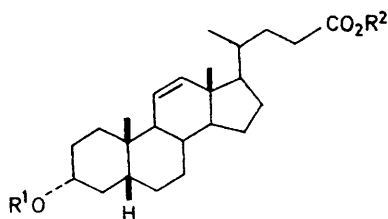


### 3 $\alpha$ -Hydroxy-5 $\beta$ -chol-11-en-24-oic Acid, a New Fungal Metabolite

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The title compound (1) has been isolated from the culture filtrate of a *Curvularia* species.

DURING the original isolation<sup>1</sup> of curvularin and dehydrocurvularin<sup>2</sup> from a *Curvularia* species, Calam and Robinson obtained a small amount of a third crystalline metabolite. We have now established that this is the bile acid derivative (1), which has not previously been obtained from a natural source.<sup>3</sup>



- (1) R<sup>1</sup> = R<sup>2</sup> = H  
 (2) R<sup>1</sup> = Ac, R<sup>2</sup> = H  
 (3) R<sup>1</sup> = H, R<sup>2</sup> = Me

The compound, C<sub>24</sub>H<sub>38</sub>O<sub>3</sub>, shows n.m.r. signals characteristic<sup>4,5</sup> of a 3-hydroxy- $\Delta^{11}$ -steroid and proved to be identical with an authentic sample of synthetic 3 $\alpha$ -hydroxy-5 $\beta$ -chol-11-en-24-oic acid.<sup>6</sup> The acetate (2) and the methyl ester (3) prepared from the metabolite were homogeneous and were identical with authentic specimens. The mass spectrum of the acid (1) bears a marked resemblance to that of deoxycholic acid, fragmentation following the usual pathways.<sup>7</sup>

Although bacteria are able to cleave the side-chain of cholesterol *via* C<sub>24</sub> acid intermediates<sup>8</sup> there are no indications that fungi can themselves produce bile acids.<sup>9</sup> The *Curvularia* was grown on an aqueous medium containing glucose, mineral salts, and a peptone (Lab-Lemco, Oxoid Ltd.) resulting from the enzymic hydrolysis of meat, and we think it likely that the metabolite resulted from the action of the fungus on a precursor such as cholic acid or deoxycholic acid which was fortuitously present in the peptone.

#### EXPERIMENTAL

M.p.s were determined on a Kofler hot-stage apparatus. I.r. spectra were obtained for KBr discs and n.m.r. spectra

were measured with a Varian HA-100 spectrometer (100 MHz) for solutions in deuteriochloroform with tetramethylsilane as internal standard. Mass spectra were obtained with an A.E.I. MS30 spectrometer and a Perkin-Elmer F-11 gas chromatograph was used for analytical g.l.c.

**3 $\alpha$ -Hydroxy-5 $\beta$ -chol-11-en-24-oic Acid (1).**—The metabolite crystallised from benzene to give plates (90 mg), m.p. 166°, [ $\alpha$ ]<sub>D</sub><sup>19</sup> +25.9° (*c* 2.2 in CHCl<sub>3</sub>) [Found: C, 76.8; H, 10.3%; *M* (by vapour phase osmometry in CHCl<sub>3</sub>), 388. C<sub>24</sub>H<sub>38</sub>O<sub>3</sub> requires C, 76.95; H, 10.25%; *M*, 374.6],  $\nu_{\max}$ , 3420 (OH), 2660 (hydrogen-bonded OH), and 1729 cm<sup>-1</sup> (carboxylic C=O),  $\tau$  3.91, 4.57, and *ca.* 7.72 (3H, AMXm, *J*<sub>AM</sub> 10, *J*<sub>AX</sub> 2, *J*<sub>MX</sub> 3 Hz, -CH=CH-CH=), 4.26br (1H, s, =CHOH), *ca.* 6.35 (1H, *W*<sub>3</sub> *ca.* 20 Hz, axial =CHOH), and 9.12 and 9.28 (each 3H, s, Me at C-10 and C-13), *m/e* 374 (*M*<sup>+</sup>, 9%), 356 (100), 341 (35), 255 (45), 228 (40), and 213 (25). It was identical (i.r., n.m.r., and mixed m.p.) with an authentic specimen, m.p. 164–166° (lit.,<sup>6</sup> 165–166, 165–165.5, 168–169°).

**3 $\alpha$ -Acetoxy-5 $\beta$ -chol-11-en-24-oic Acid (2).**—A mixture of the hydroxy-acid (1) (15.5 mg), acetic anhydride (0.2 ml), and dry pyridine (1.0 ml) was kept at room temperature for 12 h and then mixed with water (0.3 ml). After 3 h the mixture was evaporated under reduced pressure and the residue was crystallised from methanol to give the *acetoxy-acid* (2) (12 mg), m.p. 126–127° [Found: *m/e*, 356.2717. C<sub>24</sub>H<sub>36</sub>O<sub>2</sub> (*M* - MeCO<sub>2</sub>H) requires 356.2715],  $\nu_{\max}$ , *ca.* 2700 (hydrogen-bonded OH), 1730 (ester C=O), and 1710 cm<sup>-1</sup> (carboxylic C=O),  $\tau$  3.90, 4.58, and *ca.* 7.70 (3H, AMXm, *J*<sub>AM</sub> 10, *J*<sub>AX</sub> 2, *J*<sub>XM</sub> 3 Hz, -CH=CH-CH=), *ca.* 5.26 (1H, *W*<sub>3</sub> *ca.* 20 Hz, axial =CHOAc), 7.99 (3H, s, CH<sub>3</sub>-CO<sub>2</sub>R), and 9.12 and 9.28 (each 3H, s, Me at C-10 and C-13), *m/e* 416 (*M*<sup>+</sup>, 0.2%), 356 (66), 341 (29), 255 (71), 228 (63), and 213 (39). It was identical (i.r., n.m.r., and mixed m.p.) with the acetate prepared from an authentic specimen of the hydroxy-acid.

**Methyl 3 $\alpha$ -Hydroxy-5 $\beta$ -chol-11-en-24-oate (3).**—A mixture of the hydroxy-acid (1) (8 mg), dicyclohexylcarbodi-imide<sup>10</sup> (9 mg), anhydrous methanol (5 ml), and anhydrous pyridine (1 ml) was kept for 12 h at room temperature and evaporated. Extraction of the residue with chloroform gave a gum which showed a single peak [*t*<sub>R</sub> 1.63 relative to cholesterol (*t*<sub>R</sub> 1.00)] when analysed by g.l.c. using a column

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(2 m) of F.S.-1265 fluorosilicone oil (1.5%) on AW-DMCS Chromosorb W (80—100 mesh) at 225°. T.l.c. on silica gel in benzene-ethyl acetate gave the methyl ester (3), which crystallised from hexane in needles, m.p. 101—103° (lit.,<sup>6</sup> 101—102°) (Found:  $M^+$ , 388. Calc. for  $C_{25}H_{40}O_3$ :  $M$ , 388), *m/e* 388 ( $M^+$ , 3.5%), 370 (56), 355 (20), 255 (56),

228 (35), and 213 (25). This was identical (g.l.c., mixed m.p.) with an authentic specimen, m.p. 103—104°.

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