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EPICUTICULAR WAX OF *CIRSIUM ARVENSE**

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Key Word Index—*Cirsium arvense*; Compositae; Canada thistle; epicuticular wax composition; hydrocarbons; esters; Ψ-taraxasterol acetate; 1,3-ditetradecanoyl-2-hexanoylglycerol.

Abstract—Epicuticular wax of *Cirsium arvense* contains hydrocarbons (12%), esters (35%), free acids (3%), free alcohols (10%), triterpene acetates (8%) and 1,3-ditetradecanoyl-2-hexanoylglycerol (8%) as major components. Minor components are triterpene alcohols (3%) and nonacosan-10-ol (2%). The esters contain triterpene alcohol esters (19%) as well as esters of alkanols.

INTRODUCTION

The family Compositae is one of the largest plant families with *ca* 900 genera and 13 000 species [1], but epicuticular wax analyses have been reported for very few species [2]. Principal components of waxes from *Baccharis cordifolia* [3], an *Artemisia* species [4], *Sonchus asper* [5] and *Senecio odoris* [6] have been analysed. Wax from *Cirsium arvense* (Canada thistle), which is a serious introduced weed in cultivated areas of North America, has now been analysed. The plant has a glossy appearance, suggesting the absence of constituents such as secondary alcohols and β-diketones which are known to cause glaucousness. Preliminary GLC analysis of the whole wax indicated that a number of unusual components were present.

RESULTS AND DISCUSSION

The composition of the wax is shown in Table 1, esters were the largest group of components. The hydrocarbon chain lengths ranged from C_{23} to C_{33} (Table 2); there was an appreciable proportion of heptacosane, which is a less common component of wax hydrocarbons [2]. Long-chain esters had the composition: C_{38}, 1%; C_{40}, 3%; C_{42}, 9%; C_{44}, 19%; C_{46}, 22%; C_{48}, 14%; C_{50}, 25%; C_{52}, 3%; C_{54}, 1%; C_{56}, 1% and unidentified components (6) 2%. The acids and alcohols (including 19% of triterpene alcohols which are discussed later) from the esters contained major C_{22} and C_{24} components thus accounting for the C_{44}–C_{48} esters. A large part of the C_{50} ester probably consisted of C_{20} acid esters of the triterpene alcohols.

Table 1. Composition and yield of epicuticular wax from *Cirsium arvense**

Component	%
Hydrocarbons	12
Esters	35
Free acids	3
Free alcohols	10
10-Nonacosanol	2
Triterpene acetates	8
Triterpene alcohols	3
1,3-Ditetradecanoyl-2-hexanoylglycerol	8
Unidentified	22
Yield (% dry wt)	0.8

*In wt% determined by CC.

Free acids contained a principal C_{24} component with lesser amounts of C_{26} and C_{28} but free alcohols ranged from C_{24} to C_{30} and were thus mainly appreciably longer in chain length than the combined alcohols. Besides occurring as esters, free triterpene alcohols and also their acetates were present; compositions are shown in Table 3. The proportions of the triterpenes varied considerably; α-amyrin, prominent as an ester, was the major free triterpene but was absent as an acetate, and lupeol, which formed the major ester and acetate was absent from the free triterpene alcohols. Ψ-Taraxasterol was a minor component as an ester but prominent as the acetate and free alcohol.

The first three triterpenes are not uncommon constituents of plant waxes [2] and Ψ-taraxasterol is a major constituent (both free and acetylated) of wax from *Sonchus asper* [5]. It has also been found in seed oil of another thistle (*Carduus nigrescens*) [7] and in extracts of roots or whole plants in the Compositae [8, 9].

Nonacosan-10-ol was a minor constituent of the wax but one readily identified by GC/MS analysis of the TMSi ether [10]. This alcohol is frequently present in wax of gymnosperm species [2] and in species of the families Papaveraceae, Ranunculaceae, Liliaceae and Rosaceae [11].

An unusual triacylglycerol was a more important component and appeared as one of the most prominent single peaks in GLC analysis of the whole wax.

*NRCC No. 20085.

Table 2. Composition of wax fractions from *Cirsium arvense*

No. of carbon atoms	Hydrocarbons	Hydrolysis products of esters		Free acids	Free alcohols
		Acids	Alcohols*		
14	—	1	—	2	—
16	—	15	—	2	—
18	—	5	—	1	—
20	—	13	2	2	—
22	—	29	27	6	—
23	1	—	1	—	—
24	—	26	29	44	14
25	3	—	—	—	—
26	—	5	7	19	28
27	21	—	1	—	—
28	—	4	7	15	30
29	43	—	1	—	—
30	—	2	2	3	22
31	23	—	—	—	—
32	—	1	—	1	—
33	5	—	—	—	—
Unidentified†	4 (10)	9 (7)			

*Triterpene alcohols were also obtained by ester cleavage; see Table 3.
†Number of components in parentheses.

Table 3. Composition of esterified and free triterpene alcohols from wax of *Cirsium arvense*

	As esters	As acetates	Free
β-Amyrin	30	14	25
α-Amyrin	31	—	50
Lupeol	37	56	—
Ψ-Taraxasterol	2	30	25

GC/MS analysis of the isolated triacylglycerol showed that only a C_{34} component (or components) occurred and that two C_{14} acyl groups and one C_6 acyl group were present. Two structures were possible: 1,3-ditetradecanoyl-2-hexanoylglycerol (**1**) or 1,2-ditetradecanoyl-3-hexanoylglycerol (**2**); a mixture of **1** and **2** was also possible. Compounds **1** and **2** were, therefore, synthesized by the general procedure of Mitchell [12] and the mass fragmentations are shown in Fig. 1.

It has been reported that mass spectra may distinguish between symmetrical structures, such as **1**, and unsymmetrical structures such as **2** [13–15]. Thus, in the mass spectrum of **1**, ions were found with *m/z* 383 and 369 due to loss of the acyloxy group $CH_3(CH_2)_{12}CO_2^+$ and of the acyloxymethylene group $CH_3(CH_2)_{12}CO_2^+CH_2$, respectively. The ion *m/z* 495 $[M^+ - CH_3(CH_2)_4CO_2]$ would also be expected but not the ion *m/z* 481 $[M^+ - CH_3(CH_2)_4CO_2CH_2]$; in structure **2**, however, since the hexanoyl group is attached to a primary position the ion *m/z* 481 would be expected. Spectra of lower MW triacylglycerols have been interpreted in this way to indicate their structures [16].

In the natural product, only ions corresponding to structure **1** were observed, but ion *m/z* 495 had a relative intensity of only 3% and since that with *m/z* 481, if present, would have been smaller, it might have been missed (particularly if the natural product was in fact a mixture of **1** and **2**). The relatively low intensity of diagnostic ions in the mass spectra of triacylglycerols has been noted before [15].

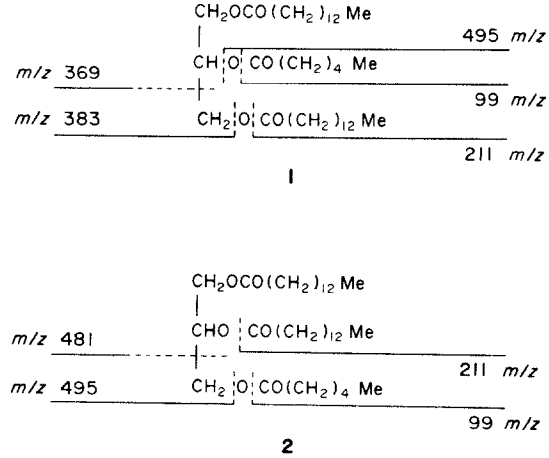

Fig. 1. MS fragmentation of 1,3-ditetradecanoyl-2-hexanoylglycerol (**1**) and 1,2-ditetradecanoyl-3-hexanoylglycerol (**2**).

The mass spectra of **1** and **2** were similar but that of **2** did show a small ion with *m/z* 481 (rel. int. 0.7%), the only other clear difference was in the relative intensities of ions with *m/z* 99 [$CH_3(CH_2)_4\overset{+}{C}O$] and 211 [$CH_3(CH_2)_{12}\overset{+}{C}O$]. In the spectrum of **1**, the base peak was 99 and the relative intensity of ion *m/z* 211 was 40% but in that of **2**, the base peak was 43 and the relative intensities of 99 and 211 were 60% and 66%, respectively. Thus, it appears that cleavage of an acyl group occurs more readily from a secondary position than from a primary position. The mass spectrum of the thistle triacylglycerol was in all respects indistinguishable from that of compound **1**.

The ^{13}C NMR spectra of the natural compound and of **1** and **2** were also measured and the readily assignable chemical shifts are shown in Table 4. The shifts of the C-2 carbons of the acyl groups in **1** and **2** are sufficiently different that the two compounds can be distinguished. In triacyl glycerols containing only long-chain fatty acids, the signal due to acyl C-2 attached to position 2 of glycerol is at lower field, by *ca* δ 0.15, than that of C-2 attached to positions 1 and 3 [17–19].

Thus, in the spectrum of **1**, with both C_{14} groups at glycerol primary positions, only one tetradecanoyl C-2 signal at δ 34.08 is seen and the hexanoyl C-2 signal, on the secondary glycerol position is at δ 34.20. In the spectrum of **2**, however, there are three acyl C-2 signals, two due to tetradecanoyl C-2 carbons, at δ 34.26 and 34.08, at positions 2 and 1 of glycerol, respectively, and one due to hexanoyl C-2, at δ 34.03, at position 3 of glycerol. The shifts of the long-chain acyl C-2 carbons are the same as those previously reported [18, 19]. The signals due to hexanoyl carbons are all at higher field than those due to the corresponding tetradecanoyl carbons.

The spectrum of the thistle triacylglycerol was indistinguishable from that of compound **1** showing that most, if not all of this component was the symmetrical glyceride. This identification confirms the GC/MS result which can only refer to volatile constituents. The melting point of the natural compound (31–35°) indicated small amounts of persistent impurities but it was close to that of **1** (35–36°) and above that of **2** (25.2–27°).

This appears to be the first time that a symmetrical triacylglycerol of this type with two long-chain acyl groups and a hexanoyl group has been identified in natural products. The chain lengths of the acyl groups total 34 carbons but mixtures of triacylglycerols with acyl groups totalling 32–38 have been isolated from a number of grass waxes (Ref. [20] and A. P. Tulloch, unpublished work). The base peak at *m/z* 99 in the mass spectrum of these compounds suggests that they too have a hexanoyl group at position 2 of glycerol.

The results of this analysis of wax from *C. arvense* indicate striking resemblances between it and wax from quite unrelated plant families. Major proportions of esters, with C_{40}–C_{50} chain lengths, are also found in wax of *Portulaca oleraceae* [21] and in wax of *Linum usitatissimum* [22] and, like the thistle, *P. oleraceae* has a glossy appearance. The chain-length range of the free alcohols, C_{24}–C_{30}, is similar to that of alcohols of wax from *L. usitatissimum* [22] and to those from panicoid grasses (Refs. [23–25] and A. P. Tulloch, unpublished work). The common triterpenes, lupeol and the amyrins, both free and as esters of long-chain acids also appear in some grass waxes [20, 24] and in other waxes [2]. The unusual triacylglycerol, with the hexanoyl group at position 2 of glycerol also occurs in grass waxes (Ref. [20] and A. P. Tulloch, unpublished work). Although the triterpene alcohol Ψ-taraxasterol may be a common component of waxes from species of Compositae, there is as yet no indication that this family has a characteristic wax composition different from that of species from other families.

EXPERIMENTAL

Plants of *C. arvense* (L.) Scop. were collected in September while flowering, and epicuticular wax extracted by 10 sec treatment with redistilled hexane. Wax was chromatographed on an Si gel column and eluted with hexane containing increasing proportions of Et_2O [26]. Fractions were examined by TLC ($CHCl_3$) and GC (Dexsil 300) [27]. Hydrocarbons, esters and free acids (as Me esters) and alcohols (as acetates) were identified by GC analysis after

Table 4. Chemical shifts of carbons of triacylglycerol from wax of *Cirsium arvense* and of triacylglycerols **1** and **2** (in $CDCl_3$)

From *Cirsium arvense*		1,3-Ditetradecanoyl-2-hexanoylglycerol (**1**)		1,2-Ditetradecanoyl-3-hexanoylglycerol (**2**)	
Tetradecanoyl* carbons	Hexanoyl carbons	Tetradecanoyl* carbons	Hexanoyl carbons	Tetradecanoyl* carbons	Hexanoyl carbons
2 34.10	2 34.21	2 34.08	2 34.20	2 34.26,† 34.08‡	2 34.03
3 24.92	3 24.61	3 24.91	3 24.60	3 24.91	3 24.57
12 31.96	4 31.25	12 31.96	4 31.24	12 31.96	4 31.28
13 22.72	5 22.33	13 22.71	5 22.32	13 22.71	5 22.32
14 14.13	6 13.91	14 14.11	6 13.89	14 14.11	6 13.88

In ppm from TMS.

*Other carbons not assigned.

†C-2 of tetradecanoyl group attached to position 2 of glycerol.

‡C-2 of tetradecanoyl group attached to position 1 of glycerol.

addition of authentic compounds. The triterpene acetates and part of the esters were eluted together (hexane–Et_2O, 99:1) and were separated by prep. TLC (hexane–$CHCl_3$, 1:1). Esters were converted to Me esters and alcohols by acid methanolysis and these products separated by CC [21]. Alcohols from the esters, which contained triterpene alcohols (19%, GC), as TMSi derivatives [28] and the triterpene acetates were analysed by GC/MS using a 30-m capillary column coated with silicone OV 101, the temp. was programmed from 150 to 300°. Relative emergence temps. of the TMSi ethers of α- and β-amyrins and of lupeol were the same as those previously described [20]. Ψ-Taraxasterol TMSi ether had MS [70 eV, *m/z* (rel. int.)]: M^+ missing, 408 (2), 369 (3), 218 (2), 209 (4), 203 (9), 191 (16), 190 (30), 189 (100), 73 (58) and the emergence temp. relative to the TMSi ether of triacontanol was 1.045. MS of the triterpene acetates were [70 eV, *m/z* (rel. int.)]: β-amyrin M^+ 468 (1), 408 (0.5), 218 (100), 203 (74), 189 (36); α-amyrin M^+ 468 (0.5), 218 (97), 203 (39), 189 (61), 95 (100); lupeol M^+ 468 (1), 408 (0.5), 218 (26), 203 (20), 189 (68), 107 (100); Ψ-taraxasterol M^+ 468 (2), 408 (2), 249 (3), 218 (3), 204 (12), 203 (14), 189 (93), 121 (100); the relative emergence temps. were β-amyrin acetate 1.0, α-amyrin acetate 1.02, lupeol acetate 1.02, ψ-taraxerol acetate 1.06. As acetates, α-amyrin and lupeol were not readily distinguished by GC/MS and the presence of lupeol was determined by ^1H NMR. The MS of ψ-taraxasterol acetate was the same as that of an authentic sample.

10-Nonacosanol was eluted with hexane–Et_2O (49:1) and identified by GC/MS of the TMSi ether [70 eV, *m/z* (rel. int.)]: M^+ missing, 481 M − 15 (1), 369 (30), 229 (74), 73 (100). Free acids, triterpene alcohols and triacyglycerol were eluted together with hexane–Et_2O (24:1), Me esters of the acids were separated by rechromatography after CH_2N_2 treatment. The mixture of triterpene alcohols and triacylglycerol was acetylated and then separated by rechromatography. The triterpene acetates were analysed as above. The triacylglycerol was analysed by GC/MS using a 50 m capillary column coated with OV-1, temp. was programmed from 150 to 300° and kept at 300° for 20 min, only one component was found with MS (70 eV, *m/z* rel. int.): M^+ missing, 495 (2), 383 (24), 369 (3), 285 (10), 227 (9), 211 (32), 173 (16), 158 (8), 99 (100). The fraction was crystallized from hexane at −10° and gave a solid mp 31–35°; ^{13}C NMR in Table 4.

1,3-Ditetradecanoyl-2-hexanoylglycerol (**1**) was synthesized by the general method [12] from 1,3-dichloropropan-2-ol, reaction with hexanoic anhydride in pyridine gave the hexanoate and further reaction with Na tetradecanoate in dimethyl formamide at 150° gave the symmetrical triacylglycerol in low overall yield. The product was crystallized from hexane at −10° and had mp 35.5–36°. MS [70eV, *m/z* (rel. int.)]: M^+ missing, 495 (3), 383 (37), 369 (4), 285 (12), 227 (7), 211 (40), 173 (20), 158 (9), 99 (100); ^{13}C NMR in Table 4: Found: C, 72.99; H, 11.55. $C_{37}H_{70}O_6$ requires: C, 72.74; H, 11.55%. 1,2-Ditetradecanoyl-3-hexanoylglycerol (**2**) was prepared from 3-chloropropan-1,2-diol, acylation with tetradecanoic anhydride gave the ditetradecanoate and reaction with Na hexanoate, as above gave the crude product. Extensive purification by CC (Si gel) gave the triacylglycerol, mp 25.5–27° (from hexane). MS [70eV, *m/z* (rel. int.)]: M^+ missing 495 (3), 494 (3), 383 (39), 369 (2), 339 (8), 285 (12), 227 (9), 211 (68), 173 (17), 158 (9), 99 (62), 43 (100); ^{13}C NMR in Table 4; Found: C, 72.47; H, 11.40. $C_{37}H_{70}O_6$ requires: C, 72.74; H, 11.55%.

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