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EPICUTICULAR WAX OF *AGROPYRON SMITHII* LEAVES*

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Key Word Index—*Agropyron smithii*; Gramineae; western wheatgrass; leaf wax; composition; β-diketones; 25-oxohentriacontane-14,16-dione; 25-hydroxyhentriacontane-14,16-dione; 26-hydroxyhentriacontane-14,16-dione; ^{13}C NMR spectra.

Abstract—Wax on the leaves of *Agropyron smithii* contains hydrocarbons (3%, C_{27}–C_{33}), esters (4%, C_{26}–C_{56}), free acids (5%, C_{14}–C_{36}), hentriacontane-14,16-dione (17%), 25-oxohentriacontane-14,16-dione (3%), 25-hydroxyhentriacontane-14,16-dione (30%), 26-hydroxyhentriacontane-14,16-dione (18%) and unidentified material (20%). Free and combined acids and combined alcohols did not contain a characteristic major component and free alcohols were absent. A method of estimating proportions of isomeric hydroxy-β-diketones by ^{13}C NMR spectroscopy of the hydroxy acids obtained from hydrolysis is described.

INTRODUCTION

In previous studies of leaf wax of members of the Gramineae waxes from several wheat varieties [1–3], from oats [4] and from rye [5] were analyzed. The present investigation is part of a study of waxes of perennial grasses which can be grown in Western Canada. *Agropyron smithii* Rydb. (*Gramineae*), western wheatgrass, is a creeping perennial grass which is native to and occurs widely over most of the western part of North America [6]. Since the leaves of *A. smithii* are usually bluish-green [6], it seemed likely that it would be more waxy than other grasses investigated earlier.

RESULTS AND DISCUSSION

Most parts of the plant were glaucous including both sides of the leaf blade, the leaf sheath and the culm. In cereal crops previously examined [1–5], glaucousness was generally restricted to the leaf sheath. The percentage of wax present (Table 1) was 0.7%, higher than that found for cereals which ranged from 0.4 to 0.6% wax [1–5]. β-Diketones are the major components and form 68% of the wax (Table 1). A high β-diketone content was also indicated by the intensity of the A at 273 nm [7]. Hydroxy-β-diketones, 48% of the wax, form the major part of the β-diketones. The highest percentage of hydroxy β-diketone that has been previously observed in a plant wax was 15% in rye [5].

Amounts of hydrocarbons and esters are relatively small and free alcohols could not be detected. Earlier studies [1–5, 8] showed that glaucous parts of the plants, particularly the leaf sheath, contained high proportions of β-diketones and that the green leaf blade contained high proportions of free alcohols; thus free alcohol percentage was low when that of β-diketone was high. In *A. smithii* free alcohol content is very low probably because almost the entire plant is glaucous.

Table 2 shows the chain length distribution in the wax components. Hydrocarbons have the usual composition with major C_{29} and C_{31} components, and esters and the acids and alcohols obtained by acid methanolysis have a wide chain length range without any long chain major components. The alcohol composition is unusual in this respect since wax esters from other members of the Gramineae give alcohols with a single major component, usually C_{26} or C_{28} [1–5] (free alcohols contain the same major component). Hexacosanol would be expected to be the major alcohol since it is the principal component of the free and combined alcohols of *A. intermedium* [9] and *A. cristatum* (A. P. Tulloch and L. L. Hoffman, unpublished work). The combined alcohols are also unusual in containing an appreciable percentage of α- and β-amyrins so that the longer chain esters are presumably mainly esters of these triterpene alcohols.

Free acids also have a wide range of chain lengths with unusually high percentages of C_{28} and C_{30} acids; the C_{26} acid which would be expected to be a major component was a very minor constituent. Wax of *A. smithii* may differ from other grass waxes in lacking a major characteristic free acid and free alcohol because the β-diketone content of the wax is so high.

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

Component	%
Hydrocarbons	3
Esters	4
Free alcohols	—
Free acids	5
β-Diketone	17
Oxo β-diketone	3
Hydroxy β-diketones	48
Unidentified	20
Yield (% of dry wt)	0.7
$E^{1\%}_{1\,cm}$ at 273 nm (isooctane)	160

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*NRCC No. 15283.

The only unsubstituted β-diketone present in the wax is hentriacontane-14,16-dione, the same as that previously identified in waxes of wheat [1–3], rye [5], barley [10] and oats [4]. 25-Oxohentriacontane-14,16-dione, which is one of the major β-diketones of wax of *A. intermedium* [9], is a minor component and was identified by MS comparison with 25-oxo β-diketone from *A. intermedium* wax.

The hydroxy β-diketones of the wax are a mixture of 25-hydroxy (63%) and 26-hydroxy (37%) hentriacontane-14,16-diones. These structures were indicated by MS, a peak at *m/e* 409 was formed by cleavage of the 26,27 bond of the 26-hydroxy isomer and one at *m/e* 395 by cleavage of the corresponding 25,26 bond of the 25-hydroxy isomer [5, 11]. The relative intensities of these two peaks indicated 22% 26-hydroxy β-diketone but since MS previously gave an inaccurate estimation of composition of mixtures of hydroxy β-diketones [4], the structures and amounts of the isomers were investigated further by examination of the acidic products yielded by alkaline hydrolysis. The products, myristic acid and hydroxyhexadecanoic acids, confirmed the 14,16-dione structure and also the presence of a hydroxyl group on the C_{16} side of the β-diketone. Since ^{13}C NMR spectroscopy of hydroxy tetradecanoates obtained by hydrolysis of mixed hydroxy β-diketones from oat wax showed proportions of isomers quite accurately [4], Me 10- and 11-hydroxyhexadecanoates were synthesised and their ^{13}C NMR spectra examined. Chemical shifts of the hydroxy isomers are listed in Table 3 and assignments were made as previously described [12, 13]. The difference between the chemical shift of one of the carbons β to the CHOH group in the 11-hydroxy ester and the other β-carbons is used to estimate proportions of the isomers in mixtures. The β-carbon signals in the 10-hydroxy isomer, due to C-8 and C-12, coincide at 25.60 ppm, the signal due to C-9 in the 11-hydroxy isomer has the same shift but that due to C-13, since it is the third CH_2 group from the Me end group [12, 13], is upfield at 25.32 ppm. This difference is quite sufficient to calculate relative proportions. There is also a smaller difference of 2 Hz, between the C-14 shifts of the two isomers due to the different directions of the γ and δ effects of the OH group [13], which can be used to confirm the calculation from β-carbon signals.

The biosynthetic significance of the presence of two hydroxy β-diketones has been considered in discussion of the wax from *A. intermedium* [9]. The composition of wax from *A. smithii* is similar to that of *A. intermedium* in that the same major β-diketones are present, although the proportions of oxo- and hydroxy-β-diketones are considerably different. A close resemblance would not be expected since *A. smithii* is the only octaploid species of *Agropyron* in North America and does not hybridize with other *Agropyron* species [6]. The wax has a more distant resemblance to wax of *Triticum durum* since both contain the 25-hydroxy-β-diketone [2]. *Agropyron* and *Triticum* are related genera, both in the tribe *Triticeae*

Table 2. Composition of wax fractions from *Agropyron smithii*

No. of carbon atoms	Hydro-carbons	Esters	Hydrolysis products of esters: Acids	Hydrolysis products of esters: Alcohols*	Free acids
14	—	—	1	1	9
16	—	—	10	4	5
18	—	—	9	4	—
20	—	—	15	7	3
22	—	—	13	6	3
23	1	—	—	—	—
24	—	—	25	4	15
25	1	—	—	—	—
26	—	3	4	9	3
27	6	—	—	—	—
28	—	3	5	16	10
29	36	—	—	—	—
30	—	3	5	5	27
31	42	—	—	—	—
32	—	4	1	—	6
33	6	—	—	—	—
34	—	6	—	—	1
36	—	4	—	—	1
38	—	6	—	—	—
40	—	8	—	—	—
42	—	9	—	—	—
44	—	10	—	—	—
46	—	9	—	—	—
48	—	9	—	—	—
50	—	3	—	—	—
52	—	3	—	—	—
54	—	1	—	—	—
56	—	3	—	4	—
Unidentified†	8 (4)	17 (5)	12 (6)	4 (2)	17 (7)

* Combined alcohols also contain 40% of α- and β-amyrin (3:2). † Number of components in parentheses.

Table 3. ^{13}C NMR chemical shifts of methyl 10- and 11-hydroxyhexadecanoates*

Carbon	Shifts (ppm relative to TMS) 10-hydroxy	11-hydroxy
2	34.05	34.04
3	24.91	24.91
4	29.10	29.09
5	29.16	29.18
6	29.37	29.33
7	29.61	29.50
8	25.60	29.65
9	37.46	25.61
10	—	37.43
11	37.46	—
12	25.60	37.43
13	29.37	25.32
14	31.83	31.91
15	22.58	22.62
16	14.05	14.01

* Shift of carboxyl and CHOH carbons omitted.

[14], so that similarities between some species of the two genera are not unexpected.

Though specific uses for grass waxes have not yet been developed, waxes with high hydroxy-β-diketone contents might be useful due to the number of functional groups. It is likely that more waxy varieties containing up to 2% of wax (percentage of dry wt) could be obtained by selection from the natural population.

EXPERIMENTAL

Glaucous plants of *Agropyron smithii*, growing wild in the vicinity of Saskatoon, Saskatchewan, were collected during August and extracted with hexane as previously described [1]; spikes were removed from the plants before extraction. Previously described methods were used for TLC [2], GLC [8] and ^{13}C NMR [9] analyses. Wax was separated by column chromatography on Si gel and components were eluted with hexane containing increasing proportions of Et_2O (cf. separation of rye leaf wax [5]). The compositions of hydrocarbons and free acids were determined by GLC as described in ref. [1].

Esters and β-diketone. These components were separated as described for rye wax [5]. Me esters and alcohols were obtained from the esters by acid methanolysis and separated on Si gel [15]. The first part of the alcohol fraction to be eluted (40% of total alcohols) consisted only of triterpene alcohols which were shown by PMR and MS [15] to be α- and β-amyrins. GLC of the acetylated amyrins [15] showed the ratio of β- to α-amyrin was 2:3. The remaining long chain alcohols were analysed by GLC after acetylation. The β-diketone was identified as hentriacontane-14,16-dione by mp and MS [5].

Oxo β-diketone. This component was identified as 25-oxohentriacontane-14,16-dione by MS comparison with the major oxo β-diketone from wax of *Agropyron intermedium* [9]. The PMR spectrum showed that 10-oxohentriacontane-14,16-dione was absent [9].

Hydroxy β-diketones. The hydroxy β-diketone fraction had MS (probe) 70 eV *m/e* (rel. int.): 480 M^+ (8), 462 (13), 444 (13), 409 (10), 395 (35), 380 (2), 366 (5), 348 (5), 297 (10), 294 (9), 281 (10), 279 (15), 268 (17), 253 (30), 250 (13), 237 (10), 218 (15), 211 (35), 100 (23), 43 (100). Hydroxy β-diketones were subjected to alkaline hydrolysis and the acidic products were separated into non-oxygenated esters (shown by GLC to be principally Me tetradecanoate) and hydroxy esters as described earlier [4]. Using the ^{13}C NMR method reported below the hydroxy esters were shown to be a mixture of 10-hydroxyhexadecanoate (63%) and 11-hydroxyhexadecanoate (37%).

Synthesis of Me 10- and Me 11-hydroxyhexadecanoates. Me 10-oxohexadecanoate was prepared by the general method of refs [16, 17] from 9-methoxycarbonylnonanoyl chloride and pentylmalonic acid; the yield was 56% and the mp 36° (Found: C, 71:7; H, 11.5. $C_{17}H_{32}O_3$ requires C, 71.8; H, 11.3%). Me 11-oxohexadecanoate was prepared by the same method from 10-methoxycarbonyldecanoyl chloride and butylmalonic acid; mp 37°, yield 53%. (Found: C, 72.0; H, 11.4. $C_{17}H_{32}O_3$ requires C, 71.8; H, 11.3%). Hydroxy esters were prepared in almost quantitative yield by reduction of oxo esters with $NaBH_4$ in MeOH at 25°. Me 10-hydroxyhexadecanoate had mp 44° (Found: C, 71.1; H, 11.9. $C_{17}H_{34}O_3$ requires C, 71.3; H, 12.0%); GC–MS 70 eV *m/e* (rel. int.): M^+ missing, 201 (18), 172 (23), 169 (100), 98 (37), 87 (68); ^{13}C NMR in Table 3. Me-11-hydroxyhexadecanoate mp 42.5° (Found: C, 71.1; H, 11.9. $C_{17}H_{34}O_3$ requires C, 71.3; H, 12.0%); GC–MS 70 eV *m/e* (rel. int.): M^+ missing, 215 (10), 186 (15), 183 (45), 101 (8), 95 (25), 87 (100); ^{13}C NMR in Table 3. To calculate % of 11-hydroxy C_{16} in mixtures with 10-hydroxy C_{16}, it is assumed that the integral of signals due to C-9 and C-13 of 11-OH C_{16} are equal and if the integral of the composite signal due to C-9 of 11-OH, C-8 and C-12 of 10-OH is x and the integral of the signal due to C-13 of 11-OH is y, then % of 11-OH C_{16} = $100y/[y + 1/2(x - y)]$. Using this method, % of 11-OH in a mixture of synthetic 10- and 11-OH isomers, which contained 20% 11-OH, was estimated to be 21%. Comparing intensities of peaks at 186 and 172 in GC–MS of this mixture, indicated that it contained 18.7% 11-OH. However, comparison of peaks at 215 and 201 and at 183 and 169 indicated only 14.2 and 13.7% 11-OH respectively.

Unidentified material. About one third of this material was gradually eluted over a large number of fractions before hydroxy-β-diketones and the remainder was a dark gum, frequently found in grass waxes, eluted after hydroxy-β-diketones. It is unlikely that any single major component is present in this unidentified fraction.

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