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BIOSYNTHETIC STUDIES OF MARINE LIPIDS-XXIX.[1] DEMONSTRATION OF STEROL SIDE CHAIN DEALKYLATION USING CELL-FREE EXTRACTS OF MARINE SPONGES

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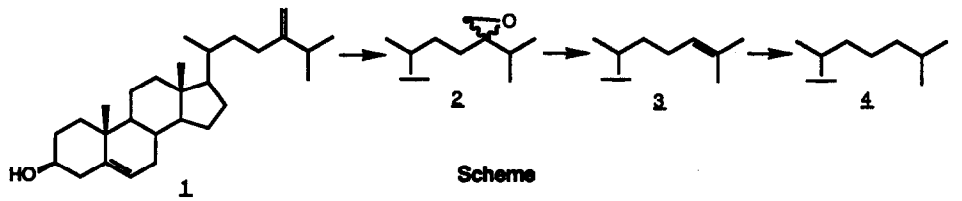
Abstract: Cell-free extracts of eight marine sponges were used in biosynthetic studies to examine the course and scope of sterol side chain dealkylation.

As shown in the accompanying article,[1] the use of cell-free extracts of sponges in biosynthetic studies with S-adenosyl-methionine (SAM) can overcome many of the difficulties encountered in working with whole organisms. We now report the preparation of cell-free extracts from five shallow-water sponges (-10-25m) collected by SCUBA, and three frozen deep-sea sponges (-400m) collected in the Monterey Canyon by the Monterey Bay Aquarium Research Institute's submersible, and their use with labeled sterols rather than labeled SAM in a sterol biosynthetic investigation. Due to obvious difficulties in simulating the natural environment of deep-sea sponges in incorporation experiments by conventional techniques with whole sponges, the use of cell-free extracts is essential to biosynthetic investigations of such materials which are becoming increasingly available owing to the use of manned and unmanned submersibles.

The recent report[2] that certain sponges can degrade 24-alkylated sterol side chains to cholesterol was surprising as these same sponges are also capable of side chain alkylation as well as de novo sterol biosynthesis.[3] We now describe the use of cell-free extracts[4] to assay for the presence of the enzymes responsible for the individual steps of sterol side chain dealkylation of [3-^{3}H] 24-methylenecholesterol (1) to cholesterol (4) in seven species of



Scheme

the Demospongiae and one of the Hexactinellida.

As summarized in the Table, cell-free extracts of all the sponges examined transformed 24-methylenecholesterol (1) to cholesterol (4) via intermediates 2 and 3. Mechanistically these experiments are important as they confirm the conversion of 24-methylenecholesterol (1) to its 24(28) epoxide (2) analogous to the path demonstrated in insects.[5] Our initial work with whole sponges[2] demonstrated the conversion of ^{3}H-labelled 24-methylenecholesterol and its epoxide (2) to desmosterol (3) and cholesterol (4), thus offering direct evidence for the second and third steps of the Scheme as well as inferring the existence of epoxide 2 as an intermediate. The present cell-free experiments now provide direct evidence for the epoxidation of 24-methylene-

Table: Incubations with 1 uCi (2.2x10^{6} dpm) [3-^{3}H] 24-methylenecholesterol (1)

SPONGE	Recovered activity in precursor (dpm)	Recovered activity in dpm		
		2	3	4
Acarnus erithicus [a]	1.07x10^{6}	4,500	2,300	2,100
Polymastia pachymastia [a]	1.25x10^{6}	8,700	2,700	2,500
F. Hymeniacidonidae[a]	1.07x10^{6}	7,900	2,800	2,300
Aplysina fistularis [a]	8.31x10^{5}	5,600	1,900	900
Tethya aurantia [a]	8.94x10^{5}	3,100	2,700	4,500
Polymastia sp.[b]	1.25x10^{6}	5,000	600	1,400
unidentified demosponge [b]	1.09x10^{6}	3,500	600	1,200
Hexactinellida[b]	1.10x10^{6}	4,400	4,900	2,100
Control (no sponge)	1.40x10^{6}	45	40	50

[a] Sponges collected by SCUBA at -10 -30m; [b] Sponges collected by submersible at -400m.

cholesterol (1) and thus for all steps in the Scheme.[6] The experiments summarized in the Table represent a relatively rapid assay for the dealkylation pathway and -- given the range of sponges examined -- indicate the generality of sterol side chain dealkylation in sponges.

For the preparation of cell-free extracts, the following were rapidly added to a Waring blendor (to prevent freezing, the blendor remained on at low speed): 250 mL buffer[7] (pH 7.7), inhibitors (pepstatin, leupeptin and phenylmethylsulfonyl fluoride),[8] 100 g sponge (freshly cut into small pieces), 50 g polyvinylpolypyrrolidone and 100 mL liq. N_2. Once homogeneous, the mixture was squeezed through cheese cloth to produce the crude extract. This was centrifuged at 10,000 x g (1 h) and the supernatant centrifuged a second time at 150,000 x g (1 h). Incubations were performed with 50 ul of the homogenized pellet from the high speed centrifugation (re-suspended in 2 mL buffer) with 1 uCi [3-^{3}H] 24-methylenecholesterol, 0.1 mg NADPH, 10 ul H_2O and 1 mg Tween 20 at 27^{o}C for 4 h. Incubations were terminated by extraction with ethyl acetate and addition of "cold" sterols. The sterol mixture was purified by chromatography over silica followed by normal and reversed phase HPLC. In a control experiment (incubation of [3-^{3}H] 24-methylenecholesterol with all reagents except the sponge extract) the precursor was recovered unchanged.

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References.

1. For preceeding paper, see: J.-L. Giner and C. Djerassi accompaning article.
2. S. Malik, R.G. Kerr and C. Djerassi J. Am. Chem. Soc. 110 (1988) 6895.
3. (a) R.G. Kerr, I.L. Stoilov, J.E. Thompson and C. Djerassi Tetrahedron 45 (1989) 1893; (b) C.J. Silva, L. Wunsche and C. Djerassi manuscript in preparation.
4. Sterol side chain dealkylation has been studied in insects using cell-free extracts; see: Y. Fujimoto, M. Morisaki and N. Ikekawa Biochemistry 19 (1980) 1065.
5. J.A. Svoboda in Isopentenoids in Plants (W.D. Nes, G. Fuller and L. Tsai eds.) Marcel Dekker, New York, 1984 p. 367.
6. The conversion of [3-^{3}H] 24(28)-epoxy-24-methylenecholesterol (2) to desmosterol (3) and cholesterol (4) using a cell-free extract of Tethya aurantia has also been demonstrated.

7. The buffer contains 50mM N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES), 50mM tris(hydroxymethyl)-aminomethane (Tris), 5mM dithiothreitol, 5mM ethylenediaminetetra-acetic acid and 1 g/L bovine serum albumin. The pH was adjusted to 7.7 with Tris.
8. Pepstatin, leupeptin and phenylmethylsulfonyl fluoride were added to prevent protease degradation of the protein(s) being purified. For a general discussion of the stabilization and concentration of proteins, see: T.G. Cooper in "The Tools of Biochemistry" Wiley Interscience, New York (1977) chapter 10.

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