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Mucosin: a New Bicyclic Eicosanoid from the Mediterranean Sponge *Reniera mucosa*

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Abstract: Mucosin (**1**), a new carboxylic acid containing an unusual bicyclo [4.3.0.] nonane skeleton, has been isolated from the Mediterranean marine sponge *Reniera mucosa*. The structure of **1** is suggested on the basis of spectroscopic data. © 1997 Published by Elsevier Science Ltd.

Eicosanoids with related fatty acids are of basic relevance to the equilibrium of normal physiology in mammalian systems. Surprisingly, marine organisms are an extraordinary source of unusual eicosanoid-like substances[3] which have been isolated mainly from algae[4] but also from invertebrates.[5] Many eicosanoids have been found in sponges even though their biochemical and ecological role is until now unknown. We now report the isolation and structural characterization of a new eicosanoid sponge metabolite, mucosin (**1**), containing an unusual bicyclo [4.3.0] nonane skeleton.

Mucosin has been found in specimens of *Reniera mucosa* Griessinger, 1971 collected from January to March 1995 in different geographical areas: Blanes (Spain), Grotte de Jarrè (France), Massalubrense (Italy), and Procida (Italy).

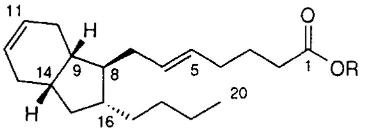
All samples of *Reniera mucosa* (totally 1.350 Kg, wet weight) were immediately frozen after collection and, after some months (3-5), were separately extracted with acetone. The diethyl ether soluble fraction from the acetone extract was fractionated on silica gel column eluting with petroleum ether and increasing amounts of diethyl ether (from 1% to 50%). All the previously reported[6-8] paniceins were collected in the less polar fractions. In addition, the TLC analysis (petroleum ether/diethyl ether, 1:1) revealed the presence, in the more polar fractions, of a metabolite (*Rf* = 0.8) with a characteristic chromatic reaction when the TLC plate was sprayed with $Ce(SO_4)_2$. The spot acquired, after some minutes, a violet colour that increased in intensity during the first 10 hours. The crude mixture (800 mg), obtained by combining all the fractions containing the intriguing compound, was subjected to NMR analysis. The pattern of the ^{1}H-NMR signals immediately suggested the presence of a mixture of free fatty acids. But, some atypical ^{1}H-NMR (δ 5.67) resonances prompted a deeper study.

The crude mixture was treated with CH_2N_2 and fractionated by HPLC (*n*-hexane/ethyl acetate, 99.8:0.2) obtaining, after the elution of a series of methyl esters of common fatty acids, a good amount (8.5 mg) of pure methyl ester of mucosin (**2**). Compound **2** (TLC petroleum ether/diethyl ether, 9:1, *Rf* = 0.8), which preserved the characteristic chromatic reaction of its acid (**1**), was subjected to extensive spectral analysis.

Compound **2** is optically active, $[\alpha]_D = -35.5°$ (*n*-hexane, c = 0.8), with the molecular formula $C_{21}H_{34}O_2$ (HREIMS on the molecular peak at *m/z* 318.2576, required 318.2559). The IR band at 1739 cm^{-1}

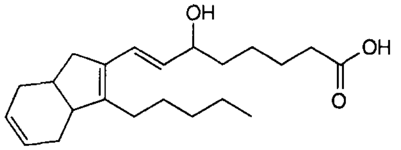
supported the presence of a carbonyl ester that was confirmed by the ^{13}C-NMR signal at δ 174.2 (C-1). The ^{1}H-NMR spectrum displayed signals easily attributable to the methyl ester (δ 3.66), to four olefinic protons at δ 5.67 (2H, H-11 and H-12), 5.45 (H-6) and 5.34 (H-5) and to a terminal fatty acid methyl (δ 0.88, H_3-20). The protons of the methylene near the carbonyl were connected, through the cross-peaks observed in the ^{1}H-^{1}H COSY, to the Δ^{5-6} double bond. In fact, the protons at δ 2.31 (H_2-2) were coupled to those at δ 1.69 (H_2-3) and, in turn, to the allylic ones at 2.02 (H_2-4). The allylic protons at C-7 (δ 2.12) were simplified after a spin decoupling experiment at 5.45 (H-6). Bearing in mind the HREIMS fragment at *m/z* 261.1925 (M^+ - C_4H_9), the ^{1}H-^{1}H connectivities observed starting from H_3-20 (δ 0.88), to H_2-19 (δ 1.28), H_2-18 (δ 1.37), H_2-17 (δ 1.56) and, finally, the expected number of unsaturations (5), mucosin should be characterized by two linear fatty acid chains, with respectively 4 and 7 carbons, linked to a central bicyclic nucleus. Other MS fragments at *m/z* 176 and 119 confirmed this hypothesis.

The bicyclic partial structure contains two olefinic protons (δ 5.67, H-11 and H-12) coupled to four allylic protons (H_2-10 δ 1.70 and 2.25; H_2-13 δ 1.72 and 2.19), four methine protons (H-8, δ 1.12; H-9, δ 1.11; H-14, δ 1.50; H-16, δ 1.59) and two methylene protons (H_2-15, δ 1.33 and 1.55). All protons were connected to the corresponding carbons by a HSQC experiment, suggesting a bicyclo [4.3.0] nonane system. HMBC experiments (table 1) strongly supported this suggestion displaying diagnostic correlations: H_2-18 --> C-16, H_2-7 --> C-9, H-8 --> C-9 and C-10. The *E* stereochemistry of the double bond at $\Delta^{5,6}$ was determined both by the downfield ^{13}C-NMR resonance of C-4 (δ 31.7) and by the NOE effect between H_2-7 and H-5, whereas the relative stereochemistry at the chiral C-8, C-9, C-14 and C-16 was suggested by a series of diagnostic correlations observed in NOESY and ROESY experiments. In fact, H_2-7 showed a steric interaction with both H-16 and H-9 according to a *trans* configuration of the two alkyl chains at C-16 and C-8. A series of cross-peaks were observed for H-9, that interacts with H-14, H_2-7 and H-16 in addition to the expected interactions with the two allylic protons (δ 1.70, 2.25) at C-10.



1 R = H

2 R = CH_3



3

The carbon skeleton of mucosin (**1**) is new, even though very recently[9] a related C-22 bicyclic lipid, dictyosphaerin (**3**), has been isolated from the southern marine green alga *Dictyosphaeria sericea.*. Other metabolites, plakotenin[10] and manzamenones[11], containing a bicyclo [4.3.0] nonane ring system have been isolated from an Okinawan *Plakortis* sponge. Biosynthetically, plakotenin should derive from a linear polypropionate precursor, whereas manzamenones could be generated by coupling two fatty acid precursors. Analogously, mucosin (**1**) should be derived from arachidonic acid through an intramolecular cycloaddition, involving $\Delta^{8,9}$ and $\Delta^{14,15}$, and isomerization of $\Delta^{5,6}$. The genera *Plakortis* and *Reniera* belong to the same class, Demospongiae, but to orders, Homosclerophida and Haplosclerida, which are taxonomically quite different. However it is interesting to observe that sponges belonging to the genus *Reniera* contain a series of unusual

Table I. ^{1}H and ^{13}C NMR Data of Mucosin Methyl Ester (2) in $CDCl_3$.

Position	δ_H[a]		J (Hz)	δ_C[b]	^{13}C coupled with ^{1}H (HMBC correlations)	^{1}H correlated with ^{1}H (NOESY and ROESY experiments)
1				174.2		
2	2.31	t	7.5	33.2	C-1, C-3, C-4	
3	1.69	m		24.5	C-1, C-2, C-4, C-5	
4	2.02	q	7.2	31.7	C-2, C-3, C-5, C-6	H-6
5	5.39	dt	15.6; 7.2	129.8	C-3, C-4, C-6, C-7	H-3, H-7
6	5.45	dt	15.6; 6.9	130.0	C-4, C-5, C-7, C-8	H-4, H-8
7	2.12	m		36.3	C-5, C-6, C-8, C-9, C-16	H-5, H-9, H-16
8	1.12	m		52.1	C-6, C-7, C-9, C-10, C-14, C-16	
9	1.11	m		47.1	C-8, C-10, C-13, C-14	H-7, H-10b, H-13a, H-14, H-16
10a	1.70	m		31.5	C-8, C-9, C-11, C-12	H-11, H-14
10b	2.25	m		31.5	C-9, C-11, C-12	H-9, H-11
11	5.67	m		127.0	C-9, C-10	H-10a, H-10b
12	5.67	m		127.0	C-13, C-14	H-13a, H-13b
13a	1.72	m		32.0	C-9, C-11, C-12, C-14	H-9, H-12
13b	2.19	m		32.0	C-9, C-11, C-12, C-14	H-12, H-14, H-15b
14	1.50	m		39.9	C-9, C-10, C-13	H-9, H-10a, H-13b
15a	1.33	m		36.7	C-13, C-14, C-16	
15b	1.55	m		36.7	C-8, C-9, C-14, C-16	H-13b
16	1.59	m		42.1	C-7, C-8, C-9	H-7, H-9
17	1.15	m		36.5	C-16, C-19	
18	1.40	m		36.4	C-16, C-17, C-19	
19	1.28	m		22.6	C-17, C-18, C-20	
20	0.88	t	6.7	13.8	C-18, C-19	
-OMe	3.66	s		51.4	C-1	

[a] Assignments were determined by DQF-COSY

[b] Assignments were determined by gHSQC experiment.

All experiments were performed on Bruker AMX 500 and DRX 600 spectrometers and structurally unrelated metabolites as such as aryl carotenoids (zeaxanthin, renieratene, isorenieratene, renierapurpurina) from *R. japonica*,[12] isoquinoline quinones (renierone, mimosamycin, remieramycins A-D) from a *R.* sp,[13] pentacyclic alkaloids (sarains) from *R. sarai*,[14] polyacetylenes compounds (renierins) from *R.* fulva,[15] and sequiterpenoids linked to aromatic rings (paniceins) found in *Halichondria panicea*,[6] *Reniera mucosa* [8] and *Reniera fulva*.[7] Some of these taxonomical data seem to be conflicting. In particular those referred to *Reniera fulva* and to paniceins. In fact, it seems that different collections of *R. fulva* [7, 15] contain completely different metabolites whereas paniceins are present in three different sponges. Probably, before drawing careless conclusions it is necessary to reanalyze the taxonomy of these sponges. In fact, all specimens (from France, Italy and Spain) of sponge *R. mucosa* analyzed in this work are from a taxonomic point of view identical to that previously classified [6] as *H. panicea.* Because of this it is highly probable that paniceins and mucosin (**1**) can chemically characterise *R. mucosa.*

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