Phytochemistry, 1973, Vol. 12, pp. 835 to 847. Pergamon Press. Printed in England.

VOLATILE ACIDS OF SUN-CURED GREEK *NICOTIANA TABACUM**

B. KIMLAND, A. J. AASEN, S.-O. ALMQVIST, P. ARPINO† and C. R. ENZELL
Chemical Research Department, Swedish Tobacco Co., S-10462 Stockholm 17, Sweden

(*Received* 24 *October* 1972. *Accepted* 13 *November* 1972)

Key Word Index—*Nicotiana tabacum*; Solanaceae; Greek tobacco; volatile acids; organic acids; aromatic acids; terpenoid acids.

Abstract—The volatile acids of sun-cured Greek tobacco have been studied. Examination of this material by GC–MS supplemented by other spectroscopic methods and in some instances by synthesis, has permitted the identification of nearly a hundred compounds. About half of them have not been encountered previously in tobacco or tobacco smoke, and the majority of the new compounds are straight and branched-chain unsaturated acids and aromatic acids. Five of the oxygenated acids are evidently seco- or nor-terpenoids.

INTRODUCTION

THE ORGANIC acids, which comprise 5–8% of the particulate phase of tobacco smoke,[1] have received considerable attention because of their physiological effects and their contribution to the leaf aroma and smoke flavour. Short chain fatty acids have been found to be cilio-toxic,[2] while long chain fatty acids display a tumorpromoting effect when co-painted with smoke condensate on the backs of mice.[3] The importance of organic acids to the organoleptic properties of tobacco smoke is apparent when one considers that they are normally present in appreciable amounts, and many of them, especially the short chain ones, possess a penetratingly acrid and pungent odor. Several have been patented as tobacco additives to improve the tobacco flavour, or even to substitute Turkish tobacco in blended cigarettes, e.g. 3-methylbutanoic acid,[4] 3-methylpentanoic acid[4] and 2-hydroxy-3-methylpentanoic acid.[5]

RESULTS AND DISCUSSION

The volatile acidic fraction of sun-cured Greek tobacco (*Nicotiana tabacum* L.), isolated as recently described,[6] was initially separated into weaker and stronger acids by employing either partition between aqueous bicarbonate and ether or by chromatography on KOH-

---

* Part XVI in the series "Tobacco Chemistry". For Part XV see AASEN, A. J., KIMLAND, B., ALMQVIST, S.-O. and ENZELL, C. R. (1972) *Acta Chem. Scand.* **26**, 2573.

† Present address: Institut de Chimie, Université Louis Pasteur, Strasbourg, France.

[1] BUYSKE, D. A., WILDER, P. and HOBBS, M. E. (1957) *Anal. Chem.* **29**, 105.

[2] WYNDER, E. L. and HOFFMANN, D. (1963) *Deut. Med. Wochschr.* **88**, 623.

[3] WYNDER, E. L. and HOFFMANN, D. (1961) *Cancer* **14**, 1306.

[4] STEDMAN, R. L. and STILLS, C. D. (1965) *U.S. Pat.* 3,180,340, April 27.

[5] ONISHI, I., MORISHITA, I., SAKURAI, K., TAKAHARA, H. and FUKUZUMI, T. (1970) *Jap. Pat. Spec.* 7,032,919, Oct. 23.

[6] KIMLAND, B., AASEN, A. J. and ENZELL, C. R. (1972) *Acta Chem. Scand.* **26**, 2177.

treated silica gel.[7] Each of the two more strongly acidic fractions thus obtained was divided into two portions; one was methylated with diazomethane and the other with methanol containing catalytic amounts of mineral acid. GLC analysis of the four ester fractions showed that these alternative procedures gave almost identical products, except that partial losses of shorter chain ($< C_5$) methyl esters were observed when refluxing methanol was used, and that the anhydride of 2-ethyl-3-methylmaleic acid rather than the dimethyl ester was formed on methylation with diazomethane. In addition to obtaining derivatives that are more readily separated by GLC, a clear advantage in esterifying the acids is that the MS of the methyl esters have been more extensively studied.[8,9]

The methyl esters obtained on methylation with methanol were chromatographed on a silica gel column to give a less polar fraction, which GC–MS analysis showed to contain only alkanoic and alkenoic acids, and a second more polar fraction. The less polar material was separated on a $AgNO_3$-impregnated silica gel column[10] into five fractions, A1–A5, while the more polar was divided into three fractions, B1–B3. All of these fractions were examined by GC–MS and a number of constituents could be identified by comparison of their MS with those of authentic material, and by co-chromatography on capillary columns.

TABLE 1. VOLATILE ACIDS IN GREEK TOBACCO

No.	Compound	Method of identification*	Ref.	Previously detected: Smoke Ref.	Leaf Ref.
Saturated acids					
I	Propanoic	MS, GC	11	1	12
II	*n*-Butanoic	MS, GC	11	1	12
III	*Iso*butanoic	MS†, GC		1	13
IV	*n*-Pentanoic	MS, GC	11	1	13
V	2-Methylbutanoic	MS†, GC			13
VI	3-Methylbutanoic	MS†, GC		14	15
VII	*n*-Hexanoic	MS, GC	11	1	15
VIII	3-Methylpentanoic	MS†, GC		16	13
IX	4-Methylpentanoic	MS†, GC		14	17
X	2,3-Dimethylbutanoic	MS†, GC			
XI	*n*-Heptanoic	MS, GC	11	1	15
XII	4-Methylhexanoic‡	MS			
XIII	5-Methylhexanoic	MS†, GC			
XIV	*n*-Octanoic	MS, GC	11	1	17
XV	Octanoic (branched)	MS			

---

[7] MCCARTHY, R. D. and DUTHIE, A. H. (1962) *J. Lipid Res.* **3**, 117.

[8] RYHAGE, R. and STENHAGEN, E. (1963) in *Mass Spectrometry of Organic Ions* (edited by MCLAFFERTY, F. W.), p. 399, Academic Press, New York; and references therein.

[9] BUDZIKIEWICZ, H., DJERASSI, C. and WILLIAMS, D. H. (1967) *Mass Spectrometry of Organic Compounds*, Holden-Day, San Francisco.

[10] NORIN, T. and WESTFELT, L. (1963) *Acta Chem. Scand.* **17**, 1828.

[11] RYHAGE, R. and STENHAGEN, E. (1959) *Arkiv Kemi* **13**, 523.

[12] CHAKRABORTY, M. K. and WEYBREW, J. A. (1963) *Tob. Sci.* **7**, 122.

[13] KABURAKI, Y. and SATO, Y. (1962) *Nippon Nogeikagaku Kaishi* **36**, 865; *idem.* (1965) *Chem. Abs.* **62**, 6815b.

[14] STEDMAN, R. L., BURDICK, D. and SCHMELTZ, I. (1963) *Tob. Sci.* **7**, 166.

[15] SCHMELTZ, I., MILLER, R. L. and STEDMAN, R. L. (1963) *J. Gas Chromatog.* **1**, 27.

[16] STEDMAN, R. L., BURDICK, D., CHAMBERLAIN, W. J. and SCHMELTZ, I. (1964) *Tob. Sci.* **8**, 79.

[17] SCHMELTZ, I., STEDMAN, R. L. and MILLER, R. L. (1963) *J. Assoc. Offic. Agric. Chem.* **46**, 779.

TABLE 1—*continued*

				Previously detected:	
No.	Compound	Method of identification*	Ref.	Smoke Ref.	Leaf Ref.
Saturated acids—continued					
XVI	*n*-Nonanoic	MS, GC	11	1	17
XVII	2-Methyloctanoic	MS			
XVIII	*n*-Decanoic	MS, GC	11	1	18
XIX	7-Methylnonanoic	MS			
XX	*n*-Undecanoic	MS, GC	11	19	20
XXI	8-Methyldecanoic	MS			
XXII	*n*-Dodecanoic	MS, GC	11	21	22
XXIII	*n*-Tridecanoic	MS, GC	11		
XXIV	*n*-Tetradecanoic	MS, GC	11	21	12
XXV	Tetradecanoic (branched)	MS			
XXVI	*n*-Pentadecanoic	MS, GC	11	23	20
XXVII	12-Methyltetradecanoic	MS		24	
XXVIII	*n*-Hexadecanoic	MS, GC	11	21	12
XXIX	Hexadecanoic (branched)	MS			
XXX	*n*-Heptadecanoic	MS, GC	11	23	20
XXXI	2-Methylhexadecanoic	MS†, GC			
XXXII	Heptadecanoic (branched)	MS			
XXXIII	*n*-Octadecanoic	MS, GC	11	21	25
Monoenoic acids					
XXXIV	2*E*-Butenoic	MS†, GC§			13
XXXV	Pentenoic (two isomers)‖	MS			
XXXVI	2*E*-Hexenoic	MS†, GC§			26¶
XXXVII	3*Z*-Hexenoic	MS, IR, NMR			
XXXVIII	4ζ-Hexenoic	MS	27		
XXXIX	3-Methyl-3*E*-pentenoic	MS, NMR			
XL	3-Methyl-3*Z*-pentenoic	MS			
XLI	Hexenoic (two isomers)‖	MS			
XLII	2*E*-Heptenoic	MS†, GC§			26¶
XLIII	4*Z*-Heptenoic	MS, IR, NMR			
XLIV	5-Methyl-2ζ-hexenoic‡,**	MS	28		
XLV	Heptenoic (five isomers)‖	MS			
XLVI	5*Z*-Octenoic	MS, IR, NMR			
XLVII	7-Octenoic	MS, NMR			
XLVIII	Octenoic (three isomers)‖	MS			
XLIX	3*Z*-Nonenoic‡,**	MS, NMR			
L	6*Z*-Nonenoic	MS, IR, NMR			

---

[18] Ivanov, N. and Ognyanov, I. (1965) *Compt. Rend. Acad. Bulgare Sci.* **18,** 1015; *idem.* (1966) *Chem. Abs.* **64,** 11566c.

[19] Moknachev, I. G. and Astakhova, L. G. (1968) *Tabak* **3,** 31.

[20] Mold, J. D., Means, R. E. and Ruth, J. M. (1966) *Phytochem.* **5,** 59.

[21] Clemo, G. R. (1958) *Tetrahedron* **3,** 168.

[22] Onishi, I. and Yamamoto, K. (1957) *Bull. Agric. Chem. Soc. Japan* **21,** 90.

[23] van Duuren, B. L. and Kosak, A. I. (1958) *J. Org. Chem.* **23,** 473.

[24] Rodgman, A. and Cook, L. C. (1969) *22nd Tob. Chem. Res. Conf., Richmond, Oct.* 1968; *idem.* (1969) *Coresta* 65.

[25] Swain, A. P. and Stedman, R. L. (1962) *J. Assoc. Offic. Agric. Chem.* **45,** 536.

[26] Creasy, P. J. and Saxby, M. J. (1969) *Phytochem.* **8,** 2427.

[27] Rohwedder, W. K., Mabrouk, A. F. and Selke, E. (1965) *J. Phys. Chem.* **69,** 1711.

[28] McFadden, W. H. and Buttery, R. G. (1970) in *Topics in Organic Mass Spectrometry* (edited by Burlingame, A. L.) Wiley-Interscience, New York.

TABLE 1—*continued*

No.	Compound	Method of identification*	Ref.	Previously detected: Smoke Ref.	Leaf Ref.
Monoenoic acids—continued					
LI	Decenoic (three isomers)‖	MS			
LII	Hexadecenoic‖	MS, GC††	29	21	
LIII	Heptadecenoic‖	MS	29		
LIV	Octadecenoic‖	MS, GC‡‡	29	21	25
Dienoic acids					
LV	2ζ,4ζ-Hexadienoic	MS, GC§	27	30	
LVI	Octadienoic (branched)‖	MS			
LVII	2*E*,6*Z*-Nonadienoic‡	MS, UV, IR, NMR			
LVIII	Nonadienoic (two isomers)‖	MS			
LIX	3,7-Dimethyl-2*E*,6-octadienoic‡	MS, GC§	31		
LX	Hexadecadienoic‖	MS	29		
LXI	Octadecadienoic‖	MS, GC§§	29	21	25
Trienoic acids					
LXII	Tridecatrienoic‖	MS	29		
LXIII	Tetradecatrienoic‖	MS	29		
LXIV	Hexadecatrienoic‖	MS	29		
LXV	Octadecatrienoic‖	MS, GC‖‖	29		
Aromatic acids					
LXVI	Benzoic	MS, GC, IR, NMR	32	1	33
LXVII	*m*-Methylbenzoic	MS, GC	32	21	
LXVIII	Phenylacetic	MS, GC, IR, NMR	34	30	13
LXIX	3-Phenylpropanoic	MS, GC	35		
LXX	*trans*-Cinnamic	MS, GC	32		
LXXI	*cis*-Cinnamic	MS			
LXXII	4-Phenyl-3ζ-butenoic	MS†, GC§			
LXXIII	Salicylic	MS, GC	32	36	
Oxygenated, aliphatic					
LXXIV	2ζ-Hydroxy-3ζ-methylpentanoic	MS, IR, NMR	37		37
LXXV	4ζ-Hydroxy-3ζ-methyl-pentanoic**,¶¶	MS, GC, NMR	38, 39		
LXXVI	5-Methyl-4-oxohexanoic	MS, GC, IR, NMR			
LXXVII	2*S*-Isopropyl-5-oxohexanoic	MS, IR, NMR			40

---

[29] HALLGREN, B., RYHAGE, R. and STENHAGEN, E. (1959) *Acta Chem. Scand.* **13**, 845.

[30] OSMAN, S. and BARSON, J. (1966) *Phytochem.* **5**, 511.

[31] THOMAS, A. F., WILLHALM, B. and MÜLLER, R. (1969) *Org. Mass Spectrom.* **2**, 223.

[32] *Dow Uncertified Mass Spectral Data* (edited by GOHLKE, R. S.), Framington, Mass. (1963).

[33] FUKUZUMI, T., TAKAHARA, H., KANEKO, H. and ONISHI, I. (1965) *Nippon Nogeikagaku Kaishi* **39**, 199; *idem.* (1965) *Chem. Abs.* **63**, 15234c.

[34] McCLOSKEY, J. A., LEEMANS, R. A. J. M. and PROCHASKA, P. O. (1970) *Arch. Mass Spect. Data* **1**, 44.

[35] McCLOSKEY, J. A., LEEMANS, R. A. J. M. and PROCHASKA, P. O. (1970) *Arch. Mass Spect. Data* **1**, 96.

[36] STECK, W., YANG, C.-H. and WENDER, S. H. (1965) *Chem. & Ind.* 560.

[37] FUKUZUMI, T., TAKAHARA, H. and KANEKO, H. (1966) *Agric. Biol. Chem.* **30**, 513.

[38] HONKANEN, E., MOISIO, T. and KARVONEN, P. (1969) *Acta Chem. Scand.* **23**, 531.

[39] LAPORTE, J.-F. and RAMBAUD, R. (1966) *Compt. Rend.* **262**, 1095; see also: Note added in proof.

[40] FUKUZUMI, T., KANEKO, H. and TAKAHARA, H. (1967) *Agric. Biol. Chem.* **31**, 607.

TABLE 1—*continued*

No.	Compound	Method of identification*	Ref.	Previously detected: Smoke Ref.	Previously detected: Leaf Ref.
Oxygenated, aliphatic—continued					
LXXVIII	4-Oxononanoic‡,**	MS, GC, IR, NMR			
LXXIX	3-Isopropyl-6-oxo-2*E*-heptenoic	MS, UV, IR, NMR			
LXXX	3ζ-Isopropyl-6-oxo-4*E*-heptenoic	MS, IR, NMR			
LXXXI	3ζ-Hydroxy-3ζ-methyl-6ζ-isopropyl-4*E*-octenoic	MS, IR, NMR			
Others					
LXXXII	2-Furoic	MS, GC	41	42	13
LXXXIII	3,4-Dihydroxybenzaldehyde***	MS†, GC		43	44
LXXXIV	2-Ethyl-3-methylmaleic‡,**,†††	MS, IR, NMR	45		
LXXXV	2-Hydroxy-3,5,5-trimethyl-1,4-cyclohexadion-2-ene**,‡‡‡	MS, IR, NMR			

* Identified as the methyl ester unless stated otherwise. 'GC' = inseparable when co-injected with authentic material on a capillary column. † MS compared with that of commercially available material. ‡ Independently identified in leaf by Demole *et al.*[46] § Identical retention time with that of the *trans*-isomer. ‖ Positions and configurations of double bonds are undetermined. ¶ Latakia tobacco (fumigated). ** Independently identified in leaf by Roberts *et al.*[47] †† Inseparable from methyl palmitoleate. ‡‡ Inseparable from methyl oleate. §§ Inseparable from methyl linoleate. ‖‖ Inseparable from methyl linolenoate. ¶¶ Identified as the *cis*-lactone.[39] *** Identified as the dimethyl ether. ††† Identified both as the dimethyl ester and the anhydride. ‡‡‡ Identified as the methyl ether.

Since several of the major components of fractions A3, A5, B1, B2 and B3 could not be identified on the basis of their MS alone, they were isolated by preparative GLC and their structures subsequently elucidated by means of additional spectral data. The compounds identified, including the anhydride 2-ethyl-3-methylmaleic anhydride isolated from the polar fraction of the diazomethane-methylated acids, are compiled in Table 1 and gas chromatograms showing their relative concentrations are reproduced in Fig. 1. Most of the spectral data including selected NMR assignments for each of the isolated compounds is given in the Experimental, and only data related to more subtle structural features, e.g. NOE—are discussed below.

Saturated Acids

The majority of the saturated acids listed in Table 1 are previously known constituents of tobacco and tobacco smoke, and they account for the larger part of the acidic material of the present tobacco. Compounds XII, XIX, XXI and XXVII were assigned the *anteiso*-structures on the basis of the MS of their methyl esters, which are closely similar to those of the corresponding straight chain compounds except for displaying more abundant

---

[41] *Eight Peak Index of Mass Spectra*, Mass Spectrometry Data Centre, Awre, Aldermaston.

[42] SCHMELTZ, I. and SCHLOTZHAUER, W. S. (1962) *Tob. Sci.* **6**, 90.

[43] YANG, C.-H. and WENDER, S. H. (1962) *Tob. Sci.* **6**, 158.

[44] YANG, C.-H. and WENDER, S. H. (1964) *Phytochem.* **3**, 17.

[45] STOLL, M., WINTER, M., GAUTSCHI, F., FLAMENT, I. and WILLHALM, B. (1967) *Helv. Chim. Acta* **50**, 628.

[46] DEMOLE, E. and BERTHET, D. (1972) *Helv. Chim. Acta* **55**, 1866, 1898.

[47] ROBERTS, D. L. and ROHDE, W. A. (1972) *Tob. Sci.* **16**, 107.

M – 29, M – 57 and M – (29 + 32) ions.[48] Methyl esters carrying a methyl substituent in position 2, 3 or 4 also exhibit M – 57 ions caused by the expulsion of the C(2)–C(4) part of the chain, but their lower mass regions differ significantly from those of the present compounds.[49] The MS of XV, XXV, XXIX and XXXII resemble those of the corresponding straight chain esters, but these compounds display shorter retention times suggesting branching which, however, could not be established from the MS alone.

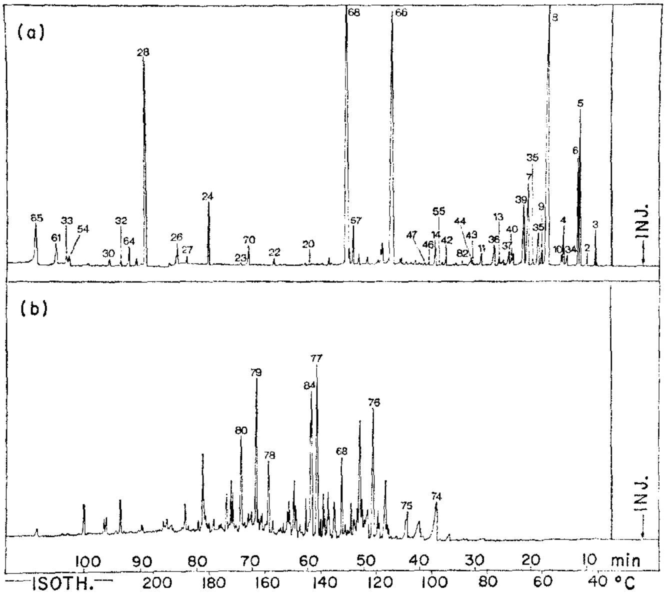


Fig. 1. Gas chromatograms of methylated volatile acids of Greek tobacco. (a) Total nonpolar methyl esters (CH_2N_2); (b) total polar methyl esters (CH_3OH/H^+).

Peak numbers correspond to the Roman numerals in Table 1.

Unsaturated, Aliphatic Acids

It is well known that double bond positions, and configurations in unsaturated aliphatic esters possessing more than seven carbon atoms in the chain, usually cannot be determined exclusively by MS.[8] However, normal α,β-unsaturated, long-chain methyl esters constitute a favourable case in that they exhibit a characteristic m/e 113 ion which is associated with 5,6-cleavage.[8] High field NMR spectroscopy and the use of shift reagents[50] have recently proved successful in the analysis of unsaturated, long-chain fatty acid methyl esters[51–53] and triglycerides.[54,55] Discrimination between *cis* and *trans* configuration of disubstituted ethylenes is usually possible on the basis of the magnitude of the vicinal olefinic spin–spin coupling constants.[56] Even when this constant cannot be obtained easily from the spectrum,

---

[48] Ryhage, R. and Stenhagen, E. (1960) *Arkiv. Kemi* **15**, 291.

[49] Nguyen Dinh-Nguyen (1968) *Arkiv Kemi* **28**, 289.

[50] Hinckley, C. C. (1969) *J. Am. Chem. Soc.* **91**, 5160.

[51] Frost, D. J. and Barzilay, J. (1971) *Analyt. Chem.* **43**, 1316.

[52] Wineburg, J. P. and Swern, D. (1972) *J. Am. Oil Chem. Soc.* **49**, 267.

[53] Boudreaux, G. J., Bailey, A. V. and Tripp, V. W. (1972) *J. Am. Oil Chem. Soc.* **49**, 200.

[54] Pfeffer, P. E. and Rothbart, H. L. (1972) *Tetrahedron Letters* 2533.

[55] Almqvist, S.-O., Andersson, R., Shahab, J. and Olsson, K. (1972) *Acta Chem. Scand.* **26**, 3378.

[56] Jackman, J. M. and Sternhell, S. (1969) *Applications of Nuclear Magnetic Resonance Spectroscopy*, Pergamon Press, Oxford.

the induced shift ratios[52] can provide information about the configuration. Thus, in *cis* compounds, the olefinic proton most distant from the coordination point, and the allylic methylene group adjacent to it, generally display similar induced shift ratios, while in *trans* compounds this olefinic proton is shifted much more. The nuclear Overhauser effect has been found valuable for the determination of the stereochemistry of tri- and tetra-substituted ethylenes.[57] Additional information on the substitution and configuration of the double bonds of unsaturated esters was also obtained from their IR spectra.[58]

The straight chain hexenoates XXXVI, XXXVII and XXXVIII were readily identified since the published MS of the four positional isomers display noticeable differences.[27] The presence of a vinylic ethyl group in one of these, 3*Z*-hexenoate (XXXVII), as well as in 4*Z*-heptenoate (XLIII), 5*Z*-octenoate (XLVI), 6*Z*-nonenoate (L) and 2*E*,6*Z*-nonadienoate (LVII) was apparent from the chemical shift[56] of the methyl group (triplet at δ *ca.* 0·95). IR absorption in the 700–730 cm^{-1} region[58] and vicinal spin–spin coupling constants[56] ($J_{CH=CH}$ *ca.* 10 Hz), which could be measured after addition of a shift reagent[50] and spin decoupling experiments, strongly suggested a *cis* configuration for this double bond. One of these compounds, LVII, also contained a conjugated, *trans* disubstituted double bond, evident from its spectral data (976 cm^{-1}, 206 nm, $J_{CH=CH}$ 16 Hz).

The methyl esters of 7-octenoic (XLVII) and 5*Z*-octenoic acid (XLVI) were isolated as a mixture, as were the esters of 3*Z*-nonenoic (XLIX) and 6*Z*-nonenoic acid (L). Tentative structural assignments could, however, be made on the basis of the MS of the pure compounds (GC–MS), which suggested two pairs of positional isomers, and from the NMR and IR spectra of the mixtures. The NMR signals of XLIX were readily obtained after subtracting the spectrum of L, a pure sample of which had been isolated. Characteristic multiplets[59] at δ 4·92, δ 4·96 and δ 5·79 indicated the presence of the R–CH=CH_2 group in 7-octenoate (XLVII), while the chemical shift of the doublet at δ 3·05 in the spectrum of the nonenoates suggested that the C(2)H_2 group was adjacent to both the double bond and the ester function. Addition of shift reagent and spin decoupling experiments established the configuration of the double bond of XLIX as *cis*.

The structure of methyl 3-methyl-3*E*-pentenoate (XXXIX) followed from its NMR and MS. The *trans* configuration of the double bond was established on the basis of a positive nuclear Overhauser effect[57] observed in the integral of the olefinic proton when the methylene group was irradiated. The corresponding *cis* isomer (XL) was also present as judged by the fact that it gave a MS almost identical to that of the *trans* compound.

The presence of the monoterpene acid 3,7-dimethyl-2*E*,6-octadienoic acid (LIX) was obvious since its methyl ester was identical (MS, co-chromatography) to authentic material prepared from geraniol.[60]

Of the methyl esters of the straight chain monoenoic (LII and LIV), dienoic (LX and LXI) and trienoic acids (LXIII, LXIV and LXV), four (LII, LIV, LXI and LXV) exhibited identical MS and identical retention times (co-injection) to the esters of palmitoleic, oleic, linoleic, and linolenic acids respectively and are hence obviously identical to these ubiquitous compounds.

Only GC–MS data was obtained for two pentenoates (XXXV), two hexenoates (XLI,

---

[57] Noggle, J. H. and Schirmer, R. E. (1971) *The Nuclear Overhauser Effect—Chemical Applications*, Academic Press, New York.

[58] Nakanishi, K. (1964) *Infrared Absorption Spectroscopy—Practical*, Nankodo, Tokyo.

[59] Wenkert, E. and Beak, P. (1961) *Chem. & Ind.* 1574.

[60] de Haan, J. W. and van de Ven, L. J. M. (1971) *Tetrahedron Letters* 2703.

probably branched), five heptenoates (XLV), three octenoates (XLVIII), three decenoates (LI) and two nonadienoates (LVIII). They were present in too low a concentration for isolation.

Aromatic Acids

The structure of methyl *m*-methylbenzoate (LXVII) followed from its MS and from the fact that this compound and the *p*-isomer, which exhibits a nearly identical MS, could easily be separated by GLC. The presence of *cis*-cinnamic acid (LXXI) was indicated by the MS of its methyl ester which was nearly identical to that of methyl *trans*-cinnamate (LXX).

Oxygenated, Aliphatic Acids

The presence of 4ζ-hydroxy-3ζ-methylpentanoic (LXXV), 5-methyl-4-oxohexanoic (LXXVI) and 4-oxononanoic acid (LXXVIII) was established by direct comparison of the lactone of LXXV and the methyl esters of LXXVI and LXXVIII with authentic material. The attachment of the isopropyl group to position 2 rather than to position 4 in the 5-oxohexanoate derivative LXXVII was demonstrated by decoupling experiments: a small long-range coupling between the $C(4)H_2$ and the $C(6)H_3$ groups, i.e. 'isopropylidenic coupling',[61] confirmed the structure methyl 2-isopropyl-5-oxohexanoate. Its optical activity corresponds to the *S*-isomer which has been found previously in tobacco leaf by Fukuzumi *et al.*[40] In the conjugated keto acid 3-isopropyl-6-oxo-2*E*-heptenoic acid (LXXIX), the configuration of the double bond was established as *E*, since a positive nuclear Overhauser effect[57] could be observed for the olefinic proton signal when the methyl groups of the isopropyl group were irradiated.

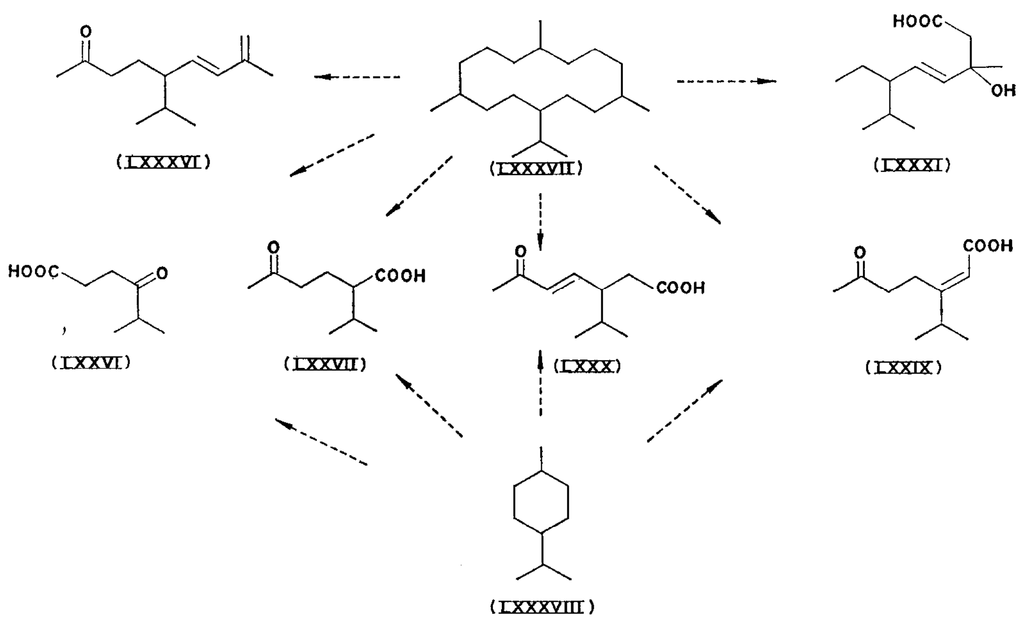
Although the molecular ion in the MS of methyl 3ζ-hydroxy-3ζ-methyl-6ζ-isopropyl-4*E*-octenoate (LXXXI) was not visible, the structure was determined from the IR and NMR data and from accurate mass measurements of the M − 18 ion ($C_{13}H_{22}O_2$). The configuration of the double bond, which could only be deduced after addition of shift reagent,[50] was shown to be *trans* ($J_{CH=CH}$ 16 Hz). IR absorption at 978 cm^{-1} confirmed this assignment. Extrapolation of LIS-data to zero concentration of added shift reagent indicated a chemical shift for the allylic proton C(6)H of δ 1·58, which is consistent with the attachment of an isopropyl group to a vinylic carbon atom.[62,63] The mutual spin–spin coupling between the olefinic C(5)-proton and the allylic C(6)-proton was demonstrated by decoupling experiments.

Several of the acids, namely 5-methyl-4-oxohexanoic (LXXVI), 2*S*-isopropyl-5-oxohexanoic (LXXVII), 3-isopropyl-6-oxo-2*E*-heptenoic (LXXIX), 3ζ-isopropyl-6-oxo-4*E*-heptenoic (LXXX) and 3ζ-hydroxy-3ζ-methyl-6ζ-isopropyl-4*E*-octenoic acid (LXXXI), are of special interest since they evidently are nor- or seco-terpenoids. These acids, like many other tobacco constituents such as solanone (LXXXVI), solanol,[47] and norsolanadione,[47,64] may therefore be regarded as nor-derivatives of diterpenoids possessing the thunbergane skeleton (LXXXVII), (see Scheme 1). Although this view is strengthened by

---

[61] BARFIELD, M. and CHAKRABARTI, B. (1969) *Chem. Revs.* **69**, 757.
[62] BOTHNER-BY, A. A., NAAR-COLIN, C. and GÜNTHER, G. (1962) *J. Am. Chem. Soc.* **84**, 2784.
[63] RUMMENS, F. H. A. (1972) *J. Magn. Res.* **6**, 550.
[64] KIMLAND, B., AASEN, A. J. and ENZELL, C. R. To be published.

the presence of a number of such macrocylic diterpenoids in tobacco,[47,65,66] it should be noted that four of them, LXXVI, LXXVII, LXXIX and LXXX may equally well be viewed as nor- or seco-monoterpenoids of the *p*-menthane series (LXXXVIII).



Others

3,4-Dihydroxybenzaldehyde (LXXXIII) and 2-hydroxy-3,5,5-trimethyl-1,4-cyclohexadion-2-ene (LXXXV) were identified as the corresponding methyl ethers after methylation. Although the present evidence does not preclude that the monomethyl ether of LXXXIII is the tobacco constituent rather than the diol (LXXXIII), the latter is preferred since it occurs in the strongly acidic fraction and has previously been detected in both tobacco leaves[44] and tobacco smoke.[43] 2-Ethyl-3-methylmaleic acid (LXXXIV) was isolated as the dimethyl ester when employing methanol, and as the anhydride when using diazomethane as the methylating agent. Since both acid and base were used in the isolation procedure, it is not clear whether the free acid or the anhydride, or both, are present in the tobacco. The same situation applies to 4ζ-hydroxy-3ζ-methyl-pentanoic acid (LXXV) and the corresponding lactone.

EXPERIMENTAL

Materials and methods. The solvents, silica gel, and drying agents were purified as previously described.[67] NMR spectra were recorded on a Varian A60-A instrument linked to a C-1024 CAT, and a Varian HA-100D attached to a Varian Fourier Transform 100 unit using $CDCl_3$ as solvent and TMS as internal reference. IR spectra were measured as films on a Digilab FTS-14 instrument. UV spectra were recorded using EtOH as solvent. Rotations were determined on a Perkin–Elmer 141 polarimeter. Accurate mass determinations were carried out at the Laboratory for Mass Spectrometry, Karolinska Institutet, Stockholm. GC–MS was

---

[65] Rowland, R. L. and Roberts, D. L. (1963) *J. Org. Chem.* **28,** 1165.

[66] Rowland, R. L., Rodgman, A., Schumacher, J. N., Roberts, D. L., Cook, L. S. and Walker, Jr., W. E. (1964) *J. Org. Chem.* **29,** 16.

[67] Appleton, R. A., Enzell, C. R. and Kimland, B. (1970) *Beitr. Tabakforsch.* **5,** 266.

carried out on a modified[68] LKB 9000 instrument operated at 70 eV. The capillary columns (50 m × 0·25 mm) used in the present study were made by coating stainless steel tubes with Emulphor or glass tubes with Ucon Oil HB 2000, using the dynamic packing method.[69] Analytical (capillary) and preparative GC was performed on a Varian 1700 instrument fitted with both a capillary and a regular injection block and a flame ionization detector. The preparative separations were carried out on a 3 m × 3·2 mm glass column packed with 5% Carbowax 20 M on Chromosorb G and the fractions were collected at −70° in U-shaped teflon tubes (i.d. 5 mm) equipped with an electrostatic precipitator.[70]

Isolation. The isolation of 55 g volatile acidic material from 295 kg sun-cured Greek tobacco (*Nicotiana tabacum* L.) has been described previously.[6] The phenols could be removed from the acidic material by either of two methods utilizing their weekly acidic properties. *Method* 1. A portion of the acidic material (1·4 g) was dissolved in Et_2O and extracted with saturated aq. $NaHCO_3$. The aqueous phase was acidified and extracted with Et_2O. Removal of the solvent by distillation at atm. pressure through a Vigreux column (these distillation conditions were used throughout this study; the wts are not accurate because solvents were not completely removed to avoid loss of very volatile material) furnished a fraction (1·1 g) containing the stronger acids. Distillation of the epiphasic solution gave a phenolic fraction (0·17 g; 15%), which has not been investigated further. *Method* 2. A portion of the acidic material (5·4 g), dissolved in a small quantity of Et_2O, was placed on a silica gel column (50 g) which had been pretreated with isopropanol–KOH as detailed by McCarthy *et al.*[7] The weakly acidic material was washed out with Et_2O to yield a phenolic fraction (0·67 g; 17%), and subsequent elution with 2% HCO_2H in Et_2O gave a more strongly acidic fraction (contaminated with some HCO_2H). Each of the two more strongly acidic fractions thus obtained were divided into two portions; one was methylated in boiling MeOH solution containing a catalytic amount of H_2SO_4 (5 hr reflux), and the other by treatment with fresh CH_2N_2 in Et_2O. In the first case the methyl esters were isolated by Et_2O-extraction of the H_2O-diluted reaction mixture, whilst removal of the solvent and some methyl formate by distillation was sufficient in method 2. The methyl ester fractions were subsequently studied by GC–MS. Volatile acidic material (13·5 g) was separated, according to method 1, into a phenolic fraction (1·7 g), and a more acidic fraction (11·3 g) which was methylated (CH_3OH/H^+) and separated on a silica gel column (800 g) into two fractions by eluting with 5% Et_2O in pentane (8 l.) and Et_2O (10 l.) respectively. The less polar esters (8·3 g) were rechromatographed on a silica gel column impregnated with $AgNO_3$[10] to give five fractions (A1–A5; wts 3·7, 2·1, 0·7, 0·5 and 0·5 g respectively). The more polar esters (1·1 g) were separated on a silica gel column into three fractions (B1–B3; wts: 0·15, 0·20 and 0·72 g respectively). All fractions were subsequently studied by GC–MS. When authentic material was available the identifications were confirmed by recording their MS under identical conditions, and by co-chromatography on capillary columns. A number of the larger components of fractions A3, A5, B1, B2 and B3 were isolated by preparative GLC; their spectral data are given below. Half of the acidic material obtained according to method 2, which had been methylated with CH_2N_2, was similarly separated on a silica gel column into a less polar (2·1 g) and a more polar fraction (0·25 g). 2-Ethyl-3-methylmaleic anhydride was isolated from the latter fraction by preparative GLC.

Spectral data and syntheses. In the cases where the shift reagent Eu $(DPM)_3$ was used, relative induced chemical shifts are given (*r*).[52] MS data not readily available in the literature for some of the compounds are given below. The following abbreviations are used for describing NMR data: *s*—singlet; *d*—doublet; *t*—triplet; *q*—quartet; *qn*—quintet; *m*—multiplet. *Methyl* 2,3-*dimethylbutanoate* (X). MS: 130 = M^+ (0·5), 88 (100), 43 (35), 57 (30), 59 (22), 71 (20), 41 (14), 56 (13). *Methyl* 4-*methylhexanoate* (XII). MS: M^+ at *m/e* 144 not seen, 74 (100), 87 (85), 55 (59), 43 (53), 41 (46), 57 (42), 71 (37), 88 (36). *Methyl* 5-*methylhexanoate* (XIII). MS: M^+ at *m/e* 144 not seen, 74 (100), 43 (39), 87 (23), 101 (22), 59 (22), 41 (22), 69 (22), 55 (12). *Methyl* 2-*methyloctanoate* (XVII). MS: 172 = M^+ (3), 88 (100), 101 (45), 41 (25), 43 (25), 57 (25), 55 (20), 69 (16), 59 (13). *Methyl* 7-*methylnonanoate* (XIX). MS: M^+ at *m/e* 186 not seen, 74 (100), 87 (76), 55 (41), 41 (33), 43 (26), 57 (21), 69 (19), 83 (19), 97 (19). *Methyl* 8-*methyldecanoate* (XXI). MS: M^+ at *m/e* 200 not seen, 74 (100), 87 (60), 55 (48), 41 (41), 69 (35), 143 (34), 43 (33), 57 (26). *Methyl* 12-*methyltetradecanoate* (XXVII). MS: 256 = M^+ (9), 74 (100), 87 (83), 43 (40), 55 (39), 41 (37), 57 (36), 69 (24), 75 (21). *Methyl* 2-*methylhexadecanoate* (XXXI). MS: 284 = M^+ (10), 88 (100), 101 (37), 43 (17), 55 (13), 57 (12), 41 (12), 69 (11).

Methyl 3z-*hexenoate* (XXXVII), isolated from fraction A5. MS: 128 = M^+ (27), 41 (100), 74 (71), 68 (70), 69 (60), 59 (37), 55 (23), 43 (21), 71 (16); IR: 1740 (s), 1436 (m), 1331 (m), 1258 (m), 1191 (m), 1165 (s), 1116 (w), 1020 (m), 700 (m) cm^{-1}; NMR: δ 0·98 ($C(6)H_3$, *t*, *J* 7·5 Hz), δ 2·05 ($C(5)H_2$, *qn*, *J ca.* 7·5 Hz), δ 3·09 ($C(2)H_2$, *d*, *J ca.* 6 Hz), δ 3·68 (OMe, *s*), δ 5·54 (C(3)H and C(4)H, *m*). Addition of $Eu(DPM)_3$: *r* = 1 ($C(2)H_2$, *d*), *r* = 0·69 (C(3)H, *m*), *r* = 0·18 (C(4)H, *m*), *r* = 0·24 ($C(5)H_2$, *qn*), *r* = 0·01 ($C(6)H_3$, *t*), *r* = 0·92 (OMe, *s*). Irradiation at *r* = 1 effected simplification of the multiplet at *r* = 0·69 to a doublet with 10 Hz.

[68] ROERAADE, J. and ENZELL, C. R. (1969) *Acta Chem. Scand.* **22**, 2380.

[69] DIJKSTRA, G. and DE GOEY, J. (1958) in *Gas Chromatography* (edited by DESTY, D. H.), p. 56, Butterworths, London.

[70] KRATZ, P., JACOBS, M. and MITZNER, B. M. (1959) *Analyst* **84**, 671.

Methyl 3-*methyl*-3E-*pentenoate* (XXXIX), isolated from fraction A3, MS: 128 = M$^+$ (54), 41 (100), 69 (86), 68 (59), 96 (38), 59 (36), 67 (22), 97 (18); NMR: δ 1·62 (C(5)H$_3$, *m*, *J ca.* 1·5 Hz and *J* 6·5 Hz), δ 1·76 (C(3)Me, *qn*, *J* 1·5 Hz), δ 3·05 (C(2)H$_2$, *s*), δ 3·66 (OMe, *s*), δ 5·43 (C(4)H, *q* with further splittings, *J* 1·5 Hz and *J* 6·5 Hz). Irradiation at δ 1·76 and δ 3·05 respectively resulted in zero and 20% enhancement of the NMR-signal of the olefinic proton (δ 5·43).

Methyl 3-*methyl*-3Z-*pentenoate* (XL), MS: 128 = M$^+$ (40), 41 (100), 69 (87), 68 (53), 96 (36), 59 (32), 67 (18), 43 (17).

Methyl 4Z-*heptenoate* (XLIII), isolated from fraction A5. MS: 142 = M$^+$ (10), 74 (100), 41 (62), 43 (50), 68 (46), 69 (40), 55 (40), 110 (32), 67 (31); IR: 1740 (s), 1438 (s), 1360 (m), 1169 (s), 1117 (m), 992 (w), 915 (w), 889 (w), 844 (w), 725 (m) cm^{-1}; NMR: δ 0·95 (C(7)H$_3$, *t*, *J* 7·5 Hz), δ 2·06 (C(6)H$_2$, *qn*, *J ca.* 7·5 Hz), δ 2·34 (C(2)H$_2$ and C(3)H$_2$, *m*), δ 3·65 (OMe, *s*), δ 5·34 (C(4)H and C(5)H, *m*). Addition of Eu(DPM)$_3$: *r* = 1 (C(2)H$_2$, *t*, *J ca.* 7·5 Hz), *r* = 0·71 (C(3)H$_2$, *q*, *J ca.* 7 Hz), *r* = 0·36 (C(4)H, *q* with further splittings, *J ca.* 9 Hz), *r* = 0·17 (C(5)H, *m*), *r* = 0·14 (C(6)H$_2$, *qn*, *J ca.* 7·5 Hz), *r* = 0·05 (C(7)H$_3$, *t*, *J* 7·5 Hz), *r* = 0·95 (OMe, *s*). Irradiation at *r* = 0·71 removed a coupling in the signal at *r* = 0·36 giving a doublet with *J* 10 Hz.

Methyl 7-*octenoate* (XLVII), and *methyl* 5Z-*octenoate* (XLVI) isolated from fraction A5 as a mixture in the ratio 1:3. IR of the mixture: 1740 (s), 1632 (w), 1438 (m), 1205 (s), 1165 (s), 720 (w) cm^{-1}; GC–MS of the mixture, compound XLVII: 156 = M$^+$ (1·5), 74 (100), 55 (80), 41 (50), 43 (43), 82 (46), 59 (45), 96 (44), 124 (26); compound XLVI: 156 = M$^+$ (5), 74 (100), 55 (58), 41 (56), 43 (56), 82 (52), 67 (41), 124 (28), 96 (24), 59 (23); NMR of the mixture, compound XLVII: δ 4·92 (C(8)H$_C$, J_{AC} 10 Hz, J_{BC} 1·5 Hz), δ 4·96 (C(8)H$_B$, J_{AB} 18 Hz, J_{BC} 1·5 Hz), δ 5·79 (C(7)H$_A$, J_{AB} 18 Hz, J_{AC} 10 Hz, J_{AX} 6·5 Hz); compound XLVI: δ 0·95 (C(8)H$_3$, *t*, *J* 7·5 Hz), δ 1·67 (C(3)H$_2$, *qn*, *J ca.* 7 Hz), δ 2·03 (C(4)H$_2$ and C(7)H$_2$, *m*), δ 2·31 (C(2)H$_2$, *t*, *J* 7·5 Hz), δ 3·65 (OMe, *s*), δ 5·34 (C(5)H and C(6)H, *m*).

Methyl 3Z-*nonenoate* (XLIX), was obtained as a 1:1 mixture with methyl 6Z-nonenoate (L) from fraction A5. MS (GC–MS): 170 = M$^+$ (6), 74 (100), 41 (78), 55 (70), 96 (66), 138 (44), 59 (42), 43 (36), 69 (33), 81 (32); IR: 1740 (s) cm^{-1}; NMR (after subtraction of the signals belonging to L, *vide infra*): δ 0·90 (C(9)H$_3$, distorted *t*, *J ca.* 7 Hz), δ *ca.* 1·4 (C(6)H$_2$, C(7)H$_2$ and C(8)H$_2$, *m*), δ *ca.* 2·05 (C(5)H$_2$, *m*), δ 3·05 (C(2)H$_2$, *d*, *J* 5 Hz), δ 3·62 (OMe, *s*), δ 5·55 (C(3)H and (C(4)H, *m*). Addition of Eu(DPM)$_3$: *r* = 1 (C(2)H$_2$, *d*), *r* = 0·69 (C(3)H, *m*, *J ca.* 1 Hz, 7 Hz and 10 Hz), *r* = 0·19 (C(4)H, *m*, *J ca.* 1, 7 and 10 Hz), *r* = 0·20 (C(5)H$_2$, *q*, *J ca.* 7 Hz). Irradiation at *r* = 1 simplified the signals at *r* = 0·69 and *r* = 0·19 to a doublet with further splitting, and two triplets respectively. The splittings of the doublet were *ca.* 1 and 10 Hz, while the triplets were separated by 10 Hz. The splitting of each triplet was *ca.* 7·5 Hz. Irradiation at *r* = 0·20 effected similar simplification of the signal at *r* = 0·69 to two triplets, and *r* = 0·19 to a doublet with further splittings. The appearance of these multiplets were similar to those produced with irradiation at *r* = 1.

Methyl 6Z-*nonenoate* (L), isolated from fraction A5. MS: 170 = M$^+$ (7), 74 (100), 55 (75), 41 (71), 96 (65), 138 (47), 59 (43), 43 (38), 87 (36), 69 (35); IR: 1741 (s), 1438 (m), 1362 (w), 1201 (m), 1160 (m), 720 (w) cm^{-1}; NMR: δ 0·98 (C(9)H$_3$, *t*, *J* 7·5 Hz), δ *ca.* 1·5 (C(3)H$_2$ and C(4)H$_2$, *m*), δ *ca.* 2·04 (C(5)H$_2$ and C(8)H$_2$, *m*), δ 2·36 (C(2)H$_2$, *t*, *J* 7·5 Hz), δ 3·65 (OMe, *s*), δ *ca.* 5·35 (C(6)H and C(7)H, *m*). Addition of Eu(DPM)$_3$: *r* = 1 (C(2)H$_2$, *t*), *r* = 0·67 (C(3)H$_2$, *qn*, *J ca.* 7 Hz), *r* = 0·33 (C(4)H$_2$, *m*), *r* = 0·14 (C(5)H$_2$, *m*), *r* = 0·05, average, (C(6)H and (C(7)H, *m*), *r* = 0·04 (C(8)H$_2$, *m*), *r* = 0·015 (C(9)H$_3$, *t*), *r* = 0·95 (OMe). Irradiation at *r* = 0·67 produced a singlet at *r* = 1.

Methyl 2E,6Z-*nonadienoate* (LVII), isolated from fraction A5. MS: 168 = M$^+$ (1·5), 100 (100), 41 (81), 69 (64), 68 (17), 67 (14), 53 (9), 109 (8); IR: 1729 (s), 1660 (m), 1438 (m), 1271 (m), 1203 (m), 1164 (m), 1127 (w), 1071 (w), 1043 (w), 1025 (w), 976 (m), 851 (w), 730 (w) cm^{-1}; UV: λ_{max} 206 nm (EtOH); NMR: δ 0·96 (C(9)H$_3$, *t*, *J* 7·5 Hz), δ 2·03 (C(8)H$_2$, *qn*, *J ca.* 7 Hz), δ *ca.* 2·2 (C(4)H$_2$ and C(5)H$_2$, *m*), δ 3·71 (OMe, *s*), δ *ca.* 5·35 (C(6)H and C(7)H, *m*), δ 5·82 (C(2)H, *d*, *J* 16 Hz), δ 6·98 (C(3)H, doublet of triplets, *J* 7 and 16 Hz). Irradiation at δ 2·03 simplified the triplet at δ 0·96 to a singlet. Addition of Eu(DPM)$_3$: *r* = 1 (OMe, *s*), *r* = 0·98 (C(2)H, *d*), *r* = 0·92 (C(3)H, doublet of triplets), *r* = 0·16 (C(4)H$_2$, *q*, *J ca.* 7 Hz), *r* = 0·08 (C(5)H$_2$, *q*, *J ca.* 7 Hz), *r* = 0·05, average (C(6)H and C(7)H, *m*), *r* = 0·03 (C(8)H$_2$, *qn*, *J ca.* 7 Hz), *r* = 0·015 (C(9)H$_3$, *t*, *J ca.* 7 Hz).

Methyl 3,7-*dimethyl*-2E,6-*octadienoate* (LIX): Geraniol[60] was oxidized to the corresponding methyl ester according to the procedure of Corey *et al.*[71]

Methyl 2ζ-*hydroxy*-3ζ-*methylpentanoate* (LXXIV), isolated from fraction B 2. MS: M$^+$ at *m/e* 146 not seen, 90 (100), 45 (76), 87 (73), 41 (40), 57 (28), 69 (26), 43 (13), 58 (11); IR: identical to authentic;[37] $[\alpha]^{20}_{D}$ −18·5° (*c* 0·48 in CHCl$_3$); NMR: δ 0·81 (C(3)Me, *d*, *J* 6·8 Hz), δ 0·93 (C(5)H$_3$, *t*, *J* 7 Hz), δ 1·1–1·6 (C(4)H$_2$, *m*), δ 1·82 (C(3)H, *m*), δ 2·77 (OH, δ dependent on concentration), δ 3·77 (OMe, *s*), δ 4·18 (C(2)H, broad s), Irradiations at δ 1·82, δ 2·77 and δ 4·18 respectively effected the following alterations: the doublet at δ 0·81 collapsed to a singlet, a doublet (*J* 2·5 Hz) appeared at δ 4·18, and the multiplet at δ 1·82 was simplified to a sextet (*J ca.* 7 Hz). The C(4)H$_2$-protons appeared to be non-equivalent. Addition of Eu(DPM)$_3$ caused line-broadening.

The lactone of 4ζ-*hydroxy*-3ζ-*methylpentanoic acid* (LXXV), obtained from fraction B3 as a 1:1 mixture

---

[71] Corey, E. J., Gilman, N. W. and Ganem, B. E. (1968) *J. Am. Chem. Soc.* **90**, 5616.

with an unknown constituent. GC–MS of the mixture furnished the MS of the lactone of LXXV: 114 = M^+ (6), 42 (100), 45 (58), 43 (41), 70 (35), 55 (27), 41 (26), 71 (21), 99 (13); IR of the mixture disclosed absorption at 1780 and 1740 cm^{-1}; NMR: (only signals assigned to the lactone of LXXV are given) δ 1·14 (C(3)Me, *d*, *J* 6·5 Hz), δ 1·38 (C(4)Me, *d*, *J* 6 Hz), δ *ca.* 2·2 (C(2)H_A, m), δ *ca.* 2·6 (C(2)H_B, *m*), δ *ca.* 4·17 (C(4)H, *m*).

Methyl 5-*methyl*-4-*oxohexanoate* (LXXVI), isolated from fraction B2. MS: 158 = M^+ (1·5), 115 (100), 43 (45), 55 (37), 87 (19), 59 (17), 127 (15), 71 (13), 41 (13); accurate mass determination: $C_8H_{14}O_3$, found 158·0936, calc. 158·0943; IR: 1740 (s), 1715 (s), 1469 (w), 1439 (m), 1382 (w), 1365 (m), 1211 (m), 1168 (m), 1091 (w), 1075 (w), 1022 (w), 982 (w), 962 (w), 839 (w) cm^{-1}; NMR: δ 1·12 (C(5)Me and C(6)H_3, *d*, *J* 7 Hz), δ 2·5–2·9 (C(2)H_2 and C(3)H_2 constituting an A_2B_2-system—nearly symmetrical—overlapping the C(5)H-proton), δ 3·67 (OMe, *s*). Synthesis: dimethyl succinate (5 g) was added slowly to a solution of isobutylmagnesium bromide (29 g) in Et_2O (300 ml), and subsequently stirred at ambient temp. for 1 hr. 0·5 N H_2SO_4 was added and the Et_2O layer collected. The product (4·1 g) was chromatographed on a silica gel column to give 2,7-dimethyl-3,6-octadione (0·26 g) and methyl 5-methyl-4-oxohexanoate (1·1 g) in addition to unreacted dimethyl succinate. The physical properties of the synthetic methyl 5-methyl-4-oxohexanoate were identical in all respects with those of the ester derived from the acid isolated from the tobacco.

Methyl 2S-*isopropyl*-5-*oxohexanoate* (LXXVII), isolated from fraction B3. MS: 186 = M^+ (0·7), 43 (100), 69 (43), 101 (43), 126 (40), 41 (25), 129 (24), 116 (22), 111 (22); accurate mass determination: $C_{10}H_{18}O_3$, found 186·1262, calc. 186·1256; IR: 1733 (s), 1720 (s), 1437 (m), 1370 (m), 1200 (m), 1158 (s), 1046 (w), 1000 (w), 940 (w), 851 (w) cm^{-1}; NMR: δ 0·91 (C(2)C-Me, *d*, *J* 7 Hz), δ 0·94 (C(2)C-Me, *d*, *J* 7 Hz), δ *ca.* 1·80 (C(2)CH, *m*), δ 1·88 (C(3)H_2, *q*, *J* 7 Hz), δ *ca.* 2·1 (C(2)H, *m*), δ 2·12 (C(6)H_3, *s*), δ 2·41 (C(4)H_2, *t*, *J* 7 Hz), δ 3·67 (OMe, *s*). Irradiation at δ 2·41 effected sharpening of the singlet at δ 2·12. $[\alpha]_D^{25}$ −8·6° (*c* 1·7 in $CHCl_3$).

Methyl 4-*oxononanoate* (LXXVIII), isolated from fraction B1. MS: 186 = M^+ (0·4), 43 (100), 98 (93), 115 (71), 130 (59), 55 (58), 71 (57), 99 (57), 41 (27); accurate mass determination: $C_{10}H_{18}O_3$, found 186·1248, calc. 186·1256; IR: 1740 (s), 1719 (s), 1440 (m), 1370 (m), 1200 (m), 1168 (m), 1126 (w), 1080 (w), 1029 (w), 851 (w) cm^{-1}, i.e. identical with that of an authentic specimen; NMR: δ 0·88 (C(9)H_3, distorted triplet, *J ca.* 7 Hz), δ 1·28 (C(7)H_2 and C(8)H_2, *m*), δ *ca.* 1·6 (C(6)H_2, *m*), δ 2·43 (C(5)H_2, triplet with broad lines, *J ca.* 8 Hz), δ 2·65 (C(2)H_2 and C(3)H_2, A_2B_2-system—symmetrical), δ 3·66 (OMe, *s*).

Methyl 3-*isopropyl*-6-*oxo*-2E-*heptenoate* (LXXIX), isolated from fraction B2, MS: 198 = M^+ (1), 43 (100), 123 (63), 95 (60), 166 (39), 41 (21), 109 (20), 81 (20), 67 (17); accurate mass determinations: $C_{11}H_{18}O_3$, found 198·1262, calc. 198·1256, $C_{11}H_{16}O_2$, found 180·1155, calc. 180·1150; IR: 1718 (s), 1641 (m), 1439 (m), 1390 (w), 1361 (w), 1321 (w), 1275 (w), 1227 (m), 1170 (s), 1010 (m), 920 (w), 870 (w) cm^{-1}; UV: λ_{max} 221 nm (EtOH); NMR: δ 1·08 (C(3)C-$(Me)_2$, *d*, *J* 7 Hz), δ 2·17 (C(7)H_3, *s*), δ 2·39 (C(3)CH, septet, *J* 7 Hz), δ 2·6 (C(5)H_2) and δ 2·8 (C(4)H_2), A_2B_2-system—symmetrical, δ 3·68 (OMe, *s*), δ 5·70 (C(2)H, *s*). Irradiation at δ 1·08 increased the intensity of the signal resonating at δ 5·70 by 8%.

Methyl 3ζ-*isopropyl*-6-*oxo*-4E-*heptenoate* (LXXX), isolated from fraction B3. MS: 198 = M^+ (0·5), 43 (100), 124 (33), 95 (25), 97 (24), 81 (24), 123 (22), 113 (22), 166 (20), 125 (20); accurate mass determinations: $C_{11}H_{18}O_3$, found 198·1266, calc. 198·1256, $C_{11}H_{16}O_2$, found 180·1157, calc. 180·1150; IR: 1739 (s), 1677 (s), 1629 (m), 1438 (m), 1365 (m), 1257 (m), 1164 (m), 1110 (w), 990 (m) cm^{-1}; NMR: δ 0·89 (C(3)C-Me, *d*, *J* 7 Hz), δ 0·94 (C(3)C-Me, *d*, *J* 7 Hz), δ 1·77 (C(3)CH, *m*, *J ca.* 7 Hz), δ 2·23 (C(7)H_3, *s*), δ 2·1–2·7 (C(2)H_2 and C(3)H, *m*), δ 3·64 (OMe, *s*), δ 6·06 (C(5)H, *d*, *J* 16 Hz), δ 6·66 (C(4)H, *q*, *J* 8 and 16 Hz): $[\alpha]_D^{25}$ −5·3° (*c* 0·9 in $CHCl_3$).

Methyl 3ζ-*hydroxy*-3ζ-*methyl*-6ζ-*isopropyl*-4E-*octenoate* (LXXXI), isolated from fraction B2. MS: M^+ at *m/e* 228 not seen, 43 (100), 97 (39), 111 (28), 55 (27), 112 (24), 69 (23), 41 (23), 71 (14); accurate mass determination: $C_{13}H_{22}O_2$ (M − 18), found 210·1620, calc. 210·1620; IR: 3500 (broad), 1730 (s), 1440 (m), 1384 (m), 1369 (m), 1338 (m), 1209 (m), 1175 (m), 1128 (w), 1012 (w), 978 (m), 939 (w) cm^{-1}; NMR: δ 0·78 (C(8)H_3, *t*, *J* 7 Hz), δ 0·79 and δ 0·84 (C(6)C-$(Me)_2$, *d*, *J* 7 Hz), δ 1·2–1·8 (C(6)H, C(6)CH and C(7)H_2, *m*), δ 1·31 (C(3)Me, *s*), δ 2·56 (C(2)H_2, *s*), δ 3·66 (OMe, *s*), δ 3·82 (OH, broad *s*), δ 5·42 (C(4)H and C(5)H, *m*). Addition of Eu$(DPM)_3$: *r* = 1·28 (C(2)H_A, *d*, *J* 16 Hz), *r* = 1·21 (C(2)H_B, *d*, *J* 16 Hz), *r* = 1 (C(3)Me, *s*), *r* = 0·87 (C(4)H, *d*, *J* 16 Hz), *r* = 0·68 (C(5)H, *q*, *J* 9 and 16 Hz), *r* = 0·26 (C(6)H, *m*), *r* = 0·13 (C(8)H_3, *t*, *J* 7 Hz), *r* = 0·11 (C(6)C-$(Me)_2$, *d*, *J* 7 Hz), *r* = 0·31 (OMe, *s*). Extrapolation to zero concentration of Eu$(DPM)_3$ indicated the chemical shift of C(6)H as δ 1·58. The coupling between C(5)H and C(6)H was indicated by splittings of 9 Hz in the multiplet at *r* = 0·26, and demonstrated in decoupling experiments, i.e. irradiations at *r* = 0·26 and 0·68 respectively removed the mutual spin–spin coupling; $[\alpha]_D^{25}$ −0·8° (*c* 0·5 in $CHCl_3$). NMR indicated a diastereomeric mixture 1:4.

Dimethyl 2-*ethyl*-3-*methylmaleate* (LXXXIV) isolated from fraction B2. MS: 186 = M^+ (9), 154 (100), 155 (90), 67 (47), 95 (41), 126 (40), 127 (36), 59 (33); NMR: δ 1·07 (C(2)$CH_2\underline{C}\underline{H}_3$, *t*, *J* 7·5 Hz), δ 1·95 (C(3)Me, *t*, *J* 0·8 Hz), δ 2·37 (C(2)CH_2, quartet of quartets, *J* 0·8 and 7·5 Hz), δ 3·75 and δ 3·77 (OMe, *s*). 2-*Ethyl*-3-*methylmaleic anhydride* was isolated from the fraction containing the polar methyl esters obtained after methylation with CH_2N_2. MS: 140 = M^+ (63), 67 (100), 112 (67), 53 (60), 68 (43), 41 (20), 69 (13), 66 (13), 51 (13); IR: 1846 (m), 1765 (s), 1389 (w), 1289 (m), 1250 (m), 1125 (w), 910 (s), 735 (m) cm^{-1}; NMR: δ 1·2 (C(2)$CH_2\underline{C}\underline{H}_3$, *t*, *J* 7·5 Hz), δ 2·09 (C(3)Me, *t*, *J* 0·8 Hz), δ 2·51 (C(2)CH_2, quartet of quartets, *J* g 0·8 and 7·5 Hz).

2-*Methoxy*-3,5,5-*trimethyl*-1,4-*cyclohexadion*-2-*ene* (LXXXV), isolated from fraction B1. MS: 182 = M^+ (20), 83 (100), 167 (40), 140 (40), 98 (24), 154 (19), 41 (15), 55 (14), 139 (14); accurate mass determination: $C_{10}H_{14}O_3$, found 182·0939, calc. 182·0943; IR: 1735 (m, imp. ?), 1698 (s), 1669 (s), 1604 (m), 1450 (m), 1385 (m), 1375 (m), 1338 (m), 1318 (m), 1288 (m), 1200 (m), 1142 (m), 998 (m) cm^{-1}; UV: λ_{max} 278 nm (EtOH); NMR: δ 1·21 (C(5)-$(Me)_2$, *s*), δ 1·89 (C(3)-Me, *s*), δ 2·68 (C(6)H_2, *s*), δ 3·66 (4H, imp. ?), δ 3·96 (OMe, *s*).

Acknowledgements—Grateful appreciation is expressed to Miss A.-M. Eklund for valuable technical assistance, Mr. J. Roeraade for providing capillary columns, Professor K. Olsson, the Agricultural College of Sweden, Uppsala, for placing their NMR instrument at our disposal and Dr. D. L. Roberts, R. J. Reynolds Tobacco Co., Winston-Salem, and Dr. E. Honkanen, Biochemical Research Institute, Helsinki, for gifts of 4-oxononanoic acid and 4-hydroxy-3-methylpentanoic acid lactone. Drs. E. Demole and D. Berthet, Firmenich & Cie., Genève, and Drs. D. L. Roberts and W. A. Rohde, R. J. Reynolds Tobacco Co., Winston-Salem, are acknowledged for disclosing their latest results prior to publication.

Note added in proof. In an attempt to confirm the assignments given by Laporte *et al.*[39] for the configurations of the lactones of 4ζ-hydroxy-3ζ-methylpentanoic acid (LXXV), NOE-experiments were carried out on both the *cis* and the *trans* isomers (synthetic) by irradiating the 3-methyl group and observing the intensity of the 4-proton. The isomer corresponding to that from tobacco showed a 36% increase while the other increased 21%. The positive NOE-eflect for the latter isomer, presumably the *cis*-isomer, might be explained by having a staggered conformation bringing the 3-methyl group in closer proximity to the 4-proton. These results should be interpreted with great caution since there are many pitfalls in performing such experiments.[57] They show, however, the desirability of independent proof of the original assignments.[39]