Metabolism in Porifera. IX. Studies on the biological conversion of cholesterol into 19-nor-cholestanol by the sponge *Axinella polypoides*[1]

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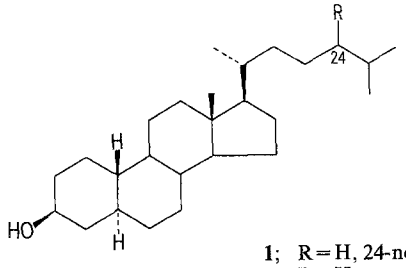
Summary. The conversion of cholesterol into 19-nor-5α-cholestan-3β-ol by the sponge *Axinella polypoides* involves a partial loss (40%) of the 3α-hydrogen atom; moreover administration to the sponge of [4-^{14}C]cholesterol tritiated at C-4β and C-7 showed that the 4β- and 7-hydrogen atoms are retained in this conversion. A competitive uptake experiment, [4-^{14}C]cholesterol vs. [7-$^{3}H_2$]5α-cholestanol, showed that the sponge utilized exclusively cholesterol for the production of 19-nor-5α-cholestan-3β-ol.

The array of uncommon sterols isolated from sponges[2,3], having novel side-chain alkylation patterns[4,5] and also nuclear modifications[6,7], has raised obvious questions regarding their bio origin.

We have recently shown that the sponge *Axinella polypoides*, which lacks the conventional sterols and contains an unique series of 19-nor-stanols (**1**-**8**)[6], can convert very efficiently administered cholest-5-en-3β-ol into 19-nor-5α-cholestan-3β-ol (**2**)[8].

This paper presents results of an investigation undertaken to examine the mechanism of this bioconversion using ^{3}H:^{14}C double labelled substrates. A competitive uptake experiment, [4-^{14}C]cholest-5-en-3β-ol vs. 5α-[7-$^{3}H_2$]cholestan-3β-ol, designed to elucidate the role of the Δ^5-double bond of cholest-5-en-3β-ol in this biological conversion, is also described.



1; R = H, 24-nor
2; R = H
3; R = Me
4; R = Et
5; R = H, Δ^{22}-trans
6; R = Me, Δ^{22}-trans
7; R = Me, $\Delta^{24(28)}$
8; R = Et, Δ^{22}-trans

Materials and methods. [4-^{14}C]Cholest-5-en-3β-ol (53 mCi/mmole), and [7-$^{3}H_2$]cholest-5-en-3β-ol (15.6 Ci/mmole) were supplied by the Radiochemical Centre, Amersham (Bucks, Great Britain).

5α-[7-$^{3}H_2$]Cholestan-3β-ol was prepared by hydrogenation of [7-$^{3}H_2$]cholest-5-en-3β-ol (500 μCi) with PtO_2 and conc. H_2SO_4 in ethyl acetate[9], and purified[10] to a final sp. act. of 6.65×10^8 dpm/mg.

[3α-$^{3}H_2$]Cholest-5-en-3β-ol was prepared by sodium borotritide (The Radiochemical Centre, 100 mCi; 293 mCi/mmole) reduction of cholest-3,5-dien-3-yl acetate (20 mg) in 95% ethanol (2 ml) according to Dauben and Easthman[11], and purified, after addition of cold cholest-5-en-3β-ol (10 mg), to a final constant sp. act. of 1.70×10^8 dpm/mg.

[4β-^{3}H]Cholest-5-en-3β-ol was prepared by the method of Ireland et al.[12]: 6β-chlorocholest-4-en-3β-yl benzoate (150 mg) was converted with lithium aluminium tritide (5 mCi; 171 mCi/mmole) in dry diethyl ether (0.2 ml) to [4β-^{3}H] cholest-5-en-3β-ol, which was purified, after addition of carrier cholest-5-en-3β-ol (40 mg), to a final constant sp. radioact. of 1.20×10^8 dpm/mg. Radioactivity measurements were made on a Nuclear Chicago Mark 1 Spectrometer using the channel ratio method.

Feeding experiments (10 days incubations) were performed as previously described[8]. Cholest-5-en-3β-ol and 19-norstanols were recovered in that order from the light petroleum extract of the lyophilized tissues by repetitive silica gel column chromatography (benzene/diethyl ether).

Results and discussion. In a first series of experiments, *A. polypoides* was fed with [4-^{14}C, 3α-^{3}H]- and [4-^{14}C, 4β-^{3}H]cholest-5-en-3β-ol. The results are reported in table 1. In the conversion of [4-^{14}C, 4β-^{3}H]cholest-5-en-3β-ol into 19-nor-5α-cholestan-3β-ol, the ^{3}H:^{14}C ratio of the recovered 19-nor-compounds was substantially identical with that of the administered precursor, indicating that 4β label had been retained during the transformation. That migration of tritium had not occurred during this transformation was demonstrated by chemical degradations. Hydrogenation of the Δ^{22} double bonds of the labelled 19-nor-stanols, followed by transformation into 3-ketones by Jones oxidation, failed to change the ^{3}H:^{14}C ratio signifi-

Table 1. ^{14}C-Radioactivities and ^{3}H: ^{14}C ratios of precursor cholest-5-en-3β-ol and metabolites 19-nor-stanols and their transformation products

Compounds	Experiment A: [4-^{14}C, 3α-^{3}H]			Experiment B: [4-^{14}C, 4β-^{3}H]			Experiment C: [4-^{14}C, 7-$^{3}H_2$]		
	^{14}C Activity Total dpm × 10^6	dpm/mg	^{3}H: ^{14}C ratio	^{14}C Activity Total dpm × 10^6	dpm/mg	^{3}H: ^{14}C ratio	^{14}C Activity Total dpm × 10^6	dpm/mg	^{3}H: ^{14}C ratio
Cholest-5-en-3β-ol									
Administered	55	1.9×10^7	2.83	55	9×10^6	2.99	110	1.5×10^9	2.69
Recovered	13	1.3×10^5	2.78	1.5	1.5×10^4	2.85	10	1×10^5	2.49
19-nor-stanols									
Natural mixture	18	1.5×10^4	1.71	0.7	1.9×10^3	2.65	16	2.1×10^4	2.48
After hydrogenation and Jones oxidation		1.5×10^4	0.06		1.2×10^3	2.30			
After base equilibration					1.2×10^3	0.11			

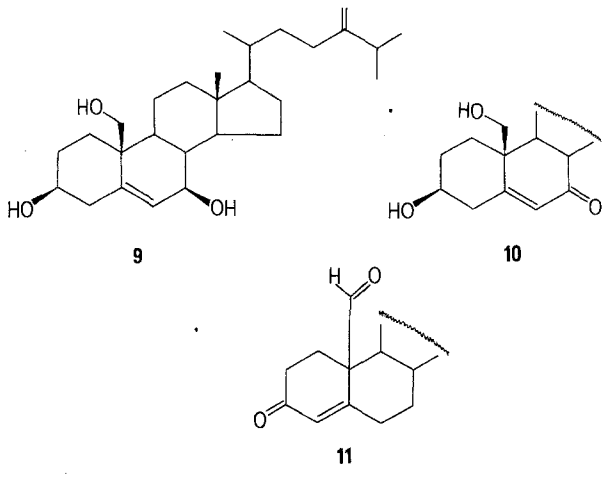
cantly; exposure of the 3-keto compounds to enolization in base (CH_3OH/CH_3ONa at reflux 3 h) resulted in an almost complete loss of tritium. It has been established that, in the biosynthesis of an oestrogen from an androgen of the type Δ^5-3β-hydroxy, the oxidative removal of the 19-methyl group is coupled with oxidation at the position 3 and migration of the Δ^5-double bond to the Δ^4-position[13,14]. Recent results obtained in our laboratory have shown that the conversion of cholest-5-en-3β-ol into cholest-4-en-3-one occurs in the sponge *A. verrucosa* through the stereospecific removal of 4β-H[15]. The results of the experiment B (table 1), showing that the 4β-tritiated substrate suffered no loss of tritium, suggest that the biosynthesis of 19-nor-stanols in the sponge *A. polypoides* is mechanically different from the process involved in the formation of oestrogens from androgens. Further, the results of the incubations using [4-^{14}C, 3α-^{3}H]cholest-5-en-3β-ol as substrate, which gave 19-nor-stanols with a $^3H:^{14}C$ ratio of 1.71:1, representing only 40% loss of tritium (table 1), support the view that a different pathway for the removal of the 19-methyl group from a sterol substrate occurs in sponges. The tritium recovered in the 19-nor-stanols was completely lost upon oxidation of the 3β-hydroxy group to 3-ketone. This partial loss of tritium at C-3 might indicate that the biosynthesis of 19-nor-stanols in the sponge. *A. polypoides* occurs through at least 2 different pathways, including one which involves the oxidation at C-3; the possibility of a re-introduction of tritium via a compartimentalized pool of NADPH[16] during the metabolic transformations might also account for the partial retention of tritium at C-3.

The recent isolation of 24-methylene cholest-5-en-3β,7β,19 triol (**9**) from the soft coral *Litophyton viridis*[17] has stimulated us to investigate whether such a compound could be a possible intermediate in the formation of 19-nor-stanols, as suggested by the same authors, through, for example, a 7-keto derivative (**10**) paralleling the oestrogen biosynthesis which requires a Δ^4-3-keto-19-aldehyde (**11**) intermediate[18]. So we fed the sponge with [7-$^3H_2$]cholest-5-en-3β-ol mixed with an appropriate amount of [4-^{14}C]cholest-5-en-3β-ol. The results of the incubation listed in table 1 (C) indicate that C-7 tritium was completely retained during the bioconversion, thus excluding the involvement of an oxidative step at C-7 in the pathway to give 19-nor-5α-cholestan-3β-ol from cholest-5-en-3β-ol in sponges.

To investigate the role of the Δ^5-double bond of cholest-5-en-3β-ol in this conversion, a competitive uptake experiment, [4-^{14}C]cholest-5-en-3β-ol vs. 5α-[7-$^3H_2$]cholestan-3β-ol, has been designed. *A. polypoides* was fed with a mixture of [4-^{14}C]cholest-5-en-3β-ol and 5α-[7-$^3H_2$]cholestan-3β-ol in ca. 1:1 molar ratio. As summarized in table 2, the radioactivity associated with 19-nor-stanols was exclusively due to ^{14}C, while the ^3H: ^{14}C ratio observed for the substrates recovered unchanged was about double that of the administered ones. These figures establish that the sponge has utilized exclusively cholest-5-en-3β-ol for production of 19-nor-5α-cholestan-3β-ol, and 5α-cholestan-3β-ol is not an intermediate in this conversion. So, the presence of the Δ^5-double bond in the sterol nucleus seems to be a prerequisite for the removal of the 19-methyl group. That demethylation can precede saturation of the Δ^5-double bond seems to be also supported by the recent discovery of 19-nor-cholest-5-en-3β-ol from the gorgonian *Pseudoplexaura porosa*[19].

Table 2. ^3H: ^{14}C Ratios of the 19-nor-stanols isolated from *A. polypoides* after administration of [4-^{14}C]cholest-5-en-3β-ol (3.2×10^8 dpm/mg) and [7-$^3H_2$]5α-cholestan-3β-ol (6.65×10^8 dpm/mg)

	^3H (dpm)	^{14}C (dpm)	^3H: ^{14}C
Administered substrates	7.2×10^7	2.8×10^7	2.5
Recovered substrates	5.6×10^6	1.1×10^6	5.1
Recovered 19-nor-stanols	-	4.8×10^6	0.0



1 Part of this work has been presented at the Nato Conference on Marine Natural Products (Jersey, Great Britain, October, 1976). - This contribution is part of the Programma finalizzato 'Oceanografia e Fondi marini - sottoprogetto Risorse biologiche' C.N.R. Italy.

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