CHIMIKA CHRONIKA

NEW SERIES

AN INTERNATIONAL EDITION
OF THE ASSOCIATION OF GREEK CHEMISTS

3-4/90

CMCRCZ 19(3-4), 125-248(1990)

Volume 19, No 3-4, p.p. 125-248 September-December 1990

ISSN 0366-693X

CHIMIKA CHRONIKA
NEW SERIES
AN INTERNATIONAL EDITION

Pubished 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 eacch volume, or sent by request. Subscriptions are taken by volume at 1000 drachmas for membres and 2000 drachmas for Corporations in Greece and 28 U.S. dollars to all other countries except Cyprus, where subscriptions are made on request.

Phototypesetted and Printed in Greece by LICHNOS LTD GRAPHIC ARTS
24, PL. THEATROU 105 52 ATHENS tel. 3214766

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

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

Chimika Chronika, New Series, 19, 125-130 (1990)

ON THE INTERACTION OF ADJACENT CYANO AND NITROSO GROUPS

V. PAPAGEORGIOU and S. ZLATANOS

Laboratory of Organic Chemistry, College of Engineering, University of Thessaloniki, Thessaloniki 54006, GREECE

(Received June 30, 1986)

SUMMARY

The substitution of the halogen atom by a cyano group in 1-halo-2-nitroso compounds and the subsequent interaction of the adjacent cyano and nitroso groups have been investigated. Evidence is supplied that hydrogen atoms geminal to the halogen and the nitroso groups influence the mechanism of the substitution reaction to the extent that in their absence a pinacol rearrangement takes place. Interaction of the vicinal cyano and nitroso groups occurs whenever these groups are not bonded to terminal carbon atoms. The interaction leads to a pyrazolic ring as a stable intermediate which subsequently rearranges to 5-amino-isoxazole derivatives. Attempts to form 1-cyano-2-nitroso compounds in one-step 1,2-addition employing nitrosyl cyanide afforded oxazetidine derivatives. Mechanism is proposed to account for the products obtained.

Key words: Cyano and Nitroso groups, N-cyano-oxazetidine, Nitrosylcyanide.

INTRODUCTION

The reaction of olefins with nitrosyl containing inorganic and organic compounds concerns pure and applied chemistry. The 1,2-addition of NOCl to carbon-carbon double bond and the subsequent replacement to the chlorine atom by the cyano group may induce rearrangements[1,2] and could lead to isoxazole derivatives and other important heterocyclic compounds[3,4] paving the way for two-step large scale production of pharmaceuticals from olefins. Pertinent to the ineraction of the -NO and -CN groups in adjacent carbon atoms is the presence of hydrogen atoms since they appear to have a profound influence on the mechanism of the reaction. The use of ONCN in synthetic organic methods[5,6] opens up the possibility of synchronous 1,2-addition of the -NO and -CN groups suggesting possible elimination of undesirable effects by other reactants.

This paper is a continuation of previous investigations on the substitution of halogen atoms by the cyano group in 1-halo-2-nitroso compounds and the interaction of vicinal nitroso and cyano groups.[1,2] In this it is also investigated the addition reaction of nitrosyl cyanide to olefins.

EXPERIMENTAL

Reagents

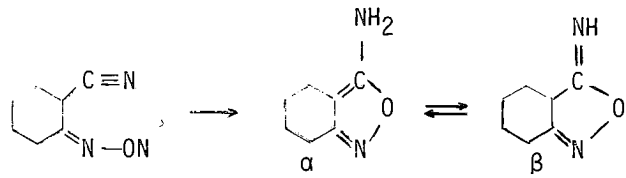
The experimental details of the reactions of 1-halo-2-nitroso cyclohexane and 2-halo-3-nitroso-2,3-dimethylbutane with KCN have been described previously[1,2]. 1-Chloro-2-nitroso-1,2-diphenylethane was prepared by literature methods[3].

Preparation of 2,2-3,3-tetramethyl-N-cyano-oxazetidine

Nitrosyl chloride was added[5] to a stirred suspension of dry silver cyanide (2 mol) in dichloromethane at -30°C. After about 4 min, when the reaction flask was filled with a bluish gas, 2,3-dimethyl-2-butene was added. The reaction mixture was allowed to warm up in about 30 min by which time the bluish gas disappeared. TLC indicated the presence of several compounds and the major component was separated by preparative gas chromatography on a Carbowax 20 column. Attempts to purify further the pale yellow viscous liquid by fractional distillation failed since the heat applied enhanced its tendency to polymerise even under reduced pressure. IR (Nujol):2260 ($C\equiv N$), 1360 cm^{-1} ms, m/z :140(2.5%, $M^{+\cdot}$), 125(5.4%, $M-CH_3$), 110(100%, $M-2CH_3$), 84(69%, $M-2CH_3-CN$) Found:C, 60.15; H, 8.61; N, 20.1. $C_7H_{12}N_2O$ requires: C, 60; H, 8.57; N, 20%.

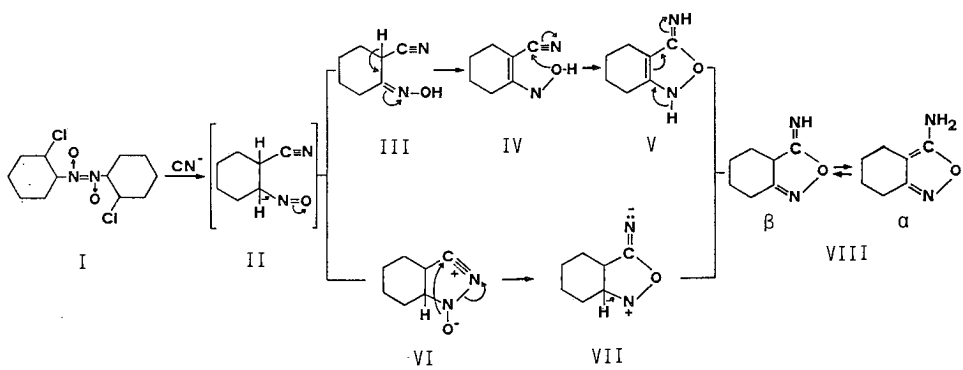
RESULTS AND DISCUSSION

Initially cyclohexene was selected as an appropriate unsaturated system to form the 1-cyano-2-nitroso derivative in order to investigate the interaction of these groups in adjacent carbon atoms. The first step, the formation of 1-halo-2-nitrosocyclohexane is a straightforward process[7]. Two reports appeared almost simultaneously[1,4] dealing with the reaction of 1-chloro-2-nitrosocyclohexane dimer with KCN. Although the investigators supply evidence that the chlorine atom is replaced by the cyano group, the intermediacy of 1-cyano-2-nitrosocyclohexane has not been proved. Dines and Scheinbaum[4] suggested that in the presence of triethylamine the reaction proceeds via an oxime which then readily isomerises to 3,4-cyclohexano-5-amino-isoxazole ($VIII_\alpha$). However, the conversion of 1-cyano-2-oximinocyclohexane (III) to the isomeric forms of 3,4-cyclohexeno-5-amino-isoxazole was already reported in the literature[8].



III VIII

The experimental evidence we have gathered[1,2] on the interaction of vicinal -CN and -NO groups bonded to carbon with labile hydrogen atoms favours the formation of a pyrazole derivative of polar structure VI which is subsequently converted to isomers $VIII_{\alpha,\beta}$ (Scheme 1). The formation of the pyrazolic ring bears an analogy with the well-documented interaction between pairs of adjacent -N=N- and -NO groups[9-11].

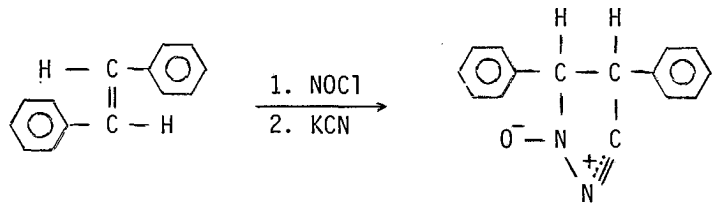


Scheme 1

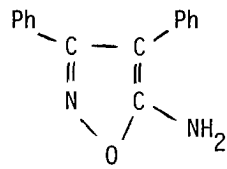
Furthermore the fragmentation pattern in the mass spectrum of the isomers $VIII_{\alpha,\beta}$ differs substantially from that of the solid which, however, converts to $VIII_{\alpha,\beta}$ when left in the dark for several days. Since Dines and Scheinbaum[4] did not obtain compound VI when they treated 1-chloro-2-nitroso-cyclohexane with KCN in the presence of triethylamine, it is suggested that the reaction might proceed via alternative routes (Scheme 1).

Dines and Scheinbaum[4] strating from either *cis* or *trans* 2-butene obtained 3,4-dimethyl-5-amino-isoxazole in two steps. However, their attempts to convert 1-cyano-2-methyl-propionaldioxime to the desired 3,3-dimethyl-5-amino-isoxazole did not prove successful. On the basis of

these observations they suggested that an obvious condition for the formation of 5-amino-isoxazoles in the presence of hydrogen on the vicinal carbon atoms bearing the -CN and -NO groups provided that the carbon atoms are not terminal. In order to test the validity of the above suggestion we have chosen *trans*-stilbene and 2,3-dimethyl-2-butene. Treatment of *trans*-stilbene with NOCl and subsequently with KCN afforded compound X.[2] The complete conversion of X to 3,4-diphenyl-5-amino-isoxazole took several days, the mass spectrum of which lacked the $[M-28]^+$ peak.



IX X

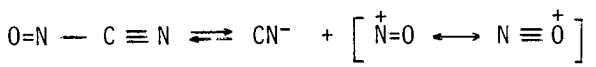


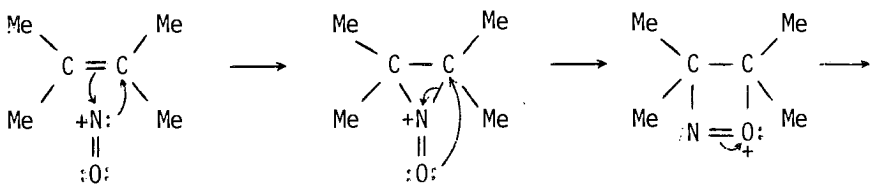
XI

The confirmation of the important role of the labile hydrogen atoms suggested that the pyrazolic ring structure might be more stable in the absence of hydrogen such as in the case of 2,3-dimethyl-2-cyano-3-nitrosobutane.

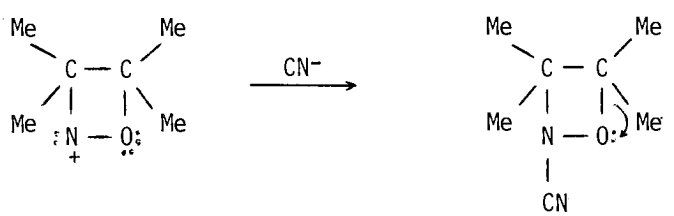
The mode of action of the cyanide ion and the failure to form the 2-cyano-3-nitroso derivative of 2,3-dimethyl-butane suggested that other reaction pathways should be sought for its preparation. Kirby and coworkers[5,6] prepared ONCN and established that it is a powerful electrophilic dienophile. However, as far as we know, no reports exist describing 1,2-addition although there is such a possibility in view of the structural similarities (12-14) of this compound with NOCl and, more important, with CF_3NO. Thus, the reaction of 2,3-dimethyl-2-butene with ONCN afforded a yellowish product in the mass spectrum of which appear

peaks at m/z=140 $[M]^+$, 125 $[M\text{-}CH_3]^+$, 110 $[M\text{-}2CH_3]^+$ and 84 $[M\text{-}2CH_3\text{-}CN]^+$. The presence of cyano-group is revealed by the strong absorption at 2260 cm^{-1} while the band in the fingerprint region at 1360 cm^{-1} is attributed to the oxazetidine ring[15]. Contrary to expectations, the evidence suggests that the most likely structure is that of 2,2,3,3-tetramethyl-N-cyano-1,2-oxazetidine (XVI). Thus, although an 1,2-addition takes place it only involves the NO group. A plausible explanation is the outlined mechanism.





XII XIII XIV



XV XVI

The viscosity of the liquid increased after a few hours with subsequent loss of intensity of the 1350 cm^{-1} band, while no change was evident in the absorption intensity of the $-C\equiv N$ band. Changes were also observed in the mass spectrum which, when considered in conjuction with the ir spectrum, suggest that distruction of the oxazetidine ring occurs and a polymer is formed with -N(CN)-O-C-C backbone. Although the oxazetidine derivative and the polymer merit further investigation, it is beyond the scope of the present study.

Περίληψη

Αλληλεπίδραση γειτονικών κυανο και νιτρωδο ομάδων

Μελετήθηκαν η αντικατάσταση αλογόνου από την κυανο ομάδα σε 1-αλογονο-2-νιτρωδο ενώσεις και η μετέπειτα αλληλεπίδραση των γειτονικών κυανο- και νιτρωδο- ομάδων. Παρέχονται στοιχεία ότι η παρουσία υδρογόνου στα άτομα άνθρακα που έχουν αλογόνο ή την κυανο- ομάδα επηρεάζει τον μηχανισμό της αντίδρασης. Η αλληλεπίδραση γειτονικών κυανο- και νιτρωδο- ομάδων επιτελείται εφόσον δεν είναι προσαρτημένες σε ακραία άτομα άνθρακα. Από την αλληλεπίδραση προκύπτει πυραζολικός δακτύλιος ως σταθερό ενδιάμεσο προϊόν που στη συνέχεια μετασχηματίζεται σε παράγωγα του 5-αμινο ισοξαζολίου. Προσπάθεια σχηματισμού 1-κυανο-2-νιτρωδο-ενώσεων σε ένα στάδιο με 1,2-προσθήκη του ONNC έδωσε παράγωγα της οξαζετιδίνης. Προτείνονται μηχανισμοί σχηματισμού των λαμβανομένων προϊόντων.

REFERENCES

1. Papageorgiou V.P.: *Ph. D. Thesis*, Univ. of Thessaloniki, 1970.
2. Papageorgiou V.P.: *Chim. Chron.*, 3, 47, (1974).
3. Tilden W. and Forster J.: *J. Chem. Soc.*, 65, 327 (1894): Beilstein V, p. 632.
4. Dines M.B. and Scheinbaum M.L.: *Tet. Letters*, 4817 (1969).
5. Horsewood P. and Kirby G.W.: *Chem. Comm.*, 1139 (1971).
6. Horsewood P. and Kirby G.W.: *J.C.S. Perkin I*, 1587 (1980).
7. Ponder B.P., Walton T.E. and Pollock W.J.: *J. Org. Chem.*, 33, 3957 (1968).
8. Von Anwers K.: *Ann.*, 441, 88 (1925).
9. Goldstein H. and Stamm R.: *Helv. Chim. Acta*, 35, 1470 (1952).
10. Bauer H., Bedford G. and Katritzky A.: *J. Chem. Soc.*, 751, (1964).
11. Diehl P., Chist H. and Mallory F.: *Helv. Chim. Acta*, 45, 504 (1962).
12. Mark H.F., Gaylord N.G. and Bikales N.M.: "*Encyclopedia of Polymer Science and Technology*", Wiley John, V. 9, p. 322-336, N. York, 1968.
13. Gowenlock B.G., Johnson C.A.F., Keary C.M. and Pfab J.: *J. Chem. Soc. Perkin 2*, 1975, 351.
14. Dickinson R., Kirby G.W., Sweeny J.G. and Tyler J.K.: *J. Chem. Soc. Faraday 2*, 74, 1393 (1978).
15. Falk R.A. and Readio J.D.: *J. Org. Chem.*, 34, 4089 (1969).

Chimika Chronika, New Series, 19, 131-136 (1990)

COMPOSITION OF INDUSTRIAL WHEYS FROM FETA AND KEFALOGRAVIERA CHEESES AND MYZITHRA SERUM

C.SCHILLER-KOLLIA, I.G.ROUSSIS AND M.G.KONTOMINAS

Department of Chemistry, University of Ioannina, Ioannina 45110, Greece

(Received July 24, 1986)

SUMMARY

The solid residue, ash, lactose, fat, and nitrogen content and the elements K, Ca, Mg, P, Cu, Fe, Zn, Mn, Sr, Al, Pb, Ba, and As, using ICP-emission spectroscopy, were determined in industrial wheys from feta and kefalograviera cheeses as well as in myzithra (cheese from whey) serum. The two cheese wheys did not show significant differences in composition between them. The myzithra serum contained essentially no fat and a significant amount of nitrogen eventhough lower than that of the two cheese wheys. Myzithra serum, similar to the cheese wheys, contained significant amounts of K, Na, Ca, Mg, P and Zn. It is therefore proposed that the serum can be incorporated along with the cheese wheys for further industrial utilization.

Key words: whey, serum, composition, elements

INTRODUCTION

The whey produced during cheese making practises contains various constituents of high nutritional value[1,2] while as an industrial waste it is responsible for a significant amount of resulting environmental pollution[3].

The various methods of whey utilization[2,4], especially the current trends employing microorganisms, such as those producing single cell protein[5,6], ethanol[7,8] and other products[9,10] demand exact knowledge of its composition including macro- and trace- elements.

The objective of this paper was to report values of the main constituents and elements of industrial wheys of one soft cheese (feta), one hard cheese (kefalograviera) and of the serum of a cheese whey (myzithra) as well as to apply Inductively Coupled Plasma (ICP)-emission spectroscopy to the analysis of cheese wheys. The samples were selected so, to investigate possible differentiation in

composition between the two wheys as well as to determine whether the myzithra serum could be further utilized.

EXPERIMENTAL

Materials

Samples were supplied by the dairy plant "DODONI' S.A. in Ioannina in various seasons during the year. The feta cheese (soft cheese) and the kefalograviera cheese (hard cheese) were produced from ewe's milk which contained a small amount of goat's milk. The myzithra cheese (cheese from whey) was produced from kefalograviera whey with the addition of about 10% whole milk (ewe´s milk plus some goat's milk).

TABLE I. Spectral lines and detection limits in sample using ICP-emission spectroscopy.

Element	Spectral lines (mm)	Detection limit in sample (ppm)
Zn	202.548	5
P	213.618	10
Pb	220.353	0.1
As	234.984	0.05
Al	308.215	0.05
Cu	324.754	0.5
Mg	383.826	5
Sr	407.771	0.1
Ba	455.404	0.01
Mn	257.610	0.01
Fe	259.940	2
Na	588.995	10
Ca	315.887	5
K	766.490	20

The solid residue was determined gravimetrically at 100-105°C until constant weight. Fat was determined by the Gerber method using specifically designed butyrometers for whey and defatted milk. Nitrogen was determined by the Kjeldahl

method[2]. Lactose was determined by the IDF method[11] for milk using chloramine T after deproteination. Ash was determined by dry ashing at 550°C[12]. Elements were determined by ICP-emission spectrometry. This method which provides an excellent sensitivity along with multielement determination capabilities[13], has been previously applied to milk analysis[14,15]. A Philips ICP-source model PV 8490 was used. Spectrometric conditions were as follows:

burner: Universal type
Plasma Argon: 21 1/min
carrier Argon: 1.2 1/min
sample uptake: 1.9 ml/min
nebulizer: cross-flow with peristaltic pump
Spectral lines and detection limits in the sample are given in Table I.

RESULTS AND DISCUSSION

Results for the solid residue, ash, lactose, fat and nitrogen content are given in Table II. Data reported for the two wheys (Table II) are within the range of values reported in the literature[2,16-18]. Above values indicate no statistically significant differences between feta and kefalograviera wheys (p=0.20).

TABLE II. Solid residue, ash, lactose, fat and nitrogen content of wheys and serum of myzithra.

	Whey of Feta (WF) % (b.w.)		Whey of Kefalograviera (WK) % (b.w.)		Serum of myzithra (SM) % (b.w.)	
Constituent	average	range	average	range	average	range
solid residue %	7.79±0.39*	7.48±8.34	7.63±0.17	7.37-7.74	7.50±0.22	7.24-7.76
Ash %	0.49±0.02	0.48±0.53	0.50±0.02	0.48-0.53	0.50±0.01	0.41-0.64
Lactose %	4.75±0.21	4.63±5.07	4.89±0.11	4.75-5.00	4.87±0.08	4.77-4.97
Fat %	0.52±0.23	0.25-0.80	0.42±0.12	0.23-0.55	0.06±0.04	0.02-0.10
Nitrogen %	0.232±0.009	0.223-0.245	0.224±0.012	0.212-0.237	0.144±0.024	0.103-0.0174

*Errors given are the standard deviation for n=4

1.2: Statistically significant differences are the following:

1.Fat: WF-SF and WK-SM, P=0.01

2.Nitrogen: WF-SM and WK-SM, P=0.01

Solid residue, ash and lactose values for the myzithra serum were similar to those of the two wheys. The fat and protein are retained by the myzithra curd[2]. In accordance to this, the fat content of the myzithra serum was negligent and the nitrogen content lower than that of the two wheys. The differences in fat and nitrogen content between the myzithra serum and the two cheese wheys was statistically significant (p=0.01). However the nitrogen content of myzithra serum was found to be high. This fact along with the relative high solid residue in the myzithra serum are probably owed to the addition of milk in the raw material (whey of kefalograviera). Data in Table II leads to the remark that the fat content of the whey of a hard cheese (kefalograviera) is lower than the respective amount of a soft cheese (feta) in contrast to the reported values in the literature[2]. However such a comparison is not strictly valid since samples analyzed in this work were produced from raw materials not specifically standardized, while it is also known that the whey composition depends on various factors such as season of production, feeding conditions, origin of milk etc.[17,19].

The values for K, Na, Ca, Mg, P, Cu, Fe, Zn as well as for Mn, Sr, Al, Pb, Ba and As are given in Table III. Data indicated similar values of elements for the two cheese wheys and the myzithra serum. Results shown in Table III

TABLE III. Elements of wheys and serum of myzithra.

	Whey of Feta (WF) ppm		Whey of Kefalograviera (WK) ppm		Serum of myzithra (SM) ppm	
Element	average	range	average	range	average	range
K	1240±164.6*	1059-1380	1127.6±30.6	1096-1157	1235±207.6	1074-1443
Na	426.3±43.4	380-466	442±226	427-468	468.0±369	427-497
Ca	311.7±50.5	281-370	319±325	296-343	284.7±40.4	257-331
Mg	90.0±16.4	76-108	86±6.2	81-93	95±11.8	82-105
P	441.3±49.7	399-496	424.3±29.8	390-443	416.7±30.3	382-438
Cu	4.4±1.1	3.4-5.6	3.43±0.4	3.2-3.9	2.45±0.07	2.4-2.5
Fe	16.3±8.7	9-26	13.7±1.2	13-15	<2	
Zn	108±61.7	38-152	61.3±28.5	42-94	61.3±25.5	35-86

For the elements Mn, Sr, Al, Pb, Ba and As the concentrations were lower than 1ppm.

*Errors given are the standard deviation for n=3.

are generally within the range of those reported in the literature[2,18,20-22] which seem to be rather wide. This is logically expected considering that the concentration of the elements in the whey depends on the origin of milk used for cheesemaking, on seasonal variation, lactation, etc. while possible contamination of milk from the containers should not be excluded[23,24].

With regards to the pollution potential of the examined waste by-products, preliminary BOD5 determinations using the Winkler azide modification method[25] gave values in the range of 30,000-80,000 mg/l which apparently confirm their great pollution potential.

CONCLUSIONS

Present results for the examined industrial waste by-products lead to the following preliminary conclusions:

a. The wheys of feta (soft cheese) and kefalograviera (hard cheese) do not show differences in composition that would justify separate handling for further utilization.

b. The myzithra serum has similar lactose and element content to that of the two cheese wheys and also a significant amount of nitrogen, eventhough lower than those of the two wheys. It can therefore be incorporated with two wheys for further industrial utilization.

c. The values for lactose, nitrogen and elements of all the three cheese waste by-products show that these are good sources of carbon, nitrogen and inorganic elements for the growth of appropriate microorganisms.

ΣΥΣΤΑΣΗ ΤΟΥ ΟΡΟΥ ΤΩΝ ΤΥΡΙΩΝ ΦΕΤΑ ΚΑΙ ΚΕΦΑΛΟΓΡΑΒΙΕΡΑ ΚΑΘΩΣ ΚΑΙ ΤΟΥ ΟΡΟΥ ΤΗΣ ΜΥΖΗΘΡΑΣ

ΠΕΡΙΛΗΨΗ

Προσδιορίστηκαν τα: στερεό υπόλειμμα, τέφρα, λακτόζη, λιπος, άζωτο και τα στοιχεία K, Na, Ca, Mg, P, Cu, Fe, Zn, Mn, Sr, Al, Pb, Ba και As στον ορό των τυριών φέτα και κεφαλογραβιέρα καθώς και στον ορό της μυζήθρας με φασματοσκοπία εκπομπής ICP. Η σύσταση των ορών των δύο τυριών δεν εμφάνισε σημαντικές διαφορές. Ο ορός της μυζήθρας περιέχει αμελητέα ποσότητα λίπους και σημαντική ποσότητα αζώτου αν και χαμηλώτερη απ'ότι οι οροί των δύο τυριών. Ο ορός της μυζήθρας περιείχε σημαντικά ποσά K, Na, Ca, Mg, P και Zn ανάλογα εκείνων των ορών των δύο τυριών. Προτείνεται ότι ο ορός της μυζήθρας μπορεί να ενσωματωθεί στους ορούς των δύο τυριών για περαιτέρω βιομηχανική αξιοποίηση π.χ. υπόστρωμα ανάπτυξης μικροοργανισμών.

ACKNOWLEDGEMENTS

Advice on determination of elements, offered by Mr. P.Dolezei is greatly appreciated.

REFERENCES

1. Webb, B.H., Johnston A.G. and Alford J.A., *Fundamental of dairy chemistry* 2nd ed. The AVI Publ. Co., Westport, Conn. (1974).
2. Zerfiridis, G., *Study on the cheesemaking procedure of a new type of cheese from cow's cheese whey.* Ph.D.Thesis. University of Thessaloniki, Greece (1976).
3. Bourdonnaye, A.de la., *Laitiere Francaise* Sept. No 323: 541-567 (1974).
4. International Dairy Federation: Bulletin No 212. *Trends in whey utilization. Brussels* (1987).
5. Moon, N.J., Hammond E.E. and Glatz, B.A., *J.Dairy Science,* 61, 1357-1361 (1978).
6. El-Samragy, Y.A and Zall, R.R., *J.Dairy Science,* 71, 1135-1140 (1988).
7. Janssens, J.H. Bernard, A., and Bailey R.B., *Biotechnology and Bioengineering.,* Vol.26, 1-5 (1984).
8. Zakrzewski, E.,Zmarlicki S., *Milchwissenschagt* 43(7), 435-437 (1988).
9. Ennis, B.M., Maddox, I.S. and Schoutens, G.H., *New Zealand J., Dairy Science and Techn.,* 21, 99-109 (1986).
10. Roy, D., Goulet J. and Leduy, A., *Journal of Dairy Science* 70, 506-513 (1987).
11. International Dairy Federation: Standard No 28A. *Determination of the lactose content of milk,* Brussels (1984).
12. Association of Official Agricultural Chemists: *Official Methods of Analysis* 10th ed. Washington D.C. (1965).
13. Benton Jones J.JR., *Analysis of Food Contaminates,* edited by John Gilbert. Elsevier. Appplied Science Publishers Ltd, London (1984).
14. Cook, K.K., 94th, *Annual meeting of the Assoc. of Official Anal.Chemists* 91983).
15. Kluckner, R.D., Int.Winter Conference. San Juan, Puerto Rico (1980).
16. Veinoglou B., Anifantakis E., *Dairying,* Vol.II. Athens, Greece (1981).
17. Hansen, P.S. and Jensen, G.K., *Dairy Science Abstracts* No 4274, (1978).
18. Brabec, J., *Dairy Science Abstracts,* No 2251 (1978).
19. Nani, R., *Dairy Science Abstracts,* No 7246 (1979).
20. Polyanskii, K.K., Cherenkova, Yu.G., Trishechenko, B.A. and Krarchencko, E.F., *Dairy Science Abstracts,* No 4848 (1979).
21. Wong, N.P., La Croix, D.E. and Vestal, J.H., *J.Dairy Science,* 60(10), 1650 (1977).
22. Glass, L. and Hendrick, T.I., *J.Dairy Science,* 60, 190 (1977).
23. Capella, P., Losi, G., Rastelli, R., Lerici, C.R., *Scienza e technologia delgi Aliment* 4(5), 295-303 (1974).
24. Voudouris, E., *Food Technology,* Vol.II, *Dairying,* Ioannina, Greece (1982).
25. American Public Health Association: *Standard Methods for the examination of water and waste water.* 16th edition, Washington (1985).

Chimika Chronika, New Series, 19, 137-151 (1990)

EFFECT OF VERY LOW FLUORIDE CONCENTRATIONS ON THE ELECTROCHEMICAL POTENTIAL OF THE ORAL CARIOGENIC BACTERIUM *STREPTOCOCCUS MUTANS* - A RADIOTRACER STUDY

N. PSARROS and H. DUSCHNER

Fachbereich Physikalische Chemie - Kernchemie, Philipps-Universität, Hans-Meerwein-Straße, W-3550 Marburg

(Received March 3, 1991)

SUMMARY

The effect of fluoride on the metabolism of *S.mutans* was studied by radiotracer determinations of the intra/extracellular distribution coefficients, the electrical potential $\Delta\psi$ across the cell membrane and the uptake of fluoride in the bacterial cytoplasma, cell membrane and cell wall. *S.mutans* accumulates fluoride from slightly acidic environment. Increasing external fluoride concentrations affect the pH gradient and the Dy. Lysozyme treated bacteria are more susceptible to fluoride than whole cells. Fluoride-rich protein fractions after anion exchange chromatography give evidence for various interactions of fluoride with bacterial proteins. The results indicate that fluoride affects the bacterial metabolism at low concentrations, beginning in the ppm range.

Key words: S.mutans FA-1, fluoride, dimethyloxazolidinedion, intra/extracellular distribution

INTRODUCTION

The radiotracer isotope ^{18}F can be obtained in ionic form, carrier free or with high specific activity. The production is based on specific nuclear reactions and subsequent radiochemical separation[1]. Because of the relatively short half-life of the nuclide (109 min), the production site (accelerator) should be situated in the vicinity of the applying laboratory. Under these conditions ionic fluoride can be labelled in a wide range of concentrations. The total amount of fluoride induced by the nuclear reaction is far below the natural background concentrations found in physiological media (some 0.01 ppm). Consequently, the addition of ^{18}F tracer is without effect on the natural background concentrations of fluoride. Thus, the intra/extracellular distribution can be studied without inter-

ference by the added radiotracer. Although such low concentrations are far away from affecting vital functions of *S.mutans*, the intra/extracellular distribution data obtained under these conditions are inevitable for better understanding fluoride interactions at higher concentrations.

Fluoride is assumed to interfere with the metabolism *S.mutans*. Information on the effect of fluoride on the metabolic efficacy of the cell can be obtained from its capacity to maintain intra/extracellular pH gradients (internal alkaline[2]) and the electrical potential (interior negative). Indirect evidence for these data can be derived from the intra/extracellular distribution of selected ^{14}C-labelled indicator ions, which should be taken up by the cell non-specifically. Consequently, these ions should have large solvation radii and low surface charge density and should be not metabolized by the bacteria. This model seems to apply for the ions used in the present investigations: the anion of the weak acid 5,5-dimethyloxazolidine-2,4-dion (DMO^-) as indicator for the pH gradient and the tetraphenylphosphonium cation (TPP^+) as indicator for the electrical gradient ($\Delta\psi$) Because of its lipophilic character this cation penetrates the boundary between extracellular and intracelular spaces (cell membrane) and accumulates in the negatively charged interior of the bacteria[3]. Under these conditions, intra/extracellular distribution coefficients of fluoride can be discussed with respect to its impact on cellular functions of *S.mutans*.

MATERIALS AND METHODS

Growth of the bacteria

S.mutans FA-1 (serotype b; *S.rattus*) obtained in lyophilized form (Behring-Werke, Marburg) was grown as described previously[1]. The bacteria used for the experiments were in the stationary phase of growth. With regard to the characteristics of fluoride uptake, no difference between bacteria from the logarithmic and stationary phase of growth was observed in a previous experiment[1].

Lysozyme treatment

Lysozyme treated cells were obtained according to Chassy[4]. Bacteria were incubated with a solution containing 0.1 M Tris-buffer (pH 8.2), 1 mg/ml lysozyme (20,000 U/mg) and 12% polyethyleneglykol (MW 200) for stabilization. Lysis was not observed during this process.

Intracellular water volume

The intracellular water volume was determined by the HTO/^{14}C-dextrane exclusion method[5]. It was 47.5 ± 8% of the cell wet weight for whole bacteria and 47 ± 6% for lysozyme treated bacteria.

Determination of the pH gradient

Cells were incubated for 5 minutes at 37°C with buffered solutions (pH 3.4 – 9.2) containing glucose ($8*10^{-4}$ M), $4.5*10^{-2}$ M NaCl (whole cells) or 0.38 M polyethyleneglykol (lysozyme treated cells) and the radioactive labelled species. The concentrations of fluoride (added as NaF) used for the incubations were: natural background (approx. 10^{-8} M), 10^{-6} M and 10^{-4} M, that of dimethyloxazolidinedion was 10^{-4} M. These values represent the total concentrations in solution, irrespective from the degree of dissociation. The pK_a values of the two acids are 3.2 and 6.2 respectively. Nearly carrier free ^{18}F in ionic form was obtained by a procedure developed in this this laboratory[1]. All other radioactive species were used in commercially available form (Amersham-Buchler).

After equilibration (5 min), cells and supernatant were separated by centrifugation (11,000 g). By this procedure a good pellet with low amounts of intercellular water was obtained. The intracellular concentrations of the investigated species were not affected by squeezing. After centrifugation, 1 ml aliquots of the supernatant were transferred to polyethylene vials containing 10 ml of scintillation cocktail (Quickszint 212^{R}). The remaininig supernatant was removed by sucking with a water jet pump. The pellets were then suspended in 1 ml 0.15 M NaCl and transferred also into polyethylene vials containing the scintillation cocktail. The activities of the radionuclides were determined by liquid scintillation spectrometry. The intra/extra-cellular distribution coefficients of the ionic species were calculated from these data using the Henderson-Hasselbalch equation[6].

Determination of the gradient of the electrical potential ($\Delta\psi$)

The electrical potential ($\Delta\psi$) between intra- and extracellular spaces of whole and lysozyme treated cells was evaluated from the distribution coefficients of the ^{14}C-labelled tetraphenylphosphonium cation. Bacteria were incubated (20 min; 37°C) with 10^{-4} M solutions of ^{14}C-labelled tetraphenylphosphonium chloride containing none, 10^{-6} M and 10^{-4} M fluoride. $\Delta\psi$ values were calculated from the distribution data with a modified form of the Nernst equation[3,7]. Problems arise in estimating the amount of the non-specifically accumulated TPP^+ in the lipid layer of the membrane and in other lipophilic compartments of the cell[3,8]. These amounts of TPP^+ were assumed to be constant during the present

expreriments, so that the data obtained indicate relative differences of $\Delta\psi$ values, which are due to the different experimental conditions.

Chromatography of proteins and fluoride in the cytosol, the cell membrane and the cell wall

For the determination of the amount of fluoride bound to cytosolic proteins, lysozyme treated cells were incubated (20 min; 37°C) with ^{18}F labelled solutions of sodium fluoride (10^{-4} M; pH 8.2). Cells were separated from the supernatant by centrifugation at 20,000 g for 30 minutes. The supernatant was desalted by gel filtration on a Sephadex G-25 column (Pharmacia) with $0.5*10^{-2}$ M Tris-buffer pH 7.6. The ^{18}F-labelled fractions were collected and fractionated on a DEAE-Sephacel anion exchange column (Pharmacia) with a gradient of stepwise increasing sodium chloride concentrations (0 - 0.7 M NaCl in $0.5*10^{-2}$ M Tris-buffer pH 7.6). the eluate was collected in fractions of 2 ml. The ^{18}F activities were monitored by liquid scintillation counting of 0.5 ml aliquots. The protein concentrations were monitored during the gel filtration by measuring the extinction at 280 nm. The protein concentrations in the Sephacel fractions were determined using the Lowry protein assay method[9]. Thus, the determined optical densities are a measure for the total protein concentration. In a similar experiment performed without previous fluoride incubation, the fractions of the eluent were tested for enolase activity using the assay described by Bergmeyer[10].

For the determination of fluoride bound to membrane proteins, the membrane fraction was dissolved in $0.5*10^{-2}$ M Tris-buffer pH 7.6 containing 0.1% Triton-X-100 and processed as described above. In this case 0.1% Triton was also added to the eluent.

The cell wall fraction was obtained from the supernatant remaining after the lysozyme treatments. For this experiment whole cells were incubated with 10^{-4} M ^{18}F-labelled NaF. Lysozyme was nearly completely removed from the supernatant by precipitation with ammonium sulfate (half-saturated solution). After centrifugation (10 min; 20,000 g) the supernatant was fractionated on a DOWEX 1X8 anion exchange column with a NaCl gradient of stepwise increasing concentration (0 - 0.5 M).

RESULTS

Distribution coefficients and $\Delta\psi$

Intra/extracellular distribution coefficients (D) of DMO^- determined with whole and lysozyme treated cells increase steeply with decreasing external pH (Fig. 1). Whole cells seem to be slightly more effective in accumulating the ion than those treated with lysozyme. The intra/extracellular pH gradient is levelled at neutral pH, so that the distribution coefficients

approach constant values. These are between 3 and 4 for whole cells and between 1 and 2 for lysozyme treated cells.

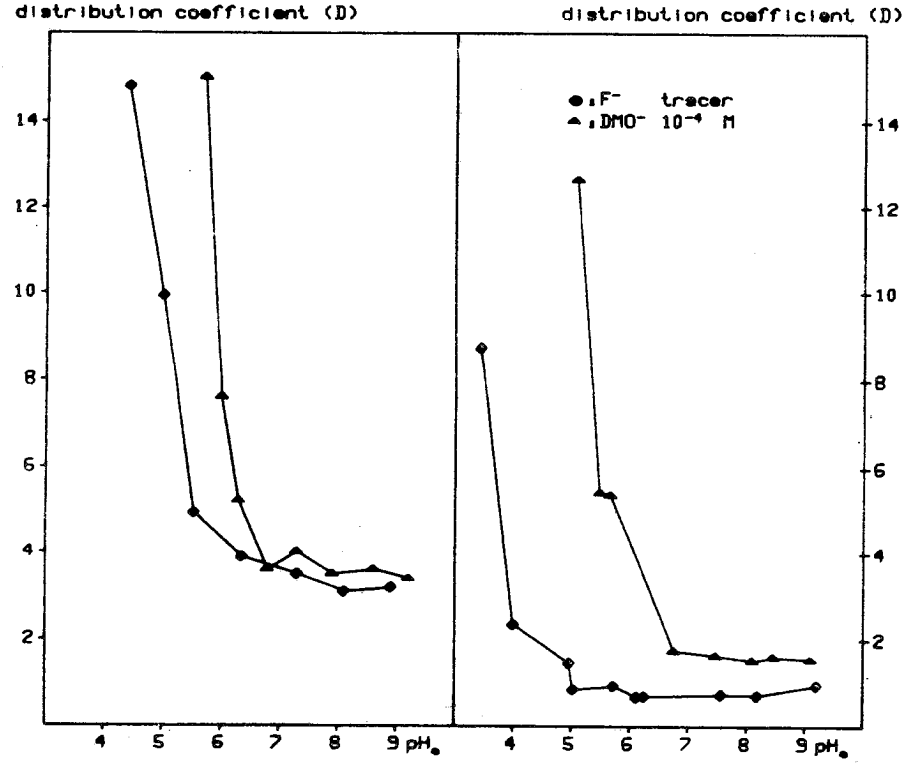


FIG. 1: *Intra/extracellular distribution coefficients (D) of fluoride and dimethyloxazolidine-dion anion versus external pH (linear plot; pH range: 4.0 – 9.2). Left: whole bacteria; right: lysozyme treated bacteria.*

As long as the import of the weak acid into the cell and its dissociation in the cytoplasma are not affected by secondary processes, the ratio of the intra/extracellular anion concentration can be expressed by the following equation[6]:

$$\log D = \frac{[A^-]i}{[A^-]e} = pHi - pHe = \Delta pH \quad (I)$$

According to eq. I, the logarithms of the anion accumulation coefficients should be a linear function with respect to the pH gradient between intra- (pH_i) and extracellular (pH_e)

spaces of the cell. Actually, the logarithms of the DMO^- distribution coefficients (pH_e below 6.5) are correlated linearly to the extracellular pH (Fig. 2).

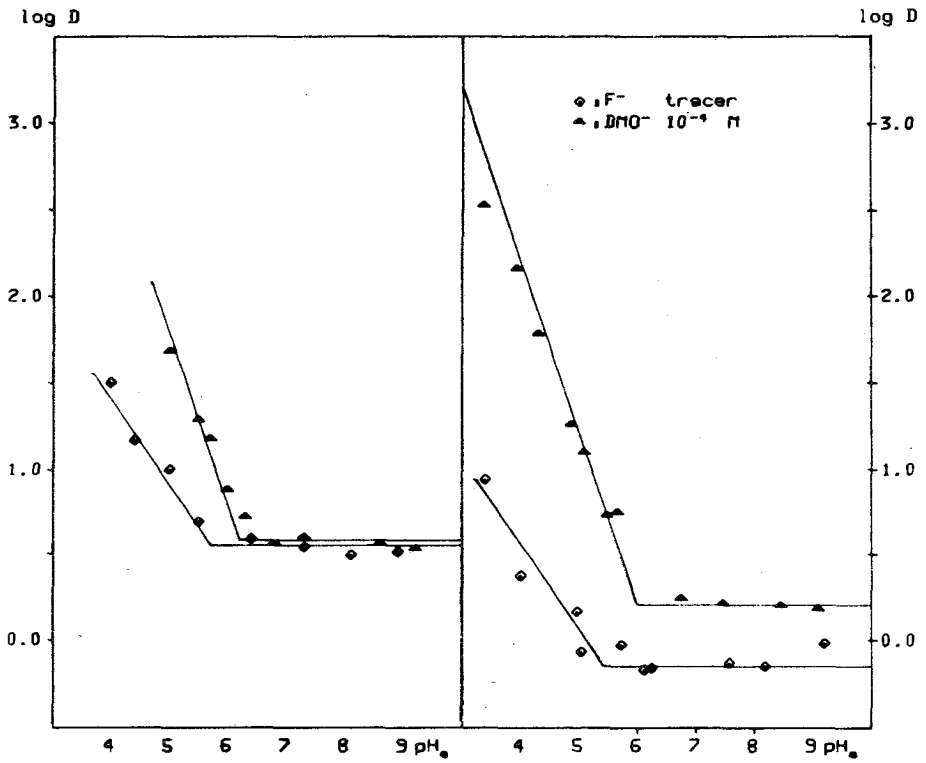


FIG. 2: *Intra/extracellular distribution coefficients (D) of fluoride and dimethyloxazolidine-dion anion versus external pH (logarithmic plot; pH range: 3.4 – 9.2). Left: whole bacteria; right: lysozyme treated bacteria.*

When, in a first approach, the intracellular pH is assumed to be unaffected by the acidity of the external solutions, the plot is in accordance with equation I. As expected for monobasic acids the slope of the linear regression is about −1. Also according to eq. I the log D values should equal zero when the pH of the external solution approaches the intracellular pH (i.e. when $\Delta pH = 0$) The experimental results show, however, that log D values of about zero are observed only for lysozyme treated cells. Whole cells seem to accumulate fluoride even from neutral media. (log D about 0.6).

The distribution coefficients of natural background amounts of fluoride (Fig. 1) are somewhat lower than those of DMO^-. The effect is more pronounced for lysozyme treated than for whole cells. The log D plots can be again approached by linear regressions with a

slope of -0.5 (Fig. 2). This slope is distinctly lower than that of the DMO^- distribution coefficients. Obviously, in contrast to DMO^- the intra/extracellular distribution of fluoride cannot be described by equation I. Therefore, the results of the fluoride distribution experiments are given as linear plots only.

When the external concentrations of fluoride exceed the natural background level, intact and lysozyme treated cells show distinctly different capabilities to accumulate the ion (Fig. 3).

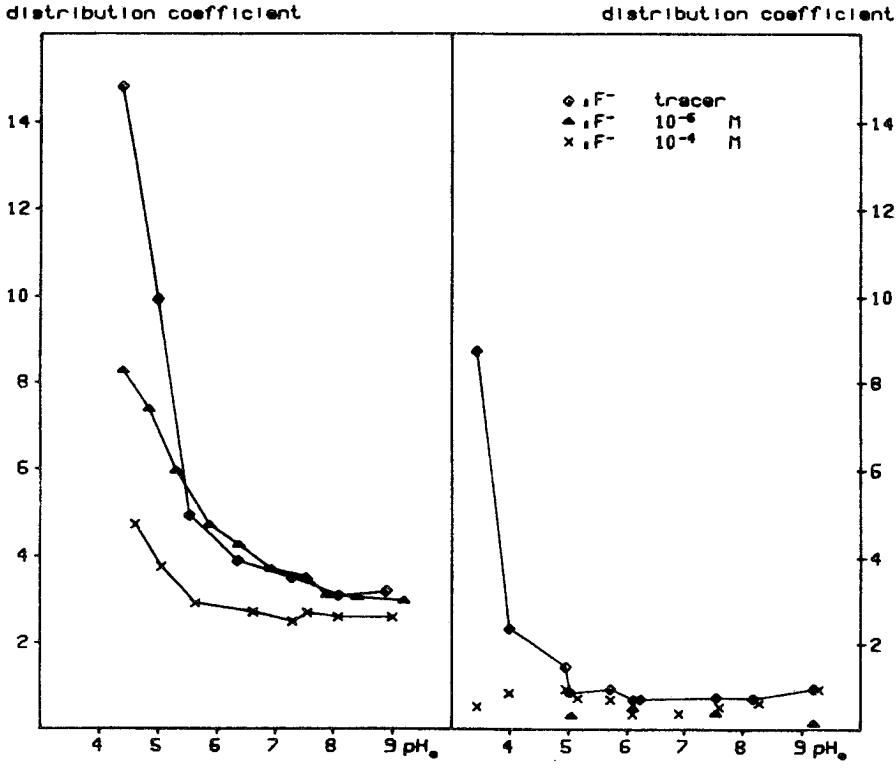


FIG. 3: *Intra/extracellular distribution coefficients (D) of fluoride versus external pH at various external fluoride concentrations (linear plot; pH range: 4.0 - 9.2). Left: whole bacteria; right: lysozyme treated bacteria.*

Already at an external fluoride concentration of 10^{-6} M, lysozyme treated bacteria are unable to accumulate fluoride. The distribution coefficients are close to 1. The capacity of the whole cells to accumulate fluoride decreases when the extracellular concentrations increase from the natural background level to 10^{-6} or 10^{-4} M. This tendency is most pronounced when the ion is taken up from slightly acidic solutions.

Some information on the interference of fluoride with the $\Delta\psi$ across the bacterial membrane can be obtained from the determined distribution coefficients of the tetraphenylphosphonium cation. Both the variation of the external fluoride concentrations and the variation of the pH_e seems to affect the $\Delta\psi$ in whole cells. Without added fluoride (i.e. at natural background concentrations) the $\Delta\psi$ value is about −20 mV at a pH_e of 4.0. Although the values are fluctuating, a slightlty decreasing tendency is observed when the pH_e grows basic (Fig. 4). At constant pH_e the $\Delta\psi$ increases significantly with increasing extracellular fluoride concentrations. At pH_e 4.0 the $\Delta\psi$ of lysozyme treated cells is identical to that of whole cells, when the external solution is free of fluoride (about −20 mV). In contrast to whole cells, however, a sharp maximum is observed at a pH_e between 5.0 and 6.0. When these cells are incubated with small amounts of fluoride (10^{-6} M), the electrical potential collapses to values below −10 mV irrespective of the external pH.

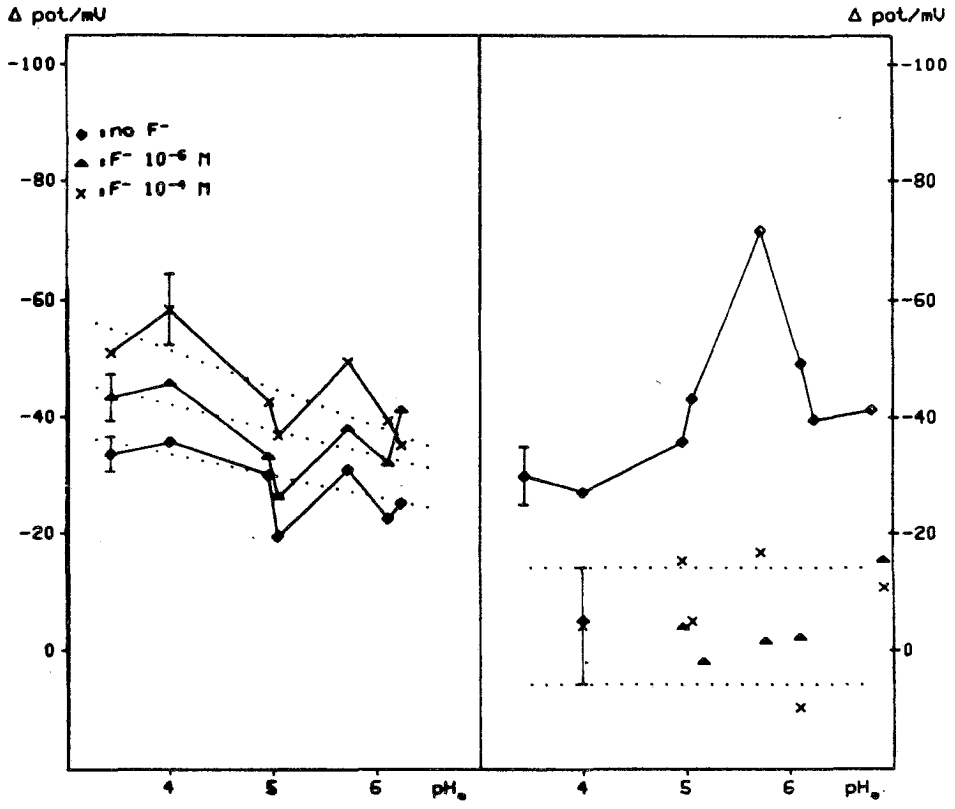


FIG. 4: *Electrical potential ($\Delta\psi$) versus external pH at various external fluoride concentrations. Left: whole bacteria; right: lysozyme treated bacteria.*

Permanent bound fluoride

Permanent bound fluoride[11] is found in the cell wall, the cell membrane and the cytosol. From the total amount of fluoride ^{18}F activity taken up by the cells at pH_e 7.6, about 2% were found in the cell wall, 12% in the membrane and 86% in the cytosol.

In the protein fraction of the cytosol the major amount of fluoride (more than 95%) is eluted at low chloride concentrations. This is evidence for very weak protein-fluoride interactions. With increasing chloride concentrations some fractions with high fluoride concentrations are coeluted with protein-containing fractions. Enolase (EC 4.2.1.11) was identified in the 0.4 M (400 mM) NaCl fraction (Fig. 5). Native-PAGE electropherograms of corresponding fluoride-containing and fluoride-free enolase fractions showed the same band structure. Autoradiograms of the fluoride-labelled bands could not be obtained. It is not clear if fluoride dissociates from the proteins during electrophoresis or if the fluoride radioactivity was not sufficient enough for an autoradiogram because of the short half-life of ^{18}F.

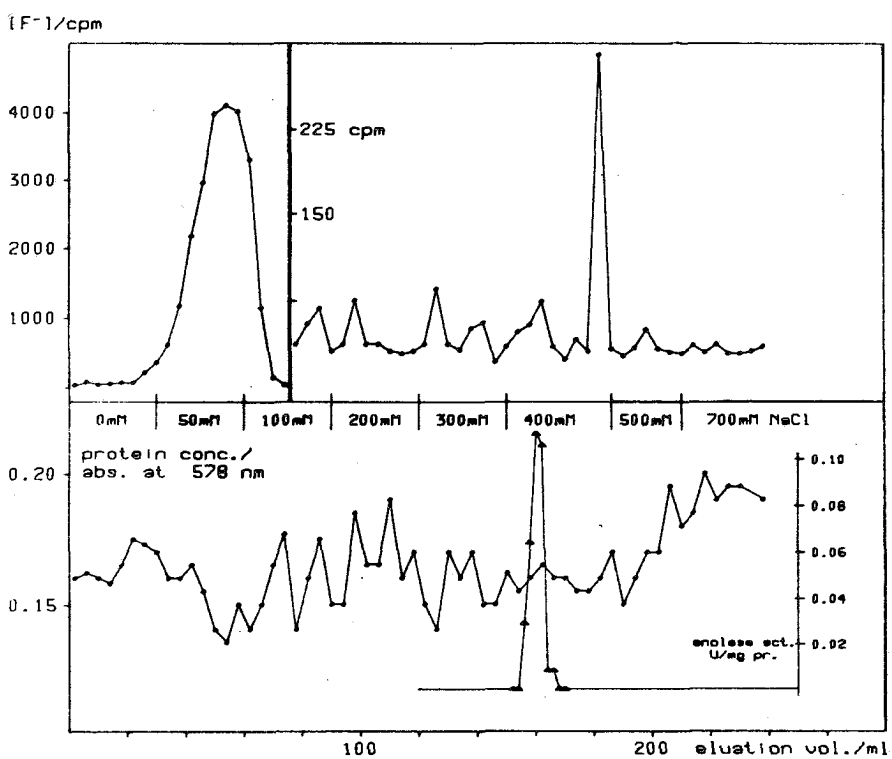


FIG. 5: *Elution diagram of the cytoplasmic fraction.*

The amount of fluoride eluted at low NaCl concentrations is smaller in the membrane fraction than it is in the cytoplasmic fraction. The number of proteins coeluted with fluoride is also smaller. On the other hand some fluoride-containing fractions do not contain any ptorein (Fig. 6).

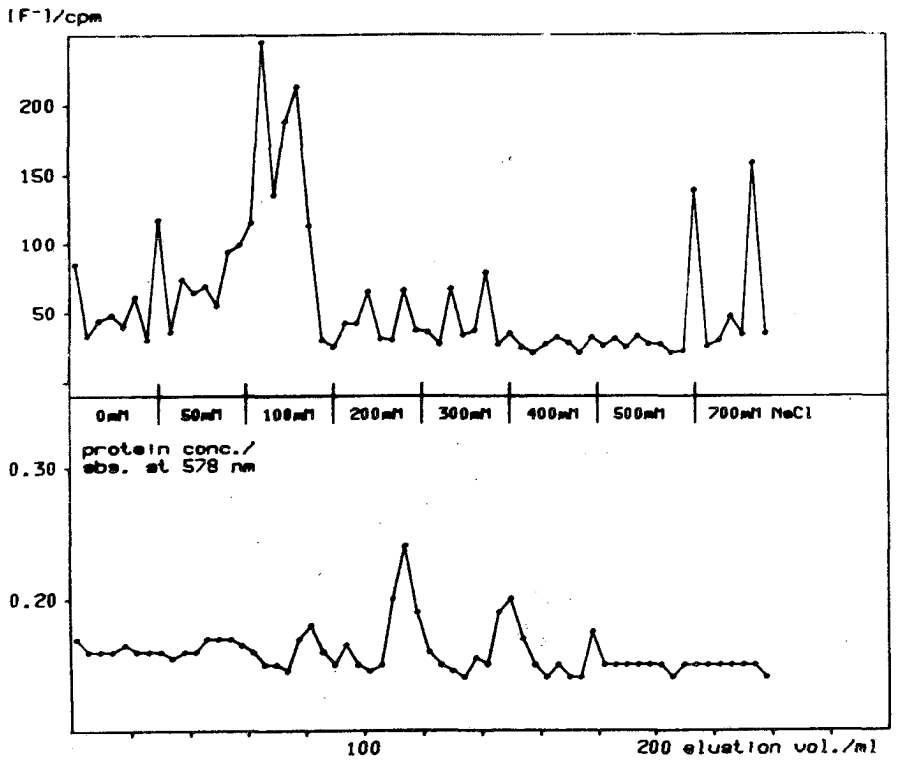


FIG. 6: *Elution diagram of the membrane fraction.*

The major amount of the fluoride in the cell wall fraction was eluted with chloride-free buffer. The fractions contained also protein. At 0.2 M (200 mM) NaCl two distinct fluoride peaks without coincident protein appeared. They presumably contain cell wall fragments (Fig. 7).

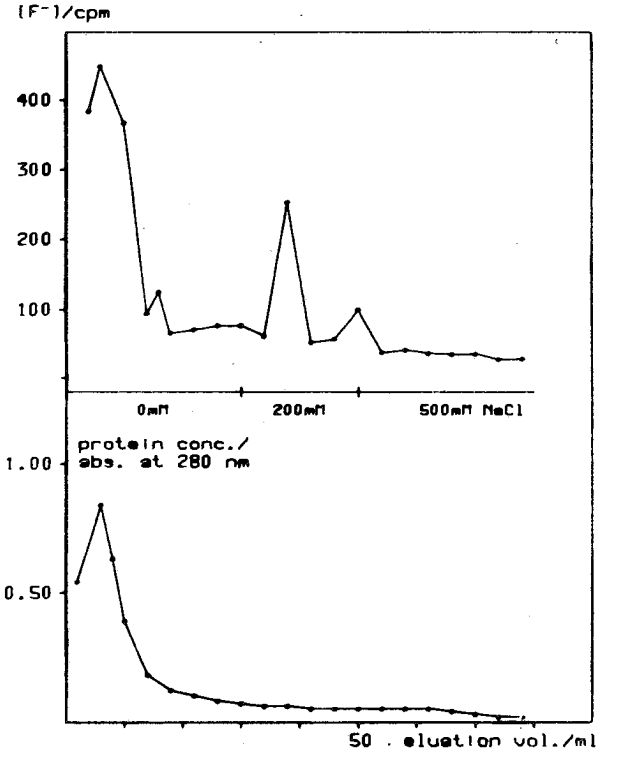


FIG. 7: *Elution diagram of the cell wall fraction.*

DISCUSSION

According to eq. I the logarithms of the intra/extracellular distribution coefficients of the anions of weak acids should be a linear function of the intra/extracellular pH gradient, and consequently of the external pH, constant internal pH assumed. The pH_e dependency of the log D values of DMO^- is in good correspondence to eq. I (Fig. 2) vor pH_e values below 6.0. When the extracellular pH approaches pH_i (at about pH 6.5) the distribution coefficients converge to a limit of about 4.0 for whole and about 1.0 for lysozyme treated bacteria. The somewhat higher D values of the whole bacteria at neutral pH are caused possibly by the uptake of minor quantities of DMO molecules by the cell wall.

By incubating *S.mutans* with nearly carrier-free solutions of ^{18}F-fluoride (Fig. 1) the intra/extracellular distribution of the natural background concentrations of the ion can be studied. Studies at these minute concentrations are necessary, because ppm quantities of fluoride are reported to interfere with cellular functions of oral streptococci[12 - 17]. Under these conditions the behaviour of the fluoride distribution coefficents is already divergent from that of DMO^-. At identical external pH fluoride is accumulated by *S.mutans* less effectively than DMO^-, the distribution coefficients achieved by lysozyme treated bacteria beeing far lower than those of whole cells. The log D values in both types of cells can be approached by linear regression with a slope of about -0.5 (Fig. 2) indicating, that the fluoride distribution does not follow eq. I and that the pH_i cannot be calculated from fluoride distribution data. From the fluoride distribution behaviour can be deduced, that the intracellular fluoride exists also in a form which is not identical with the undissociated molecule (HF) of the free fluoride ion.

In order to obtain information about the binding sites of fluoride in the bacterial cell, the proteins of the cell wall, the cell membrane and the cytoplasma were chromatographically fractionated after disintegration of bacteria incubated with ^{18}F at pH 7.6. The main fraction of fluoride (86%) taken up by the cells is located in the cytoplasma (Fig. 5). About 98% of the fluoride initially adhering to the cytoplasmic proteins are eluted from the exchange column at low sodium chloride concentrations without large amounts of coeluted protein. This indicates very weak fluoride-protein interactions. The remaining 2% of fluoride are eluted from the column with increasing sodium fluoride concentrations. In six fractions ^{18}F activity is coeluted with protein, e.g. enolase, which could be identified by enzyme test.

Minor fractions (7%) of fluoride taken up by the cells are linked to proteins of the membrane (Fig. 6). Two fluoride fractions obtained at NaCl concentrations above 0.6 M (600 mM) are not correlated with protein fractions. These fractions may contain ionic polysaccharides of yet unknown composition. Fluoride-containing cell wall fractions eluted at higher NaCl concentrations also seem to contain only cell wall fragments, since they do not show absorption at 280 mn.

The influx of a weak acid into the cell is mediated via the internal alkaline pH gradient[2]. It should be independent from the external concentration of the acid, provided that the coimported protons are extruded. However, in slightly acidic external solutions, the fluoride distribution coefficients in *S.mutans* whole cells decrease considerably when the extracellular fluoride concentrations exceed the natural background level (Fig. 3). This decrease is correlated to the fluoride concentration and the external acidity, indicating that

the influx of protons from slightly acidic solutions containing only 10^{-6} M fluoride cannot be compensated completely by the bacteria. Compared with the whole cells, lysozyme treated cells are far more sensitive to fluoride.

Additional evidence for the extreme sensitivity of lysozyme treated cells to imported fluoride is the collapse of the $\Delta\psi$ when the external concentrations exceed the natural background level (Fig. 4). In contrast to the lysozyme treated cells, the $\Delta\psi$ of the intact bacteria increases at higher extracellular fluoride concentrations, thus compensating the decrease of the pH gradient in the overall balance of the electrochemical potential.

CONCLUSION

Whole and lysozyme treated cells of *S.mutans* respond differently when incubated with DMO^- and F^-. This is evidence for specific interaction mechanisms of fluoride with the bacteria. Although both species are transmembrane proton conductors, protons imported from acidic media in form of H^+DMO^- are compensated more effectively than those imported in form of H^+F^-. The proton efflux is mediated via the well known glycolytic pathway with lactic acid as final stage[18,19] and/or a proton translocating ATPase[6,20,21].

The nutrients which propel this process, are taken up via mechanisms located in the cell membrane, i.e. the proton-motive force-driven port and the PEP-phosphotransferase system. The internal alkaline pH gradient and the internal negative electrical potential are maintained by these processes. DMO can be assumed not to interfere with the bacterial metabolism at concentrations used in the present work[22]. Therefore protons imported together with DMO^- can be compensated by the cell. The efficacy of proton export decreases after treatment of the cells with lysozyme, which possibly affects the PEP-phosphotransferase mechanism of glucose uptake. The remaining proton-motive force-driven port is easily deactivated either by direct poisoning[16] or by dissipation of the proton-motive force as a consequence of the inhibition of the glycolytic enzyme enolase. However, since the streptococcal enolase has a K_i for fluoride in the range of 4.0 - $8.0 * 10^3$ M regardless of the fluoride sensitivity of the strains[23], the assumption of enolase inhibition at fluoride concentrations of 10^6 M seems not probable.

ΠΕΡΙΛΗΨΗ

Η επίδραση πολύ χαμηλών συγκεντρώσεων ιόντων φθορίου στο ηλεκτροχημικό δυναμικό του στοματικού τερηδονογόνου βακτηριδίου Streptococcus mutans
Ν. ΨΑΡΡΟΣ και Η. DUSCHNER

Η επίδραση των ιόντων φθορίου στο ηλεκτροχημικό δυναμικό του *S.mutans* μελετήθηκε με ραδιοϊσοτοπικό προσδιορισμό των συντελεστών κατανομής ανάμεσα στο εσωτερικό και το εξωτερικό των βακτηριδίων, του ηλεκτρικού δυναμικού Δψ της κυτταρικής μεμβράνης και της πρόσληψης ιόντων φθορίου από το κυτταρικό τείχος, την κυτταρική μεμβράνη και το κυτόπλασμα των βακτηριδίων.

Ο *S.mutans* συσσωρεύει ιόντα φθορίου από ένα ελαφρώς όξινο περιβάλλον. Αυξανόμενες συγκεντρώσεις ιόντων φθορίου έχουν αρνητική επίδραση στην ικανότητα των βακτηριδίων να διατηρούν ένα αλκαλικό εσωτερικό pH και ένα ηλεκτρικό δυναμικό. Βακτηρίδια που έχουν υποστεί διεργασία με το ένζυμο λυσοζύμη δείχνουν μεγαλύτερη επιδεκτικότητα στην επίδραση των ιόντων φθορίου από άθικτα. Χρωματογραφική ανάλυση του κυτοπλάσματος είχε σαν αποτέλεσμα την συλλογή πρωτεϊνικών κλασμάτων περιεχόντων φθόριο.

Τα αποτελέσματα της εργασίας μας δείχνουν ότι τα ιόντα φθορίου επηρεάζουν αρνητικά τον μεταβολισμό του *S.mutans* ήδη σε συγκεντρώσεις της τάξης των ppm.

REFERENCES

1. Duschner H., Bergmann H., and Psarros N.: *D. Zahnärztl. Zeitschr.*, **40**, 482 (1985)
2. Padan E., Zilberstein D., and Schuldiner S.: *Biophys. Biochim. Acta.*, **650**, 151 (1981)
3. Keevil C.W., and Hamilton I.R.: *Anal. Biochem.*, **139**, 228 (1984)
4. Chassy B.M.: *Biochem. Biophys. Res. Comm.*, **68**, 603 (1976)
5. Psarros N.: *"Wechselwirkungen von Fluorid mit dem Metabolismus des oralen kariogenen Bakteriums* Streptococcus mutans *FA-1 (S.rattus)"*. Intemann Gauting/München (1988)
6. Riebeling V., Thauer R., and Jungermann K.: *Eur. J. Biochem.*, 55, 445 (1975)
7. Kashket E.R., and Barker S.L.: *J. Bacteriol.*, **130**, 1017 (1977)
8. Schild L., and Schönfeld P.: *Biomed. Biochim. Acta*, **45**, 1215 (1986)
9. Lowry O.H., Rosenbrough N.J., Farr A.L., and Randall R.J.: *J. Biol. Chem.*, **193**, 265 (1951)
10. Bergmeyer H.U.: *"Methods in enzymatic analysis"* Verlag Chemie Weinheim (1974)
11. Yotis W.W., and Brennan P.C.: *Caries Res.*, **17**, 444 (1983)
12. Hamilton I.R.: *Can. J. Microbiol.*, **15**, 1013 (1968)
13. Kanapka J.A., Khandelwal R.L., and Hamilton I.R.: *Archs. Biochem. Biophys.*, **144**, 596 (1971)
14. Kanapka J.A., and Hamilton I.R.: *Archs. Biochem. Biophys.*, **146**, 167 (1974)
15. Eisenberg A.D., Bender G.R., and Marquis R.E.: *Archs. oral Biol.*, **42**, 133 (1980)
16. Bender G.R., Thibodeau E.A., and Marquis R.E.: *J. Dent. Res.*, **64**, 90 (1985)
17. Germaine G.R., and Tellefson L.M.: *Infect. Immun.*, **51**, 119 (1986)
18. Ten Brink B., and Konings W.N.: *Eur. J. Biochem.*, **111**, 59 (1980)
19. Keevil C.W., Williamson M.I., and Marsh P.D.: *Archs. oral Biol.* **29**, 871 (1986)
20. Hamilton I.R., St. Martin E.J.: *Infect. Immun.*, **36**, 567 (1986)
21. Kobayashi H.: *J. Biol. Chem.*, **260**, 72 (1985)

22. Harold F.M., Pawlasová E., and Baadra J.R.: *Biochim. Biophys. Acta*, **196**, 235 (1970)
23. Bunick F., and Kashket S.: *Infect. Immun.*, **34**, 856 (1981)

Chimika Chronika, New Series, 19, 153-164 (1990)

CHEMICAL CONSTITUENTS OF THE ROOT BARK OF GOSSYPIUM HIRSUTUM L.

P.IOANNIDIS and S.PHILIANOS

Laboratory of Pharmacognosy, University of Athens, 20 Hippocratous Str., GR 10680 Athens-GREECE

(Received June 8,1989)

SUMMARY

From the root bark of Gossypium hirsutum L.(Malvaceae) the following chemical constituents were isolated:4-desmethylsterols, methylsterols, triterpene alcohols, fatty acids,gossypol derivatives,sugars,amino acids,serotonin and mucilage. In the preliminary pharmacological study, the effect of the aqueous leaves extract on mice and rabbit uteri was examined.

Key words: Gossypium hirsutum L.,Malvaceae, root bark leaves. Sterols, gossypol derivatives, serotonin, amino acids,sugars, fatty acids,mucilage.

INTRODUCTION

The cotton plant is known world-wide for its oil and its fibres. In medicine, the root bark of Gossypium was used in therapeutics since 1840, when Bouchelle demonstrated its use as a popular abortive[3]. From experimental study on lower mammalians it was found that it exerts a slight stimulating action on the uterus,similar to that of ergot[1-3].

Dose: 113.4g of root bark boiled in 946ml water till reduced to 473ml;59ml of infusion taken every 20 or 30 min.,[2]. As a popular remedy it is reported to be oxytocic, hemostatic and vasoconstrictor[2],the active component of which has not as yet been isolated[4].Externally it has been used as a lotion against hemorroids[2].The scope of this paper is to study the chemical constituents of this drug and determine the active component so as to explain its use and its oxytocic action.

EXPERIMENTAL

Plant material.The cotton root bark and the leaves of the plant were collected respectively in Septemper and June in the Levadia region (Greece). These samples were dried in a cool dry place.

1.Preliminary tests

Free amino acid. Root bark material (40g) was finely powdered and extracted on a steam bath four times with a 8:2 methanol-water volume solution. The combined extracts were evaporated in vacuo and subsequently dissolved in water. This solution was analysed according to the Cleaver and Cassidy method[12]. Free amino acids were detected by Paper Chromatography and by Thin Layer Chromatography on cellulose plates[9].

Sugars.Root bark material (120g) was extracted twice with portions of water (200ml each) on a steam bath. The extracts were filtered, concetrated in vacuo and used for P.C.[8,9], T.L.C. and for the preparation of a clear solution analysed by High Performance Liquid Chromatography[6].

Mucilage. Root bark material (2g) was finely powdered and mixed with water (60ml), ethyl alcohol (480ml) was added to the filtrate and the precipitate filtered through a Gooch crucible which was subsequently dried and weighed.This precipitate was hydrolysed.The principal hydrolysis products were identified[5,6,8].

2.Petroleum ether extract

Root bark material (500g) was extracted with petroleum ether. Evaporation of the solvent yelded a residue. The unsaponified matter[17] was fractionated by Column Chromatography[14] and subsequently by T.L.C.[15]. Three bands corresponding to the sterols,the methylsterols and the dimethylsterols were scraped extracted and filtered.

Quantitative determination of sterols was effected througs Gas Chromatography(F.I.D.,glass column SE-30,carrier gas nitrogen,flow rate 30ml/min column temperature 230^{o}C), by injecting a standard solution of a certain sterol(e.g. cholestanol) and then the sterol solutions eluted from the bands.

Dimethylsterols were analysed by G.C.[16,18,19]. Desmethylsterols and methylsterols were individually analysed as sterol acetates[16] by G.C.-Mass Spectroscopy (beam energy 78eV[25-28]). In the saponified matter; fatty acids were characterised as methyl esters by G.C.[20].

3. Diethyl ether extract

A sample (500g) of dried root bark was extracted with diethyl ether. After evaporation of the solvent, a small portion of the residue was drawn off and then analysed by two-dimensional T.L.C. and sprayed with the phloroglycine-HCl reagent[23].

Spectrophotometric determination of mixed gossypols

This determination was based on the formation of a colored complex with p-anisidine.[13] The total gossypols content was measured at the U.V. absorption maxima of the coloured complex compared with the observed absorption of gossypol solution of known concentration.

Isolation of gossypol derivatives

The isolation of the gossypol derivatives and their purification was based on the formation of their complexes with borax[24]. The gossypols with a cis-dihydroxyl group form complexes with borax[24], which are soluble in water. Thus gossypol and 6-methoxygossypol migrate from the organic phase, a mixture of ethyl acetate in hexane (1:3), to the aqueous phase. 6,6'-Dimethoxygossypol with the rest of the lipids remains in the organic phase. From the aqueous phase, the 6-methoxygossypol was extracted with an ethyl acetate-hexane (1:1) mixture.

Portions (500g) of dried root bark (drying was carried out in darkness and at low temperature), were extracted twice with diethyl ether. The combined extracts were subsequently evaporated in vacuo and extracted with 200ml of a 1:3 ethyl acetate-hexane mixture[21].

Isolation of gossypol from the dry root bark. The solution of ethyl acetate in hexane was extacted four times with 80ml portions of an aqueous solution containing 9% sodium chloride

and 1% borax.The organic phase was kept for the isolation of 6,6'-dimethoxygossypol.The aqueous extracts were combined and extracted (4x) with portions of an ethyl acetate-hexane (1:1,150 ml) mixture. The organic phase contained 6-methoxygossypol. The aqueous phase was adjusted to pH 3[21] and the precipitate of gossypol was dissolved in an ethyl acetate:petroleum ether (b.p. 40-60°C) mixture (1:3) and washed with saturated sodium chloride solution to remove the last traces of acid. The organic phase was then dried over sodium sulfate,filtered and evaporated in vacuo.The residue was dissolved in ethyl acetate (20 ml) and chromatographed over a silica gel column, eluted with a mixture of ethyl acetate:petroleum ether (1:3,200 ml).The total eluates were combined and evaporated in vacuo and the resulting residue was dissolved in a small volume of chloroform.Recrystallisation of the precipitate was effected from chloroform.

Isolation of 6-methoxygossypol.The organic phase containing the 6-methoxygossypol in ethyl acetate:hexane (1:1)[21], was washed twice with a 0.1 N hydrochloric acid and then with a saturated sodium chloride solution to remove the last traces of the acid. The organic phase was then dried over sodium sulfate,filtered and evaporated in vacuo.The residue was dissolved in a small volume of ethyl acetate:petroleum ether (1:3),which was chromatographed over a column of silica gel, eluting with ethyl acetate:petroleum ether (200ml, 1:3). The eluates were combined and evaporated in vacuo,the residue was dissolved in a small volume of benzene and mixed with an equal volume of hexane.Recrystallisation of the precipitate was effected from benzene:hexane (1:1).

Isolation of 6.6'-dimethoxygossypol. The ethyl acetate:hexane (1:3) phase[21] was washed twice with 0.1 N HCl solution and then with saturated NaCl solution to remove the last traces of the acid.The organic phase was dried over sodium sulfate,filtered and finally evaporated in vacuo at 30°C.The residue was dissolved in benzene and chromatographed over a column of silica gel.The column was eluted with ethyl acetate (200ml) and the total eluates were combined and evaporated in

vacuo[21].The residue was dissolved in a small volume of benzene and mixed with an equal volume of hexane. The resulting crystals were filtered and washed with hexane. The precipitate was subjected to T.L.C. on silica gel eluting with diethyl ether:petroleum ether (1:3). The region betwen Rf 0.17 and 0.25 was scraped and separately extracted repeatedly with diethyl ether.The extracts were combined and evaporated in vacuo,the residue was dissolved in a small volume of benzene and then mixed with an equal volume of hexane.Recrystallisation of the residue was effected from benzene:hexane (1:1).

Isolation of serotonin

Samples of the root bark (100g) and leaves (100g) were extracted separately with a methanol:water solution 9:1 at 5°C over a period of 14 hrs,the filtrate was purified through an aluminium oxide column, and concentrated in vacuo at 35°C.

Chromatographic analysis[22]. The leaf and root bark extracts were separately chromatographed on Whatman N°1 paper with four different solvent with a parallel standard of serotonin.Subsequently the chromatogramme is sprayed with 4-dimethylaminobenzaldehyde[8] and three other different reagent[22].

Preparative paper chromatography of the extracts on Whatman N°3 MM using four different solvents was repeated many times at a low temperature.

Spectrophotometry.The absorption spectrum was obtained in the U.V. on a 9:1 methanol:water solution of serotonin and also on a solution of the substance isolated from the leaves and from the root-bark.

Preliminary pharmacological tests

The apparatus for isolated organs using a pH 7 Tyrode liquid at a temperature of 36°C and a wave monitor recorded the movement of the smooth muscular uterus fibres. The preliminary study on the effect of the aqueous leaves extract was carried out on isolated mice and rabbit uteri.

The aqueous extract was prepared by treating 160g of leaves according to the procedure described above (isolation

of serotonin). The total column eluates were combined and condensed under vacuum at 35°C till dryness.The residue was dissolved in a total volume of 2ml water and used for the preliminary pharmacological tests.A measured volume of this aqueous leaves extracts was separately chromatographed by P.C. with a parallel standard of serotonin at various concentrations.

RESULTS

Tests for saponin[7],flavonoids[10] and essential oil[11] were negative in the root bark.

Amino acids. Leucine,tryptophane,valine, serine, lysine, alanine,arginine and glutamic acid.

Sugars.Glucose,fructose,saccharose,maltose,raffinose.

Mucilage.The hydrolysis of mucilage revealed the presence of 55% glucose and 30% galactose.

Petroleum ether extract: 2.2% of the dry root bark.

Unsaponified matter: 0.22% of the drug.Comparison of the analytical data against reference material showed the presence of the following constituents:

a.Desmethylsterols. Cholesterol 2.9%, 1.7mg(1), m/e 368, 353,260,247,255,228,213,campesterol 6.5%,3.8mg(1.3),m/e 382, 367,274,261,255,228,213,stigmasterol 13.7%,8mg(1.42),m/e 394, 379,286,273,253,255,228,213,351,296,β-sitosterol 76.9%,45.1mg (1.63),m/e 396,381,288,275,255,228,213.

b.Methylsterols.Lophenol 12.6%,0.26mg(1.27),m/e 442,428, 382,367,274,329,269,obtusifoliol 10.3%,0.22mg(1.47),m/e 468, 453,408,393,342,425,grammisterol 20.3%,0.43mg(1.16),m/e 454, 439,394,379,327,287,370, cycloeucalenol 4%,0.08mg(1.7),citrostadienol 50.1%,1.05mg(2.13),m/e 468,453,408,393,327,301,310.

c.Dimethylsterols.Lanosterol 2%, 0.09mg (1.54),cycloartenol 17.5%,0.75mg(1.75),24-methylenecycloartanol 46.6%,2mg (1.98),probably cyclobranol 33.9%,1.46mg(2.28).(Numbers cited in parentheses indicate the retention time relative to that of cholesterol acetate. The previous number indicates the weight of the sterols contained in a 100g of dry root bark.

In the saponified matter: (0.59% of the dry root bark);

Presence,lauric o.6%,myristic 1.2%,palmitic 21.1%,palmitoleic 1.9%,stearic 6.4%,oleic 50.4%,linoleic 11.6%, and linolenic 6.8%[20] acids.

Diethyl ether extract. After evaporation of the solvent, the weight of the brownish red residue represented 1.2% of the dry weight drug.The investigation of gossypol derivatives in the ether extract was effected by T.L.C.,where, after spraying with the phloroglycine-HCl reagent,a light red to purple colour characteristic of hemigossypol as well as of the methyl ethers of gossypol and gossypol appeared.

Spectrophotometric determination of mixed gossypols. The U.V. absorption curve of the colored complex showed two characteristic maxima at 440 and 460 nm.The total gossypols content was measured at the absorption maxima of the coloured complex compared with the observed absorption of gossypol solution of known concentration;it was thus found to be 0.8% of the dried drug.

Isolation of gossypol from the dry root bark.Recrystallisation of the precipitate from chloroform yielded pure crystals m.p.184^{0}C.See table 1 and figures 1,2.

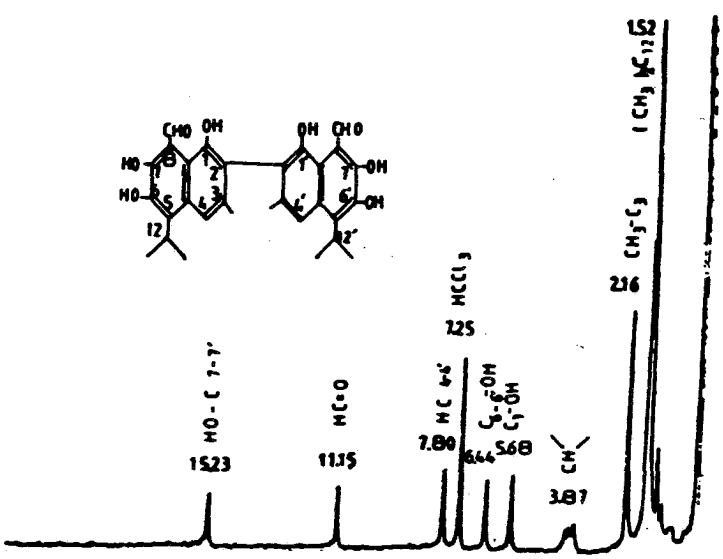


FIG.1: *Proton Magnetic Resonance spectrum of gossypol dissolved in deuteriated chloroform.*

TABLE 1: Spectral Data of Gossypol Derivatives.

	I.R. CM^{-1}	U.V. (nm)		N.M.R.(δ)		
	CHO C-OH			G.	M.G.	D.M.G.
G.	1625,3345	a.236,283,289,	$(Me)_2C$+	1.52	1.55	1.51
		376.	Me-3	2.16	2.15	2.15
		b.212,266,404.	$(Me)_2CH$‡	3.87	3.92	3.91
		c.240,293,386.	MeO-C-6	--	3.99	4
			MeO-C-6'	--	--	4
M.G.	1615,3360	a.235,288,369.	HO-C-1	5.67	5.83	5.74
		b.236,265,298,	HO-C-1'	5.67	5.77	5.74
		391.	HO-C-6	6.44	6.41	--
		c.240,290,376.	HO-C-6'	6.44	--	--
			CHO	11.15	11.14	11.16
D.M.G.	1612	a.231,253,287,	CHO	11.15	11.11	11.16
		360.	HO-C-7	15.23	15.15	14.59
		c.249,296,401.	HO-C-7'	15.23	14.54	14.59

a.absorption maxima in methanol (nm), b.absorption maxima after treatment with a 5% ethanol solution of aluminium chloride, c. absorption maxima after treatment with a 1% aqueous borax solution[21].
G= gossypol,M.G.= 6-methoxygossypol,D.M.G.=6,6'-dimethoxygossypol.

Isolation of 6-methoxygossypol. Recrystallisation of the residue from benzene:hexane (1:1),yielded pure crystals m.p. 146^0C.See table 1 and figures 2,3 .

Isolation of 6,6'-dimethoxygossypol.Recrystallisation of the residue from benzene:hexane (1:1),yielded pure gold - yellow crystals m.p. 181^0C.See table 1 and figures 2,4.

Isolation of serotonin.The leaf and root bark extracts showed the presence of serotonin after spraying with four different reagents.Preparative paper chromatography of the extracts on Whatman $N^0$3MM with four different solvents yielded a substance displaying the same U.V. spectrum as serotonin ie.,a maximum at 275 nm[22].

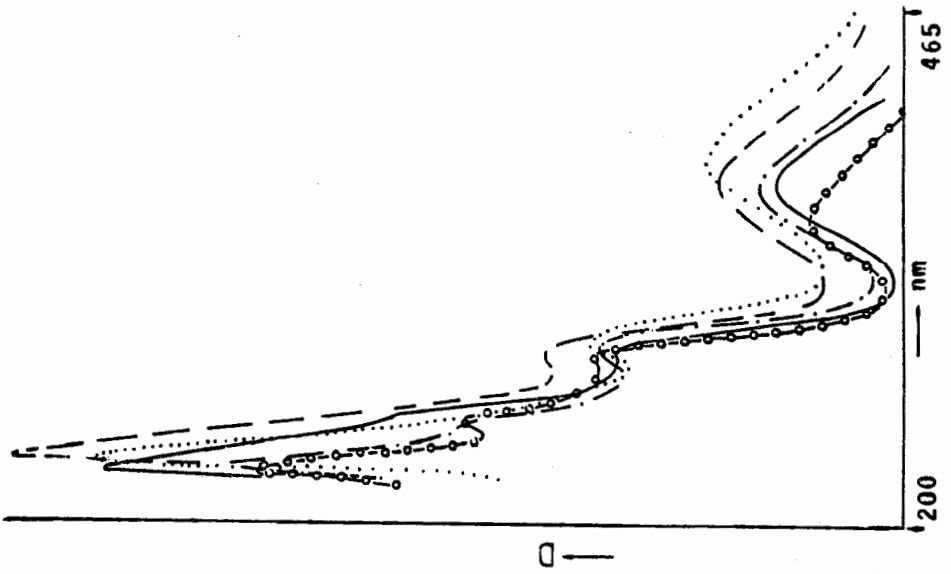


FIG.2: *Visible and U.V. absorption spectra of the three gossypols dissolved in methanol as well as of the respective complexes formed after addition of a few drops of 1% aqueous borax solution.*
———= *gossypol*,-·-·-·-= *6-methoxygossypol*,-o-o-o-=*6,6'-dimethoxygossypol*,.....= *gossypol and borax*,----= *6-methoxygossypol and borax.*

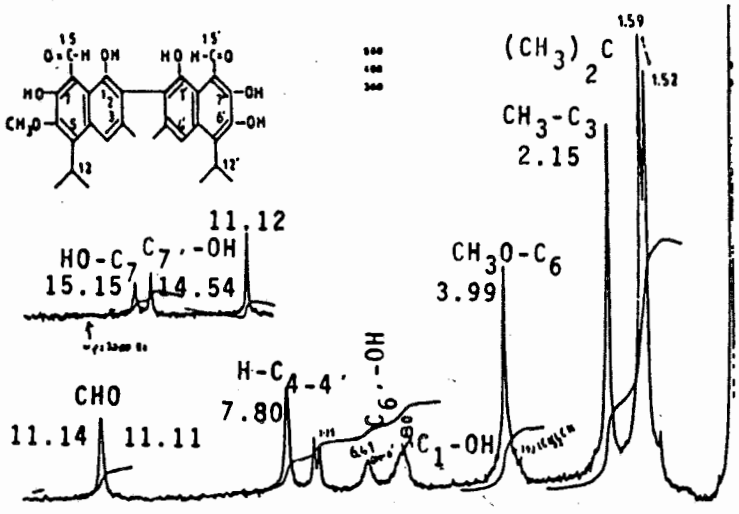


FIG.3: *Proton Magnetic Resonance spectrum of 6-methoxygossypol dissolved in deuteriated chloroform.*

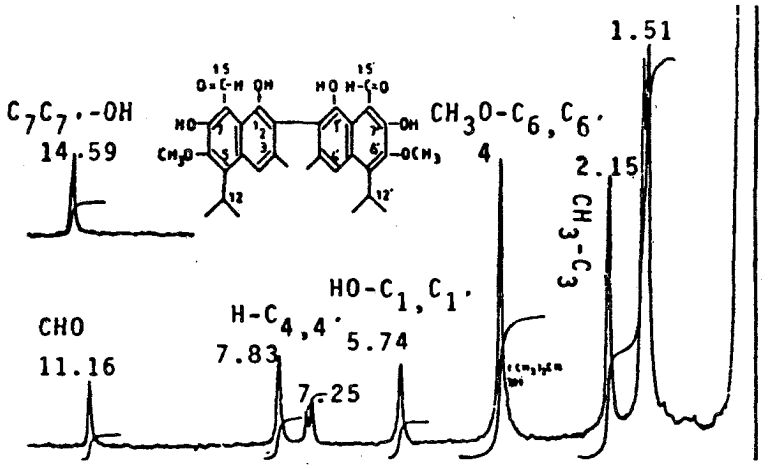


FIG.4: *Proton Magnetic Resonance spectrum of 6,6'-dimethoxy-gossypol dissolved in deuteriated chloroform.*

Preliminary pharmacological tests.This study showed,that, the aqueous leaves extract (in which serotonin was present at a molarity of $4.54x10^{-4}$), causes contractions of the smooth muscular fibres of both mice and rabbit utery, under the condition that, serotonin is identified in this extract. See fig. 5,6.

Serotonin free leaves extracts give negative results. The presence of serotonin was verified by P.C. using the 4-dimethylaminobenzaldehyde reagent[8,22].

Acknowledgments

The authors would like to express their thanks to lecturer Ch. Caragheorghiou at the Pharmacology Laboratory of the Faculty of Medicine (Athens Univercity).

ΠΕΡΙΛΗΨΗ

ΧΗΜΙΚΑ ΣΥΣΤΑΤΙΚΑ ΤΟΥ ΦΛΟΙΟΥ ΤΗΣ ΡΙΖΑΣ ΤΟΥ GOSSYPIUM HIRSUTUM L.

Από τον φλοιό της ρίζας του Gossypium hirsutum;Malvaceae, απομονώθηκαν τα παρακάτω χημικά συστατικά:4-δεσμεθυλοστερόλες, μεθυλοστερόλες,διμεθυλοστερόλες,λιπαρά οξέα,παράγωγα των γκοσ-

συπολών,σάκχαρα,αμινοξέα,σεροτονίνη και βλέννα.Στη προκαταρκτική φαρμακολογική μελέτη,εξετάσθηκαν τα αποτελέσματα του υδατικού εκχυλίσματος των φύλλων στην απομονωμένη μήτρα του ποντικού και του κουνελιού.

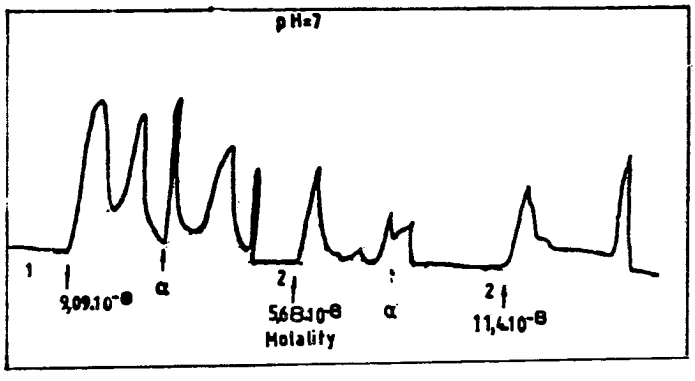


FIG.5: *Pharmacological action of the aqueous leaves extract on an isolated mouse uterus.Doubling the dose not result in a two-fold amplitude and duration of the convulsions since the smooth muscular fibres are subject to fatigue due to the fact that they belong to small mamals.*

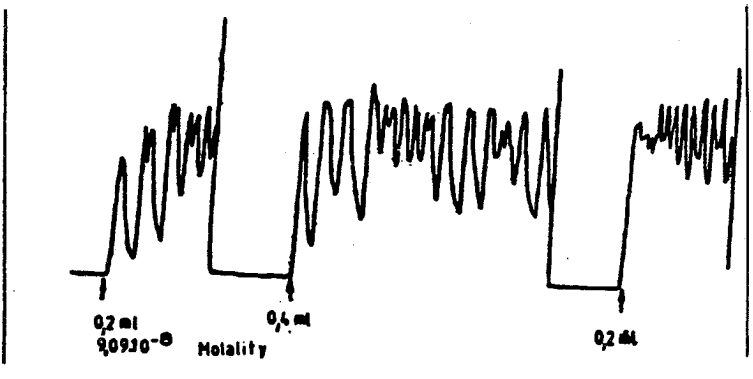


FIG.6: *Pharmacological action of aqueous leaves extract on an isolated rabbit uterus. The movement of the smooth muscular fibres of the rabbit uterus were recorded by means of a wave monitor recorder.Doubling of the dose doubled the duration as well as the amplitude of muscular fibres contraction.*

REFERENCES

1. Schmidt-Wetter R., *Taschenbuch der Pharmakognosie*, p. 178, Sherpe-Verlag zu Krefeld (1950).
2. Morton J., *Folk Remedies of the Low Country*, E.A. p. 67, Seemann, Miami (1973).
3. *The Dispensatory of the United States of America* 25th ed., part II, p. 1651 (1955).
4. Paris R., Moyse H., *Precis de Matière Medicale*, p. 254, Masson, (1967).
5. Kwan Y., Nurok U. and Zlatkis A., *J. Chromatogr.*, 174, 187, (1979).
6. Cegla G. and Bell K., *J. Am. Oil Chem. Soc.*, 54, 150 (1977).
7. *Pharmacopée Française* 9th ed., Vol., 2, p. 306 (1972).
8. Stahl E., *Thin Layer Chromatography*. Springer Verlag, (1969).
9. Linskens H., *Papierchromatographie in der Botanic* p. 164, Berlin (1959).
10. Mabry T., Markham K. and Thomas M., *The systematic Identification of Flavonoids* p. 3, Berlin-Heidelberg-New York (1970).
11. *Greak Pharmacopoea* p. 1221 (1974).
12. Cleaver C. and Cassidy M., *J.Am.Chem.Soc.*, 72, 1147 (1950).
13. Wolff J., *Manuel d'Analyse des Corpes Gras*. Azoulay Paris (1968).
14. Kriticos P., Philianos S., *Sci. Phar.*, 31, 4, 290 (1963).
15. Gegiou D., *Chimica Chronica, New Series* 9, 285 (1980)
16. Patterson G., *Anal. Chem.*, 43, 1165 (1971).
17. *Official and Tentative Methods of the American Oil Chemists' Society (A.O.C.S.)*, Ca, Ga-40 (1972).
18. Itoh T., Tamura T., Lida T. and Matsumoto T., *Steroids* 23, 687 (1974).
19. Menounos P., Staphylakis K. and Gegiou D., *Phytochemistry* 25, 761 (1986).
20. *A.O.C.S., Tentative Method* Ce 1-62 (1970).
21. Stipanovic R., Bell A., Mace M. and Howell C., *Phytochemistry*, 14, 1077 (1975).
22. Bulard C., Leopold C., *Compt. Rend. Acad. Sci.*, 247, 1382, Paris (1958).
23. Stipanovic R., Bell A., O'Brien D. and Lukefahr M., *Phytochemistry*, 17, 151 (1978).
24. Bell A., Stipanovic R., Howell C. and Fryxell P., *Phytochemistry*, 14, 225 (1975).
25. Galli G. and Maroni S., *Steroids* 10, 189 (1967).
26. Knights B., *J. Gas Chromat.*, 5, 273 (1967).
27. Wyllie S. and Djerassi C., *J. Org. Chem.*, 33, 305 (1968).
28. Itoh T., Tamura T. and Matsumoto T., *Fette Seifen Anstrichm.*, 80, 382 (1978).

Chimika Chronika, New Series, 19, 165-175 (1990)

A METHOD FOR THE THEORETICAL EVALUATION OF THE LENGTH OF THE AMINO-AND CARBOXYTERMINAL PARTS OF THE CORE HISTONE POLYPEPTIDES

APOSTOLOS G. KARAMAOUNAS

Research and Development Laboratories N.Petsiavas S.A. (Pharmaceuticals) 11 Nikodimou Str. and Voulis, 105 58 Athens, Greece.

(Received June 10, 1988 - Revised May 1991)

SUMMARY

This paper is based on experimental results obtained by different investigators in an attempt to study the core histone structure. It constitutes a theoretical approach of the same problem and makes use of two postulates: a) That the methyl or/and acetyl substitutions occuring exclusively in the aminoterminal parts of core histone polypeptides are destined to inhibit certain interactions between neighboring molecules and b) That the ratio of substituted residues to the total number of corresponding residues offers the possibility to estimate the length of the two parts of the molecule. The precision of this first approach is increased by tracing the relative hydropathy of the chain within the estimated lenght range.

Key words: Amino acid residues, aminoterminal (a/t), cleavage, chromatin, core histones, core particle, carboxyterminal (c/t), eucaryotic,histones, hydropathy, linker DNA, nucleosome, nucleosome fiber, nucleosome train, postsynthetic modifications, tails, tetrade rule.

INTRODUCTION

It has been generally accepted that the aminoterminal parts of the core histone polypeptides are situated on the surface of the molecules and by many investigators (1-4,11) have been considered to be responsible for the winding of DNA around the 8meric complex of the four core histones 2xH2A, 2xH2B, 2xH3 and 2xH4.

The aminoterminal (a/t) parts of histones are rich in the basic amino acids lysine and arginine(8,10,12-14), their total positive charge considered as being attracted by an equal negative charge, due to the

ionized hydroxyls of the DNA phosphate groups. This attraction is supposed to result in the helicoidal winding of the DNA around the histone 8mer, forming the unit known as "core particle" (20). It seems however probable that this electrostatic attraction is not the only factor for the winding of DNA (10,22).

A percentage of the lysines presents their ε-amino groups substituted with acetyl-or methyl groups and, in the case of Lys 9 of H3, with both of these substituents. Acetyl substituted are also Ser 1 of H2A and H4 (17-19). The substitutions constitute postsynthetic modifications and are permanent and fixed (14).

The substitutions never occur in the interior of the histone molecules.

Roughly speaking the histone molecule can be divided in two distingt parts:

1. The strongly polar a/t part, very ptobably rather voluminous because of attraction of water molecules, which, competing with CO< and NH< groups of the chain for the formation of hydrogen bonds, reduce the possibility of creation of higher order stuctures.

2. The much less hydrophilic (polar) c/t part, having a rather globular character (8,10,13,14).

However, the strong polarity of the a/t parts is due not only to the presence of Lys, Arg, Glu etc. residues, but also to other amino acids, which, even to a lesser extent, are considered as polar (for. ex. Gly or Ser).

On the other hand, polar residues are also abundant in the c/t parts which, by tradition, were always considered as non polar.

We find it therefore rather incorrect to speak of "polar a/t and non polar c/t parts" and would consider it more realistic to apply a general method for the quantitative expression of the degree of polarity of the two parts, to evaluate, in other words, their"hydropathy". term suggested by (31).

The introduction of such a criterion, combined with the method described below, could also provide information on the localization of the site on the primary sequence where the strongly polar a/t part leaves the surface of the molecule and continues its conformation as c/t part.

ESTIMATION OF THE LENGTH OF THE A/T PARTS

As mentioned in the summary it is postulated that the Me or/and Ac substitutions of certain Lys and Ser residues are presumably destined to inhibit sterically, or in another, yet unknown way, possible interactions between the histones themselves or between the histones and the DNA. The substitutions affect exclusively Lys and Ser residues of the a/t part. Same residues belonging to the c/t part are never modified. On the other hand Lys residues seem at first look to be randomly distributed over the whole chain. We shall revert to this point in the discussion at the end of the paper.

These considerations lead to the assumption that to every Lys (or Lys + Ser in the case of H2A and H4 where serines are taken also into account because those under Nr.1 in the sequences are also Ac-substituted) residue "belongs" a fragment of the chain of variable length so that the ratio of the number of Lys + Ser (modified or not) up to the most distant modified Lys, to the total number of corresponding residues in the molecule gives approximately the measure of"exposure" of the a/t part on its surface or better, within its surrounding hydration envelope.

This statement can be represented by the simple relation:

$$\frac{L^* + L_a}{L} = \frac{R_a}{R} \qquad (1)$$

where R_a= number of a/t residues, L^*= number of modified Lys and Ser, L_a= number of unmodified Lys included in the chain section from the NH_2-end up to the most distant modified Lys, L = total number of Lys and Ser residues and R = total number of residues in the molecule.

The following Fig.1 depicts how we imagine this statement:

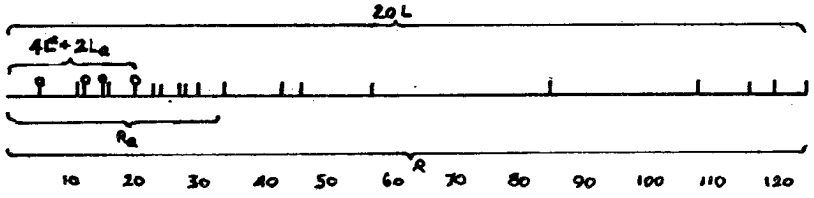


FIG.1: *Schematic representation of the statement (1) as applied to calf thymus H2B. Only the Lys are depicted as small vertical lines projecting upwards from the ground line, representing the chain. The modified Lys are symbolized by* ♀ .

It has to be emphasized however that the values for R_a obtained throught formula (1) must be taken as average values ($\bar{R}_a$) since, as stated previously, the distribution of the L's along the polypeptide chains is considered as random.

Obviously the $\bar{R}_a$'s thus obtained would be much more reliable if the distribution of the L's within the sequence were periodic, i.e. if there was one L every determined set of R's. For example, in the case of H2B, if there was one Lys every 125:20= 6,25 residues. Naturally this is not the case, as seen in the Appendix I.

Therefore relation (1) was revised in order to take into account the degree of deviation from the periodic distribution and the expression:

$$R_a = (L^* + L_a)(\frac{R}{L} \pm s) \qquad (2)$$

where

$$s = \pm\sqrt{\frac{\sum(n_L - 1)^2}{L - 1}} \qquad (3)$$

is suggested.

In case of normal (periodic) distribution, in every set would correspond one Lys (or Lys+Ser). n_L is the number of Lys (or Lys+Ser) actually present in each set. Obviously $0 \leqslant n_L \leqslant R/L$. For $n_L = 1$ in all the sets we would have s=0 and the formula (1) would hold.

The ratio R/L gives the number of residues per set and, practically is not an integer, so that the sequence can not be devided in "equiresidual" sets. It was therefore necessary to consider the nearest smaller integer and distribute the resulting small difference -if any- proportionally over all the n_L's present in each set.

TABLE I: Statistical variation of R_a about the average value $\bar{R}_a$. Based on the data of 8,12-14 for the calf thymus core histones. $\bar{R}_a$ values computed according to formula (1). See also Appendix I.

Histone	$\bar{R}_a$	R_a min	R_a max	-Δ	+Δ
H2A	14	12	17	2	3
H2B	37	33	41	4	4
H3	62	58	66	4	4
H4	47	42	53	5	6

There are thus several points within the $\bar{R}_a \pm \Delta$ range that can represent the ending of the a/t and the beginning of the c/t part.

In order to localize the point corresponding to this property the assumption was made that it must lie anyway within the above range, but at a site where the relative hydropathy "p" attains its highest negative value. As relative hydropathy is defined the algebraic sum of the hydropathy indices divided by a certain number (span) of residues.

This criterion was chosen because at the site of highest negative "p" the chain is still "attracted" by the surrounding water molecules and this attraction results in a weakening of all the secondary intramolecular interactions, whereas after that point, these forces are again strengthened because of the overall reduced polarity of the c/t part, resulting in expelling of the major part of the water. This is, according to the writer's opinion, the site where the a/t chain leaves the surface of the molecule and continues its conformation as c/t part,forming the globular c/t body.

The site with highest negative "p" is computed according to the method described by (31) for spans of 9 residues.

The relative hydropathies for the four calf thymus core histones are plotted in the Appendix II. The $\bar{R}_a \pm \Delta$ range is marked by two vertical lines and the point with highest negative hydropathy within this range by an arrow.

As it can be seen from the plots, these points correspond to:

13 residues for H2A
33 " " H2B
60 " " H3 and
43 " " H4

and represent, according to our opinion, the actual lengths (R_a) of the a/t parts of the core histone polypeptides.

DISCUSSION

We remark first of all that the computed a/t lengths give an average of 37 residues per core histone a/t parts, which is in good agreement with the results of trypsin cleavage experiments of chromatin (5-7,9), though somewhat bigger. In any case we observe that H3 and H4 have very long aminoterminal parts. These parts have been considered by certain

authors as responsible for the formation of the skeleton of the nucleosome fibers (20,22,27-28) and by others for the interactions between 8mer and DNA (23-25).

As suggested previously the a/t parts must be poor in ordered structures because of attrection of water molecules. This point of view is further supported by the fact that, at least in the case of H2B, H3 and H4 their a/t parts are 3 times as rich in proline residues as are their c/t parts (totally 9 a/t against 3 c/t prolines) and it is known that the presence of proline residues in the chain is not favourable for the formation of α-helices (38). This fact could eventually explain why the core histones are generally poor in α-helices (32-33,39) and it could be deduced that this structure is accumulated rather in the c/t parts. As stated above, this rule is not valid for H2A, as confirmed by crystallografic studies (39).

As previously postulated, the distribution of lysines over the whole core histone molecule is random. This postulate seems, however, to be incorrect. In fact we observe that most lysines belonging to the a/t parts as computed above, follow a regular pattern: they are positioned in such a way as to occupy every fourth place within the sequence.

We call this regularity: "the tetrade rule".

In the case of c/t parts, as computed by subtraction of R_a's from the total number of residues per molecule, this rule is obeyed sporadically by very few of the lysines present, if at all.

The following Table II is sufficiently persuasive:

TABLE II. The tetrade rule in a/t and c/t parts of core histones. Modified lysines are characterized by the symbol *.

Histone	Aminoterminal part			Carboxyterminal part		
	Total Lys	Lys obeying the tetrade rule	%	Total Lys	Lys obeying the tetrade rule	%
H2A	3	5*,9,13	100	11	95,99 125,129	36
H2B	11	11,15 12*,16,20*,24,28 23,27	82	9	116,120	22
H3	9	14*, 18* 23*, 27*	44	3	---	0
H4	7	8*,12*, 16*,20*	57	4	---	0

It is interesting to note that in the case of H3 and H4 the a/t lysines obeying the tetrade rule are all modified.
It is obvious,there can not be a matter of coincidence. We think that these peculiarities can not be deprived of biological importance and require a profound investigation.

ACKNOWLEDGMENT

I wish to extend my warmest thanks to Dr.Irene Mavrides from "DEMOCRITOS" N.R.C. Athens/Greece for critical reading of the manuscript and helpful suggestions.

ΠΕΡΙΛΗΨΗ

ΜΕΘΟΔΟΣ ΓΙΑ ΤΗΝ ΘΕΩΡΗΤΙΚΗ ΕΚΤΙΜΗΣΗ ΤΩΥ ΜΗΚΟΥΣ ΤΩΝ ΑΜΙΝΟ -ΚΑΙ ΚΑΡΒΟΞΥΤΕΛΙΚΩΝ ΤΜΗΜΑΤΩΝ ΤΩΝ ΠΟΛΥΠΕΠΤΙΔΙΩΝ ΤΩΝ ΕΣΩΤΕΡΙΚΩΝ ΙΣΤΟΝΩΝ

Στήν ἐργασία αυτή γίνεται προσπάθεια, με βάση πειραματικά δεδομένα διαφόρων ερευνητῶν,νά μελετηθεῖ η δομή τῶν εσωτερικῶν ιστονῶν. Πρόκειται για θεωρητική προσέγγιση τοῦ προβλήματος κατά την οποία γίνεται χρήση δύο ἀξιωμάτων: α)"Οτι οι υποκαταστάσεις με μεθύλιο ἤ/και ἀκετύλιο ορισμένων καταλοίπων αμινοξέων που ἀνήκουν αποκλειστικά στο αμινοτελικό ἄκρο των ιστονικῶν πολυπεπτιδίων έχουν προορισμό την παρεμπόδιση ορισμένων αλληλεπιδράσεων με γειτονικά μόρια και β)"Οτι ο λόγος των υποκατεστημένων καταλοίπων προς το σύνολο των αντίστοιχων καταλοίπων στο μόριο προσφέρει τη δυνατότητα της εκτίμησης του μήκους των δύο τμημάτων, αμινοτελικοῦ και καρβοξυτελικοῦ.

Η ακρίβεια της πρώτης αυτῆς προσέγγισης αύξάνει μέ την παρακολούθηση της σχετικῆς υδροπάθειας (υδροφιλίας ἤ υδροφοβίας) της πολυπεπτιδικῆς αλυσίδας μέσα στά ὅρια της πρώτης εκτίμησης, ὅπως αυτά προκύπτουν από τη στατιστική μελέτη της απόκλισης από το υπολογιζόμενο μέσο μήκος του αμινοτελικοῦ τμήματος.

APPENDIX I

AMINO ACID SEQUENCES OF THE FOUR CALF THYMUS CORE HISTONES*

NH_2

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
H2A	Ser[1]	Gly	Arg	Gly	Lys[1]	Gln	Gly	Gly	Lys	Ala	Arg	Ala	Lys	Ala	Lys	Thr	Arg	Ser	Ser	Arg
H2B	Pro	Gln	Pro	Ala	Lys[1]	Ser	Ala	Pro	Ala	Pro	Lys	Lys[1]	Gly	Ser	Lys[1]	Lys	Ala	Val	Thr	Lys[1]
H3	Ala	Arg	Thr	Lys	Gln	Thr	Ala	Arg	Lys[3]	Ser	Thr	Gly	Gly	Lys[1]	Ala	Pro	Arg	Lys[1]	Gln	Leu
H4	Ser[1]	Gly	Arg	Gly	Lys[1]	Gly	Gly	Lys[1]	Gly	Leu	Gly	Lys[1]	Gly	Gly	Ala	Lys[1]	Arg	His	Arg	Lys[2]

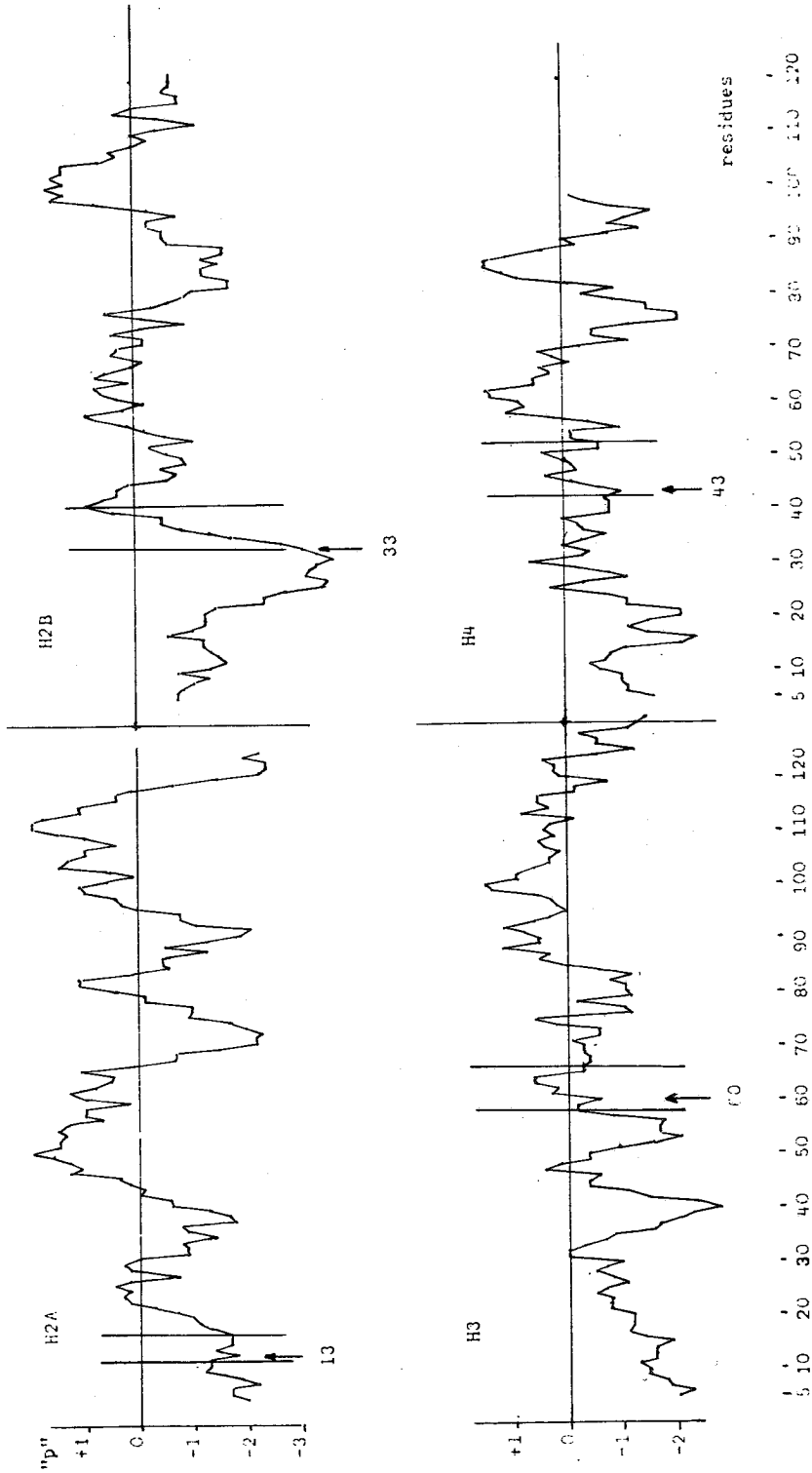
	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
H2A	Ala	Gly	Leu	Gln	Phe	Pro	Val	Gly	Arg	Val	His	Arg	Ile	Leu	Arg	Lys	Gly	Asn	Tyr	Ala
H2B	Ala	Glu	Lys	Lys	Asp	Gly	Lys	Lys	Arg	Lys	Arg	Ser	Arg	Lys	Glu	Ser	Tyr	Ser	Val	Tyr
H3	Ala	Thr	Lys[1]	Ala	Ala	Arg	Lys[2]	Ser	Ala	Pro	Ala	Thr	Gly	Gly	Val	Lys	Lys	Pro	His	Arg
H4	Val	Leu	Arg	Asp	Asn	Ile	Gln	Gly	Ile	Thr	Lys	Pro	Ala	Ile	Arg	Arg	Leu	Ala	Arg	Gly

	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
H2A	Glu	Arg	Val	Gly	Ala	Gly	Ala	Pro	Val	Tyr	Leu	Ala	Ala	Val	Leu	Glu	Tyr	Leu	Thr	Ala
H2B	Val	Tyr	Lys	Val	Leu	Lys	Gln	Val	His	Pro	Asp	Thr	Gly	Ile	Ser	Ser	Lys	Ala	Met	Gly
H3	Tyr	Arg	Pro	Gly	Thr	Val	Ala	Leu	Arg	Glu	Ile	Arg	Arg	Tyr	Gly	Lys	Ser	Thr	Glu	Leu
H4	Gly	Val	Lys	Arg	Arg	Ile	Ser	Gly	Leu	Ile	Tyr	Glu	Glu	Thr	Arg	Gly	Val	Leu	Lys	Val

```
      61  62  63  64  65  66  67  68  69  70  71  72  73  74  75  76  77  78  79  80
H2A   Glu Ile Leu Glu Leu Ala Gly Asn Ala Ala Arg Asp Asn Lys Lys Thr Arg Ile Ile Phe
H2B   Ile Met Asn Ser Phe Val Asn Asp Ile Phe Glu Arg Ile Ala Gly Glu Ala Ser Arg Leu
H3    Leu Ile Arg Lys Leu Pro Phe Gln Arg Leu Val Arg Glu Ile Ala Gln Asp Phe Lys Thr
H4    Phe Leu Glu Asn Val Ile Arg Asp Ala Val Thr Tyr Thr Glu His Ala Lys Arg Lys Thr

      81  82  83  84  85  86  87  88  89  90  91  92  93  94  95  96  97  98  99 100
H2A   Arg His Leu Gln Leu Ala Ile Arg Asn Asp Glu Glu Leu Asn Lys Leu Leu Gly Lys Val
H2B   Ala His Tyr Asn Lys Arg Ser Thr Ile Thr Ser Arg Glu Ile Gln Thr Ala Val Arg Leu
H3    Asp Leu Arg Phe Gln Ser Ser Ala Val Met Ala Leu Gln Glu Ala Cys Glu Ala Tyr Leu
H4    Val Thr Ala Met Asp Val Val Tyr Ala Leu Lys Arg Gln Gly Arg Thr Leu Tyr Gly Phe

     101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
H2A   Thr Ile Ala Gln Gly Gly Val Leu Phe Asn Ile Gln Ala Val Leu Leu Phe Lys Lys Tyr
H2B   Leu Leu Phe Gly Glu Leu Ala Lys His Ala Val Ser Glu Gly Thr Lys Ala Val Thr Lys
H3    Val Gly Leu Phe Glu Asp Thr Asn Leu Cys Ala Ile His Ala Lys Arg Val Thr Ile Met
H4    Gly Gly

     121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
H2A   Glu Ser His His Lys Ala Lys Gly Lys
H2B   Tyr Thr Ser Ser Lys
H3    Pro Lys Asp Ile Gln Leu Ala Arg Arg Ile Arg Gly Glu Arg Ala
H4
                                                                  COOH
```

The indices represent: [1]acetyl, [2]methyl, [3]acetyl+methyl

* according to (8,12,13,14).



APPENDIX II: Hydropathy tracing of calf thymus core histones

REFERENCES

1. Simpson,R.T. *Proc.Nat.Acad.Sci.USA*,73 4400-4 (1976)
2. Kornberg,R.D. *Science* 184 868-71 (1974)
3. Oudet,P. et al. *Phil.Tr.R.Soc.London,Ser.B.* (1977)
4a. Thomas,J.O.-Kornberg,R.D. *Pr.Nat.Ac.Scie.USA* 72 2626-30 (1975)
4b. Thomas,J.O.-Kornberg,R.D. *FEBS Lett.* 58 353-58 (1975)
5. Weintraub,H.-Van Lente,F. *Pr.Nat.Ac.Sci.USA* 71 4149-65 (1974)
6. Richards,F.M.-Vithayatil,P.J. *J.Biol.Chem.* 234 1459-65 (1959)
7. Taniuchi,H. et al. *J.Biol.Chem.* 244 4600-6 (1969)
8a. Ogava,Y. et al. *J.Biol.Chem.* 244 4387-92 (1969)
8b. DeLange,R.J.-Smith,E.L. *Acc.Chem.Res.* 5 368-73 (1972)
8c. DeLange,R.J. *Pr.Nat.Ac.Sci.USA* 69 882 4 (1972)
9. Platt,T. et al. *J.Biol.Chem.* 248 110-21 (1973)
10. Malchy,B.-Kaplan,H. *J.Mol.Biol.* 82 537-45 (1974)
11. Camerini-Otero,R.D. *Cell* 8 333-47
12a. Yeoman,L.C. et al. *J.Biol.Chem.*247 8018-23 (1972)
12b. Sautière,P. et al. *Eur.J.Biochem.* 41 563-76(1974)
13. Iwai,K. et al. *J.Biochem.(Tokyo)* 72 357-67 (1972)
14. Olson,M.O. et al. *Bioch.Bioph.Res.Comm.* 46 50-55 (1972)
15. Liew,C.C. et al. *Nature(London)*226 414-7
16a. Sperling,R.-Amos,L.A. *Pr.Nat.Ac.Sci.USA* 74 3772-6 (1977)
16b. Wachtel,E.J.-Sperling,R. *Nucl.Ac.Res.* 6 139-51 (1079)
17a,b,c. in the reviews under No 35,36 and 21 below
18a. In the review of Ogawa,Y.et al. as No 8a above
18b. Patty,L. et al. *J.Biol.Chem.* 248 6834-40 (1973)
19a,b. in the reviews under No 35 and 36 below.
20. Kornberg,R.D.*Ann.Rev.Biochem.* 46 931-54 (1977)
21. Isenberg,I. *Ann.Rev.Biochem.* 48 159-91 (1979)
22. Sperling,R.-Wachtel,E.J. *Adv.Prot.Chem.* 34 1-60 (1981)
23. Bina-Stein,M.-Simpson,R.T. *Cell* 11 609-18 (1977)
24. Camerini-Otero,R.D.-Felsenfeld,G. *Nucl.A.Res.* 4 1159-81 (1977b)
25. Oudet,P. et al. *Cold Spr.Har.Symp.Quant.Biol.*42 287-300 (1978)
26. Thomas,J.O.-Butler,R.J.G.*Cold Spr.Har.Symp.Quant.Biol.*42 119-125 (1978)
27. DeLange,R,J. et al. *Biochemistry* 18 1942-6 (1979)
28. Martinson,H.G. et al. *Biochemistry* 18 1075-82 (1979)
29. Camerini-Otero,R,D.-Felsenfeld,G. *Pr.Nat.Ac.Sci.USA* 74 5519-23 (1977a)
30. McGhee,J.D.-Felsenfeld,G. *Ann.Rev.Biochem.* 49 1115-56 (1980)
31. Kyte,J.-Doolittle,R.F. *J.Mol.Biol.* 157 105-32 (1982)
32. D'Anna j.A.Jr.-Isenberg, I. *Biochemistry* 13 4492-7 (1974b)
33. Moss,T. et al. *Eur.J.Biochem.* 71-337-50 (1076b)
34. Klug,A. *Angew.Chemie (Int.Ed.in engl.)* Vol 22-No 8, 565-636 (VIII 1983)
35. Dixon,G.H. *Organization and Expression of Chromosomes (V.G.Ed)* Dahlem Konferenzen, Abakon Verlagsges.,Berlin (1976)
36. Allfrey,V.G. *Chromatin and Chromosome Structure* (H.J.Li and R.A.Eckhardt,Ed.) Academic Press, N.Y.(1977)
37. Sperling,R.-Wachtel,E.J. *Molecular Mechanisms of Biological Recognition* (M.Balaban Ed.) Elsevier/North Holland Biomedical Press,Amsterdam (1979)
38. Stryer L. "*Biochemistry*" W.H.Freeman and Co, San Francisco (1981)
39. Burlingame,R.W. et al. *Science* 228 546-553 (1985)

Chimika Chronika, New Series, 19, 177-191 (1990)

INFLUENCE OF CO_2 PARTIAL PRESSURE AND IONIC STRENGTH ON Cd REACTIONS WITH CALCITE.

P.PAPADOPOULOS and D.L. ROWELL

Institute of Soil Science, 141 23 Lycovrissi, Athens, Greece.
Soil Science Department, The University, London Rd, Reading, UK.

(Received February 22, 1990)

SUMMARY

An attempt was made to elucidate the reactions of Cd with calcite (BDH, A.R. grade) by changing the experimental conditions, i.e. the CO_2 partial pressure from $10^{-3.49}$ atm to 10^{-2} atm using 1% CO_2 and the ionic strength of the equilibrium solution using 5mM $Ca(NO_3)_2$ instead of H_2O.

Both these changes resulted in increased Ca levels in solution and reduced pH values compared with the $CaCO_3$-H_2O - Cd system open to the atmosphere, ("pure system").

Reduced affinity of Cd for the $CaCO_3$ surface was observed in both cases (lower Kd values) and this was more pronounced in the system with 5mM $Ca(NO_3)_2$ probably because of increased Ca competition.

In the 1% CO_2 system deviation from ideal surface solid solution formation between $CdCO_3$ and $CaCO_3$ was observed probably as a result of aqueous phase interactions whereas in the 5mM $Ca(NO_3)_2$ system an almost ideal surface solid solution between $CdCO_3$ and $CaCO_3$ was formed and in this later case the Thorstenson and Plummer equation for expressing pIAP values of solid solutions was valid.

Chemisorption of Cd on $CaCO_3$ surface sites is the dominant mechanism for low Cd additions whereas surface precipitation of $CdCO_3$ as a coating on $CaCO_3$ is the dominant mechanism for higher Cd additions with pH and Ca concentration changes. These experiments clearly indicate the specific affinity of Cd for the $CaCO_3$ surface which is expected from theoretical considerations.

Key words: Cd sorption, Calcite, Solubility product constant, Surface solid solution.

INTRODUCTION

Reactions of Cd with naturally occuring solids continue to attract research interest because they help to a better understanding of the chemistry of complex natural systems for this element, for which it is generally accepted that increased uptake through the soil-plant system can result in various diseases in human beings.[1]

Attention has been focused on Cd reactions with oxide minerals[2], organic matter[3,4], clay minerals [5,6,7]. Only recently the specific role of calcite in Cd retention in calcareous and limed soils and sediments has been examined in some detail[8,9,10].

A high affinity of Cd for the calcite surface was found in accordance with theory[10] and for low Cd additions in a preequilibrated $CaCO_3$ - H_2O system, open to the atmosphere, an almost ideal surface solid solution between $CdCO_3$ and $CaCO_3$ was formed. In this case the solubility product constants obtained from the Thorstenson and Plummer[11] equation derived for magnesian calcites, could be a useful way to relate carbonate reacted Cd with solution composition.

Increased ionic strength in a solution usually results in a lower affinity of an ion for a surface and decreasing sorption because of lower activity, increased competition and in some cases complexation.

So, Garcia and Page[12] found a decrease in the amount of Cd sorbed an a clay surface with increasing ionic strength. Cline and OConnor[13] working with Cd sorption and mobility in sludge amended soils observed reduced metal sorption with increasing ionic strength. They suggested that this decrease is the result of reduced metal activity, increased competition for sorbing sites and complexation. Franklin and Morse[14,15] studied the reactions of Mn and Cu with calcite in pure water and seawater and found lower distribution coefficients for sorption from seawater than from pure water.

This decrease was attributed to Mg competition and decreased metal activity in seawater.

In the present study we tried to elucidate the Cd-$CaCO_3$ reaction by changing the experimental conditions, i.e. the CO_2 partial pressure from $10^{-3.49}$ to 10^{-2} atm and the ionic strength 5mM $Ca(NO_3)_2$ instead of H_2O. Both these cases result in increased Ca concentrations and reduced pH values compared with the Cd-$CaCO_3$-H_2O system at $10^{-3.49}$ atm and occur under natural soil conditions.

MATERIALS and METHODS

A.R. $CaCO_3$ (BDH) with a specific surface area of $0.15 m^2 g^{-1}$ measured by krypton adsorption was used throughout these experiments. 4g of $CaCO_3$ was placed in a 150ml Azlon high density polypropylene bottle together with 50ml of either deionized -distilled water or 50ml of 5mM $Ca(NO_3)_2$ at $25 \pm 1^{o}C$. Air -1% CO_2 mixture saturated with water was passed through the suspensions for the experiments with increased CO_2 partial pressure and air saturated with water for the experiments with increased ionic strength by using 5mM $Ca(NO_3)_2$. After this preequilibrium stage which took about two days, Cd solutions were added as nitrate salts[16] in the range of 10^{-6} to 10^{-2} M, gravimetrically.

The same air mixtures were passed through the suspensions for three more days and the bottles were shaken by hand from time to time. The clear solutions after centrifugation at 2500g for 20 min and acidification to about pH ~ 2 with HNO_3 (Aristar) were used for metal determinations. Three replicates were run for each level of added Cd, two for chemical determinations and the other one for pH measurements.

Reacted Cd was calculated from the difference between the initial and equilibrium concentrations.

The pH was measured by using a Radiometer Ion 85 Analyzer with combined electrode and NBS buffers. Cd and Ca concentrations were measured by AAS using a Perkin Elmer

3030 for metal concentrations above 0.01 μg ml^{-1} combined with a graphite furnace HGA -500 and AS -40 autosampler for lower concentrations. More details about experimental conditions and calculations of metal activities from the association constants for metal complexes and the Davies equation [17] by using a computer program are described elsewhere [18].

RESULTS and DISCUSSION

The results of Cd-$CaCO_3$ interactions were displayed in sorption isotherms[19] using a log-log scale to cover the whole range of Cd concentrations and in solubility diagrams[20,21].

Effect of increased CO_2 partial pressure

It has been showed that Cd is chemisorbed into calcite with Cd replacing Ca until the solution has the composition expected for an equilibrium $CdCO_3$-H_2O system, indicating that the $CaCO_3$ surface has been covered by $CdCO_3$. Further addition of Cd causes $CdCO_3$ to precipitate with pH and Ca concentration changes[10].

The adsorption limit, i.e. the point where $CdCO_3$ precipitation appears to become the dominant mechanism is between 0.6 and 1.2 μmol Cd g^{-1} $CaCO_3$ whereas from the measured calcite specific surface area (0.15 m^2g^{-1}) and the unit cell dimensions (a_{rh}=6.376 Å, Lippman[22]), a value of about 1.22 μmol of surface Ca atoms g^{-1} $CaCO_3$ was found available for displacement. This probably indicates that the effective $CaCO_3$ surface area is smaller than that found by krypton adsorption although it is almost impossible from thermodynamic considerations to be exact regarding the experimental adsorption limit in agreement with Corey[23].

Increasing CO_2 partial pressure from $10^{-3.49}$ to 10^{-2} atm in a $CaCO_3$-CO_2-H_2O system, open to the atmosphere as in the present study, results in increased dissolution of $CaCO_3$[24]. So in the solution there are more Ca ions and total carbonate (CO_{2aq}+HCO_3^- +CO_3^{2-}) and the pH of the system decreases from 8.30 to about 7.30. If the solubility product

constant (K_{so}) of calcite holds in this equilibrium system, then Ca levels in solution increase from about 17 µg ml^{-1} to about 65 µg ml^{-1}. Also, increased CO_2 partial pressure results in a four fold increase in $CdHCO_3^+$ and a two - fold decrease in $CdCO_3^o$ species in solution[16,25]. Total Cd activity coefficients, $\frac{(Cd^{2+})}{(Cd)\,total}$[26], decrease from about 0.70 to 0.55 for the low Cd additions. The above chemical reactions indicate that increased carbonate levels in solution probably encourage aqueous phase interactions.

The reaction isotherm (Fig.1,b) follows an almost straight line up to about 0.14 µmol Cd g^{-1} $CaCO_3$ and then the slope increases up to about 1.40 µmol Cd^{-1} $CaCO_3$ (Kd values from Table 1).

This is very close to the point at which precipitation of $CdCO_3$ begins as indicated from the solubility diagram (Fig.2,b) and hence Cd activity in solution is controlled by the $CdCO_3$ solubility line.

Points below 1.40 µmol Cd $g^{-1}CaCO_3$ can be treated as the sorption part of the isotherm[27] and above as the precipitation part with the formation of $CdCO_3$ which controls Cd activity in solution. Values of Kd from Table 1 have a mean value of 7.9 for low Cd additions and 23 for higher ones. Above 1.40 µmol Cd g^{-1} Kd values become less than 1 because after $CaCO_3$ surface coverage, CO_3^{2-} ions needed for $CdCO_3$ precipitation are depleted.

Low Kd values probably indicate a reduced affinity of Cd for the $CaCO_3$ surface because Kd is a measure of retention of solute by the solid matrix[28]. At high Cd additions higher Kd values are observed until surface precipitation of pure $CdCO_3$ becomes the dominant mechanism. This surface precipitaton is indicated by the abrupt pH decrease and Ca undersaturation with respect to $CaCO_3$ (Fig.2,b), and these changes are possible to occur only when the $CaCO_3$ surface is excluded from reaction taking into account the strong buffer capacity of the $CaCO_3$-CO_2-H_2O system when H^+ or OH^- ions are added[24,29].

TABLE I.Kd values in lm^{-2} for Cd-$CaCO_3$ reaction under different experimental conditions.

Pure system	With 1% CO_2	With 5mM $Ca(NO_3)_2$
40.0	6.15	4.21
23.3	6.90	4.34
18.6	8.69	5.06
17.2	8.95	5.17
15.7	8.89	5.12
17.5	16.15	4.66
18.5	20.50	3.15
18.9	20.67	3.33
20.7	23.06	3.42
19.3	25.36	2.91
25.9	29.43	0.53
31.1	22.53	0.13
32.0	26.95	0.08
57.8	2.19	0.05
20.8	0.12	0.02
0.91	0.04	0.01
0.31	0.03	0.01
0.05		
0.03		

The sorption limit for this reaction, 1.40 μmol Cd g^{-1} $CaCO_3$, seems to be slightly more that the one based on surface area measurements and unit cell dimensions, as mentioned before (1.22 μmol Cd g^{-1} $CaCO_3$). The sorption limit for the pure system is between 0.6-1.2 μmol Cd g^{-1} $CaCO_3$[10] and this probably indicates that in the 1% CO_2 system Cd is removed from solution in addition to sorption or by a different mechanism because of complexation. At 1% CO_2, with more carbonate in solution precipitation (complexes) rather begins before reaching the $CdCO_3$ solubility line.

Values for thermodynamic distribution coefficients

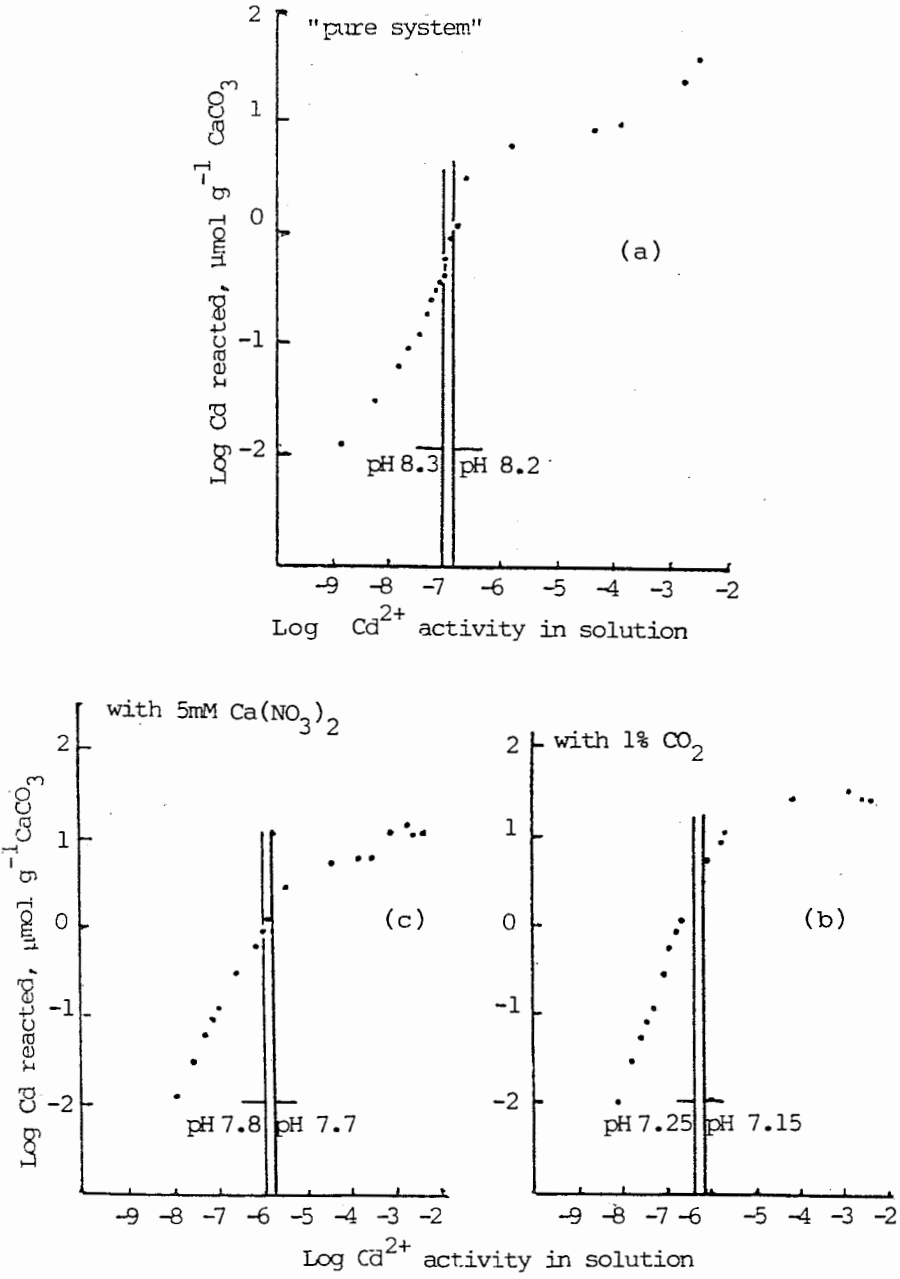


FIG.1. The reaction isotherm of Cd and $CaCO_3$ under different experimental conditions. Vertical lines represent the Cd^{2+} activity in equilibrium with $CdCO_3$ at different pH values.

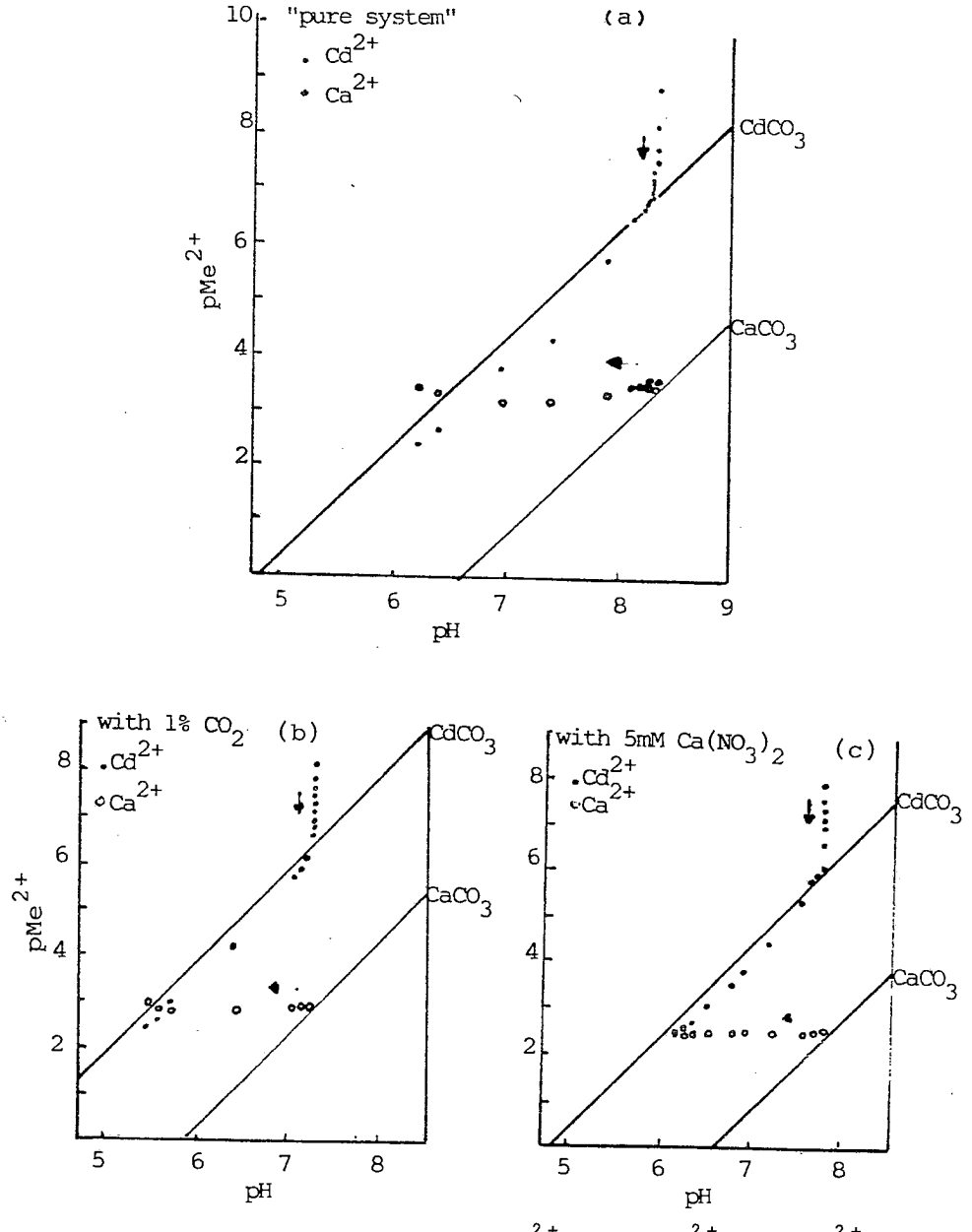


FIG.2. Solubility diagrams ($pH-pM^{2+}$) for Cd^{2+} and Ca^{2+} in solutions equilibrated with $CaCO_3$. Arrows indicate the direction of increased Cd additions. Some points are not plotted for Ca at low additions of Cd.

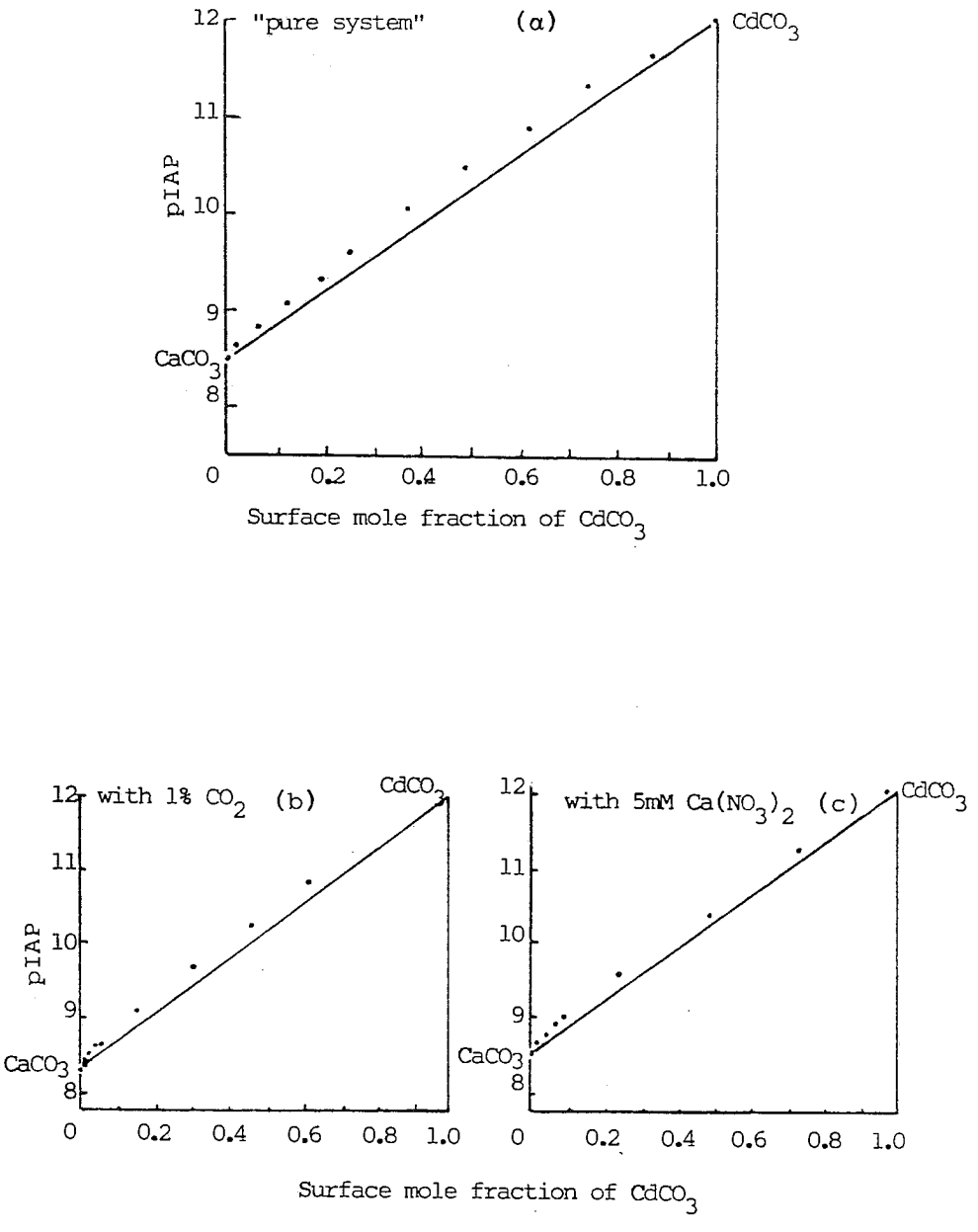


FIG.3: The relationship between pIAP and mole fraction of Cd on the surface of $CaCO_3$..

(Terminology) of solid solutions[30] for surface sites only, D*, have a mean value of about 1000 for low Cd additions and 4200 for higher ones. The homogeneous distribution coefficient, D, for the $CaCO_3$ system based on the ratio of their solubility products ($pk_{soCaCO3}$=8.48, $pk_{soCdCO3}$=12.00) is about 3310. The mean values of 1000 and 4200 found in this study indicate a deviation of this system from ideal surface solid solution formation probably because of secondary reactions. At the low Cd additions, D* is smaller that D indicating that the solid solution factor based on activity coefficients on the solid surface[29] acts to lower the solution of $CdCO_3$ in $CaCO_3$ significantly from that expected if an ideal surface solid solution has been formed in agreement with the Kd values of the reaction isotherm (Fig.1,b).

Treatment of the data for low Cd additions using the Thorstenson and Plummer[11] equation derived for magnesian calcites and converted in its Cd form ($K_{CdCO3-CaCO3}=(Ca^{2+})^{1-x}.(Cd^{2+})^{x}.(CO_3^{2-})$ assuming a sorption limit of 1.40 μmol Cd $g^{-1}CaCO_3$ produces the Fig.3b. There is deviation of the points from the straight line connecting the pk_{so} values for calcite (8.48) and otavite (12.00) probably as a result of the deviation of this system from ideal behaviour for the range of Cd concentrations used as discussed before.

Effect of increased ionic strength

The reaction isotherm and solubility diagram from this experiment are in Figs. 1c and 2c respectively. Generally, the same pattern of reactions as the previous systems ("pure", with 1% CO_2) exists.

The reaction isotherm is almost linear up to about 1.25 μmol Cd g^{-1} $CaCO_3$. This is almost identical to the value of 1.22 μmol Cd g^{-1} $CaCO_3$ as sorption limit based on $CaCO_3$ surface area measurements and unit cell dimensions. Kd values (Table 1) for this part of the isotherm have a mean value of about 4.2. This, according to definition of Kd,

indicates a lower affinity of Cd for the $CaCO_3$ surface under these conditions compared with the "pure" system (Kd=20.9) and with 1% CO_2 (Kd=7.9 and 23). Ca concentrations which were about 17 μg ml^{-1} for the "pure" system and about 65 μg ml^{-1} for the system with 1% CO_2 become about 210 μg ml^{-1} for this system. Also, Cd-carbono species are not significant as they were with 1% CO_2. Because total Cd activity coeficients for this system, at low Cd additions, have a mean value of about 0.57 (were 0.70 and 0.55 for the "pure" and 1% CO_2 systems, respectively), this lower affinity can be attributed to increased Ca competition with Cd for the same sites on the surface.

This experiment clearly indicates the specific affinity of Cd for the calcite surface because Cd reacts with $CaCO_3$ even in the presence of 5000 times more Ca and with a surface that is positively charged under these conditions (p.z.c~9)[31]. The solubility diagram (Fig.2.c) for this system indicates undersaturation with respect to $CdCO_3$ for low Cd additions and $CdCO_3$ formation as a surface coating on $CaCO_3$ for higher Cd additions (above 1.25 μmol Cd g^{-1} $CaCO_3$).

D* values for this system have a mean value of about 3230 which is very close with the value 3310 based on thermodynamic calculations from the $CaCO_3$ and $CdCO_3$ solubility product constants (pk_{so} 8.48 and 12 for $CaCO_3$ and $CdCO_3$ respectively). This indicates the formation of an ideal surface solid solution between $CdCO_3$-$CaCO_3$. Calculations according to Thorstenson and Plummer[11] equation led to Fig.3c for a sorption limit of 1.25 μmol Cd g^{-1} $CaCO_3$.

CONCLUSIONS

The experiments in this study (with 1% CO_2 and 5mM $Ca(NO_3)_2$) generally indicate reduced Cd affinity for $CaCO_3$ surface for low Cd additions (lower Kd values comparing with the "pure"system)[10]. However, the three systems have the same general pattern of reactions. So, the dominant mechanism for low Cd additions is chemisorption on $CaCO_3$ surface sites

($Cd^{2+}+CaCO_{3(s)}=CdCO_{3(ads)}+Ca^{2+}$) with insignificant pH change, and surface precipitation of Cd as $CdCO_3$ on $CaCO_3$ surface ($Cd^{2+}+HCO_3^-=CdCO_{3(s)}+H^+$) at the higher Cd additions.

The high affinity of Cd for the calcite surface which is a specific adsorption[32] is expected from the following:

a. the Paneth-Fajans rule[33]

b. the similarity between Cd^{2+} and Ca^{2+} effective ionic radii (0.095 and 0.100 nm respectively)[34]

c. $CdCO_3$ and $CaCO_3$ are isotypes[22]

d. the similar electron configuration of Cd and Ca ions[35]

e. theories about heterogeneous nucleation and precipitation[36].

More details can be found in reference 37. The concept of a surface solid solution based on coprecipitation theories and the Thorstenson and Plummer[11] equation seems to be a useful approach in studying Cd mobility and reactivity in calcareous soils and sediments where calcite is a predominant constituent.

TERMINOLOGY

S=Kd. C, Linear adsorption isotherm

S amount of solute adsor bed at equilibrium, μmol m^{-2}

C concentration in solution, μmol l^{-1}

Kd distribution coefficient, lm^{-2}.

$$D=\frac{(Ca^{2+}).X_{cdCO_3}}{(Cd^{2+}).X_{CaCO_3}}=\frac{K_{soCaCO_3}}{K_{soCdCO_3}}$$

distribution coefficient for solid solution of $CdCO_3$ to $CaCO_3$

(Ca^{2+}), (Cd^{2+}) Ca and Cd concentrations in solution

X_{CdCO_3}, X_{CaCO_3} mole fractions of $CdCO_3$ and $CaCO_3$ in the solid solution.

D* homogeneous distribution coefficient for surface sites only. In this case X_{CdCO3} and X_{CaCO_3} are mole fractions for surface sites only.

$K_{so}=\frac{(Ca^{2+}).(CO_3^{2-})}{(CaCO_3)_{(s)}}$ Solubility product constant for $CaCO_{3(s)}$

(Ca^{2+}), (CO_3^{2-}) ion activities in solution
$(CaCO_3)$ $CaCO_3$ activity
When $(CaCO_3)=1$ then K_{so}=IAP (ion activity product)
Thorstenson and Plummer (1977) equation for magnesian calcites:
$K_{Mg\text{-}calcite} = (Ca^{2+})^{1-x} \cdot (Mg^{2+})^{x} \cdot (CO_3^{-2})$,
thermodynamic stoichiomentric solubility product for magnesian calcites
X mole fraction of $MgCO_3$ in calcite
(Ca^{2+}), (Mg^{2+}), (CO_3^{2-}) activities in solution
This equation in its Cd form is:
$K_{CdCO_3\text{-}CaCO_3} = (Ca^{2+})^{1-x} \cdot (Cd^{2+})^{x} \cdot (CO_3^{2-})$.
x mole fraction of $CdCO_3$ on the calcite surface.

ΠΕΡΙΛΗΨΗ

ΕΠΙΔΡΑΣΗ ΤΗΣ ΜΕΤΑΒΟΛΗΣ ΤΗΣ ΜΕΡΙΚΗΣ ΠΙΕΣΗΣ CO_2 ΚΑΙ ΤΗΣ ΙΟΝΙΚΗΣ ΙΣΧΥΟΣ ΣΕ ΑΝΤΙΔΡΑΣΕΙΣ Cd ΜΕ ΑΝΘΡΑΚΙΚΟ ΑΣΒΕΣΤΙΟ

Στην εργασία αυτή μελετήθηκαν οι αντιδράσεις του Cd με ανθρακικό ασβέστιο ($CaCO_3$ BDH A.R βαθμού ειδικής επιφάνειας 0.15 $m^2\ g^{-1}$) κάτω απο ειδικές πειραματικές συνθήκες δηλαδή με μεταβολή της μερικής πίεσης του CO_2 απο $10^{-3.49}$ (κανονική ατμόσφαιρική πίεση CO_2) σε 10^{-2} atm με τη χρήση 1% CO_2 και της ιονικής ισχύος του διαλύματος ισορροπίας με 5mM $Ca(NO_3)_2$ αντί H_2O.

Οι μεταβολές αυτές ειχαν σαν αποτέλεσμα την αύξηση των συγκεντρώσεων Ca στο διάλυμα ισορροπίας και τη μείωση των τιμών pH συγκρινόμενες με το σύστημα ισορροπίας $CaCO_3$-CO_2-H_2O,ανοικτό στην ατμόσφαιρα ("pure" system).

Και στις δυο περιπτώσεις παρατηρήθηκε μειωμένη τάση αντίδρασης του Cd με την επιφάνεια του $CaCO_3$ (χαμηλότερες τιμές του συντελεστή κατανομής Kd) σε σχέση με το καθαρό σύστημα με χαμηλότερη αυτή του συστήματος με 5mM $Ca(NO_3)_2$ πιθανόν λόγω αυξημένου ανταγωνισμού απο τα ιόντα Ca του διαλύματος ισορροπίας.

Στο σύστημα με 1% CO_2 παρατηρήθηκε απόκλιση απο τον σχηματισμό ιδανικού επιφανειακού στερεού διαλύματος μεταξύ $CdCO_3$ και $CaCO_3$ πιθανόν σαν αποτέλεσμα αντιδράσεων στην υγρή φάση (σχηματισμός συμπλόκων κλπ). Στο σύστημα με 5mM $Ca(NO_3)_2$ σχηματίσθηκε ένα σχεδόν ιδανικό επιφανειακό

στερεό διάλυμα και στην περίπτωση αυτή είχε εφαρμογή η εξίσωση Thorstenson και Plummer για το γινόμενο διαλυτότητας στερεών διαλυμάτων.

Ο επικρατέστερος μηχανισμός για τις χαμηλές προσθήκες Cd είναι η χημειορόφηση των ιόντων Cd σε ενεργές θέσεις της επιφάνειας του $CaCO_3$, ενώ για τις υψηλές προσθήκες Cd ο επικρατέστερος μηχανισμός είναι η καταβύθιση $CdCO_3$ σαν κάλυμμα στην επιφάνεια του $CaCO_3$ με ταυτόχρονες αλλαγές στις τιμές του pH και των συγκεντρώσεων Ca στα διαλύματα.

Με τα πειράματα αυτά αποδεικνύεται η μεγάλη τάση αντίδρασης του Cd με το $CaCO_3$ γεγονός που ερμηνεύεται και απο θεωρητικής πλευράς.

REFERENCES

1. Friberg,L.: Cadmium in the Environment, Academic Press, N.York (1974).
2. Kinniburgh, D.G. and Jackson,M.L.In:Adsorption of Inorganics at Solid-Liquid Interfaces, eds. M.A. Anderson and A.S. Rubin, pp.91-160. Ann Arbor Science, Ann Arbor, Mich(1981)
3. Hodgson, J.E.: Advan. Agron. 15, 115 (1963).
4. Stevenson, F.J. Cycles of soil: Carbon,Nitrogen,Phosphorus Sulfur, Micronutrients. John Wiley, New York. (1986).
5. Farrah, H. and Pickering, W.E.: Aust. J.Chem. 30, 1417. (1977).
6. Tiller, K.G., Gerth, J. and Brummer,G.:Geoderma 34,1 (1984,a).
7. Tiller, K.G., Gerth, J.and Brummer, G.:Geoderma 34, 17 (1984,b).
8. Mc Bride, M.B.: Soil Sci. Soc.Am.J. 44, 26 (1980).
9. Davies, J.A., Fuller, C.C and Cook, A.D.: Geochim. Cosmochim. Acta 51, 1477 (1987).
10. Papadopoulos P. and Rowell, D.L.: J.Soil Sci. 39, 23(1988)
11. Thorstenson, D.C. and Plummer, L.N.: Amer.J.Sci.277. 1202 (1977).
12. Garcia-Miragaya and Page, A.L.:Soil Sci. Soc. Am.J. 40, 658 (1976).
13. Cline, G.R. and O'Connor, G.A.:Soil Sci. 138 (3) 248(1984).
14. Franklin, M.L. and Morse, J.W.:Ocean Sci. Eng. 7(2),147 (1982).
15. Franklin, M.L. and Morse, J.W.: Mar. Chem. 12, 241.(1983).
16. Slavin, W., Carnrick, G.R. and Manning, D.C.:Anal. Chem.56 163 (1984).
17. Davies, C.W.: Ion association. Butterworths, London (1962).
18. Papadopoulos P.: PH.D. Thesis, University of Reading(1985).
19. Giles, C.M.,Mc.Ewan, T.H.Nakhwa, S.N. and Smith D.:J.Chem. Soc., London, 3973 (1960).
20. Lindsay, W.L.: Chemical equilibria in Soils. John Wiley and Sons. (1979).
21. Sposito, G.: The thermodynamics of soil solutions.Oxford Clarendon Press (1981).
22. Lippman, F.: Sedimentary Carbonate Minerals. Springer - Verlag. (1973).

23.Corey, R.B.: In: Adsorption of Inorganics at Solid-Liquid Interfaces, eds, M.A.Anderson and A.J.Rubin, pp.161-182. Ann. Arbor Science, Ann Arbor, Mich. (1981).
24.Butler, J.N.: Carbon Dioxide Equilibria and their Applications, Addison - Wesley Publishing Company (1982).
25.Baes, C.F. and Mesmer, E.E.: The Hydrolysis of Cations, John, Wiley and Sons, New York, (1976).
26.Millero, F.J. and Schereiber, D.R.:Amer. J.Sci. 282 1508 (1982).
27.Sposito, G.: The Surface Chemistry of Soils, Oxford University Press (1984).
28.Travis, C.C. and Etnier, E.L.:J.Environ. Qual. 10, 8(1981).
29.Stumm, W. and Morgan, J.J.: Aquatic Chemistry, An Introduction emphasizing chemical equilibrium in natural waters 2nd Edition. Wiley - Interscience (1981).
30.Vaslow, F. and Boyd, G.E.: J.Am. Chem. Soc. 74, 4691(1952).
31.Subranamian, P. and Agar, G.E.: J.Colloid Interface Sci. 24, 433 (1967).
32.James, R.D. and Healy, T.W.: J.Colloid Interface Sci. 40. 65 (1972,c).
33.Adamson A.W.:Physical Chemistry of Surfaces. 4th Edition, John Wiley and Sons, New York (1982).
34.Shannon, R.D.: Acta Crystallogr. A32, 751 (1976).
35.Reeder, R.J.: In: Carbonates: Mineralogy and Chemistry, R.J. Reeder ed. pp.1-47. Mineralogical Society of America (1983).
36.Mullin, J.W.: Crystallization, Butterworths (1972).
37.Papadopoulos, P. and Rowell, D.L.: J.Soil Sci.40, 39 (1989).

Chimika Chronika, New Series, 19, 193-202 (1990)

AMINE DERIVATIVES OF GLYCIDYL ESTERS: SYNTHESIS AND TECHNOLOGICAL APPLICATIONS

S.N.ZLATANOS

Laboratory of Organic Chemistry, College of Chemical Engineering, Aristotle University of Thessaloniki, GREECE

(Received December 11, 1990)

SUMMARY

In the present work a new series of amine derivatives of glycidyl-esters and alkyl-glycidyl-esters were studied. The ring opening of glycidyl ester epoxides was effected by treatment with diethylamine. The attack occurs at the terminal carbon of the epoxide and the product formed was the a isomer. The reaction proceeds in one step with excellent yields. The compounds studied show satisfactory bacteriocidal activity. Some of them might be used as food additives and cosmetics.

Key words : Alkyl-glycidyl-esters, Lauric acid-glycidyl-esters, diethylamine

INTRODUCTION

Studies on the structure and energy strain of ethylene oxide (1), the smallest member of the epoxides, showed that it is very reactive and gives easily ring opening reactions, releasing the energy strain of the ring. The epoxide bonds have substantial ionic character and the oxygen is easily attacked by electrophiles whereas the carbon atoms are sensitive to nucleophilic attack.

The epoxide entity, one of the most extensively studied ambident substrates (2), reveals some interesting structural features. The orientation of the nucleophilic ring-opening (2) is controlled by stereochemical and electronic (inductive, conjugative) effects. Clearly, stereochemical factors direct the attack to the least hindered site whereas electronic factors of the substituents may direct the attack to either site of the ring. It follows that the nature of the final product will depend on the prevailance of steric or electronic factors, during the ring-opening step, in the formation of the intermediate or transition state involved. Experiments have shown (2) that basic or neutral conditions favour the isomer formed by attack at the least hindered site as the major or the sole product.

Subject to the above considerations is the position of the epoxide ring in the molecule. Glycidyl esters fall in the category of terminal epoxides and their ring-opening reaction forms the subject of this paper.

The present study was dictated by the fact that the monoglycerides of fatty acids exhibit antibacterial properties and as such they find application as food preservatives (3).

Earlier reports on fatty acids and their monoglycerides have revealed that the latter inhibit bacterial growth in contrast with the diglycerides, which are poor inhibitors and the triglycerides, which rather favour the bacterial growth (4). A report on the antimicrobial action of the lauric acid monoglyceride has shown that it is very active against GRAM-(+) bacteria and coliform bacteria but it is inactive against GRAM-(-) bacteria (5).

In trying to take advantage of the antibacterial action of monoglycerides, it was thought that similar results might be obtained by the glycidyl esters, as the latter are structural analogues of the former (one epoxide ring is equivalent to two hydroxy-groups). Furthermore, the antibacterial action of the glycidyl esters might be further enhanced by the addition of one molecule of diethylamine, a compound of known biological significance. It was, thus, anticipated that the resulting molecule would exhibit the combined activity of both.

The ring-opening of epoxides by amines was reported first by Graham (6). Later Al'bitskaya and Petrov (7) reported the same reaction using aqueous solutions of the amines. The reaction is complete in 1-2 days and the yield is 60-70 %. The yield was further improved by carrying out the reaction at 120°C with a pressure of 3 bar as reported by Rutzen(8).

C_4-C_{18} straight chain glycidyl esters have been employed for the present purpose as well as mono-alkyl-substituted phthalic acid glycidyl esters.

EXPERIMENTAL

Principal starting Materials

Phthalic anhydride : ca 98 % m.p. 130-132°C (Merck)
Diethylamine : ca>98 % (GC); b.p. 55-57° (Fluka)
Phthalic acid monoalkylester : This was prepared from sodium alkoxide and phthalic acid anhydride. GC of Me-ester showed < 99 % purity. The

rest of the fatty acids and their salts used in this work were purchased from Merck.

Preparation of Glycidyl esters

Caprylic acid glycidyl ester:

A mixture of sodium caprylate 33.8 g (0.2 mole), epichlorhydrin 376g (4 mole) and tetraethylammonium iodide 0.64g, (≃ 2 % with respect to the acid salt) was stirred for 1 h at 115°C. The sodium chloride precipitate was filtered off, the excess epichlorohydrin was removed by distillation and the glycidyl ester was extracted from the residue with ether to give 38 g of ester (yield 93 %).

Oleic acid and lauric acid glycidyl esters

Prepared as described above.

Iso-decatrianoic acid glycidyl ester

Isodecatrianoic acid 35.4 g (0.615 mole) was dissolved in a 1:1 ethanol-ether solvent mixture and the solution was titrated against ethanolic sodium hydroxide (phenolphthalein change). Extraction with ether followed by evaporation of the aqueous layer afforded 39 g (0.165 mole) of the salt.

The glycidyl ester was prepared from the salt as described above and 41.3 g of ester (yield 92 %) were obtained.

Isodecaoctaonic acid glycidyl ester

Prepared as described previously.

Butyric acid glycidyl ester

Butyric anhydride 30 g (0.19 mole) was used instead of the acid for the preparation of the sodium butyrate 40 g (0.36 mole) and 38 g of ester (yield 73 %) were obtained.

Preparation of monoalkyl-substituted phthalic acid glycidyl esters

Prepared according to an earlier reported method (9).

Diethylamine-hydroxy-derivatives of glycidyl esters

2-hydroxy-3(N,N-diethylamino) propylcaprylate

Caprylic acid glycidyl ester 38 g (0.19 mole) was heated at 66°C and then diethylamine 16.65 g (0.23 mole) was added dropwise under mechanical

stirring. The reaction was slightly exothermic and the temperature rose to 80°C over a period of 5 1/2 h. Excess diethylamine was then distilled off in vacuo (at 50°C/5 torr). The residue was repeatedly washed with water (ten times with 50 ml portions) to remove traces of diethylamine.

Crystallization from pentane at -20°C resulted in a colourless liquid at room temperature, whose purity was checked by TLC.

Thin Layer Chromatography (TLC)

TLC plates were prepared from silica gel (Merck). The thickness of the plates was 0.25 mm. The samples were dissolved in chloroform, and the developing mixture was chloroform, ethyl acetate and methanol (65:10: 25). The visualization of the spots was achieved using a wide-mouth glass bottle containing a few crystals of iodine.

The product was, then, purified by column chromatography (Kieselgel 60, 70-230 Mesh) using a chloroform:ethylacetate:methanol (6.5:1:2.5) mixture to afford 46 g (0.19 mole) of pure product.

The rest of the diethylamine derivatives of glycidyl esters were prepared and purified similarly (Table 1).

DATA

Methyl 2-hydroxy-3(N,N-diethylamino)propylphthalate 7.

^{1}H nmr: δ(CD_2Cl_2)1.0(t,6H,-N($CH_2\underline{CH_3}$)$_2$, 2.6(m,6H,-$\underline{CH_2}$N($\underline{CH_2}CH_3$)$_2$, 3.75(m, 2H,CHOH), 3.85(s,3H,-OCH_3), 4.25(t,2H,-$COOCH_2$), 7.6(m,4H,C_6H_4—),ppm. Ms, m/z:309($M^{+\cdot}$), 294, 278, 266, 237, 219, 192, 163, 135, 105. Found:C,62.50; H, 7.61; N,4.45. $C_{16}H_{23}NO_5$ requires:C, 62.12; H, 7.49; N, 4.53%.

Octyl 2-hydroxy-3(N,N-diethylamino)propylphthalate 10.

^{1}H nmr: δ(CD_2Cl_2), 1.0[t,9H,-N($CH_2\underline{CH_3}$)$_2$, and -$(CH_2)_6\underline{CH_3}$], 1.3[s,broad, 12H,-$(CH_2)_6$], 2.5[m,6H,-$\underline{CH_2}$N($\underline{CH_2}CH_3$)$_2$], 3.75(m,2H,CHOH), 4.2[m,4H,-(COOC H_2)$_2$], 7.6(m,4H,C_6H_4—)ppm. Ms, m/z: 407($M^{+\cdot}$), 390, 376, 364, 290, 278, 222, 192, 160, 130, 105.

2-Hydroxy-3(N,N-diethylamino)laurate 3.

^{1}H nmr: δ($CDCl_3$) 1.0[t,3H,-$(CH_2)_9\underline{CH_3}$], 1.1[t,6H,-N($CH_2\underline{CH_3}$)$_2$], 1.35 [s,broad,18H,-$(\underline{CH_2})_9CH_3$], 2.6[m,8H,-$\underline{CH_2}$N($\underline{CH_2}CH_3$)$_2$,-$\underline{CH_2}$COO—], 3.8(m,2H, CHOH), 4.2(m,2H,-$COOCH_2$) ppm. Ms, m/z: 329($M^{+\cdot}$), 312, 300, 296, 272, 257, 230, 216, 202, 189, 183, 157, 146. Found: C, 69.07; H, 12.06; N, 4.38 $C_{19}H_{39}NO_3$requires: C, 69.25; H, 11.93; N, 4.25 %.

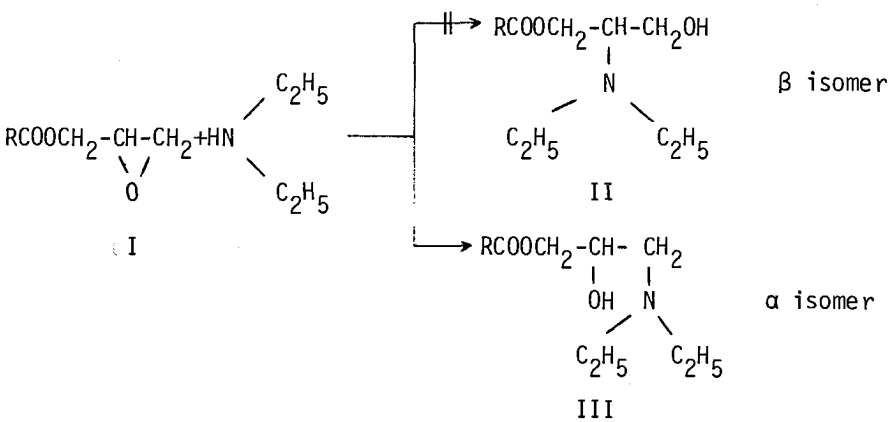
All the other compounds of Table 2 gave analogous spectroscopic data.

RESULTS AND DISCUSSION

The glycidyl esters were prepared from their corresponding acid salts by reaction with epichlorohydrin while the phthalic acid glycidyl esters were prepared according to an earlier reported method (9).

The reaction products were analysed for purity by TLC and their structure was determined by NMR spectroscopy and mass spectrometry.

The reaction of glycidyl esters (I) with diethylamine is shown in scheme 1.



Scheme 1

The best conditions to obtain high yields is to use a molar ratio of ester to amine equal to 1:1.2 and to keep the temperature constant at 70° C. The reaction was exothermic and the temperature after 5 h rose from 75 to 95°C(Table 1).

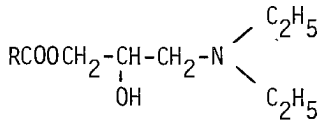
The method employed, may be regarded as superior to earlier ones, taking into consideration on the high accessibility of the product amines (III)(Scheme 1), in that it involves simple reaction conditions and gives high yields of the amines (III) (Table 1).

From the experimental results it is clear that the reaction of diethylamine with lauric glycidyl ester leads mainly to the formation of the α isomer in high yield (Scheme 1).

The alkyl groups in the diethylamine cause +I inductive effect (electron donors) increasing the electron density on the nitrogen atom and consequently its nucleophilic character.

A mechanism that might explain the above reaction is shown in Scheme 2.

Table 1. Temperature change as a function of reaction time and product yields of fatty acid glycidyl esters and diethylamine derivatives of monoalkyl-substituted phthalic acid glycidyl esters of type.



III

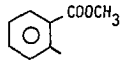
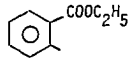
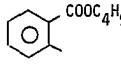
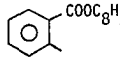
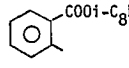
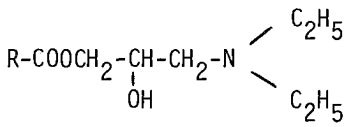
Compound No	R	Reaction Time	Temperature °C		Yield %
			Start (To)	End (Tt)	
1	n-C_3H_7–	5	68	77	87
2	n-C_7H_{15}–	5 30'	66	78	89
3	n-$C_{11}H_{23}$–	5 30'	69	94	90
4	n-$C_{17}H_{33}$–	5	70	80	90
5	CH_3–CH(CH_3)–$(CH_2)_9$–	5	68	77	87
6	CH_3–CH(CH_3)–$(CH_2)_{14}$–	5	70	75	88
7		5 30'	70	82	90
8		5 30'	68	85	90
9		5 30'	69	84	88
10		5 35'	70	80	87
11		5 35'	70	86	87

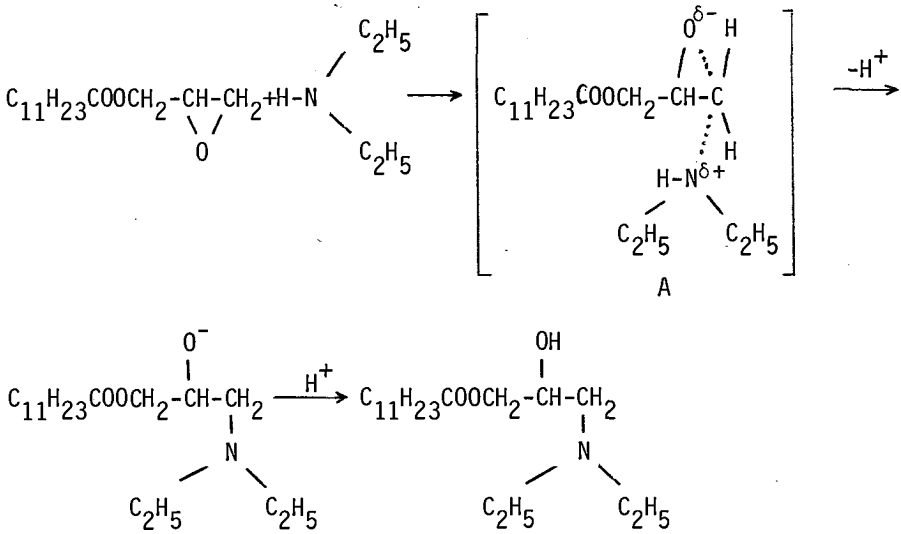
Table 2. Analytical data of products of the reaction of monoalkylglycidyl-ester of phthalic acid with diethylamine of the type:



Compound No	Acid number		Saponification number		n_D^{20}
	Exptl.	Calc.	Exptl.	Calc.	
1	0.2	0	261.5	258	1.4237
2	0.1	0	206	205	1.4454
3	0	0	171.3	170.2	1.4851
4	0.2	0	134.7	136.2	1.4626
5	0.25	0	166.1	163.2	1.5023
6	0.25	0	137.2	135.6	1.5172
7	0.2	0	359.1	362.4	1.5139
8	0.34	0	343.3	346.7	1.5210
9	0.37	0	324	319	1.5682
10	0.38	0	265	275	1.4985
11	0.3	0	267	275	1.4972

Under the adopted conditions, the reaction proceeds, most probably, by the S_N2 mechanism. The intermediate A was formed by nucleophilic attack of a molecule of diethylamine on the carbon atoms of the epoxide.

The major isolated α isomer indicated the prefered site of ring-opening. Steric crowding is an important issue in this context.



Scheme 2

Besides, five more diethylamine derivatives alkyl-glycidyl-phthalic esters were prepared analogously. The yields and the analytical data of the products are shown in Tables 1 and 2. The spectroscopic data showed that in all cases the main product was the α isomer.

The compounds 3, 7, 10 were tested for antimicrobial activity against Staphylococcus aureus, E. coli, Ps cepacia, Candida albicans and Aspergillus niger at various concentrations (Table 3) 1% solution in preservative (1% solution means concentrations 1 while 1+1 = 0,5%).

As it can be seen from Table 3 only compounds 3 and 10 were found to be active.

Compound 3 showed distinct antimicrobial activity whereas 7 was found to be completely inactive. The sensitivity of the microorganisms to compound 3 and 10 followed the order: Ps cepacia ≃ Aspergillus niger > E.coli ≃ Candida albicans > Staphylococcus aureus.

Compound 3 can be suggested as a good food and perfume preservative due to its strong bacteriocidal action against E. coli.

Table 3. Microtests for microbial growth inhibition for food and perfumes preservatives.

Compound Nº	Dilution	Inhibition Zone (cm)															
		Staphylococcus aureus			Escherichia coli			Ps. cepacia			Candida albicans			Aspergillus niger			
3	1	0.1	0.1	0.1	0.2	0.2	0.2	0.3	0.3	0.3	0.2	0.2	0.2	0.3	0.3	0.3	
		0.1	0.1	0.1	0.2	0.2	0.2	0.3	0.3	0.3	0.2	0.2	0.2	0.3	0.3	0.3	
	1+1	0.1	0.1	0.1	0.2	0.2	0.2	0.3	0.3	0.3	0.1	0.1	0.1	0.2	0.2	0.2	
		0.1	0.1	0.1	0.2	0.2	0.2	0.3	0.3	0.3	0.1	0.1	0.1	0.2	0.2	0.2	
	1+2	0.1	0.1	0.1	0.2	0.2	0.2	0.3	0.3	0.3	<0.1	<0.1	<0.1	0.1	0.1	0.1	
		0.1	0.1	0.1	0.2	0.2	0.2	0.3	0.3	0.3	<0.1	<0.1	<0.1	0.1	0.1	0.1	
	1+4	<0.1	<0.1	<0.1	0.1	0.1	0.1	0.2	0.1	0.1	–	–	–	<0.1	<0.1	<0.1	
		<0.1	<0.1	<0.1	0.1	0.1	0.1	0.2	0.2	0.1	–	–	–	<0.1	<0.1	<0.1	
	1+9	–	–	–	<0.1	<0.1	<0.1	0.1	0.1	0.1	–	–	–	–	–	–	
		–	–	–	<0.1	<0.1	<0.1	0.1	0.1	0.1	–	–	–	–	–	–	
7	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
	1+1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
	1+2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
	1+4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
	1+9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
10	1	0.2	0.2	0.2	0.1	0.1	0.1	0.2	0.2	0.2	0.1	0.1	0.1	–	–	–	
		0.2	0.2	0.2	0.1	0.1	0.1	0.2	0.2	0.2	0.1	0.1	0.1	–	–	–	
	1+1	0.1	0.1	0.1	0.1	0.1	<0.1	0.1	0.1	0.1	–	–	–	–	–	–	
		0.1	0.1	0.1	<0.1	<0.1	<0.1	0.1	0.1	0.1	–	–	–	–	–	–	
	1+2	–	–	–	–	–	–	0.1	0.1	0.1	–	–	–	–	–	–	
		–	<0.1	<0.1	–	–	–	0.1	0.1	0.1	–	–	–	–	–	–	
	1+4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
	1+9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	

Dilution : 1% solution in preservative (1% solution means concentration 1, while 1+1= 0,5 %)

The dilution of the substance is effected in 1% aqueous solution of Tween 40. This solvent is not antimicrobial.

Dashes imply no inhibition to microbial growth.

ΑΜΙΝΟΠΑΡΑΓΩΓΑ ΤΩΝ ΓΛΥΚΙΔΥΛΕΣΤΕΡΩΝ : ΣΥΝΘΕΣΗ ΚΑΙ ΤΕΧΝΟΛΟΓΙΚΕΣ ΕΦΑΡΜΟΓΕΣ

Σ.Ν.ΖΛΑΤΑΝΟΣ

Εργαστήριο Οργανικής Χημείας, Πολυτεχνική Σχολή, Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη 54006, ΕΛΛΑΔΑ

ΠΕΡΙΛΗΨΗ

Στην εργασία αυτή έγινε η σύνθεση και η μελέτη μιας νέας σειράς αμινοπαραγώγων των γλυκιδυλεστέρων και των αλκυλογλυκιδυλεστέρων. Η διάνοιξη του εποξειδικού δακτυλίου επιτυγχάνεται με κατεργασία διαιθυλαμίνης, η δε προσβολή γίνεται στον τελικό άνθρακα του εποξειδίου το δε προϊόν που σχηματίζεται είναι το α-ισομερές. Η αντίδραση εξελίσεται σ'ένα στάδιο με πάρα πολύ καλές αποδόσεις.

Οι ενώσεις που μελετήθηκαν δείχνουν ικανοποιητική βακτηριοστατική δράση, μερικές δε απ'αυτές θα μπορούσαν να χρησιμοποιηθούν σαν πρόσθετα σε τρόφιμα και καλλυντικά.

REFERENCES

1. Swern, D., *J.Am.Oil Chem. Soc.*, 47, 424 (1970)
2. Parker, R.E. and Isaaks, N.S., *Chem. Rev.*, 59, 737 (1959)
3. Shibasaki, I., Yuta K., Matsura H., Iwasaki T., *Jpn Tokyo Koho*, 79 34, 061, 24 Oct. (1979)
4. Pavell, H.J., May, J.T., *J.Gen. Appl. Microbiol.*, 27, (2) 185 (1981)
5. Kabara, J.J., *J.Soc.Cosmet.Chem.*, 31 (1), 1-10 (1980)
6. Graham, A.R., Millidge A.F., and Young D.P., *J.Chem. Soc.*, III 2180 (1954)
7. Al'bitskaya, V.M. and Petrov A.A., *Zhur. Obshchei Khim.*, 28, 901-4 (1958)
8. Rutzen H., *Fette-Seifen-Anstrichmittel*, 3, 87 (1982)
9. Zlatanos, S.N. and Sagredos, A.N., *J. Am. Oil Chem. Soc.*, 67, 661 (1990)

Chimika Chronika, New Series, 19, 203-217 (1990)

GAS OIL STEAM CRACKING

A. ZABANIOTOU and C. FLICOTEAUX

Department of Chemical Engineering, School of Engineering, University of Thessaloniki, GR. 54006 Thessaloniki, Greece and
Laboratoire de Génie et Informatique Chimiques, Ecole Centrale des Arts et Manufactures de Paris, Grand Voie des Vignes, 92295 Châtenay Malabry Cedex, France.

(Received December 15, 1990)

SUMMARY

Gas oil pyrolysis experiments in the presence of steam were performed in a laboratory-scale tubular Inconel 600 reactor of 4 m long and 4 mm diameter. The purpose was to determine the effect of temperature on the production of olefines and especially on ethylene and to estimate liquid products composition. The best yield of ethylene , 31% by weight was obtained at 760°C, residence time 0.33 sec and mass ratio steam to gas oil equal to 2. Liquid pyrolysis products consist of high carbon aromatics, especially above 700°C pyrolysis temperature.Coke deposits on the walls and the exit of the reactor was important.

Key words : Gas oil; steam cracking; pyrolysis; olefins; ethylene, liquids.

INTRODUCTION

Energy consumption is a very important problem especially in the field of raw material cost and petrochemical production. The price icrease of petrochemicals allows to consider not only light petroleum fractions for petrochemical industry, but also heavy petroleum fractions. There is tendency today, to utilize kerosene, gas oil and heavier distillate fractions as a raw material for ethylene production.

The product distribution in the effluent from a pyrolysis reactor depends on four principal variables:

1. Feedstock composition
2. Conversion or severity level
3. Temperature and residence time
4. Hydrocarbon partial pressure.

The composition of the products of gas oil pyrolysis is influenced by the distribution of products from primary decomposition of the saturates in the feed and by the extent to which this distribution is modified by secondary reactions.

The present study was undertaken to show the influence of temperature and steam on the product distribution and composition of gas oil pyrolysis.

EXPERIMENTAL

Raw material.

Gas oil for experiments was supplied by the "Total Company" (HarFleur, France). Physical characteristics were as follows : boiling range 243-384 C; refractive index $n^{20}D=34.5$; density $d^{20}4=0.8596$. Chemical characteristics and composition are presented in Table I.

Apparatus.

Our experiments were conducted with a continuous reactor (3). It is a cracking coil Inconel 600 reactor 4m long with 4-mm internal diameter. It consists of 3 parts: the first two parts are the preheating gas oil part and the preheating steam part. The third one is the cracking zone (3,4).

The coil is round on a 18/8 stainless steel pipe of 60/64-mm diameter. The reactor is heated internally by an electrical resistance coiled on a coralum H threaded tube, of 178/25-mm diameter. The electrical resistance is protected by a quartz tube 30/33-mm diameter, 30 mm long.

Pyrolysis temperature was measured by means of three Chromel-Alumel thermocouples inserted in the cracking zone. Two other thermocouples are used to measure temperaturew of preheating gas oil and steam .

The products of pyrolysis from the reactor were led to the condenser, where they were ice-cooled to about 0 C. The pyrolysis gases were determined in the MARIOTTE bottles at atmospheric pressure .

Experimental method.

The experiments were carried in the range from 600 to 850 °C and at atmospheric pressure to investigate the effect on the composition of the pyrolysis products.

During the experiment , dilution was made at a constant weight ratio of 2Kg water for every kilogram of gas oil. Before each experiment the reactor was let to be stabilized for about 2hr of the experiment temperature. After the stabilization at the pyrolysis temperature water is pumped through the reactor for 15' minutes and after that gas oil was let to enter the reactor. The time of cracking was about 95 minutes.

After each cracking Nitrogen of 5 lt/hr was let to pass the reactor. At the same time, the gaseous and liquid products were sampled to measure their compositions.

TABLE I: Characteristics of gas oil used.

Family		% wt.
Paraffins		39.0
Uncondensed Naphthenes		20.3
Condensed Naphthenes		10.4
Aromatics		
Alkybenzene	C_nH_{2n-6}	7.0
Indane + tetralin	C_nH_{2n-8}	3.9
Indene	C_nH_{2n-10}	1.7
Naphtalene	C_nH_{2n-12}	5.2
Acenaphthene + diphenyl	C_nH_{2n-14}	2.0
Acenaphthene + fluorene	C_nH_{2n-16}	1.2
Phenathrene + anthracene	C_nH_{2n-18}	1.5
	total 22.5	
Sulfur compounds		
Benzothiophene	$C_nH_{2n-10}S$	5.4
Dibenzothiophene	$C_nH_{2n-16}S$	2.4
		total 7.8
Mean No of C in family :	Saturated	16.7
	Naphthenic	13.3
	Aromatic	14.8
Elemental Analysis:	C	85.35
	H	13.30
	N	<0.10
	O	<0.10
	S	1.40
Mean molecular weight :		238.7
Mean molecular formula:		$C_{17.19}H_{32.43}$

Effluent gas product analysis was performed by gas chromatography. Table II shows the analytical conditions of gas chromatography.

TABLE II : Gas chromatography analysis of gas oil steam cracking gases.

Objects of analyses	H_2, CH_4	CH_4,C_2H_6, C_2H_4, C_3H_8, C_3H_6, C_4H_8, C_5H_{10}.
Type of gas chromatographer	Hermann-Moritz CP_3	Gira
Detector	Thermal conductivity	Thermal Conductivity
Column	Molecular sieve 5A°	Porasil B (80/100 mesh)
Column length	2 m	4 m
Column diameter	2.25 mm	2.25 mm
Column temperature	45°C	90°C
Carrier gas	N_2	N_2

TABLE III : Fractional distillation of liquid products of gas oil steam cracking at 650 C.

Fraction	B.P (°C)	% wt.
Distillate 1	<200	10.98
Distillate 2	200-270	22.25
Residue	>270	64.16
Losses		2.61

The condensed liquid products were measured. The separation of water from hydrocarbons was achieved by centrifugation and addition of salt. The determination of liquids composition has been carried out by I.F.P (French Institute of Petroleum).

The various methods employed were simulated distillation by gas chromatography, fractional distillation, liquid and gas chromatography coupled with mass spectrometry. The results of the analysis have been reproducible with an accuracy higher than 5% and are shown in Tables III - X.

TABLE IV : Mass spectrometry analysis of the distillate **1** from fractional distillation of the liquid products of gas oil steam cracking at 650 °C.

	vol.% of distillate 1	wt. % of liquid products
Paraffins	15.56	
Monocycloparaffins	32.00	
Dicycloparaffins	17.98	
		8.86 total
Aromatics	30.70	
Indane + Tetralin	3.90	
Naphthalene	0.10	
		4.71 total
	100.00 total	13.57 total

Mean molecular weight : 100.5

In the calculation of residence time (t) the following relation was considered:

$$t = \frac{V}{(Mg+Ml+Ms)\frac{RT}{P}}$$

where:

V= reactor volume

Mg = moles of gaseous products

Ml = moles of liquid products

Ms = moles of steam

R = 6.23 L/ K.mol

P = pressure, 76 cm

T = temperature

t = residence time, sec.

The yield of each component in gaseous products was defined as follows:

$$Yi = \frac{Vg.Xi.Pi}{W}$$

where:

W = Mass flow rate of gas oil, Kg/hr

Vg = Volumetric flow rate of gaseous products, l/hr
p = density, Kg/m3
X = concentration of products (vol.%, wt%)
Y = yield of products (wt%)

TABLE V : Mass spectrometry analysis of the distillate **2** from fractional distillation of the liquid products of gas oil steam cracking at 650 C.

	wt.% of distillate 2	wt.% of liquid products
Paraffins	26.05	5.80
Uncondensed Naphthene	21.60	4.81
Condensed Naphthene	13.39	2.98
Alkylbenzene	11.26	2.50
Indane + tetralin	6.50	1.45
Indene	6.96	1.55
Naphthalene	9.22	2.05
Acenaphthene + diphenyl	0.04	0.01
Acenaphthene + fluorene	0.00	0.00
Anthracene	0.02	0.00
Benzothiophene	4.95	1.10
Dibenzothiophene	0.00	0.00
	99.99 total	22.25 total
Mean atoms of C:	Aromatics	11.6
	Saturated	13.0
	Naphthanic	11.8
Molecular weight		174.0

TABLE VI : Liquid chromatography analysis of the residue from fractional distillation of the liquid products of gas oil steam cracking at 650 °C.

	wt.% of residue	wt.% of liquid products
Saturated hydrocarbons	40.06	25.70
Aromatic hydrocarbons	49.72	31.90
Resins	10.22	6.56
	100.00 total	64.16 total

Mean molecular weight : 217.32

TABLE VII : Mass spectrometry analysis of the saturated hydrocarbons of the residue from fractional distillation of gas oil steam cracking liquid products at 650°C.

Familly	% vol.	% wt. of saturated hydrocarbons	% wt. of liquid products
Paraffins (mostly -iso)	59.78	23.95	15.36
Naphthene with 1 ring	15.83	6.34	4.06
Naphthene with 2 rings	12.16	4.87	3.12
Naphthene with 3 rings	7.27	2.91	1.27
Naphthene with 4 rings	4.62	1.85	1.19
Naphthene with 5 rings	0.34	0.14	0.09
Naphthene with 6 rings	0.00	0.00	0.00
Monoaromatics	0.00	0.00	0.00
	100.00 total	40.06 total	25.69 total

Mean molecular weight : 207.8

TABLE VIII: Mass spectrometry analysis of the aromatic hydrocarbons of the residue from fractional distillation of gas oil steam cracking liquid products at 650°C.

No of C / Family	$C_{11}C_{12}$	C_{13}	C_{14}	C_{15}	C_{16}	C_{17}	C_{18}	C_{19}	C_{20}	C_{21}	C_{22}	C_{23}	C_{24}	% wt. of aromatics	% wt. of residue	wt. of liquids
C_nH_{2n-32} and, or $C_nH_{2n-22}S$						0.42	0.44	0.50	0.24	0.13	0.08			1.61	0.80	0.51
C_nH_{2n-30} and, or $C_nH_{2n-20}S$					0.40	0.37	0.37	0.28	0.24	0.24	0.24	2.17		1.08	1.08	0.69
C_nH_{2n-28} and, or $C_nH_{2n-20}S$					0.44	0.46	0.48	0.37	0.34	0.27	10.25			2.66	1.32	0.85
C_nH_{2n-26} and, or $C_nH_{2n-16}S$	0.54	2.02	3.08	3.72		2.30	1.24	0.56	0.44	0.51	0.22	0.22		15.38	7.65	4.92
C_nH_{2n-24} and, or $C_nH_{2n-14}S$	0.23	0.31	0.66	0.66	0.69	0.53	0.53	0.53	0.49	0.40	0.35			5.38	2.67	1.71
CnH_{2n-22} and, or $C_nH_{2n-12}S$	0.33	0.51	0.80	0.90	1.00	0.67	0.65	0.40	0.37	0.34	0.34			6.31	3.14	2.01

TABLE VIII:(cont.)

C_nH_{2n-20} and, or $C_nH_{2n-10}S$	1.31	1.32	1.33	1.13	1.42	0.97	0.97	0.97	0.64	0.64	0.56	0.44			11.71	5.82	3.74
C_nH_{2n-18}				0.49	1.61	2.17	1.48	1.08	0.77	0.45	0.35	0.33	0.33	0.25	9.3	4.62	2.97
C_nH_{2n-16}				0.90	1.30	1.30	1.25	0.78	0.77	0.55	0.46	0.46	0.32	0.27	8.36	4.16	2.67
C_nH_{2n-14}				0.95	1.25	1.35	1.20	1.15	1.00	0.59	0.52	0.41	0.36	0.36	9.14	4.54	2.91
C_nH_{2n-12}		2.65		2.25	1.50	1.12	0.60	0.54	0.44	0.30	0.38	0.35	0.25	0.26	10.72	5.33	3.42
C_nH_{2n-10}		0.39		0.45	0.51	0.41	0.41	0.41	0.41	0.37	0.37	0.37	0.32	0.21	4.65	2.31	1.48
C_nH_{2n-8}		0.45		0.55	0.57	0.79	0.53	0.51	0.51	0.49	0.49	0.49	0.44	0.44	6.28	3.12	2.00
C_nH_{2n-6}		0.35		0.37	0.40	0.53	0.62	0.73	0.58	0.58	0.58	0.57	0.49	0.47	6.31	3.14	2.01
													TOTAL		100.00	49.72	31.90

Mean molecular weight : 221

TABLE IX : Comparison of mass spectrometry analysis between gas oil used for the experiments and liquid products fraction of 200+ obtained from steam cracking at 750°C.

Composition % wt.		Gas oil	Liquid products fraction 200+
Paraffins		39.0	28.56
Uncondensed Naphthene		20.3	14.13
Condensed Naphthene		10.4	7.59
Alkybenzene	C_nH_{2n-6}	7.0	3.87
Indane + tetralin	C_nH_{2n-8}	3.9	4.86
Indene	C_nH_{2n-10}	1.7	4.86
Naphthalene	C_nH_{2n-12}	5.2	9.44
Acenaphthene + diphenyl	C_nH_{2n-14}	2.0	2.10
Acenaphthene + fluorene	C_nH_{2n-16}	1.2	2.16
Phenathrene + anthracene	C_nH_{2n-18}	1.5	2.45
Benzothiophene	$C_nH_{2n-10}S$	5.4	8.37
Dibenzothiophene	$C_nH_{2n-16}S$	2.4	5.47

TABLE X : Mass spectrometry analysis of liquid products from gas oil steam cracking at 700° C.

Family	type	wt.%
Condensed Naphthenes	$C_{13}H_{24}$	5.0
Aromatics		
Alkybenzenes:		
	C_7H_8	
	C_8H_{10}	
	C_9H_{12}	
	$C_{10}H_{14}$	
	$C_{11}H_{16}$	12.0 (total)
Indane + tetralin:	$C_{15}H_{22}$	7.6
Indene:	$C_{15}H_{20}$	7.8

TABLE X (cont)

Naphthalene:		
	$C_{10}H_8$	
	$C_{11}H_{10}$	
	$C_{12}H_{12}$	
	$C_{13}H_{14}$	26.5 (total)
Acenaphthene + diphenyl:	$C_{15}H_{16}$	7.0
Acenaphthene + fluorene:	$C_{15}H_{14}$	8.8
Phenathrene + anthracene:	$C_{15}H_{12}$	9.4
Benzothiophenes:		
	$C_{12}H_{14}S$	
	$C_{13}H_{16}S$	
	$C_{14}H_{18}S$	
	$C_{15}H_{20}S$	9.4 (total)
		TOTAL 95.0

Mean Molecular weight : 170

Experimental results.

Figure 1 shows the influence of pyrolysis temperature on liquid and gaseous products distribution. The optimal gaseous products conversion is about 66% wt. at 760°C pyrolysis temperature. At the same temperature liquid products conversion is about 16% wt.

Figure 2 shows the evolution of deposits on the walls and the exit of the reactor with temperature. After 760°C coke deposits increase monotonically resulting the increasing of the pressure drop inside the reactor.

Coke deposits on the reactor walls reducing the overall heat transfer coefficient and increasing the pressure drop across the reactor are due to the catalytic reactions on the metallic walls. Coke deposit contains carbon and hydrogen (5) and is not deposited uniformly along the reactor (6).

The gaseous products consist of light paraffins (CH_4, C_2H_6, C_3H_8), olefins (C_2H_4, C_3H_6, C_4H_8, C_5H_{10}), diolefin (C_4H_6) and hydrogen. Oleffins distribution of of the gas oil pyrolysis is given in Figure 3 and of parafins , CH_4 and H_2 in Figure 4.

The optimal yields of C_2H_4 and C_3H_6 31% and 11.4% by weight, were obtained at 760° C and 700°C respectively ,in the Inconel reactor, for a steam to gas oil mass ratio equal to 2 and a residence time of ~0.33 sec.

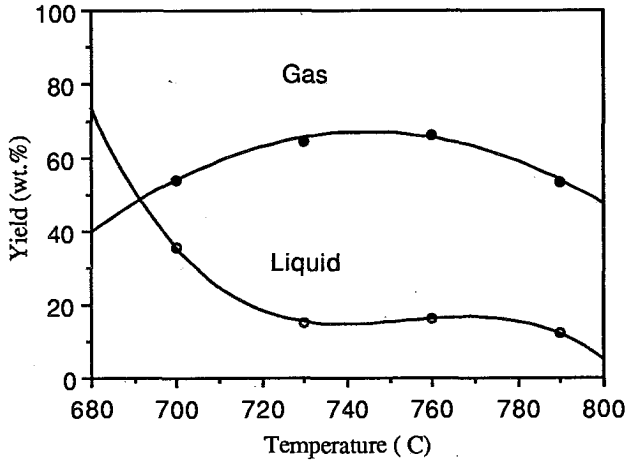


FIG. 1. Influence of temperature of gas oil steam cracking on liquid and gaseous product yields in an Inconel reactor for a dilution ratio ~2.

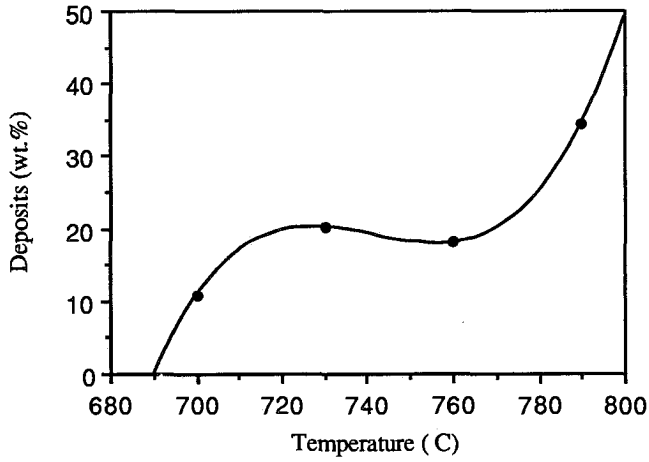


FIG. 2. Deposits versus temperature evolution of atmospheric gas oil cracked in an Inconel reactor for a dilution ratio ~2.

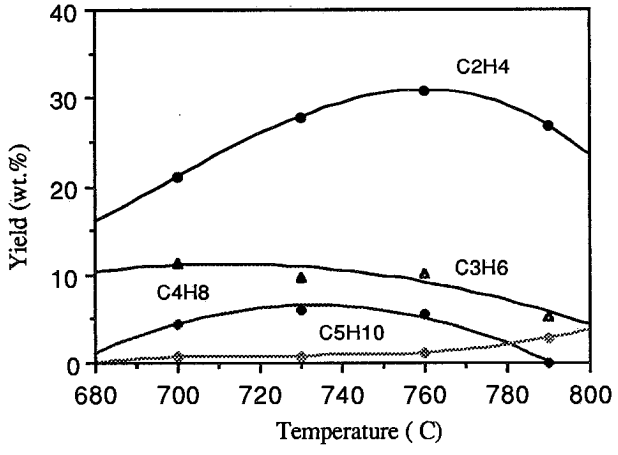


FIG 3. Influence of temperature of an atmospheric gas oil steam cracking on yields of olefines , in a Inconel reactor for a dilution ratio ~2.

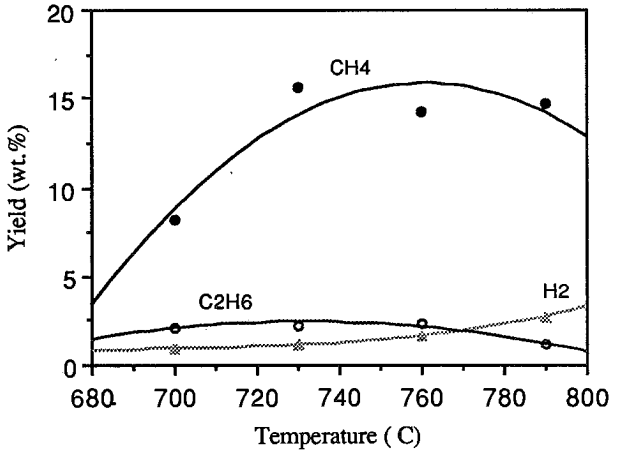


FIG 4. Influence of temperature on paraffins and H2 distribution of an atmospheric gas oil cracked in a Inconel reactor for a dilution ratio ~2.

The yield of C4H8 increase with temperature and reach a maximum of 6% at 730°C. For temperatures higher than the above the yield of C_4H_8 declined. C_5H_{10} increases monotonically when the temperature increases.

As Figure 4 shows, the yield of CH_4 increases with temperature to a maximum of ~16% at 740 C. In opposite, H_2 shows a high rate increasing in the yield for temperatures >760 C and allow rate increasing for temperatures <760 C. Yields of C_2H_6 shows a maximum of 2.2% at 730 C.

Liquid products yield decreases with temperature as Figure 1 shows. They consist of high carbon aromatics and their appearance changes with the pyrolysis temperature increase from transparent become black at higher temperature. At 650 C they consist of paraffins, 27 % of paraffins contained in the raw material are converted in products; of cycloparafins, 30% of condensed naphthenes contained in the gas oil are converted and aromatics. As we can notice (Table X) ,above 700 C they are mostly consisted of aromatics (95% by weigh), and all paraffins contained in raw material are converted in products.

ΠΕΡΙΛΗΨΗ

ΔΙΑΣΠΑΣΗ ΜΕ ΑΤΜΟ ΤΟΥ ΑΕΡΙΕΛΑΙΟΥ

Πειράματα πυρόλυσης των αεριελαίων παρουσία ατμού πραγματοποιήθηκαν σε εργαστηριακή κλίμακα μέσα σε σωληνωτό αντιδραστήρα inconel 600, μήκους 4 μέτρων και διαμέτρου 4 χιλιοστομέτρων. Σκοπός ήταν ο καθορισμός της επίδρασης της θερμοκρασίας επί της παραγωγής ολεφινών και ιδιαίτερα αιθυλενίου και να ευρεθεί η σύνθεση των υγρών προϊόντων. Η καλλίτερη απόδοση αιθυλενίου, 31% κατά βάρος προέκυψε σε 760°C, χρόνος παραμονής 0.33 δευτερόλεπτα και σχέση μάζας ατμού προς αεριελαίου ισούται προς 2. Τα υγρά προϊόντα της πυρόλυσης συνίστανται από αρωματικές ενώσεις υψηλής περιεκτικότητας σε άνθρακα, ιδιαίτερα άνω της θερμοκρασίας πυρόλυσης 700°C. Αποθέματα κωκ επί των τοιχωμάτων και της εξόδου του αντιδραστήρα ήταν σημαντικά.

Conclusion.

The aim of this study was to obtain experimental data on the pyrolysis of an atmospheric gas oil in an Inconel reactor of 4m long. Experiments indicated that the best yield of C_2H_4, 31% by weight were obtained at 760 C for a mass ratio of steam to gas oil equal to 2 and residence time of 0.33 sec. The maximum yield of C3H6 (14%) was obtained at 700 C.

Yields of H_2 , CH_4 are important, caused by the secondary reactions. Coke deposits also, appeared very important on the walls and the exit of the Inconel 600 reactor limiting the heat transfer and increasing the pressure drop. These deposits produced by the secondary treactions showed the catalytic role of the metal walls (4,7).

The main component of pyrolysis oil are aromatic hydrocarbons, consisted mostly of naphthalene, alkylbenzene, indane ,tetralin which are not found in large quantities in the raw material gas oil.

REFERENCES

1. M. Hirato, S. Yoshioka and M. Tanaka, Hitachi Rev., 20 (1971) 326.
2. D. Kunzru and P. Kumar, Ind. Eng. Chem. Process Des. Dev., 24 (1985) 774.
3. D. Depeyre, C. Flicoteaux, F. Arbabzadeh and A. Zabaniotou, Ind. Eng. Chem. Res., 28 (1979) 967.
4. B. Blouri, J. Giraud, S. Nouri and D. Herault, Ind. Eng. Chem. Process Des. Dev.,20 (1981) 307.
5. B. Blouri, D. Depeyre, M. Diop dit Sall and F. Ouassel, Lavoisier , 1 (1987) 215.
6. P. Kumar and D. Kunzru, The Canadian Journal of Chem. Eng., 63 (1985) 598.
7. D. Depeyre, C. Flicoteaux and C. Caldaire, Ind. Eng. Chem. Process Des. Dev. 24 (1985) 1251.

Chimika Chronika, New Series, 19, 219-224 (1990)

SYNTHESE DE QUELQUES NOUVEAUX ARYLOXY —ACETAMIDES ET— PROPANOLAMINES SUBSTITUÉS SUR L'AZOTE.

A. PAPADAKI-VALIRAKI, TH. SIATRA-PAPASTAIKOUDI, G. PAPAIOANNOU

Laboratoire de Pharmacie Chimique de l'Université d'Athènes 104, rue Solonos, 10680 Athènes, Grèce.

(Received July 8, 1986)

RÉSUMÉ

Les auteurs ont préparé un certain nombre d'aryloxy-acétamides et —propanolamines, substitués sur l'azote et caractérisés par la présence en position 2- et 6- du noyau d'une fonction méthyl et d'un groupement méthoxy respectivement, en vue d'étudier leur action antiarrhythmique éventuelle.

Key words

Aryloxy-acétamides et -propanolamines substitués sur l'azote.

PARTIE THÉORIQUE

L'action antiarrhythmique du procainamide[1] et de diverses propanolamines telles que le propranolol[2], l'aténolol[3], le practolol[4] etc. est bien connue. Au cours de ce travail nous avons préparé un certain nombre d'aryloxy-acétamides et -propanolamines, substitués sur l'azote et caractérisés par la présence en position 2- et 6- du noyau d'une fonction méthyle et d'un groupement méthoxy respectivement, en vue d'étudier leur action antiarrhythmique éventuelle. Les produits en question correspondent aux formules générales I et II.

où $-N\begin{matrix}R_1\\R_2\end{matrix}$ $=\overline{N\text{-}CH_2(CH_2)_3CH_2}$

$=\overline{N\text{-}CH_2(CH_2)_2CH_2}$

$=\overline{N\text{-}CH_2CH_2OCH_2CH_2}$

$=\text{-}NH\text{-}CH(CH_3)_2$

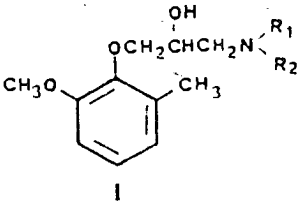
$\text{-}NH\text{-}CH(CH_3)CH_2C_6H_5$

où $R=H$

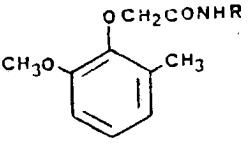
$=CH_2\text{-}\overline{N\text{-}CH_2CH_2OCH_2CH_2}$

$=CH_2CH_2N\begin{matrix}CH_3\\CH_3\end{matrix}$

La synthèse de ces dérivés a été effectuée suivant le schéma cidessous.



I



II

où $-N<^{R_1}_{R_2}$ $=-N-CH_2(CH_2)_3CH_2$

$=N-CH_2(CH_2)_2CH_2$

$=-N-CH_2CH_2OCH_2CH_2$

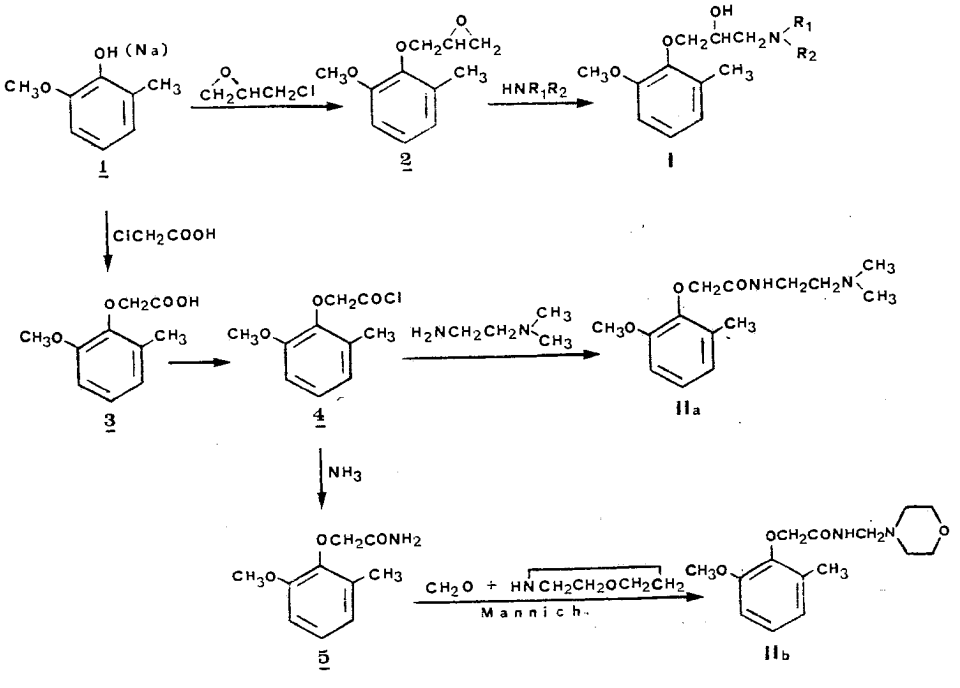
$=-NH-CH(CH_3)_2$

$-NH-CH(CH_3)CH_2C_6H_5$

où R=H

$=CH_2-N-CH_2CH_2OCH_2CH_2$

$=CH_2CH_2N<^{CH_3}_{CH_3}$



Comme produit du départ pour les composés des formules I et II a été employé le méthyl-**6** guaiacol (*1*), obtenue par réduction de l'o-vanilline, à l'aide d'hydrazine, suivant le procédé de Wolf-Kishner[5].

Le méthyl-**6** guaiacol (*1*) est traité par l'épichlochydrine, en milieu alcalin, pour obtenir le produit intermédiaire *2* (epoxyéther non isolé), qui conduit aux dérivés de formule générale I par action de diverses amines[6].

Pour obtenir les dérivés de formule générale II, on traite le guaiacol *1* par l'acide chloracétique[7], ce qui donne l'acide méthyl-2 méthoxy-6 phénoxyacétique (*3*). M Hirota et G. Hirano[8] mentionnent ce dernier acide sans pourtant indiquer ni méthode de préparation ni constantes. L'acide *3* est transformé au chlorure correspondant (*4*), par action de chlorure de thionyle, puis au produit de formule générale II, dans lequel R = $(CH_2)_2N(CH_3)_2$, par action de diméthylamino-éthylamine[7]. Le chlorure *4*, traité à l'ammoniaque, donne l'amide *5* (il n'est pas décrit dans la littérature) et ce dernier, par application de la réaction de Mannich[9], le composé de formule générale II, dans lequel R = $\overline{N\text{-}CH_2CH_2\text{-}O\text{-}CH_2CH_2}$.

PARTIE EXPERIMENTALE

La structure des produits préparés a été vérifiée par l'analyse élémentaire et les spectres IR et RMN.

Les points de fusion ont été pris sur un appareil Büchi, en capillaires ouverts et ne sont pas corrigés, les spectres IR au spectrophotomètre Perkin-Elmer 177 et les spectres RMN à l'aide d'un spectrophotomètre Varian 90 MHz et Varian 60MHz. Les analyses élémentaires ont été effectuées par le Service Central de Microanalyse du C.N.R.S. (Paris), que nous remercions vivement. Les valeurs trouvées étaient conformes à la théorie ±4%.

Methyl-6 guaiacol. Il est préparé par réduction de l'o-vanilline à l'aide d'hydrazine, suivant le procédé de G. Lock et coll[6] avec un rendement de 75%. Avant d'être employé pour l'étape suivante, le produit est purifié par passage à travers une colonne de Kieselgel 60 et en éluant par le benzène. F = 42°C (Litt. F = **42**°C).

(Methyl-2 méthoxy-6) phénoxy-1 pipéridino-3 propanol-2. (I, NR_1R_2 = $\overline{-N\text{-}CH_2(CH_2)_3CH_2}$).

A la solution de 6,9g (0,05mole) de méthyl-6 guaiacol et de 2g (0,05mole) d'hydroxyde de sodium dans 20ml d'eau, on ajoute, goutte à goutte et sous agitation, 8, 6ml (0,1 mole) d'épichlorhydrine. L'addition du réactif terminée, le mélange est chauffé au reflux pendant 3h. Le produit de la réaction est refroidi, puis extrait à l'éther et les solution éthérées réunies sont lavées avec une solution d'hydroxyde é de potassium à 10% puis à l'eau jusqu' à neutralisation. La solution éthérée, séchée sur la sulfate de magnésium anhydre, puis évaporée à sec, abandonne un résidu huileux (époxyéther), qui est employé tel quel pour l'étape suivante. Le résidu est dissous dans 30ml de

benzène anhydre et la solution obtenue est additionnée de 12,75g (0,15mole) de pipéridine. Le mélange est chauffé au reflux pendant 3h, puis lavé à l'eau jusqu' à neutralisation, séché sur le sulfate de magnésium anhydre te évaporé à sec. On obtient 10,5g de produit (rend. 75%),sous forme de base, qui est transformée en chlorhydrate (crystallin, F = 133°C).
$C_{16}H_{25}NO_3$: NMR($CDCl_3$, 60MHz) δ:0,68-1,83 ppm (m, 6H, 3, 4, 5-piperidine-H), 1,95-2,85 ppm (m, 6H, 2, 6-Piperidine-H, CH_2N), 2,25 ppm (s, 3H, CH_3-Ar), 3,10-4,50 ppm (complex m, 4H, OCH_2CH, OH), 3,77 ppm (s, 3H, CH_3O), 6,40-7, 15 ppm (m, 3H, aromatic).

Suivant ce même procédé ont été préparés tous les produits, dont les analyses et constantes sont rassemblées au tableau I.

Dans le cas du T5145, le produit final a été isolé par chromatographie sur colonne d'alumine et en éluant par le mélange éther de pétrole-éther.

Acide méthyl-2 méthoxy-6 phénoxyacétique 3

Dans le mélange de 13,8g (0,1 mole) de méthyl-6 guaiacol et de 45ml de lessive de soude à 33% (0,5 mole) on ajoute 18,9g (0,2 mole) d'acide chloracétique et une petite quantité d'eau (jusqu'à dissolution complète du sel phénolique formé), puis on fait chauffer le tout sur bain marie pendant une heure (au reflux), on refroidit et on acidifie avec l'acide chlorhydrique concentré. L'acide (*3*), qui précipite, est essoré, lavé á l'eau et recristallisé dans le mélange benzène-éther de pétrole. Rend. 82%, F = 102°C.

(Méthyl-2 méthoxy-6 phénoxy) acétamide 5.

Le mélange de 9,8g (0,05 mole) d'acide méthyl-2 méthoxy-6 phénoxyacétique (*3*) et de 42g (27ml, 0,35 mole) de chlorure de thionyle est chauffé sur bain marie pendant 2-3h au reflux. L'éxcès du chlorure de thionyle est ensuite éliminé par évaporation sous pression réduite à l'aide de benzène anhydre. Le chlorure de l'acide *3* ainsi obtenu est ajouté par petites quantités dans un excès d'ammoniaque et l'amide formé est essoré, lavé et recristallisé dans l'éthanol. Rend. 54% (5,5g) F = 99°C.

Analyse $C_{10}H_{13}NO_3$ Calc.% C 61,53 H 6,71
Tr. % 61,38 6,70

α-(Méthyl-2 méthoxy-6 phénoxy)N-morpholinométhyl-acétamide (II, R = $-CH_2N(CH_2)_2$-O-CH_2-CH_2).

On charge un ballon 1,95g (0,01 mole) de l'amide *5*, 0,51g (0,017 mole) de paraformaldéhyde, 2,1g (0,017 mole) de chlorhydrate de morpholine, 2-3 gouttes d'acide chlorhydrique concentré et 15ml d'éthanol. Le mélange est chauffé au reflux pentant 5h, puis le solvant est éliminé sous pression réduite et le residu jaunâtre alcalinisé à l'aide d'une solution d'hydroxide de sodium à 10% et extrait à l'éther. les extraits éthérés réunis, lavés à l'eau jusqu' à élimination de l'excès de la morpholine, séchés et évaporés à sec sous pression réduite, abandonnent 1,44g de base (rend. 49%), qui est transformée en chlorhydrate (F = 133°C, acétone).

$C_{15}H_{22}N_2O_4$ RMN ($CDCl_3$ 90 MHz) δ:2,30 (s, 3H, CH_3), 2,65 (t, 4H, A_2X_2, J_{AX} = 5Hz, H morpholiniques-3,5), 3,71 (t, 4H, A_2X_2, I_{AX} = 4,5 Hz, H morpholiniques-2,6) 3,88 (s, 3H, CH_3O), 4,26 (d, 2H, AX_2, I_{AX} = 6, 5Hz, NH-CH_2N), 4,45(s, 2H, OCH_2CO), 6,70-7,12 (m, 3H, aromatic) 7,35-7,80 (s, large, 1H, NH).

Analyse $C_{15}H_{23}ClN_2O_4$ Calc. % C 54,46 H 7,01
Tr. % 54,34 6,96

α-(méthyl-2 méthoxy-6 phénoxy)-N-diméthylaminoéthyl-acétamide (II, R=-$(CH_2)_2$-N-$(CH_3)_2$

La solution de 5,5g (0,026 mole) de chlorure de méthyl-2 méthoxy-6 phénoxy-acétyle dans 20 ml d'acétate d'éthyle, refroidie à -5°C, est additionnée, goutte à goutte et à une température n'excédant pas le 0°C, d'une dolution de 3,6g (0,028 mole) de β-diméthylaminoéthylamine dans 10ml d'acétate d'éthyle, puis le mélange est laissé au repos pendant 1-2h à la température ambiante. Le chlorhydrate de l'a mide ainsi formé est essoré et recristallisé dans le mélange acétone-éther. Rend. 44% (3,4g de sel.). F = 157-8°C. $C_{14}H_{22}N_2O_3$ NMR ($CDCl_3$, 60Mz) δ: 1,75-2,94 ppm (m, 2H, $CH_2N\begin{matrix}CH_3\\CH_3\end{matrix}$), 2,25 ppm (s, 6H, $(CH_3)_2$N), 3, 10-3,65 ppm (m, 2H, $CONHCH_2$), 3,78 ppm (s, 3H, CH_3O), 4,37 (s, 2H, OCH_2CO), 6, 5-7, 8 ppm [m, 4H, aromatic, NH).

Analyse $C_{14}H_{23}N_2O_3Cl$ Calc. % C 55,54 H 7,60
Tr. % 55,35 7,43

SUMMARY

Synthesis of some new N-substituted aryloxy-acetamides and -propanolamines.

In this study some N-substituted (2-methyl-6-methoxy) phenoxy-axetamides and -propanolamines were prepared. The acetamides were prepared from (2-methyl-6-methoxy) phenoxyacetic acid which was converted into the corresponding chloride. The latter either reacted with dialkylaminoalky-

TABLEAU I: (Méthyl-2 méthoxy-6) phénoxy-1 amino-3 propanols-2 substitués sur l'azote.

R_1 N R_2	Rendement % (en base)	No	Sel	Formule brute	F°C	Analyses Calc. % C	Calc. % H	Tr. % C	Tr. % H
-N-$CH_2(CH_2)_3CH_2$	75	T_{5124}	Chlorhydrate	$C_{16}H_{26}NO_3Cl$	133	**60,84**	8,30	60,82	8,23
-N-$CH_2(CH_2)_2CH_2$	80	T_{5137}	Chlorhydrate	$C_{15}H_{24}NO_3Cl$	78	**59,09**	8,90	58,80	8,36
-N-$CH_2CH_2OCH_2CH_2$	70	T_{5136}	Chlorhydrate	$C_{15}H_{24}NO_4Cl$	88	**56,68**	7,61	56,60	7,39
-N-$CH_2CH_2OCH_2CH_2$	—	T_{5127}	Picrate	$C_{21}H_{26}N_4O_{11}$	167	**49,41**	5,13	49,45	5,01
-HN-$CH(CH_3)_2$	78	T_{5125}	Chlorhydrate	$C_{14}H_{24}NO_3Cl$	93	**58,02**	8,35	57,55	8,41
*C_6H_5-CH_2CH-NH-CH_3	60	T_{5145}	Chlorhydrate	$C_{20}H_{28}NO_3Cl$	158	**65,65**	7,71	65,57	7,70

(*) La base est purifiée par chromatographie sur colonne d'alumine et en éluant par le mélange éther de petroléther (2:1).

lamine or was converted into amide which was subjected to the Mannich reaction. The propanolamines were prepared from 6-methylguaiacol by its reaction with epichlorhydrin and then with different amines. The above compounds were synthesized to test for antiarrhythmic action.

ΠΕΡΙΛΗΨΗ

Σύνθεση μερικών νέων Ν-υποκατεστημένων αρυλοξυ-ακεταμιδίων και προπανολαμινών.

Στην εργασία αυτή παρασκευάστηκαν μερικά υποκατεστημένα στο άζωτο (2-μεθυλο-6-μεθοξυ)-φαινοξυ ακεταμίδια και προπανολαμίνες. Τα ακεταμίδια παρασκευάστηκαν από το (2-μεθυλο-6-μεθοξυ)-φαινοξυοξικό οξύ δια μετατροπής του σε χλωρίδιο και επιδράσεως με διαλκυλαμινοαλκυλαμίνη ή μετατροπής του χλωριδίου σε αμίδιο και εφαρμογής της αντιδράσεως Mannich. Οι προπανολαμίνες παρασκευάστηκαν από την μεθυλο-6-γουαϊακόλη, δι' επιδράσεως με επιχλωρυδρίνη και στη συνέχεια με διάφορες αμίνες.

Τα παράγωγα αυτά παρασκευάστηκαν για να εξεταστούν για τυχόν αντιαρρυθμική δράση.

BIBLIOGRAPHIE

1. Mark, L.C., Kayden, H.J., Steele, J.M., Cooper, J.R., Rowenstein, E.R, Brodie, B.B. *J. Pharm. Exp. Ther. 102,* 5 (1951).
2. Black, J.W., Crowther, A.F., Shanks, G.R., Smith, L.H., Dornhorst, A.C. *Lancet 1* 1080 (1964).
3. Barrett, A.M., Hull, R. LeCount D.J. Squire, C.J., Carter, J. *Germ. Pat.*2,007,751 (1970) *Chem. Abstr. 73* 120318 (1970).
4. Danilewicz, J.C., Kemp. J.E.G.,: *J. Med. Chem. 16,* 168 (1973).
5. Lock, G.: *Monatsh 85,* 802 (1954).
6. Tsatsas, G., Siatra, Th., Varonos, D., Spyraki, Ch.: *Ann. Pharm. Fr. 31,* 305 (1973).
7. Guioca-Cedopoulou V., Tsatsas G., Papaioannou, G., Zarolinski J.F., Browne R.K., Possley, L.H.: *Ann. Pharm.* Fr. *28,* 707 (1970).
8. Hirota, M., Hirano G.: *Bull. Chem. Soc. Jap. 45,* 1448 (1972).
9. Papadaki-Valiraki, A., Papaioannou G., *Ann. Pharm. Fr., 42,* 257 (1984).

Chimika Chronika, New Series, 19, 225-232 (1990)

AMINO-2-[(MÉTHOXY-2-MÉTHYLPHÉNOXY-6) MÉTHYL]-5-THIADIAZOLES-1,3,4 N-SUBSTITUÉS.

A. PAPADAKI-VALIRAKI*, TH. SIATRA-PAPASTAIKOUDI*, P. TSITSA* A. DELITHEOS**.

* *Laboratoire de Pharmacie Chimique, Université d' Athènes, 104 rue Solonos, Athènes, Grèce.*

** *Laboratoire de Pharmacologie Experimentale, Université d' Athènes Goudi, Athènes, Grèce.*

(Received April 28, 1988)

SUMMARY

N-SUBSTITUD 2-AMINO-5-[(2-METHOXY-6-METHYLPHENOXY)METHYL]-1,3,4-THIADIAZOLES.

The synthesis of some new N-substituted 2-Amino-5 [(2-methoxy-6-methylphenoxy) methyl]1,3,4-Thiadiazoles and the synthesis of the 2-[c2-methyl-6-methoxyphenoxy)methyl]-6-p-bromopheny-imidazo-[2,1-b]1,3,4thiadiazole are described.

Five from these compounds have been rested for their antimicrobial activity.

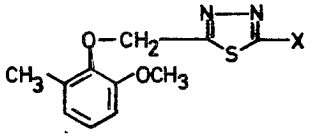
Key Words: activité antimicrobienne, N-substitué 2 amino-5-alkoxy-thiadiazoles.

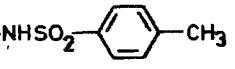
RÉSUMÉ

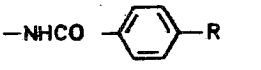
Les auteurs décrivent la synthèse de quelques amino-2-[(méthoxy-2-méthyl-6-phénoxy) méthyl]5-thiadiazoles-1,3,4 N-substitués, ainsi que la synthèse du [méthyl-2-méthoxy-6-phénoxy)méthyl]2-p-bromephényl-6-imidazo [2,1-b]-thiadiazole-1,3,4. Cinq de ces produits ont été testés pour leur action antimicrobienne éventuelle.

Le noyau du thiadiazole-1,3,4 bisubstitué en positions 2- et 5-, est retrouvé dans plusieurs produits dont l'action pharmacologique varie suivant la nature des substituants (antispasmodique[1,2], anticholinergique[3], antimicrobienne[4,5], antifungorale[6,7], antivirale[8]). Ceci nous a incités à préparer une série de dérivés du thiadiazole-1,3,4 qui ont été testés pour leur action antimicrobienne éventuelle.

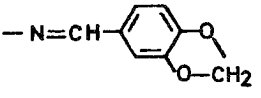
Les produits préparés correspondent à la formule générale:

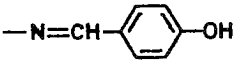


dans laquelle X =  I

 (R = Cl, CH_3, OCH_3) II, III, IV

—NHCO — Adam V

 VI

 VII

Comme matièrè première pour la synthèse des produits précédents nous avons employé l'amino-2-[(méthoxy-2-méthyl-6-phénoxy)méthyl]-5-thiadiazole-1,3,4 (*3*), qui n'est pas décrit dans la littérature et dont la préparation a été réalisée suivant le schèma 1:

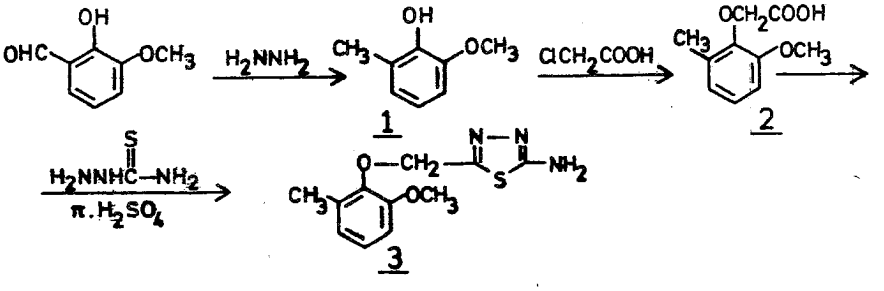


Schéma 1

La réduction de l'o-vanilline à l'aide d'hydrazine (selon Wolf-Kishner[9]) conduit au méthyl-6-guaiacol (*1*), qui, sous l'action de l'acide chloracétique en milieu alcalin, donne l'acide méthyl-2-méthoxy-6-phénoxyacétique (*2*). Ce dernier, chauffé à 80-90°C avec le thiosémicarbazide, en présence d'acide sulfurique cncentré (suivant un procéde de Chubb et Nissenbaum[11], qui est appliqué à des acides aliphatiques), donne l'amino-2-thiadiazole-1,3,4 substitué en position-5 (*3*). Nous avons préféré ce procédé car il nous fournissait le produit désiré avec un rendement satisfaisant, ce qui n'était pas le cas avec les autres procédés consistant au chauffage du chlorure de l'acide avec le thiosémicarbazide, en présence de trichlorure de phosphore[12,13].

L'action du p-toluènesulfochlorure sur le composé *3*, en présence de pyridine, conduit au produit I. Les produits II-V ont été obtenus par action des chlorures appropriés, fraîchement préparés, sur le composé *3*, en présence de triéthylamine dans le chlorure de méthylène[14]. Les produits VI et VII ont été obtenus selon le procédé de préparation des bases de Shiff, soit, en chauffant le composé *3* avec les aldéhydes appropriés en présence d'éthanol et ont été isolés sous forme pure après passage à travers une colonne de gel de silice (éthanol) et élution au benzène.

Le fait que quelques dérivés analogues de l'imidazo[2,1-β]-thiazole présentent une action antelminthique intéressante[15] nous a donné l'idée de préparer un dérivé de l'imidazo[2,1-β] thiachiazole-1,3,4 le produit 5 selon le schéma suivant:

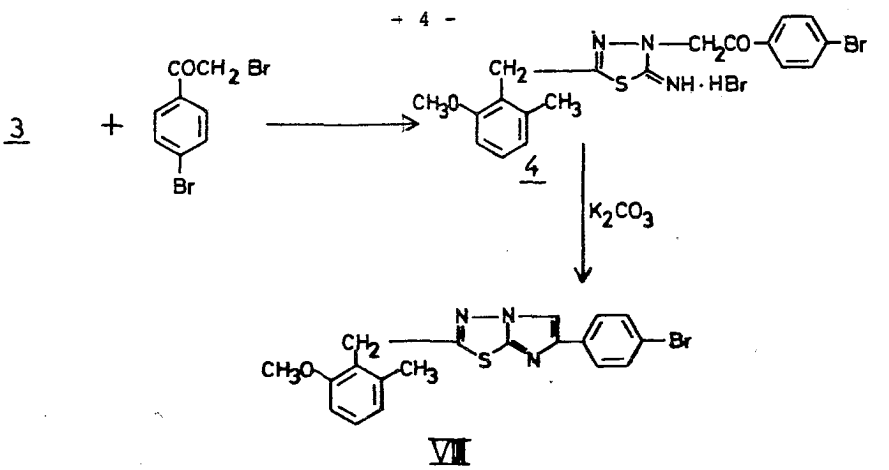


Schéma 2

La synthèse du composé 4 a été réalisée suivant un procédé décrit par M.A. Eldawy et al[16] pour les produits analogues, tandis que le dérivé 5 est obtenu en traitant le composé *4* par le corbonate neutre de potassium anhydre.

PARTIE EXPERIMENTALE

La structure des produits préparés a été vérifiée par l'analyse élémentaire et les spectres IR et RMN.

Les points de fusion ont été pris sur un appareil Büchi, en capillaires ouverts et ils ne sont pas corrigés, les spectres IR au spectrophotomètre Perkin-Elmer 177 et les spectres RMN à l'aide d'un spectrophotomètre Varian 80MH). Les analyses élémenttaires ont été effectuées par le Service Central de Microanalyse du CNRS (Paris) que nous remercions vivement; les valeurs trouvées étaient conformes à la théorie ±4%.

Amino-2-[(méthoxy-2-méthyl-6-phénoxy)méthyl]-5-thiadiazole-1,3,4 (3)

Le mélange de 6g de l'acide *2* (0,03 mole), de 2,28 g de thiosemicarbazide (0,025 mole) et de 3,15 ml l'acide sulfurique concentré est chauffé pentant 7 heures à 85-90°C, sous agitation, puis traîté à chaud avec de petites quantités d'eau. Les solutions aqueuses réunies sont alcalinisées à l'ammoniaque et le produit solide, qui se sépare, est essoré, lavé à l'eau et recristallisé dans l'ethanol. On obtient 4,2 g du produit *3*. F° 203-4. Rndt. 56%.

Analyse: $C_{11}H_{13}N_3SO_2$ Calc. % C: 52,57 H: 5,21 N: 16,72

Tr. % C: 53,01 H: 5,28 N: 16,50

Spectre IR Absorption à 3290 cm^{-1}($-NH_2$).

(p-Toluènesulfonamido)-2-[(méthoxy-2-méthyl-6-phénoxy)méthyl]-5-thiadiazole-1,3,4 (3a)

La solution de 0,95 g de p-toluènesulfochlorure (0,005 mole) dans 2,5 ml de pyridine anhydre est ajoutée, lentement et sous agitation, à une solution de 1,25 g de produit *3* (0,005 mole) dans 2,5 ml de pyridine. Le mélange est abandonné pentant la nuit à la température ambiante, puis additionné d'acide chlorhydrique 6N en excès. Le produit brut qui se sépare est redissous dans une solution aqueuse d'hydroxide de sodium, précipité de nouveau à l'aide d'acide chlorhydrique, essoré, lavé et recristallisé dans l'éthanol. On obtient 0,5 g du composé 3F° 119-120. Rndt. 31%.

Analyse: $C_{18}H_{19}N_3O_4S_2$ Calc. % C: 53,32 H: 4,72 S: 15,81

Tr. % C: 53,39 H: 4,66 S: 15,61

(p-Chlorbenzoylamino)-2-[(méthoxy-2-méthyl-6-phénoxy)méthyl]-5-thiadiazole-1,3,4 (3b).

La solution de 1,25 g (0,005 mole) de produit *3* et de 0,6 g de triéthylamine (0,006 mole) dans 10 ml de chlorure de méthyléne est additionnée goutte à goutte de 1,05 g (0,006 mole) de chlorure de p-chlorobenzoyle à la température ambiante. Le mélange est abandonné pendant 30 min., puis additionné d'une petite quantité d'eau et agité. Après décantation de la couche aqueuse, on

ajoute sous agitation une nouvelle quantité d'eau, qui est à son tours éliminée par décantation. L'addition d'une petite quantité de benzène au résidu provoque la séparation d'un produit solide, qui est recristallisé (difficilement car très peu soluble) dans le mélange méthanol/éthanol. Les produits qui figurent dans le tableau I ont été obtenus suivant le procédé qui vient d' être décrit.

Les spectres IR de ces produits ont montré des bandes de forte absorption à 1670-1650 cm^{-1} (C = O) et à 3150-3140 cm^{-1} (NH).

$C_{18}H_{16}N_3SO_3Cl$(3b) RMN($CDCl_3$) δ(ppm):2,31(s, 3H, CH_3), 3,90(s, 3H, OCH_3), 5,35(s, 2H, CH_20), 6,65-7,64 (m, 8H, H aromatiques, NH).

Le tableau I comprend les constantes, les rendements et les résultats de l'analyse élémentaire des produits ainsi préparés (3b, 3c, 3d, 3ε).

Base de Schiff du produit 3 avec le méthylénedioxy-3, 4-benzaldéhyde (*3a*).

Le mélange de 1,25 g de produit *3* (0,005 mole) et de 1,5 g de méthylénedioxy-3, 4-benzaldéhyde (0,01 mole) dans 100 ml d'éthanol est chauffé à reflux pentant 6 heures. Après l'évaporation de l'éthanol on fait passer la solution éthérée du résidu à travers une colonne de gel de silice. L'élution est faite à l'aide de benzène. On obtient 1 g de produit pur, sous forme de cristaux jaunes, qui recristallisé dans l'éthanol, fond à 107-8° C. Rndt. 53%.

Analyse: $C_{19}H_{17}N_3O_4S$ Calc. % C: 59,52 H: 4,47
Tr. % C: 59,62 H: 4,75

RMN ($CDCl_3$) δ 2,22 ppm (S, 3H, CH_3), 3,80 ppm (S, 3H, CH_3O), 5,27 ppm (S, 2H, OCH_2), 5,99 ppm (S, 2H, OCH_2O), 6,60-7,80 ppm (dm, 6H, H aromatiques), 8,73 ppm (S, 1H, CH = N).

Base de Schiff du produit 3 avec le p-hydroxybenzaldéhyde (3b')

Obtenue suivant le procédé décrit plus haut, elle se présente sous forme d'aiguilles jaunes, qui fondent à 161°C. Rndt. 40%.

Analyse: $C_{18}H_{17}N_3O_3S$ Calc. % C: 60,83 H: 4,82
Tr. % C: 61,35 H: 4,90

Bromhydrate d'imino-2-p-bromophénacyl-3-[(méthyl-2-méthoxy-6-phénoxy) méthyl]-5-thiadiazole-1,3,4 (4)

La solution de 2,78 g de bromure de p-bromophénacyle (0,01 mole) dans 20 ml d'alcool absolu est additionnée de 2,51 g de produit *3* (0,01 mole) et le mélange est chauffé à reflux pendant 1 heure. Après refroidissement, on ajoute 80 ml d'éther anhydre et le précipité formé est essoré, lavé et recristallisé dans le mélange méthanol/éthanol. On obtient 3,75 g de produit (Rndt 83%), qui fond à 194°C.

Analyse: $C_{19}H_{19}N_3O_3SBr_2$ Calc. % C: 43,28 H: 3,63 Br: 29,93
Tr. % C: 43,14 H: 3,58 Br: 29,36

Le spectre IR montre une forte absorption à 1710 cm^{-1} (C = 0).

[(Méthyl-2-méthoxy-6-phénoxy)méthyl]-2-bromophényl-6-imidazo[2,1-β]-thiadiazole-1,3,4 (5)

1 g du produit précédent (4) est traité avec un excès de carbonate neutre de potassium anhydre et une pétite quantité d'eau. Le mélange est extrait à l'éther et au benzène, les solvants sont éliminés par évaporation et le résidu est recristallisé dans l'éthanol. F^0 155. Le spectre IR a montré l'absence de groupement cétone.

Analyse: $C_{19}H_{16}N_3O_2SBr$ Calc. % C: 53,15 H: 3,76 Br: 18,38

Tr. % C: 53,05 H: 3,78 Br: 18,42

RMN($CDCl_3$) δ(ppm) 2,30 (s, 3H, CH_3), 3,86 (s, 3H, CH_3O), 5,23 (s, 2H, CH_2O), 6,58-7,28 (m, 3H, 2-(CH_3), 6-(CH_3O) C_6H_3), 7,35-7,83 (q, 4H, AA'BB', $J_{AA'} \simeq J_{BB'} \simeq$ OHz, $J_{AB} \simeq J_{A'B'} \simeq$ 8Hz, P-Br-C_6H_4), 7,91 (s, 1H, H-5).

Action antimicrobienne.

Les composés N^0 II, III, IV, V et VI ont été testés pour leur activité antimicrobienne vis à vis des espèces suivantes: Staphylococus aureus (ATCC 25923), Esherichia coli (ATCC 25922) et Pseudomonas aeruginosa (ATCC 27853). (Elles sont utilisées comme espèces originales pour le test de sensibilité des différents microbes aux antibiotiques selon la méthode Bauer-Kirby).

Le teste de l'activité antimicrobienne a été effectué comme suivant: Des disques petri (diamétre 9 mm) sont ranplis avec 10 ml de milieu nutritif solide (Tryptone soya Agar-Oxoid). Le milieu est ensemencé avec une suspension microbienne qui provient d'une dilution 1/100 d'une incubation de l'espèce pendant 24 h. A la surface des disques Petri sont déposés des disques de papier filtre Whatman N^0 1 diamètre 6 mm préalablement imbibés avec 10 ml de solution de la substance à l'étude dans la diméthylsulfoxyde (DMSO). Après incubation pendant 24 h à 37^0 C on observe les zones d'inhibition obtenues autour des disques.

Résultats:

Aucun de composés étudiés et pour aucune espèce utilisée n'adonné une zone d'inhibition significative. Le composé 3a' a donné pour la Pseudomonas aeruginosa une faible zone d'inhibition qui est considerée sans valeur.

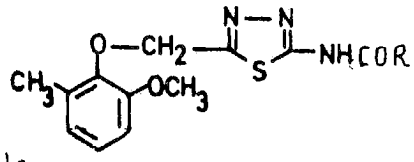
ΠΕΡΙΛΗΨΗ

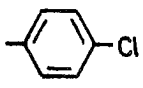
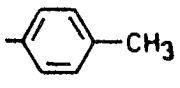
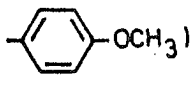
Ν-Υποκατεστημένα-2-Αμινο-5-(2-μεθοξυ-6-μεθυλοφαινοξυ)μεθυλο-1,3,4-θειαδιαζόλια.

Περιγράφεται η σύνθεση μερικών νέων Ν-υποκατεστημένων 2-Αμινο-5[(2-μεθοξυ-6-μεθυλοφαινοξυ)μεθυλο]-1,3,4-θειαδιαζολίων καθώς και η σύνθεση του 2-[(2-μεθυλο-6-μεθοξυφαινοξυ)μεθυλο]6-p-βρωμοφαινυλο-ιμιδαζο[2,1-β]-1,3,4-θειαδιαζολίου.

Πέντε από τα παράγωγα αυτά δοκιμάστηκαν για τυχόν αντιμικροβιακή δράση.

TABLEAU I: Arylamido-2-[(méthyl-2-méthoxy-6-phénoxy)méthyl]-5-thiadiazoles-1,3,4.



	R	Formule brute	F°	Rndt.%		Analyse Calc.%	Analyse Tr.%
II		$C_{18}H_{16}N_3SO_3Cl$	265	63	C,H,Cl	55,45 4,14 9,09	55,05 4,17 8,73
III		$C_{19}H_{19}N_3O_3S$	227	65	C,H	61,77 5,18	61,41 5,22
IV		$C_{19}H_{19}N_3O_4S$	244	68	C,H	59,21 4,97	58,84 4,98
V	— Adam	$C_{22}H_{27}N_3O_3S$	211	65	C,H	63,90 6,58	63,68 6,45

BIBLIOGRAPHIE

1. Capleo B.C., Myers, M., Myers L.P. Saville J.E., Smith A.C.B., Stillings M.R., Tulloch I.F., Walter D.S., Welbourn A.P. J. Med. Chem. 1986, ***29,*** 2273.
2. Stillings M.R., Welbourn A.P., Walter D.S. J. Med. Chem. 1986 ***29,*** 2280.
3. Muhi-Eldeen Z., Ali-Hadi, Ali Al-Shamma, Salman S.R., Sameh I., Falih N. Eur. J. Med. Chem-Chim. Ther. 1986, 219.
4. Joshi N.N., Nadkarmy V.V. J. Ind. Chem. Soc. 1977, ***54,*** 1081.
5. Ovsepyan T.R., Avetisyan A.Kh., Aroyan A.A., Paronikyan, R.V. Arm. Khim Zhr 1977, *30*, 70.
6. Shams-El Dine S.A., Hazzaa AAB, Pharmazie 1974, ***29,*** 761.
7. Singh, N.B., Singh, H., Singh, S. J. Ind. Chem. Soc. 1975, ***52,*** 1200.
8. Tonevo. M., Eleke K., Chemotherapy (Basel) 1974, ***20,*** 350.
9. Lock, G. Monatsh 1954, ***85,*** 802.
10. Guioca-Dedopoulou V., Tsatsas G., Papaioannou G., Zarolinski J.F., Browne R.K., Possley, L.H., Ann. Pharm. Fr., 1970, ***28,*** 707.
11. Chubb L.F., Nissenbaum J. Can. J. Chem. 1959, ***37,*** 1121.
12. Ishikawa M., Kikkawa I., Ann. Repts. Shionogi Research Lab. 1954, ***1,***416.
13. Takatori K., Fuzise S., Nippon Kagaku, C.A. ***54,*** 515, (1960).
14. Ogata M. Matsumoto H., Shimizu S., Kida S., Wada T., Sato K., J. Med. Chem. 1986, ***29,***417.
15. Hashimoto Y., Kanos, Jap. 7406099 (1974) CA *82* 11375v (1975).
16. Eldawy, M.A., Shams et-Dine S.A. El-Brembaly K.M., Pharmazie 1979, 34(3) 144.

Chimika Chronika, New Series, 19, 233-241 (1990)

SHORT PAPER

NEW ORGANOBORON COMPLEXES AS CATALYSTS IN ASPHALT BLOWING

N.D. ECONOMOU[1], V.P.PAPAGEORGIOU[2], A.F. NICOLAIDES[3]

Aristotle University of Thessaloniki-School of Engineering

[1]*Dept.of Civil Engineering, Division of Structural Engineering*

[2]*Dept. of Chemical Engineering, Division of Chemistry*

[3]*Dept. of Civil Engineering, Division of Transport and Organization*

(Received June 17, 1988)

SUMMARY

In this paper preparation and use of new organoboron complexes of β-diketones (acetylaceton, 2,4-hexanedione) with dicarbonic-or hydroxy-acids (citric acid, salicylic acid, oxalic acid) as catalyst in asphalt oxidation have been described.

Blowing of asphalt sample (Arabian light) treated with catalysts (Organoboron Complexes) gave interesting results compared to the blowing of the same sample but in absence of the mentioned catalyst.

The optimisation achieved concerns: reduction of blowing time, reduction of losses and also increase of product penetration.

On the other hand, no change in the softening point was observed.

Key words: Asphalt, bitumen, air blowing, oxidation, boron complexes, catalysts.

INTRODUCTION

The major objective of this project is optimisation of asphalt properties in order to receive better and cheeper industrial products.

Today, the largest source of asphalt[1] (bitumen) is crude petroleum oil (Fig. 1). The crude petroleum enters in a tube at high turbulent flow and very quickly reaches the appropriate temperature for the initial distillation processes. It then enters in a fractionating tower where the lighter among the volatile components vaporize and are drawn off for further refining into naphta, gasoline, kerosene and a wide variety of other petroleum products.

The described fractionating process gives residue which can also further be refined by distillation or undergo solvent extraction to produce asphalt. It can also be processed by blowing air[2-4] through it at an elevated temperature to produce air blown asphalt.

The above process produces asphalt with desirable properties[5-6] appropriate for a wide variety of industrial products. These includes

roofing asphalts, pipe-coating enamels, undersealing asphalts, undercoatings and many other useful products.

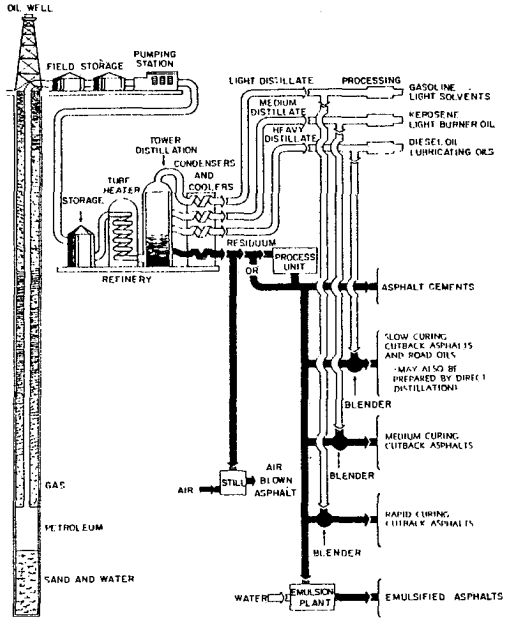


FIG. 1. *PETROLEUM ASPHALT FLOW CHART (From "Introduction to Asphalt"[1], Asphalt Institute)*

As illustrated[6] in Fig. 2, air blowing process (continuous or batch) involves the processing of a vacuum residue (base stock or flux) through an oxidation tower within the range of 230-270^{o}C while air is passed through the hot flux.

The batch air blowing we followed for the experimental trials is graphically illustrated[6] in Fig. 3.

The properties of produced blown asphalts are discribed by two basic physical characteristics of asphalt, softening point and penetration.

Softening point[7] is defined as the temperature at which an asphalt diskcast in shouldered ring and heated at a controlled rate in a water or glycerin bath-softens and sags downwards a distance of 25 mm under the weight of a steel ball of 9.5 mm diameter (Fig. 4). Asphalt does not have a definite melting point but changes gradually from a solid to a

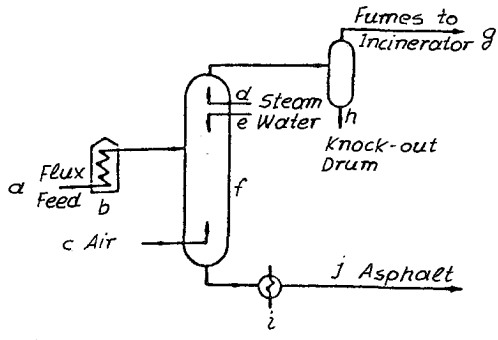


FIG. 2. *AIR BLOWING a. Flux feed; b. Heat; c. Air; d. Steam; e. Water; f. Oxidation tower; g. Fumes to incinerator; h. Knock out drum; i. Heat exchange; j. Blown asphalt.*

fluid during an increase in temperature.

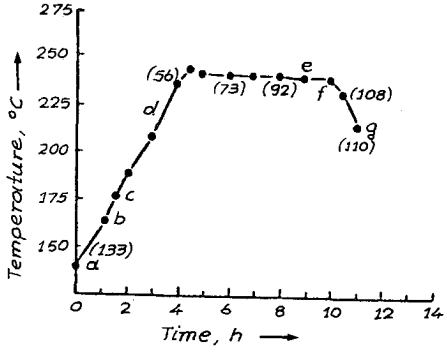


FIG. 3. *a. Begin charge and heat; b. End charge; c. Air injection start; d. Water injection start; e. Air injection reduced, hold test; f. Batch approved, circulate; g. Air and water off, unload.*

Penetration[8] is defined as the distance in tenths of a millimeter that a standard needle vertically penetrates a properly prepared sample of asphalt under known conditions of loading time and temperature (Fig. 5). The harder materials are indicated by the lower number.

The mechanism of oxidation in air blowing of asphalt samples is extremely complicated[9-11] and no detailed information is available. As they believe reactions of the following type can occur:

Air blowing of asphalt in the presence of various catalysts in the

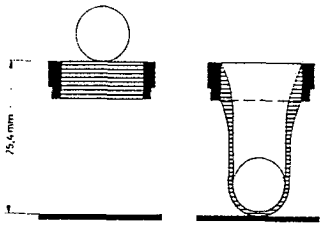


FIG. 4. *SOFTENING POINT TEST*

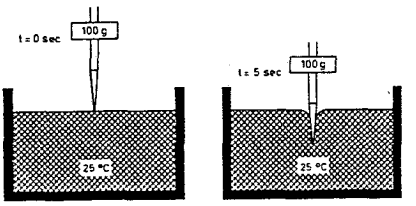
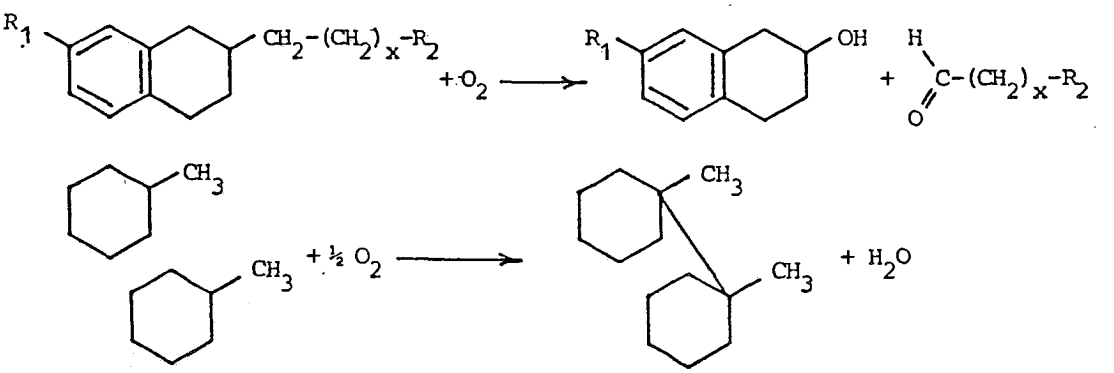


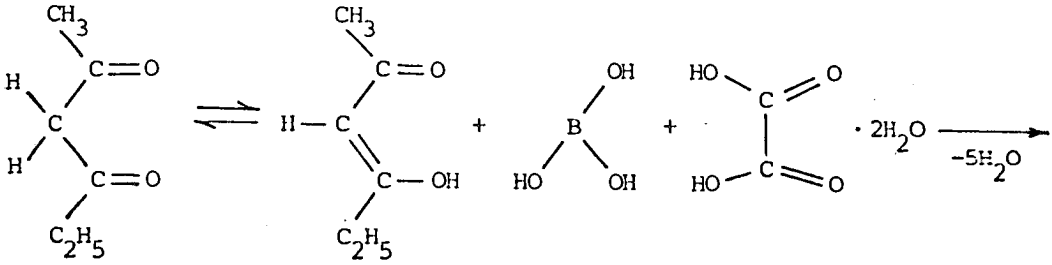
FIG. 5. *PENETRATION TEST*

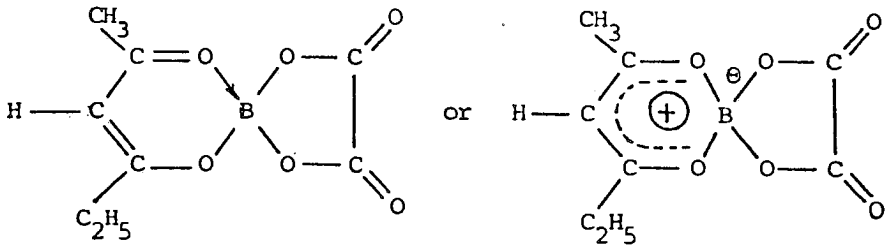
most of the cases gave: reduction of blowing time and change of the softening point/penetration ratio compared to that of air blowing in the absence of catalysts. The most well known catalysts are[12] iron(III) chloride and phosphorous pentoxide. Boron fluoride[12], boric acid[13] and acetylacetone complexes[14] had been also used. Working further on air blowing of asphalt in the presence of catalysts we tested new organoboron complexes.



Since Dilthey[15] and coworkers had studied the reaction of boron compounds with -diketones for the first time, there have been referring many corresponding compounds[16,17]. In our study[18] for synthesis and technological applications of new boron complexes with β-diketones and organic acids (dicarbonic of hydroxy ones) we have synthesized the compounds I, II and III. Their synthesis was based[19] on the reaction of boric acid

with β-diketone and a dicarbonic or hydroxyl-acid:
acetylacetone + boric acid + citric acid.....I
acetylacetone + boric acid + salicylic acid.....II
2,4 hexanedione + boric acid + oxalic acid.....III





III III

These complexes were selected because of their relatively cheep raw materials, good yields and their different structures.

RESULTS - DISCUSSION

We can summerize the results of trial application of new organaboron complexes I, II, III as catalysts in asphalt blowing in the following table:

Complex	Blowing time (min)	Losses (%)	Softening point (^{o}C)	Penetration (0.1 mm,25^{o}C,100g,5sec)
	0		44	89
*	540	6.8	80	15
I	505	6.5	78	19
II	480	6.3	77	20
III	490	6.5	77	19

* no complex was used

From the data it is clear that all the three organoborn complexes we used, gave the same results.

The addition of 0.5% of a new organoboron complex in asphalt blowing gives the following results compared with blowing in the abscence of catalysts:

1. reduction of blowing time
2. reduction of losses during blowing
3. increase of penetration of produced blown asphalt
4. no essential difference in softening point of produced blown asphalt.

Consequently we can draw two major considerations:

a. the reduction of blowing time and losses give a good suggestion for industrial use of new organoboron complexes in asphalt blowing because of saving energy, increase of produced blown asphalt quantity and reduction of pollution from the losses.

b. the increase of penetration of produced blown asphalt can give specified grades of roofing and waterproofing asphalts.

EXPERIMENTAL

A mixture of 80/20: asphalt 80-100 pen (cut 1050-1080), vacuum gas oil (cut 70-1050) from refining crude oil (Arabian light) in Thessaloniki refinery, was used as flux for the trials.

The oxidation vessel is illustrated in Fig. 6.

The conditions employed for the experiments are given below:

Flux quantity for blowing: 25 kg

New organoboron complex (catalyst) quantity: 0,5% in flux;air feeding: 5.3-6.0 $cm^3\ min^{-1}\ g^{-1}$

Temperature during blowing: 235-240^{o}C.

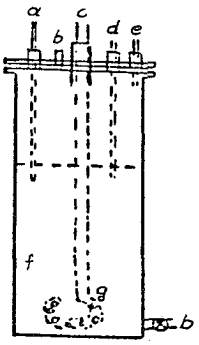


FIG. 6. *OXIDATION VESSEL a. Thermometer; b. Sampling points; c. Air; d. Air-termperature regulator; e. Losses to knock-out drum for measuring; f. Flux; g. Introduction of air into the flux through holes.*

PREPARATION OF ORGANOBORON CATALYST

0.67 mol of β-diketone, 0.67 mol of boric acid and 0.67 mol of organic acid were added together with 500 ml of xylene in a three-necked two-litre flask. A Dean Stark trap was adjusted to the flask for measuring of separated water. The mixture was heated under stirring and refluxed until the total amount of water was separated. Boron complexes were purified by recrystallization (from acetone for I, from toluene for II, from ether/acetone for III).

Data for I (colorless crystalls):

yield: 68%, m.p.:209-12°C.

anal. calc. (%) for $C_{11}H_{13}BO_9$: C 44.04, H 4.37, B 3.60; found (%) C 43.85, H 4.30, B 3.50.

IR (in KBr): 1725(C=O), 1575-1540 (C⋯C⋯C), 1360 (B-O) cm^{-1}.

UV (in ethanol): $\lambda_{max}(\varepsilon)$ = 288 (44.04) nm.

^1H NMR (in CD_3COCD_3): δ = 2.30 (s, CH_3CO), 2.72 (s, $C(CH_2)_2$), 6.33 (s, CH) ppm.

Data for II (colorless crystalls):

yield: 67%, m.p. 132-5°C.

anal. calc. (%) for $C_{12}H_{11}BO_5$: C 58.58, H 4.51, B 4.39; found (%) C 58.83, H 4.55, B 4.28.

IR (in KBr): 1705(C=O), 1575-1550(C⋯C⋯C),1380(B-O) cm^{-1}.

UV (in ethanol): $\lambda_{max}(\varepsilon)$ = 288 (44.01)nm.
^{1}H NMR (in CD_3COCD_3): δ = 2.19(s, CH_3CO), 6.38 (s, CH of β-dik.)ppm.
Data for III (colorless crystalls):
yield: 38%, m.p. 132-3^oC.
anal. calc. (%) for $C_8H_9BO_6$: C 45.33, H 4.28, B 5.10; found (%) C 45.63, H 4.27, B 5.01.
IR(in KBr): 1825, 1785 (C=O), 1570-1540(C⋯C⋯C, 1370(B-O) cm^{-1}.
UV(in ethanol): $\lambda_{max}(\varepsilon)$ = 288 (44.02) nm.
^{1}H NMR (in CD_3COCD_3): δ = 1.23(t, 3H, CH_3CH_2), 2.50(s, CH_3CO), 6.62 (s, CH), 9.78 (q, 2H, CH_3CH_2)ppm.

ACKNOWLEDGEMENT

We wish to thank BITOUMINA S.A. THESSALONIKI for supporting this work by giving us their kind permission to use Factory's Lab and Chemicals.

ΠΕΡΙΛΗΨΗ

ΝΕΑ ΟΡΓΑΝΟΒΟΡΙΚΑ ΣΥΜΠΛΟΚΑ ΩΣ ΚΑΤΑΛΥΤΕΣ ΣΤΗΝ ΟΞΕΙΔΩΣΗ ΑΣΦΑΛΤΟΥ

Ν.Δ.ΟΙΚΟΝΟΜΟΥ[1], Β.Π.ΠΑΠΑΓΕΩΡΓΙΟΥ[2], Α.Φ.ΝΙΚΟΛΑΙΔΗΣ[3]

Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης-Πολυτεχνική Σχολή
[1]Τμήμα Πολιτικών Μηχανικών, Τομέας Επιστήμης & Τεχνολογίας των Κατασκευών
[2]Τμήμα Χημικών Μηχανικών, Τομέας Χημείας
[3]Τμήμα Πολιτικών Μηχανικών, Τομέας Συγκοινωνιών & Οργάνωσης

Στην εργασία αυτή περιγράφονται η παρασκευή και η χρήση νέων οργανοβορικών συμπλόκων των β-δικετονών (ακετυλακετόνης και 2,4-εξανοδιόνης) με δικαρβονικά ή υδρυξυ- οξέα (κιτρικό οξύ, σαλικυλικό οξύ, οξαλικό οξύ), σαν καταλύτες στην οξείδωση της ασφάλτου.

Η οξείδωση (φύσημα με αέρια) δείγματος ασφάλτου από Arabian light παρουσία των οργανοβορικών συμπλόκων σαν καταλύτες, έδωσε ενδιαφέροντα αποτελέσματα σε σύγκριση με την οξείδωση του ίδιου δείγματος αλλά χωρίς τον καταλύτη.

Η βελτίωση αφορά την μείωση του χρόνου οξείδωσης, την μείωση των απωλειών και επίσης την αύξηση της διεισδυτικότητας του προϊόντος.

Αντίστοιχα δεν παρατηρήθηκε αλλαγή στο σημείο μάλθωσης.

REFERENCES

1. Asphat Institute, MS-5, "*Introduction to Asphalt*", p.2, Asphalt Institute, Maryland, (1977)
2. Byerly, F.X. U.S. Patent 524, 130 (1894)
3. Abraham, H. "*Asphalt and Allied Substances*", vol.I, p.477, 6th Ed. Van Nostrand, New York, 1960

4. Traxler, R.N. "*Asphalt, Its Composition, Properties and Uses*", p.121, Reinhold Publ. Corp., New York, 1961
5. Corbett, L.W. and Sworbric, R.E. ASTM. STP 347, 39 (1963)
6. Corbett, L.W. and Urban R. "*Asphalt and Bitumen*" in Ulmann's Encyclopedia of Industrial Chemistry, vol. A3, p.169, VCH Verlagsgesellschaft mbH, Weinheim, 1985
7. ASTM Annual Book of Standards "*Roofing, Waterproofing and Bituminous Materials*", vol.04.04: D 36-76, ASTM, Philadelphia, 1983
8. ASTM Annual Book of Standards "*Roofing, Waterproofing and Bituminous Materials*", vol. 04.03: D 5.78, ASTM, Philadelphia, 1983
9. Corbett, L.W. and Swarbrick, R.E. *Proc. Assoc.Asp. Paving Technologists*, 29, 104 (1960)
10. Depke, F.M. "*Bitumen-und Teerlacke*" s.34, COLOMB, Stuttgart, 1970
11. Hoiberg, A.J. *Proc. Eng. Chemistry*, 19, 450 (1980)
12. Hoiberg, A.J. *Proc. Assoc. Asp. Paving Technologists*, 19, 225 (1950)
13. Lenz, A.P. U.S. Patent 2.375.117 (1945)
14. Moschopedis, S.E. and Speight, J.G. *Fuel*, 57, 235 (1978)
15. Dilthey, W. et al., *Liebigs Ann Chem.*, 334, 300 (1905)
16. Balaban, A. *Colloq. Intern. Centr. Nat. Rech. Scient.* 233 (1970)
17. Mikhailow, B.M. *Pure and Applied Chemistry*, 49, 749 (1977)
18. Economou, N. "*Synthesis, Structure and Technological Application of New Boron Complexes with β-diketons and organic dicarbonic- or hydroxy-acids*" Dissertation, College of Engineering, Aristotle University of Thessaloniki, 1987
19. Heimann, W. and Sagredos, A. *Chem. Ber.*, 98, 1949 (1965)

Chimika Chronika, New Series, 19, 243-248 (1990)

SHORT PAPER

2,3- DISPHOSPHOGLYCERATE; A POTENT PHYSIOLOGICAL INHIBITOR OF PLATELET AGGREGATION, INDUCED BY PLATELET ACTIVATING FACTOR (PAF), IN VITRO.

ANGELOS EVANGELOU, SPIROS KARKABOUNAS AND DEMOS TSOUKATOS.*

Laboratories of Exp. Physiology, Faculty of Medicine and *Biochemistry, School of Sciences, University of Ioannina, P.O. Box 1186, GR-45110 Ioannina Greece.

(Received March 8, 1989.)

Key words : Platelet aggregation, Rabbit Platelet Rich Plasma, Aggregatory effect PAF, 2,3 DPG.

INTRODUCTION

There are evidences that 2,3-disphosphoglycerate (2,3 DPG), an organic phosphate of the erythrocytes, is a potent in vitro inhibitor of platelet aggregation induced by ADP, epinephrine, norepinephrine and collagen (1). On the other hand, 2,3 DPG, acting in concert with Phospholipase-A, enhances in vitro, platelet aggregation, induced by ADP, epinephrine and norepinephrine, as well as, induces in vitro platelet agrregation, in the presence of suboptimal concentration of Na-arachidonate (2,3).

Platelet Aggregating Factor (PAF), is a potent mediator of inflammatory and allergic reaction, which induces an irreversible aggregation of platelets, acting in a very different way than the above mentioned aggregatory substances (4). There are no, as well, sufficient evidences for any physiological inhibitor of PAF, so far (5).

MATERIALS AND METHODS

Rabbit patelets rich plasma (PRP) was prepared according to methods previously described (4). PRP was aspirinated in order to inhibit cycloxygenase and treated by CP/CPK a cleavage of ADP. With this procedure PRP is a very good model for in vitro study of PAF action. (4). PAF was diluted in a solution of BSA/saline in concentrations of 5mg/ml and 10 mg/ml and platelet aggregation was measured in aliquots of 0.5 ml PRP in a platelet ligh transmission aggregometer (model 330, Chrono-log Corp) in 37o C under stirring at 1200 rpm. 2,3 DPG was dissolved in Tris buffer (1M, pH 7.4).

Results and discussion

Results are shown on figure 1 and table 1.

2, 3 DPG, inhibited platelet aggregation induced by 25 pg and 50pg of PAF to a degree proportional to the cocentration of the substance. (Figure 1).

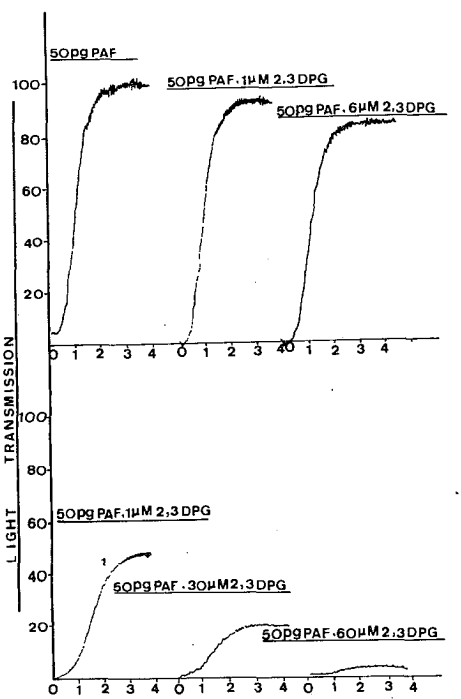


Figure 1: Inhibition by various concentrations of 2,3 DPG, of rabbit platelet aggregation induced by 50 pg of PAF. Note the complete inhibition by 60μM of 2,3 DPG.

Inhibition of the aggregatory effect of PAF, was manifested from concentrations of 18μM of 2,3 DPG, while complete inhibition was succeeded in concentrations of 60μM of 2,3 DPG. (Fig. 1, table 1).

SUBSTANCE	CONCENTRATION	AGGREGATION
2,3 DPG	6μM	0
2,3 DPG	18μM	0
2,3 DPG	30μM	0
2,3 DPG	60μM	0
PAF	5pg	3
PAF	15pg	39
PAF	25pg	66
PAF	50pg	100
PAF 50pg + 2,3DPG	6μM	85
PAF 50pg + 2,3DPG	18μM	48
PAF 50pg + 2,3DPG	30μM	19
PAF 50pg + 2,3DPG	60μM	0
PAF 50pg + 2,3DPG	6μM	66
PAF 50pg + 2,3DPG	18μM	35
PAF 50pg + 2,3DPG	30μM	14
PAF 50pg + 2,3DPG	60μM	0

Table I: Percentenge of rabbit platelet aggregation induced by various concentations of 2,3 DPG and PAF and by simultaneous administration of the two substances.

Inhibition of Paf induced platelet aggregation by 2,3 DPG, remains to be explained.

Suggestions concerning action of 2,3 DPG on cycloxygenase and/or thromboxane synthetase (3) can not explain our results since in aspirinated platelets cycloxygenase has been inhibited by aspirin (4).

It is possible that 2,3 might interact with heme proteins on the outside of platelets plasma membrane. This interaction might be critical

to platelet responsivenes and be influenced to a significant extend by 2,3 DPG. On the other hand 2,3 DPG may as well, inhibit intraplatelet glycolysis, which is a very important proccess for platelet aggregation, induced by PAF (6). There are evidences that PAF plays a role in thrombosis of vascular disease (7) and platelets of patients with β-Thalassemia are very sensitive to PAF induced aggregation (8). Since during hemolysis plasma 2,3 DPG is increased our findings are indicating that this substance may be a key component, in preventing PAF induced thrombosis, during such episodes.

SUMMARY

The aggregatory effect of Platelet Activating Factor (PAF) on rabbit's platelets rich plasma (PRP), was tested in the presence of various concentriations of 2,3-diphosphoglycerate, (2,3 DPG).
2,3-DPG inhibited the aggregatory effect of 25 pg and 50pg of PAF, in a degree proportional to its concentration, from slight, up to complete inhibition when concentration of 2,3 DPG reached 60μM.
2,3 DPG may, thus, be a key component, in preventing thrombosis during intravascular hemolysis.

ΠΕΡΙΛΗΨΗ

2,3-Διφωσφογλυκερικό οξύ: Ενας ισχυρός φυσικός αναστολέας της συγκόλλησης αιμοπεταλίων που προκαλείται από τον Παράγοντα ενεργοποίησης αιμοπεταλίων (PAF).
Αγγελος Ευαγγέλου, Σπύρος Καρκαμπούνας και Δημόκριτος Τσουκάτος *.
Εργαστήρια Πειρ. Φυσιολογίας, Ιατρικού Τμήματος και Βιοχημείας * Χημικού Τμήματος, του Πανεπιστημίου Ιωαννίνων.

Ο παράγοντας Ενεργοποίησης Αιμοπεταλίων (PAF) είναι μία από τις ισχυρότερες ουσίες πρόκλησης συσσώρευσης αιμοπεταλίων, για την οποία δεν έχει βρεθεί μέχρι σήμερα αποτελεσματικός ενδογενής αναστολέας.
Η πρόσθεση 2,3-διφωσφογλυκερικού οξέος (2,3-DPG), σε πλούσιο σε αιμοπετάλια πλάσμα κονίκλου (PRP) διαπιστώθηκε ότι προκαλεί διάφορου βαθμού αναστολή της συγκολλητικής δράσης του PAF στα αιμοπετάλια.

Η ανασταλτική δράση του 2,3-DPG στη συσσωρευτική δράση του PAF φαίνεται να είναι ποσοτική, εμφανίζεται από συγκεντρώσεις 6 μM 2,3 - DPG και γίνεται πλήρης σε συγκεντρώσεις 60 μM 2,3-DPG. Τα ευρήματα αυτά συνηγορούν για την πιθανή προστατευτική δράση του 2,3-DPG στην πρόκληση θρομβώσεων από το PAF, κατά την διάρκεια αιμολυτικών επεισοδίων.

REFERENCES

1. Iatridis, S.G., Iatridis, P.G., Markidou, S.G., and Ragatz, B.H. 2,3-Diphosphoglycerate. A physiological Inhibiotor of Plateler Aggregation Science 187, 259-261, (1975).
2. Iatridis, P.G., and Iatridis, S.G. The combined effect of 2,3-diphosphoglycerate, phospholipase-A and arachidonic acid on platelet aggregation induced by ADP, epinephrine, nor-epinephrine and collagen. XVII Congress of the International Society of Hematology. Paris, France (1978).
3. Iatridis, P.G., Hadd, H., Kotrotsou, M. and Iatridis, S.G. The combined effects of 2,3 DPG and Na-Arachidonate on Platelet Aggregation and on TxA2-formation. Thromb. Res. 42, 177- 185, (1986).
4. Gazenave J.P., Benveniste J., Mustrad J.F.: Aggregation of rabbit platelets by platelet activating factor, is independent or release reaction and the arachidonic patway and inhibited by membraneactive drugs. Lab. Invest. 41, 275-285 (1979).
5. Tsoukatos, D., Demopoulos, C.A., Tselepis, A., Moschidis, M., Donos,A., Evangelou, A. and Benveniste, J. Effects of Cardiolipins on Rabbit Platelet Aggregation in relation to Paf. 2nd International Conferance on Platelet Aggregating Factor (PAF). Catlinburg, Tennessee, USA (1986).
6. Tsagaris, G. Th., Demopoulos, C.A., Kapoulas, V.M. and Tsoukatos, D. The role of glycolysis on platelet aggregation induced by AGEPC (Greek). 1st Hellenic Meeting on Platelet Aggregating Factor. Ioannina, Greece (1987).

7. Benveniste J., Chingard M. : A role of PAF-acether in platelet dependent vascular diseases? Circulation 72, 713-717 (1985).

8. Andrikopoulos M., Demopoulos C.A and Staphopoulou-Sparou R.: Studies on Paf-induced aggregation on Platelets of patients with β-Thalassemia. (Greek). 1st Hellenic Meeting on Platelet Aggregating Factor (Paf). Ioannina, Greece (1987).

Keyword index (Volume 19) 1990

Author index (Volume 19) 1990

CONTENTS

September - December 1990 Volume 19 No 3 - 4