Phytochemistry, 1966, Vol. 5, pp. 1349 to 1352. Pergamon Press Ltd. Printed in England

SHORT COMMUNICATION

A GAS-LIQUID CHROMATOGRAPHIC AND SPECTROMETRIC IDENTIFICATION OF 2-METHYLDOTRIACONTANE

A CONSTITUENT OF THE LEAF WAX OF *AEONIUM LINDLEYI* (CRASSULACEAE)

G. Eglinton, R. J. Hamilton,* W. B. Kelly† and R. I. Reed

Chemistry Department, The University, Glasgow W.2

(*Received* 23 *March* 1966)

Abstract—A hydrocarbon, isolated gas-liquid chromatographically from the leaf wax of *Aeonium lindleyi* (Crass.) has been shown by an i.r. and mass spectrometric examination to be 2-methyldotriacontane.

Mold, Stevens, Means and Ruth[1] have shown that the paraffins of the tobacco leaf are *n*-, iso- and *ante*-iso-alkanes, thus confirming and extending the work of Carruthers and Johnstone.[2] A tentative identification[3] of the presence of iso-alkanes in the leaf wax of *Aeonium lindleyi* (Crass.) had previously been made on the basis of their retention data on gas-liquid chromatography. Confirmation of this identification has now been achieved by means of i.r. spectroscopy and mass spectrometry.

RESULTS AND DISCUSSION

James and Martin[4] first noted that a plot of the GLC retention times for members of a homologous series against carbon number on a semi-logarithm scale gave a straight line. It was found that the GLC retention times of the compounds in the alkane fraction of *A. lindleyi* leaf wax formed two straight lines when plotted in this way and it was assumed that the two straight lines were formed from the retention times of *n*- and iso-alkanes by analogy with the work of Downing, Kranz and Murray.[5] However, it was realized that other alkane isomers might have retention times which corresponded to *n*- or iso-alkanes, and the data in Table 1 suggest that 2-methyl and 3-methyl pentadecane[6] (iso- and *ante*-iso-C_{16}) should be inseparable under our conditions. A polar phase is unlikely to afford much improvement.

The total alkane fraction from *A. lindleyi* was accordingly subjected to preparative GLC and the compounds present in the two peaks corresponding in retention time to the reference samples of *n*-C_{33} and iso-C_{33} were collected and subsequently shown to be 99 per cent pure by GLC on an analytical column.

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* Department of Chemistry and Biology, Regional College of Technology, Liverpool.

† Research Department, Unilever Ltd., Colworth House, Bedford House, Bedford.

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Table 1. Gas–liquid chromatographic relative retention times for alkanes

				Relative retention times		
				5% Apiezon L	0·5% Apiezon L	
Carbon numbers	Alkane	b.p. (°C)	Pressure (mm Hg)	154°*	124°*	184°*
16	*n*-Hexadecane	170	760	1·00	1·00	
16	2-Methylpentadecane	165	760	0·82		
16	3-Methylpentadecane	166	760	0·80	0·83	
16	5-Methylpentadecane	163	760	0·72	0·74	
16	5-*n*-Butyldodecane	156	760	0·56	0·52	
25	*n*-Pentacosane	259	15			0·66
26	*n*-Hexacosane	262	15			1·00
26	3-Ethyltetracosane	255	10			0·82
26	1-Cyclopentylheneicosane	264	10			1·52
26	1-Cyclohexyleicosane	265	10			1·52
26	2-Cyclohexyleicosane	256	10			1·35
27	*n*-Heptacosane	270	15			1·52
28	*n*-Octacosane	278	15			2·37

Flow rates were approximately 60 ml/min: boiling points are from Refs. 6 and 8–10; * GLC column temperatures.

The i.r. spectra of the total alkane fraction and of these two components were measured over the CH bending region and compared with similar data for the reference alkanes. The curves in Fig. 1 and the values in Table 2 show that the absorption in the 1400–1500 cm^{-1} region is of a similar pattern for all of the acylic alkanes. Where a cyclohexane ring is present, however, a prominent band at 1449 cm^{-1} ($\epsilon = 160$) appears, due to the bending absorption of the methylenes in the ring. In the 1300–1400 cm^{-1} region the usual *n*-alkane pattern—a medium-strong band at 1379 and much weaker bands at 1367, 1352 and 1340 cm^{-1}—is no longer found if the compound contains a gem-dimethyl group, the absorption at 1379 cm^{-1} being less than that of the two new bands at 1384 and 1367 cm^{-1}. The suspected iso-C_{33} and *n*-C_{33} alkanes collected from *A. lindleyi* proved to have i.r. spectra indistinguishable from those of the pure iso and *n*-reference compounds over the region 1300–1500 cm^{-1}. Similarly, the spectra of the total alkane fractions from *A. lindleyi* and *A. Smithii* showed that a substantial proportion of gem-dimethyl groups is present in these instances.

Mass spectral data were more definitive and clearly supported the identity of the synthetic and natural iso-C_{33} alkanes. The parent peak was about 25 per cent of the abundance of the (parent—C_3H_7)+ ion, which results from the ready cleavage of the molecule to furnish this ion and the C_3H_7 radical.[7] The remainder of the breakdown pattern derives from the regular linear chain of thirty carbons. The *n*-C_{33} alkanes, synthetic and natural, were identical.

CONCLUSIONS

The i.r. absorption and mass spectroscopic data for the two hydrocarbons isolated from the leaf wax of *Aeonium lindleyi* are in agreement with the GLC data that two hydrocarbons

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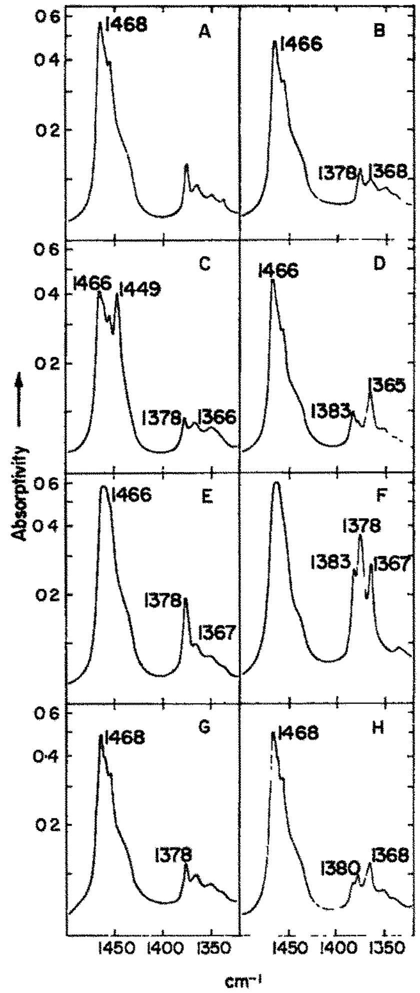

FIG. 1. INFRA-RED ABSORPTION FOR ALKANES AND PLANT ALKANE FRACTIONS IN THE C—H BENDING REGION (1500–1320 CM^{-1}) IN CCl_4 SOLUTION (2 MM CELLS).

A. *n*-hexacosane (0·122 M); B. *n*-dotriacontane (0·0082 M); C. 1-cyclohexyleicosane (0·012 M); D. 2-methyldotriacontane (0·0076 M); E. 3-ethyltetracosane (0·0126 M); F. 2,6,10,15,19,23-hexamethyltetracosane (squalane, 0·0092 M); G. alkane fraction from *Monanthes pallens* (3·80 mg in 1 ml); H. alkane fraction from *Aeonium smithii* (3·88 mg in 1 ml).

are *n*-C_{33} and iso-C_{33}. It seems likely, therefore, that the major constituents of the alkane fraction of the leaf wax *A. lindleyi* are *n*-alkanes and that the compounds which accompany the odd carbon number *n*-alkanes are iso-alkanes. It was not possible, due to lack of material, to test the authors' previous suggestion[3] that the even carbon number hydrocarbons which accompany the even-numbered *n*-alkanes in plant waxes might be largely *ante*-iso-alkanes. If this should prove to be the case the situation would then parallel that for tobacco wax!

EXPERIMENTAL

The leaf wax of *Aeonium lindleyi* (Crass.) was extracted with chloroform and the hydrocarbon fraction obtained by alumina chromatography as previously described.[3] Two

TABLE 2. INTENSITIES OF THE PRINCIPAL C—H BENDING BANDS $\delta(CH_2)$–$\delta(CH_3)$ IN THE INFRA-RED SPECTRA OF ALKANES IN THE REGION 1500–1330 cm^{-1}

Carbon number	Alkane or alkane fraction	1467	1458	1384	1379	1367	1352	1340
16	*n*-Hexadecane	155	110		45	20	15	10
16	2-Methylpentadecane	175	105	50	35	60	15	15
16	3-Methylpentadecane	150	120		70	*k*	15	10
16	5-Methylpentadecane	155	120		75	*k*	15	15
16	7-*n*-Propyltridecane	150	110		65	*k*		15
16	5-*n*-Butyldodecane	165	130		75	*k*		15
26	*n*-Hexacosane	230	155		50	30	20	20
26	3-Ethyltetracosane	225	*k*		70	35	25	15
26	1-Cyclopentylheneicosane	180	135		30	25	20	15
26	1-Cyclohexyleicosane	170	130*		35	30	25	
26	2-Cyclohexyleicosane	160	125*		60	30	20	
28	*n*-Octacosane	240	160		50	30	25	20
30	*n*-Triacontane	250	170		50	35	25	20
30	Squalane	260		105	150	110		25
32	*n*-Dotriacontane	270	180		50	35	25	20
33	2-Methyldotriacontane	285	170	50	35	70	30	20
Total alkane fraction† from								
	A. smithii	265	170	35	45	55	25	20
	M. laxiflora	280	185	*m*	55	40	30	25
	A. caespitosum	330	220	*m*	60	55	35	25
	A. spathulatum	280	185	*m*	55	50	35	25
	M. pallens	275	185		50	40	30	20

Actual ν_{max} values are given to ± 2 cm^{-1} except for a few compounds where they are slightly outside this ± 2 cm^{-1} limit. ϵ_a data for carbon tetrachloride solutions, generally as approx. 0·01 M solutions in 2-mm cells (cf. Fig. 1) approximated to the nearest 5 units (l/mole × cm), and are calculated relative to a solvent–solvent baseline.

* Prominent additional band at 1449 cm^{-1} (ϵ_a approx. 160) due to bending adsorptions of the methylenes of the cyclohexene ring.

† ϵ_a values for these alkane fractions are calculated on an average mol. wt. value estimated from the GLC percentages.[3]

k—This peak is not resolved.

m—No peak can be seen in this region. *A. Aeonium*, *M. Monanthes* spp.

alkanes, which were believed to be iso-C_{33} and *n*-C_{33} from their gas–liquid chromatography (GLC) relative retention times, were isolated from this fraction by preparative GLC at 230° on a 4 ft × 2 cm column of Celite (80–100 mesh) coated with 1% SE30 (Pye Argon Gas Chromatograph). Repeated passage of the crude alkane mixture (1–2 mg in chloroform per pass) was necessary to obtain sufficient quantity of the individual hydrocarbons.

The pure reference alkanes, the alkane fractions of certain leaf waxes and the alkanes which had been isolated as above were examined (i) by gas–liquid chromatography using a Pye Argon Gas Chromatograph and Apiezon "L" as stationary phase (Table 1), (ii) by i.r. spectroscopy over the CH bending region (1500–1330 cm^{-1}) using a Unicam SP 100 prism grating double-beam spectrophotometer (Table 2 and Fig. 1) and (iii) by mass spectrometry using an A.E.I. MS2 spectrometer with heated ion source and direct injection.

Acknowledgements—The authors thank Professor J. P. Wibaut of the University of Amsterdam and Professor W. Dixon of the Pennsylvania State University for some of the pure standard hydrocarbons and acknowledge with thanks the gift of 2-methyldotriacontane from Shell Ltd., Thornton Research Station. The authors are indebted to Mrs. F. Lawrie for the i.r. measurements and assistance in processing the data.