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EPICUTICULAR WAX OF *POA AMPLA* LEAVES*

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Key Word Index—*Poa ampla*; Gramineae; big bluegrass; epicuticular wax; leaves; composition; hentriacontane-14,16-dione; 5-oxohentriacontane-14,16-dione; 4-, 5- and 6-hydroxyhentriacontane-14,16-dione.

Abstract—Leaf wax of a glaucous variety of *Poa ampla* contains hydrocarbons (5%, C_{23}–C_{35}), esters (9%, C_{36}–C_{56}), free acids (3%, C_{16}–C_{34}), free alcohols (6%, mainly C_{26}); hentriacontane-14,16-dione (14%), 5-oxohentriacontane-14,16-dione (1%); hydroxy β-diketones (56%) and unidentified material (6%). The hydroxy β-diketones, which are more abundant in this wax than in others, were shown by ^{13}C NMR to consist of 4-hydroxy (15%), 5-hydroxy (70%) and 6-hydroxy (15%) hentriacontane-14,16-diones.

INTRODUCTION

The genus *Poa* (bluegrasses) is one of the larger genera in the family Gramineae and many species are important forage grasses. Except for a brief report concerning wax of *P. colensoi* [1] there have been no investigations of waxes from *Poa* species. Because of the possibility of obtaining useful waxes from grasses and also of using wax composition in grass classification, a complete analysis of leaf wax of *Poa ampla* (Merr.) has been made. *P. ampla* (big bluegrass) is a native of western North America which usually occurs as a glaucous variety; it is a valuable drought resistant pasture grass.

RESULTS

The wax was found to contain 14% of β-diketone and 56% of hydroxy β-diketones, the highest percentage of hydroxy β-diketones found in any wax examined to date [2] (Table 1). As expected from the high β-diketone content the wax showed strong UV absorption at 273 nm. Wax content, 0.6% of dry wt, was slightly higher than that usually found for grasses (0.4–0.5%) [3–5].

Table 1. Composition and yield of epicuticular wax from *Poa ampla**

Component	%
Hydrocarbons	5
Esters	9
Free acids	3
Free alcohols	6
β-Diketone	14
Oxo β-diketone	1
Hydroxy β-diketones	56
Unidentified fractions	
Eluted after β-diketones	1
Eluted after hydroxy β-diketones	3
Lost on column	2
Yield (% dry wt)	0.6
$E^{1\%}_{1\,cm}$ at 273 nm (isooctane)	175

* Determined from weights of components obtained by silicic acid column chromatography.

Table 2 lists the chain lengths of the wax fractions. Hydrocarbons ranged from C_{23}–C_{35} with C_{31} as major component. Esters showed a range of chain lengths from C_{36} to C_{56} without dominant component, which result from esterification of C_{16}–C_{32} alcohols with C_{16}–C_{30} acids and are similar to those found in esters of other grass waxes [3–5]. Combined alcohols also contained *ca* 14% of lupeol. Hexacosanol formed only 48% of combined alcohols but 85% of the free alcohols. Free acids had the wide chain length range C_{14}–C_{34} usually observed in grass waxes [2, 5].

The β-diketone fraction consisted only of hentriacontane-14,16-dione which has previously been found in wax from 6 species in the grass tribe Hordeae and also in wax from oats [2]. MS of the hydroxy β-diketones showed that the major component was probably 5-hydroxyhentriacontane-14,16-dione but other isomers also appeared to be present. Since it was found previously that MS does not measure proportions of isomers accurately [4], these components were also examined by alkali hydrolysis. The acidic products were hexadecanoic acid and a mixture of hydroxytetradecanoic acids confirming that the hydroxyl group was present in the C_{13} side of the molecule. The ^{13}C NMR spectrum of the methyl esters of the hydroxytetradecanoic acids obtained showed that 11-hydroxy (from 4-hydroxy β-diketone), 10-hydroxy (from 5-hydroxy β-diketone) and 9-hydroxy (from 6-hydroxy β-diketone) esters were present. Recent investigations of the ^{13}C NMR spectra of all the isomeric hydroxyoctadecanoates [6] has made it possible to assign many of the signals in the spectrum of the mixed hydroxytetradecanoates unambiguously. The intensities of the signals showed that the composition of the hydroxy β-diketone mixture was approximately 4-hydroxy (15%), 5-hydroxy (70%) and 6-hydroxy (15%). It is useful to note that signals due to C-13 and C-12 of each hydroxytetradecanoate have characteristic shifts and clearly establish the presence of these isomers. Also the shift of C-12 of 9-hydroxytetradecanoate is sufficiently different from that of C-12 of the 8-hydroxy isomer that the absence of the latter signal is good evidence for the absence of this particular isomer (and hence 7-hydroxy β-diketone) from the mixture.

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Table 2. Composition of wax fractions from *Poa ampla*

No. of carbon atoms	Hydrocarbons	Esters	Hydrolysis products of esters: Acids	Hydrolysis products of esters: Alcohols	Free acids	Free alcohols
14	—	—	—	—	1	—
16	—	—	4	1	27	—
18	—	—	3	2	1	—
20	—	—	16	2	2	—
22	—	—	24	9	17	—
23	1	—	—	—	—	—
24	—	—	32	10	19	4
25	1	—	—	—	—	—
26	—	—	14	48	9	85
27	4	—	—	—	—	—
28	—	—	5	6	6	5
29	16	—	—	—	—	—
30	—	—	2	4	4	3
31	66	—	—	—	—	—
32	—	—	—	4	6	3
33	9	—	—	—	—	—
34	—	—	—	—	3	—
35	1	—	—	—	—	—
36	—	3	—	—	—	—
38	—	10	—	—	—	—
40	—	8	—	—	—	—
42	—	11	—	—	—	—
44	—	10	—	—	—	—
46	—	15	—	—	—	—
48	—	14	—	—	—	—
50	—	13	—	—	—	—
52	—	8	—	—	—	—
54	—	5	—	—	—	—
56	—	3	—	—	—	—
Unidentified	5 (5)	—	—	14 (1)*	5 (8)	—

* Unidentified alcohol, mostly lupeol (GC–MS).

Two of the hydroxy β-diketones present in *P. ampla* wax were previously found in wax from oats, which contained a minor amount of hydroxy β-diketones, consisting of 5-hydroxy (58%), 6-hydroxy (35%) and 7-hydroxy (8%) [4]. 4-Hydroxy β-diketone has not been found before but 4-hydroxy-25-oxohentriacontane-14,16-dione was a minor component of wax from spikes of *Agropyron intermedium* [7]. The presence of a major amount of the 5-hydroxy β-diketone in wax of *P. ampla* is of biosynthetic interest since, if it is assumed that hydroxy β-diketones are formed by hydroxylation of β-diketones, then perhaps the formation of this isomer is similar to that of the most common hydroxy β-diketone, 25-hydroxyhentriacontane-14,16-dione, since in both the site of hydroxylation is separated from the β-diketone grouping by 8 methylene groups. The only other hydroxy β-diketone with the hydroxyl at C-5 is 5-hydroxytritriacontane-12,14-dione which is a very minor constituent of wax of *Festuca ovina* [8].

An oxo β-diketone was also present as a minor component (Table 1) and MS showed that it was most probably 5-oxohentriacontane-14,16-dione. The only other common oxo β-diketone which has been previously isolated is 25-oxohentriacontane-14,16-dione from wax of *Agropyron intermedium* [7]; its structure was determined by chemical degradation so that its MS fragmentation could be used to interpret that of the 5-oxo isomer, found in the present investigation. Almost all the ions supported the 5-oxo structure, in particular the intense ion at *m/e* 379, produced by cleavage of the 6,7 bond, corresponds with the ion at *m/e* 351 in the MS of the 25-oxo isomer which similarly results from cleavage of the 23,24 bond. The weak ion at *m/e* 393 could indicate the presence of a small percentage of the 4-oxo isomer but in view of the previously mentioned limitations of MS in mixture analysis, the presence of this isomer remains uncertain. There was no fragmentation suggesting the presence of other isomers. Thus the oxo β-diketone in *P. ampla* is oxygenated at the same carbon as the major hydroxy β-diketone. In wax of *A. intermedium* the hydroxy β-diketone consists of two isomers, 25-hydroxy (85%) and 26-hydroxy (15%), but the oxo β-diketone is entirely the 25-oxo isomer [7].

It has been suggested that the chain lengths of some wax components, particularly the free alcohols, might have some value in grass classification [2] and since the grass under investigation belongs to a genus not previously examined it is useful to compare its composition with those of possibly related genera. The genus *Poa* belongs to the grass tribe Festuceae and wax of one species from each of the genera *Dactylis*, *Festuca* and *Lolium* (all in the same tribe) also contain hexacosanol as major free alcohol [2]. Wax of one species of *Bromus*, also in the tribe Festuceae, however, contained octacosanol [5]. In these species only wax of *F. ovina* contained β-diketone, which was principally tritriacontane-12,14-dione [2, 5], different from the *Poa* β-diketones both in chain length and position of the carbonyl groups. There is a similarity, however, in that the hydroxyl group of the hydroxy β-diketones of both waxes are attached to C-5. Since according to one estimate [9] the tribe Festuceae contains 40 genera and 700 species, too few species have been examined so far to make useful generalizations. There is one respect, though in which wax from *P. ampla* resembles almost all the other β-diketone containing grass waxes and that is that the high β-diketone content is accompanied by a low free alcohol content [2].

EXPERIMENTAL

P. ampla strain P8903 was grown outside from seed supplied by J. L. Schwendiman, Plant Materials Center, USDA, Pullman, Washington. Plants did not flower until the second year of growth but leaf blades were glaucous within 6 weeks after germination. Wax was extracted with hexane [10] from grass cut during the second year of growth, spikes were excluded from the extraction.

Wax was fractionated by column chromatography on Si gel with hexane containing increasing proportions of Et_2O as eluent [11]. The following fractions were obtained: hydrocarbons, a mixture of esters and β-diketone, oxo β-diketone and unidentified minor components, free acids, free alcohols, hydroxy β-diketones with some alcohols, hydroxy β-diketones and unidentified gum. These were examined by TLC and GLC as before [2]. Hydrocarbons, free acids and alcohols were identified by GLC after addition of authentic compounds. β-Diketone was separated as the Cu complex [12] and esters were purified as before [11] and identified by GLC.

Esters were subjected to methanolysis and the Me esters and alcohols separated on a Si gel column [13]. Me esters were analysed by GLC and the alcohols (as TMSi ethers) by GC–MS (Dexsil column). A triterpenoid component in the alcohol fraction gave the fragment ions expected from the TMSi ether of lupeol [14].

The purified β-diketone was hydrolysed (NaOH) and the resulting acids and Me ketones separated [10]. GLC showed the former (as Me esters) to be C_{14} and C_{16} and the latter to be C_{15} and C_{17}. 5-Oxohentriacontane-14,16-dione was isolated from a crude fraction by several crystallizations from EtOAc, mp 75–76°; MS (probe) 70 eV *m/e* (rel. int.): 478 M^+ (11), 460 (10), 436 (2), 421 (2), 393 (1), 379 (17), 309 (4), 296 (7), 282 (20), 281 (18), 278 (7), 267 (8), 239 (13), 225 (28), 183 (31), 113 (16), 85 (78), 57 (100).

Alcohols were partly removed from mixtures with hydroxy β-diketones by crystallization from $CHCl_3$ and completely by rechromatography of the mother liquors. Combined hydroxy β-diketone fractions were crystallized twice from EtOAc, mp 74–75°, $[\alpha]_D^{25}$ + 1.2, $[\alpha]_{546}^{25}$ + 1.6, $[\alpha]_{436}^{25}$ + 2.1 ($CHCl_3$; *c* 4.9); (Found: C, 77.6; H, 12.8. $C_{31}H_{60}O_3$ requires: C, 77.4; H, 12.6%): GC–MS 70 eV *m/e* (rel. int.) 480 M^+ (1), 462 (1), 444 (3), 437 (1), 423 (5), 409 (2), 309 (6), 281 (18), 251 (8), 239 (25), 100 (100).

Part of the hydroxy β-diketones was hydrolysed and separated into Me ketones and acids and the acids converted to Me esters and separated (Si gel column) into Me hexadecanoate and Me hydroxytetradecanoates [10]. Hexadecanoic acid was obtained by hydrolysis of the Me ester and its mp was not depressed by that of an authentic sample. The Me hydroxytetradecanoates had $[\alpha]_D^{25}$ + 1.2, $[\alpha]_{546}^{25}$ + 1.8, $[\alpha]_{436}^{25}$ + 2.5 ($CHCl_3$; *c* 4.1) and ^{13}C NMR ($CDCl_3$): 14.08 (terminal CH_3), 18.84 (C-13 of 11-OH), 22.65 (C-13 of 9-OH), 22.78 (C-13 of 10-OH) 24.94 (C-3), 25.34 (C-11 of 9-OH), 25.62 (C-9 of 11-OH, C-8 of 10-OH and C-7 of 9-OH), 27.86 (C-12 of 10-OH), 29.12–29.62 (unresolved signals), 31.92 (C-12 of 9-OH), 34.08 (C-2), 37.19 (C-11 of 10-OH), 37.46 (C-10 of 11-OH, C-9 of 10-OH and C-8 and C-10 of 9-OH), 39.68 (C-12 of 11-OH). The composition of the mixture was the average of values obtained from the intensities of the signals due to the α carbons and from the intensities of the signals due to the β carbons (all on the terminal Me side of the CHOH).

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