Four New Briarane Diterpenoids from the Gorgonian Coral *Junceella fragilis*

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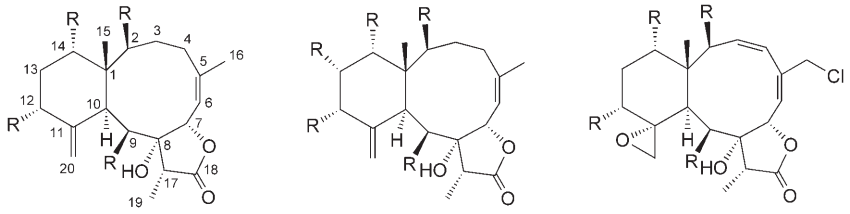
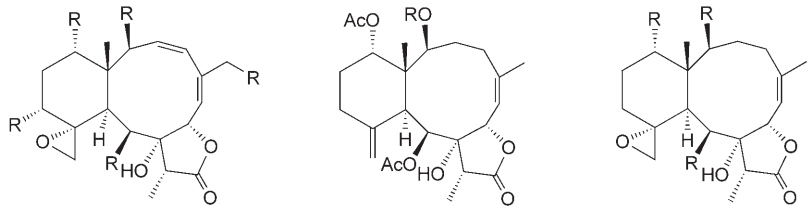
Chemical investigation of the gorgonian coral *Junceella fragilis*, collected by scuba diving in Taiwan, resulted in the isolation of four new briarane-type diterpenoids, frajunolides A–D (**1**–**4**), along with three known briaranes. Their structures were elucidated on the basis of spectroscopic studies, especially 1- and 2D-NMR as well as HR-MS experiments. The inhibitory effect of all isolated metabolites towards superoxide-anion generation and elastase release by human neutrophils in response to formylmethionyl-leucyl-phenylalanine/dihydrocytochalasin B (FMLP/CB) was evaluated.

Introduction. – The search for novel anti-inflammatory agents from marine organisms has been intense in the last three decades. Marine creatures constitute a large part of the ecosystem, and they recently became a potential source of secondary metabolites of wide structural diversity, being of medicinal and economic interest. The gorgonians of the genus *Junceella* grow in the Indo-West Pacific regions as whip-shaped, unbranched colonies with variable color [1]. They assemble a variety of highly oxidized briarane-type diterpenoidal γ-lactones with bicyclic six- and ten-membered rings (3,8-cyclized cembranoids) [2–5]. Most of these briaranes are endowed with biological activities such as cytotoxic [6–9], anti-inflammatory [10], immunomodulatory [11], antiviral [12], and insecticidal [13][14] effects. Several briarane derivatives have been isolated from the gorgonian *Junceella fragilis* RIDLEY (Ellisellidae) [15–18].

In continuation of our chemical studies of the local fauna [19][20], we chemically investigated *J. fragilis* collected off the eastern coast of Taiwan. Chromatographic fractionation of an acetone extract of the title species now led to the isolation of four new briarane-type diterpenoids named frajunolides A–D (**1**–**4**), together with the known constituents junceellolide E (**5**) [16], umbraculolide A (**6**) [21], and 11α,20α-epoxy-4-deacetoxyjunceellolide D (**7**) [15]. Their structures were determined on the basis of spectroscopic analysis, especially NMR and HR-ESI-MS, and their inhibitory effects on superoxide-anion generation and elastase release by human neutrophils in response to formylmethionyl-leucyl-phenylalanine/dihydrocytochalasin B (FMLP/CB) was investigated.



1 R = AcO **2** R = AcO **3** R = AcO



4 R = AcO **5** R = EtCO **6** R = Ac **7** R = AcO

Results and Discussion. – 1. *Structure Elucidation*. The HR-ESI mass spectrum of **1** revealed the $[M+Na]^+$ ion peak at *m/z* 573.2292, consistent with the molecular formula $C_{28}H_{38}O_{11}$ and ten degrees of unsaturation. The IR spectrum displayed absorption bands diagnostic of OH, five-membered lactone, and ester functionalities. Both the ^{1}H- and ^{13}C-NMR spectroscopic data (*Tables 1* and *2*, resp.) indicated the presence of four AcO units, a lactone ring, and two C=C bonds, suggesting two additional rings.

The NMR spectra of **1** revealed four AcO groups at δ(H) 1.89, 1.97, 2.09, 2.19 [δ(C) 21.0 (2 C-atoms), 21.1, 21.6; 169.2, 169.9, 170.2, 170.5]. The ^{13}C-NMR signal at δ(C) 176.0 (C(18)) was assigned to a five-membered lactone ring, together with an oxymethine at δ(C) 77.6 (C(7)) and an oxygenated quaternary C-atom at δ(C) 83.1 (C(8))[1]. The *singlet*s at δ(H) 5.19 and 5.06, along with the CH_2 signal at δ(C) 110.1, were ascribed to an exocyclic methylidene group. A trisubstituted C=C bond was evident from δ(H) 5.71 (*d*, H–C(6), δ(C) 120.3 (C(6)), and δ(C) 145.0 (*s*, C(5)). Three Me signals appeared at δ(H) 1.23 (*s*), 2.07 (*s*), and 1.09 (*d*), and were assigned to H–C(15), H–C(16), and H–C(19), respectively, with the aid of HMBC correlations (*Fig. 1*).

The spectroscopic data of **1** were consistent with a tetra-acetylated briarane skeleton comprising a five-membered lactone ring, an exocyclic methylidene, and one trisubstituted C=C bond [4][11]. The ^{1}H,^{1}H-COSY spectrum of **1** exhibited connectivities of H–C(2)/H–C(3)/H–C(4), H–C(6)/H–C(7), H–C(12)/H–C(13)/H–C(14), and H–C(17)/H–C(19). The HMBC spectrum displayed correlations between H–C(7)/C(8), H–C(9)/C(7), H–C(12)/C(20), H–C(14)/C(2), H–C(15)/C(2), H–C(16)/C(6), and H–C(20)/C(10), in addition to the multiple correlations of

[1]) Arbitrary atom numbering.

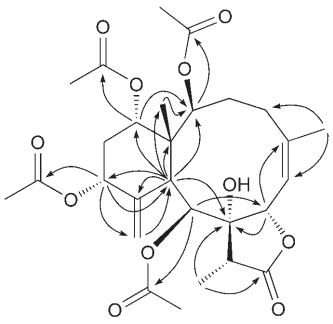


Fig. 1. *Key HMBC (H → C) correlations for* **1**

H–C(10) with C(1), C(2), C(8), C(11), C(12), C(14), and C(15), respectively. The above HMBC correlations were in good agreement with the proposed briarane structure, and located the C=C bond at C(5), the methylidene at C(11), the OH group at C(8), and the lactone ring between C(18) and C(7). The four oxygenated CH at δ(H) 4.79, 5.37, 5.35 , and 4.66 were bonded to the C-atoms at δ(C) 74.4, 70.5, 67.3, and 73.8, and assigned, respectively, to C(2), C(9), C(12), and C(14). The four AcO groups were positioned by tracing HMBC cross-peaks between each carbonyl C-atom to the respective methine H-atom.

The relative configuration of **1** was determined through analogy with naturally occurring briarane diterpenes, and from its NOESY spectrum. Naturally occurring briaranes have the Me(15) group in β-orientation and H–C(10) in α-orientation [12]. The absence of any NOESY correlation between H–C(10) and Me(15) confirmed that the orientations of these two H-atoms were α and β, respectively. The NOESY correlation between H–C(6) and Me(16) was consistent with a (*Z*)-configured C=C bond at C(5). The NOESY correlations between Me(15) and both H–C(12) and H–C(14), and between H–C(7) and H–C(17), together with the absence of any correlation between H–C(10) and either H–C(12) or H–C(14), implied β-orientations for H–C(7), H–C(12), H–C(14), and H–C(17). In addition, the correlation of H–C(10) and both H–C(2) and H–C(9), and of H–C(9) and Me(19) favored the α-orientation of H–C(2), H–C(9), and Me(19). Hence, The structure of **1** was determined as *rel*-(1*S*,2*S*,5*Z*,7*S*,8*S*,9*S*,10*S*,12*R*,14*S*,17*R*)-2,9,12,14-tetraacetoxy-8-hydroxybriara-5,11(20)-dien-18,7-olide[2]), and named *frajunolide A*.

The HR-ESI mass spectrum of **2** revealed the molecular formula $C_{30}H_{40}O_{13}$, with eleven degrees of unsaturation. The NMR spectra of **2** (*Tables 1* and *2*) revealed a very close relationship with **1**, showing the same functionalities, but five instead of four AcO units. Detailed inspection of the ^{1}H- and ^{13}C-NMR data of **2** indicated the presence of five AcO *singlet*s at δ(H) 1.97 (9 H), 2.09 (3 H), and 2.21 (3 H) [δ(C) 20.8 (3 C), 21.1, 21.6], and five C=O moieties at δ(C) 169.3, 169.6 (2 C), 170.4, and 170.6. Five oxymethines resonated at δ(H) 4.94, 5.53, 5.53, 5.25–5.28, and 5.01, and were assigned to H–C(2), H–C(9), H–C(12), H–C(13), and H–C(14), each showing HMBC cross-peaks with their respective carbonyl C-atoms. The additional AcO group accounted for the increase of 58 mass units in the molecular weight of **2** compared to **1**,

[2]) For systematic names, see *Exper. Part*.

Table 1. *^{1}H-NMR Data of* **1**–**4**. At 300 MHz in $CDCl_3$; δ in ppm, J in Hz. Arbitrary atom numbering. Asterisks (*) indicate overlapping signals.

Position	**1**	**2**	**3**[a])	**4**
2	4.79 (br. *s*)	4.94 (*d*, *J* = 6.2)	5.65*	5.71 (*d*, *J* = 9.6)
3	2.38–2.45 (*m*)	2.42–2.49 (*m*)	5.64*	5.64 (br. *d*, *J* = 9.5)
	1.70–1.75 (*m*)	1.70–1.74 (*m*)		
4	2.57–2.63 (*m*)	2.57–2.63 (*m*)	6.34–6.38 (*m*)	6.28 (*d*, *J* = 9.8)
	2.14–2.18 (*m*)	2.14–2.10 (*m*)		
6	5.71 (*d*, *J* = 9.9)	5.55*	6.05 (*d*, *J* = 9.0)	5.74 (*d*, *J* = 8.3)
7	5.24 (*d*, *J* = 9.9)	5.28–5.32 (*m*)	4.97 (*d*, *J* = 9.0)	4.99 (*d*, *J* = 8.3)
9	5.37 (*d*, *J* = 5.9)	5.53*	4.81 (*d*, *J* = 4.5)	4.81 (*d*, *J* = 4.5)
10	3.54 (*d*, *J* = 5.9)	3.68 (*d*, *J* = 3.1)	3.67 (*d*, *J* = 4.5)	3.69 (*d*, *J* = 4.5)
12	5.35 (*d*, *J* = 7.7)	5.53*	4.50 (*t*, *J* = 3.0)	4.48 (br. *s*)
13	2.52–2.58 (*m*)	5.22–5.28 (*m*)	2.23–2.29 (*m*)	2.31–2.36 (*m*)
	1.60–1.64 (*m*)		1.87–1.93 (*m*)	1.89–1.93 (*m*)
14	4.66 (*d*, *J* = 4.3)	5.01 (*d*, *J* = 3.6)	4.90 (*t*, *J* = 3.0)	4.92 (br. *s*)
15	1.23 (*s*)	1.17 (*s*)	1.05 (*s*)	1.05 (*s*)
16	2.07 (*s*)	2.03 (*s*)	4.61 (*s*)	5.37 (*d*, *J* = 15.8)
				4.81 (*d*, *J* = 15.8)
17	2.46 (*q*, *J* = 7.2)	2.55 (*q*, *J* = 7.0)	2.29–2.33 (*m*)	2.28–2.34 (*m*)
19	1.09 (*d*, *J* = 7.2)	1.13 (*d*, *J* = 7.0)	1.17 (*d*, *J* = 7.0)	1.16 (*d*, *J* = 7.0)
20	5.19 (*s*)	5.55*	3.55 (*d*, *J* = 2.0)	3.54 (*d*, *J* = 2.3)
	5.06 (*s*)	5.33 (*s*)	2.80 (*d*, *J* = 2.0)	2.78 (*d*, *J* = 2.3)
2-AcO	1.97 (*s*)	1.97 (*s*)	1.98 (*s*)	1.96 (*s*)
9-AcO	2.19 (*s*)	2.21 (*s*)	2.20 (*s*)	2.17 (*s*)
12-AcO	2.09 (*s*)	2.09 (*s*)	2.11 (*s*)	2.12 (*s*)
13-AcO		1.97 (*s*)		
14-AcO	1.89 (*s*)	1.97 (*s*)	2.03 (*s*)	2.04 (*s*)
16-AcO				2.14 (*s*)

[a]) Recorded at 500 MHz.

as well as for the additional degree of unsaturation. The strong NOESY correlations (*Fig. 2*) between Me(15) and both H–C(14) and H–C(13), between H–C(13) and H–C(14), and between H–C(12) and H–C(13) as well as Me(15) indicated β-orientation to the former H-atoms, and α-orientation to the three adjacent AcO groups. Consequently, the structure of **2** was identified as *rel*-(1*S*,2*S*,5*Z*,7*S*,8*S*,9*S*,10*S*,12*S*,13*S*,14*R*,17*R*)-2,9,12,13,14-pentaacetoxy-8-hydroxybriara-5,11(20)-dien-18,7-olide, and named *frajunolide B*.

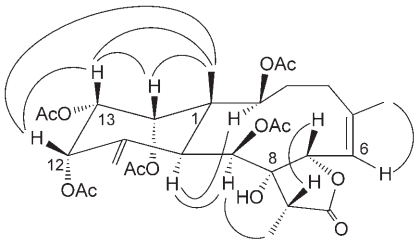


Fig. 2. *Key NOESY correlations for* **2**

Table 2. *^{13}C-NMR Data* of **1**–**4**. At 75 MHz in $CDCl_3$; δ in ppm. Arbitrary atom numbering.

Position	**1**	**2**	**3**[a])	**4**
1	46.8 (*s*)	46.3 (*s*)	47.1 (*s*)	47.2 (*s*)
2	74.4 (*d*)	73.6 (*d*)	74.2 (*d*)	74.5 (*d*)
3	31.9 (*t*)	30.9 (*t*)	132.1 (*d*)	132.6 (*d*)
4	29.0 (*t*)	28.9 (*t*)	128.0 (*d*)	127.6 (*d*)
5	145.0 (*s*)	145.3 (*s*)	140.0 (*s*)	139.8 (*s*)
6	120.3 (*d*)	119.4 (*d*)	126.0 (*d*)	122.7 (*d*)
7	77.6 (*d*)	78.1(*d*)	78.7 (*d*)	78.9 (*d*)
8	83.1 (*s*)	82.2 (*s*)	81.1 (*s*)	81.2 (*s*)
9	70.5 (*d*)	70.2 (*d*)	63.9 (*d*)	64.1 (*d*)
10	42.3 (*d*)	40.0 (*d*)	32.8 (*d*)	32.9 (*d*)
11	148.3 (*s*)	145.7 (*s*)	59.0 (*s*)	59.2 (*s*)
12	67.3 (*d*)	72.1 (*d*)	73.1 (*d*)	73.3 (*d*)
13	34.7 (*t*)	66.9 (*d*)	28.5 (*t*)	28.6 (*t*)
14	73.8 (*d*)	73.6 (*d*)	73.5 (*d*)	73.6 (*d*)
15	15.7 (*q*)	14.9 (*q*)	13.9 (*q*)	14.1 (*q*)
16	27.8 (*q*)	27.3 (*q*)	44.9 (*t*)	63.3 (*t*)
17	42.3 (*d*)	43.3 (*d*)	44.1 (*d*)	44.3 (*d*)
18	176.0 (*s*)	175.8 (*s*)	175.2 (*s*)	175.4 (*s*)
19	6.4 (*q*)	6.5 (*q*)	6.3 (*q*)	6.4 (*q*)
20	110.1 (*t*)	115.2 (*t*)	49.0 (*t*)	49.0 (*t*)
2-AcO	170.5 (*s*), 21.0 (*q*)	170.6 (*s*), 20.8 (*q*)	169.6 (*s*), 21.1 (*q*)	169.4 (*s*), 21.2 (*q*)
9-AcO	169.2 (*s*), 21.6 (*q*)	169.3 (*s*), 21.6 (*q*)	170.2 (*s*), 21.5 (*q*)	170.3 (*s*), 21.6 (*q*)
12-AcO	169.9 (*s*), 21.1 (*q*)	169.6 (*s*), 20.8 (*q*)	170.0 (*s*), 21.2 (*q*)	170.3 (*s*), 21.2 (*q*)
13-AcO		169.6 (*s*), 21.1 (*q*)		
14-AcO	170.2 (*s*), 21.0 (*q*)	170.4 (*s*), 20.8 (*q*)	169.9 (*s*), 21.1 (*q*)	170.2 (*s*), 21.2 (*q*)
16-AcO				170.3 (*s*), 20.9 (*q*)

[a]) Recorded at 125 MHz.

The HR-ESI mass spectrum of **3** disclosed the molecular formula $C_{28}H_{35}ClO_{12}$, consistent with eleven degrees of unsaturation. The presence of a Cl-atom was evidenced from the $[M-35]^+$ fragment ion and the $[M(^{37}Cl)+Na]^+$ isotope fragment, with the typical relative peak intensity in the mass spectrum [22]. The ^{1}H-NMR spectrum of **3** (*Table 1*) showed the presence of four AcO units and three olefinic H-atoms, none of which belonged to an exocyclic methylidene group. The ^{1}H-NMR spectrum contained two *gem*-coupled signals at δ(H) 3.55, 2.80 (2*d*, $J=2.0$ Hz, CH_2(20)), together with the corresponding δ(C) value at 49.0 (C(20)) and an oxygenated quaternary C-atom at δ(C) 59.0 (C(11)), indicating the presence of an exocyclic epoxide (spirocyclic oxirane) [3][9]. The HMBC spectrum of **3** showed ^2J-correlations between C(11) and each of H–C(10), CH_2(20), and H–C(12), which confirmed that the epoxide ring contained C(11) and C(20) (*Fig.* 3).

The olefinic signal at δ(H) 6.05 (*d*, $J=9.0$ Hz) was assigned to H–C(6), based on its spin-coupling with H–C(7) at δ(H) 4.97. The ^{1}H,^{1}H-COSY spectrum showed correlations between the olefinic CH at δ(H) 5.64 (H–C(3)) with two methines at δ(H) 5.65 (H–C(2)) and 6.34–6.38 (H–C(4)). The HMBC correlations of H–C(4) with the quaternary C-atom at δ(C) 140.0 (C(5)) and the methine at δ(C) 126.0 (C(6))

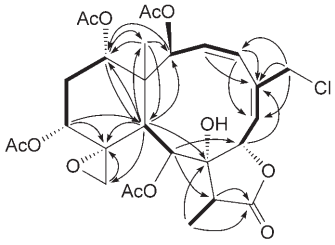


Fig. 3. *Key HMBC* (→) *and COSY* (▬) *correlations for* **3**

positioned the additional C=C bond at C(3). The CH_2 unit at δ(C) 44.9 was more shielded than expected for an oxygenated C-atom, and was correlated to the *singlet* at δ(H) 4.61 (2 H) in the HMQC spectrum. The latter CH_2 signal was ^3J-correlated with both C(4) and C(6), proving the attachment of the chloromethyl group at C(5) (*Fig.* 3).

The chemical shifts for C(11) and C(20) (δ(C) between 55 and 61 and between 47 and 52, resp.), and the NOESY correlations between CH_2(20) and both Me(15) and H–C(12) were in accordance with β-orientation of CH_2(20), (11*R*)-configuration regarding the epoxy ring, and chair conformation of the cyclohexane ring (*Fig. 4*) [23]. Other NOESY correlations were identical to those of **1**. Consequently, the structure of **3** was determined as *rel*-(1*R*,2*S*,7*S*,8*S*,9*S*,10*S*,11*R*,12*R*,14*S*,17*R*)-(2,9,12,14-tetraacetoxy-16-chloro-11,20-epoxy-8-hydroxybriara-3,5-dien-18,7-olide, and named *frajunolide C*.

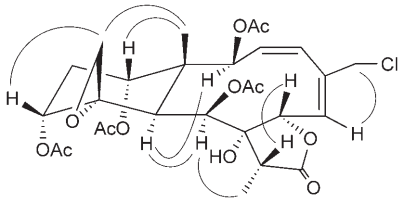


Fig. 4. *Key NOESY correlations for* **3**

Compound **4** was assigned the molecular formula $C_{30}H_{38}O_{14}$, indicating twelve degrees of unsaturation, and the absence of a Cl-atom. The NMR spectra (*Tables 1* and *2*) revealed the presence of a briarane skeleton similar to that of **3**, but with different NMR data regarding position 16. The two *gem*-coupled H-atoms at δ(H) 5.37, 4.81 (2*d*, J = 15.8 Hz) were bonded to a C-atom at δ(C) 63.3, and displayed ^2J-correlations to a quaternary C-atom at δ(C) 139.8 (C(5)) as well as ^3J-correlations with an olefinic CH at δ(C) 122.7 (C(6)) and an AcO C=O group at δ(C) 170.3. This confirmed the attachment of an AcO group at C(16). The remaining HMBC and NOESY correlations were identical with those observed for **3**. Therefore, the structure of **4** was elucidated as *rel*-(1*R*,2*S*,7*S*,8*S*,9*S*,10*S*,11*R*,12*R*,14*S*,17*R*)-2,9,12,14,16-pentaacetoxy-11,20-epoxy-8-hydroxybriara-3,5-dien-18,7-olide, and named *frajunolide D*.

2. *Biological Tests.* The *in vitro* anti-inflammatory activities of the isolated briaranes **1**–**7** on superoxide-anion generation and elastase release by human neutrophils in response to FMLP/CB was evaluated. The results are collected in *Table 3*, genistein being used as positive control (data not shown). As can be seen, compounds **3**, **5**, and **7** exhibited mild anti-inflammatory activities at a concentration of 10 μg/ml.

Table 3. *Inhibitory Effects of* **1**–**7** *on Superoxide-Anion Generation and Elastase Release by Human Neutrophils in Response to FMLP/CB*

Compound	Inhibition [%][a])	
	Superoxide anion	Elastase release
Flajunolide A (**1**)	13.93 ± 5.94	6.47 ± 3.30
Flajunolide B (**2**)	14.50 ± 5.64*	5.16 ± 6.18
Flajunolide C (**3**)	19.76 ± 5.12**	14.47 ± 5.16*
Flajunolide D (**4**)	5.61 ± 2.93	2.54 ± 2.05
Junceellolide E (**5**)	38.69 ± 6.46**	29.74 ± 7.12**
Umbraculolide A (**6**)	11.35 ± 2.54*	2.37 ± 6.80
11α,20α-Epoxy-4-deacetoxyjunceellolide D (**7**)	27.85 ± 4.55**	8.91 ± 4.84

[a]) At a drug concentration of 10 μg/ml. Results are presented as mean ± S.E.M. ($n = 3-4$). Statistical significance: $P < 0.05$ (*), $P < 0.01$ (**) rel. to control (DMSO).

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Experimental Part

General. Column Chromatography (CC): silica gel *60* (200–300 mesh *Merck*). TLC: silica gel GF_{254} (*Merck*). Optical rotation: *Jasco DIP-1000* spectropolarimeter. IR Spectra: *Hitachi T-2001* spectrophotometer, in CH_2Cl_2 soln; in cm^{-1}. NMR Spectra: *Bruker FT-300* or *Varian Unity-INOVA-500* FT-NMR spectrometers; δ in ppm, J in Hz. HR-ESI-MS: *Jeol JMS-HX-110* mass spectrometer; in m/z.

Animal Material. The gorgonian *Junceella fragilis* RIDLEY (Ellisellidae) was collected in February 2005 in Tai-Tong County (Taiwan) by means of scuba diving at a depth of 15 m. The fresh gorgonian was immediately frozen after collection, and kept at $-20°$ until processed. A voucher specimen (WSG-5) was deposited at the School of Pharmacy, College of Medicine, National Taiwan University, Taiwan.

Extraction and Isolation. The outer grey layer of the gorgonian (wet weight 2.5 kg) was extracted with acetone (3×) at r.t. The solvent was removed under vacuum, and the crude extract (20 g) was partitioned between AcOEt and H_2O (1:1). The AcOEt-soluble portion (15 g) was subjected to CC (SiO_2; hexane/AcOEt 10:1 → 0:1) to afford 13 fractions (*Fr. 1–13*). *Fr. 10* (770 mg) was purified by CC (SiO_2; hexane/AcOEt 30:1 → 0:1) to yield junceellolide E (**5**; 51 mg) and umbraculolide A (**6**; 114 mg), as well as 11α,20α-epoxy-4-deacetoxyjunceellolide D (**7**; 50 mg) after further purification by RP-HPLC (MeOH/H_2O 7:3). *Fr. 13* was purified by CC (SiO_2; hexane/AcOEt 10:1 → 0:1) to afford eleven subfractions (*Fr. 13a–13k*). *Fr. 13g* (66 mg) was separated by RP-HPLC (MeOH/H_2O 3:2) to yield **1** (23 mg). Analogous purification of *Fr. 13h* (85 mg) by RP-HPLC (MeOH/H_2O 3:2) yielded **2** (12 mg) and **3** (4.3 mg), and RP-HPLC purification of Fr. *Fr. 13k* afforded **4** (6.5 mg).

Frajunolide A (=*(1*R**,3a*S**,4Z,8*S**,8a*S**,9*S**,11*R**,12a*S**,13*S**,13a*S**)-1,2,3a,6,7,8,8a,9,10,11,12,12a,13,13a-Tetradecahydro-13a-hydroxy-1,5,8a-trimethyl-12-methylidene-2-oxobenzo[4,5]cyclodeca[1,2-*b*]furan-8,9,11,13-tetrayl Tetraacetate*; **1**). Colorless, amorphous powder. $[\alpha]_D^{26} = -87.8$ ($c = 1.0$, CH_2Cl_2). IR (CH_2Cl_2): 3454, 1782, 1739, 1668. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-MS: 573.2292 ($[M+Na]^+$, $C_{28}H_{38}NaO_{11}^+$; calc. 573.2312).

Frajunolide B (=*(1*R**,3a*S**,4Z,8*S**,8a*S**,9*R**,10*S**,11*S**,12a*S**,13*S**,13a*S**)-1,2,3a,6,7,8,8a,9,10,11,12,12a,13,13a-Tetradecahydro-13a-hydroxy-1,5,8a-trimethyl-12-methylidene-2-oxobenzo[4,5]cyclodeca[1,2-*b*]furan-8,9,10,11,13-pentayl Pentaacetate*; **2**). Colorless, amorphous powder. $[\alpha]_D^{26} = -12.7$ ($c = 1.0$, CH_2Cl_2). IR (CH_2Cl_2): 3467, 1771, 1741, 1670. ^{1}H- and ^{13}C-NMR, resp.: see *Tables 1* and *2*, resp. HR-ESI-MS: 631.2329 ($[M+Na]^+$, $C_{30}H_{40}NaO_{13}^+$; calc. 631.2367).

Frajunolide C (=*(1*R**,3a*S**,4E,6Z,8*S**,8a*R**,9*S**,11*R**,12*R**,12a*S**,13*S**,13a*S**)-5-(Chloromethyl)-1,3a,8,8a,9,10,11,12a,13,13a-decahydro-13a-hydroxy-1,8a-dimethyl-2-oxo-2*H*-spiro[benzo[4,5]cyclodeca[1,2-*b*]furan-12,2′-oxirane]-8,9,11,13-tetrayl Tetraacetate*; **3**). Colorless, amorphous powder. $[\alpha]_D^{26} =$

-13.2 ($c = 0.4$, CH_2Cl_2). IR (CH_2Cl_2): 3485, 1773, 1734, 1684. ^{1}H-and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-MS: 621.1713 ($[M+Na]^+$, $C_{28}H_{35}{}^{35}ClNaO_{12}^+$; calc. 621.1715), 623 ($C_{28}H_{35}{}^{37}ClNaO_{12}^+$), 563 ($[M-{}^{35}Cl]^+$)).

Frajunolide D (=*(1*R*,3a*S*,4*E*,6*Z*,8*S*,8a*R*,9*S*,11*R*,12*R*,12a*S*,13*S*,13a*S*)-5-[(Acetoxy)-methyl])-1,3a,8,8a,9,10,11,12a,13,13a-decahydro-13a-hydroxy-1,8a-dimethyl-2-oxo-2*H*-spiro[benzo[4,5]-cyclodeca[1,2-b]furan-12,2′-oxirane]-8,9,11,13-tetrayl Tetraacetate*; **4**). Colorless, amorphous powder. $[\alpha]_D^{26} = -15.4$ ($c = 0.6$, CH_2Cl_2). IR (CH_2Cl_2): 3501, 1778, 1739, 1670. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-MS: 645.2124 ($[M+Na]^+$, $C_{30}H_{38}NaO_{14}^+$; calc. 645.2159).

Biological Assay. Human neutrophils were obtained by means of dextran sedimentation and *Ficoll* centrifugation. Superoxide generation and elastase release were carried out according to procedures described previously [23]. Briefly, superoxide-anion production was assayed by monitoring the superoxide-dismutase-inhibitable reduction of ferricytochrome *c*. Elastase-release experiments were performed with MeO-Suc-Ala-Ala-Pro-Val-*para*-nitroanilide as the elastase substrate.

REFERENCES

[1] K. Fabricius, P. Alderslade, 'Soft Corals and Sea Fans', Australian Institute of Marine Science, Townsville MC, 2001, p. 230.
[2] S. J. Wratten, D. J. Faulkner, *Tetrahedron* **1979**, *35*, 1907.
[3] H. Y. He, D. J. Faulkner, *Tetrahedron* **1991**, *47*, 3271.
[4] A. D. Rodriguez, C. Ramirez, O. M. Cobar, *J. Nat. Prod.* **1996**, *59*, 15.
[5] P. J. Sung, J. H. Sheu, J. P. Xu, *Heterocycles* **2002**, *57*, 535.
[6] J. Rodriguez, R. M. Nieto, C. Jimenez, *J. Nat. Prod.* **1998**, *61*, 313.
[7] P. J. Sung, J. H. Su, G. H. Wang, S. F. Lin, C. Y. Duh, J. H. Sheu, *J. Nat. Prod.* **1999**, *62*, 457.
[8] S. L. Wu, P. J. Sung, M. Y. Chiang, J. Y. Wu, J. H. Sheu, *J. Nat. Prod.* **2001**, *64*, 1415.
[9] S. H. Qi, S. Zhang, Y. M. Wen, Z. H. Xiao, Q. X. Li, *Helv. Chim. Acta* **2005**, *88*, 2349.
[10] J. Shin, M. Park, W. Fenical, *Tetrahedron* **1989**, *45*, 1633.
[11] M. T. Hamann, K. N. Harrison, A. R. Carroll, P. J. Scheuer, *Heterocycles* **1996**, *42*, 325.
[12] C. Subrahmanyam, R. Kulatheeswaran, R. S. Ward, *J. Nat. Prod.* **1998**, *61*, 1120.
[13] R. L. Hendrickson, J. H. Cardellina II, *Tetrahedron* **1986**, *42*, 6565.
[14] K. A. El Sayed, D. C. Dunbar, T. L. Perry, S. P. Wilkins, M. T. Hamann, J. T. Greenplate, M. A. Wideman, *J. Agric. Food Chem.* **1997**, *45*, 2735.
[15] M. Garcia, J. Rodriguez, C. Jimenez, *J. Nat. Prod.* **1999**, *62*, 257.
[16] P. J. Sung, S. L. Wu, H. J. Fang, M. Y. Chiang, J. Y. Wu, L. S. Fang, J. H. Sheu, *J. Nat. Prod.* **2000**, *63*, 1483.
[17] P. J. Sung, T. Y. Fan, L. S. Fang, J. H. Sheu, S. W. Wu, G. H. Wang, M. R. Lin, *Chem. Pharm. Bull.* **2003**, *51*, 1429.
[18] P. J. Sung, M. R. Lin, L. S. Fang, *Chem. Pharm. Bull.* **2004**, *52*, 1504.
[19] Y. C. Lin, Y. L. Huang, A. T. Khalil, M. H. Chen, Y. C. Shen, *Chem. Pharm. Bull.* **2005**, *53*, 128.
[20] Y. B. Cheng, J. Y. Jang, A. T. Khalil, Y. C. Shen, *J. Nat. Prod.* **2006**, *69*, 675.
[21] C. Subrahmanyam, R. Kulatheeswaran, R. S. Ward, *J. Nat. Prod.* **1998**, *61*, 1120.
[22] E. O. Pordesimo, F. J. Schmitz, L. S. Ciereszko, M. B. Hossain, D. van der Helm, *J. Org. Chem.* **1991**, *56*, 2344.
[23] T. L. Hwang, Y. L. Leu, S. H. Kao, M. C. Tang, H. L. Chang, *Free Radic. Biol. Med*. **2006**, *41*, 1433.

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