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LIPIDS FROM ROOTS OF *ONOSMA HETEROPHYLLA*

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Key Word Index—*Onosma heterophylla*; Boraginaceae; lipids; waxes; fatty substances; GC/MS analysis.

Abstract—The lipids (waxes and fatty substances) extracted from roots of *Onosma heterophylla* are reported. The wax fraction consists of esters of palmitic acid and its homologues with higher alcohols. β-Sitosterol was detected in this fraction in minor quantities. GC/MS analysis of the fatty substances fraction showed that its main constituents were esters of saturated as well as mono-, di- and triunsaturated fatty acids. Paraffins were also detected in traces. The presence of methyl, ethyl and iso-propyl esters of fatty acids, discovered for the first time in the roots of a higher plant, are considered to be of particular importance in the biosynthesis of fatty substances.

INTRODUCTION

The lipophilic red pigments of Boraginaceae species exhibit remarkable biological and pharmacological properties [1–4] attributable to the presence of isohexenylnaphthazarins, also known as alkannins and shikonins. One of the most important therapeutic properties is the healing action [5] of the oily extracts from *Anchusa*, *Onosma*, *Lithospermum* and *Cynoglossum* species. These extracts contain isohexenylnaphthazarins and lipids. Alknnins have been the subject of recent investigations [6–10], whereas very little is known about the lipids [11–13].

In this paper we report on the fatty substances and the waxes occurring in the roots of *Onosma heterophylla*, a shrub which grows wild in Northern Greece.

RESULTS AND DISCUSSION

Air dried roots of *O. heterophylla* were extracted with cold *n*-hexane. Evaporation of the solvent left a red residue, which was subsequently treated with cold methanol and then filtered to separate the waxes. Treatment of the methanolic filtrate with copper acetate removed the accompanying pigments (isohexenylnaphthazarins) as insoluble Cu-chelates. The filtrate contained the fatty substances.

The wax fraction was subjected to GC/MS analysis. The fragmentation pattern suggests that it consists of esters of higher fatty acids with higher alcohols, in particular palmitic acid as well as its homologues (C_{18}, C_{20}, C_{22}, C_{24} and C_{26}). The esterified alcohols were found to correspond to molecular formulae $C_{26}H_{53}OH$, $C_{28}H_{57}OH$, $C_{30}H_{61}OH$ and $C_{32}H_{65}OH$.

The mass spectra of the esters (Table 1) exhibit prominent peaks attributable to $[M+1]^+$ at *m/z* 621, 649, 677 and 705. The results suggest that the waxes of the roots of *O. heterophylla* have a similar chemical composition to those of *Alkanna tinctoria* [13], another member of the Boraginaceae.

A separate GC/MS analysis on the wax fraction also revealed the presence of β-sitosterol. This was identified by comparison with literature data [14].

Analysis of the fatty substances occurring in the root extract gave unexpected results and these were confirmed by repeated GC/MS runs on independent samples. The mass spectral data suggest the absence of free fatty acids and indicate the presence of esters of higher fatty acids instead (Table 2).

The occurrence of these esters is of particular importance since they are connected with the biosynthesis of fatty substances in plants and, to the best of our knowledge, no such esters have been found in higher plants. Traces of methyl and ethyl esters of fatty acids have been detected in lipid extracts of the lower fungi [15], animal and human livers, insects [16], freshwater algae [17] and bacteria [18]. They are present in rare cases in the seed lipids of higher plants [19]. The results indicate that methyl, ethyl and isopropyl groups participate in the formation of esters. The treatment of the preliminary hexane extract with cold methanol excludes any possibility of transesterification taking place during the analytical procedure. Moreover, the simultaneous presence of ethyl and isopropyl esters leaves no room for doubt that the methyl esters are indigenous to the lipids of the roots of *O. heterophylla*.

Table 1. Esters of the wax fraction of *Onosma heterophylla*

Molecular ion $[M+1]^+$	Ester
621	$C_{15}H_{31}COOC_{26}H_{53}$
649	$C_{15}H_{31}COOC_{28}H_{57}$
	$C_{17}H_{35}COOC_{26}H_{53}$
677	$C_{15}H_{31}COOC_{30}H_{61}$
	$C_{17}H_{35}COOC_{28}H_{57}$
	$C_{19}H_{39}COOC_{26}H_{53}$
705	$C_{15}H_{31}COOC_{32}H_{65}$
	$C_{17}H_{35}COOC_{30}H_{61}$
	$C_{19}H_{39}COOC_{28}H_{57}$
	$C_{21}H_{43}COOC_{26}H_{53}$

Table 2. Components of the fatty substances fraction of the roots of *Onosma heterophylla*

Peak No.	R_t (min)	MS *m/z*	Component
1	10.7	170[M]$^+$, 127, 99, 85, 71	*n*-Dodecane
2	15.0	184[M]$^+$, 127, 99, 85, 71	*n*-Decatriane
3	25.3	214[M]$^+$, 185, 183, 171, 129, 115, 74	Methyl dodecanoate
4	36.6	242[M]$^+$, 213, 211, 199, 74	Methyl tetradecanoate
5	39.8	252[M]$^+$, 221, 195, 178, 115, 74	Methyl 4-methyl-tetradeca-9,12-diene-oate
6	40.4	254[M]$^+$, 223, 197, 115, 74	Methyl 4-methyl-tetradec-9-ene-oate
7	45.3	268[M]$^+$, 237, 236, 194, 152, 87, 74	Methyl hexadec-9-ene-oate
8	46.7	270[M]$^+$, 241, 239, 227, 87, 74	Methyl hexadecanoate
9	49.4	284[M]$^+$, 241, 239, 101, 88	Ethyl hexadecanoate
10	50.9	298[M]$^+$, 241, 239, 102, 87, 74	Isopropyl hexadecanoate
11	52.8	292[M]$^+$, 261, 236, 194, 87, 74	Methyl octadeca-9,12,15-triene-oate
12	53.7	294[M]$^+$, 263, 220, 178, 87, 74	Methyl octadeca-9,12-diene-oate
13	54.3	296[M]$^+$, 265, 222, 180, 87, 74	Methyl octadec-9-ene-oate
14	55.4	298[M]$^+$, 269, 267, 255, 87, 74	Methyl octadecanoate

EXPERIMENTAL

Plant material was collected from the south coast of lake M. Prespa (Northern Greece). Its identity was established morphologically, anatomically and by chromosomic examination and was shown to be the species *heterophylla* of the genus *Onosma* (chromosomic number $2n = 26$, $n = 13$).

Extraction and isolation. Air dried roots (200 g) were extracted repeatedly for 3 hr with *n*-hexane at room temp. The solvent was removed *in vacuo* at a temp. below 50° and a deep red waxy material (6.2 g) was obtained. MeOH (50 ml) was added to 5.3 g of this material (the preliminary hexane extract) and the mixture stirred magnetically for 10 min. It was then filtered, washed with MeOH (2 × 10 ml) and the washings added to the filtrate. The insol. material (1.2% of the wax fraction) was subjected to GC/MS analysis.

The combined MeOH filtrates were subsequently treated with a soln of copper acetate (in MeOH) to remove the isohexenylnaphthazarins. After 30 min stirring at room temp. the mixture was filtered to remove the insol. Cu-chelates. The solvent was removed *in vacuo* and a yellow residue (0.8% fatty substances fraction) was obtained, which was then subjected to GC/MS analysis.

GC/MS analysis of wax fraction. The intact fraction was subjected to GC/MS analysis using a 0.37 m × 2 mm (i.d.) column packed with 3% SE-30 (Chromosorb G) and operated at 200–350° (incr. 10°/min) with He, at 25 ml/min. Injection port temp. 380°. Mass spectra were taken at an ionizing voltage of 70 eV.

GC/MS analysis of fatty substances fraction. This analysis was performed on a 1% SE-30 (Chromosorb G) column, 4 m × 2 mm (i.d.). He flow rate 20 ml/min, temp. 100–240° (incr. 2°/min). Injection port temp. 200°. Mass spectra were taken at an ionizing voltage of 70 eV.

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