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METABOLITES FROM THE MARINE SPONGE SPONGIONELLA GRACILIS. THREE FURTHER NOR-DITERPENES, ONE OF THEM BASED ON A NOVEL CARBOCYCLIC SKELETON.

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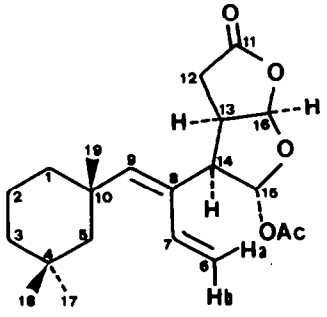
Abstract. - Three new nor-diterpenes have been isolated from the Mediterranean sponge Spongionella gracilis and their structures have been determined by spectroscopic and chemical evidences. The structures of two of the new metabolites (3 and 4) are strictly related to gracilin A (2), a previously isolated nor-diterpene from the same source, whereas the third compound, spongionellin (1), possesses a new carbocyclic skeleton. The biogenesis of nor-diterpenes from S. gracilis is briefly discussed.

A chemical study of the sponge Spongionella gracilis was undertaken in the frame of a systematic survey on marine invertebrates living in the Mediterranean sea near the southern coast of Italy. Our own interest in this sponge was increased by the discovery that this organism is a source of nor- and bis-nor-diterpenes, which are very rare metabolites from marine environment [1-3].

In this report we describe the structural determination of three minor nor-diterpenes from the same sponge among which spongionellin (1) represents the first example of a new degraded diterpenes skeletal class.



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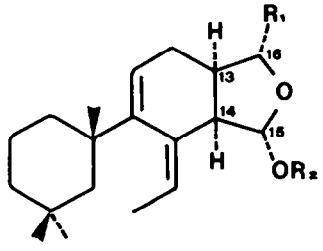
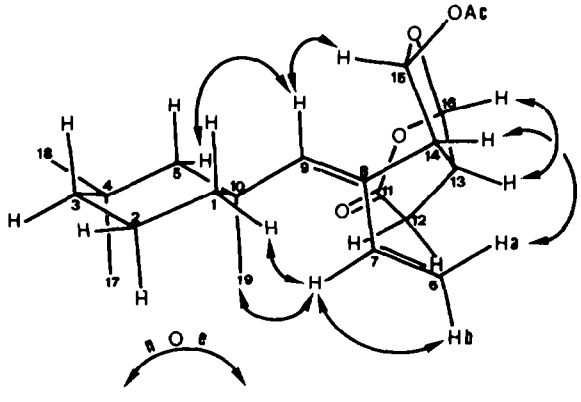


2 R_1 = OAc R_2 = Ac
3 R_1 = H R_2 = Ac
4 R_1 = H R_2 = H

A collection of Spongionella gracilis was made in the Bay of Naples during the Summer of 1985. Fresh tissues of the animals were extracted in the dark with chloroform-methanol (1:1) and the condensed extracts were subjected to repeated silica gel chromatographies; the final purification of single compounds was achieved by HPLC.

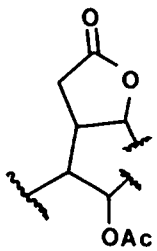
Spongionellin (1), $[\alpha]_D$ +1.2 (c 1.0, $CHCl_3$), is a colourless amorphous solid, having the molecular formula $C_{21}H_{30}O_5$ [from HRMS and ^{13}C-NMR (Table 1)]. The IR spectrum contained bands attributable to a five membered lactone (ν_{max} 1780 cm^{-1}) and an acetate group (1740 cm^{-1}), while it excluded the presence of hydroxyl functionalities. The fragment in the mass spectrum of 1 at m/z 302 (M^+ -AcOH) was in accord with the acetate group, also evident by inspection of ^{1}H-NMR spectrum (singlet at δ 2.08) and ^{13}C-NMR spectrum (signal at δ 169.1) which displayed a further carbonyl resonance due to the lactone function at δ 171.7. UV absorption at λ_{max} 234 nm (ε 11370) indicates the presence of a conjugated diene which is shown to be disubstituted by examination of both ^{13}C-NMR[δ 114.3 (-CH_2,C_6), 134.4 (-CH, C_7), 128.8 (-C-, C_8) and 143.3 (-CH, C_9)] and ^{1}H-NMR spectra where the resonance attributable to 9-H (δ 5.55, bs), 7-H (δ 6.96, bdd, J = 16.9 and 11.7 Hz) and 6-H_2 (δ 5.11, bd, J= 16.9 Hz and 5.19, bd, J= 11.7 Hz) were readily inter-related by double resonance experiments. A detailed analysis of the 500 MHz ^{1}H-NMR spectrum of 1, assisted by extensive spin-spin decoupling experiments, defined the presence in 1 of the substructure A, for which there is also evidence in the ^{13}C-NMR spectrum (a complete assignment, based upon ^{13}C-^{1}H shift correlated 2D-NMR experiment, is reported in Table 1). Consideration of the chemical shifts of 15-H (δ 6.51) and 16-H (δ 6.14) and of the pertinent carbon atoms (δ 100.4, C_{15} and 106.9, C_{16}) in the ^{1}H- and ^{13}C-NMR spectra of 1, respectively, in conjugation with the absence in the latter spectrum of other signals attributable to sp^3 carbons linked to oxygen atoms, clearly concluded that the fifth oxygen implied by the molecular formula of 1 must be comprised by C_{15} and C_{16}. Furthermore, the presence of an allylic coupling between 14-H and 9-H was indicative of the C_8-C_{14} connection, thus allowing the partial structure B to be derived. Taking into account that the elemental formula of 1 implies a further degree of unsaturation and that the rest of the molecule includes two non-hydrogen-bearing sp^3-carbons, four methylenes and three methyls on quaternary carbons, the completion of the gross structure was accomplished as follows.



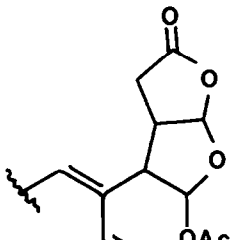
One of the methylene groups must be isolated since it resonates in the ^{1}H-NMR spectrum as an AB system centered at δ 1.34 [the part A displays each line of the doublet

splitted into a triplet (J = 1.2 Hz) by long range couplings (see below)] . The other three methylenes in question are contiguous, as indicated by homonuclear decoupling experiments, which allowed a complete analysis of each signal (Table 1). The values of the coupling constants among the protons of the above methylene groups clearly indicated that they are incorporated in a six membered ring. Particularly, the resonances of 1-Heq, 3-Heq and 5-Heq show the long range couplings, typically observed among equatorial protons in cyclohexanes in the chair conformation. The above data are compatible only with the part structure C also present in gracilin A (2), a previously isolated metabolite from the same sponge , thus unavoidably leading to structure 1 (devoid of stereochemistry) for the new metabolite.

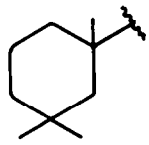
LAH reduction of 1, followed by acetylation afforded as expected, the triacetate 5, thus confirming the correct assignment of formula 1. The structure of compound 5 was indicated by spectral data and, particularly, by NMR analysis (see experimental).



A



B


C

The E configuration of Δ^{8} double bond, as well as the relative stereochemistry of the chiral centers at C-13, C-14, C-15 and C-16 as depicted in structure 1 were deduced from nOed's data (Table 1) which show in addition that the preferred conformation of the diene mojety is trans-coplanar with respect to C_7-C_8 single bond as expected by observation of a ^{5}J coupling between 9-H and 6-H_b. The correct assignment of the chirality of the carbon atoms belonging to the oxygen containing portion of the molecule was secured by the comparison of the NMR data with those of norrisolide (6), a metabolite from Dendrilla sp., containing the same fused γ-lactone-tetrahydrofurane ring system and whose structure was determined by X-ray diffraction experiment [4].

The configuration at C-10 and the absolute stereochemistry of spongionellin as well as of gracilin A have not been determined. Therefore the stereochemistry drawn was an arbitrary choice which seems most likely on the ground of a biosynthetic hypothesis for the nor-diterpenes from Spongionella gracilis (see below).

Compound 3 (gracilin E), $[\alpha]_D$ -55.6 (c 0.8, $CHCl_3$), had the molecular formula $C_{21}H_{32}O_3$ (HREIMS) . Its spectral properties [λ_{max} 224 nm (ϵ 6530); ν_{max} 1750 and 1235 cm^{-1}; ^{1}H- and ^{13}C-NMR (see Table 2)] indicated a structure closely related to gracilin A (2) with a methylene at C-16 replacing the -CHOAc group. In accordance with this structural hypothesis, LAH reduction of 3 afforded a diol (7) identical in all respects to that obtained from gracilin A (2) in the same experimental conditions[2].

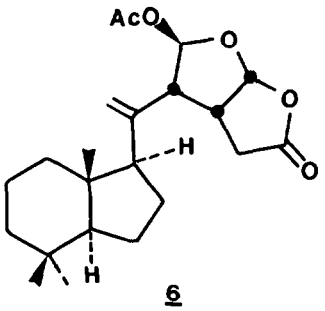
This experiment did not account for the stereochemistry at C_{15} which was deduced

from the value of the coupling constant between 15-H and 14-H, identical to that found in gracilin A, and by the absence of nOe effect between this pair of protons.

The last novel compound, gracilin F (4), was isolated as a colourless oil, $[\alpha]_D$ +0.5 (c 1.2, $CHCl_3$) having the molecular formula $C_{19}H_{30}O_2$ (HREIMS).

Table 1. Nuclear Magnetic Resonance Data for 1*

Assignment	δ ^{13}C	δ ^{1}H	J, Hz
1ax		1.20[c] (ddd)	1ax-1eq = 13.5; 1ax-2ax = 12.0; 1ax-2eq = 5.0;
	39.2		1eq-2ax = 3.0; 1eq-2eq = 3.0; 1eq-5eq = 1.2;
1eq		2.00 (bddd)	2ax-3ax = 12.0; 2ax-3eq = 3.8; 2eq-3eq = 3.8;
			3ax-3eq = 13.2; 3eq-5eq = 1.2; 5ax-5eq = 14.0;
2	19.7	1.44 (m)	6a-7 = 16.9; 6b-7 = 11.7; 12a-12b = 18.4;
3ax		1.08[c] (ddd)	12a-13 = 9.6; 12b-13 = 3.7; 13-14 = 9.2
	39.4		13-16 = 5.9; 14-15 = 3.7
3eq		1.31 (bddd)	
4	31.3		
5ax		1.16[c] (d)	
	53.8		
5eq		1.53 (dt)	
6a		5.11 (bd)	
	114.3		
6b		5.19 (bd)	
7	134.4	6.96 (bdd)	
8	128.8		
9	143.3	5.55 (bs)	
10	37.7		
11	171.7		
12a		2.51 (dd)	
	30.1		
12b		2.44 (dd)	
13	40.5	3.46 (dddd)	
14	48.1	3.36 (bdd)	
15	100.4	6.51 (d)	
16	106.9	6.14 (d)	
17	28.4[a]	0.89[b] (s)	
18	32.8[a]	0.91[b] (s)	
19	30.6	1.10 (s)	
$\underline{CH_3}CO$	20.9	2.08 (s)	
$CH_3\underline{C}O$	169.1		



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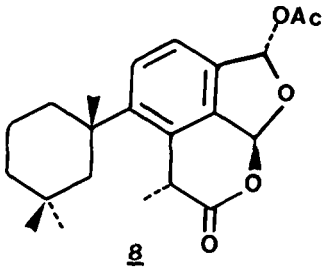

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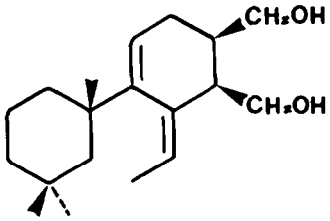

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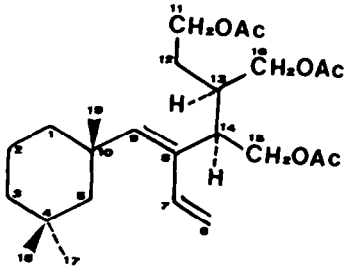

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* δ values ($CDCl_3$) are in ppm from TMS. Assignments are based on ^{13}C-^{1}H shift correlated 2D-NMR spectroscopy.

a-b. Values with identical superscript within each column may be interchanged.

c. Superimposed on other signals.

Comparison of the pertinent spectral data [^{1}H- and ^{13}C-NMR (see Table 2), MS, IR and UV (see experimental)] with those of gracilin E (3) clearly indicated that the new compound was the corresponding emiacetal. Conclusive identification was achieved by acetylation of 4 which afforded a product indistinguishable from natural 3.

Metabolites based on the spongian skeleton have been isolated from Spongia (Dictyoceratida) and Aplysilla (Dendroceratida)[5]. Therefore it is not unreasonable to suppose that nor-diterpenes from S. gracilis (Dictyoceratida) could biogenetically derive from a spongian precursor as already proposed for other diterpenes showing structural analogies [4,6,7]. A plausible biogenetic pathway is reported in the Scheme.

Table 2. Nuclear Magnetic Resonance Data for 3 and 4 (in $CDCl_3$).

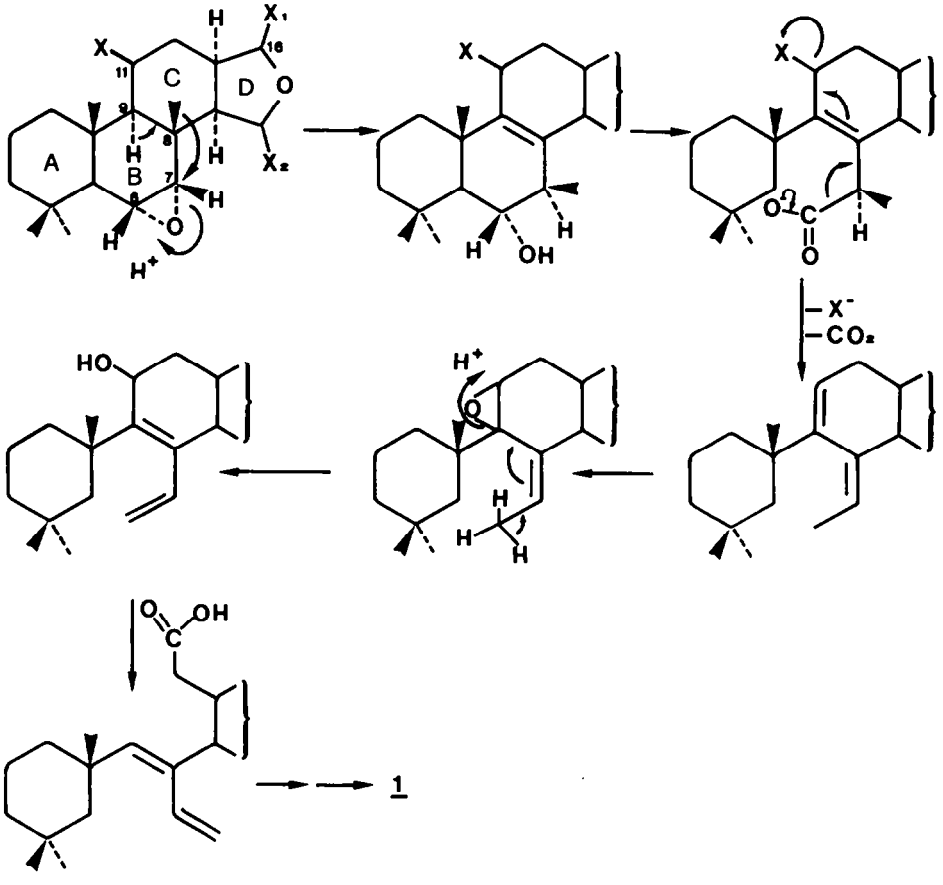
	3			4		
Assignment	$\delta^{13}C$ *	δ^1H	J_{HH}, Hz	$\delta^{13}C$ *	δ^1H	J_{HH}, Hz
1ax	38.6[a]	1.09 (ddd)[c]	13.2,13.2,3.7	38.5[e]	1.07 (ddd)[g]	13.2,13.2,3.7
1eq		°			°	
2	19.5	1.47 (m)		19.5	1.46 (m)	
3ax	39.3[a]	°[c]		39.3[e]	°[g]	
3eq		1.56 (bddd)[c]	13.2,3.0,3.0		1.56 (bddd)[g]	13.2,3.0,3.0
4	31.3			31.3		
5ax	52.5	1.40 (d)	14.0	52.6	1.38 (d)	14.0
5eq		1.51 (dt)	14.0,1.2,1.2		1.52 (dt)	14.0,1.2,1.2
6	17.6	1.63 (d)	7.2	17.6	1.63 (d)	7.2
7	121.8	5.35 (q)	7.2	121.4	5.36 (q)	7.2
8	135.8			135.8		
9	155.1			155.1		
10	38.4			38.5		
11	124.3	5.89 (dd)	4.4,7.3	124.5	5.87 (dd)	4.4,7.3
12a	28.2	2.00 (ddd)	14.7,7.3,2.2	28.0	1.96 (ddd)	14.2,7.3,2.3
12b		1.78 (ddd)	14.7,4.4,5.9		1.77 (ddd)	14.2,4.4,5.9
13	38.4	2.78 (m)		38.9	2.79 (m)	
14	59.0	3.09 (dd)	9.6,1.2	60.6	2.97 (dd)	9.6,2.2
15	103.3	5.98 (d)	1.2	103.1	5.14 (d)	2.2
16a	75.3	4.11 (dd)	8.8,8.8	73.4	4.14 (dd)	8.8,8.8
16b		3.60 (dd)[d]	8.8,4.4		3.46 (dd)[h]	8.8,4.4
17	27.6[b]	0.88 (s)[d]		27.6[f]	0.88 (s)[h]	
18	35.8[b]	1.01 (s)[d]		35.8[f]	1.01 (s)[h]	
19	25.8	1.26 (s)		25.9	1.26 (s)	
$\underline{CH_3}CO$	21.3	2.05 (s)				
$CH_3\underline{CO}$	170.1					

* Assignments based on DEPT sequence and comparison with a model compound (gracilin A).
a-h. Values with identical superscript within each column may be interchanged.
°. Superimposed on other signals in the region 1.38-1.32 .

The first two steps of the sequence of reactions, leading to opening of ring B, are common to the proposed biogenesis of aplysulphurin (8), a metabolite isolated from Aplysilla sulphurea (Dendroceratida) [7]: migration of the 8-Me group to C-7, induced by opening of a 6,7-

-epoxide, generates a C_8-C_9 double bond and an -OH at C-6, whose oxidation with concomitant cleavage of the C_5-C_6 bond and supply of a hydrogen to C-5 produces a carboxyl group. Subsequent decarboxylation, with concomitant migration of C_8-C_9 double bond and elimination of a X^- group, gives the nor-diterpene skeleton found in gracilin-A,E and F. The skeleton of spongionellin could be generated through an epoxidation of the C_9-C_{11} unsaturation, followed by opening of the oxirane ring inducing the formation of a conjugated diene. Opening of ring C could be analogous to that of ring B through oxidation at C_{11} and concomitant cleavage of C_9-C_{11} bond, which would produce a carboxyl group capable of lactonizing to C_{16}.

Scheme



EXPERIMENTAL

IR spectra were recorded on a Perkin-Elmer Model 399 spectrophotometer and UV spectra on a Perkin-Elmer Model 550S spectrophotometer.

Optical rotations were taken on a Perkin-Elmer Model 141 polarimeter with a 10 cm cell.

^{1}H-NMR experiments were performed on a Bruker WM-500 spectrometer and ^{13}C-NMR spectra were recorded on a Bruker WM-250 spectrometer in $CDCl_3$ solutions; all chemical shifts are reported with respect to Me_4Si (δ = 0).

The samples used for nOe measurements were previously degassed by bubbling argon through the solution for 40 min.

^{13}C-^{1}H shift correlated 2D-NMR spectrum was performed using a Bruker microprogram. The shift correlation with polarization transfer via ^{1}J coupling experiment was carried out adjusting the fixed delays to give maximum polarization for J_{C-H} = 135 Hz.

Low resolution mass spectra were recorded at 70 eV on an AEI MS 30 mass spectrometer. High resolution mass spectra were recorded on an AEI MS 902 spectrometer.

High performance liquid chromatographies were performed on a Lichrosorb Si-60 (25 cm x 4 mm) column using a Varian 2010 pump equipped with a differential refractometer and n-hexane/ethyl acetate solvent mixtures as eluent.

Isolation of spongionellin (1), gracilin E (3) and gracilin F (4). S. gracilis was collected by hand at about 10 m depth in September 1985, along the coast of the Bay of Naples, near Capo Miseno. Freshly collected animals (16.0 g, dry weight after extraction) were cut into small pieces and extracted three times with $CHCl_3$-MeOH (1:1) at room temperature in the dark. The combined lipid extracts were taken to dryness and the residue (3.5 g) was chromatographed on a silica gel column (180 g), eluted with increasing amounts of Et_2O in n-hexane. The fractions eluted with n-hexane-Et_2O (9:1) yielded crude gracilin E (3), which was purified by HPLC using n-hexane-ethyl acetate (85:15). The fractions eluted with n-hexane-Et_2O (7:3) gave a mixture of 1 and 4, which was resolved into the single components by HPLC (eluent n-hexane-ethyl acetate 7:3).

Spongionellin, 8 mg, $[\alpha]_D$ +1.2 (c 1.0, $CHCl_3$); IR ($CHCl_3$) 1780 and 1740 cm^{-1}; UV (MeOH) λ_{max} 234 nm (ϵ 11370); ^{1}H- and ^{13}C-NMR (see Table 1); MS, m/z 362 (M^+), 302 (M^+-AcOH), 287 (M^+-AcOH-CH_3); HRMS, found m/z 362.2080, $C_{21}H_{30}O_5$ requires 362.2085.

Gracilin E (3), $[\alpha]_D$ -55.6 (c 0.8, $CHCl_3$); IR ($CHCl_3$) 1750 and 1235 cm^{-1}; UV (MeOH) λ_{max} 224 nm (ϵ 6530); ^{1}H- and ^{13}C-NMR (see Table 2); MS, m/z 332 (M^+), 272 (M^+-AcOH), 257 (M^+-AcOH-CH_3); HRMS, found m/z 332.2360, $C_{21}H_{32}O_3$ requires 332.2343.

Gracilin F (4), $[\alpha]_D$ +0.5 (c 1.2, $CHCl_3$); IR ($CHCl_3$) 3500-3100 cm^{-1}; UV (MeOH) λ_{max} 224 nm (ϵ 6700); ^{1}H- and ^{13}C-NMR (see Table 2); MS, m/z 272 (M^+-H_2O), 257 (M^+-H_2O-CH_3); HRMS, found m/z 290.2229, $C_{19}H_{30}O_2$ requires 290.2238.

Reduction and acetylation of spongionellin (1).

A solution of spongionellin (1, 6 mg) in dry ethyl ether (4 ml) was added to a suspension of lithium aluminum hydride (30 mg) in dry ethyl ether (4 ml) and the mixture stirred at room temperature for 10 min. The excess reagent was destroyed by addition of ethyl acetate, then a few drops of water were added and the product was extracted with ethyl ether. The crude product was acetylated with Ac_2O-pyridine (1:1) at room temperature overnight. The usual work up and purification by TLC (eluant: n-hexane-EtOAc 1:1), gave the triacetate 5 (3 mg): IR ($CHCl_3$) 1740, 1235 cm^{-1}; UV (MeOH) λ_{max} 234 nm (ϵ 11200); ^{1}H-NMR ($CDCl_3$) δ 0.87 (6H, s, 17-H_3 and 18-H_3), 1.08 (3H, s, 19-H_3), 1.60 (2H, m, superimposed on other signals, 12-H_2), 2.01 (3H, s, acetate), 2.04 (3H, s, acetate), 2.04 (1H, m, submerged by acetate signals, 13-H), 2.82 (1H, bq, J= 7.3 Hz, 14-H), 4.03 (2H, AB system further coupled superimposed on 16-H_2 and 11-H_2 signals, J= 11.3 and 5.5 Hz, 15-H_2), 4.19 (2H, AB system further coupled superimposed on 15-H_2 and 11-H_2 signals, J = 11.3 and 5.8 Hz, 16-H_2), 4.12 (2H, m, superimposed on 15-H_2 and 16-H_2 signals, 11-H_2), 5.12 (1H, bd, J= 11.7 Hz, 6-H_a), 5.17 (1H, bd; J= 17.9 Hz, 6-H_b), 5.40 (1H, bs, 9-H), 6.80 (1H, bdd, J= 17.9 and 11.7 Hz, 7-H); MS, m/z 376 (M^+-AcOH), 361 (M^+-AcOH-CH_3), 316 (M^+-2AcOH), 301 (M^+-2AcOH-CH_3), 256 (M^+-3AcOH), 241 (M^+-3AcOH-CH_3).

Reduction of gracilin E (3).

A solution of gracilin E (3, 8 mg) in dry ethyl ether (4 ml) was added to a suspension of lithium aluminum hydride (30 mg) in dry ethyl ether (3 ml) and the mixture was stirred for 10 min at room temperature. After destroying the excess reagent by dropwise addition of ethyl acetate, a few drops of water were added and the product was extracted with ethyl ether. Removal of solvent afforded an impure product which was purified by TLC on a silica gel plate (0.25 mm thickness) developing with Et_2O-n-hexane (8:2). The product obtained (7, 4 mg), was identical in all respects to that obtained from gracilin A in the same experimental conditions.

Acetylation of gracilin F (4).

Gracilin F (4, 4 mg) was acetylated in pyridine (1 ml) with Ac_2O (1 ml) at room

temperature overnight. The usual work up and purification by TLC (eluant: n-hexane-EtOAc 85:15), yields the acetylated product, whose chromatographic and spectral properties were identical to those of natural 3.

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