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CUTIN ACIDS FROM BRYOPHYTES: AN ω-1 HYDROXY ALKANOIC ACID IN TWO LIVERWORT SPECIES*

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Key Word Index—*Sphagnum*; moss; *Conocephalum conicum*; *Astarella lindenbergiana*; liverworts; Bryophyta; phylogeny; cutin; hydroxy acids; alkanes; MS; 15-OH hexadecanoic acid.

Abstract—The cutin acids of two species of *Sphagnum* moss and two species of liverwort from the order Marchantiales have been analysed. The liverwort species contain as a bound lipid, 15-hydroxyhexadecanoic acid, hitherto unreported in higher plants. The relative abundances of 8,16-, 9,16- and 10,16-dihydroxyhexadecanoic acids may be of value in the chemotaxonomy of *Sphagnum* mosses. Based on their cutin acid compositions, mosses and liverworts may be less related than generally supposed, the *Sphagnum* cutins resembling more those of angiosperms and gymnosperms. The *n*-alkane content of *Sphagnum* is very low, but there are quantitative differences between species.

INTRODUCTION

The chemistry of plant cutins has received considerable attention [1, 2] and it appears that the principal constituents are hydroxy- and epoxyhydroxyalkanoic acids of C_{16} and C_{18} chainlength, predominantly interlinked by ester-functions [2, 3]. More recently, hydroxyoxoalkanoic acids have also been reported as bound cutin lipids [4, 5]. Cutin acids have also been reported as constituents of geological sediments, and hence are of paleochemotaxonomic, as well as contemporary taxonomic interest [6,7]. An earlier study of gymnosperm, fern and lycopod cutins suggests their potential as a taxonomic and phylogenetic criterion in these groups [8].

The evolutionary relationship of the Bryophyta to other plant phyla is obscure [9]. The moss genus *Sphagnum* has frequently presented a problem to taxonomists. The present study suggests that cutin acid composition may throw some light upon both the taxonomy and phylogeny of the Bryophyta.

RESULTS

Mosses

Fresh leaf material, stripped free of stem tissue, was exhaustively extracted with organic solvents and, in the case of *Sphagnum palustre*, with cellulolytic reagents [10, 11]. The residue was subsequently refluxed in alcoholic KOH, and the resultant lipids analysed by a previously reported method [8].

Major differences were observed in the dihydroxyhexadecanoic acids of *Sphagnum* species examined; the 10,16-isomer is predominant in *S. palustre*, whereas in *S. cuspidatum*, the 8,16-isomer predominates over 9,16- and 10,16-isomers (Table 1). The characteristic MS of Me ester-TMSi ethers of hydroxyalkanoic acids facilitate the quantitative analysis of non-separable isomers [12–15]. The *m/e* 245 and 303 ions result from the α-cleavage of the methine-function bearing the O-TMSi group in Me 8,16-diTMSioxyhexadecanoate. Ion pairs *m/e* 259/289 and 273/275 similarly result from the α-cleavage pattern of 9,16- and 10,16-isomers [8, 11]. The identity of the dihydroxyhexadecanoic acids is further corroborated by the chromatographic behaviour of their derivatives [8, 11, 12]. Although a major constituent of *S. palustre*, 16-hydroxyhexadecanoic acid is absent from the cutin acid fraction of *S. cuspidatum*. Hydroxyhexadecanedioic acids are present in the cutin acids of both moss species, although hydroxypentadecanedioic acids have only been identified in *S. cuspidatum*. The MS of diMe TMSioxypentadecanedioates and TMSioxyhexadecanedioates derived from the *Sphagnum* species are closely similar to published data, as is their chromatographic behaviour [8]. The GC–MS data of the peak identified as diMe 6-TMSioxypentadecanedioate corresponded closely with those of the synthetic derivative [12].

The *n*-alkanes extracted from both species of *Sphagnum* revealed an envelope of the type normally associated with the leaf wax hydrocarbons of higher plants (Table 2), in which odd chain-length alkanes predominate. The dominant homologues however are of shorter chain length (*ca* C_{23}) than alkanes generally encountered in higher plants (*ca* C_{27-31}) [1].

Liverworts

The ether-soluble acids (Table 1) derived from two thallose liverwort species included 15-hydroxyhexadecanoic acid. While only confirmed as a bound lipid of *Astarella* thallus, this acid is definitely established as a

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Table 1. Gas chromatographic–mass spectrometric analysis of ether-soluble carboxylic acids* liberated upon saponification of solvent-extracted bryophyte tissues

Ether-soluble carboxylic acid	*Sphagnum palustre* % total bound acids	*Sphagnum cuspidatum* % total bound acids	*Astarella lindenbergiana* %total bound acids	*Conocephalum*† *conicum* % total bound acids
PHENOLIC				
o-hydroxybenzoic	—	—	6.3	—
p-hydroxybenzoic	—	15.2	1.9	—
m-coumaric	—	2.9	2.8	—
p-coumaric	3.4	1.7	6.7	—
ALKANOIC				
hexadecanoic	2.4	9.1	2.6	26.8
octadecanoic	—	—	—	3.3
octadecenoic	0.5	—	—	—
octadecadienoic	0.5	—	0.9	—
eicosanoic	—	2.2	—	4.2
docosanoic	—	5.1	—	4.4
tricosanoic	—	—	—	0.8
tetracosanoic	—	—	—	7.2
HYDROXYALKANOIC				
2-hydroxyhexadecanoic	—	—	—	4.8
15-hydroxyhexadecanoic‡	—	—	72.3	11.7
16-hydroxyhexadecanoic	36.3	—	—	6.0
2-hydroxyoctadecanoic	—	—	—	3.3
2-hydroxydocosanoic	—	—	—	tr.
2-hydroxytricosanoic	—	—	—	2.0
2-hydroxytetracosanoic	—	—	—	9.5
HYDROXYALKANEDIOIC				
6-hydroxypentadecanedioic•	—	2.4	—	—
7-hydroxypentadecanedioic•	—	1.5	—	—
8-hydroxypentadecanedioic•	—	0.6	—	—
7-hydroxyhexadecanedioic•	4.6	10.2	—	—
8-hydroxyhexadecanedioic•	4.5	6.5	—	—
DIHYDROXYALKANOIC				
8,16-dihydroxyhexadecanoic•	2.2	12.6	—	—
9,16-dihydroxyhexadecanoic•	5.2	4.0	—	—
10,16-dihydroxyhexadecanoic•	27.8	6.6	—	—
10,15-dihydroxyhexadecanoic§	—	—	3.9	—
Unidentified	—	—	—	8.4
Unidentified lipids	3.8	19.3	2.6	7.4

* Identified by GC–MS of Me ester-OTMSi ether derivatives [6, 8, 11, 13, 14, 28, 29]. † Isolated cuticle membrane. ‡ Novel occurrence in a higher plant. § Novel structure, tentative assignment based on GC–MS evidence. • Non-separable isomers on GLC; quantitative estimate obtained from MS fragment ions e.g. *m/e* 273/275, 259/289.

cutin constituent in *Conocephalum*. The 15-hydroxyhexadecanoic acid was characterised as its Me ester-TMSi ether by GLC co-injection and GC-MS comparison with the derivative of the authentic compound. The base peak of the spectrum, *m/e* 117 is due to the stable ion (I) [16], and indicates the presence of a CH_3-CHOTMSi-group. The M^+, as is typical of Me TMSioxyalkanoates, is extremely weak, although the M^+-15, M^+-31, M^+-47 series is present, with a strong metastable ion supporting the loss of a 32 A.M.U. fragment from the M^+-15 ion. An ion at *m/e* 314 results from the expulsion of CH_3CHO from the M^+, resulting in the ion (II) (Fig. 1). The characteristic ions *m/e* 146 and 159 indicate that the derivative is a Me ester containing a single TMSi moiety [13, 14].

A minor constituent of the ether-soluble acid fraction of *Astarella* thallus-bound lipids (Table 1) appears from GC–MS to be a single compound, tentatively identified as 10,15-dihydroxyhexadecanoic acid. The MS has been rationalised as follows: a major ion at *m/e* 117 is probably $MeCH{=}\overset{+}{O}{-}SiMe_3$ which suggests a 15-TMSioxy derivative [16]. The intensity of this stable ion is less than it is in the case of Me 15-TMSioxyhexadecanoate because of competing fragmentation pathways due to a 10-TMSioxyfunction. An ion at *m/e* 431 corresponds to the M^+-15 of a Me diTMSioxyhexadecanoate. The ion at *m/e* 402 resulting from the loss of CH_3CHO from the M^+ probably arises in an analogous manner to the *m/e* 314 ion in Me 15-TMSioxyhexadecanoate (Fig. 1). The ions at *m/e* 273 and 275 are due to cleavage α- to a 10-TMSioxy-bearing methine function. The further decomposition of the 273 ion in Me 10,16-diTMSioxyhexadecanoate involving the loss of methoxytrimethylsilane, is well established and was also observed. An ion at *m/e* 185 is probably formed by loss of trimethylsilanol from the *m/e* 275 ion. The weak ion at *m/e* 244, which generally occurs in Me alkanoates bearing an isolated 10-TMSioxy function, is probably of the structure $.(CH_2)_8C(OMe){=}\overset{+}{O}{-}SiMe_3$ [8]. Additionally, significant ions at *m/e* 147 and 159 confirm that the compound is a Me ester containing two O-TMSi functions [12, 14]. The retention index of the derivative (2400 on SE-30) was in accord with the structural assignment, in that the related compounds show the following retention data

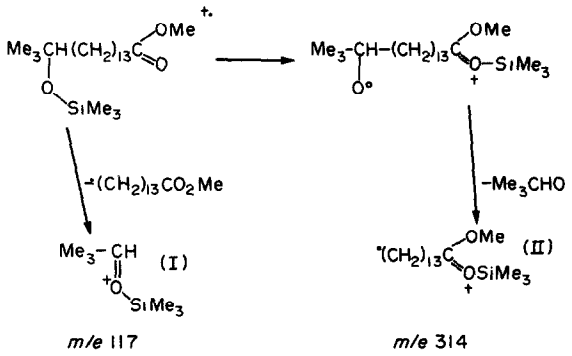

Fig. 1.

upon the same phase [12], Me 15-TMSioxyhexadecanoate (2210) Me 16-TMSioxyhexadecanoate (2280) Me 10,16-diTMSioxyhexadecanoate (2475).

Insufficient material was available for further chemical investigation. In neither liverwort were any of the primary OH-bearing dihydroxyhexadecanoic acids found, though 16-hydroxyhexadecanoic acid was identified in *Conocephalum* cutin hydrolysate.

Of the four bryophytes examined, it was only possible to isolate intact cutin membranes from *Conocephalum*. IR analysis revealed that major alkyl ($\bar{\nu}$ 2925, 2854 cm^{-1}) and ester ($\bar{\nu}$ 1730 cm^{-1}) moieties were present in *Conocephalum* cutin, while an absorption ascribed to minor phenolic substituents ($\bar{\nu}$ 1610 cm^{-1}) was also observed. Hydrolysis of the cutin membrane gave a *ca* 70% yield of ether-soluble constituents. The series of 2-hydroxy alkanoic acids found in *Conocephalum* cutin was not present in the three other bryophytes studied.

DISCUSSION

It has been suggested that the development of a cuticle may have been a crucial factor in the colonisation of land by plants [17]. Bryophytes have traditionally been regarded as the most primitive land plants and Buch [18] has reported the occurrence of cuticles in bryophytes in which the water supply comes from below. Such plants are thus well adapted to water conservation and this feature has been given some importance in the classical taxonomy of both mosses and liverworts [9]. Stewart and Follet [19] have also reported a leaf cuticle in the genus *Sphagnum*.

While in the present study the cutin membranes of *Sphagnum* mosses were not isolated in the usual way [10,11], the hydroxyalkanoic acids released upon saponification of the solvent-stripped leaf materials are unlikely to derive from any other leaf constitutents. Suberin, a polymer similar to cutin, could be a source of the ether-soluble acids, although dihydroxyhexadecanoic acids have thus far not been reported as major suberin constitutents [20]. 15-Hydroxyhexadecanoic acid was not firmly established as a cutin constituent in *Astarella*, but it occurs as a bound lipid in this species and in the cutin of the related *Conocephalum*. The occurrence of ω-1 hydroxyalkanoic acids in bryophytes is of some paleochemotaxonomic interest, since the degradation of Green River Shale kerogen with chromic acid results in small amounts of ω-1 oxoalkanoic acids [21]. The only other known occurrence of ω-1 hydroxyalkanoic acids is in a yeast [22]. Whether there is any relationship with kerogen oxo-acids is open to question, especially since 9,16- and 10,16-dihydroxyhexadecanoic acids, normally associated with cutin have also been isolated from Green River Shale [23].

The cutin acids of *Sphagnum palustre* (Table 1) are similar to those found in higher plants, except for the higher ratio of 16-hydroxyhexadecanoic to dihydroxyhexadecanoic acids. Such a quantitative variation is probably explained by differing relative activities of enzymes concerned with the hydroxylation of 16-hydroxyhexadecanoic acid derivatives in these plants. Minor amounts of hydroxyhexadecanedioic acids (Table 1) present in the hydrolysate possess a similar OH substitution pattern to that of dihydroxyhexadecanoic acids suggesting a biogenetic relationship. *S. palustre* and *S. cuspidatum* cutin acids are predominantly the C_{16}-type. The striking differences in the secondary hydroxyl pattern of dihydroxyhexadecanoic acids (Table 1) and in the *n*-alkane pattern (Table 2) suggests that cutin and hydrocarbon compositions may provide valuable complementary taxonomic criteria in the Sphagnales, the constituent taxa of which exhibit close morphological similarities [9]. The abundance of the 8,16-isomer is unusual and has only been reported in the cutin of a few other plant species [24]. While no phylogenetic significance can yet be attached to this variation in isomer abundance, it is possible that its further study in a number of *Sphagnum* species could allow clarification of their sug-generic evolutionary relationship.

The tentative identification of the 10,15-dihydroxyhexadecanoic acid poses a point of further interest; the biosynthesis studies by Kolattukudy [2, 25] suggest that the ubiquitous 10,16-dihydroxyhexadecanoic acid component of plant cutins is formed by hydroxylation of 16-hydroxyhexadecanoic acid. It is possible that in the liverworts an analogous enzyme has evolved which allows further hydroxylation of 15-hydroxyhexadecanoic acid. If this is the case, the liverworts may have evolved their own C_{16}-type cutin in a manner paralleling that of most cutin-containing plants.

In addition to the 15-hydroxyhexadecanoic acid, *Conocephalum* cutin contains a series of 2-hydroxy alkanoic acids, the most abundant of which is the C_{16}-homologue. Higher homologues are also present, accompanied by

Table 2. Chain length distribution in *n*-alkanes extracted from *Sphagnum palustre* and *S. cuspidatum* foliar tissue. The abundance of each homologue is expressed as a percentage of total *n*-alkanes

n-alkane homologue	C_{21}	C_{22}	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}	C_{34}
Sphagnum palustre	8.5	0.8	43.2	1.8	21.6	0.8	4.1	0.5	3.6	0.7	9.4	0.3	3.4	0.2
Sphagnum cuspidatum	10.0	0.8	52.9	1.8	25.4	0.9	5.8	0.7	1.4	0.2	—	—	—	—

alkanoic acids and alcohols of the same chain-length. The cutin of *Conocephalum* contains lipids which are more frequently associated with wax [1, 2]. Whether they are, in fact, chemical constituents of the cutin or are simply in very close physical association with it, remains uncertain. Other lower plant cutins are reported to contain tightly-held wax alcohols; hence the phenomenon may not be unique [8, 13, 25].

The similarity of cutin acids found in higher plants to those of the mosses herein examined, together with the substantial differences from those of the liverworts, suggests that *Sphagnum* mosses may be more closely related to the higher plants and lycopods than are the liverworts so far investigated [2, 8, 12].

EXPERIMENTAL

Hydrocarbons. Shoots of *S. cuspidatum* Ehrh. ex Hoffm. emend (fr. wt 130 g) were collected from Westhay Moor, Somerset Levels, and lyophilised (dry wt 26.8 g). An aliquot (15.8 g) was Soxhlet-extracted in toluene–MeOH (1:1), and the solvent removed (*vacuo*) to yield a green oil (0.519 g). Column chromatography on Si gel M.F.C. (28 g), eluting with *n*-hexane (100 ml), afforded upon evaporation a colourless wax (1.6 mg) suggested by TLC to consist of saturated hydrocarbons. Shoots of *S. palustre* L, obtained from Trellech Bog, Monmouthshire were extracted (fr wt 165 g) in a similar manner; an aliquot of the lipid extract (60 mg) was subjected to preparative-TLC using Si gel plates and development with *n*-hexane. The alkane band was located by brief exposure to I_2 vapour. Hydrocarbons represented *ca* 0.002% of the fr wt in both moss species. GLC of the hydrocarbons was carried out using a FID instrument temp programmed at 4°/min from 100° to 300°. The columns were stainless steel (3 m length, 1.6 mm o.d.), packed with Gas Chrom 'Q' coated with 1% OV-1 and N_2 carrier was maintained at 11 ml/min. All peaks coinjected with authentic alkane standards.

Cutin acids. (a) Solvent-extracted *S. palustre* tissue was worked up in bulk, employing a method similar to that reported previously [7,9–13]. Cellulose was removed by digestion with $ZnCl_2$–HCl reagent; the resulting slurry was filtered on glass wool, rinsed with dil HCl, H_2O, Me_2CO and MeOH. The residue was finally Soxhlet-extracted with toluene–MeOH (12 hr) to yield a residual buff coloured powder (1.53 g). An aliquot of the powder (0.71 g) was refluxed (12 hr) in 4% methanolic KOH (30 ml). Et_2O-soluble acids were worked up by a previously reported method [9]. Me esters, prepared by the method of ref. [27] were silylated with N,O-*bis*-(trimethylsilyl)-acetamide in pyridine. Me ester TMSi ethers were subjected to GC–MS analysis using a single-stage Watson–Bieman separator. A linear scan mode (10–600 amu in 4 sec) was used. Packed columns of stainless steel (3 m length, 1.6 mm o.d.) containing 1% OV-17 or OV-101 were employed, temp. programming at 4°/min from 100–280°.

An aliquot of the Et_2O-soluble acid Me esters from *S. palustre* was separated by preparative-TLC on Si gel, employing Et_2O–hexane–MeOH (40:10:1) as developing solvent. The dihydroxy ester band (R_f 0.35) was located by brief exposure to I_2 vapour. IR (CCl_4 soln) of the eluted band showed major alkyl ($\bar{\nu}$ C–H 2930 and 2860 cm^{-1}, *s*), ester ($\bar{\nu}$ C=O 1735 cm^{-1} *s*) and minor OH absorption at 3400 cm^{-1} ($\bar{\nu}$ OH).

The dihydroxy ester fraction was silylated and subjected to preparative GLC using a stainless steel column (24 m length, 6 mm o.d.) packed with 2% SE-30 coated upon Gas Chrom Q. N_2 carrier gas at 40 ml/min was used, isothermally at 240°. High resolution MS carried out on the Me diTMSioxyhexadecanoate component, confirmed the elemental composition of its *m/e* 245 and 273 fragment ions.

Calculated	Found	Composition
245.156	245.157	$C_{12}H_{27}O_3Si^+$
273.189	273.189	$C_{14}H_{29}O_3Si^+$

GC–MS identifications were largely based upon previously published data [4, 6]. (b) *Conocephalum conicum* (L) Underw. was Soxhlet-extracted (24 hr) with MeOH and toluene–MeOH (1:1). Subsequent treatment with $ZnCl_2$ reagent for a prolonged period liberated fragments of cutin. Owing to the nature of the thallus, no quantitative recoveries were possible [4]. IR (KBr disc) of the membranes showed a broad band at 3400 cm^{-1} ($\bar{\nu}$ OH) and absorption at 2925 and 2854 cm^{-1} ($\bar{\nu}$ C–H, *s*, alkyl) 1730 ($\bar{\nu}$ C=O, *s*, ester 1610 ($\bar{\nu}$ C=C, *w*, aromatic) with minor bands at 1510, 1460, 1160, 1060 and 803 cm^{-1}. The small quantity of cutin available (2 mg) was saponified with 4% methanolic KOH, to yield, upon work-up in the usual manner [4] an Et_2O-soluble lipid fraction (1.1 mg) which was subsequently methylated and silylated. The Me ester TMSi ethers were subjected to GLC and GC–MS using a 3.5 m length, 3 mm o.d. silanised glass column packed with 2% SE-30 on Gas Chrom Q. He carrier was maintained at 30 ml/min while temp programming at 4°/min, 100–250°. The identity of 15-hydroxyhexadecanoic acid was confirmed by coinjection with an authentic sample (Me ester TmSi ether). (c) Freshly picked *Astarella lindenbergiana* (Corda) Lindberg thallus (2.4 g) was washed free of adhering soil particles and lyophilised (8 hr). Resulting tissue (0.52 g) was sliced and Soxhlet-extracted with $CHCl_3$–MeOH (2:1) for 12 hr. Residual tissue was refluxed with 5% ethanolic KOH, the supernatant taken to dryness (*vacuo*) and dissolved in H_2O (3 ml). The acidified soln (H_3PO_4 to pH 2) was extracted with Et_2O (×4). The combined Et_2O layers were washed with H_2O (×3) and acids extracted into 6% $NaHCO_3$ soln (×3). The combined $NaHCO_3$ solns yielded upon evaporation a pale yellow oil (5.3 mg). The acids were derivatised in the usual manner and subjected to GC–MS analysis. The monohydroxy Me ester fraction was isolated by Si gel TLC after development with Et_2O–hexane–MeOH (40:10:1). The eluted band R_f 0.55 was silylated and GC–MS confirmed the identity of the 15-hydroxyhexadecanoate component (Me ester TMSi ether).

Mass spectra. Mosses—Me diTMSioxyhexadecanoates. *Sphagnum palustre*: M^+-15, *m/e* 431 (7); 415 (2.1); 399 (1.1); 309 (4); 303 (4); 289 (8); 275 (58); 273 (100); 259 (20); 245 (11); 244 (14); 185 (5); 169 (11.5); 159 (9); 155 (4); 147 (17); 73 (>100); 75 (82); 89 (12); 103 (30); 109 (10); 129 (33). *Sphagnum cuspidatum*: M^+-15, *m/e* 431 (6); 415 (2); 399 (4.5); 309 (14); 303 (37); 289 (14); 275 (25); 273 (51); 259 (33); 245 (100); 244 (8); 230 (5); 216 (17); 213 (14); 199 (10); 185 (18); 169 (39); 159 (25); 155 (31); 147 (46); 141 (59); 73 (>100); 75 (80).

Liverworts. Me 15-TMSioxyhexadecanoate derived from *Conocephalum* and *Astarella* cutin: M^+-15, *m/e* 343 (2.5); 327 (1.2); 314 (7.3); 311 (3.4); m*282, 268 (0.3); 159 (4.5); 146 (3); 117 (100); 73 (34); 75 (23.5). *Astarella* minor lipid, tentatively identified as Me 10,15-diTMSioxyhexadecanoate (identical MS after GC–MS on SE 30 (R.I. 2400) OV-17 and Apiezon L phases): M^+-15, *m/e* 431 (2); 402 (4.5); 387 (1.8); 341 (7.2); 309 (1.8); 299 (4.5); 297 (8); 275 (10); 273 (15); 244 (2); 225 (3); 185 (5.5); 169 (10); 159 (9); 147 (8); 129 (21); 117 (86); 109 (10); 103 (12); 95 (28); 89 (15); 81 (17); 75 (42); 73 (100).

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REFERENCES

1. Martin, J. T. and Juniper, B. E. (1970) *The Cuticles of Plants*, Edward Arnold, London.
2. Kolattukudy, P. E. and Walton, T. J. (1972) "The Biochemistry of Plant Cuticular Lipids", in *Progress in the Chemistry of Fats and other Lipids*, Vol. 13 Part 3 (ed. R. T. Holman) pp. 121–175. Pergamon Press, Oxford.
3. Holloway, P. J. (1973) *Phytochemistry* **12.** 2913.
4. Deas, A. H. B., Baker, E. A. and Holloway, P. J. (1974) *Phytochemistry* **13.** 1901.
5. Holloway, P. J. (1974) *Phytochemistry* **13.** 2201.
6. Eglinton, G., Hunneman, D. H. and Douraghi-Zadeh, K. (1968) *Tetrahedron* **24.** 5929.
7. Hunneman, D. H. and Eglinton, G. (1969) in *Advances in Organic Geochemistry 1968*, p. 157. Pergamon Press, Oxford.
8. Hunneman, D. H. and Eglinton, G. (1972) *Phytochemistry* **11.** 1989.
9. Watson, E. V. (1963) *The Structure and Life of Bryophytes*, Academic Press, New York.
10. Holloway, P. J. and Baker, E. A. (1968) *Plant Physiol.* **43.** 1878.
11. Eglinton, G. and Hunneman, D. H. (1968) *Phytochemistry* **7.** 313.
12. Caldicott, A. B. (1973) Ph.D. thesis, University of Bristol.
13. Hunneman, D. H. (1971) Ph.D. thesis, University of Bristol.
14. Eglinton, G., Hunneman, D. H. and McCormick, A. (1968) *Org. Mass Spectrom.* **1.** 593.
15. Kleiman, R. and Spencer, G. F. (1973) *J. Am. Oil Chem. Soc.* **50.** 31.
16. Diekman, J., Thompson, J. B. and Djerassi, C. (1967) *J. Org. Chem.* **50,** 3904.
17. Crafts, A. S. (1961) *The Chemistry and Mode of Action of Herbicides*, ch. 5, p. 28. Interscience, New York.
18. Buch, H. (1947) *Soc. Sci. Fenn. Comm. Biol.* **9.** 1.
19. Stewart, J. M. and Follett, E. A. C. (1966) *Can. J. Botany* **44.** 421.
20. Holloway, P. J. (1972) *Chem. Phys. Lipids.* **9.** 171.
21. Burlingame, A. L. and Simoneit, B. R. (1969) *Nature* **222.** 741.
22. Tulloch, A. P. and Spencer, J. F. T. (1968) *Can. J. Chem.* **46.** 1523.
23. McIntosh, A. G. (1968) B.Sc. Thesis, University of Glasgow.
24. Holloway, P. J., Deas, A. H. B. and Kabaara, A. M. (1972) *Phytochemistry* **11.** 1443.
25. Kolattukudy, P. E. (1970) *Biochem. Biophys. Res. Commun.* **41.** 299.
26. Holloway, P. J. (1974) *Phytochemistry* **13.** 2201.
27. Schlenk, H. and Gellerman, J. L. (1960) *Anal. Chem.* **32.** 1412.
28. Horman, I. and Viani, R. (1971) *Org. Mass. Spectrom.* **5.** 203.
29. Hallgren, B., Ryhage, R. and Stenhagen, E. (1959) *Acta Chem. Scand.* **13.** 845.