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# CONFIGURATION AT C-24 OF STEROLS FROM THE LIVERWORT *PALAVICINNIA LYELLII*

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**Key Word Index**—*Palavicinnia lyellii*; Bryophyta; 4-desmethylsterols; 24β-methylcholest-5-en-3β-ol; 24α-methylcholest-5-en-3β-ol; 24α-ethylcholest-5-en-3β-ol.

**Abstract**—The 4-desmethylsterol fraction of the liverwort *Palavicinnia lyellii* is composed of 36% 24β-methylcholest-5-en-3β-ol (dihydrobrassicasterol), 16% 24α-methylcholest-5-en-3β-ol (campesterol), 33% 24α-ethylcholest-5-en-3β-ol (sitosterol) and 15% 24ξ-ethylcholesta-5,22-dien-3β-ol.

The sterol content of several mosses and liverworts were previously reported [1–3], but the absolute configuration at C-24 which is of chemotaxonomic significance was not examined [4]. The configuration at C-24 of the sterols from the photosynthetic thallose liverwort *Palavicinnia lyellii* is determined in this study.

The unsaponifiable fraction of the extract of *Palavicinnia lyellii* was chromatographed on neutral alumina, and the 4-desmethylsterols were analysed by GC which indicated the presence of two major sterol components. The 4-desmethylsterols were further purified by Si gel TLC and then separated by prep. HPLC.

The first peak to elute from the prep. HPLC was identified by GC as 24-methylcholesterol ($RR_t$ = 1.32). The mass spectrum contained ions at *m/z* (rel. int.) 400 $[M]^+$ (50), 385 $[M-Me]^+$ (12), 382 $[M-H_2O]^+$ (19), 367 $[M-Me-H_2O]^+$ (16), 289 $[M-H_2O-C_7H_9]^+$ (53), 273 [M − side chain]$^+$ (44), and 255 $[M-H_2O$ − side chain]$^+$ (100) characteristic of a 24-methyl-$\Delta^5$-sterol [4, 5]. Analytical HPLC provided a peak with an $\alpha_c$ = 1.08 which is identical to that of 24-methylcholesterol. The $^1$H NMR spectrum at 360 MHz of this 24-methylsterol component indicated that this sample was composed of a mixture of 70% 24β-methyl- and 30% 24α-methylcholesterol (Table 1). The $^1$H NMR signals from the mixture of *P. lyellii* were quantitated using the C-27 and C-21 proton absorption peaks of the 24α- and 24β-methylcholesterols as previously documented [6].

The second peak to elute from the prep. HPLC was identified by GC ($RR_t$ = 1.43) and analytical HPLC ($\alpha_c$ = 1.16) as 24-ethylcholesta-5,22-dien-3β-ol. The mass spectrum contained ions at *m/z* (rel. int.) 412 $[M]^+$ (25), 369 [M − 43]$^+$ (33), 351 $[M-H_2O-43]^+$ (8), 299 $[M-H_2O-Me-(C_{23}-C_{27})-1]^+$ (17), 271 [M − side chain − 2H]$^+$ (100), 255 $[M-H_2O$ − side chain]$^+$ (83) characteristic of 24-ethyl-$\Delta^{5,22}$-sterol [4, 5]. Insufficient material was available for $^1$H NMR analysis.

The third peak to elute from the prep. HPLC was identified by GC as 24-ethylcholesterol ($RR_t$ = 1.65). The mass spectrum contained ions at *m/z* (rel. int.) 414 $[M]^+$ (7), 329 [M − 85]$^+$ (14), 303 [M − 111]$^+$ (18), 273 [M − side chain]$^+$ (43) and 255 $[M-H_2O$ − side chain]$^+$ (100) characteristic of a 24-ethyl-$\Delta^5$-sterol [4, 5]. Analytical HPLC provided a peak with an $\alpha_c$ = 1.24 which is identical to that of 24-ethylcholesterol. The $^1$H NMR indicated that only the 24α-ethylcholest-5-en-3β-ol was present (Table 1).

*P. lyellii* contains a mixture of 24β-methyl-and 24α-methylcholest-5-en-3β-ol as its dominant sterol component (52%). The dominance of the methyl sterol fraction and particularly the 24β-methyl sterol is unusual in terrestrial plants but is found in some green algae [7]. The occurrence of only the 24α-ethylcholest-5-en-3β-ol indicates that this is a 'main line' plant from a sterol evolution perspective [4]. The sterol composition of the liverworts would suggest that they represent a transition

Table 1. $^1$H NMR of liverwort sterols

| | 24β-Methylcholesterol | | 24α-Methylcholesterol | | 24α-Ethylcholesterol | |
|---|---|---|---|---|---|---|
| Proton position | *P. lyellii* | Authentic* | *P. lyellii* | Authentic† | *P. lyellii* | Authentic‡ |
| 3H, H-18 (*s*) | 0.68 | 0.68 | 0.68 | 0.68 | 0.68 | 0.68 |
| 3H, H-19 (*s*) | 1.01 | 1.01 | 1.01 | 1.01 | 1.01 | 1.01 |
| 3H, H-21 (*d*, *J* = 6 Hz) | 0.92 | 0.92 | 0.91 | 0.91 | 0.92 | 0.92 |
| 6H, H-26 and H-27 (*d*, *J* = 6 Hz) | 0.85, 0.77 | 0.85, 0.77 | 0.85, 0.80 | 0.85, 0.80 | 0.83, 0.81 | 0.83, 0.81 |
| 3H, H-28 (*d*, *J* = 6 Hz) | 0.78 | 0.78 | 0.82 | 0.82 | — | — |
| 3H, H-29 (*t*, *J* = 7 Hz) | — | — | — | — | 0.85 | 0.85 |

*Synthesized from ergosterol [8].
†From Applied Science Laboratories, Inc.
‡From cabbage.

from algae to the lower vascular plants in sterol biosynthetic capability. This is particularly interesting since the liverworts are considered by virtue of their anatomy and reproductive strategy to represent an early evolutionary branch of the land plants.

## EXPERIMENTAL

*Organism. Palavicinnia lyellii* was collected in large mats of nearly pure culture from the pine barrens of New Jersey, U.S.A. The thallus was cleaned of all soil and detritus.

*Extraction and separation.* Lipids of *P. lyellii* (61.6 g) were extracted with $Me_2CO$ for 48 hr in a Soxhlet. The solvent was evaporated and the extracts were saponified at reflux for 4 hr with 5% KOH in 50% MeOH. Extraction with $Et_2O$ ( × 3) yielded a neutral lipid (278 mg). This was chromatographed on a 3% deactivated $Al_2O_3$ column with a solvent system of dry $Et_2O$ graded into hexane, and finally with MeOH. The sterol fraction was then chromatographed on Si gel TLC with $C_6H_6$–EtOAc (9:1). The 4-desmethylsterols (9.7 mg) had $R_f = 0.17$, were detected by dichlorofluorescein under UV and were eluted with dry $Et_2O$. GC was performed on a 6 m × 2 mm 1% XE-60 column at 240° with He at 45 ml/min. All $RR_t$'s are relative to cholesterol. Prep. HPLC was performed on a Whatman M-20 column (25 × 2.5 cm) packed with Whatman ODS-3 chromatographed at 28° with MeOH–MeCN (1:9) 7.5 ml/min. Analytical HPLC was performed on a $C_{18}$ μBondpak column (30 cm × 3 mm) chromatographed at 55° with MeCN–*iso*-PrOH (8:2) at 1 ml/min. The $\alpha_c$'s (K′ sample/K′ cholesterol) [9] are reported only from the analytical column. Detection for both HPLC techniques was by UV at 205 nm. EIMS (probe) was performed at 70 eV. $^1$H NMR was performed at 360 MHz at ambient temp. in $CDCl_3$ with TMS as an int. standard.

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## REFERENCES

1. Marsili, A. and Morelli, I. (1968) *Phytochemistry* **7**, 1705.
2. Marsili, A. and Morelli, I. (1970) *Phytochemistry* **9**, 651.
3. Matsuo, A., Nakayma, N., Maeda, M., Hayashi, S. and Yasuda, S. (1973) *Phytochemistry* **12**, 2413.
4. Nes, W. R. and Mc Kean, M. L. (1978) *Biochemistry of Steroids and Other Isopentenoids*. p. 411. University Park Press, Baltimore.
5. Knights, B. A. (1967) *J. Gas Chromatogr.* **5**, 273.
6. Chiu, P. L. and Patterson, G. W. (1981) *Lipids* **15**, 203.
7. Patterson, G. W. (1971) *Lipids* **6**, 120.
8. Barton, D. H. R. and Robinson, C. H. (1954) *J. Chem. Soc.* 3045.
9. DiBussolo, J. M. and Nes, W. R. (1982) *J. Chromatogr. Sci.* **20**, 193.

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# 7-DEHYDROSITOSTEROL FROM *RAUWOLFIA SERPENTINA*

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**Key Word Index**—*Rauwolfia serpentina*; Apocynaceae; 7-dehydrositosterol.

**Abstract**—7-Dehydrositosterol has been isolated from the roots of *Rauwolfia serpentina*.

From previous reports [1–3], it appears that the sterols of *Rauwolfia serpentina* are a mixture of stigmasterol and sitosterol. In this paper we report the isolation of 7-dehydrositosterol from the roots of *Rauwolfia serpentina*.

A homogeneous sterol, $C_{29}H_{48}O$, $[\alpha]_D^{30} - 2.2°$ ($CHCl_3$), $[M]^+$ at *m/z* 412, mp 138° was isolated from the neutral fraction of *R. serpentina* roots. The sterol showed a UV spectrum [$\lambda_{max}$ nm: 292, 281, 270.5, 261 (with log ε: 3.76, 3.94, 3.92 and 3.83)] characteristic of a homoannular diene system. The IR spectrum of the sterol showed bands at 3400 (hydroxyl), 1635 (substituted double bond), 1365 and 1375 $cm^{-1}$ (C-methyl and gem-dimethyl). The $^1$H NMR spectrum (90 MHz in $CDCl_3$) showed the presence of two vinylic protons (δ 5.32–5.5, 1H, doublet and δ 5.07–5.3, 1H, complex multiplet) of the homodiene system. The base peak in the mass spectrum of the sterol at *m/z* 353 also showed the presence of the $\Delta^{5,7}$-diene system [4]. The peak at *m/z* 271 (35.9%) could be accounted for by cleavage of the C-17, C-20 bond of a sterol [5].

A shift of the $^1$H NMR signal from (δ 3.4–3.75, 1H, *br m*) to (δ 4.45–4.9, 1H, *br m*) was readily discernible in the spectrum of its monoacetate, mp 126°, IR $\nu_{max}^{KBr}$ 1720 $cm^{-1}$,