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Bromosphaerodiol, a minor bromo compound from the red alga *Sphaerococcus coronopifolius*[1]

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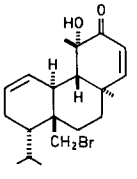
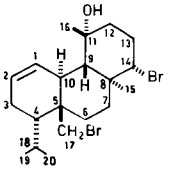
Summary. A minor bromoditerpene, bromosphaerodiol, has been isolated from the chloroform extract of the red marine alga *Sphaerococcus coronopifolius* and its structure has been established by chemical and spectroscopic methods.

2 bromoditerpenes with a tricyclic skeleton, sphaerococcenol A[2] (**I**) and bromosphaerol[3] (**II**), have been isolated recently from the red alga *Sphaerococcus coronopifolius* (Good. and Woodw.) C. Ag., family Sphaerococcaceae. Careful re-examination of the minor lipids of this alga has now led to the isolation of a closely related compound, bromosphaerodiol, for which formula **III** has been established on the basis of its chemical and spectroscopic behaviour.



I



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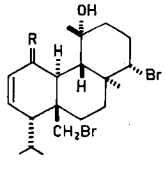
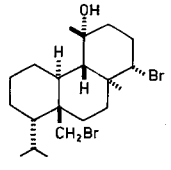
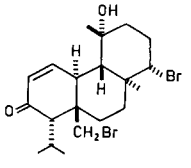


III R = OH, H



IV



V

VI R = O

Material and methods. S. coronopifolius (5 kg fresh wt) was collected near Portopalo, Sicily, during the spring of 1976. The chloroform extract of the freeze-dried, pulverized alga was column chromatographed on silica gel using increasing concentrations of methanol in chloroform as the eluent. The appropriate fractions, which emerged from the column with 4% methanol, were rechromatographed on PLC (silica gel; chloroform-methanol 95:5) and **III** recrystallized from methanol. Yield 48 mg, m.p. 170–172 °C, $[\alpha]_D$ – 44.3° (c 1 in chloroform).

Catalytic hydrogenation of **III** (10 mg) was performed in ethanol at 70 °C and 3 at for 12 h in the presence of platinum oxide. The crude product was recrystallized from ethanol thus giving **IV** (5 mg), m.p. 112–114 °C $[\alpha]_D$ + 10.2°.

Bromosphaerol **II** (624 mg) was oxidized with CrO_3-Py in dichloromethane at room temperature for 3 days. The crude product was chromatographed (PLC) yielding, along with a minute amount of **V**, 27 mg of **VI** and 549 mg of starting material.

Reduction of **VI** (20 mg) was performed with excess sodium borohydride in ethanol at room temperature for 1 h. After chromatographic purification (PLC) pure **III** was obtained in 80% yield.

The determination of the absolute configuration of the C-1 centre in **III** was carried out by the Horeau method[4] which gave a preponderance of (–) α-phenylbutyric acid (optical yield 8%).

Results and discussion. Compound **III** has the molecular formula $C_{20}H_{32}O_2Br_2$ (M^+ 462, 464, 466; measured mass 462.0773; calculated for $C_{20}H_{32}O_2Br_2^{79}$ 462.0770), ν_{max}^{KBr} 3400 cm^{-1} (OH). The NMR-spectrum ($CDCl_3$, δ scale) displayed signals at 5.80 (2H, bs, H-2 and H-3) 4.22 (1H, m, H-14), 3.90 (AB system, J 9 Hz, H-17), 3.52 (1H, bd, J 11 Hz, H-1), 1.32 (3H, s, H-15), 1.27 (3H, s, H-16), 1.02 and 0.97 (3H each, d's, J 6.5 Hz, H-19 and H-20). The mass spectrum showed diagnostically important peaks at m/e (% base peak) 462, 464, 466 (0.05; M^+), 444, 446, 448 (19; M^+–H_2O), 429, 431, 433 (10; M^+–H_2O–CH_3), 419, 421, 423 (0.12; M^+–C_3H_7), 401, 403, 405 (52; M^+–H_2O–C_3H_7), 365, 367 (78; M^+–H_2O–Br), 351, 353 (67; M^+–H_2O–CH_2Br), 321, 323 (100; M^+–H_2O–C_3H_7–HBr); 285 (26; M^+–H_2O–HBr–Br), 281, 283 (67; M^+–H_2O–C_5H_8BrO), 271 (27; M^+–H_2O–HBr–CH_2Br), 253 (26; M^+–$2H_2O$–HBr–CH_2Br), 241 (63; M^+–H_2O–C_3H_7–2HBr).

1 This investigation was supported by Consiglio Nazionale delle Ricerche, Roma.

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The above data strongly suggested that **III** was closely related to bromosphaerol. Chemical correlation between the 2 compounds was attained as follows. Catalytic hydrogenation of **III** yielded a product which proved to be identical with an authentic sample of **IV** derived from bromosphaerol[3].

The structure of bromosphaerodiol was definitively elucidated by its partial synthesis from bromosphaerol. Oxidation of **II** afforded, in addition to minor amounts of **V**, ketone **VI**, m.p. 131–133 °C, $[\alpha]_D$ – 97.7° (c 1 in chloroform), M^+ m/e 460, 462, 464, $\nu_{max}^{CHCl_3}$ 3350 (OH), 1680 and 1650 cm^{-1} (α,β-unsaturated ketone), λ_{max} 233 nm (ε 5190), δ ($CDCl_3$) 6.72 (1H, A-part of an ABX pattern, J_{AB} 10.5 Hz, J_{AX} 5 Hz, H-3), 6.25 (1H, B-part of an ABX pattern, J_{BX} nonexistent or very small, H-2), 4.02 (1H, dd, J 12 and 3 Hz, H-14), 3.56 (2H, AB system, J 11 Hz, H-17), 2.70 (1H, m, H-4), 1.33 (3H, s, H-15), 1.03 (3H, s, H-16), 1.09 and 0.99 (3H each, d's, J 7 Hz, H-19 and H-20). Irradiation at δ 2.70 converted the signal at δ 6.72 to the A-part of an AB system, while irradiation at δ 2.21 (tentatively the frequency of H-18) not only simplified the multiplet at δ 2.70 but at the same time caused the doublets due to the methyls of the isopropyl group to collapse into singlets.

Sodium borohydride reduction of **VI** afforded in high yield a compound which was identified as bromosphaerodiol on the basis of its physical (m.p., $[\alpha]_D$, NMR, MS) and chromatographic properties. This result firmly establishes structure **III** for bromosphaerodiol and at the same time determines its relative stereochemistry (apart from the C-1 centre), since the relative stereochemistry of **II** is known[3]. The quasi-equatorial nature of the OH group at C-1 was indicated by the value of the coupling constant (11 Hz) between H-1 and H-10 in the NMR spectrum of **III**.

The Horeau method[4] applied to **III** allowed to determine the chirality at C-1 as S, and this determines the absolute stereochemistry of **III** and consequently of **II**.

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Metabolism in Porifera. VII. Conversion of [7,7-$^3H_2$]-fucosterol into calysterol by the sponge *Calyx niceaensis*

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Summary. The sponge *Calyx niceaensis* metabolizes administered [7,7-$^3H_2$]-fucosterol to produce labelled calysterol, the principal sterol component of the sponge, possessing the unique feature of a cyclopropene ring bridging $C_{23,24}$.

Sponges contain a great variety of sterols[1] including biogenetically unprecedent aplysterol and 24,28-didehydroaplysterol[2], first examples of 26-alkylation in steroid biosynthesis, from the sponges of the genus *Verongia*[3], and 19-nor-stanols and 3β-hydroxymethyl-A-nor-5α-steranes, from the sponges *Axinella polypoides*[4] and *A. verrucosa*[5], respectively. Previous radiolabelling experiments using labelled mevalonate and/or acetate in the sponges *V. aerophoba*[6], *A. polypoides*[7] and *A. verrucosa*[8] resulted in no radioactive sterols, but *A. polypoides* and *A. verrucosa* converted very efficiently cholesterol into 19-nor-cholestanol[7] and 3β-hydroxymethyl-A-nor-5α-cholestane[8], respectively. So we could conclude that sponges are unable to synthesize de novo their sterols but, at least in the case of 2 *Axinella* species, they modify the sterols taken up from the diet.

A further remarkable structural variant in steroid biosynthesis, in which the attachment of 'extra' carbon atoms to the normal cholesterol skeleton at C-23 is exemplified, is now represented by calysterol (**1**), the principal sterol component (ca. 90% of the total sterol content) of the sponge *Calyx niceaensis*[9], where it occurs accompained by 2 minor yet unusual sterols, cholesta-5-ene-23-yn-3β-ol (**2**) and 23-ethylcholesta-5,23-dien-3β-ol (**3**)[10].

Table 1. Incorporation of label from [1-^{14}C]-acetate and [CH_3-^{14}C]-methionine into fatty acids and calysterol (1) by *Calix niceaensis**

	[1-^{14}C]-acetate** (0.125 mCi)		[CH_3-^{14}C]-methionine*** (0.05 mCi)	
	Weight (g)	dpm/mg	Weight (g)	dpm/mg
Lyophilized animals	48	–	99	–
Fatty acid methyl esters	0.14	913	0.095	7.615
Crude sterol fraction	0.28	309	0.32	1.137
Calysterol (after acetylation in the hot and chromatography)	0.10	35	0.085	177
Calysterol (after conversion to the diols mixture and chromatography)	0.02	23	0.012	243

*Labelled precursors were fed to the animals by addition of 5 ml of aqueous solution to the aquarium (50 l); 10 days after the administration, the animals were taken, washed and frozen at –20 °C. ** Specific activity 62 mCi/mmole. *** Specific activity 56 mCi/mmole.

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