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BIOSYNTHESIS OF STEROLS IN THE SEA CUCUMBER STICHOPUS CALIFORNICUS.

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Various reports deal with the biosynthesis of Δ^5 and Δ^7 sterols in sea cucumbers.[1] While Numura[2] et al. failed to observe incorporation of acetate-1,2-^{14}C into sterols of Stichopus japonicus, Goad et al.[3] and Voogt and Over[4] could show that Cucumaria elongata, C. planci, Holothuria tubulosa, and S. regallis can biosynthesize sterols from 2-^{14}C-mevalonate[3] and sodium acetate-^{14}C,[4] respectively. In continuation of our work on the biotransformation of lanosterol to the sea cucumber sapogenin holotoxinogenin,[5] we have now examined some of the later stages of the biosynthesis of sterols in the same sea cucumber and report that S. californicus can biosynthesize sterols de novo from $^3H_3C\text{-}COO^-K^+$ and can transform 3-^{3}H-lanosterol (1), 1,2-$^3H_2$-cholesterol (2), and 3-^{3}H-Δ^7-cholestenol (3) to Δ^5 and Δ^7 sterols. The incubations and sterol isolations were conducted as described earlier.[1,5] The nature of the sterols was established by comparison of GC retention times of the free sterols and their acetates over O.V. 25(3% on Gas Chrom Q) and O.V. 3(3% on Gas Chrom Q) with those of authentic samples and by a combination of GC-MS of the acetates. In order to determine the extent of incorporation of the radioactive substrates into the various sterols, the radioactive acetates were diluted with cold sterol acetates (1:1) derived from S. californicus (with the exception of 3-^{3}H-lanosterol incubation derived sterols) and preparatively gas chromatographed (Table I). 3-^{3}H-Lanosterol (1) was prepared by reduction of lanostenone with $LiAl^3H_4$ (25 mCi) in ether whereas 3-^{3}H-cholest-7-en-3β-ol (3) was prepared by reduction of cholest-7-en-3-one with NaB^3H_4 (25 mCi) in isopropanol. Cholestanol (9) (stanols) and 4 (Δ^7-stenols) which cochromatographed were separated from cholesterol (Δ^5-sterols) by TLC on $AgNO_3$-silica gel (using chloroform-ethanol 98:2). Alternatively stanols, Δ^7-sterols and Δ^5-sterols were separated by treatment of the sterol mixture with m-chloroperbenzoic acid in chloroform followed by thin layer chromatography[6] over silica gel developed with ethyl acetate - chloroform (7-13) (see Table II). In all incubations the radioactive sterol acetates

were diluted with cholesterol, Δ^7-cholestenol (4) and cholestanol (9) and saponified with methanolic KOH prior to $AgNO_3$-silica gel thin layer chromatography or epoxidation. That the sterols derived from 3-^{3}H-lanosterol (1) and 3-^{3}H-Δ^7-cholestenol (3) incubations retained tritium at C-3 was established by CrO_3/Py oxidation of the sterol mixtures. In either case less than 6% of the radioactivity was retained in the total ketone portion (crude) and probably represents the sterol pool arising from the catabolic products of the radioactive substrates. Our results (Tables I and II) lead to the following conclusions which offer considerable insight into our present knowledge[1] of sterol biosynthesis by holothuroids:

1. S. californicus can biosynthesize sterols de novo from $^3H_3C\text{-}COO^-K^+$ and can transform 1, 2 and 3 to Δ^5 and Δ^7-unsaturated sterols. The efficiency of incorporation is 1>2>3>$^3H_3C\text{-}COO^-K^+$ (Table I) - the isolation of labeled cholesterol from incubation with 1 being particularly noteworthy.[9]

2. The sea cucumber can transform cholesterol (6)[1,7] to Δ^7-cholestenol (4), and vice versa. The latter process proceeds with retention of tritium at position 3 (Table II); Δ^7-unsaturated sterols obtained from incubation of either 2 or 3 were radioactive. It is possible[1] that the conversion of 6 to 4 proceeds via the $\Delta^{5,7}$-diene 5. The converse of this process ($\Delta^7 \rightarrow \Delta^{5,7} \rightarrow \Delta^5$) is ubiquitous in mammals.

3. The sea cucumber can alkylate the cholesterol side chain at position 24 to furnish both Δ^5- and Δ^7-24-methyl and ethyl sterols (Table I), thus excluding a purely dietary origin.

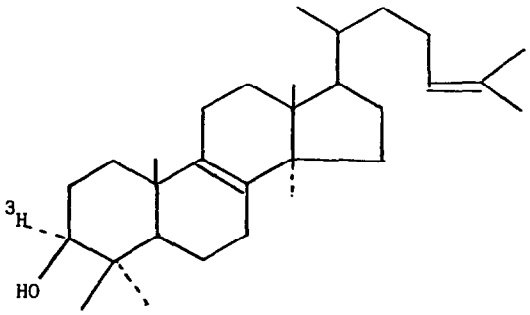
4. Our results conclusively show that the sea cucumber can convert cholesterol (6) and Δ^7-cholestenol (4) to cholestanol (9); the latter contained substantial radioactivity (Table II) thus excluding the transformation sequence 4→5→6→7→8→9 so prevalent in mammals.[8] Direct bio-reduction of the double bond is a conceivable alternative.

Table II. Thin Layer Chromatography of m-Cl-perbenzoic Acid Treated Sterols from Stichopus Californicus.

Band	Rf. ($EtOAc\text{-}CHCl_3$) 7-13	Substrates: Total Radioactivity of TLC Bands (d/min)		
			2	3
A	0.67	Stanols	9.44×10^6	8.32×10^5
B	0.52	Δ^7-Sterols	1.04×10^7	1.06×10^7
C	0.37	Δ^5-Sterols	1.16×10^7	7.58×10^6

Table I. Biosynthesis of Sterols in Sea Cucumber Stichopus Californicus.

G.C. Peak	R.Rt. OV-25	Ref. OV-25	Acetates Nature of Sterols	$^{3}H_3C\text{-}COO^{-}K^{+}$ %Compd	d/min/mg	1,2-$^{3}H_2$-Cholesterol (2) %Compd	d/min/mg	3-^{3}H-Δ^{7}-Cholestenol (3) %Compd	d/min/mg	3-^{3}H-Lanosterol (1) %Compd	d/min/mg
1	1.00	1.00	Cholesterol Dihydrocholesterol	35	1.70×10^{5}	34	2.70×10^{6}	26	2.30×10^{5}	25	2.40×10^{7}
2	1.12	1.13	Δ^{7}-Cholesten-3β-ol 24ξ-methyl-$\Delta^{5,22}$-cholestadien-3β-ol	16	3.1×10^{5}	17	2.10×10^{6}	26	1.70×10^{6}	26	1.60×10^{6}
2a	1.21		unknown							8	8.80×10^{6}
3	1.33	1.34	24ξ-Methyl-$\Delta^{7,22}$-cholestadien-3β-ol	26	2.5×10^{5}	25	1.28×10^{6}	23	1.69×10^{6}	10	1.30×10^{6}
3a	1.41		unknown							14	1.00×10^{6}
4	1.51	1.51	24ξ-Methyl-Δ^{7}-cholesten-3β-ol;	15	4.60×10^{5}	15	1.03×10^{6}	11	1.84×10^{6}	9	2.80×10^{6}
		1.57	Ergostanol								
5	1.71	1.69	24ξ-Ethyl-$\Delta^{5,22}$-cholestadien-3β-ol;								
6	1.87	1.87	24ξ-Ethyl-Δ^{5}-cholesten-3β-ol; 24ξ-ethyl-cholestanol	8	1.40×10^{6}	9	2.5×10^{6}	12	4.90×10^{5}	7	9.0×10^{6}
			Total Sterols (Amount)		(30 mg)$_6$		(30 mg)$_7$		(7 mg)$_6$		(28 mg)$_8$
			Specific Radioactivity (d/m/mg)		4.43×10^{8}		3.03×10^{8}		5.20×10^{7}		3.34×10^{9}
			Total Radioactivity (d/m)		1.13×10^{11}		7.58×10^{8}		3.12×10^{10}		9.36×10^{10}
			Amount of Substrate (d/m)		2.20×10^{11} (100 mCi)		1.10×10^{10} (5 mCi)		4.20×10^{10} (3/4th derived from 25 mCi $NaB^{3}H_4$)		4.20×10^{10} (3/4th derived from 25 mCi $LiAl^{3}H_4$)
			% Incorporation		0.067		6.880		0.078		22.20



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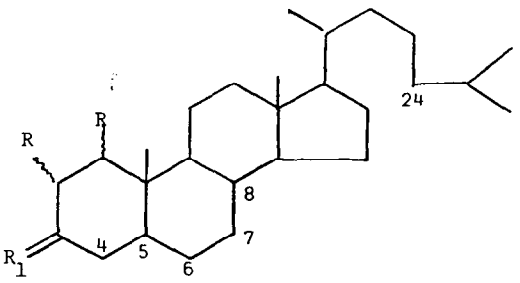

Structures 2 to 9

2 Δ^5 : R=^{3}H R_1= 

3 Δ^7 : R_1=H R_1= 

4 Δ^7 : R=H R_1= 

5 $\Delta^{5,7}$: R=H R_1= 

6 Δ^5 : R=H R_1= 

7 Δ^4 : R=H R_1= O

8 R=H R_1= O

9 R=H R_1= 

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REFERENCES

1. For detailed review see L. J. Goad in "Biochemical and Biophysical Perspectives in Marine Biology" (D. C. Malins and J. R. Sargent, eds.), Academic Press, New York, 1976, pp. 282-300.

2. T. Nomura, Y. Tsuchiya, D. Andre, and M. Barbier, Bull. Jap. Soc. Scient. Fish., 35, 299 (1969)

3. L. J. Goad, I. Rubinstein, and A. G. Smith, Proc. Roy. Soc. Lond. B., 180, 223 (1972).

4. P. A. Voogt and J. Over, Comp. Biochem. Physiol., 45b, 71 (1973).

5. Y. M. Sheikh and C. Djerassi, J. Chem. Soc. Chem. Comm., 1057 (1976).

6. A. G. Smith and L. J. Goad, Biochem. J., 146, 25 (1975); ibid., 33 (1975).

7. For conversion of cholesterol to Δ^7-cholestenol in star fish see U. M. H. Fagerlund and D. R. Idler, Can. J. Biochem. & Physiol., 38, 997 (1960); A. G. Smith and L. J. Goad, FEBS Lett., 12, 233 (1971).

8. Reduction of Δ^5-3β-ols to saturated 3β-ols via. the Δ^4-3-oxo grouping is a common reaction in the formation of steroid hormones in mammals. See H. H. Rees and T. W. Goodwin "Biosynthesis of Triterpenes, Steroids and Carotenoids", in Specialists Periodical Report on Biosynthesis, Vol. 1, Ch. 3, pp. 85-90, The Chemical Society, London, 1972.

9. The conversion of lanosterol to cholesterol involves the elimination of the C-14 and C-4 attached methyl groups. It is generally accepted that the 4-methyl groups are eliminated via a 3-keto-4-carboxylic acid (see ref. 8). Retention of tritium in cholesterol and its homologs biosynthesized from 1 is thus suggestive of an alternative mechanism in the sea cucumber or in symbionts residing within the animal.