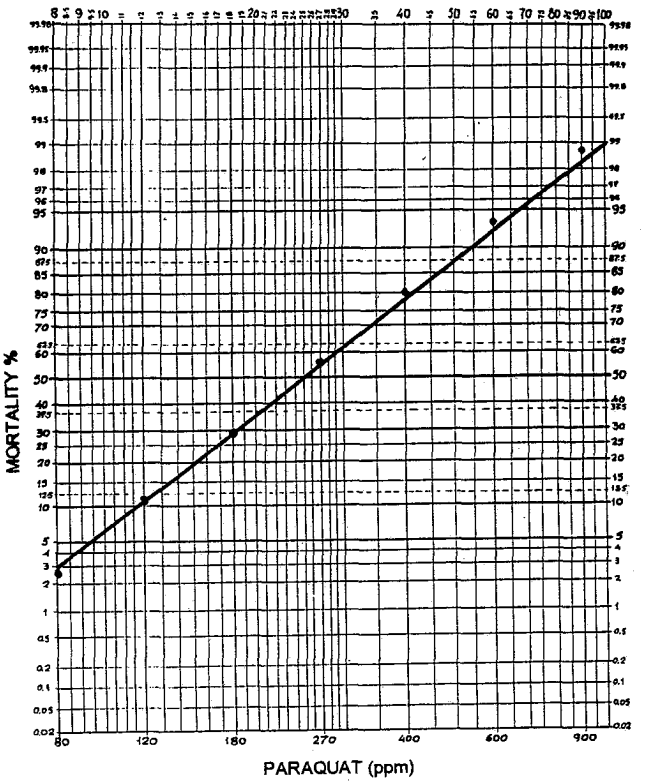


FIG. 1: *Probit diagram of the paraquat toxicity on mice, derived from the data of Table I according to Litchfield and Wilcoxon*[26].

II, soon after injection of paraquat its concentration gradually increases to several tissues being higher in the blood. But at 24 hours after injection the paraquat content is highest in the lung (and secondly in the kidneys), this increase continuing for at least 48 hours (provided that the animal survives). Interestingly, the paraquat concentrations in the lungs (see Table II) are very close to the value of 25 mg/kg, which seems to be the fatal limit. This indicates that data on the acute toxicity of paraquat (and probably of most substances) in *Tetrahymena* may be quite comparable to the respective toxicity in higher animals and man. The same indication results also by comparing the slopes of the probit lines of Fig. 1 and 2, which are very close to each other. This permits direct correlations of toxicities or potencies[26], thus minimizing the number of the experimental animals required in toxicological studies.

Microscopical observations on *Tetrahymena* cells intoxicated with different paraquat concentrations may be summarized as follows: At paraquat concentrations of 8 ppm the motility of the cells was highly increased; at 16 ppm it was normal, while at

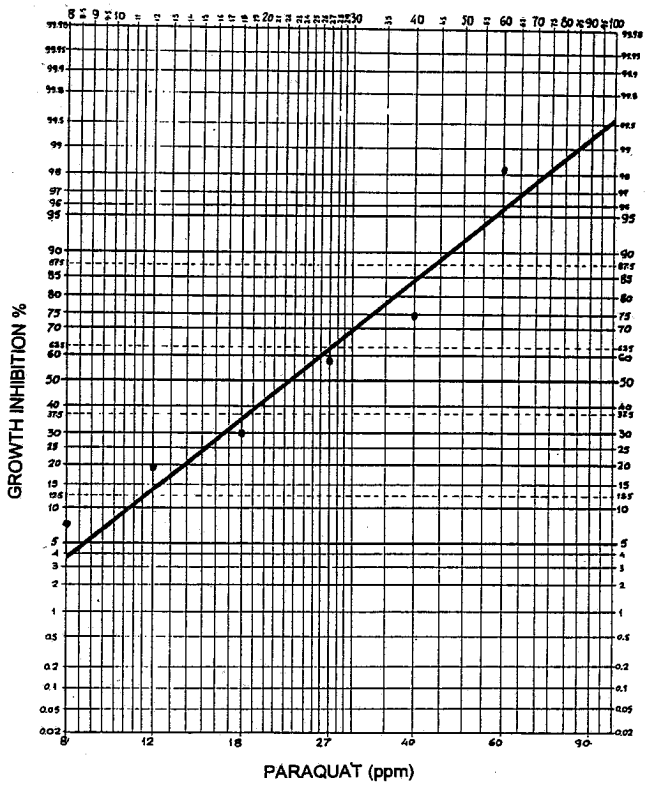


FIG. 2. *Probid diagram of the toxicity of paraquat on Tetrahymena, derived from the data of Table II.*

higher concentrations it was gradually reduced and at paraquat concentrations of 50 ppm or higher the cells were very slowly moving with non-directional movement. Concomitant with reduced motility was dose-dependent alteration of the shape of cells, initially in the posterior part until they became completely spherical. Interestingly, cells harvested and re-suspended in 1% dextrose solution showed highly increased motility even at paraquat concentrations of 50 ppm. However, this was accompanied by less resistance of the cells to the toxic effects and at paraquat concentrations of 80-90 ppm there was a 100% mortality within 30-60 min. This indicates that the paraquat uptake by *Tetrahymena* cells is faster in 1% dextrose, in accordance with older findings supporting that paraquat uptake by lung cells is combined with active transport mechanisms[9].

Even more interesting are some observations relating paraquat toxic effects with the age of *Tetrahymena* cells. Namely, addition of paraquat to early stationary phase

TABLE II: Paraquat concentration in mice tissues. Contents are expressed in ppm (or mg/kg).

Paraquat Dose (mg/kg)	Tissue or Organ	Time after paraquat injection			
		4 h	24 h	48 h	7 days
18	Lung	4	12	16	6
18	Kidneys	5	3	4	3
18	Liver	2	2	-	-
18	Urine	-	3	-	-
18	Blood	6	2	-	-
40	Lung	9	22	-	-
40	Kidneys	-	12	-	-
40	Liver	7	5	-	-
40	Urine	-	4	-	-
40	Blood	10	7	-	-

cells (age: 72 h) caused much less mortality compared to logarithmic phase cells. Furthermore, by successive additions of paraquat, 20 ppm every 24 h, stationary phase cells survived for at least 24 h at paraquat concentrations of 80 ppm. This is also in accordance with the fact that paraquat fatal effects depend on the rate of its uptake by the target cells of the lung[3].

In conclusion, *Tetrahymena* cultures are very valuable tool in toxicological studies, providing very useful information which at least is helpful in order to reduce the number of animal testing.

ΠΕΡΙΛΗΨΗ

Συγκριτική μελέτη της τοξικότητας του paraquat σε ποντίκια και Tetrahymena pyriformis

Σύγκριση της οξείας τοξικότητας του paraquat σε ποντίκαι και σε καλλιέργειες του πρωτοζώου *Tetrahymena pyriformis* (κλάδος Ε) κατέληξε σε όμοιες τιμές (LD_{50}=25 mg/kg στα ποντίκια μετά από ενδομυική χορήγηση, EC_{50}=22 ppm στις καλλιέργειες του πρωτοζώου). Η συγκέντρωση paraquat στους πνεύμονες των ποντικιών έφθασε σε ίδια επίπεδα 24-48 ώρες μετά την ένεση (16-22 mg/kg ή ppm). Τα δεδομένα αυτά, μαζί με εκείνα που προκύπτουν από μικροσκοπικές παρατηρήσεις των μεταβολών της κινητικότητας και του σχήματος των κυττάρων της Τετραϋμένας δείχνουν ότι οι καλλιέργειεες αυτού του πρωτοζώου αποτελούν ένα αξιόλογο σύστημα τοξικολογικών μελετών, ικανό να περιορίσει πολύ μέχρι πάρα πολύ τον αριθμό των απαιτουμένων πειραματοζώων.

REFERENCES

1. Weidel, H. and Russo, M., *Monatsh. Chem.* **3**,850 (1882).
2. Haley, T., *Clin. Toxicol.* **14**,1 (1979).
3. Athanaselis, S. and Koutselinis, A., *Hell. Arm. Forces Med. Rev.* **20**(Suppl. 1),13 (1986).
4. Autor, A.P., *Biochemical mechanisms of paraquat toxicity*, p.240, Academic Press, New York (1977).
5. Smith, J.G., *Human Toxicol.* **7**,15 (1988).
6. Bus, J.S. and Gibson, J.E., *Environ. Health Perspect.* **55**,37 (1984).
7. Fogt, F. and Zilker, T., *Human Toxicol.* **8**,465 (1981).
8. Ledwith, A., In *Biochemical mechanisms of paraquat toxicity*, p.21, Academic Press, London (1977).
9. Rose, M.S., Smith, L.L. and Wyatt, J., *Nature* **252**,314 (1974).
10. Rose, M.S. and Smith, L.L., In *Biochemical mechanisms of paraquat toxicity*, p.71, Academic Press, London (1977).
11. Kimbrough, R.D. and Gaines, T.B., *Toxic Appl. Pharmacol.* **17**,679 (1970).
12. Vijeyaratham, G.S. and Corrin, B., *J. Pathol.* **103**,123 (1971).
13. Smith, P. and heath, D., *J. Pathol.* **114**,177 (1974).
14. Swan, A.A.B., *Brit. Med. J.* **4**,551 (1967).
15. Daniel, J.W. and Gage, J.C., *Brit. J. Int. Med.* **23**,133 (1966).
16. Hutner, S.H., *J. Protozool.* **11**:1 (1964).
17. Sanders, M. and Nathan, H.A., *J. Gen. Microbiol.* **21**,264 (1959).
18. Johnson, I.S., Simpson, D.J. and Cline, J.C., *Cancer Res.* **22**, 617 (1962).
19. Clancy, C.F., *Am. J. Trop. Med. Hyg.* **17**,359 (1968).
20. Schultz, T.W. and Dumont, J.N., *J. Protozool.* **24**,164 (1977).
21. Bringmann, G. and Kuhn, R., *Gesund. Ing.* **80**,239 (1959).
22. Burbanck, W.D. and Spoon, D.M., *J. Protozool.* **4**,739 (1967).
23. Carter, J.W. and Cameron, I.L., *Water Res.* **7**,951 (1973).
24. Kapoulas, V.M., Thompson, G.A.T. Jr and Hanahan, D.J., *Biochim. Biophys. Acta* **176**, 237 (1969).
25. Calderbank, A. and Yuen, S.H., *Analyst* **90**,99 (1965).
26. Litchfield, T.T. and Wilcoxon, F., *J. Pharm. Exp.* **96**,99 (1949).

Chimica Chronica, New Series, 22, 213-224 (1993)

TANNIN CHEMISTRY OF NINE CRETAN CAROB VARIETIES

S. MARAKIS, M.LAMBRAKIS and S.DIAMANTOGLOU

University of Athens, Institute of General Botany, 157 84 Athens, Greece.

(Received June 9, 1993)

SUMMARY

Chemistry and resistance to fungal attack of the ripe desseded carob pod tannins of nine cretan varieties (u-1, u-2, u-3, u-4, u-5 (ungrafted) and g-1, g-2, g-3, g-4 (grafted)), were studied. The percentage of total tannins ranges between 4.2 - 13.3% on desseded carob pod dry weight. The carob pods of the ungrafted varieties were richer in tannins than those of the grafted varieties. The main constituents of carob polyphenols were found to be condensed tannins. The ethyl acetate-soluble thioglycolic acid degradation products, of the examined tannins, were identified as (+) catechin, (-) epicatechin, (-) epigallocatechin, (-) epigallocatechin gallate and (-) epicatechin gallate with exception the (-) epicatechin which was not found in the tannins of the u-5 and g-4 varieties. The (+) catechin-3-gallate was detected for the first time in carob tannins and in fact only in the u-4, u-5, g-2 and g-4 varieties. Strong acid hydrolysis of tannins resulted in the production of delphinidin, cyanidin and pelargonidin, in all carob varieties apart from the last one, which was not identified in tannins of g-1 variety. The alkaline treatment of tannins gave gallic acid, phloroglucinol, catechol and pyrogallol except the last two phenols that were not identified in g-1 tannins. The tannins of the nine carob varieties presented different resistance to fungal activity. This resistance in most carob tannins was similar to that of *Mimosa* tannins.

Key words: Tannins, carob tannins, carob polyphenols, carob beans, tannin degradation, tannase, condensed tannins, (+) catechin, (-) epicatechin, gallocatechin

INTRODUCTION

The carob tree (*Ceratonia siliqua* L.) naturally grows on barren soils (often unproductive for any other type of crop) in most warm regions of the Mediterranean, mainly near the coasts. The species also occurs in Phodesia, parts of the USA, Australia, South America and other

parts with similar climate to that of Mediterranean countries[1,2]. Greece is the fourth largest carob bean (fruit of *Ceratonia siliqua*) producing country (35000 t/a) in the world[1].

Carob fruit consists of about 90% pod and 10% seeds[3]. The ripe deseeded carob pod of nine cretan carob varieties contains high levels (6-13% on dry weight) of total tannins[4,5]. Würsch[6] found that carob pods from Portugal, Italy and Cyprus contained higher tannin amounts (20-27%).

The main constituents of carob polyphenols were found to be condensed tannins, containing the flavan nucleus[7]. The major leucoanthocyanins of deseeded carob pod are highly polymerized leucodelphinidins[8]. Studies on the tannins of wild strawberry leaves and avocado seeds, support the suggestion that condensed tannins are composed of flavan-3-ol and flavan-3,4-diol subunits[9,10].

The structure of condensed tannins has, until recently, not been fully investigated due to the lack of suitalble degradation methods. Beets et al[11] and Tamir et al[12] successfully employed thioglycolic acid (TGA) to degrade the tannins from common heather (*Calluna vulgaris*) and carob pod, respectively. The authors suggested the procedure to be suitable for the degradation of condensed tannins in general.

The tannins (fractions, structure etc) of different carob varieties have not been investigated yet. Tamir et al[12] studied the structure of carob condensed tannins but the authors did not report the carob variety from which tannins were isolated.

This paper describes the chemistry and resistance to fungal attack (activity) of the carob tannins of nine cretan varieties.

EXPERIMENTAL

Carob varieties

Marakis et al[5,13] recognized nine cretan carob varieties: five ungrafted "Agries" (u-1, u-2, u-3, u-4, u-5) and four grafted "Imeres" (g-1, g-2, g-3, g-4).

Tannin isolation from ripe carob pod

A 100 g quantity of deseeded carob pod of each variety was lyophilized and subsequently milled in a mill bearing a 2 mm sieve. 50 g of this pod powder were suspended into 300 ml of deionized water and autoclaved for 30 min at 121°C. The slurry passed through a cheese cloth and its

residue resuspended in 250 ml of deionized water and autoclaved once again. Then the two filtrates were mixed (E_A extract). 0.02 g of sodium pyrosulfite was added to prevent tannin oxidation. The total tannins were isolated from E_A extract after precipitation with saturated solution of lead acetate. The precipitate was filtered and washed with distilled water with continuous agitation. On gradually adding Dowex-50W (H-form) to the suspension, the Pb^{++} was bound to the cation exchanger and the free tannins go into solution. A little ethanol was added to prevent reprecipitation.

Tannin fractions

The separation of the soluble (Sol) and insoluble (Insol1 and Insol2) fractions in ethyl acetate was carried out by the method of Vuataz et al[14]. The remaining in the water layer, Insol1 and Insol2 fractions, were precipitated by saturated solution of lead acetate in pH 5.5 and 8.5, respectively. The tannin fraction precipitated by Tween 80 (TPT fraction) was prepared according to Marakis and Diamantoglou method[15].

Thioglycolic acid (TGA) degradation of condensed tannins

It was made according to Betts et al[11] and Tamir et al[12] procedures.

Mineral acid hydrolysis

It was carried out by 2M HCl[16,17].

Alkaline fusion of tannins

It was made by the method of Roux[18].

Identification of the tannin degradation products

It was made by the methods of Block et al[19], Harborne[20,21], Roux and Maihs[22], Hathway[23], Tamir et al[12], Strumeyer and Malin[24]. In most cases, paper chromatography - Whatman No 1 paper was used. 3 mm paper was used for preparative chromatography of acid hydrolysis products. The two-dimensional solvent system was: n-BuOH-HOAc-H_2O (BAW, 60:15:25) and 2% AcOH. For the detection of anthocyanidins, one-dimensional chromatograms were developed with HOAc-HCl-H_2O ("Forestal" 30:3:10).

Spectra analyses

UV and vis. spectra of the compounds were determined in EtOH or MeOH plus 0.01 M hydrochloric acid[12]. Aluminium chloride shifts were determined by the method of Jurd[25]. IR spectra of the TGA degradation products were obtained using the KBr pellet technique.

Molecular Mass (M) estimation of the tannins

It was carried out according to the procedure described by Tamir et al[12].

Relative Astringency (R.A) estimation of the carob tannins

It was made by the Bate-Smith method[26].

Culture media of fungi

For the estimation of the fungal growth, on substrates containing tannins as sole carbon source, the media A and B were used. Medium-A composition (g/l): Freeze-dried tannins of each cretan carob variety, 20; $(NH_4)_2SO_4$, 5; K_2HPO_4, 1; $MgSO_4.7H_2O$, 0.5; KCl, 0.5; $ZnSO_4$, 0.01; $CuSO_4.5H_2O$, 0.005; biotin, 0.04; thiamine, 1; pyridoxine hydrochloride, 0.5 and nicotinic acid, 0.5. The pH was adjusted to 5.5. Medium-B composition: As medium A but carob tannins were replaced by *Mimosa* tannins (20 g/l).

The media were sterilized by autoclaving (15 min, 121°C).

Batch cultivation

Fungal cultures were carried out according to Marakis and Diamantoglou[15].

Tannase preparation

The purified tannase was prepared from mycelium extract of *Penicillium frequentans*, grown on the tannic acid medium, as Yamada et al[27] described , *P. frequentans* was isolated from moldy carob beans[28].

Fungal tannase activity

Tannase activity was determined by the methods of Yamada et al[27] and Marakis[29].

RESULTS AND DISCUSSION

Greece produces about 45000 tonns per year of carob beans, the 90% of which comes from the island Crete. This low carob bean yield could be easily fivefolded, if only the existing ungrafted trees were grafted by a good quality carob variety.

The total tannin contents (% on carob pod dry weight) and some tannin fractions (% on total tannins) of the nine cretan carob varieties are presented in Table I.

Total tannins

The total tannin precentage of the deseeded carob pod varies, in different carob varieties, from 4.4% (g-1 variety) to 13.3% (u-5 variety). Ungrafted varieties were richer in tannins than grafted ones. This possibly indicates the protective role, tannins have, for the natural

TABLE I. Total tannins (% on desseded carob pod dry weight) and their fractions (% on total tannins) of nine cretan carob varieties.

Carob varieties	Ungrafted varieties					Grafted varieties			
Tannins	u-1	u-2	u-3	u-4	u-5	g-1	g-2	g-3	g-4
Total tannins*	6.7	6.2	5.6	6.1	13.3	4.4	4.7	5,2	4.7
Fraction Sol.	80.4	75.1	60.9	71.8	67.4	67.6	72.3	62.9	66.8
" Insol1	10.7	18.3	33.0	22.8	31.8	27.6	22.7	33.2	33.2
" Insol2	8.9	6.6	6.1	5.4	0.8	4.0	5.0	3.4	ND
" TPT	16.9	18.1	17.8	24.5	9.9	7.1	10.1	12.3	7.9

*Total tannin content of the deseeded carob pod of carob varieties u-1, u-2, u-3, u-4, g-1, g-2, g-3, g-4 has been determined by Marakis et al (1987).
ND=Not detected. Sol=fraction soluble in ethyl acetate. Insol1, Insol2= fractions insoluble in ethyl acetate, precipitated by saturated solution of lead acetate in pH 5.5 and 8.5 respectively. TPT=fraction precipitated by Tween 80.

"wild" varieties, against to microbial attack (activity) and herbivores[15].

The tannin percentages (4.4-13.3%) of the most cretan carob varieties are similar to those reported by Tamir and Alumot[12] and Würsch et al[30], except of Würsch[6] data, where a 20-27% percentage was reported. The observed fluctuation of the carob pod tannin contents may be due to different facts, such as, the carob variety, the producing country or the method used for tannin determination.

Tannin fractions

Carob tannins are generally divided into two groups, according to their behaviour upon extraction of an aqueous solution with ethyl acetate: a) Sol fraction passing into the organic phase and b) Insol1, Insol2 fractions remaining in the water layer. The fraction Sol is 2-8 and 9-19 fold higher than Insol1 and Insol2 fractions, respectively. Grafted varieties are generally richer in Insol1 fraction but poorer in

Insol2, compared to ungrafted varieties. The very low percentage (0.8%) of Insol2 tannins in the u-5 variety and the absence of this tannin fraction from g-4 variety remains someway enigmatic. In our exploratory visits in carob tree plantations, we observed that rats were preferably eating the carob beans and tree bark of the u-5 and g-4 varieties, while they were avoiding other adjacent carob varieties. We estimated that the above preference of the rats was due to the different relative astrigency (R.A) of the cretan carob beans. The R.A determination of the nine carob varieties revealed that: a) The R.A. was generally higher in the ungrafted carob varieties than the grafted ones. b) The R.A value of the u-5 and g-4 varieties was about 4 fold smaller than the one of the other examined carob varieties. This may be due to the highly polymerized tannins of these carob varieties, because the more highly the tannins are polymerized the lower the R.A is, as it occurs in grape tannins[31]. This appears to be true for the cretan carob tannins, since the M (3400 and 3700) of the tannins of the u-5 and g-4 varieties is higher than the other (2600-3000).

Combining the participation percentages of the Sol, Insol1 and Insol2 fractions in the total carob tannins we conclude that the ungrafted varieties u-4 and u-5 present similar percentage values to the grafted varieties g-2 and g-4, respectively.

The TPT fraction consisting of low degree polymerized gallocatechin derivatives ((+) catechin-3-gallate), is degraded and used by a few fungi with high tanninolytic ability and adaptation to tanninous materials (environments)[15].

The "Agria" (ungrafted varieties) carob beans are richer in TPT fraction than the "Imera" (grafted varieties) carob beans. Perhaps for this reason the fungal growth is poorer in E_A extract of "Agria" carob pods compared to the "Imera", although, both substrates contained the same sugar and tannin amounts.

Tannin analysis

Ripe carob pod tannins, mainly condensed, are composed of flavan-3-ol and flavan-3,4-diol subunits (Table II). (+) Catechin and (-) epicatechin gallate esters were found to be the ethyl acetate-soluble thioglycolic acid degradation products of all cretan carob varieties. Tamir et al[12] identified (-) epicatechin gallate, (-) epigallocatechin gallate and (-) epigallocatechin as the products produced by TGA degradation

of ethyl acetate soluble tannin fraction. This led us to conclude that carob pod condensed tannins are probably formed from various subunits of the above flavan-3-ols and their gallate esters. It was the first time that, (+) catechin-3-gallate, from ripe carob pod, was determined exclusively in tannins of the u-4, g-2/u-5, g-4 carob varieties. The first isolation of this ester from a plant source (*Bergenia* species) was reported by Haslam[17]. The hydrolysis of (+) catechin-3-gallate, by *P.frequentans* tannase, gave gallic acid and (+) catechin.

TABLE II. Tannin analysis of nine cretan carob varieties.

Carob varieties / Phenols	Ungrafted varieties					Grafted varieties			
	u-1	u-2	u-3	u-4	u-5	g-1	g-2	g-3	g-4
Flavan-3-ols and their gallate esters:									
(+) Catechin	+	+	+	+	+	+	+	+	+
(-) Epicatechin	+	+	+	+	ND	+	+	+	ND
(-) Epigallocatechin	+	+	+	+	+	+	+	+	+
(-) Epigallocatechin gallate	+	+	+	+	+	+	+	+	+
(-) Epicatechin gallate	+	+	+	+	+	+	+	+	+
(+) Catechin-3-gallate	ND	ND	ND	+	+	ND	+	ND	+
Flavan-3,4-diols:									
Delphinidin	+	+	+	+	+	+	+	+	+
Cyanidin	+	+	+	+	+	+	+	+	+
Pelargonidin	+	+	+	+	+	ND	+	+	+
Other phenols:									
Gallic acid	+	+	+	+	+	+	+	+	+
Phloroglucinol	+	+	+	+	+	+	+	+	+
Catechol	+	+	+	+	+	ND	+	+	+
Pyrogallol	+	+	+	+	+	ND	+	+	+

ND=Not Detected

Both this result and the similar participation percentages of the Sol, Insol1 and Insol2 fractions, in the total tannins, of the u-4, g-2/u-5, g-4 varieties, mentioned above, support that the grafted g-2, g-4 varieties originated from u-4 and u-5 ungrafted ("wild") trees, through grafting. Haslam[17] reported that a polymeric proanthocyanidin localized in the roots of *Bergenia* species consists entirely of (+) catechin-3-

gallate. A polymerization scheme (pathway), which results in C-C bonds, was proposed. This scheme may be applicable to the ripe carob tannins which are probably formed from various catechin units in contrast to the uniformly built *Bergenia* root tannins.

The three compounds obtained by 2M HCl hydrolysis of E_A carob extract tannins were identified as cyanidin, delphinidin and pelargonidin. These compounds may be derived from the polymeric catechin, the copolymer of catechin and leucoanthocyanidin, or the polymeric leucoanthocyanidin[32]. The above three anthocyanidins were determined by Tamir et al[12] in carob tannins. Delphinidin, a highly polymerized compound, consists of the main component of carob leucoanthocyanidins in u-5 and g-4 varieties. The presence of flavan-3-ols and flavan-3,4-diols, in the rich in tannins carob pods, was expected, because these phenols (of great metabolic significance) occur widely in the higher plants (leaves, fruits etc).

The products of tannin fusion with KOH were identified chromatographically: as gallic acid, phloroglucinol,catechol and pyrogallol. Pyrogallol is also produced by gallic acid decarboxylation. Nachtomi and Alumot[33] did not determine free monomers of phenols in ripe carob beans. We suppose that, the relatively high amounts of gallic acid, determined in the nine cretan carob varieties, is due to the enzymic oxidation of the flavanols.

The grafted variety g-1 presented a peculiar image with regard to its tannin structure (profile), as four tannin monomers ((+) catechin-3-gallate, pelargonidin, catechol and pyrogallol) were absent from this carob variety tannins. The fruit characteristics of this variety were entirely different compared to those of other cretan varieties[5,13].

Fungal growth on carob tannins

The growth of 37 fungal species, isolated from tanninous materials[28], presented significant differentiation in media containing tannins of the nine cretan varieties and *Mimosa* tannins (catechols) as sole carbon sources. So, a limited number of fungal species, with high tanninolytic ability, presented a low growth in ungrafted variety tannins with exception the u-5 variety tannins. On the other hand, a higher number of fungi grew in media containing tannins of grafted carob varieties. Particularly, media containing tannins of u-5 and g-4 varieties supported the

growth of the most fungal species. In these media we also observed the richer mycelial growth of the examined fungi. This may be due to low relative astringency or to the absence of (-) epicatechin from the tannins of the u-5 and g-4 carob varieties. Because, as relative experiments have indicated, the fungi: *P. frequentans* (Pefr) and *Aspergillus carbonarius* (AsDT10), both with high tanninolytic ability, appeared to have very low mycelial growth, in medium containing (-) epicatechin as carbon source, compared to that containing (+) catechin. It is also possible that, the absence of (-) epicatechin from the u-5 and g-4 and the low lignin content of these carob varieties attracts the preference of rats to eating the fruits and the tree barks of these carob varieties. Consequently, the tannins of the u-5 and g-4 varieties are not suggested for leather tanning. While the tannins of u-1, u-2, u-3, u-4 and g-3 carob varieties could be used for this purpose because these tannins indicated similar, and in some cases, superior resistance, to microbial attack in comparison to the *Mimosa* tannins used for leather tanning today.

ΠΕΡΙΛΗΨΗ

Ταννίνες χαρουπάλευρου εννέα ποικιλιών κρητικών ξυλοκεράτων.

Η χημική σύσταση και η αντοχή προς τη μικροβιακή δραστηριότητα των ταννινών του χαρουπάλευρου εννέα ποικιλιών κρητικών ξυλοκεράτων (u-1, u-2, u-3, u-4, u-5 (άγριες), g-1, g-2, g-3, g-4 (ήμερες)) μελετήθηκαν. Η περιεκτικότητα του ξηρού χαρουπάλευρου σε ολικές ταννίνες κυμαίνεται, ανάλογα με την ποικιλία, μεταξύ 4.2-13.3%. Τα άγρια ξυλοκέρατα είναι πλουσιότερα σε ταννίνες σε σχέση με τα ήμερα. Τα κύρια συστατικά των χαρουπο-πολυφαινολών είναι οι συμπυκνωμένες ταννίνες. Εξετάζοντας τη δομή των ταννινών και τα διάφορα κλάσματα αυτών (κλάσμα καταβυθιζόμενο από Tween 80 (TPT), κλάσμα διαλυτό (Sol) και κλάσματα (Insol1 και Insol12) αδιάλυτα στο οξικό αιθύλιο) διαπιστώσαμε μια σημαντική διαφοροποίηση μεταξύ των ταννινών των εννέα κρητικών χαρουποποικιλιών. Ετσι: Τα άγρια ξυλοκέρατα είναι πλουσιότερα σε TPT ταννίνες από τα ήμερα. Γι'αυτό ίσως οι ταννίνες των άγριων ποικιλιών είναι ανθεκτικότερες στη μυκητιακή δραστηριότητα παρά οι ταννίνες των ήμερων, καθόσον, το TPT κλάσμα δε διασπάται εύκολα από τους μύκητες[15]. Το κλάσμα Sol είναι 2-8 και 9-19 φορές υψηλότερο από τα κλάσματα Insol1 και Insol2, αντίστοιχα. Γενικά οι ήμερες χαρουποποικιλίες είναι πλουσιότερες σε ταννίνες Insol1, αλλά πτωχότερες σε Insol2 σε σχέση με τις άγριες ποικιλίες. Τα προϊόντα διάσπασης του Sol κλάσματος, όλων των ποικιλιών, βρέθηκε να είναι: (+) κατεχίνη, (-) επικατεχίνη, (-) επιγαλλοκατεχίνη γαλλική και (-) επικατεχίνη γαλλική, με εξαίρεση την (-) επικατεχίνη, η οποία δεν ανιχνεύτηκε στις ποικιλίες u-5 και g-4. Η (+) κατεχίνη-3-γαλλική προσδιορίστηκε για πρώτη φορά σε χαρουποταννίνες και μόνο στις ποικιλίες u-4, u-5, g-2 και g-4. Ισχυρή όξινη υδρόλυση των ταννινών είχε ως αποτέλεσμα την παραγωγή: δελφινιδίνης, κυανιδίνης και πελαργονιδίνης με εξαίρεση την τελευταία, η οποία δεν προσδιορίσθηκε στις ταννίνες της g-1. Η κατεργασία (σύντηξη) των τα-

νινών με ΚΟΗ έδωσε γαλλικό οξύ, φλωρογλυκινόλη, κατεχόλη και πυρογαλλόλη. Οι δύο τελευταίες ενώσεις δεν προσδιορίστηκαν στις ταννίνες της g-1 ποικιλίας.

Οι ταννίνες των εννέα κρητικών ποικιλιών παρουσίασαν διαφορετική αντίσταση στη μυκητιακή δραστηριότητα. Η αντοχή αυτή στις περισσότερες χαρουποταννίνες ήταν παρόμοια με εκείνη των ταννινών της *Mimosa*, οι οποίες, σήμερα, χρησιμοποιούνται ευρέως στη βυρσοδεψία.

REFERENCES

1. Mitrakos, K.: *"The carob"*. A report to Tate and Lyle, Ltd, University of Reading, England (1968).
2. Imrie, F.K.E. and Vlitos, A.J.: *Single-Cell Protein II.* (2nd edition) pp. 223-243. The MIT Press, Cambridge, Massachusetts and London, England (1975).
3. Calixto, F.S. and Canellas, J.: *Appl. Microbiol.* 33, 1319 (1982).
4. Marakis, S. and Karagouni, A.D.: *Biotech. Letters* 7(11), 831 (1985).
5. Marakis, S., Lambrakis, M. and Diamantoglou, S. In: *Proceedings 5e Congres Mediterranen des Techniciens et chimistes des Industries du Cuir*, pp.71-82, Grece-Athenes, Mars (1993).
6. Würsch, P. In: *Proceedings of IInd Internat.* Carob Symposium, pp.621-629, Spain, Valencia, 29 Sept. - 1 Oct. (1987).
7. Nachtomi, E. and Alumot, E.: *J.Sci., Food Agric.* 14, 464 (1963).
8. Joslyn, M.A., Nishira, H. and Ito, S.: *J.Sci., Food Agric.* 19, 543 (1968)
9. Creasy, L.L. and Swain, T.: *Nature* 208, 151 (1965).
10. Geissman, T.A. and Dittmar, H.F.K.: *Phytochem.* 4, 359 (1965).
11. Betts, M.J., Brown, B.R., Brown, P.E. and Pike, W.T.: *Chem.Communications* pp. 1110-1112 (1967).
12. Tamir, M., Nachtomi, E. and Alumot, E.: *Phytochem.* 10, 2769 (1971).
13. Marakis, S., Kalaitzakis, J. and Mitrakos, K. In: *Proceedings of IInd Internat.* Carob Symp. , pp. 195-208, Spain, Valencia, 29 Sept. 1 Oct. (1987).
14. Vuataz, L., Brandenberger, H. and Egli, H.: *J.Chromatog.* 2, 173 (1959).
15. Marakis, S. and Diamantoglou, S.: *Cryptogamie Mycol.* 11(4), 243 (1990).
16. Ito, S. and Oshima, Y.: *Agric. Biol. Chem.* 26, 156 (1962).
17. Haslam, E.: *J. Chem. Soc.* (C), 1824 (1969).
18. Roux, D.G.: *J. Am. Leather Chem. Ass.* 53, 384 (1958).
19. Block, R.J., Durrum, E.L. and Zweig, G.: *Paper Chromatography and Paper Electrophoresis*, Chap. 5. p. 94, Academic Press, New York (1955).
20. Harborne, J.B.: *Biochem. J.* 70, 22 (1958).
21. Harborne, J.B.: *J.Chromatog.* 1, 473 (1958).
22. Roux, D.G. and Maihs, A.E.: *J.Chromatog.* 4, 65 (1960).
23. Hathway, D.E.: *Chromatographic and Electrophoretic Techniques*, chap. 17, p.322, Heinemann, London (1963).
24. Strumeyer, D.H. and Malin, M.J.: *J.Agric.Food Chem.* 23(5), 909 (1975).
25. Jurd, L.: *Phytochem.* 8, 445 (1969).
26. Bate-Smith, E.C.: *Phytochem.* 12, 907 (1973).
27. Yamada, H., Adachi, O., Watanabe, M. and Sato, N.: *Agric. Biol. Chem.* 32(9), 1070 (1968).

28. Charpentie, M.J. et Marakis, S.: *Cryptogamie Mycol.* 1, 165 (1980).
29. Marakis, S.: *PhD. Thesis*, University of Athens, Department of Biological Science, p. 73 (1980).
30. Würsch, P., Del Vedovo, S., Rosset, J. and Smiley, M.: *Lebensm. Wiss. U. Technol.* 17, 351 (1984)
31. Harvalia, A. et Bena-Tzourou, I.: *Hellenica Oenologica Chronica*, Vol. 2. p. 13 (1982).
32. Bate-Smith, E.C. and Swain, T.: *Chem. and Ind.* 1953, 377 (1953).
33. Nachtomi, E. and Alumot, E.: *J.Sci.Food Agric.* 11, 153 (1963).

Chimica Chronica, New Series, 22, 225-236 (1993)

POTENTIOMETRIC STUDIES ON THE TERNARY COMPLEX SYSTEMS CONTAINING N-(2-ACETAMIDO)-IMINO DIACETIC ACID AND ALIPHATIC ACIDS

M.M.A. Hamed, I.T. Ahmed and M.R. Mahmoud

Chemistry Department, Faculty of Science, Assiut University, Assiut, Egypt.

(Received December 28,1992)

SUMMARY

Solution equilibria of the ternary systems M^{+n}(Co(II), Ni(II), Cu(II), Y(III), La(III), Ce(III), UO_2(II))-N-(2-acetamido)-iminodiacetic acid $(HADA)^{-1}$-some di- and tricarboxylic aliphatic acids (succinic, malic, tartaric, citric, H_2L or H_3L) have been studied potentiometrically. From the potentiometric titration curves the formation of the 1:1:1 ternary complexes are inferred. The stability constants of the different binary and ternary complexes formed in such systems have been determined at t=25 $\pm$ 0.1°C and μ=0.1 mol dm^{-3} (KNO_3). It is deduced that the mixed complex is more stable than the corresponding binary aliphatic acid anion complex. The order of stability of the binary or mixed ligand complexes in terms of nature of aliphatic di- and tricarboxylate anions and metal ion has been examined and discussed.

Key Words: Ternary complexes, ADA, aliphatic acids, stability constants.

INTRODUCTION

The coordination chemistry of N-(2-acetamido)-imino diacetic acid (H_2ADA) is of interset due to its biochemical significance. Thus H_2ADA is considered as an important complexing agent in the field of metal ion buffers suitable for use in biochemistry at physiological pH=7.0.

Moreover, it is widely used as an important analytical chelating agent for spectrophotometric determination of some metal ions[1-4] (especially Co(II), Cu(II), U(IV) and Bi(III)). For these important applications many investigators studied the binary metal complexes of H_2ADA[5-8]. On the other hand few publications were published on ternary metal complexes containing such compound[9,10]. Accordingly, the work of the present article is devoted to carry out a systematic potentiometric study on the ternary system: some metal ions M^{+n} (Co(II), Ni(II), Cu(II), Y(III), La(III), Ce(III), UO_2(II)): $HADA^{-1}$: some di- and tricarboxylic aliphatic acids (succinic, malic, tartaric, citric, H_2L or H_3L). The formation constants of the binary and ternary metal complexes have been determined adopting the Irving and Rossotti technique[11]. The aim of this work is to obtain some information about the ternary complex formation in such systems. Moreover, stability of the formed ternary complex in relation to that of the corresponding binary complex as well as in terms of nature of metal ion and aliphatic acid moiety was examined and discussed.

EXPERIMENTAL

Materials and solutions

Matel salts ($CoCl_2.6H_2O$, $NiCl_2.6H_2O$, $CuCl_2.2H_2O$, YCl_3, $La(NO_3)_3.6H_2O$, $Ce(NO_3)_3.6H_2O$, $UO_2(NO_3)_2.6H_2O$) were used as A.R. products. Stock solutions of these metal salts were prepared and standardized as recommended. H_2ADA was analytical grade (BDH) with purity not less than 98% and was used without further purification. Since the solubility of the free acid H_2ADA in pure aqueous media is very small, the monosodium salt was prepared by titration of H_2ADA with standard carbonate-free sodium hydroxide solution. The required concentration was then obtained by accurate dilution. All aliphatic acids used were of extra pure products. Other chemicals used (HNO_3, NaOH, KCl and KH phthalate) were of analytical reagent grade. All solutions were prepared using CO_2-free distilled water.

Potentiometric titrations

Numerous titrations with a relatively high concentrated standard carbonate-free sodium hydroxide solution of different M^{+n}-$HADA^{-1}$ and/or aliphatic acid mixtures in 1:1:1 molar ratio (5×10^{-3} mol dm^{-3} for each) were performed at 25 ± 0.1°C. The constant temperature was achieved by using an air thermostat box. A constant ionic strength was obtained with 0.1 mol dm^{-3} KNO_3 and total volume was kept constant at 50 ml. pH's were measured with Orion pH-meter model 701 A (accurate to ± 0.005 pH unit). The different solutions titrated can be represented according to the following scheme:

HNO_3 (a); HNO_3 + $HADA^{-1}$ (b); HNO_3 + $HADA^{-1}$ + M^{+n} (c);

HNO_3 + aliphatic acid (d); HNO_3 + aliphatic acid + M^{+n} (e);

HNO_3 + $HADA^{-1}$ + aliphatic acid + M^{+n} (f).

RESULTS AND DISCUSSION

Representative typical titration curves obtained according to the sequence described in the experimental section are displayed in Fig. 1. It is evident that the different 1:1 binary $[M(ADA)]^{n-2}$ complexes are formed at low pH's (pH 2.8-3.6). This is achieved from the appeared divergence of the 1:1 binary M^{+n}-$HADA^{-1}$ titration curve from that of the corresponding free $(HADA)^{-1}$ solution. This behavior strongly suggest that the ligand $(HADA)^{-1}$ is characterized by high tendency to form metal complexes. In this respect it is worthy to indicate that binary complex solutions Y(III), Co(II), Ni(II), Cu(II), La(III), Ce(III), UO_2(II) with $(HADA)^{-1}$ show precipitation at high pH's (6-10.40) depending on the nature of metal ion. This can be likely ascribed to the behaviour that such complexes undergo hydrolysis reaction where hydroxo complexes are probably formed. Accordingly in such cases a further study was not possible beyond the precipitation point. Moreover, except in case of Cu(II) the titration curves of the different 1:1

M^{+n}-$(HADA)^{-1}$ complex solutions do not show any buffer zones at high pH's (up to ~10) denoting no possibility for the deprotonation of the amide proton in the formed $[M(ADA)]^{n-2}$ complex[5,7].

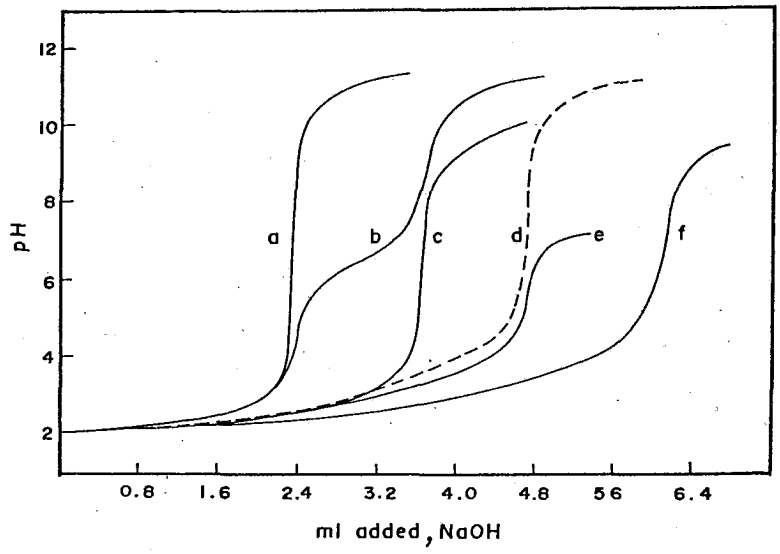


FIG 1: Titration curves for [Co(II)-ADA-tartaric acid] at 25°C and μ = 0.1 mol dm^{-3} KNO_3 with 0.2062 mol dm^{-3} NaOH.

a) 9.54 x 10^{-3} mol dm^{-3} HNO_3 + 0.1 mol dm^{-3} KNO_3
b) Solution (a) + 5.0 x 10^{-3} mol dm^{-3} ADA
c) Solution (b) + 5.0 x 10^{-3} mol dm^{-3} Co(II)
d) Solution (a) + 5.0 x 10^{-3} mol dm^{-3} tartaric acid
e) Solution (d) + 5.0 x 10^{-3} mol dm^{-3} Co(II)
f) Solution (e) + 5.0 x 10^{-3} mol dm^{-3} ADA.

Examination of the different titration curves of the examined binary M^{+n}-aliphatic acids complexes reveals that the pH at which the binary complex is formed largely depends on both the nature of aliphatic acid and metal ion. Generally, for the same metal ion- aliphatic acid anion complex, the pH at

which complex formation starts is increased as the aliphatic acid anion is changed in the direction: citrate → tartarate → malate → succinate. This donates low tendency of aliphatic acid anion towards complex formation in the same sequence. This suggestion is substantiated by the observed decrease in the magnitude of horizontal displacement of curve e from d in the same direction. With respect to the nature of metal ion, one observes that with the same aliphatic acid anion, binary complex formation with metal ions Y(III), Cu(II), UO_2(II), begins at pH's lower than that with the other metal ions applied (Co(II), Ni(II), La(III), Ce(III)). However, M^{+n}-succinic acid complex solutions (except in case of UO_2(II)) as well as La(III), Ce(III)-tartaric complex solutions show precipitation at low pH's (3.5 and 4.5 respectively). Other binary M^{+n}-aliphatic acid complex solutions under investigation do not show precipitation up to pH $\geqslant$ 6. However, in all cases calculations could not be possible beyond the precipitation point.

The titration curves of the different investigated 1:1:1 ternary complexes are strongly overlap with the corresponding ones of the $[M(ADA)]^{n-2}$ solutions at low pH's. Generally, above certain pH value which is largely dependent on the nature of both aliphatic acid and metal ion, a divergence of the ternary titration curve from that of the corresponding $[M(ADA)]^{n-2}$ one is occured. This shows the coordination of aliphatic acid anion with the binary $[M(ADA)]^{n-2}$ complex in stepwise manner as represented by the following equation:

$$M(ADA)^{n-2} + H_2L \text{ or } H_3L \rightleftharpoons M(ADA)(L)^{n-4} \text{ or } M(ADA)(L)^{n-5}$$

Accordingly, it may be assumed that aliphatic acid anion would combine with $[M(ADA)]^{n-2}$ binary complex species in ternary systems as it does with $[M(H_2O)_x]^{+n}$ in a binary system. The horizontal distance between curves c and f was measured and used for the calculation of $\bar{n}_{mix}$ (average number of the secondary ligand aliphatic acid anions attached per

$[M(ADA)]^{n-2}$. The equation used for the calculation of $\bar{n}_{mix}$ was the same as in the original paper[11].

$$\bar{n}_{mix} \quad \frac{(V_f - V_c)[n^o + E^o + T_L^o(Y - \bar{n}_H)]}{(V_o + V_c)\bar{n}_H T_M^o} \qquad \ldots\ldots (1)$$

Here T_M^o is the concentration of $[M(ADA)]^{n-2}$ which is equal to the concentration of M^{+n} used; Y = number of dissociable protons of aliphatic acid (Y = 2 in case of succinic, malic, tartaric and 3 in case of citric acid); V_o = original volume (50 ml); V_c, V_f are the volume of alkali consumed to reach the same pH value in curves c and f. All other symbols have their usual meanings[11]. $\bar{n}_H$ for the secondary ligand, aliphatic acid, at different pH values were available from determination of the aliphatic acids formation constants values as described later. From the values of $\bar{n}_{mix}$ so obtained, free secondary ligand exponent, pL^-_{mix} was calculated using the equation:

$$pL^-_{mix} = \log\left(\frac{\sum_{Y=0}^{Y=2 \text{ or } 3} \beta_Y^H \left(\frac{1}{10^B}\right)^Y}{T_L^o - \bar{n}_{mix} T_M^o} \cdot \frac{V_o + V_f}{V_o}\right) \qquad \ldots\ldots (2)$$

β_Y^H = second and thrid formation constant values for the applying aliphatic acids.

B = the pH meter reading.

The second acid dissociation constant value pK_{a_2} for the mono sodium salt of N-(2-acetamido)-iminodiacetic acid $(HADA)^{-1}$, has been determined under identical conditions from the titration curves a and b using the Irving-Rossotti formulate[11]. The obtained value (6.51) is in agreement with the corresponding literature one[12]. The acid dissociation constant values of aliphatic acids have been determined from the titration curves a and d. The obtained values are

in good agreement with the corresponding ones reported in the literature[13]. The formation constants of the different binary complexes $M(ADA)^{n-2}$ and ML^{n-2} or ML^{n-3} were determined. This was made by applying the original equations of Irving and Rossotti[11] to the binary complex solution systems (curves b, c and d, e for $[M(ADA)]^{n-2}$ and $[ML]^{n-2}$ or $[ML]^{n-3}$ respectively). The different log $K^{M}_{M(ADA)}$, log K^{M}_{ML} and log $K^{M(ADA)}_{M(ADA)(L)}$ values were obtained from the corresponding experimental formation curves making use of the average value and striaght line methods. The values obtained along with the error as obtained by applying the least-squares method are listed in Tables 1,2.

TABLE 1: Formation constant values of the different $[M(ADA)]^{n-2}$ complexes at 25°C and μ=0.1 mol dm^{-3} KNO_3.

M^{+n}	log $K^{H}_{M(ADA)}$		Mean
	i	ii	
Y(III)	7.30	7.65	7.48 ± 0.06
Co(II)	6.80	6.75	6.78 ± 0.05
Ni(II)	7.00	7.05	7.03 ± 0.05
La(III)	6.70	6.70	6.70 ± 0.11
Ce(III)	6.85	6.85	6.85 ± 0.03
UO_2(II)	7.05	7.00	7.03 ± 0.03

i- Average value method, ii- Straight line method.

TABLE 2: Formation constant values for the binary complexes $[ML]^{n-2}$ or $[ML]^{n-3}$ ($\log K^{M}_{ML}$) and those for the mixed ligand complexes $[M(ADA)(L)]^{n-4}$ or $n-5$ ($\log K^{M(ADA)}_{M(ADA)(L)}$) at 25°C and μ = 0.1 mol dm^{-3} KNO_3.

Aliphatic acid	$\log K^{M}_{ML}$							$\log K^{M(ADA)}_{M(ADA)(L)}$						
	Y(III)	Co(II)	Ni(II)	Cu(II)	La(III)	Ce(III)	UO_2(II)	Y(III)	Co(II)	Ni(II)	Cu(II)	La(III)	Ce(III)	UO_2(II)
Citric acid	8.86 ± 0.03	6.47 ± 0.07	6.71 ± 0.02	6.95 ± 0.04	6.52 ± 0.05	6.55 ± 0.04	8.37 ± 0.01	9.56 ± 0.02	7.21 ± 0.02	7.25 ± 0.04	7.56 ± 0.05	7.11 ± 0.03	7.16 ± 0.03	9.00 ± 0.02
Succinic acid	-	-	-	-	-	-	5.26 ± 0.03	4.91 ± 0.08	4.41 ± 0.04	4.54 ± 0.13	4.54 ± 0.10	4.72 ± 0.05	4.60 ± 0.02	5.87 ± 0.03
Malic acid	4.18 ± 0.03	3.25 ± 0.09	3.67 ± 0.02	4.00 ± 0.03	4.49 ± 0.05	4.39 ± 0.02	5.19 ± 0.06	4.85 ± 0.02	4.41 ± 0.06	4.48 ± 0.07	4.50 ± 0.04	4.70 ± 0.02	4.53 ± 0.09	5.63 ± 0.10
Tartaric acid	4.12 ± 0.05	3.10 ± 0.01	3.30 ± 0.01	4.00 ± 0.01	-	-	5.05 ± 0.06	4.69 ± 0.01	4.43 ± 0.10	4.43 ± 0.05	4.50 ± 0.01	4.38 ± 0.01	4.43 ± 0.03	5.16 ± 0.05

The stability of the ternary complex on being compared to that of the corresponding binary aliphatic acid anion complex is expressed in terms of Δlog K (cf. Table 3). Examination of Tables(2,3) clearly indicates that the formation constant corresponding to the association of aliphatic acid anion with $[M(ADA)]^{n-2}$ is higher than that corresponding to the reaction of the same aliphatic acid anion with $[M(H_2O)_x]^{+m}$ i.e the former has higher stability relative to the latter one (Δlog K is positive). This behaviour can be likely ascribed to the presence of two extra five membered chelate rings in the ternary complex as a result of the coordination of the primary N,O,O tridentate $(ADA)^{-2}$ ligand on compared to that in the corresponding 1:1 binary $[ML]^{n-2}$ or $[ML]^{n-3}$ complex where each of the succinate, malate, tartarate forms one, seven membered chelate ring while citrate ion forms two, seven membered chelate.

TABLE 3: Δlog K values for the different $[M(ADA)(L)]^{n-4}$ and $[M(ADA)(L)]^{n-5}$ ternary complexes and the corresponding $[ML]^{n-2}$ or $[ML]^{n-3}$ binary complexes.

Aliphatic acid	Δlog K						
	Y(III)	Co(II)	Ni(II)	Cu(II)	La(III)	Ce(III)	UO_2(II)
Citric acid	+0.70	+0.74	+0.54	+0.61	+0.59	+0.61	+0.63
Succinic acid	-	-	-	-	-	-	+0.51
Malic acid	+0.67	+1.16	+0.81	+0.50	+0.21	+0.14	+0.44
Tartaric acid	+0.57	+1.33	+1.13	+0.50	-	-	+0.11

With respect to the effect of the di- and tricarboxylate aliphatic acid moiety on the stability of the formed binary or ternary complexes of the same metal ion, one can deduce the following stability order: citrate ion (pK_{a_1} = 3.13, pK_{a_2} = 4.76, pK_{a_3} = 6.40) >> succinate ion (pK_{a_1} = 4.21, pK_{a_2} = 5.64) > malate ion (pK_{a_1} = 3.40, pK_{a_2} = 5.05) > tartarate ion (pK_{a_1} = 3.04, pK_{a_2} = 4.37)[13]. The remarkably high stability of the binary or ternary complex containing the tricarboxylate citrate ion can be ascribed to the behaviour that this ligand act as good σ-donor on compared to the dicarboxylate succinate or malate or tartarate ions. Furthermore, the tricarboxylate citrate ion has high tendency to act as O^-,O^-,O^- tridentate ligand leading to formation of two metal chelated rings (both are seven membered). The observed decrease in stability of the same metal ion binary or ternary complex as the nature of the dicarboxylate aliphatic acid moiety is changed along the sequence, succinate → malate → tartarate is in accordance with the decrease in basicity of such ions in the same direction i.e behave as weak σ-donor along the same sequence. Furthermore, carfuel examination of the same ADA and/or aliphatic acid anion metal complex interms of nature of the coordinated metal ion reveals that the stability of such complexes follows the order Y(III) > Cu(II) > Ni(II) > Co(II) (for d-transition metal ions) and the order UO_2(II) > Ce(III) > La(III) (for the inner transition metal ions). This behaviour is in accordance with the usual order of such metal ions complexes[14-16].

ΠΕΡΙΛΗΨΗ

Ποτενσιομετρική μελέτη των τριαδικών συστημάτων συμπλόκων περιεχόντων N-(2-ακεταμιδο)-ιμινο διοξικό οξύ και αλειφατικά οξέα

Εμελετήθηκαν ποτενσιομετρικά διαλύματα σε ισορροπία των τριαδικών συστημάτων M^{+n} (Co(II),Ni(II),Cu(II),Y(III), La(III),Ce(III),UO_2(II)-N-(2-ακεταμιδο)-ιμιδοδιοξικό οξύ ((HADA)$^{-1}$- μερικών δι- και τρικαρβοξυλικά αλειφατικά οξέα

(μυρμικικό,μηλικό,τρυγικό,κιτρικό, H_2L ή H_3L). Από τις καμπύλες της ποτενσιομετρικής ογκομετρήσεως συμπεραίνεται ο σχηματισμός των 1:1:1 τριαδικών συμπλόκων. Οι στεθερές σταθερότητας των διαφόρων δυαδικών και τριαδικών συμπλόκων των σχηματισθέντων εις αυτά τα συστήματα, προσδιορίσθηκαν εις $t=25\pm0.1^{o}C$ και $\mu=0,1$ mol $dm^{-3}(KNO_3)$. Εξάγεται ότι το μικτό σύμπλοκο είναι πλέον σταθερό από το αντίστοιχο δυαδικό ανιοντικό σύμπλοκό αλειφατικού οξέος. Η τάξη σταθερότητας των δυαδικών ή μικτών υποκαταστατών συμπλόκων σε σχέση με την φύση των αλειφατικών δι-και τρικαρβοξυλικών ανιόντων και μεταλλοϊόντων εξετάζεται και συζητείται.

REFERENCES

1. Gonzalez-Portal, A.; Bermejo-Martinez, F. and Baluja-Santos, C.: Acta. Quim. Compostelana **4**, 73 (1980).
2. Lojo-Rocamonde, S.; Gonzalez-Portal, A. and Bermejo-Martinez, F.: Acta. Quim. Compostelana, **5**, 74 (1981).
3. Lojo-Rocamonde, S.; Gonzalez-Portal, A. and Bermejo-Martinez, F.: Acta. Quim. Compostelana **6**, 36 (1982).
4. Gonzalez-Portal, A.; Bermejo-Martinez, F.; Baluja-Santos, C. and Diez-Rodriguez, M.C.: Microchem. J. **31**, 368 (1985).
5. Nakon, R.: Anal. Biochem. **95**, 527 (1979).
6. Lance, E.A. and Nakon, R.: Inorg. Chim. Acta. **55**, L1 (1981).
7. Lance, E.A.; Rhodes III, C.W. and Nakon, R.: Anal. Biochem. **133**, 492 (1983).
8. Paar, D.P.; Rhodes III, C. and Nakon, R.: Inorg. Chim. Acta. **80**, L11 (1983).
9. Nakon, R.; Krishnamoorthy, C.R.; Townshend and Grayson, J.: Inorg. Chim. Acta. **124**, L5 (1986).
10. Mahmoud, M.R.; Azab, H.A.; Mansour, H. and Mohamed, A.H.: Chem. Scripta **29**, 347 (1989).
11. Irving, H.M. and Rossotti, H.S.: J. Chem. Soc. 3397 (1953); 2904 (1954).
12. Perrin, D.D. and Dempsey, B.: Buffers for pH and Metal Ion Control, 1 st ed., p. 160, Chapman and Hall, London (1974).

13. Purie, J.: Handbook of Analytical Chemistry pp. 276, 278, 280 Mir Publisher Moscow (1975).
14. Grinberg, A.A. and Yatsimerski, K.B.: Bull. A. Cad. Sci. URSS Div. Chim. Sci., 239 (1952).
15. Irving, H. and Williams, R.J.P.: J. Chem. Soc. 3192 (1953).
16. Cotton, F.A. and Wilkinson, G.: Advanced Inorganic Chem. pp. 1076, 1078, 1083, 1084, Wiley-East, New Delhi (1972).

SHORT PAPER

PROTEIN-RICH DIET DECREASES RESISTANCE OF MICE TO PARAQUAT POISONING

ZUHAIR M. ABDEL-KADER

University of Athens, School of Natural Sciences, Laboratory of Food Chemistry, Athens, Greece

(Received October 30, 1993)

SUMMARY

The influence of dietary protein on the resistance of mice to paraquat poisoning was studied by intramuscular injection of paraquat solutions to mice fed for 7 days with high- or low-protein diets (400 and 50 g/kg respectively). Paraquat doses up to 200 mg/kg body weight, increasing by a geometrical factor of 2, were used in two initial experiments with total number of 40 mice in each. In a final experiment with 134 animals, paraquat doses up to 90 mg/kg body weight were increasing by a geometrical factor of 1.5. Deaths of animals kept on the protein-rich diet were completed within 48 hours after injection, but within 72 hours in animals kept on low-protein diet. The respective LD_{50} values were 18 mg/kg and 25 mg/kg, this difference being statistically significant at the 95% probability level ($p<0.05$). Possible explanations of the above effects are discussed.

Key words: Dietary protein, paraquat, poisoning, mice

INTRODUCTION

Paraquat (1,1'-dimethyl-4,4'-bipyridinium ion) is a powerful herbicide used world-wide in farms, plantations and private gardns as a non-selective contact weedkiller. Some countries limit the use of paraquat due to the harmful and often lethal effects of intoxication[1].

The toxicity of paraquat is due to the formation of superoxide free radicals[2] and singlet oxygen which attack the membrane lipids with formation of lipid hydroperoxides[2,3]. The biochemistry and clinical effects of paraquat intoxication in humans and animals are well documented in the medical literature[4]. This is due to the wealth of data relating to chemical reactions based on the aforementioned free radicals[5] and to many case reports describing irreversible respiratory failure following ingestion or inhalation of paraquat[6].

Absorption of paraquat from gastrointestinal tract shows considerable variation

(10-90%) and about 90% of the absorbed amount is excreted in the urine within 48-72 hours[7]. Also, considerable variation has been shown regarding the resistance of animal species against paraquat lethal effects[8]. Thus, the following LD_{50} values were estimated: Mice 150 mg/kg, cat 30 mg/kg, chicken 200 mg/kg, sheep 70 mg/kg and ox 36-54 mg/kg[8].

Several studies have shown that the toxic effects of paraquat depend on the presence of oxygen[1,9-13] although in order to prevent the development of superoxide free radicals total exclusion of segments of the lung from external ventilation is required[14].

On the other hand, dietary factors are known to influence the toxic effects of most herbicides and insecticides, especially of the chlorinated hydrocarbons, either by increasing or by decreasing the resistance of the animal organisms[15-25]. In most cases, low protein diets (or even starvation) decrease the toxicity due to lowering of the rates of production of toxic metabolites. The opposite effect was observed in dogs intoxicated with chloroform[21-22], while DDT intoxication did not seem to be influenced by the protein content of the food[15].

In the present work the dependence of paraquat toxicity on mice from the protein content of their diet was investigated.

MATERIALS AND METHODS

Male mice (souris) 15-20 days old and weighting 13-19 g were used in this study. They were reproduced and grown in the animal house of the Laboratory of Experimental Pharmacology, School of Medicine, University of Athens.

Low-protein and high-protein feeds, with the respective compositions given in Table I, were purchased form Zootrophiki Co. (Athens, Greece).

The animals were housed two per cage and maintained at room temperature (18-21°C) with controlled light-dark cycles (12 hours each).

In each series of experiments, the total number of animals was divided into two equal groups (one for each type of diet), and each group was subdivided into subgroups with equal number of animal for each dose of paraquat. The animals of the first group were fed for 7 days with low-protein diet and those of the second group were fed with high-protein diet for the same period of time. Then, the animals were intoxicated by intramuscular injection of 0.1 ml per 10 g body weight of appropriate solutions of paraquat in saline, corresponding to doses up to 200 mg/kg body weight in two preliminary series of experiments, or up to 90 mg/kg body weight in the final series of experiments. Namely:

In two series of initial experiments using 40 mice in each series, doses of 0, 25, 50, 100 and 200 mg paraquat were administered to subgroups of 4 animals. These doses

TABLE I: Composition of low- and high-protein diets of experimental animals.

Components	Low-protein diet	High-protein diet
Ingredients (g/kg)		
Cane sugar	700	100
Molass	100	200
Skimmed milk	100	40
Corn oil	40	500
Soyabean meal	-	100
Wheat flour	-	40
Minerals	40	20
Vitamins	20	
Nutrients' content		
Protein (g/100 g)	5	40
Fat (g/100 g)	4	7
Carbohydrate (g/100 g)	70	37
Energy (KJ/100 g)	1400	1550

correspond to 0.1 ml per 10 g body weight of the following solutions of paraquat in saline: 0, 2.5, 5.0, 10.0 and 20.0 mg/ml, i.e. prepared by consecutive dilutions of the latter with an equal volume of saline solution (dilution factor 2).

In the final series of experiments using a total number of 134 mice, with subgroups of 8 animals the doses were 0, 8, 12, 18, 27, 40, 60 and 90 mg paraquat per kg body weight (corresponding to solutions of 0, 0.8, 1.2, 1.8, 2.7, 4.0, 6.0 and 9.0 mg/ml; dilution factor 1.5).

Estimation of median lethal doses (LD_{50}) and statistical evaluations of the results were made by the probit analysis method of Litchfield and Wilcoxon[26]. Briefly, the method involves plotting the probability units ("probits") of % effect (mortality) against the logarithms of doses ("logits"). A straight line, best fitting to "observed" values -especially the indermediate ones- defines the "expected" (theoretical) values for each dose, including those corresponding to (observed) 0% or 100% mortality. For the latter, "corrected observed" values are then found from a Table. A nomograph connecting the expected value and the difference between each observed (or corrected) effect and the corresponding expected effect gives the chi-square contribution of each point. The sum of chi-squares is multiplied by the average number of animals per subgroup and divided by the degrees of freedom (number of doses minus 2); this value being lower than a critical chi-square value, the data are not

significantly heterogeneous, and the LD_{50} is read from the line of the graph as the dose for 50% effect (mortality). Another two nomographs facilitate the calculations of the confidence limits of LD_{50} and of slopes of lines, as well as of the tests for parallelism of two lines and for relative potencies.

RESULTS

Initially, two series of preliminary experiments were performed using a total number of 40 mice in each series and paraquat doses up to 200 mg/kg body weight, as mentioned above. The results of the second series of these initial experiments (Table II) indicated that under these conditions the LD_{50} values are of the order of 25 mg/kg, but animals fed with a low-protein diet seemed to be more resistant to paraquat poisoning. This is especially evident by comparing the number and the rate of deaths at the doses of 50 and 100 mg/kg.

TABLE II. Mortality data of preliminary experiments (Number of deaths/total number of animals).

Dietary protein	Time (h)	Paraquat doses (mg/kg) 0	25	50	100	200
40%	0	0/4	0/4	0/4	0/4	0/4
	24	0/4	2/4	2/4	4/4	4/4
	48	0/4	2/4	4/4	4/4	4/4
	72	0/4	3/4	4/4	4/4	4/4
5%	0	0/4	0/4	0/4	0/4	0/4
	24	0/4	2/4	1/4	3/4	4/4
	48	0/4	2/4	3/4	3/4	4/4
	72	0/4	2/4	4/4	3/4	4/4

Based on these observations, the final series of experiments was performed with a 2-fold number of animals per subgroup in combination with 8 paraquat doses between 0 and 90 mg/kg (see Materials and Methods).

Table III summarizes the mortality data at 24, 48 and 72 hours after intramuscular injection of paraquat. After this time, no further deaths occurred during the next 10 days. From these data it is evident that both, the rate and total number of deaths were lower in the animals fed with a low-protein diet. In accordance to these qualitative observations, statistical evaluation of these results by the probit analysis method of Litchfield-Wilcoxon[26] showed that LD_{50} values 72 hours after intoxication were 18

TABLE III: Composition of low- and high-protein diets of experimental animals.

Dietary protein	Time (h)	Paraquat doses (mg/kg) 0	8	12	18	27	40	60	90
40%	0	0/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8
	24	0/8	0/8	1/8	1/8	2/8	5/8	6/8	8/8
	48	0/8	0/8	2/8	4/8	6/8	7/8	8/8	8/8
	72	0/8	1/8	2/8	4/8	6/8	7/8	8/8	8/8
5%	0	0/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8
	24	0/8	0/8	0/8	1/8	2/8	4/8	5/8	8/8
	48	0/8	0/8	0/8	1/8	3/8	5/8	7/8	8/8
	72	0/8	0/8	1/8	2/8	5/8	6/8	8/8	8/8

mg/kg and 25 mg/kg for mice fed with a protein-rich and low-protein diet respectively (see Fig. 1). This difference was statistically significant at the 95% probability level ($p<0.05$).

DISCUSSION

The results described above clearly indicate that in mice kept on a high-protein diet the resistance to paraquat poisoning is lower in comparison to mice fed with a low-protein diet. Expressed in terms of LD_{50} values, which were found 18 mg/kg and 25 mg/kg respectively (72 h after intoxication), this difference is statistically significant at the level of $p=0.05$ in a series of experiments using 8 animals per dose of paraquat.

However, taking into account that the same trend was also shown in another two series of initial experiments (Table II), as well as the significantly lower rate of mortality of mice fed with the low-protein diet, observed in all the series of experiments, it may be concluded with certainty that dietary protein exerts an enhancing effect on the toxicity of paraquat.

Several possible mechanisms may be speculated for the above effects of dietary protein. First, a possible metabolite of paraquat, the 4-carboxy-1-methylpyridilium chloride, is more active in all toxic effects of paraquat[2]. Thus, an increase of enzyme synthesis due to high levels of dietary protein may enhance the paraquat toxicity. On the other hand, and increased NADPH production concomitant with increased biosynthetic activity, may enhance the rate of paraquat reduction followed by its re-oxidation combined with the formation of superoxide free radicals. Finally, an increase of cellular activity due to high levels of dietary protein may enhance the rate of paraquat

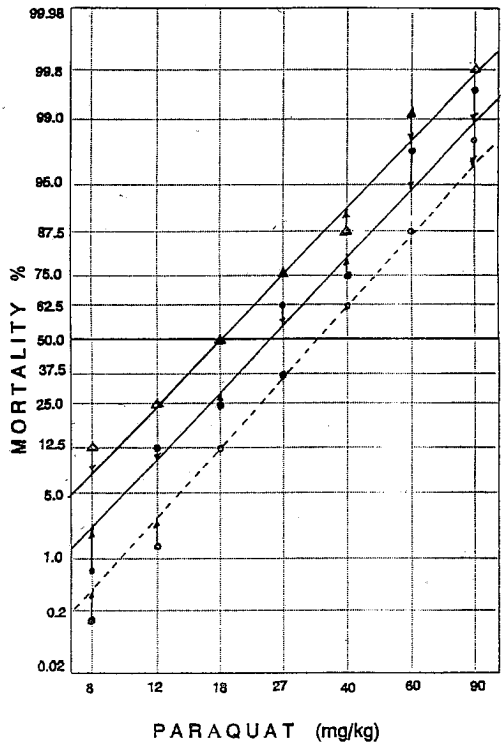


FIG. 1: *Logit probit diagrams of the dose-effect data of Table III corresponding to mortality of mice fed with high-protein diet 48-72 h after intoxication (upper line), or fed with low-protein diet 48 h (lower line) and 72 h (middle line) after intoxication with paraquat. Triangles and circles indicate the observed (or statistically corrected) values corresponding to the lines indicated by arrows. The respective LD_{50} values are directly estimated as the paraquat doses corresponding to 50% mortality.*

uptake by cells, thus enhancing its toxic effects. Relevant to this are the finding of Rose et al[27] indicating that paraquat uptake by lung cells is combined with active transport mechanisms.

ΠΕΡΙΛΗΨΗ

Πλούσια σε πρωτεΐνες δίαιτα ελαττώνει την αντίσταση των ποντικών σε δηλητηρίαση από paraquat

Η επίδραση των πρωτεϊνών της τροφής στην αντίσταση των ποντικών σε δηλητηρίαση από paraquat μελετήθηκε με πειράματα ενδομυικής ένεσης διαλυμάτων paraquat σε ποντίκια, μετά από 7ήμερη διατροφή τους με τροφή πλούσια ή φτωχή σε πρωτεΐνες (400 και 50 g/kg αντίστοιχα). Σε δύο προκαταρκτικά πειράματα (με 40 ποντίκια συνολικά στο καθένα) χορηγήθηκαν δόσεις paraquat μέχρι 200 mg/kg βάρους σώματος, αυξανόμενες γεωμετρικά (με γεωμετρικό λόγο 2). Στο τελικό πείραμα

DIETARY PROTEIN IN PARAQUAT POISONING

χρησιμοποιήθηκαν 134 πειραματόζωα, με δόσεις paraquat 0-90 mg/kg αυξανόμενες με γεωμετρικό λόγο 1,5. Οι θάνατοι των πειραματοζώων που είχαν τραφεί με πλούσια σε πρωτεΐνη δίαιτα περατώθηκε μέσα σε 48 ώρες, ενώ εκείνων που είχαν τραφεί με φτωχή σε πρωτεΐνες τροφή, περατώθηκε σε 72 ώρες. Οι αντίστοιχες τιμές LD_{50} βρέθηκαν 18 mg/kg και 25 mg/kg αντίστοιχα. Η διαφορά αυτή ήταν στατιστικά σημαντική με πιθανότητα 95% ($p<0,05$). Διατυπώνονται απόψεις για τις πιθανές εξηγήσεις των παραπάνω αποτελεσμάτων.

REFERENCES

1. Fogt, F. and Zilker, T., *Human Toxicol.* **8**,465 (1981).
2. Autor, A.P., *Biochemical mechanisms of paraquat toxicity*, p.240, Academic Press, New York (1977).
3. Smith, J.G., *Human Toxicol.* **7**,15 (1988).
4. Bus, J.S. and Gibson, J.E., *Environ. Health Perspect.* **55**,37 (1984).
5. Farrington, J.A., Ebert, M., Land, E.J. and Fletcher, K., *Biochim. Biophys. Acta* **314**,372 (1973).
6. Smith, P. and heath, D.D., *CRC Review of Toxicology* **4**,411 (1976).
7. Daniel, J.W. and Gage, J.C., *Brit. J. Int. Med.* **23**,133 (1966).
8. Swan, A.A.B., *Brit. Med. J.* **4**,551 (1967).
9. Aurelio, P., *The Toxicity of paraquat, diquat and morphamquat*, p.108, Hans Juber, Bern-Stuttgart-Vienna (1978).
10. Fisher, H.K., Clements, J.A. and Wright, R.R., *Am. Rev. Resp. Dis.* **107**,246 (1973).
11. Fisher, A., Kerr, S., Alper, R. and Kefalides, N.A., *Am. Rev. Resp. Dis.* **121**(Suppl.),364 (1980).
12. Onyema, H.P. and Oehme, F.W., *Veterinary and Human Toxicol.* **26**,1 (1984).
13. Suzuki, K., Takasu, N., Arita, S., Maenosono, A., Ishimatsu, S., Nishina, M., Tanaka, S. and Kohama, A., *Human Toxicol.* **8**,33 (1989).
14. Zilker, T. and Fogt, F., *Congress der Wiener Intensivmedizinischen Tage, Vienna, Feb.* (1988).
15. Boyd, E.M. and Chen. C.P. *Arch. Environ. Health* **17**,156 (1968).
16. Boyd, E.M. and Decastro, E.S., *Bull. World Health Org.* **38**,141 (1968).
17. Donaldson, W.E., Sheets, J. and Jackson, M.D., *Poultry Sci.* **47**,237 (1968).
18. Krishnamurthy, K., Urs, T.S. and Kayara, J.P., *Indian J. Exp. Biochem.* **3**,168 (1965).
19. McLean, A.E.M. and McLean, E.K., *Proc. Nutr. Soc.* **26**,13 (1967).
20. McLean, A.E.M. and McLean, E.K., *Brit. Med. Bull.* **25**,278 (1969).
21. Miller, L.L., Ress, J.F. and Whipple, G.H., *Am. J. Med. Sci.* **200**,739 (1940).
22. Miller, L.L. and Whipple, G.H., *Am. J. Med. Sci.* **199**,204 (1940).
23. Seawright, A.A. and McLean, E.K., *Biochem. J.* **105**,1055 (1967).
24. Stickel, W.H., Dodge W.E., Sheldon, W.G., Dewitt, J.B. and Stickel, L.F., *J. Wildlife Management* **29**,147 (1965).
25. Wong, D.T. and Terriere, L.C., *Biochem. Pharmacol.* **14**,375 (1965).
26. Litchfield, T.T. and Wilcoxon, F., *J. Pharm. Exp.* **96**,99 (1949).
27. Rose, M.S., Smith, L.L. and Wyatt, J., *Nature* **252**,314 (1974).

Chimica Chronica, New Series, 22, 245-254 (1993)

2-[2-{(4-CHLOROPHENYL-METHYL-PHENYL)-SILYLOXY}-ETHYL]-1-METHYLPYRROLIDINE, THE SILA-ANALOGUE OF THE ANTIHISTAMINIC DRUG 2-[2-{1-(4-CHLOROPHENYL)-1-PHENYLETHOXY}-ETHYL]-1-METHYLPYRROLIDINE

P. ASLANIDIS

Department of General and Inorganic Chemistry, University of Thessaloniki, Thessaloniki 540 06, Greece.

(Received March 18, 1993)

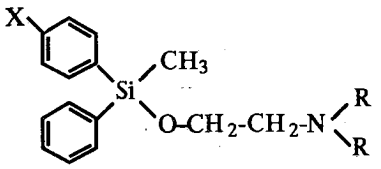
ABSTRACT

The sila-analogue of the antihistaminic drug 2-[2-{1-(4-chlorophenyl)-1-phenylethoxy}-ethyl]-1-methylpyrrolidine ("clemastine") from the class of benz-hydryl ethers and some of its derivatives of type **5** are prepared by successive substitution of the two chlorine atoms in dichloro-methyl-phenylsilane, as shown in scheme 1. Hydrolysis of these compounds in the presence of hydrochloric acid results in the formation of the corresponding disiloxanes. The new compounds and their precursors are characterized by elemental analyses, ^{1}H-NMR and mass-spectra.

Key words: Silapharmaka, 2-[2-{(4-Chlorophenyl-methyl-phenyl)-silyloxy}-ethyl]-1-methylpyrrolidine, Hydrolysis, ^{1}H-NMR, Mass spectra.

INTRODUCTION

It is generally known that no organosilicon compounds have been detected in living matter till now due to lack of *in vivo* processes to build up Si-C or Si-H bonds. However, such compounds, prepared in the laboratory, can exhibit biological activity when applied orally[1]. In recent years a large number of organosilicon compounds, having structures analogous to those of well known drugs have been synthesized and therapeutically or prophylactically used. In most cases the sila-pharmacon and its carbon analogue showed essential equivalence in biological activity and toxicity, but significant differences were found in their pharmacokinetic parameters[2-5]. In fact, a shorter duration of activity and lower toxicity were found for the silicon compounds as a consequence of their hydrolysis in the body fluids.



X= H, Cl, Br, CH_3

NR_2= $N(CH_3)_2$, $N(C_2H_5)_2$

I

Compounds containing Si-O-C units readily undergo hydrolytic decomposition. Thus, replacement of a carbon atom in the C-O-C unit of drugs by silicon could be useful in cases where a sila-pharmacon with a short duration of action is desired. With respect to this point, extensive investigations have been carried out by Wannagat and coworkers[6,7] in the field of sila-benzhydrylethers of type **I**.

Comparative pharmacological tests have shown that the silicon compounds exhibid strong histaminolytic and anticholinergic effects but only for a short period of 15-30 minutes.

The present paper deals with the synthesis and characterization of silicon analogues of the antihistaminic drug 2-[2-{1-(4-chlorophenyl)-1-phenyl-ethoxy}-ethyl]-1-methylpyrrolidine ("clemastine"), from benzhydrylethers **I**. Additionally, the behaviour of these compounds upon hydrolysis is described.

EXPERIMENTAL

Materials

Reactions involving organometallic reagents were carried out under dry nitrogen with the usual precautions for the rigorous exclusion of moisture and air. Diethyl ether used as solvent was dried by refluxing over lithium tetrahydro-alumininate and distilled from it immediately prior to its use. Petroleum ether (bp 40° C) was dried and stored over metallic sodium. Chloro-dimethylamino-methyl-phenylsilane (**2**), chloro-(4-chlorophenyl)-methyl-phenylsilane (**4a**), chloro-methyl-diphenylsilane (**4b**) and chloro-methyl-(4-methylphenyl)-phenylsilane (**4c**) were prepared by the literature methods[8-11]. 4-Chlorophenylmagnesium bromide and 4-methylphenyl-magnesium bromide were prepared from Grignard grade magnesium and 4-chlorobromobenzene and 4-bromotoluene respectively. Dichloro-methyl-phenylsilane, chloromethyl-diphenylsilane, 4-chlorobromobenzene, 4-bromo-toluene, dimethylamine and 2-(2-hydroxyethyl)-1-methylpyrrolidine were obtained commercially.

General techniques

Infrared spectra were recorded on a Perkin-Elmer 467 spectrophotometer, while mass spectra were obtained using a V.G. TS-250 spectrometer. Proton NMR were run on a Bruker AW 80 spectrometer. Proton resonances are given in ppm downfield from internal tetramethylsilane and were obtained using $CDCl_3$ as solvent. Microanalyses for C, H and N were performed on a Perkin-Elmer 240 B elemental analyzer. Molecular weights were determined by mass spectroscopic studies. A Büchi GKR-51 apparatus was used for kugelrohr distillation of viscous products. All boiling points reported here are uncorrected.

*(4-chlorophenyl)-dimethylamino-methyl-phenylsilane (**3a**)*

*a) From chloro-dimethylamino-methyl-phenylsilane (**2**):*

A solution of chloro-dimethylamino-methyl-phenylsilane (35.9 g, 180 mmol) in 50 ml ether was added dropwise at 0° C (ice cooling) during 30 min to a stirred etheral solution of 4-chlorophenylmagnesium bromide [prepared from 4-chlorobromobenzene (36 g, 187 mmol) and excess of magnesium in 150 ml ether]. The reaction mixture was stirred for 1 h and then refluxed for an additional 2 h. The precipitated magnesium salts were filtered off and the ether solution was concentrated yielding a yellow oil which after fractional distillation under vacuum (0.1 Torr) gave the product as a colorless liquid (27.3 g, 55%).

*b) From chloro-(4-chlorophenyl)-methyl-phenylsilane (**4a**):*

0.18 mol of 4-chlorophenylmagnesium bromide, prepared from 36 g 4-bromochlorobenzene and excess of magnesium in 150 ml ether, was added dropwise to a cooled (0° C) solution of dichloro-methyl-phenylsilane (100 ml of a solution containing 37.5 g, 0.18 mol) in ether. After stirring at 0° C for 1 h and refluxing for a 2 h period, the reaction mixture was worked-up as described above yielding 34.5 g (69%) chloro-methyl-(4-chlorophenyl)-phenylsilane. A solution of 20 g (75 mmoles) of the freshly distilled product, dissolved in 150 ml petroleum ether (b.p. 40° C), was introduced into a two-necked, round-bottomed 250 ml flask equipped with a gas inlet, magnetic stirrer and addition funnel. After cooling at 0° C gaseous dimethylamine was passed through the solution over a 20 min period in the course of which dimethylammonium hydrochloride precipitated. The resulting mixture was stirred for 2 h at room temperature and then was refluxed for 30 min. After removing the salt by filtration under nitrogen the solvent was evaporated under reduced pressure and the residue purified by fractional distillation to yield 17.4 g (84%) of the highly sensitive oil, b.p. 140° C/0.1 Torr.

^{1}H NMR ($CDCl_3$): δ=0.52 (s, 3H; $SiCH_3$), 2.54 (s, 6H; $N(CH_3)_2$), 7.2-7.6 (m, 9H; $C_6H_5 + C_6H_4$).

MS (EI, 70 eV): m/z (%)= 275 (M^+, 15), 260 (75), 231 (41), 216 (100), 197 (37), 154 (22), 105 (31), 78 (8), 69 (70), 51 (9).

Anal. calc. for $C_{15}H_{18}NClSi$: C, 65.31; H, 6.57; N, 5.07. Found: C, 65.64; H, 6.76; N, 4.89.

*Dimethylamino-methyl-(4-methylphenyl)-phenylsilane (**3c**)*

A solution of 35.9 g (0.18 mol) of chloro-dimethylamino-methyl-phenylsilane in 50 ml ether was added dropwise at 0° C during 30 min to a stirred equimolar solution of 4-methylphenylmagnesium bromide. The reaction mixture was worked up as described above yielding 22.9 g (50%) of the sensitive colorless liquid, b.p. 124° C/0.1 Torr.

^{1}H NMR ($CDCl_3$): δ=0.60 (s, 3H; $SiCH_3$), 2.35 (s, 3H; Ph-CH_3), 2.60 (s, 6H; $N(CH_3)_2$), 7.0-7.8 (m, 9H; $C_6H_5 + C_6H_4$).

MS (EI, 70 eV): m/z (%)= 255 (M^+, 22), 240 (66), 211 (48), 196 (100), 163 (44), 120 (20), 105 (23), 78 (12).

Anal. calc. for $C_{16}H_{21}NSi$: C, 75.23; H, 8.28; N, 5.48. Found: C, 75.95; H, 8.43; N, 5.30.

*2-[2-{(4-chlorophenyl-methyl-phenyl)-silyloxy}-ethyl]-1-methylpyrrolidine (**5a**)*

*a) From (4-chlorophenyl)-dimethylamino-methyl-phenylsilane (**3a**):*

2-(2-hydroxyethyl)-1-methylpyrrolidine (5.17 g, 40 mmol) was added to a stirred solution of 9.1 g (40 mmol) (4-chlorophenyl)-dimethylamino-methyl-phenylsilane in 150 ml petroleum ether. Gaseous dimethylamine was formed immediately and was removed by a stream of dry nitrogen. The mixture was stirred overnight under nitrogen atmosphere and then refluxed until no evolution of dimethylamine occurred. Removal of the solvent in the rotary evaporation produced a brownish-yellow residue which was purified by Kugelrohr distillation. Yield 12.8 g (89%).

*b) From chloro-(4-chlorophenyl)-methyl-phenylsilane (**4a**):*

Into a two-necked, round-bottomed 500 ml flask equipped with a nitrogen inlet, magnetic stirrer and addition funnel was introduced a solution of 15.5 g (120 mmol) 2-(2-hydroxyethyl)-1-methylpyrrolidine in 300 ml petroleum ether. Chloro-(4-chlorophenyl)-methyl-phenylsilane (10.7 g, 40 mmol) in petroleum ether (50 ml) was added, dropwise through the addition funnel, under nitrogen, to the stirred mixture

over a period of 30 min. A grayish-white precipitate was formed immediately. After the completion of the addition, the mixture was refluxed for 2 h and then suction filtered. The filtrate was distilled and the residue purified by Kugelrohr distillation to afford 4.3 g (30%) of 2-[2-{-(4-chlorophenyl)-methyl-phenylsilyloxy}-ethyl]-1-methylpyrrolidine, b.p.189° C/0.1 Torr.

^{1}H NMR ($CDCl_3$): δ=0.62 (s, 3H; $SiCH_3$), 1.4-2.2 [m, 6H; C-CH_2-CH_2-C (ring) + C-CH_2-C (chain)], 2.22 (s, 3H; NCH_3), 2.82-3.15 (m, 3H; >CH-N + N-CH_2-C), 3.72 (t, 2H; O-CH_2-C), 7.2-7.6 (m, 9H; C_6H_5 + C_6H_4).

MS (EI, 70 eV): m/z (%)= 360 (M^+, 3), 230 (5), 84 (100), 82 (7), 70 (6), 55 (3), 42 (13).

Anal. calc. for $C_{20}H_{26}NClOSi$: C, 66.73; H, 7.28; N, 3.89. Found: C, 66.48; H, 7.09; N, 4.00.

*2-[2-(diphenyl-methyl-silyloxy)-ethyl]-1-methylpyrrolidine (**5b**)*

Using procedure a) described above for the preparation of **5a**, 2-[2-(di-phenyl-methyl-silyloxy)-ethyl]-1-methylpyrrolidine (12.8 g, 95%), b.p. 163° C/ 0.1 Torr, was prepared from dimethylamino-diphenyl-methylsilane (10.0 g 41.4 mmoles) and 2-(2-hydroxyethyl)-1-methylpyrrolidine (5.35 g, 41.4 mmoles) in 150 ml petroleum ether.

^{1}H NMR ($CDCl_3$): δ=0.58 (s, 3H; $SiCH_3$), 1.2-2.3 [m, 6H; C-CH_2-CH_2-C (ring) + C-CH_2-C (chain)], 2.21 (s, 3H; NCH_3), 2.79-3.18 (m, 3H; >CH-N + N-CH_2-C), 3.76 (t, 2H; O-CH_2-C), 7.2-7.6 (m, 10H; C_6H_5).

MS (EI, 70 eV): m/z (%)= 325 (M^+, 10), 310 (8), 197 (5), 84 (100), 77 (7), 70 (10), 55 (6), 42 (25).

Anal. calc. for $C_{20}H_{27}NOSi$: C, 73.80; H, 8.36; N, 4.30. Found: C, 73.44; H, 8.18; N, 4.10.

*2-[2-{methyl-(4-methylphenyl)-phenyl-silyloxy}-ethyl]-1-methylpyrrolidine (**5c**)*

Using the same procedure as for ***5a***, the title compound was prepared from dimethylamino-methyl-(4-chlorophenyl)-phenylsilane (3c) (10.0 g, 39 mmoles) and 2-(2-hydroxyethyl)-1-methylpyrrolidine (5.0 g, 49 mmol) in petroleum ether. The yield of colorless oil was 12.2 g (92%), b.p. 168° C/0.1 Torr.

^{1}H NMR ($CDCl_3$): δ=0.52 (s, 3H; $SiCH_3$), 1.30-2.10 [m, 6H; C-CH_2-CH_2-C (ring) + C-CH_2-C (chain)], 2.20 (s, 3H; Ph-CH_3), 2.30 (s, 3H; NCH_3), 2.77-3.20 (m, 3H; >CH-N + N-CH_2-C), 3.70 (t, 2H; O-CH_2-C), 7.0-7.6 (m, 9H; C_6H_5 + C_6H_4).

MS (EI, 70 eV): m/z (%)= 339 (M^+, 8), 325 (12), 212 (4), 84 (100), 91 (14), 77 (11).

Anal. calc. for $C_{21}H_{29}NOSi$: C, 74.28; H, 8.61; N, 4.12. Found: C, 73.95; H, 8.50; N, 4.30.

Hydrolysis

Small amounts of compounds **5**, dissolved in 50 ml ether, were treated with 4-5 ml diluted hydrochloric acid and the aqueous phase was separated and extracted with 50 ml of ether. The combinated ether phases were washed with 50 ml of water an dried over anhydrous sodium sulfate. After removal of the solvent under reduced pressure, the residue was identified by NMR spectroscopy as the corresponding disiloxane.

RESULTS AND DISCUSSION

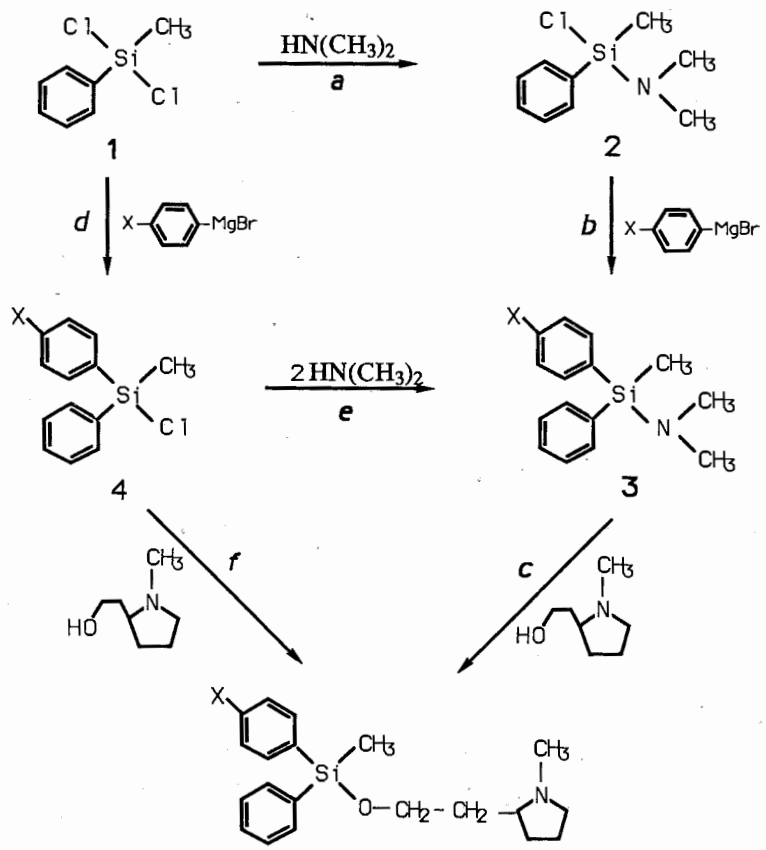
Synthesis

Due to the high reactivity of the Si-Cl bond, chlorosilanes possess a high potence in synthetic applications of which nucleophilic substitution is practically unlimited. Under suitable conditions successive substitution of the two chlorine atoms is possible in the case of dichlorosilanes. Therefore, dichloro-methyl-phenylsilane was employed for the synthesis of sila-clemastine and its derivatives.

The synthesis of compounds **5a-5c** was relatively straightforward and followed established procedures (scheme 1). The first route involves Grignard reaction of dichloro-methyl-phenylsilane with p-chlorophenylmagnesium bromide to introduce the aryl group (reaction **d**) followed by treatment of the intermediate **4** with 2-(2-hydroxyethyl)-1-methylpyrrolidine to introduce the ethoxyamine substituent (reaction **f**). The disadvantage of this short procedure was the unsatisfactory yield due the formation of hydrochloric acid during the second step, although pyridine or excess of 2-(2-hydroxyethyl)-1-methylpyrrolidine was used in order to trap this byproduct.

This problem could be bypassed using a more elegant and convenient method for the formation of the Si-O bond, namely the alcoholic cleavage of the Si-N bond in silylamines **3**. These were easily obtained by treatment of compounds **4** with gaseous dimethylamine in excellent yields, or, allternatively, by partial aminolysis of dichloro-methyl-phenylsilane (reaction **a**) and subsequent Grignard reaction of intermediates **2** (reaction **b**). The attractive feature of this latter route to **3** was the use of chloro-dimethylamino-methyl-phenylsilane (**2**) as the common precursor for the preparation of all products of type **5**. However, partial aminolysis of Si-Cl bond in dichlorosilanes demands low temperatures in order to invoke the cleavage of the

second Si-Cl bond. Therefore, treatment of dichloro-methyl-phenylsilane with dimethylamine was done at -30° C in ether solution. On the other hand no precautions were required during the subsequent introduction of the p-substituted phenyl group.



3-5	a	b	c
x	Cl	H	CH_3

Scheme 1

In summary, among the routes to obtain the sila-analogues **5a-c** of the antihystaminic drug 2-[2-{1-(4-chlorophenyl)-1-phenylethoxy}-ethyl]-1-methyl-pyrrolidine the sequence **1→2→3→5** seemed to be the more advantageous one.

Some of the compounds depicted in scheme 1 (**3b, 4a-c**) have been described previously[10, 11]. They were characterized by comparison of their physical data with the reported values. The novel compounds **3a, 3c** and **5a-c** were obtained as colorless oils and were stored under nitrogen. Further investigations involving determination of the antihistaminic potency of the prepared sila-pharmaca are in progress.

The infrared spectra of the compounds were generally dominated by strong absorptions assigned to the aryl groups and to the heterocyclic ring. Worth menthioning were the characteristic peak at 1250 cm^{-1} for the $\delta(Si\text{-}CH_3)$ vibration[12] and the very strong absorption at 1100 cm^{-1} attributed to the $\delta(Si\text{-}O)$ vibration[13].

The ^1H NMR spectra of the products were diagnostically of major importance, due the presence of various resonances of characteristic chemical shifts, as can be exemplary demonstated by the spectrum of **5a** (figure 1):

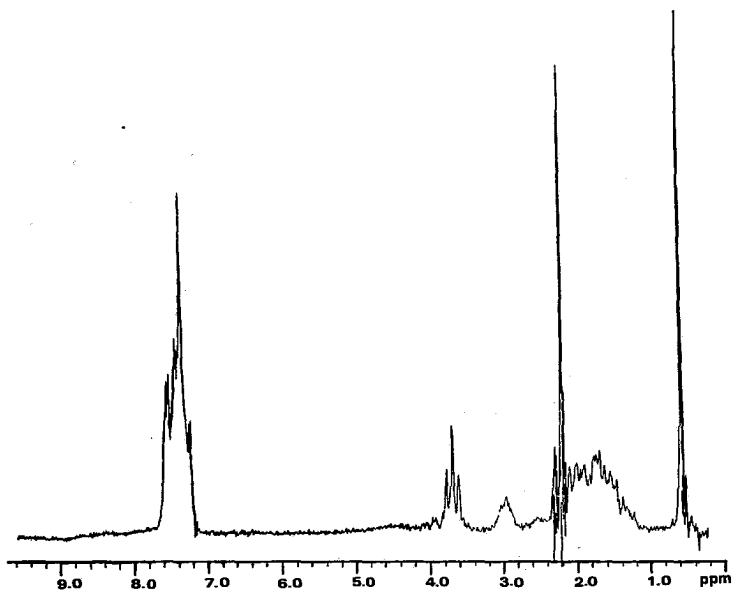
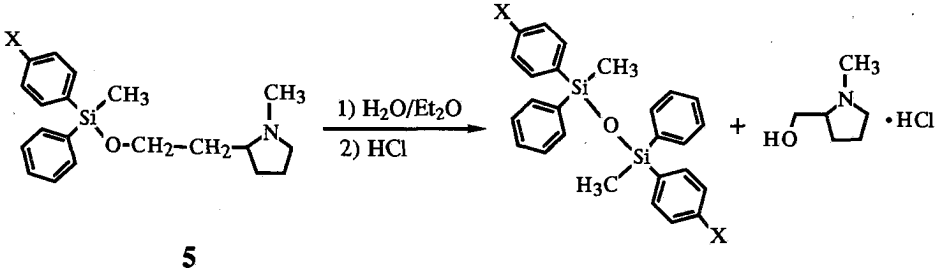


Figure 1: ^1H NMR spectrum of **5a**

In the remarkably simple mass spectra of compounds **5a-c** the molecular ion peak is quite small, but clearly present in all cases. Although there are some differences in relative intensities between the spectra of **5a-c**, the base peak in all the compounds are located at m/e=84, corresponding to the $[CH_3\text{-}N\text{-}CH_2\text{-}CH_2\text{-}CH]^+$ ion. No peaks are observed at m/e=(M-84), but the characteristic ions $[RR'R''Si]^+$ occur at m/e= 230 and 197 for **5a** and **5b** respectivelly.

Hydrolysis

In contrast to their highly moisture sensitive precursors of type **3** or **4** compounds **5a-c** were unusually stable to hydrolysis. Investigation of the possibility of hydrolysing **5b** was carried out using aqueous diethyl ether or tetrahydrofurane, but, after stirring for 12 hours, the starting material did not decompose to a considerable extent. However, the cleavage of the Si-O bond readily occured in the presence of strong acids e.g. HCl, whereby the primarily formed silanol underwent condensation to the corresponding disiloxane (scheme 2):



5 **6**

The isolated disiloxane was identified by comparison of its ^1H NMR data with these of the original compound prepared by condensation of the di-methylamino derivative **3b** with diphenyl-methyl-silanol.

Acknowledgement

Support of this work by the Stiftung Volkswagenwerk, Hannover, Germany, is gratefully acknowledged.

Περίληψη

ΣΙΛΑΝΟ-ΠΑΡΑΓΩΓΑ ΤΟΥ ΑΝΤΙΪΣΤΑΜΙΝΙΚΟΥ ΦΑΡΜΑΚΟΥ 2-[2-{(4-ΧΛΩΡΟΦΑΙΝΥΛΟ)-1-ΦΑΙΝΥΛΟΑΙΘΟΞΥ}-ΑΙΘΥΛΟ]-ΜΕΘΥΛΠΥΡΡΟΛΙΔΙΝΗ

Στην παρούσα εργασία περιγράφεται η σύνθεση σιλανο-παραγώγων της 2-[2-{(4-χλωροφαινυλο)-1-φαινυλοαιθοξυ}-αιθυλο]-1-μεθυλπυρρολιδίνης, ενός αντιϊσταμινικού φαρμάκου γνωστού με την όνομασία "clemastine". Η σύνθεση επιτυγχάνεται με δύο τρόπους και σε τρία στάδια σύμφωνα με τις αντιδράσεις του σχήματος 1. Οι ενδιάμεσες ενώσεις του τύπου **3** και **4** είναι εξαιρετικά ευαίσθητες, ενώ αντίθετα οι ενώσεις του τύπου **5** είναι ασυνήθιστα σταθερές και δεν εμφανίζουν αξιόλογη υδρόλυση, ακόμη και μετά από μακρόχρονη παραμονή τους σε υδατικά διαλύματα. Οι τελευταίες υδρολύονται παρουσία υδροχλωρικού οξέος σχηματίζοντας σιλανόλες, οι οποίες συμπυκνώνονται αμέσως προς τα αντίστοιχα δισιλοξάνια (σχήμα 2). Οι νέες ενώσεις του τύπου **3** και **5** ταυτοποιούνται με στοιχειακή ανάλυση και φασματοσκοπία NMR και μαζών.

REFERENCES

1. Voronkov, M.G., *Pure Appl. Chem.*, **13**, 35 (1966).
2. Fessenden, R.J. and Coon, M.D., *J. Med. Chem.*, **8**, 604 (1965).
3. Fessenden, R.J. and Ahlfors, C., *J. Med. Chem.*, **10**, 810 (1967).
4. Kröning,G., Schulz, E. and Sprung, W.-D., *Pharmazie* **10**, 765 (1975)
5. Rice, L.M., Sheth, B.S. and Wheeler,J.W., *J. Heterocyclic Chem.* **10**, 731 (1973)
6. Tacke, R. and Wannagat, U., *Mh. Chem.*, **106**, 1005(1975).
7. Tacke, R. and Wannagat, U., *Mh. Chem.*, **107**, 111(1976)
8. Washburne, S.S. and Peterson, W.R., *J. Organomet. Chem.* **21**, 59 (1970).
9. Washburne, S.S. and Peterson, W.R., *Inorg. Nucl. Chem. Lett.* **5**, 17 (1970).
10. Popeleva, G.S., Savushkina, V.I., Andrianov, K.A. and Golubtsov, S.A., *Zh. Obshch. Khim.* **32**, 557 (1962).
11. Bazouin, A. and Lefort, M., *Ger. Offen.* 2,014,885, C.A. **73**, 131129 d 1970).
12. Popowski, E., Kosse, P. and Kelling, H., *Z. anorg. allg. Chem.*, **594**, 179 (1991).
13. Wright, N. and Hunter, M.J., *J. Am. Chem. Soc.*, **69**, 803 (1947).

Author Index (Volume 22, 1993)

Errata

The title of the paper by A. Ioannou, M. Doula, A. Dimirkou, P. Papadopoulos in page 141 of the 3/93 issue was accidentally omitted. This should be INTERFACIAL POLARIZATION IN SOLIDS.

The references of the paper by M. Mitrakas and C. Sikalidis in page 172 of the 3/93 issue were not completed. The omitted number of them, 13-17 are as follows:

13. Garagounis K., *Sapropelic Mud Production after Peat Processing with Thermal Mineral Water,* p. 55, Final Research Report, General Secretariat of Research and Technology, Athens (1988) (In Greek).
14. Ciferri R., *Atti XXXVI Congr. Naz. Ass. Med. Ital. Talass. e Ter. Fisica,* Vol. II, Perugia-Chianciano (1959) (In Italian).
15. Angelidis Z. and Mitrakas M., *Preliminary Study of Pikrolimnis's Mineral Water and Mud,* Internal report, Hellenic Association of Municipalities and Communities of Curative Springs and Spas (1987).
16. Valsamaras D. and Gavriilidis G., *Hydrological Report on Lisbori's and Thermi's of Lesbos Thermal Mineral Springs Protection,* Internal reports, Hellenic Association of Municipalities and Communities of Curative Springs and Spas (1990).
17. Agostini G., *Atti XLII Congr. Naz. Ass. Med. Ital. Idrocl. Talass. e Ter. Fisica,* p. 279, Acqui (1971) (In Italian).

Keyword index (Volume 22) 1993

CHIMIKA CHRONIKA, *NEW SERIES*

CONTENTS

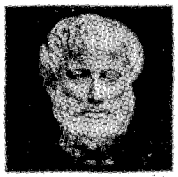
October - December 1993 Volume 22 No 4

This year Macedonia

Macedonia

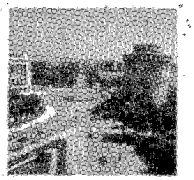
For 4,000 years* steeped in the history of Greece

Statue of Aristotle. Stagira.



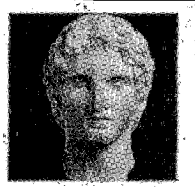
Aristotle, the tutor of Alexander the Great, was born in Stagira in Macedonia in 384 BC. Together with Plato, he is regarded as one of the greatest philosophers the world has known. Aristotle was a true academic, concerned with Physics, Astronomy, Rhetoric, Literature, Political Science and History.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

The White Tower of Thessaloniki.



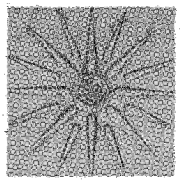
Thessaloniki, the heart of Macedonia, is a modern city with 1,000,000 inhabitants. It is strategically located at the crossroads of Europe with Asia. Having spread the Word at Philippi, the Apostle Paul continued his teachings in Thessaloniki. Its important monuments from antiquity and byzantium up to 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.



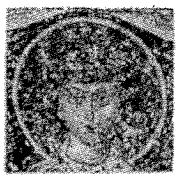
This 16 pointed star of Vergina was uncovered during the archaeological excavations at Vergina. This symbol of the Greek Macedonian Dynasty decorated the golden tomb of Philip II. The Star of Vergina, extracted from the soil of Macedonia, has since become the symbol of Hellenism.

The OlympianAphrodite (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