Phytochemistry, Vol. 22, No. 7, pp. 1605–1613, 1983.
Printed in Great Britain.

0031–9422/83 $3.00 + 0.00
Pergamon Press Ltd.

EPICUTICULAR WAXES FROM *AGROPYRON DASYSTACHYUM*, *AGROPYRON RIPARIUM* AND *AGROPYRON ELONGATUM**

ALEXANDER P. TULLOCH

National Research Council of Canada, Prairie Regional Laboratory, Saskatoon, Saskatchewan, Canada, S7N 0W9

(*Received* 26 *November* 1982)

Key Word Index—*Agropyron dasystachyum*; *A. riparium*; *A. elongatum*; Gramineae; epicuticular wax; 25-hydroxyhentriacontane-14,16-dione; 26-hydroxyhentriacontane-14,16-dione; 10-oxohentriacontane-14,16-dione; 25-oxohentriacontane-14,16-dione; 4-hydroxy-25-oxohentriacontane-14,16-dione; 18-hydroxy-7,16-hentriacontanedione.

Abstract—*Epicuticular waxes from whole plants of Agropyron dasystachyum var. psammophylum, A. riparium and A. elongatum* contain hydrocarbons (5–8%), long chain esters (12–15%) and free acids (2–5%). The major esters are C_{34}–C_{56} esters derived from C_{16}–C_{30} acids and alcohols (1-hexacosanol is the major alcohol) but C_{31}, C_{33} and C_{35} esters (3–11%) are also present. The latter esters are C_{18} and C_{20} acid esters of C_{13} and C_{15} 2-alkanols. *A. dasystachyum* wax contains 2% free alcohols, that of *A. riparium* contains 17% and that of *A. elongatum* 11% (1-hexacosanol is the major alcohol in each). Diesters (2%), C_8–C_{12} diols esterified by (*E*)-2-alkenoic acids, are present in *A. riparium* wax. Hentriacontane-14,16-dione is present: 29% in *A. dasystachyum wax* and 32% in *A. riparium* wax, but only 5% in *A. elongatum* wax. 25-Oxohentriacontane-14,16-dione forms 14% of *A. dasystachyum* wax and 27% of *A. elongatum* wax but the oxo β-diketones of *A. riparium* wax (5%) consist of both 10-oxo- and 25-oxohentriacontane-14,16-diones in the ratio 4:1. Hydroxy β-diketones of the waxes are 25- and 26-hydroxyhentriacontane-14,16-diones; in *A. dasystachyum* (20%) the ratio is 3:1, in *A. elongatum* (20%) the ratio is 9:1 but in *A. riparium* (5%) it is *ca* 1:2. The configuration of the hydroxyl group in the 26-hydroxy β-diketone is opposite to that in the 25-hydroxy derivative. The unusual composition of the oxygenated β-diketones of *A. riparium* confirms that this species should be regarded as separate from *A. dasystachyum*. Wax from *A. elongatum* also contains 4-hydroxy-25-oxohentriacontane-14,16-dione (4%) and an unusual oxo-β-ketol, 18-hydroxy-7,16-hentriacontanedione (2%), both these components are probably derived biosynthetically from the 25-oxo β-diketone which is the major component of this wax. Syntheses of racemic 18-hydroxy-7,16-hentriacontanedione and of a model β-ketol, 25-hydroxy-27-pentacosanone, are described.

INTRODUCTION

The grass genus *Agropyron* contains a number of species which were, at one time, regarded as distinct species but which, more recently, have been considered as just varieties of one species. Thus, *Agropyron dasystachyum* (Hook) Scribn. (northern wheatgrass) and *Agropyron riparium* Scribn. and Smith (streambank wheatgrass) were regarded as separate but related species [1]. Later, however, they were described as varieties of *A. dasystachyum*: *A. dasystachyum* (Hook). Scribn. var. *dasystachyum* and *A. dasystachyum* (Hook.) Scribn. var. *riparium* (Scribn. and Smith) Bowden, respectively [2, 3]. More recently, a third variety, *A. dasystachyum* (Hook). Scribn. var. *psammophilum* (Gillet and Senn) Voss, has been described [4, 5] and is found on sand dunes in contrast to the other two varieties which occur on heavy clay soils [1, 4].

During an analysis of the principal components of epicuticular waxes from 123 species in the tribe Triticeae, including 18 species in the genus *Agropyron*, by GC/MS, wax from '*A. dasystachyum*' (unspecified varieties) was found to have an unusual oxygenated β-diketone composition [6]. The hydroxy β-diketone was mainly 26-hydroxyhentriacontane-14,16-dione and the oxo β-diketone mainly 10-oxohentriacontane-14,16-dione. Since the hydroxy β-diketones from other species of *Agropyron* and from the related genera *Anthosachne*, *Elytrigia* and *Roegneria* and also from *Elymus* and *Leymus*, altogether 22 species which contain hydroxy β-diketone, all contain mainly 25-hydroxyhentriacontane-14,16-dione [6], a complete investigation of wax from '*A. dasystachyum*' was undertaken. For this purpose *A. riparium* cv Sodar, which was developed from a species collected in Oregon and which has been stated to be *A. dasystachyum* [7], was selected. It is convenient here, as discussed later, to continue to regard *A. riparium* as a distinct species. Wax from *A. dasystachyum* var. *psammophilum* (the only variety available) was also examined.

25-Oxohentriacontane-14,16-dione, first observed in wax from *A. intermedium* [8], has since been found in wax from 12 species of the Triticeae [6], and in some species it was the largest single component. Complete analysis of a wax with a major amount of this oxo β-diketone was desirable and has now been carried out with wax from *A. elongatum* (Host) Beauv. cv Orbit (tall wheatgrass). This species is a caespitose grass, originally introduced from southern U.S.S.R., and developed as forage for wet alkaline and also semiarid conditions [9, 10]. Complete analyses of these waxes should help to define the applications of wax compositions in grass classification. Also, isolation of new oxygenated β-diketones may clarify the biosynthetic relationships between these components.

*NRCC No. 20988.

RESULTS AND DISCUSSION

Table 1 shows the composition of waxes from the three species. The amount of wax obtained was close to 1% of the dry wt for *A. riparium* but only 0.5–0.6% for the other two; percentages of *ca* 0.6% are common for grasses with β-diketone containing waxes [6, 11, 12]. Hydrocarbon, ester and free acid contents were similar in all three but free alcohol contents were appreciably different. Free alcohol content was lowest in *A. dasystachyum* which had the highest β-diketone content. Oxygenated β-diketone content was greatest in *A. elongatum* and smallest in *A. riparium*.

Hydrocarbon compositions (Table 2) were very similar to each other and to those of waxes from other species in the Triticeae [8, 11, 13–16], with nonacosane and hentriacontane as major components.

Esters (Table 3) had a very wide chain length range with C_{46} the largest single component, although forming only 12–17% of the total. Unusual odd-numbered esters, C_{31}, C_{33} and C_{35}, were found and GC/MS showed that these were 2-tridecyl octadecanoate and icosanoate and 2-pentadecyl icosanoate, respectively. Esters of 2-alkanols have been found previously in wax from barley spikes [17]. Their mass spectra, unlike those of esters of 1-alkanols, show four prominent fragments: from an ester RCO_2R' these are ions corresponding to $RCO_2H_2^+$, RCO_2H^+, RCO^+ and $[R'-1]^+$ [17]. Thus, pentadecyl icosanoate gives the ions *m/z* 313 $[RCO_2H_2]^+$, 312 $[RCO_2H]^+$, 295 $[RCO]^+$ and 210 $[R'-1]^+$. Esters from *A. riparium* also contained minor amounts of unsaturated esters derived from (*E*)-2-alkenoic acids. Such esters have previously been found in wax from wheat [13–15] and rye [16].

As expected, alcohols obtained on hydrolysis of esters (Table 4) contained C_{13} and C_{15} 2-alkanols which were identified by GC/MS of the TMSi ethers. The $[M-15]^+$ fragments had *m/z* 257 and 285, respectively, and both had the base peak at *m/z* 117 [Me $\dot{C}$HOTMSi] [18]. The presence of methyl (*E*)-2-docosenoate and tetracosenoate in methyl esters of acids from esters of *A. riparium* was also confirmed by GC/MS. These acids were previously identified as TMSi ethers [19]; as methyl esters they have a characteristic ion at *m/z* 113, presumably $CH_2{=}CH{-}CH{=}CH{-}\overset{+OH}{\overset{\|}{C}}{-}OMe$, formed by proton transfer from the γ-carbon rather than the α-carbon. Major acids from esters were C_{20} and C_{22} and the major alcohol was 1-hexacosanol, but both acids and alcohols had a wide range of

Table 1. Composition and yield of epicuticular waxes from *Agropyron* species*

Components	*A. dasystachyum* var. *psammophylum*	*A. riparium*	*A. elongatum*
Hydrocarbons	5	8	5
Esters	12	13	15
Diesters	—	2	—
Free acids	5	3	2
Free alcohols	2	17	11
β-Diketone	29	32	5
Oxo β-diketones	14	5	27
Hydroxy β-diketones	20	5	20
Hydroxyoxo β-diketone	—	—	4
Oxo β-ketol	—	—	2
Unidentified	13	15	9
Yield (% dry wt)	0.58	0.90	0.48
$E^{1\%}_{1\,cm}$ at 273 nm (iso-octane)	160	116	140

*In wt % determined by CC.

Table 2. Composition of hydrocarbons from waxes of *Agropyron* species*

Carbon No.	*A. dasystachyum* var. *psammophylum*	*A. riparium*	*A. elongatum*
23	1	—	1
25	2	1	3
27	6	2	6
29	39	35	40
31	42	46	39
33	8	13	8
35	—	1	—
Unidentified†	2(6)	2(3)	3(7)

*Components less than 0.5% have been omitted.

†Number of components in parentheses; includes components with emergence temperatures which suggested branched chain structures or an even-number of carbons.

Table 3. Compositions of esters from waxes of *Agropyron* species*

Carbon No.	*A. dasystachyum* var. *psammophylum*	*A. riparium*	*A. elongatum*
31†	—	1	1
32	—	3	—
33†	4	1	1
34	4	3	3
35†	7	1	2
36	8	3	4
38	5	3	4
40	7	5	6
42	7	9	10
42:1‡	—	3	—
44	8	10	15
44:1‡	—	5	—
46	12	17	16
46:1‡	—	2	—
48	8	14	10
50	7	5	2
52	5	4	3
54	4	2	2
56	3	1	3
58	—	—	2
Unidentified§	11(17)	8(7)	16(11)

*Components less than 0.5% have been omitted.
†Esters of 2-alkanols.
‡Esters of (*E*)-2-alkenoic acids.
§Number of components in parentheses; includes components with emergence temperatures which suggested branched chain structures or an odd-number of carbons.

Table 4. Composition of acids and alcohols obtained by hydrolysis of esters from waxes of *Agropyron* species*

	A. dasystachyum var. *psammophylum*		*A. riparium*		*A. elongatum*	
Carbon No.	Acids	Alcohols	Acids	Alcohols	Acids	Alcohols
13†	—	3	—	—	—	1
14	1	—	—	—	1	—
15†	—	9	—	3	—	3
16	8	10	8	6	8	3
18	6	11	7	7	5	2
20	28	1	20	7	21	5
22	20	2	16	8	21	8
22:1‡	—	—	5	—	—	—
24	18	13	11	9	16	16
24:1‡	—	—	8	—	—	—
26	4	28	9	42	7	54
28	4	3	8	10	5	2
30	4	7	6	4	4	1
32	1	3	—	—	—	—
Triterpenes	—	7	—	—	—	3
Unidentified§	6(13)	3(6)	2(4)	4(11)	12(16)	2(4)

*Components less than 0.5% have been omitted.
†2-Alkanols.
‡(*E*)-2-Alkenoic acids.
§See last footnote to Table 3.

chain lengths accounting for that of the parent esters.

A small amount of diesters was present in wax from *A. riparium*. They had the same structure and composition as those found earlier in waxes from rye [16] and wheat [20] and consisted of C_8–C_{12} diols esterified by equal proportions of (*E*)-2-docosenoic and tetracosenoic acids. Structures were confirmed by GC/MS of the methanolysis products; spectra of the methyl esters were as described above and of the *bis*-TMSi ethers of the diols were the same as those previously reported [21]. Major free acids of all three waxes (Table 5) were C_{22}–C_{30} components but C_{28} and C_{30} predominated in wax from *A. elongatum*. A small amount of (*E*)-2-docosenoic and tetracosenoic occurred in acids from *A. riparium*. 1-Hexacosanol was the principal free alcohol as it was in waxes from the majority of *Agropyron* and *Roegneria* species which have been examined [6].

β-Diketones accounted for most (42–63%) of the remainder of the waxes. In this investigation an improved procedure for purification of these components involving rechromatography on a copper acetate–silica gel column has been used [22]. β-Diketones isolated by CC are usually mixed with esters and were formerly recovered by repeated copper complex formation [13, 23]; esters were finally purified by rechromatography after conversion of remaining β-diketones to semicarbazones [16]. This new procedure also separated a mixture of diesters and 25-oxohentriacontane-14,16-dione from *A. riparium* wax. During examination of β-diketones from *A. elongatum*, the iodoform reaction, first employed to degrade labelled β-diketones [22] to a mixture of two acids, rather than to the mixture of methyl ketones and acids given by alkaline cleavage [13, 24], was used and the expected pairs of acids were obtained from the β-diketone, 25-oxo and 25-hydroxy β-diketone.

β-Diketone from all three waxes was hentriacontane-14,16-dione, the same as was found in waxes from well over 100 species of the tribe Triticeae [6, 25]. There were, however, marked differences between the compositions of the oxygenated β-diketones. Only 25-oxohentriacontane-14,16-dione could be detected in waxes of *A. dasystachyum* and *A. elongatum*, but wax of *A. riparium* contained 10-oxo β-diketone as well and the ratio of 10-oxo to 25-oxo β-diketone was 4:1. The two β-diketones are largely separated by CC, and can be estimated in mixtures by ^{1}H NMR [8]. Both were first found in wax from *A. intermedium* where the ratio of 25-oxo to 10-oxo β-diketone was 17:5 [8].

25-Hydroxyhentriacontane-14,16-dione was the major hydroxy β-diketone in wax of *A. elongatum* and *A. dasystachyum* but 26-hydroxy β-diketone was also present, *ca* 10% in *A. elongatum* and 25% in *A. dasystachyum*. Both were previously found in wax of *A. intermedium* [8] and *A. smithii* [11] and the 26-hydroxy isomer was the minor component. GC/MS (of the *bis*-TMSi ether [19]) showed that in wax from *A. riparium*, however, the 26-hydroxy isomer was the major component. Examination of hydroxy esters, obtained by cleavage of hydroxy β-diketones, by ^{13}C NMR [11] showed that the ratio of 26-hydroxy isomer to 25-hydroxy was *ca* 13:7.

Hydroxy β-diketones from *A. elongatum* and *A. dasystachyum* had small positive specific rotations as have all hydroxy β-diketones, from waxes of the Triticeae, for which specific rotations have been measured previously [13–16]. Mixed hydroxy β-diketones from *A. riparium*, however, had a small negative rotation as did the hydroxyhexadecanoates obtained by alkaline cleavage. The magnitude of rotation was lower than that usually observed suggesting that although 26-hydroxy β-diketone is levorotatory, the 25-hydroxy isomer has given the resulting mixture a smaller than usual rotation. The negative rotation shows that 26-hydroxy β-diketone has the opposite, or *R*, configuration from all previously reported hydroxy β-diketones with 31 and 33 carbons, except for 5-hydroxytritriacontane-12,14-dione from wax of *Andropogon* species. It suggests that the enzyme

Table 5. Composition of free acids and free alcohols from waxes of *Agropyron* species*

	A. dasystachyum var. *psammophylum*		*A. riparium*		*A. elongatum*	
Carbon No.	Free acids	Free alcohols	Free acids	Free alcohols	Free acids	Free alcohols
14	2	—	5	—	7	—
16	4	—	12	—	6	—
18	—	—	—	—	1	—
20	4	—	2	—	2	—
22	16	—	10	—	7	—
22:1†	—	—	3	—	—	—
24	25	1	16	2	13	3
24:1†	—	—	5	—	—	—
26	6	94	8	82	11	83
28	17	3	16	11	20	4
30	18	2	12	2	22	3
32	5	—	5	—	7	—
34	1	—	1	—	1	—
Unidentified‡	2(6)	—	5(11)	3(8)	3(7)	7(6)

*Components less than 0.5% have been omitted.

†(*E*)-2-Alkenoic acids.

‡See last footnote to Table 2.

responsible for hydroxylation at C-26, assuming that to be the biosynthetic pathway, differs from that responsible for hydroxylation at C-25 in more than just the possession of a different degree of specificity regarding the hydroxylation site.

During CC of wax from *A. elongatum*, an unusually large amount of partially crystalline components was eluted after the hydroxy β-diketone. GC and TLC indicated two components, and one of these, which had the smaller R_f on TLC and the longer GC R_t, was converted by the iodoform reaction to a mixture of 11-hydroxytetradecanoate and 10-oxohexadecanoate and was, thus, probably 4-hydroxy-25-oxohentriacontane-14,16-dione; the other component was not affected by the reaction. The two constituents were separated on the copper acetate–Si gel column when the copper complex of the β-diketone emerged last.

^{1}H NMR of the β-diketone showed the presence of hydroxyl and carbonyl substituents; comparison of the ^{13}C NMR spectrum with those of appropriate hydroxy and oxo esters [26, 27] showed that the hydroxyl group was on C-4 from one end of the chain and the oxo group was on C-7 from the other end. These substituents are not close enough to the β-diketone group to affect the signals of neighbouring carbons so that the spectrum does not indicate the position of this group [A. P. Tulloch, unpublished work]. Mass spectrometry of the *bis*-TMSi ether (Fig. 1) showed all the ions expected [12, 19] for the derivative of 4-hydroxy-25-oxohentriacontane-14,16-dione except for that with *m/z* 553. Like most of the other hydroxy β-diketones which have the hydroxyl group near the end of the C-14 part of the chain [28, 29] of this hydroxyoxo β-diketone had a small positive rotation indicating the *S* configuration. This disubstituted β-diketone had not previously been isolated in pure form but it was present as a mixture with 25- and 26-hydroxy-10-oxo β-diketones from spike wax of *A. intermedium* [8]. GC/MS examination of wax isolated from only the spikes of *A. elongatum* indicated that it contained *ca* twice as much hydroxyoxo β-diketone as did wax from the whole plant.

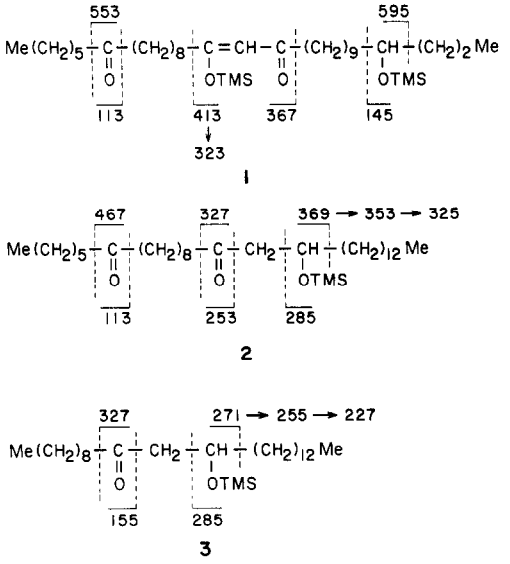

Fig. 1. Mass spectral fragmentation of TMSi ethers of hydroxyoxo β-diketone and oxo β-ketol from wax of *Agropyron elongatum* and of synthetic β-ketol. (**1**) 4-Hydroxy-25-oxohentriacontane-14,16-dione di TMSi ether. (**2**) 18-Hydroxy-7,16-hentriacontanedione TMSi ether. (**3**) 12-Hydroxy-10-pentacosanone TMSi ether.

The other component of the mixture with hydroxyoxo β-diketone was shown by GC/MS of the TMSi ether to be the oxo β-ketol, 18-hydroxy-7,16-hentriacontanedione (Fig. 1). Except for the ion with *m/z* 467 all the expected ions, corresponding to fragmentation on both sides of the three functional groups, were observed. The spectrum proved that the hydroxyl was at C-14 and not at C-16, if the positions of the hydroxyl and carbonyl groups were reversed an ion with *m/z* 211 would be expected in place of the ions of *m/z* 253 and 285.

Thus, fragments resulting from cleavage of the C-25–C-26 bond of the TMSi derivatives of both the β-diketone and the β-ketol, *m/z* 553 from the former and *m/z* 467 from the latter, were missing from the mass spectrum. These fragments probably underwent further breakdown due to the presence of the O-TMSi group. In the mass spectrum of the TMSi ether of the oxo β-ketol, ions with *m/z* 353 and 325, formally loss of CH_4 and CO, respectively, probably resulted from further fragmentation of ion *m/z* 369 by a process involving migration of the Si containing group to the neighbouring carboxyl group. Analogous fragmentation has been reported in the mass spectra of TMSi ethers of β-hydroxyesters [30]. This type of fragmentation was also prominent in the spectrum (Fig. 1) of the TMSi ether of the model β-ketol, 12-hydroxy-10-pentacosanone (see below), where ions *m/z* 255 and 227 are presumably derived from ion *m/z* 271. Otherwise the mass spectrum of the ether of the model ketol contained the four expected fragment ions and agreed with the above interpretation of the spectrum of the oxo β-ketol derivative.

The ^{13}C NMR spectrum (Table 6) was also of interest, particularly in the effect of hydroxyl substitution on the shifts of the carbonyl carbon and on carbons α to it. 12-Hydroxy-10-pentacosanone, a β-ketol lacking the isolated oxo group, was synthesized to confirm the ^{13}C signal assignments. The carbon between the oxygenated carbons had a shift of δ49.04, downfield from the usual position for a carbon α to a carbonyl group [27] by Δδ 6.1; but the carbonyl carbon was also downfield from the expected position by Δδ 1.1, since the hydroxyl group in 3-hydroxyoctadecanoate has α- and β-effects of Δδ +7.11 and −0.79, respectively [26]. The α-carbon on the other side of the carbonyl group experienced a downfield displacement of Δδ +0.9, presumably because intramolecular hydrogen bonding brought the hydroxyl group closer to it. No previous studies of ^{13}C NMR spectra of β-ketols have been made.

Comparison of corresponding signals of the two β-ketols (Table 6) showed that all were the same, except for those of C-14 and C-15 in the oxo β-ketol which were slightly upfield of those of C-8 and C-9 in the other ketol. This is a long-range effect of the carbonyl at position 7 and confirmed that the two carbonyl groups were separated by eight methylene groups. Long-range η and θ effects of −0.09 and −0.06 were observed across seven and eight carbons in spectra of oxo-octadecanoates [27]. Shifts of the first seven carbons of the oxo β-ketol were very close to those of C-12–C-18 of methyl oxo-octadecanoate [27] and showed that the carbonyl is at position 7. Thus, ^{13}C NMR indicated the structure of the oxo β-ketol as far

Table 6. Chemical shifts of corresponding carbons of β-ketols [in δ-values (ppm) from tetramethylsilane]

Carbon No.	18-Hydroxy-7,16-hentriacontanedione*	Carbon No.	12-Hydroxy-10-pentacosanone
1	14.06	1	14.14
2	22.55	2	22.72
3	31.67	3	31.92
4	29.00		
5	23.93†		
6	42.90‡		
7	211.57		
8	42.80‡		
9	23.86†		
10–13, 21–28	29.14–29.75	4–7, 15–22	29.25–29.73
14	23.63	8	23.72
15	43.70	9	43.77
16	212.50	10	212.52
17	49.04	11	49.04
18	67.75	12	67.75
19	36.58	13	36.59
20	25.54	14	25.55
29	31.98	23	31.99
30	22.74	24	22.74
31	14.15	25	14.14

*From wax of *A. elongatum*.
†,‡Assignments may be interchanged.

as C-20 but could not establish the length of the rest of the chain.

To confirm the structure further, 18-hydroxy-7,16-hentriacontanedione was synthesized and the ^{13}C and mass spectra and the TLC behavior were all indistinguishable from those of the natural compound. This synthetic oxo β-ketol is not identical with the natural one because the latter contains a chiral centre and has an appreciable specific rotation ($[\alpha]_D^{25} - 7.02°$). This is much larger than that observed for long chain compounds with an isolated hydroxyl and is to be attributed to the effect of the nearby carbonyl group, thus, methyl 3-hydroxyoctadecanoate had $[\alpha]_D^{25} - 15°$ [31].

Comparison of other physical properties of the oxo β-ketol with those of the positional isomer, 25-hydroxy β-diketone, showed that these were considerably different when the β-diketone grouping was lacking. Thus, the mp of the β-ketol was 15° higher and it behaved as a more polar compound on TLC and CC.

The ketols were prepared by regiospecific aldol condensation with lithium enolates of appropriate methyl ketones [32]; 12-hydroxy-10-pentacosanone was prepared from 2-undecanone and tetradecanal and 18-hydroxy-7,16-hentriacontanedione from 2,11-heptadecanedione and tetradecanal. 2,11-Heptadecanedione was synthesized by anodic coupling of 7-oxotridecanoic acid and 5-oxohexanoic acid. This is the same diketone as that obtained by alkaline cleavage of 25-oxohentriacontane-14,16-dione and comparison with the diketone, isolated from cleavage products of oxo β-diketone from *A. elongatum*, showed them to be identical.

β-Ketols seem to have been reported previously from only one plant wax when a mixture of 16-hydroxy-14-nonacosanone and 13-hydroxy-15-nonacosanone was isolated from cabbage wax [33, 34]. The β-ketols from the two waxes, however, probably have quite different biosynthetic origins. Since ketones are major components of cabbage wax (β-diketones have never been reported in that wax), β-ketols could be formed by β-hydroxylation of the ketone [35]. On the other hand, since the oxo β-ketol and 25-oxo β-diketone (which may also be described as 7,16,18-hentriacontanetrione) were oxygenated at the same carbons, it seemed most likely that the oxo β-ketol was formed by specific reduction of the C-14 carbonyl group. There is no evidence for the other possibility [35] that β-ketols are intermediates in synthesis of β-diketones [36].

In conclusion, the results of this analysis of waxes from three species of *Agropyron* showed that there were very marked differences in the compositions of oxygenated β-diketones from *A. riparium* and *A. dasystachyum*, in fact, *A. riparium* had a composition, high 10-oxo relative to 25-oxo and high 26-hydroxy relative to 25-hydroxy, which is quite different from those of any of the 12 species in *Agropyron* and closely related genera which contained both oxo- and hydroxy β-diketones [6]. This pronounced difference in wax composition supported the earlier taxonomic conclusion which regarded *A. riparium* as a separate species [1]. This is a good example of the use of wax composition to distinguish between species. *A. riparium* also differed from *A. dasystachyum* in containing diesters; (*E*)-2-docosenoic and tetracosenoic acids (which were the acid components of the diesters) occur, additionally, as free acids and monoesters. These components were less unusual, however, than the β-diketone composition, since diesters and their component acids (either free or as monoesters) also occur in waxes from *Triticum* species [13, 15, 20] and from a *Secale* species [16].

Another result was the finding that esters of C_{13} and C_{15} 2-alkanols occurred in other waxes from the Triticeae

besides barley spike wax [17]. This type of ester might, however, be restricted to wax from spikes, since in the present investigation whole plants were extracted, whereas in most earlier investigations spikes were excluded from extraction [11, 13–16].

Finally, analysis of *A. elongatum* wax showed that when there was an unusually high oxo β-diketone content, then other components, presumably biosynthetically derived from them, could also be present. Thus, 4-hydroxy-25-oxohentriacontane-14,16-dione, probably formed by hydroxylation at C-4, and the 25-oxo β-ketol, derived by reduction of the C-14 carbonyl group, were isolated.

EXPERIMENTAL

Plant material. Seeds of *A. riparium* cv Sodar were obtained from the U.S. Department of Agriculture, National Plant Materials Center, Beltsville, Maryland, and seeds of *A. elongatum* cv Orbit from T. Lawrence, Canada Agriculture Research Station, Swift Current, Saskatchewan. Seeds were planted outside; neither species flowered during the first year of growth but *A. riparium* plants were visibly glaucous ($E^{1\%}_{1cm}$ at 273 nm was 62). *A. elongatum* plants showed no sign of glaucousness. Grasses were cut, including spikes, while flowering in year 3 of growth. *A. dasystachyum* var. *psammophilum* was collected from an area of sand hills 20 miles south of Saskatoon, Saskatchewan, at the flowering stage on 29 June 1979. It was identified by Dr. V. Harms at the W. P. Fraser Herbarium, University of Saskatchewan. Wax was extracted from whole plants by a 10 sec immersion in hexane.

Wax analysis. Wax was chromatographed on a column of Si gel and eluted with hexane containing increasing amounts of Et_2O [16]; fractions were examined by GC as before [28]. Fractions were also examined by TLC (Si gel) although, as previously reported [8, 14], the relative R_f values of β-diketones and alkanols were considerably affected by the solvent. In $CHCl_3$ containing 1.5% EtOH and 5% EtOAc, R_f values were: 25-oxo β-ketol, 0.44; hydroxyoxo β-diketone, 0.36; 1-hexacosanol, 0.35; but when no EtOH was present R_f values were: oxo β-ketol, 0.23; hydroxyoxo β-diketone, 0.20; 1-hexacosanol, 0.30. The effects of solvent on TLC of β-ketols has been reported earlier [34].

Hydrocarbons, free acids and alcohols. Identified by GC comparison with authentic compounds. Other components and hydrolysis products were also examined by GC/MS using a 30 m × 0.3 mm fused silica column chemically bonded with DB-5 [polymethyl (5% phenyl) siloxane], linear velocity of H_2 was 60 cm/sec, samples were injected at 40°, the temp. immediately raised to 125° and programmed at 4°/min to 300°.

Separation of β-diketones and hydroxy β-diketones. Mixtures of β-diketones with esters and of hydroxy β-diketones with alcohols were purified by CC on a $Cu(OAc)_2$–Si gel column [22]. The $Cu(OAc)_2$–Si gel adsorbent was prepared by mixing Si gel (200–400 mesh, Bio Sil A from Bio-Rad Laboratories) (80 g) with a soln of $Cu(OAc)_2 \cdot H_2O$ (25 g) in H_2O (100 ml), removing the H_2O on a rotary evaporator and drying at 120° for 24 hr. A hexane soln of the CC fraction, containing β-diketone, was applied to the $Cu(OAc)_2$–Si gel column (10 g adsorbent/g mixture) prepared in hexane, and esters or alcohols eluted with hexane–Et_2O (9:1). The β-diketone–Cu complex was then eluted with $CHCl_3$–EtOH (17:3) at 50°. If the column still retained some complex it was extruded and extracted in a Soxhlet using the same solvent until recovery was complete. β-Diketone was subsequently isolated by treatment of the Cu complex with aq. 2 M HCl and $CHCl_3$ [23]. Mixtures of esters and β-diketones from all three waxes (including up to 8 g of mixture) of alcohols and hydroxy β-diketone from *A. riparium* and *A. elongatum*, and of diesters and 25-oxo β-diketone from *A. riparium* were successfully separated.

Esters. From all three waxes, esters were examined by GC/MS but only esters with chain lengths below C_{42} could be eluted. MS of esters of 2-alkanols were: [70 eV, *m/z* (rel. int.)] C_{31}, $[M]^+$ missing, 285 (8), 284 (15), 267 (14), 182 (30), 55 (100); C_{33}, $[M]^+$ missing, 313 (10), 312 (18), 295 (10), 182 (21), 55 (100); C_{35}, $[M]^+$ missing, 313 (17), 312 (27); 295 (16), 210 (21), 55 (100). Esters were subjected to methanolysis and Me esters and alcohols separated [37]. These alcohols were examined, as TMSi ethers, by GC/MS; MS of TMSi ethers of 2-alkanols were: [70 eV, *m/z* (rel. int.)] 2-tridecanol, $[M]^+$ missing, 257 (5), 117 (100), 75 (44); 2-pentadecanol, $[M]^+$ missing, 285 (4), 117 (100), 75 (41); authentic 2-pentadecanol TMSi ether had a very similar MS. The (*E*)-2-alkenoates in the Me esters had characteristic MS, as described below.

Diesters from A. riparium. Subjected to methanolysis and the mixture of dols and Me esters isolated. The composition of the diols, obtained by GC analysis as the acetates, was C_8, 11%; C_9, 33%; C_{10}, 34%; C_{11}, 18%, C_{12}, 4%. Structures of the diols were confirmed by GC/MS of the *bis*-TMSi ethers, the C_{10} *bis*-TMSi ether had a typical MS [70 eV, *m/z* (rel. int.)]: $[M]^+$ missing, 303 $[M-15]^+$ (1), 228 (2), 213 (4), 177 (9), 149 (28), 147 (73), 83 (100), 75 (89), 73 (86) (cf. ref. [21]). Me (*E*)-2-docosenoate had MS: 352 $[M]^+$ (4), 320 $[M-32]^+$ (10), 141 (12), 113 (46), 87 (52), 43 (100); and Me (*E*)-2-tetracosenoate: 380 $[M]^+$ (2), 348 (5), 141 (15), 113 (53), 87 (48), 43 (100); authentic synthetic ester [20] had a very similar spectrum.

β-Diketones. From all three waxes β-diketones were shown to be hentriacontane-14,16-dione by GC/MS of the TMSi enol ethers [12, 19]. 25-Oxo-β-diketone from *A. dasystachyum* and *A. elongatum* was also identified in the same way [19]. The ^1H NMR spectrum was also the same as that of 25-oxohentriacontane-14,16-dione from *A. intermedium* [8]. Mass fragmentography with integration of selected ion profiles was also applied to the oxo β-diketone from *A. dasystachyum* and gave [*m/z* (integral)]: 325 (100), 339 (2), 353 (16), 367 (76), confirming the absence of the 10-oxo isomer. β-Diketone from *A. elongatum* was degraded by the iodoform reaction [22] and gave C_{14} and C_{16} acids, 25-oxo β-diketone gave only C_{14} and oxo C_{16} acids. Oxo β-diketone was hydrolysed with NaOH–EtOH and acidic and neutral products separated [8, 13]. The Me ketones were chromatographed on silicic acid, and 2-pentadecanone eluted with hexane–Et_2O (49:1), and 2,11-heptadecanedione eluted with hexane–Et_2O (91:9). This diketone was distilled and crystallized from hexane, mp and mmp with synthetic 2,11-diketone (see below) were 69.5–70.5°. During CC of wax from *A. riparium*, 10-oxohentriacontane-14,16-dione was eluted (with hexane–Et_2O, 97:3) after the mixture of diesters and 25-oxo β-diketone. 10-Oxo β-diketone was identified by GC/MS of the TMSi enol ether [19] and distinguished from the 25-oxo isomer by ^1H NMR [8].

Hydroxy β-diketones. Fractions obtained by CC on $Cu(OAc)_2$–Si gel were crystallized from EtOAc. Hydroxy β-diketone from *A. elongatum* had mp 75.5–76.0°, $[\alpha]^{25}_{589}$ + 0.39°, $[\alpha]^{25}_{546}$ + 0.53°, $[\alpha]^{25}_{436}$ + 0.74°, $[\alpha]^{25}_{365}$ + 1.65° ($CHCl_3$; *c* 2.8); iodoform degradation gave the C_{14} acid and a hydroxy C_{16} acid. Comparison of ions *m/z* 173 and 187 in the peak corresponding to the *bis*-TMSi ether of hydroxy β-diketone, in GC/MS of whole wax, indicated the 25-hydroxy isomer, 90%, and 26-hydroxy isomer, 10%. Hydroxy β-diketone from *A. dasystachyum* also had the same mp and $[\alpha]^{25}_{589}$ + 0.30°, $[\alpha]^{25}_{546}$ + 0.45°, $[\alpha]^{25}_{436}$ + 0.99°, $[\alpha]^{25}_{365}$ + 2.0° ($CHCl_3$; *c* 2.0). GC/MS and mass fragmentography of the *bis*-TMSi enol ether gave [*m/z* (integral)]: 173 (32), 187 (100), 539 (96), 553 (23) showing that it was a mixture of 25- and 26-hydroxy isomers in the ratio, 3:1. Hydroxy β-diketone from *A. riparium* had mp 80.0–80.5° and $[\alpha]^{25}_{589}$ − 0.39°, $[\alpha]^{25}_{546}$ − 0.43°, $[\alpha]^{25}_{436}$ − 0.55°, $[\alpha]^{25}_{365}$ − 0.78° ($CHCl_3$; *c* 5.1). During GC/MS of the *bis*-TMSi enol ether the MS of a single scan at the centre of

the GC peak showed ions for the 26-hydroxy isomer only [70 eV, *m/z* (rel. int.)]: 624 $[M]^+$ (1), 609 (10), 553 (21), 441 (10), 351 [441 − 90]$^+$ (14), 325 (40), 173 (32), 73 (100). Comparison of the intensities of the relevant signals in the ^{13}C NMR spectrum of hydroxy esters from alkaline cleavage [11] indicated a ratio of 11-hydroxyhexadecanoate – 10-hydroxyhexadecanoate of 13:7. The mixed Me hydroxyhexadecanoates, after purification by distillation of the acetates, had $[\alpha]_{589}^{25}$ −0.16°, $[\alpha]_{546}^{25}$ −0.19°, $[\alpha]_{436}^{25}$ −0.40°, $[\alpha]_{365}^{25}$ −1.34° ($CHCl_3$; *c* 4.2). GC/MS partially separated the TMSi ethers; [70 eV, *m/z* (rel. int.)] Me 10-hydroxyhexadecanoate (30%), relative emergence temp. 1.000, $[M]^+$ missing, 343 $[M-15]^+$ (1), 273 (58), 187 (100); Me 11-hydroxyhexadecanoate TMSi ether (70%), relative emergence temp. 1.008, $[M]^+$ missing, 343 (1), 287 (60), 173 (100).

Oxo β-ketol. During CC of wax from *A. elongatum* (12.9 g), hydroxy β-diketones, followed by unidentified gummy fractions, were eluted with hexane–Et_2O (4:1). Elution with hexane–Et_2O–Me_2CO (15:5:1) then yielded a partially crystalline fraction (1.2 g). This mixture (TLC) was applied to a $Cu(OAc)_2$ Si gel column (50 g), and elution with hexane Et_2O (3:1) gave, first, 1-hexacosanol (0.15 g), and then, 18-hydroxy-7,16-hentriacontanedione (oxo β-ketol, 0.2 g). Elution with $CHCl_3$–EtOH (20:3) followed by Soxhlet extraction gave a crude Cu complex (0.73 g) which yielded a purified complex after crystallization from hexane (150 ml). 4-Hydroxy-25-oxohentriacontane-14,16-dione was extracted on decomposition of the complex with 2 M HCl; after crystallization twice from EtOAc the mp was 86.0–86.5°, $[\alpha]_{589}^{25}$ +0.41°, $[\alpha]_{546}^{25}$ +0.49°, $[\alpha]_{436}^{25}$ +0.86°, $[\alpha]_{365}^{25}$ +1.22° ($CHCl_3$; *c* 4.9); ^1H NMR (90 MHz, CCl_4): δ 5.33 (1H, *s*, H-15), 3.47 (1H, *m*, H-4), 2.23 (8H, *q*, H-13, H-17, H-24, H-26), 1.3 (*m*, CH_2 of chain), 0.85 (6H, *t*, H-1, H-31); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.05 (C-31), 14.18 (C-1), 18.88 (C-2), 22.53 (C-30), 23.40, 23.44 (C-12, C-18 of keto form), 23.87, 23.91 (C-23, C-27), 25.68, 25.73, 25.77 (C-6 and C-12, C-18 of enol form), 28.99 (C-28), 29.06–29.72 (unassigned signals), 31.65 (C-29), 37.58 (C-5), 38.44 (C-13, C-17 of enol form), 39.77 (C-3), 42.88 (C-24, C-26), 43.81, 43.84 (C-13, C-17 of keto form), 57.24 (C-15 of keto form), 71.75 (C-4), 99.09 (C-15 of enol form), 194.55 (C-14, C-16), 211.58 (C-25). (Found: C, 75.6; H, 11.8. $C_{31}H_{58}O_4$ requires: C, 75.3; H, 11.8%.) GC/MS of the *bis*-TMSi enol ether [70 eV, *m/z* (rel. int.)]: 638 $[M]^+$, (0.5), 595 (4), 413 (7), 367 (14), 323 (413–90, 7), 145 (28), 113 (12), 73 (100). GC/MS analysis of the products of the iodoform degradation showed [70 eV, *m/z* (rel. int.)]: Me 11-hydroxytetradecanoate TMSi ether $[M]^+$ missing, 287 (25), 145 (100), 73 (73) and Me 10-oxohexadecanoate 284 $[M]^+$ (0.5), 214 (8), 199 (7), 167 (3), 157 (12), 139 (9), 125 (21), 113 (24), 43 (100) (cf. MS of authentic Me 10-oxohexadecanoate [8]). The oxo β-ketol was crystallized from hexane and had mp 92–93°; $[\alpha]_{589}^{25}$ −7.02°, $[\alpha]_{546}^{25}$ −8.22°, $[\alpha]_{436}^{25}$ −13.37°, $[\alpha]_{365}^{25}$ −19.40° ($CHCl_3$; *c* 1.75); IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3550 (w), 2940 (s), 2860 (s), 1702 (m); ^1H NMR (90 MHz, CCl_4): δ 3.87 (1H, *m*, H-18), 2.30 (8H, *m*, H-6, H-8, H-15, H-17), 1.25 (*m*, CH_2 of chain), 0.86 (6H, *t*, H-1, H-31); ^{13}C NMR: Table 6; GC/MS of the TMSi ether [70 eV, *m/z* (rel. int.)] $[M]^+$ missing, 537 $[M-15]^+$ (2), 369 (8), 353 (1), 327 (8), 325 (12), 285 (7), 253 (14), 159 (7), 113 (21), 75 (47), 43 (100). (Found: C, 77.6; H, 12.6. $C_{31}H_{60}O_3$ requires: C, 77.4; H, 12.6%.)

Synthesis of reference compounds. 12-Hydroxy-10-pentacosanone. Di-isopropylamine (0.130 g, 1.3 mmol) was allowed to react in dry THF (5 ml), under N_2, with butyl lithium in hexane (1.5 ml of 1 M soln) at −60°, warmed to room temp. and then cooled to −78°. 2-Undecanone (0.221 g, 1.3 mmol) was added and stirred at −78° for 25 min, a soln of tetradecanal (0.27 g, 1.27 mmol) in THF (5 ml) was added and the reaction continued at −78° for a further 25 min. The soln was poured into H_2O, acidified with 2 M HCl and the product extracted with Et_2O. Chromatography on Si gel yielded first starting materials (eluted with hexane–Et_2O, 97:3) and the required β-ketol (0.169 g) (eluted with hexane–Et_2O, 91:9). After crystallization from hexane, the mp was 74–75°; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3520 (w), 2940 (s), 2860 (s), 1700 (w); ^1H NMR (90 MHz, CCl_4, shaken D_2O): δ 3.88 (1H, *m*, H-12), 2.36 (4H, *m*, H-9, H-11), 1.25, 0.88 (6H, *t*, H-1, H-25); ^{13}C NMR: Table 6; GC/MS of the TMSi ether [70 eV, *m/z* (rel. int.)] $[M]^+$ missing, 439 $[M-15]^+$ (33), 327 (18), 285 (12), 271 (36), 255 (8), 227 (19), 155 (88), 73 (73), 43 (100). (Found: C, 78.8; H, 13.3. $C_{25}H_{50}O_2$ requires: C, 78.5; H, 13.2%.)

2,11-Heptadecanedione. 7-Oxotridecanoic acid was prepared from 1-morpholinocyclohexene and heptanoyl chloride [38] and crystallized from Me_2CO, mp 59–62° (lit. [39], 63°). A soln of 5-oxohexanoic acid (8 g, 61.4 mmol) and 7-oxotridecanoic acid (7 g, 30.7 mmol) in MeOH (70 ml) containing NaOMe (from 0.05 g, 2 mmol Na) was electrolysed between Pt electrodes for 6 hr (average current 1.1 A) until just alkaline. HOAc (1 ml) was added and the solvent removed, products were taken-up in Et_2O, washed with $NaHCO_3$ and H_2O and dried (Na_2SO_4). After removal of solvent the product was distilled at 0.05 mm and most of the required diketone was in the fraction bp 130–145°. This fraction was chromatographed on Si gel. Elution with hexane–Et_2O (19:1) gave first by-products followed by the diketone (1.943 g). After crystallization from hexane the mp was 70–71°; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2940 (s), 2860 (s), 1708 (s); ^1H NMR (90 MHz, CCl_4): δ 2.3 (6H, *m*, H-3, H-10, H-12), 2.03 (3H, *s*, H-1). (Found: C, 76.6; H, 12.1. $C_{17}H_{32}O_2$ requires: C, 76.1; H, 12.0%.)

18-Hydroxy-7,16-hentriacontanedione. A soln of 2,11-heptadecanedione (0.523 g, 1.95 mmol) in THF (5 ml) was added to lithium di-isopropylamide (prepared as above) in THF (5 ml) and hexane (2.8 ml) at −78° and the mixture stirred for 30 min. Tetradecanal (0.403 g, 1.97 mmol) in THF (5 ml) was added and the reaction continued at −78° for a further 30 min. Product was recovered, after decomposition with H_2O and acid, by $CHCl_3$ extraction. Chromatography on Si gel and elution with hexane containing increasing amounts of Et_2O separated unreacted starting materials (0.250 g), crude oxo β-ketol (0.156 g, eluted with hexane–Et_2O, 3:1) and more polar by-products (0.425 g). Crystallization of this crude product from iso-octane gave 18-hydroxy-7,16-hentriacontanedione (0.037 g). After recrystallization from hexane, the mp was 92.5–93.0°; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3540 (w), 2940 (s), 2860 (s), 1702 (m); ^1H NMR (90 MHz $CDCl_3$): δ 4.0 (1H, *m*, H-18), 2.45 (8H, *m*, H-6, H-8, H-15, H-17); ^{13}C NMR was the same as that of the natural oxo β-ketol in Table 6; GC/MS of the TMSi ether [70 eV, *m/z* (rel. int.)]: $[M]^+$ missing, 537 $[M-15]^+$ (4), 353 (1), 327 (10), 325 (15), 285 (10), 253 (16), 159 (8), 113 (21), 75 (63), 43 (100). (Found: C, 77.5; H, 12.5. $C_{31}H_{60}O_3$ requires: C, 77.4; H, 12.6%.)

Acknowledgements—I am grateful to L. L. Hoffman for experimental assistance, W. C. Haid for ^1H NMR, M. Mazurek for ^{13}C NMR and L. R. Hogge and D. J. Olson for GC/MS analysis.

REFERENCES

1. Hitchcock, A. S. (1951) *Manual of the Grasses of the United States* 2nd edn, No. 200. U.S. Dept Agric. Misc. Publ., Washington.
2. Bowden, W. M. (1965) *Can. J. Botany* **43**, 1421.
3. Hitchcock, C. L., Cronquist, A. and Ownbey, M. (1969) *Vascular Plants of the Pacific Northwest* Part. 1, p. 454. University of Washington Press, Seattle.
4. Voss, E. G. (1972) *Michigan Flora* Part 1, p. 163. Cranbrook Institute of Science, Bloomfield Hills.
5. Boivin, B. (1981) *Flora of the Prairie Provinces* Part. V, p. 94. Université Laval, Québec.
6. Tulloch, A. P., Baum B. R. and Hoffman, L. L. (1980) *Can. J. Botany* **58**, 2602.

7. Dewey, D. R. (1975) *Bot. Gaz.* **136**, 122.
8. Tulloch, A. P. and Hoffman, L. L. (1976) *Phytochemistry* **15**, 1145.
9. Hanson, A. A. (1972) *Grass Varieties in the United States* p. 4. U.S. Dept Agric., Washington.
10. Lawrence, T. (1967) *Can. J. Plant Sci.* **47**, 611.
11. Tulloch, A. P. (1976) *Phytochemistry* **15**, 1153.
12. Tulloch, A. P. and Hoffman, L. L. (1979) *Phytochemistry* **18**, 267.
13. Tulloch, A. P. and Weenink, R. O. (1969) *Can. J. Chem.* **47**, 3119.
14. Tulloch, A. P. and Hoffman, L. L. (1971) *Phytochemistry* **10**, 871.
15. Tulloch, A. P. and Hoffman, L. L. (1973) *Phytochemistry* **12**, 2217.
16. Tulloch, A. P. and Hoffman, L. L. (1974) *Phytochemistry* **13**, 2535.
17. von Wettstein-Knowles, P. and Netting, A. G. (1976) *Lipids* **11**, 478.
18. Ubik, K., Stransky, K. and Streibl, M. (1975) *Collect. Czech. Chem. Commun.* **40**, 1718.
19. Tulloch, A. P. and Hogge, L. R. (1978) *J. Chromatogr.* **157**, 291.
20. Tulloch, A. P. (1971) *Lipids* **6**, 641.
21. McCloskey, J. A., Stillwell, R. N. and Lawson, A. M. (1968) *Analyt. Chem.* **40**, 233.
22. Netting, A. G. and von Wettstein-Knowles, P. (1976) *Arch. Biochem. Biophys.* **174**, 613.
23. Horn, D. H. S., Kranz, Z. H. and Lamberton, J. A. (1964) *Aust. J. Chem.* **17**, 464.
24. Horn, D. H. S. and Lamberton, J. A. (1962) *Chem. Ind.* 2036.
25. Tulloch, A. P. (1976) in *Chemistry and Biochemistry of Natural Waxes* (Kolattukudy, P. E., ed.) p. 235. Elsevier, Amsterdam.
26. Tulloch, A. P. (1978) *Org. Mag. Reson.* **11**, 109.
27. Tulloch, A. P. (1977) *Can. J. Chem.* **55**, 1135.
28. Tulloch, A. P. and Hoffman, L. L. (1973) *Lipids* **8**, 617.
29. Tulloch, A. P. (1978) *Phytochemistry* **17**, 1613.
30. Eglinton, G., Hunneman, D. H. and McCormick, A. (1968) *Org. Mass. Spectrom.* **1**, 593.
31. Tulloch, A. P. and Spencer, J. F. T. (1964) *Can. J. Chem.* **42**, 830.
32. Stork, G., Kraus, G. A. and Garcia, G. A. (1974) *J. Org. Chem.* **39**, 3459.
33. Schmid, H. H. O. and Bandi, P. C. (1971) *J. Lipid Res.* **12**, 198.
34. Holloway, P. J. and Brown, G. A. (1977) *Chem. Phys. Lipids* **19**, 1.
35. Kolattukudy, P. E., Croteau, R. and Buckner, J. S. (1976) in *Chemistry and Biochemistry of Natural Waxes* (Kolattukudy, P. E., ed.) p. 289. Elsevier, Amsterdam.
36. Mikkelsen, J. D. and von Wettstein-Knowles, P. (1978) *Arch. Biochem. Biophys.* **188**, 172.
37. Tulloch, A. P. (1974) *Lipids* **9**, 664.
38. Hünig, S., Lücke, E. and Benzing, E. (1958) *Chem. Ber.* **91**, 129.
39. Keskin, H. (1963) *Rev. Fac. Sci. Univ. Istanbul Ser. C.* **23**, 31.