

CAVERNOSINE, A NOVEL ICHTHYOTOXIC TERPENOID LACTONE

FROM THE SPONGE *FASCIOSPONGIA CAVERNOSA* ⁽¹⁾.

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ABSTRACT : Cavernosine, a novel ichthyotoxic terpenoid lactone has been isolated from the sponge *Fasciospongia cavernosa*. Its structure has been determined by spectral analysis, chemical degradation and correlation with β -ionone.

During this last decade, a great variety of secondary metabolites have been isolated from marine sponges ⁽³⁾. It has been repeatedly suggested that some of these compounds may act as deterrents against potential predators ^(4,5). Thus it is not surprising that many of them show ichthyotoxic activity. This aspect of the ecology of sponges has been briefly discussed in a recent paper ⁽⁶⁾.

In the course of our investigations on ecologically significant constituents of sponges, we now wish to present the structure elucidation of cavernosine (1), a novel terpenoid lactone. This compound is responsible, at least in part, for the toxicity of the dichloromethane extract of the marine sponge *Fasciospongia cavernosa* against the freshwater fish *Lebistes reticulatus* (LD₅₀=20mg/l). Successive silica gel column chromatographies of the CH₂Cl₂ extract, monitored by toxicity bioassays, yielded a toxic fraction containing most of the original activity. This fraction shows two main spots on tlc. After further purification, it was found that ichthyotoxicity was associated with each of these spots. The more polar spot corresponds to a mixture of 5,8-sterol peroxides which was not further examined ⁽⁷⁾. The less polar spot corresponds to a single derivative, homogeneous by GLC, which was named cavernosine.

Cavernosine (1) exhibits a parent mass spectral peak at m/z 280 (HRMS 280.20383 ; calculated for C₁₇H₂₈O₃ : 280.20385) and characteristic fragment ions at m/z 265 (M⁺ -CH₃), 262 (M⁺ -H₂O), 247 (M⁺ -H₂O-CH₃) and 177 (M⁺ -C₄H₇O₃ ; HRMS 177.16433, calculated for C₁₃H₂₁ : 177.16432). An hydroxyl function (ν_{OH} 3490 cm⁻¹) and a γ -lactone ring ($\nu_{C=O}$ 1785 cm⁻¹) account for all three oxygen atoms of the molecule. Since 1 is recovered on treatment with Ac₂O/pyridine at room temperature, the hydroxyl group must be tertiary. The isoprenoid character of 1 was suggested by the presence in its ¹H NMR spectrum of four 3H singlets attributable to four quaternary methyl groups [two on sp³ carbon atoms (δ 0.98 and 0.99), one on an sp² carbon atom (δ 1.58) and one on an sp³ carbon atom bearing an oxygen atom (δ 1.34)].

Moreover, the lactonic 1H double doublet at δ 4.35 was shown by double irradiation experiments to be coupled only with a methylene group. The latter is itself coupled to a 2H multiplet at δ 2.55 attributable to a methylene adjacent to the carbonyl group, thus implying that the γ -lactone is solely substituted at the γ -position. Partial structure A may thus be proposed.

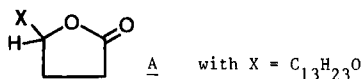
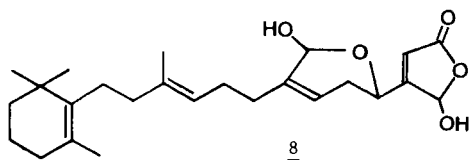
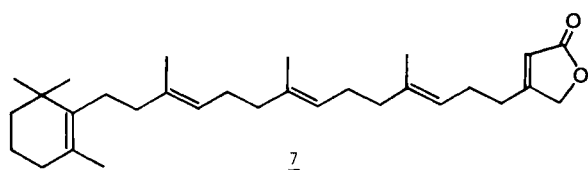
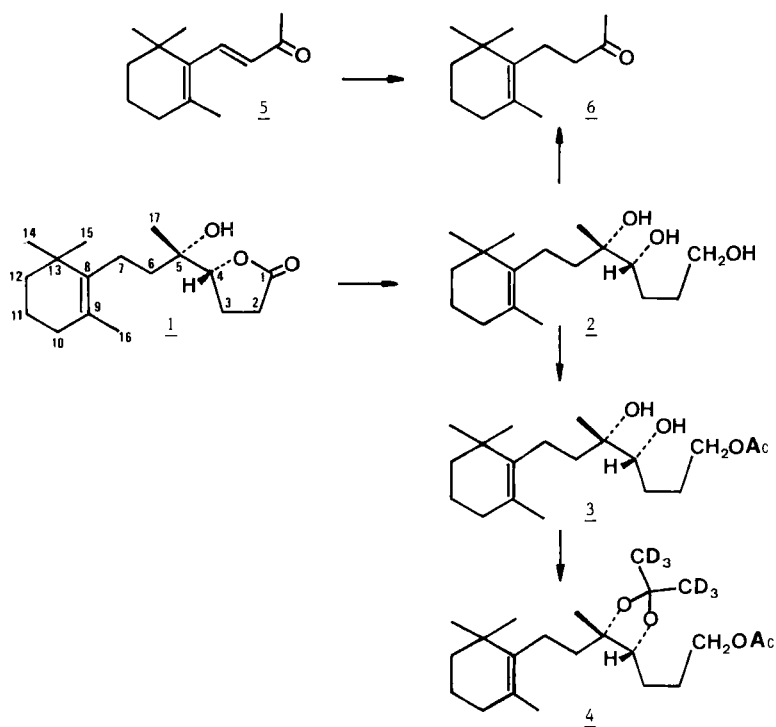


Table 1. Comparison of the ¹³C NMR spectra of 1, 7 and 8 (δ ,ppm).

Carbon atom number	Cavernosine <u>1</u>	Mokupalide (8) <u>7</u>	Manoalide (9) <u>8</u>
1	177.3	-	-
8	136.4	135.7	136.7
9	127.6	126.6	127.3
4	85.7	-	-
5	73.1	-	-
12	39.9	39.5	40.3
2	37.2	-	-
13	35.1	34.9	35.2
10	32.8	32.7	33.1
17	28.9	-	-
14,15	[28.7 28.7]	[28.6 28.6]	[28.9 28.9]
7	23.2	25.6	26.5
3,6	[22.0 21.9]	- -	- -
16	19.7	19.6	20.1
11	19.5	19.5	20.1

The structure of the remaining portion of the molecule was established as follows. First, comparison of the ¹³C NMR spectrum of 1 with those of mokupalide (7) (8) and manoalide (8) (9) clearly indicates that these three compounds possess the same trimethylcyclohexene ring (Table 1). Secondly, LiAlH₄ reduction of cavernosine yielded triol 2, which on NaIO₄ treatment provided the dihydro- β -ionone 6. The latter was found to be identical (GLC, tlc, IR, MS and ¹H NMR) with an authentic sample prepared by selective reduction of β -ionone (5) following the procedure of OJIMA et al. (10,11). These results demonstrate that cavernosine may be represented by structure 1 (without stereostructure). ¹H NMR analysis of the acetonide 4, prepared by selective acetylation of the primary alcohol of 2 (12) followed by formation of the d₆-acetonide using Amberlyst resin as catalyst (13), established a cis-relationship between the methyl group at C-5 and the HC-4 proton. Indeed, irradiation of the



¹³C-methyl signal at δ 1.27 caused a 20% NOE enhancement of the integrated area of the HC-4 proton (14). Therefore, the new terpenoid lactone isolated from *F. cavernosa* must be an *erythro* isomer represented by structure 1 (or its enantiomer).

Cavernosine (1) is reminiscent of a series of sponge monocarbocyclic terpenoids such as muqubilin from *Prianos* sp. (15), manoalide (8) from *Luffariella variabilis* (9,16) and mokupalide (7) from an unidentified sponge (8), the structures of which are closely related to that of vitamin A. Biogenetically they may be regarded as derived from polyprenoid chains by monocyclization followed by subsequent enzymatic oxidation of the linear moiety of the precursor.

As far as we know, only a few chemical studies have been devoted to species of the genus *Fasciospongia* (Spongiidae). N-acyl-2-methylene- β -alanine methyl esters have been reported from a Red Sea collection of *F. cavernosa* (17), some furanosesterterpenes from *F. fovea* and an Australian *Fasciospongia* sp. (18), and geranylarnesol from *F. fovea* again (18).

EXPERIMENTAL

The NMR spectra were recorded in CDCl₃ solution. Chemical shifts are quoted in δ values (ppm) downfield from TMS as internal standard. All described compounds were homogeneous in tlc (silica gel) and capillary GLC (OV-1, 190°).

Isolation of cavernosine (1).

Typically, sun-dried specimens of *Fasciospongia cavernosa* collected around Laing Island (Papua-New Guinea), were finely ground and 750 g of the resulting powder were then extracted with CH₂Cl₂. This afforded 23.5 g of an extract which was chromatographed on a silica gel column (eluent C₆H₆/AcOEt 9:1 to 6:4). The fractionation was monitored through toxicity bioassays (6). The active fraction was treated with CH₂N₂ in ether and again chromatographed on a silica gel column (eluent : C₆H₆/AcOEt 8:2). This led to an active fraction free of fatty acids showing two main spots in tlc. Further attempts to separate these two derivatives by silica gel column chromatographies only yielded small amounts of pure compounds. Therefore the fraction was submitted to acetylation (acetic anhydride/pyridine 1:1). Chromatography (silica gel, eluent : C₆H₆/AcOEt 7:3) of the acetylated residue yielded 103 mg of cavernosine 1 and 60 mg of a mixture of monoacetylated 5,8-sterol peroxides as shown by ¹H NMR and MS.

Cavernosine : oil ; $[\alpha]_{435} = -1.8^\circ$ (CHCl₃, c=1.19) ; C₁₇H₂₈O₃ (by HRMS : calculated 280.20385, measured 280.20383) ; IR (film) : 3490 and 1785 cm⁻¹ ; MS : 280 (M⁺, 20), 265 (M⁺-CH₃, 10), 262 (M⁺-H₂O, 3), 247 (M⁺-H₂O-CH₃, 8), 177 (M⁺-C₄H₇O₃, 30 ; HRMS 177.16433, calculated for C₁₃H₂₁ : 177.16432), 137 (35), 136 (35), 135 (35), 123 (100), 121 (70) and 107 (55) ; ¹H NMR (270 MHz) : 0.98 (3H,s), 0.99 (3H,s), 1.34 (3H,s), 1.58 (3H,s), 2.55 (2H,m) and 4.35

(1H,dd, J=7.5 and 7.5 Hz). ^{13}C NMR (15.08 MHz) : see Table 1.

LiAlH₄ reduction of 1.

A solution of 1 (20 mg) in dry THF (5 ml) containing LiAlH₄ (50 mg) was stirred for 4 h at room temperature. Addition of a saturated MgSO₄ solution and extraction with CH₂Cl₂/EtOH 3:2 yielded 19 mg of crude triol 2 which was purified by flash chromatography (19) (silica gel, eluent : hexane/acetone 7:3).

2 : oil ; IR (film) : 3450 cm⁻¹ ; MS : 284 (M⁺, 1), 266 (M⁺-H₂O, 0.5), 248 (M⁺-2H₂O, 0.5), 233 (M⁺-2H₂O-CH₃, 0.5), 195 (5), 177 (40), 163 (5), 137 (60), 123 (60), 121 (62), 109 (59), 107 (100) ; ^1H NMR (270 MHz) : 0.98 (3H,s), 1.00 (3H,s), 1.21 (3H,s), 1.59 (3H,s), 3.42 (1H, d, J=10Hz), 3.70 (2H,m).

Selective acetylation of 2 (12).

2 g of neutral alumina (Macherey-Nagel) were added to a solution of 2 (74 mg) in dry AcOEt (10 ml). The mixture was refluxed for 24 h, filtered on cotton wool, washed with AcOEt/CH₃OH 9:1 and evaporated to dryness under reduced pressure. The residue was chromatographed (silica gel, eluent : hexane/acetone 8:2) and yielded 34 mg of the diol-monoacetate 3.

3 : oil ; IR (film) : 3450 and 1735 cm⁻¹ ; MS : M⁺ 326 ; ^1H NMR (60 MHz) : 1.00 (6H,s), 1.23 (3H,s), 1.61 (3H,s), 2.06 (3H,s), 3.43 (1H,m) and 4.15 (2H,dd, J=6 and 6Hz).

Isopropylidene acetal of 3 (13).

Compound 3 (32 mg) dissolved in (CD₃)₂CO (1.5 ml) was stirred in the presence of 50 mg of Amberlyst resin (H⁺ form) for 20 min. at room temperature. Filtration and evaporation of the solvent gave an oil which was subjected to flash chromatography (silica gel, eluent : hexane/acetone 9:1) to yield the pure acetal 4 (24 mg).

4 : oil ; IR (film) : 2240 and 1745 cm⁻¹ ; MS : M⁺ 372 ; ^1H NMR (100 MHz) : 0.97 (3H,s), 0.98 (3H,s), 1.27 (3H,s), 1.58 (3H,s), 2.04 (3H,s), 3.75 (1H,m), 4.14 (3H,dd, J=6 and 6Hz). Irradiation at 1.27 results in a 20% ±3 enhancement of the integrated area of the signal attributable to HC-4 (3.75), the integrated area of the 2H signal at 4.14 (H₂C-1) being taken as reference (average of three different runs). No alteration of the 3H singlet at 1.27 (H₃C-17) was observed when the 1H signal at 3.75 was saturated (14).

Sodium periodate oxidation of 2

A solution of NaIO₄ (10 mg) in MeOH/H₂O 9:1 (5 ml) was added to triol 2 (5 mg) dissolved in the same solvent (5 ml). The suspension was stirred at room temperature for 2 h. After usual work-up and flash chromatography (silica gel, eluent : hexane/acetone 8:2) 2 mg of 6 were obtained. Its physical and spectral properties (IR, ^1H NMR, MS, tlc and GLC) were found to be identical to that of the dihydro-derivative obtained by treatment of β-ionone (5)

with the mixture $\text{Et}_3\text{SiH}/(\text{Ph}_3\text{P})_3\text{RhCl}$ (I) using the procedure described by OJIMA et al (10,11).

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