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EPICUTICULAR WAX OF *AGROPYRON INTERMEDIUM**

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Key Word Index—*Agropyron intermedium*; Gramineae; intermediate wheatgrass; leaf wax; composition; β-diketones; 25-oxohentriacontane-14,16-dione; 10-oxohentriacontane-14,16-dione; 25-hydroxyhentriacontane-14,16-dione; 26-hydroxyhentriacontane-14,16-dione; dioxo β-diketones; hydroxyoxo-β-diketones; mass spectra.

Abstract—Wax on leaves of *Agropyron intermedium* contains hydrocarbons (11%, C_{27}–C_{33}), esters (11%, C_{32}–C_{60}), free alcohols (18%, C_{26}) 25-oxohentriacontane-14,16-dione (17%), 10-oxohentriacontane-14,16-dione (5%), 25-hydroxyhentriacontane-14,16-dione (12%) and 26-hydroxyhentriacontane-14,16-dione (2%). Wax on spikes contains additional components, C_{25}–C_{33} *cis* 9-alkenes (32% of hydrocarbons), and more β-diketones, 25-hydroxy (17%) and 26-hydroxy (3%) hentriacontane-14,16-diones, 10,25-dioxohentriacontane-14,16-dione (1%) and 4-hydroxy-25-oxo-(2%), 25-hydroxy-10-oxo-(1.3%) and 26-hydroxy-10-oxo-(0.7%) hentriacontane-14,16-diones; free alcohols were very minor components (1%, C_{24}–C_{32}).

INTRODUCTION

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 commercial applications. Wax of different chemical composition, and so of different physical form [1,2] may affect transpiration and also leaf surface properties to varying extents [3,4]. Though most plant waxes in commercial use are obtained from tropical or semi-tropical plants, wax with useful properties might be derived from plants of temperate regions. Also a detailed knowledge of composition of plant waxes from a number of different genera in a family might be taxonomically useful.

Previously waxes from the cereal crops, wheat [5–7], oats [8] and rye [1] were investigated but it appeared that perennial grasses might be more convenient sources of waxes than straw from cereal crops. Grass could be cut when wax yield was greatest, wax extracted with solvent and the dewaxed grass used to feed animals. A similar process has been proposed for esparto grass [9].

Waxes of a number of drought resistant perennial grasses are now being investigated, most of the grasses are glaucous or 'grey' varieties since these may have more epicuticular wax. This paper describes analysis of wax from *Agropyron intermedium* (Host) Beauv. (*Gramineae*), a native of Europe [10], which has been introduced into North America and developed for pasture and hay production. This investigation has been particularly extensive since the wax contains an unusual variety of substituted β-diketones, some of which appear restricted to a particular part of the plant.

RESULTS

A. intermedium did not flower during the first summer of growth and plants were not visibly glaucous, but in the second year numerous culms with spikes appeared. The leaf sheath and to a lesser extent the abaxial side of the leaf blade were now moderately glaucous, a similar distribution of glaucousness being observed on wheat [6,11], rye [1] and oats [8]. In addition, glumes, lemmas and rachis of the large spike (*ca* 20 cm long) were also noticeably glaucous. Wax was therefore extracted from leaves and stems and also, separately, from spikes; in previous investigations of cereal crops, heads were excluded from extraction. Compositions of wax from these two portions of flowering plants and also from vegetative plants are shown in Table 1. Since UV absorption of wax from spikes indicated a very high β-diketone content [12] and also since TLC showed that free alcohols were very low (making separation easier) this wax was completely analysed as well as wax from leaves and stems.

Yield of wax from the more glaucous flowering plants was the same as that from vegetative plants suggesting that glaucousness in this case indicates not more wax but a different composition. Glaucousness in grasses seems to be almost always associated with high β-diketone content [1,11,13]. This is also true for *A. intermedium* since wax from green vegetative plants contains only about 15% β-diketone and 60% free alcohol but wax from flowering plants contains 47% β-diketones and 18% free alcohol and spike wax contains 58% β-diketones and only 1% free alcohol.

Hydrocarbons form about 10% of each wax, the compositions of hydrocarbons of wax from leaves and stems of both flowering and vegetative plants are almost the same with major C_{29} and C_{31} components (Table 2), which is fairly similar to hydrocarbon composition of wax from cereal crops [1,5–7]. Hydrocarbons of spike wax are, however, appreciably different, C_{27} is a much larger component and 32% unsaturated hydrocarbons are present. IR and NMR indicated that these are *cis* monoenes and permanganate–periodate oxidation gave C_{16}–C_{24} acids in about the same proportions as the C_{25}–C_{33} unsaturated hydrocarbons showing that the double bond is principally located at the 9,10 position.

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Table 1. Composition and yield of epicuticular wax from *Agropyron intermedium*

Components	Flowering plants (second year of growth) Plant part Spike*	Leaves and stems*	Vegetative plants (first year of growth) Leaves†
Hydrocarbons	10	11	10
Esters	6	11	7
Free alcohols	1	18	60
Free acids	2	—	—
β-Diketone	11	11	‡
Oxo-β-diketones	22	22	‡
Hydroxy-β-diketones	20	14	‡
Dioxo-β-diketones	1	—	—
Hydroxyoxo-β-diketones	4	¶	—
Unidentified	23	12	‡
Yield (% dry wt)	0.6	0.5	0.5
$E^{1\%}_{1cm}$ at 273 nm (isooctane)	175	112	37

* Determined by column chromatography. † Determined by GLC. ‡ Not estimated. ¶ Hydroxyoxo-β-diketones apparently present but probably do not exceed 1%.

Unsaturated hydrocarbons have been occasionally reported in plant waxes, rose hip wax contains *cis* 5-alkenes with 9-alkenes as very minor components [14].

The long chain esters of each wax have wide chain length ranges (Table 3) but shorter chain esters are more abundant in spike wax. Me esters obtained by methanolysis of the esters from both waxes of flowering plants are fairly similar (Table 4), the chain length range is C_{16}–C_{30} and C_{24} are the largest components. Alcohols from esters of leaf and stem wax are hexacosanol with much lesser amounts of other chain lengths but alcohol from spike wax esters contain no such major component, triterpene alcohols may be present but were not identified.

Free acids were detected only in spike wax and have the usual very wide chain length range, C_{14}–C_{34}, (Table 5). Free alcohols of wax from leaves and stems of both types of plant contain over 90% of C_{26} alcohol but the very small amount of free alcohols in spike wax does not contain a major characteristic alcohol but ranges from C_{24} to C_{32}. This untypical composition is similar to that observed for free alcohols of wax of the flag leaf sheath (which also has a high β-diketone content) of Selkirk wheat [11].

Table 2. Composition of hydrocarbons from wax of *Agropyron intermedium*

Carbon No.	Flowering plants Spike	Leaves and stems	Vegetative plants Leaves*
21	1	—	—
23	3	1	—
25	6	1	—
25:1	2	—	—
27	20	4	7
27:1	8	—	—
29	20	39	36
29:1	6	—	—
31	14	41	41
31:1	16	—	—
33	2	14	16
33:1	2	—	—

* From GLC analysis of whole wax.

Table 3. Composition of esters from wax of *Agropyron intermedium*

Carbon No.	Flowering plants Spike	Leaves and stems	Vegetative plants Leaves*
30	6	—	—
32	7	1	—
34	7	2	—
36	4	1	—
38	4	2	3
40	6	4	7
42	10	11	22
44	12	9	20
46	9	15	23
48	7	12	13
50	1	10	6
52	3	8	5
54	2	6	—
56	2	5	—
58	—	4	—
60	—	1	—
Unidentified†	20 (9)	9 (8)	—

* From GLC analysis of whole wax. † Number of components in parentheses.

The remainder of the waxes consist mainly of β-diketones and the structure of these components was established using compounds isolated by column chromatography of spike wax. Corresponding components were then isolated from wax of leaves and stems of flowering plants and identified by comparison. The unsubstituted β-diketone is hentriacontane-14,16-dione, the same compound that occurs in waxes of several cereal crops [1,5–8,15].

A new β-diketone 25-oxohentriacontane-14,16-dione comprising 17% of spike, leaf and stem waxes was isolated and its structure established from the acidic products of alkaline hydrolysis. Myristic and 10-oxohexadecanoic acids were formed and the structure of the latter

Table 4. Composition of acids and alcohols obtained by hydrolysis of esters of wax from flowering plants of *Agropyron intermedium*

Carbon No.	Acids Spike	Stem and leaves	Alcohols Spike	Stem and leaves
12	—	—	4	—
14	—	—	12	—
16	13	6	1	—
18	10	5	5	1
20	25	19	2	1
22	8	14	6	4
24	30	26	28	12
26	8	15	17	77
28	2	9	5	2
30	4	5	—	1
Unidentified*	—	1 (1)	20 (1)	2 (1)

* Number of components in parentheses.

Table 5. Composition of free acids and free alcohols from wax of *Agropyron intermedium*

Carbon No.	Flowering plants: Spike Acids	Flowering plants: Spike Alcohols	Flowering plants: Leaves and stems* Alcohols	Vegetative plants: Leaves*† Alcohols
14	14	—	—	—
16	12	—	—	—
18	8	—	—	—
20	2	—	—	—
22	3	—	—	—
24	23	16	3	2
26	9	33	91	95
28	16	9	2	2
30	13	16	1	1
32	5	8	—	—
34	1	—	—	—
Unidentified‡	4 (4)	18 (10)	3 (6)	—

* Free acids were not detected. † Determined by GLC of whole wax. ‡ Number of components in parentheses.

(as the Me ester) was established by MS comparison with authentic 10-oxo C_{16} Me ester. A second oxo-β-diketone, 10-oxohentriacontane-14,16-dione, was eluted immediately after the first one and formed 5% of the waxes. GC–MS analysis (as Me esters) showed that the acids obtained by hydrolysis were palmitic and 5-oxotetradecanoic.

The PMR spectrum of 10-oxohentriacontane-14,16-dione confirmed its structure and contained a quartet at δ1.82 due to the protons on C-12 which are β to the two carbonyls at C-10 and C-14. Also the singlet due to the proton on the double bond (C-15) of the enolic form is displaced 0.02 ppm to lower field (compared with the corresponding signal in the spectrum of the 25-oxo isomer) by the C-10 carbonyl. These spectral differences can be used to estimate proportions of the two isomers in mixtures.

Oxo-β-diketones can be mistaken for free primary alcohols by TLC, since in $CHCl_3$ containing 1% EtOH they have almost the same R_f value but with $CHCl_3$ containing 1.5% EtOH the R_f value of oxo-β-diketones is greater than that of primary alcohol. Further increases in EtOH content of the solvent improves the resolution of the two classes. TLC analysis therefore, should ideally be performed using $CHCl_3$ with various proportions of EtOH.

Hydroxy-β-diketones were the other major β-diketones comprising 20% of spike wax and 14% of leaf and stem wax. MS peaks appeared at *m/e* 395 indicating 25-hydroxyhentriacontane-14,16-dione [1,16] and also at *m/e* 409 which suggested the presence of the 26-hydroxy isomer. Since MS analysis may not indicate the proportions of isomeric hydroxy β-diketones [8], they were hydrolysed and the hydroxy C_{16} esters obtained examined by ^{13}C NMR. It was shown during analysis of the hydroxy β-diketones of wax from *A. smithii* [17] that this method could be used to analyse mixtures of Me 10- and 11-hydroxyhexadecanoates. In this way it was estimated that spike wax contained 17% of the 25-hydroxy isomer and 3% of the 26-hydroxy isomer and leaf and stem wax contained 12% of the former and 2% of the latter isomer.

The other β-diketones were minor components of the waxes (Table 1) but their structures were determined since they were new. The dioxo-β-diketone was shown by MS (discussed below) to be 10,25-dioxohentriacontane-14,16-dione, the PMR spectrum with a quartet at 1.82 ppm and an enolic proton at 5.33 ppm supported this structure. The hydroxyoxo-β-diketones are a mixture of three isomers: 4-hydroxy-25-oxo, 25-hydroxy-10-oxo and 26-hydroxy-10-oxohentriacontane-14,16-diones in the ratio 3:2:1 so that the percentages in the wax are about 2.0, 1.3 and 0.7‰ respectively. Hydrolysis of the mixture gave two oxo acids and two hydroxy acids which were separated by column chromatography as Me esters. GC–MS showed the oxo esters to be Me 5-oxotetradecanoate and 10-oxohexadecanoate which had already been obtained separately from the isomeric oxo esters previously discussed. The ^{13}C NMR spectrum contained signals in complete agreement (except for one unassigned signal at 15.25 ppm, presumably due to an impurity) with the proposed structures. Chemical shifts were assigned by analogy with chemical shifts of isomeric oxooctadecanoates which have been studied previously [18]. Hydroxy esters were shown by GC–MS to be methyl 11-hydroxytetradecanoate and 10- and 11-hydroxyhexadecanoates. The ^{13}C NMR spectrum agreed with these structures and also indicated the proportions of 10- and 11-hydroxy C_{16} esters. The ratio of 11-hydroxy to 10-hydroxy ester was much larger (1:2) than it was for the hydroxy β-diketone hydrolysis products (17:83). Again assignment of the ^{13}C shifts was made by analogy with spectra of isomeric methyl hydroxyoctadecanoates [18]. The PMR spectrum showed two singlets of equal intensity at 5.31 and 5.33 ppm (enolic proton) indicating a 1:1 ratio of the isomer with an oxo group at C-25 to the 2 isomers with the oxo group at C-10.

Since MS fragmentation patterns are not always completely predictable and may not completely establish the structure of new compounds, discussion of the MS of the new β-diketones has been postponed until the structures were established by other means. These spectra are discussed now so that MS can be used to identify these compounds when they are isolated from other waxes. Figure 1 illustrates the principal fragmentation patterns. In the spectrum of the 25-oxo-β-diketone, the only ion which indicates that the oxo group is at C-25 is *m/e* 113 produced by α cleavage of the 24,25 bond and no ions from cleavage of the 25,26 bond were observed and those from cleavage of the 26,27 bond were very weak. Ion *m/e* 351 is much more prominent and apparently results from 23,24 bond cleavage with the charge remaining on the fragment which does not contain the CO group close to the point of cleavage. In the absence of high resolution spectra, this fragment might be wrongly taken to indicate an oxo group at another position such as C-22. This type of cleavage does not seem to occur with simple long chain ketones [19] but has been reported in MS of other compounds which contain a second carbonyl group such as Me 7-, 8-, 9- and 15-oxooctadecanoates [20]. Also in the MS of Me 10-oxohexadecanoate, in which carbonyl groups are separated by the same number of methylene groups as they are in 25-oxo β-diketone (see Experimental), the peaks *m/e* 157 and 125 (157-32) correspond to this type of β cleavage. Furthermore, peaks *m/e* 185 and 153 (185-32), of the same origin appear in the MS of Me 12-oxooctadecanoate and these change to *m/e* 187 and 155 in the MS of Me 9-dideuterio-12-oxohexadecanoate

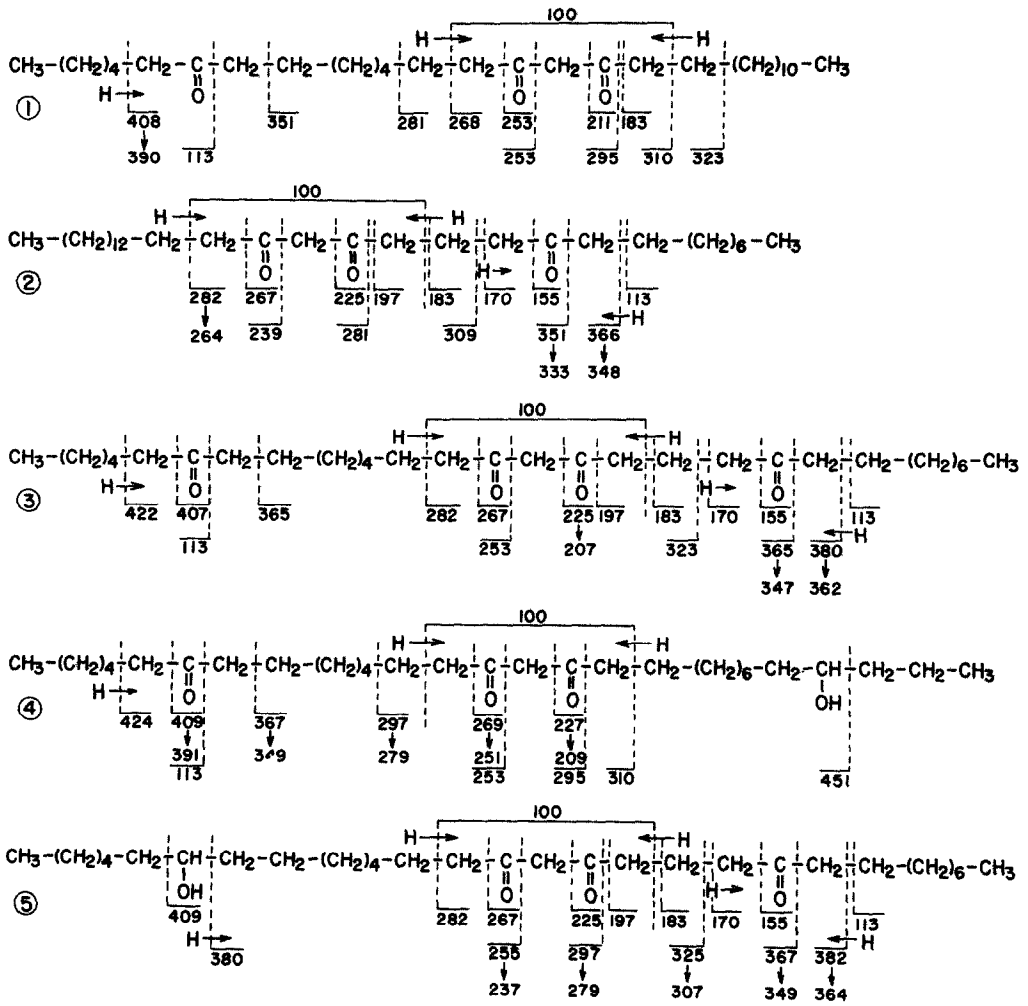

Fig. 1. MS fragmentation of oxygenated β-diketones from wax of *Agropyron intermedium*. ① 25-Oxohentriacontane-14,16-dione ② 10-Oxohentriacontane-14,16-dione ③ 10,25-Dioxohentriacontane-14,16-dione ④ 4-Hydroxy-25-oxohentriacontane-14,16-dione ⑤ 25-Hydroxy-10-oxohentriacontane-14,16-dione.

(A. P. Tulloch, unpublished work). The remainder of the MS is quite well known and indicates the position of the β-diketone group [16].

The MS of the 10-oxo-β-diketone is less unusual and clearly confirms the assigned structure and β-cleavage as discussed above occurs giving the peak *m/e* 309. The corresponding peak, *m/e* 323, in the MS of 25-oxo β-diketone was much smaller. The MS of the 10,25-dioxo-β-diketone contains the features of both the spectra previously discussed and is in complete agreement with the assigned structure. The MS of the mixed hydroxyoxo-β-diketones can be completely interpreted from the proposed structures as shown in the figure. A small peak at *m/e* 423 shows the presence of the 26-hydroxy-10-oxo β-diketone. The intensities of the peaks, however, do not indicate the relative proportions of isomers at all accurately.

DISCUSSION

The occurrence of monoenoic hydrocarbons is an unusual feature of the spike wax though these uncommon components have been previously reported in flowers (*Aloe* species [21] and fruits (rose hips [14]). The biosynthetic interrelationships, however, are one of the most interesting features of this wax. It has been suggested previously that hydroxy β-diketones arise by enzymatic hydroxylation of the β-diketone [6] and it is likely that hydroxylation at each different position requires a different enzyme. Thus the same enzyme in durum wheat [6] and rye [1] could cause hydroxylation at C-25, two other enzymes could cause hydroxylation at C-8 and C-9 in *Triticum compactum* [5] and *T. aestivum* [7] and three others could cause hydroxylation at C-5, C-6 and C-7 in oats [8,22]. Now it appears that *A. intermedium* contains another enzyme causing hydroxylation at C-26.

The isolation of the oxo-β-diketones is a new aspect of the biosynthesis and it can be assumed that specific dehydrogenation of the 25-CHOH group only gives the 25-oxo β-diketone (there was no indication of the presence of 26-oxo isomer). It has been suggested that specific dehydrogenation of one of a pair of secondary alcohols produces a single ketone in *Brassica oleracea* [23]. The 10-oxo-β-diketone may be formed by an analogous route even though the corresponding 10-hydroxy compound

was not found. Presumably after specific hydroxylation at C-10, the entire product is dehydrogenated to the C-10 oxo compound. C_{29} secondary alcohols and ketones with the oxygen attached at C_{10} have been found in other plant waxes [24]. Formation of the 10,25-dioxo-β-diketone probably involves application of both oxygenation processes to the precursor β-diketone.

Another hydroxylation mechanism is involved in the biosynthesis of the hydroxyoxo-β-diketones. These may be formed by hydroxylation of the oxo-β-diketones, 25-oxo at C-4 and 10-oxo at C-25 and C-26, since no 4-hydroxy-β-diketone was found (11-hydroxytetradecanoic acid in the hydrolysis products from the hydroxy-β-diketones would have been easily detected by GLC or ^{13}C NMR). Hydroxylation at C-4 may not, however, require another enzyme since positions C-4 and C-26 are both separated from a β-diketone carbonyl by nine CH_2 groups so that the enzyme which normally hydroxylates at C-26 could perhaps also hydroxylate the 25-oxo-β-diketone at C-4.

The concentration of unusual oxygenated β-diketones in spike wax suggests that wax from heads of durum wheat, rye and barley could be usefully investigated. It appears that barley spike wax contains considerably more hydroxy-β-diketones than does leaf wax [13]. A concentration of oxygenated components in flower and fruit waxes has also been observed in species of the family *Rosaceae* [19].

Wax of *A. intermedium*, not unexpectedly, shows more resemblance to wax of *A. smithii* [17], the same major β-diketones being present, than to waxes of wheat, rye and barley which are in the same tribe of the *Gramineae*. It resembles rye wax, however, in that 25-hydroxy β-diketone and also the major C_{26} alcohol are present in both [1]. 25-Hydroxy β-diketone is also present in durum wheat wax but here the major alcohol is octacosanol [6]. Investigation of wax from other species of Agropyron would be interesting since a species containing only the enzyme which hydroxylates β-diketones at C-26 might be found.

EXPERIMENTAL

Seeds of *A. intermedium* cv. Chief (which was developed from introductions from U.S.S.R.) were obtained from Dr. R. P. Knowles, Canada Department of Agriculture, Saskatoon, and sown outside. Only vegetative growth took place during the first summer but leaves were cut 125 days after germination and extracted with hexane as previously described [5]. The grass produced numerous spikes in July of the second year of growth and, just before exsertion of the anthers (to avoid contamination from pollen), the spikes and the leaves and stems were cut and extracted separately.

Chromatographic analyses. TLC was carried out in $CHCl_3$ containing 1% EtOH [6] when 25-oxo-β-diketone and C_{26} alcohol had almost the same R_f (*ca* 0.15); in $CHCl_3$ containing 1.5% EtOH R_f's were: 25-oxo-β-diketone, 0.24; C_{26} alcohol, 0.18; in $CHCl_3$ containing 5% EtOAc [25] R_f's were: hydroxy-β-diketone, 0.52, dioxo-β-diketone, 0.36; C_{26} alcohol, 0.35; hydroxyoxo-β-diketone, 0.17. Wax extracted from leaves of vegetative plants was analysed by GLC without preliminary column separation [11,25]. Wax from spikes and from leaves and stems of flowering plants was fractionated by column chromatography on Si gel using hexane with increasing proportions of Et_2O as eluant [1]. Where possible fractions were identified and analyzed by GLC as before [1,11].

^{13}C NMR. Natural abundance spectra were obtained at 25.2 MHz with proton noise de-coupling (2 KHz band width). Spectra were measured in $CDCl_3$ soln at 1000 Hz sweep width and acquisition time 4 sec. Chemical shifts are in ppm from TMS.

Hydrocarbons. GLC analysis indicated that unsaturated components were present in hydrocarbons from the spike only [26]. Oxidation of part of this hydrocarbon fraction with permangate–periodate in *tert*-BuOH containing 40% H_2O [27] gave, after CH_2N_2 treatment, Me esters of C_9, C_{16} (5%), C_{18} (20%), C_{20} (14%), C_{22} (51%) and C_{24} (10%) acids together with the saturated hydrocarbons. The IR spectrum did not contain a peak in the 960–970 cm^{-1} region.

Esters and β-diketone. These were separated from mixed fractions by Cu complex formation of the β-diketone [12] and pure esters obtained by removal of the remainder of the β-diketone as the semicarbazone [1]. Methanolysis and GLC analysis of the products was carried out as described earlier [2]. The β-diketone was identified by MS [1].

Free acids and alcohols. In fractionation of wax from spikes, part of the free acids was separated as Me esters from the oxo-β-diketone as described below and the remainder was isolated by re-chromatography, after CH_2N_2 treatment, of a mixed fraction eluted with hexane-Et_2O 19:1. Free alcohols were also obtained during the re-chromatography. No free acids were detected during chromatography of wax from leaves and stems but a pure free alcohol fraction was obtained without re-chromatography being required.

Oxo-β-diketones. During column chromatography of wax (7.6 g) from spikes, fractions totalling (1.43 g) were eluted with hexane–Et_2O (97:3, 3 l.; that is after elution of β-diketone but before elution of free alcohols and most of the free acids) which gave one major peak on GLC. Crystallization from EtOAc gave 25-oxohentriacontane-14,16-dione mp 76–76.5°; (Found: C, 77.8; H, 12.1. $C_{31}H_{58}O_3$ requires: C, 77.8; H, 12.2%); IR (5% in CCl_4): strong bands at 1712 cm^{-1} (CO) and 1602 cm^{-1} ($COCH_2CO$); UV (*iso*octane): $E^{1\%}_{1cm}$ at 273 nm, 271; PMR (100 MHz, CCl_4): δ2.20 (*q*, protons α to CO groups), 5.31 (*s*, enol form of β-diketone); MS (probe) 70 eV *m/e* (rel. int.): 478 M^+ (2), 460 (2) 408 (1), 390 (1), 351 (6), 323 (1), 310 (4), 295 (2), 281 (2), 268 (3), 253 (14), 211 (11), 183 (11), 113 (27), 100 (25), 43 (100). The oxo β-diketone was subjected to alkaline hydrolysis and neutral and acidic products separated [5]. Column chromatography of Me esters of the acids gave Me myristate (identified after GLC and hydrolysis, as myristic acid, mp and mmp 53–54°) and Me 10-oxohexadecanoate (mp and mmp with authentic material 36.5-37°). The two samples of 10-oxohexadecanoate had indistinguishable MS (probe) 70 eV *m/e* (rel. int.): 284 M^+ (7), 253 (16), 214 (47), 199 (23), 182 (9), 157 (39), 139 (19), 128 (64), 125 (35), 113 (50), 43 (100). Further elution of the column with the same solvent (5 l.) gave a fraction (0.65 g) containing 10-oxohentriacontane-14,16-dione together with some free fatty acids (shown by GLC after CH_2N_2 treatment). Treatment of part (0.56 g) of this fraction with CH_2N_2 and re-chromatography gave Me esters (0.075 g, eluted with hexane–Et_2O (99:1) and 10-oxo-β-diketone (0.31 g). Crystallization from EtOAc gave 10-oxohentriacontane-14,16-dione as long needles mp 79–80°; (Found: C, 77.6, H, 12.3. $C_{31}H_{58}O_3$ requires: C, 77.8; H, 12.2%): PMR (100 MHz, CCl_4): δ1.82 (*q*, protons on C-12), 2.18 (*m*, protons α to CO groups), 5.33 (*s*, enol form of β-diketone); MS (probe) 70 eV *m/e* (rel. int.): 478 M^+ (7), 460 (4), 366 (4), 348 (3), 333 (3), 309 (6), 282 (11), 281 (5), 267 (5), 264 (3), 239 (6), 225 (18), 197 (13), 183 (5), 170 (19), 155 (8), 113 (35), 100 (14), 43 (100). Though this β-diketone appeared to be homogenous, on GLC it gave 2 peaks of varying intensity, the second with the same emergence temp as the 25-oxo isomer and the first with an emergence temp corresponding to that of a component with one less carbon atom. It is assumed that the appearance of double peaks is due to decomposition on the column. Hydrolysis of part of the 10-oxo-β-diketone gave acids which as Me esters showed on GC–MS (3% OV-1 as liquid phase) 70 eV *m/e* (rel. int.) Me 5-oxotetradecanoate, 256 M^+ (2), 155 (15; 4,5-cleavage), 144 (63; 6,7-cleavage + H), 129 (31; 5,6-cleavage), 113 (12), 112 (100; 144-32) and Me palmitate, 270 M^+ (2). GLC resolution

of these hydrolysis products on the silicone column was that expected from previous investigations of isomeric oxooctadecanoates [28]. PMR of a mixture of 10-oxo- and 25-oxo β-diketones showed separate singlets at δ5.33 and 5.31 due to the proton on the double bond of the enolic forms and the intensities were proportional to the amounts present. It was thus shown that during column chromatography of wax from spikes the 2 isomers were completely separated but when wax from leaves and stems was chromatographed only about 65% of the oxo-β-diketones was obtained as pure 25-oxo isomer. The remainder was shown by NMR to be a *ca* 1:1 mixture of the 2 isomers.

Hydroxy-β-diketones. Fractions obtained by column chromatography (elution with hexane–Et_2O (92:8)) of wax from spikes and wax from leaves and stems were both examined in the same way: MS (probe) 70 eV *m/e*: 480 M^+, 409, 395, 253, 211. Fractions were then hydrolyzed and the hydroxy ester product separated and shown by GLC to be hydroxy hexadecanoates [6]. The percentages of 10- and 11-hydroxyhexadecanoates in the products were estimated by ^{13}C NMR as described for the analysis of hydroxy-β-diketones from wax of *Agropyron smithii* [17]. Hydroxy acids derived from waxes from different parts of the plant both contained about 17% 11-hydroxy C_{16} so that the hydroxy β-diketones consisted of 83% 25-hydroxy and 17% 26-hydroxy hentriacontane-14,16-diones.

Dioxo-β-diketone. During chromatography of wax from spikes a crude gummy fraction was obtained by elution with hexane–Et_2O (9:1). Crystallization from EtOAc and then from CCl_4 gave 10,25-dioxohentriacontane-14,16-dione as *ca* 30% of the fraction; mp 95°; (Found: C, 75.2; H, 11.0; $C_{31}H_{56}O_4$ requires: C, 75.6; H, 11.5%); PMR (100 MHz, CCl_4): δ1.82 (*q*, protons on C-12), 218 (*m*, protons α to CO groups) 5.33 (*s*, proton on double bond of enolic form). MS (probe) 70 eV *m/e* (rel. int.): 492 M^+ (6), 474 (8), 422 (2), 407 (2), 380 (2), 365 (9), 362 (3), 347 (15), 323 (7), 295 (4), 282 (10), 267 (8), 264 (5), 253 (20), 225 (29), 207 (22), 197 (16), 183 (9), 170 (14), 155 (7), 113 (42), 100 (13), 43 (100).

Hydroxyoxo-β-diketones. After elution of dioxo-β-diketones an intermediate fraction was eluted with hexane–Et_2O (8:2, 1 l.) which consisted partly of a mixture of dioxo- and hydroxyoxo β-diketones (TLC). Further elution with the same solvent gave crude hydroxyoxo-β-diketones (0.67 g from 7.6 g wax from spikes). Crystallization from EtOAc gave mixed hydroxyoxo-β-diketones (0.25 g) which were almost free from impurities; mp 79-80°; PMR (100 MHz, CCl_4) δ1.82 (*q*, protons on C-12 of 25-hydroxy-10-oxo-β-diketone), 2.22 (*m*, protons α to CO), 3.45 (*m*, CHOH), 5.31 and 533 (both *s* of almost equal intensities, enolic forms of 4-hydroxy-25-oxo- and 25-hydroxy-10-oxo-β-diketones, respectively); MS (probe) 70 eV *m/e* (rel. int.): 494 M^+ (1), 476 (6), 458 (5), 451 (3), 424 (2), 423 (1), 409 (2), 391 (3), 380 (1), 364 (2), 349 (6), 310 (2), 307 (2), 295 (3), 282 (4), 279 (5), 269 (3), 267 (4), 253 (14), 251 (5), 237 (4), 225 (18), 197 (10), 183 (12), 170 (5), 155 (5), 113 (28), 100 (6), 43 (100). Hydroxyoxo-β-diketones (0.202 g) were refluxed with 4% KOH in 95% EtOH (35 ml) for 48 hr and after dilution with H_2O (100 ml), neutral products (0.078 g) were extracted with hexane. The aq sol. was acidified and acids (0.096 g) were extracted with Et_2O and after removal of Et_2O converted to Me esters with CH_2N_2. TLC (hexane–Et_2O, 4:1) indicated that the Me esters were a mixture of oxo- and hydroxyesters. Chromatography on Si gel gave oxo esters (0.035 g, elution with hexane–Et_2O, 24:1) and hydroxy esters (0.039 g, elution with hexane–Et_2O 92:8). GLC showed that the oxo esters consisted of oxotetradecanoate (32%) and oxohexadecanoate (68%). GC–MS showed that these components had spectra the same as those reported above for Me 5-oxotetradecanoate and Me 10-oxohexadecanoate. ^{13}C NMR: 14.0, 14.07 (terminal CH_3), 15.25 (unassigned signal), 18.93 (C-3 of 5-oxo C_{14}), 22.47 (C-15 of 10-oxo C_{16}), 22.64 (C-13 of 5-oxo C_{14}) 23.82, 23.85 (C_8, C_{12} of 10-oxo C_{16} and C-7 of 5-oxo C_{14}), 24.90 (C-3 of 10-oxo C_{16}), 28.92–29.66 (number of unassigned signals), 31.56 (C-14 of 10-oxo C_{16}), 31.83 (C-12 of 5-oxo C_{14}), 33.07 (C-2 of 5-oxo C_{14}), 34.05 (C-2 of 10-oxo C_{16}), 41.42 (C-4 of 5-oxo C_{14}), 42.71, 42.79, 42.85 (C-9 and C-11 of 10-oxo C_{16} and C-6 of 5-oxo C_{14}). When analysed by GLC the hydroxy esters were a mixture of hydroxytetradecanoate (39%) and hydroxyhexadecanoate (61%). GC–MS 70 eV *m/e* (rel. int.) showed methyl 11-hydroxytetradecanoate: M^+ missing, 215 (10, 11, 12 cleavage), 186 (15, 10, 11 cleavage + H), 183 (45, 215-32), 143 (25), 55 (100) and a mixture of Me 10- and Me 11-hydroxyhexadecanoates: M^+ missing, 215 (7), 201 (12), 186 (7), 183 (25), 172 (15), 169 (64), 55 (100); ratio of peak 186 to peak 172 indicated 32% 11-hydroxy C_{16} present. ^{13}C NMR: 14.04, 14.07, 14.12 (terminal Me), 18.83 (C-13 of 11-OH C_{14}), 22.61, 22.64 (C-15 of 10- and 11-OH C_{16}) 24.94 (C-3), 25.33 (13 of 11-OH C_{16}), 25.61 (C-8 and C-12 of 10-OH C_{16}, C-9 of 11-OH C_{16}, C_9 of 11-OH C_{14}), 29.12–29.65 (unassigned signals), 31.83 (C-14 of 10-OH C_{16}), 31.91 (C-14 of 11-OH C_{16}), 34.08 (C-2), 37.45, 37.49 (C-9, C-11 of 10-OH C_{16}, C-10, C-12 of 11-OH C_{16}), 39.67 (C-12 of 11-OH C_{14}); the ratio of intensities of signals due to C-14 of 11-OH C_{16} to C-14 of 10-OH C_{16} indicated about 33% 11-hydroxy C_{16} present.

Unidentified material. Ca 30% of the unidentified material was eluted gradually from the column before hydroxy-β-diketones and the rest was composed of gummy material in the mother liquors from crystallization of the disubstituted β-diketones and obtained by elution of the column with hexane–$CHCl_3$–EtOH (7:2:1).

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REFERENCES

1. Tulloch, A. P. and Hoffman, L. L. (1974) *Phytochemistry* **13,** 2535.
2. Tulloch, A. P. (1974) *Lipids* **9,** 664.
3. Hall, D. M., Matus, A. I., Lamberton, J. A. and Barber, H. N. (1965) *Australian J. Biol. Sci.* **18,** 323.
4. Troughton, J. H. and Hall, D. M. (1967) *Australian J. Biol. Sci.* **20,** 509.
5. Tulloch, A. P. and Weenink, R. O. (1969) *Can. J. Chem.* **47,** 3119.
6. Tulloch, A. P. and Hoffman, L. L. (1971) *Phytochemistry* **10,** 871.
7. Tulloch, A. P. and Hoffman, L. L. (1973) *Phytochemistry* **12,** 2217.
8. Tulloch, A. P. and Hoffman, L. L. (1973) *Lipids* **8,** 617.
9. D. D. R. Patent No. 74703 (1970).
10. Hegi, G. (1935) *Illustrierte Flora von Mittel-Europa*, Zweite Auflage, Band I, p. 492, Carl Hanser Velag, München.
11. Tulloch, A. P. (1973) *Phytochemistry* **12,** 2225.
12. Horn D. H. S., Kranz, Z. H. and Lamberton, J. A. (1964) *Australian J. Chem.* **17,** 464.
13. von Wettstein-Knowles, P. (1971) in *Barley Genetics* II (R. A. Nilan, Ed.), p. 146, Washington State University Press, Pullman, Washington.
14. Wollrab, V. (1968) *Coll. Czech. Chem. Commun.* **33,** 1584.
15. Jackson, L. L. (1971) *Phytochemistry* **10,** 487.
16. Trka, A. and Streibl, M. (1974) *Coll. Czech. Chem. Commun.* **39,** 468.
17. Tulloch, A. P. (1976) *Phytochemistry* **15,** 1153.
18. Tulloch, A. P. and Mazurek, M. (1976) *Lipids* **11,** 228.
19. Wollrab, V. (1969) *Phytochemistry* **8,** 623.
20. Ryhage, R. and Stenhagen, E. (1960) *Arkiv Kemi* **15,** 545.
21. Herbin, G. A. and Robins, P. A. (1968) *Phytochemistry* **7,** 239.

22. Dierickx, P. J. and Buffel, K. (1972) *Phytochemistry* **11,** 2654.
23. Netting, A. G. and Macey, M. J. K. (1971) *Phytochemistry* **10,** 1917.
24. Dodova-Anghelova, M. S. and Ivanov, C. P. (1973) *Phytochemistry* **12,** 2239.
25. Tulloch, A. P. (1973) *J. Am. Oil. Chem. Soc.* **50,** 367.
26. Tulloch, A. P. (1972) *J. Am. Oil. Chem. Soc.* **49,** 609.
27. Tulloch, A. P. and Craig, B. M. (1964) *J. Am. Oil Chem. Soc.* **41,** 322.
28. Tulloch, A. P. (1964) *J. Am. Oil. Chem. Soc.* **41,** 833.