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DISTRIBUTION PATTERN OF STEROLS IN LIVERWORTS BELONGING TO THE JUNGERMANNIINEAE*

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Abstract—The sterol fractions of eight leafy liverworts were analyzed by GLC and GC–MS. Five 3β-sterols, cholest-5-en-3β-ol, 24-methylcholest-5,22-dien-3β-ol, 24-methylcholest-5-en-3β-ol, 24-ethylcholest-5,22-dien-3β-ol and 24-ethylcholest-5-en-3β-ol, were detected in all samples but there were differences in the relative amounts present.

INTRODUCTION

THE RECENT application of GC–MS provides a simple and accurate method for sterol analysis and Knights has reported an attempt to relate sterol composition to taxonomic classification in one group of higher plants.[1]

The liverworts (Hepaticae) form a unique division in the plant kingdom and their plant bodies consist of gametophytes grown from the spores. Nevertheless, the sterol components have hardly been investigated. Following from our identification of five sterols in *Scapania parvitexta*,[2] we have now extended our study to the sterols of eight liverworts belonging to the suborder Jungermanniineae.

RESULTS AND DISCUSSION

From the eight liverworts listed in Table 1, solid fractions (m.p. 140–150°) were separated by elution chromatography of their methanol extracts, and the major portion of each fraction was found from IR and NMR measurements to be a mixture of sterols. GLC of each of these fractions exhibited five peaks, which were successfully assigned to five known sterols by comparison with authentic samples. Moreover, these fractions, as such and as their TMS derivatives, were analyzed by GC–MS and they were found to have the same five sterols. Table 2 shows the characteristic ions in mass spectra of the five components. Among them, *m/e* 273, 255, 229 and 213 ions appearing in all spectra are known as sterol fragments coming from the loss of side chain, and the remaining ions can be characterized

* Part XIII in the series "Chemical Constituents from Hepaticae". For Part XII see MATSUO, A., NAKAYAMA, M. and HAYASHI, S. (1973) *Bull. Chem. Soc. Japan* **46**, 1010.

[1] KNIGHTS, B. A. (1966) *The Chemistry of Natural Products Symposium*, I.U.P.A.C., Stockholm, Abstract No. 4.

[2] MATSUO, A., NAKAYAMA, M., HAYASHI, S. and YASUDA, S. (1972) *Agr. Biol. Chem.* (*Tokyo*) **36**, 2241.

respectively as M^+, $[M-15]^+$, $[M-18]^+$ and $[M-33]^+$, etc. In MS of the TMS ethers, both *m/e* 129 and $[M-129]^+$ ions characteristic for 3β, Δ^5-sterols were observed as predominant ions in all components.

TABLE 1. STEROL CONTENT OF LIVERWORTS

Family	Species*	Cholest-5-en-3β-ol	24-Methylcholest-5,22-dien-3β-ol	24-Methylcholest-5-en-3β-ol	24-Ethylcholest-5-en-3β-ol
Jungermanniaceae	*Jungermannia thermarum*[1]	3%	7%	30%	19%
	J. torticalyx[2]	3	13	25	13
Scapaniaceae	*Scapania parvitexta*[3,2]	1	14	29	17
	S. undulata[4]	1	10	31	17
	Macrodiplophyllum plicatum[5]	4	9	20	45
Lophocoleaceae	*Chiloscyphus polyanthus*[6]	7	12	18	20
	Heteroscyphus bescherellei[7]	2	8	21	30
Plagiochilacea	*Plagiochila japonica*[8]	1	7	19	35
	P. ovalifolia[9]	5	8	22	33

* Liverworts examined in this work were collected in the following places: 1—Chitani in Ehime; 2—Iwakuni in Yamaguchi; 3—Yakushima in Kagoshima; 4—Sugadaira in Nagano; 5—Rubeshibe in Hokkaido; 6—Yabakei in Oita; 7—Iwakuni in Yamaguchi; 8—Yabakei in Oita; 9—Garo in Fukushima.

Thus, the five components are cholest-5-en-3β-ol (C_{27}, Δ^5), 24-methylcholest-5,22-dien-3β-ol (C_{28}, $\Delta^{5,22}$), 24-methylcholest-5-en-3β-ol (C_{28}, Δ^5), 24-ethylcholest-5,22-dien-3β-ol (C_{29}, $\Delta^{5,22}$) and 24-ethylcholest-5-en-3β-ol (C_{29}, Δ^5). This assignment was confirmed by comparison of the retention times and MS patterns with those of authentic specimens and with authentic spectra.[3] The relative compositions of these sterols in each liverwort were calculated from the relative peak areas of the gas chromatogram (Table 1).

TABLE 2. CHARACTERISTIC IONS OF THE STEROLS

Sterols	Characteristic ions of each MS (*m/e*)
Cholest-5-en-3β-ol	386, 371, 368, 353, 273, 255, 229, 213
24-Methylcholest-5,22-dien-3β-ol	398, 383, 380, 365, 355, 337, 300, 273, 271, 255, 229, 213
24-Methylcholest-5-en-3β-ol	400, 385, 382, 367, 273, 255, 299, 213
24-Ethylcholest-5,22-dien-3β-ol	412, 397, 394, 379, 369, 351, 300, 273, 271, 255, 229, 213
24-Ethylcholest-5-en-3β-ol	414, 399, 396, 381, 273, 255, 229, 213

Most higher plants are known to contain 24-ethylcholest-5-en-3β-ol as the major component. The liverworts examined in the present work, however, contained 24-ethylcholest-5,22-dien-3β-ol as the main component, with the exception of *Macrodiplophyllum plicatum*. Cholest-5-en-3β-ol and 24-methylcholest-5,22-dien-3β-ol were generally the two least components. Relative amounts of 24-methylcholest-5-en-3β-ol and 24-ethylcholest-5-en-3β-ol varied according to the family examined: in the Jungermanniaceae and Scapaniaceae 24-methylcholest-5-en-3β-ol is the second largest component, whereas in the Lophocoleaceae and Plagiochilaceae 24-ethylcholest-5-en-3β-ol is. Species belonging to the same family generally have similar distribution patterns.

EXPERIMENTAL

M.ps are uncorrected. The IR spectra were taken in CCl_4 solutions and the NMR spectra on a 60-MHz spectrometer in $CDCl_3$ solutions using TMS as an internal standard.

[3] KNIGHTS, B. A. (1967) *J. Gas Chromatog.* 273.

Material and separation of sterol fractions. 8 species of liverworts were collected from various places in Japan, dried in the shade for 1 week, and then digested with 2 × vol. MeOH to the dried plants. Each of the extracts was chromatographed over a silica gel column (1·5 × 45 cm) with C_6H_6–EtOAc (4:1), and a fraction (m.p. 140–150°) showing one spot with R_f 0·38 on TLC with silica gel and the above solvent was collected. The fraction gave a positive Libermann–Barchard color reaction, and exhibited the characteristic IR and NMR spectra to sterols.

GLC of sterol fractions. GLC was carried out on a FID-type apparatus in connection with a glass column (3 mm × 3 m) packed with silicon OV1 (1·0%) on Shimalite (60–80 mesh). The column temp. was 255°.

Preparation of TMS derivatives. The sterol fractions (each 20 mg) obtained from 8 liverworts were converted to the TMS derivatives by treatment with 1 ml of pyridine–hexamethyldisilazane (HMDS)–trimethylchlorosilane (HMCS) (10:2:1). After standing at room temp. overnight, the solvents were evaporated in vacuum.

GC–MS analyses of sterol fractions and their TMS derivatives. GC–MS analyses of sterol fractions and their TMS derivatives were carried out with a single focus mass spectrometer under the following conditions: silicon OV1 (1·0%) on Shimalite (60–80 mesh); 250° column temp.; 70 μA total emission; 3·5 kV ion accelerating voltage; 300° ionization chamber temp.