EPICUTICULAR WAX ESTERS OF THE LEAVES OF SCUTELLARIA LATERIFLORA L.

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ABSTRACT

Subsequent to phytochemical investigation on Scutellaria lateriflora (Labiatae) from Mazandran province in Iran, the wax ester fraction of the leaves has been investigated. Using TLC, CC GLC and GLC-MS, the alkyl esters of the epicuticular wax was analysed. Total concentration of the esters was 35% and it was shown to be composed of a homologous series having various chain lengths in the range of C_{34}-C_{52} which are compriesed of C_{16}-C_{28} n-alkanols and C_{14}-C_{26} n-acids. All the components are reported for the first time in the family labiatae.

INTRODUCTION

Scutellaria lateriflora L. (Fam. Labiatae) is a plant having blue flowers which occur as one sided racemes and the calyx is companulate. It grows in northern province of Mazandaran in Iran in marshy places, along river banks and lake shores. The aerial parts of this plant has been used as a herbal remedy in the treatment of neuralgia, insomina (1), hysteria, and epilepsy (1,2). It is normally administered as either in tablet form or as tincture.

As a result of the phytochemical investigations which have been carried out previously, the following compounds are detected in S.lateriflora: scutellariarin (1,2,4,5), tannin (2,5), and catalpol glycoside (6). There are several reports concerning the analysis of wax esters in plants of some families (7,9). However, as there were no reports in the literature regarding the wax ester composition of any of species within this genus or even the family Labiatae, the data in the present article is now reported for the first time in this family.

There has been some investigations indicating the isolation of constituents from the genus Scutellaria of which the following can be mentioned; flavonoids in S.albida(10), S.Orientalis (11), S.tournefortii(12); iridoid glycosides in S.ultriculata, S.galericulata, S.versicolor, S.minor, and S.Woronowii (6); and terpensids in the essential oils of S.galericulata and S.parvula (13).

EXPERIMENTAL

Wax extraction

One kilogram of the dried, crushed leaves of the plant, which were co-

llected at flowering stage from Mazandaran province (specimen was authenticated by professor A.Zargari) were macerated in chloroform for three minutes.The epicuticular wax was recovered by removal of the solvent in vacuo.

Separation of components

A sample of wax (7g),which was previously dissolved in hexane, was applied on to a silicagel column, Merck (80-200 mesh).The column was first eluted with hexane (1.5L) to remove hydrocarbons, and alkyl esters was obtained by gradient elution with hexane-$CHCl_3$(95.5) (4L) and(90; 10) (5L).

Thin layer chromatography

TLC was preformed using 0.25 mm silicagel plates (kieselgel G.type 60, Merck) with carbon tetra chloride as running solvent,the wax samples being applied to the plates as solutions in chloroform.The plates were developed using a 0.05 % aqueous solution of Rhodamine 6G before examination under U.V.light(365nm).Using the reference standards; spermacetic wax (which is mainly composed of esters) and pure n-C_{40} ester,this technique was used to establish the presence of alkyl esters.

Isolation of n-alkanols

Using the method of Siegler et al. (14) and Emery and Gear (15),the n-alkanols were isolated from the wax esters.Two hundred grammes of the separated esters were added to a mixture of 33% aqueous potassium hydroxide (4ml),ethylene glycol (40 ml), and toluene (12 ml),and in a 250 ml round bottom flask.The solution was refluxed for 18 hours prior to removal of the toluene by evaproation. Diethyl ether (10 ml) and distilled water (10 ml) were added to residue which had been placed in a separator. After shaking, the phases were separated and the aqueous phase washed twice with diethyl ether (25 ml portions).The ethereal phases were combined and dried over anhydrous sodium sulphate.The mixture was filtered and the ether removed leaving the n-alkanols as a white solid.

Isolation and Methylation of n-Acids

A sample of wax ester (100 mg)was refluxed for three days with methanol (100 ml) and sulphuric acid (2 ml) in a 250 ml round bottom flask. The reaction mixture was concentrated to a small volume under reduced pressure and after transfer to a separator,distilled water (15 ml) and diethyl ether (15 ml) were added.After shaking,the aqueous phase was separated and washed twice with 20 ml portions of diethyl ether.The combined ether extracts were dried over anhydrous sodium sulphate before filtration and removal of the solvent using vacuum. This procedure yielded the n-acid methyl esters as a yellowish semi-solid.Inentical me-

thod was used to prepare methyl n-octadecanoate (stearic acid, (C_{18}) (BDH) and n-docosanoic acid (behenic acid, C_{22}) (kochlight) being used as reference standards. The identity and purity of these compounds were determined using GC-MS method.

Gas Liquid chromatography

Gas liquid chromatograms were run on a Perkin-Elmer model sigma 300 gas chromatograph fitted with an FID detector coupled to a sigma 15 data station using a stainless steel column (1.5 m long, 2.5 mm i.d.) packed with chromosorb p (60-80 mesh) coated with OV-17. 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 condition used were as follows: (1) Esters-OV17 (3%); column 290°C and 330°C in separate isothermal runs; injector 350°C and 400°C. Reference compounds: spermaceti and n-C_{40} ester chain, (2) alcohols and methyl esters-OV17 (3%); column: 165°C and 260°C in separate isothermal runs; injector 250°C and 300°C. Reference compounds (a) alcohols: n-hexadecanol (cetyl alcohol, C_{16}) (BDH) and n-docosanol (behenic alcohol, C_{22}) (kochlight); (b) methyl esters: methyl n-octadecanoate and methyl n-docosanoate.

Gas-Liquid chromatography-Mass spectrometry

This technique was carried out using a keratos-AEI MS 30 double beam mass spectrometer coupled to a computer. The spectra of components were taken at 70 ev. The GLC conditions and the columns used were as previously described.

RESULTS AND DISCUSSION

Leaf waxes of plants have been studied for two principal reasons; interest in the physical properties of the plant surface and interest in composition and commerical applications. Wax of different chemical composition, and so of different physical form (16,17) may affect transpiration and also leaf surface properties of varying extent (18). As wax with useful properties might be derived from plants of temperate regions, a detailed knowledge of the composition of plant waxes from different genera in a family might be taxonomically useful. Literature survey shows that reports on components of plant waxes in the family Labiatae is scarce. Therefore, the composition of wax esters of an important herbal remedy, Scutellaria lateriflora is now investigaced.

The leaves of S. lateriflora were collected from Mazandaran province in northern Iran, when it was at flowering stage, in August. The yield of wax from the leaves was 1.2% based

on the dry weight of the plant material. Preliminary examination using TLC technique revealed that the wax was composed of hydrocarbons, free acids and alcohols and high concentration of esters (R_f 0.5).

Using column chromatography the alkyl esters fraction was isolated as described under experimental. Total concentration of wax esters was 2.5g (35%). On GLC analysis, the wax ester fraction was shown to be composed of esters of varying chain lengths (Table 1). Spermaceti which is mainly composed of esters and authentic C_{40} ester were used as the reference materials. From the literature it can be seen that the longest chain in the ester mixture of spermaceti is the C_{36} homologue (hexadecyl eicosanoate) and that the C_{30} homologue (hexadecyl tetradecanoate) is present in the highest concentration. Using the retention time of these two peaks, the C_{40} sample, and log retention data it can be seen that the esters in the wax under investigation belong to a homologous series (Fig.1). Homologues with an even number of carbon atoms predominated, the range being C_{34}-C_{52}. The principal homolgues were C_{42},C_{44},C_{46},C_{48},C_{50}, and C_{52} comprising 80.2% of the total esters. These chain lengths were within the general range for plant wax esters (19).

Table 1: Retention times of esters from the wax of S.lateriflora L.

Esters	R.t. (min)	Esters	R.t. (min)
$C_{34}H_{68}O_2$	7.2	$C_{44}H_{88}O_2$	39.8
$C_{36}H_{72}O_2$	10.0	$C_{46}H_{92}O_2$	56.0
$C_{38}H_{76}O_2$	14.0	$C_{48}H_{96}O_2$	78.6
$C_{40}H_{80}O_2$	19.6	$C_{50}H_{100}O_2$	110.6
$C_{42}H_{84}O_2$	28.4	$C_{52}H_{104}O_2$	152.2

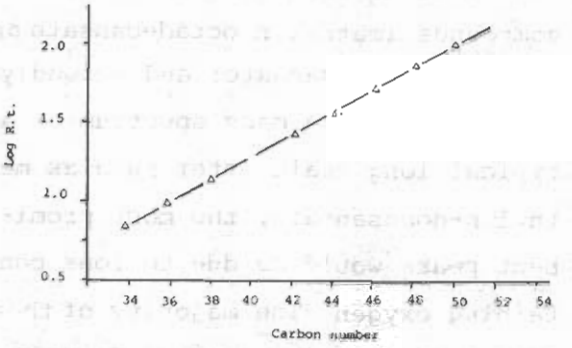

Figure 1: Plot of log retention times against carbon number for the peaks assigned to the esters of S.lateriflora L. wax.

To determine which n-alkanols and n-acids were present in the esters, the fraction was subjected to hydrolysis and methylation. Using log retention data, the n-alkanols produced on hydrolysis were shown to have chain lengths in the range of C_{16} (n-hexadecanol) to C_{28} (n-octacosanol) with n- tetracosanol (C_{24}), n-hexacosanol (C_{26}), and n-octacosanol (C_{28}) being the major homologues (Table 2).

Table 2: Composition of n-alkanols produced on the hydrolysis of the wax esters of S.lateriflora L.

Peak No.	Identity	%	Peak No.	Identity	%
1	n-Hexadecanol	1.9	5	n-Tetracosanol	20.0
2	n-Octadecanol	6.1	6	n-Hexacosanol	28.0
3	n-Eicosanol	7.4	7	n-Octacosanol	33.6
4	n-Docosanol	3.0			

The identity of these n-alkanols was achieved using their mass spectra and authentic samples such as cetyl al-

cohol (C_{16}) and behenic alcohol (C_{22}).

The methyl esters produced by hydrolysis and subsequent methylation were identified in two ways: firstly by the use of log retention time data and prepared reference compounds (methyl n-octadecansate and methyl n- docosanoate) and secondly by GC-MS. In the mass spectrum of a typical long chain ester such as methyl n-docosanoate, the most prominent peaks would be due to ions containing oxygen. The majority of these ions belong to the general formula $CH_3-O-\overset{\overset{O}{\|}}{C}-(CH_2)_n^+$ (e.g.ions m/e 311,297, 283, and particularly 87) containing the intact methoxycarbonyl group. The spectrum should also contain three additional significant peaks, caused by ions containing oxygen; the molecular ion (e.g.M^+,354), the acylium ion $-\overset{\overset{O}{\|}}{C}-(CH_2)_n-CH_3^+$ (e.g.m/e 323,M-31) and the rearranged ion $CH_3-O-\overset{\overset{CH_3}{|}}{C}=CH_2$ (m/e 74).

The mass spectra of the compounds separated by GLC all followed this general fragmentation sequence,(Table 3) and it is concluded that they are methyl esters of acids falling in the range of C_{14}(n-tetradecanoic) to C_{26} (n-hexacosanoic). The three major homologues were n-eicosanoic (C_{20}), n-docosanoic (C_{22}), and n-tetracosanoic (C_{24}) (Table 3) which are common acids in plant waxes (19).

Table 3: Composition and mass spectral data of methyl ester homologues of n-acids produced on the hydrolysis of the wax esters of S.lateriflora L.

Peak No.	M^+(%)	Fragment ions (%)	Identity	%
1	242(30)	211(20),143(55),87(85),74(100)	Methyl n-tetradecanoate	1.8
2	270(25)	239(20),143(48),87(85),74(100)	Methyl n-Hexadecanoate	7.6
3	298(40)	267(21),143(60),87(90),74(100)	Methyl n-Octadecanoate	8.2
4	326(30)	295(18),143(50),87(80),74(100)	Methyl n-eicosanoate	17.6
5	354(35)	323(20),143(54),87(83),74(100)	Methyl n-docosanoate	33.6
6	382(42)	351(25),143(62),87(92),74(100)	Methyl n-tetracosanoate	26.8
7	410(35)	379(26),143(52),87(90),74(100)	Methyl n-hexacosanoate	7.0

In plant waxes, an ester of given chain length can be composed of a combination of acids and alcohols (14,20), thus on the basis of mass spectra, the wax esters of this plant is also composed of several acid alcohol combinations. These are summarized in Table 4.

Table 4:Composition of the wax esters of S.lateriflora L.

Carbon number	Alcohol	Acid	Percentage (GLC)
C_{34}	C_{18}	C_{16}	3.5
C_{36}	C_{16}	C_{20}	6.1
C_{38}	C_{16}	C_{22}	5.2
C_{40}	C_{24}	C_{16}	6.0
C_{42}	C_{24}	C_{18}	8.6
C_{44}	C_{24}	C_{20}	10.4
C_{46}	C_{24}	C_{22}	16.4
C_{48}	C_{26}	C_{20}	20.0
	C_{24}	C_{24}	
C_{50}	C_{26}	C_{22}	14.5
	C_{28}	C_{20}	
C_{52}	C_{28}	C_{24}	9.2

Therefore, the major esters in

this wax are tetracosanyl eicosa - noate, tetracosanyl docosanoate,tetracosanyl tetracosanoate, hexacosanyl eicosanoate, hexacosanyl docosanoate,hexacosanyl tetracosanoate,octacosanyl eicosanoate, octacosanyl docosanoate, octacosanyl tetracosanoate.

CONCLUSION

The alkyl esters of the leaf wax of scutellaria lateriflora (fam.Labiatae) which is a sedative-hypnotic plant is now analysed foı the first time in the genus and in the family. The range of the ester homologues was $n\text{-}C_{34}$-$n\text{-}C_{52}$ with even number of carbon atoms being predominant. The major esters were $n\text{-}C_{46}$ and $n\text{-}C_{48}$. These components were comprised of different n-alkanols and n-acids of which the range of homologues for the former is $n\text{-}C_{16}$-$n\text{-}C_{28}$ with the range of latter being $n\text{-}C_{14}$-$n\text{-}C_{26}$. The mass spectra of the esters indicated that chains are composed of a combination of acids and alcohols. Wax esters of other species within the family labiatae are under investigation.

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