Phytochemistry, Vol. 20, No. 12, pp. 2711–2716, 1981.
Printed in Great Britain.

0031-9422/81/122711-06 $02.00/0

EPICUTICULAR WAX OF *JUNIPERUS SCOPULORUM**

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(*Received* 24 *March* 1981)

Key Word Index—*Juniperus scopulorum*; Cupressaceae; Rocky Mountain juniper; epicuticular wax composition; nonacosan-10-ol; nonacosane-4,10-diol; 13-hydroxydotriacontanoic acid.

Abstract—Epicuticular wax from *Juniperus scopulorum* contains hydrocarbons (16%), esters (11%), free acids (1%), nonacosan-10-ol (27%), nonacosane-diols (7%) and estolides (16%). The major hydrocarbon is tritriacontane; the principal esters are C_{34}–C_{46}, mainly octyl and decyl esters of C_{28}–C_{36} acids; and the diols consist of nonacosane-4,10-diol (57%), 5,10-diol (28%), 7,10-diol (11%) and 10,13-diol (4%). Hydrolysis of the estolides gave a mixture of acids, ω-hydroxy acids, α, ω-diols, alcohols and hydroxy acids. The hydroxy acids are a new class of C_{28}–C_{36} acids with the OH attached to either the eighteenth or twentieth carbon from the terminal methyl end; the major component is 13-hydroxydotriacontanoic acid. Syntheses of this acid and of nonacosane-4,10-dione and nonacosane-4,10-diol are described.

INTRODUCTION

Some species of *Juniperus* appear glaucous with a waxy bloom on both leaves and fruits but there seems to have been no complete investigation of the composition of wax from any species. Hydrolysis products of a few *Juniperus* waxes have been known for a long time however. Thus 12-hydroxydodecanoic acid (sabinic acid) was obtained from *J. sabina* and 16-hydroxyhexadecanoic acid from *J. communis* [1–3]. 24-Hydroxytetracosanoic acid, and other acids, were isolated from hydrolysed wax of *J. rigida* [4]. These hydroxy acids are present in the wax as short-chain polyesters, known as estolides [1], which can be either neutral, sometimes cyclic, structures or linear acidic compounds [5].

Conifer waxes have been divided into two groups: those containing principally estolides and those with the secondary alcohol nonacosan-10-ol as major component [6]. This alcohol is found in wax of *J. oxycedrus* [7], *J. phoenicea* [8], *J. communis* [9, 10] and *J. macropoda* [11]. In addition an unusual diol, nonacosane-5,10-diol, is found in wax of *J. oxycedrus* [7] and *J. communis* [9].

The present investigation deals with wax of *J. scopulorum* Sarg. (Rocky Mountain Juniper), which is a moderate-sized tree native to western North America. In the course of an examination of waxes from *Juniperus* species, by TLC and GLC, to determine whether they could be distinguished by wax composition, an unusual component was observed in wax of this species and also in wax from *J. horizontalis* Moench. The component had a lower R_f value (0.1 in $CHCl_3$–EtOAc, 9:1) than common wax components and appeared to be a long-chain diol or triol. Since the R_f value was much lower than that reported for nonacosanediols from wax of *Pinus radiata* (0.25 in $CHCl_3$) [12] the wax was fractionated by chromatography and the components were identified.

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RESULTS

The amount of wax, expressed either as a percentage (0.4) of the dry wt or as a distribution (80 μg/cm²), is similar to that reported for other waxy plants [12–14].

The composition of the wax is shown in Table 1 and the constituents of some of the fractions are given in Table 2. Hydrocarbons formed 16% of the wax, with tritriacontane the major component (68%), and esters with chain lengths ranging from C_{34} to C_{46} accounting for 11%. The alcohols obtained on methanolysis had quite unusually short chain lengths and consisted principally of octanol and decanol with some C_{18} and C_{22} alcohols; the acids had correspondingly much longer chain lengths, C_{16}–C_{36}, with C_{30}–C_{34} as the major components. The esters are thus mainly the octyl and decyl esters of these acids. The structures of the esters were confirmed by GC/MS. Thus the C_{38} ester peak, 564 $[M]^+$, showed fragments at *m/z* 112 and 157 corresponding to $[R^1 - 1]^+$ and $[CO_2R^1]^+$ from an ester RCO_2R^1 [15]

Table 1. Composition and yield of epicuticular wax from *J. scopulorum*

Components	Wt (%, CC)
Hydrocarbons	16
Esters	11
Free acids	1
Nonacosan-10-ol	27
Diols	7
Less polar estolides	5
More polar estolides	11
Unidentified fractions eluted during chromatography	12
Lost on column	10
Yield (% dry wt)	0.4
Amount of wax (μg/cm²)	80

Table 2. Composition of wax fractions from *J. scopulorum**

No. of C atoms	Hydrocarbons	Esters	Hydrolysis products of esters: Acids	Hydrolysis products of esters: Alcohols	Free acids
8	—	—	—	47	—
10	—	—	—	11	—
16	—	—	3	—	2
18	—	—	—	23	1
20	—	—	—	—	1
22	—	—	3	19	10
24	—	2	6	—	13
26	—	1	4	—	5
27	1	—	—	—	—
28	—	—	6	—	4
29	2	—	—	—	—
30	—	—	12	—	7
31	7	—	—	—	—
32	—	—	21	—	9
33	68	—	—	—	—
34	—	6	26	—	22
35	14	—	—	-	-
36	—	—	8	-	4
38	—	13	—	—	—
40	—	19	—	—	—
42	—	21	—	—	—
44	—	11	—	—	—
46	—	3	—	—	—
Unidentified	8 (4)†	24 (11)	11 (9)	—	22 (9)

* % (w/w), determined by GLC.

† Number of components in parentheses. Includes components with emergence temperatures which suggested branched-chain structures, or for hydrocarbons an even number of carbons, or for esters, acids and alcohols an odd number of carbons. Components present in less than 0.5% have been omitted.

where R^1 is C_8 and at *m/z* 453 corresponding to $RCO_2H_2^+$ where R is C_{29}. The C_{40} ester was shown in the same way to be octyl dotriacontanoate. The C_{34} ester, on the other hand, was of a different type; fragments of similar origin at *m/z* 252, 297 and 257 showed it to be octadecyl hexadecanoate.

Free acids had chain lengths similar to combined acids but were very minor components (1%). No terpene acids were isolated. Free primary alcohols, which are often prominent components of waxes from monocotyledons and dicotyledons [16] were also absent. Instead nonacosan-10-ol was present as the largest single component (27%); its structure followed from the two principal fragments in the MS of its TMSi ether, *m/z* 229 and 369 [12, 17]. The melting point of the alcohol and the acetate and the melting point and specific rotation of the acid phthalate were the same as those previously reported [18]. The absolute configuration of this alcohol is not known.

Estolide-containing fractions were eluted after the C_{29} alcohol, with hexane containing from 6% up to 40% ether, showing that components with a considerable range of polarities were present. For convenient analysis, however, they were grouped into two fractions: fraction A, less polar estolides; fraction B, more polar estolides. They were characterized by their methanolysis products (Table 3). As expected, the less polar estolides contained a higher proportion of unhydroxylated acids and a smaller proportion of ω-hydroxy acids than did the more polar estolides. This is similar to the results obtained from estolides of *P. radiata* [12]. The major unhydroxylated acids were C_{12} and C_{14} acids and the ω-hydroxy acids were C_{12}–C_{16} acids, but the α, ω-diols contained C_{14} and C_{16} components only. Long-chain primary alcohols and alkan-2-ols, identified by GC/MS [19], were very minor constituents of the methanolysis products. Besides these products, very long chain hydroxy esters were also isolated from fraction A (7%) and from fraction B (3%). GC/MS of the TMSi ethers showed the presence of the esters listed in Table 4. Each GC peak contained two isomeric components and the structures followed from the MS fragmentation patterns (cleavage occurs on both sides of the oxygenated carbon) [20]. Thus the mixture of TMSi derivatives of methyl 13-hydroxy- and methyl 15-hydroxy-dotriacontanoates gave fragments with *m/z* 315 $[TMSiOC^+H(CH_2)_{11}CO_2Me]$ and with *m/z* 369 $[Me(CH_2)_{18}C^+HOTMSi]$ from the ether of the 13-hydroxy ester, and analogous fragments with *m/z* 343 and 341 from the ether of the 15-hydroxy ester. To confirm the structures, the major hydroxy ester, methyl 13-hydroxydotriacontanoate, was synthesized. Reaction between eicosanoyl chloride and 1-morpholinocyclododecene [21] followed by alkali hydrolysis of the β-diketone gave methyl 13-oxodotriacontanoate; the

Table 3. Hydrolysis products from estolides

No. of C atoms	Acids A*	Acids B*	ω-Hydroxy acids A	ω-Hydroxy acids B	α,ω-Diols A	α,ω-Diols B	Alkan-1-ols A	Alkan-2-ols A
12	31	34	60	55	—	—	—	—
14	30	19	20	18	83	72	—	—
16	6	3	20	25	17	28	—	—
18	2	—	—	—	—	—	6	—
20	6	5	—	—	—	—	4	—
22	12	16	—	—	—	—	30	—
23	—	—	—	—	—	—	—	4
24	6	9	—	—	—	—	5	—
25	—	—	—	—	—	—	—	10
26	—	2	—	—	—	—	20	—
27	—	—	—	—	—	—	-	27
28	—	1	—	—	—	—	26	—
29	—	—	—	—	—	—	—	44
30	2	3	—	—	—	—	—	—
32	2	3	—	—	—	—	8	—
33	—	—	—	—	—	—	—	15
34	3	5	—	—	—	—	—	—
% Obtained on hydrolysis†	33	18	10	20	6	7	1	2

* A, less polar estolides (fraction A); B, more polar estolides (fraction B).

† Long chain hydroxy esters were also obtained from fraction A (7%) and fraction B(3%). Unidentified non-volatile components present in fraction A and fraction B were 41% and 52%, respectively.

Table 4. Composition of long-chain hydroxy esters from estolides

Position of OH group	No. of C atoms	Fraction A*	Fraction B*
9	28		18
11	28	—	3
11	30	9	32
13	30	5	3
13	32	49	25
15	32	9	5
15	34	16	11
17	34	7	3
17	36	4	—
19	36	1	—

*A, less polar estolides (fraction A); B, more polar estolides (fraction B).

hydroxy ester was obtained by borohydride reduction. The GC retention time of the acetate and the retention time and MS of the TMSi ether were the same as those of the derivatives of the principal hydroxy ester in the mixture from the less polar estolides.

The later fractions eluted contained diols, the first of these consisted almost entirely of nonacosane-4,10-diol, followed by fractions consisting of mixtures of this diol with 5,10-, 7,10- and 10,13-diols. The structures of these diols followed from the MS of their TMSi ethers which were essentially the same as those discussed for the C_{29} diols isolated from wax of *P. radiata* [12]. The four isomeric diols as TMSi ethers were resolved by capillary GC and the composition was 4,10-diol (57%), 5,10-diol (28%), 7,10-diol (11%) and 10,13-diol (4%). The unusual diol, nonacosane-10,16-diol, which was also found in wax of *P. radiata* [22] was a very minor constituent of the 4,10-diol fraction. This component probably forms no more than 0.25% of the total diols. Since the diols had markedly lower R_f values than those reported from *P. radiata* [12], the 4,10-diol was characterized and the structure confirmed by oxidation to nonacosane-4,10-dione. This product was identical with the 4,10-dione synthesized by reaction between 7-oxodecanoyl chloride and the sodium derivative of octadecylmalonic acid. Reduction of the dione gave 4,10-diol as a mixture of diastereomers; the MS of the bis TMSi ether was indistinguishable from that of the natural diol. The ^{13}C NMR spectra were also the same and, applying known long-range effects of hydroxyl groups [23] to chemical shifts, showed clearly that one hydroxyl group was on the fourth carbon from one end and the second hydroxyl group was on a carbon separated from the other carbinyl carbon by five methylene groups. Also R_f values of natural and synthetic diols were identical in three TLC solvent systems.

DISCUSSION

The components identified and listed in Table 1 accounted for 78% of the wax and unidentified, non-volatile, gums and polar compounds not recovered from the column (22%) formed the remainder. About 50% of the methanolysis products from the estolides, however, also consisted of non-volatile material, and if this is derived not from estolides but from material eluted along with them, then the percentage of estolides would be only 8% and the percentage of unidentified components would be 30%. These probably consist of a large number of relatively high MW components with a wide range of polarities. As much as 30% of unidentified substances, which could perhaps be produced by oxidation of unsaturated wax components, has also been observed in other waxes [24].

Since few, if any, other conifer waxes have been completely analysed, the present results can only be compared with the partial analyses previously reported. The major hydrocarbon is tritriacontane; this composition is similar to that of the hydrocarbons from 15 species, including two *Juniperus* species, from the family Cupressaceae [25]. The C_{33} hydrocarbon is much less prominent in hydrocarbons from the families Pinaceae [25, 26] and Podocarpaceae [27].

While the ester chain lengths are similar to those of esters from other plant waxes [16], the alcohols obtained on hydrolysis, mainly C_8 and C_{10}, are much shorter. Esters from *P. radiata*, the only other conifer wax ester analysed in this way, gave the more usual C_{20}–C_{32} acids and alcohols (but ester acids from primary needles did contain C_{12} acid) [12].

The principal wax component, nonacosan-10-ol, has, as was mentioned earlier, been found in wax of a number of *Juniperus* species and also in many other conifer waxes [6]. It is also found in waxes from several other plant families, particularly Rosaceae [16, 18], Papaveraceae and Ranunculaceae [17]. It is thought that the presence of this component in wax causes glaucousness [17]. In one of the earliest investigations, it was shown that this alcohol, from apple fruit wax, and the derived acetate had no measurable optical rotation but the acid phthalate had a small positive rotation [18]. The same results were obtained with the C_{29} alcohol in this investigation and show that the alcohols from *J. scopulorum* and from apples have the same configuration.

The major hydrolysis products of the estolides were those usually obtained from conifer estolides [12, 25, 26]. 12-Hydroxydodecanoic and 16-hydroxyhexadecanoic acids, first reported from *Juniperus* species in 1909 [1–3], and 14-hydroxytetradecanoic acid were prominent products. The α, ω-C_{14} and C_{16} diols have also been previously isolated from conifer estolides [12, 28, 29]. Long-chain alkan-2-ols, C_{23}–C_{33}, were minor components, and though this type of alcohol does not appear to have been reported before, short-chain, C_{11}–C_{17}, alkan-2-ols (as esters) have been found in wax from barley [30] and from *Eucalyptus* [31]. The estolides yielded smaller amounts of a new series of C_{28}–C_{36} hydroxy acids, with the hydroxyl group on carbons 9, 11, 13, 15, 17 or 19 depending on chain length, the hydroxyl is thus attached to either the eighteenth or twentieth carbon from the terminal methyl end; the most prominent acid in the series was 13-hydroxydotriacontanoic acid. Hydroxy acids with these chain lengths have not been reported before, but 13-hydroxydocosanoic acid is produced by the yeast *Candida bogoriensis* [32]; some of the corresponding oxo acids, 11-oxotriacontanoic [33, 34] and 13-oxodotriacontanoic [35, 36], have been obtained by hydrolysis of certain insect waxes.

The diols were a mixture of 4,10-, 5,10-, 7,10- and 10,13-diols with the first predominating; similar to the diols from *P. radiata* [12]. The 5,10-diol was previously

isolated from wax of *J. oxycedrus* [7] and *J. communis* [9] and is also present in wax of *Rhus cotinus* [37]. The diacetate of the 4,10-diol had a small negative rotation whereas the diacetate of the 5,10-diol, from *J. oxycedrus*, had a small positive rotation [7] indicating a difference in the configuration of the diols or at least in the optical centres nearest to the ends of the chains.

The composition of this wax suggests relatively simple biosynthetic relationships for several components. Thus the major C_{33} alkane may be derived directly from the C_{34} acid by decarboxylation; in other waxes major hydrocarbons frequently have chain lengths longer than the acids [16]. If acids are hydroxylated to hydroxy acids and then decarboxylated to secondary alcohols [38], then 11-hydroxytriacontanoic acid, which is the major long-chain hydroxy acid from estolides B, could be the precursor of nonacosan-10-ol. The other long-chain hydroxy acids are probably produced by hydroxylation of the C_{28}–C_{36} acids; the hydroxyl group entering at the eighteenth or twentieth carbon from the terminal methyl end. Diols may be biosynthesized by hydroxylation of nonacosan-10-ol in a less specific manner, hydroxylation could occur at either carbons 4, 5, 7 or 13. This range of hydroxyl position, especially involving carbons 4, 5 and 7 is reminiscent of the 4-, 5- and 6-hydroxy-β-diketones from *Poa ampla* [39] or the 5-, 6- and 7-hydroxy-β-diketones from oats [24].

The present results, and those reported for other *Juniperus* species, suggest that the different species have similar compositions and, in fact, no qualitative differences were observed during TLC and GLC comparison of waxes from *J. scopulorum* and *J. horizontalis*.

EXPERIMENTAL

Wax isolation. Branches up to 15 cm long, which consisted of younger green twigs on older brown branches, were cut at the end of September and extrd by immersion in cold redistilled hexane for 45 sec. The vol of a sample of twigs was determined by solvent displacement in hexane and, assuming an average diameter of 2 mm, the surface area was calculated. The wt of extracted wax corresponded to an average wax distribution of 80 μg/cm^2 and represented 0.4% of the dry wt of the twigs.

Analytical procedures. TLC was carried out on Si gel with $CHCl_3$ and $CHCl_3$–EtOAc (9:1 and 7:3). R_f values in $CHCl_3$: nonacosan-10-ol, 0.4; methyl 13-hydroxydotriacontanoate 0.1; nonacosane-4,10-diol 0.03; in $CHCl_3$–EtOAc, 9:1; methyl 13-hydroxydotriacontanoate 0.6; triacontan-1-ol 0.4; methyl 16-hydroxyhexadecanoate 0.3; pentacosane-1,16-diol 0.2; nonacosane-4,10-diol 0.1; dodecane-1,12-diol 0.08; and in $CHCl_3$–EtOAc, 7:3; pentacosane-1,16-diol 0.4; nonacosane-4,10-diol 0.3; hexacosane-1,26-diol 0.2.

GLC. 1 m × 3 mm stainless steel column packed with 1.5% Dexsil 300 on 80–100 mesh, acid washed and silanized Chromosorb W; temp. 125–400° at 3°/min. Chain lengths were identified after addition of authentic compounds and % volatile determined using int. standards of similar structure, thus long-chain hydroxy esters were estimated using methyl 13-hydroxydocosanoate [32] as standard. Most fractions were also examined by GC/MS, after trimethylsilylation [40], using a 10-m capillary column coated with OV 101; temp. 125–350°.

Chromatography. Wax (11 g) was chromatographed on Si gel (200 g) and fractions eluted with hexane containing increasing proportions of Et_2O. Hydrocarbons were eluted with hexane, esters with hexane–Et_2O (99:1), nonacosan-10-ol with hexane–Et_2O (49:1), less polar estolides fraction (A) and free fatty acids with hexane–Et_2O (47:3 to 9:1), more polar estolides (fraction B) with hexane–Et_2O (4:1 and 3:2) and diols with hexane–Et_2O–EtOH (5:4:1).

GC/MS analysis of the esters showed [70 eV, *m/z* (rel. int.)] C_{34}: 508 $[M]^+$ (1), 297 (1), 257 (16), 252 (2), 57 (100); C_{38}: 564 $[M]^+$ (1), 453 (3), 185 (4), 157 (5), 112 (40), 57 (100), C_{40}: 592 $[M]^+$ (1), 481 (2), 185 (5), 157 (5), 112 (37), 57 (100); C_{42}: $[M]^+$ 620 $[M]^+$ (0.5), 509 (2), 185 (4), 157 (5), 112 (32), 57 (100). Esters were refluxed with 5% methanolic-HCl–C_6H_6 (1:1) for 18 hr, acid neutralized with Ag_2CO_3, solvent removed at 20° and alcohols acetylated with acetyl chloride in $CHCl_3$. The mixture of Me esters and acetates was analysed by GLC (temp. 50–300°).

Me esters of free fatty acids were obtained by rechromatographing the mixture of acids and less polar estolides after CH_2N_2 treatment.

Nonacosan-10-ol was recrystallized from EtOAc and had mp 81–82° (lit. [18] 81.9–82.2°), $[\alpha]_{365}^{25}$ 0.00 ($CHCl_3$, *c* 2.0) MS, TMSi ether, 70 eV, *m/z* (rel. int.): $[M]^+$ missing, 481 $[M-15]^+$ (1), 369 (43), 229 (100), 73 (82). The acetate was crystallized from hexane and had mp 43–44° (lit. [18] 44.5–45.0°), $[\alpha]_{365}^{25}$ 0.00 ($CHCl_3$, *c* 10.4). The acid phthalate was prepared by heating the alcohol with phthalic anhydride in pyridine at 95° for 72 hr. After crystallization (EtOH) the mp was 55–56° (lit. [18] 54.5–54.7°) and $[\alpha]_{589}^{25}$ +0.41°, $[\alpha]_{546}^{25}$ +0.57°, $[\alpha]_{436}^{25}$ 0.71° and $[\alpha]_{365}^{25}$ +0.86° (*c*, 4.6 $CHCl_3$) (lit. [18] $[\alpha]_{546}^{175}$ +0.62°).

Nonacosane-4,10-diol was the only diol in the first diol fraction eluted and was purified by conversion to the diacetate and chromatography on Si gel. The diacetate was eluted with hexane–Et_2O (24:1). The diol was recovered by treatment with methanolic-HCl and crystallized (EtOH), mp 112–114°. MS, bis-TMSi ether, 70 eV, *m/z* (rel. int.): $[M]^+$ missing, 541 (1), 369 (17), 317 (17), 227 (12), 145 (62), 137 (33), 73 (49), 57 (100); ^{13}C NMR ($CDCl_3$): δ 14.09 (C-1, C-29), 18.88 (C-2), 22.70 (C-28), 25.67 (C-6, C-8, C-12), 29.36 (C-7), 29.70 (unassigned signals), 31.96 (C-27), 37.51 (C-5, C-9), 37.65 (C-11), 39.84 (C-3), 71.76 (C-4), 72.05 (C-10); (Found: C, 78.94; H, 13.65. $C_{29}H_{60}O_2$ requires: C, 79.02; H, 13.72%.) The acetate was crystallized (hexane), mp 36–37°; $[\alpha]_{589}^{25}$ −0.36°, $[\alpha]_{546}^{25}$ −0.43°, $[\alpha]_{436}^{25}$ −0.84° (*c* 5.6, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 14.03 (C-1), 14.17 (C-29), 18.64 (C-2), 21.26, 21.36 (acetate Me), 22.76 (C-28), 25.29 (C-6, C-8), 25.36 (C-12), 29.51 (C-7), 29.60–29.75 (unassigned signals), 31.98 (C-27), 34.17 (C-5, C-9, C-11), 36.39 (C-3), 74.12 (C-4); 74.38 (C-10), 170.85, 170.87 (acetate CO). The 4,10-diol was oxidized with CrO_3 in HOAc at 25° to give nonacosane-4,10-dione which was crystallized (hexane), mp and mmp with synthetic dione 91–92°. GC/MS showed that the unfractionated mixed diols had the composition: 4,10-diol (57%), 5,10-diol (28%), 7,10 diol (11%) and 10,13-diol (4%); relative emergence times: 4,10, 1.000; 5,10, 0.984; 7,10, 0.965 and 10,13, 0.957; MS of the bis-TMSi ethers of the other diols [70 eV, *m/z* (rel. int.)]: 5,10-diol, $[M]^+$ missing, 369 (28), 317 (33), 227 (10), 159 (100), 137 (36), 73 (81); 7,10-diol, $[M]^+$ missing, 409 (11), 369 (21), 317 (5), 227 (92), 187 (100), 129 (61), 73 (72); 10,13 diol, $[M]^+$ missing, 367 (26), 359 (3), 327 (40), 269 (80), 229 (100), 129 (76), 73 (90). GC/MS of the TMSi ethers of the mother liquors from crystallization of the 4,10-diol; small peak, with R_t very close to that of the 10,13-diol, due to the ether of the 10,16-diol. MS 70 eV, *m/z* (rel. int.): $[M]^+$ missing, 457 (7), 401 (14), 367 (8), 311 (13), 285 (58), 229 (63), 129 (27), 73 (100).

The less polar estolides had R_f ($CHCl_3$) in the range 0.01–0.15 and the more polar estolides in the range 0.05–0.10. Estolides were refluxed 18 hr with methanolic-HCl and products recovered and chromatographed on Si gel. Me esters were eluted with hexane–Et_2O (99:1), long-chain hydroxy esters, primary alcohols and alkan-2-ols with hexane–Et_2O (9:1 and 4:1), ω-hydroxy esters with hexane–Et_2O (3:2) and α, ω-diols with

hexane–Me_2CO (4:1). The principal component of the long-chain hydroxy ester fraction from the less polar estolides, after acetylation, had the same R_f as the acetate of synthetic Me 13-hydroxydotriacontanoate. These fractions were also examined by GC/MS after trimethylsilylation. Ethers of hydroxy esters from the more polar estolides; MS 70 eV, *m/z* (rel. int.): methyl 9-hydroxy- and 11-hydroxyoctacosanoates, $[M]^+$ missing 369 (14), 341 (3), 287 (6), 259 (61), 183 (7), 159 (100) 73 (64); methyl 11-hydroxy- and 13-hydroxy triacontanoates, $[M]^+$ missing 369 (26), 341 (4), 315 (5), 287 (63), 183 (44), 73 (100). Ethers of hydroxy esters from the less polar estolides; MS, 70 eV, *m/z* (rel. int.): methyl 13-hydroxy- and 15-hydroxydotriacontanoates, $[M]^+$ missing, 369 (18), 343 (12), 341 (9), 315 (32), 73 (100); methyl 15-hydroxy and 17-hydroxytetratriacontanoates, $[M]^+$ missing, 371 (2), 369 (24), 343 (37), 341 (9), 73 (100); methyl 17-hydroxy- and 19-hydroxyhexatriacontanoates, $[M]^+$ missing, 399 (11), 371 (63), 369 (46), 341 (15), 295 (3), 267 (3), 73 (100). This fraction, from the less polar estolides, also contained alkan-1-ols and alkan-2-ols identified by GC/MS of TMSi ether from $[M-15]^+$, and from $[M-15]^+$ and *m/z* 117 peaks respectively [19].

Synthesis of nonacosane-4,10-diol. Octadecyl methanesulphonate was prepared in the usual way [41] and crystallized (MeOH), mp 59–60°. Reaction with the Na derivative of diethyl malonate, in the usual way [42], gave octadecyl malonic acid, mp 126–127°, from C_6H_6 (lit. [43] mp 121°). A mixture of octadecylmalonic acid (10 g), C_6H_6 (60 ml) and dimethoxyethane (30 ml) was dried by azeotropic distillation [44]; *p*-toluenesulphonic acid (0.1 g) and dihydropyran (8 ml) were added and the mixture stirred for 30 min. After addition of further *p*-toluene-sulphonic acid (0.1 g) and dihydropyran (3 ml) a clear soln of the malonic ester was obtained. The soln was stirred with KOH pellets, filtered and solvents and excess reagents removed at 20° and 10 mm. The product was dissolved in C_6H_6 (100 ml), added to a suspension of Na (1.5 g) in C_6H_6 (150 ml) and stirred overnight. 7-Oxodecanoyl chloride (6.7 g) [45] in C_6H_6 (50 ml) was added and the mixture stirred for 4 hr, HOAc (18 ml) was then added and refluxed for 4 hr. After washing with $NaHCO_3$ and with H_2O the solvent was removed and the residue crystallized from hexane giving nonacosane-4,10-dione (4.9 g); mp 91–92°; ^{13}C NMR ($CDCl_3$): δ 13.81 (C-1), 14.16 (C-29), 17.37 (C-2), 22.75 (C-28), 23.56 (C-6, C-8), 23.97 (C-12), 28.85 (C-7), 29.35 (C-13), 29.43–29.76 (unassigned signals), 32.0 (C-27), 42.51 (C-5, C-9), 42.94 (C-11), 44.81 (C-3), 212.54 (C-4), 212.84 (C-10); (Found: C, 79.98; H, 12.90. $C_{29}H_{56}O_2$ requires: C, 79.95, H, 13.10%.) Reduction of the diketone by refluxing with excess $NaBH_4$ in EtOH gave nonacosane-4,10-diol; mp 92–104° after crystallization from EtOAc; MS bis-TMSi ether, 70 eV, *m/z* (rel. int.): $[M]^+$ missing, 541 (3), 451 (3), 369 (44), 317 (36), 227 (20), 145 (70), 73 (100), ^{13}C NMR ($CDCl_3$): δ 14.09 (C-1, C-29), 18.85 (C-2), 22.68 (C-28), 25.65 (C-6, C-8, C-12), 29.36 (C-7), 29.71 (unassigned signals), 31.95 (C-27), 37.50 (C-5, C-9), 37.63 (C-11), 39.81 (C-3), 71.69 (C-4), 71.99 (C-10); (Found: C, 79.51; H, 13.67. $C_{29}H_{60}O_2$ requires: C, 79.02; H, 13.72%.)

Methyl 13-hydroxydotriacontanoate. Eicosanoyl chloride (14 g) in $CHCl_3$ (30 ml) was added to 1-morpholinocyclododecene (10 g) and Et_3N (5 g) in $CHCl_3$ (20 ml) and the mixture stirred at 5° for 18 hr. After working up and hydrolysing in the usual way [21], 13-oxodotriacontanoic acid (12.3 g, after crystallization from $CHCl_3$) was obtained; mp 108.5–109.5° (lit. [35] mp 104.5–105.5°). The Me ester was prepared and purified by chromatography on Si gel; mp 84.0–84.3° (large plates from Me_2CO) (lit. [36] mp 89–90°); (Found: C, 78.12; H, 12.83. $C_{33}H_{64}O_3$ requires: C, 77.89; H, 12.68%.) The oxo ester was reduced with $NaBH_4$ in EtOH at 74° for 45 min and gave methyl 13-hydroxydotriacontanoate; mp 85–86° (from Me_2CO); MS TMSi ether, 70 eV, *m/z* (rel. int.): $[M]^+$ missing, 567 $[M-15]^+$ (2), 369 (55), 315 (100), 286 (14), 73 (69); (Found: C, 77.58; H, 13.09. $C_{33}H_{66}O_3$ requires: C, 77.58; H, 13.02%.) Acetylation of the hydroxy ester gave methyl 13-acetoxydotriacontanoate; mp 46–47° (from hexane); (Found: C, 75.88; H, 12.27. $C_{35}H_{68}O_4$ requires C, 76.03; H, 12.40.)

Acknowledgements—The authors thank L. R. Hogge for GC/MS and M. Mazurek for ^{13}CNMR.

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