The conversion of **6** to tyrosine was accomplished by hydrogenation (CH_3CO_2H, 5% Pd-C, 24 h at r.t. and pressure) of a partially purified sample, obtained by fractionating the crude material on silica gel column in chlorophorm-methanol, 1:1.

The ester **7** was synthesized from hydroxylamine hydrochloride and *p*-hydroxyphenylpyruvic acid and subsequent methylation with CH_2N_2 of the resulting oximinoacid[6].

To our knowledge, compound **6** is the second oxime so for detected from natural sources, oximino-succinic acid being known to occur in plants[7].

Summary. The occurrence from a marine sponge of 4-hydroxyphenylpyruvic acid oxime is good evidence that an oxime (**4**) is the biogenetic precursor of aerothionin (**1**), homoaerothionin (**2**) and aeroplysinin-1 (**3**), brominated metabolites isolated from Verongia sponges.

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

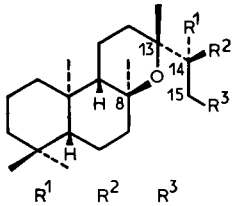
[6] *Dictionary of Organic Compounds*, 4th ed., (Eyre & Spottiswoode Publishers, London 1965), v. 3, p. 1794.

[7] A. J. Virtanen, A. A. Arhimo, J. Sundman and L. Jämes, J. prakt. Chem. *162*, 71 (1943).

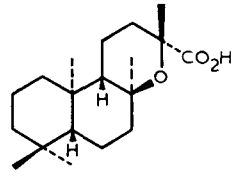
Barbatol, a New Diterpenoid from a *Sideritis arborescens* Salzm. subspecie[1]

Continuing our studies on diterpenes of genus *Sideritis* plants (family Labiatae) endemic in the Iberian Peninsula[2–4], we have examined the composition of a subspecie of *Sideritis arborescens* Salzm. collected near Barbate (Cádiz). From the total diterpene components we have now isolated a compound already described, siderol[5], plus a new diterpene[6], barbatol (**1**), $C_{20}H_{36}O_3$, m.p. 100–105° (from *n*-hexane), $[\alpha]_D^{20°}$ — 13.6° (*c*, 0.25, EtOH).

The IR-spectrum of **1** exhibits strong —OH absorption (3340 cm^{-1}) and no —CO— bands. Acetylation of **1** yields a diacetate **2** [m.p. 102–103° (from EtOH:H_2O), $[\alpha]_D^{20°}$ + 18.5° (*c*, 0.42, $CHCl_3$)], the IR-spectrum of which is devoid of —OH absorptions. It seems plausible that the third oxygen atom of barbatol is involved in an ether linkage.



	R^1	R^2	R^3
1	OH	H	OH
2	OAc	H	OAc
5	H	OH	OH
6	H	OH	OTs
7	H	OH	SCH_2Ph
8	H	OH	H
9	H	OCOPh	H



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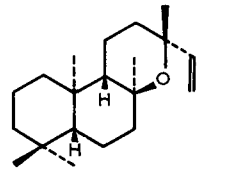

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The NMR-spectrum of **2** shows a 1H quartet at δ 5.03, X part of an ABX system (J_{XA} 8.75 Hz; J_{XB} 2.65 Hz), assigned to the geminal proton of a secondary acetoxyl group. Between δ 3.90 and 4.63 there are 8 lines, the AB part (ν_A 4.49 δ and ν_B 4.10 δ; J_{AB} 12 Hz), originated by the 2 protons of an acetylated primary alcohol. Two acetoxyl groups at δ 2.10 and 2.02, and 5 methyl singlets at δ 1.21 (6H, attached to carbon atoms bearing an ethereal oxygen[7]), 0.84 (3H) and 0.79 (6H) are also observed. These data pointed toward a structural hypothesis based on the labdane skeleton with an 8,13-cyclic ether and two hydroxyl groups on the ethyl side chain[8].

The presence of a $-CHOH-CH_2OH$ grouping attached to C–13 is substantiated by the products obtained by treating barbatol with HIO_4 in ethanol solution. Formaldehyde (identified as the dimedone derivative) and another aldehyde are formed. The latter, without further characterization, was treated with Jones' reagent affording an acid **3** [$C_{19}H_{32}O_3$, $[\alpha]_D^{20°}$ — 50° (*c*, 0.81, $CHCl_3$)] the m.p. of which [149–152° (from *n*-hexane)] is identical with a substance previously described as the 15-*nor*acid derivative obtained from (—)-13-epimanoyl oxide[9]. Moreover, m.p. and optical rotation of **3** are identical, although of opposite sign the latter, with those recorded for the enantiomeric 15-*nor*acid[7]. Thus barbatol may be a 14,15-dihydroxy derivative of *ent*-8,13β-epoxylabdane.

In order to confirm this hypothesis, (—)-13-epimanoyl oxide (**4**) was treated with osmium tetroxide in Et_2O: dioxane (1:1) solution yielding quantitatively two 14,15-diols epimeric at C–14 and easily separated on silica gel preparative plates eluted with $CHCl_3$:MeOH (19:1). The less polar component (11% of the total) and its diacetyl derivative are identical in all respects (m.p., m.m.p., $[\alpha]_D$, IR and NMR) with barbatol and its diacetate.

The absolute stereochemistry of the secondary alcohol on C–14 was established as follows. The most polar[10] diol obtained by osmylation is compound **5** [89% of the total, m.p. 108.5–109.5° (from *n*-hexane), $[\alpha]_D^{18°}$ —19.3° (*c*, 0.31, $CHCl_3$)], which under controlled conditions can be transformed into the monotosylate **6** [m.p. 38–40° (from

[1] Part XXV in the series 'Studies on diterpenes from genus *Sideritis*'. For part XXIV see B. Rodríguez, S. Valverde, R. Cuesta and A. Peña, Phytochemistry, in press.

[2] C. von Carstenn-Lichterfelde, S. Valverde and B. Rodríguez, Aust. J. Chem. *27*, 517 (1974).

[3] W. A. Ayer, J.-A. H. Ball, B. Rodríguez and S. Valverde, Can. J. Chem. *52*, 2792 (1974).

[4] R. M. Rabanal, B. Rodríguez and S. Valverde, Experientia *30*, 977 (1974).

[5] F. Piozzi, P. Venturella, A. Bellino and R. Mondelli, Tetrahedron *24*, 4073 (1968).

[6] Satisfactory elemental analysis have been obtained for all the products here described.

[7] J. A. Giles, J. N. Schumacher, S. S. Mims and E. Bernasek, Tetrahedron *18*, 169 (1962).

[8] B. Rodríguez and S. Valverde, Tetrahedron *29*, 2837 (1973).

[9] D. H. McLean and S. N. Slater, J. Soc. Chem. Ind. *64*, 28 (1945).

[10] This reaction sequence was carried out with the C-14 epimer due to lack of the natural epimer which is formed as a minor component only.

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.

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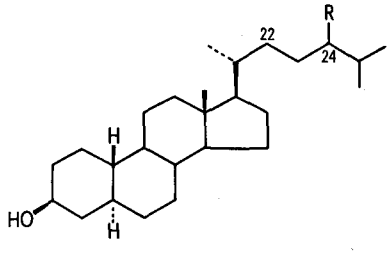
[11] A. HOREAU and A. NOUAILLE, Tetrahedron Lett. *1971*, 1939.
[12] J. R. BREWSTER, Tetrahedron *13*, 106 (1961).
[13] The authors thank Dr. J. BORJA, Botany Department, Faculty of Pharmacy, Madrid, for the collection and botanical classification of the plant material and Dr. B. M. FRAGA, Department of Organic Chemistry, University of La Laguna (Tenerife, Canary Isles) for a sample of (−)-13-epimanoyl oxide.

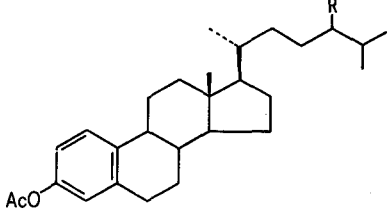
Metabolism in Porifera-V. Biosynthesis of 19-Nor-Stanols: Conversion of Cholesterol into 19-Nor-Cholestanols by the Sponge *Axinella polypoides*

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).

[1] P. DE LUCA, M. DE ROSA, L. MINALE and G. SODANO, J. chem. Soc. Perkin *I*, 2132 (1972). – P. DE LUCA, M. DE ROSA, L. MINALE, R. PULITI, G. SODANO, F. GIORDANO and L. MAZZARELLA, J. chem. Soc. Chem. Commun. *1973*, 825.
[2] L. MINALE and G. SODANO, J. chem. Soc. Perkin *I*, 1888 (1974).
[3] L. MINALE and G. SODANO, J. chem. Soc. Perkin *I*, 2380 (1974).
[4] M. DE ROSA, L. MINALE and G. SODANO, Experientia *31*, 408 (1975).
[5] M. DE ROSA, L. MINALE and G. SODANO, Comp. Biochem. Physiol., *45 B*, 883 (1973).
[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.