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MINOR BISNORDITERPENES FROM THE MARINE SPONGE *SPONGIONELLA GRACILIS* AND REVISION OF THE Δ^6 CONFIGURATION OF GRACILIN B

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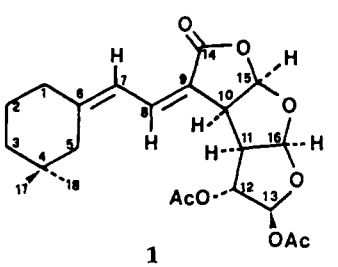
ABSTRACT.—Two new representatives of the rare class of bisnorditerpenes, gracilin C and D (**2** and **4**), have been isolated as minor metabolites from *Spongionella gracilis*, a Mediterranean sponge. The structure of these compounds, both related to that of gracilin B (**3**), the major metabolite of *S. gracilis*, were solved by spectral analyses, including 2D-nmr spectroscopy, and chemical interconversions. Stereostructure **3** has been reassigned to gracilin B instead of the previously proposed **1** on the basis of additional nOe difference experiments and ^{13}C-nmr data.

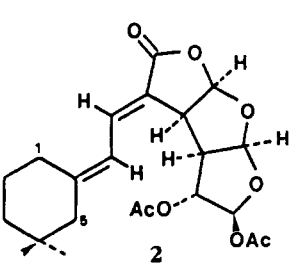
In the course of our investigations on the constituents of Mediterranean organisms, we isolated from the sponge *Spongionella gracilis* (Vosmaer, 1883) a structurally novel metabolite, gracilin B, for which we proposed structure **1** (1). Gracilin B, which is the major compound in the lipophilic extract of this invertebrate, represents, to the best of our knowledge, the first bisnorditerpene observed from a marine sponge. In a continuing study of the same marine organism, we have isolated, as minor constituents, two new degraded diterpenes which we named gracilin C and D (**2** and **4**), and whose structure determination is discussed here. In the light of the spectral data for the isomeric compounds gracilin B and C, we have reinvestigated the stereochemistry of gracilin B, and we reassigned the Δ^6 configuration of this compound as *E* (see structure **3**) rather than the previously proposed *Z*.

RESULTS AND DISCUSSION

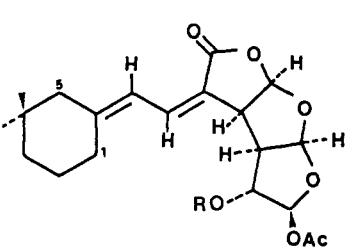
Collections of *S. gracilis* were made along the coast of the Bay of Naples in the summer of 1984. Freshly collected animals were immediately extracted with $CHCl_3$-MeOH (1:1) in the dark. Gracilin C (**2**) and D (**4**) were isolated by flash chromatog-



1



2



3 R=Ac
4 R=Propyl

raphy of the crude extract on silica gel and were purified by hplc from approximately the same fractions containing gracilin B (**3**).

Gracilin C (**2**) was a minor component of the organic extract, comprising less than 0.5% of the $CHCl_3$-soluble material. Data from hrms and ^{13}C-nmr spectroscopy (Table 1) established a molecular formula of $C_{22}H_{28}O_8$ for this compound, thus indicating it

TABLE 1. ^{13}C-nmr Data for Compounds **1-5**[a]

Carbon No.	Compounds				
	gracilin-B (**3**)	**2**	**1**	**5**	**4**
1	28.77	29.69	37.76	37.92	28.83
2	23.72	23.63	24.36	24.37	23.75
3	39.42	39.28	39.60	39.45	38.48
4	34.41	34.54	34.36	34.69	34.45
5	51.31	51.44	42.45	43.42	51.36
6	156.08	158.37	156.20	158.42	156.09
7	118.96	119.41	119.33	119.90	119.02
8	139.55	134.52	139.75	134.63	139.60
9	119.84	122.39	120.04	122.35	119.87
10	46.15	43.27	46.29	43.26	46.21
11	52.35	51.00	52.51	51.33	52.47
12	78.82	78.90	79.03	78.82	78.74
13	101.01	100.78	100.96	100.96	101.08
14	167.28	169.38	167.33	169.45	167.28
15	106.34	107.19	106.34	107.26	106.35
16	114.06	114.22	114.04	114.12	114.06
17	27.93[b]	28.34[c]	28.07[d]	28.39	27.98[e]
18	28.92[b]	28.50[c]	28.75[d]	28.39	28.95[e]
CH_3CO-	20.69 and 20.69	20.71 and 20.63	20.66 and 20.78	20.66 and 20.66	20.70
CH_3CO-	169.75 and 169.65	169.63 and 169.22	169.68 and 169.68	170.33 and 169.62	169.68
CH_3CH_2CO-					8.82
CH_3CH_2CO-					27.43
CH_3CH_2CO-					173.29

[a]δ Values ($CDCl_3$) are in ppm from TMS. Assignments are based on ^{13}C-^1H shift correlated 2D-nmr spectroscopy.

[b-e]Values with identical superscript within each column may be interchanged.

to be an isomer of gracilin B. Its ir spectrum showed absorptions [ν max (CS_2) 1760, 1745, and 1630 cm^{-1}] that suggested the presence of functionalities similar to those found in gracilin B (**3**). The mass spectrum of **2** is also very similar to that of gracilin B (**3**), with strong peaks at *m/z* 360 (M^+-AcOH), 318 (M^+-AcOH-CH_2CO), and 300 (M^+-2AcOH), indicating a facile loss of two acetate groups. The ^1H-nmr spectrum of **2** is strongly reminiscent of that of gracilin B (**3**), apart from the signals corresponding to 7-H [δ 5.99 (1H, d, J=12.4 Hz)] and 8-H [δ 7.63 (1H, dd, J=12.4 and 1.3 Hz)] which, in the previously isolated compound, appeared as an AB system centered at δ 7.22. Having in mind the ^1H-nmr spectrum of gracilin B (**3**), the chemical shifts, multiplicities, and coupling constants for all protons of gracilin C were readily defined by spin decoupling studies. Assignments are given in Tables 2 and 3. Detailed analyses of these data, in conjunction with uv absorption of **2**, λ max (MeOH) 292 nm (ε 19600), indicated that the differences in the two isomers should be confined to the conjugated part structure. That the two compounds were geometric isomers about the double bonds of the conjugated lactone was confirmed by photochemical interconversion of gracilin B (**3**) into gracilin C (**2**).

TABLE 2. ^{1}H-nmr Data for Compounds **1-5**[a]

Proton No.	Compounds				
	gracilin-B (**3**)	**2**	**1**	**5**	**4**
1	2.45 (m) 2.23 (m)	2.40 (t)	2.25 (t)	2.26 (t)	2.45 (m) 2.25 (m)
2	1.58 (m)	1.60 (m)	1.62 (m)	1.65 (m)	1.61 (m)
3	1.38 (t)	1.42 (t)	1.40 (t)	1.41 (t)	1.39 (t)
5	2.05 (s)	2.09 (s)	2.21 (s)	2.23 (bs)	2.06[b] (s)
7	AB system	5.99 (d)	AB system	6.18 (d)	AB system
8	centered at 7.22	7.63 (dd)	centered at 7.26	7.59 (dd)	centered at 7.23
10	3.95 (dd)	4.00 (ddd)	3.96 (dd)	4.00 (ddd)	3.95 (dd)
11	3.11 (dd)	3.32 (dd)	3.12 (dd)	3.27 (dd)	3.10 (dd)
12	5.09 (s)	4.88 (s)	5.07 (s)	4.90 (s)	5.10 (s)
13	6.16 (s)	6.13 (s)	6.21 (s)	6.13 (s)	6.17 (s)
15	5.99 (d)	6.07 (d)	6.02 (d)	6.08 (d)	6.00 (d)
16	6.12 (d)	6.20 (d)	6.15 (d)	6.17 (d)	6.12 (d)
17	0.88 (s)[c]	0.89 (s)[d]	0.90 (s)[e]	0.90 (s)[f]	0.88 (s)[g]
18	0.83 (s)[c]	0.86 (s)[d]	0.88 (s)[e]	0.83 (s)[f]	0.83 (s)[g]
CH_3CO- . . .	2.05 and 2.06 (s's)	2.04 and 2.11 (s's)	2.08 and 2.10 (s's)	2.06 and 2.09 (s's)	2.06 (s)
CH_3CH_2CO- .					1.10 (t)
CH_3CH_2CO- .					2.33 (q)

[a]δ Values ($CDCl_3$) are in ppm from TMS. *J* values are reported in Table 3.
[b]Superimposed on CH_3CO-.
[c-g]Values with identical superscript within each column may be interchanged.

Nuclear Overhauser enhancement difference spectral studies (nOeds) were performed with **2** in order to determine the stereochemical differences between the two isomers. As expected from the values of the chemical shifts of 7-H and 8-H (2,3), the stereochemistry of the C-8 double bond was observed to be *E*. Thus, no enhancement of 10-H was observed when 8-H was irradiated, whereas this proton was found to be within nOe proximity with those linked to C-1. The latter experiment, in conjunction with the enhancement of the 5-H_2 signal measured when 7-H was irradiated, allowed the stereochemistry of the C-6 double bond to be assigned as *E*. This result was rather surprising, since, when comparing the ^{13}C-nmr data of **2** with those of gracilin B (see Table 1), we did not observe the difference of chemical shift for the C-1 and C-5 resonances which could be expected if the two compounds had opposite configurations at the C-6 double bond as well (4). This led us to reinvestigate the stereochemistry of gracilin B. The initial assignment of the Δ^6 configuration as *Z* was based on the enhancement of 1-H_2 signals observed in a nOe difference experiment when 7-H was irradiated (1). An error could have arisen from a not perfectly selective irradiation on 7-H

TABLE 3. *J* (Hz) for Compounds **1-5**

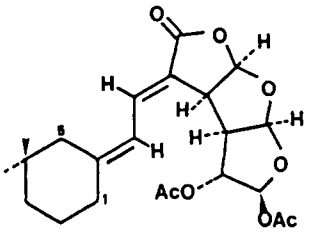
gracilin-B (**3**)	**2**	**1**	**5**	**4**
3-2=6.2	1-2=6.2	1-2=6.2	1-2=6.2	3-2=6.2
7-8=12.4	3-2=5.8	3-2=6.2	3-2=5.8	7-8=12.4
10-11=12.4	7-8=12.4	7-8=12.1	7-8=12.4	10-11=12.4
10-15=6.5	8-10=1.3	10-11=12.1	8-10=2.2	10-15=6.5
11-16=6.2	10-11=12.4	10-15=6.6	10-11=12.4	11-16=6.2
	10-15=6.5	11-16=6.2	10-15=6.5	CH_3CH_2CO=7.3
	11-16=6.2		11-16=6.0	

due to the similarity of its ^{1}H-nmr resonance to that of 8-H. Additional nOe studies confirmed this hypothesis. In fact, enhancements of the 8-H and 7-H signals were observed by irradiation at 1-H_2 and 5-H_2 resonances, respectively, thus indicating that the correct stereostructure for gracilin B should be **3** rather than **1**.

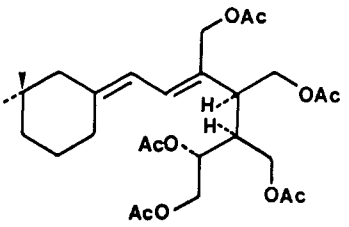
Confirmatory evidence arose from the analysis of ^{13}C-nmr data (Table 1) of the other two possible geometric isomers [6*Z*,8*Z* (**1**) and 6*Z*,8*E* (**5**)] of gracilin B prepared by its photochemical interconversion, which showed remarkable differences of chemical shifts of C-1 and C-5 resonances due to inversion of the C-6 double bond configuration.

Gracilin D (**4**) has the composition $C_{23}H_{30}O_8$ on the basis of hrms and ^{13}C-nmr spectroscopy, and showed ir and uv absorptions that clearly defined the same functionalities as in gracilin B (**3**). Compound **4** differed from **3** only in the high field region of their respective ^{1}H- and ^{13}C-nmr spectra. While three ester-type carbonyls persisted in the ^{13}C-nmr, only one acetate methyl signal was observed in the ^{1}H-nmr spectrum of **4**; instead a methylene quartet at δ 2.33 (J=7.3 Hz) and a 3H triplet at δ 1.10 (J=7.3 Hz) suggested a propionate ester. The presence of an additional sp^3 methylene (δ 27.43) in the ^{13}C-nmr spectrum of **4** and its mass spectrum [intense ions at *m/z* 374 (M^+-AcOH), 318 (M^+-AcOH-CH_3CHCO or M^+-CH_3CH_2COOH-CH_2CO), and 300 (M^+AcOH-CH_3CH_2COOH)] substantiated this hypothesis. Tables 1-3 offer a tabulated comparison of the ^{1}H- and ^{13}C-nmr data of gracilin B (**3**) and D (**4**).

The above data indicate that an acetate in **3** is replaced by a propionte in **4**. This conclusion and relationship between the two compounds was confirmed by LAH reduction of **4** followed by acetylation, which gave the same pentaacetate as that obtained from **3** under the same conditions (1), and to which, on the basis of the above considerations, structure **6** must be assigned.



5



6

The location of the propionate ester, however, remained to be established. Direct evidence for such an assignment was provided by long range ^{13}C-^{1}H shift correlated 2D-nmr spectroscopy, through which it was possible to assign the three carbonyl resonances to the respective carbon atoms. The signal at δ 173.29 was seen to correlate with the protons of the methylene quartet at δ 2.33 and of the methyl triplet at δ 1.10, whereas the carbon resonating at δ 169.68 was shown to be long range coupled with the acetate methyl at δ 2.06, thus indicating that the two signals belong to the propionate and acetate group, respectively. That the remaining carbonyl resonance (δ 167.28) was to be assigned to the lactone was secured by its correlation with 8-H. On the other hand, in the contour plot of the 2D spectrum of **4**, a cross peak correlating the carbonyl at δ 169.68 and the proton at δ 6.17 (13-H) clearly indicated the location of the acetate at C-13. Unfortunately, no correlation between the carbonyl at δ 173.29 and 12-H could be observed, perhaps due to the low intensity of the pertinent cross peak.

In order confirm the above assignments we examined the fully coupled ^{13}C-nmr spectrum of **4**. The carbonyls resonating at δ 173.29 and 169.68 appeared as two multiplets that were simplified by selective irradiations at 12-H (δ 5.10) and 13-H (δ 6.17)

resonances, respectively, thus unambigously confirming the locations of the two ester functions.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—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- and ^{13}C-nmr experiments were performed on a Bruker WM 250 spectrometer in $CDCl_3$ solutions (0.1 M) with TMS as internal reference with $\delta=0$. 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 spectra were carried out using a Bruker microprogram. The shift correlation with polarization transfer via ^1J-coupling experiments were performed adjusting the fixed delays D_3 and D_4 to give maximum polarization for $J_{C\text{-}H}=135$ Hz when ^1J couplings were used; for detecting ^{13}C-^{1}H long-range correlations D_3 and D_4 were adjusted to give maximum polarization for $J_{C\text{-}H}=6.25$ and 7.25 Hz in two different experiments.

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

Melting point was determined on a Kofler apparatus and is uncorrected.

Reverse phase hplc was carried out on a Waters instrument equipped with a differential refractometer. The column used was a μ-Bondapak C_{18} (7.8 mm × 30 cm, Waters).

BIOLOGICAL MATERIAL.—*S. gracilis* was collected by hand at about a 10-m depth in July 1984, along the coast of the Bay of Naples, near Capo Miseno. A voucher specimen is on file at our laboratories.

ISOLATION OF GRACILIN C (**2**) AND GRACILIN D (**4**).—Fresh tissue (17.5 g, dry weight after extraction) from *S. gracilis* was extracted three times with $CHCl_3$-MeOH (1:1) in the dark. The combined lipid extracts were evaporated under reduced pressure, and the residue (3.85 g) was chromatographed on a silica gel column (200 g), eluted with increasing amounts of Et_2O in *n*-hexane (from 0 to 30%), under a slight pressure of N_2. The fractions eluted with *n*-hexane-Et_2O (75:25) yielded a mixture of **2** and **4** homogenous by tlc; the successive fractions (*n*-hexane-Et_2O, 7:3) yielded the previously described gracilin B (**3**) (1) as a pure compound. The separation of **2** and **4** was carried out with a μ-Bondapak C_{18} column using Me CN-H_2O (7:3) as eluent to give 18 mg of **2** and 42 mg of **4**.

Gracilin C (**2**).—Mp 240-241° (MeOH); $[\alpha]^{25}$D +273.30 (c 1.4, $CHCl_3$); ir (CS_2) 1760, 1745, 1630 cm^{-1}; uv λ max (MeOH) 292 nm (ε 19600); ^{1}H nmr (see Tables 2 and 3); ^{13}C nmr (see Table 1); ms, *m/z* (rel. int.) 420 (M^+, 6), 360 (M^+-AcOH, 24), 318 (M^+-AcOH-CH_2CO, 100), 313 (10), 300 (M^+-2AcOH, 10), 282 (8), 271 (17), 259 (3), 254 (7), 250 (2), 243 (16), 231 (10), 218 (12), 203 (8), 189 (7), 173 (6), 161 (4), 149 (15), 109 (2); hrms *m/z* 420.1783 ($C_{22}H_{28}O_8$ requires 420.1790).

Gracilin D (**4**).—Oil; $[\alpha]^{25}$D +130.10 (c 1.1, $CHCl_3$); ir (CS_2) 1760, 1745, 1630 cm^{-1}; uv λ max (MeOH) 296 nm (ε 23150); ^{1}H nmr (see Tables 2 and 3); ^{13}C nmr (see Table 1); ms *m/z* (rel. int.) 434 (M^+, 6), 374 (M^+-AcOH, 10) 318 (M^+-AcOH-CH_3CHCO or/and M^+-CH_3CH_2COOH-CH_2CO, 100), 306 (9), 300 (M^+-AcOH-CH_3CH_2COOH, 7), 282 (5), 271 (22), 259 (6), 254 (7), 243 (17), 231 (10), 218 (7), 205 (18), 189 (6), 177 (5), 163 (6), 149 (25), 133 (5), 101 (4); hrms *m/z* 434.1941 ($C_{23}H_{30}O_8$ requires 434.1947).

REDUCTION AND ACETYLATION OF GRACILIN D (**4**).—$LiAlH_4$ (100 mg) was added to a solution of gracilin D (**4**, 20 mg) in dry Et_2O (5 ml), and the reaction mixture was stirred at room temperature. After 20 min, the excess reagent was destroyed with EtOAc, and the product was partitioned between dilute HCl and EtOAc. The organic layer was washed with H_2O, dried over Na_2SO_4, and taken to dryness. The crude mixture was acetylated with Ac_2O-pyridine (1:1) for 16 h at room temperature. The usual work up and purification by tlc on silica gel plates (0.25-mm thickness) developing with Et_2O-*n*-hexane (8:2) gave the expected pentaacetate **6** (5 mg), identical in all respects with the product obtained from gracilin B (**3**) in the same experimental conditions (1).

PHOTOCHEMICAL INTERCONVERSION OF GRACILIN B (**3**) INTO GRACILIN C (**2**), **1**, AND **5**.—A solution of gracilin B (**3**) (100 mg) in 200 ml of EtOH was irradiated at room temperature for 4 h with a halogen lamp (Osram, 220 V, 650 W). The solution was evaporated under reduced pressure, and the crude residue was observed to contain **1**, **2**, **3**, and **5**, in the approximate ratio of 1:1:1:1 by its nmr spectrum analysis. This material was purified by analytical tlc (SiO_2, eluent Et_2O-*n*-hexane, 7:3), to give the starting gracilin B (**3**) and gracilin C (**2**) as pure samples and a mixture of **1** and **5**. Compound **2** was shown to be identical to the natural product by direct comparison of their chromatographic and spectroscopic properties. Compounds **1** and **5** were finally separated by hplc on a μ-Bondapak C_{18} column (eluent 30% aqueous acetonitrile); 18 mg of each isomer were obtained.

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