Juncenolides H – K, New Briarane Diterpenoids from *Junceella juncea*

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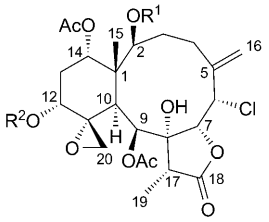
Chemical investigation of the Taiwanese gorgonian coral *Junceella juncea* resulted in the isolation of four new briarane-type diterpenoids, juncenolides H, I, J, and K (**1**–**4**). Their structures were determined on the basis of spectroscopic analysis, especially 1- and 2D-NMR. The inhibitory effects of compounds **1**–**4** on superoxide-anion generation and elastase release by human neutrophils were evaluated.

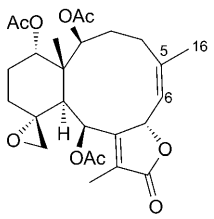
Introduction. – Marine organisms are an integral part of our environment and are intensively studied for their ecological impact, their ability to manufacture secondary metabolites with unique structures, and potential medicinal and economic importance [1]. The gorgonians of the genus *Junceella* (Ellisellidae) grow in tropical regions as whip-shaped unbranched colonies with variable colors [2]. They produce highly oxidized briarane-type diterpenoid γ-lactones with bicyclic six- and ten-membered fused-ring skeletons (3,8-cyclized cembranoids) [3][4]. Many of these briaranes possess a wide range of biological activities such as cytotoxic [5–7], anti-inflammatory [8], immunomodulatory [9], antiviral [10], and insecticidal [11][12] effects. Previous studies disclosed several briarane-type diterpenes in *J. juncea* [13–19]. Our investigation of an acetone extract of this species led to the isolation of additional new briarane-type diterpenoids, juncenolides H (**1**), I (**2**), J (**3**), and K (**4**)[1]). Their structures were determined on the basis of spectroscopic analyses, especially NMR and HR-ESI-MS. The relative configurations of **1**–**4** were determined by detailed studies of their NOESY plots. The inhibitory effect of **1**–**4** on superoxide anion generation and elastase release by human neutrophils was evaluated.

Results and Discussion. – The HR-ESI-MS of **1** revealed a *pseudo*-molecular-ion peak at *m*/*z* 623.1873 ($[M + Na]^+$), consistent with the molecular formula $C_{28}H_{37}ClO_{12}$, having ten degrees of unsaturation. The presence of a Cl-atom was confirmed by the appearance of an isotope fragment ion at *m*/*z* 625 ($[M + Na + 2]^+$) in the ESI-MS, showing the characteristic relative abundance compared to the molecular ion (1:3) [20]. The IR spectrum displayed absorption bands diagnostic of OH

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.*



1 $R^1 = R^2 = Ac$
2 $R^1 = Me_2CHCO$, $R^2 = Ac$
3 $R^1 = Ac$, $R^2 = Me_2CHCH_2CO$



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(3460 cm^{-1}), 5-membered lactone (1789 cm^{-1}), ester (1732 cm^{-1}), and C=C bond (1651 cm^{-1}) functionalities. Both ^{1}H- and ^{13}C-NMR data (*Tables 1* and *2*) indicated the presence of four acetate units, a lactone ring, and one exocyclic CH_2=C bond, accounting for seven degrees of unsaturation and suggesting three additional rings. The

Table 1. *^{1}H-NMR Data* (500 MHz, $CDCl_3$) *of* **1**–**4**[1]). δ in ppm, J in Hz.

	1	**2**	**3**	**4**
H–C(2)	5.96 (*d*, *J* = 8.4)	5.99 (*d*, *J* = 8.4)	5.59 (*d*, *J* = 8.5)	4.74 (br. *s*)
CH_2(3)	2.67–2.73 (*m*), 1.55–1.61 (*m*)	1.60–1.64 (*m*), 2.70–2.76 (*m*)	1.60–1.64 (*m*), 2.70–2.76 (*m*)	2.53–2.59 (*m*), 1.74–1.80 (*m*)
CH_2(4)	2.41–2.47 (*m*), 2.02–2.08 (*m*)	2.42–2.48 (*m*), 2.32–2.38 (*m*)	2.42–2.47 (*m*)	2.60–2.66 (*m*), 2.20–2.26 (*m*)
H–C(6)	4.62 (*d*, *J* = 3.2)	4.62 (br. *s*)	4.63 (br. *s*)	5.01 (*d*, *J* = 8.5)
H–C(7)	4.44 (br. *s*)	4.45 (br. *s*)	4.45 (br. *s*)	5.51 (*d*, *J* = 8.5)
H–C(9)	5.74 (*s*)	5.75 (*s*)	5.74 (*s*)	6.54 (*d*, *J* = 7.0)
H–C(10)	3.68 (*s*)	3.71 (*s*)	3.67 (*s*)	2.63–2.68 (*m*)
H–C(12) or CH_2(12)	4.53 (br. *s*)	4.55 (br. *s*)	4.59 (br. *s*)	2.30–2.36 (*m*), 1.10–1.16 (*m*)
CH_2(13)	2.23–2.29 (*m*), 2.02–2.08 (*m*)	2.24–2.29 (*m*), 2.01–2.07 (*m*)	2.22–2.27 (*m*), 2.03–2.09 (*m*)	2.11–2.17 (*m*), 1.80–1.86 (*m*)
H–C(14)	4.89 (br. *s*)	4.83 (br. *s*)	4.91 (br. *s*)	4.80 (*d*, *J* = 3.5)
Me(15)	1.14 (*s*)	1.16 (*s*)	1.16 (*s*)	1.16 (*s*)
CH_2(16) or Me(16)	5.78, 5.49 (2 *s*)	5.79, 5.51 (2 *s*)	5.82, 5.52 (2 *s*)	2.01 (*s*)
H–C(17)	2.95 (*q*, *J* = 7.0)	2.96 (*q*, *J* = 6.6)	2.97 (*q*, *J* = 7.0)	
Me(19)	1.24 (*d*, *J* = 7.0)	1.27 (*d*, *J* = 6.6)	1.27 (*d*, *J* = 7.0)	2.01 (*s*)
CH_2(20)	2.82 (*d*, *J* = 3.2), 2.34 (*d*, *J* = 3.2)	2.82 (*d*, *J* = 3.0), 2.35 (*d*, *J* = 3.0)	2.82 (*d*, *J* = 3.5), 2.35 (*d*, *J* = 3.5)	2.60–2.66 (*m*), 2.46–2.52 (*m*)
H–C(2′) or CH_2(2′)		2.50 (*sept.*, *J* = 7.0)	2.08–2.12 (*m*)	
Me(3′) or H–C(3′)		1.12 (*d*, *J* = 7.0)	2.05–2.11 (*m*)	
Me(4′)		1.17 (*d*, *J* = 7.0)	0.93 (*d*, *J* = 6.5)	
Me(5′)			0.93 (*d*, *J* = 6.5)	
AcO–C(2)	1.99 (*s*)		2.01 (*s*)	2.01 (*s*)
AcO–C(9)	2.21 (*s*)	1.96 (*s*)	2.22 (*s*)	2.12 (*s*)
AcO–C(12)	1.99 (*s*)	2.21 (*s*)		
AcO–C(14)	1.96 (*s*)	1.99 (*s*)	1.98 (*s*)	1.96 (*s*)
OH	3.46 (*s*)			

Table 2. *^{13}C-NMR Data* (75 MHz, $CDCl_3$) *of* **1**–**4**[1]). δ in ppm.

	1	**2**	**3**	**4**
C(1)	47.7 (*s*)	47.9 (*s*)	47.8 (*s*)	46.0 (*s*)
C(2)	73.0 (*d*)	72.6 (*d*)	73.0 (*d*)	74.1 (*d*)
C(3)	28.1 (*t*)	28.4 (*t*)	28.2 (*t*)	31.3 (*t*)
C(4)	33.5 (*t*)	33.5 (*t*)	33.5 (*t*)	29.1 (*t*)
C(5)	146.7 (*s*)	146.8 (*s*)	146.6 (*s*)	143.0 (*s*)
C(6)	53.9 (*d*)	52.6 (*d*)	51.5 (*d*)	124.7 (*d*)
C(7)	81.4 (*d*)	81.2 (*d*)	81.2 (*d*)	77.2 (*d*)
C(8)	81.2 (*s*)	81.4 (*s*)	81.3 (*s*)	155.8 (*s*)
C(9)	70.9 (*d*)	72.1 (*d*)	73.0 (*d*)	66.5 (*d*)
C(10)	35.5 (*d*)	35.6 (*d*)	35.6 (*d*)	40.5 (*d*)
C(11)	57.5 (*s*)	57.6 (*s*)	57.4 (*s*)	59.7 (*s*)
C(12)	73.5 (*d*)	73.5 (*d*)	73.0 (*d*)	22.9 (*t*)
C(13)	29.0 (*t*)	29.0 (*t*)	29.3 (*t*)	23.8 (*t*)
C(14)	73.2 (*d*)	73.3 (*d*)	73.2 (*d*)	73.9 (*d*)
C(15)	13.9 (*q*)	14.1 (*q*)	14.0 (*q*)	15.8 (*q*)
C(16)	121.1 (*t*)	121.2 (*t*)	121.2 (*t*)	27.0 (*q*)
C(17)	51.4 (*d*)	51.5 (*d*)	51.5 (*d*)	127.5 (*s*)
C(18)	174.5 (*s*)	174.6 (*s*)	174.4 (*s*)	173.6 (*s*)
C(19)	5.8 (*q*)	6.0 (*q*)	6.6 (*q*)	9.3 (*q*)
C(20)	50.3 (*t*)	50.4 (*t*)	50.5 (*t*)	58.2 (*t*)
C(1′)		176.7 (*s*)	171.6 (*s*)	
C(2′)		34.1 (*d*)	43.5 (*t*)	
C(3′)		18.3 (*q*)	25.5 (*d*)	
C(4′)		19.3 (*q*)	22.3 (*q*)	
C(5′)			22.4 (*q*)	
AcO-C(2)	170.3 (*s*), 20.8 (*q*)		170.9 (*s*), 21.2 (*q*)	170.7 (*s*), 21.0 (*q*)
AcO-C(9)	169.4 (*s*), 21.0 (*q*)	169.5 (*s*), 21.0 (*q*)	169.3 (*s*), 21.2 (*q*)	168.9 (*s*), 21.6 (*q*)
AcO-C(12)	169.4 (*s*), 21.2 (*q*)	169.5 (*s*), 21.2 (*q*)		
AcO-C(14)	170.9 (*s*), 21.0 (*q*)	170.2 (*s*), 21.2 (*q*)	170.4 (*s*), 21.2 (*q*)	169.8 (*s*), 20.9 (*q*)

NMR spectra revealed four acetates at δ(H) 2.21 (*s*, 3 H), 1.99 (*s*, 6 H), and 1.96 (*s*, 3 H) (δ(C) 170.9, 170.3, 169.4 (2 C), 21.2, 21.0 (2 C), and 20.8). The C=O signal at δ(C) 174.5 (C(18)) was assigned to a 5-membered lactone ring together with the CHO = group at δ(C) 81.4 (C(7)) and an O-bearing quaternary C-atom at δ(C) 81.2 (C(8)). The two *s* at δ(H) 5.78 and 5.49 (δ(C) 121.1) were ascribed to an exocyclic CH_2 = group by their HMBC cross-peaks with the CH_2 group at δ(C) 33.5 (C(4)) and the quaternary C-atom at δ(C) 146.7 (C(5)) (*Fig. 1*). The tertiary Me(15) group at δ(H) 1.14 was correlated with the quaternary C(1) atom at δ(C) 47.7, two CHO groups at δ(C) 73.2 (C(14)) and 73.0 (C(2)), and another quaternary C-atom at δ(C) 57.5 (C(11)). The secondary Me(19) at δ(H) 1.24 had correlations with a lactone C=O (C(18)), the oxygenated quaternary C-atom C(8), and the CH C-atom C(17). The two geminal H-atoms at δ 2.82 and 2.34 (*d*, $J = 3.2$ Hz each, CH_2(20)) together with the corresponding C-atom at δ(C) 50.3 (*t*) and the quaternary C-atom at δ(C) 57.5 (*s*, C(11)) were attributed to an exocyclic epoxide, *i.e.*, a spirocyclic oxirane ring [14]. The two remaining degrees of unsaturation were accounted for by assuming the presence of a bicyclic C-atom skeleton of a briarane diterpene [9][14]. The correlations of the

oxirare H-atoms (CH_2(20)) with the quaternary C(11) at δ(C) 57.5 and the CHO group at δ(C) 73.5 (C(12)) allowed the location of the 11,20-epoxy moiety. That the OH group (δ(H) 3.46 (br. *s*), D_2O-exchangeable) was attached to C(8) was evidenced by the HMBCs OH/C(8) and C(9). H–C(6) (δ(H) 4.62 (*d*, $J = 3.2$ Hz)) was correlated to the quaternary olefinic C(5) and the oxocyclic C(16) and the corresponding δ(C) 53.9 (C(6)) suggesting the attachment of a Cl-atom at this position. The four CHOs groups observed at δ(H) 5.96, 5.74, 4.53, and 4.89 were bonded to C-atoms at δ(C) 73.0, 70.9, 73.5, and 73.2 and assigned to C(2), C(9), C(12), and C(14), respectively, with the aid of HMBC cross-peaks between each C=O C-atom and the respective CH H-atom. The COSY plot exhibited connectivities between H–C(2)/CH_2(3)/CH_2(4), H–C(6)/H–C(7), H–C(9)/H–C(10), H–C(12)/CH_2(13)/H–C(14), and H–C(17)/Me(19) (*Fig. 1*), and the HMBCs of H–C(10)/C(12), C(11), C(20), C(2), C(15), C(14), and C(8), removed any ambiguity and corroborated the proposed briarane structure (*Fig. 1*). The relative configuration of **1** was determined on the basis of biogenetic considerations and a NOESY experiment. Naturally occurring briaranes have the Me(15) in the β-orientation and H–C(10) in the α-orientation [10], which was verified by the absence of an NOE effect between H–C(10) and Me(15). The NOESY correlations (*Fig. 1*) between Me(15)/H–C(14) and CH_2(20), and H–C(7)/H–C(6) and H–C(17) implied β-orientation of H–C(6), H–C(7), H–C(12), H–C(14), H–C(17), and CH_2(20). The correlations between H–C(10)/H–C(2), H–C(9), and Me(19), and H–C(9)/H–C(2) and OH–C(8) suggested the α-orientation of H–C(2), H–C(9), and Me(19), as well as of OH–C(8). In addition to the NOESY correlations CH_2(20)/H–C(12) and Me(15), the chemical-shift values of C(11) and C(20) fall within the ranges δ(C) 55–61 and 47–52, respectively [21], of a β-oriented axial epoxy group of at a cyclohexane moiety in chair conformation. This would give (11*R*) configuration for the epoxy ring. While the structure of **1** looked similar to that of gemmacolide C = (1*R*,2′*R*,3a*R*,4*S*,8*S*,8a*S*,9*S*,11*R*,12a*S*,13*S*,13a*R*)-8,9,11,13-tetrakis(acetyloxy)-4-chlorotetradecahydro-13a-hydroxy-1,8a-dimethyl-5-methylenespiro[benzo[4,5]cyclodeca[1,2-*b*]furan-12(2*H*),2′-oxiran]-2-one, isolated previously from *J. gemmacea* [14], the two compounds differ in configuration at C(2) and C(9), which was manifested in upfield shifts of C(9) ($\Delta\delta$(C) = 10.2), C(1) ($\Delta\delta$(C) = 16), and downfield shifts of C(7) ($\Delta\delta$(C) = 9.6), C(17) ($\Delta\delta$(C) = 15.9) in the case of **1**. Hence, the structure of juncenolide H was established as **1**.

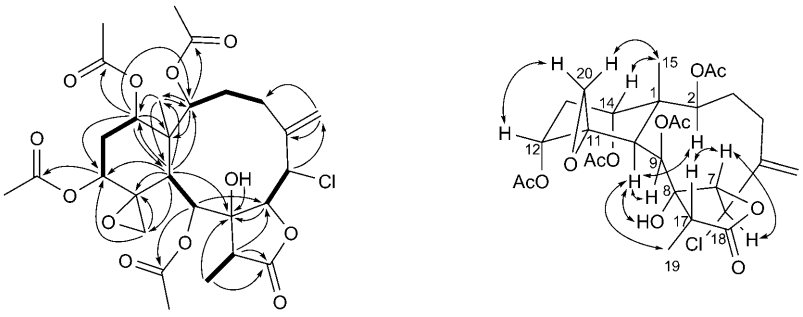

Fig. 1. *Key HMBC* (H → C), *COSY* (—), *and NOESY correlations* (↔) *for* **1**

Compound **2** was isolated as optically active, colorless crystals. The molecular formula was determined to be $C_{30}H_{41}ClO_{12}$ from HR-ESI-MS data (*m*/*z* 651.2180 ($[M + Na]^+$)), which also showed a pair of peaks at *m*/*z* 651.2180 and 653.2151 (3 : 1), indicating the presence of one Cl-atom. Its IR bands revealed the presence of a five-membered-ring lactone (1778 cm^{-1}) and ester groups (1738, 1732, and 1720 cm^{-1}). Comparison of its ^{1}H- and ^{13}C-NMR data (*Tables 1* and *2*) with that of **1** indicated the presence of only three acetates at δ(H) 2.21, 1.99, and 1.96 (3 *s*) (δ(C) 170.2, 169.5 (2 C), 21.2 (2 C) and 21.0). Extra signals appeared in the form of an isobutyric acid ester (=2-methylpropanoate) with a CH *sept.* (δ(H) 2.50) coupled with two Me groups (δ(H) 1.12 and 1.17 (2*d*); δ(C) 19.3 (*q*), 18.3 (*q*), 34.1 (*d*), and 176.7 (*s*)). The key HMBC (*Fig. 2*) H–C(2)/C=O (δ(C) 176.7) firmly established the attachment of the isobutyrate at C(2). The signal of the C=O group at δ(C) 174.6 was assigned to that of a five-membered lactone, together with the O-bearing CH at δ(C) 81.2 (C(7)) and at δ(H) 4.45. The CH signal at δ(C) 52.6 (δ(H) 4.62 (br. *s*)) were attributed to a chlorinated CH moiety (C(6)), which was verified by a HMQC experiment. The other signals were similar to those of **1**, including signals arising from an exocyclic CH_2=group (δ(H) 5.79 and 5.51; δ(C) 121.2 (*t*)) and an axial exocyclic oxirane (δ(H) 2.82 and 2.35; δ(C) 50.4 (*t*) and 57.6 (*s*)), and the tertiary Me(15) *s* (δ(H) 1.16) and the secondary Me(19) *d* (δ(H) 1.27). HMBCs H–C(9)/δ(C) 169.5, H–C(12)/δ(C) 169.5, H–C(14)/δ(C) 170.2 established the points of attachment of the remaining three AcO groups. Further HMBCs confirmed the connectivities of a briarane skeleton for **2**. NOESY Correlations (*Fig. 2*) revealed the relative configuration of the attached functional groups of **2**, which is similar to that of compound **1**.

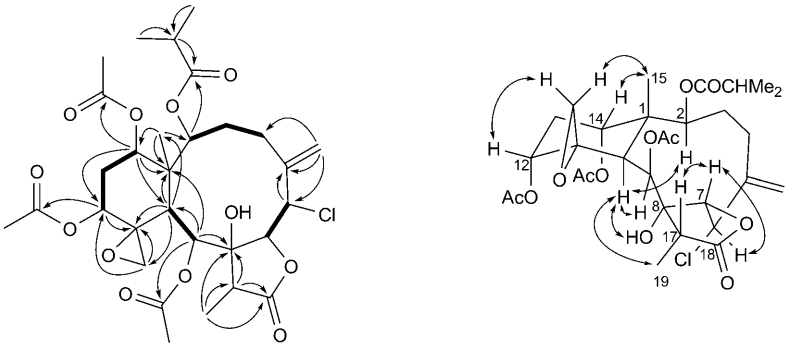

Fig. 2. *Key HMBC* (H → C), *COSY* (—), *and NOESY correlations* (↔) *for* **2**

Compound **3** was isolated as optically active, colorless crystals. The HR-ESI-MS of **3** displayed a *quasi*-molecular-ion peak at *m*/*z* 665.2344 ($[M + Na]^+$, $C_{31}H_{43}ClNaO_{12}^+$; calc. 665.2341). Its IR data showed the absorption bands diagnostic of OH, 5-membered lactone, and ester groups. Features similar to those of **2** were observed in the ^{1}H- and ^{13}C-NMR data of **3**. The presence of three acetates at δ(H) 2.22, 2.01, and 1.98 (3 *s*) (δ(C) 21.2 (*q*, triple intensity), 169.3, 170.9, and 170.4) was established; they were located at C(2), C(9), and C(14), respectively, by HMBC experiments (*Fig. 3*). An isovaleric acid ester (= 3-methylbutanoate) was revealed by a CH signal at δ(H) 2.05–2.11 (*m*), which showed spin-spin coupling with two Me groups (δ(H) 0.93 (*d*,

$J = 6.5$ Hz, 6 H)) as observed in a COSY plot. This isovalerate moiety was located at C(12) by HMBCs (*Fig. 3*). Similarly to compound **2**, the ester C=O at δ(C) 174.4 had to be assigned to a five-membered lactone ring along with the oxygenated CH at δ(C) 81.2. The NOESY experiments (*Fig. 3*) showed interactions between Me(15)/H−C(14)/H−C(12)/CH_2(20), H−C(6)/H−C(7)/H−C(17), and H−C(10)/OH−C(8)/H−C(9)/H−C(2), indicating that the acetates at C(2) and C(9) are in β-orientation, whereas the acetate at C(14), the Cl-atom at C(6), and the isovalerate at C(12), as well as the OH group at C(8) are in α-position. Thus, compound **3** was established as an analogue of **1** with an isovalerate group attached at C(12).

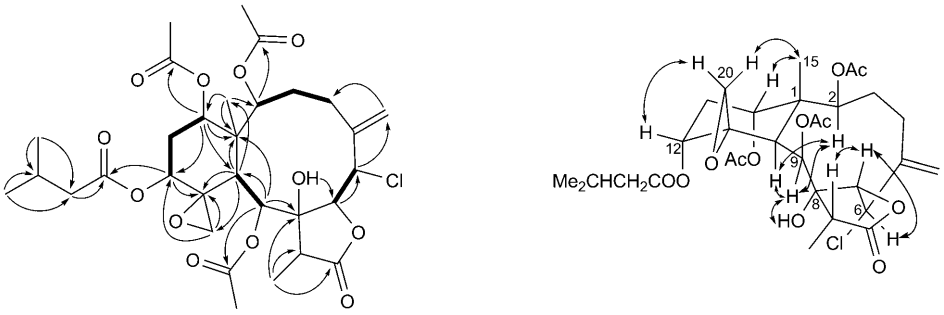

Fig. 3. *Key HMBC* (H → C), *COSY* (—), *and NOESY correlations* (↔) *for* **3**

The molecular formula of compound **4**, $C_{26}H_{34}O_9$, as determined by HR-ESI-MS, was devoid of isotope patterns. The IR absorption bands showed similar features to those of **2** indicating that they had the same functionalities. The ^{1}H- and ^{13}C-NMR spectra revealed the presence of three acetate Me signals at δ(H) 2.01, 2.12, and 1.96 (3 *s*) (δ(C) 21.0 (*q*), 21.6 (*q*), 20.9 (*q*), 170.7, 168.9, and 169.8), the locations of which were assigned by HMBCs (*Fig. 4*) at C(2), C(9), and C(14), respectively. An additional C=O (δ(C) 173.6) was assigned to the C=O of a lactone ring, which was conjugated with a C=C bond as revealed by the HMBCs Me(19)/C(17), C(8), and C(18). The signals of two geminal H-atoms at δ(H) 2.49 and 2.63 (CH_2(20)) were connected by HMBC with C(20) at δ(C) 58.2 (*t*) and the quaternary C(11) at δ(C) 59.7 (*s*), indicating the presence of an exocyclic epoxy group at C(11). The Me(16) group at δ(H) 2.01 was correlated with a CH_2 group (δ(C) 29.1 (*t*, C(4))), a quaternary C-atom (δ(C) 143.0 (*s*, C(5))), and a CH group (δ(C) 124.7 (*d*, C(6))), the latter two implying that an endocyclic C=C bond was located between C(5) and C(6). This C=C bond is constrained in a (*Z*)-configuration as revealed by NOESY correlations (*Fig. 4*) between H_β−C(4) and H−C(7). The COSY plot of **4** displayed the correlations H−C(2)/CH_2(3)/CH_2(4)/Me(16), H−C(6)/H−C(7), H−C(9)/H−C(10)/CH_2(20), and H−C(12)/CH_2(13)/H−C(14), all consistent with the position of each functional group at the bicyclic ring. Assuming that Me−C(1) has the same β-configuration as in **2**, the NOESY correlation Me(15)/H−C(14) would locate H−C(14) on the β-face. The NOESY correlations H−C(10)/H−C(9)/H−C(12), H−C(10)/H−C(2)/H_α−C(3), and H_β−C(3)/H−C(7) suggested the α-configuration of H−C(9) and H−C(2), and the β-orientation of H−C(7). Thus, it was established that compound **4** is a new analogue

of **1** and **2** with an endocyclic C(5)=C(6) bond being adjacent to a but-2-eno-4-lactone ring.

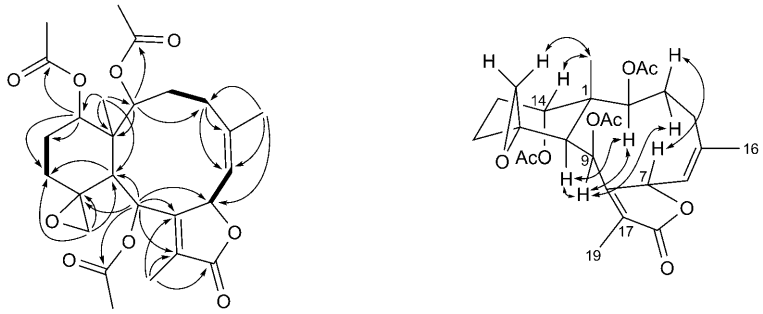

Fig. 4. *Key HMBC* (H → C)*, COSY* (—)*, and NOESY correlations* (↔) *for* **4**

The anti-inflammatory activities of **1**–**4** were evaluated on human neutrophils *in vitro* for their inhibition of elastase release and of the generation of superoxide anion. Among the isolated diterpenoids, juncenolide H (**1**) showed a mild inhibitory effect on superoxide-anion generation and elastase release by human neutrophils in response to FMLP/CB to an extent of 28.87 and 15.12%, respectively (*Table 3*).

Table 3. *Inhibitory Effect of* **1**–**4** *on Superoxide-Anion Generation and Elastase Release by Human Neutrophils in Response to FMLP/CB* (Formyl-Met-Leu-Phe/cytochalasin B)[a])

	Superoxide anion (inh. [%])	Elastase (inh. [%])
Juncenolide H (**1**)	28.87 ± 2.40[b])	15.12 ± 6.33
Juncenolide I (**2**)	1.30 ± 4.33	15.57 ± 5.81
Juncenolide J (**3**)	− 3.59 ± 2.69	2.12 ± 3.38
Juncenolide K (**4**)	− 5.69 ± 3.25	17.48 ± 5.42
Genistein[c])	65.00 ± 5.71	51.60 ± 5.89

[a]) Percentage of inhibition (inh. %) at a concentration of 10 μg/ml. Results are presented as mean ± s.e.m. ($n = 3–4$). [b]) $P < 0.001$ as compared with the control DMSO. [c]) Positive control.

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Experimental Part

General. Prep. TLC: precoated silica-gel plates (SiO_2; *silica gel 60 F-254*, 1 mm; *Merck*). Column chromatography (CC): SiO_2 *60* (*Merck*) or *Sephadex LH-20* (*Amersham Pharmacia Biotech AB*, Sweden). HPLC: *Hitachi* system; *LiChrospher® Si 60* (5 μm, 250–10; *Merck*) for normal phase and *LiChrospher® 100 RP-18e* (5 μm, 250–10; *Merck*) for reversed-phase. Optical rotations: *Jasco-DIP-1000* polarimeter. IR and UV Spectra: *Hitachi-T-2001* and *Hitachi-U-3210* spectrophotometers, resp. ^{1}H- and ^{13}C-NMR, COSY, HMQC, HMBC, and NOESY Experiments: *Bruker-FT-300* spectrometer; chemical shifts δ in ppm rel. to Me_4Si as an internal standard, coupling constants J in Hz. EI-MS and HR-ESI-MS: *Jeol-JMS-HX-110* mass spectrometer; in m/z (rel. %).

Animal Material. The gorgonian *Junceella juncea* PALLAS (Ellisellidae) was collected in Tai-Tong County, Taiwan, by scuba diving at a depth of 15 m, in February 2003. The fresh gorgonian was

immediately frozen after collection and kept at $-20°$ until processed. A voucher specimen (WSG-5) was deposited with the School of Pharmacy, College of Medicine, National Taiwan University, Taiwan.

Extraction and Isolation. The outer grey layer of the gorgonian (180 g) was extracted with acetone (3×500 ml) at r.t., and the acetone extract was concentrated. The crude extract (2 g) was partitioned between AcOEt and H_2O (1:1). The AcOEt-soluble portion (1.3 g) was subjected to CC (SiO_2, hexane/AcOEt $10:1 \rightarrow 0:1$; TLC (GF_{254}) monitoring): *Fractions 1–11. Fr. 3* (192 mg) was separated by CC (*Sephadex LH-20*, CH_2Cl_2/MeOH 1:1): *Frs. 3.1–3.3. Fr. 3.1* (30 mg) was subjected to reserved-phase HPLC (MeOH/H_2O 7:3): **1** (10 mg).

Another batch of gorgonian (2 kg) was extracted with acetone and CH_2Cl_2 (each 3×51) at r.t., and the extract was concentrated. The crude extract (13 g) was partitioned between AcOEt and H_2O (1:1). The AcOEt-soluble portion (8 g) was separated by CC (*Sephadex LH-20*, CH_2Cl_2/MeOH 1:1): *Frs. A–C. Fr. B* (4.92 g) was subjected to CC (SiO_2, hexane/CH_2Cl_2/MeOH $250:250:1 \rightarrow 5:5:1$): *Frs. B.1–B.17. Fr. B.11* (131 mg) was subjected to HPLC (CH_2Cl_2/MeOH 105:1) to give two fractions. These two fractions were each subjected to reversed-phase HPLC (MeOH/H_2O/MeCN 70:25:5 and 65:30:5, resp.): **3** (2.5 mg) and **4** (4.0 mg), resp. *Fr. B.14* (83 mg) was subjected to HPLC (CH_2Cl_2/MeOH 125:1) and then to reversed phase HPLC (MeOH/H_2O/MeCN 65:35:5): **2** (17 mg).

Juncenolide H (= rel-*(1*R,*2*S,*6*R,*7*S,*8*R,*9*S,*10*R,*11*S,*12*S,*14*R,*17*S*)-2,9,12,14-Tetrakis(acetyloxy)-6-chloro-11,20-epoxy-8-hydroxybriaran-5(16)-en-7(18)-lactone* = rel-*(1*R,*2′*R,*3a*R,*4*S,*8*S,*8a*S,*9*S,*11*R,*12a*S,*13*S,*13a*R*)-8,9,11,13-Tetrakis(acetyloxy)-4-chlorotetradecahydro-13a-hydroxy-1,8a-dimethyl-5-methylenespiro[benzo[4,5]cyclodeca[1,2-*b*]furan-12(2*H*),2′-oxiran]-2-one*; **1**): Colorless amorphous powder. $[\alpha]_D = +40.6$ ($c = 1.0$, CH_2Cl_2). IR (CH_2Cl_2): 3460 (OH), 2939 (CH), 1789 (lactone), 1732 (ester), 1651 (C=C). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS: 623 ($[M + Na]^+$). HR-ESI-MS: 623.1873 ($[M + Na]^+$, $C_{28}H_{37}ClNaO_{12}^+$; calc. 623.1871).

Juncenolide I (= rel-*(1*R,*2′*R,*3a*R,*4*S,*8*S,*8a*S,*9*S,*11*R,*12a*S,*13*S,*13a*R*)-9,11,13-Tris(acetyloxy)-4-chlorotetradecahydro-13a-hydroxy-1,8a-dimethyl-5-methylene-2-oxospiro[benzo[4,5]cyclodeca[1,2-*b*]furan-12(2*H*),2′-oxiran]-8-yl 2-Methylpropanoate*; **2**): Colorless amorphous powder. $[\alpha]_D = +18$ ($c = 0.2$, CH_2Cl_2). IR (CH_2Cl_2): 3440 (OH), 2943 (CH), 1778 (lactone), 1732 (ester), 1645 (C=C). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS: 651 ($[M + Na]^+$). HR-ESI-MS: 651.2180 ($[M + Na]^+$, $C_{30}H_{41}ClNaO_{12}^+$; calc. 651.2184).

Juncenolide J (= rel-*(1*R,*2′*R,*3a*R,*4*S,*8*S,*8a*S,*9*S,*11*R,*12a*S,*13*S,*13a*R*)-8,9,13-Tris(acetyloxy)-4-chlorotetradecahydro-13a-hydroxy-1,8a-dimethyl-5-methylene-2-oxospiro[benzo[4,5]cyclodeca[1,2-*b*]furan-12(2*H*),2′-oxiran]-11-yl 3-Methylbutanoate*; **3**): Colorless amorphous powder. $[\alpha]_D = +14$ ($c = 0.2$, CH_2Cl_2). IR (CH_2Cl_2): 3450 (OH), 2935 (CH), 1784 (lactone), 1738 (ester), 1656 (C=C). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS: 665 ($[M + Na]^+$). HR-ESI-MS: 665.2344 ($[M + Na]^+$, $C_{31}H_{43}ClNaO_{12}^+$; calc. 665.2341).

Juncenolide K (= rel-*(2′*R,*3a*S,*4*Z,*8*S,*8a*S,*9*S,*12a*S,*13*S*)-8,9,13-Tris(acetyloxy)-3a,6,7,8,8a,9,10,11,12a,13-decahydro-1,5,8a-trimethylspiro[benzo[4,5]cyclodeca[1,2-*b*]furan-12(2*H*),2′-oxiran]-2-one*; **4**): Colorless amorphous powder. $[\alpha]_D = -85$ ($c = 0.2$, CH_2Cl_2). IR (CH_2Cl_2): 3459 (OH), 2950 (CH), 1777 (lactone), 1734 (ester), 1659 (C=C). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS: 513 ($[M + Na]^+$). HR-ESI-MS: 513.2100 ($[M + Na]^+$, $C_{26}H_{34}NaO_9^+$; calc. 513.2098).

Inhibitory Effect on Superoxide-Anion Generation and Elastase Release by Human Neutrophils. Human neutrophils were obtained by means of dextran sedimentation and *Ficoll*® centrifugation. Superoxide generation and elastase release were carried out according to the procedures described previously [22]. Superoxide-anion production was assayed by monitoring the superoxide dismutase-inhibitable reduction of ferricytochrome *c*. Elastase-release experiments were performed using MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide as the elastase substrate.

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