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**.

4 A. Horeau, Tetrahedron Lett. *1961*, 506; *1962*, 965.

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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2 P. De Luca, M. De Rosa, L. Minale and G. Sodano, J. chem. Soc. Perkin I *1972*, 2132.
3 M. De Rosa, L. Minale and G. Sodano, Comp. Biochem. Physiol. *46B*, 823 (1973).
4 L. Minale and G. Sodano, J. chem. Soc. Perkin I *1974*, 1888.
5 L. Minale and G. Sodano, J. chem. Soc. Perkin I *1974*, 2380.
6 M. De Rosa, L. Minale and G. Sodano, Comp. Biochem. Physiol. *45B*, 883 (1973).
7 M. De Rosa, L. Minale and G. Sodano, Experientia *31*, 758 (1975).
8 M. De Rosa, L. Minale and G. Sodano, Experientia *31*, 408 (1975).
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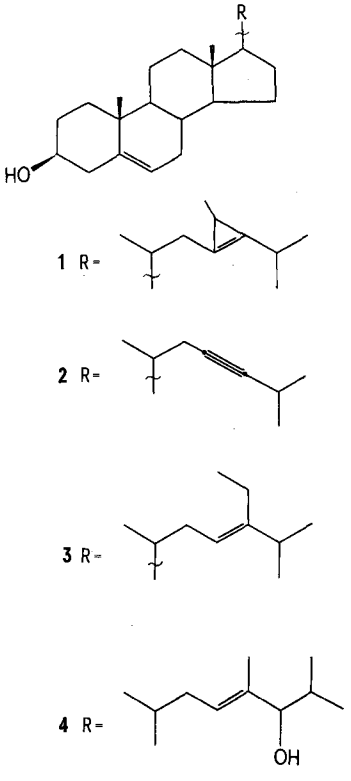
10 E. Steiner, C. Djerassi, E. Fattorusso, S. Magno, L. Mayol, C. Santacroce and D. Sica, Helv. chim. Acta (in press).

Table 2. Incorporation results after administration of labelled C-29 sterols to *Calix niceaensis**

Precursor	Total fed (dpm)	Lyophilized animals (g)	Total sterols (mg)	(dpm/mg)	Radioactivity recovered (%)	Calysterol (mg)	(dpm/mg)**	Radioactivity*** in calysterol (%)
[7,7-$^3H_2$]-Fucosterol	13.5×10^6	30	155	45.625	53	56	1.160	2.7
[7,7-$^3H_2$]-Stigmasterol	25.1×10^6	260	1031	9.143	37	460	20	0.21
[7,7-$^3H_2$]-β-Sitosterol	1.2×10^6	80	346	1.696	94	75	9	0.28

* Labelled precursor were fed to the animals by addition of 1 ml ethanolic solutions to the aquaria (50 l); incubation period: 10 days; [7,7-$^3H_2$]-fucosterol, [7,7-$^3H_2$]-stigmasterol and [7.7-$^3H_2$]-β-sitosterol had the following specific activities: (dpm/mg) 4.84×10^6, 6.96×10^6 and 2.99×10^5, respectively. ** After the final purification. *** Respect to that present in the total fraction and assuming that calysterol accounts for 90% of the total sterol recovered.



A sterol such as the acetylenic **2** might be a biochemical precursor of the cyclopropene-containing calysterol by transmethylation from S-adenosylmethionine and the present investigation was therefore undertaken firstly to examine this possibility.

Because of substantially negative results of these experiments, the sponge was fed with the most common naturally occurring C_{29} sterols in order to investigate the possibility that calysterol originates by modification of exogenous sterols taken from the diet.

Materials and methods. Feeding experiments. In separate experiments, *Calyx niceaensis*, maintained in well-aerated sea water at 14 °C, was fed with [1-^{14}C]-acetate and [CH_3-^{14}C]-methionine by addition of aqueous solutions to the aquaria. Free sterols and fatty acids were recovered by chromatography on silica gel of the light petroleum extract of the lyophilized tissues. Elution with benzene gave the free fatty acids, which were converted into methyl esters (diazomethane) and further purified by chromatography on silica gel followed by distillation at 250 °C (for details see De Rosa et al.[6]) and counted. The sterol fraction was eluted with benzene-ether (9:1) and then crystallized from methanol. Further purification of calysterol was carried out by acetylation in the hot (acetic anhydride – pyridine – sterols, 1 ml – 2 drops – 50 mg; 5 h reflux) which resulted in the formation of a mixture of diacetates (arising from calysterol by opening of the cyclopropene ring), which was separated by silica gel chromatography from sterol monoacetates deriving from **2**, **3** and conventional sterols present in the original mixture. The major component of the diacetate mixture was shown to be **4** on the basis of its spectral properties[11]. The diacetates were converted to the corresponding diols (20% methanolic KOH; 6 h reflux), which were counted after chromatography on silica gel and crystallization. 3 similar separate feeding experiments were effected by adding labelled fucosterol, stigmasterol and β-sitosterol in ethanolic solutions to the aquaria; after incubations sterols were recovered from the lyophilized animals as before, and added to the appropriate carrier and purified as before.

Labelled sterol substrates. The substrates were labelled with tritium in the position 7 using the procedure of Knapp et al.[12], subject to slight modifications. 3α,5α-Cyclo-steran-6-ones were activated by ^{3}H-exchange by refluxing for 15 h the ketones (30 mg) in ^3H_2O (0.2 ml, 1 Ci), dioxane (0.8 ml) and triethylamine (0.1 ml) in a sealed viale. Tritiated materials were recovered by lyophilization and converted into the corresponding alcohols by $LiAlH_4$ reduction. The free sterols were regenerated by treatment with zinc-acetic acid, followed, after dilution with appropriate carriers (ca. 50 mg), by saponification of the resulting acetates, and recrystallized to constant radioactivity.

Results and discussion. The results of the feeding experiments are given in tables 1 and 2. Acetate (table 1) is incorporated efficiently into fatty acids, but utilized only to a very small extent for the biosynthesis of calysterol (**1**), suggesting that there is no de novo sterol biosynthesis. Similar results also occurred when the sponge was fed with methyl-labelled methionine: the small amount of radioactivity associated with calysterol (**1**) when compared with that associated with fatty acids is suggestive for the absence in the sponge of a biosynthesis of **1** by direct addition of 2 'C_1 units' derived from adenosylmethionine to a C_{27} sterol substrate such as for example **2**. In view of these and previous results, indicating the ability of sponges to modify dietary sterols, we decided to feed *Calyx niceaensis* with a series of labelled C_{29} sterols, as fucosterol, stigmasterol and β-sitosterol, to examine the possibility of transformation of exogenous sterols for calysterol production in the sponge.

The incorporation data (table 2) indicate that the sponge can metabolize injected [7,7-$^3H_2$]-fucosterol to produce

11 An account of this work will be published elsewhere.

12 F. F. Knapp, J. B. Graig, L. J. Goad and T. W. Goodwin, J. C. S. Chem. Commun. *1971*, 707.

labelled calysterol, while apparently stigmasterol and β-sitosterol cannot act as precursor of this unique sterol. Purification of calysterol through the diacetates, the diols and again the diacetates in the experiment with [7,7-$^3H_2$]-fucosterol shows that the radioactivity associated with the calysterol derivatives is substantially constant in all the steps of the purification, whilst in the case of the experiments with [7,7-$^3H_2$]-stigmasterol and [7,7-$^3H_2$]-β-sitosterol, the radioactivity decreases to the final values given in table 2.

In a trial experiment, 'cold' calysterol was mixed with labelled fucosterol and acetylated in the hot: The calysterol derivatives, purified as above, were found to be devoid of significant radioactivity.

Although these findings are not conclusive if fucosterol is indeed the true calysterol precursor, nevertheless they add further support to the suggestion that the sponges are unable to synthesize their sterols de novo but they modify sterols taken up from the diet.

Completion of the natural groups of ergot alkaloids: Syntheses and pharmacological profiles of β-ergosine and β-ergoptine*

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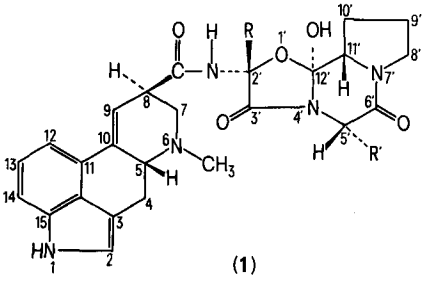
Summary. The syntheses and pharmacological potencies of β-ergosine and β-ergoptine, the missing links in the natural groups of ergot peptide alkaloids are described.

Ergot alkaloids occuring in nature can be divided into 3 groups by their substitution in position 2′ of the peptide moiety (see depicted general structure **1** and corresponding table).



(1)

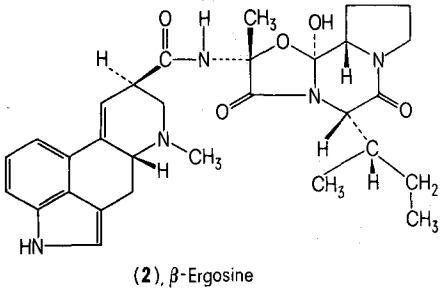
R′	Ergotamine group R=CH_3	Ergoxine group R=C_2H_5	Ergotoxine group R=$CH(CH_3)_2$
CH_2–C_6H_5	Ergotamine	Ergostine	Ergocristine
CH_2–$CH(CH_3)_2$	Ergosine	Ergoptine*	α-Ergokryptine
$CHCH_3$–C_2H_5	β-Ergosine*	β-Ergoptine*	β-Ergokryptine
$CH(CH_3)_2$	Ergovaline*	Ergonine*	Ergocornine

* Not yet found in nature.

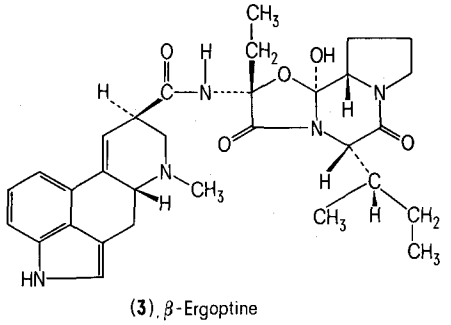
4 natural alkaloids are found in the ergotoxine group characterized by an isopropyl residue in position 2′ of the peptide moiety: ergocristine, α-ergokryptine, β-ergokryptine and ergocornine. In the 2 other groups, smaller numbers of natural alkaloids occur: in the ergotamine group ergotamine and ergosine, in the ergoxine group ergostine only.

In order to complete the 2 latter groups ergovaline[1], ergoptine and ergonine[2] have formerly been synthetized. Discovery[3] and synthesis[4] of the 4th natural member of the ergotoxine group, β-ergokryptine, prompted us to synthetize the corresponding analoga of the ergotamine and ergoxine group and to investigate their pharmacological activities.

In line with the existing nomenclature, the name β-ergosine (**2**) is proposed for the alkaloid of the ergotamine group and the name β-ergoptine (**3**) for the alkaloid of the ergoxine group.



(**2**), β-Ergosine



(**3**), β-Ergoptine

The syntheses of the 2 alkaloids have been accomplished in strict analogy to those of ergosine[1], respectively ergoptine[2], with the only difference that L-isoleucyl-L-proline-lactam[4] was used as essential building block instead of L-leucyl-L-proline-lactam. The syntheses and absolute configurations of S-(+)-methyl-benzyloxy-malonic-acid-monoethylester-chloride[5] used for the synthesis of β-ergosine, respectively S-(+)-ethyl-benzyloxy-malonic-acid-monoethylester-chloride[6] used for the synthesis of β-ergoptine were known. Hence follow automatically the absolute configurations of the 2 new alkaloids given in formulas **2** and **3**, since it is known that the syntheses