THE WAX HYDROCARBONS OF *SCUTELLARIA LATERIFLORA* L.

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Although the value of alkanes in chemotaxonomy has been discussed by several authors (1–5), only the waxes of two species from the family Labiatae have been investigated (6–7). Therefore, as part of a phytochemical investigation of *Scutellaria lateriflora* L., an analysis of the wax hydrocarbons has been carried out to assess whether alkanes might offer an additional or alternative chemotaxonomic character to the volatile oils and iridoids previously considered (8, 9).

RESULTS AND DISCUSSION

The yield of wax from the aerial parts of *S. lateriflora* was 1.2% calculated on the dry weight of the plant material. The hydrocarbon fraction, which constituted 20% of the wax, was separated from the wax esters and acids by column chromatography (6, 7, 10). A preliminary glc analysis of this fraction indicated that the amount of branched alkanes present was small. The low concentration of branched alkanes (1.2% of the total hydrocarbon) was confirmed after separation with molecular sieve. The composition of the *n*-alkane and branched alkane fractions as determined by glc analysis is given in table 1 and table 2, respectively. No unsaturated hydrocarbons were detected.

The hydrocarbons fall in the range C_{23} to C_{37}, the odd carbon number *n*-alkanes being predominant as is the general rule for plant waxes (2, 11). The four major components in the *n*-alkane fraction are *n*-nonacosane (C_{29}, 7%), *n*-hentriacontane (C_{31}, 14%), *n*-tritriacontane (C_{33}, 38%) and *n*-pentatriacontane (C_{35}, 24%). The latter *n*-alkane is not one of the major hydrocarbons in the wax of either *Rosmarinus officinalis* L. (1.8%) or *Marrubium vulgare* L. (2.3%) (6, 7). Although *n*-heptatriacontane (C_{37}) is present only as a small percentage (1%) of the alkane total, it was not detected in the waxes of the two species previously examined.

TABLE 1. n-*Alkanes of the wax from* Scutellaria lateriflora *L.*

Number of carbon atoms	%	Number of carbon atoms	%
C_{23}	trace[a]	C_{30}	1
C_{24}	trace	C_{31}	14
C_{25}	1	C_{32}	3
C_{26}	trace	C_{33}	38
C_{27}	3	C_{34}	6
C_{28}	1	C_{35}	24
C_{29}	7	C_{36}	1
		C_{37}	1

(Weight % determined by glc; calculated as % of total area on the basis of three runs).
[a]Trace: the peaks produced by these hydrocarbons were too small for measurement and have not been included in the total.

The concentration of branched alkanes is low (1.2%), which is considered to be normal for plant waxes (12), whereas the figure calculated for *R. officinalis* is 18% (7). The glc analysis of this fraction showed the presence of three homologous series which were identified as 3,9-dimethyl-(72%), 2-methyl-(18%) and 3-methyl-(10%) alkanes. Therefore, in the wax of this plant the 3,9-dimethyl alkanes are predominant. In *M. vulgare* and *R. officinalis*, the 3,9-dimethyl alkanes constitute, respectively, only

TABLE 2. *Branched alkanes of the wax from* Scutellaria lateriflora *L.*

Number of carbon atoms	3,9-Dimethylalkane	2-Methylalkane	3-Methylalkane
23	trace[a]	trace	trace
24	1	trace	trace
25	trace	trace	trace
26	2	trace	trace
27	3	1	1
28	5	1	1
29	2	2	1
30	27	trace	1
31	3	2	1
32	23	1	3
33	1	5	trace
34	5	trace	2
35	trace	6	trace

(Weight % determined by glc; calculated as % of total area on basis of three runs.)

[a]Trace: the peaks produced by these hydrocarbons were too small for measurement and have not been included in the total.

22% and 2% of the branched alkane total and do not predominate.

The qualitative and quantitative differences observed within the wax hydrocarbons of these species suggest that *n*-alkanes and branched alkanes might be used as chemotaxonomic characters in the Labiatae. To investigate this hypothesis, further species from these and other genera of the family are now being studied.

EXPERIMENTAL

PREPARATION OF THE WAX.—Six hundred grams of the dried, crushed, aerial parts of *Scutellaria lateriflora* L., obtained commercially[1], were placed in a fat-free cellulose Soxhlet thimble and macerated overnight in petroleum ether (40°–60°) prior to continuous extraction with this solvent for eight hours. The wax was recovered by removal of the solvent under reduced pressure.

SEPARATION OF COMPONENTS.—A sample of wax (7.00 g) was applied to a silica gel (Fisons, 80–200 mesh, 200 g) column. The hydrocarbons (1.4 g) were eluted with petroleum ether (40°–60°) (1.5 liters). Chloroform (2.5 liters) was used to recover the esters and free acids from the column.

Molecular sieve was used to separate branched alkanes from the *n*-alkanes (6, 7, 13). A sample of hydrocarbon fraction (1.0 g) was dissolved in 50 ml of warm redistilled iso-octane. The solution was shaken for 6 hours with molecular sieve (Fisons, 5Å, 8–12 mesh; 28.0 g, previously activated at 400°C for 8 hours) and then allowed to stand for a further 16 hours in contact with the sieve. The sieve was removed by filtration and washed twice with 25 ml iso-octane; the branched alkanes were recovered from the combined filtrate by evaporation under reduced pressure. The residue was redissolved in 50 ml iso-octane and the whole procedure repeated. The final yield of branched alkanes was 0.012 g.

THIN LAYER CHROMATOGRAPHY.—Tlc was performed on silica gel plates (Kieselgel G type 60 Merck, 0.25 mm thickness 20 cm) with carbon tetrachloride as solvent. The plates were developed with a 0.05% aqueous solution of Rhodamine 6G prior to examination under uv light (365 nm) (14). By comparison with known standards this technique was used to establish the presence of hydrocarbon, ester, and acid fractions in the wax and to check the purity of the hydrocarbon fraction obtained by column chromatography.

The hydrocarbon fraction was examined for the presence of unsaturated hydrocarbons on silica gel plates impregnated with silver nitrate (20%) (7). After being sprayed with a 0.05% aqueous solution of fluorescein sodium, the plates were examined under uv light (365 nm).

[1]Potters (Herbal Supplies) Ltd., England. Batch 0707. The authenticity of the sample was verified by macroscopical and microscopical examination, comparisons having been made with literature reports (17) and material obtained from the herbarium of the Manchester Museum. The sample consisted of the aerial parts of the plant collected at the fruiting stage.

GAS LIQUID CHROMATOGRAPHY.—Gas liquid chromatograms were run on a Perkin-Elmer model F11 gas chromatograph fitted with an F.I.D. detector with a stainless steel column (1.5 m long, 2.5 mm i.d.) packed with Chromosorb P (60–80 mesh, non acid washed) coated with O.V. 17 (10%). The carrier gas was nitrogen, flow rate 30 ml/min. A column temperature of 280° was used for the analysis of the total hydrocarbon fraction; column temperatures of 240° and 280° were used for the branched alkane fraction in separate isothermal runs. The *n*-alkanes were identified from their log retention times, *n*-tricosane (C_{23}) and a paraffin wax sample as standards (15, 16). Branched alkanes were identified from their log retention data by comparison with authentic samples of 2-methyl pentacosane, 3-methyl heptacosane and 3,9-methyl octacosane (6, 7). The relative areas of the peaks were obtained by multiplying peak heights by width at half height.

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