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CONTENTS

NEW SERIES OF SUBSTITUTED PHENETHYLAMINES AND THEIR PHARMACOLOGICAL ACTIVITY

A. PAPADAKI-VALIRAKI, V. GUIOCA, G. PAPAIOANNOU, G. TSATSAS.
Laboratory of Pharmaceutical Chemistry, University of Athens, Athens, Greece.
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Summary

A series of phenethylamines having hydroxy and/or alcoxy groups in 2,3-positions and with or without subsitution on the amino group, were synthesized and their nonoxalate soluble salts were evaluated in a preliminary screening in mice. In further study, the four most effective compounds, N-Diethyl-(2,3-dihydroxy) phenethylamine, N-benzyl -(2,3-dibenzyloxy)-phenethylamine, N-cyclohexyl-(2,3-dibenzyloxy) phenethylamine and (2-benzyloxy-3-methoxy) phenethylamine, compared with mescaline, tended to be more potent than mescaline in depressing locomotor activity, biting response, body temperature and neuromuscular coordination.
Key words: Substituted Phenethylamines

Introduction

It is known that, among the various sympathomimetic drugs the effects of which depend upon release of norephinephrine from stores in adrenergic nerve terminals, the 3,4-dihydroxy-phenethylamines (i.e. catecholamines) are the most potent. According to Daly *et al*[1], however, for a catecholamine to exert a sympathomimetic activity it is not necessary to have the hydroxy groups in 3 and 4 positions of the benzene ring and similar compounds containing hydroxy groups in 2 and 3 positions proved to be sympathomimetic as well. We undertook the task to prepare some more series of phenethylamine having hydroxy and/or alcoxy groups in 2,3-positions and with or without substitution on the amino group, because only few of these compounds have already been described in the literature. The sympathomimetic action of the prepared compounds has also been studied.

Chemistry.

The compounds we have prepared correspond to the general formula (I).

$$CH_2CH_2X$$

$$\begin{array}{c} \text{—OR}_1 \\ \text{—OR}_2 \end{array} \qquad \qquad (I)$$

where $X = -NH_2$, $-N(CH_3)_2$, $-N(C_2H_5)_2$,

$-NHCH_2C_6H_5$, $-NCH_2(CH_2)_3CH_2$, $-NCH_2CH_2OCH_2CH_2$,

$-NHCH(CH_2)_4CH_2$, $-NCH_2(CH_2)_2CH_2$.

In respect to the nature of R_1 and R_2, the above compounds can be classified in five series, i.e.:

Ia :	$R_1 = C_2H_5$, $R_2 = CH_3$
Ib :	$R_1 = CH_2C_6H_5$, $R_2 = CH_3$
Ic :	$R_1 = H$, $R_2 = CH_3$
Id :	$R_1 = R_2 = CH_2C_6H_5$
Ie :	$R_1 = R_2 = H$
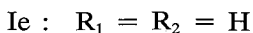
For the preparation of the above compounds the following materials have been used;

a) The suitable 2,3-dialcoxy-benzaldehydes,[2,3,4,5] which were converted either to 2,3-dialcoxy-phenethylamines without any subsition on the amino group (Ia, Ib, Id, X=NH$_2$) through the formation of the corresponding nitrostyrenes, or to 2,3-dialcoxy- phenethylchlorides (I, X=Cl), which reacting with various amines give the phenethylamines of the formula I, with substitution on the amino group (Ia, Ib, Id, X=substituted amino group), and,

b) The 2-benzyloxy-3-methoxy phenethylamines (Ib) and the 2,3-dibenzyloxy-phenethylamines (Id), substituted or not on the amino group, which through a hydragenolysis of the benzyl groups (Pd/C 10%) of their pure hydrochlorides give the corresponding 2-hydroxy-3-methoxy- and 2,3-dihydroxy-phenylethylamines (Ic, Ie).

The series of reactions can be presented as follows:

Compounds Ia, Ib, Id.

$$Ar-CH=CHNO_2^{(10,11)} \leftarrow ArCHO \xrightarrow{\begin{array}{c} C_6H_5CONHCH_2COOH \\ \hline CH_3COONa(CH_3CO)_2O \end{array}} Ar-CH=\underset{\underset{\underset{C_6H_5}{C}}{\overset{\backslash/}{N}}}{\overset{}{C}}-\overset{O^{(8)}}{\overset{}{C}}=O$$

$\downarrow$ LiAlH$_4$

Ia, Ib, Id
X=NH$_2$

For the compounds Ia, Ar = (benzene ring with OC$_2$H$_5$ and OCH$_3$) = Ar$_1$

For the compounds Ib, Ar = (benzene ring with OCH$_2$C$_6$H$_5$ and OCH$_3$) = Ar$_2$

For the compounds Id, Ar = (benzene ring with OCH$_2$C$_6$H$_5$ and OCH$_2$C$_6$H$_5$) = Ar$_3$

$\downarrow$ NaOH

ArCH$_2$COCOONa
$\downarrow$ 1.H$_2$O$_2$
$\downarrow$ 2.HC1
ArCH$_2$COOH[6,7]
$\downarrow$ ROH
ArCH$_2$COOR[8,9]
$\downarrow$ LiA1H$_4$
ArCH$_2$CH$_2$OH[9]
$\downarrow$ SOCl$_2$
ArCH$_2$CH$_2$Cl[9]
$\downarrow$ Amines

Ia, Ib, Id

X=substituted amino group.

Compounds Ic, Ie.

Ib or Id (Hydrochlorides)$\xrightarrow{\text{Pd/C 10\%}}$ Ic, HCl or Ie, HCl.

The compounds so prepared are described in more details in a precedent work.[12]

Biological Evaluation. Female mice of the Swiss-Webster strain weighting 20-25 gm, supplied by Simonsen Laboratories, Inc. (Gilroy, California) were used. In order to determine the effective dose range all the test compounds and mescaline sulfate (a reference compound) were initially evaluated (in one or three mice per dose) at a series of doses ranging from 100 mg/Kg (of the salts) to the inactive dose. The compounds were emulsified in a solution consisting of 0,9% saline and 10% Tween 80, and injected intraperitoneally at the standard rate of 10 ml/kg. Approximately 37% of the compounds produced lethal effects at the dose level of 100 mg/kg and most of the drugs were not active at 8mg/kg.

After the preliminary study the four most effective compound (T 3235, T 3276, T 3277 and T 3362), see table I, and mescaline, were selected to be further evaluated in dose-response experiments conducted with a battery of six relatively objective and sensitive tests. The six short tests: (1) open field locomotor activity,[13] (2) provoked biting response,[14] (3) body temperature obtained with a rectal probe, (4) neuromuscular coordination assayed with a rotarod,[15] (5) grip strength recorded with a Chatillon scale, and (6) pain-sensitivity measured with a hot plate[16] regulated at $55°C \pm 0,5°$, were successively administered to each animal during a seven-minute observation period. The animals were given the series of tests 30 minutes after the intraperitoneal injection and also 60 minutes post injection. In order to conduct the dose-response studies the five compounds the following doses (of the salts, Table I) were evaluated: Compound T 3235, 4, 8, 16 mg/kg; Compound T 3276, 8, 16, 32 mg/kg; Compound 3277, 4, 8, 16, 25, 50 mg/kg; Compound 3362, 4, 8, 16 mg/kg, and mescaline sulfate, 16, 25, 50, 100 mg/kg. Ten animals were tested at each dose level and 30 control animals, injected with the vehicle were also observed.

The five compounds produced a decrease in locomotor activity, in biting response, in body temperature, and in neuromuscular coordination of a considerable number of animals. However, the drugs had no obvious effects on grip strength and pain-sensitivity. None of the agents produced an increase in any response.

Statistical analyses were conducted to examine dose-response relations. The response of an experimental animal to each test was analysed as showing a decrease or no appreciable difference in response relative to a control value (i.e., a criterion, formulated from the data obtained from the responses of the 30 control animals). On the basis of the criterion established for the locomotor activity test, the total number of treated mice that showed a decrease in ambulatory activity was computed for each dose level of each compound and expressed as a percentage. The dose-response curves show that the four test compounds and mescaline reduced spontaneous activity in a dose-related manner. Similarly, the dose-response curves plotted from the data obtained from the tests of biting response, of body temperature, and of neuromuscular coordination show that the depressant effects of the five drugs were dose-dependent.

The median effective dose (ED_{50}) of each compound (i.e., an estimated dose by which 50% of the animals are expected to show a reduction in locomotor activity) and the 95% confidence limits were calculated according to the method of Litchfield and Wilcoxon.[17] Similarly, from the data obtained from the biting, body temperature, and neuromuscular coordination tests, the ED_{50}'s of the five compounds were reckoned and listed with their structures in Table (I).

     A. PAPADAKI-VALIRAKI, V. GUIOCA, G. PAPAIOANNOU, G. TSATSAS.

TABLE I. *Structure - Activity Relation of the Four Most Effective Test Compounds and Mescaline*

$$CH_2CH_2\,R_1$$

$$R_5 \overset{R_2}{\underset{R_4}{\diagup}} \overset{}{R_3} \cdot \mathbf{X}$$
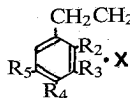
Compound No.	R_1	R_2	R_3	R_4	R_5	X
T 3235	$N\begin{smallmatrix}C_2H_5\\C_2H_5\end{smallmatrix}$	OH	OH	H	H	HCl
T 3276	$NHCH_2C_6H_5$	$OCH_2C_6H_5$	$OCH_2C_6H_5$	H	H	HCl
T 3277	NH◯	$OCH_2C_6H_5$	$OCH_2C_6H_5$	H	H	HCl
T 3362	NH_2	$OCH_2C_6H_5$	OCH_3	H	H	$\begin{smallmatrix}COOH\\CH_2\\|\\CH_2\\COOH\end{smallmatrix}$
Mescaline	NH_2	H	OCH_3	OCH_3	OCH_3	$\frac{1}{2}(H_2SO_4)\,H_2O$

Depressant Effects of the compounds: ED_{50} and 95%
Confidence Limits in micromoles /kg

Compound No.	Locomotor Activity	Biting Response	Body Temperature	Neuromuscular Coordination
T 3235	50 (28-89)	168 (54-520)	65 (32-130)	65 (24-176)
T 3276	95 (43-209)	94 (36-245)	38 (14-102)	70 (32-153)
T 3277	66 (49-90)	62 (20-192)	15 (8-28)	65 (19-221)
T 3362	30 (17-54)	41 (21-82)	17 (10-29)	30 (16-57)
Mescaline	500 (23-1100)	130 (68-247)	74 (47-116)	500 (31-1195)

The relative potencies of the compounds (as measured by the depressant action on locomotor activity) were assessed by testing the significance of potency ratios. In Table II the compounds are arranged in descending order of potency in the first column and across the top row from left to right. The results show that the four test compounds were significantly more potent than mescaline in decreasing ambulatory activity of the mice. The results of the statistical analysis of relative potencies of the compounds with respect to the depressant effects on biting response, body temperature, and neuromuscular coordination are summarized in Tables III, IV and V, respectively. Although the ED_{50}'s of the four test compounds

TABLE II: *Relative Potency of the Compounds in Terms of Their Effects on: Locomotor Activity*

Compound (ED$_{50}$)	Statistics	T3235 (30)	T3277 (50)	T3276 (66)	Mescaline (95)
T3362 (30)	Potency Ratio*	2	2	3	17
	Significance**	No	No	Yes	Yes
	Confidence Limits***			(1-8)	(6-45)
T3235 (50)	Potency Ratio		1	2	10
	Significance		No	No	Yes
	Confidence Limits				(4-26)
T3277 (66)	Potency Ratio			1	8
	Significance			No	Yes
	Confidence Limits				(3-18)
T3276 (95)	Potency Ratio				5
	Significance				Yes
	Confidence Limits				(2-16)

TABLE III: *Biting Response*

Compound (ED$_{50}$)	Statistics	T3277 (62)	T3276 (95)	T3235 (168)	Mescaline
T3362 (41)	Potency Ratio	2	2	4	3
	Significance	No	No	Yes	No
	Confidence Limits			(1-15)	
T3277 (62)	Potency Ratio		2	3	2
	Significance		No	No	No
	Confidence Limits				
T3276 (95)	Potency Ratio			2	1
	Significance			No	No
	Confidence Limits				
T3235 (168)	Potency Ratio				1
	Significance				No
	Confidence Limits				

TABLE IV: *Body Temperature*

Compound (ED$_{50}$)	Statistics	T3362 (17)	T3276 (38)	T3235 (65)	Mescaline
T3277 (15)	Potency Ratio	1	3	4	5
	Significance	No	No	Yes	Yes
	Confidence Limits			(2-11)	(2-11)
T3362 (17)	Potency Ratio		2	4	4
	Significance		No	Yes	Yes
	Confidence Limits			(2-9)	(2-9)
T3376 (38)	Potency Ratio			2	2
	Significance			No	No
	Confidence Limits				
T3235 (65)	Potency Ratio				1
	Significance				No
	Confidence Limits				

TABLE V: *Neuromuscular Coordination.*

Table V

Compound (ED$_{50}$)	Statistics	T3235 (30)	T3277 (50)	T3276 (66)	Mescaline (95)
T3362 (30)	Potency Ratio	2	2	2	17
	Significance	No	No	No	Yes
	Confidence Limits				(6-49)
T3235 (50)	Potency Ratio		1	1	8
	Significance		No	No	Yes
	Confidence Limits				(2-29)
T3277 (66)	Potency Ratio			1	8
	Significance			No	Yes
	Confidence Limits				(2-35)
T3276 (95)	Potency Ratio				7
	Significance				Yes
	Confidence Limits				(2-23)

* Potency Ratio = (ED$_{50}$ of a Compound Listed in the Top Row) + (ED$_{50}$ of the Comparative Compound in the First Column)
** Significance at the 5% Level
***95% Confidence Limits

calculated from the data obtained from the biting test were different from that of mescaline, the statistical test revealed that the differences were not significant (Table III). Analysis of body temperature pointed out that Compounds 3277 and 3362 were significantly more potent than mescaline as hypothermic agents (Table IV). The last table indicates that all the tests compounds were significantly more potent than mescaline in decreasing neuromuscular coordination. In general, the four test compounds tended to be more potent than mescaline in depressing locomotor activity, biting response, body temperature, and neuromuscular coordination.

With regard to the structures of the test compounds, the structure of Compound T 3362 shows the most resemblance to that of mescaline. However, when the potencies of the test drugs are considered, the potency of Compound T 3362 is least similar to that of mescaline.

Acknowledgments

Περίληψις

Νέαι ὑποκατεστημέναι φαιναιθυλαμίναι καὶ φαρμακολογικὴ δρᾶσις αὐτῶν
Ἡ παροῦσα ἐργασία ἀφορᾷ εἰς τὴν σύνθεσιν καὶ τὴν φαρμακολογικὴν μελέτην νέων φαιναιθυλαμινῶν. Συγκεκριμένως παρεσκευάσθησαν:
1) 2,3 -διαλκοξυ-φαιναιθυλαμίναι μετὰ ἢ ἄνευ ὑποκαταστάτου ἐπὶ τοῦ ἀζώτου, ἐκ τῶν καταλλήλων 2,3-διαλκοξυβενζαλδεϋδῶν αἱ ὁποῖαι μετατρέπονται εἰς τὰς μὴ ὑποκατεστημένας ἐπὶ τοῦ ἀζώτου φαιναιθυλαμίνας μέσω τοῦ ἀντιστοίχου

νιτροστυρενίου, ἐνῷ εἰς τὰς ὑποκατεστημένας, μέσω τῶν ἀντιστοίχων 2,3-διαλκοξυφαιναιθυλοχλωριδίων δι' ἐπιδράσεως ἐπ' αὐτῶν διαφόρων ἀμινῶν. 2) 2-ὑδροξυ-3-μεθοξυ καὶ 2,3-διυδροξυφαιναιθυλαμῖναι μετὰ ἢ ἄνευ ὑποκαταστάσεως ἐπὶ τοῦ ἀζώτου, κατόπιν ἀποβενζυλιώσεως διὰ καταλυτικῆς ὑδρογονώσεως παρουσίᾳ Pd/C τῶν ἀντιστοίχων 2-βενζυλοξυ-3-μεθοξυ καὶ 2,3-διβενζυλοξυαμινῶν.

Τὰ ληφθέντα προϊόντα ὑπεβλήθησαν εἰς προκαταρκτικὴν φαρμακολογικὴν μελέτην δοκιμασθέντα ἐπὶ μυῶν. Τὰ πλέον δραστικὰ ἐκ τούτων, δηλαδὴ ἡ N-βενζυλο - (2,3-διβενζυλοξυ) - φαιναιθυλαμίνη, ἡ N - κυκλοεξυλο-(2,3-διβενζυλοξυ) φαιναιθυλαμίνη, ἡ N-διαιθυλο-(2,3-διυδροξυ) φαιναιθυλαμίνη καὶ ἡ 2-βενζυλοξυ-3-μεθοξυ-φαιναιθυλαμίνη, εἰς περαιτέρω μελέτην καὶ συγκρινόμενα μετὰ τῆς μεσκαλίνης, ἀπεδείχθησαν ἰσχυρότερα ταύτης ὅσον ἀφορᾷ εἰς τὴν καταστολὴν τῆς κινητικῆς δράσεως, τῆς ἀνταποκρίσεως εἰς τὴν δῆξιν, τοῦ νευρομυϊκοῦ συντονισμοῦ καὶ τῆς θερμοκρασίας τοῦ σώματος.

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CONDUCTOMETRIC BEHAVIOR AND ION-PAIR FORMATION OF SYMMETRICAL TETRAALKYLAMMONIUM HALIDES IN VARIOUS SOLVENT MIXTURES

D.A. JANNAKOUDAKIS, G.C. RITZOULIS
University of Thessaloniki, Greece
(Received March 16, 1977)

Summary

The conductance of symmetrical tetraalkylammonium halides was measured in the following solvent mixtures: methanol-water, methanol-N,N'-dimethylformamide and N,N'-dimethylformamide-benzene at 25°C. All conductance data were evaluated by the Fuoss-Onsager-Skinner equations for associated and unassociated electrolytes and the resulting values of limiting equivalent conductance, association constant and "ion-size" parameter were reported. In all solvent mixtures used, the limiting equivalent conductances of the tetraalkylammonium ions decrease as the crystallographic radii increase. Although the dielectric constant of N,N'-dimethylformamide is low, the presence of N, N'-dimethylformamide in the solution prevents the ion-pair formation owing to its dipolar aprotic nature.

Key words: equivalent conductance, association constant, N,N'-Dimethylformamide, Methanol, Benzene.

Introduction

During the last few years the use of nonaqueous solvents and solvent mixtures as media for various electrochemical investigations has been of increasing interest, on the other hand the symmetrical tetraalkylammonium ions are of great theoretical interest being large in size and having a great variation of size, symmetrical shape, low charge and some of their salts are soluble in many solvents.

A great deal of research into the properties of the above mentioned salts in a large variety of solvents and solvent mixtures has been done. The high sensitivity of conductance measurements enables us to obtain precise results which lead to an explanation of the ionic behavior and solvent properties.

In the present investigation a systematic conductometric study has been undertaken on the ionic behavior of quaternary symmetrical tetraalkylammonium halides in three solvent systems, methanol (MeOH)-water, methanol-N,N'-dimethylformamide (DMF) and benzene-N,N'-dimethylformamide (DMF). These mixtures are very often used in electrochemistry. For each solvent system the Fuoss-Onsager-Skinner equation has been applied to calculate the conductometric parameters: the limiting equivalent conductance (Λ_o), the "ion size" parameter (å) and the association constant (K_A).

Experimental section

A conductance bridge, Beckman Model PC-18A was used for the conductance measurements, using a frequency of 3000 c/sec. It was fundamentaly an alternating current Wheaston bridge having an oscilloscope to determine the balance point.

The cell was held at constant temperature in an oil-filled thermostat. The bath temperature was 25 ±0,002°C as indicated by a Beckmann thermometer set against a N.B.S. thermometer.

The two conductance cells used, were similar to that described by Dagget Bair and Kraus.[1] Their constants were calibrated using Jones and Bradshaw[2] standard values of conductivity of potassium chloride solution at 25°C and the constants were found equal to 1,024 cm^{-1} and 1,031 cm^{-1}. The dielectric constants (D) of the various mixtures used were determined by means of a Dipolmeter type DM 01 which is based on the heterodyne principle.

Densities were measured at 25°C using a Sprengel pycnometer while for the measurement of viscosities we used an Ubbehlode viscometer, calibrated with conductivity water (η = 8,93.10^{-3} poise, d = 0,99707g.cm^{-3} at 25°C).[3]

The various solvent mixtures used in the conductance measurements were prepared by v/v. Stock solutions of the electrolytes were prepared for the respective solvent mixtures. Conductance runs were made by adding certain quantities of stock solution[4,5] into the cell where there was a known quantity of solvent. Special care was taken to avoid contact between the solution and the free atmosphere.

The *Me$_4$NBr tetramethylammonium bromide* (Merk AG, für die polarographie) was recrystallized twice from aceton-methanol (4.1) by adding an equal volume of acetone and dried at 110°C for three days.[6]

The *Me$_4$NI tetramethylammonium iodide* (Fluka AG Puriss), was recrystallized twice from conductivity water and dried in a vacuum desiccator.[6]

The *Et$_4$NBr tetraethylammonium bromide* (Karlo Erba R.P.), was recrystallized twice from boiling methanol-acetone (1:3) by adding an equal volume of acetone, dried and stored over P$_2$0$_5$.[6]

The *Et$_4$NI tetraethylammonium iodide* (Fluka AG puriss), was recrystallized twice from aceton-methanol and dried under vacuum.[6]

The *Bu$_4$NI tetra-n-butylammonium iodide* (Fluka AG puriss), was recrystallized twice from conductivity water and dried.

The *methanol used* for the experiments was purified as recommended by Evers and Knox.[7] It was refluxed for 24 hours with magnesium, distilled and then refluxed again with AgNO$_3$ for 24 hours in absence of moisture. The methanol was distilled again and shaked for 24 hours with activated alumina and was distilled again. The middle cut was used. It had a specific conductivity of 0,8-2.10^{-7} mho.cm^{-1} in accordance with the bibliography.[8,9]

The *N,N'-dimethylformamide* (Fluka AG) was shaken with Linde type 4A molecular sieves and then distilled under reduced pressure.

The *benzene* (Fluka AG) was purified by shaking with 4X Linde and distillation.

Results

The physical properties of various solvent mixtures used such as density (d), viscosity (η) and dielectric constant (D) are summarized in Table I.

TABLE I. *Physical Properties of Solvent Mixtures at 25°C.*

	Methanol – Water		
MeOH % v/v	d (g. cm^{-1})	$\eta \times 100$ poise	D
100	0.7866	0.544	32.62
90	0.8227	0.873	40.20
80	0.8590	1.158	45.82
70	0.8831	1.350	50.95
60	0.9073	1.500	55.66
40	0.9430	1.555	63.40
20	0.9700	1.295	71.72
	N,N'-dimethylformamide-Benzene		
DMF % v/v			
100	0.9455	0.790	38.01
90	0.9375	0.779	33.40
75	0.9278	0.747	27.20
60	0.9186	0.722	21.10
	Methanol-N,N'-dimethylformamide		
MeOH % v/v			
100	0.7866	0.544	32.62
90	0.8049	0.561	34.01
75	0.8318	0.584	35.51
50	0.8670	0.633	37.12
25	0.9101	0.705	37.70
10	0.9305	0.760	37.72

The various equivalent conductances and the corresponding concentrations for Me$_4$NI are given as an example in Table II.

The data were analyzed by the Fuoss-Onsager-Skinner[10] conductance equations. In the cases where association was detected, the Fuoss-Onsager-Skinner equation was used in the following form,

$$\Lambda = \Lambda_o\text{-}Sc^{1/2}\ \gamma^{1/2} + E'_1c\gamma\ln\ (6E'c\gamma) + Lc\gamma\text{-}K_Ac\gamma f^2\Lambda,$$

where Λ is the observed equivalent conductances, Λ_o the limiting equivalent conductance, S the Onsager coefficient, c the concentration of the salt, γ the fraction of salt present as free ions, $E' = E'_1\Lambda_o\text{-}E'_2$ the Fuoss-Onsager coefficient, L an explicit function of the "ion-size" å, f the activity coefficient and K_A the association constant.[10,11]

The data were analyzed on a Univac 1106 computer in order to find the values of $\Lambda°, K_A$ and å which minimize the sum of squares of $\Delta\Lambda_i$,[12,13]
where

$$\Delta\Lambda_i = \Lambda_{obs}\text{-}\Lambda_{calc}$$

Thus we calculated the values of the systems parameters where association was observed. (The value eg. $K_A = 17$ which corresponds to Me$_4$NI in methanol

TABLE II. *Equivalent Conductances of Me₄NI in Methanol-Water Mixtures at 25°C.*

$C \times 10^4$ gm.equiv.lit^{-1}	Λ Mho.cm.^{2}equiv^{-1}	$C \times 10^4$ gm.equiv.lit^{-1}	Λ Mho.cm.^{2}equiv
		Me₄NI	
100% in MeOH		90% v/v in MeOH	
10.5645	120.92	3.3225	91.77
17.6075	117.63	4.9354	91.05
28.1720	113.60	7.5551	89.93
35.2115	111.47	10.0927	89.15
45.7796	108.97	12.5519	88.51
52.8226	106.93	17.2495	87.36
70.4302	104.12	21.6740	86.50
		25.8485	85.83
		31.6861	84.86
		37.0676	84.07
80% v/v in MeOH		70% v/v in MeOH	
2.9232	77.72	4.6248	70.08
5.7888	76.82	9.1006	69.23
8.5995	76.18	13.4342	68.66
13.1640	75.36	17.6324	68.10
17.5854	74.59	25.6471	67.35
21.8703	74.02	33.1904	66.72
30.0553	72.99	40.3026	66.15
37.7645	72.22	47.0198	65.66
45.0381	71.60	53.3738	65.24
55.2093	70.74	59.3934	64.83
64.5859	70.09	65.1043	64.45
70.4403	69.67		
60% v/v in MeOH		40% v/v in MeOH	
10.5521	66.38	7.0030	69.37
13.2548	66.00	9.3551	69.09
17.7068	65.58	11.6346	68.82
22.0214	65.20	13.8448	68.62
30.2629	64.60	15.9889	68.43
38.0253	64.11	18.0697	68.25
45.3492	63.67	20.0900	68.10
55.5906	63.14	22.0525	67.96
65.0320	62.69	23.9594	67.85
70.9268	62.41		
20% v/v in MeOH			
2.7335	85.46		
4.0605	85.16		
6.2157	84.67		
8.3034	84.48		
10.3267	84.29		
12.2885	84.11		
14.1915	83.90		
16.0384	83.75		
17.8316	83.58		
19.5743	83.44		
22.9115	83.16		
26.0687	82.90		
29.0593	82.71		

means that between the activity of tetraalkylammonium ions and the activity of iodine ions there is a relationship of the form

$$\frac{\alpha_{Me_4NI}}{\alpha_{Me_4N+} \cdot \alpha_{I-}} = 17).$$

In the cases where no association appears or the values of association constant

are not significant the calculations were made by means of the equation:

$$\Lambda = \Lambda_o - Sc^{1/2} + E'c\ln(6E'_1c) + Lc$$

which is a two parameter equation [14-16]. The estimation of Λ_o was made graphically using the expresion $\Lambda' = \Lambda_o + L(\alpha)c$. Applying for example this technique to the system Me_4NI in 60% v/v methanol-water Λ_o was found to be equal to 68.70 as indicated in Fig.1. Using the full equation for the same system the values for $\Lambda_o = 68.69$, å $= 2.3$, $K_A = 1.0$ were obtained.
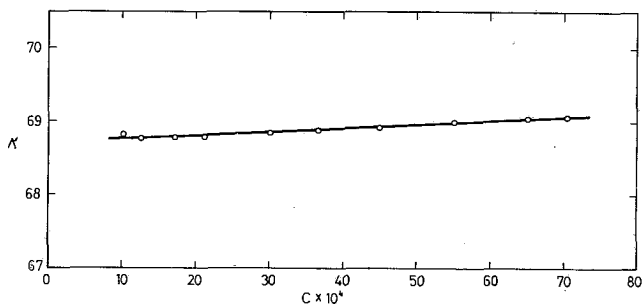
· FIG. 1. *A plot of Λ'vs concentration of Me₄NI in 60% v/v methanol-water.*

As it is shown in the above example, the agreement between the values of Λ_o obtained by the two methods is very satisfactory. The results of calculations in the case of Me_4NI in methanol-water are also given as an example in Table III.

TABLE III. *Derived Conductance Parameters for Me₄NI in MeOH-Water Mixtures at 25°C.*

MeOH % v/v	Λ_o	K_A	å	$\sigma_\Lambda = (\dfrac{\Sigma\Delta\Lambda^2}{N})^{1/2}$
100	131.44	17	3.4	0.27
90	94.80	14	4.8	0.06
80	79.61	7	3.7	0.02
70	72.00	4	3.0	0.04
60	68.69	1	2.3	0.03
40	71.08	—	—	
20	86.56	—	—	

Following the same procedure the conductance parameters of all the systems examined, were calculated and the results were tabulated in Tables IV-VI.

TABLE IV. *Derived Conductance Parameters for Tetraalkylammonium Salts in MeOH-H₂O at 25°C.*

MeOH % v/v	Λ_o	K_A	å	σ_Λ
		Et₄NI		
100	122.50	15	3.3	0.04
90	87.08	8	3.0	0.06
80	73.23	3	3.1	0.06
60	62.58	—	—	—
40	63.12	—	—	—
20	77.22	—	—	—

TABLE IV. (Continue)

MeOH % v/v	Λ_o	K_A	å	σ_A
		Bu$_4$NI		
100	102.15	18	3.4	0.06
90	73.84	9	2.8	0.03
80	62.18	6	3.0	0.05
60	53.70	—	—	—
40	54.85	—	—	—
20	67.30	—	—	—
		Me$_4$NBr		
100	124.77	14	3.1	0.05
90	90.50	10	3.6	0.03
80	76.09	—	—	—
60	67.48	—	—	—
40	70.60	—	—	—
20	86.60	—	—	—
		Et$_4$NBr		
100	116.70	11	3.6	0.05
90	82.96	—	—	—
80	71.20	—	—	—
60	61.28	—	—	—
40	63.50	—	—	—
20	78.14	—	—	—

TABLE V. *Derived Conductance Parameters for Tetraalkylammonium Salts in Methanol-DMF at 25°C.*

MeOH % v/v	Λ_o	K_A	å	$\Lambda_o\eta$	σ_A
		Et$_4$NI			
100	122.50	15	3.8	0.67	0.04
90	118.12	5	2.8	0.66	0.04
75	112.95	—	—	0.66	—
50	104.00	—	—	0.66	—
25	94.34	—	—	0.66	—
10	89.52	—	—	0.68	—
0	87.80	—	—	0.69	—
		Bu$_4$NI			
100	102.15	18	3.4	0.56	0.06
90	99.04	14	2.8	0.56	0.05
75	94.31	—	—	0.55	—
50	87.93	—	—	0.56	—
25	81.71	—	—	0.57	—
10	78.84	—	—	0.59	—
0	77.76	—	—	0.61	—
		Et$_4$NBr			
100	116.70	11	3.6	0.64	0.05
90	113.24	—	—	0.63	—
75	108.63	—	—	0.64	—
50	102.52	—	—	0.64	—
10	88.81	—	—	0.64	—
0	88.64	—	—	0.67	—

TABLE VI. *Derived Conductance Parameters of Tetraalkylammonium Salts in DMF-Benzene at 25°C.*

DMF % v/v	Λ_o	K_A	å	σ_Λ
		Et₄NI		
100	87.80	—	—	—
90	89.45	9	3.3	0.02
75	91.44	42	4.5	0.04
60	91.70	182	8.6	0.12
		Bu₄NI		
100	77.76	—	—	—
90	78.98	2	2.9	0.06
75	79.87	11	3.3	0.02
60	80.46	46	3.4	0.08
		Et₄NBr		
100	88.64	—	—	—
90	90.49	17	3.1	0.05
75	91.56	48	3.5	0.03
60	91.72	180	3.8	0.10

Discussion

According to the results obtained from the study of the ionic and conductometric behavior of tetraalkylammonium iodides and bromides in the various solvent mixtures used we arrived to the following conclusions.

The results are in a very good agreement whith the Fuoss-Onsager-Skinner equation in all systems used. All salts examined show a perceptible association in methanol solutions, which disappears when relatively small quantities of water were added. This effect is due to the increase of dielectric constant of the solvent. As it is shown in Table III there is an association in the region of methanol content up to 80%. Above this percentage, that is in water content above 20%, all the salts studied show a complete dissociation.

The limiting equivalent conductance of tetraalkylammonium salts in methanol-water mixtures changes significantly with the increase of water. It obtains its highest value in pure methanol and decreases as the amount of water increases up to about 45% in methanol. Further addition of water increases again the value of Λ_o. This effect is due to the change of viscosity which shows a maximum in the region where a minimum of the limiting equivalent conductance appears.

As it is shown the product $\Lambda_o \cdot \eta$ shows no constancy. This fact is due to the different size of radii of solvated ions in the various methanol-water mixtures as well as to the hydrogen bonds formed between the molecules of the solvent.

The limiting equivalent conductance Λ_o, of tetraalkylammonium salts having common anion, varies inversely proportional to the size of cation. This effect is due to the fact that the mobility of Bu_4N^+ is less than that of Et_4N^+ which in turn is less than the mobility of Me_4N^+ in all methanol-water mixtures studied.

In methanol-DMF solutions the salts examined show practically no association up to 10% in DMF although the dielectric constant is relatively low, while in methanol-water mixtures the same salts show significant association even for higher values of dielectric constant. This fact suggests that the association can not be attributed exclusively to the dielectric constant but also to the chemical factor

of the solvent system. Thus it is possible for the same value of dielectric constant and for the same electrolyte to have different values of association constant depending on the chemical nature of the solvent.

The limiting equivalent conductance in methanol-DMF solutions decreases as the amount of DMF increases. This is due to the variation of the viscosity. The product $\Lambda_o \cdot \eta$ is relatively constant, and thus we can conclude that the radii of solvated ions remain practically unchanged.

Also for the above studied systems Λ_o increases as the size of cation decreases. This is due to the fact that the mobility of Bu_4N^+ is less than that of Et_4N^+.

In DMF-benzene solvent mixtures, the association is very significant because of the relatively low dielectric constant, which the above solvent mixtures present. The values of association constant increase as the amount of benzene increases because of the decrease of the dielectric constant of the solvent.

Λ_o increases with the amount of benzene. This effect is due to the change of viscosity of the solvent systems used. The product $\Lambda_o \cdot \eta$ practically remains constant, and thus we can conclude that the radii of solvated ions remain practically constant.

Also it can be said that for the tetraalkylammonium salts having common anion, Λ_o increases as the size of cation decreases. This is due again to the lower mobility of Bu_4N^+ compared to that of Et_4N^+.

Περίληψη

'Αγωγιμομετρικὴ συμπεριφορὰ καὶ σχηματισμὸς ζευγῶν ἰόντων σὲ συμμετρικὰ τετρααλκυλαμμωνιοάλατα μέσα σὲ διάφορα μικτὰ διαλυτικὰ συστήματα.

Στὴν ἐργασία αὐτὴ μελετᾶται ἡ ἀγωγιμότητα συμμετρικῶν τετρααλ-κυλοαμμωνιοαλάτων στὰ ἑπόμενα διαλυτικὰ συστήματα: μεθανόλη-νερό, μεθανόλη-N-N'-διμεθυλοφορμαμίδιο καὶ N,N'-διμεθυλοφορμαμίδιο-βενζόλιο στοὺς 25°C.

Όλα τὰ ἀγωγιμομετρικὰ δεδομένα ἀναλύθηκαν μὲ τὶς ἐξισώσεις FUOSS-ONSAGER-SKINNER καὶ βρέθηκαν οἱ τιμὲς τῆς ἰσοδύναμης ἀγωγιμότητας στὴν ἄπειρη ἀραίωση, Λ_o, τῆς σταθερᾶς συζεύξεως, K_A καὶ τῆς ἰονικῆς παραμέτρου å τῶν ἁλάτων αὐτῶν.

Σ'ὅλα τὰ διαλυτικὰ συστήματα ποὺ μελετήθηκαν βρέθηκε ὅτι ἡ ἰονικὴ ἀγωγιμότητα τῶν τετρααλκυλαμμωνιοϊόντων ἐλαττώνεται μὲ τὴν αὔξηση τῆς κρυσταλλογραφικῆς τους ἀκτίνας.

Διαπιστώθηκε ὅτι ἡ ὕπαρξη τοῦ N,N'-διμεθυλοφορμαμιδίου στὸ διάλυμα παρεμποδίζει τὸ σχηματισμὸ ζευγῶν ἰόντων, παρὰ τὸ γεγονὸς ὅτι ἡ διηλεκτρικὴ σταθερὰ αὐτοῦ εἶναι σχετικὰ μικρή. Τοῦτο ἀποδίδεται στὴ σχετικὰ μεγάλη διπολικὴ ῥοπὴ τοῦ N,N'-διμεθυλοφορμαμιδίου.

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STUDY ON THE SORPTION OF CdSO$_4$ BY δ-MANGANESE OXIDE

A. KOVATSIS, K. FYTIANOS and B. NATHANAEL
Chair of Chemical and Industrial Toxicology and Department of Legal Medicine -Toxicology, Aristotelian University of Thessaloniki Greece
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Summary

This study was undertaken to investigate the sorption of Cd by β-MnO$_2$ and δ-MnO$_2$ at 22 and 45°C with wide ranges of pH aqueous systems. We also investigated the influence of other cations on the sorption of CdSO$_4$ on δ-MnO$_2$. The β- and δ- forms of MnO$_2$ were prepared and characterized by X-ray diffraction, chemical analysis and surface area. The sorption isotherms of Cd^{2+} by δ-MnO$_2$ at pH 5.4 was carried out for equilibrium time of one hour. On the contrary Cd^{2+} has not given any sorption on β-MnO$_2$ even up to 120 h experiment. When Cd^{2+} was sorbed on δ-MnO$_2$, Mn was found in the solution phase.

In conclusion we summarize that Cd^{2+} may be sorbed by δ-MnO$_2$ over the entire pH range 5-10. At pH 5.4 the rate was 5.41×10^{-4} g-ion/g. On the contrary β-MnO$_2$ does not sorbe Cd^{2+}. This is a great importance in the case that we are planning to use manganese oxides for scavenging Cd^{2+} from its solutions alone or in the presence of other cations.

Key Words: Sorption, δ-MnO$_2$: Elimination of Cd^{2+}.
Abbreviations: x/m: MCd^{2+} sorbed /gMnO$_2$
C: equilibrium solution concentration of Cd (not sorbed) M/L

Introduction

The role of hydrous manganese oxides in the control of metal ions in aqueous solutions, especialy heavy metal ions, has been a continuing subject of study in many areas of research.

Lockwood and Chen[1] reported that of the various adsorbents, manganese dioxides appear to be the most effective with iron oxide to a lesser degree.They studied the adsorption of Hg(II) by hydrous manganese oxides suspended in solution with wide range of pH, chloride concentration and ionic strength. Manganese, on the other hand, though not as abundant as iron, is nevertheless nearby ubiquitous in its appearance in natural waters, and the role of it in removing metals from ocean water seems to be important.[2] Jenne,[3] in reviewing the available information on the controls on Mn, Fe, Co, Ni, Cu and Zn in soils sediments and fresh water, concluded that the hydrous oxides of Mn and Fe, in general, furnish the principal controls on fixation of these heavy metals in soils and fresh water sediments.

Manganese oxide with activated carbon tried as hydrogen sulfide adsorbent agent and there is in progress a work on the basic nature of the MnO$_2$- sawdust to produce a more active filter material.[4] Rosselt *et al.*[5] studied the adsorption of magnesium and calcium on manganese oxide. Kiang, Li and Rothfus[6] studied the

kinetic of absorption of sulfur dioxide by manganese dioxide. Morgan and Stumm[7] explained the high sorption capacities of δ-MnO_2 for metal ions as due either to surface complex formation or ion exchange. Loganathan and Burau[8] examined sorption of Co, Zn, Ca and Na by δ-MnO_2 at 24°C and pH 4. The adsorptive behavior of 14 radionucleotides from seawater on MnO_2 precipitated in situ was examined and comparisons made with the sorptive capacity of $Fe(OH)_2$ for the same nuclides.[9] Gabano et al[10] studied in detail the adsorption properties of γ-MnO_2 in electrolyte solutions of many metals.

The main purpose of this work was to evaluate the role of manganese oxides in the scavenging and transport of Cd^{2+} in water alone or in the present of other cations. It is a well known fact nowadays that Cd and other metal-salts pollute water and cause other secondary effects upon water quality and aquatic life.[11-15] These salts mainly come from industrial effluents and there is of a great importance problem to eliminate or adsorb them on several sorbents.

Experimental

Reagents. Reagents grade chemicals were used without further purification. To prepare manganese oxides we used $Mn(NO_3)$. $4H_2O$ (Merck) and $KMnO_4$ (Mallinckrodt Chem. Woorks). The water used was bi-distilled water and the vessels were either pyrex glass or polyethylene.

In order to cover the pH range 2.6-13.0 we use two buffers solutions. The first was prepared according to Michaelis by mixing appropriate volumes of M/7 sodium acetate, M/7 barbital sodium and N/10 hydrochloric acid (pH range 2.6-9.4). The second was prepared according to Sörensen from 1M solution of glycine, N/10 sodium chloride and N/10 sodium chloride and N/10 sodium hydroxide (pH range 8.4-13.0)

Preparations and Equipment. The β-MnO_2 was prepared according to Lux [16] by heating of $Mn(NO_3)_24H_2O$ at 190°C for 24h and at 450°C also for 24h.

The δ-MnO_2 was prepared according to Buset et al.[17] by the following reaction:

$$2KMnO_4 + 8HCl = 2\ MnO_2 + 3\ Cl_2 + H_2O + 2\ KCl$$

X-ray diffraction data on the β and δ-MnO_2 sample were obtained by using a 114.6mm diameter powder diffraction camera and Moka radiation in a Philips X-ray Generator. The Moka was generated at 30 KV and 25mA(β-MnO_2) and at 40KV and 25 mA(δ-MnO_2). The sample were irradiated 4h. The X-ray powder pattern of the oxides indicated a good degree of crystallinity with 5or 6 lines. The d-spacings at 3.06,2.38, and 1.62Å permit a sufficient characterization of β-$MnO._2$ The spacings at 2.33, 1.35, and 6.35 Å were obtained for δ-MnO_2 X-ray diffraction lines of δ-MnO_2 shows absence of K in the prepared product.

Surface area of the oxides was determined by BET-N_2 method [18] using an area-meter apparatus (Ströhlein Co, W. Germany). By this method the β-MnO_2 had a surface of 0.83 m^2/g and the δ-MnO_2 had a surface of 22.12 m^2/g.

The average oxidation state of Mn in the oxides used in our experiments was determined iodometrically according to Kessick.[19] This showed that the composition of the two oxides were β-$MnO_{1,93}$ and δ-$MnO_{1,86}$. Chemical determination of Mn and Cd performed with a 303 model Perkin Elmer Atomic Absorption Spectrophotometer (AAS) with a Hitachi-Perkin Elmer recorder and three slot burner.

Determinations of PH performed with WtW PH-meter model 390 (Wissenschaftlich-Technische Werkstation GMBH Germany).

Procedure. Sorption experiments were carried out in constant temperatures at 22.0±0.5 and 45.0±0.5 C°. The equilibrium time for the sorption of Cd ion by δ-MnO$_{,2}$ was 1 hour. Sorption of Cd^{2+} was done at pH 5.4. In the standard procedure 0,1 gr of δ-MnO$_2$ was dispersed in 50 ml solution of 400 ppm Cd.$^{2+}$ After mechanical agitation at the equilibrium time of 1 hour, the suspensions were filtrated and analysed by AAS for Cd and Mn. The same experiment for β-MnO$_2$ has not given any adsorption even for 120h agitation. This may be attributed to the great surface difference and other parameters.

In order to investigate the interference of other cations (Pb, Cu, Mg, Zn) to the whole procedure of sorption, we added these cations in several consentrations.

Results

Sorption and ionic strength effects.

The sorption of Cd^{2+} on δ-MnO$_2$ at pH 5.5 and 22.0±0.5C° as function of time is shown in fig. 1. The experiment shows that after one hour the extention of the sorption has not given reasonable difference for time up to 160 h. So during the time of 1 hour, more than 90 % of the sorption has been completed under the experimental conditions.

The fig. 2. illustrates the influence of ionic strength (potassium nitrate) on the sorption of cadmiun at pH 5.4. The sorption reduced in the presence of 1 M KNO$_3$ by comparison with the no added salt.

pH effect. In fig. 3 the equilibrium sorption of Cd^{2+} is shown as function of pH over the pH range of 2.6-10.5. This is an indication of the increase of Cd^{2+} sorbed as pH increase. The general flat shape between pH 5 and IO is evident.

Mn release. The solution phase contained Mn^{2+} when Cd^{2+} was agitating on either β-MnO$_2$ or δ-MnO.$_2$ The following fig. 4 and 5 shows the release of Mn^{2+} during the sorption studies for δ-MnO.$_2$

These indicate that the Mn in solution phase probably came form the δ-MnO$_2$ structure. The rate in the equilibrium sorption of Cd^{2+} sorbed by δ-MnO$_2$ was 5.415×10^{-4} g-ion/g.

For the β-MnO$_2$ we have not observed either sorption or pH change even up to 120 h agitation. The following fig. 6 shows the Mn^{2+} release as function of pH 2,5-7,3.

Cation competition. In order to investigate the influence of other cations as Pb,$^{2+}$ Cu,$^{2+}$ Zn^{2+} and Mg^{2+} on Cd sorption on δ-MnO$_2$ we performed the same previously described experiments adding the above cations. Before that we studied the sorption of these metals independently on δ-MnO.$_2$

The fig 7 shows the sorption of different metals on δ-MnO$_2$ at 22° C. The influence of the presence of the above studied metals on the sorption of Cd on δ-MnO$_2$ is shown in fig. 8.

From the fig 7 and 8, is shown that as much as higher is the sorption of the different metals on δ-MnO$_2$, so much greater is the influence on Cd sorption on δ-MnO.$_2$

Conclusions.

From the above results we summarize that Cd^{2+} may be sorbed by δ-MnO$_2$ over the entire pH range 5-10. The sorption of Cd^{2+} by δ-MnO$_2$ at pH 5,4 was carried out at equilibrium time of 1 h and the equilibrium of sorption was found 5.415×10^{-4} g-ion/g.
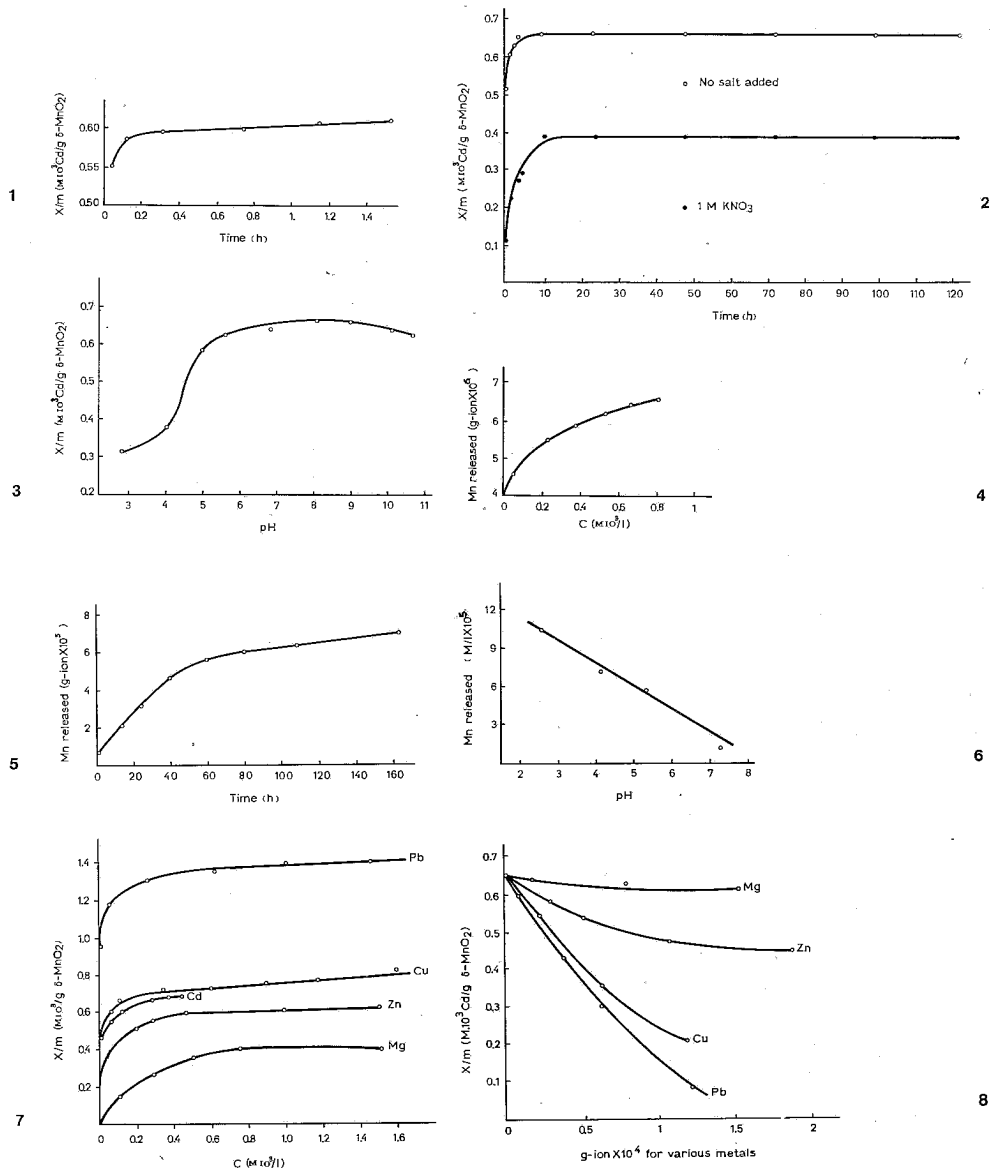
FIG 1. *The sorption of Cd^{2+} on δ-MnO$_2$ as function of time.*
FIG. 2 *Time function of salt-induced changes in Cd sorption in pH 5.4*
FIG. 3 *Sorption isotherms of Cd^{+2} on δ-MnO$_2$ at pH 5.4*
FIG. 4 *Mn released at equilibrium sorption of Cd^{2+} on 0.1g δ-MnO$_2$*
FIG. 5 *Mn released during sorption of Cd on δ-MnO$_2$ from the study at this sorption*
FIG. 6 *Mn released from β-MnO$_2$ as function of pH in range 2.5-7.3*
FIG. 7 *Sorption isotherms of Pb, Cu, Cd, Zn, and Mg at pH 5.4 on δ-MnO.$_2$*
FIG. 8 *Cations influence on Cd^{+2} sorption on δ-MnO$_2$ at pH 5.4*

On the contrary β-MnO₂ has not the ability to sorbe Cd^{2+} even up to 120h experiment. This may be attributed at least to the great difference of the specific areas of the types of oxides (δ-MnO₂: $22.12m^2/g$ and β-MnO₂: $0.83m^2/g$).

When Cd^{2+} was sorbed by δ-MnO,₂ Mn was found in the solution phase. We have the same observation for Mn release from β-MnO₂.

We also observed change in release Mn as function of range between 2.5-7.3 (fig. 6) for δ-MnO₂.

The effect of ionic strength on the sorption of Cd^{2+} on manganese oxide was strong. So the rates of sorption in the presence of IM KNO₃ have been reduced about 40% by comparison with no added salt.

The presence of other cations in the solution, have as result a reduction on Cd^{2+} sorption on δ-MnO₂. This influence is so greater, as the rate of each metal sorption on δ-MnO₂ is greater. The sorption isotherm (fig. 7) indicates that sorption affinities and capacities of δ-MnO₂ for the four cations followed the order: Pb>Cu>Zn>Mg. These results are in agreement with the following reported capacity order: Pb>Cu>Zn>Mg, for their sorption on γ-MnO₂.

This research may be the basis for experiments to scavenge Cd^{2+} from industrial effluenses where other cations are present.

Acknowledgments:

Περίληψη

Μελέτη τῆς ῥόφησης τοῦ CdSO₄ σὲ δ-MnO₂

Στὴν ἐργασία αὐτὴ ἐρευνοῦμε τὴ ῥόφηση τῶν ἰόντων καδμίου πάνω σὲ β-MnO₂ καὶ δ-MnO₂ σὲ θερμοκρασίες 22 καὶ 45° καὶ σὲ μιὰ εὐρεῖα περιοχὴ pH ὑδατικῶν διαλυμάτων. Ἐπίσης ἐρευνήθηκε ἡ ἐπίδραση ἄλλων κατιόντων πάνω στὴ ῥόφηση τοῦ CdSO₄ σὲ δ-MnO.₂ Οἱ μορφὲς β- καὶ δ- τοῦ MnO₂ παρασκευάστηκαν καὶ χαρακτηρίστηκαν μὲ διάθλαση ἀκτίνων Χ, χημικὲς ἀναλύσεις καὶ μὲ τὸ ἀνάπτυγμα τῆς ἐπιφανείας τους. Οἱ ἰσόθεμες ῥοφήσεως τοῦ καδμίου στὸ δ-MnO₂ σὲ pH 5.4 πραγματοποιήθηκαν σὲ χρόνο ἰσορροπίας μιᾶς ὥρας. Ἀντίθετα, τὰ ἰόντα καδμίου δὲν ἔδειξαν καμμιὰ ῥόφηση πάνω στὸ β-MnO₂ ὅταν τὸ πείραμα κράτησε μέχρι 120 ὥρες. Κατὰ τὴ ῥόφηση τοῦ καδμίου στὸ δ-MnO₂ ἐμφανιζόταν μαγγάνιο στὴ φάση τοῦ διαλύματος. Ἐπίσης ἐρευνήθηκε ἡ ἐπίδραση ἰόντων Pb, Cu, Zn καὶ Mg πάνω στὴ ῥόφηση καδμίου στὸ δ-MnO₂ καὶ βρέθηκε ὅτι ὁ μόλυβδος ἔχει ἀφ᾽ ἑνὸς μὲν τὴ μεγαλύτερη ῥόφηση πάνω στὸ δ-MnO₂ ἀφ᾽ ἑτέρου δὲ τὴν μεγαλύτερη ἀρνητικὴ ἐπίδραση πάνω στὴ ῥόφηση τοῦ καδμίου στὸ δ-MnO.₂

Συμπερασματικὰ συνοψίζουμε ὅτι τὰ ἰόντα καδμίου μποροῦν νὰ ῥοφηθοῦν πάνω στὸ δ-MnO₂ γιὰ μιὰ περιοχὴ pH 5-10. Στὸ pH 5.4 ὁ βαθμὸς τῆς ῥόφησης εἶναι $5,415×10^{-4}$ γραμμοϊόντα/γραμ. Ἀπεναντίας τὸ β-MnO₂ δὲν ῥοφᾶ ἰόντα καδμίου ὅταν τὸ πείραμα κρατᾶ μέχρι 120 ὥρες. Τοῦτο εἶναι σημαντικὸ γιὰ τὴν περίπτωση ποὺ σχεδιάζεται ἡ χρησιμοποίηση ὀξειδίων μαγγανίου γιὰ τὴν ἀπομάκρυνση ἰόντων καδμίου ἀπὸ διαλύματα ποὺ περιέχουν μόνο ἰόντα καδμίου ἢ καὶ ἄλλα κατιόντα τὰ ὁποῖα μποροῦν νὰ χαλάσουν τὴν ποιότητα τοῦ νεροῦ τῆς πόλης ἢ τῆς βιομηχανίας.

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BUTYLATED HYDROXYTOLUENE AND TARTARIC ACID AS ANTI-OXIDANTS IN SOYBEAN OIL

E.C. VOUDOURIS, M.E. KOMAITIS and S.D. GIANPAPAS
Laboratory of Food Chemistry, University of Athens, Athens, Greece.
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Summary

A comparative study on the oxidation of several samples of soybean oil containing various combinations of antioxidants in different amounts was carried out. The state of oxidation of the samples was checked spectrophotometrically and by determining the peroxide value and the refractive index. The results showed that the presence of BHT in proportions 0.01, 0.15, 0.20‰ and tartaric acid 0.1% improve the keeping quality of the refined soybean oil.

Key words: BHT, Tartaric acid, Synergists, Soybean oil.
Abbreviations: BHT: Butylated hydroxytoluene BHA: Butylated hydroxyanisole TA: Tartaric Acid.

Introduction

The oxidation of fats and oils causes serious damages in the taste and in the nutritional value. Usually, the vegetable oils contain active natural antioxidants (tocopherol), which protect them against oxidation. By refining these oils valuable components are destroyed. Therefore, it has been common practice to add synthetic antioxidants to the refined oils to improve their resistance to oxidation.[1]

BHA and BHT constitute the 80% of the total antioxidants used in foods.[2] These antioxidants are more effective when they are used together with some organic acids such as citric and tartaric acid.[3] The latter function as chelating agents, which scavenge metals to minimize their effect in the oxidative rancidity reactions.[1]

In a previous work[4] the use of antioxidants and synergists in olive oil was examined. In the present study various systems of synergistic antioxidants in different proportions were tested in order to find the most successful system for the preservation of soybean oil.

Soybean oil differs from the virgin olive oil, because it is refined and contains larger quantities of linoleic and linolenic acid that makes it more susceptible to oxidation.

In the soybean oil after the deodorization an undesirable odour and flavour, different from that caused by rancidity, appears. This deterioration is believed to be a result of oxidation or at least associated with this phenomenon.[5]

Experimental

Instruments

Spectrophotometer Unicam UV. Unicam Instruments, Cambridge, England Refractometer Jena, Modell II.

Reagents

a. Burylated hydroxytoluene (Bayer Kristall);

b. Tartaric acid P.A. Merck;

c. Cyclohexane purified for spectrophotometric purposes; (SpectrAR · Mallinckrodt);

Methods

The determination of the peroxide value was performed according the method described by the American Oil Chemical Society, Official and Tentative Methods.[6]

The spectrophotometric examination of the samples in the UV part of the spectrum and the determination of the conjugated dienes were carried out according to the method described in the Official and Tentative Methods.[7,8]

Raw soybean oil was subjected to analysis and gave the following results:

Saponification number	192
Iodine number	131
Peroxide value	3.75 meq. / kg. sample
Refractive index (at 40°C)	1.4680
Acidity (expressed as oleic acid)	0.30%
Conjugated dienes	0.15%

Samples of 50g of soybean oil were placed in a 250 ml beaker and different quantities of antioxidant and synergistic antioxidant were added. The samples were placed in an oven at 75°C.[9] Soybean oil without antioxidant was also placed into the oven. Every 24 hours the peroxide value and the refractive index were measured. The samples were also subjected to spectrophotometric examinations.

1 Soybean oil (Control)
2 Soybean oil+0,1% T.A*
3 Soybean oil+0,2‰ BHT
4 Soybean oil+0,02‰ BHT
   + 0,1% T.A*
5 Soybean oil+0.05‰ BHT
   + 0,1% T.A*
6 Soybean oil+0,1‰ BHT
   +0,1% T.A*
7 Soybean oil+0,15‰ BHT
   +0,1% T.A*
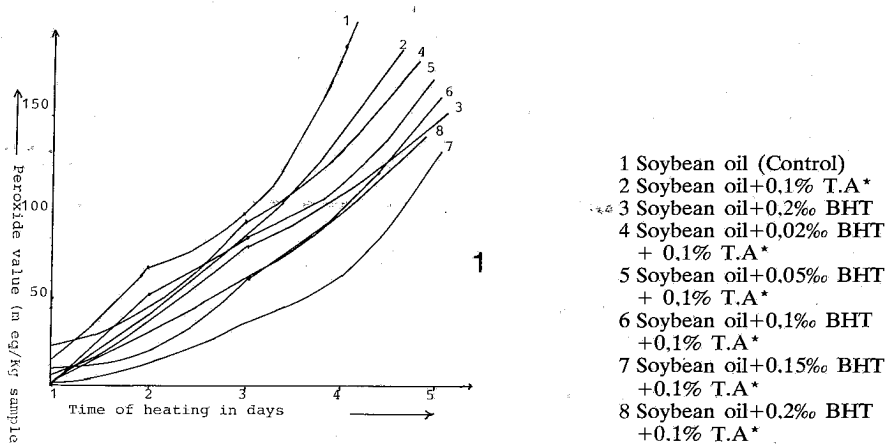8 Soybean oil+0.2‰ BHT
   +0,1% T.A*

FIG.1. *Change of peroxide value with the time of heating*

Results and Discussion

The results of these examinations are shown in tables (I), (II) and (III) and figures 1-6.

TABLE I: *Peroxide values of the oxidized samples*

No.	Samples	Peroxide values (meq/kg sample)				
		Time of heating (days)				
		1	2	3	4	5
1	Soybean oil	24.10	71.92	99.90	183.46	323.54
2	Soybean oil + 0.1% T.A*	16.19	46.47	88.60	139.86	302.68
3	Soybean oil + 0.2‰ BHT	12.87	44.37	82.28	110.75	148.87
4	Soybean oil + 0.02‰ BHT+0.1% T.A*	30.95	50.54	95.83	132.40	258.68
5	Soybean oil + 0.05‰ BHT+0.1% T.A*	14.41	57.46	87.71	113.43	200.81
6	Soybean oil + 0.1‰ BHT+0.1% T.A*	18.14	28.54	64.93	102.65	235.36
7	Soybean oil + 0.15‰ BHT+0.1% T.A*	11.01	22.36	42.89	66.99	130.65
8	Soybean oil + 0.20‰ BHT+0.1% T.A*	16.91	37.49	67.70	98.69	209.68

*T.A Tartaric acid

TABLE II: *Change in the refractive index of the oxidized samples*

No of sample	Time of heating (days)				
	1	2	3	4	5
1	1.46800	1.46875	1.46875	1.46925	1.47010
2	1.46810	1.46870	1.46885	1.46910	1.46945
3	1.46820	1.46825	1.46880	1.46890	1.46910
4	1.46825	1.46870	1.46875	1.46850	1.46945
5	1.46825	1.46850	1.46885	1.46970	1.46910
6	1.46825	1.46840	1.46870	1.46880	1.46930
7	1.46820	1.46840	1.46850	1.46855	1.46875
8	1.46825	1.46875	1,46875	1.46890	1.46905

TABLE III: *Peroxide values, conjugated dienes and spectrophotometry in UV after three days of heating*

No.	Sample	Peroxide values meq/kg sample	Conjugated dienes %	Spectrophoto-metry in UV K_{232}**
1	Soybean oil	99.90	1.15	1,44
2	Soybean oil + 0.1% T.A*	88.60	1.03	1.29
3	Soybean oil + 0.2‰ BHT	82.28	1.05	1.30
4	Soybean oil + 0.02‰ BHT+0.1% T.A*	95.83	1.22	1.51
5	Soybean oil + 0.05‰ BHT+0.1% T.A*	87.71	1.11	1,24
6	Soybean oil + 0.1‰ BHT+0.1% T.A*	64.93	0.89	1,11
7	Soybean oil + 0.15‰ BHT+0.1% T.A*	42.89	0.67	0.87
8	Soybean oil+ 0.2‰ BHT+0.1% T.A*	67.60	0.88	1.11

* T.A Tartaric acid
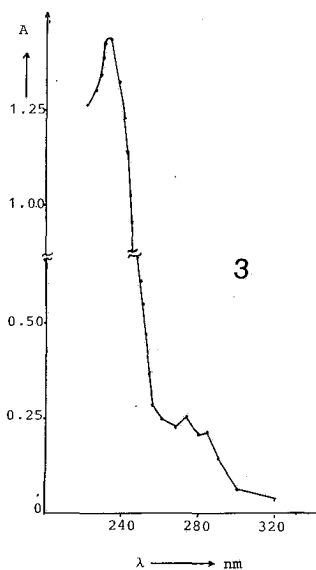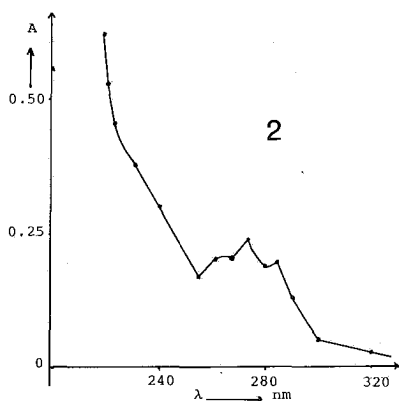**K Specific absorption coefficient

FIG.2. *Absorption spectrum of soybean oil*

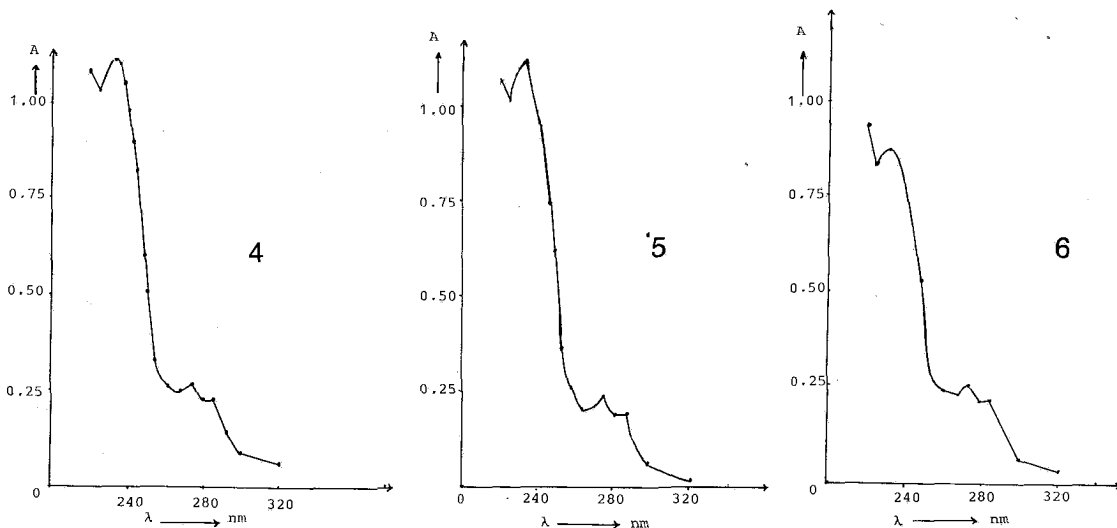FIG.3 *Absorption spectrum of soybean oil after 3 days of heating*

FIG.4 *Absorption spectrum of soybean oil+0,1‰ BHT+0,1% tartaric acid after 3 days of heating*

FIG.5. *Absorption spectrum of soybean oil+0,20‰ BHT+0,1% tartaric acid after 3 days of heating*

FIG.6. *Absorption spectrum of soybean oil+0,15‰ BHT+0,1% tartaric acid after 3 days of heating*

In all spectra, the concentration of the soybean oil was 0,1g/100ml of cyclohexane. The absorbance A was measured in silica cuvettes with a path length of 1cm.

During the oxidation the sample 1 changed colour after the first 24 hours. It became darker, but in the fifth day it was discoloured. The flavour became more intence. In the other samples the same phenomenon was observed even though in lesser extent. The discolouring of the samples was reduced in the order 1,2,5,3,4,6,8,7.

From table I it can be seen that the best results, as regard the inhibition of oxidation, were produced by the mixture 0,15‰ BHT and 0,1% tartaric acid (sample 7). This was also confirmed by the spectrum (Fig.6), in which the absorbance at 232nm is in the region of 0,9 whilst in the other figures the absorbance is greater than 1.0.Thus, for the sample 1, which does not contain antioxidants, the absorbance is 1,45.

The refractive index (table II) for sample 7 didnot change too much. This means, that in the soybean oil the refractive index increases with oxidation, whilst the presence of BHT and tartaric acid acts against this increase.

From table III it is obvious that the number of conjugated dienes as well as the specific absorption at 232nm is smaller in sample 7.

From these results it can be concluded that addition of 0.15‰ BHT and 0.1% tartaric acid in soybean oil inhibits better the oxidation. Good results gave and the samples 8 and 6.

Conclusion:

Use of BHT in proportions 0.1, 0.15 or 0.20‰ and 0.1% tartaric acid facilitates the preservation of the soybean oil, under the conditions of the experiment.

Περίληψη

Βουτυλική ύδροξυ-τολουόλη καὶ τρυγικὸ ὀξὺ ὡς ἀντιοξειδωτικὰ τοῦ σογιελαίου.

Στὴν παροῦσα ἐργασία ἔγινε συγκριτικὴ μελέτη ἐπὶ τῆς ὀξειδώσεως διαφόρων δειγμάτων σογιελαίου στὰ ὁποῖα εἶχαν προστεθεῖ διάφοροι ποσοτικοὶ συνδυασμοὶ ἀντιοξειδωτικῶν καὶ συνεργῶν ἀντιοξειδωτικῶν. Ὁ ἔλεγχος τῆς πορείας τῆς ὀξειδώσεως ἔγινε μὲ φασματοφωτομετρικοὺς προσδιορισμοὺς καὶ προσδιορισμοὺς τοῦ ἀριθμοῦ ὑπεροξειδίων καὶ τοῦ δείκτου διαθλάσεως.

Διαπιστώθηκε ὅτι ὁ συνδυασμὸς BHT στὶς ἀναλογίες 0.1, 0.15 καὶ 0.20‰ καὶ τρυγικοῦ ὀξέος 0.1% δίνει τὰ καλύτερα ἀποτελέσματα.

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Short Papers

ΜΕΛΕΤΗ ΕΠΙ ΤΟΥ ΕΛΛΗΝΙΚΟΥ ΜΕΛΙΤΟΣ Ι. ΝΟΘΕΙΑ ΔΙ' ΙΜΒΕΡΤΟΣΑΚΧΑΡΟΥ. ΙΙ. ΣΥΣΤΑΣΙΣ ΤΩΝ ΕΛΕΥΘΕΡΩΝ ΑΜΙΝΟΞΕΩΝ

ΠΑΝΑΓΙΩΤΗΣ Ι.ΜΑΥΡΙΚΟΣ, ΙΩΑΝΝΗΣ Ν.ΔΑΡΑΤΣΙΑΝΟΣ, ΜΑΡΙΑ Θ.ΚΑΤΣΟΥΛΗ καὶ ΔΙΟΝΥΣΙΟΣ Γ.ΜΑΡΚΕΤΟΣ
Ἐργαστήρια Διευθύνσεως Μελετῶν-Ἐρευνῶν τοῦ Γενικοῦ Χημείου τοῦ Κράτους, Ἀν.Τσόχα 16, Ἀθῆναι
(Ἐλήφθη τὴν 11 Φεβρουαρίου 1977)

Περίληψις

Συμφώνως πρὸς τὴν παροῦσαν μελέτην παρέχεται ἡ δυνατότης διαπιστώσεως νοθείας μὴ θερμανθέντος μέλιτος δι' ἐνζυματικῶς ἰμβερτοποιημένου καλαμοσακχάρου, ἀφ' ἑνὸς μὲν ἐκ τῆς ἀσαφείας τὴν ὁποίαν παρουσιάζει ἡ ἀνάπτυξις τῶν ἐλευθέρων ἀμινοξέων κατὰ τὴν χρωματογραφίαν ἐπὶ χάρτου ἢ λεπτῆς στιβάδος, ἥτις ἀποδίδεται εἰς τὴν παρουσίαν πεπτιδίων προερχομένων ἐκ τοῦ χρησιμοποιηθέντος ἐνζύμου, ἀφ' ἑτέρου δὲ ἐκ τῆς σχετικῶς ηὐξημένης τιμῆς τῆς HMF.

Μελετῶνται ἐξ ἄλλου, ποιοτικῶς καὶ ποσοτικῶς, τὰ ἐλεύθερα ἀμινοξέα εἰς δείγματα Ἑλληνικοῦ μέλιτος.

Ὁρολογία, Συντμήσεις: HMF: 5-ὀξυμεθυλοφουρφουρόλη, 5-hydroxymethylfurfural.
Γ.Χ.Κ.: Γενικὸν Χημεῖον τοῦ Κράτους.
A.O.A.C.: Association of Official Analytical Chemists.

Εἰσαγωγὴ

Ἡ παροῦσα μελέτη ἀποτελεῖ μέρος γενικωτέρας μελέτης ἀναληφθείσης ὑπὸ τῶν Ἐργαστηρίων τῆς Διευθύνσεως Μελετῶν - Ἐρευνῶν τοῦ Γ.Χ.Κ. καὶ ἀναφερομένης εἰς τὴν σύστασιν τοῦ Ἑλληνικοῦ μέλιτος καὶ βασιλικοῦ πολτοῦ, ὡς καὶ εἰς τοὺς τρόπους ἐλέγχου καὶ καταπολεμήσεως τῆς συγχρόνου νοθείας τῶν δύο τούτων βασικῶν μελισσοκομικῶν προϊόντων.

Προσφάτως Ἑλληνικαὶ τινες βιομηχανίαι ἐπεξεργάζονται διαλύματα καλαμοσακχάρου, διὰ διαφόρου προελεύσεως ἰμβερτασῶν, ἐπιτυγχάνουσαι οὕτω ἰμβερτοποίησιν τούτου ὑπὸ ἠπίας σχετικῶς συνθήκας, πρὸς παρασκευὴν σιροπίων. Τοιαῦτα πυκνὰ διαλύματα σιροπίων δύνανται εὐχερῶς νὰ χρησιμοποιηθοῦν διὰ τὴν νοθείαν τοῦ μέλιτος καὶ μάλιστα, ὡς τοῦτο δείκνυται εἰς τὴν παροῦσαν μελέτην, χωρὶς τὴν δυνατότητα τῆς ἀνιχνεύσεώς των διὰ τῆς ἀντιδράσεως Fiehe, λόγῳ τῆς μικρᾶς περιεκτικότητός των εἰς HMF.

Τὰ ἀμινοξέα τὰ ὁποῖα ἀπομονοῦνται ἀπὸ τεχνητῶς ἰμβερτοποιηθέντα σακχαροῦχα διαλύματα, παρουσιάζουν ἀσάφειαν κατὰ τὴν χρωματογραφικὴν τῶν ἀνάπτυξιν.[1,2] Τοῦτο προφανῶς ὀφείλεται εἰς πεπτίδια προερχόμενα ἐκ τοῦ χρησιμοποιηθέντος ἐνζύμου, τῆς ἰμβερτάσης, τὰ ὁποῖα δὲν διαχωρίζονται τῶν ἀμινοξέων μέσῳ τῆς ἰονοανταλλακτικῆς στήλης καὶ ἀκολουθοῦν ταῦτα εἰς τὴν περαιτέρω χρωματογραφικὴν ἀνάπτυξίν των ἐπὶ χάρτου ἢ λεπτῆς στιβάδος.

Εἰς τὴν παροῦσαν μελέτην περιγράφεται μέθοδος ἐλέγχου τῆς νοθείας τοῦ μέλιτος διὰ καταλλήλως ἰμβερτοποιημένων σιροπίων. Ἐξετάζεται ἐπίσης τὸ εἶδος τῶν ἐλευθέρων ἀμινοξέων δέκα δειγμάτων αὐθεντικοῦ μέλιτος θύμου, παραγωγῆς 1974-1975, ληφθέντων ἐκ διαφόρων περιοχῶν τῆς Νήσου Κρήτης, ἐνδεικτικῶς δὲ προσδιορίζεται ἡ εἰς ἐλεύθερα ἀμινοξέα περιεκτικότης δύο δειγμάτων μέλιτος τῇ βοηθείᾳ ἀναλυτοῦ ἀμινοξέων.

Πειραματικὸν μέρος-Ἀποτελέσματα

Ἀνίχνευσις καὶ ποσοτικὸς προσδιορισμὸς ἀμινοξέων

Ποσότης μέλιτος 15g, ἀραιοῦται διὰ προσθήκης περίπου 30ml ὕδατος καὶ ἀφίεται νὰ διέλθη μέσῳ στήλης ἰονοανταλλακτικῆς ῥητίνης Dowex 50 ἢ ἑτέρας παρομοίας, ὕψους 25cm καὶ διαμέτρου 2,5cm. Ἡ ταχύτης διελεύσεως ῥυθμίζεται εἰς 10 περίπου σταγόνας ἀνὰ λεπτόν. Μετὰ τὴν διέλευσιν τοῦ διαλύματος ἡ στήλη ἐκπλύνεται μὲ 500ml ἀπεσταγμένου ὕδατος μὲ τὴν αὐτὴν ταχύτητα διελεύσεως. Ἡ ἔκλουσις τῶν ἀμινοξέων ἐπιτυγχάνεται μὲ 500ml ἀμμωνίας 1N, τὸ δὲ ἐκλουσθὲν ὑγρὸν συμπυκνοῦται εἰς περιστρεφόμενον συμπυκνωτὴν (flash evaporator) ὑπὸ κενὸν καὶ εἰς θερμοκρασίαν 40°C. Τὸ συμπυκνωθὲν διάλυμα τῶν ἀμινοξέων παραλαμβάνεται διὰ διαλύματος ἰσοπροπυλικῆς ἀλκοόλης 10% εἰς ὀγκομετρικὴν φιάλην τῶν 10ml. Πρὸς διαχωρισμὸν καὶ ἀναγνώρισιν τῶν ἀμινοξέων χρησιμοποιεῖται χρωματογραφία δύο κατευθύνσεων τόσον ἐπὶ χάρτου Whatmann No. 1, ὅσον καὶ λεπτῆς στιβάδος ἐκ κυτταρίνης. Εἰς ἀμφοτέρας τὰς περιπτώσεις ὡς ὑγρὸν ἀναπτύξεως χρησιμοποιεῖται τὸ σύστημα: χλωροφόρμιον - μεθανόλη - ἀμμωνία 75% (2:2:1)[3] ἢ τὸ σύστημα: βουτανόλη - 2 - ὀξικὸν ὀξὺ - ὕδωρ (60:15:25) διὰ τὴν πρώτην κατεύθυνσιν καὶ τὸ σύστημα: φαινόλη - ὕδωρ (4:1) διὰ τὴν δευτέραν κατεύθυνσιν.

Ἡ ἐμφάνισις ἐπιτυγχάνεται μὲ διάλυμα 0,2g νινυδρίνης εἰς 100ml ἀκετόνης, εἰς τὸ ὁποῖον, ὀλίγον πρὸ τῆς χρησιμοποιήσεώς του, προστίθεται πυριδίνη εἰς ἀναλογίαν 2%.

Ἡ ἀνίχνευσις τῆς γλυκίνης γίνεται μὲ διάλυμα ο-φθαλαλδεΰδης. Μετὰ τὴν ἐξάτμισιν τῆς ἀκετόνης τὸ χρωματογράφημα θερμαίνεται ἐπὶ 2-3 λεπτὰ εἰς πυριατήριον εἰς 105°C. Διὰ τοῦ ἀνωτέρω τρόπου ἐγένετο ἡ ἀνίχνευσις τῶν ἀκολούθων 14 ἀμινοξέων εἰς ἄπαντα τὰ δείγματα:

Προλίνη, Φαινυλαλανίνη, Τυροσίνη, Ἀσπαραγινικὸν ὀξύ, Γλουταμινικὸν ὀξύ, Βαλίνη, Λευκίνη, Ἰσολευκίνη, Λυσίνη, Σερίνη, Γλυκίνη, β-Ἀλανίνη, Ἱστιδίνη, Θρεονίνη.

Εἰς δύο τῶν ἀνωτέρω δειγμάτων, προσδιωρίσθησαν περαιτέρω τὰ ἀμινοξέα τῇ βοηθείᾳ ἀναλυτοῦ ἀμινοξέων (Technicon Amino-acid Analyzer System, Model No. NC-1) διὰ χρησιμοποιήσεως νορλευκίνης ὡς ἐσωτερικοῦ προτύπου. Ἡ σύστασις τοῦ μέλιτος εἰς ἐλεύθερα ἀμινοξέα, τὰ ὁποῖα προηγουμένως ἀπεμονώθησαν δι' ἰονοανταλλακτικῆς ῥητίνης κατὰ τὴν περιγραφεῖσαν τεχνικήν, ἐμφαίνεται εἰς τὸν Πίνακα Ι.

Προσδιορισμὸς HMF[4]

Τὸ πρὸς ἐξέτασιν δεῖγμα διαυγάζεται μὲ ἀντιδραστήριον Carrez. Ἡ HMF δι' ἐπιδράσεως βαρβιτουρικοῦ ὀξέος καὶ p-τολουϊδίνης παρέχει ἐρυθρὰν χρῶσιν, τῆς ὁποίας ἡ ἀπορρόφησις μετρεῖται εἰς 550nm.

Μέθοδος ἀναλύσεως: Ἡ ἀνάλυσις ἐκτελεῖται ἐπὶ 15g μέλιτος, ἐφ' ὅσον ἡ περιεκτικότης τοῦ δείγματος δὲν ὑπερβαίνει τὰ 40ppm. Διὰ μεγαλυτέρας συγκεντρώσεις HMF λαμβάνεται ἀντιστοίχως μικροτέρα ποσότης δείγματος. Ἡ ζυγισθεῖσα ποσότης ἀραιοῦται εἰς ὄγκον 50ml καὶ διαυγάζεται διὰ διαλύματος Carrez. Εἰς περίπτωσιν παρουσίας ἐλευθέρου SO_2, τὸ ὁποῖον δυνατὸν νὰ ἔχη

ΠΙΝΑΞ I. Ἀμινοξέα ἀπομονωθέντα ἐκ δειγμάτων ἀγνοῦ μέλιτος θύμου δι' ἀναλυτοῦ ἀμινοξέων.

Ἀμινοξὺ	Συγκέντρωσις (μg/g)	
	Δεῖγμα Α	Δεῖγμα Β
Προλίνη	296,20	321,10
Φαινυλαλανίνη	215,63	240,30
Τυροσίνη	35,33	34,80
Κυστίνη	16,83	16,70
Λυσίνη	14,54	15,05
Ἀργινίνη	11,05	10,30
Σερίνη	9,21	9,25
β-Ἀλανίνη	9,11	9,28
Βαλίνη	7,02	6,93
Ἱστιδίνη	6,24	6,30
Γλυκίνη	5,50	5,43
Γλουταμινικὸν ὀξὺ	4,01	4,09
Ἀσπαραγινικὸν ὀξὺ	3,55	3,68
Θρεονίνη	3,45	3,33
Ἰσολευκίνη	2,91	3,02
Λευκίνη	2,51	2,64

προστεθῆ εἰς τὸ ἰμβερτοσάκχαρον, ὀξειδοῦται τοῦτο πρὸ τῆς προσθήκης τοῦ διαλύματος Carrez τῇ βοηθείᾳ διαλύματος ἰωδίου. Ἡ ἀνάπτυξις τοῦ χρώματος γίνεται εἰς 2ml τοῦ διαυγασθέντος μέλιτος διὰ προσθήκης διαλυμάτων ρ-τολουϊδίνης καὶ βαρβιτουρικοῦ ὀξέος. Ἡ μεγίστη ἔντασις τοῦ χρώματος ἀναπτύσσεται ἐντὸς 3 ἕως 5 λεπτῶν. Μετρεῖται ἡ ἀπορρόφησις τοῦ διαλύματος εἰς 550nm ὡς πρὸς διάλυμα συγκρίσεως, εἰς τὸ ὁποῖον, ἀντὶ βαρβιτουρικοῦ ὀξέος, ἔχει προστεθῆ ἀπεσταγμένον ὕδωρ ἴσου ὄγκου. Αἱ συγκεντρώσεις τῆς HMF ὑπολογίζονται διὰ συγκρίσεως μὲ πρότυπον καμπύλην. Εἰς τὸν πίνακα II ἐμφαίνονται αἱ περιεκτικότητες τῆς HMF δειγμάτων ἀμιγοῦς μέλιτος, ὡς καὶ τοιούτων ἐνεχόντων ἐνζυματικῶς ἢ δι' ὀξέων ἰμβερτοποιημένον καλαμοσάκ-χαρον.

Διαπιστοῦται οὕτω ὅτι αἱ χαμηλαὶ τιμαὶ τῆς HMF εἰς τὸ ἀμιγὲς μέλι αὐξάνονται κατὰ τινας δεκάδας εἰς τὸ μῖγμα μέλιτος μετὰ ἰμβερτοποιημένου ἐνζυματικῶς καλαμοσακχάρου. Ἡ ἀντίδρασις Fiehe ὅμως ἐξακολουθεῖ νὰ παραμένῃ ἀρνητική. Ἀντιθέτως εἰς τὰ δείγματα μέλιτος μετὰ ἰμβερτοποιημένου δι' ὀξέων καλαμοσακχάρου, ἡ συγκέντρωσις τῆς HMF αὐξάνεται σημαντικῶς, ἡ δὲ ἀντίδρασις Fiehe ἐμφανίζεται σαφῶς θετική.

Εἰς τὸ μῖγμα μέλιτος - ἐνζυματικῶς ἰμβερτοποιημένου καλαμοσακχάρου δὲν καθίσταται εὐκρινὴς ὁ διαχωρισμὸς τῶν ἀμινοξέων ἀλλὰ λαμβάνονται συγκεχυμένα χρωματογραφήματα. Τοῦτο ἀποδίδεται εἰς τὰ πεπτίδια τοῦ ἐνζύμου τῆς ὑδρολύσεως, τὰ ὁποῖα διέρχονται μετὰ τῶν ἀμινοξέων διὰ τῆς ῥητίνης.

Ἡ ἀνωτέρω ἐκδοχὴ ἐπιβεβαιοῦται πειραματικῶς, ἐφ' ὅσον ὑποβληθῇ εἰς ὑδρόλυσιν τὸ ἐκ τῆς ῥητίνης ἐκλουσθὲν διάλυμα (μῖγμα ἀμινοξέων-πεπτιδίων) δι' ὑδροχλωρικοῦ ὀξέος 17% ὑπὸ κάθετον ψυκτῆρα καὶ ἐπὶ δύο ὥρας.

ΠΙΝΑΞ ΙΙ: *Συγκέντρωσις HMF εἰς δείγματα ἀμιγοῦς μέλιτος καὶ μέλιτος ἐνέχοντος ἰμβερτοποιημένον καλαμοσάκχαρον*

Ἀριθμὸς καὶ εἶδος δείγματος	HMF (ppm)	Ἀντίδρασις Fiehe*
No 1 (ἀμιγὲς)	2	ἀρνητικὴ
No 2 (ἀμιγὲς)	7	»
No 3 (ἀμιγὲς)	4	»
No 4 (ἀμιγὲς)	9	»
No 5 (ἀμιγὲς)	2	»
No 6 (ἀμιγὲς)	13	»
No 1 (ἐνέχον 25% καλαμοσάκχαρον ἰμβερτ. δι' ὀξέος)	680	θετικὴ
No 1 (ἐνέχον 25% καλαμοσάκχαρον ἰμβερτ. ἐνζυματικῶς)	32	Ἀρνητικὴ
No 1 (ἐνέχον 50% καλαμοσάκχαρον ἰμβερτ. δι' ὀξέος)	1.200	Θετικὴ
No 1 (ἐνέχον 50% καλαμοσάκχαρον ἰμβερτ. ἐνζυματικῶς)	49	Ἀρνητικὴ

* Διὰ τὴν ἀντίδρασιν Fiehe ἠκολουθήθη ἡ μέθοδος ἡ ὑποδεικνυομένη ὑπὸ AOAC[5]

Τὸ οὕτω ὑδρολυθὲν προϊόν, διερχόμενον ἐκ νέου δι' ἀναγεννημένης ὀξίνης ρητίνης καὶ ἐκλουόμενον δι' ἀμμωνίας, συμπυκνοῦται εἰς μικρὸν ὄγκον καὶ χρωματογραφεῖται ἐπὶ χάρτου. Διὰ τῆς τεχνικῆς ταύτης τὰ ἀμινοξέα παρουσιάζονται καλῶς ἀνεπτυγμένα ὁ δὲ διαχωρισμός των εἶναι εὐκρινής.

Ἐνζυματικὴ ὑδρόλυσις καλαμοσακχάρου

Ἐχρησιμοποιήθη πρὸς τοῦτο τὸ ὑπὸ τὴν ὀνομασίαν "Dry Invertase" κυκλοφοροῦν εἰς τὸ ἐμπόριον ἐκχύλισμα φρουκτοζιδάσης τοῦ Γαλλικοῦ Οἴκου "Etablissement Rapidaz".

Τὸ προϊὸν τοῦτο φέρεται ὡς εἰδικὸν ἔνζυμον κατάλληλον διὰ παρασκευὴν σιροπίων, ὡς ὑδρολύον τὸ διάλυμά καλαμοσακχάρου πρὸς d-φρουκτόζην καὶ d-γλυκόζην. Ἡ ὑδρόλυσις ἐπιτυγχάνεται συμφώνως πρὸς τὰς ὀδηγίας τοῦ παρασκευαστοῦ ὡς ἀκολούθως:

Διάλυμα καλαμοσακχάρου 50% φέρεται εἰς pH 5 διὰ προσθήκης κιτρικοῦ ὀξέος καὶ θερμαίνεται εἰς 55°C, ἐνῶ συγχρόνως προστίθεται τὸ ἔνζυμον εἰς ἀναλογίαν 0,55% ἐπὶ τοῦ καλαμοσακχάρου. Τὸ μῖγμα ἀναδεύεται ἐπὶ 16 ὥρας, ἐν συνεχείᾳ ἡ θέρμανσις διακόπτεται καὶ τὸ μῖγμα διηθεῖται πρὸς λῆψιν τοῦ ἰμβερτοποιημένου σιροπίου.

Ἡ ἰμβερτοποίησις τοῦ καλαμοσακχάρου ἀνέρχεται διὰ τῆς μεθόδου ταύτης εἰς ποσοστὸν 99,8% (προσδιορισμὸς τοῦ ἰμβερτοσακχάρου κατὰ Lane-Eynon)[6].

Συμπεράσματα

Δείγματα ἀμιγοῦς μέλιτος θύμου, μὴ θερμανθέντος, προερχόμενα ἐκ διαφόρων περιοχῶν τῆς νήσου Κρήτης, ἐξετάζονται ὡς πρὸς τὰ ἀμινοξέα των τῇ βοηθείᾳ χρωματογραφίας χάρτου καὶ λεπτῆς στιβάδος. Ἅπαντα τὰ δείγματα δεικνύουν τὴν αὐτὴν εἰκόνα ἀναπτύξεως. Εἰς δύο ἐκ τούτων προσδιορίζονται ἐνδεικτικῶς τὰ ἀμινοξέα μὲ ἀναλυτὴν ἀμινοξέων. Τὰ ὑπερισχύοντα ἀμινοξέα

εἶναι ἡ προλίνη, ἡ φαινυλαλανίνη καὶ ἡ τυροσίνη. Τὰ δύο ταῦτα δείγματα ἐμφανίζονται γενικῶς λίαν πλούσια εἰς ἀμινοξέα ἐν συγκρίσει μὲ δείγματα μέλιτος Ἰταλικῆς προελεύσεως,[1] ἡ δὲ περιεκτικότης τῶν εἰς τυροσίνην συγκριτικῶς πάλιν μεγαλυτέρα. Ἐξ ἄλλου εἰς τὰ ἐξετασθέντα δείγματα ἀμιγοῦς μέλιτος προσδιορίζεται ἡ HMF, ἡ συγκέντρωσις τῆς ὁποίας εὑρίσκεται χαμηλὴ καὶ μὴ ὑπερβαίνουσα τὰ 13ppm. Ἡ τιμὴ αὕτη αὐξάνεται ἐλαφρῶς εἰς περίπτωσιν νοθείας τοῦ μέλιτος δι' ἐνζυματικῶς ἱμβερτοποιηθέντος καλαμοσακχάρου, χωρὶς ἐν τούτοις νὰ μεταβάλλεται ἡ ἀντίδρασις Fiehe, ἡ ὁποία ἐξακολουθεῖ νὰ παραμένῃ ἀρνητική. Εἰς τὰ δείγματα ταῦτα τοῦ νοθευμένου δι' ἐνζυματικῶς ἱμβερτοποιηθέντος καλαμοσακχάρου μέλιτος, ἡ ὑπὸ τὰς περιγραφείσας πειραματικὰς συνθήκας ἀπομόνωσις τῶν ἀμινοξέων καὶ ἡ ἐν συνεχείᾳ χρωματογράφησίς των ἐπὶ χάρτου ἢ λεπτῆς στιβάδος παρέχει συγκεχυμένα χρωματογραφήματα.

Λαμβάνονται ἐν τούτοις καθαρὰ ἀναπτύγματα ἀμινοξέων, ἐφ' ὅσον προηγουμένως ὑποβληθοῦν εἰς ὑδρόλυσιν τὰ βασικὰ συστατικά, τὰ ὁποῖα ἀπομονοῦνται κατὰ τὴν χρωματογραφίαν στήλης καὶ τὰ ὁποῖα ἐν συνεχείᾳ ἐπαναδιέρχονται διὰ τῆς ἰσχυρῶς κατιονικῆς ῥητίνης καὶ ἐπαναχρωματογραφοῦνται.

Ἐκ τῶν ἀνωτέρω συνάγεται ὅτι ἡ in vitro ἐνζυματικὴ ἱμβερτοποίησις καλαμοσακχάρου διὰ τὴν νοθείαν μέλιτος, δυνατὸν νὰ δημιουργῇ μὲν μικρὰν ποσότητα HMF, ἐμπλουτίζει ὅμως ἐπίσης τὸ ἱμβερτοποιημένον σακχαροῦχον διάλυμα εἰς πεπτίδια, τὰ ὁποῖα ἐμποδίζουν τὴν καθαρὰν ἀνάπτυξιν τῶν ἀμινοξέων κατὰ τὴν χρωματογράφησίν των.

Abstract

A study on the greek honey. I. Adulteration with inverted sugar. II. Free aminoacids composition.

The detection of the adulteration in genuine honey, non previously heated, by enzymatically produced inverted sugar is studied in the present work.

The column separated free aminoacids of honey samples are subjected to bidimentional thin layer or paper chromatography. The chromatograms obtained from adulterated samples are confused and difficult to interpret, contrary to the ones of genuine samples with distinct and clearly identified aminoacids. This is attributed to protein fractions derived from invertase. It has also been found that the adulterated honey samples show a substantially elevated HMF value.

In addition, the free aminoacids in two genuine thymous honey samples from Crete island have been identified and their content has been measured, using an aminoacid analyzer. High values for the aminoacids proline, phenylalanine and tyrosine have been found.

Key words: Aminoacid analyzer, 5- hydroxymethylfurfural, peptides, enzymatic inversion, thin layer chromatography, paper chromatography.

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