n-hexane), $[\alpha]_D^{20°}$ −31.4° (*c*, 0.48, $CHCl_3$)]; compound **6** on reaction with Na and benzylmercaptan in DMF affords the benzylthioether **7** (a syrup). Desulfuration (Raney/Ni) of **7** yields the alcohol **8** [m.p. 51–53° (from EtOH:H_2O), $[\alpha]_D^{18°}$ −11.2° (*c*, 0.73, $CHCl_3$)]. HOREAU's method of partial resolution[11] applied to **8** affords (−)-α-phenyl butyric acid defining as 14 S the absolute configuration. On the other hand, application of BREWSTER's[12] 'benzoate rule' to compounds **8** and **9** [m.p. 168–169° (from EtOH:H_2O), $[\alpha]_D^{18°}$ + 11.9° (*c*, 1.54, $CHCl_3$)] also define as S the absolute stereochemistry of C–14.

Therefore barbatol (**1**), epimer at C–14 of compound **5**, is *ent*-8,13β-epoxylabdane-14 S, 15-diol.

Résumé. Un nouveau diterpène, barbatol (**1**), a été isolé d'une sous-espèce de la *Sideritis arborescens* Salzm. (Labiées) et sa structure a été établie comme étant *ent*-8,13β-epoxylabdane-14 S, 15-diol.

C. VON CARSTENN-LICHTERFELDE, B. RODRÍGUEZ and S. VALVERDE[13]

Instituto de Química Orgánica General, C.S.I.C., Juan de la Cierva, E–3 Madrid–6 (Spain), 21 February 1975.

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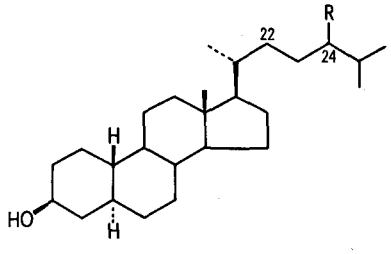
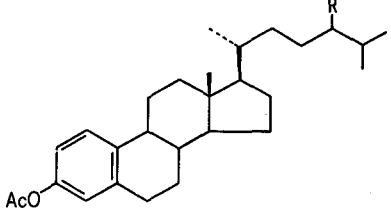
Metabolism in Porifera-V. Biosynthesis of 19-Nor-Stanols: Conversion of Cholesterol into 19-Nor-Cholestanols by the Sponge *Axinella polypoides*

Sponges have proved to be sources of unusual sterols, including new patterns of side-chain alkylation[1] and modified tetracyclic nuclei[2,3].

We have previously shown that *A. verrucosa*, which contains 3β-hydroxymethyl-A-nor-5α-steranes as sole sterol components, readily transforms the cholesterol nucleus into the A-nor-cholestane nucleus. The very low incorporation of radioactivity from acetate into these



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



9 R=H
10 R=Me
11 R=Et

stanols led to the conclusion that in the sponge the A-nor-stanols arise mainly by modification (ring-A contraction) of dietary sterols[4].

In this paper we are concerned with the origin of 19-nor-stanols (**1**–**8**) in the sponge *Axinella polypoides*, in which the usual sterols are also absent[2].

In two separate experiments, *A. polypoides*, maintained in well-aerated sea water at 14 C, was fed with labelled acetate and cholesterol by addition of aqueous (acetate) and ethanolic (cholesterol) solutions to the aquaria.

Sterols were recovered as a free sterol fraction by chromatography on silica gel of the light petroleum extract of the lyophilized tissues, while fatty acids were obtained from the subsequent chlorophorm-methanol extract by saponification procedure and then purified, after conversion into methyl esters, by chromatography on silica followed by distillation at 250 °C (experimental details are given in ref.[5]). Crude sterols recovered from sponges fed with acetate were crystallized and further purified, after conversion to acetates, by chromatography on silica followed by crystallization. The free sterols from the cholesterol incubations, after crystallization from methanol, were hydrogenated over Pt/C and subsequently oxidized with dichromate to yield the corresponding 3-ketones[6]. The latter were added to carrier 5α-cholesten-3-one, brominated in acetic acid to the 2,4-dibromo-derivatives which were dehydrobrominated with lithium carbonate-lithium bromide in dimethylformamide to give a mixture of phenols (derived from 19-nor-stanols) and cholesta-1,4-dien-3-one[7]. The mixture was then submitted to a silica gel preparative TLC (benzene-ether 9:1). The phenol fraction (Rf 0.8) was purified to constant specific activity by crystallization, acetylation and further silica gel preparative TLC (benzene).

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[6] 19-Nor-stanols have the same TLC Rf on silica gel as cholesterol[2].
[7] Experimental details are given in reference[2]. In a typical experiment 200 mg of labelled 19-nor-steran-3-ones were added to 25 mg of carrier 5α-cholestan-3-one.

Table I. Incorporation of label from [1-^{14}C]-acetate into fatty acids and 19-nor-stanols by *A. polypoides*[a]

	Weight (g)		dpm/mg[b]	
	48 h	290 h	48 h	290 h
Lyophilized animals	80	75		
Fatty acid methyl esters	0.41	0.52	3,070	4,160
Crude stanol fraction	0.17	0.15	—	—
After recrystallization	0.1	0.09	134	132
After conversion to acetates and purification by chromatography and crystallization	0.06	0.06	59	67

[a] [1-^{14}C]-acetate (62 mCi/mmole; 0.25 mCi) was fed to the animals by addition of 5 ml aqueous solution to the aquarium (50 l). 48 h after the administration, ca. half of the animals were taken, washed and frozen at −20° and the remaining animals were killed after 290 h incubation
[b] The radioactivity was measured by a Beckmann LS-250 liquid scintillation system.

Acetate was readily incorporated into fatty acids, but utilized only to a very low extent for the biosynthesis of 19-nor-stanols (Table I), suggesting that there is little or no de novo sterol biosynthesis. In view of the same very low radioactivity recovered in the stanols from both 48 h and 290 h feeding experiments, we presume that it must be due to contamination and, accordingly, the sponge, under such conditions, is unable to synthesize sterols from acetate. When specimens of *A. polypoides* were incubated with [26-^{14}C]-cholesterol, radioactivity was efficiently incorporated into the 19-nor-stanols (Table II) and almost all of this radioactivity corresponded to the 19-nor-cholestanol fraction, as shown by preparative GLC of the derived phenyl acetates mixture and determination of the radioactivity of the resolved individual components (**9**–**11**)[8].

These results indicate that *A. polypoides* readily removes the 10-methyl group from cholesterol substrate and suggest that in the sponge these unique 19-nor-stanols arise by modification (removal of 10-methyl group) of dietary sterols[9].

Table II. Incorporation of [26-^{14}C]-cholesterol into 19-nor-stanols by *A. polypoides*[a]

Period of incubation	Lyophilized animals (g)	Total fed (dpm)	Total sterol recovered		Radioactivity recovered (%)	Radioactivity recovered (%)	
			(mg)	(dpm)		Precursor[b]	19-Nor-stanols[c]
48 h	38	5.5×10^8	220	2.03×10^6	0.37	80	20
290 h	25	5.5×10^8	305	11.3×10^6	2.04	22	78

[a] [26-^{14}C]-cholesterol (61 mCi/mmole) was fed to the animals by addition of 2 ml ethanolic solution to the aquarium (50 l). 48 h after the administration, ca. half of the animals were taken, washed with water and frozen at −20 °C, and the remaining animals were killed after 290 h incubation. [b] Radioactivity was measured on cholesta-1,4-dien-3-one (see text). [c] Radioactivity was measured on the derived phenols and phenyl acetates purified to constant specific activity (see text).

[8] Portion of the labelled phenyl acetates from 19-nor-stanols deriving from 48 h incubation with [26-^{14}C]-cholesterol was added to a mixture of carrier phenyl acetates to obtain a specific radioactivity of 4×10^2 dpm/mg and subjected to preparative GLC. A Carlo Erba gaschromatograph, model G-V, equipped with a flame ionization detector was used and the separation was performed using a 2-m glass column (i.d. 6 mm) packed with 1% OV-1 Gas-Chrom 80–100 mesh and operated at 260° with N_2 at 120 ml/min. The first peak corresponding to 19-norcholesta-1,3,5(10)-trien-3-yl acetate (**9**) (31.6% of the total mixture) has a specific radioactivity of 1.2×10^3 dpm/mg.

[9] Acknowledgments. We thank the Zoological Station (Naples) for provision of the sponges and use of Laboratory facilities. The technical assistance of Mr. A. Crispino is also acknowledged.

Riassunto. La spugna *Axinella polypoides* trasforma il [26-^{14}C]-colesterolo nel 19-nor-colestanolo (**1**), mentre non utilizza l'[1-^{14}C]-acetato per la sintesi dei 19-nor-stanoli (**1**–**8**). Si suggerisce che questi unici stanoli si originano da steroli dietarici per rimozione del metile in 10.

M. De Rosa, L. Minale and G. Sodano

Laboratorio per la Chimica di Molecole di Interesse Biologico del C.N.R., Via Toiano 2, Arco Felice (Napoli, Italy), 21 February 1975.

Preparation of Synthetic Rotenoids

In a previous report, the synthesis of a new dehydrorotenoid was described by ringclosure of deoxybenzoine derivatives[1]. Now we wish to report the preparation of new rotenoids by thermal condensation of 4-ethoxycarbonyl-3-chromanones with *O*-heterocyclic phenols, affording dehydrorotenoids which were transformed into rotenoids by catalytic hydrogenation[2,3].

Heating of a mixture of 4-ethoxycarbonyl-3-chromanones (**1**) (0.15 mmol) with the phenols (**2**, **5**) (0.1 mmol) at 160–170 °C/12 mm Hg for 4 h gave the dehydrorotenoids (**3**, **6**), which were easily isolated from the reaction mixture by trituration with ether. When the reaction was carried out in boiling diphenyl ether, the yields were slightly decreased.

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