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ALKANES OF TUBERS OF *DIOSCOREA DELTOIDEA*

ROLAND HARDMAN and K. R. BRAIN*
Pharmacognosy Group, School of Pharmacy, Bath University of Technology, Bath BA2 7AY

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Abstract—The hydrocarbon content of the dried tuber of *Dioscorea deltoidea* was examined; straight-chain alkanes predominated. External alkanes were very low (0·001%, w/w) whilst internal ones were high (0·019%, w/w). Additional and different alkanes (0·001%, w/w) were produced on acid hydrolysis and large amounts of other alkanes (0·042%, w/w) by incubation with water before acid hydrolysis. Incorporation of acetate-2-^{14}C (0·038%), L-valine-^{14}C (U) (0·019%) and L-leucine-^{14}C (U) (0·002%) into the hydrocarbon of the tuber during incubation showed that biosynthesis was occurring and a dynamic role for hydrocarbons in plant metabolism is indicated.

INTRODUCTION

ALKANES are widely distributed in the plant kingdom as major constituents of surface waxes where they are said to have a protective function.[1] In addition alkanes have been reported in other sites in plants[2,3] but their function is unknown.

Examination of the seed hydrocarbons of the sapogenin-affording species *Balanites aegyptiaca* (*B. roxburghii*) and *B. pedicellaris* has shown a changing pattern of alkane during fruit ripening[4] and incubation of tissue of sapogenin-yielding plant material with the branched chain saturated hydrocarbon squalane increases the sapogenin yield.[5]

The purpose of the present investigation was to define the hydrocarbons normally present in the sapogenin-yielding tuber of *Dioscorea deltoidea* (Dioscoreaceae) and to determine the effect of the normal sapogenin extraction procedure on the hydrocarbon fraction.

RESULTS AND DISCUSSION

External Hydrocarbon

An estimate of the amount of hydrocarbon present in the cuticular layer was obtained by rapidly defatting the external surface of whole pieces of dried tuber with chloroform. This yielded 0·001% of hydrocarbon (Table 1a and Fig. 1a) in which there were considerable amounts of all the *n*-alkanes between C_{18} and C_{26}, with smaller amounts of C_{27}–C_{33}.

Total 'Free' hydrocarbon

The total 'free' hydrocarbon (external and internal) was determined by extracting the powdered tuber to exhaustion with light petroleum (b.p. 40–60°). This gave 0·02% of 'free'

* Present address: Welsh School of Pharmacy, UWIST, Cardiff CF1 3NU, Wales.

[1] A. G. DOUGLAS and G. E. EGLINTON, in *Comparative Phytochemistry* (edited by T. SWAIN), p. 57, Academic Press, London (1966).
[2] P. G. GULZ, *Phytochem.* **7**, 1009 (1968).
[3] R. E. GRICE, H. D. LOCKSLEY and F. SCHEINMANN, *Nature* **218**, 892 (1968).
[4] R. HARDMAN, C. N. WOOD and E. A. SOFOWORA, *Phytochem.* **9**, 1087 (1970).
[5] R. HARDMAN, to National Research Development Corporation, *Brit. Pat.* 1,136,626 (1968).

TABLE 1. DISTRIBUTION OF ALKANES IN TUBER TISSUE OF *D. deltoidea*

Carbon No.	mole % of total alkane					
	(a)	(b)	(c)		(d)	
14	—	—	—		0·4	
15	—	—	—		0·4	
16	—	0·4	—		1·0	
17	—	2·7	—		1·5	
18	3·0	4·5	0·4		1·8	
19	4·0	3·9	0·3		0·3	
20	4·3	3·8	0·5		1·8	
21	3·0	3·6	0·4		2·0	
22	2·5	2·1	0·7		2·8	
23	2·8	3·1	1·4		3·4	
24	2·2	1·2	1·1		3·8	0·2*
25	3·1	2·5	1·9		3·2	0·3*
26	1·9	0·8	1·8		2·7	0·4*
27	1·3	1·0	2·1	0·7*	1·7	0·5*
28	1·2	1·4	2·4		1·3	0·2*
29	1·3	0·9	2·7		0·8	
30	0·7	0·4	2·0		0·6	
31	0·1	0·6	2·0		0·4	
32	0·02	—	1·4		0·2	
33	0·04	—	0·9		0·2	
34	0·02	—	0·4		0·1	
35	—	—	0·2		—	
36	—	—	0·2		—	
37	—	—	0·1		—	
38	—	—	0·1		—	

GLC analysis using stainless steel, 5% SE30 on AW DMCS Chromosorb W, programmed from 120° to 300° at 2°/min.

(a), External hydrocarbon (0·001%); (b) total 'free' hydrocarbon (external + internal) (0·020%); (c), hydrocarbon afforded by acid hydrolysis (0·001%); (d), hydrocarbon afforded on incubation and acid hydrolysis (0·042%).

* Branched chain species.

hydrocarbon (Table 1b and Fig. 1b) and GLC examination showed approximately equal quantities of the C_{18}–C_{21}, C_{23} and C_{25} *n*-alkanes, together with smaller amounts of all the other *n*-alkanes from C_{16} to C_{31}. This distribution differed from that of the external (surface) hydrocarbon in that the chain-lengths were, on the whole, shorter, and in addition the external hydrocarbon accounted for only 5% of the total 'free' hydrocarbon.

Herbin and Robins[6] have demonstrated profound differences in kind between the hydrocarbon patterns for the external and internal leaf lipids of *Solanum grandiflora*, *Allamanda cathartica*, *Brassica oleracea*, and *Monstera deliciosa*. Whilst the external hydrocarbon showed the C_{29} and C_{31} *n*-alkanes as the major components the internal lipids showed a 'flat' distribution at lower chain lengths of C_{23}–C_{31}, with no alternation of odd and even peak heights. Kaneda[7] has examined the leaf hydrocarbon of *Spinacia oleracea* and isolated 0·06% of external alkane, of which 58–75% was *n*-C_{31}, and 0·0004% of internal alkane in which the chain lengths were as for the external lipids except that

[6] G. A. HERBIN and P. A. ROBINS, *Phytochem.* **8**, 1985 (1969).
[7] T. KANEDA, *Phytochem.* **8**, 2039 (1969).

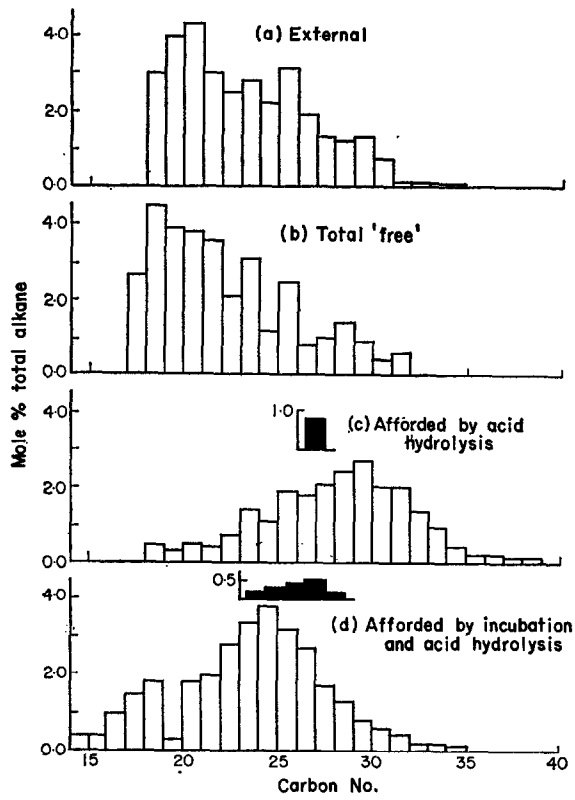

FIG. 1. DISTRIBUTION OF ALKANES IN TUBER TISSUE OF *D. deltoidea*.
GLC analysis using stainless steel, 5% SE30 on AW DMCS Chromosorb W, programmed from 120° to 300° at 2°/min. Shaded areas = distribution of branched chain species.

there was also material of lower chain length (C_{16}–C_{28}) which showed no alternation of peak heights.

Most of the work on plant hydrocarbons has centred on leaf lipids and no examination of underground organs has been reported. It can be seen that the quantity of external hydrocarbon in the tuber of *Dioscorea deltoidea* is very low, but this is in accord with the accepted function of cuticular wax in decreasing the passage of water, a factor disadvantageous in an underground organ. On the other hand, the internal hydrocarbon was fifty times greater than that reported by Kaneda[7] for spinach leaf although it showed a similar lack of alternation of odd and even carbon numbers.

Hydrocarbon Afforded by Acid Hydrolysis

A portion of the defatted plant material from the above tuber tissue extraction was subjected to the acid-hydrolysis procedure normally used for the isolation of steroidal sapogenin, and the hydrocarbon fraction isolated to give a further 0·001% of material. The distribution of alkane in this fraction (Table 1c and Fig. 1c) was completely different from that obtained by the defatting process. The maximum was at C_{29} with an even gradation in

peak size of the alkanes from C_{18} to C_{38}. In addition about 2·5% of the response was due to a peak falling between the C_{26} and C_{27} *n*-alkanes.

This hydrocarbon fraction, which was isolated after acid treatment, must have been bound in some way to prevent its initial extraction. Whilst it is possible to postulate physical separation or bonding in a form inaccessible to the hydrocarbon solvent, it seems also possible that this material arose during hydrolysis from precursors.

Hydrocarbon Afforded on Incubation and Acid Hydrolysis

The remainder of the defatted plant material was incubated in the presence of an excess of water at 37° for 96 hr, before being subjected to the sapogenin extraction procedure and isolation of the hydrocarbon (Table 1d and Fig. 1d). In this case the yield was 0·042% and, although the general shape of the distribution graph was similar to that obtained from the portion which was not incubated before hydrolysis, the maximum was shifted to C_{24} and there were considerable amounts of alkanes of lower chain length (C_{14}–C_{18}) and branched species were evident between C_{23} and C_{28}. As the amount of hydrocarbon which could be isolated from the defatted tuber powder was forty times greater after incubation it seemed likely that synthesis of hydrocarbon could be taking place during the incubation period.

The biosynthesis of plant surface hydrocarbons has been extensively studied by Kolattukudy[8] who has shown an elongation-decarboxylation pathway involving the addition of C_2 units to a fatty acid chain. It has already been noted[4] that the seed hydrocarbons of *Balanites* change on ripening and that as ripening proceeds it is the lower, and not the higher, chain-lengths which predominate. On incubation of defatted powdered tuber of *D. deltoidea* there is a rapid production of over double the normal quantity of hydrocarbon but the distribution of chain lengths is markedly different to that of the normal external and internal hydrocarbons.

When acetate-2-^{14}C was incubated in the presence of dried powdered tuber tissue of *D. deltoidea* at 37° for 96 hr incorporation into the hydrocarbon fraction was 0·038%, whilst the less direct precursors L-valine-^{14}C (U) and L-leucine-^{14}C (U) showed the lower incorporations of 0·019% and 0·002%, respectively. The relatively high specific activities of these small quantities of hydrocarbon and the changes in the pattern observed under different conditions support the hypothesis of a dynamic role for hydrocarbons in plant metabolism.

EXPERIMENTAL

Plant Material

The tuber was obtained as *Dioscorea deltoidea* Wallich from Seth Panchi Ram & Company, Kuth Growers, Manali, Kulu Hills, India. Macroscopically the rhizome agreed closely with the description of Prain and Burkill[9] for *D. deltoidea*. The dried material was reduced in a disintegrator to a coarse powder with a moisture content of 5·5%.

General Procedures

Solvents were redistilled before use, no stop-cock grease was employed and all standard precautions against contamination of the plant extracts with petroleum hydrocarbons were observed.

Isolation of External Hydrocarbon

Dried tuber, 200 g, in whole form, was placed in a 2 l. beaker. $CHCl_3$, 1 l., was poured over it and the whole swirled for 30 sec before filtration. The filtrate was evaporated to dryness and the residue was taken up in 2 ml of *n*-hexane and applied to an alumina column, 5 × 1 cm, packed in *n*-hexane. The column was

[8] P. E. KOLATTUKUDY, *Biochem.* **4**, 1844 (1965), **5**, 2265 (1966); *Phytochem.* **6**, 963 (1967); *Science* **159**, 498 (1968); *Plant Physiol.* **43**, 375 (1968).

[9] D. PRAIN and I. BURKILL, *Ann. R. Bot. Gdn. Calcutta* **14**, 1 (1936).

eluted with *n*-hexane and the first 2 ml of eluate collected and evaporated to dryness to yield 2 mg of a waxy solid.

Isolation of Total 'Free' Hydrocarbon (External + Internal)

Powdered tuber, 1100 g, was extracted in a soxhlet with 15 l. of petroleum (b.p. 40–60°) for 96 hr. Removal of the solvent under vacuum yielded 9·5 g of a brownish oil which was saponified by boiling under reflux for 1 hr with 100 ml of N/2 alc. KOH. Water, 200 ml, was added and the liquid extracted, whilst warm, with 5 × 200 ml of ether. The ethereal solution was washed with water, N/2 aq. KOH, and again three times with water. All the ethereal extracts were bulked and evaporated to dryness under reduced pressure to give 3·01 g of brownish-yellow unsaponifiable matter. This was chromatographed on an alumina column, 10 × 2·5 cm, and eluted with *n*-hexane. The first fraction, 287 mg, was a colourless oil. It was shown to have the TLC characters of a saturated hydrocarbon when compared with reference tetracosane on silica gel G plates eluted with *n*-hexane. In addition it showed a fluorescence in u.v. light, when sprayed with 0·1%, w/v solution of Rhodamine 6G, similar to the reference tetracosane.

Isolation of Hydrocarbon Afforded by Acid Hydrolysis

The powdered defatted material obtained from the isolation of the total 'free' hydrocarbon was dried under vacuum for 16 hr before division into two equal portions. One of these was hydrolysed with 4·8 l. of 2N HCl for 2 hr. The mixture was cooled, filtered at the pump and the insoluble matter was neutralised with 5 l. of 10% NH_4OH. The washed residue was dried before extraction to exhaustion with petroleum (b.p. 40–60°) and work-up as for the total 'free' hydrocarbon to yield 206 mg of an oily semi-solid.

Isolation of Hydrocarbon Afforded by Incubation and Acid Hydrolysis

The second portion of defatted tuber was incubated in the dark at 37° for 96 hr with 4 l. of water before addition of acid and work-up as above to yield 220 mg of an oily liquid.

GLC Examination of Hydrocarbon Fractions

All the analyses were carried out on a Varian Aerograph 1527-C on dual 52 cm × 0·32 cm o.d. stainless steel 5% SE30 on AW DMCS Chromosorb W columns programmed from 120° to 300° at 2°/min. Injection temp. 235°; detector temp. 325°; detector dual f.i.d.; carrier N_2. A tentative carbon number was assigned to each peak on the basis of the retention times of co-injected standards and the peak areas measured from peak height × peak width at half peak height.

Incorporation of Radioactive Precursors

Sodium acetate-2-^{14}C, 25 μc, was incubated in water with 2·5 g tuber powder for 96 hr, after which the mixture was subjected to acid hydrolysis and the hydrocarbon fraction was isolated as described above, to yield 5 mg with a specific activity of 4 × 10^3 dis/min/mg.

Similarly L-valine-^{14}C (U), 50 μc, and L-leucine-^{14}C (U), 50 μc, were used in incubations with tuber powder and extracted in the same manner to yield 6·44 mg of hydrocarbon of specific activity 3·3 × 10^3 dis/min/mg and 4·60 mg of specific activity 4·74 × 10^2 dis/min/mg, respectively.

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