Investigations on the Ring Contraction Step in the Biosynthesis of A-nor-stanols by the Marine Sponge *Axinella Verrucosa*[1]

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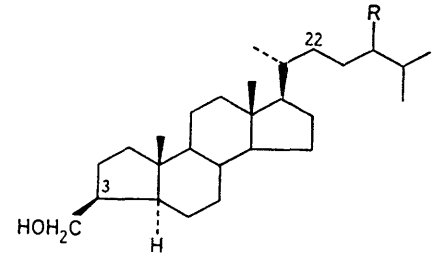
Summary The conversion of cholesterol into 3β-hydroxymethyl-A-nor-5α-cholestane by the sponge *Axinella verrucosa* involves the loss of 3α- and 4β-hydrogen atoms; the sponge also converts cholesterol into cholest-4-en-3-one and this conversion proceeds with loss of the 4β-hydrogen atom.

WE have previously demonstrated the conversion of cholesterol into 3β-hydroxymethyl-A-nor-5α-cholestane (**1**) by the sponge *Axinella verrucosa*,[2] which contains as the sole sterol components a novel group of A-nor-stanols (**1**—**6**).[3]

In this conversion C-4 of cholesterol furnishes C-3 of (**1**) and we have suggested that the ring contraction involves the formation of a C–C linkage between C-4 and C-2 of cholesterol, while C-3 furnishes the hydroxymethyl carbon.[4] We now present results on the mechanism of the ring contraction using $^{3}H:^{14}C$ doubly labelled substrates.

[3α-^{3}H]- and [4β-^{3}H]-Cholesterol were synthesized and separately administered, together with [4-^{14}C]-cholesterol, to the sponge.



(**1**) R = H
(**2**) R = H, Δ^{22}
(**3**) R = Me
(**4**) R = Me, Δ^{22}
(**5**) R = Et
(**6**) R = Et, Δ^{22}

TABLE. ^{14}C Radioactivity and $^{3}H:^{14}C$ ratios of metabolites isolated from *Axinella verrucosa* after administration of [3α-^{3}H,4-^{14}C]- and [4β-^{3}H, 4-^{14}C]-cholesterol

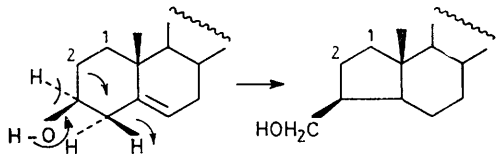
Administered substrate	[3α-^{3}H,4-^{14}C]-cholesterol		[4β-^{3}H,4-^{14}C]-cholesterol	
	^{14}C radioactivity/ total d.p.m.	$^{3}H:^{14}C$ ratio	^{14}C radioactivity/ total d.p.m.	$^{3}H:^{14}C$ ratio
Administered cholesterol	55×10^6	2·61	55×10^6	2·99
Recovered cholesterol	$17{\cdot}8 \times 10^6$	2·59	$32{\cdot}8 \times 10^6$	2·86
A-nor-stanols	$3{\cdot}3 \times 10^6$	0·04	$2{\cdot}2 \times 10^6$	0·20
Cholest-4-en-3-one	$9{\cdot}6 \times 10^4$	0·05	$1{\cdot}3 \times 10^5$	0·26

[3α-^{3}H]Cholesterol was prepared by sodium borotriti-ide reduction of cholesta-3,5-dien-3-yl acetate according to the method of Dauben and Eastham[5] and purified to a final constant specific activity of $1{\cdot}7 \times 10^8$ d.p.m. mg^{-1}; [4β-^{3}H]-cholesterol was prepared by the method of Ireland, Wrigley, and Young[6] and purified to a final constant specific activity of $1{\cdot}2 \times 10^8$ d.p.m. mg^{-1}.

After incubation for 290 h (incubation conditions are given in ref. 2), the sterols were recovered from the light petroleum extract of the lyophilized tissue. Since preliminary analyses revealed the presence of a radioactive fraction of negligible mass with an R_f value corresponding to cholest-4-en-3-one, in addition to the expected A-nor-stanol products and starting cholesterol, the sterol mixture was diluted with carrier cholesterol and cholest-4-en-3-one and then chromatographed on a silica gel column first with benzene as eluent to remove the less polar material and then with diethyl ether–benzene (5:95, *v/v*) to remove cholest-4-en-3-one, the A-nor-stanols, and cholesterol in that order. Each fraction was further purified and recrystallized to constant activity and isotopic ratio.

The results (Table) show that the A-nor-stanols obtained from both [3α-^{3}H,4-^{14}C]- and [4β-^{3}H,4-^{14}C]-cholesterol exhibited an almost complete loss of tritium. Thus the formation of 3β-hydroxymethyl-A-nor-5α-cholestane involves the loss of 3α- and 4β-hydrogen atoms from cholesterol.†

Such a ring contraction is reminiscent of the ring B contraction occurring in gibberellin biosynthesis which is initiated by the abstraction of *ent*-6α-hydrogen from *ent*-7α-hydroxykaurenoic acid.[7] Further, the formation of ^{14}C-labelled cholest-4-en-3-one from [4β-^{3}H,4-^{14}C]cholesterol reveals that the sponge has the necessary enzyme system for



SCHEME. Biogenetic conversion of cholesterol into 3β-hydroxymethyl-A-nor-cholestane; the arrows summarize the hydrogen losses and bond migration demonstrated by tracer studies.

conversion of 3β-hydroxy-5-ene compounds into the corresponding 3-oxo-4-ene compounds and that this conversion occurs with the stereospecific elimination of the 4β-hydrogen atom. It also suggests that a Δ^4-3-oxo intermediate might be involved in the biosynthesis of the A-nor-stanols. This hypothesis requires experimental verification and it is hoped that further clarification of this and other aspects of the ring contraction mechanism will result from work now in progress. The Scheme summarizes the present available evidence on the A-nor-stanols biosynthesis.

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† All the radioactivity associated with the A-nor-stanols mixture was due to (**1**), as previously shown (ref. 2).

[1] For previous paper in the series 'Metabolism in Porifera' see L. Minale, R. Riccio, O. Scalona, G. Sodano, E. Fattorusso, S. Magno, L. Mayol, and C. Santacroce, *Experientia*, 1977, **33**, 1550. This contribution is part of Programma Finalizzato 'Oceanografia e Fondi marini'—C.N.R.
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