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A STUDY ON THE SYNTHESIS OF ASYMMETRICALLY SUBSTITUTED 1-(α-AROYLOXYARYLIDENAMINO)- 4,5-DIPHENYL-1, 2, 3-triazoles

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Summary

The synthesis of asymmetrically substituted 1-(α-Aroyloxyarylidenamino)-4,5-diphenyl- 1, 2, 3-triazoles (triazolyl - isoimides) (III) prepared in 40-70% yield, under specific conditions, from 1-(N-aroylamino)- 1, 2, 3-triazoles (II) and substituted benzoyl chlorides has been studied. The mechanism of formation and the spectral data (UV, IR, NMR, MS) of all new compounds are also reported.

Key words: 1-(α-Aroyloxyarylidenamino)-4,5-diphenyl-1, 2, 3 triazoles, synthesis, spectral data.

Introduction

The major objective of this project is the synthesis of asymmetrically - in aroyl groups of the isoimide function-substituted 1-(α-aroyloxyarylidenamino)-4,5-diphenyl- 1, 2, 3-triazoles (triazolyl-isoimides) (III), which can serve as a model system for a further study on the isomerization mechanism (1) of triazolyl-isoimides to the corresponding imides. In this paper we focus on the synthesis and properties of the triazolyl-isoimides (III).

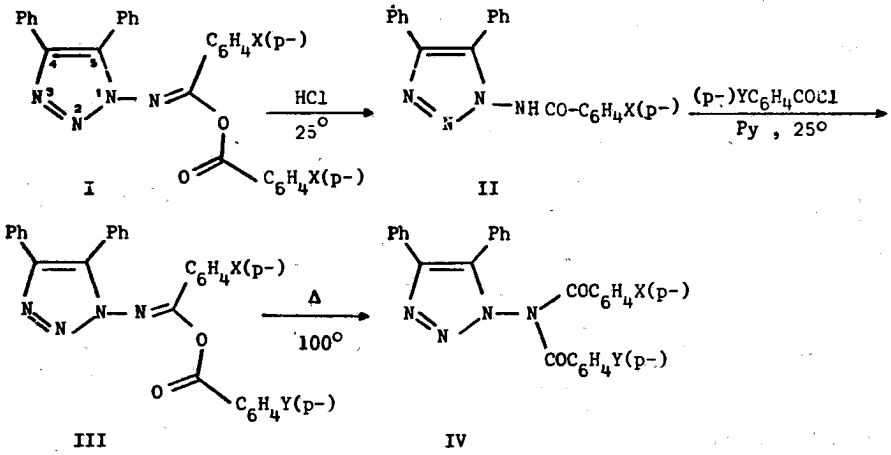
It is worth mentioning that a special interest connected with this type of triazolyl- isoimides (III) arises from their difficulty to be prepared by oxidation of asymmetrically substituted bis-aroyl-hydrazones, method used for the synthesis of symmetrically substituted triazolyl isoimides (2), since previous attempts for the synthesis of such hydrazones were unsuccessful (3). Unsuccessful was also the synthesis of these isoimides (III) via imidoylhalides and sodium or potassium benzoate (4), which is an established method for the preparation of symmetrically substituted triazolyl-isoimides (I).

Results and discussion

It has been shown previously by El Khadem and his co-workers (5) that some isoimides are obtained by direct benzoylation of heterocyclic amines. In our initial attempts we were able to prepare the unsubstituted 1-(α-benzoyloxybenzylidenamino)-4,5-diphenyl-1, 2, 3-triazole (I, where X=H) by treating either 1-amino- or 1-benzoylamino-triazole (II where X=H) with benzoylchloride.

It has been shown that this isoimide was identical in all respects with that prepared by independent synthesis *via* oxidation of benzil-bis-benzoyl-hydrazone (6). A modification of the above method using 1-aroylamino-1, 2, 3-triazoles and several p-substituted benzoylchlorides was promissing for the synthesis of asymmetrically substituted triazolyl-isoimides (III). The procedure employed for the synthesis of this type of isoimides (III) is given in Scheme 1.

Scheme 1



(A) X=H, Y=CH_3
(B) X=CH_3, Y=H
(C) X=H, Y=Cl
(D) X=Cl, Y=H
(E) X=OCH_3, Y=H
(F) X=OCH_3, Y=Cl
(G) X=Cl, Y=CH_3

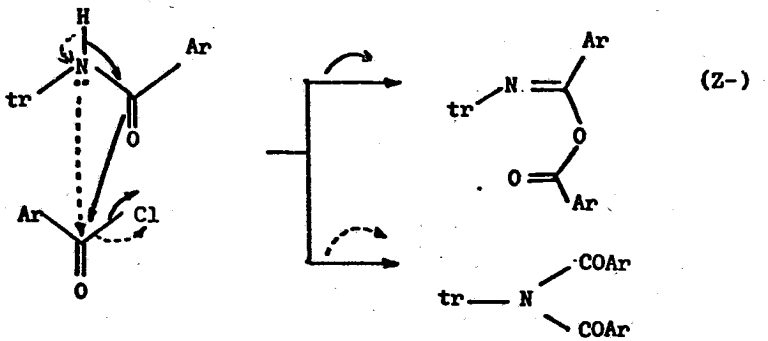
The 1-(N-aroylamino)-4,5-diphenyl-1, 2, 3-triazoles (II) were obtained by treating at room temperature the symmetrically substituted isoimides (I) with concentrated hydrochloric acid (7). Aroylation of the amides (8) in the presence of pyridine (Py) at room temperature lead to the preparation of the isoimides (III) in good yields (40-70%).

The progress of the reaction (formation of triazolyl-isoimides from 1-(N-aroylamino)-triazoles] was followed by infrared spectroscopy, observing the dissappearance of νCO at 1690 cm^{-1} for the aroylamino-group and the appearance of a higher carbonylic absorbtion at 1740-1760 cm^{-1}, characteristic of this isoimide function (9) with an additional absorption for the stretching vibration of C=N at 1630-1640 cm^{-1}, which is absent in the starting material. The high purity of the starting materials and the presence of pyridine are mandatory for a successful result. The pyridine possibly neutralizes the hydrochloric acid formed, which otherwise can lead to the isomerization of

triazolyl-isoimides (III) to the imides (IV). At this point it is worth mentioning the recently observed (10) formation of isomaleinimides under strictly non-acidic conditions and the isolation of the isomeric maleinimides under acidic-conditions.

The aroylation of the amides (II) can take place either at the nitrogen leading to imides (IV) (thermodynamic control) or at the oxygen leading to the less stable isoimides (III) (kinetic control). Under the present experimental conditions the reaction almost exclusively leads to the formation of the isoimides (III). This however is only observed, when the substituent in 5-position (Scheme 1) of the triazole ring is phenyl, otherwise mixtures of isoimides-imides are obtained, not easily isolated from the reaction mixture (11).

For the competing nucleophilic attack (N-aroylation, O-aroylation) the following mechanistic scheme can be proposed:



tr= 1,2,3-triazole ring

Since from this aroylation Z-configurated isoimides in respect to C=N bond are obtained, as showed from X-ray analysis (12,13) and from dipole moment measurements (14), the configuration of the amides (II) can be presumed. This configuration could also explain the observed steric influence of the 5-phenyl-group.

The most basic center in the molecule is the nitrogen of the N-benzoyl-amino group; therefore a nucleophilic attack from the nitrogen to the carbonyl carbon atom of the aroylchlorides should be expected to give the most thermodynamically stable N-derivative, the triazolyl-imide (IV). This formation of O-derivative, triazolyl-isoimide (III), is a kinetically controlled product.

In all studied cases where the substituents in 4,5-positions of the triazole ring are both methyl, either hydrogen in position 4- and methyl in position 5- or phenyl in position 4- and methyl in position 5-, the major product isolated was the triazolyl-imide (IV) in a mixture with a sufficient amount of triazolyl-isoimide (III).

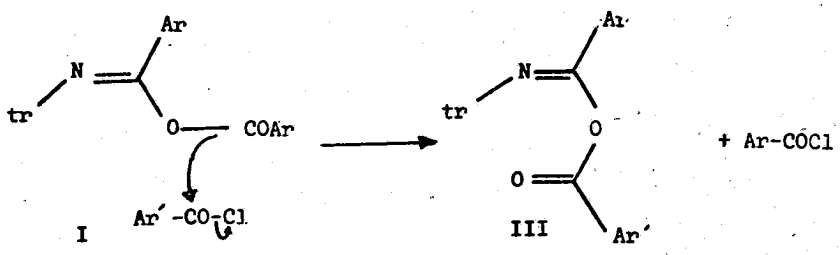
The kinetically controlled O-derivative (triazolyl-isoimide) is exclusively formed from 4,5-diphenyl-derivatives (II).

The preferable nucleophilic attack by the oxygen, in the case of 5-phenyl

substituted triazole derivatives, might be due to steric effects (less hindered position) as evidenced considering the stereomodels and this attack leads to the formation of isoimides.

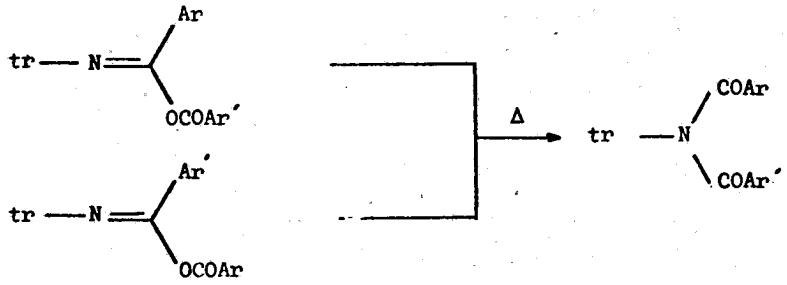
As a conclusion, the synthesis of triazolyl-isoimides is a kinetically controlled aroylation at relatively low temperatures and short reaction times, which also demands the presence of a bulky group (phenyl) in the 5- position of the triazole ring.

Asymmetrically substituted triazolyl-isoimides (III) were also prepared from symmetrically substituted triazolyl-isoimides (I) and an excess of aroyl-chloride. A possible mechanism is shown below:

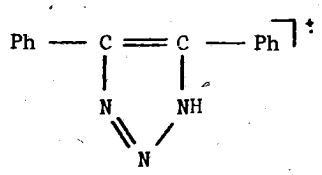


However, the route from 1-benzoylamino-triazoles (III) was found to be the best one. The structures assigned to the triazolyl-isoimides (III) were based on their spectral data (IR, NMR, MS) and on their elemental analyses.

It is well known that isoimides easily rearrange to imides (4) and there is a considerable interest in the thermal rearrangement of asymmetrically substituted triazolyl-isoimides from mechanistic point of view (11). It is worth mentioning that by heating the isoimides IIIA and IIIB the same imide is obtained:



The fragmentation pattern in the mass spectra of the isoimides (III) is analogous to that proposed for the symmetrically substituted isoimides (I) (15) and the most important fragments are reported in Table 1. In every studied case the ion:



was found, but no detection of metastable peak for the formation of this was observed. From the mass spectra it was easy to specify the position of the substituent in the molecule, studying the ion $ArCOO^+$. It is of interest to note however, that besides the ion $ArCO^+$ the ion $Ar'CO^+$ is also observed, which can be explained assuming a rearrangement of the isoimide to imide during the fragmentation process. The presence of $Ar'CO^+$ into the mass spectrometer is consistent with previously reported cases (15).

Experimental

General. M.p.'s are uncorrected and were determined with a Kofler hot-stage apparatus, ultraviolet absorbtion spectra were obtained in methanol (spectral grade) with a Perkin-Elmer 137 137 UV, using 1-cm quartz cells. IR spectra were recorded with a Beckman IR-4 spectrometer for Nujol mulls. NMR spectra were run in deuteriochloroform solution using tetramethylsilane as internal standard with a Varian A-60A spectrometer. Mass spectra were recorded with a Hitachi-Perkin-Elmer RMU-6L Mass spectrometer with ionization energy 70 eV.

The analyses were performed with a Perkin-Elmer Analyzer, Model 240. All reported yields were based on isolated products. Samples used for spectroscopic studies and analyses were purified by preparative TLC or colum chromatography (Merck, Silica gel, 60-230 mesh).

The synthesis of symmetrically substituted triazolyl-isoimides (I) is a known procedure previously reported (1). Purified (16) benzoylchloride was refluxed with thionyl chloride for 2 hours and repeatedly distilled under vacuum (b.p. 56-57°/6 Torr). p-Methylbenzoylchloride (17) (b.p. 94-96°/10 Torr) and p-chlorobenzoylchloride (18) (b.p. 114-115°/20 Torr) were also purified by the same way as benzoylchloride.

Hydrolysis of triazolyl-isoimides (I) to 1-(N-aroylamino)-triazoles (II)

According to a general procedure (19) these compounds were obtained by treating at room temperature the isoimides (I) with concentrated hydrochloric acid. After 30 min the reaction mixture was neutralized with 20% sodium bicarbonate solution and extracted with methylenechloride. Evaporation of the solvent yielded 1-(N-aroylamino)-triazoles, which were recrystallized from chloroform.

1-(N-p-Methylbenzoylamino)-4,5-diphenyl-1, 2, 3-triazole

Following the above procedure from isoimide (I, with $X=CH_3$) (0.5g) and hydrochloric acid (3 ml) the benzoylamino-triazole (II with $X=CH_3$) was obtained (200 mg, 80%, m.p. 238-240°). UV λ_{max} 244 nm. IR νCO 1695 and νNH 3150 cm^{-1}. NMR (DMSO-D_6) τ: 2.15 (d, 2H, J: 8.5 Hz), 2.54 (m. 12H), 7.62 (s, CH_3). Anal.: calcd. for $C_{22}H_{18}N_4O$: C 74.55, H 5.12, N 15.81%; found C 74.65, H 5.17, N 15.96%.

1-(N-p-Chloro-benzoylamino)-4, 5-diphenyl-1, 2, 3-triazole

From triazolyl-isoimide (I, with X=Cl) (2g) and hydrochloric acid (12 ml) the benzoylamino-triazole (II, with X=Cl) was obtained (900 mg, 61%, m.p. 217-219°). UVλ_{max} 245nm, IR νCO 1695, νNH 3150 cm^{-1}. NMR ($CDCl_3$) τ: 2.34 (d, 2H, J: 8.5Hz), 2.57 (m, 12 H). Anal.: calcd. for $C_{21}H_{15}ClN_4O$: C 67.19, H 4.03, N 14.95%; found: C 66.90, H 4.08, N 15.15%.

1-(N-p-Methoxybenzoylamino)-4,5-diphenyl-1, 2, 3-triazole

From triazolyl-isoimide (I, with X=OCH_3) (900 mg) and hydrochloric acid (4 ml) upon heating the benzoylamino-triazole (II, with X=OCH_3) was obtained (300 mg, 45%, m.p. 183-184°). UV λ_{max} 258 nm, IR νCO 1660, νNH 3280 cm^{-1}. NMR ($CDCl_3$) τ: 2.24 (d, 2H, J: 8.5 Hz) 2.64 (m, 10H), 3.24 (d, 2H, J: 8.5Hz), 6.27 (s, OCH_3). Anal.: calcd. for $C_{22}H_{18}N_4O_2$: C 71.41, H 4.9, N 15.14%; found: C 71.01, H 5.01, N 15.59%.

Synthesis of asymmetrically substituted triazolyl-isoimides (III)

A general procedure is described. 1-(N-Benzoylamino)-triazole (II) of high purity, an excess of freshly distilled benzoylchloride and dry pyridine were mechanically stirred at room temperature for 12 h. The reaction mixture was filtered and the precipitate was thorougly washed with petroleum ether and ether to remove the excess of benzoylchloride and recrystallized from a mixture of chloroform-methanol to give the asymmetrically substituted triazolyl-isoimide (III). An alternative route from symmetrically substituted triazolyl-isoimides (I), excess of benzoylchloride and presence of pyridine under the same conditions of high purity of the reactans and prolonged stirring at room temperature, yielded the same isoimides (III) also in good yields. In this case, the purification of the product was performed in the same way as described above.

1-(α-p-Methylbenzoyloxy-benzylidenamino)-4,5-diphenyl-1, 2, 3-triazole (IIIA)

From benzoylamino-triazole (II, with X=H) (200 mg), 1-p-methylbenzoyl-chloride (1.5 ml) and pyridine (0.2 ml) the triazolyl-isoimide (IIIA) was obtained (120 mg, 45%, m.p. 166-167.5°). UV λ_{max} 252 nm. IR νCO 1745 and νC=N 1640 cm^{-1}. NMR ($CDCl_3$) τ: 7.52 (s, CH_3), 1.90 (d, 2H, J: 9Hz), 2.02 (m, 2H), 2.55 (m, 15H). Anal.: calcd. for $C_{29}H_{22}N_4O_2$: C 75.96, H 4.84, N 12.22%; found: C 75.84, H 5.10, N 11.91%.

1-(α-Benzoyloxy-p-methylbenzylidenamino)-4,5-diphenyl-1, 2, 3-triazole (IIIB)

From benzoylamino-triazole (II, with X=CH_3) (100 mg), benzoylchloride (1 ml) and dry pyridine the triazolyl-isoimide (IIIB) was obtained (80 mg, 62%, m.p. 164-165°). UV λ_{max} 243 nm and shoulder at 285 nm. IR νCO 1745, νC=N 1635 cm^{-1}. NMR ($CDCl_3$) τ: 7.59 (s, CH_3), 1.79 (m, 2H), 2.14 (d, 2H, J: 8.5 Hz) and 2.55 (m, 15H). Anal.: calcd. for $C_{29}H_{22}N_4O_2$: C 75.96, H 4.84, N 12.22%; found: C 75.31, H 4.85, N 11.89%. The above isoimide was also prepared from symmetrically substituted isoimide (I, with X=CH_3) (300 mg), benzoylchloride (3 ml) and pyridine (0.2 ml) by stirring at room temperature

for 56 h (200 mg, 69%, m.p. 164-165°).

1-(α-p-Chlorobenzoyloxy-benzylidenamino)-4,5-diphenyl-1, 2, 3-triazole (IIIC)

From 1-benzoylamino-4,5-diphenyl-1, 2, 3-triazole (II, with X=H) (200 mg), p-chlorobenzoylchloride (1.5 ml) and pyridine (0.2 ml) the triazolyl-isoimide (IIIC) was obtained (120 mg, 47%, m.p. 185.5 - 187°). UV λ_{max} 253 nm. IR νCO 1755, νC=N 1640 cm^{-1}. NMR ($CDCl_3$) τ: 1.79 (d, 2H, J: 8.5 Hz), 2.02 (m, 2H), 2.52 (m, 15H). Anal.: calcd. for $C_{28}H_{19}ClN_4O_2$: C 70.28, H 3.90, N 11.71%; found: C 69.94, H 4.11, N 11.45%.

1-(α-Benzoyloxy-p-chlorobenzylidenamino)-4,5-diphenyl-1, 2, 3-triazole (IIID)

From benzoylamino-triazole (II, with X=Cl) (100 mg) benzoylchloride (1 ml) and pyridine (0.1 ml) the triazolyl-isoimide (IIID) was obtained (55 mg, 60.5%, m.p. 170-171°). UV λ_{max} 244 and shoulder at 282 nm. IR νCO 1745, νC=N 1640 cm^{-1}. NMR ($CDCl_3$) τ: 2.07 (d, 2H, J: 8.5 Hz), 1.79 (m, 2H), 2.52 (m, 15H). Anal.: calcd. for $C_{28}H_{19}ClN_4O_2$: C 70.28, H 3.90, N 11.71%; found: C 71.10, H 4.21, N 11.71%. The isoimide (IIID) was also prepared by treating the symmetrically substituted triazolyl-isoimide (I, with X=Cl) (100 mg) with benzoylchloride (1ml) and pyridine (0.1 ml) at room temperature for 40 h (60 mg, 66%, m.p. 170-171°).

1-(α-Benzoyloxy-p-methoxybenzylidenamino)-4,5-diphenyl-1, 2, 3-triazole (IIIE)

From benzoylaminotriazole (II, with X=OCH_3) (100 mg), benzoylchloride (1 ml) and pyridine (0.1 ml) the triazolyl-isoimide (IIIE) was obtained (60 mg, 47%, m.p. 175-176°). UV λ_{max} 233, 310 nm. IR νCO 1740, νC=N 1635 cm^{-1}. NMR ($CDCl_3$) τ: 6.12 (s, OCH_3), 3.02 (d, 2H, J: 9Hz), 2.03 (d, 2H, J: 9Hz), 1.72 (m, 2H), 2.49 (m, 13H). Anal.: calcd. for $C_{29}H_{22}N_4O_3$: C 73.48, H 4.68, N 11.82%; found: C 73.56, H 4.51, N 11.44%. The same isoimide was also obtained by treating the isoimide (I, with X=OCH_3) (70 mg) with benzoylchloride (1 ml) and pyridine (0.1 ml) at room temperature for 40 h (40 mg, 62%, m.p. 175-176°).

1-(α-p-Chlorobenzoyloxy-p-methoxybenzylidenamino)-4,5-diphenyl-1, 2, 3-triazole (IIIF)

From benzoylamino-triazole (II, with X=OCH_3) (25 mg), p-chlorobenzoylchloride (0.25 ml) and pyridine (0.1 ml) the triazolyl-isoimide (IIIF) was obtained (15 mg, 44%, m.p. 182-183°). UV λ_{max} 250, 310 nm. IR νCO 1740, νC=N 1635 cm^{-1}. NMR ($CDCl_3$) τ: 6.14 (s, OCH_3), 3.04 (d, 2H, J: 8.5 Hz), 2.47 and 1.97 (m, 16H). Anal.: calcd. for $C_{29}H_{11}ClN_4O_3$: C 68.56, H 4.17, N 11.03%; found: C 68.24, H 4.11, N 11.06%.

1-(α-p-Methylbenzoyloxy-p-chlorobenzylidenamino)-4,5-diphenyl-1, 2, 3-triazole (IIIG)

From benzoylamino-triazole (II, with X=Cl) (100 mg), p-methylbenzoylchloride (1.5 ml) and pyridine (0.1 ml) the triazolyl-isoimide (IIIG) was obtained (65 mg, 68%, m.p. 169-171°). UV λ_{max} 245 nm. IR νCO 1740, νC=N

1630 cm^{-1}. NMR ($CDCl_3$). τ: 7.54 (s, CH_3), 1.85 (d, 2H, J: 8.5 Hz), 2.09 (d, 2H, J: 8.5 Hz), 2.55 (m, 14H). Anal.: calcd. for $C_{29}H_{11}ClN_4O_2$: C 70.79, H 4.30, N 11.39%; found: C 71.09, H 4.21, N 11.71%.

Thermal rearrangement of asymmetrically substituted triazolyl-isoimides (III) to diaroyl-imides (IV)

The rearrangment proceeds in very high yield by heating the isoimides at 150° for 3 h without any solvent. The products were recrystallized from a mixture of ethyl acetate-petroleum ether.

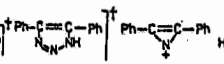
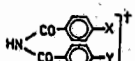
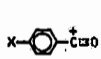
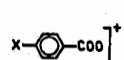
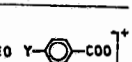
1-(N-Benzoyl-N-p-methylbenzoyl-amino)-4,5-diphenyl-1, 2, 3-triazole (IVB)

The triazolyl-isoimide (IIIA) (200 mg) gave the triazolyl-imide (IVB) (160 mg, 80%, m.p. 235-238°). UV λ_{max} 244 nm. IR νCO 1700 cm^{-1}. NMR ($CDCl_3$) τ: 7.70 (s, CH_3), 2.55 (m, 19H). Anal.: calcd. for $C_{28}H_{22}N_4O_2$: C 75.96, H 4.84, N 12.22%; found: C 75.53, H 4.95, N 11.69%. The thermal rearrangement of triazolyl-isoimide (IIIB) gave the same imide (IVB) with that of isoimide (IIIA). (Their IR spectra were superimposed).

1-(N-Benzoyl-N-p-chlorobenzoyl-amino)-4,5-diphenyl-1, 2, 3-triazole (IVC)

The triazolyl-isoimides (IIIC) and (IIID) (100 mg) gave the same imide (IVC) (90 mg, 90%, m.p. 188-192°). UV λ_{max} 245 nm. IR νCO 1700 cm^{-1}, NMR ($CDCl_3$) τ: 2.55 (m.). Anal.: calcd. for $C_{28}H_{19}ClN_4O_2$: C 70.28, H 3.90, N 11.71%; found: C 69.45, H 3.94, N 11.53%.

TABLE 1: *The major fragments of asymmetrically substituted triazolyl-isoimides (III)*

Compound	Type of ions											
	M^+	M^+-28	$Ph-C\equiv C-Ph$									
IIIA X=H Y=CH_3	458(A)	430(14)	178(60.5)	221(8)	192(22)	239(4)	77(36)	105(83)	121(–)	91(53)	119(100)	135(A)
IIIB X=CH_3	458(A)	430(7)	178(16)	221(5.5)	192(19)	239(3)	77(20)	105(69)	121(10)	91(25)	119(100)	135(–)
IIIC X=H	478(A)	450(7)	178(32)	221(9)	192(18)	258(25)	77(33)	105(100)	121(–)	113(5)	139(57)	155(3.5)
IIID X=Cl Y=H	478(A)	450(9.5)	178(22)	221(7)	192(19)	258(A)	77(34)	105(100)	121(3)	113(4)	139(47)	155(–)
IIIE X=OCH_3 Y=H	474(A)	460(6)	178(45.5)	221(4.5)	192(10)	254(A)	77(30)	106(58)	121(A)	108(5)	135(100)	151(–)
IIIΦ X=OCH_3 Y=Cl	508(A)	480(5)	178(38)	221(5)	192(9)	288(A)	108(5)	135(100)	151(–)	113(4)	139(39)	155(A)
IIIG X=Cl	492(A)	464(A)	178(52)	221(17)	192(54)	272(A)	113(6)	139(70)	155(–)	91(27)	119(83.5)	135(4)

m/e: Number in parenthesis represents relative intensity %. (–): Dash in parenthesis means absence of the corresponding ion.
A: Fragment of small relative intensity.

Περίληψη

«Μελέτη ἐπὶ τῆς συνθέσεως ἀσυμμέτρως ὑποκατεστημένων 1-(α-ἀροϋλοξυαρυλιδεναμινο)-4,5-διφαινυλο-1, 2, 3-τριαζολίων»

Μελετᾶται ἡ σύνθεση τῶν τριαζολυλο-ισοϊμιδίων (III), τὰ ὁποῖα ἀποτελοῦν νέες ἑνώσεις ἀλλὰ καὶ νέα τάξη τριαζολυλο-ισοϊμιδίων. Παρασκευάστηκαν κατὰ δύο διαφορετικοὺς τρόπους, ἀπὸ βενζοϋλαμινο-τριαζόλια καὶ ὑποκατεστημένα βενζοϋλοχλωρίδια ἢ ἀπὸ συμμετρικῶς ὑποκατεστημένα τριαζολυλο-ισοϊμίδια καὶ βενζοϋλοχλωρίδιο, μὲ ἱκανοποιητικὲς ἀποδόσεις (40-70%). Παλαιότερες προσπάθειες συνθέσεώς τους ἀπὸ τὴν ὀξείδωση τῶν διβενζοϋλο-υδραζονῶν, τῶν α-δικαρβονυλικῶν ἑνώσεων ἀπέτυχαν, καθὼς καὶ ἡ ἀπ' εὐθείας σύνθεσή τους ἀπὸ βενζοϊκὰ ἅλατα καὶ ἰμιδοϋλοαλογονίδια, γνωστὴ μέθοδο συνθέσεως συμμετρικῶς ὑποκατεστημένων ἰσοϊμιδίων. Ἡ σύνθεσή τους ἐπετεύχθη μὲ κινητικῶς ἐλεγχόμενη ἀροϋλίωση σὲ χαμηλὲς σχετικῶς θερμοκρασίες τῶν 1-(N-βενζοϋλαμινο)-τριαζολίων ἢ δι' ἐπιδράσεως βενζοϋλοχλωριδίων ἐπὶ συμμετρικῶς ὑποκατεστημένων ἰσοϊμιδίων παρουσία πυριδίνης.

Ἡ θέρμανση δύο ἰσομερῶν μὲ τὸν αὐτὸ ὑποκαταστάτη στὴν βενζοϋλοξυομάδα τοῦ ἑνὸς καὶ στὴν βενζυλιδενάμινο- τοῦ ἄλλου ὁδηγεῖ στὸ σχηματισμὸ τοῦ ἰδίου μικτοῦ διαροϋλο-ιμιδίου (IV).

Μελέτη τῶν φασματοσκοπικῶν τους ἰδιοτήτων ἔδειξε ὅτι πρόκειται γιὰ ἑνώσεις ἀνάλογες πρὸς τὰ συμμετρικῶς ὑποκατεστημένα τριαζολυλο-ισοϊμίδια (II). Ἐνδιαφέρον παρουσιάζει ἡ μελέτη τῶν φασμάτων μαζῶν αὐτῶν τῶν ἑνώσεων.

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PHYTOCHEMICAL STUDY OF *MENTHA PIPERITA* CULTIVATED IN GREECE. Part I: ESSENTIAL OILS

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Summary

Qualitative and quantitative changes of the essential oils of *Mentha piperita* cultivated in Greece, during blossom period are studied.

Key Words: *Labiatae, Mentha piperita, essential oils,* mono-and sesquiterpenes.

Introduction

The genus *Mentha* (Fam. *Labiatae)* includes thirty species, eleven of which are found in Greece[1]. *Mentha piperita L.* is a hybrid of *Mentha spicata L.* and *Mentha aquatica L.*

Ninety five percent of the plant's essential oils are found in the plant leaves and include at least seventy five mono- and sesquiterpenoids.[2] Various other constituents such as flavonoids,[3] sterols,[3] free aminoacids,[4] azulenes,[5] vitamin E,[6] are also found.

The essential oils of *M. piperita L.* (Peppermint oil) are used as flavouring in toothpastes and chewing-gums (50%), sugar products (15%), pharmaceutical preparations (15%) and various other products (20%). Dried leaves of the plant are used as a hot infusion which has curative properties.[7]

The chemical composition of the essential oils of many varieties of *M. piperita* has been thoroughly investigated (Table I). However, the paucity of data concerning the nature of the essential oils of peppermint varieties, which in the recent years are cultivated under the specific climatic and soil conditions of Greece led us in this study. Furthermore, this research was undertaken in an effort to establish the optimum conditions for harvest time and distillation of the essential oils.

TABLE I. *Chemical composition of the volatile oil of Mentha piperita L.*

No	Component	%	No	Component	%	No	Component	%
1	Acetone[2]	t	28	trans-2-Hexenol*[2]	t	55	α-Amorphene[2]	0.1
2	2-Methylpropanal*[9]		29	Tetradecane[2]		56	γ-Muurolene[26]	t
3	Butanal*[2]	t	30	1-Octen-3-ol[2]	t	57	Ledrence[2]	t
4	Ethanol[2]		31	trans-Sabinene hydrate[22]	0.8	58	Germacrene D[2]	0.9
5	2-Methylfuran[2]		32	Menthone[17]	24.2	59	α-Muurolene[26]	0.5
6	3-Methylbutanal*[9]	0.1	33	Menthofuran[23]	1.2	60	Piperitone*[20]	0.1
7	2-Methylbutanal[2]		34	Isomenthone*[24]	3.5	61	Citronellol[2]	
8	2-Ethylfuran[2]	t	35	Pentadecane[2]		62	δ-Cadinene*[14]	t
9	α-Thujene[10]	0.7	36	Copaene[2]	t	63	γ-Cadinene[29]	
10	α-Pinene*[11]		37	Linalool*[25]	0.6	64	Octadecane[2]	t
11	Camphene[12]	t	38	α-Bourbonene[26]		65	α-Cadinene[2]	t
12	β-Pinene[12]	0.8	39	cis-Sabinene hydrate[27]		66	Limonen-10-yl acetate[2]	t
13	Sabinene[13]	0.4	40	trans-p-menth-2-en-1-ol[2]	t	67	Nonadecane[2]	t
14	Myrcene[14]	0.1	41	α-Gurjunene[2]	3.7	68	Calamenene[30]	t
15	3-Methylbutanol[15]	0.2	42	Menthyl acetate*[28]		69	Dihydrolimonen-10-ol[2]	t
16	α-Terpinene[16]	t	43	Neomenthol*[12]		70	Eicosane[2]	t
17	Limonene[17]	1.2	44	β-Copaene[2]	4.9	71	Phenylethyl 3-methyl-butyrate[2]	t
18	1,8-Cineol*[17]		45	Neoisomenthyl acetate[2]		72	Caryophyllene oxide[2]	t
19	β-Phellandrene[18]	5.6	46	Terpinen-4-ol*[2]	1.2	73	Ledol[2]	0.1
20	trans-2-Hexenal[9]		47	Hexadecane[2]		74	Henicosane[2]	t
21	cis-Ocimene[19]	0.1	48	Caryophyllene*[13]	0.8	75	Thymol[31]	t
22	δ-Terpinene[16]	0.5	49	β-Ylangene[2]		76	Eugenol[2]	t
23	p-Cymene[20]	0.1	50	Menthol[28]	45.8		Total	99.7
24	Terpinolene[19]	0.1	51	trans-β-Farnesene[2]	0.1			
25	Hexanol[2]	t	52	Pulegone[20]	1.0			
26	cis-3-Hexenol[14]	0.2	53	α-Terpineol*[2]	0.1			
27	3-Octanol*[21]		54	Heptadecane[2]				

t Trace, less than 0.1%

* Compound with the major present in composite peaks

Results - Discussion

In this study, the qualitative and quantitative differences in chemical composition of the essential oils in the aromatic plant *M. piperita* were studied, at various stages during the blossom period. Specifically, the changes in menthol, menthone and menthofuran were examined.

For this purpose, identified stolons of *M. piperita* (variety *Mitcham*) were cultivated in an area specifically chosen, so that all the climatic and soil conditions were the most suitable for *M. piperita* growth.[8] Soil characteristics of the area used in this study are given below:

Texture	: Sandy clay loam
Equivalent $CaCO_3$	: 33%
Active $CaCO_3$	: 16%
Saturation percentage	: 55%
pH	: 7.5
Exchangeable cations (meq/100 g soil)	: Ca^{++} 10.05, Mg^{++} 4.66, Na^{+} 0.15, K^{+} 0.52

The essential oils yield of peppermint in this study, averaged 0.68%. This yield is higher than those obtained of peppermint crops in other parts of Greece, which gave yields from 0.4 to 0.5%. This increase in yield can, according to our opinion, be attributed to favorable climatic conditions of the experimental field and the advantageous influence[32] of soil calcium and magnesium on normal plant growth and formation of essential oils.

The climatic conditions are shown in Table II.

The aboveground part of the plants (leaves, flowers and stems) which were obtained every one week during the various stages of blooming, starting at the beginning of bloom (1st week), were subjected to vapour distillation and the essential oil yield was estimated (Fig. 1).

Although vapour distillation is not the ideal method for the collection of the essential oils, it was decided that it should be used because it is the only method which is used on a industrial scale.

The essential oils of the flowers and leaves at the beginning of the blooming as well of the whole (above ground) plant at the beginning and full blooming were subjected to a gas chromatography analysis (Fig. 2).

Identification of the peaks of the gas chromatograms was accomplished by a combined GLC-MS analysis (Table III, IV, V and VI).

The structures of the components of the essential oils are shown in Fig. 3.

The results of this study indicate the following:

1. The menthofuran content in the flowers is significantly higher (17.6%) than in the leaves (5.87%). This fact is of great importance, because

TABLE II. *Climatic data for the years 1974-1978 of the area used for peppermint cultivation*

Month	J	F	M	A	M	J	J	A	S	O	N	D
Mean air temperature (°C)	4.0	6.9	10.2	14.3	19.4	23.5	26.0	24.2	21.0	15.5	9.4	4.6
Mean soil temperature (°C)	2.5	6.3	10.6	15.6	22.2	26.3	29.9	27.4	22.8	16.1	9.4	3.8
Mean rainfall (mm)	26.3	38.6	25.3	32.2	70.6	54.7	33.0	36.2	19.5	47.7	45.3	41.3

Maximum annual temperature: 38.4°C Minimum annual temperature: −8.0 °C Mean annual rainfall: 470.7 mm

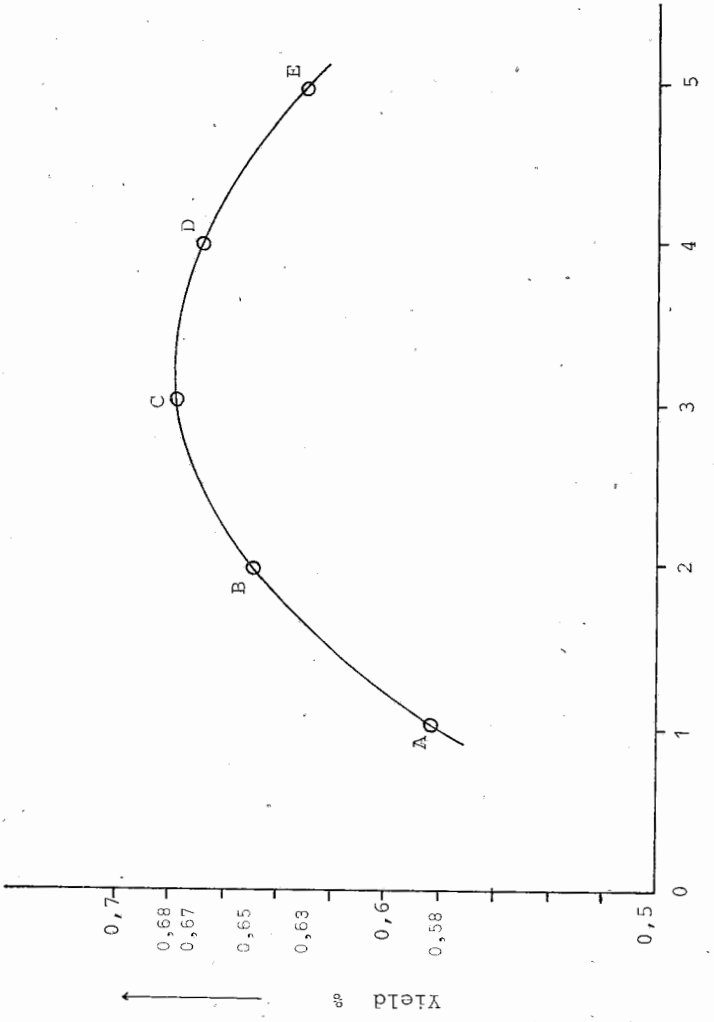

FIG. 1: *Change of the yield of peppermint oil during the bloom period, of the aboveground part of the plants.*
A: Beginning of the blooming.
C: Full blooming.
E: End of the blooming.

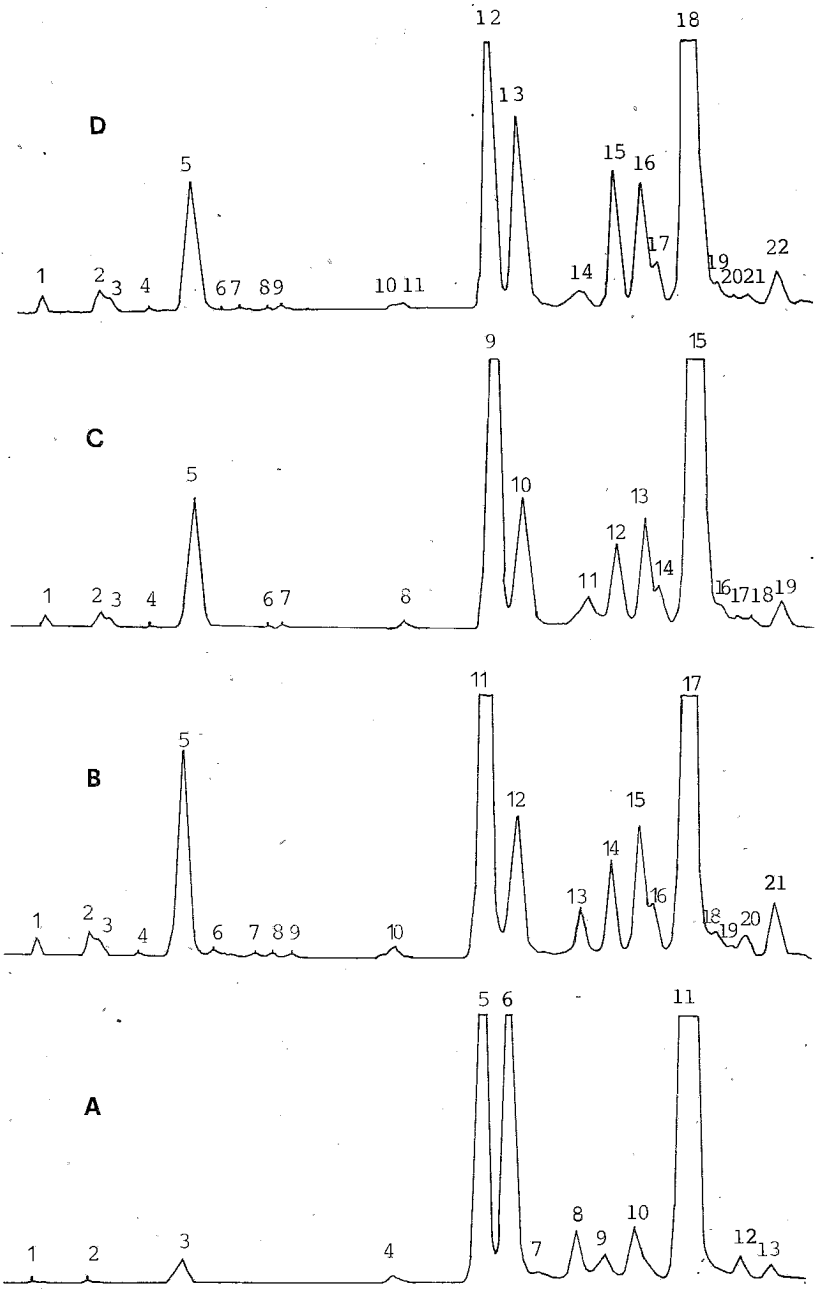

FIG. 2: *GL Chromatograms of the Greek peppermint oil found in various parts of the plant during of the blooming.*

A: From flowers at the beginning of the blooming (Table III).

B: From leaves at the beginning of the blooming (Table IV).

C: From whole plant at the beginning of the blooming period (Table V).

D: From whole plant during their full blooming (Table VI).

TABLE III. *Chemical composition of essential oils found in flowers at the beginning of the blooming period.*

No of peak	R_t (min.)	M^+	m/e	Components	%
1	3.99	136	93, 92, 39, 41, 77, 91, 27, 79 100%→	α-Pinene	0.01
2	6.21	136	93, 41, 69, 39, 27, 79, 77, 53 100%→	β-Pinene	0.02
3	9.99	136	68, 93, 67, 39, 41, 27, 53, 79 100%→	Limonene	1.25
		154	43, 41, 81, 71, 27, 38, 55, 69 100%→	1,8-Cineol*	
4	18.33	172	43, 87, 112, 70, 55, 41, 58, 56 100→	3-Octyl acetate	0.23
		130	59, 29, 43, 55, 27, 41, 31, 83 100%→	3-Octanol*	
5	21.97	154	71, 93, 111, 43, 86, 69, 55, 68 100%→	γ-Terpineol	20.12
		154	112, 69, 139, 154, 111, 70, 97, 83 100%→	Menthone*	
6	22.99	150	108, 112, 150, 69, 84, 79, 99, 41 100%→	Menthofuran	17.60
7	24.18	154	112, 69, 41, 55, 43, 139, 70, 56 100%→	Isomenthone	0.30
8	25.71	154	71, 43, 41, 93, 55, 69, 80, 67 100%→	Linalool	1.93
9	26.79	198	43, 95, 138, 81, 41, 39, 55, 82 100%→	Menthyl acetate	1.38
10	28.00	156	95, 83, 57, 43, 41, 55, 96, 82 100%→	Neomenthol*	2.76
		204	41, 79, 92, 39, 53, 77, 67, 94 100%→	Caryophyllene	
11	29.96	156	71, 81, 95, 55, 82, 138, 41, 69 100%→	Menthol	49.00
12	32.25	204	161, 105, 41, 91, 81, 119, 93, 204 100%→	Germacrene D	0.88
13	33.43	152	82, 110, 95, 41, 39, 137, 109, 54 100%→	Piperitone	0.59

* Compound with the major present in composite peaks

TABLE IV. *Chemical composition of essential oils found in leaves at the beginning of the blooming period.*

No of peak	R_t (min.)	M^+	m/e	Component	%
1	3.85	136	93, 92, 39, 41, 77, 91, 27, 79 100%→	α-Pinene	0.27
2	6.05	136	93, 41, 69, 39, 27, 79, 77, 53 100%→	β-Pinene	0.55
3	6.37	136	93, 41, 77, 91, 79, 27, 39, 69 100%→	Sabinene	0.29
4	7.93	136	41, 93, 69, 39, 27, 53, 79, 77 100%→	Myrcene	0.08
5	9.81	136	68, 93, 67, 39, 41, 27, 53, 79 100%→	Limonene	6.72
		154	43, 41, 81, 71, 27, 38, 55, 69 100%	1,8-Cineol*	
6	11.01	136	93, 91, 77, 136, 121, 39, 43, 27 100%→	γ-Terpinene	0.09
7	12.61	134	119, 134, 91, 28, 120, 117, 77, 41 100%→	p-Cymene	0.06
8	13.29	136	93, 121, 136, 39, 41, 79, 91, 27 100%→	Terpinolene	0.08
9	14.01	172	70, 43, 57, 85, 41, 29, 103, 27 100%→	Amyl Valerate	0.01
		172	43, 87, 112, 70, 55, 41, 58, 56 100%→	3-Octyl acetate	0.45
10	18.20	130	59, 29, 43, 55, 27, 41, 31, 83 100%→	3-Octanol*	
11	21.91	154	71, 93, 111, 43, 86, 69, 55, 68 100%→	γ-Terpineol	22.3
	21.91	154	112, 69, 139, 154, 111, 70. 97, 83 100%→	Menthone*	
		150	108, 112, 150, 69, 84, 79, 99, 41 100%→	Menthofuran*	
12	23.16	154	112, 69, 41, 55, 43, 139, 70, 56 100%→	Isomenthone	5.87
13	25.73	154	71, 43, 41, 93, 55, 69, 80, 67 100%→	Linalool	1.47
14	26.90	198	43, 95, 138, 81, 41, 39, 55, 82 100%→	Menthyl acetate	2.96
15	28.04	156	95, 83, 57, 43, 41, 55, 96, 82 100%→	Neomenthol	4.33
16	28.58	204	41, 79, 92, 39, 53, 77, 67, 94 100%→	Caryophyllene	1.30
17	30.03	156	71, 81, 95, 55, 82, 138, 41, 69 100%→	Menthol	47.64
18	31.10	152	81, 67, 41, 82, 152, 109, 39, 68 100%→	Pulegone	0.77

		154	59, 93, 121, 81, 43, 136, 68, 92 100%→	α-Terpineol	0.30
19	31.72				
		198	81, 69, 95, 67, 43, 82, 123, 138 100%→	Citronellyl acetate*	
20	32.29	204	161, 105, 41, 91, 81, 119, 93, 204 100%→	Germacrene D	0.70
21	33.45	152	82, 110, 95, 41, 39, 137, 109, 54 100%→	Piperitone	2.02

* Compound with the major present in composite peaks

TABLE V. *Chemical composition of essential oils of the whole plant (flowers, leaves and stems) at the beginning of the blooming period.*

No of peak	R_t (min.)	M^+	m/e	Components	%
1	4.00	136	93, 92, 39, 41, 77, 91, 27, 79 100%→	α-Pinene	0.24
2	6.26	136	93, 41, 69, 39, 27, 79, 77, 53 100%→	β-Pinene	0.43
3	6.61	136	93, 41, 77, 91, 79, 27, 39, 69 100%→	Sabinene	0.23
4	8.16	136	41, 93, 69, 39, 27, 53, 79, 77 100%→	Myrcene	0.01
		136	68, 93, 67, 39, 41, 27, 53, 79 100%→	Limonene	
5	10.04				5.76
		154	43, 41, 81, 71, 27, 38, 55, 69 100%→	1,8-Cineol*	
6	12.86	134	119, 134, 91, 28, 120, 117, 77, 41 100%→	p-Cymene	0.02
7	13.53	136	93, 121, 136, 39, 41, 79, 91, 27 100%→	Terpinolene	0.09
		172	43, 87, 112, 70, 55, 41, 58, 56 100%→	3-Octyl acetate	
8	18.39				0.31
		130	59, 29, 43, 55, 27, 41, 31, 83 100%→	3-Octanol*	
		154	71, 93, 111, 43, 86, 69, 55, 68 100%→	γ-Terpineol	
9	22.00				19.42
		154	112, 69, 139, 154, 111, 70, 97, 83 100%→	Menthone*	
		150	108, 112, 150, 69, 84, 79, 99, 41 100%→	Menthofuran*	
10	23.20				6.83
		154	112, 69, 41, 55, 43, 139, 70, 56 100%→	Isomenthone	
11	25.75	154	71, 43, 41, 93, 55, 69, 80, 67 100%→	Linalool	1.72
12	26.92	198	43, 95, 138, 81, 41, 39, 55, 82 100%→	Menthyl acetate	3.19
13	28.05	156	95, 83, 57, 43, 41, 55, 96, 82 100%→	Neomenthol	4.17

14	28.62	204	41, 79, 92, 39, 53, 77, 67, 94 100%→	Caryophyllene	1.17
15	30.07	156	71, 81, 95, 55, 82, 138, 41, 69 100%→	Menthol	53.00
16	30.12	152	81, 67, 41, 82, 152, 109, 39, 68 100%→	Pulegone	0.50
		154	59, 93, 121, 81, 43, 136, 68, 92 100%→	α-Terpineol	0.06
17	31.77				
		198	81, 69, 95, 67, 43, 82, 123, 138 100%→	Citronellyl acetate*	
18	32.29	204	161, 105, 41, 91, 81, 119, 93, 204 100%→	Germacrene D	0.14
19	33.52	152	82, 110, 95, 41, 39, 137, 109, 54 100%→	Piperitone	1.07

* Compound with the major present in composite peaks

TABLE VI. *Chemical composition of essential oils of the whole plant (flowers, leaves and stems) during their full blooming.*

No of peak	R_t (min.)	M^+	m/e	Components	%
1	4.07	136	93, 92, 39, 41, 77, 91, 27, 79 100%→	α-Pinene	0.40
2	6.38	136	93, 41, 69, 39, 27, 79, 77, 53 100%→	β-Pinene	0.68
3	6.74	136	93, 41, 77, 91, 79, 27, 39, 69 100%→	Sabinene	0.40
4	8.33	136	41, 93, 69, 39, 27, 53, 79, 77 100%→	Myrcene	0.08
		136	68, 93, 67, 39, 41, 27, 53, 79 100%→	Limonene	6.39
5	10.19				
		154	43, 41, 81, 71, 27, 38, 55, 69 100%→	1,8-Cineol*	
6	11.43	136	93, 91, 77, 136, 121, 39, 43, 27 100%→	γ-Terpinene	0.01
7	11.97	134	119, 134, 91, 28, 120, 117, 77, 41 100%→	p-Cymene	0.01
8	12.99	136	93, 121, 136, 39, 41, 79, 91, 27 100%→	Terpinolene	0.01
9	13.68	172	70, 43, 57, 85, 41, 29, 103, 27 100%→	Amyl valerate	0.12
10	18.08	172	43, 87, 112, 70, 55, 41, 58, 56 100%→	3-Octyl acetate	0.04
11	18.49	130	59, 29, 43, 55, 27, 41, 31, 83 100%→	3-Octanol	0.08
		154	71, 93, 111, 43, 86, 69, 55, 68 100%→	γ-Terpineol	14.46
12	22.08				
		154	112, 69, 139, 154, 111, 70, 97, 83 100%→	Menthone*	

		150	108, 112, 150, 69, 84, 79, 99, 41 100%→	Menthofuran*	
13	23.18				10.22
		154	112, 69, 41, 55, 43, 139, 70, 56 100%→	Isomenthone	
14	25.51	154	71, 43, 41, 93, 55, 69, 80, 67 100%→	Linalool	0.05
15	27.01	198	43, 95, 138, 81, 41, 39, 55, 82 100%→	Menthyl acetate	5.38
16	28.09	156	95, 83, 57, 43, 41, 55, 96, 82 100%→	Neomenthol	5.30
17	28.67	204	41, 79, 92, 39, 53, 77, 67, 94 100%→	Caryophyllene	1.42
18	30.10	156	71, 81, 95, 55, 82, 138, 41, 69 100%→	Menthol	50.50
19	31.11	152	81, 67, 41, 82, 152, 109, 39, 68 100%→	Pulegone	0.84
		154	59, 93, 121, 81, 43, 136, 68, 92 100%→	α-Terpineol	
20	31.80				0.35
		198	81, 69, 95, 67, 43, 82, 123, 138 100%→	Citronellyl acetate*	
21	32.34	204	161, 105, 41, 91, 81, 119, 93, 204 100%→	Germacrene D	0.37
22	33.54	152	82, 110, 95, 41, 39, 137, 109, 54	Piperitone	1.37

* Compound with the major present in composite peaks

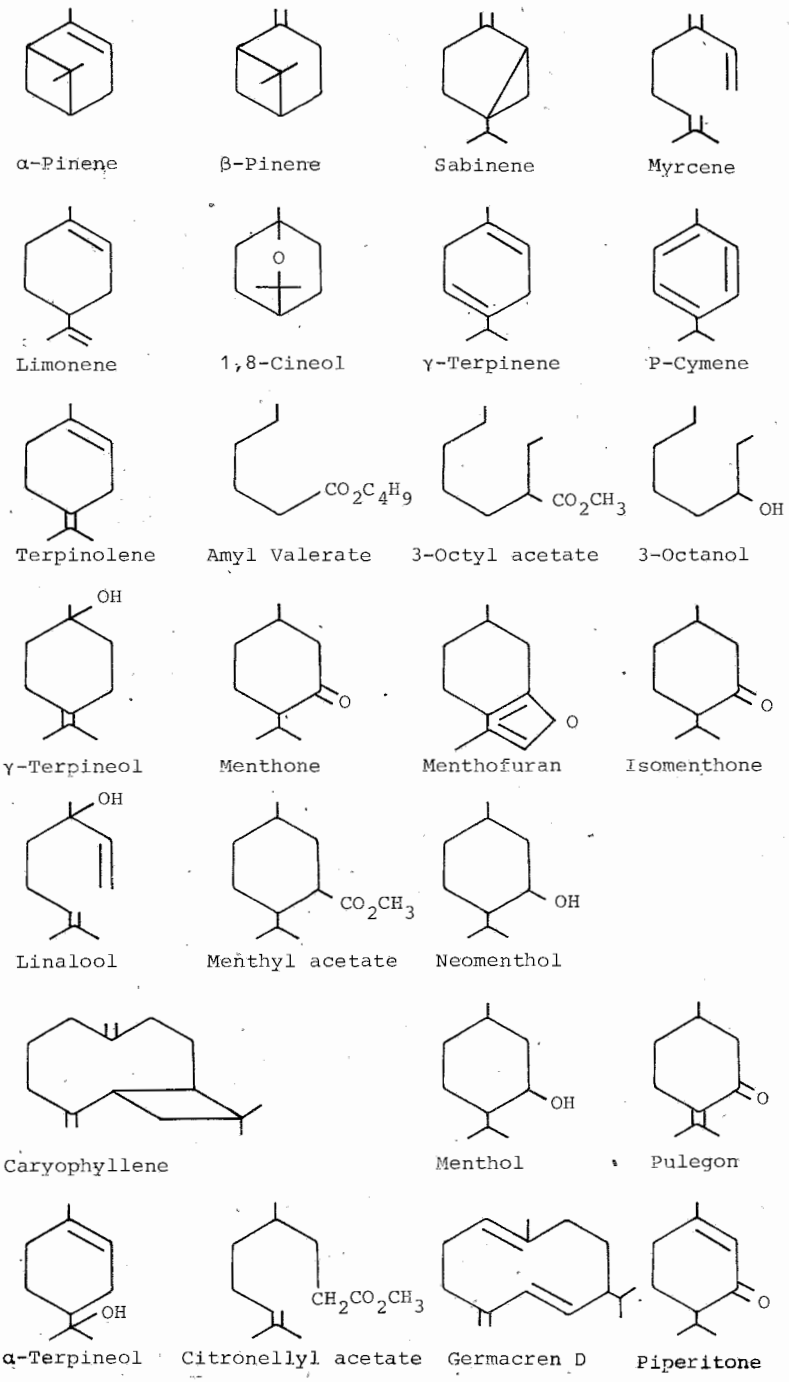

FIG. 3: *Structures of the chemical compositions found in Greek peppermint oil.*

menthofuran play an important role as a quality determining factor of peppermint oil.

2. It was confirmed that a great number of compounds, such as myrcene, p-cymene, γ-terpinene, terpinolene, sabinene, amyl valerate and pulegone, were present in the leaves but not in the flowers.

3. For the first time, traces (0.01%) of amyl valerate are reported in the leaves.

4. The essential oils content in the plant is higher at full bloom (0.68%). At this stage also, menthol and neomenthol content is very high (55.88%), menthyl acetate content reaches a maximum (5.38%), while menthone content is very low (14.45%). This advantage is in favour of the peppermint oil quality. Thus, this study gives an indication that the best harvesting season for obtaining the maximum essential oil yield is the time when peppermint plant is in full bloom (3rd week, Fig 1).

Results are a contribution to the identification of Greek peppermint oils and help in the comparison of them with some of the commercially available oils (Table VII).

TABLE VII. *Comparative presentation of the most important chemical characteristics between the Greek peppermint oil and some of the most known commercial peppermint oils.*

Components	Greek	American			English	Marokian	Bulgarian
		Washington	Midwest	Idamint			
α-Pinene	X	X	X	X	X	X	X
β-Pinene	X	X	X	X	X	X	X
Sabinen	X	X	X	X	X	X	X
Myrcene	x	x	x	x	X	x	x
Limonene	Xx	Xx	Xx	Xx	Xx	Xx	Xx
Cineol	XX	XX	XX	XX	XX	XX	XX
3-Octanol	x	x	x	x	X	x	x
tr. -Sabinene hydrate	–	X	X	X	X	X	X
Menthofuran	XX	XX	Xx	Xx	Xx	XX	XX
iso-Pulegol	–	–	–	–	–	–	–
neo-Menthol	XX	Xx	Xx	Xx	XX	Xx	Xx
Menthone.	XXX	XXX	XXX	XXX	XXX	XXX	XXX
Menthol	XXX	XXX	XXX	XXX	XXX	XXX	XXX
Menthyl acetate	XX	XX	XX	XX	Xx	XX	XX
tr. -β-Farnesene	–	X	X	X	X	X	X
Caryophyllene	Xx	Xx	Xx	Xx	Xx	Xx	Xx
Pulegone	X	Xx	X	Xx	X	Xx	Xx
Carvone	–	X	x	–	–	–	–
Germacrene D	X	Xx	Xx	Xx	Xx	X	X
Piperitone	Xx	X	X	X	X	Xx	X

x = <0.2%
X = 0.2-1%
Xx = 1-4%
XX = 4-10%
XXX = >10%

Experimental

The plant material which was used in the present study was obtained after an experimental cultivation of identified stolons of *M. piperita* (variety *Mitcham*) which were supplied by the Agricultural Association of Serrai. This experimental cultivation took place on an area of four acres located in the district of the village Leukothea of Serrai.

The plant material soon after its collection was placed into plastic bags and remained overnight for partial moisture removal. Afterwards, was subjected to a vapour distillation in a Tounaire type 220 apparatus.

The resulting oil was dried over anhydrous magnesium sulphate and was subjected to a gas chromatography analysis in a Hewlett - Packard 5830 A instrument. A 3mX3.18mm ID glass column was used, packed with 5% of Carbowax 20m (Chromosorb W-AW-DMCS, 80-100 mesh). The injector temperature was 170°C, the flame ionization detector was heated to 300°C and the column temperature was programmed between 75-230°C at the rate of 3°C/min. Helium (99.999%) was the carrier gas flowing at 30ml/min. A Hewlett - Packard 5989 A mass spectrometer connected to the gas chromatograph was used for the mass spectra.

The identification of the oil constituents was based on gas chromatographic evidence and analysis of mass spectra.

Περίληψη

Φυτοχημικὴ μελέτη τῆς Mentha Piperita L. ποὺ καλλιεργήθηκε στὴν Ἑλλάδα. Μέρος Ι: Αἰθέρια ἔλαια

Σ' αὐτὴ τὴν ἐργασία μελετῶνται οἱ ποιοτικὲς καὶ ποσοτικὲς διαφοροποιήσεις ποὺ παρατηροῦνται στὴ χημικὴ σύσταση τῶν αἰθερίων ἐλαίων τῶν διαφόρων μερῶν (ἄνθη, φύλλα, βλαστός) τοῦ ἀρωματικοῦ φυτοῦ *Mentha piperita L.*, οἱ ὁποῖες συμβαίνουν κατὰ τὴ διάρκεια τῆς ἀνθοφορίας του.

Γι' αὐτὸ τὸ σκοπὸ ἔγινε εἰδικὴ πειραματικὴ καλλιέργεια τοῦ φυτοῦ κάτω ἀπὸ συγκεκριμένες ἐδαφολογικὲς καὶ κλιματολογικὲς συνθῆκες. Ἔτσι, ἐκτὸς ἀπὸ τὰ ἄλλα, κατορθώθηκε νὰ προσδιορισθῆ ὁ πλέον ἐνδεδειγμένος χρόνος γιὰ τὴ συλλογὴ καὶ ἀπόσταξη τοῦ φυτικοῦ ὑλικοῦ, ὥστε νὰ λαμβάνεται καλύτερης ποιότητας ἔλαιο.

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PHYTOCHEMICAL STUDY OF *MENTHA PIPERITA* CULTIVATED IN GREECE. PART II: UTILIZATION OF PLANT RESIDUE

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Summary

The chemical composition of peppermint plant (*M. piperita L.*) residues after essential oils extraction is studied. In addition, their nutritional value is evaluated and their use as a feed stuff is suggested.

Key Words: *Labiatae, Mentha piperita L.*, mint meal, feed stuff.

Introduction

The material remaining after processing peppermint plant (*M. piperita L.*) for obtaining the essential oils[1] contains both pharmaceutical use substances[1] such as azulenes, vitamin E, carotenoids and high nutritional value constituents[2,3], such as free aminoacids and proteins. Nevertheless, no attempt has been made in our country for the utilization of peppermint waste which is otherwise discarded. This implies a benefit loss for the producer and a potential environment degradation because of residue disposal.

For the aforementioned reasons, it was considered worth-while to study the utilization of peppermint residues and evaluate of their use as a potential feed stuff because of their chemical composition on the one hand and their influence on broiler growth rate on the other.

Results and Discussion

Plant material remained after essential oils extraction was dried, ground and assayed for determining the basic components that constitute the nutritional value of a feed stuff. Results of chemical analyses (Table I) indicate the similarity of mint meal to that of alfalfa[4,5] which is one of the most basic fodders. These results led us in the biological evaluation of mint meal as a factor affecting growth rate of broilers. One hundred and forty eight broilers (hybrid *Hubbard*) one day of age were used in the experimentation and they were separated into two groups, seventy four individuals in each group (50% male and 50% female). Group A, the controls, receiving alfalfa meal in their ration, and group B, the treated individuals fed with mint meal in their ration.

TABLE I: *Chemical composition of mint and alfalfa meal*

Components		Mint meal*	Alfalfa meal**
Dry material	(g/Kg)	950	904
«Protein» Nitrogen (N×6.25)	»	125	194
Celluloses (according to Sharrer)	»	280	232
Fats	»	30	24
Ash	»	125	100
Non nitrogen extractable substances	»	390	354
Macroelements			
Calcium	(%)	1.34	1.50
Potassium	»	1.87	1.50
Sodium	»	0.15	0.50
Magnesium	»	0.80	—
Trace elements			
Manganese	(mg/Kg)	106.00	44.00
Iron	»	2756.00	280.00
Copper	»	26.00	13.20
Zinc	»	27.00	18.20
Cobalt	»	2.50	0.11
Necesary aminoacids (percent of the total proteinaceous substances)			
Valine		8.00	4.60
Methionine		5.10	0.60
Isoleuvine		4.50	6.50
Leucine		7.80	6.40
Tyrosine		2.50	2.80
Lysine		9.50	6.50
Arginine		4.10	4.70
Threonine		3.40	3.50
Histidine		1.70	1.70
Phenylalanine		2.50	4.80
Cystine		—	2.30

* Data obtained as described in the experimental section
** Data obtained from the literature[4,5]

TABLE II: *Percent composition of poultry rations used in the experimentation*

Ration of group A (control)	%	Ration of group B	%
Concentrate «Nutrikem» (type 1603)	10	Concentrate «Nutrikem» (type 1603)	10
Soybean meal	22	Soybean meal	22
Alfalfa meal	5	Mint meal	6
Corn meal	43	Corn meal	42
Wheat meal	20	Wheat meal	20

It was thus pursued to find whether addition of mint meal in broiler's ration, in about the same amount to that of alfalfa meal, had any inhibitory action on growth rate and on Feed Conversion Index (F.C.I.). Percent composition and chemical analysis of the rations used for the groups are given in Tables II and III. Average increase of body weight within the two groups during the experimentation is given in Table IV and Figure 1.

TABLE III: *Chemical composition of poultry rations used in the experimentation.*

Components		Ration of group A	Ration of group B
Dry material	(g/Kg)	882	890
«Protein» Nitrogen (N×6.25)	»	231	222
Celluloses (according to Sharrer)	»	43	45
Fats	»	33	35
Ash	»	58	58
Non nitrogen extractable substances	»	517	530
Macroelements			
Calcium	(%)	1.11	1.11
Potassium	»	2.20	2.20
Sodium	»	0.25	0.22
Magnesium	»	0.27	0.30
Trace elements			
Manganese	(mg/Kg)	40.30	44.50
Iron	»	306.00	452.00
Copper	»	2.40	3.30
Zinc	»	80.00	82.00
Cobalt	»	0.50	0.70

TABLE IV: *Average increase of body weight (g) of groups A and B during the experimentation*

Tested groups	Days of experimentation 1st	18th	28th	54th	72th	Total increase of body weight
Group A						
Average body weight ($\bar{X}$)	36	343	633	1650	2750	2714
Group B						
Average body weight ($\bar{X}$)	33	327	630	1630	2731	2698
Percent increase $\frac{\bar{X}-\bar{X}_{ist}}{\bar{X}_{ist}}$.100 of group A	–	852.7	1658.3	4483.3	7538.9	–
Percent increase $\frac{\bar{X}-\bar{X}_{ist}}{\bar{X}_{ist}}$. 100 of group B	–	890.9	1809.7	4839.4	8172.7	–

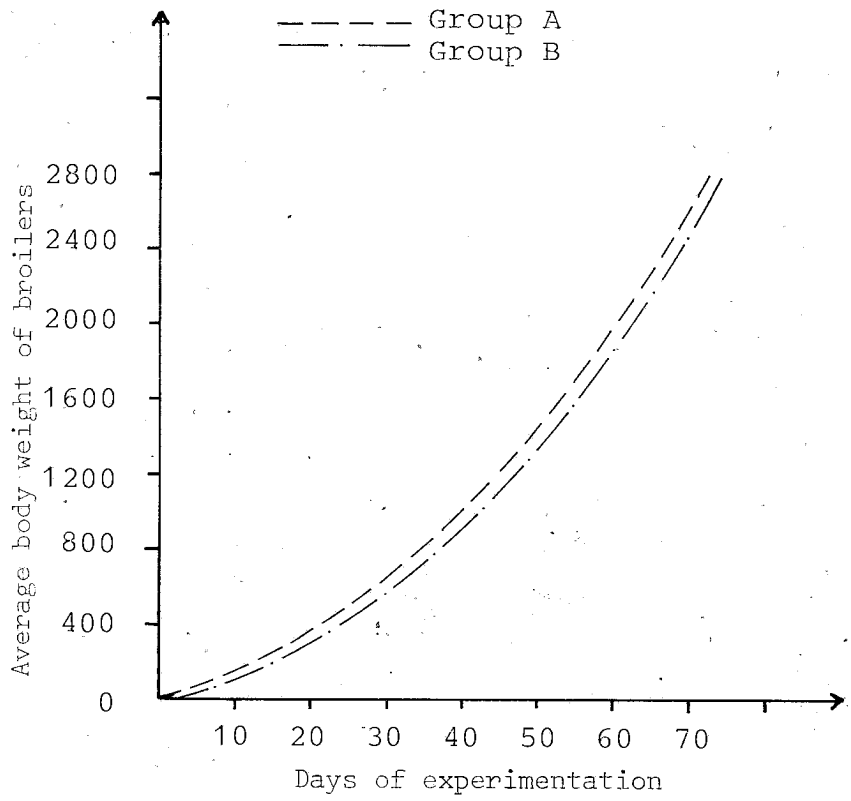

FIG. 1: *Growth rate curves of broilers belonging to groups A and B.*

Growth rate curve of Figure 1 and data of Table IV show that increase of average body weight reached up to 7538.9% at the end of the experiment for group A whereas the corresponding increase for group B was 8172.7%. In other words, group B increase was higher than group A by a 633.8%.

Values of Feed Conversion Index (F.C.I.) for each group of tested animals are given in Table V. Data of Table V show that values of F.C.I. for group B are superior to those for group A, a fact suggesting that addition of mint meal to poultry rations can be proven more advantageous than addition of alfalfa meal.

Experimental

Inorganic compounds determination of mint meal

Macro and trace elements analyses in mint meal were done by using the dry ashing technique for plant tissue decomposition, dissolving the ash in HCl acid and determining each element by Atomic Absorption Spectroscopy using a Perkin Elmer model 503 instrument[6].

TABLE V: *Values of Feed Conversion Index (F.C.I.)* during the experimentation*

Tested groups	Days of experimentation			
	18th	28th	54th	72nd
	F.C.I.			
Group A (control)	1.40	1.95	2.32	2.14
Group B	1.30	1.87	2.23	2.09
$\frac{(F.C.I.)_B - (F.C.I.)_A}{(F.C.I.)_A} \cdot 100$	−7.14	−4.10	−3.88	−2.34

* $F.C.I. = \frac{\text{Feed consumed (Kg)}}{\text{Body weight gained (Kg)}}$

Organic components determination

1. Total nitrogen was determined by the Kjeldahl procedure[7].
2. Celluloses analysis was carried out by the Sharrer method[8].
3. Total fats were determined by the Soxhlet method using ether as the extracting agent[8].
4. Aminoacids were determined after hydrolysis of proteins with 6N HCl acid for 24 hrs in an oil bath at 137±2 °C[9]. Qualitative and quantitative analysis of aminoacids was done using an automatic aminoacid analyser, Phoenix Model K-8000.

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Περίληψη

Φυτοχημικὴ μελέτη τῆς Mentha Piperita L. ποὺ καλλιεργεῖται στὴν Ἑλλάδα. Μέρος ΙΙ: Ἀξιοποίηση τῶν φυτικῶν ὑπολειμμάτων

Σ' αὐτὴ τὴν ἐργασία μελετᾶται ἡ ἀξιοποίηση τῶν φυτικῶν ὑπολειμμάτων ποὺ ἀπομένουν μετὰ τὴν παραλαβὴ τῶν αἰθερίων ἐλαίων ἀπὸ τὸ φυτὸ *M. piperita L.* Γι' αὐτὸ τὸν σκοπὸ τὰ παραπάνω ὑπολείμματα ὑποβλήθηκαν σὲ χημικὴ ἀνάλυση προκειμένου νὰ προσδιορισθοῦν ὅλοι ἐκεῖνοι οἱ χημικοὶ παρά-

γοντες ποὺ καθορίζουν τὴ θρεπτικὴ ἀξία, ὥστε νὰ ἐκτιμηθῆ ἡ δυνατότητα χρησιμοποιήσεώς τους ὡς ζωοτροφῆς. Ἐπακολούθησε βιολογικὸ πείραμα, τὰ ἀποτελέσματα τοῦ ὁποίου καθιερώνουν τὸ μεντάλευρο σὰν ζωοτροφὴ ἀνάλογης θρεπτικῆς ἀξίας πρὸς τὸ μηδικάλευρο.

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MÉTHYLATION DES AMINO-4 ARYL-5 PYRIMIDINES»

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Résumé

Méthylation des amino-4-aryl-5-pyrimidines

La méthylation des amino-4 aryl-5 pyrimidines a lieu exclusivement en position -1 du noyau pyrimidinique, et donne les méthyl-1 dihydro-1,4 aryl-5 imino-4 pyrimidines.

La détermination de la structure des dérivés N-méthyl-pyrimidiniques s'effectue par voie chimique, par hydrolyse alcaline, qui donne les N-méthylpyrimidones correspondantes connues, ainsi que par l'étude des leur spectres de RMN.

Les méthyl-1 dihydro-1,4 aryl-5 imino-4 pyrimidines obtenues, sont des composés instables contrairement à leur sels avec le sulfate de diméthyle et notamment avec l' acide iodhydrique, qui sont stables.

Key words: Méthylation, Pyrimidines.

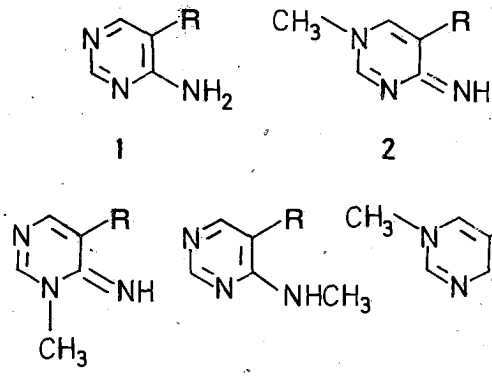
Abréviations

TFAM = Trisformylaminométhane

Introduction

La méthylation des amino-4-aryl-5-pyrimidines **1** peut conduire à la formation de trois dérivés méthylés isomères, deux N-méthylés sur les deux azotes intranucléaires en position 1 et 3 du noyau pyrimidinique, les méthyl-1 dihydro-1,4 aryl-5 imino-4 pyrimidine **2** et méthyl-3 dihydro-3,4 aryl-5 imino-4 pyrimidine **3** respectivement et un troisième du groupement amine en position 4, la méthylamino-4 aryl-5 pyrimidine **4**[1].

La méthylation de l' amino-4 phényl-5 pyrimidine **1a** par l'iodure de méthyle a été effecture par Davies et Piggott[2]. La réaction n' a pas donné comme produits les imino-4 pyrimidines **2a** et **3a** ettendues, ni la méthylamino-4 pyrimidine **4a** mais la méthyl-1 dihydro-1,4 phényl-5 oxo-4 pyrimidine **5a.** Par la suite, en modifiant les conditions de la réaction, la méthylation de l' aminopyrimidine **1a** a été faite avec le sulfate de diméthyle. Après décomposition par la soude du méthyl sulfate intermédiaire formé, le seul produit isolé de la réaction est la méthyl-1 dihydro-1,4 phényl-5 imino-4 pyrimidine **2a**[3,4].



1 2

3 4 5

6 7

a: R= C_6H_5- b: R= p-NO_2-C_6H_4-

c: R= p-NH_2-C_6H_4- d: R= a-$C_{10}H_7$-

Dans ce mémoire, on effectue la méthylation de l' amino-4 p-nitrophényl-5 pyrimidine **1b**[5], de l' amino-4 p-aminophényl-5 pyrimidine **1c**[5] et l' amino-4 a-naphthyl-5 pyrimidine **1d**[6] avec le sulfate de diméthyle et l' iodure de méthyle et on étudie ensuite la structure des produits méthylés obtenus (tableau I).

Partie Expérimentale

Les points de fusion sont pris au banc Koffler et ils ne sont pas corrigés. Les microanalyses ont été effectuées sur «Perkin Elmer» modèle 240. Les spectres UV ont été déterminés dans l' éthanol, au moyen d' un spectrophotomètre «Perkin Elmer» modèle 124. Les spectres IR sont enregistrés sur «Perkin Elmer» modèle 257 à partir d' échantillons dispersés dans le nujol. Les spectres de RMN ont été mesurés sur un appareil «Varian A-60 A» à 60 Mc/s. Les déplacements chimiques sont exprimés en τ par rapport au tétraméthylsilane utilisé comme référence interne.

Préparation des méthyl sulfates

Méthylsulfate de la méthyl-1 amino-4 p-nitrophényl-5 pyrimidine 6b

A une solution de 2.16 g (0.01 mole) de l' amino-4 p-nitrophényl-5 pyrimidine **1b** dans 120 ml de dioxane on ajoute goutte à goutte une solution de 4 ml (0.04 mole) de sulfate de diméthyle dans 20 ml de dioxane, à 50°. On maintient le mélange réactionnel à 50° pendant 1h, et on refroidit. On sépare

TABLEAU I: *Produits de la méthylation des amino-4 aryl-5 pyrimidines et de leur hydrolyse par la soude*

Pyrimidine de départ	Agent de méthylation		Dérivés N_1-méthylés	Produits de l'hydrolyse par la soude des dérivés N_1-méthylés
	$(CH_3)_2SO_4$	CH_3J		
amino-4 p-nitro-phényl-5 pyrimidine 1b	méthyl sulfate du composé 1b	sel d' ammonium quaternaire du composé 1b	méthyl-1 dihydro-1,4 p-nitrophényl-5 imino-4 pyrimidine, 2b	méthyl-1 dihydro-1,4 p-nitrophenyl-5 oxo-4 pyrimidine 5b
amino-4 p-amino-phényl-5 pyrimidine 1c	la méthylation n' a pas eu leu	mélange non séparé	—	—
amino-4 α-naphthyl-5 pyrimidine 1d	le produit de la méthylation n' a pas été isolé	sel d' ammonium quaternaire du composé 1d	méthyl-1 dihydro-1,4 α-naphthyl-5 imino-4 pyrimidine 2d	méthyl-1 dihydro-1,4 α-naphthyl-5 oxo-4 pyrimidine 5d

par filtration le précipité blanc, on l' extrait à l' alcool absolu chaud, on chasse le solvant par évaporation et on obtient 2.7 g du méthyl sulfate **6b** qui est recristallisé dans l' éthanol. Cristaux jaunâtres, F = 240-241°, (Rdt= 80%).

Analyse $C_{12}H_{14}N_4O_6S$

Calc. %:	C 42.11	H 4.12	N 16.37
Tr.	42.13	4.10	16.51

Spectre UV (éthanol) : λmax 256 nm (ε= 18300)
Spectre IR (nujol): : bande NH_2 à 3310, 3160, 3120 cm^{-1}
Spectre RMN (TFAM) : 6.02 τ S, 5.80 τ S.

Préparations des iodures 7

0.005 mol de l' amino-4 pyrimidine 1.80 ml de méthanol et 4 ml d' iodure de méthyle sont chauffés pendant 2 h à reflux. On concentre et on recristallise dans un mélange éthanol - éther.

a) Iodure du méthyl-1 amino-4 p-nitrophényl-5 pyrimidine 7b

A partir de l' aminopyrimidine **1b** on obtient l' iodure **7b** F= 281 - 282°, p= 1.2 g, Rdt= 70%.

Analyse $C_{11}H_{11}N_4O_2I$

Calc. %:	C 36.89	H 3.09	N 15.64
Tr.	36.80	2.97	15.68

Spectre UV (éthanol) : λmax 255 nm (ε= 16400)
Spectre IR (nujol) : bande NH_2 à 3.400, 3.260, 3.110 cm^{-1}
Spectre RMN (TFAM) : 5.78 τ S.

b) Iodure du méthyl-1 amino-4 naphthyl-5 pyrimidine 7d

A partir de l' aminopyrimidine **1d** on obtient l' iodure **7d**, F= 278.5-279.5 C, p= 1.5g, Rdt= 83%.

Analyse $C_{15}H_{14}N_3I$

Calc. %:	C 49.61	H 3.89	N 11.57
Tr.	49.71	3.92	11.41

Spectre UV (éthanol) : λmax 258 nm (ε= 15200)
Spectre IR (nujol) : bande NH_2 à 3360, 3280, 3110 cm^{-1}
Spectre RMN (TFAM) : 5.78 τ S.

Décomposition des sels 6 et 7. Préparation des méthyl-1 dihydro-1,4 imino-4 pyrimidines 2

A une solution de 0.001 mole de sel **6** ou **7** dans 20 ml d' eau, on ajoute à froid 5 ml d' une solution aqueuse de soude à 1%. On extrait immédiatement par le chloroforme, sèche sur sulfate de sodium et on distille le solvant.

Méthyl-1 dihydro-1,4 p-nitrophényl-5 imino-4 pyrimidine 2b

a) A partir du méthyl sulfate **6b** on obtient 0.2 g de l' iminopyrimidine **2b,** sous forme de cristaux jaunes, qui recristallisent dans un mélange chloroforme-éther, F= 192 - 193° (d), (Rdt= 85%).

Analyse $C_{11}H_{10}N_4O_2$

Calc. %:	C 57.38	H 4.38	N 24.34
Tr.	57.41	4.41	24.32

Spectre UV (éthanol) : λmax 254 nm (ε= 17200), 260 nm (ε= 16900)
Spectre IR (nujol) : bande NH à 3260 cm^{-1}
Spectre RMN ($CDCl_3$) : 6.54 τ S.

b) A partir de l' iodure **7b** on obtient 0.14 g de l' iminopyrimidine **2b,** F= 192 - 193° (d), p= 0.14 g, Rdt= 62%.

Méthyl-1 dihydro-1,4 -naphthyl-5 imino-4 pyrimidine 2d

A partir de l' iodure **7d** on obtient l' iminopyrimidine **2d** qui recristallise dans un mélange de chloroforme et d' éther et est filtré dans une atmosphère d' azote.

L' opération doit se faire très vite car le composé **2d** s' altère facilement. F= 176 - 178°, p= 0.1 g, Rdt= 42%.

Analyse $C_{15}H_{13}N_3$

Calc. %:	C 76.57	H 5.57	N 1776
Tr.	76.80	5.64	17.86

Spectre UV (éthanol) : λmax 260 nm (ε= 18200)
Spectre IR (nujol) : bande NH à 3230 cm^{-1}
Spectre RMN ($CDCl_3$) : 6.55 τ S.

Hydrolyse des méthyl sulfates 6 et des iodures 7

Dans des conditions douces:

0.001 mole des sels **6** ou **7** dans 30 ml d' une solution aqueuse de soude 0.1 N rédgissent pendant 24 h à la température ambiante.

a) A partir du méthyl sulfate **6b** après filtration et lavage avec un peu d' éthanol on obtient 0.18 g de l' oxopyrimidine **5b**[7], F= 305 - 307°, (Rdt= 78%).

b) A partir de l' iodure **7b** après filtration et lavage avec un peu d' éthanol on obtient 0.15 g de l' oxopyrimidine **5b**[7], F= 307 - 309°, (Rdt= 65%).

c) A partir de l' iodure **7d** après extraction par le chloroforme et distillation du solvant on obtient 0.2 g de l' oxopyrimidine **5d**[7], F= 211 - 213°, (Rdt= 85%).

Dans des conditions intenses

0.001 mole des sels **6** ou **7** dans 3.6 ml d'une solution aqueuse de soude 1N est chauffé au bain-marie pendant 10 mn.

a) A partir du méthyl sulfate **6b** après filtration on obtient 0.07 g de l' ox-

opyrimidine **5b**, (Rdt= 30%).

b) A partir de l' iodure **7b**, après filtration on obtient 0.1 g de l' oxopyrimidine **5b**, (Rdt= 43%).

c) A partir de l' iodure **7d** après extraction par le chloroforme et distillation du solvant on obtient 0.18 g de l' oxopyrimidine **5d**[7], (Rdt= 80%).

Résultats et discussion

Parmi les trois pyrimidines mentionnées ci-dessus, seule l' aminopyrimidine **1b** a pu être méthylée par le sulfate de diméthyle. Ainsi, nous avons obtenu le méthyl sulfate **6b**, qui a été décomposé par une solution aqueuse de soude très diluée. Des trois isomères possibles, un seul dérivé N-méthylé a été formé, qui a été isolé et identifié comme étant la méthyl-1 dihydro-1,4 p-nitrophényl-5 imino-4 pyrimidine **2b**.

Les tentatives de méthylation de l' aminopyrimidine **1c** sont restées sans succès bien qu' on ait effectué plusieurs essais en modifiant chaque fois les conditions de la réaction.

La méthylation de l' aminopyrimidine **1d** a donné un solide blanc, très instable qui, au fur et à mesure devenait de plus en plus gris et résineux. Comme dans ce composé nous avons constaté la présence de l' anion sulfate, nous avons supposé qu' il était un méthyl sulfate de l' aminopyrimidine **1d**. Après décomposition par la soude, nous avons obtenu un composé résineux qui ne contenait pas d' anion sulfate et qui après chromatographie sur couche mince a montré qu' il était un mélange complexe, impossible à séparer.

La méthylation des mêmes pyrimidines **1b**, **1c**, et **1d**, avec l' iodure de méthyle a donné pour les composés **1b** et **1d** deux sels d' ammonium quaternaire, les **7b** et **7d** respectivement tandis que la **1c** a donné un mélange résineux qu' il n' a pas été possible de séparer. Après décomposition des sels **7b** et **7d** par une solution aqueuse de soude diluée, nous avons obtenu respectivement la méthyl-1 dihydro-1,4 p-nitrophényl-5 imino-4 pyrimidine **2b** et la méthyl-1 dihydro-1,4 α-naphthyl-5 imino-4 pyrimidine **2d**.

En général, nous avons remarqué que les méthyl sulfates, les sels d' ammonium quaternaire, et les sels avec l' acide iodhydrique, sont dans la plupart des cas, des composés stables. Par contre, les dérivés N-méthylés correspontants s' altèrent souvent par la lumière ou l' air et il faut travailler dans une atmosphère de gaz inerte ou à l' abri de la lumière.

Pour établir la structure des dérivés méthylés nous avons procédé à leur étude par voie chimique et par spectroscopie. Nous avons d' abord comparé les dérivés N-méthylés formés, et leurs isomères méthylamino-4. Dans le cas, par exemple, de la nitrophénypyrimidine **1b**, nous avons trouvé qu' il n' était pas identique à la méthylamino-4 p-nitrophényl-5 pyrimidine[5]. Il a été trouvé ainsi, qu' aucune des méthylations effectuées au cours de ce travail ne portait sur l' azote du groupement amine.

Ensuite, nous avons traité les méthyl sulfates ou les sels d' ammonium quaternaire de chacun des dérivés méthylés, ou les dérivés méthylés, avec une solution aqueuse de soude concentrée.

Il est connu que l' hydrolyse des dérivés N_1-méthylés des amino-4 pyrimidines par la soude concentrée, donne les oxo-4 pyrimidines correspondantes[8,9], alors que les dérivés N_3-méthylés subissent le réarrangement Dimroth et donnent les isomères méthylamino-4 pyrimidines[9 à 11].

En utilisant le même prosédé[8,9], après l' hydrolyse par la soude, de la méthyl-1 p-nitrophényl-5 imino-4 pyrimidine **2b** et de la méthyl-1 α-naphthyl-5 imino-4 pyrimidine **2d**, nous avons obtenu respectivement la méthyl-1 p-nitrophényl-5 oxo-4 pyrimidine[12] **5b** et la méthyl-1 α-naphthyl-5 oxo-4 pyrimidine[13] **5d**. Ce fait, constitue une première indication de la structure des dérivés N-méthylés.

En dehors de ce procédé et dans le but de confirmer d' une façon plus certaine la structure des composés ci-dessus, nous avons étudié les spectres de RMN des N-méthylpyrimidines et des N-méthylpyrimidones[7] correspondantes.

Il a été montré[7,13] que les valeurs de déplacement diamagnétique,

$$\Delta^{CDCl_3}_{C_6H_6}$$

des protons du groupement méthyle des dérivés N_1-méthylés (1.48 - 1.90 ppm), sont plus importantes que celles des isomères N_3-méthylés (0.95 - 1.10 ppm).

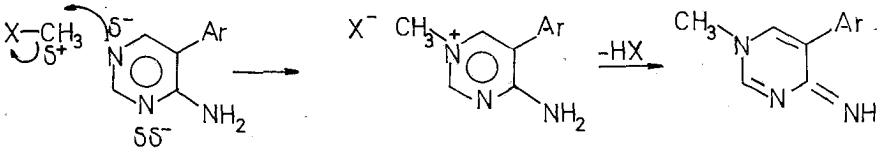
En effectuant la mesure des déplacements diamagnetiques des protons du groupement méthyle des dérivés N-méthylés dans le chloroforme deutéré et dans le benzène nous avons constaté que les valeurs trouvées sont du même ordre de grandeur que celles des dérivés N_1-méthylés. Il en est de même pour les déplacements paramagnétiques des mêmes composés dans le chloroforme deutéré et dans l' acide trifluoracétique (Tableau II)[13].

TABLEAU II: *Valeurs de déplacement diamagnétique,* $\Delta^{CDCl_3}_{C_6H_6}$, *et paramagnétique* $\Delta^{CDCl_3}_{TFA}$ *en ppm des protons du groupement méthyle des dérivés N-Méthylés.*

$\Delta^{CDCl_3}_{C_6H_6}$		$\Delta^{CDCl_3}_{TFA}$	
N_1-méthylprimidones	N_1-méthylpyrimidones	N_1-méthylpyrimidines	N_1-méthylpyrimidones
2a + 1.45	5a + 1.67	2a − 0.70	5a − 0.62
2b + 1.32	5b + ★	2b − 0.67	5b − ★
2d + 1.54	5d + 1.64	2d − 0.75	5d − 0.67

★ Composés insolubles dans le $CDCl_3$ et le C_6H_6.

En étudiant les résultats de la méthylation des amino-4 pyrimidines **1** nous pouvons conclure qu' il est bien possible, que la répartition de la densité électronique de la molécule de ces composés est donnée par les formules **8**, de façon qu' il soit possible l' attaque de la position -1 du noyau pyrimidinique, par l' agent de méthylation.



Summary

Methylation of 4-Amino-5-arylpyrimidines

4-Amino-5-arylpyrimidines are methylated with dimethylsulfate and methyliodide exclucively in 1-position of the pyrimidine ring giving 1-methyl-1,4-dihydro-5-aryl-4-iminopyrimidines.

The structure of the obtained N-methyl derivatives was elucidated by alcaline hydrolysis leading to the corresponding N-methylprimidones and by their spectral data.

The N-methyl derivatives obtained, by standing at the atmosphere air are changed whereas their methylsulfate and especially hydroiodide saltes are stable.

Περίληψη

Μεθυλίωση τῶν 4-ἀμινο-5-αρυλοπυριμιδινῶν

Ἡ μεθυλίωση τῶν 4-ἀμινο-5-αρυλοπυριμιδινῶν μὲ θειϊκὸ διμεθύλιο καὶ μεθυλιωδίδιο, γίνεται ἀποκλειστικὰ στὴ θέση 1- τοῦ πυριμιδινικοῦ δακτυλίου καὶ δίνει τὶς 1-μεθυλο-1,4-διϋδρο-4-ιμινο-5-αρυλοπυριμιδίνες.

Ἡ διευκρίνιση τῆς συντάξεως τῶν λαμβανομένων N-μεθυλοπαραγώγων γίνεται χημικῶς μὲ ἀλκαλικὴ ὑδρόλυση ἡ ὁποία δίνει τὶς ἀντίστοιχες N-μεθυλοπυριμιδόνες καὶ μὲ τὴ μελέτη φασματοσκοπικῶν δεδομένων, κυρίως μὲ τὰ φάσματα πυρηνικοῦ μαγνητικοῦ σρντονισμοῦ ἀπὸ τὴ μέτρηση τῆς διαμαγνητικῆς μετατοπίσεως σὲ διαφορετικοὺς διαλύτες.

Οἱ λαμβανόμενες 1-μεθυλο-1,4-διϋδρο-5-αρυλο-4-ιμινοπυριμιδίνες εἶναι ἑνώσεις ἀσταθεῖς ποὺ ἀλλοιώνονται εὔκολα κατὰ τὴν παραμονή τους στὸν ἀέρα, ἀντίθετα πρὸς τὰ ἅλατά τους μὲ θειϊκὸ διμεθύλιο καὶ κυρίως μὲ ὑδροϊωδικὸ ὀξύ, ποὺ εἶναι ἑνώσεις σταθερές.

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ΔΙΑΚΡΙΣΗ ΑΙΘΑΝΟΛΗΣ ΑΠΟ ΖΥΜΩΣΗ ΑΠΟ ΣΥΝΘΕΤΙΚΗ ΑΙΘΑΝΟΛΗ. ΔΙΑΚΥΜΑΝΣΗ ΤΟΥ ^{14}C ΣΕ ΕΛΛΗΝΙΚΟΥΣ ΟΙΝΟΥΣ ΚΑΙ ΑΠΟΣΤΑΓΜΑΤΑ ΟΙΝΟΥ

ΓΚΕΓΚΙΟΥ ΝΤ.*, ΜΠΟΤΣΙΒΑΛΗ Μ., ΓΕΩΡΓΟΥΛΗ Μ. καὶ ΧΑΤΖΗΔΑΚΗ Ε.
Γενικὸ Χημεῖο τοῦ Κράτους, Δ/νση Μελετῶν - Ἐρευνῶν,
Ἀν. Τσόχα 16, Ἀθῆναι 602.

Περίληψη

Μελετήθηκε μέθοδος διακρίσεως αἰθανόλης ἀπὸ ζύμωση ἀπὸ συνθετικὴ αἰθανόλη βάσει τῆς εἰδικῆς ραδιενέργειας τοῦ ^{14}C μὲ φασματόμετρο σπινθηρισμοῦ ὑγρῶν. Χρησιμοποιήθηκαν ταχεῖες μέθοδοι ἐξωτερικοῦ προτύπου ποὺ ἔδωσαν πολὺ ἱκανοποιητικὰ ἀποτελέσματα. Μετρήθηκε ἐπίσης ἡ διακύμανση τοῦ ^{14}C σὲ ἑλληνικοὺς οἴνους καὶ ἀποστάγματα οἴνου. Ἡ μέση τιμὴ τῆς εἰδικῆς ραδιενέργειας τῶν ἑλληνικῶν προϊόντων παραγωγῆς 1974 ἦταν 19,2 dpm ^{14}C/gC καὶ τῶν προϊόντων παραγωγῆς 1976, 18 dpm ^{14}C/gC. Οἱ τιμὲς αὐτὲς εἶναι σὲ πλήρη συμφωνία μὲ τὶς σχετικὲς εὐρωπαϊκὲς μετρήσεις.

Εἰσαγωγή

Ἡ αἰθανόλη παράγεται εἴτε ἀπὸ ὑδατάνθρακες μὲ ζύμωση εἴτε ἀπὸ ὑδρογονάνθρακες συνθετικά. Ἡ βιομηχανικὴ παρασκευὴ τῆς συνθετικῆς αἰθανόλης γίνεται μὲ ἐνυδάτωση αἰθυλενίου. Τὸ αἰθυλένιο προέρχεται ἀπὸ πυρόλυση πετρελαίων ἢ ἀπὸ ἀέρια γαιανθράκων. Λόγῳ τοῦ διαφορετικοῦ τρόπου παρασκευῆς ἡ αἰθανόλη ἀπὸ ζύμωση διαφέρει ἀπὸ τὴ συνθετικὴ αἰθανόλη στὶς προσμίξεις ποὺ τὴ συνοδεύουν. Ἡ συνθετικὴ αἰθανόλη δὲν περιέχει η-προπανόλη, ἰσοβουτανόλη, ἀμυλικὲς ἀλκοόλες καὶ ἐστέρες τους. Ἡ αἰθανόλη ἀπὸ ζύμωση δὲν περιέχει διαιθυλαιθέρα, ὑδρογονάνθρακες, πρακτικῶς τριτοταγῆ βουτανόλη καὶ ἰσοπροπανόλη, τὴν ὁποία μόνο μερικὰ εἴδη οἰνικῶν προϊόντων περιέχουν σὲ πολὺ μικρὰ ποσοστά. Ἔρευνες γιὰ τὴ διάκριση τῶν δύο προϊόντων ἔχουν βασιστεῖ στὶς διαφορετικὲς αὐτὲς προσμίξεις. Συγκεκριμένα ἔχει βρεθεῖ ὅτι τὸ μέγιστο ποσοστὸ τῆς ἰσοπροπανόλης στὴ συνθετικὴ αἰθανόλη εἶναι 15mg/100ml, ἐνῶ στὴν αἰθανόλη ἀπὸ ζύμωση, ὅταν ὑπάρχει, δὲν ξεπερνάει τὰ 3mg/100ml[1]. Γιὰ τὸν προσδιορισμὸ τῆς τριτοταγοῦς βουτανόλης δὲν ἔχει βρεθεῖ ἱκανοποιητικὴ χρωματογραφικὴ στήλη γιὰ τὸ διαχωρισμό της.

Καλύτερος τρόπος γιὰ τὴ διάκριση τῶν δύο προϊόντων μπορεῖ νὰ θεωρηθεῖ ὁ προσδιορισμὸς τοῦ περιεχομένου ^{14}C.[2-6] Τὸ CO_2 τῆς ἀτμόσφαιρας περιέχει, ἐκτὸς ἀπὸ τὸ σταθερὸ ἰσότοπο ^{12}C, τὸ σταθερὸ ἰσότοπο ^{13}C καὶ μικρὰ ποσὰ τοῦ ἀσταθοῦς ἰσοτόπου ^{14}C. Ὁ ^{14}C σχηματίζεται στὰ ἀνώτερα στρώματα τῆς ἀτμόσφαιρας ἀπὸ πυρῆνες ἀζώτου μὲ τὴν ἐπίδραση κοσμικῆς ἀκτινοβολίας. Μετὰ τὸ σχηματισμό του μπαίνει στὸν κύκλο τοῦ CO_2 ὡς $^{14}CO_2$. Ἀπὸ πολλὲς χιλιετηρίδες ἔχει ἀποκατασταθεῖ ἰσορροπία ἀνάμεσα στὸ

σχηματιζόμενο καὶ στὸ ραδιενεργῶς ἀποικοδομούμενο ^{14}C. Ἔτσι, τὸ σύνολο τῆς ζώσας ὀργανικῆς ὕλης, ἐφ' ὅσον βρίσκεται μέσα στὸν κύκλο τοῦ CO_2, ποὺ περιέχει τὸ ἴδιο ποσοστὸ ^{14}C. Πεθαίνοντας ἡ ὀργανικὴ ὕλη παύει νὰ μετέχει τοῦ κύκλου CO_2 καὶ νὰ ἐμπλουτίζεται σὲ ^{14}C καὶ λαβαίνει χώρα μόνο μεταστοιχείωση τοῦ ἀσταθοῦς ἰσοτόπου μὲ ἥμισυ χρόνο ζωῆς 5760 ἔτη. Ἡ μεταστοιχείωση τοῦ ^{14}C σὲ ^{14}N γίνεται μὲ ἐκπομπὴ ἀκτινοβολίας-β.

Ἡ αἰθανόλη ἀπὸ ζύμωση, δεδομένου ὅτι προέρχεται ἀπὸ ζώσα ὀργανικὴ ὕλη, περιέχει τὸ φυσιολογικὸ ποσοστὸ ^{14}C. Ἀντίθετα ἡ συνθετικὴ αἰθανόλη δὲν περιέχει ^{14}C, δεδομένου ὅτι τὰ πετρέλαια, ὅπως εἶναι παραδεκτό, προέρχονται ἀπὸ ζῶντες ὀργανισμοὺς ποὺ ἔχουν πεθάνει πρὶν ἀπὸ ἑκατομμύρια ἔτη. Στὴν ἐργασία αὐτὴ μελετήθηκε ἡ μέθοδος ἀπαριθμήσεως τῆς ἀκτινοβολίας-β μὲ Φασματόμετρο Σπινθηρισμοῦ Ὑγρῶν σὲ αἰθανόλη ἀπὸ δείγματα ἑλληνικῶν οἴνων καὶ ἀποσταγμάτων οἴνου μὲ σκοπὸ τὴ διάκριση τῆς αἰθανόλης αὐτῆς ἀπὸ τὴ συνθετικὴ αἰθανόλη. Ἐπίσης μετρήθηκε τὸ ἐπίπεδο τῆς εἰδικῆς ραδιενέργειας τοῦ ^{14}C τῆς περιεχόμενης στὰ ἑλληνικὰ αὐτὰ προϊόντα αἰθανόλης, γιὰ τὰ ἔτη 1974 καὶ 1976.

Μιὰ ἀκόμη προσπάθεια γιὰ τὴ διάκριση τῆς αἰθανόλης ἀπὸ ζύμωση ἀπὸ τὴ συνθετικὴ αἰθανόλη ἔγινε μὲ τὴ μέτρηση τοῦ σταθεροῦ ἰσοτόπου ^{13}C σὲ αἰθανόλη ἀπὸ ζύμωση διαφόρων πρώτων ὑλῶν καὶ σὲ συνθετικὴ αἰθανόλη.[7] Τὰ ἀποτελέσματα αὐτὰ δὲν μπορεῖ νὰ θεωρηθοῦν καλύτερα ἀπὸ τὴ μέτρηση τῆς εἰδικῆς ραδιενέργειας τοῦ ^{14}C.

Πειραματικὸ μέρος

Ἀντιδραστήρια

Διάλυμα σπινθηριστῶν: 8,0g 2,5-διφαινυλοξαζόλης (PPO) καὶ 0,5g p-δις (4-μεθυλο, 5-φαινυλοξαζολυλο) βενζόλιο (διμεθυλο-POPOP) σὲ 1l τολουολίου γιὰ σπινθηριστές. Τὰ ἀνωτέρω ἀντιδραστήρια ἦταν τῆς Packard Instrument Co.

Συνθετικὴ αἰθανόλη: καθαρότητας 99,9% τῆς BP Chemicals International Ltd.

Ἐξωτερικὰ πρότυπα διαλύματα: κάθε πρότυπο διάλυμα περιέχει τὴν ἴδια ραδιενέργεια (^{14}C: $1,0\times10^5 \pm 2,0\%$ dpm). Τὰ διαλύματα περιέχουν σπινθηριστῆ μὲ βάση τὸ τολουόλιο, τολουόλιο ἰχνοθετημένο μὲ ^{14}C καὶ νιτρομεθάνιο ὡς παράγοντα ἀποσβέσεως. Ἡ συγκέντρωση τοῦ σπινθηριστῆ εἶναι PPO 4,0g/l καὶ διμεθυλο-POPOP 0,25g/l τολουολίου. Ὁ ὁλικὸς ὄγκος κάθε διαλύματος εἶναι 15ml. Τὰ πρότυπα διαλύματα ἦταν τῆς Packard Instrument Co.

Ὄργανα

Φασματόμετρο Σπινθηρισμοῦ Ὑγρῶν Packard TRI-CARB τύπος 3385 (discriminator 50-1000, gain 5,7%) καὶ τύπος 3330 (discriminator 50-1000, gain 6,3%).

Γυάλινα φιαλίδια μετρήσεως μὲ χαμηλὴ περιεκτικότητα σὲ κάλιο καὶ πῶμα ἀπὸ πολυαιθυλένιο.

Προχοΐδα τῶν 25ml καὶ διαβαθμισμένα σιφώνια τῶν 10ml.

Ἀποστακτικὴ συσκευὴ μὲ στήλη vigreux ὕψους 80cm καὶ ἐσωτερικῆς διαμέτρου 2,5cm.

Μέθοδος

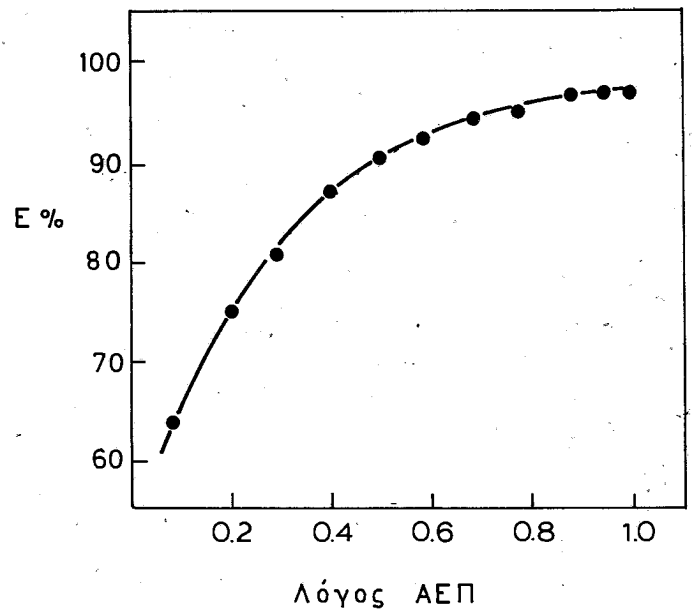
Παραλαβὴ αἰθανόλης: Ἡ παραλαβὴ τῆς αἰθανόλης ἀπὸ τὰ δείγματα γίνεται μὲ ἀπόσταξη. Ἡ στήλη ἀποστάξεως κατασκευάστηκε στὸ ἐργαστήριο, ἔτσι ὥστε μὲ μιὰ μόνο ἀπόσταξη κάτω ἀπὸ ἀτμοσφαιρικὴ πίεση νὰ λαμβάνεται αἰθανόλη 94-95°. Πρὶν ἀπὸ τὴν ἀπόσταξη τὰ δείγματα πρέπει νὰ ὑποστοῦν ἐξουδετέρωση. Ἡ ἐξουδετέρωση γίνεται μὲ KOH παρουσία φαινολοφθαλεΐνης, ἐκτὸς ἀπὸ τὴν περίπτωση τῶν ἐρυθρῶν οἴνων ὅπου ἡ ἐξουδετέρωση γίνεται χωρὶς δείκτη, γιατὶ ἡ χρωστική τους λειτουργεῖ ὡς δείκτης. Ὁ ὄγκος τοῦ χρησιμοποιούμενου γιὰ τὴν ἀπόσταξη δείγματος ἐξαρτᾶται ἀπὸ τὸν ἀλκοολικὸ βαθμὸ τοῦ προϊόντος. Γιὰ νὰ μετρηθεῖ, μετὰ τὴν ἀπόσταξη, ὁ ἀλκοολικὸς βαθμὸς μὲ ἀραιόμετρο, χρειάζονται τουλάχιστον 160 ml αἰθανόλης. Συλλέγεται τὸ κλᾶσμα ποὺ ἀποστάζει στοὺς 78-79°. Τὸ πρῶτο κλάσμα τῆς ἀποστάξεως 15ml περίπου ἀπορρίπτεται. Σὲ τρεῖς ὧρες περίπου συλλέγονται 120 ml αἰθανόλης.

Προετοιμασία δειγμάτων αἰθανόλης καὶ ἀπαρίθμηση ἀκτινοβολίας: Ὁ θόρυβος τοῦ ὑποστρώματος μετριέται μὲ τὴ βοήθεια τυφλοῦ συνθετικῆς αἰθανόλης. Τόσο ἡ συνθετικὴ αἰθανόλη ὅσο καὶ τὰ ἐξεταζόμενα δείγματα αἰθανόλης μετριοῦνται εἰς διπλοῦν καὶ προετοιμάζονται ὡς ἑξῆς: σὲ κάθε ἕνα ἀπὸ τὰ γυάλινα φιαλίδια προστίθεται μὲ σιφώνιο ποσότητα 7,5 ml αἰθανόλης 92° καὶ μὲ προχοΐδα 7,5 ml διαλύματος σπινθηριστῶν. Πωματίζεται τὸ φιαλίδιο καὶ ἀναμιγνύεται τὸ περιεχόμενό του. Τὰ ἕτοιμα γιὰ ἀπαρίθμηση δείγματα φυλάγονται στὸ ψυγεῖο.

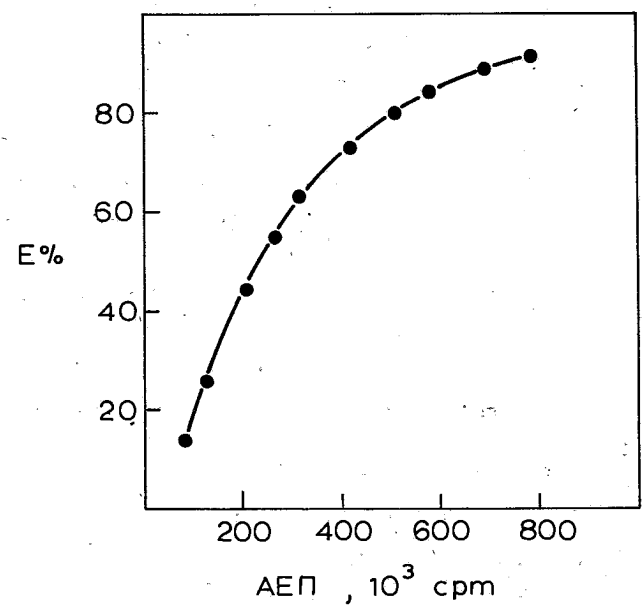
Γιὰ τὴν ἀπαρίθμηση τῆς ἀκτινοβολίας-β χρησιμοποιήθηκε ἡ μέθοδος ἐξωτερικοῦ προτύπου (TRI-CARB 3330) καὶ ἡ μέθοδος λόγου διαύλων ἐξωτερικοῦ προτύπου (TRI-CARB 3385).

Μὲ τὸν τύπο 3330, στὴ διάρκεια ἀπαριθμήσεως 100min συλλέγονται 4000 κρούσεις περίπου γιὰ τὰ δείγματα αἰθανόλης ἀπὸ ζύμωση. Ἡ ἀπόδοση τῆς ἀπαριθμήσεως εἶναι 43,5 - 44,5% καὶ ὁ θόρυβος τοῦ ὑποστρώματος 37 cpm. Μὲ τὸν τύπο 3385 συλλέγονται 5000 κρούσεις μὲ σφάλμα 1,5%. Ὁ ἀπαιτούμενος χρόνος ἀπαριθμήσεως γιὰ δείγματα αἰθανόλης ἀπὸ ζύμωση εἶναι 65 min περίπου, ἡ ἀπόδοση τῆς ἀπαριθμήσεως 87 - 87,6% καὶ ὁ θόρυβος τοῦ ὑποστρώματος 28 cpm.

Οἱ καμπύλες ἀποδόσεως ποὺ κατασκευάσθηκαν μὲ τὴ βοήθεια τῶν ἐξωτερικῶν πρότυπων διαλυμάτων δίνονται στὰ Σχήματα 1 καὶ 2 γιὰ τοὺς δύο τύπους φασματομέτρων.



ΣΧΗΜΑ 1: *Καμπύλη ἀποδόσεως ἀπαριθμήσεως E (cpm/dpm)% ἔναντι τοῦ Λόγου Αὐτόματου Ἐξωτερικοῦ Προτύπου (Λόγος ΑΕΠ). — (TRI-CARB* 3385).



ΣΧΗΜΑ 2: *Καμπύλη ἀποδόσεως ἀπαριθμήσεως E (cpm/dpm)% ἔναντι τῶν κρούσεων ἀνὰ λεπτὸ Αὐτόματου Ἐξωτερικοῦ Προτύπου (ΑΕΠ). — (TRI-CARB 3330).*

Ὑπολογισμὸς εἰδικῆς ραδιενέργειας

Ἀπὸ τὶς κρούσεις ἀνὰ λεπτὸ (cpm) καὶ τὴν ἀπόδοση % ὑπολογίζεται ἡ εἰδικὴ ραδιενέργεια σὲ διασπάσεις ^{14}C ἀνὰ λεπτὸ ἀνὰ γραμμάριο ἄνθρακα (dpm ^{14}C/gC) βάσει τοῦ τύπου:

$$\frac{\text{dpm } ^{14}C}{\text{g C}} = \frac{\left(\frac{cpm_\Delta}{E_\Delta} - \frac{cpm_T}{E_T}\right) \cdot 1{,}917 \cdot 100}{B \cdot V \cdot E}$$

ὅπου:
cpm_Δ: τιμὴ σὲ cpm δείγματος,
cpm_T: τιμὴ σὲ cpm συνθετικῆς αἰθανόλης (θόρυβος ὑποστρώματος),
E_Δ: ἀπόδοση % δείγματος,
E_T: ἀπόδοση % συνθετικῆς αἰθανόλης,
B: ἀλκοολικὸς βαθμὸς δείγματος στοὺς 15,5° C (92°),
V: ὄγκος δείγματος σὲ ml (7,5 ml),
E: εἰδικὸ βάρος ἀπόλυτης αἰθανόλης στοὺς 15,5° C (0,7939) καὶ
1,917: ποσότητα αἰθανόλης σὲ g ποὺ ἀντιστοιχεῖ σὲ 1g ἄνθρακα.

Ἀποτελέσματα καὶ συζήτηση

Τὰ δείγματα, κυρίως τοῦ οἴνου, ἔπρεπε νὰ ὑποστοῦν ἐξουδετέρωση πρὶν ἀπὸ τὴν ἀπόσταξη, γιατὶ διαφορετικὰ παρουσιάζουν μεγάλη ἀπόσβεση, ἡ ὁποία ἀνάλογα μὲ τὸ μέγεθός της εἶχε ὡς ἀποτέλεσμα τὴν αὔξηση τοῦ σφάλματος τῆς μετρήσεως καὶ σὲ ὁρισμένες περιπτώσεις ἡ μέτρηση ἦταν προβληματική.

Στὴν πράξη ἡ ἀπόσβεση μπορεῖ νὰ ὁριστεῖ ὡς ἡ μείωση τῆς ἀποδόσεως λόγῳ ὑπάρξεως στὸ φιαλίδιο ὑλικῶν μολύνσεως. Σχεδὸν κάθε ξένη οὐσία μπορεῖ νὰ παράγει μικρὴ ἢ μεγάλη ἀπόσβεση. Ἡ ἀντιμετώπιση τῆς μειωμένης ἀποδόσεως στὴν ἀπαρίθμηση, λόγῳ ἀκριβῶς τῆς ἀποσβέσεως, γίνεται μὲ διάφορες μεθόδους. Στὴν ἐργασία αὐτὴ χρησιμοποιήθηκαν, ὅπως ἀναφέρεται καὶ ἀνωτέρω, οἱ μέθοδοι ἐξωτερικοῦ προτύπου. Οἱ δύο αὐτὲς μέθοδοι εἶναι πολὺ γρήγορες καὶ ἐνδείκνυνται γιὰ ἀναλύσεις μεγάλου ἀριθμοῦ δειγμάτων, ὅπως ἦταν αὐτὲς ποὺ εἶχαν ν' ἀντιμετωπίσουν τὰ ἐργαστήριά μας, γιὰ τὸν ἔλεγχο τῶν εἰσαγόμενων καὶ τῶν ἐξαγόμενων προϊόντων.

Ἂν καὶ οἱ περισσότεροι παράγοντες ἀποσβέσεως παράγουν τὶς ἴδιες καμπύλες ἀποσβέσεως, ἐν τούτοις εἶναι δυνατὸ νὰ προκύψουν σφάλματα, ὅταν τὰ ἐξεταζόμενα δείγματα καὶ τὰ ἐξωτερικὰ πρότυπα διαλύματα δὲν ἔχουν τὴν ἴδια χημικὴ σύσταση. Γιὰ νὰ ἐλεγχθεῖ πιθανὸ σφάλμα, λόγῳ τῆς διαφορετικῆς χημικῆς συστάσεως τῶν ἐξεταζόμενων δειγμάτων καὶ τῆς σειρᾶς τῶν προτύπων διαλυμάτων, παρασκευάστηκε πρότυπο διάλυμα (5×10^4dpm) ποὺ περιεῖχε, ὡς παράγοντα ἀποσβέσεως, συνθετικὴ αἰθανόλη (50%). Ἡ ἀπόδοση τοῦ διαλύματος αὐτοῦ ἦταν σὲ πλήρη συμφωνία μὲ τὴν ἀπόδοση γιὰ τὰ ἐξεταζόμενα δείγματα αἰθανόλης. Ἐπίσης, δεῖγμα αἰθανόλης ἀπὸ ζύμωση ποὺ εἶχε μετρηθεῖ σὲ γαλλικὸ ἐργαστήριο (Union Nationale des Distillateurs d'Alcool, Paris) μὲ τὴ μέθοδο ἐσωτερικοῦ προτύπου, μετρήθηκε καὶ στὸ ἐργαστήριό μας καὶ ἔδωσε τὰ ἴδια ἀποτελέσματα.

Ἡ χρησιμοποιούμενη συνήθως μέθοδος ἐσωτερικοῦ προτύπου σύμφωνα μὲ τὴν ὁποία κάθε δεῖγμα αἰθανόλης μετριέται χωρὶς καὶ μὲ τὴν προσθήκη ἰχνοθετημένης μὲ ^{14}C πρότυπης οὐσίας, ἂν καὶ ἀπὸ τὴν ἄποψη τῆς ἀποσβέσεως παρουσιάζεται πλεονεκτική, ἔχει τὸ μειονέκτημα νὰ ἐξαρτᾶται ἀπὸ τὴν ἀκρίβεια προσθήκης τῆς ἰχνοθετημένης οὐσίας, ἡ ὁποία προστίθεται σὲ μικροποσότητες. Ἐπίσης εἶναι χρονοβόρα καὶ παρουσιάζει τὶς δυσχέρειες ποὺ συνοδεύουν τὸ χειρισμὸ ραδιενεργῶν οὐσιῶν.

Ὁ Πίνακας Ι περιλαμβάνει τ' ἀποτελέσματα τῶν μετρήσεων πάνω σὲ διάφορα δείγματα οἴνου καὶ ἀποστάγματα οἴνου ἀπὸ διάφορες περιοχὲς τῆς χώρας παραγωγῆς 1974 καὶ ὁ Πίνακας ΙΙ περιλαμβάνει τ' ἀποτελέσματα ἀπὸ δείγματα ἑλληνικῶν οἴνων παραγωγῆς 1976.

ΠΙΝΑΚΑΣ Ι: *Εἰδικὴ ραδιενέργεια αἰθανόλης ἀπὸ ἑλληνικοὺς οἴνους καὶ ἀποστάγματα οἴνου παραγωγῆς 1974.*

α/α	Προϊόν	dpm ^{14}C/gC μέση τιμὴ δύο μετρήσεων
1	Οἶνος	18.3
2	»	18.8
3	ἀπόσταγμα οἴνου	19.2
4	»	20.5
5	»	20.1
6	»	19.5
7	»	18.5
8	»	20.1
9	»	19.6
10	οἶνος	19.9
11	»	19.3
12	»	19.6
13	»	19.5
14	»	20.1
15	ἀπόσταγμα οἴνου	18.5
16	»	19.8
17	»	19.3
18	»	17.9
19	»	18.7
20	»	19.3
21	»	18.5
22	»	18.9
23	»	17.8
24	»	18.9
25	οἶνος	19.5
26	»	18.6
27	»	19.5
28	»	19.3
29	»	19.3

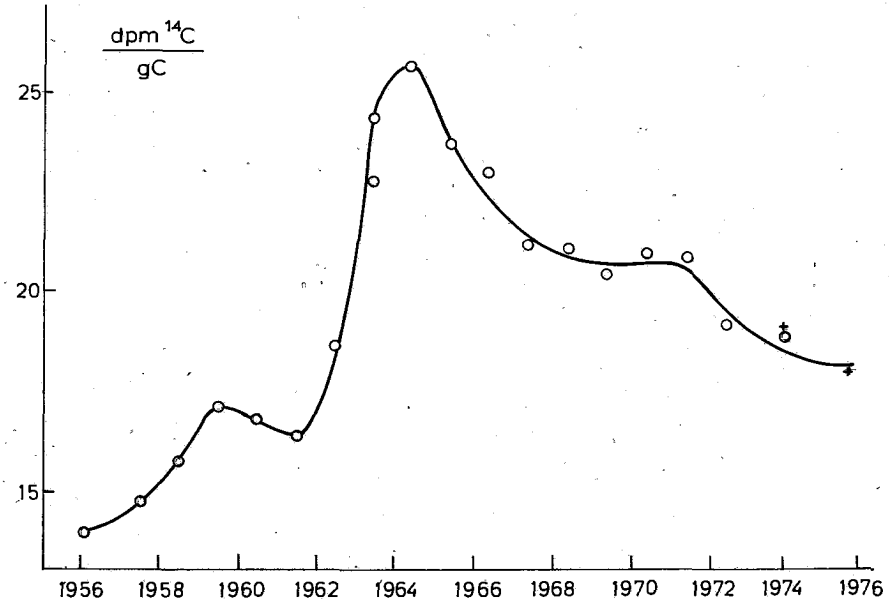
Μέση τιμή: 19,2 dpm ^{14}C/gC
Σταθερὴ ἀπόκλιση: 0,659
Συντελεστὴς διακυμάνσεως: 3,4%

ΠΙΝΑΚΑΣ ΙΙ: *Εἰδικὴ ραδιενέργεια αἰθανόλης ἀπὸ ἑλληνικοὺς οἴνους καὶ στοιχεῖα τῶν οἴνων παραγωγῆς 1976.*

α/α	Περιοχή	Ποικιλία	Ἀλκοολικὸς βαθμὸς 20°C	Ὀλικὴ ὀξύτης σὲ H_2SO_4, g/l	Πτητικὴ ὀξύτης σὲ CH_3COOH, g/l	dpm ^{14}C/gC μέση τιμὴ δύο μετρήσεων
1	Κάντζα	σαβατιανὸ	12,9	3,9	0,50	16,9
2	Μαρκόπουλο	»	12,8	3,2	0,38	18,1
3	Κάντζα	μανδηλάρια	12,9	2,5	0,47	17,2
4	Πικέρμι	σαβατιανό	11,5	4,4	0,30	17,8
5	Πεζά-Κρήτης	κοτσιφάλι	12,4	6,5	0,36	17,6
6	»	κρᾶμα ἐρυθρῶν	12,3	5,6	0,42	17,9
7	»	ροζακί	11,4	4,4	0,33	17,4
8	»	σουλτανί	12,6	5,3	0,33	17,3
9	»	βιλάνα	12,6	6,7	0,45	17,7
10	»	κρᾶμα λευκό	12,7	5,8	0,45	18,1
11	»	ροζακί	12,2	4,8	0,26	18,3
12	»	σουλτανί	12,4	6,9	0,30	17,7
13	»	μαντηλάρι	12,8	5,3	0,48	19,2
14	Σητεία-Κρήτης	διάτικο	15,1	4,8	0,70	18,9
15	Δάφνες »	κρᾶμα	12,0	6,0	0,41	17,7
16	» »	»	11,8	6,2	0,40	17,6
17	» »	διάτικο	13,8	4,5	0,53	17,8
18	» »	»	13,5	4,9	0,49	18,4
19	Τύρναβος	μοσχάτο	11,9	3,8	0,40	18,6
20	Ραψάνη	κρᾶμα	11,5	6,8	0,40	17,7
21	Ζάκυνθος	»	12,5	5,6	0,50	18,7
22	Ζαχάρω	χλωροσταφιδίτης	12,8	7,4	0,62	18,2
23	Πύργος	φιλέρι + »	12,4	5,2	0,65	18,7
24	Εὔβοια	καντούρα	11,5	5,0	0,18	18,0
25	»	»	10,7	5,8	0,27	17,0
26	Ζίτσα	delica	9,8	9,0	0,48	17,5
27	Γαστούνη	κρᾶμα	12,5	6,3	0,75	19,4
28	Μαντινεία	»	10,3	8,0	0,51	18,6
29	»	φιλέρι	11,7	7,1	0,51	17,8
30	»	μοσχοφίλερο	11,3	5,9	0,55	17,1
31	Ἐπίδαυρος	κρᾶμα	12,9	6,1	0,69	17,1
32	Πυλία-Μεσσηνίας	ροδίτης	11,6	5,6	0,63	18,5
33	Μαλέμε-Χανιά	ρωμέικο	12,5	4,8	0,40	18,8
34	»	»	11,9	7,5	0,78	18,2
35	»	»	12,7	6,3	0,43	18,3
36	»	»	12,1	6,2	0,58	18,9

Μέση τιμή: 18,0 dpm ^{14}C/gC
Σταθερὴ ἀπόκλιση: 0,633
Συντελεστὴς διακυμάνσεως: 3,5%

Ὅπως φαίνεται στὸ Σχῆμα 3, πρὶν ἀπὸ τὶς πυρηνικὲς δοκιμές, εἶχε ἀποκατασταθεῖ στὴ γῆ ἰσορροπία μεταξὺ τοῦ σχηματιζόμενου καὶ τοῦ ραδιενεργῶς ἀποικοδομούμενου $^{14}CO_2$ καὶ ἡ εἰδικὴ ραδιενέργεια τοῦ ^{14}C ἦταν 14dpm/gC περίπου. Μὲ τὴν ἔναρξη τῶν πυρηνικῶν δοκιμῶν ἡ τιμὴ τῆς εἰδικῆς ραδιενέργειας ἀνέρχονταν ἀπὸ χρόνο σὲ χρόνο κι' ἔφτασε τὴ μέγιστη τιμὴ 25dpm/gC περίπου τὸ 1964. Ἀπὸ τότε πέφτει πάλι, γιατὶ ἀποφασίστηκε διεθνῶς ἡ διακοπὴ τῶν πυρηνικῶν δοκιμῶν. Τὸ 1974, ὅπως φαίνεται στὸν Πίνακα I, ἡ μέση τιμὴ τῆς εἰδικῆς ραδιενέργειας, ὅπως μετρήθηκε στὰ δείγματα τῶν ἑλληνικῶν οἴνων καὶ ἀποσταγμάτων οἴνου, ἦταν 19,2 dpm^{14}C/gC. Ἡ τιμὴ αὐτὴ συμφωνεῖ μὲ τὴν τιμὴ τοῦ Σχήματος 3 ποὺ ἀναφέρεται στὶς γερμανικὲς μετρήσεις. Τὸ 1976 ἡ μέση τιμὴ τῆς εἰδικῆς ραδιενέργειας, ὅπως φαίνεται ἀπὸ τὸν Πίνακα II, εἶναι μικρότερη, δηλαδὴ 18 dpm^{14}C/gC. Ἡ τιμὴ αὐτὴ ἐπίσης συγκρίνεται καλὰ μὲ τὴν τιμὴ 18,1 dpm/gC τῶν γερμανικῶν προϊόντων.[8]



ΣΧΗΜΑ 3: *Διακύμανση περιεχόμενου ^{14}C σὲ διάφορα προϊόντα ἐτῶν παραγωγῆς 1956 ἕως 1976. ο Rauschenbach καὶ Simon.*[2]
+ δικές μας μετρήσεις.

Ἀπὸ τὴν πτώση τῆς τιμῆς τῆς εἰδικῆς ραδιενέργειας τοῦ ^{14}C φαίνεται ὅτι, ἐφ' ὅσον δὲν ἐπαναληφθοῦν οἱ πυρηνικὲς δοκιμὲς ἡ τιμὴ θὰ ἰσορροπήσει πάλι στὰ ἐπίπεδα τοῦ 1956. Ἐπίσης ἀπὸ τὴ σύγκριση τῶν ἑλληνικῶν ἀποτελεσμάτων μὲ τ' ἀποτελέσματα τῶν γερμανικῶν μετρήσεων φαίνεται ὅτι ἡ τιμὴ τῆς εἰδικῆς ραδιενέργειας τοῦ ^{14}C εἶναι στὸ ἴδιο ἐπίπεδο τοὐλάχιστον στὴν Εὐρώπη. Τὸ συμπέρασμα αὐτὸ ἐνισχύεται καὶ ἀπὸ γαλλικὲς καὶ ἰταλικές[2] καὶ ἀπὸ πορτογαλικὲς μετρήσεις.[9]

Τὸ ἐπίπεδο τῆς εἰδικῆς ραδιενέργειας ἐπηρεάζεται σὲ πολὺ μικρότερο βαθμό, τοπικά, ἀπὸ τὴν παρουσία βιομηχανιῶν ποὺ λόγῳ τῶν καυσαερίων τους ἀραιώνουν τὸ φυσικὸ διοξείδιο τοῦ ἄνθρακα (ἐπίδραση Suess)[5]. Συγκριτικὲς μετρήσεις ἐν τούτοις ποὺ ἔγιναν στὴ Γερμανία σὲ προϊόντα ποὺ προέρχονταν

ἀπὸ περιοχὴ 50m ἀπὸ αὐτοκινητόδρομο καὶ 800m ἀπὸ τὸν ἴδιο αὐτοκινητόδρομο μέσα σὲ βαυαρικὸ δάσος δὲν ἔδειξαν σημαντικὲς διαφορές. Στὴν ἴδια κατεύθυνση δείχνει καὶ ἡ σύγκριση τῶν μετρήσεών μας μὲ τὶς ἄλλες εὐρωπαϊκὲς μετρήσεις.

Summary

Dinstinction of fermentation ethanol from synthetic ethanol. Variation of ^{14}C in greek wines and wine distillates

A method to distinguish fermentation ethanol from synthetic ethanol based on the specific radioactivity of ^{14}C by liquid scintillation spectrometry was studied. External standard methods were used which gave quite satisfactory results. The variation of ^{14}C in greek wines and wine distillates was also measured. The mean value of the specific radioactivity of the greek products produced in 1974 was 19,2 dpm ^{14}C/gC and of the products produced in 1976, 18dpm ^{14}C/gC. The values are in good agreement with relative european measurements.

Key Words: Analytical methods. Wine and wine distillates. Liquid Scintillation Counting. Specific radioactivity of ^{14}C.

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Εὐχαριστίες

Εὐχαριστοῦμε τὸν Δρα Γ. Ἀκογιούνογλου τοῦ Κ.Π.Ε. «Δημόκριτος» γιὰ τὴν χρησιμοποίηση τοῦ φασματομέτρου TRI-CARB 3385 πρὶν τὸ ἐργαστήριό μας προμηθευτεῖ τὸ φασματόμετρο TRI-CARB 3330 καὶ γιὰ τὶς μετέπειτα συγκριτικὲς μετρήσεις καὶ τὸν κ. Σ. Δαούση τοῦ Κ.Π.Ε. «Δημόκριτος» γιὰ τὴν τεχνική του βοήθεια.

Τέλος εὐχαριστοῦμε τὴ Φοροτεχνικὴ Δ/νση τοῦ Γεν. Χημείου τοῦ Κράτους γιὰ τὴ συνεργασία της στὴ συλλογὴ τῶν δειγμάτων.

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STUDY ON THE ANTIBIOTIC FRACTION OF *ALKANNA TINCTORIA TAUSCH*

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Summary

Silicic acid chromatography of the fraction from the roots of *A. tinctoria* has yielded two naphthaquinones, 5,8-dihydroxy-2-(4′-methylpent-3′-enyl) -1,4-naphthaquinone (arnebin-7 or deoxy-alkannin) and 5,8-dihydroxy-2- (1′-acetoxy-4′-methylpent-3′-enyl) -1,4-naphthaquinone (alkannin acetate) along with 5,8-dihydroxy-2- (1′-methylcrotonoyloxy-4′-methylpent-3′-enyl) -1,4-naphthaquinone (alkannin angelate) and 5,8-dihydroxy-2- (1′-isovaleryloxy-4′ -methylpent-3′-enyl) -1,4-naphthaquinone (alkannin isovalerate), which were reported earlier,[1] possessing antibiotic and cytotoxic activities.

Key Words: *Alkanna tinctoria,* Naphthaquinones, Antimicrobial effects.

Introduction

The Hellenic Health Authorities have recently issued a free sale certificate for the proprietary medicine ***Histoplastin Red®***. As far as we know, this is the first preparation of its kind in the world and is considered to fill a considerable gap in the therapeutic arsenal, because it provides effective treatment in cases of indolent ulcers (ulcus cruris) while it also exhibits remarkable antibiotic action.

The active ingredient of the above preparation is an oily extract of the roots of *A. tinctoria*. One of the authors has already reported[2,3,4] the chemical composition of the root extract of the above plant. In this paper the antibiotic fraction of this extract studied.

Results and Discussion

The antimicrobial action of certain pigments of structure similar to these pigments, like the arnebins[5] and shikonins[6], in addition to our observation[7] on the therapeutic result obtained by treatment with ***Histoplastin Red®*** ointment, led us to the detailed study of the antibiotic fraction of *A. tinctoria* root extract.

For this purpose the roots were extracted with *n*-hexane. After evaporation of the solvent, the semi-solid residue was extracted with cold methanol to eliminate waxes[2]. Then the pigments were precipitated as Cu-complexes, and simultaneously separated from the fluorescent fraction[4]. The Cu-complexes were decomposed with hydrochloric acid and the pigment fraction was taken-up into ether.

The two fractions, pigments and fluorescent substances, obtained as above, were checked for their antimicrobial action. It was proved[8] that only pigment fraction exhibits an antimicrobial effect against *Staphylococcus aureus SG511* and *Staphylococcus epidermidis* (Fig. 1).

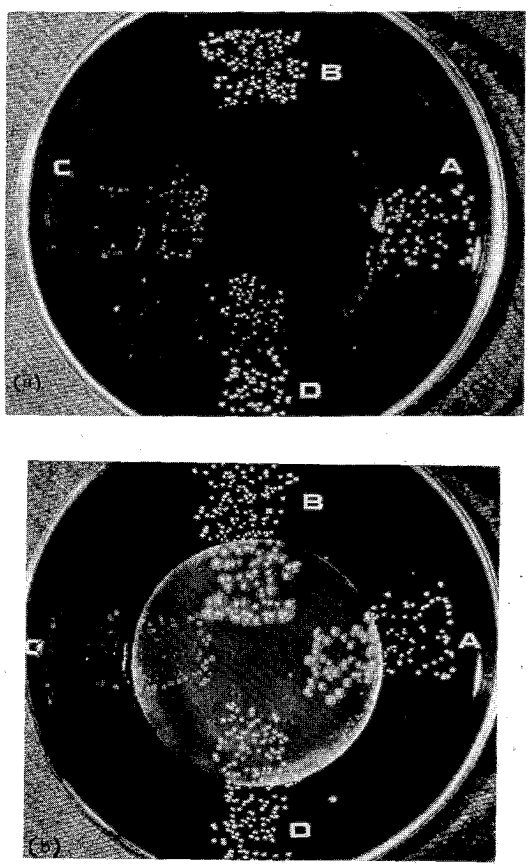


FIG. I (a) Antibacterial control of the fraction of the pigments of *Alkanna tinctoria*. Concentration 20mg/5ml acetone. Infusion of 0.2ml/15.9cm² of disc surface.

A. *Staphylococcus aureus SG511*	: complete inhibition
B. *Staphylococcus epidermidis*	: complete inhibition
C. *Escherichia coli*	: complete growth
D. *Candida albicans*	: complete growth

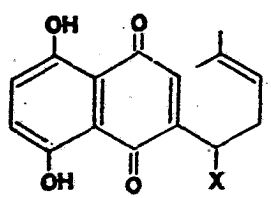
(b) Antibacterial control of the fraction of the fluorescent substances of *Alkanna tinctoria*. Concentration 21.8mg/5ml acetone. Infusion of 0.2ml/15.9cm² of disc surface.

A. *Staphylococcus aureus SG511*	: complete growth
B. *Staphylococcus epidermidis*	: complete growth
C. *Escherichia coli*	: complete growth
D. *Candida albicans*	: complete growth

In a further step, the antimicrobial effect of individual constituents of the pigment fraction was studied. The results are indicated in Table I. The antimicrobial control was affected according to the Heiss[9] method which is suitable for the study of non-water soluble substances (cosmetic creams, deodorants etc.).

Apart from the pigments which have already reported[1,3,4], it may be possible that other pigments exist in smaller proportion and with similar R_f. This fact, in connection with their biological interest[7,10], has prompted us to the detailed reexamination of the pigment fraction. Thus, after repeated column chromatography (very slow flow rate, Table II) two more pigments were isolated.

The first of them R_f 0.45, m.p. 94-95°C, analysed for $C_{16}H_{16}O_4$, M^+272, gave a deep blue solution upon treatment with caustic alkali. Its UV-Vis spectra were similar to the corresponding spectra of the already known pigment esters of alkannin. However, its IR spectrum hasn't shown an esteric structure. Its NMR spectrum has shown, apart from the known peaks[11], a broad peak (4H) centered at 2.42 δ indicating methylene protons coupled and deshielded by olefinic groups. This pigment, therefore, has the structure I, of the already known arnebin-7 (deoxyalkannin)[12].



I. X=H

II. X=OCOCH$_3$

The second pigment R_f 0.27, m.p. 103-104°C, analysed for $C_{18}H_{18}O_6$, M^+ 330, gave a deep blue solution upon treatment with caustic alkali. Its IR spectrum has shown a strong absorption at 1735 cm^{-1} (ester carbonyl) whenever all the other absorptions, as well as its UV-Vis spectra have shown that it is a typical ester of alkannin. Moreover, its NMR spectrum, among the other known peaks[11], included a singlet peak at 2.15 δ (3H) which was attributed to the protons of acetyl group. This pigment, therefore, has the structure of alkannin acetate (II).

TABLE I: Results of the antibacterial study of the components of the antibiotic fraction of *Alkanna tinctoria*.

Substances	Concentration*	*Staphylococcus aureus SG 511*	*Staphylococcus epidermidis*	*Escherichia coli*	*Candida albicans*
Benzene extract of *A. tinctoria*	50mg/5ml acetone	0	0	3	3
Benzene extract of *A. tinctoria*	50mg/5ml isopropyl myristate	0	0	3	0
Fraction of the pigments of *A. tinctoria*	20mg/5ml acetone	0	0	3	3
Fraction of the fluorescent substances of *A. tinctoria*	21.8mg/5ml acetone	3	3	3	3
Alkannin	18.1mg/5ml acetone	0	0	3	0
β, β-Dimethyl-acrylic ester of alkannin	21.5mg/5ml acetone	0	0	3	3
Histoplastin Red®	0.3g/15.9 cm²	0	0	3	3
	0.15g/15.9 cm²	1-2	1-2	3	3
Acetone	0.2ml/15.9 cm²	3	3	3	3

* From each solution a quantity of 0.2 ml/15.9 cm² of disc surface was used.
0 = complete inhibition 1 = isolated colonies 2 = microcolonies 3 = complete growth

TABLE II: Column chromatography of the pigment fraction (0.927g) *of Alkanna tinctoria.*

Fraction	ml	Eluant	Mass (g)	TLC
1	100	*n*-hexane/benzene (60:40)		
2	100	*n*-hexane/benzene (60:40)		
3	100	*n*-hexane/benzene (50:50)	0.048	
4	50	*n*-hexane/benzene (50:50)	(~5.2%)	R_f 0.45
5	50	*n*-hexane/benzene (40:60)		
6	50	*n*-hexane/benzene (40:60)		
7	25	*n*-hexane/benzene (30:70)		
8	100	*n*-hexane/benzene (30:70)		
9	100	*n*-hexane/benzene (30:70)	0.565	
10	100	*n*-hexane/benzene (30:70)	(60.9%)	R_f 0.41
11	100	*n*-hexane/benzene (30:70)		
12	50	*n*-hexane/benzene (30:70)		
13	25	*n*-hexane/benzene (30:70)		
14	25	*n*-hexane/benzene (20:80)		
15	100	*n*-hexane/benzene (20:80)		
16	100	*n*-hexane/benzene (20:80)	0.256	R_f 0.41
17	100	Benzene	(27.6%)	R_f 0.27
18	100	Benzene		
19	100	Benzene		
20	100	Benzene		
21	100	Benzene		R_f 0.41
22	50	Benzene	0.033	R_f 0.27
23	25	Benzene	(3.5%)	R_f 0.103
24	25	Chloroform		
25	100	Chloroform		
26	100	Chloroform		

Experimental

Melting points are uncorrected and were determined with a Kofler hot-stage apparatus. The following instruments were used in the determination of spectra: Cary (UV-Vis), Perkin-Elmer (IR), Varian EM 360 (tetramethylsilane as internal reference). Mass spectra (chemical ionization, with isobutane as the bombarding gas) were measured on a Finningan 3200 spectrometer with source temperature 150°C.

Roots of *A. tinctoria* were provided us by the Greek Pharmaceutical Company CHROPI S.A. (Neon Phaleron, Athens).

Isolation of the Pigments

The powdered dry roots (500 g) were extracted at room temperature with 4×1000ml *n*-hexane for 24h, under continuous stirring in a nitrogen atmosphere. The four extracts were combined. Evaporation of *n*-hexane afforded 13.4 g of a deep red semi-solid residue (yield 2.8%). The semi-solid was extracted with 1000 ml cold methanol for 30 min. The mixture was filtered. The material left on the filter was washed with methanol (3×200 ml). The methanol washings were added to the filtrate. From the combined methanolic solutions the mixture of the pigments was obtained as insoluble Cu-chelates by addition of cupric acetate. The Cu-chelates were decomposed with 10% hydrochloric acid to give a mixture of free pigments. The mixture of the pigments was fractionated through a column 2 cm i.d., 32 cm high, packed with silicic acid 100 mesh ASTM (Mallinckrodt). TLC was carried out on Merck Kieselgel 60 F_{254} plates. Solvent system: benzene/ chloroform/ acetone (50:50:1).

The first fractions obtained from the column (Table II) contained 48 mg crude anhydroalkannin (arnebin-7 or deoxyalkannin) which was purified by repeated recrystallizations from *n*-hexane to yield 25 mg pure red crystals m.p. 94-95° C (Lit.[12] 95° C). Anal. Calcd.: $C_{16}H_{16}O_4$, C 70.58%, H 5.88%. Found: C 70.32%, H 6.01%. Mol. wt. 272. Uv-Vis λ^{EtOH}_{max} nm (log ε): 275 (3.82), 482 (3.75), 513 (3.80), 550 (3.45). IR ν^{KBr}_{max} cm^{-1}: 3030, 1610, 1560, 1210. NMR ($CDCl_3$): 1.53 and 1.63 δ [6H, each s., = C $(CH_3)_2$], 2.28-2.57 δ (4H, m., Ar-CH_2-CH_2-), 5.07 δ [1H, t., -CH= CMe_2], 6.83 δ (1H, s., proton on quinone ring), 7.16 δ (2H, s., protons on aromatic ring), 12.32 and 12.54 δ (2H, each s., -OH).

Fractionation on the column was continued to yield 206 mg crude alkannin acetate, which was purified by repeated recrystallizations from *n*-hexane to furnish pure alkannin acetate (142 mg), m.p. 103-104° C (Lit.[5] 104-105° C). Anal. Calcd.: $C_{18}H_{18}O_6$; C 65.45%, H 5.45%. Found: C 65.22%, H 5.55%. Mol. wt. 330. UV-Vis λ^{EtOH}_{max} nm (log ε): 275 (3.80), 485 (3.82), 516 (3.85), 560 (3.65). IR ν^{KBr}_{max} cm^{-1}: 1740, 1615, 1575. NMR ($CDCl_3$): 1.60 and 1.70 δ [6H, each s., = C $(CH_3)_2$], 2.15 δ (3H, s., -$COCH_3$), 2.57 δ (2H, dt., -CH(O)-CH_2-), 5.15 δ (1H, t., -CH= CMe_2), 6.05 δ (1H, t., Ar-CH(O)-), 6.94 δ (1H, s.,

proton on quinone ring), 7.17 δ (2H, s., protons on aromatic ring), 12.36 and 12.52 δ (2H, each s., -OH).

Περίληψη

Μελέτη τοῦ ἀντιβιοτικοῦ κλάσματος τῆς *Alkanna tinctoria Tausch*

Σ' αὐτὴ τὴν ἐργασία μελετᾶται τὸ κλάσμα ἐκχυλίσεως τῶν ριζῶν τῆς *A. tinctoria* ποὺ περιέχει τὶς ἀντιβιοτικὲς οὐσίες. Ἡ μελέτη ἐπεκτείνεται καὶ στὸ φαρμακευτικὸ ἰδιοσκεύασμα ποὺ ἔχει σὰν δραστικὸ συστατικὸ τὸ ἀντιβιοτικὸ κλάσμα τοῦ παραπάνω φυτοῦ.

Μὲ ἀφορμὴ τὶς πολὺ ἐνδιαφέρουσες φαρμακολογικὲς καὶ βιολογικὲς ἰδιότητες τοῦ παραπάνω κλάσματος ἔγινε ἐπανεξέταση τῆς χημικῆς του συστάσεως, ἀπὸ τὴν ὁποία προέκυψε ἡ ἀπομόνωση καὶ ταυτοποίηση δύο ἀκόμη χρωστικῶν, τῆς 5,8-διυδροξυ-2- (4'-μεθυλοπεντ-3'-ενυλ) -1,4-ναφθοκινόνης (arnebin-7 ἢ deoxyalkannin) καὶ τῆς 5,8-διυδροξυ-2- (1'-ακετοξυ-4'-μεθυλοπεντ-3'-ενυλ) -1,4-ναφθοκινόνης (ὀξικοῦ ἐστέρα τῆς ἀλκαννίνης).

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A STUDY OF SILICON – TELLURIUM COMPOUNDS ($Si_{1+x}Te_2$)

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Summary

In order to investigate the structure of compounds formed in the binary system of Si-Te, large single crystals were grown by the Bridgmann technique with various starting stoichiometric proportions. A new dissolution procedure was developed which involved sample heating in a teflon beaker in the presence of H_2O_2 and NaOH solution. Chemical analysis showed a Si and Te percentage corresponding to the formula $Si_{1+X}Te_2$ with $0<x\leqq0.33$. The structure of these compounds was studied by transmission electron microscopy combined with electron diffraction patterns. The structure for crystals with the stoichiometric compositions in the above range was found identical to that found for $Si_{1.33}Te_2$ (= Si_2Te_3) under the growing conditions used. Deviations from stoichiometry do not change the structure and can be explained on the basis of the statistical occurence of Si atoms in the sublattice of Te atoms.

Key words: Silicon-tellurium compounds, silicon-tellurium dissolution, electron microscopy, electron diffraction, semiconductor.

Introduction

Most of the elements belonging to groups IV and VI of the periodic table form compounds with stoichiometric proportions of 1:1 or 1:2. In contrast, silicon-tellurium compounds can be described by the general formula $Si_{1+X}Te_2$ with the value of x ranging from 0 to 0.33. It is for that reason that different investigators[1-7] reported different chemical formulas for Si-Te crystals, which otherwise exhibited the same physical properties.

Silicon telluride ($Si_{1+X}Te_2$) is of great interest in practical applications because it is a semiconductor with a large energy gap, low Hall mobility and relatively high electrical resistivity. It is usually formed between Si-CdTe interfaces of Si-vidicon targets.

The structure and the composition of this compound is controversial, therefore it was considered worthwile to study its structure by using TEM methods under various stoichiometric compositions.

Weiss et al.[8] was the first to examine the crystal assemblage of $SiTe_2$ and suggested a srystal structure similar to that of CdI_2 with unit cell dimensions a_o= 4.28 Å, c_o = 6.71 Å and space group $P\bar{3}m$. Taketoshi et al.[9], also, investigated the $SiTe_2$ structure and reported unit cell parameters a= 7.428 Å and c= 6.733 Å. This unit cell configuration with $a=a_o\sqrt{3}$ and $c=c_o$ was

put forward to account for superlattice diffraction spots of ($\frac{1}{3}$ $\frac{1}{3}$ 0) and ($\frac{2}{3}$ $\frac{2}{3}$ 0) type present on (001) reciprocal lattice plane. This type of superstructure, which was attributed to a slight displacement of the tellurium atoms in the (001) crystal planes, was also observed during the study of the structure defects of single $SiTe_2$ crystals by means of the electron microscope[10]. In that work it was confirmed that the c parameter of the crystal equalled $2c_0$ and that the material behaved as a polytype. Ploog et al.[11] studied single crystals with the composition $Si_{1.33}Te_2$ (= Si_2Te_3) using x-rays and reported unit cell parameters $a = 7.43$ Å ($= a_0\sqrt{3}$) and $c = 13.482$ Å ($= 2c_0$) and space group $P\bar{3}1C$ with $z = 4$. Silicon atoms form Si_2 units occupying the 2/3 of the cation sites of the CdI_2 type structure with Si-Si bond distance aproximately 2.3 Å. Each silicon atom is surrounded by three tellurium atoms and one silicon atom in a tetrahedral configuration with the Si-Te bond distance approximately equal to 2.55 Å. Each tellurium atom is bonded to only two silicon atoms with the Si-Te-Si bond angle approximately equal to 93°. Tellurium atoms form a hexagonal close-packed array, whereas the Si-Si pairs can occupy any of the 28 probable vacant sites available for the eight silicon atoms in the unit cell (Fig. 1).

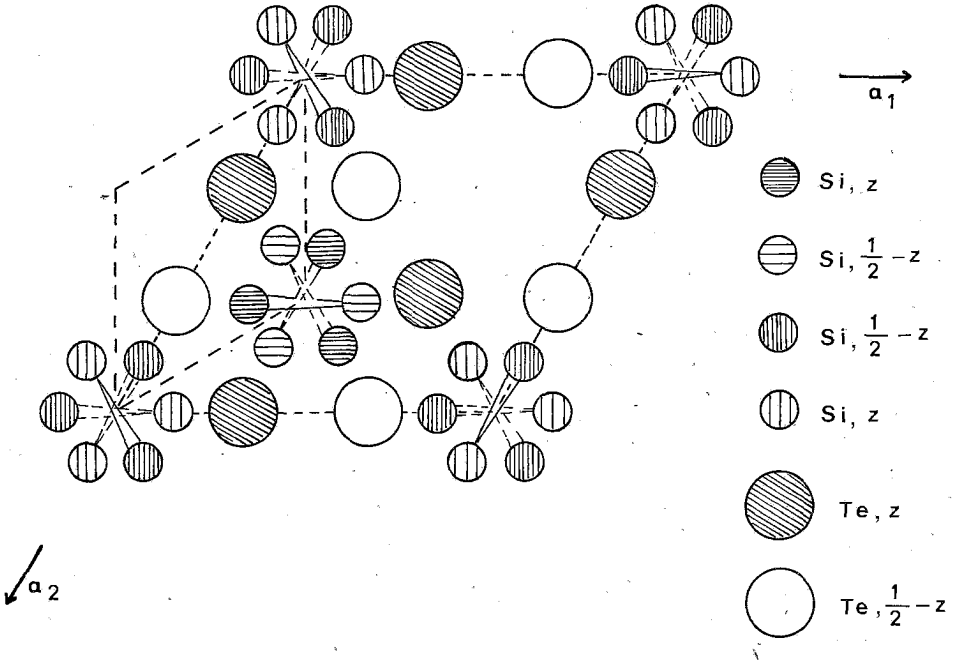


FIG. 1: *Projection of Si_2Te_3 unit cell on the (001) plane. All possible orientations of Si-Si pairs are shown. For clarity only half of the cell, parallel to c axis is projected.*

Since, these unit cell parameters were in agreement with those of $SiTe_2$ crystals[10] and the DTA measurements and the IR spectra were exactly identical to those reported by Ploog et al.[11], it was considered worthwhile to study

crystals of the Si-Te system with variable stoichiometry and at the same time investigate and examine their crystallographic parameters by means of electron microscopy.

Experimental

Crystal preparation

Large single crystals of varying stoichiometry were grown by the Bridgmann technique. Substances used for that purpose were of extremely high purity (silicon 99.999% and tellurium 99.999%) in proportions (1+x)Si: 2Te, where x= 0, 0.2, 0.33 and 1.0. The two components in the form of small fragments were placed in a quartz ampoule 10 cm long and 2 cm diameter, and the ampoule was sealed under a 10^{-5} Torr vacuum. The lowest part of the ampoule containing the material was placed in a vertical furnace with a linear scale temperature gradient starting from 1000°C. The quartz ampoule remained in the highest temperature site for 24 hours in order to achieve complete melt homogeneity. After the 24 hours had elapsed the ampoule was lowered, by means of a suitable mechanism, at a lowering rate of 33 mm/d down to the temperature of 800°C. At this point the power of the furnace was turned off and the ampoule was left for about 10 hours to cool off to room remperature and then removed from the furnace for further examination.

Single crystals prepared by this method showed a deep red color, cleavage at the (001) plane and they were approximately 3 cm long and 2 cm wide. Their single crystallinity was checked by means of Laue diagrams and electron diffraction. It was thus verified that the crystals were well developed with a small number of structural defects.

Determination of crystal stoichiometry

Single crystals that had been checked previously for their crystallinity were used for determining the proportions of silicon and tellurium in the compound.

Sodium carbonate fusion in platinum crucible for assaying silicon telluride crystals was not used lest some tellurium losses might occur as a result of high fusion temperature and crystal griding preceding the fusion. The acids (HNO_3, $HClO_4$, H_2SO_4, etc.) for tellurium dissolution followed by NaOH treatment for silicon dissolution was not considered since we considered that the sensitivity of the method was not what was needed. A new technique was developed, instead, for dissolving the crystals. This technique involved sample heating in a teflon beaker in the presence of H_2O_2 and NaOH solution. This dissolution technique is more advantageous than the mentioned ones in that, it is rapid, it is conducted under relatively mild conditions and no interferences are encountered in Si and Te determination.

A 0.1 to 0.2 g sample is placed in a 100 ml teflon beaker and 10 ml of 30% H_2O_2 solution are added. The beaker is heated on a sand bath (120-150°C) for the oxidation of tellurium, resulting in a crystal lattice collapse and the release of silicon in a very fine colloidal state. After 10 min heating 10 ml of 40% NaOH solution are added for complete silicon dissolution and the

heating is continued until final solution volume of 5 ml. Then, the beaker is removed from the sand bath, cooled and after the addition of 5 ml of 30% H_2O_2 and 5 ml of 40% NaOH the beaker is heated again till everything has gone into solution (indicated by a clear and uncolored solution). Complete dissolution takes about 20-30 min. and yields water soluble $Na_2TeO_6H_4$ and Na_2SiO_3 as final products. The beaker is then removed from the sand bath, cooled and after the addition of 20 ml of distilled water and 22 ml of 6 N HCl for partial neutralization of the NaOH used, heated again in the sand bath (100 - 120°C) for solution clearing and complete decomposition of the peroxide. The beaker is then cooled and its contents are quantitatively transfered to a 500 ml volumetric flask. Before dilution to volume with distilled water a few drops of 6 N HCl are added for the solution acidification (pH 2 - 4). It is essential that the pH should be maintained at this region to avoid silicon precipitation as SiO_2 in lower pH's.

Quantitative determination of silicon was done by the molybdosilicid acid method[12] using a Varian model 635 spectrophotometer with transmittance read at 390 mμ. Tellurium was determined by Atomic Absorption Spectroscopy using a Perkin Elmer model 503 instrument at the 214.3 m spectral resonance line with air-acetylene flame[13].

Results

Chemical analyses of four different crystals prepared with stoichiometry closely corresponding to the formula $SiTe_2$ gave the average percentage of 10.52 and 89.34 for Si and Te respectively. This composition reflects a stoichiometry of the type:

$$Si_{1.07}Te_{2.00}$$

Two crystals prepared with stoichiometry corresponding to the formula $Si_{1.2}Te_{2.0}$ gave the average percentage of 11.30 and 88.62 for Si and Te respectively. In this case, the stoichiometry can be formulated by:

$$Si_{1.16}Te_{2.00}$$

Two other crystals prepared with stoichiometry corresponding to the formula $Si_{1.5}Te_{2.0}$ gave the average percentage of 12.68 and 87.02 for Si and Te respectively. In this case the stoichiometry can be formulated by:

$$Si_{1.32}Te_{2.00}$$

Finally, two other crystals prepared with stoichiometry corresponding to the formula $Si_{2.0}Te_{2.0}$ gave the average percentage of 12.77 and 87.16 for Si and Te respectively. In this case, this composition reflects a stoichiometry of the type:

$$Si_{1.33}Te_{2.00}$$

Chemical analyses revealed that in all crystals obtained, the stoichiometry was of the type $Si_{1+x}Te_2$, with x ranging from 0 to 0.33. Crystals with x

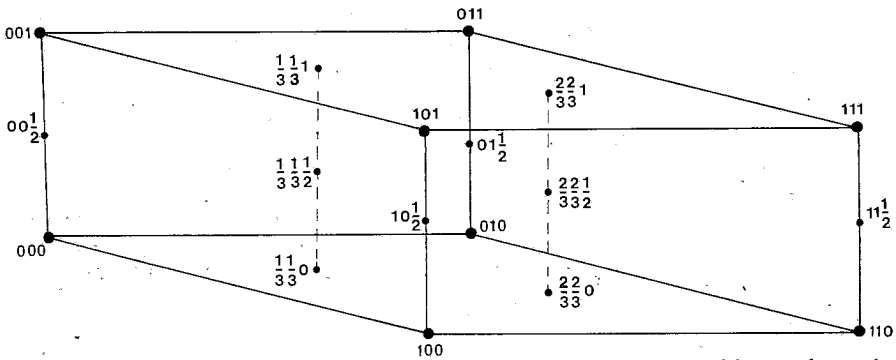
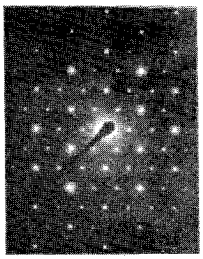
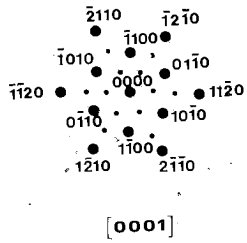
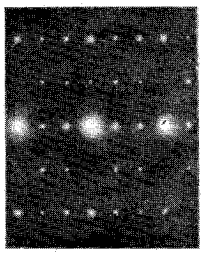


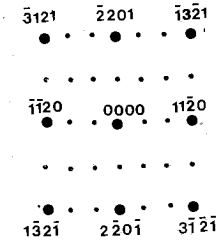
FIG. 2: *The unit cell of reciprocal lattice constructed from various reciprocal lattice planes observed by electron diffraction.*



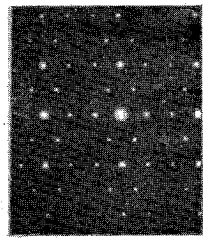


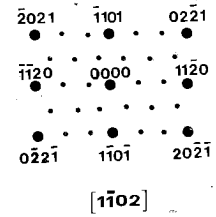
[0001]





[$1\bar{1}04$]





[$1\bar{1}02$]

FIG. 3: *Three of the electron diffraction patterns used to construct the reciprocal lattice unit cell shown in Fig. 2. Only the basic diffraction spots are indexed and the electron beam direction is given for each pattern.*

greater than 0.33 were not observed in spite of the use of silicon in proportions with $x = 1$ in the preparation procedure.

Conclusions

Attempts to prepare crystals with stoichiometry either of SiTe or $SiTe_2$ type showed that in all cases the stoichiometry of the crystals obtained ranged from $SiTe_2$ to $Si_{1.33}Te_2$ (= Si_2Te_3). Measurements of crystal parameters and electron diffraction patterns revealed a reciprocal lattice (Fig. 2 and 3), which provides sufficient evidence that the crystal structure is the one proposed by Ploog et al.[11]. Results of this research can be accounted for on the grounds of Ploog's suggested model for $Si_{1.33}Te_2$ type stoichiometry. According to thie model tellurium atoms form a close-packed hexagonal arrangement and the Si-Si pairs fill statistically the 2/3 of the probable octahedral vacant sites between alternate tellurium planes. Consequently, a percentage of Si-Si pairs is statistically permissible to be missing without disturbing the crystal structure and thus crystals with varying stoichiometry and identical structure can be formed.

Περίληψη

Μελέτη τῶν ἑνώσεων πυριτίου - τελλουρίου ($Si_{1+x}Te_2$)

Μὲ σκοπὸ τὴν μελέτη τῆς δομῆς τῶν στερεῶν ἑνώσεων στὸ δυαδικὸ σύστημα Si-Te παρασκευάσθηκαν μὲ τὴν τεχνικὴ τοῦ Bridgmann μεγάλοι μονοκρύσταλλοι μὲ διάφορες ἀρχικὲς στοιχειομετρικὲς ἀναλογίες. Μία νέα τεχνικὴ διαλυτοποιήσεως ἐπέτρεψε τὴν ἁπλούστευση τῆς χημικῆς ἀναλύσεως τῶν ἑνώσεων ποὺ ἀντιστοιχοῦσαν στὸν τύπο: $Si_{1+x}Te_2$ μὲ $0 < x \leqq 0.33$. Ἡ δομὴ τῶν ἑνώσεων αὐτῶν μελετήθηκε μὲ πρότυπα περίθλασης ἀπὸ τὸ ἠλεκτρονικὸ μικροσκόπιο. Μὲ τὶς συνθῆκες παρασκευῆς ποὺ χρησιμοποιήθηκαν, ὅλες οἱ ἑνώσεις εἶχαν τὴν δομὴ τοῦ $Si_{1.33}Te_2$ (= Si_2Te_3). Οἱ ἀποκλίσεις ἀπὸ τὴν στοιχειομετρικὴ ἀναλογία δὲν ἀλλάζουν τὴν δομὴ καὶ μποροῦν νὰ ἐξηγηθοῦν ἀπὸ τὴν στατιστικὴ κατανομὴ τῶν ἀτόμων τοῦ πυριτίου μέσα στὸ ὑπόπλεγμα τῶν ἀτόμων τοῦ τελλουρίου.

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Short Paper.

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BUBBLE MEASUREMENTS IN ELECTROLYTIC FLOTATION

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Key words: Gas bubbles, measurement, photographs, electrolysis, flotation, wastes, separation.

In recent years the idea of the utilization of rising gas bubbles for the separation of suspended matter from a dispersion (i.e. flotation) has been applied in effluent treatment. One of the techniques by which finely divided gas bubbles can be produced is electrolysis. The measurement of gas bubbles during electroflotation showed an average value of approximately 50 μm in normal conditions.

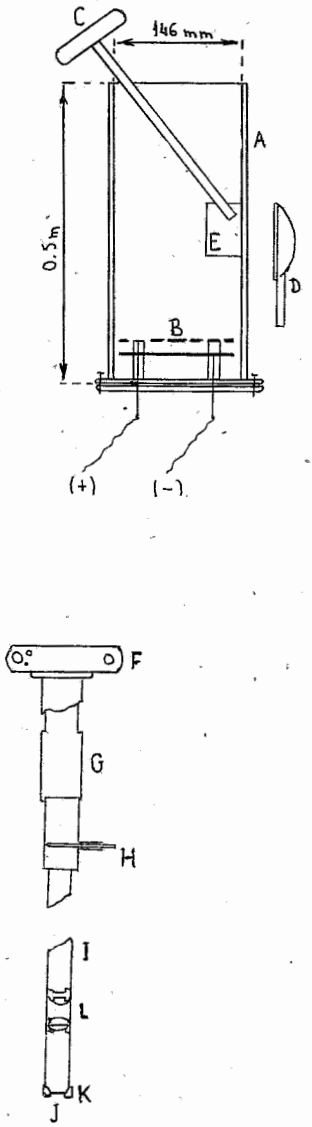
One of the first goals of the scientific studies, regarding the electrolytically produced bubbles, was the bubble growth, as this presumambly could lead to improvements in commercial electrolytic processes [1]. A further stimulus to the study was that electrical measurements could be made with great accuracy and relative ease and mathematically, bubble growth by mass transfer is analogous to bubble growth by heat transfer. Hence, it was thought that information concerning one phase change might contribute to knowledge of the other. There are several attempts by chemical engineers to relate electrolytic gas evolution to nucleate boiling. However, it was proved[2] that microconvenction does not abet volume flux in electrolysis.

To present a mathematical statement for the problem of mass diffusion, which is of interest for electrolytic bubbles, the controlling step of bubble growth should be found. In fact, despite the considerable literature on the subject, there is no universal agreement as to the precise mechanism, which indeed may vary with the electrode surface and other operating conditions[3].

Information concerning the contact angle was also of significance to theoretical workers. It was thought, originally, that there should be an effect on bubble growth for two reasons. For one, the liquid/gas interfacial area for a given volume of gas in the form of a spherical segment is a function of the contact angle. The second reason concerns the movement of liquid solution which occurs as a bubble expands. However, in the literature[1,4] contradiction appears concerning this problem.

All the above mentioned theory was dealing with electrolytically generated bubbles in a rather general form. Regarding the application of electrolysis on flotation, the existing studies were concentrated in the measurement of hydrogen bubbles only[5,6].

The present work had the aim of measuring both the hydrogen and oxygen bubbles under electroflotation conditions. A 0.05% p.v. sulphuric acid solution was used, so that the current density could be varied in the range of 100 to 300 A/m^2. The camera used in this study was constructed earlier[7] for a solvent extraction project, and the arrangement is shown in Figure 1. The electrodes used were from stainless steel; more details can be found in reference[8].



A Flotation vessel
B Electrode cell
C Photographic camera
D Flash unit
E Window
F Camera body
G Extension tube
H Sliding plate shutter
I Immersion probe
J Glass disc
K Gasket
L Lenses

FIGURE 1: *Arrangement of vessel and camera.*

When the data were plotted on probability paper, it was found difficult, especially in the case of 300 A/m², to fit them in a straight line, as it is shown in Figure 2. This was due to the fact that hydrogen and oxygen bubbles were measured at the same time.

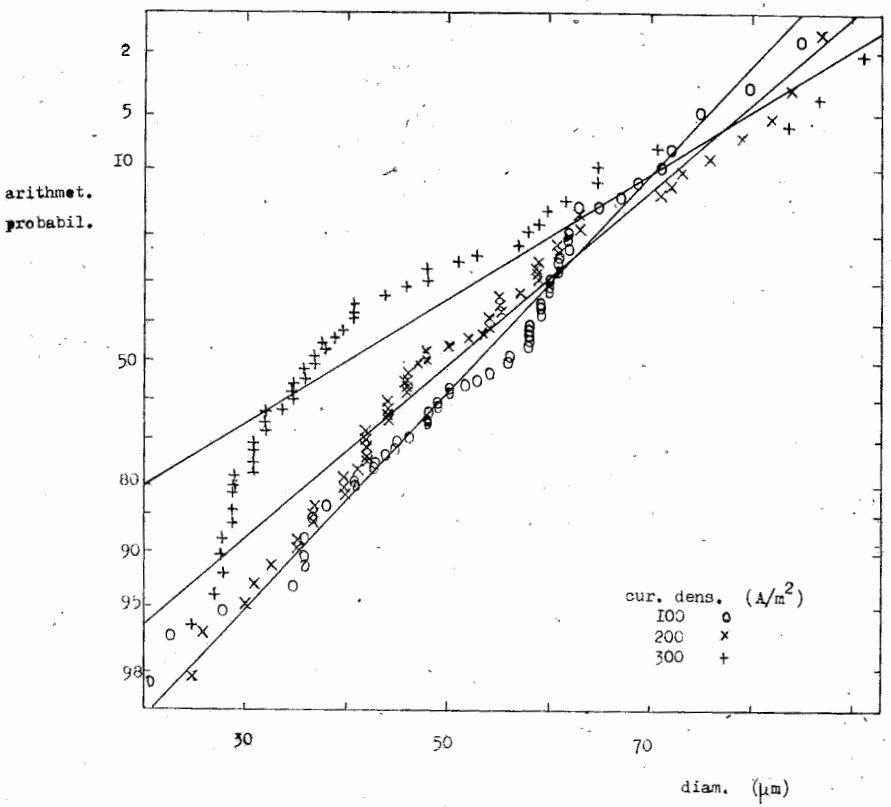


FIGURE 2: *Bubbles measurements during electroflotation.*

Gas bubbles evolved at the electrode under different conditions may have different sizes. For instance, in an alkaline electrolyte the oxygen is evolved on the anode in the form of relatively large bubbles which rise rapidly and the electrolyte remains clear; while the hydrogen is evolved on a nickel or platinum cathode in the form of very fine bubbles that create milky turbidity within the cathodic space[4]. In an acid electrolyte the difference in sizes is not so great; in such a case the hydrogen bubbles are larger than the oxygen bubbles.

The problem can be seen clearly in Figure 3, where two maxima are observed. In this histogram, diameters at the range boundaries were included in the lower range.

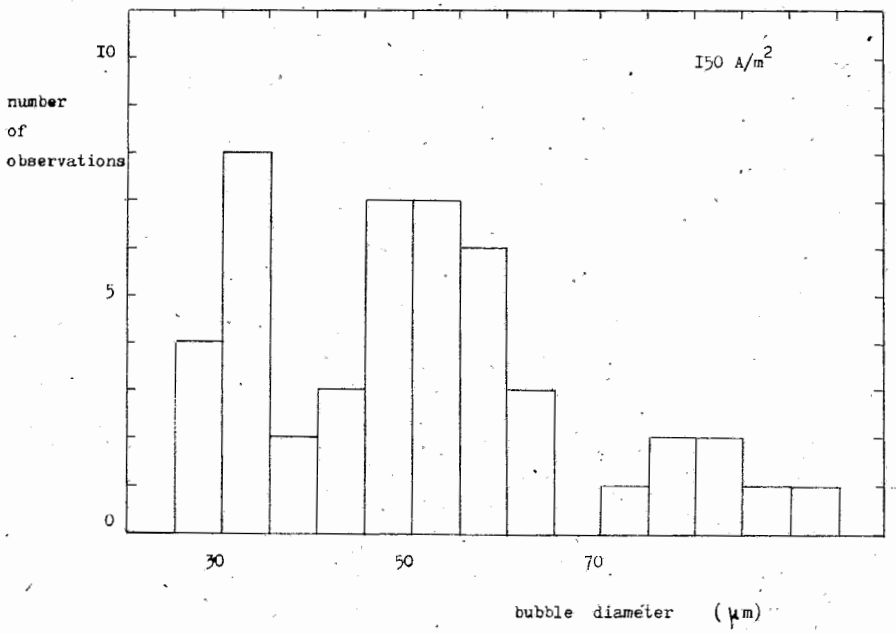


FIGURE 3: *Histogram of electrolytically produced bubbles.*

All the bubbles measured were in the range of 20 to 90 μm. Those bubbles around the upper limit are believed to be the outcome of coalescence. Measurement in a different liquid height, around 0.25 m from the top (while the previous measurements were at 0.10 m), gave a comparable result. Also, measurement with no electrolyte at 100 A/m^2 gave a mean diameter of 50 μm.

The addition to the solution of electrolytes could change the values of the surface tensions at a given potential, and this may cause either an increase or a decrease in the wettability of the metal surface. The increase of electrode wettability as a result of an increase in the double layer charge on its surface was said[4] to be of great importance in electrochemical processes.

The effect of added solutes on the size of hydrogen bubbles was studied also elsewhere[9]. The authors reported a decrease in bubble size effected by the addition of one mole of sodium chloride.

Further conclusions from the photographic measurements are shown in Figure 4, where the size distribution at different current densities was plotted against the percentage of the observed bubbles. It is noticed that the statistical mean and the dispersion in the empirical distribution with respect to bubble size decrease as the applied current density is increased.

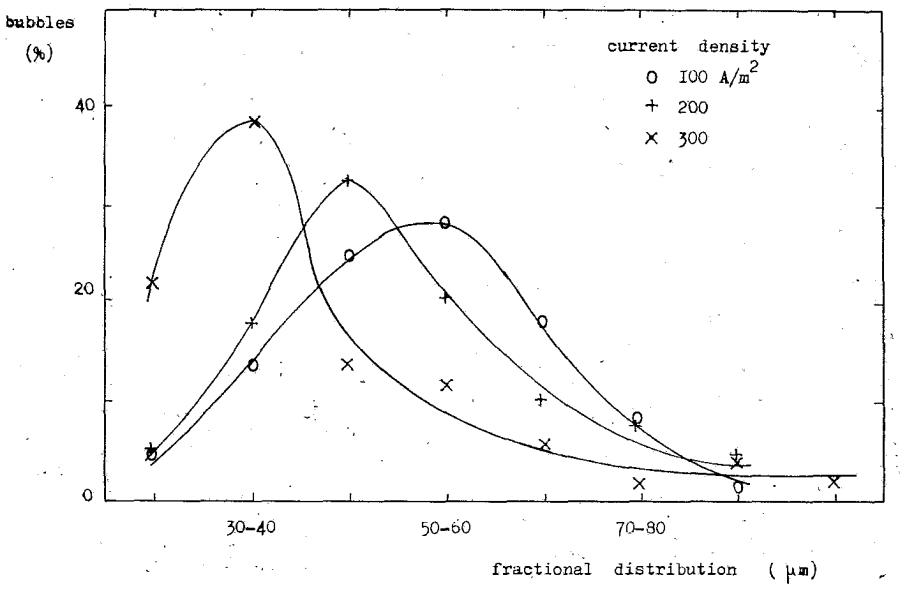


FIGURE 4: *Observed distribution of gas bubbles at different current densities.*

So the gas bubbles were found to become smaller with increasing current density and in the meantime, the number of bubbles was found to increase, as expected. It is noted that it was found[6], for hydrogen bubbles, that they remain at approximately the same level at fairly high densities of the order of 1,000 A/m^2 and more. Apparently at very high current densities the influence of charge of potential on the bubble size diminishes. Concluding, in the range invertigated, our results are found in agreement with the literature.

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Περίληψη

Μέτρηση τῶν Φυσαλίδων στὴν Ἠλεκτρολυτικὴ Ἐπίπλευση

Ἡ ἐπίπλευση εἶναι μιὰ μέθοδος διαχωρισμοῦ κι ἐφαρμόζεται, ἐκτὸς τοῦ τομέα τῆς ὑδρομεταλλουργίας, στὰ ὑγρὰ ἀπόβλητα. Ὑπάρχουν διάφορες τεχνικὲς παρασκευῆς λεπτῶν, ἀέριων φυσαλίδων· μιὰ ἀπ' αὐτές, ἡ πιὸ σύγχρονη, εἶναι ἡ ἠλεκτρόλυση. Σκοπὸς τῆς σύντομης αὐτῆς ἐργασίας εἶναι ἡ περιγραφὴ πειραμάτων ποὺ ἀπόβλεπαν στὴ μέτρηση τῶν φυσαλίδων ὑδρογόνου καὶ ὀξυγόνου μὲ φωτογράφηση καὶ μετὰ προβολή, σὲ διάφορες συνθῆκες ἠλεκτροεπίπλευσης. Μεταβλητὲς παράμετροι ἦταν ἡ πυκνότητα τοῦ ρεύματος, τὸ ὕψος τῆς φωτογράφησης μέσα στὸ ὑγρό, καὶ ἡ χρήση ἠλεκτρολυτῶν. Σὲ κανονικὲς συνθῆκες ἠλεκτροεπίπλευσης (δηλ. 100 A/m^2) βρέθηκε ὅτι ἡ μέση τιμὴ τῆς διαμέτρου τῶν φυσαλίδων ἦταν περίπου 50 μm. Ἐπίσης, οἱ φυσαλίδες βρέθηκαν νὰ μικραίνουν σὲ μέγεθος καθὼς ἡ πυκνότητα τοῦ ρεύματος αὐξάνονταν, ἐνῶ βέβαια ὁ ἀριθμός των, στὸ μεταξύ, αὐξάνονταν ἐπίσης.

ERRATA

Table II in page 190 of the previous issue must be changed as follows:

TABLE II. *NMR and MS spectral data of Compounds 4(a-j).*

Compound	^{1}H-NMR (CCl_4) δ ppm	MS m/e (relative intensity, ion)
4a	7.95-7.32 (m, 10H); 6.73 (s, 1H, C_4-H).	221 (63, M^+), 193 (6), 144 (16, M-C_6H_5), 116 (4, M-C_6H_5CO), 105 (100, C_6H_5CO), 77 (54, C_6H_5), 51 (16).
4b	7.97-7.30 (M, 8H); 6.53 (s, 1H, C_4-H).[a]	289 (32, M^+), 254 (11, M-Cl), 212 (11, M-C_6H_5), 184 (4, M-C_6H_5CO), 105 (100, C_6H_5CO), 77 (45), 51 (12).
4c	7.75-7.30 (m, 5H); 2.37 (s, 3H, C_5-CH_3); 2.06 (s, 3H, C_4-CH_3).	173 (100, M^+), 158 (45, M-CH_3), 131 (24, M-CH_2CO) 130 (36, M-CH_3CO), 77 (26), (51 (14), 43 (14, CH_3CO)
4d	7.88-7.32 (m, 5H); 6.22 (s, 1H, C_4-H), 2.80 (q, 2H, J=7.5 Hz). 1.36 (t, 3H, J=7.5 Hz).	173 (87, M^+), 145 (31), 144 (100, M-C_2H_5), 117 (15) 116 (39, M-C_2H_5CO), 77 (58), 57 (12, C_2H_5CO), 51 (28).
4e	7.37 (br.s, 3H); 2.42 (s, 3H, C_5-CH_3); 1.78 (s, 3H, C_4-CH_3).	241 (100, M^+), 226 (91, M-CH_3), 199 (64 M-CH_2CO) 198 (53, M-CH_3CO), 174 (17), 173 (22), 172 (27), 171 (28), 43 (49, CH_3CO).
4f	7.34 (br.s, 3H); 5.98 (s, 1H, C_4-H); 2.87 (q, 2H, J=7.5 Hz), 1.38 (t, 3H, J=7.5 Hz).	241 (31, M^+), 226 (7, M-CH_3), 212 (100 M-C_2H_5), 184 (22, M-C_2H_5CO), 173 (14), 57 (8, C_2H_5CO).
4g	8.18 (br.s, 1H, C_5-H); 7.77-7.32 (m, 5H); 2.60 (q, 2H, J=7.5 Hz). 1.23 (t, 3H, J=7.5 Hz).	173 (59, M^+), 158 (99, M-CH_3), 146 (16), 145 (15, M-28), 144 (36, M-C_2H_5), 130 (62), 118 (71), 104 (21, M-C_2H_5C=CO), 103 (28), 77 (100).
4h	8.29 (br.s, 1H, C_5-H); 7.40 (br.s, 3H); 2.30 (q, 2H, J=7.5 Hz); 1.13 (t, 3H, J=7.5 Hz).	241 (60, M^+), 226 (100, M-CH_3), 214 (9), 213 (13, M-28), 212 (13, M-C_2H_5), 206 (15, M-Cl), 198 (49), 178 (67), 173 (12), 172 (20, M-C_2H_5C=CO), 171 (13).
4i	7.83-7.30 (m, 5H); 2.87-2.48 (m, 4H); 2.03-1.69 (m, 4H).	199 (100, M^+), 198 (60), 171 (18), 170 (44), 143 (75), 130 (20), 129 (23), 119 (46), 117 (15), 103 (35), 102 (21), 101 (22), 77 (68), 75 (40), 51 (32).
4j	7.37 (br.s, 3H); 2.92-2.63 (m, 2H); 2.45-1.67 (m, 6H).	267 (100, M^+), 239 (9), 238 (7), 232 (36, M-Cl), 213 (29), 211 (45), 204 (64), 198 (25), 174 (18), 173 (13), 172 (26).

a: Lit.[14] δ=7.35-6.65 (m, ArH), 6.10 ppm (s, 1H, C_4-H) (in $CDCl_3$)