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QUANTITATIVE GAS CHROMATOGRAPHIC METHOD FOR THE ANALYSIS OF ALIPHATIC HYDROCARBONS, TERPENES, FATTY ALCOHOLS, FATTY ACIDS AND STEROLS IN TOBACCO

R. F. SEVERSON*, J. J. ELLINGTON, R. F. ARRENDALE and M. E. SNOOK

Tobacco Laboratory, Science and Education Administration-Federal Research, U.S. Department of Agriculture, P.O. Box 5677, Athens, Ga. 30604 (U.S.A.)

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SUMMARY

A rapid and reproducible method is described for the analysis of the major tobacco lipids, including the C_{25}–C_{34} paraffinic hydrocarbons, neophytadiene, phytol, docosanol, squalene, α-tocopherol, β-amyrin, cycloartenol, 24-methylenecycloartanol, palmitic acid, stearic acid, the unsaturated C_{18} acids, cholesterol, stigmasterol, campesterol, sitosterol and solanesol. The method is based upon the short-column silicic acid chromatography of saponified lipids from a hexane extract of tobacco. The free lipids are separated into non-polar, polar and terpenoid fractions and quantitated by gas chromatography, with the use of internal standards. The applicability of the method has been demonstrated by the highly reproducible analyses of a cigarette blend, conventional flue-cured tobaccos and two close-grown tobaccos. The method should also be applicable to analyses of lipids in other natural products, including foodstuffs.

INTRODUCTION

Many of the components in the hexane-soluble fraction of tobacco, the so-called tobacco lipids, and their pyrolysis products contribute to the flavor and aroma of tobacco smoke[1]. Also, pyrolysis studies have shown that the hexane-soluble components in tobacco are major precursors of the tumorigenic polynuclear aromatic hydrocarbons in tobacco smoke[2,3]. Consequently, our laboratory has been involved in developing rapid and reproducible methods for the quantitation of the major lipid components in tobacco. Chortyk *et al.*[4] developed a method utilizing silicic acid and gas chromatography (GC) for the determination of the paraffinic hydrocarbons and neophytadiene in leaf and smoke. Ellington *et al.*[5] used ion-exchange chromatography and GC to analyze tobacco fatty acids of C_{12} to C_{34} chain lengths. Schlotzhauer *et al.*[6] utilized thin-layer chromatography for the class separation of tobacco lipids. Severson *et al.*[7] found that solanesol, the most abundant component in the

* To whom correspondence should be addressed.

tobacco lipid fraction, could be quantified by short-column, high-temperature GC. Based on the above studies, Ellington *et al.*[8] developed a rapid method for the GC quantitation of neophytadiene, the hydrocarbons, the major fatty acids, the sterols and solanesol. They used a Unisil silicic acid* (Clarkson, Williamsport, Pa., U.S.A.) column to separate the hexane-soluble portion of saponified tobacco into two groups, non-polar and polar lipids (Fig. 1).

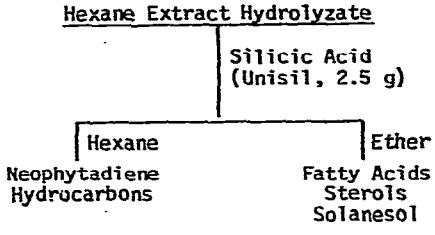

Fig. 1. Separation procedure of Ellington *et al.*[8] for hexane extract of saponified tobacco.

Although these methods are very useful for the quantification of the above lipid components, they failed to isolate and quantify many of the terpenes and paraffinic alcohols known to be in tobacco. We now describe how we extended the method for a more complete determination of the major GC-volatile hydrocarbons, terpenes, fatty alcohols, fatty acids and sterols in flue-cured tobacco.

EXPERIMENTAL

Solvents and standards

All of the solvents (Burdick & Jackson, Richmond, Calif., U.S.A., distilled-in-glass grade) were redistilled before use. Dimethylformamide (DMF), N,O-bis(trimethylsilyl)acetamide (BSA) and Tri-Sil Z (trimethylsilylimidazole in dry pyridine) were silylation grade (Pierce, Rockford, Ill., U.S.A.). Fatty acid and sterol standards were obtained from Applied Science Labs. (State College, Pa., U.S.A.) and docosane, phytol, the cosanols, and the Dexsil 300 GC from Analabs (North Haven, Conn., U.S.A.). SP-2250 was supplied by Supelco (Bellefonte, Pa., U.S.A.). Solanesol, obtained from an industrial source, was purified according to Severson *et al.*[7]. Squalene, α-D-tocopherol and β-amyrin were obtained from Pfaltz and Bauer (Flushing, N.Y., U.S.A.). Cycloartenol and 24-methylenecycloartanol were obtained from Dr. W. Herbert Morrison, Field Crops Utilization and Marketing Research Laboratory, S.E.A.-F.R., U.S.D.A., Athens, Ga., U.S.A.

The following tobacco samples were analyzed: Eastern Carolina (1968; commercial flue-cured, redried, aged); South Carolina (1971; commercial flue-cured, redried, aged); NC2326 (1976; experimental, flue-cured), Coker 139 (1976; experimental, flue-cured) and Speight G-28 (1976; experimental, close-grown, flue-cured), Tobacco Research Laboratory, S.E.A.-F.R., U.S.D.A., Oxford, N.C., U.S.A.; Cigarette Blend (1977; commercial blend used in 85-mm non-filter cigarettes).

* Reference to a company or product name does not imply approval or recommendation by the U.S. Department of Agriculture (U.S.D.A.).

Tobacco preparation and dry weight

Tobacco samples were equilibrated in the open under laboratory conditions for 2 days and then ground in a Wiley mill to pass through a 22-mesh screen. For moisture determination, *ca.* 200 mg of ground tobacco were heated for 3 h at 95 ± 0.05° in a vented oven[9]. Moisture was determined before each base hydrolysis.

Hydrolysis

The base-hydrolysis procedure[8] was modified to allow quantitative recovery of components. About 1 g of ground tobacco (22 mesh) was hydrolyzed under nitrogen for 2 h by refluxing with 40 ml of 2 *N* KOH in 85% ethanol in a 300-ml saponification flask. The reaction mixture was cooled in an ice-bath, 50 ml of water were added and the solution was slowly adjusted to pH 2 with concentrated HCl. The mixture was filtered through a 12.5-cm fast-flow folded filter-paper into a 250-ml separating funnel and the hydrolysis flask and filter were washed with 50 ml of benzene–85% ethanol (1:1). Saturated aqueous KCl (25 ml) was added and the mixture was extracted with hexane (3 × 50 ml; 1 × 25 ml).

Preparation of hexane extract for silicic acid chromatography

The hexane extract of the hydrolyzate was concentrated to dryness on a rotary evaporator at 40° under a water-aspirator vacuum. Two consecutive 5-ml portions of benzene were added and the mixture was taken to dryness under vacuum after each addition for removal of residual water or ethanol. After determining the weight of the residue, the appropriate amounts of the internal standards were added (700 μg of docosane, 300 μg of pentacosanol and 3 mg of nervonic acid per 50 mg of hexane extract).

Silicic acid chromatography

A modified (Fisher and Porter) 250 mm × 9 mm I.D. chromatographic column equipped with a sintered glass disc, tapered stop-cock and a 1-l globe-shaped reservoir was slurry packed with 5.0 g of Unisil silicic acid (100–120 mesh, activated at 150° for 2 h) in hexane. A 30–40-mg aliquot of the hexane extract containing internal standard was layered on the column and eluted with 100 ml of hexane to yield the non-polar lipid fraction. The terpene fraction was then removed with 1900 ml of benzene–hexane (1:3) and the polar lipid fraction with 400 ml of benzene–diethyl ether (3:1). The column was operated under a nitrogen pressure of 3–5 p.s.i.

Gas chromatography

Non-polar fraction. The hexane-eluted fraction was reduced in volume to *ca.* 5 ml, transferred to a tapered test-tupe and dried under a stream of nitrogen. The residue was dissolved in 100 μl of isooctane and 3–5-μl portions were analyzed in a Hewlett-Packard 5750 gas chromatograph on an 8 ft. × 1/8 in. stainless-steel (SS) column containing 5% Dexsil 300 GC on Chromosorb W AW (100–120 mesh) (temperature program: 100–325° at 4°/min; injection port, 290°; flame-ionization detector, 350°). Peak areas were measured by an Autolabs System IV electronic integrator.

Terpene fraction. The benzene–hexane fraction was reduced in volume to *ca.* 5 ml on a rotary evaporator, transferred to a tapered test-tube and reduced in

volume under a stream of nitrogen to *ca.* 0.5 ml. About 50% of this solution was transferred to a tapered test-tube and dried at 40° by a stream of nitrogen. A 50-μl volume of Tri-Sil Z and 30 μl of BSA were added and the solution was heated for 30 min at 76°. The resulting trimethylsilyl (TMS) derivatives were analysed (3–7-μl samples) in a Hewlett-Packard 5830 reporting gas chromatograph on a 2.4 m × 2 mm I.D. glass column containing 3% SP-2250 on Supelcon AW DMCS (100–120 mesh) (temperature program: 90–325° at 2°/min; injection port, 275°; flame-ionization detector, 350°). All of the peak areas were corrected for differences in detector response, and all the triterpenes were assumed to yield detector responses equivalent to that of β-amyrin. When standards of GC purity were not available, the response factor of the nearest-eluted standard was used.

Polar lipid fraction. The benzene–diethyl ether fraction was reduced in volume on a rotary evaporator at 40° to *ca.* 5 ml, transferred to a tapered test-tube and reduced to *ca.* 1.0 ml under a stream of nitrogen at 40°. About 30% of the solution was transferred to a tapered test-tube, blown to dryness under nitrogen at 40° and 25 μl each of DMF and BSA were added. The test-tube was then sealed with a PTFE-lined cap, and the mixture heated for 15 min at 76°. A 2–5-μl portion was analyzed in a Hewlett-Packard 5830 reporting gas chromatograph on a 46 cm × 2 mm glass column of 5% Dexsil 300 GC on Chromosorb W AW (100–120 mesh) (temperature program: 100–325° at 4°/min; injection port, 290°; flame-ionization detector, 350°). A standard mixture of palmitic, oleic, stearic and nervonic acids, cholesterol, stigmasterol, sitosterol and solanesol was used to determine detector responses. All of the components were assumed to have response values equal to that of the nearest-eluted standard.

RESULTS AND DISCUSSION

Based on GC profiles obtained in our laboratory for neutral fractions of cigarette smoke condensate (CSC) and tobacco pyrolyzates[10], silicic acid chromatography was expected to separate the terpene–fatty alcohol fraction from the non-polar hydrocarbons and neophytadiene and also from the polar sterols, fatty acids and solanesol. As shown in Fig. 2, we had found that the petroleum ether eluate (F-PE) contained neophytadiene and the long-chain, aliphatic hydrocarbons or

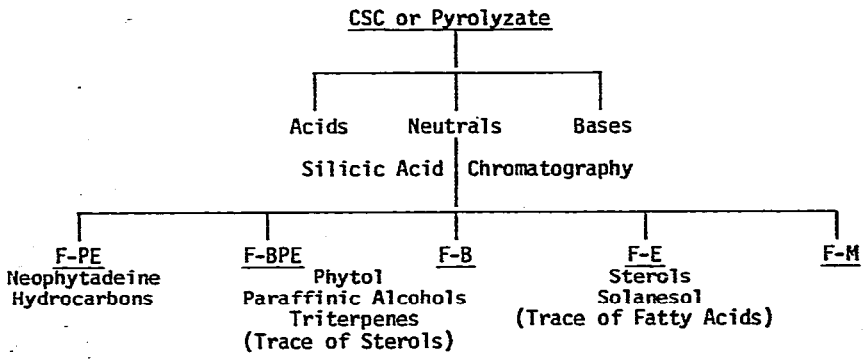

Fig. 2. Silicic acid separation previously used to compare neutral fractions of cigarette smoke condensate (CSC) and tobacco pyrolyzates[10]; PE, petroleum ether; BPE, 25% benzene PE; E, ethyl ether; M, methanol; F, fraction.

paraffins. The benzene fraction (F-B) contained components whose GC retention times agreed with phytol, docosanol and the triterpene, β-amyrin. The sterols, solanesol and trace amounts of fatty acids eluted in the ether fraction, labeled F-E. However, profile analyses clearly showed overlapping GC elution times for the hydrocarbon waxes, sterols and diterpenes and would make GC quantitation impossible for unresolved mixtures of sterols and terpenes. In addition, the diterpenes, neophytadiene and phytol, and the paraffinic alcohols, coeluted with the fatty acids.

The above results suggested that certain modifications of the Unisil silicic acid method of Ellington *et al.*[8] could improve separation of the alcohols and di- and triterpenes from the non-polar and polar lipids of tobacco. After numerous experiments with various eluant mixtures, we found that a small, 5-g Unisil silicic acid column satisfactorily separated the tobacco lipids into three distinct lipid fractions, which could then be readily analyzed by GC.

We found that only 50 ml of hexane readily eluted all the hydrocarbons and neophytadiene (Table I). The saturated alcohols were removed by elution with 1600 ml of hexane–benzene (3:1), whereas 1900 ml were required to elute completely the terpenes, phytol and β-amyrin. Since pentacosanol was not present in the hexane extract of hydrolyzed leaf, it was used as an internal standard for GC quantitation of the alcohols and terpenes of tobacco. The sterols began to elute slowly after 2000 ml of hexane–benzene or they could be rapidly removed with 25% diethyl ether in benzene. Thus, the sterols could be eluted after the terpenes, phytol and β-amyrin by about 10 column volumes of hexane–benzene or preferably by 300 ml of 25% diethyl ether in benzene. Similarly, solanesol and the fatty acids were also rapidly eluted with 25% diethyl ether in benzene. Nervonic acid or other materials, which coelute with it on GC, were not present in the hexane extract of tobacco. It was, therefore, chosen as the internal standard for the GC quantitation of the sterols, solanesol and fatty acids. The elution volumes for sterols and fatty acids were also checked with ^{14}C-labeled lipids added to tobacco prior to base hydrolysis (Table II). The data agreed well with the elution volumes observed by GC monitoring (Table I). For the leaf extract, all of the [^{14}C]cholesterol was recovered in the benzene–diethyl ether fraction The 1.2% of activity found in the first 1500 ml of hexane–benzene for cholesterol–[^{14}C]palmitate, for a 1-h base hydrolysis, was due to unhydrolyzed ester. However, a 2-h hydrolysis completely saponified the ester. These data show that sterol-ester hydrolyses were complete in 2 h and that recovery of the components was >95%.

The elution volumes in Tables I and II were observed for samples up to 50 mg of hexane extract. Larger amounts caused overloading of the column and early elution of the sterols. Fig. 3 summarizes our final procedure to separate the hydrolyzed lipids into groups suitable for GC analyses. Tobacco samples (1 g) provided sufficiently large quantities of hexane extract for subsequent saponification. Chromatography of the free lipids on a small Unisil silicic acid column yielded three fractions: the hexane-eluted non-polar lipids (neophytadiene, hydrocarbons and docosane); the terpene fraction (phytol, paraffinic alcohols, triterpenes and pentacosanol), removed with 25% benzene in hexane; and the polar lipids (the fatty acids, sterols and solanesol, together with the nervonic acid standard) eluted with the benzene–diethyl ether (3:1) solvent. These compounds were all readily gas chromatographed on a short 18-in. column of Dexsil 300 GC with rapid programming of the oven temperature. This allowed rapid monitoring of the silicic acid fractions. Since the

TABLE I
PERCENT ELUTION OF SELECTED TOBACCO LIPIDS PLUS STANDARDS (STD.) FROM A 5-g SILICIC ACID (UNISIL) COLUMN

Component	*Solvent volume (ml)*											
	Hexane		*Benzene–hexane (1:3)*							*Benzene–diethyl ether (3:1)*		
	50	*100*	*1500*	*1600*	*1700*	*1800*	*1900*	*2000*	*2100*	*100*	*200*	*300*
Neophytadiene extract	100.0											
Hydrocarbon extract (C_{25}–C_{34})	100.0											
Docosane	100.0											
Std. + extract	100.0											
Phytol			45.7	78.9	89.9	96.0	100.0					
Std. + extract			62.5	88.8	95.0	97.6	100.0					
Extract			85.1	90.1	96.2	99.2	100.0					
Docosanol			95.4	100.0								
Std. + extract			96.2	100.0								
Extract			96.7	100.0								
Pentacosanol			98.2	99.8	100.0							
Std. + extract			96.5	100.0								
α-Tocopherol												
Std. + extract			96.2	97.8	98.8	100.0						
β-Amyrin			95.9	98.1	99.1	100.0						
Std. + extract			96.7	98.3	99.2	97.7	100.0					
Extract			95.0	98.4	99.0	99.5	100.0					
Cholesterol								0.6	1.4	97.6	99.4	100.0
Std. + extract									0.4	98.0	99.7	100.0
Extract										98.5	99.3	100.0
Stigmasterol								0.6	1.4	97.4	99.7	100.0
Std. + extract									0.5	98.6	99.7	100.0
Extract										98.0	99.5	100.0
Sitosterol								0.6	1.3	97.7	99.5	100.0
Std. + extract										98.1	99.4	100.0
Extract										98.4	99.5	100.0
Palmitic acid										96.7	99.3	100.0
Std. + extract										96.8	99.5	100.0
Extract										96.6	99.2	100.0
Oleic acid										97.6	99.4	100.0
Std. + extract										97.7	99.5	100.0
Extract										97.6	99.4	100.0
Stearic acid										96.6	99.5	100.0
Std. + extract										96.7	99.5	100.0
Extract										96.4	99.4	100.0
Nervonic acid										98.4	99.5	100.0
Std. + extract										98.9	99.6	100.0

uniform Unisil adsorbent is available in 500-g batches, almost 100 samples could be run on this standardized amount. The procedure is relatively rapid, since in 12 h one person could readily hydrolyze three samples, and extract, separate and prepare the lipids for GC analysis.

TABLE II

RECOVERY OF ^{14}C-LABELED LIPIDS ADDED TO TOBACCO BEFORE BASE HYDROLYSIS

Values given are the percentages of ^{14}C in the silicic acid fraction based on total counts recovered after chromatography of hydrolyzate containing the hexane-soluble compounds.

Eluting solvent volume (ml)	*[^{14}C]Cholesterol*	*[^{14}C]Palmitic acid*	*Cholesterol–[^{14}C]palmitate*	
	2-h Hydrolysis		*2-h Hydrolysis*	*1-h Hydrolysis*
Hexane (0–100)	—	—	—	—
Hexane–benzene (3:1)				
0–1500	—	—	—	1.2
1600–2000	—	—	—	—
Benzene–diethyl ether (3:1)				
0–300	99.7	100.0	100.0	98.8
300–400	0.3	—	—	—
	Total recovery (%) *			
	96.5**	99.5***	97.0***	95.0***

* Based on ^{14}C levels added before base hydrolysis.
** Average of three determinations.
*** One determination.

A typical gas chromatogram of the hydrocarbon components in the non-polar lipid fraction is shown in Fig. 4. The neophytadiene and paraffinic hydrocarbons listed have been previously characterized[4].

The diterpenes, paraffinic alcohols and the triterpenes in the terpene fraction were analyzed as TMS derivatives on a SP-2250 column and a typical chromatogram is shown in Fig. 5. The compounds were confirmed by GC retention data and by GC–mass spectrometric (MS) data. Most of the components in this fraction failed to

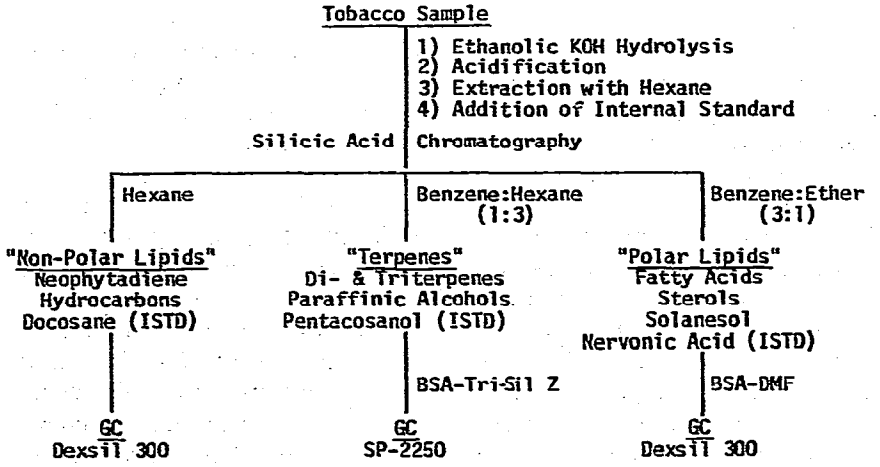

Fig. 3. Chromatographic procedure developed to separate hydrolyzed tobacco lipids.

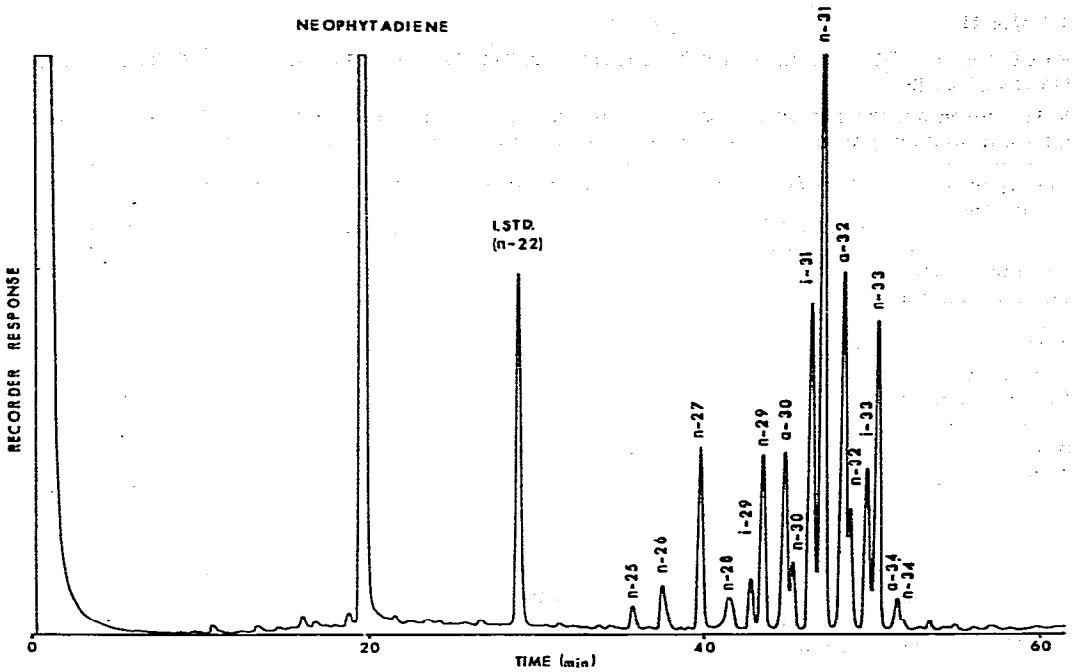

Fig. 4. Gas chromatogram of non-polar lipid fraction, containing neophytadiene and aliphatic hydrocarbons (*n* = normal, *i* = iso, *a* = anteiso).

yield significant molecular ions on mass spectrometry. Therefore, for unambiguous identifications, it was necessary to compare reference GC–MS spectra on the derivatized standards.

The components in the polar lipid fraction were analyzed as TMS derivatives on an 18-in. Dexsil 300 GC column. A typical chromatogram is shown in Fig. 6. This short column rapidly produced quantitative data on the fatty acids, sterols and solanesol. If data on individual fatty acids are required a portion of the polar lipid fraction can be treated with diazomethane and the methyl esters of the acids can be analyzed on a Silar-10C column. Quantitative data on each of the four major tobacco sterols can be obtained using a SP-5250 column. The components in this fraction have now been identified by GC–MS and were previously characterized[7,8].

Data on replicate hydrolyses and GC analyses of selected components in the three silicic acid fractions are given in Tables III–V. Reproducibility was excellent for the components in the non-polar lipid fraction (Table III) and the polar lipid fraction (Table V). The replicate data on the components in the terpene fraction (Table IV) were more variable, the average relative standard deviation being *ca.* 10%.

The method was used to determine lipid levels in various tobaccos grown under different production methods. The Eastern Carolina, South Carolina and NC2326 flue-cured tobaccos were grown according to conventional methods, using about 6000 plants per acre. For these tobaccos, whole leaf material was analyzed. The Coker 139 and Speight G-28 were grown by a more recent tobacco-production method involving close-growing of plants at six times the normal density, or at about

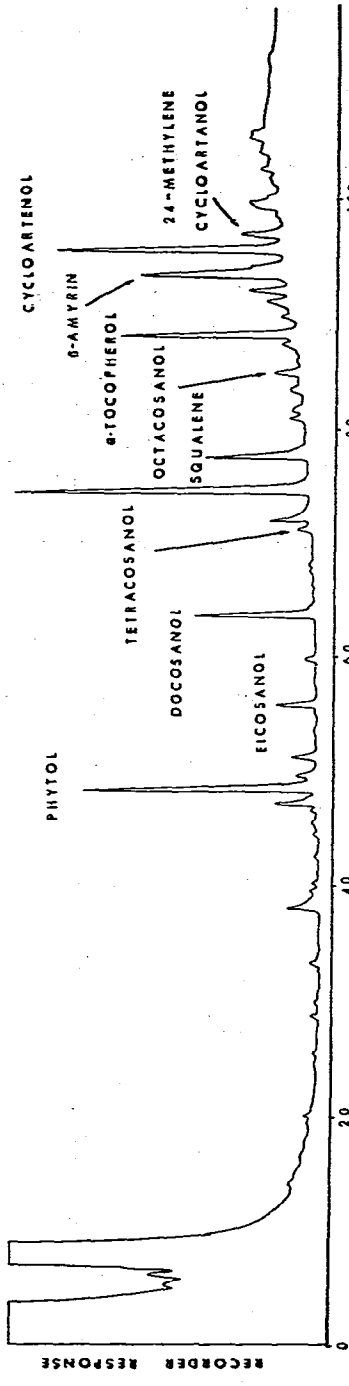

Fig. 5. Gas chromatogram of the TMS derivatives of compounds in the terpene fraction.

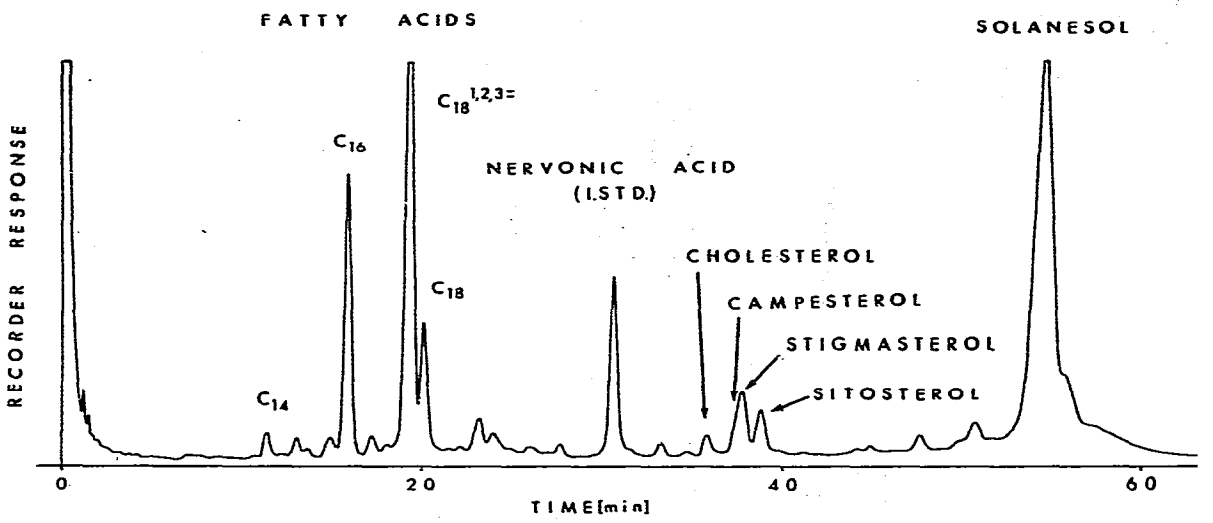

Fig. 6. Gas chromatogram of the TMS derivatives of components in the polar lipid fraction.

TABLE III

REPLICATE ANALYSES OF THE NON-POLAR LIPID FRACTION FROM NC2326 TOBACCO

The relative retention time was with respect to docosane internal standard on a 8-ft. Dexsil 300 column (100–325° at 4°/min).

*Compound**	*Rel. retention time*	*Percent hydrocarbon fraction*** *in run number*			*Mean ± S.D.*
		1	*2*	*3*	
n-C_{25}	1.225	0.96	0.96	0.94	0.95 ± 0.02
n-C_{26}	1.294	0.53	0.42	0.36	0.44 ± 0.09
n-C_{27}	1.357	6.20	6.15	6.20	6.18 ± 0.02
a-C_{28}	1.403	0.40	0.42	0.39	0.40 ± 0.03
n-C_{28}	1.421	0.99	0.85	0.91	0.91 ± 0.07
i-C_{29}	1.460	2.47	2.52	2.48	2.48 ± 0.04
n-C_{29}	1.482	7.54	7.56	7.54	7.55 ± 0.01
a-C_{30}	1.528	7.71	7.75	7.88	7.78 ± 0.09
n-C_{30}	1.543	2.66	2.78	2.66	2.70 ± 0.07
i-C_{31}	1.581	12.78	12.99	12.99	12.92 ± 0.12
n-C_{31}	1.604	25.45	25.45	25.43	25.41 ± 0.06
a-C_{32}	1.645	12.00	12.31	12.28	12.20 ± 0.17
n-C_{32}	1.658	4.13	4.03	4.10	4.09 ± 0.05
i-C_{33}	1.693	5.66	5.42	5.39	5.49 ± 0.15
n-C_{33}	1.714	9.38	9.26	9.41	9.35 ± 0.08
a-C_{34}	1.751	1.01	0.98	0.97	0.99 ± 0.02
n-C_{34}	1.800	0.17	0.17	0.16	0.17 ± 0.01
		*Dry weight of tobacco (%)****			
Neophytadiene	0.693	0.175	0.170	0.176	0.174 ± 0.003
Total hydrocarbons		0.154	0.154	0.154	0.154 ± 0.000

* a = Anteiso-methyl branched, i = iso-methyl branched.
** Calculated assuming unitary detector response.
*** Calculated assuming a detector response identical to that of docosane.

TABLE IV

REPLICATE ANALYSES OF THE TERPENE FRACTION FROM NC2326 TOBACCO

The retention time is relative to that of pentacosanol on an 8-ft. SP-2250 column.

Compound	*Rel. retention time*	*Percent weight of dry leaf* in run number*			*Mean ± S.D.*
		1	*2*	*3*	
Phytol	0.650	0.0132	0.0131	0.0121	0.0128 ± 0.0006
Docosanol	0.851	0.0109	0.0090	0.0086	0.0095 ± 0.0012
Squalene	1.036	0.0101	0.0090	0.0085	0.0092 ± 0.0011
Octacosanol	1.131	0.0033	0.0034	0.0036	0.0034 ± 0.0002
α-Tocopherol	1.177	0.0049	0.0050	0.0038	0.0046 ± 0.0007
β-Amyrin	1.246	0.0258	0.0204	0.0220	0.0227 ± 0.0028
Cycloartenol	1.274	0.0269	0.0209	0.0223	0.0234 ± 0.0031
24-Methylenecycloartanol	1.291	0.0047	0.0045	0.0047	0.0046 ± 0.0001

* Corrected for difference in detector response.

35,000 plants per acre. Whole plants were harvested and cured intact. Thus, the Coker 139 sample consisted of *ca.* 34% leaf and 65% stalk and the Speight G-28 sample consisted of 36% leaf and 60% stalk; the remainders were due to flowers.

As shown in Table VI, all the tobaccos had similar hydrocarbon distributions. However, the total hydrocarbon levels in the close-grown tobacco were *ca.* 50% lower than those in the conventional tobaccos. These data are consistent with our previous observations[11] that the leaves of close-grown tobaccos contained 90% more hydrocarbons than the stalk.

The fatty acid, sterol and paraffinic alcohol contents of the various tobaccos are compared in Table VII. Again, the levels of sterols and docosanol were *ca.* 50% lower for the total close-grown plants than for the leaves of the conventional tobaccos. The sterols were present in all plant parts but were at greatly reduced levels in the stalks and flowers of the close-grown tobaccos[11]. Unlike the levels of sterols and hydrocarbons, fatty acid levels in the close-grown tobaccos did not show the expected

TABLE V

REPLICATE ANALYSES OF THE POLAR LIPID FRACTION FROM NC2326 TOBACCO

Retention times of TMS derivatives, relative to nervonic acid, on an 18-in. Dexsil 300 column (100–325° at 4°/min).

Compound	*Rel. retention time*	*Percent weight of dry leaf* in run number*			*Mean ± S.D.*
		1	*2*	*3*	
Palmitic acid	0.503	0.194	0.193	0.197	0.195 ± 0.002
Oleic, linoleic and linolenic acids	0.595	0.473	0.470	0.478	0.474 ± 0.004
Stearic acid	0.660	0.061	0.064	0.064	0.063 ± 0.002
Cholesterol	1.159	0.019	0.016	0.015	0.017 ± 0.002
Stigmasterol, campesterol	1.220	0.088	0.093	0.087	0.089 ± 0.003
Sitosterol	1.255	0.061	0.063	0.063	0.062 ± 0.001
Solanesol	1.711	1.900	1.970	2.070	1.980 ± 0.080

* Corrected for differences in detector response.

TABLE VI

COMPARISON OF HYDROCARBON LEVELS (%) IN VARIOUS TOBACCOS

Compound	*Conventional flue-cured leaves*			*Cigarette blend*	*Close-grown plants*	
	E. Carolina	*S. Carolina*	*NC2326*		*Coker 139*	*Speight G-28*
n-C_{25}	0.8	1.3	1.0	0.9	1.1	1.1
n-C_{26}	0.4	0.4	0.4	1.5	0.4	0.5
n-C_{27}	7.1	7.4	6.2	6.5	5.4	6.8
a-C_{28}	0.4	0.3	0.4	0.3	0.4	5.3
n-C_{28}	0.9	1.2	0.9	1.2	0.6	0.8
i-C_{29}	2.0	1.8	2.5	1.7	2.6	2.2
n-C_{29}	5.9	6.4	7.6	6.2	5.6	6.4
a-C_{30}	6.6	6.7	7.8	6.8	8.6	8.8
n-C_{30}	2.4	2.9	2.7	2.0	1.7	1.7
i-C_{31}	13.0	12.8	12.9	11.8	13.4	10.8
n-C_{31}	20.0	22.1	25.4	23.2	22.6	20.9
a-C_{32}	13.4	14.0	12.2	15.9	12.8	13.1
n-C_{32}	4.4	4.7	4.1	4.6	4.2	4.1
i-C_{33}	6.6	5.9	5.5	4.5	6.2	5.2
n-C_{33}	9.4	9.6	9.4	11.2	13.5	11.3
a-C_{34}	1.7	1.1	1.0	0.5	0.7	0.7
n-C_{34}	0.2	1.3	0.2	0.1	0.3	0.5
	Percent dry weight of tobacco					
Total hydrocarbons	0.234	0.170	0.154	0.162	0.087	0.093

decrease. Although the fatty acids were distributed throughout the whole tobacco plant, the highest levels occurred in the flowers[11], thus contributing to the total amount seen in the combined extract.

TABLE VII

COMPARISON OF FATTY ACID, STEROL AND PARAFFINIC ALCOHOL LEVELS AS PERCENT DRY WEIGHT OF TOBACCO

	Conventional flue-cured leaves			*Cigarette blend*	*Close-grown plants*	
	E. Carolina	*S. Carolina*	*NC2326*		*Coker 139*	*Speight G-28*
Fatty acids						
C_{16} 1,2,3=*	0.267	0.312	0.195	0.187	0.134	0.174
C_{18}	0.443	0.793	0.474	0.408	0.240	0.436
C_{18}	0.127	0.062	0.063	0.104	0.046	0.054
Sterols						
Cholesterol	0.048	0.016	0.017	0.013	0.009	0.010
Campesterol, stigmasterol	0.180	0.098	0.089	0.056	0.041	0.054
Sitosterol	0.132	0.071	0.062	0.035	0.023	0.027
Alcohols						
Docosanol	0.011	0.012	0.010	0.0089	0.0062	0.0061
Octasanol	0.0073	0.0086	0.0034	0.0019	0.0008	0.0009

* Oleic, linoleic and linolenic acids.

The terpene levels in the various tobaccos are compared in Table VIII. Neophytadiene was a major component in the conventionally grown tobaccos, but was found in very low levels in the close-grown plants. The phytol levels in the close-grown tobaccos were comparable to those in the conventional tobaccos. This observation is consistent with the low levels of neophytadiene in the close-grown tobacco. In the close-growing method, the shaded conditions and clean-cut harvesting conditions do not allow the leaves to undergo their normal yellowing process, by which the bound phytol is biochemically oxidized to neophytadiene. The levels of the triterpenes were about one third of the levels in the close-grown tobaccos. Also, a very low level of the C_{45} isoprenoid, solanesol, was found in the close-grown tobaccos. These observations are indicative of a change in leaf biochemistry due to the shaded and crowded conditions in the close-grown fields. The reduction of sterols and of the high-molecular-weight terpenes in the close-grown tobaccos can be considered desirable, since these compounds have been shown to be potent precursors of the tumorigenic polynuclear aromatic hydrocarbons of cigarette smoke[3].

TABLE VIII

COMPARISON OF TERPENE LEVELS (AS PERCENT DRY WEIGHT OF TOBACCO)

Compound	*Conventional flue-cured leaves*			*Cigarette blend*	*Close-grown plants*	
	E. Carolina	*S. Carolina*	*NC2326*		*Coker 139*	*Speight G-28*
Neophytadiene	0.131	0.134	0.174	0.093	0.006	0.007
Phytol	0.029	0.016	0.013	0.016	0.015	0.019
Squalene	0.0100	0.0094	0.0099	0.0063	0.0037	0.0048
α-Tocopherol	0.0241	0.066	0.0045	0.014	0.0018	0.0019
β-Amyrin	0.0258	0.024	0.023	0.012	0.0047	0.0057
Cycloartenol	0.0425	0.028	0.024	0.017	0.0083	0.011
24-Methylene-cycloartanol	0.0063	0.0051	0.0046	0.0030	0.0020	0.0025
Solanesol	3.11	2.24	1.99	1.05	0.09	0.12

We have described a rapid and accurate method for the determination of tobacco lipids. This procedure can readily be used to screen various tobaccos for their major total lipids and/or to obtain lipid fractions for use in other studies. We believe this chromatographic method could also be applied to these lipids occurring in foods or in natural plant products in our environment.

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