Tobacco Chemistry

3** : Unsaturated Hydrocarbon Constituents of Greek Tobacco*

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Previous studies of the unsaturated hydrocarbon constituents of tobacco have revealed the presence of remarkably few representatives of this class of substance (1). Of the olefinic compounds, only the presence of neophytadiene (2) and some tetraterpenes, including certain carotenes (3), in normal tobacco is well established. However, squalene has been stated to be present in Burley tobacco (4) and was shown to occur in tobacco tissues grown *in vitro* in the absence of oxygen (5). Moreover, cembrene (thunbergene) has been isolated from tissue slices of *N. tabacum* after inhibition of sterol biosynthesis by addition of tris(2-diethylamino-ethyl)phosphate trihydrochloride and β -diethylamino-ethyl-diphenylpropylacetate chloride (6). As regards the aromatic hydrocarbon constituents, evidence for the presence of only benzene, toluene, isomeric xylenes (7), and some polynuclear aromatic hydrocarbons (8–10) of which the simplest representatives are phenanthrene and anthracene (8), seems to have been presented. However, it has been pointed out that the presence of polynuclear material is almost certainly the result of contamination during growth and/or subsequent industrial processing (10).

In contrast, a large number of olefins and aromatic hydrocarbons have been encountered in tobacco smoke (1, 11) and these are hence generally regarded as being formed in the smoking process (12, 13). The amounts of benzene, naphthalene and corresponding alkyl derivatives present in the smoke have been estimated to vary from 46 to 0.17 μg per cigarette (13) and those of polynuclear aromatic hydrocarbons from 0.1 to 0.01 μg per cigarette (14). An authoritative account dealing with the constituents of tobacco and smoke has recently been given by Stedman (1).

In view of the limited information available regarding unsaturated hydrocarbons present in tobacco it was of interest to examine these in further detail. The present paper, which represents a continuation of an investigation of tobacco constituents (15), deals with the more volatile components of Greek tobacco belonging to this group of compounds.

EXPERIMENTAL

Instruments and Material

Gas chromatography was performed on a Varian 1700 instrument equipped with a capillary injector and* a flame ionisation detector (FID). Gas chromatography in combination with mass spectrometry was carried out on a modified LKB 9000 instrument, in which the original gas chromatograph had been replaced by a home made unit equipped with a capillary injector, a device for introduction of make-up gas, a splitter and a FID. The signal from the FID could be detected a few seconds prior to that of the total ion current sensor of the mass spectrometer due to different travelling time after the splitter (16). The capillary column used in the present study was made by coating a stainless steel tube (50 m $\times$ 0.5 mm, Handy and Harman grade 316-S) with Apiezon L (Associated Electrical Industries, England), dissolved in hexane (10%), using the dynamic method (17). The stainless steel Soxhlet type apparatus used in the extraction of the tobacco was carefully cleaned by refluxing successively with ethanol, ether and n-hexane; no impurities could be detected gas chromatographically in the distillation residue on concentrating the purified n-pentane used in a subsequent blank extraction.

Of the organic solvents used, n-pentane (technical, b. p. 34–38°) was purified by successive treatment with fuming sulphuric acid (30% SO_3), a saturated solution of potassium permanganate in dilute sulphuric acid, and water. It was dried (CaO) and distilled twice using a packed column (60 $\times$ 3.5 cm, reflux ratio 0.5) disregarding each time a forerun (5%) and a residue (10%). No impurities, except trace amounts of hexane and heptane, could be detected in the residue resulting on concentrating 1 liter to 0.01 ml when examined gas chromatographically under conditions similar to those used in the investigation of the tobacco compounds. The ether (technical, b. p. 33–37°) was dried (CaCl_2) and distilled as above with the exception that the last distillation was made after addition of a small amount of calcium hydride to the distillation pot. The purity was the same or better than that of the purified n-pentane. The acetic acid (pro analysi) was shown gas chromatographically to contain no impurities extractable

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with ether after neutralisation with sodium hydroxide. The ethanol (spectroscopic grade) used in the reaction was shown to lack absorption above 220 nm.

The silica gel (Merck, 0.05–0.20 mm) used for preparative chromatography was purified, after increasing the water content to 10 per cent and packing in a glass column, by eluting with ethanol (2 kg EtOH/kg SiO₂) and subsequently with purified ether until no impurities could be detected in the eluate by gas chromatography after 10 000-fold concentration.

Of the inorganic drying agents, sodium sulphate (pro analysi, 5 kg) was extracted 4 times with 2 liter batches of purified ether, but, as this proved insufficient, it was transferred to a column and eluted with ether until no impurities could be detected gas chromatographically in the residue (0.01 ml) resulting on concentrating two liters of the eluate. The remaining ether was then removed under vacuum and the dry sodium sulphate transferred and stored in a glass bottle. The small amounts of calcium oxide used were heated to 900° immediately prior to use. The dilute aqueous solutions of sodium hydroxide (10%) and sulphuric acid (10%) were prepared from pro analysi chemicals and distilled water. These, the saturated aqueous sodium carbonate solution and all water used in the investigation were extracted twice with purified ether prior to use. The carbon dioxide used in the distillation was of instrument quality (> 99.99% CO₂) and was purified immediately before the distillation by filtration through an active carbon filter. The Girard T reagent was recrystallized from ethanol and was shown to be free from impurities by performing a blank experiment and examining the concentrated extracts by gas chromatography. Plates for silver nitrate-silica gel chromatography and adsorbent for silver nitrate-silica gel column chromatography were prepared according to a procedure given by *Norin and Westfelt* (18). In the latter case purified silica gel was used, and the aqueous silver nitrate (pro analysi) solution used for impregnation was extracted several times with purified ether prior to application.

The tobacco investigated had been grown in Serres, Greece, in 1968 and represented a part of the commercially available crop.

Isolation

The leaves (31.0 kg) containing 10.3 per cent water were crumbled and extracted with purified n-pentane (70 l) for 81 hrs, and subsequently with purified ether (50 l) for 90 hrs in a stainless steel Soxhlet type apparatus. The ether extract was concentrated to 1.5 liters by careful removal of ether through a packed column (60 × 3.5 cm, reflux ratio 0.2) and slowly added with stirring to purified n-pentane (20 l). The n-pentane soluble part was combined with the n-pentane extract and concentrated to 1.5 liters as above. In the further work all solutions of tobacco compounds were concentrated using a Vigreux column (1 m) and, to avoid loss of material of low volatility, some solvent was left in the residue. The weights given for the various fractions are hence inaccurate.

The concentrated extract was distilled in vacuo (85°, 0.01–0.001 mm Hg) according to a recently described method (19) involving the use of carbon dioxide as carrier gas and total condensation of this and the distillate at liquid nitrogen temperature. In view of the large amounts of extract the published method was however modified in that the distillation trap outlet was not closed during the distillation but connected to an evacuating system. The distillation yielded a volatile material (160 g) and a residue (812 g), which has not been investigated further.

The distillate was diluted with ether and extracted several times with dilute sulphuric acid. The combined aqueous solutions were made alkaline with dilute sodium hydroxide and extracted with ether. The ether solution was washed with water, dried (Na₂SO₄), and carefully concentrated to give a fraction containing bases (84 g). The aqueous solution on saturating with sodium chloride and subsequent extraction with ether yielded further basic material (6.0 g). The distillate was then extracted several times with dilute sodium hydroxide and the resulting aqueous solutions were combined, acidified with dilute sulphuric acid, and extracted with ether. The ether solution was washed with water, dried (Na₂SO₄), and the solvent evaporated to give an acidic fraction (5.5 g). Neither this fraction nor those comprising the bases have been studied further.

The remaining neutral material was washed with water, dried (Na₂SO₄), and carefully concentrated to give a low boiling neutral fraction (19 g). This material (19 g) was added to a mixture of absolute ethanol (270 ml), acetic acid (30 ml) and Girard T reagent (30 g) and boiled under reflux for 1 hr. The cooled reaction mixture was diluted with water (1050 ml) containing sodium hydroxide to give the resulting solution a pH of 6.8. Extraction with ether (6 × 60 ml) and successive treatment of the combined ether extract with a saturated aqueous sodium carbonate solution and water, followed by drying (Na₂SO₄) and careful removal of solvent, gave a neutral fraction (13 g).

The remaining aqueous solution was acidified with dilute sulphuric (10%, 260 ml), allowed to stand at room temperature for 2 hrs and then extracted with ether. The ether solution was washed with a saturated sodium carbonate solution and water, dried (Na₂SO₄), and concentrated by removal of solvent to give a carbonyl fraction (2.8 g) which is presently being examined.

The neutral fraction remaining after removal of carbonyls (13 g) was chromatographed on silica gel (600 g). n-Pentane eluted a hydrocarbon fraction (4.1 g) and the remainder of the material (4.4 g), which is presently being investigated, was eluted with ether. The hydrocarbons (4.1 g) were adsorbed on silver nitrate impregnated silica gel (180 g, AgNO₃:SiO₂ = 0.3:1). Elution with n-pentane gave a saturated hydrocarbon fraction (1.0 g) and further elution with n-pentane containing increasing amounts of ether gave an unsaturated hydrocarbon fraction (3.0 g). The saturated hydrocarbon fraction has been investigated earlier (15) and was not processed further.

Examination of the Unsaturated Hydrocarbon Fraction

The unsaturated hydrocarbon fraction (2.8 g) was adsorbed on silica gel and elution with *n*-pentane (425 ml) furnished neophytadiene (2.5 g), which was identified by means of infrared, nmr and mass spectra. Further elution with ether/*n*-pentane (1:100, 500 ml) gave a fraction A (0.30 g) containing unsaturated hydrocarbons.

Fraction A was examined by gas chromatography and the best result was achieved with a capillary column (50 m $\times$ 0.5 mm) coated with Apiezon L using a carrier gas pressure of 8 psi giving a flow of 4 ml N_2 /min. The temperature of the injection block and the detector was 280°. The samples were injected with a split ratio of 1:20. The column temperature was maintained at 50° for 8 minutes after injection and then programmed to increase linearly at a rate of 2°/min. It was finally held at 250° until completion of the run. The resulting chromatogram is given in Fig. 1.

Using this column in the gas chromatographic unit of the LKB 9000 instrument but substituting nitrogen for helium as carrier gas (4 ml/min) and reducing the injection volume to half, the split ratio to 1:4 and the programming rate to 1°/min gave a somewhat better separation and made it possible to obtain good spectra of the compounds corresponding to the numbered peaks in the chromatogram (Fig. 1). The separator, connecting the gas chromatographic unit with the mass spectrometer, was kept at 250° and the ion source at 290°. The energy of the impinging electrons was 20 eV except when mass spectra were recorded, when it was automatically raised to 70 eV. The scanning time from 10 to 500 mass units was 4.2 seconds. Mass spectra were recorded close to the maxima of the GLC-peaks; for inadequately resolved peaks three or more spectra were recorded to improve the information obtained. To allow correction for background effects, scans were also made between GLC-peaks at suitable intervals.

Elimination of Olefins

Fraction A (35 mg) dissolved in ether (1 ml) was treated with osmium tetroxide for 6 days at room temperature. Hydrogen sulphide was then slowly bubbled through the solution until no further material precipitated. The solution and the ether extracts of the precipitate were filtered through a short cellulose column prewashed with ether, and subsequently through a short aluminium oxide column similarly pretreated to give an aromatic hydrocarbon fraction. This was examined by gas chromatography and gas chromatography-mass spectrometry under the same conditions as discussed above.

Mass Spectrometric Data

Peaks of diagnostic significance in the spectra of the alkylbenzenes and alkylnaphthalenes of interest are summarized in Tables 1 and 2, while the spectra of the other compounds, which have been compared directly with literature data (20-23) and available reference material, are not included. In Tables 1 and 2 the

relative intensities are expressed as a percentage of the molecular peak which is arbitrarily assigned the value 100.

Table 1. Relative intensities of certain diagnostically significant ions in the mass spectra of some alkylbenzenes from tobacco.

The peak number refers to the gas chromatogram in Fig. 1.

	M.W.	Peak no.	M	M-1	M-15	M-29	M-43
C ₂ -alkylbenzenes	106	2	100	16	210	19	13
	106	3	100	64	172	29	14
	106	4	100	56	156	22	11
C ₃ -alkylbenzenes	120	6	100	16	320	50	40
	120	8	100	12	270	44	33
	120	9	100	26	173	15	18
	120	10	100	29	200	23	26
C ₄ -alkylbenzenes	134	11	100	—	150	157	64
	134	14	100	21	183	—	37
	134	15	100	18	196	7	29

Table 2. Relative intensities of certain diagnostically significant ions in the mass spectra of some alkylnaphthalenes.

The peak number refers to the gas chromatogram in Fig. 1.

	M.W.	Peak no.	M	M-1	M-15	M-29	M-43
C ₂ -alkylnaphthalenes	156	24	100	—	214	—	—
	156	25	100	35	50	—	—
	156	26	100	31	56	—	—
	156	28	100	35	68	—	—
	156	29	100	27	75	—	—
	156	30	100	30	52	—	—
	156	31	100	30	56	—	—
	156	32	100	22	97	—	—
C ₃ -alkylnaphthalenes	170	33	100	—	7	292	27
	170	34	100	—	193	27	13
	170	35	100	—	159	12	8
	170	36	100	—	144	20	12
	170	37	100	—	178	44	11
	170	38	100	25	68	3	3
	170	39	100	27	67	3	3
	170	40	100	37	74	7	3
	170	41	100	22	70	2	2
	170	42	100	30	130	—	—
C ₄ -alkylnaphthalenes	170	43	100	39	72	16	5
	170	44	100	19	106	6	6
	170	47	100	29	112	12	6
	184	45	100	—	40	200	10
	184	46	100	—	67	433	45
	184	48	100	4	100	84	7
	184	49	100	—	107	50	7
	184	50	100	—	125	19	6
	184	51	100	46	168	43	22
	184	52	100	15	90	10	10

DISCUSSION AND RESULTS

The volatile unsaturated hydrocarbons of tobacco grown in Serres, Greece, in 1968 were isolated as detailed in the experimental section whilst taking extreme precautions to avoid contamination during the isolation procedure. The compounds were separated by chromatography on silica gel to give neophytadiene as the main constituent together with a fraction, designated A, comprising the remaining unsaturated hydrocarbons. Examination of this fraction by gas chromatography and by gas chromatography in combination with mass spectrometry revealed that it contained at least 170 components and that the vast majority of these are aromatic compounds. The gas chromatogram of fraction A is given in Fig. 1.

In order to obtain further information about fraction A and to simplify the interpretation of the mass spectra and the gas chromatogram, the compounds containing olefinic linkages were removed selectively and the resulting material reexamined. This was effected by bis-hydroxylation of the olefinic double bonds using osmium tetroxide and hydrogen sulphide. The aromatic components, being unreactive towards this reagent, were readily isolated by chromatography.

Examination of the mass spectrometric and gas chromatographic data obtained by the above techniques shows that the compounds can conveniently be divided into four groups namely, olefines, alkylbenzenes, naphthalenes, and other aromatic compounds. A more detailed discussion of the identification of the individual compounds and other structural information obtained is outlined below and the results are summarized in Table 3. Indication as to which of the identified compounds have previously been encountered in tobacco and/or smoke is also given.

Olefins

The compounds corresponding to the peaks 1, 5, 7, 12, 13, 17, 20, 57 and 58, and in part to peaks 6 and 11 were eliminated by the osmium tetroxide treatment and are hence olefins. The most prominent of these compounds [57] was identified as neophytadiene, while the others, which are present in very low concentration, did not give spectra corresponding to any of the available references nor did they provide sufficient information to allow any definite structural assignments. It is evident however that two of the compounds are monoterpenes [7, 11], one is a sesquiterpene [20], and one is a further diterpene [58].

Alkylbenzenes

A fair number of the unsaturated hydrocarbons could be identified as alkylbenzenes on the basis of the mass spectrometric results. More detailed structural information about these compounds was obtained by considering their fragmentation pattern using rules recently summarized by Grubb and Meyerson (23), relationships derived from published spectra (20–22), and by comparison of gas chromatographic retention times.

It is of interest that neither the simplest member of the group, toluene, nor benzene could be detected and that all alkylbenzenes are present in low concentration. It is obvious, however, that due to their high volatility benzene and toluene could have been lost in the work up if originally present in the tobacco in low concentrations.

C₂-alkylbenzenes: Ethylbenzene (peak 2) is readily distinguished mass spectrometrically from isomeric xylenes, and the latter can only be differentiated by means of retention data. Peak 3 corresponds to p- and/or m-xylene, which are not separated on the column used, and peak 4 to o-xylene. All of these compounds have previously been found in smoke, but only the xylenes in tobacco.

C₃-alkylbenzenes: Of the eight theoretically possible C₃-alkylbenzenes, which mass spectrometrically can readily be classified as n-propyl-, isopropyl-, methyl-ethyl- and trimethylbenzene, at least four occur in tobacco. It follows when retention data are taken in conjunction with the mass spectrometric results that peak 6 corresponds to p- and/or m-ethyltoluene, peak 8 to o-ethyltoluene, peak 9 to 1,2,4-trimethylbenzene and peak 10 to 1,2,3-trimethylbenzene. None of these have previously been encountered in tobacco but all have been detected in smoke.

C₄-alkylbenzenes: It is clear from the mass spectrometric results that peak 11 corresponds to diethylbenzene and peaks 14 and 15 to tetramethylbenzenes. Although the M-Et/M-Me intensity ratio indicates (23) that the first compound is the meta rather than the ortho or para isomer, the lack of retention data makes it impossible to establish with certainty the substitution pattern in any of the three compounds. However, no C₄-alkylbenzenes have previously been found in tobacco and only one, p-cymene, has been detected in smoke.

C₆–C₈-alkylbenzenes: In view of the number of possible isomers and the enhanced chances of several isomers having the same or nearly the same retention times it should only be noted here that peak 27 corresponds to a C₆-alkylbenzene, peaks 34, 36 and 37 are partly due to C₇-alkylbenzenes, and peaks 44–46 partly to C₈-alkylbenzenes. No compounds of this category have been detected in either tobacco or smoke previously.

Naphthalenes

It follows from the mass spectrometric results that the vast majority of the unsaturated hydrocarbons are alkylnaphthalenes. Since these fragment in a manner analogous to that of the alkylbenzenes, more detailed structural information about the former could be obtained in a similar way from these data and by considering retention data when available (25, 26).

The most abundant of the unsaturated hydrocarbons, except for neophytadiene, is naphthalene, which corresponds to peak 18. The next two prominent peaks in the chromatogram, 21 and 22, are due to 2- and

Table 3. Unsaturated hydrocarbon constituents of Greek tobacco.

Peak no.	Molecular weight	Compound	GC retention authenticated	Presence previously demonstrated (1,24) in	
				smoke	to-bacco
1.	124	C ₉ H ₁₆			
2.	106	ethylbenzene	x ^a	+	
3.	106	p- and/or m-xylene	x ^a	+	+
4.	106	o-xylene	x ^a	+	+
5.	122	C ₉ H ₁₄			
6.	120+138	p- and/or m-ethyltoluene + C ₁₀ H ₁₈ 1:1	x ^a	+	
7.	136	monoterpene			
8.	120	o-ethyltoluene	x ^a	+	
9.	120	1,2,4-trimethylbenzene	x ^a	+	
10.	120	1,2,3-trimethylbenzene	x ^a	+	
11.	134+136	diethylbenzene + monoterpene 1:1			
12.	132	C ₁₀ H ₁₂			
13.	132	C ₁₀ H ₁₂			
14.	134	tetramethylbenzene			
15.	134	tetramethylbenzene			
16.	132	tetralin	x ^a	+	
17.	146	C ₁₁ H ₁₄			
18.	128	naphthalene	x ^a	+	
19.	146	5- and/or 6-methyltetralin			
20.	204	sesquiterpene			
21.	142	2-methylnaphthalene	x ^a	+	
22.	142	1-methylnaphthalene	x ^a	+	
23.	154	biphenyl	x ^a	+	
24.	156	1-ethylnaphthalene	x ^b		
25.	156	2,6- and/or 2,7-dimethylnaphthalene	x ^b	+	
26.	156	1,7-dimethylnaphthalene	x ^b		
27.	162	C ₈ -alkylbenzene			
28.	156	1,6- and/or 1,3-dimethylnaphthalene	x ^b	+	
29.	156	2,3-dimethylnaphthalene	x ^b		
30.	156	1,4-dimethylnaphthalene	x ^b		
31.	156	1,5-dimethylnaphthalene	x ^b		
32.	156	1,2-dimethylnaphthalene	x ^b		
33.	170	n-propylnaphthalene			
34.	170+176	C ₇ -alkylbenzene + isop.- and/or methylethyl-naphthalene 1:1			
35.	170	isop.- and/or methylethyl-naphthalene			
36.	170+176	C ₇ -alkylbenzene + isop.- and/or methylethyl-naphthalene 1:1			
37.	170+176	C ₇ -alkylbenzene + isop.- and/or methylethyl-naphthalene 1:1			
38.	170	trimethylnaphthalene			
39.	170	trimethylnaphthalene			
40.	170+184	trimethylnaphthalene + C ₈ -alkyl-naphthalene 10:1			
41.	170	trimethylnaphthalene			
42.	170	trimethylnaphthalene			
43.	170	trimethylnaphthalene			
44.	170+190	trimethylnaphthalene + C ₈ -alkylbenzene 4:1			
45.	184+190	methylpropyl- and/or s-butyl-naphthalene + C ₈ -alkylbenzene 5:1			
46.	170+184 +190	trimethyl- + methylpropyl- and/or s-butyl-naphthalene + C ₈ -alkylbenzene 1:1:1			
47.	170	trimethylnaphthalene			
48.	184	diethylnaphthalene			
49.	184	diethylnaphthalene			
50.	184	C ₈ -alkyl-naphthalene			
51.	184+204	C ₈ -alkylnaphthalene + C ₁₅ H ₂₄ 5:1			
52.	184	tetramethylnaphthalene			
53.	184+204	tetramethylnaphthalene + C ₁₅ H ₂₄ 5:1			
54.	184	tetramethylnaphthalene			
55.	184+204	tetramethylnaphthalene + C ₁₅ H ₂₄ 2:1			
56.	198+184	C ₈ - + C ₈ -alkylnaphthalene 1:1			
57.	278	neophytadiene	x ^a	+	+
58.	272	diterpene			
59.	184	C ₈ -alkyl-naphthalene			
60.	178	anthracene and/or phenanthrene			
61.	198	C ₈ -alkyl-naphthalene			
62.	198	C ₈ -alkyl-naphthalene			
63.	212	C ₈ -alkyl-naphthalene			

a) Identification confirmed by direct gas-chromatographic comparison with authentic materials.

b) Gas-chromatographic retention data from literature (25, 26).

1-methylnaphthalene respectively. These three compounds have previously been encountered in tobacco smoke but not in tobacco.

C₂-alkylnaphthalenes: Of the twelve theoretically possible C₂-alkylnaphthalenes at least eight are present in tobacco and of the latter the mass spectrometric results show that one is an ethylnaphthalene and the others are dimethylnaphthalenes. Using available retention data it could be concluded that peak 24 is due to 1-ethylnaphthalene, peak 25 to 2,6- and/or 2,7-dimethylnaphthalene, peak 26 to 1,7-dimethylnaphthalene, peak 28 to 1,6- and/or 1,3-dimethylnaphthalene, peak 29 to 2,3-dimethylnaphthalene, peak 30 to 1,4-dimethylnaphthalene, peak 31 to 1,5-dimethylnaphthalene and peak 32 to 1,2-dimethylnaphthalene. None of these compounds, several of which are significant constituents of the unsaturated hydrocarbon fraction, have previously been found in tobacco. The 1,6-, 1,8-, 2,6- and 2,7-dimethylnaphthalenes have however been encountered in tobacco smoke, and of these only 1,8-dimethylnaphthalene has not been detected in the tobacco now investigated.

C₃-alkylnaphthalenes: The mass spectrometric results show that peak 33 corresponds to n-propylnaphthalene, peaks 34–37 to isopropyl- and/or methylethylnaphthalenes and peaks 38–44 and 47 to trimethylnaphthalenes. Since no mass spectrometric or gas chromatographic retention data for C₃-alkylnaphthalenes of known structure has been available no further structural information could be obtained about these compounds, of which several are significant constituents of the unsaturated hydrocarbon fraction. No C₃-alkylnaphthalene has however previously been found in tobacco and only one, 1,3,6-trimethylnaphthalene, has been reported present in tobacco smoke.

C₄-alkylnaphthalenes: Although, as in the case of the C₃-alkylnaphthalenes, there is no mass spectrometric or retention data concerning C₄-alkylnaphthalenes available in the literature, it may be concluded from the mass spectrometric results that peaks 45 and 46 are partly due to n-propylmethyl- and/or s-butyl-naphthalenes, peaks 48 and 49 exclusively to diethylnaphthalenes, and peaks 52–55 mainly to tetramethylnaphthalenes. The peaks 50, 51 and 59 are evidently also due mainly to C₄-alkylnaphthalenes, but as these do not conform to any of the established patterns they are probably mixtures of isomers having substituents of different length. No C₄-alkylnaphthalenes have been encountered previously in either tobacco or smoke although some of these are fairly prominent constituents of fraction A.

C₅- and C₆-alkylnaphthalenes: It may be concluded from the mass spectrometric results that the peaks 56, 61 and 62 are due mainly to C₅-alkylnaphthalenes and peak 63 mainly to C₆-alkylnaphthalenes, but that these are mixtures of isomers having sidechains of different length and hence the possibility of obtaining further

structural information from the mass spectra is excluded. Moreover, these are less abundant than most of the lower homologs discussed above, and no compounds of this category have previously been reported present in either tobacco or smoke.

Other Aromatic Constituents

Fraction A was also found to comprise four further constituents which did not belong to any of the above groups. Thus peak 16 was shown to be due to tetralin, peak 19 to 5- and/or 6-methyltetralin, peak 23 to biphenyl and peak 60 to anthracene and/or phenanthrene. The identification of tetralin and biphenyl was also supported by retention data; mass spectra and retention times for anthracene and phenanthrene are nearly identical under the conditions used and a distinction could not be made with certainty. While the tetralins are present in very low concentration, the other two compounds are significant constituents of fraction A. With the exception of methyltetralin, all have previously been found in tobacco smoke, whilst phenanthrene and anthracene have also been found in tobacco.

CONCLUSIONS

Neophytadiene and the other isoprenoid olefines are anticipated plant products, while most of the aromatic constituents are not. It might be inferred, following earlier proposals and findings regarding polynuclear aromatic hydrocarbons (10), that the presence of the aromatic compounds is a result of contamination during growth, curing, storage and/or transport. However, it seems premature to exclude the possibility that these compounds are formed from tobacco constituent precursors on exposure to light and atmospheric oxygen subsequent to harvest, particularly as the tobacco investigated is sun-cured, comes from a non-industrial area, and was extracted immediately on arrival in Sweden. In fact, since little is known about compounds present in concentrations below the part-per-million-level the presence of these compounds even in the growing leaf can obviously not be excluded (cf. ref. 27).

Although no attempt has been made in the present investigation to obtain quantitative information about the various constituents, it is of some interest to compare the level at which these occur in tobacco with the corresponding figures for smoke (13) in spite of the fact that the latter derives from a different type of tobacco. It can be concluded that the concentration of the alkylbenzenes, when compared on the basis of equal weight of tobacco, is several orders of magnitude greater in smoke than in tobacco, while the common alkyl naphthalenes are evidently present in similar concentrations. It has been shown that 3,4-benzopyrene present in tobacco is transferred to the smoke to the extent of about ten per cent (10) and it seems probable therefore that most of the present compounds are transferred to a similar or higher extent, as they have lower boiling points.

Finally it should be emphasized that the rigorous purification of the materials used in the isolation work was undertaken on account of the fact that the commercially available products contained a large number of aromatic contaminants. In many of the earlier publications on unsaturated hydrocarbons from tobacco and smoke little attention seems to have been paid to this problem and as a consequence the origin of the compounds found is not in all cases certain.

SUMMARY

Tobacco from Serres, Greece, has been studied with respect to the content of unsaturated hydrocarbons. The isolation of these constituents involved as a key step distillation with the aid of a new technique using carbon dioxide as carrier gas and total condensation of this and the distillate at liquid nitrogen temperature. In addition to the main constituent, neophytadiene, the unsaturated hydrocarbon fraction was shown to contain a series of aromatic compounds. These were examined by means of gas chromatography on capillary columns and also by gas chromatography in combination with mass spectrometry. Fractions were examined before and after elimination of smaller amounts of accompanying olefinic material with the aid of osmium tetroxide. The aromatic constituents were found to be alkylated benzene and naphthalene derivatives, of which many have been identified unambiguously. The vast majority of these have not previously been encountered in tobacco. Several have, however, been detected in smoke and the present results therefore show that these compounds are not necessarily formed solely in the pyrolysis process.

ZUSAMMENFASSUNG

Aus Serres (Griechenland) stammender Tabak wurde auf seinen Gehalt an ungesättigten Kohlenwasserstoffen untersucht. Zur Isolierung dieser Inhaltsstoffe wurde als entscheidender Schritt eine Destillation nach einem neuartigen Verfahren durchgeführt, bei dem Kohlendioxid als Trägergas benutzt und zusammen mit dem Destillat bei der Temperaturstufe flüssigen Stickstoffs vollständig kondensiert wird. Es wird nachgewiesen, daß die Fraktion der ungesättigten Kohlenwasserstoffe neben dem hauptsächlichen Inhaltsstoff, Neophytadien, eine Reihe von aromatischen Verbindungen enthält. Diese Verbindungen wurden gaschromatographisch unter Verwendung von Kapillarsäulen und auch durch Kombination von Gaschromatographie und Massenspektrometrie untersucht. Die Fraktionen wurden vor und nach der Eliminierung von verhältnismäßig kleinen Mengen olefinischer Begleitstoffe mit Hilfe von Osmiumtetroxid untersucht. Es zeigte sich, daß die aromatischen Inhaltsstoffe alkylierte Benzol- und Naphthalinderivate sind, die größtenteils eindeutig identifiziert wurden. Die überwiegende Mehrheit dieser Inhaltsstoffe hat sich bisher im Tabak noch nicht gezeigt. Einige wurden jedoch im

Rauch festgestellt; aus den Ergebnissen dieser Arbeit läßt sich daher schließen, daß sie nicht unbedingt während der Pyrolyse gebildet werden.

RESUME

On a étudié les hydrocarbures non saturés présents dans le tabac de Serres, Grèce. Leur isolement faisait intervenir une distillation sous vide partiel à 85° réalisée suivant une nouvelle technique utilisant CO₂ comme gaz vecteur, et la condensation totale du CO₂ et du distillat à la température de N₂ liquide. Outre son composé principal, le néophytadiène, la fraction hydrocarbures non saturés contenait une série de composés aromatiques. Ils ont été étudiés par chromatographie gazeuse avec colonnes capillaires et aussi par chromatographie gazeuse couplée avec spectrométrie de masse. Les fractions ont été examinées avant et après élimination des petites quantités de substances oléfiniques qui les accompagnaient à l'aide de O₈O₄. On a constaté que les constituants aromatiques étaient des dérivés benzéniques et naphthaléniques alkylés dont plusieurs ont été parfaitement identifiés. Pour la plupart, ils n'avaient pas encore été signalés comme présents dans le tabac. Cependant, plusieurs avaient été décelés dans la fumée et il apparaît donc que ces composés ne sont pas nécessairement formés seulement au cours de la pyrolyse.

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