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STEROLS IN PORPHYRIDIUM CRUENTUM

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On the basis of a negative Liebermann-Burchard reaction, it has been reported[1] that sterols are absent from the unicellular red alga, Porphyridium cruentum (Order Bangiales). Since this would make the organism unique amongst the investigated Rhodophyceae, we have re-examined this alga and report the presence of sterols.

P. cruentum (Cambridge Culture Collection 1380/1A) was grown on a defined medium[2], and was routinely checked for possible contamination. Cells (100 g. wet weight) were extracted by direct saponification and the 4,4-dimethyl, 4α-methyl and 4-desmethyl sterols separated from the unsaponifiable material (326 mg.).

4-Desmethyl sterols (10 mg.) G.L.C. analysis (QF-1, SE-30, HiEff-8BP) revealed the presence of three components, which corresponded to trans-22-dehydrocholesterol (63%), cholesterol (3%) and ergosterol (34%). Separation of the acetates on $AgNO_3$-silica gel produced three bands corresponding to the acetates of (a) cholesterol, (b) 22-dehydrocholesterol and (c) ergosterol. G.L.C. analysis (QF-1 and SE-30) of band (a) indicated the presence of four components co-chromatographing with the acetates of (i) cholesterol (19%) (ii) brassicasterol (5%), (iii) stigmasterol (10%) and (iv) β-sitosterol (65%), respectively. Band (a) was subjected to G.L.C.-mass spectrometry (JXR): peak (i) showed ions at m/e 428, 368 (base peak), 353, 260, 255, 247, 213 in accordance with the spectrum of authentic cholesteryl acetate;[3,4] peak (ii) was very small, the only significant ion being observed at m/e 380 [M^+-acetate]; peak (iii) showed ions at m/e 394 [M^+-acetate] (base peak), 379, 351, 255, 253, 228, 213; peak (iv) had a fragmentation pattern with ions at m/e 456, 396, 381, 329, 303, 288, 275, 255 and 213. The latter two spectra agree with the spectra[4] of stigmasteryl and β-sitosteryl acetates, respectively. The levels of alkylated sterols were so low as to be undetectable in the total sterol mixture, and the configuration at C-24 of the alkylated sterols could not therefore be determined.

Band (b) consisted of a single component co-chromatographing on G.L.C. with trans-22-dehydrocholesteryl acetate, m.p. 123-126° C (lit[5,6] 125-128° C), I.R. $\gamma_{max.}$ 965 $cm.^{-1}$ (trans disubstituted double bond), 800 $cm.^{-1}$ (trisubstituted double bond); G.L.C.-M.S. m/e 366 [M^+-acetate] (base peak), 351, 313, 282, 258, 255, 253, 245, 213, 111, 81, 69 and 55. This is in agreement with the mass spectrum reported[6,7] for 22-dehydrocholesterol. N.M.R.[8] ($CDCl_3$) (cf. 6), singlets at 0.69 (C-18 methyl), 1.02 (C-19-methyl) and 2.02 p.p.m. (acetate); doublets centred at 0.86 (J=6 c.p.s., C-26 and C-27 methyls) and 1.00 p.p.m. (J = 7 c.p.s. C-21 methyl); multiplets centred at 4.60 (C-3 proton) and 5.34 p.p.m. (C-6, C-22, C-23 protons). The position of the C-19 methyl resonance confirms the presence of a Δ^5 bond.

G.L.C. of band (c) indicated the presence of two components, the major peak (ii) (93% of total) co-chromatographing with ergosteryl acetate and the minor component (peak i) with both the acetates of cholesterol and cholesta-5,7,22-trien-3β-ol. Its behaviour on $AgNO_3$- impregnated T.L.C. and its mass spectrum rule out its identity with cholesteryl acetate. G.L.C. - M.S.: peak (i) m/e 424, 409, 364 (base peak), 349, 323, 321, 313, 311, 253, 251, 211 and 143; peak (ii) 438, 423, 378 (base peak), 363, 337, 335, 313, 311, 253, 251, 211 and 143. These spectra are in accord with those reported[9,10] for the acetates of cholesta-5,7,22-trien-3β-ol and ergosterol. The N.M.R., and I.R. spectra were also in agreement with the above structures.

In summary, the 4-desmethyl sterols of P. cruentum include trans-22-dehydrocholesterol, 24ξ-methyl-cholesta-5,7,22-trien-3β-ol, cholesterol, 24ξ-ethyl-cholest-5-en-3β-ol, 24ξ-ethyl-cholesta-5,22,-dien-3β-ol, cholesta-5,7,22-trien-3β-ol and possibly 24ξ-methyl-cholesta-5,22-dien-3β-ol.

The 4α-methyl sterol fraction (3.7 mg.) consists of four components, three of which appear to be novel sterols, and are under further investigation.

4,4-dimethyl sterols (0.4 mg.) G.L.C. together with G.L.C.-M.S. showed the presence of 24,25-dihydrolanosterol, cycloartenol, 24-methylene cycloartanol together with an unknown component.

Since cycloartenol may replace lanosterol as the first product of 2,3-oxidosqualene-cyclization in plants (for review see 11), it was of interest to determine the nature of the cyclization product in P. cruentum, especially in view of the co-occurrence of dihydrolano-

sterol and cycloartenol.

P. cruentum cells suspended in 0.1M potassium phosphate buffer p.H.7.4 were ruptured by passage through a French pressure cell and the broken cell preparation (containing 320 mg. protein) incubated anaerobically at 25° for 16 hr. with 3,22 [^{3}H]-2,3-oxidosqualene (1.77×10^{6} d.p.m.). The reaction mixture was saponified and the non-saponifiable fraction subjected to T.L.C. The 4,4-dimethyl sterol band contained 1.45×10^{5} d.p.m. (16.4% of the available isomer of substrate), whereas there was only 0.3% incorporation into the corresponding fraction from a control incubation containing boiled cell preparation.

An aliquot of the 4,4-dimethyl sterols after addition of non-radioactive dihydrolanosterol, lanosterol, cycloartenol and 24-methylene cycloartanol was subjected to preparative G.L.C. on 3% XE-60. Samples were collected at 1 min. intervals, as they eluted from the column, in glass capillary tubes at ambient temperature, and radioassayed. Greater than 90% of the recovered radioactivity co-chromatographed with cycloartenol whilst negligible radioactivity was associated with any other peak.

On recrystallization of aliquots of the 4,4-dimethyl sterol fraction with carrier cycloartenol and lanosterol, respectively, the cycloartenol rapidly attained constant specific radioactivity (2043, 1872, 1973, 1876, 1883 d.p.m./mg.), whereas approximately 97% of the radioactivity had been removed from the lanosterol after the sixth recrystallisation (135, 523, 223, 154, 61, 81, 42 d.p.m./mg.).

Since cycloartenol and 24-methylene dihydrolanosterol co-chromatograph on G.L.C. (XE-60) and could also conceivably co-crystallise, the cycloartenol after the fourth recrystallization was subjected to T.L.C. on $AgNO_3$-silica gel, developing twice with chloroform. Greater than 95% of the recovered radioactivity was present in the cycloartenol band, with less than 3% in the 24-methylene dihydrolanosterol region.

The occurence of the C_{27} sterol, trans-22-dehydrocholesterol as the major component of P. cruentum sterols with lesser amounts of C-24 alkylated sterols is in agreement with other recent reports in Rhodophyceae of the preponderance of C_{27} sterols often accompanied by much lesser amounts of the C_{28} and C_{29} members [for review see 12]. The identification of cycloartenol and its formation as the only significant product of anaerobic cyclization of 2,3-oxidosqualene, indicates its central role in Rhodophyceae. This is in agreement with the previous[13] tentative identification of cycloartenol in a red alga by T.L.C. properties. It has been suggested[14] that P. cruentum may be morphologically amongst the simplest free-living

eukariotic organisms, and is, therefore, probably amongst the most "primitive" organism in which cycloartenol has been reported. The lack of anaerobic in vitro formation of any 4,4-dimethyl sterols besides cycloartenol from 2,3-oxidosqualene is in agreement with similar observations[15] in Ochromonas malhamensis and may simply reflect lack of cofactors or inactivity of the appropriate enzymes under the conditions employed. In the intact organism, the cycloartenol is probably further metabolized to 24,25-dihydrolanosterol and 24-methylene cycloartanol, which could be precursors of the C_{27} and C-24 alkylated sterols, respectively.

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