EPICUTICULAR WAX ALKANES OF SCUTELLARIA LATERIFLORA L.LEAVES

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ABSTRACT

The dried aerial parts of Scutellaria lateriflora L.(family Labiatae),have been used for many years as a domestic remedy for epilepsy,hysteria,and nervous tension states.Although several phytochemical investigations have been made regarding the constituents present in this species(i.e. flavonoids,iridoids),none have been recorded concerning the cuticular wax.

Using thin layer chromatography (TLC), column chromatography,gas-liquid chromatography(GLC),and GLC-mass spectrometry (GLC-MS)the alkanes of the leaves of S. lateriflora collected from Mazandaran province in northern Iran have been analysed.The wax yield was 1.2%.The alkanes fall in the range of C_{23} to $C_{37}$the odd carbon number n-alkanes being predominant. The concentration of branched alkane is low (1.2%).Three homologous series of branched alkanes were identified as 3,9-dimethyl alkanes,2-methyl alkanes and 3-methyl alkanes.

INTRODUCTION

As stated in the British Herbal Pharmacopoiea(1),the scullcap used in herbal medicine consists of the dried aerial parts of Scutellaria lateriflora L.(subfamily Scutellarioideae),within the family labiatae.The plant grows in northern province of Mazandaran in Iran in marshy places and along river banks and lake shores.Detailed morphological description of S.lateriflora are given by several workers(1,2),the main diagnostic features of the plant being that the blue flowers occur as one sided racemes.

This herbal remedy has been used on its own or in combination with Humulus and or passiflora in the treatment of neuralgia,insomnia,hysteria and epilepsy (1-3).

In previous phytochemical investigations,many compounds have been isolated from different plants of the genus Scutellaria which consists of 200 species. The center of attention in these studies has been the isolation and identification of flavonoids and iridoid glycosides, in particular,scutellarin(1,2,4,5)and catalpol glycoside (6).As there were no reports in the literature regarding the components of the leaf waxes of the plants within this genus,the wax alkanes of the leaves of this important medicinal plant is now investigated.

EXPERIMENTAL

Preparation of the wax ;

Six hundred grammes of the dried, crushed leaves of the plant,which were collected at flowering stage and was authenticated by professor A.Zargari,were macerated in chloroform for one minute. The wax was recovered by removal of the solvent under reduced pressure in a previously dried and accurately weighed Buchi flask.

Separation of components;

A sample of wax (7g)was applied on to a silicagel column,Merck,(80-200 mesh). The hydrocarbons(1.4 g) were eluted with petroleum ether (40°-60°).

Thin layer chromatography;

TLC was performed using silicagel plates (kieselgel) G type 60 Merck,(0.25 mm thickness 20 cm)with carbon tetrachloride as running solvent the wax samples being applied to the plates as solution in chloroform.The plates were developed using a 0.05% aqueous solution of Rhodamine 6 G before examination under U.V. light (365nm),Using the reference standards:n-tricosane (C_{23}),n-heptacosane (C_{27}),and spermaceti wax,this technique was used to establish the presence of the 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 hydrocar-

bons using silicagel plates impregnated with silver nitrate (20%).

After spraying with a 0.05% aqueous solution of fluorescein sodium the plates were examined under U.V.light(365 nm).

Separation of n-Alkanes and Branched al - kanes;

Branched alkanes were separated from n-alkanes using molecular sieve(7-9).One gramme of the hydrocarbon fraction was dissolved in 50 ml of warm redistilled iso-octane.The solution was shaken for 6 hours with molecular sieve,Pisons,(5Å ,8-12 mesh;28.0g,previously activated at 400°C for 8 hours) and then allowed to stand for a further 16 hours in contact with the sieve.The sieves were removed by filtration and washed twice with 25 ml iso-octane,the branched alkanes being reco - vered from the combined filtrate by evaporation under reduced pressure.The residue was redissolved in 50 ml iso-octane and the procedure repeated.The yield of branched alkanes was 0.012g.

Gas-Liquid chromatography;

Gas liquid chromatograms were run on a Perkin-Elmer model sigma 300 gas chromatograph coupled to a Perkin-Elmer sigma 15 Data station,fitted with an flame ionization detector,using a stainless steel column(1.5 m long,2.5 mm i.d.)packed with Chromosorb P(60-80 mesh)coated with OV17.The carrier gas was nitrogen having a flow rate of 30 ml/minute.The materials for analysis were dissolved in a small volume of either petroleum ether or ether before injection.The stationary phase concentrations and other conditions used were as follows;

(A)The hydrocarbon fraction-OV17(10%);column 280°C,injector 400°C.Reference compounds :n-docosane(C_{22}),n-tricosane(C_{23}) and n-heptacosane(C_{27}).(Separated from beeswax and identified using MS).

(B) Branched alkane fraction-OV17(10%); column 240°C and 280°C in separate iso - thermal runs;injector 300°C and 400°C. Reference compounds 2-methyl pentacosane, 3-methyl heptacosane,and 3,9-methyl oc - tacosane.

Gas chromatography-Mass spectrometry;

This technique was carried out using a Pye-Unicam Series 104 gas chromatography equipped with a flame ionization using a glass column containing 3% OV17 on Chromosorb P.Column temperature,and other conditions were the same.The carrier gas was helium at a flow rate of 40 ml/minute. The effluent from the column was fed directly into the separator of a keratos-AEI MS 30 double beam mass spectrometer (separator temperature of 220°C).

RESULTS AND DISCUSSION

The yield of wax from the leaves of S. lateriflora was 1.2% based on the dry weight of the plant material.The presence of wax constituents was demonstrated by the use of TLC,showing a typical separation of the wax compounds found in S.lateriflora L.This technique has certain limitations as it does not separate the specific constituents with in an individual class of compound.However,by applying argentative silicagel plates,it can be used to detect the presence of unsaturated alkanes (alkenes)(10,11).No unsaturated hydrocarbons were detected in the wax using this method.

Using column chromatography the alkane fraction was separated.The isolated wax hydrocarbons were analysed by applying gas liquid chromatography , a specimen trace being shown in Fig.1.Two of the n-alkanes were identified using the refe - rence compounds n-tricosane(C_{23})and n-heptacosane (C_{27}).The others were identified by plotting their log retention times against carbon number(12,13).As all the points fall in a straight line(Fig,2.).It

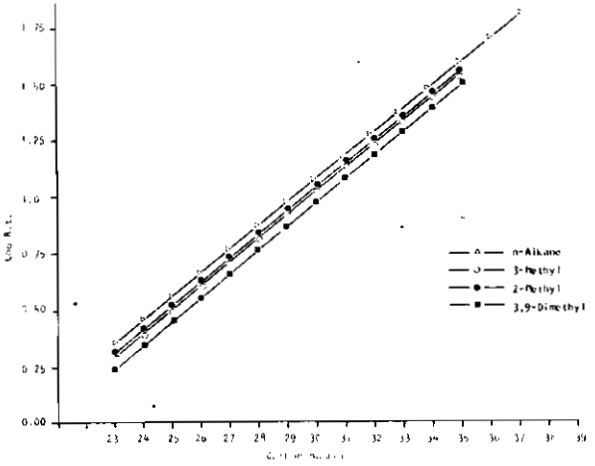

Figure 2:Plot of log retention times against carbon number for the peaks assigned to the n-and branched alkanes of S.lateriflora L.

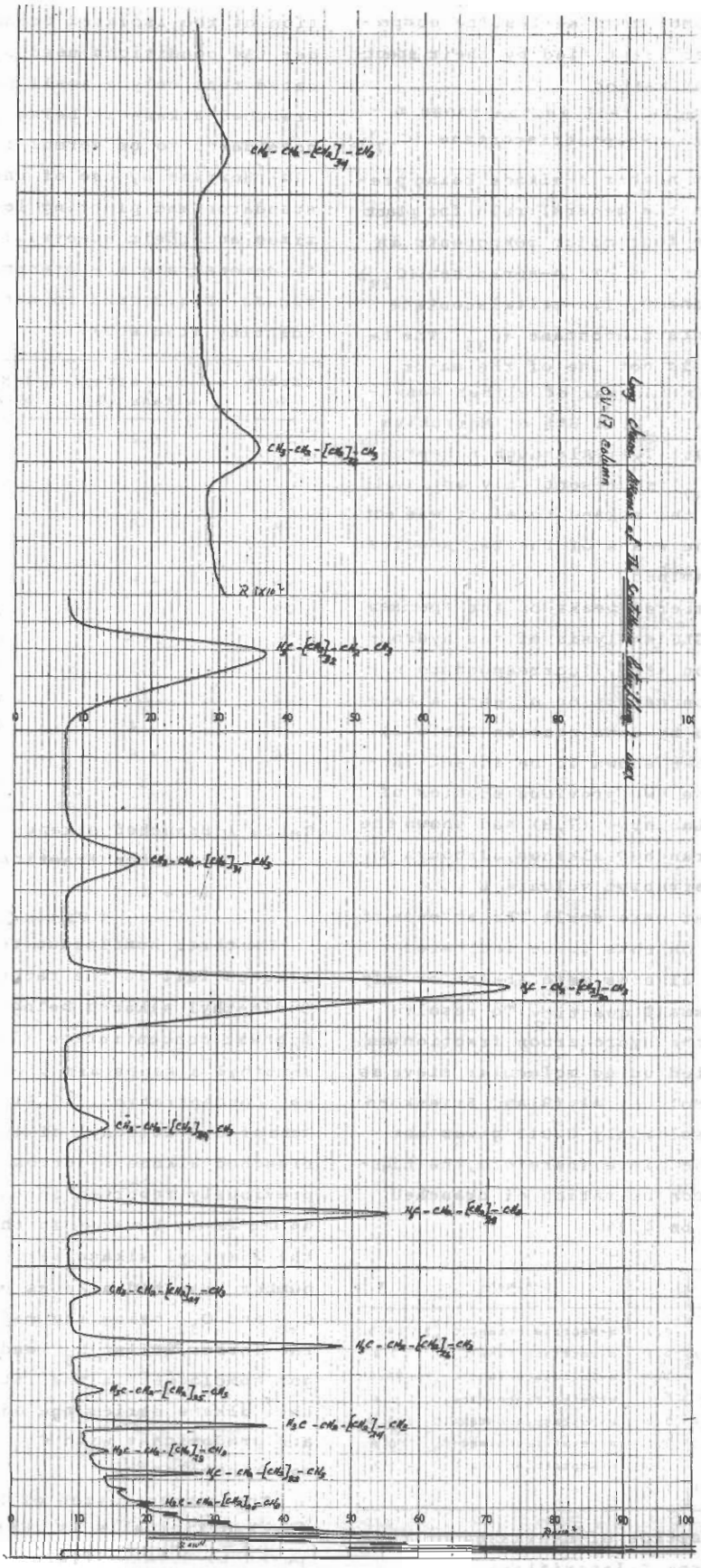

Fig. 1: Gas chromatogram of the wax hydrocarbon fraction of Scutellaria lateriflora L.
Column: OV17 (10%) at 280°

is,suggested that the components belong to the same homologous series.The components were also identified by their spectra using GLC-MS method.

The hydrocarbons fall in the range n-tricosane(C_{23}) to n-heptatriacontane (C_{37}), the odd carbon number n-alkane being predominant as is the general rule for plant waxes (14).The four major components in the n-alkane series are n-nonacosane(C_{29}), n-hentriacontane(C_{31}),n-tritriacontane (C_{33})and n-pentatriacontane (C_{35}).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%)(7,8).Although n-heptatriacontane(C_{37})is present only as a small percentage of the alkane total,it was not detected in the waxes of the two species previously examined.

All the numbered peaks on the specimen trace of the GLC analysis of the hydrocarbon fraction (Fig.1)correspond to n-alkanes.The concentration of each alkane was calculated by data station(Table 1). Alkenes had been shown to be absent using TLC,but the two previous studies of the waxes of Labiatae (7,8) had shown the presence of branched alkanes,although in the case of Marrubium vulgare L.,the amounts present were small.The absence of further peaks on this trace indicated that branched alkanes were absent or only present in a small quantity.To resolve this problem,the hydrocarbon fraction was further separated using molecular sieve,as described by Mold et al.(9)and Brieskorn (7,8).As a result,only 0.012 g was not retained by the sieve,Therefore,the highest possible concentration of branched alkanes would be 1.2%.

n-Alkane	%	n-Alkane	%
n-Tricosane(C_{23})	0.1	n-Hentriacontane(C_{31})	14.0
n-Tetracosane(C_{24})	0.1	n-Dotriacontane(C_{32})	2.4
n-Pentacosane(C_{25})	1.0	n-Tritriacontane(C_{33})	38.0
n-Hexacosane(C_{26})	0.4	n-Tetratriacontane(C_{34})	6.4
n-Heptacosane(C_{27})	3.0	n-Pentatriacontane(C_{35})	24.0
n-Octacosane(C_{28})	0.5	n-Hexatriacontane(C_{36})	0.8
n-Nonacosane(C_{29})	7.0	n-Heptatriacontane(C_{37})	1.0
n-Triacontane(C_{30})	1.2		

Table 1:Composition of n-alkanes of the wax from S.lateriflora L.

A gas liquid chromatographic examination of the isolated branched alkanes under the conditions used as before indicated that only a small percentage of branched alkane is being present,which is considered to be normal for plant waxes (14).However,by use of the reference standards and plotting log retention times and their spectra,it was possible to demonstrate the presence of these compounds (Fig.2) and to determine their identity (table 2).

Carbon No	3,9-Dimethyl alkane (%)	3-Methyl alkane(%)	3-Methyl alkane (%)
23	0.2	0.2	T
24	0.7	T	0.6
25	0.3	0.6	0.2
26	1.8	0.1	0.4
27	3.0	1.3	0.8
28	4.8	1.0	1.1
29	1.7	1.5	1.0
30	26.8	0.6	1.4
31	3.3	1.4	0.7
32	22.6	0.7	2.5
33	1.4	4.6	T
34	4.8	T	1.9
35	T	5.7	T

Table 2:Branched alkanes of the wax hydrocarbon fraction of S.laterif-lora L.

T.Values $<0.1\%$

The three homologous series of branched alkanes fall in the range C_{23}-C_{35},the 3,9-dimethyl alkanes being present in the highest concentration (71.4%)whilst the 3-methyl alkanes are present in the lowest concentration (17.8%).Such a high content of 3,9-dimethyl alkanes within a branched alkane fraction has not been previously reported in the waxes of plants in the family labiatae, In this series and the 3-methyl alkane series,even carbon number branched alkanes predominate,with C_{30} and C_{32} being the major homologues in the former,whilst C_{32} and C_{34} are the major homologues in the latter.In the 2-methyl alkane series,the odd carbon numbers are predominant with C_{33} and C_{35} being the major homologues.

The concentration of branched alkanes for R.officinalis is 18%(7) which compared to S.lateriflora is high.The GLC analysis of this fraction showed the pre-

sence 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-di - methyl alkanes constitute,respectively, only 22% and 2% of the branched alkane total and do not predominate.

CONCLUSIONS

This study indicates that the struc - ture of n- alkanes of the leaf wax is in accordance with other plant waxes.However, detection of C_{35} in high concentration in S.lateriflora wax is interesting as this n-alkane very rarely occurs in plants in considerable amounts.

The low concentration of branched al-kanes and other qualitative and quanti - tative differences between the wax hyd - rocarbons of Scutellaria lateriflora L. and those of the Rosmarinus officinalis L.and Marrubium vulgare L.(7,8),suggest that n-alkanes and branched alkanes might be used in this family as chemotaxonomic characters.

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