Metabolism in Porifera VI. Role of the 5,6 Double Bond and the Fate of the C-4 of Cholesterol During the Conversion into 3β-Hydroxymethyl-A-nor-5α-Steranes in the Sponge *Axinella verrucosa*

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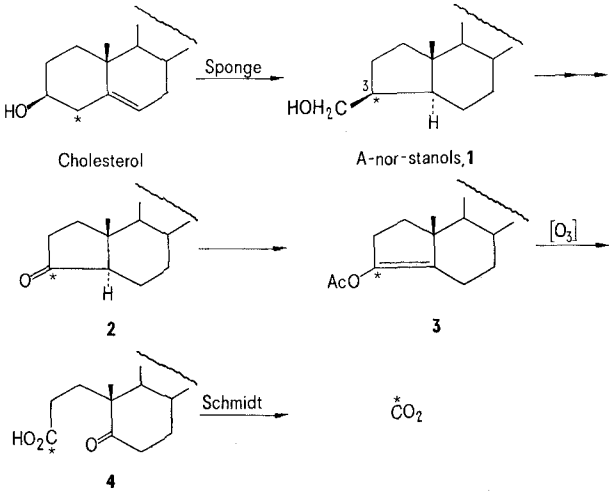
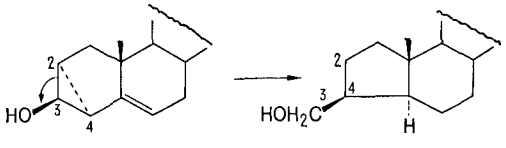
Summary. During the conversion of cholesterol into 3 β-hydroxymethyl-A-nor-5 α-cholestane by the sponge *Axinella verrucosa*, the carbon-3 of this latter originate from carbon-4 of cholesterol. Cholestanol moreover does not seem an intermediate in this conversion.

The marine sponge *Axinella verrucosa* contains exclusively stanols with a 3β-hydroxymethyl-A-nor-5α-cholestane nucleus (**1**) carrying conventional saturated and Δ^{22}-unsaturated C_8, C_9 and C_{10} side chains[1]. It has been reported that the sponge does not incorporate acetate into A-nor-steranes, but it converts readily [4-^{14}C]-cholesterol into 3β-hydroxymethyl-A-nor-5α-cholestane, and in the ring contraction carbon 4 of the cholesterol nucleus is not lost, nor does it furnish the 3 β-hydroxymethyl carbon of the A-nor-cholestane skeleton[2]. We have now examined this transformation in more detail and we show that the label is located at position 3 of the A-nor-cholestane skeleton.

Treatment with isopropenyl acetate and sulphuric acid of the nor-ketones (part structure **2**; mg 100, specific activity 4.31×10^4 dpm/mg) mixture derived from the 3β-hydroxymethyl-A-nor-steranes with specific activity 4.22×10^4 dpm/mg recovered from the sponge fed with [4-^{14}C]-cholesterol[3] gave the enolacetate mixture (part structure **3**, 70 mg; NMR singlets at δ 2.1, 1.0 and 0.67 for CH_3CO-, 10- and 13-CH_3's, respectively). This was converted, on ozonolysis at −20°, to the seco-ketoacids **4** (24 mg after purification on silica gel column chromatografy; no molecular ions in the m.s., peaks for M^+-H_2O at *m/e* 414, 400 and 386, and base peaks at *m/e* 360, 346 and 332 originating by loss of $CH_2CH_2CO_2H$ and H transfer; NMR singlets in $CDCl_3$ at 1.14 and 0.74 for 10- and 13-CH_3's; also characterized by m.s. of the corresponding methyl esters (CH_2N_2), M^+/e 446, 432 and 418 and base peaks at *m/e* 360,346 and 332). Schmidt degradation of **4** (12 mg), performed in a standard manner[4], gave labelled CO_2, recovered as $BaCO_3$ which was directly counted in the presence of Packard Cab-O-Sil. The radioactivity recovered in $BaCO_3$ accounted for ca. 60% of the ^{14}C in **4**. In the same reaction conditions, 4-acetilbutyric acid similarly afforded CO_2 in ca. 60% yield. On the basis of these results it is apparent that the ring contraction involves the formation of carbon-carbon linkage between C(4) and C(2) of cholesterol, while the carbon-3 furnishs the hydroxymethyl carbon.



Carbon-3 of the A-nor-steranes is derived from the carbon-4 of cholesterol.



Biogenetic conversion of cholesterol into 3β-hydroxymethyl-A-nor-cholestane.

Next, experiments have also been performed to establish the role of the 5,6 double bond of cholesterol in this conversion.

Axinella verrucosa was fed with a 1:1 mixture of [4-^{14}C]-cholesterol and [7α-^{3}H] -cholestanol, this latter prepared by hydrogenation of [7α-^{3}H] -cholesterol according to SMITH and GOAD[5]. After 240 h incubation, sterols were recovered from the light petroleum extract of the lyophilized tissues and, after addition of carrier cholesterol, were chromatographed on silica[6]. The cholesterol-cholestanol fractions were hydrogenated on platinum-charcoal and crystallized to constant specific activity and constant ^{3}H: ^{14}C ratio (Table), while the A-nor-steranes fraction was processed as reported in reference[2] (crystallization, hydrogenation of the Δ^{22} double bond, chromic acid oxidation to the corresponding carboxylic acids and crystallization) and counted (Table). The ^{3}H: ^{14}C ratio observed for the A-nor-steranes was ca. half of the ratio in the administered substrates and accordingly the ^{3}H: ^{14}C ratio for the recovered unchanged substrates was higher. So it seems apparent that the

^{3}H: ^{14}C ratios of the 3β-hydroxymethyl-A-nor-steranes isolated from *A. verrucosa* after administration of [4-^{14}C]-cholesterol (3.2×10^8 dpm/mg) and [7α-^{3}H]-cholestanol (6.65×10^8 dpm/mg)

	^{3}H (dpm)	^{14}C (dpm)	^{3}H/^{14}C
Administered substrates	7.2×10^7	2.8×10^7	2.54
Recovered A-nor-steranes	1.65×10^6	1.13×10^6	1.46
Recovered substrates	9.54×10^6	3.15×10^6	3.02

The incubation conditions are given in reference[2]; lyophilized animals weighted 19.5 g from which 170 mg of recrystallized A-nor-stanols mixture were recovered. 100 mg of carrier cholesterol were added before chromatographic purification of the crude sterol mixture.

sponge utilizes preferably cholesterol during the biosynthesis of the A-nor-steranes and cholestanol, although it can act as precursor of these latter, does not seem an intermediate in the conversion of cholesterol into 3β-hydroxymethyl-A-nor-5 α-cholestane.

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[2] M. DE ROSA, L. MINALE and G. SODANO, Experientia *31*, 408 (1975).
[3] The incubation conditions are given in reference [2]; the conditions of extraction and isolation of stanols are given in references [1] and [2]; the labelled stanols, after dilution with carrier A-nor-stanols, were hydrogenated on palladium-charcoal and converted to the nor-ketones (experimental details in reference [2]).
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[6] The 3β-hydroxymethyl-A-nor-5α-steranes are less polar than cholesterol (Rf on silica gel TLC in chloroform 0.45 as against Rf 0.4).

Ontogenesis of Monoamine Oxidase in the Thyroid Gland of Rats

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Summary. During postnatal development, MAO activity in the thyroid gland of male rats increases until day 16, when adult values are reached.

Monoamine oxidase (MAO, E.C.1.4.3.4) is a degradating enzyme catalyzing oxidation of primary and secondary amines. MAO has been found in various tissues of different animal species[1]. It is present also in the thyroid gland[2]. The final products of MAO activity are aldehyde and hydrogen peroxide. Hydrogen peroxide enzymatically generated in the thyroid under the catalytic influence of peroxidases is needed for oxidation of iodide to a more reactive state (I^+)[3]. The H_2O_2-peroxidase system participates also in the synthesis of thyroxine and triiodothyronine[4]. BAUDHUIN[5] has found that the activity of MAO in the thyroid gland is substantially higher in comparison with other tissues and localized in both mitochondria and microsomes. It is not known whether this enzyme has a direct effect on the biogenesis of thyroid hormones. However the significance of MAO in the thyroid is supported by the presence of catecholamines in thyroid tissue.

The aim of the present work was to study the activity of MAO in the thyroid gland of rats during postnatal development.

Materials and methods. Wistar albino male rats were used. The animals were killed by decapitation. Thyroids were removed, placed into ice-cold 0.25 *M* sucrose and homogenized in a glass-Contes homogenizer. Details of the assay for MAO have been described by WURTMAN and AXELROD[6]. The reaction mixture contained 25 μl (6.25 nmol) of tryptamine C^{14} (specific activity 2.7 mCi/mmol, Amersham, England), 250 μl of 0.5 *M* potassium phosphate buffer (pH 7.4) and various amount of tissue homogenate in a final volume 0.3 ml. The reaction, carried out at 37 °C, was stopped after 20 min with 2 *N* HCl and the radioactive products were extracted into 10 ml of toluene; 4 ml of toluene extracts were measured for radioactivity in 10 ml of Bray's solution by a Packard scintillation counter. The activity of MAO during ontogenesis was assayed in 0.5 mg samples in triplicates and two series of experiments were performed[7].

Results. Figure 1 shows the effect of varying concentrations of thyroid tissue upon the rate of C^{14}-indolacetic acid formation. The amount of acid was proportional to concentration in the range between 0.1 and 1 mg. The

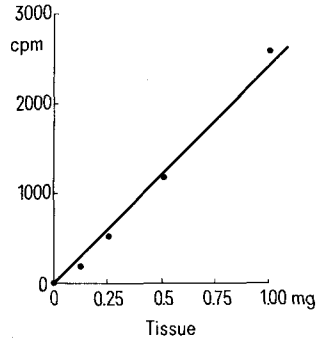

Fig. 1. C^{14}-indolacetic acid production during 20 min incubation vs. amount of tissue.

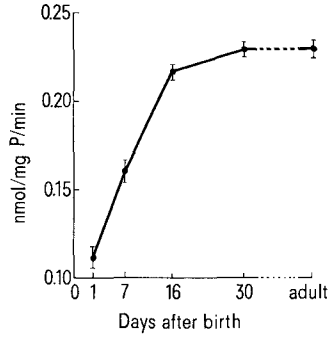

Fig. 2. MAO activity during development in 0.5 mg thyroid incubated for 20 min. Means of 10 values ± SE (results of experiments 1 and 2 were pooled).

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