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Sterols: Isolation from a Blue-Green Alga

Abstract. *A crystalline mixture of sterols was isolated from the filamentous blue-green alga* Phormidium luridum. *The mixture consisted of unsaturated 24-ethylcholesterols possessing* Δ^7-, $\Delta^{5,7}$-, *and* Δ^5-*bonds together with their* Δ^{22}-*derivatives and a small amount of cholesterol. The major component was 24-ethyl-*Δ^7-*cholestenol. Squalene and phytol were also evident.*

Due to their procaryotic nature, blue-green algae are thought to represent a primitive form of plant possessing a number of unusual properties (*1*) including the apparent lack of sterols (*2*). In an attempt to deduce where sterol formation is blocked, we investigated the isopentenoid constituents of the blue-green alga *Phormidium luridum* var. *olivaceae* Boresch (*3*).

Phormidium luridum was grown on an alkaline mixture of salts [Medium C of Kratz and Myers (*4*)] and flushed with air supplemented with 4 percent CO_2 (*5*). There was no contamination of our culture by eucaryots, or of our growth medium or our chemicals by sterols. Soxhlet extraction was carried out with acetone on 100 g (fresh weight) of whole cells. After saponification of the acetone-soluble material in 10 percent ethanolic KOH, the ligroin-soluble portion was chromatographed on alumina. The monohydroxyl region of the chromatogram was chromatographed again on alumina, yielding a crystalline material which proved to be sterol. Recrystallization from methanol gave 3.0 mg of colorless plates or leaflets, m.p. 136° to 141°C (Kofler hot stage).

Gas-liquid chromatography on XE-60 indicated that the crystals were a mixture with two major components (> 80 percent) and four minor components. The retention times relative to cholesterol (RRT) of the major components were 1.67 and 1.96 in an abundance ratio of two to three. The RRT's of the minor components were 1.00, 1.47, 1.80, and 2.11 with the last two substances being the principal ones. Ultraviolet analysis of the crystalline mixture showed the typical spectrum (maximum absorbancy 272, 282, and 294 nm) of a $\Delta^{5,7}$-sterol, and the extinction coefficients indicated that the substance amounted to 14 percent of the total.

In the mass spectrum of the crystals there were three parent peaks at mass/charge 414, 412, and 410 (in decreasing order of peak heights). Comparison of the amounts in these various analyses indicate that the substance with RRT 1.96 had a mass of 414, that with RRT 1.67 a mass of 412, and that with RRT 1.80 a mass of 410. Ultraviolet absorption spectra indicate that this third most abundant component must have had a $\Delta^{5,7}$-system. Thin-layer chromatography of the mixture on silica gel-G gave no spot in the 4,4′-dimethyl region (such as lanosterol gives), but a strong spot was evident in the normal sterol region. Peaks at *m/e* 231 and 229 for typical steroidal loss of the side chain plus 42 mass units (*6*) confirmed the absence of 4,4′-substitution and furthermore eliminated the possibility of a 14-methyl group. Based on the molecular weights, then, the sterols must have had a 24-ethyl group and one, two, and three double bonds ($C_{29}H_{50}O$ = 414; $C_{29}H_{48}O$ = 412; and $C_{29}H_{46}O$ = 410).

The ultraviolet spectrum of our mixture after correction for 14 percent $\Delta^{5,7}$-sterol had an unusually high end absorption (extinction coefficient ε at 215 nm of 2100) which was more characteristic (*7*) of Δ^7 (ε_{215} 3000) than of Δ^5 (ε_{215} 700). This was corroborated by the infrared spectrum which showed a frequency (ν_{max}) of 1033 cm^{-1} for Δ^7 rather than ν_{max} 1050 cm^{-1} for Δ^5 (*8*). A shoulder near ν_{max} 1050 cm^{-1}, however, indicated some Δ^5-component as was also suggested by the lowered ε_{215}, and the relationship of the intensities of the gas-liquid chromatographic and mass spectral peaks. The infrared spectrum had a weak band at 968 cm^{-1} corresponding to some *trans*-Δ^{22} (*9*).

This information is consistent with the following structures: component with RRT 1.96 is 24-ethyl-Δ^7-cholestenol ($C_{29}H_{50}O$); component with RRT 1.67 is 24-ethyl-$\Delta^{7,22}$-cholestadienol ($C_{29}H_{48}O$); component with RRT 1.80 is 24-ethyl-$\Delta^{5,7,22}$-cholestatrienol ($C_{29}H_{46}O$). An authentic sample of 24-ethyl-$\Delta^{7,22}$-cholestadienol had RRT 1.67. From the known contributions (*10*) of Δ^{22}- and $\Delta^{5,7}$-unsaturation the calculated RRT's of the Δ^7- and $\Delta^{5,7,22}$-derivatives are 1.94 and 1.80. All three values agree with the structures assigned. The RRT's of the other components at 1.00, 1.47, and 2.11 similarly indicate they represent cholesterol (authentic RRT 1.00), 24-ethyl-$\Delta^{5,22}$-cholestadienol (authentic RRT 1.44) and 24-ethyl-$\Delta^{5,7}$-cholestadienol (calculated RRT 2.09). In addition, authentic 24-ethyl-Δ^5-cholestenol had RRT 1.67 and, for the reasons mentioned above, it must have occurred together with the $\Delta^{7,22}$-component (RRT 1.67).

We have also isolated and similarly identified phytol from *P. luridum* by mass spectroscopy, thin-layer chromatography, and nuclear magnetic resonance spectroscopy, and a trace of squalene was apparent by gas-liquid chromatography.

This is further evidence that the usual biosynthetic pathway to sterols is not always inoperative in blue-green algae.

NOEL J. DE SOUZA
WILLIAM R. NES
Department of Biological Sciences, Drexel Institute of Technology, Philadelphia, Pennsylvania 19104

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