

Bacterial Triterpenes

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Summary The first isolation of a pentacyclic triterpene, hopene-b, from a bacterium is reported.

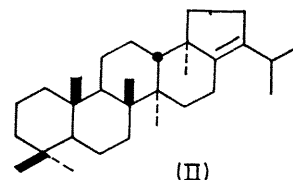
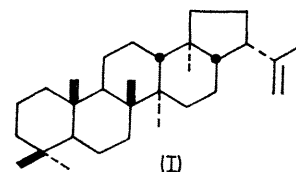
WE report the isolation of a pentacyclic triterpene, *viz.* hopene-b (I)¹ [diploptene, hop-22(29)-ene], from a bacterium, the first occasion on which any such product of squalene cyclization has been characterized from a prokaryotic organism.

The bacterium is a novel species of marked thermophilic and acidophilic character isolated from natural habitats in the volcanic area of Naples, and has optimal growth at temperatures of 58–60 °C and pH *ca.* 2.6–2.8. We have already reported the characterization of 11-cyclohexylundecanoic and 13-cyclohexyltridecanoic acids as the major fatty acids of this organism.² For the work on the non-saponifiable lipids the organism has been grown on 0.15% Difco beef extract in 0.2 M-citric acid-phosphate buffer (pH 2.6).

After 18 h the cells were centrifuged down and lyophilized; 40 g of cells, extracted for 12 h with light petroleum, afforded 0.3 g of oil, which after t.l.c. on SiO₂ in cyclohexane gave 112 mg of crude crystalline solid, *R_F* 0.9 (0.28% of the dry weight of cells), m.p. *ca.* 190°. G.l.c. of this material (5% SE-30, 290°) showed the major component (86%) to have a retention time (4.0) identical with that of authentic hopene-b. Further purification by t.l.c. on SiO₂-AgNO₃ in 95:5 light petroleum-benzene afforded hopene-b (*R_F* 0.3), m.p. 205 °C (lit. *ca.* 210 °C), [α]_D (CHCl₃) +60° (lit. +62°), identical with authentic material as characterized by i.r., n.m.r., and mass spectra.

The hopene-b is accompanied by four minor components,

of which one has been identified, by the same criteria, as hopene-1 (II).^{3,4}



The general absence of cyclized squalene derivatives in bacteria is well known and is a characteristic feature of prokaryotic organisms.⁵ However, significant quantities of squalene (0.5%) and derived 4,4-dimethyl-, 4-methyl-, and 4-desmethyl-steroids (0.2%) have been found in methane-grown *Methylococcus capsulatus* and some other instances;⁶ nevertheless in most prokaryotes the synthesis of such substances is not detectable and they are present, if at all, in such minute quantity that an external origin may often be suspected. In the present case, no trace of any similar compounds could be detected when Difco beef

extract equivalent to about five times the quantity of bacteria studied was extracted exhaustively with Et₂O and the extract examined by mass spectrometry and g l c , in contrast, hopene-b was readily confirmed in bacteria grown on 0.3% soluble starch-salts brought to pH 3.0 with H₂SO₄. Triterpenoids of the hopane series have so far been recorded only from green plants, and most commonly from ferns and lichens.⁷ In our own organism, the simultaneous presence of highly anomalous components in both

the saponifiable and the non-saponifiable lipids is quite possibly related to the somewhat extreme environment (pH 2.1, 60 °C) from which it was isolated, and the corresponding peculiarities of membrane structure which such characteristics may well require.

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