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STEROLS OF THREE LICHEN SPECIES: *LOBARIA PULMONARIA*, *LOBARIA SCROBICULATA* AND *USNEA LONGISSIMA*

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Key Word Index—*Lobaria pulmonaria*; *Lobaria scrobiculata*; Peltigerales; *Usnea longissima*; Parmeliales; lichens; sterol composition; solvent extractable sterols; base extractable lipid pools.

Abstract—Sterols were extracted from the lichens *Lobaria pulmonaria*, *Lobaria scrobiculata* and *Usnea longissima* with chloroform-methanol (2:1) (solvent-extractable fraction) followed by saponification of the residual lichen material to give a tightly-bound sterol fraction. The compounds were principally ergosterol, episterol, fecosterol and lichesterol with minor quantities of C_{27}, C_{28} and C_{29} monoenes and dienes of the phytosterol type.

INTRODUCTION

The present study was undertaken due to a combined interest in lichens and in the occurrence and biosynthesis of natural sterols. A detailed survey on the current state of investigations into substances from these symbiotic associations of algae and fungi has stressed the importance of continued work on a chemical monography of all species [1]. Recently the sterols of *Xanthoria parietina* [2] and *Pseudevernia furfuraceae* [3] have been described in detail, the first delineation of the sterol composition of lichens.

The present work reports the sterol composition of three lichen species, *Lobaria pulmonaria*, *Lobaria scrobiculata* and *Usnea longissima* and confirms the presence of two distinct lichen sterol pools. Several groups have reported that a solvent extractable and an alkali extractable lipid fraction can be isolated from fungi and algae [4, 5] and this has also been observed with lichens. Our results also reveal a complex mixture of C_{27}, C_{28} and C_{29} sterols which include lichesterol (ergosta-5,8,22-trien-3β-ol) and fecosterol [ergosta-8,24(28)-dien-3β-ol], compounds reported in mutants of *Neurospora crassa* [6] and *Saccharomyces serivisiae* [7].

RESULTS

The solvent-extractable sterols from the three lichen species examined were obtained as steryl acetates following saponification of the chloroform–methanol (2:1) extract, column and preparative TLC to obtain the 4-demethylsterol fraction, and a second preparative TLC (System 1) of the acetates of this fraction. The *Lobaria* species sterols and the *Usnea longissima* sterols were 0·2 and 0·04% respectively of the dry wt of the ground lichen material. Preliminary analysis of the steryl acetate fractions by GLC indicated complex mixtures. Individual sterol components were identified after separation of the mixture into zones by argentation TLC (System 2).

Zone 1 (Table 1) co-chromatographed with ergosteryl acetate. The spectral data (MS, NMR, UV) confirmed the assignment. Ergosterol formed 25·0% and 24·7% and 21·1% of the sterol mixtures of *Lobaria pulmonaria*, *L. scrobiculata* and *Usnea longissima* respectively.

Zone 2 steryl acetate co-chromatographed with episteryl acetate. Spectral data [M^+ 440, with significant ions at *m/e* 440, 425, 380, 365, 355, 341, 313, 225, 253 and 213; δ 0·54 (3H, *s*, (-18), 0·81 (3H, *s*, C-19), 1·03 (9H, *d*, J = 7Hz, C-21, C-26 and

Table 1. Solvent-extractable sterols from three lichen species

Sterol acetate	TLC zone	TLC R_f	GLC RR_c*	% Composition		
				L. pulmonaria	*L. scrobiculata*	*U. longissima*
Ergosta-5,7,22-trien-3β-ol (ergosterol)	1	0·20	4·30	25·0	24·7	21·1
Ergosta-7,24(28)-dien-3β-ol (episterol)	2	0·30	5·30	31·1	32·8	11·3
Ergosta-8,24(28)-dien-3β-ol (fecosterol)	3A	0·37	4·80	36·0	23·8	0·4
Ergosta-5,8,22-trien-3β-ol (lichesterol)	3B	0·40	4·05	1·5	6·8	56·3
24-Methylcholesta-5,22-dien-3β-ol	4A	0·47	3·85	2·1	0·8	0·4
24-Ethylcholesta-5,22-dien-3β-ol	4B	0·50	4·80	0·9	1·7	5·6
Cholest-5-en-3β-ol	5A	0·52	3·30	0·4	0·6	0·4
24-Methylcholest-5-en-3β-ol	5B	0·52	4·35	0·8	0·9	3·1
24-Ethylcholest-5-en-3β-ol	5C	0·58	5·45	2·2	7·9	1·4

* GLC retention time relative to cholestane.

C-27), 5·5 (1H, *m*, C-7), 4·70 (1H, *m*, C-28)] were consistent with the literature values for episteryl acetate.

Zone 3 from the *Lobaria* species (called 3A) was shown by GLC to be predominantly a compound of RR_c 4·80. Further argentation TLC gave this compound (as shown by GLC) in >90% purity. The MS was very similar to episteryl acetate (*m/e* 440, 425, 380, 365, 355, 341, 313, 255, 253 and 213). The NMR spectrum (δ 0·61 (3H, *s*, C-18), 0·96 (3H, *s*, C-19), 0·95 (3H, *d*, J = 7Hz, C-21), 1·03 (6H, *d*, J = 7Hz, C-26 and C-27), 4·68 (1H, *m*, C-28) was identical to that published for fecosteryl acetate [6, 7].

Zone 3B steryl acetate was found predominantly in *U. longissima* (56·3% of the solvent-extractable sterols). The physical characteristics (R_f 0·40, RR_c 4·05, mp 123–126°) were the same as those of lichsteryl acetate [2, 6, 8]. The mass spectral data (*m/e* 438, 378, 363, 337, 313, 353 and 351, 253, 211) confirmed two nuclear double bonds and the ion at *m/e* 337 suggested a ring B diene [9]. The NMR data supported the lichesteryl (ergosta-5,8, 22-trien-3β-yl) acetate structure, showing *trans*-olefinic protons at δ 5·19 (2H, *m*, C-22 and C-23) and the proton of a trisubstituted double bond at δ 5·44 (1H, *m*, C-6). The second nuclear double bond was tetrasubstituted and was assigned at 8(9) due to the methyl proton resonances at δ 0·67 (3H, *s*, C-18) and δ 1·20 (3H, *s*, C-19), lower than in sterols with an 8(14) double bond [10]. The side-chain methyl resonances at δ 1·02 (3H, *d*, J = 6Hz, C-21), 0·82 (3H, *d*, J = 7Hz, C-26), 0·84 (3H, *d*, J = 7Hz, C-27) and 0·92 (3H, *d*, J = 7Hz, C-28) were also consistent with the lichesteryl acetate structure.

Zones 4 and 5 contained two and three sterols respectively which represented minor proportions of the sterol mixtures. After extensive argentation TLC the acetate derivatives were characterized by GLC and MS. The configuration of the C-24 methyl or ethyl group could not be deduced from the results obtained. In order of decreasing polarity the compounds isolated were 24-methylcholesta-5, 22-dien-3β-yl acetate (RR_c 3·85, *m/e* 440, 380, 296, 282, 255, 228, 213), 24-ethylcholesta-5, 22-dien-3β-yl acetate (RR_c 4·80, *m/e* 394, 379, 351, 296, 282, 255, 228, 213), cholest-5-en-3β-yl acetate (*m/e* 428, 368, 260, 255, 247, 228, 213), 24-methylcholest-5-en-3β-yl acetate (*m/e* 442, 382, 274, 261, 255, 228, 213) and 24-ethylcholest-5-en-3β-yl acetate (*m/e* 456, 396, 381, 255, 213). No evidence of stanols or Δ^7-stenols could be found from the TLC or GLC of the sterol mixtures.

The three species examined in this work gave tightly-bound sterol fractions significantly different in composition from the direct solvent extractable material. The yield from this sterol pool was very low. Both *Lobaria* species yielded less than 0·005% tightly bound sterols while the *U. longissima* contained not more than 5×10^{-4}% of these sterols. Identification of these micro quantities by TLC and GLC comparison with the solvent-extractable sterols indicated that the same sterols were present but in significantly different proportions. Both *Lobaria* species contained 1–2% the total sterols in the tightly-bound pools. Both the solvent extractable and tightly bound fractions contained about 25% ergosterol; the amounts of episterol (*ca* 15%) and fecosterol (*ca* 3%) were significantly lower in the latter fraction. In contrast to the lowered fecosterol and episterol

levels there were significantly higher concentrations of 24-ethylcholest-5-en-3β-ol (increased from 1·5 to 12%) and 24-methylcholest-5-en-3β-ol (increased from 0·8 to 13%) in *L. scrobiculata*. The sterol composition of the bound fraction from *U. longissima* also showed enrichment in 24-ethylcholesta-5,22-dien-3β-ol (33%), a decrease in ergosterol (9%) and lichesterol (23%) and approximately the same concentration of episterol (11%).

The sterol content of *U. longissima* was similar to that of *Xanthoria parietina* [2], however in the present study we did not detect any stanols or Δ^7-stenols as found in *Pseudevernia furfuracea* [3]. It is interesting to note that lichesterol, first reported as a lichen sterol, is present in all three lichen species as well as in mutant strains of *Neurospora crassa* [6] and *Saccharomyces cerevisiae* [7]. It was proposed that lichesterol is an immediate precursor of ergosterol and that the mutant strains are deficient in the appropriate Δ^8–Δ^7 isomerase required to effect this biological transformation. The corresponding wild type strains of *N. crassa* and *S. cerevisiae* and other fungi do not contain any lichesterol whereas lichens are the only organism which apparently accumulates this biosynthetic intermediate.

EXPERIMENTAL

Lobaria pulmonaria, *Lobaria scrobiculata* and *Usnea longissima* were collected at Musquoidoboit River in Nova Scotia, Canada in December of 1973. A second sample of *Lobaria scrobiculata* was taken in March of 1974 at Chester, Nova Scotia.

Lipids were extracted from air-dried, ground material with $CHCl_3$–MeOH (2:1) by refluxing for 2 hr. The tissue was filtered, washed with fresh solvent, and the combined filtrate concentrated and saponified to give the solvent extractable sterol fractions. A tightly-bound lipid fraction was obtained by digestion of the residual tissue with MeOH–H_2O–KOH(80:10:10) under reflux for 2 hr. The tissue was again filtered and the lipids were extracted into Et_2O.

Preparative TLC (0·2 × 1 meter plates, 1 mm layer Si gel $HF_{254\,\&\,366}$) in hexane–Et_2O–EtOH (40:10:1) (System 1) gave the 4-demethylsterol fraction (R_f 0·2). This was acetylated and re-run in the same TLC system (R_f 0·7) to give a crystalline steryl acetate fraction.

Separation of the steryl acetates was achieved by repeated $AgNO_3$-TLC on 0·1 mm layers of Si gel $HF_{254\,\&\,366}$ containing 15% (w/w) $AgNO_3$ in C_6H_6–hexane (3:2) (System 2). Fractions were monitored by GLC on 1·5% Ov-17. NMR spectra were recorded using $CDCl_3$ as the solvent and TMS as an internal standard.

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