From the above experiments, it is clear that the proposed primary sequence may be close to the natural product. However, other alternatives, such as a cyclic formulation or even N-acetyl and N-formyl derivatives, can fit the known chemical data. Note that a molecular weight measurement on the original substance is still necessary in order to support a possible monomeric cyclic peptide arrangement (for which several possibilities can be written)[12]. Whether these and other compounds will yield the same enzymatic cleavage fragments, much less the biological activity of the natural product, is uncertain at this time[13,14].

Summary. A peptide isolated from rats habituated to a sound stimulus has been given the structure L-Glu-Ala-Gly-Tyr-Ser-Lys-OH. A synthesis of this compound afforded a product that different from the natural material on the basis of chromatographic and physiological comparisons. The proposed sequence must therefore be in error.

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[12] Since the free N^{ε}-amino group in lysine will routinely give a positive ninhydrin reaction, then the natural peptide must possess blocking groups at both the glutamic acid and lysine residues.

[13] After this work was completed, a second synthesis appeared: H. LACKNER and N. TIEMANN, Naturwissenschaften *61*, 217 (1974). Although the product possessed a broader mp of 170–177°, the chromatographic properties were said to be identical with our peptide. Unfortunately, a direct comparison was not possible between the Seattle and Göttingen peptides, due to a lack of additional material from the latter group. In any event, little biological activity was found and it was concluded that a mistake existed in the original structural investigation.

[14] G. UNGAR, personal communication (June 5, 1975). The possible presence of some cofactor has been suggested as an explanation for the higher activity of the natural product.

Occurrence of 4-Hydroxyphenylpyruvic Acid Oxime in the Marine Sponge *Hymeniacidon sanguinea*

Sponges of the family Verongidae provide a series of closely related compounds which may be considered as metabolites of 3,5-dibromotyrosine, including aerothionin (**1**)[1], homoaerothionin (**2**)[1] and the nitrile aeroplysinin-1 (**3**)[2].

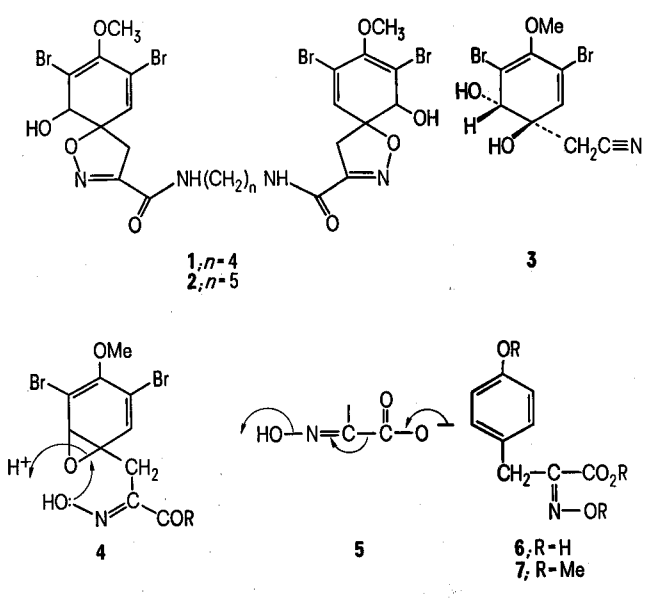
The spiro system in **1** and **2** could arise in various ways, including nucleofilic attack by an oxime function on an arene oxide as shown in **4**. Following certain suggestions that nitriles may be derived in vivo from α-amino-acids by way of α-keto- and α-oximino-acids[3], it has been speculated that the oxime **4** could be also a likely precursor of the nitrile aeroplysinin-1, as indicated in **5**[1].

We now have good support for the hypothesis of an oxime precursor of these compounds, by isolating from a marine sponge, *Hymeniacidon sanguinea*, the oximino-pyruvic acid **6**.

Fresh sponge (50 g, dry weight after extraction), collected near Roscoff[4] (France), was extracted (×3) with cold acetone for 3 days; solvent was removed and the aqueous residue was extracted with ether and *n*-butanol. After evaporation, 2 g *n*-butanol soluble material was dissolved in H_2O and applied to a column (2×20 cm) of Dowex 50W-X2, H^+ form. After washing with 1 *N* HCl, the crude 4-hydroxyphenyl-pyruvic acid oxime (**6**, 0.5 g) was eluted with H_2O. The NMR-spectrum (deuteriated acetone) showed 2 broad doublets (J 8Hz) centered at δ 6.7 and 7.2 for the aromatic protons consistent with a 1,4-disubstituted benzene system and a b singlet at δ 3.8. The UV, λ_{max} 279 nm (MeOH) bathochromically shifted by addition of alkali to 290 nm, was indicative for a phenol structure.

Further purification[5] was carried out on the permethyl-derivative, prepared with CH_2N_2 (in methanol, 1 h at r.t.) or with methyl iodide and silver oxide in chlorophorm at r.t. PLC on silica gel (Merck F_{254}; eluent: benzene) of the product gave **7** (in ca. 50% yield based on the crude material), as oil, M^+/e 237; λ_{max}^{MeOH} 225 and 275 nm (ε, 9,900; 2,370); $\nu_{max}^{liquid\ film}$ 1725, 1610 and 1510, 1040, 840 and 815 cm^{-1}; δ (100 MHz, CCl_4, ppm from TMS), 7.04 (2H, d, J 8 Hz), 6.65 (2H, d, J 8 Hz), 4.00 (3H, s, OCH_3) and overlapping 2 sharp singlets centered 3.75 for 2 OCH_3 and the benzylic CH_2.

The structure of 3-(4-hydroxyphenyl)-2-oximinopropionic acid for this sponge metabolite was definitively proved by converting **6** to tyrosine, and synthesizing the methyl 3-(4-methoxyphenyl)-2-methoximino-propionate (**7**).



[1] K. MOODY, R. H. THOMSON, E. FATTORUSSO, L. MINALE and G. SODANO, J. chem. Soc. Perkin *1*, 18 (1972).

[2] E. FATTORUSSO, L. MINALE and G. SODANO, J. chem. Soc. Perkin *1*, 16 (1972); L. MAZZARELLA and R. PULITI. Gazz. Chim. ital. *102*, 391 (1972).

[3] B. B. STOWE, Fortschr. Chem. org. Nat. Stoffe *17*, 248 (1959). A. AHMAD and I. D. SPENCER, Can. J. Chem. *38*, 1625 (1960).

[4] We are grateful to the Station Biologique de Roscoff (Nord-Finistère, France) for their hospitality which enabled us to collect the sponge.

[5] Difficulty was experienced in purification of the natural compound because its facile conversion to the *p*-hydroxyphenylacetic acid.

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.

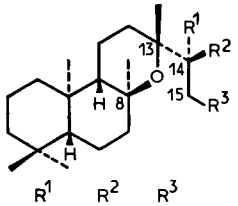
[6] *Dictionary of Organic Compounds*, 4th ed., (Eyre & Spottiswoode Publishers, London 1965), v. 3, p. 1794.

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

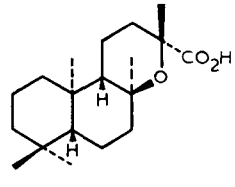
Barbatol, a New Diterpenoid from a *Sideritis arborescens* Salzm. subspecie[1]

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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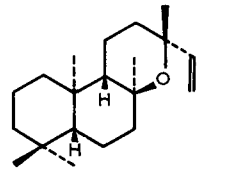

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The NMR-spectrum of **2** shows a 1H quartet at δ 5.03, X part of an ABX system (J_{XA} 8.75 Hz; J_{XB} 2.65 Hz), assigned to the geminal proton of a secondary acetoxyl group. Between δ 3.90 and 4.63 there are 8 lines, the AB part (ν_A 4.49 δ and ν_B 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.

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[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).

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[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.