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STEROLS AND TRITERPENOIDS—XI[1]

ISOLATION OF ARUNDOIN AND SAWAMILLETIN* FROM CUBAN SUGAR CANE WAX

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Abstract—A mixture of triterpene methyl ethers obtained from the leaf wax of Cuban sugar cane (*Saccharum officinarum L.*) has been shown to consist predominantly of arundoin (fernenol methyl ether) and sawamilletin (taraxerol methyl ether), by means of preparative GLC, mass spectrometry and direct comparison with authentic specimens. Evidence for the presence, in trace amounts, of a third component having identical gas liquid chromatographic retention times with bauerenol methyl ether, was also obtained by GLC, but it did not prove feasible to further characterize this substance. Retention times, relative to 5α-cholestane are reported for nine triterpene methyl ethers which were subjected to GLC on 0·5% Apiezon L, 1·5% SE-30, 1·5% QF-1 and 1·0% CDMS columns. The mass spectra of triterpene methyl ethers are discussed.

ALTHOUGH not confined to grasses,[2] triterpene methyl ethers would seem to be of relatively common occurrence within the family Gramineae. Thus germanicol methyl ether (miliacin, I) occurs[3,4] as a constituent of the grasses *Panicum miliaceum* L.† and *Syntherisma sanguinalis* Dulac, var. *ciliaris* Honda; taraxerol methyl ether (sawamilletin, IV) has been isolated[3,5] from *Echinochloa crusgalli* L.; β-amyrin methyl ether (isosawamilletin, III)[6] and α-amyrin methyl ether (VI)[7] have been obtained from a species originally designated *Arundo conspicua* Forst. f.[8] but now identified as

* Recently, H. Ito, T. Obara and S. Abe *J. Chem. Soc. Japan* **86**, 540 (1965), have reported the identity of sawamilletin with crusgallin indicating the priority of the latter name for this compound.

† Species names, with the authorities cited, are those used by the authors of the original chemical papers quoted. No attempt has been made to substitute modern equivalents.

[1] Part XI of the Gatersleben Series. Part X. M. von Ardenne, G. Osske, K. Schreiber, K. Steinfelder and R. Tümmler, *J. Insect Physiol.* **11**, 1365 (1965).

[2] E.g. J. W. Rowe and C. L. Bower, *Tetrahedron Letters* No. 32, 2745 (1965); S. Matsunaga, J. Okada and S. Uyeo, *Chem. Comm.* 525 (1965).

[3] H. Ito, *J. Chem. Soc. Japan* **59**, 274 (1938); S. Abe and T. Obara, *Nippon Kagaku Zasshi* **80**, 1487 (1959).

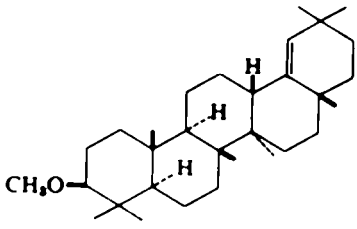
[4] H. Ito, *J. Faculty Agr. Hokkaido Imp. Univ.* **37**, 1 (1934); S. Abe, *Bull. Chem. Soc. Japan* **33**, 271 (1960); N. Sugiyama and S. Abe, *Nippon Kagaku Zasshi* **82**, 1051 (1961); S. Abe, *Ibid.* **82**, 1054, 1057 (1961).

[5] S. Abe, *Nippon Kagaku Zasshi* **80**, 677, 1491 (1959).

[6] G. Eglinton, R. J. Hamilton, M. Martin-Smith, S. J. Smith and G. Subramanian *Tetrahedron Letters* No. 34, 2323 (1964).

[7] T. A. Bryce, G. Eglington, R. J. Hamilton, M. Martin-Smith and G. Subramanian, *Phytochemistry*, in the press.

[8] E.g. T. F. Cheeseman, *Manual of the New Zealand Flora* (2nd Edition) Wellington, Government Printer (1925).



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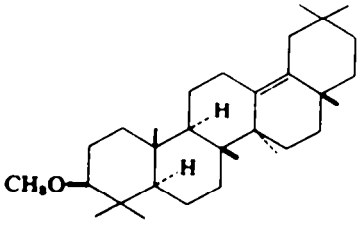

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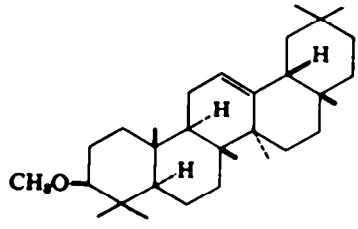

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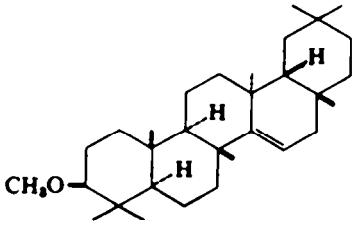

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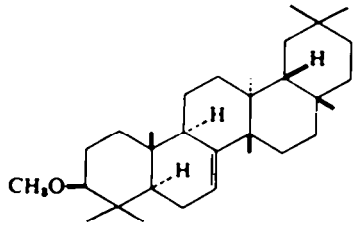

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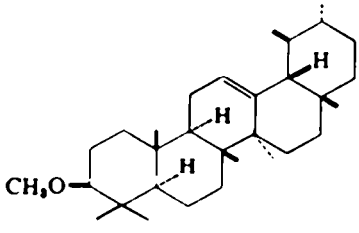

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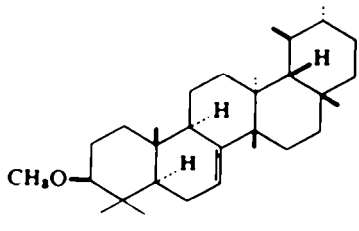

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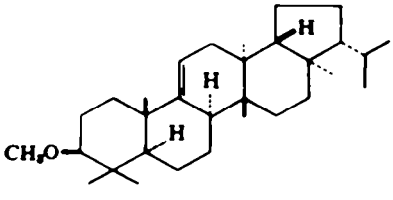
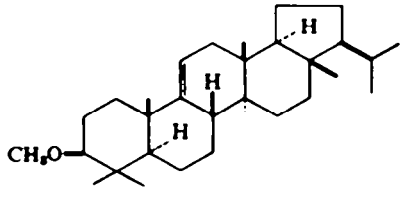


VIII



IX

Cortaderia toetoe Zotov;[9,10] arundoin (VIII),[11] the methyl ether of fernenol,[12,13] has been isolated from *Cortaderia toetoe*,[9] *Cortaderia fulvida* (Buch.) Zotov,[10] *Cortaderia richardii* (Endl.) Zotov,[10] *Imperata cylindrica* P. Beauv. var. *media* Hubbard (= var. *koenigii* Durand et Schinz)[11,14] and *Zoysia japonica* Steud.;[13] and isoarborinol methyl ether (cylindrin), the structure of which as IX follows from the recent structural elucidation of arborinol,[15] has been obtained from *Imperata cylindrica*[11,14] and from *Zoysia japonica*.[13] We now wish to report the isolation of arundoin and sawamilletin from the wax of Cuban sugar cane (*Saccharum officinarum* L.), which, like *Imperata cylindrica* belongs to the tribe Andropogoneae of the Gramineae. This characterization of triterpene methyl ethers in sugar cane wax would appear to be the first to be recorded, despite extensive studies on the constituents of the wax from various strains of *Saccharum officinarum*.[16]

After preliminary saponification of the oily portion of the sugar cane leaf wax and removal of a light petroleum-insoluble sterol fraction from the resulting neutral material, repeated chromatography of the material soluble in light petroleum over silica gel gave, as the fourth fraction to be characterized,[17] after crystallization from benzene–methanol, material of m.p. 194–205° and $[\alpha]_D$ +7·9° (Chf), which was preliminarily designated as "Substance W" (the over-all yield was 2·3% of the unsaponifiable material). Substance W appeared homogenous on TLC employing Kieselgel G (Merck) as adsorbant and light petroleum (b.p. 70–80°)–ethyl acetate–benzene 7:2:1 as solvent system (R_f 2·9 and 1·8; standard = cholesterol and cycloartenol, respectively), but by developing with light petroleum only, it was possible to distinguish two very closely running components which could not be separated on a preparative scale. Substance W exhibited strong absorption in the IR at 1104 cm^{-1}, an absorption which we have found is typical of triterpene methyl ethers. However, the IR absorption in KCl disc in the 1375 cm^{-1} and 1460^{-1} regions was not sharp as is usual with pure triterpene methyl ethers, which normally give characteristic absorptions that can be used to aid in the identification of any particular triterpene methyl ether.[18] Furthermore, Substance W showed positive reactions both in the Liebermann–Burchard test (cherry-red colour) as well as in the tetranitromethane test. These observations therefore strongly suggested that Substance W was in all probability a mixture of triterpene methyl ethers—the lack of resolution on TLC having

[9] V. D. Zotov, *N. Z. J. Bot.* **1**, 78 (1963).
[10] M. Martin-Smith, G. Subramanian and H. E. Connor, *Phytochemistry* in the press.
[11] K. Nishimoto, M. Ito, S. Natori and T. Ohmoto, *Tetrahedron Letters* No. 27, 2245 (1965).
[12] S. K. Kundu, A. Chatterjee and A. S. Rao, *Tetrahedron Letters* No. 10, 1043 (1966).
[13] K. Nishimoto, M. Ito, S. Natori and T. Ohmoto, *Chem. Pharm. Bull. Tokyo* **14**, 97 (1966).
[14] T. Ohmoto, K. Nishimoto, M. Ito and S. Natori, *Chem. Pharm. Bull. Tokyo* **13**, 224 (1965).
[15] O. Kennard, L. Riva di Sanseverino, H. Vorbrüggen and C. Djerassi, *Tetrahedron Letters* No. 39, 3433 (1965).
[16] *Inter alia* J. A. Lamberton and A. H. Redcliffe, *Austral. J. Appl. Sci.* **11**, 473 (1960) and earlier papers; R. T. Balch, *Wax and Fatty By-Products From Sugar Cane* Technol. Rep. Ser. no 3. Sugar Research Foundation, New York (1947); D. E. Whyte and B. Hengeveld, *J. Amer. Oil Chem. Soc.* **27**, 57 (1950); N. Wiedenhof, *Ibid.* **36**, 297 (1959); D. R. Kreger, *Rec. Trav. Bot. Neerl.* **41**, 606 (1948); D. H. S. Horn and M. Matic, *J. Sci. Fd. Agric.* **8**, 571 (1957); S. Bose and K. C. Gupta, *Proc. Ann. Conv. Sugar Technologists' Assoc. India* **29**, 70 (1961) in *Chem. Abstr.* **60**, 13423 (1964).
[17] G. Osske and K. Schreiber, *Tetrahedron* **21**, 1559 (1965).
[18] cf. H. Snatzke, F. Lampert and R. Tschesche, *Tetrahedron* **18**, 1417 (1962).

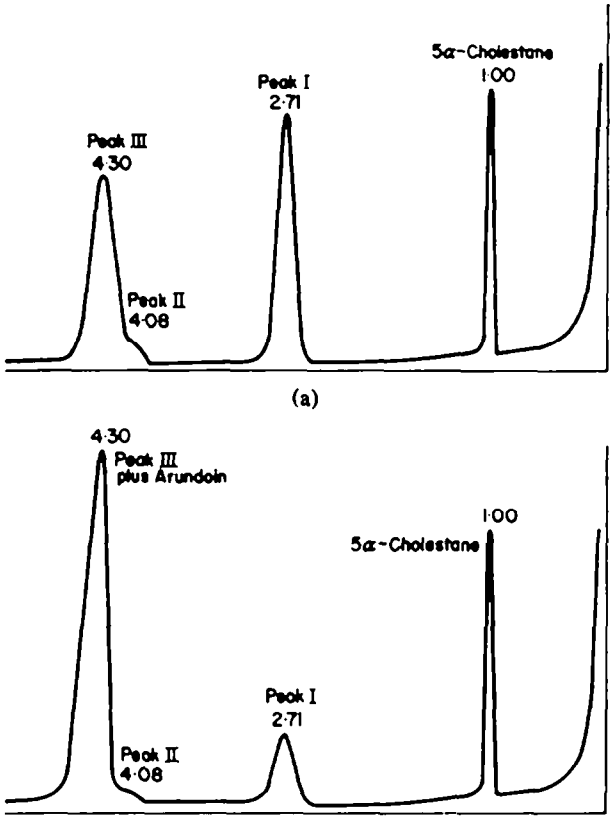


FIG. 1. Reproduction of the gas liquid chromatographic traces obtained on a 0·5% Apiezon L column at 240° with:
(a) the mixture of triterpene methyl ethers from Cuban sugar cane wax,
(b) the mixture of triterpene methyl ethers from Cuban sugar cane wax with added arundoin.
The figures over the peaks are retention times relative to 5α-cholestane = 1·00.

TABLE 1. RELATIVE GAS LIQUID CHROMATOGRAPHIC RETENTION TIMES OF COMPONENTS OF "SUBSTANCE W" FROM CUBAN SUGAR CANE WAX*

Component	0·5% Apiezon L. Column Temp 240°	1·5% SE-30. Column Temp 240°	1·5% QF-1. Column Temp 225°	1·0% CDMS. Column Temp 240°
5α-Cholestane (internal reference standard)	1·00 (11–14 mins)	1·00 (3–4 mins)	1·00 (2½–2¾ mins)	1·00 (2½–3 mins)
Peak I	2·71	2·43	2·74	3·65
Peak II	4·08	—	—	—
Peak III	4·30	3·21	3·50	5·47

* The stationary phase was supported on Gas Chrom Z, 100–120 mesh; column temps as shown; detector temp 248°; carrier gas argon, 60 ml/min. The relative retention times shown represent the mean of at least three determinations.

analogy in the similar lack of resolution reported[19,20] for triterpene alcohols. Accordingly Substance W was subjected to GLC.

Preliminary analytical studies employing 0·5% Apiezon L as stationary phase and argon as carrier gas resolved Substance W into three components. Two of these components were indicated by prominent well resolved peaks (Peaks I and III, Fig. 1a) whilst the third component (Peak II, Fig. 1a) was indicated by a small, incompletely resolved peak on the low retention side of Peak III i.e. the main peak of higher retention time. However, analytical GLC employing 1·5% SE-30, 1·5% QF-1 or 1·0% CDMS as stationary phases with argon as carrier gas revealed the presence of only two peaks.* Similarly only two peaks were apparent on GLC employing 2·5% SE-30 and 2% XE-60 as stationary phases with nitrogen as carrier gas.

Since the gas liquid chromatographic retention times of triterpenoids,[21,22] like those of steroids,[23] are conventionally determined relative to the retention time of 5α-cholestane, further experiments were conducted with added 5α-cholestane as internal standard in order to obtain relative retention values for the components of substance W. The results, representing the average of several determinations, for the stationary phases 0·5% Apiezon L, 1·5% SE-30, 1·5% QF-1 and 1·0% CDMS on the support Gas Chrom Z of 100–120 mesh are summarised in Table 1.

In order to facilitate the identification of these ethers, it became necessary to determine the relative retention times of selected authentic triterpene methyl ethers, since, although there have been published several papers on the GLC of various triterpenoids,[21,22,24] including triterpene trimethylsilyl ethers,[21,22] there would appear to have been little work reported on triterpene methyl ethers. Accordingly the retention times of nine triterpene methyl ethers (readily available from natural sources or from methylation of the parent alcohol by adaptation of the method of Morice

* The use of both non-polar (Apiezon L grease and silicone gum SE-30) and polar (fluorosilicone polymer QF-1 and polyester CDMS) stationary phases was in an empirical search for the most efficient resolution of the triterpene methyl ethers. Eglinton *et al.*[24a] showed the versatility of Apiezon L in the GLC of compounds of MWs 250–500 with relatively high m.ps., while the suitability of SE-30 and polyester phases for the GLC of steroids was shown by Horning *et al.*[23a,f] Clayton[23b] used a polyester column with steroid methyl ethers. The main factors influencing GLC retention times are mol wt, polarity and conformational restriction.[23] In the present work, where mol wt is constant and conformations rigid, it was not possible to predict which stationary phase would be the most sensitive to differences in molecular shape in combination with the small differences in polarity attendant upon double bond position. The high temperatures necessary for GLC of triterpene methyl ethers ruled out the use in the stationary phase of silver nitrate, which might otherwise have given a separation based on double bond environment.

[19] T. Murakami, H. Itokawa, F. Uzuki and N. Sawada, *Chem. Pharm. Bull. Tokyo* **13**, 1346 (1965).
[20] M. Shimizu, F. Uchimaru and G. Ohta, *Chem. Pharm. Bull. Tokyo* **12**, 74 (1964).
[21] N. Ikekawa, S. Natori, H. Itokawa, S. Tobinaga and M. Matsui, *Chem. Pharm. Bull. Tokyo* **13**, 316 (1965).
[22] N. Ikekawa, S. Natori, H. Ageta, K. Iwata and M. Matsui, *Chem. Pharm. Bull. Tokyo* **13**, 320 (1965)
[23] E.g. [a] R. B. Clayton, *Biochemistry* **1**, 357 (1962); [b] C. J. W. Brooks and L. Hanaineh, *Biochem. J.* **87**, 151 (1963); [c] B. A. Knights, *J. Gas Chromatog.* **2**, 160 (1954); [d] E. C. Horning, W. J. A. VandenHeuvel and B. G. Creech, in *Methods of Biochemical Analysis* (Edited by D. Glick) Vol. IX, p. 69. Interscience, New York (1963); [e] W. J. A. VandenHeuvel, C. C. Sweeley and E. C. Horning, *J. Amer. Chem. Soc.* **82**, 3481 (1960); [f] E. O. A. Haahti, W. J. A. VandenHeuvel and E. C. Horning, *J. Org. Chem.* **26**, 626 (1961).
[24] *Inter alia* [a] G. Eglinton, R. J. Hamilton, R. Hodges and R. A. Raphael, *Chem. & Ind.* 955 (1959); [b] P. Capella, E. Fedeli and M. Cirimele, *Ibid.* 1590 (1963).

and Simpson[25]), relative to 5α-cholestane, were determined on the same four stationary phases as listed for the components of substance W in Table 1. The results are summarized in Table 2.

Examination of Table 2 reveals that none of the four stationary phases employed gave any clear distinction between the five ethers (compounds I–V) derived from the oleanane or *friedo* rearranged oleanane skeleton and indeed the virtually identical retention times of these five ethers were further substantiated when various mixtures of these compounds, taken two at a time, were subjected to GLC. Thus a single symmetrical peak, giving no indication of the presence of two components, was shown by mixtures of β-amyrin methyl ether with taraxerol methyl ether, of β-amyrin methyl ether with germanicol methyl ether, of taraxerol methyl ether with germanicol methyl ether, and of δ-amyrin methyl ether with germanicol methyl ether on all four stationary phases. Of the four stationary phases employed, only Apiezon L gave any resolution of a mixture of bauerenol methyl ether and arundoin, and then resolution was only partial as apparent from Fig. 2. Cylindrin and α-amyrin methyl ether were clearly resolvable from all seven of the other triterpene methyl ethers on all columns except the QF-1 column where cylindrin could not be distinguished from bauerenol methyl ether or arundoin.

That the lack of resolution of the methyl ethers of the oleanane group on GLC was not a result of backbone rearrangement of the different compounds, to give the same thermodynamically favoured compound as a common species, was demonstrated by employing preparative GLC of selected representatives, under comparable conditions and collecting eluted material. In this way, utilizing the diagnostic mass spectral cracking patterns of pentacyclic triterpenes,[26,27] it was shown that taraxerol methyl ether and β-amyrin methyl ether emerged unchanged from 1·5% SE-30 and 1% Apiezon L columns at 240°, whilst employing infrared spectral characteristics it was shown that multiflorenol methyl ether emerged unchanged from a 1% Apiezon L column at 240°.

In connection with the similar retention times of the five methyl ethers of the oleanane group, it is of interest that very close retention time values, relative to 5α-cholestane, have been reported[21] for β-amyrin (3·23) and taraxerol (3·14) on 1·3% SE-30 columns and for their trimethylsilyl ethers on 2% CNSi, 1·5% QF-1 and 1% NGS columns—the values for these trimethylsilyl ethers being 3·34 and 3·15; 3·12 and 3·12; and 3·42 and 3·33 respectively. The spread of retention time values, relative to 5α-cholestane, on the SE-30 columns as given in Table 2 viz., 2·44–3·43 is somewhat lower than the range of 3-6 previously reported[21] for monosubstituted pentacyclic triterpenes on SE-30 columns where the substituents are hydroxyl, carbonyl, acetoxyl, methoxycarbonyl etc., but this would be in accord with the relatively non-polar nature of the methoxyl group.

Calculation of "group retention factors" as has been done in the steroid field[23,28] is not feasible for the triterpene methyl ethers listed in Table 2, on account of the differences in carbon skeleton which accompany the differences in double bond position. Indeed, within the nine triterpene methyl ethers studied, seven different

[25] I. M. Morice and J. C. E. Simpson, *J. Chem. Soc.* **198**, (1942).
[26] C. Djerassi, H. Budzikiewicz and J. M. Wilson, *Tetrahedron Letters*, No. 7, **263** (1962).
[27] H. Budzikiewicz, J. M. Wilson and C. Djerassi, *J. Amer. Chem. Soc.* **85**, 3688 (1963).
[28] R. B. Clayton, *Nature, Lond.* **190**, 1071 (1961); **192**, 524 (1961).

TABLE 2. RELATIVE GAS LIQUID CHROMATOGRAPHIC RETENTION TIMES OF AUTHENTIC TRITERPENE METHYL ETHERS*

Compound		0·5% Apiezon L. Column Temp 240°	1·5% SE-30. Column Temp 240°	1·5% QF-1. Column Temp 225°	1·0%CDMS. Column Temp 240°
5α-Cholestane (internal reference standard)		1·00 (11–14 mins)	1·00 (3–4 mins)	1·00 (2½–2¾ mins)	1·00 (2½–3 mins)
Germanicol Methyl Ether (Miliacin)	I	2·83	2·54	2·76	3·79
δ-Amyrin Methyl Ether (Isomiliacin)	II	2·80	2·44	2·78	3·79
β-Amyrin Methyl Ether (Isosawamilletin)	III	2·79	2·45	2·89	3·77
Taraxerol Methyl Ether (Sawamilletin)	IV	2·74	2·45	2·75	3·67
Multiflorenol Methyl Ether	V	2·74	2·46	2·83	3·77
α-Amyrin Methyl Ether	VI	3·20	2·73	3·17	4·25
Bauerenol Methyl Ether	VII	4·11	3·24	3·42	5·59
Fernenol Methyl Ether (Arundoin)	VIII	4·31	3·20	3·52	5·50
Isoarborinol Methyl Ether (Cylindrin)	IX	4·95	3·43	3·47	6·25

* The stationary phase was supported on Gas Chrom Z, 100–120 mesh; column temps as shown; detector temp 248°; carrier gas argon, 60 ml/min. The relative retention times shown represent the mean of at least three determinations.

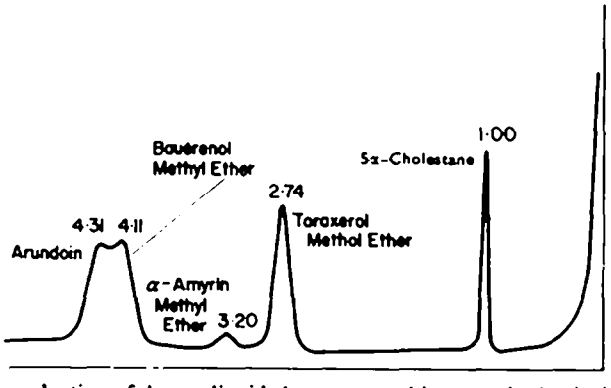


FIG. 2. Reproduction of the gas liquid chromatographic trace obtained with a mixture of taraxerol methyl ether, α-amyrin methyl ether, bauerenol methyl ether and arundoin on a 0·5% Apiezon L column at 240° showing the incomplete separation of bauerenol methyl ether and arundoin. The figures over the peaks are retention times relative to 5α-cholestane = 1·00.

nuclear systems are represented. Thus, three of the compounds (the methyl ethers of germanicol, I, δ-amyrin, II and β-amyrin, III) possess an unrearranged oleanane skeleton, one (the methyl ether of taraxerol, IV) is a derivative of D-*friedo*oleanane,[29] one (the methyl ether of multiflorenol, V) is a derivative of D:C-*friedo*oleanane, one (the methyl ether of α-amyrin, VI) is a derivative of ursane, one (the methyl ether of bauerenol, VII) is a derivative of D:C-*friedo*ursane, one (arundoin, VIII) is a derivative of E:C-*friedo*isohopane, and one (cylindrin, IX) is an E:C-*friedo* derivative of the as yet unnamed parent skeleton from which arborinol can be presumed to arise in Nature.[13,15] Nevertheless it is of interest that the ratio of the relative retention times of β-amyrin methyl ether and multiflorenol methyl ether of the oleanane series is not the same as the ratio of the relative retention times of the corresponding ursane analogues, α-amyrin methyl ether and bauerenol methyl ether, on any of the stationary phases.

Comparison of the data in Table 1 with the data in Table 2 immediately suggests that substance W from Cuban sugar cane wax could contain arundoin (Peak III), bauerenol methyl ether (Peak II) and one or more triterpene methyl ethers belonging to the oleanane group (Peak I). Indeed, addition of authentic arundoin to substance W intensified Peak III on all stationary phases, addition of bauerenol methyl ether intensified Peak II on the Apiezon L column and addition of taraxerol methyl ether, β-amyrin methyl ether or multiflorenol methyl ether intensified Peak I on all columns.

Preparative GLC of substance W, although not permitting the isolation of the material responsible for Peak II of the analytical Apiezon L column on account of its very low abundance in the mixture, was successful in permitting the isolation of the material corresponding to Peak III and of the material corresponding to Peak I. These fractions were then analysed by means of mass spectrometry.

Employing a direct inlet system the material corresponding to Peak III gave a mass spectrum identical with that of authentic arundoin obtained with a direct inlet system (Fig. 3b), whilst the samples showed identity of IR spectra in KCl disc and absence of any mixed melting point depression—thus confirming arundoin to be the component of substance W responsible for Peak III on the gas liquid chromatographic traces. The mass spectrum of the material corresponding to Peak I was strictly comparable to that of authentic taraxerol methyl ether (sawamilletin) (Fig. 3c), whilst direct comparison of the samples showed identity of IR spectra in KCl disc and absence of any mixed m.p. depression—thus confirming taraxerol methyl ether as a component of substance W. These results suggest, moreover, that if any other triterpene methyl ethers of the oleanane group are co-occuring with taraxerol methyl ether in Cuban sugar cane wax, then they must be present in very small amounts. Certainly the absence in the mass spectrum of the material corresponding to Peak I of any ion of *m/e* 234 (the expected[27] base peak in the mass spectrum of multiflorenol methyl ether) would indicate the absence of this compound. Similarly the observed identical relative abundances of the ions *m/e* 203, 204, 205 and 218 in the mass spectra of the material corresponding to Peak I and of authentic taraxerol methyl ether would rule out the presence of any appreciable quantities of germanicol methyl ether (prominent peaks at *m/e* 203, 204, 205[26]), δ-amyrin methyl ether (base peak *m/e* 205[27]) or β-amyrin methyl ether (base peak *m/e* 218[27]), although the possibility that one or more of these compounds is present in trace amounts can not be ruled out.

[29] *Friedo* nomenclature of S. Allard and G. Ourisson, *Tetrahedron* **1**, 277 (1957).

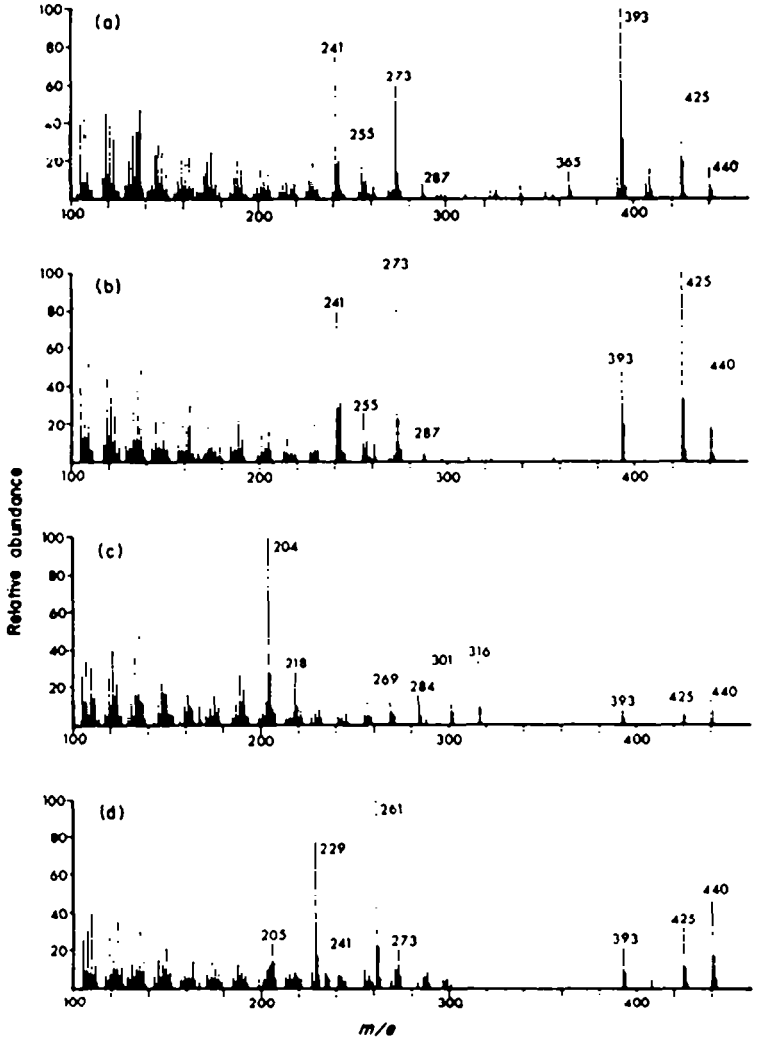


FIG. 3. Line diagrams of the mass spectra of triterpene methyl ethers.
(a) Arundoin using heated inlet system.
(b) Arundoin using direct inlet system.
(c) Taraxerol methyl ether using direct inlet system.
(d) Bauerenol methyl ether using direct inlet system.

Thus arundoin and sawamilletin (taraxerol methyl ether) have been conclusively identified as constituents of Cuban sugar cane wax and bauerenol methyl ether is probably a third minor constituent. The co-occurence of β-sitosterol,[17] stigmasterol,[17] campesterol,[17] 24-methylene-lophenol,[17] 24-ethylidene-lophenol,[17] sawamilletin and arundoin in *Saccharum officinarum* L. is of biogenetic interest, since it would not be inconsistent with the hypothesis that three distinct modes of cyclization[80] of all *trans* squalene could be occurring side by side viz. chair, boat, chair, boat conformational

[80] cf. L. Ruzicka, *Proc. Chem. Soc.* 341 (1959); T. G. Halsall and R. T. Aplin, *Fortschritte der Chemie Organischer Naturstoffe* (Edited by L. Zechmeister) Vol. 22, pp. 153–202. Springer-Verlag, New York (1964).

TABLE 3. MAJOR PEAKS IN THE MASS SPECTRA OF TRITERPENE METHYL ETHERS

Compound	Predicted* Mass spectral peaks	Observed mass spectral peaks (Relative ion abundances given as % of Base Peak = 100)		Metastable peaks for loss of methanol from ion of series A to corresponding ion of series B	
		Series A corresponding to predicted ions	Series B arising by loss of methanol	Observed	Calculated
	m/e	*m/e*	*m/e*		
	440 (Parent)	440 (15%)	408 (16%)	378·3	378·3
	425 (h)	425 (60%)	393 (100%)	363·4	363·4
Arundoin (VIII)		365 (14%)	332 (4%)	?	303·8
(Heated inlet system)	355 (l)	355 (1%)	323 (3%)	294·0	293·9
cf. Fig. 3a.	287 (m)	287 (8%)	255 (31%)	226·5	226·6
	273 (b)	273 (58%)	241 (76%)	212·8	212·8
	261 (l)	261 (5%)	229 (14%)	201·0	200·9
	m/e	*m/e*	*m/e*		
	440 (Parent)	440 (48%)			
	425 (h)	425 (100%)	393 (50%)	363·4	363·4
Arundoin (VIII)	355 (l)	355 (1%)	323 (4%)	294·0	293·9
(Direct inlet system)	287 (m)	287 (10%)	255 (26%)	226·5	226·6
cf. Fig. 3b.	273 (b)	273 (100%)	241 (80%)	212·8	212·8
	261 (l)	261 (9%)	229 (19%)	201·0	200·9
	m/e	*m/e*	*m/e*		
	440 (Parent)	440 (14%)			
	425 (l)	425 (8%)	393 (6%)	363·4	363·4
Taraxerol methyl ether (IV)	316 (m)	316 (33%)	284 (15%)	255·2	255·2
(Direct inlet system)	301 (m)	301 (29%)	269 (20%)	240·4	240·4
cf. Fig. 3c.	218 (m)	218 (28%)			
	204 (b)	204 (100%)			
	189 (m)	189 (26%)			
	m/e	*m/e*	*m/e*		
	440 (Parent)	440 (46%)			
	425 (m)	425 (32%)	393 (21%)	363·4	363·4
Bauerenol methyl ether (VII)	287 (l)	287 (6%)	255 (10%)	226·6	226·6
(Direct inlet system)	273 (l)	273 (20%)	241 (18%)	212·8	212·8
cf. Fig. 3d	261 (b)	261 (100%)	229 (77%)	201·0	200·9
	234 (l)	234 (8%)	202 (8%)	?	174·4
	205 (m)	205 (24%)			
	m/e	*m/e*	*m/e*		
	440 (Parent)	440 (7%)	408 (1%)	378·5	378·3
	425 (l)	425 (2%)	393 (1%)	363·4	363·4
	222 (l)	222 (3%)	190 (15%)	162·6	162·6
	221 (l)	221 (7%)	189	161·6	161·6
β-Amyrin methyl ether (III)	218 (b)	218 (100%)			
(Direct inlet system)	205 (l)	205 (3%)			
	203 (m)	203 (36%)			
	189 (m)	189 (14%)			
	133 (l)	133 (10%)			

TABLE 3 (*Contd.*)

	m/e	*m/e*	*m/e*		
	440 (Parent)	440 (4%)	408 (1%)	378·5	378·3
	425 (l)	425 (1%)	393	363·4	363·4
	222 (l)	222 (5%)	190 (10%)	162·6	162·6
α-Amyrin methyl ether (VI)	221 (l)	221 (11%)	189	161·6	161·6
(Direct inlet system)	218 (b)	218 (100%)			
	205 (l)	205 (2%)			
	203 (m)	203 (14%)			
	189 (m)	189 (14%)			
	133 (l)	133 (8%)			
	m/e	*m/e*	*m/e*		
	440 (Parent)	440 (70%)			
	425 (h)	425 (100%)	393 (61%)	363·4	363·4
Cylindrin (IX)	355 (l)	355 (9%)	323 (13%)	294·0	293·9
(Direct inlet system)	287 (m)	287 (16%)	255 (30%)	226·6	226·6
	273 (b)	273 (87%)	241 (52%)	212·8	212·8
	261 (l)	261 (10%)	229 (22%)	201·0	200·9

* Calculated from fragmentation patterns established by Budzikiewicz *et al.*[27]

l = low intensity
m = medium intensity
h = high intensity
b = base peak

The abundances quoted for the ions arising by loss of methanol are not corrected for contributions from fragments of the same mass arising from other modes of breakdown.

sequence for the sterols, chair, chair, chair boat conformational sequence for sawamilletin and chair, chair, chair, chair, boat conformational sequence for arundoin. An analogous situation has been reported for *Ficus macrophylla* Desf.[31] where the same three cyclizations of all *trans* squalene would account for the presence of cycloartenol, lupeol and butyrospermol, and moretenol respectively.

The mass spectra of the triterpene methyl ethers as determined in the present work were in excellent agreement with the cracking pattern to be predicted from the work of Djerassi *et al.*[26,27] except for the presence in relatively high abundance of an additional series of ions (series B) of mass 32 units less than the ions containing the methoxyl group of the predicted series (series A). That this extra series of ions is related to the predicted[26,27] methoxyl-bearing ions by a one-stage loss of methanol is confirmed by the appearance of the appropriate metastable ions in the spectra. The predicted ions, the observed ions and the observed metastable ions pertinent to this loss of methanol are shown in Table 3.

Although not the most favoured route of mass spectral fragmentation, similar loss of methanol also occurs as a minor route of fragmentation with simple methyl ethers as apparent from the tabulated data of McLafferty.[32] Thus, in addition to providing further illustration that the fragmentation of the triterpene nucleus is a more favoured process than fragmentation about a functional group, as is seen with the triterpene alcohols[26,27] where the characteristic cleavage of simple aliphatic alcohols at the

[31] M. N. Galbraith, C. J. Miller, J. W. L. Rawson, E. Ritchie, J. S. Shannon and W. C. Taylor, *Austral. J. Chem.* **18**, 226 (1965).

[32] F. W. McLafferty, *Analyt. Chem.* **29**, 1782 (1957).

C—COH bond[33] is subordinate to the nuclear fragmentations, the triterpene methyl ethers provide an example of a usually less important fragmentation process gaining prominence.

It is of considerable interest that when the mass spectrum of arundoin was determined using a heated inlet system (Fig. 3a), in place of the direct inlet system routinely employed, loss of methanol became more pronounced. Thus a prominent peak becomes apparent at *m/e* 408 (parent minus 32) with an observed metastable ion at 378·3 (calculated for *m/e* 440 → 408, 378·3) whilst the ions at *m/e* 393, *m/e* 255 and *m/e* 241 become more abundant than the ions from which they are derived through loss of methanol. Also of interest is the appearance of an ion *m/e* 365 in the mass spectrum of arundoin when the heated inlet system is employed. That this peak is derived by loss of the isopropyl side chain from ring E of arundoin in the ion *m/e* 408 is apparent from the existence of a metastable ion at 326·5 (calc. 326·5). The absence of this peak in the mass spectrum of arundoin determined using a direct inlet system, together with the lack of resolution of the isopropyl group methyl proton absorptions in the NMR spectrum of arundoin using a 40 megacycle instrument were factors which contributed to the earlier wrong assignment of structure to arundoin,[6] since it is well established that loss of side chain is one of the characteristic fragmentations undergone by steroids and triterpenes in the mass spectrometer.[34,35]

An interesting facet of the NMR spectra of the triterpene methyl ethers which were determined in the course of the present work is the high field at which the signal from the 3α proton occurs. In the original interpretation[6] of the NMR spectrum of arundoin, the absorption at 6·62 τ was concluded to have an intensity of 4 protons, but careful re-examination of the NMR spectrum of arundoin in conjunction with a study of the NMR spectra of the methyl ethers of α-amyrin, β-amyrin, bauerenol and multiflorenol shows that the absorption at ca. 6·60 τ is of intensity 3 protons and so attributable to the O—CH_3 group, whilst a low diffuse absorption, intensity one proton, centred near 7·4 τ and ca. 30 c/s broad must arise from the O—CH< proton. This high field absorption by the 3α proton, which has also been observed in the NMR spectra of arundoin and cylindrin by Dr. S. Natori to whom we are indebted for this information, is to be compared with the diffuse low intensity absorption of the 3α proton at 6·8 τ in α-amyrin and β-amyrin and at ca. 5·6 τ in 3β-acetoxy triterpenes,[36] and must be attributed to a high degree of shielding by the methoxyl group.

EXPERIMENTAL

All $[\alpha]_D$'s were measured at 20° using a Bellingham Stanley Pepol 60 instrument. The mass spectra: A.E.I. M.S.9 double-focusing mass spectrometer using a direct inlet system, except in the case of arundoin where the mass spectrum was determined both with the direct inlet system and with a heated inlet system. The energy of ionizing electrons was 70 V, the ionizing current was 100 μA and the source temp was 150°. GLC: A standard Pye Panchromatograph, giving preheating of the argon carrier gas and fitted with standard glass tubes, containing the column packing, of 5 feet in length and internal diameter ca. $\frac{1}{16}$ inch. The detector was the standard Lovelock argon ionization type, fitted with a ^{90}Sr source and the current from the detector was fed into a Honeywell Brown (Newhouse, Lanarkshire, Scotland) pen recorder with sensitivity 0–10 mV.

[33] R. A. Friedel and A. G. Sharkey, *Analyt. Chem.* **28**, 940 (1956).
[34] R. I. Reed and P. de Mayo, *Chem. & Ind.* 1481 (1956).
[35] H. Budzikiewicz and C. Djerassi, *J. Amer. Chem. Soc.* **84**, 1430 (1962).
[36] M. Sharma, R. E. Glick and R. O. Mumma, *J. Org. Chem.* **27**, 4512 (1962).

Direct injections (0·2–0·3 μl. of a chf soln of the comps under investigation) were made on to the column through a silicone-rubber 'blind hole' stopper with a 1 μl. syringe (Hamilton Co. Inc. Whittier, Calif., U.S.A.). Standard conditions were as follows: column temp, 240 ± 1°; detector temp, 248 ± 1° except in the case of the QF-1 columns where it was 225 ± 1°; argon flow rate, 60 ml/min at outlet (inlet pressure 10–12 lb/in²); nominal detector voltage, 1000 V; sensitivity setting, 1×10^{-6} amp.

The instrument employed in the preparative gas liquid chromatographic work was an Aerograph-A.90.P$_3$ (Wilkens Instrument and Research Inc., Walnut Creek, California) using He as the carrier gas and fitted with standard Cu-tubes, containing the column packing, of 10 feet in length and internal diameter ca. ¼ inch. The detector was of the thermal conductivity type and the current from the detector was fed into a Kent Mark 3 recorder with sensitivity 0–10 mV.

Direct injections (15 to 20 μl.) of a chf soln of the compds under investigation were made on to the column through a silicone-rubber 'blind hole' stopper with a 50 μl. syringe (Hamilton Co. Inc., Whittier, California, U.S.A.). Standard conditions were as follows: column temp, 280 ± 1°; detector temp, 315 ± 1°, He gas flow rate, 100 ml/min, at outlet, filament current 195 milliamps, attenuation 32.

Preparation of columns. Column packings for the Pye Panchromatograph were prepared on the silane-treated support, Gas-Chrom Z (Applied Science Laboratories Inc., State College, Pennsylvania, U.S.A.) of 100–120 mesh. The coating with stationary phase was achieved by weighing out the required quantity of the desired stationary phase, viz. Apiezon L grease (Edwards High Vacuum Ltd., Manor Royal, Crawley, Sussex, U.K.); silicone polymer, SE-30, (General Electric Co., Schenectady, N.Y., U.S.A.); fluorosilicone polymer, QF-1 (FS-1265) (Wilkens Instrument and Research Inc., Walnut Creek, California, U.S.A.); or cyclohexane dimethanol succinate, CDMS (Applied Science Laboratories Inc., State College, Pennsylvania, U.S.A.), dissolving in AnalaR chf and adding the support to the soln so obtained. The chf was then removed by distillation *in vacuo* at 100° with the minimum of agitation and the coated supporting phase further dried for 1 hour *in vacuo* at 100°. Column packings so prepared contained 0·5% (w/v) Apiezon L, 1·5% (w/v) SE-30, 1·5% (w/v) QF-1 and 1·0% (w/v) CDMS.

The glass tubes were then filled with the required column packing with repeated gentle tapping. Before any freshly packed column was used for chromatography it was stabilized by heating at 250° for 24 hr in a slow stream of argon.

Column packings for the Aerograph-A.90.P$_3$ were prepared on the silane-treated support Gas-Chrom Z (Applied Science Laboratories Inc., State College, Pennsylvania, U.S.A.) of 100–120 mesh. The coating of the stationary phase was achieved by suspending the support in 100 ml of 3% SE-30 in toluene and applying a gentle vacuum to remove occluded air. After 15–20 min the suspension was poured into a Buchner funnel with gentle suction—the vacuum being released as soon as filtrate ceased to flow. The moist support was transferred to a filter paper, and after air-drying it was dried in an oven at 80° for 6 hr.

The Cu-column used for the packing was treated with dichlorodimethylsilane in toluene, and then washed well with toluene and MeOH and dried before use. The column was packed by gradual addition of the coated support and repeated tapping. Columns thus prepared were coiled and stabilized before use, by heating at 300° for 24 hr in a slow stream of argon.

Determination of retention data. Measurements of retention times on the Pye Panchromatograph were made between the first displacement of the recorder pen after the injection and the point corresponding to the peak of the response to the compound concerned. The recorded response to the injection was observed 20–25 sec after the moment of injection and coincided with the return of the outlet flow rate from an elevated level (due to the press wave from the evaporation of chf) to 60 ml/min. 5α-Cholestane was included in most solutions used to measure retention times and these were expressed as ratios relative to 5α-cholestane, but in a number of experiments arundoin was employed as a secondary reference standard. Good agreement between the relative retention times determined with each standard was obtained, within the limits ±0·05 for the Apiezon L columns ±0·07 for the SE-30 columns, ±0·10 for the QF-1 columns and ±0·15 for the CDMS columns.

The efficiencies of all columns, except the Apiezon L column which gave theoretical plate values of over 2,200 for all compounds studied, were poor and it is to be concluded that SE-30, QF-1 and CDMS supported on Gas Chrom Z are not suitable stationary phases for the gas liquid chromatography of triterpene methyl ethers.

Triterpene methyl ethers. The cylindrin employed in the gas liquid chromatographic studies was kindly provided by Dr. S. Natori, National Institute of Hygenic Sciences, Tokyo, Japan, to whom the present authors wish to express their appreciation. They also wish to cordially thank Dr. S. Abe, Yamazaki Works, Japan for gifts of miliacin, isomiliacin, sawamilletin and isosawamilletin, and Dr. C. J. W. Brooks of the University of Glasgow for a gift of taraxerol.

The methyl ethers of multiflorenol and bauerenol were prepared from multiflorenol and bauerenol (isolated from the bark of *Gelonium multiflorum* A. Juss by the procedure of Sengupta and Khastgir)[37] by the same procedure as was used in the preparation of the methyl ethers of taraxerol, β-amyrin and α-amyrin, through adaptation of the method of Morice and Simpson.[35]

Triterpene alcohol (400 mg) and potassium sand (400 mg) were stirred in dry benzene (5 ml) at room temp under N for 3 hr. MeI (1 ml) in dry benzene (2 ml) was added every 2 hr with refluxing for 12 hr. MeOH was added to decompose unreacted K, water added and the benzene layer separated and washed with water. The solid residue obtained from the organic layer, on removal of the solvent was subjected to IR spectral analysis in CCl_4 soln, to ensure the absence of OH absorption, and then crystallized from AcOEt to constant m.p.

In this way were prepared in ca. 90% yield:

Multiflorenol methyl ether, m.p. 190–193°, $[\alpha]_D = +32$ ($c = 1{\cdot}9$ in chf), ϵ 4,300 at 205 mμ. (Found: C, 84·4; H, 11·4. $C_{31}H_{52}O$ requires: C, 84·5; H, 11·9%.)

Bauerenol methyl ether, m.p. 212–215°, $[\alpha]_D = -32$ ($c = 1{\cdot}2$ in chf), ϵ 4,100 at 205 mμ. (Found: C, 84·6; H, 11·9. $C_{31}H_{52}O$ requires: C, 84·5; H, 11·9%.)

Methyl ether of α-amyrin, m.p. 221–223°, $[\alpha]_D = +92$ ($c = 2{\cdot}0$ in chf). Lit.,[35] m.p. 221–222°, $[\alpha]_D = +93$ (in chf). Methyl ether of β-amyrin, m.p. 247–248°, $[\alpha]_D = +98$ ($c = 2{\cdot}0$ in chf). Lit.,[8,35] m.p. 247–248°, $[\alpha]_D = +98$ (in chf). Taraxerol methyl ether, m.p. 276–278°. Lit.,[8,5] m.p 278°.

Isolation of arundoin and sawamilletin from Cuban sugar cane wax

Substance W (4·68 g), isolated as previously described[17] by silica gel chromatography (4 columns) of the "unsaponifiable Fraction II" (92 g) obtained from the oily portion of Cuban sugar cane leaf wax (700 g), was dissolved in light petroleum (b.p. 70–80°; 100 ml) and rechromatographed over silica gel (VEB Laborchemie Apolda; 1 kg). Elution with light petroleum (b.p. 70–80°)–benzene in the ratios 7:3 (1 l) and 1:1 (3·1 l) gave 205 fractions of 20 ml. Fractions 110–205 contained Substance W (3·4 g, over-all yield 2·3% of the total unsaponifiable material), a sample of which (300 mg) was crystallized from benzene–MeOH: m.p. 196–210°, $[\alpha]_D^{18} = +7{\cdot}9$ ($c = 1{\cdot}3$ in chf).

The so-obtained substance W (40 mg) was dissolved in chf (0·2 ml) and the soln automatically injected, 15 to 20 μl. at a time, onto a 3% SE-30 column in the Aerograph-A90.P$_3$ instrument. The fractions corresponding to the two well resolved peaks on the trace were collected as they eluted from the column in capillary glass tubes. After 8 cycles, were obtained Fraction A, corresponding to Peak I in Fig. 1a, (10 mg) and Fraction B, corresponding to Peaks II and III in Fig. 1a, (12 mg).

Fraction A had m.p. 276–278° which was undepressed on admixture with authentic taraxerol methyl ether. Further identification of Fraction A as taraxerol methyl ether was achieved through comparison of IR and mass spectra.

Fraction B had m.p. 235–236° which was undepressed on admixture with authentic arundoin. The small amounts of the material corresponding to Peak II of Fig. 1 which were present had no perceptible influence on either the mass spectrum or the IR spectrum of Fraction B, which were identical with those of authentic arundoin.

[37] P. Sengupta and H. N. Khastgir, *Tetrahedron* **19**, 123 (1963).