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SURFACE WAX OF *ANDREAEA* AND *POGONATUM* SPECIES

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Key Word Index—*Andreaea rupestris*; *Pogonatum aloides*; *P. urnigerum*; Bryophyta; mosses; gametophytic stems; surface waxes; GC analysis; aliphatic compounds; chain length distributions.

Abstract—The mosses *Andreaea rupestris*, *Pogonatum aloides* and *P. urnigerum* contain surface waxes in amounts of 0.05–0.12% dry wt. The waxes consisted of esters (C_{38}–C_{54}), primary alcohols (C_{20}–C_{32}), free fatty acids (C_{16}–C_{30}), and alkanes (C_{21}–C_{31}). Additionally, aldehydes (C_{22}–C_{30}) were major constituents in the wax of *P. urnigerum*. The classes and their chain length distributions in the surface waxes of these mosses are comparable to those of epicuticular waxes of higher plants.

INTRODUCTION

Cuticles and waxy surface coverings of mosses and liverworts have been studied morphologically and from a functional point of view [1–3], but the chemical nature of true surface lipids was rarely examined [4–7]. Although data on alkanes from numerous bryophytes are now available [8], these are usually derived from analyses of extracts of whole plants. As shown from our previous work, internal alkanes in bryophytes may amount to *ca* two-thirds of the total alkanes and the profiles of external and internal alkanes differ [5]. Recently, an attempt was made to compare the surface lipids of the moss *Saelania glaucescens* with cuticular wax of higher plants [9], although the conspicuous surface covering of *Saelania* consists mainly of the diterpenoid, ent-16-kauranol, aliphatic constituents represent only a minor part of the surface lipids. For a better understanding of the relationship of these lipids to epicuticular wax of higher plants, further detailed investigations on the chemical nature of surface waxes in moss gametophytes are desirable. We therefore examined *Pogonatum urnigerum* (Hedw.) P. Beauv. and *Pogonatum aloides* (Hedw.) P. Beauv. These members of the Polytrichaceae are endohydric [1]. As an ectohydric species, *Andreaea rupestris* Hedw. was also examined. This species was also chosen because wax constituents of members of the Andreaeales have not been studied before.

RESULTS AND DISCUSSION

The highest amount of surface wax was found in *P. urnigerum* (Table 1). This is in agreement with the glaucous coloration of this species, the water-repellent leaf surfaces and the well developed wax covering as seen in the scanning electron microscope [2]. Smaller yields of surface wax were obtained from *P. aloides*. The leaves of this species are also water-repellent but do not show the glaucous character of *P. urnigerum*. While endohydric mosses possess a cuticle, and cuticular wax can be observed at least in some species, ectohydric mosses were suggested not to have a lipid surface covering [1]. However, traces of wax are visible on the surface of the ectohydric *Hookeria lucens* [2]. As is now also shown for *A. rupestris*, ectohydric mosses may contain surface waxes in small quantities, but they are not water repellent.

The waxes from *Andreaea* and *Pogonatum* spp. contained fractions commonly found in epicuticular wax of seed plants, i.e. alkanes, free fatty acids, primary alcohols, and esters (Table 1). Alkanes were

Table 1. Composition and yield of surface wax from *Andreaea* and *Pogonatum* species

Component	Yield (%) *A. rupestris*	*P. aloides*	*P. urnigerum*
Alkanes	1.3	0.1	0.4
Free fatty acids	26.1	45.7	22.9
Alcohols	13.8	3.1	12.8
Aldehydes	—	—	44.3
Esters	58.8	51.1	19.6
Total yield (% dry wt)	0.05	0.08	0.12

present only in small proportions, whilst the percentages of free fatty acids and esters were comparatively high. The major class of the wax from *P. urnigerum* was aldehydes but this fraction was not detected in *P. aloides* and *A. rupestris*. When only the aliphatic constituents of *Saelania* surface lipids [9] are considered, the fractions are comparable to those in *Andreaea* and *Pogonatum*, although their relative proportions differ considerably. In *Saelania*, free fatty acids are by far the most predominant aliphatic fraction, whilst the amounts of esters and alkanes are low and free primary alcohols are present only in trace amounts. These classes also occur in the epicuticular waxes of seed plants [10]. Even the fact, that aldehydes were detected only in the wax of *P. urnigerum* is not in conflict with this view. Aldehydes are regarded as precursors of primary alcohols in the reductive pathway of wax biosynthesis, but they are often not detectable in waxes in spite of the occurrence of primary alcohols [11]. Other constituents of mosses like diterpenoids, sterols, and triterpenoids [12] were not detected in the surface waxes of *Andreaea* and *Pogonatum*. There was also no indication of the presence of steryl esters and esters of phytol and phytenic acid reported from mosses [13]. The absence of these esters in surface waxes of *Andreaea* and *Pogonatum* coincides with results from *Dicranum elongatum* [7]. In this species, esters from surface lipids are predominantly composed of saturated fatty acids and 1-octadecanol, while internal lipids contain appreciable amounts of sterols and prenols esterified with polyunsaturated fatty acids.

Table 2. Relative amounts of the major esters in surface waxes of *Andreaea* and *Pogonatum* species

Species	Homologues (%)					
	C_{42}	C_{44}	C_{46}	C_{48}	C_{50}	C_{52}
A. rupestris	8	17	21	27	13	3
P. aloides	3	9	21	27	21	9
P. urnigerum	4	23	31	18	11	6

The esters in the surface waxes of *Andreaea* and *Pogonatum* were composed of C_{38}–C_{54} homologues with C_{46} or C_{48} as major constituents (Table 2). The even-numbered homologues were predominant. From the chain length distributions, the esters resemble those usually found in higher plant epicuticular waxes and this holds true also for the ester hydrolysis products. The dominant constituents of the acid moieties in esters are often in the C_{20}–C_{24} range, whereas free fatty acids tend to have larger proportions of chain length C_{26} and higher [11]. In the wax of *Andreaea* and *Pogonatum*, both free and esterified fatty acids contained the lower homologues as major constituents (Table 3). The higher homologues C_{26} and C_{28} were present in greater percentages only in the free fatty acids of *A. rupestris* and *P. urnigerum*. The chain length distributions of free and esterified alcohols were similar, the lower chain lengths being more prominent in the esterified alcohols. In *Andreaea*, C_{28} predominated in both fractions, while in the *Pogonatum* spp. C_{26} was the major free alcohol and C_{24} the dominant esterified one. Corresponding to their close biosynthetic relationship, free primary alcohols and aldehydes usually display similar chain length distributions [11]. This is also the case in *P. urnigerum*, although the profile of aldehydes was markedly concentrated towards the predominant C_{26} constituent compared with the more regular distribution of alcohols. In the alkanes of *P. urnigerum* and *A. rupestris* C_{25} was the main homologue accounting for more than 40% of the total fraction (Table 4). The profile of *P. urnigerum* was dominated by this constituent; in *A. rupestris* C_{27} was also present in appreciable amounts. The odd/even ratios were in a moderate range (9.0 and 8.1 respectively). A comparatively even distribution was observed in the

Table 3. Relative amounts of major fatty acids, alcohols and aldehydes in surface waxes of *Andreaea* and *Pogonatum* species

Species Fraction	Homologues (%)							
	C_{16}	C_{18}	C_{20}	C_{22}	C_{24}	C_{26}	C_{28}	C_{30}
A. rupestris								
Free fatty acids	4	6	5	21	21	12	19	1
Ester fatty acids	1	3	33	39	13	5	2	—
Free alcohols	—	—	1	8	14	22	47	2
Ester alcohols	—	—	2	16	16	22	38	1
P. aloides								
Free fatty acids	22	7	16	23	17	9	1	1
Ester fatty acids	5	2	4	34	40	12	1	—
Free alcohols	—	—	2	5	31	37	14	5
Ester alhohols	—	—	1	10	34	33	13	5
P. urnigerum								
Free fatty acids	2	3	2	32	20	27	8	2
Ester fatty acids	1	1	3	66	21	5	1	—
Free alcohols	—	—	1	8	23	37	22	6
Ester alcohols	—	—	1	16	27	21	20	12
Aldehydes	—	—	—	5	20	59	12	1

Table 4. Relative amounts of alkanes in surface waxes of *Andreaea* and *Pogonatum* species

Species	Homologues (%)										
	C_{21}	C_{22}	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}
A. rupestris	4	2	10	4	41	4	29	1	3	—	2
P. aloides	7	2	17	4	23	4	15	3	11	2	12
P. urnigerum	3	2	11	3	46	3	18	1	4	1	8

alkanes of *P. aloides* with little preponderance of C_{25} and a lower odd/even ratio (5.7). Besides C_{25}, the alkane profiles of both *Pogonatum* spp. additionally display a second smaller maximum at C_{31}. Considering this bimodal distribution, the alkane profiles differ markedly from *Polytrichum* where only one maximum occurs at C_{29} or C_{31} [5], but they resemble those found in *Sphagnum* [4, 14]. In contrast to our preliminary suggestions on bryophyte alkanes, this type of profile is obviously not restricted to the genus *Sphagnum*, but occurs in other moss groups also. On the other hand, chain length distributions of alkanes are controlled by environmental and developmental factors [15–17].

Variations of alkane profiles even between related species may therefore be less meaningful. Whether these factors also affect the formation of alkanes and other wax constituents in bryophytes is presently under investigation.

Taking into account the data now available, moss gametophytes are obviously capable of forming wax which is located at the plant surface. This was demonstrated by morphological studies [2] and is confirmed by the extraction method employed in this investigation which rules out contamination of wax extracts by significant amounts of internal lipids. Including findings in *Hookeria lucens*, the data from *Andreaea rupestris* further suggest that there is no sharp line between endohydric and ectohydric mosses with respect to the occurrence of surface wax. At least this species of Andreaeales apparently exhibits no unusual wax composition. Concerning the compound classes found, the surface waxes of mosses are obviously related to epicuticular waxes of higher plants. Even the high amount of ent-16-kauranol in *Saelania* [9] could be in agreement with this view since large proportions of diterpenoids or triterpenoids are occasionally also present in cuticular waxes of seed plants [10]. A closer examination of individual fractions from moss waxes reveals chain length distributions with pronounced proportions of lower chain lengths whilst homologues C_{28} and higher are mostly present in smaller amounts. However, C_{28} is the predominant constituent of alcohols in *Andreaea* and a higher proportion of C_{28} also occurs in the free fatty acids of this species. Additionally, bimodal alkane profiles are found in *Pogonatum*. These results may be taken as an indication of the existence of at least two elongating systems which have been postulated to be operative in wax biosynthesis of higher plants [11]. We therefore suggest the surface waxes of moss gametophytes to be comparable to epicuticular waxes of seed plants, a conclusion which was also drawn from morphological features [2].

EXPERIMENTAL

The mosses were collected during Sept. 1980 in the Schwarzwald near Klosterreichenbach, S.W. Germany (voucher specimens identified by Professor G. Buchloh, Hohenheim, are deposited in the herbarium of the Institut für Botanik, Universität Hohenheim). Uppermost parts of gametophytic stems were selected and carefully purified. Extraction of the surface waxes was carried out by dipping semi-desiccated samples (H_2O content *ca* 80% dry wt) into $CHCl_3$ for 10 sec. Longer extraction times, and extraction of completely desiccated samples, resulted in substantial contamination of extracts with internal lipids but yielded no further amounts of wax constituents. Analysis of the wax by prep. TLC and GC was done as described earlier [17, 18].

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