Notes

Briarane Diterpenes from the Indian Ocean Gorgonian *Gorgonella umbraculum*

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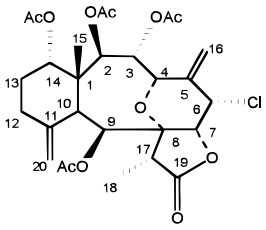
Received August 7, 1996

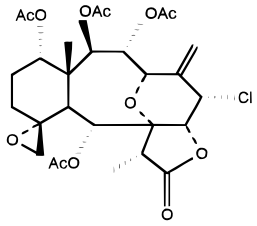
Three briarane diterpenes, junceellin (**1**), praelolide (**2**), and compound **3** (of which **3** is new), were isolated from the gorgonian *Gorgonella umbraculum* (EII & Sol), and their structures were established on the basis of their spectral data.

As part of our investigation of marine organisms of the Indian seas, we have examined the "red type" gorgonian *Gorgonella umbraculum* (EII & Sol)[1] (family Ellisellidae), and in this paper we present the isolation and structure elucidation of three briarane diterpenes **1**, **2**, and **3** from this organism.

The gorgonian was collected from the Tuticorin area of the Bay of Bengal and was extracted with EtOH; the residue from the EtOH extract was taken into EtOAc. The EtOAc solubles were chromatographed on a SiO_2 column with hexane through EtOAc eluents. Extensive rechromatography of some of the resulting fractions yielded three briarane diterpenoids, **1**, **2**, and **3**.



1



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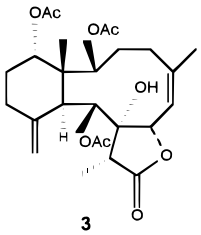

3

Compounds **1** and **2** were identified as the briarane diterpenes junceellin[2−4] and praelolide,[4−6] respectively, on the basis of their NMR data (Table 1)[7,8] and, in the case of **1,** by X-ray analysis also,[9] which agreed completely with the X-ray structure of junceellin reported by Yao et al.[3]

Compound **3**, a new diterpene, had the molecular formula of $C_{26}H_{36}O_8$. The IR spectrum showed hydroxyl, γ-lactone, and acetate bands. The ^1H and ^{13}C NMR spectra as well as the COSY and HETCOR spectra showed that **3** had a lactone carbonyl (δ 176. 1), three acetate groups, an exocyclic double bond [150.9, 113.1, 5.04 s (1H), 4.93 s (1H)], a methyl substituted (Z)-trisubstituted double bond [145.2, 120.3, 5.65 d, $J = 9.7$ Hz (1H); 26.8, 1.98 s (3H)], a tertiary hydroxyl group (82.9), four oxymethine carbons [78.0, 4.84 br (1H); 74.4, 4.66 br (1H), 74.3, 5.29 d, $J = 9.7$ Hz (1H); 71.3, 5.32 d, $J = 6.1$ Hz (1H)], an aliphatic quaternary carbon (46.8), two aliphatic methine carbons [42.5, 2.47 q, $J = 7.0$ Hz (1H); 41.8, 3.43 d, $J = 6.1$ Hz (1H)], a secondary methyl group [6.5, 1.12 d, $J = 7.0$ Hz (3H)], and a tertiary methyl group [15.6, 1.12 s (3H)]. These data indicated that **3** possessed the same carbon skeleton (including the γ-lactone system) as compounds **1** and **2**.

The trisubstituted double bond in **3** could be present at Δ^5 or Δ^{11}. In the former case, the olefinic proton (H-6) would be coupled to an oxymethine proton (H-7), but in the latter case the olefinic proton (H-12) would be coupled to an aliphatic proton (H-13). A COSY spectrum of **3** showed connectivity between the olefinic proton (δ 5.65 d) and an oxymethine proton (5.29 d). Therefore, the trisubstituted double bond in **3** was located at Δ^5; consequently, the exocyclic double bond was at $\Delta^{11(20)}$.

The COSY spectrum also showed connectivity between the H-10 proton (δ 3.43 d) and an oxymethine proton (5.32 d). Because C-1 and C-11 carbons do not have hydrogens, the oxymethine proton signal of 5.32 d must be due to the H-9 proton. Because the H-10 signal (3.43 d) is a doublet, C-9 can only have one hydrogen and therefore bears a substituent. Thus, one of the three acetate groups could be assigned to C-9 in **3.**

As mentioned above, the H-7 and H-9 protons were observed only as doublets, indicating that they were coupled only to H-6 and H-10, respectively. If there were a hydrogen at C-8 the above signals would have been further split. Thus, the C-8 carbon does not have

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S0163-3864(96)00576-9 CCC: $15.00 © 1998 American Chemical Society and American Society of Pharmacognosy
Published on Web 07/25/1998

Table 1. ^{1}H and ^{13}C NMR Chemical Shifts for **1**−**3**[a]

	1		**2**		**3**	
position	C	H	C	H	C	H
1	47.4		46.9		46.8	
2	77.5	5.43 d (6.6)	78.5	5.39 d (6.9)	78.0	4.84 br
3	78.8	6.14 dd (10.9, 6.6)	74.0	6.19 dd (10.8,6.9)	29.1[b]	2.59 m[b]
4	63.7	4.48 d (10.9)	64.0	4.47 d (10.8)	26.9 b	2.05 m[b]
5	134.2		134.4		145.2	
6	53.9	5.02 q (2.6)	54.0	4.96 d (2.8)	120.3	5.65 d (9.7)
7	79.1	4.52 d (2.6)	79.1	4.40 d (2.8)	74.3	5.29 d (9.7)
8	82.7		82.9		82.9	
9	74.5	5.95 s	72.9	5.59 s	71.3	5.32 d (6.1)
10	44.0	3.11 s	41.0	2.83 s	41.8	3.43 d (6.1)
11	147.2		56.2		150.9	
12	27.5	2.27 m	24.6	2.15 m, 1.29 m	26.4[b]	1.81 m[b]
13	32.6	1.75 m	29.3	1.89 m	31.1[b]	2.22 m[b]
14	72.8	4.97t (2.5)	71.0	4.99br	74.4	4.66 br
15	15.0	1.12 s	15.8	1.24 s	15.6	1.12 s
16	119.6	5.57 d (2.2), 5.36 d (2.2)	119.4	5.56 d (2.0), 5.35 d (2.0)	26.8	1.98 s
17	49.9	2.78 q (7.0)	49.5	2.79 q (7.0)	42.5	2.47 q (7.0)
18	7.1	1.29 d (7.0)	7.3	1.32 d (7.0)	6.5	1.12 d (7.0)
19	174.2		174.2		176.1	
20	111.9	5.10 s, 4.76 s	51.3	2.65 d (3.0), 2.45 d (3.0)	113.1	5.04 s, 4.93 s
			acetate carbonyls and accetate methyls			
	170.4	2.33 s	170.2	2.31 s	170.5	2.21 s (6H)
	170.0	2.07 s	169.9	2.09 s	170.4	1.91 s (3H)
	169.8	2.05 s	169.8	2.06 s	169.5	
	169.7	2.00 s	169.6	2.00 s	21.7	
	21.0		21.1		21.2	
	21.0		20.9		21.1	
	20.5		20.4			
	20.4		20.3			

[a] In ppm: solvent $CDCl_3$. The assignments were made on the basis of ^{1}H−^{1}H COSY, and short-range ^{13}C−^{1}H correlations. Proton spectra were recorded at 400 MHz, and carbon spectra at 100 MHz on a Bruker 400 WM instrument. Carbon assignments are supported by DEPT experiments. ^{1}H (δ) values are followed by the multiplicities and coupling constants J (Hz). [b] The signals in the same column may be interchanged.

a hydrogen but possesses a substituent. Therefore, the tertiary hydroxy group could be assigned to the C-8 position.

The chemical shifts of the four methylene carbons in **3** are observed at δ 31.1, 29.1, 26.9, and 26.4, of which the highest observed value (31.1) is less than the value expected for a methylene carbon an either side of the quaternary carbon C-1, namely C-2 or C-14. Therefore, the methylene groups could be present only at C-3, C-4, C-12, and C-13. Consequently, positions C-2 and C-14 in **3** are substituted by the remaining two acetate groups.

The relative stereochemistry of **3** was determined by analogy with naturally occurring briarane diterpenes and from its NOE difference spectra. Naturally occurring briaranes have the C-15 methyl group in the β-orientation and the H-10 in the α-orientation. Further, in the briaranes having the β-hydroxy-γ-lactone system, the OH group (at C-8) and the C-18 methyl group are α-oriented. Compound **3**, too may, be assumed to have a similar orientation at these positions.

Irradiation of H-10 produced NOEs at H-2 (13.8%), H-20 (δ 5.04, 4.9%), H-9 (3.7%), and H-6 (3.3%). H-2 is thus on the same side as H-10; consequently, the C-2 acetate is β-oriented. The NOE interaction between H-10 and H-6 suggested that the Δ^5 double bond in the 10-membered ring is oriented in such a way that the H-6 is on the same side as H-10, and the H-6 and H-7 are antiparallel. The large coupling observed (9.7 Hz) confirms the antiparallel arrangement of H-6 and H-7 and the β-orientation of H-7. Irradiation of H-16 produced NOEs at H-6 (4.5%) and H-2 (2.5%), confirming the (*Z*)-nature of the Δ^5 double bond.

Irradiation of H-15 and H-18 (both have the same chemical shift) produced NOEs at H-14 (3.1%) and H-9 (1.3%). Thus, H-14 is β-oriented, and the C-14 acetate α-oriented. The small NOE (1.3%) observed for H-9 is indicative that it is present in the α-quasiequatorial position. The overall relative stereochemistry of **3** is therefore 1R*, 2S*, 7R*, 8R*, 9S*, 10S*, 14S*, and 17R*.

Compound **3** showed weak antibacterial activity[10] against *Bacillus pumilus* at 500 μg/mL concentration level. Praelolide (**2**) was reported[4] to show antiviral activity against Herpes simplex viruses I and II. *Gorgonella umbraculum* is used in traditional medicine in the Tuticorin region; the presence of the briaranes in this organism may be responsible for its activity.

Experiemental Section

General. Si gel (100−200 mesh, ACME brand) was used for column chromatography and Si gel-G (ACME brand) for TLC. IR spectra and optical rotations were taken in $CHCl_3$. FABMS were taken in *m*-dinitrobenzyl alcohol matrix, and the Cl spectra, with ammonia.

Extraction of the Gorgonian. The dried organism was cut into small pieces and the cut material (3.5 kg) was soaked in EtOH (15 L). After soaking the material for a few days, the solvent was decanted and the material was extracted six more times with EtOH. The solvent was removed from the extract and the combined dark residue extracted repeatedly with EtOAc. Re-

moval of EtOAc from the extract gave a dark oil (55 g), which was chromatographed over a SiO_2 gel (500 g) column (60 mm × 1 m) using solvent mixtures of increasing polarity from *n*-hexane through EtOAc. Further purification of some of the fractions gave junceellin (**1**) (0.4 g), praelolide (**2**) (0.2 g), and compound **3** (0.05 g).

Junceellin (1): obtained from the 9:1 hexane−EtOAc eluents as colorless plates ($CHCl_3$); mp 240−242 °C; $[\alpha]^{30}_D$ −13° (*c* 0.95); (lit.[2], mp 272−274°); R_f 0.61 (hexane−EtOAc 8:2); IR ν_{max} 1792, 1740, 1603, 928, and 878 cm^{-1}; EIMS *m*/*z* 583 (8), 425 (25), 243 (100%); FABMS *m*/*z* 583/85 [M + 1]; CIMS [M + NH_4] *m*/*z* 600.2210 (calcd for $C_{28}H_{39}O_{11}$ NCl, 600.2213).

Praelolide (2): obtained from the 8.5:1.5 hexane−EtOAc eluents as colorless crystals ($CHCl_3$); mp 302−305 °C; $[\alpha]^{30}_D$ −28° (*c* 1.0); (lit.,[5] mp 265−267°); R_f 0.44 (hexane−EtOAc 3:2); IR ν_{max} 1792, 1742, 1603, 930, and 878 cm^{-1}; EIMS *m*/*z* 600 (18), 243 (100%); FABMS *m*/*z* 599/601 [M + 1]; CIMS [M + NH_4] *m*/*z* 616.2170 (calcd for $C_{28}H_{39}O_{12}$ NCl, 616.2162).

Compound 3: eluted from 87.5: 12.5 hexane−EtOAc eluents as colorless crystals ($CHCl_3$); mp 217−219 °C; $[\alpha]^{30}_D$ −37° (*c* 0.51); R_f 0.54 (hexane−EtOAc 7:3); IR ν_{max} 3466, 1792, 1730, 928, and 877 cm^{-1}; EIMS *m*/*z* 404 (15), 282 (15), 133 (100), 119 (84), 107 (96), 91 (98%); FABMS *m*/*z* 493 [M + 1]; CIMS [M + NH_4] *m*/*z* 510.2703 (calcd for $C_{26}H_{40}O_9N$, 510.2704).

Acknowledgment. We thank Dr. P. A. Thomas, Office-incharge, CMFRI, Vizhinjam, Kerala, India, for the identification of the gorgonian; the Regional Sophisticated Instrumentation Centre, CDRI, Lucknow, for the FABMS; the UGC New Delhi, for the COSIST and DRS program facilities at AU and CSIR, New Delhi, for a SRF to R.K. We are also grateful to Professor M B Hursthouse (EPSRC X-ray Crystallography service, Cardiff) for the X-ray analysis of **1**.

References and Notes

(1) CMFRI, Cochin, India, Bulletin no.74, August 1987.
(2) Lin, Y.; Long, K. *Zhongshan Daxue Xuebao, Ziran Kexueban* **1983**, *2*, 46.
(3) Yao, J.; Qian, J.; Fan, H.; Shih, K.; Huang, S.; Lin, Y.; Long. K. *Zhongshan Daxue Xuebao, Ziran Kexueban* **1984**, *1*, 83.
(4) Shin, J.; Park, M.; Fenical, W. *Tetrahedron* **1989**, *45*, 1633.
(5) Luo, Y.; Long. K.; Fang, Z. *Zhongshan Daxue Xuebao, Ziran Kexueban* **1983**, *1*, 83; Chem. Abstr. **1983**, 99:50572c.
(6) Dai, J.; Wan, Z.; Rao, Z.; Liang, D.; Fang, Z; Luo, Y.; Long, K. *Sci. Sin.*, (*Ser. B*) **1985**, *28*, 1132.
(7) A comparison of our samples of junceellin and praelolide with literature samples could not be made as they were not available. Professor W. Fenical of the Scripps Institution of Oceanography, San Diego, CA, informed us that in their publication[4] they did not describe new structures for junceellin and praelolide but only reiterated a structure assignment published earlier by Chinese workers.[2−6]
(8) Our assignments for these compounds at the H-2 and H-4 signals are the reverse of the literature assignments for the same.[4] Our C-4 signal assignment also differs from literature.[4] Our assignments, however, are made by COSY and HETCOR experiments unlike lieterature assignments, which were not supported by HETCOR studies but were made by comparison with known compounds.
(9) Crystallographic data of compound **1** have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK.
(10) By the agar cup-plate diffusion method[11] on nutrient agar medium (high media).
(11) Cruickshank, R.; Duguid, J. P.; Swin, R.H.A. In *The Practice of Medical Microbiology*; Churchill, Livingstone: London, 1975; Vol. 2, 12th ed., p 110.

NP960576V