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BIOSYNTHETIC STUDIES OF MARINE LIPIDS–XXVIII.[1] USE OF SPONGE CELL-FREE EXTRACTS IN THE STUDY OF MARINE STEROL BIOSYNTHESIS

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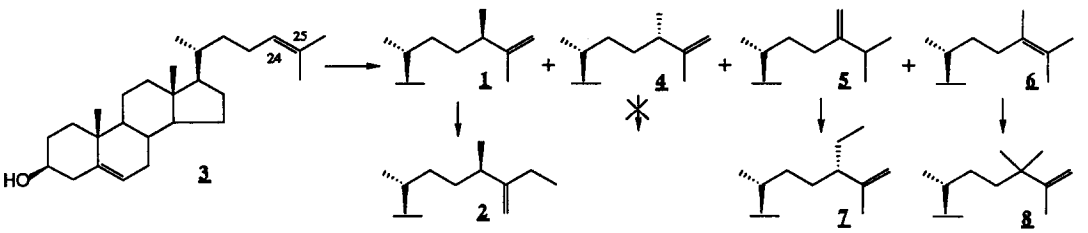
SUMMARY - Cell-free extracts from sponges facilitate the study of marine sterol biosynthesis. Experimental results from three sponges of the enzymatic alkylation of the sterol side chain are presented.

A large variety of biosynthetically interesting sterols have been isolated from marine sponges.[2] While sponges have proved tractable to feeding experiments,[3] the long periods required for metabolism of radiolabeled precursors and the logistical problems of working with sponges found in faraway seas are limiting.[4] As a solution to these problems we have developed methodology using cell-free extracts. These can be prepared using techniques developed for higher plants and marine algae.[5,6] We have kept cell-free extracts in cryogenic storage for long periods (>5 yrs.) without problems. The technique is rapid, often allowing observation of one biosynthetic step at a time, and, in the cases of methyl transfer reactions, permitting the use of commercially available radiolabeled S-adenosylmethionine (SAM) instead of labeled sterols, thus facilitating the separation of products from substrates. We present here results on sterol biosynthesis in three sponges using this technique.

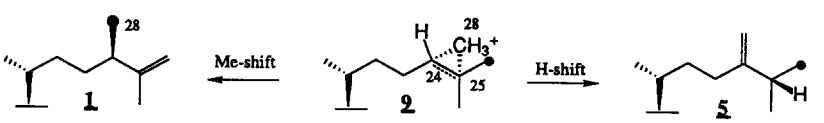
Earlier feeding experiments with Aplysina fistularis have shown epicodisterol (**1**) to be the precursor of dehydroaplysterol (**2**).[7] However the origin of **1** was unknown since it was then thought that sponges were generally incapable of de novo sterol biosynthesis. Fresh sponge (20 g) was ground at 0° with a Polytron tissue homogenizer in 80 ml buffer[8] containing 8 g polyvinylpolypyrrolidone.[9] The 10,000 x g supernatant was centrifuged at 125,000 x g to give a microsomal pellet which was suspended in a minimal volume of the same buffer and used in the subsequent experiments. Incubation

of the cell-free extract (90 μl) with ^{3}H-SAM (30 μl, 15 μCi) and desmosterol (3) (30 μl as a 0.5 mg/ml aqueous suspension in 1% Tween 80) at 27° for 4 hrs. yielded mainly epicodisterol (1, 24,000 dpm), but also smaller amounts of codisterol (4), 24-methylenecholesterol (5), and 24-methyldesmosterol (6) (c. 2,000 dpm each, identified by HPLC with the aid of cold carriers after protection as their i-methyl ethers).[10] A control experiment without added sterol precursor yielded no radiolabeled products. These compounds were also assayed as substrates under the same conditions: epicodisterol (1)



was biomethylated to dehydroaplysterol (2, 10,000 dpm); codisterol (4) did not serve as a substrate in the reaction; 24-methylenecholesterol (5) went to clerosterol (7, 16,000 dpm); 24-methyldesmosterol (6) gave 24-methylcodisterol (8, 5,000 dpm). While the information obtained is consistent with the results of the feeding experiments,[7] a more detailed picture was obtained.

The 24α stereochemistry of epicodisterol (1) is interesting because addition of the methyl group generally occurs from the β-face,[11] which is consistent with the co-production of codisterol (4). How do we then explain the configuration of 1? We can postulate the intermediacy of a bridged carbonium ion (9), as has been drawn for the biosynthesis of 24-methylenecholesterol (5).[11] If, instead of a hydride shift from C24 to C25



we have a methyl shift from C25 to C24, we should find the added methyl group

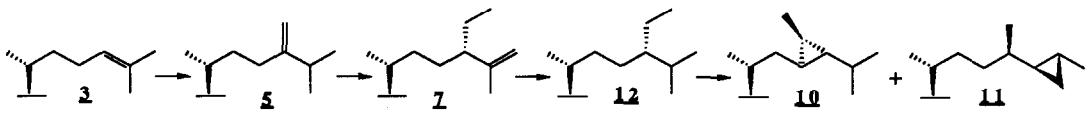
at C25. Such a methyl shift was ruled out by ozonolysis of the i-methyl ether of **1** to the 25-ketone and exchange of the acidic protons, which showed all the tritium to be at C-28. Epimerization through H-migrations between C-28 and C-24 of the protonated cyclopropane intermediate (**9**) is a possible explanation, but is considered unlikely since no tritium was found at C-24. Alternatively, the enzyme may bind the side chain in such a way as to present the opposite face of the double bond to the methyl donor (cf. **3a** to **1a**).

3a **1a**

The discovery[12] of (24-R) 24,25-methylenecholesterol (the deprotonated form of the protonated cyclopropane **9**) in the sponge Lissodendoryx topsenti provides new support for nonclassical intermediates in biomethylation. A cell-free extract was prepared by a modification of the above procedure: the sponge was ground to a powder in liquid nitrogen in a blender,[13] followed by extraction of the powder with buffer[8] using a Polytron. In cell-free extract experiments (10 μl cell-free extract, 10 μl sterol-Tween 80 suspension, and 10 μl ^{3}H-SAM (10 μCi)) desmosterol (**3**) was converted mainly to 24-methylenecholesterol (**5**, 30,000 dpm) plus smaller amounts (c. 3,000 dpm each) of codisterol (**4**) and the new cyclopropyl sterol. This shows direct formation of (24-R) 24,25-methylenecholesterol from the methyl transfer reaction and confirms results obtained from conventional feeding experiments.[12]

Many cyclopropyl sterols in sponges have been postulated to be interrelated through the rearrangement of nonclassical ions.[12] In feeding experiments[3] with Cribrochalina vasculum, 24-methylenecholesterol (**5**) was shown to be a precursor of dihydrocalysterol (**10**) and petrosterol (**11**). A cell-free extract was prepared as outlined above from frozen sponge which had been shipped in Dry Ice from the Bahamas. An acetone powder was prepared from the cell-free extract by adding 1 ml of the suspended microsomes to 10

ml acetone at -20° with stirring. The precipitated protein was centrifuged, washed with ether, dried in vacuo, and resuspended in buffer.[8] This was used for the cell-free extract experiments as outlined above. Biomethylation of desmosterol (3) using 1 μCi ^{3}H-SAM gave 24-methylenecholesterol (5, 20,000 dpm). Contrary to what was expected, when 24-methylenecholesterol (5) was assayed in the presence of 10 μCi ^{3}H-SAM, no cyclopropyl sterols (10, 11) were found, but instead, clerosterol (7, 22,000 dpm). As a working hypothesis we propose that reduction of clerosterol (7) leads to clionasterol (12) and that an aberrant 22-dehydrogenase converts this to the cyclopropyl sterols.



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