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MAJOR STEROLS OF *ACHLYA BISEXUALIS*

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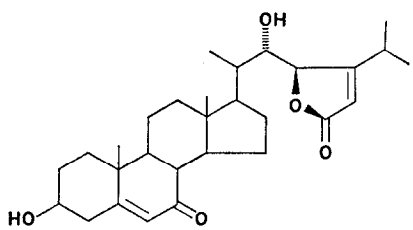
Key Word Index—*Achlya bisexualis*; Phycomycetes; sterols; fucosterol; 24-methylene cholesterol; cholesterol; 7-dehydrofucosterol.

Abstract—Fucosterol, 24-methylene cholesterol, and cholesterol have been identified as major sterols in *Achlya bisexualis*. 7-Dehydrofucosterol has also been tentatively identified.

THE FUNGAL sex hormone, antheridiol (I), has been isolated from the water mold, *Achlya bisexualis*.[1] In view of its carbon skeleton and on the basis of previous biosynthetic studies of steroidal metabolites,[2] it seems likely that the biogenetic origin of the hormone involves a C_{29} sterol skeleton. Prior to undertaking labelling experiments, some major sterols of this water mold were isolated and identified.



(I)

The mold cultures were grown on 500 ml of media as previously described[1] and the mycelium collected after 7 days. The air dried mycelium was saponified with ethanolic KOH and the lipids extracted with hexane. The non-saponifiable material was chromatographed on alumina (activity III) and squalane and other hydrocarbons (up to 80% of the non-saponifiable lipid) eluted with light petrol. Further elution with diethyl ether gave a fraction containing sterols, which were subsequently acetylated and separated by preparative TLC on silica gel impregnated with silver nitrate.

GLC of the sterol acetate mixture showed four major components, RR_t (retention time relative to cholesteryl acetate), 1·00, 1·28, 1·51 and 1·67, in a ratio of 3:17:70:10 respectively, based on peak areas, and three minor components <1% each, with RR_t, 1·11, 1·20 and 1·40. The RR_ts of the four major components correspond to previously published values for cholesteryl acetate, 24-methylene cholesteryl acetate, fucosteryl acetate and an unidentified steroid, respectively.[3]

Two distinct bands were observed on the TLC plates in daylight with R_fs 0·47–0·51 and

[1] MCMORRIS, T. C. and BARKSDALE, A. W. (1967) *Nature* **215**, 320.
[2] DE SOUZA, N. J., GIHISALBERI, E. L., REES, H. H. and GOODWIN, T. W. (1970) *Phytochemistry* **9**, 1247.
[3] PATTERSON G. W. (1971) *Anal. Chem.* **43**, 1165.

0·60–0·70. Under UV light another two bands were visible with R_fs, 0·19–0·33 and 0·70–0·74. Each of the four bands were eluted with ether and examined separately.

Band R_f 0·60–0·70 was recrystallized from ethanol as plates, m.p. 118–119°, m.m.p. with fucosteryl acetate 118–119°. On injection with fucosteryl acetate on QF-1 column only one peak was shown with RR_t = 1·51. The MS and NMR of the isolated steroid were identical in every respect with authentic fucosteryl acetate. The NMR spectrum clearly distinguished between fucosterol and its isomer, 28-isofucosterol in that the proton resonance signal of the C-25 hydrogen appears at δ 2·2 and δ 2·8, respectively.

Band R_f 0·47–0·51 was recrystallized from ethanol as plates, m.p. 131–132°, m.m.p. with 24-methylene cholesteryl acetate, 130–132°. On injection with 24-methylene cholesteryl acetate on QF-1 column only one peak was shown with RR_t = 1·28. The MS, NMR and IR of the isolated steroid were identical in every respect to authentic 24-methylene cholesteryl acetate.

Band 0·19–0·33, on GLC examination showed a major component with RR_t = 1·67 and a number of small impurities. It was therefore subjected again to preparative TLC using 10% ethyl acetate–benzene as solvent. Recrystallization from methanol gave 1 mg of colourless crystals, m.p. 127–130°. Literature m.p. for 7-dehydrofucosterol 129–131°.[4] The UV spectrum only showed the absorption maximum at 282 nm with appropriate fine structure expected[5] for a $\Delta^{5,7}$ system (*I* not measured precisely). The MS showed a peak for M^+ less $MeCO_2H$ at *m/e* 392 corresponding to a molecular weight of 452 (or 410, $C_{29}H_{46}O$, for the alcohol). The $\Delta^{5,7}$ system was evident in peaks at *m/e* 143, 157, 158 as expected.[6] The possibility of a Δ^{22} bond was examined by comparison of the MS of Δ^7-dehydrostigmasteryl acetate and the isolated sterol. The former gave a prominent peak at *m/e* 253 corresponding to loss of $MeCO_2H$ and side chain, but no peak at 296 or 297, whereas the latter gave a fragment for M^+ less $MeCO_2H$ less side chain at *m/e* 253 which was 3 times as intense as the peak *m/e* 297 corresponding to allylic cleavage at C_{22},C_{23}. Lack of material prevented further examination. We tentatively suggest therefore that this steroid is 7-dehydrofucosterol or its C_{28} isomer.

Band R_f 0·70–0·74 was difficult to distinguish even under UV light because of overlapping with fucosteryl acetate. This band was therefore sujbected again to preparative TLC using 20% hexane–benzene as solvent. The band which co-chromatographed with cholesteryl acetate was examined by GLC which showed the major component corresponding to cholesteryl acetate and two smaller peaks, about 3% each, with RR_t 1·29 and 1·51. Further attempts at separation were not made because of lack of material. Confirmation of cholesteryl acetate was obtained by GLC on 1% QF-1 and MS analysis of the major component showed a peak for M^+ less $MeCO_2H$ at *m/e* 368. On injection of this sample with cholesteryl acetate no change was shown in the MS.

Achlya bisexualis therefore contains a mixture of sterols with 27-, 28- and 29-carbon skeletons. The presence of 24-methylene cholesterol, a C_{28} sterol, and 7-dehydrofucosterol, a C_{29} sterol, may well serve as biosynthetic precursors of fucosterol, a C_{29} sterol. The sterols produced by a number of Phycomycetes have been investigated by McCorkindale *et al.*[7] Fungi of the orders Saprolegniales and Leptomitales were found to contain varying proportions of cholesterol, demosterol, 24-methylene cholesterol and fucosterol. It is

[4] NES, W. R. and GOVINDA MALYA, P. A. (1971) *J. Biol. Chem.* **246**, 561.
[5] DORFMAN, L. (1953) *Chem. Rev.* **53**, 47.
[6] GALLI, G. and MARONI, S. (1967) *Steroids* **10**, 187.
[7] MCCORKINDALE, N. J., HUTCHINSON, S. A., PURSEY, B. A., SCOTT, W. T. and WHEELER, R. (1961) *Phytochemistry* **8**, 861.

incidentally of particular interest to compare the major sterols in this fresh water mold with those of the marine brown algae (Phaeophyceae), in which the predominant sterol is fucosterol, and in some species the presence of 24-methylene cholesterol has been confirmed.[8] The presence of fucosterol in water mold provides the necessary carbon skeleton for subsequent modification of the side chain and nucleus. As has been found in the case of ergosterol synthesis in yeast,[9] a sequential series of modifications must undoubtedly be involved.

EXPERIMENTAL

Materials and instruments. Fucosterol was isolated from *Fucus gardneri*,[10] collected from the beach at Vancouver, B.C. 24-Methylene cholesterol was synthesized by the reported procedure.[11] Cholesterol was obtained commercially. *Achlya bisexualis*, 14524, was obtained from American Type Culture Collection. Spectra were obtained on the following instruments: Perkin–Elmer 457 (IR), Unicam SP800, Varian A56/60 (NMR), Hitachi–Perkin–Elmer RMU-7 double focussing mass spectrometer connected to a Varian 1400 GLC, and a Varian model 2100 gas chromatograph fitted with a 1% QF-1 column and operated as reported.[3] M.ps are uncorrected.

Extraction of steroids. The mycelium from *Achlya bisexualis* was filtered off and air dried at room temp. The dried mycelium (50 g) was macerated in a blender with 500 ml 50% EtOH–H_2O. A further 250 ml EtOH and a soln. of 75 g KOH in 100 ml H_2O were added and the mixture refluxed under N_2 for 3 hr. The soln. was centrifuged and the residue washed with EtOH. The soln. was diluted to 2× its vol. with H_2O and extracted 4× with hexane. The hexane soln. was washed until neutral with H_2O and dried over $MgSO_4$. Evaporation of the hexane *in vacuo* gave 450 mg of a yellow oil.

Column chromatography. In a typical separation 450 mg of non-saponifiable lipid was chromatographed on 10 g alumina (activity III) and the hydrocarbons (360 mg) eluted with 100 ml light petrol.; the column was then eluted with 100 ml Et_2O to give a fraction (90 mg) containing sterols. The sterol fraction was then acetylated with 5 ml pyridine–Ac_2O (2:1) at room temp. for 12 hr and worked up in the normal way.

TLC was carried out on plates 0·75 mm thick made from a slurry containing 40 g silica gel G, 18 g $AgNO_3$, 80 mg rhodamine 6G, 25 ml EtOH and 60 ml H_2O. Plates were prepared fresh each day and activated by heating at 120° for 30 min before use and developed with benzene unless stated otherwise.

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[11] IDLER, D. R. and FAGERLUND, V. H. M. (1957) *J. Am. Chem. Soc.* **79**, 1988.