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Biyoulactones A–C, New Pentacyclic Meroterpenoids from *Hypericum chinense*

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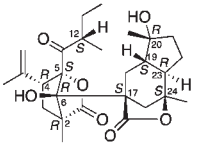
ABSTRACT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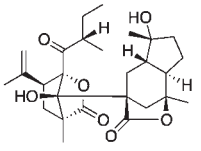
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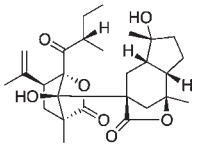
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Three novel pentacyclic meroterpenoids with a unique dilactone structure containing C–C bonded bi- and tricyclic γ-lactone moieties, biyoulactones A–C (1–3), were isolated from the roots of *Hypericum chinense*, and their structures were elucidated on the basis of spectroscopic data. The relative and absolute stereochemistry of 1 was assigned by a combination of NOESY and a single crystal X-ray diffraction analysis.

The plants of the genus *Hypericum* (family Clusiaceae) have been used as traditional remedies in several parts of the world.[1] These plants are known to contain various types of compounds (e.g., terpenoids, naphtodianthrones, xanthones, flavonoids, and prenylated acylphloroglucinols).[1] During our search for structurally interesting compounds from *Hypericum* spp., we have reported the isolation of some meroterpenoids such as yezo'otogirins A–C from *H. yezoense*[2] and yojironins A and B from *H. yojiroanum*.[3] Recently, we have also reported the isolation of four prenylated xanthones, biyouxanthones A–D, from the extracts of *H. chinense* roots.[4] In Japan, aerial parts of this plant are used as a folk medicine for treatment of female disorders.[5] Further investigation of the extracts from the roots of *H. chinense* resulted in the isolation of three novel pentacyclic meroterpenoids with a unique dilactone structure, biyoulactones A–C (**1**–**3**). In this Letter, we describe the isolation and structure elucidation of **1**–**3**.

The roots of *H. chinense* (2.5 kg, dry) were extracted with MeOH, and the extracts were partitioned successively with *n*-hexane, EtOAc, and H_2O. The EtOAc-soluble portions were subjected to a Toyopearl HW-40 column (MeOH/H_2O), a Sephadex LH-20 column (MeOH), a silica gel column (*n*-hexane/EtOAc), and a Chromatorex-DIOL column (*n*-hexane/$CHCl_3$/acetone) chromatographies to give a fraction containing a mixture of meroterpenoids. The fraction was purified by C_{18} HPLC (MeOH/H_2O) and silica gel HPLC (*n*-hexane/*i*-PrOH) to yield biyoulactones A (**1**, 0.000015%), B (**2**, 0.000022%), and C (**3**, 0.000011%). Two known compounds, chinesin I[6] and hyperielliptone HB,[7] were also isolated in the purification process of **1**–**3**.

Biyoulactone A (**1**) was isolated as an optically active colorless platelet {$[\alpha]_D^{25}$ +8.1 (*c* 0.13, MeOH)}. The HRESIMS analysis revealed the molecular formula to be $C_{27}H_{38}O_7$ (*m*/*z* 497.2508 $[M+Na]^+$, Δ–0.2 mmu). The IR spectrum showed absorption bands due to hydroxy (3376 cm^{-1}) and carbonyl functionalities (1792, 1740, and 1712 cm^{-1}). The ^1H and ^{13}C NMR spectra of **1** (Table 1) showed the signals of one hydroxy group, one 1,1-disubstituted olefin, one ketone carbonyl group, two carboxy

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groups, six sp^3 quaternary carbons, four sp^3 methines, six sp^3 methylenes, and six methyls. The analysis of the $^1H-^1H$ COSY and HMBC spectra (Figure 1) suggested the gross structures of three partial units (units A–C) as follows. The $^1H-^1H$ COSY spectrum suggested the connectivity of C-3 to C-4, while the connectivities of C-2 to C-1, C-3, C-6, and C-7 were disclosed by the analysis of HMBC correlations (Figure 1). The presence of an isopropenyl group at C-4 were revealed by HMBC cross-peaks of H_2-9 to C-4 and C-10. HMBC correlations for 6-OH to C-5 and C-6 indicated the connectivity of C-5 to C-6 and the exsistence of a hydroxy group at C-6. The connectivity of C-4 to C-5 was implied by a NOESY correlation of H-4/6-OH, since an effective HMBC correlation which suggests the connectivity was not observed. The chemical shifts of C-5 (δ_C 97.0) and C-1 (δ_C 177.3) suggested that C-1 was connected to C-5 through an ester linkage. Thus, the gross structure of unit A was proposed as shown. In unit B, the $^1H-^1H$ COSY spectrum revealed the connectivities of C-18–C-19, C-19–C-23, and C-21–C-23 (Figure 1). The presence of an octahydroindene ring (C-17–C-25) and two methyl groups at C-20 and C-24 were disclosed based on the HMBC analysis. In addition, the chemical shifts for C-20 (δ_C 76.7) and C-24 (δ_C 85.0) implied that these carbons were oxygenated. An HMBC correlation for H_2-18 to an ester carbonyl carbon (C-16) and the chemical shifts for C-16 (δ_C 180.5) and C-24 suggested the existence of a γ-lactone ring (C-16, C-17, C-24, and C-25). Therefore, the gross structure of unit B was elucidated as shown. Similarly, the presence of a 2-methylbutanoyl group (unit C) was assigned.

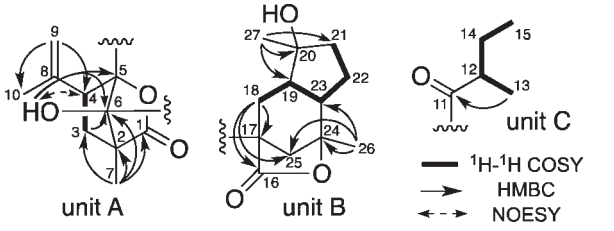

Figure 1. Selected 2D NMR correlations for units A (C-1–C-10), B (C-16–C-27), and C (C-11–C-15) of biyoulactone A (**1**).

The connectivity of C-6 (unit A) to C-17 (unit B) was implied based on an HMBC correlation for 6-OH to C-17 (Figure 2). The connectivity between C-5 (unit A) and C-11 (unit C) was deduced due to the unsaturated degree of **1**. Accordingly, the gross structure of biyoulactone A (**1**) was elucidated as shown in Figure 2.

The relative stereochemistry for units A and B were assigned as follows. In unit A, NOESY cross-peaks of 6-OH/H-3b, 6-OH/H-4, 6-OH/H_3-7, H-3b/H-4, and H-3a/H_3-10 were observed, suggesting that 6-OH, C-7, and H-4 were all β-oriented (Figure 3). In unit B, the chair conformation for the cyclohexane ring (C-17–C-19 and C-23–C-25) was disclosed by NOESY cross-peaks of H-18a/H-25a and H-18a/H-23. The coupling constant for H-19 (td, J = 12.2, 4.0 Hz) implied the *trans* junction of the octahydroindene ring, while NOESY correlations for H-19/H-18b, H-19/H_3-27, and H-18b/H_3-27 indicated the β-orientations for H-19 and C-27. The stereochemistry for C-12 (unit C) and the relative relationship between units A and B were not assigned by the NOESY analysis.

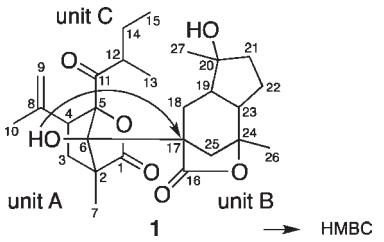

Figure 2. Gross structure of biyoulactone A (**1**).

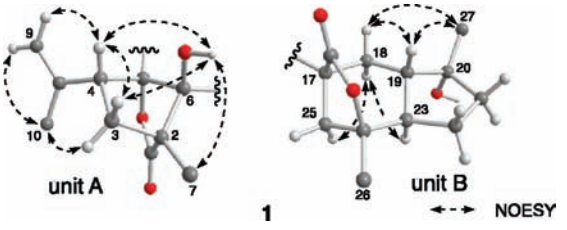

Figure 3. Selected NOESY correlations and relative stereochemistry for units A (C-1–C-10) and B (C-16–C-27) of biyoulactone A (**1**) (protons of methyl groups were omitted).

Finally, the structure of biyoulactone A (**1**) was confirmed by a single-crystal X-ray diffraction analysis.[8] The ORTEP drawing of **1** was shown in Figure 4. The analysis also revealed the absolute stereochemistry of **1** (Flack parameter, −0.25(21), calculated using 3157 Friedel pairs).[9] Therefore, the absolute configurations at 10 chiral centers of **1** were assigned as 2*R*, 4*R*, 5*S*, 6*R*, 12*S*, 17*S*, 19*S*, 20*R*, 23*R*, and 24*S*.

Biyoulactone B (**2**) was obtained as an optically active colorless amorphous solid {$[\alpha]_D^{25}$ +9.7 (*c* 0.18, MeOH)}. The HRESIMS analysis indicated that **2** had the same molecular formula, $C_{27}H_{38}O_7$ (*m*/*z* 497.2506 $[M+Na]^+$, Δ −0.4 mmu), as that of **1**. The ^1H and ^{13}C NMR data of **2** were similar to those of **1**, while differences were observed for the chemical shifts of H-4 and C-4 (Table 1). From these facts, **2** was deduced to be a stereoisomer of **1** at C-4. The proposed structure was confirmed by NOESY cross-peaks of 6-OH/H-3b, 6-OH/H_3-7, 6-OH/H_3-10, H-3b/

(8) Crystallographic data for biyoulactone A (**1**) have been deposited at the Cambridge Crystallographic Data Center (deposition number CCDC 830075).

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Table 1. ^{1}H and ^{13}C NMR Data for Biyoulactones A–C (**1**–**3**) in $CDCl_3$

	1		**2**		**3**	
position	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	177.3	–	177.2	–	177.3	–
2	56.9	–	55.7	–	55.6	–
3a	35.8	1.55 (1H, dd, J = 12.4, 4.7)	34.1	1.69 (1H, dd, J = 12.0, 8.6)	33.9	1.68 (1H, dd, J = 11.9, 8.8)
3b		2.50 (1H, dd, J = 12.4, 10.5)		2.48 (1H, dd, J = 12.0, 8.6)		2.47 (1H, dd, J = 11.9, 8.8)
4	49.7	3.81 (1H, dd, J = 10.5, 4.7)	57.4	2.76 (1H, t, J = 8.6)	57.8	2.79 (1H, t, J = 8.8)
5	97.0	–	95.9	–	96.0	–
6	90.7	–	89.7	–	89.7	–
7	11.6	1.16 (3H, s)	11.3	1.19 (3H, s)	11.3	1.19 (3H, s)
8	140.8	–	141.7	–	142.1	–
9	117.3	4.93, 4.82 (1H each, brs)	118.0	4.99, 4.88 (1H each, brs)	118.1	4.95, 4.84 (1H each, brs)
10	22.3	1.71 (3H, s)	21.9	1.84 (3H, s)	21.4	1.86 (3H, s)
11	208.5	–	206.6	–	206.2	–
12	45.6	2.73 (1H, m)	45.6	2.85 (1H, m)	45.8	2.83 (1H, m)
13	13.8	1.05 (3H, d, J = 7.0)	14.7	1.15 (3H, d, J = 7.1)	14.3	1.16 (3H, d, J = 7.0)
14	24.6	1.71, 1.41 (1H each, m)	24.2	1.73, 1.38 (1H each, m)	23.6	1.89, 1.40 (1H each, m)
15	11.3	0.91 (3H, t, J = 7.4)	11.4	0.93 (3H, t, J = 7.6)	11.4	0.96 (3H, t, J = 7.5)
16	180.5	–	180.6	–	179.8	–
17	51.7	–	52.1	–	50.6	–
18a	29.1	1.15 (1H, t, J = 12.2)	30.4	1.06 (1H, m)	31.5	1.04 (1H, t, J = 13.2)
18b		2.19 (1H, m)		2.27 (1H, dd, J = 7.3, 2.4)		2.30 (1H, dd, J = 13.2, 7.0)
19	49.7	1.08 (1H, td, J = 12.2, 4.0)	49.9	1.07 (1H, m)	46.2	1.84 (1H, m)
20	76.7[a]	–	76.8[a]	–	79.4	–
21a	40.1	1.70 (1H, m)	40.0	1.70 (1H, m)	37.5	1.77 (1H, m)
21b		1.85 (1H, dd, J = 11.6, 9.7)		1.84 (1H, m)		1.71 (1H, m)
22a	22.1	1.71 (1H, m)	22.3	1.73 (1H, m)	23.0	1.32 (1H, m)
22b		1.32 (1H, m)		1.30 (1H, m)		1.74 (1H, m)
23	48.4	1.95 (1H, td, J = 11.9, 6.3)	48.3	1.93 (1H, td, J = 12.1, 5.9)	45.3	2.35 (1H, q, J = 8.6)
24	85.0	–	84.7	–	87.6	–
25a	46.8	1.92 (1H, d, J = 12.1)	47.3	1.87 (1H, d, J = 12.0)	41.1	2.02 (2H, m)
25b		2.22 (1H, dd, J = 12.1, 2.3)		2.21 (1H, dd, J = 12.0, 2.4)		
26	22.1	1.47 (3H, s)	22.1	1.45 (3H, s)	23.9	1.41 (3H, s)
27	26.7	1.29 (3H, s)	26.8	1.29 (3H, s)	29.3	1.29 (3H, s)
6-OH		5.81 (1H, s)		5.83 (1H, s)		5.89 (1H, s)

[a] The signal was overlapped with that of $CDCl_3$, and the chemical shift was assigned by HMBC spectrum.

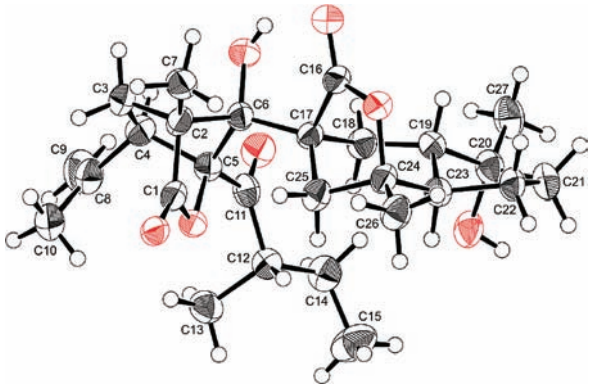


Figure 4. ORTEP drawing of biyoulactone A (**1**).

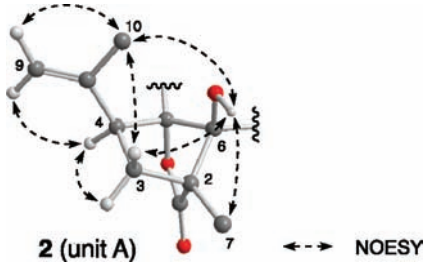
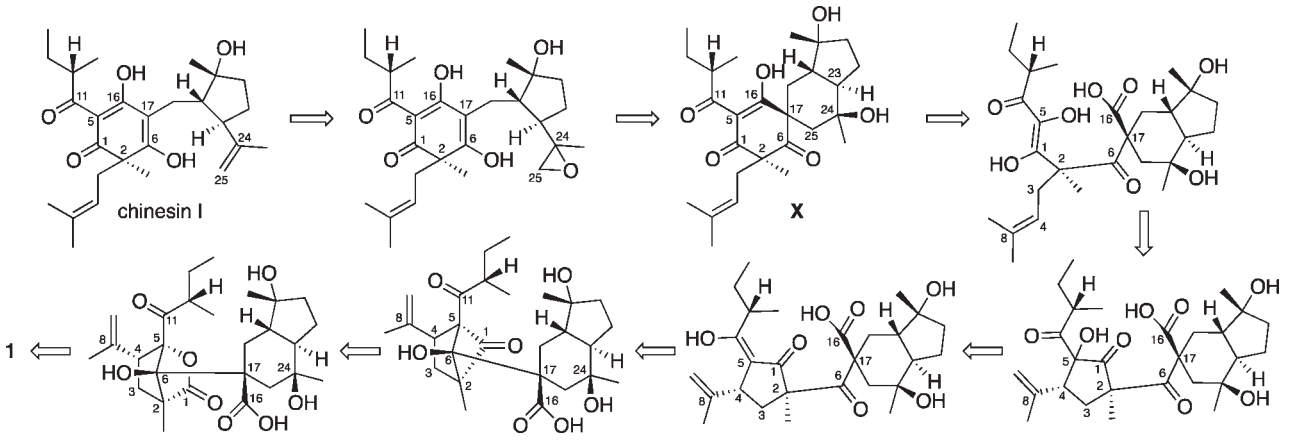


Figure 5. Selected NOESY correlations and relative stereochemistry for unit A (C-1–C-10) of biyoulactone B (**2**) (protons of methyl groups were omitted).

H_3-10, and H-3a/H-4 (Figure 5). Thus, the structure of biyoulactone B (**2**) was assigned as a 4-epimer of **1**.

Biyoulactone C (**3**), $C_{27}H_{38}O_7$ (m/z 497.2505 $[M+Na]^+$, Δ −0.5 mmu), was obtained as an optically active colorless amorphous solid {$[\alpha]_D^{25}$ +15.7 (c 0.09, MeOH)}. The analysis of the ^{1}H and ^{13}C NMR data (Table 1) implied **3** to be

Scheme 1. Possible Biogenetic Path of Biyoulactone A (**1**)



a stereoisomer of **2** on unit B (C-16−C-27). The relative stereochemistry of unit B in **3** was assigned as follows. NOESY correlations for H-18a/H-25a, H-18a/H-21a, and H-22a/H-25a suggested that these protons were oriented to the same α-side, while the *β*-orientations for H-18b, H-19, and H-23 were disclosed by NOESY cross-peaks of H-19/H-18b and H-19/H-23 (Figure 6). These correlations also indicated the chair conformation of the cyclohexane ring and the *cis* fusion of the octahydroindene ring. The *β*-orientation of C-27 was revealed by NOESY correlations for H_3-27/H-19 and H_3-27/H-23. Thus, the structure of biyoulactone C (**3**) was assigned as a 23-epimer of **2**.

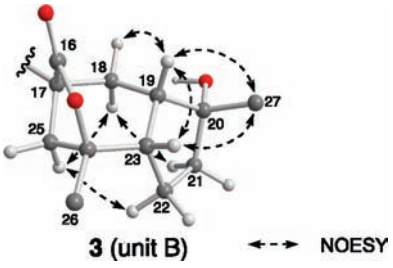


Figure 6. Selected NOESY correlations and relative stereochemistry for unit B (C-16−C-27) of biyoulactone C (**3**) (protons of methyl groups were omitted).

Biyoulactone A (**1**) is a novel pentacyclic meroterpenoid with a unique dilactone structure containing C−C bonded bi- and tricyclic *γ*-lactone moieties, while biyoulactones B (**2**) and C (**3**) are stereoisomers of **1**. The structure of **1**, including the absolute stereochemistry, and those of **2** and **3**, including the relative stereochemistry, were assigned based on the spectroscopic data.

A possible biogenetic path of biyoulactone A (**1**) is proposed as shown in Scheme 1. A plausible biogenetic intermediate (**X**) is derived by epoxidation of a double bond and intramolecular cyclization of chinesin I, a prenylated acylphloroglucinol previously isolated from the same plant origin.[6] The 23-epimer of **X**, hyperielliptone HB,[7] was also reported from *Hypericum geminiflorum*. Oxidative cleavage of a C-5−C-16 bond in **X** seems to occur and is followed by intramolecular cyclization, dehydration, further cyclization, Baeyer−Villiger oxidation, and lactonization to generate biyoulactone A (**1**). Biyoulactones A−C (**1**−**3**) did not show cytotoxicity against murine lymphoma L1210 cells and human epidermoid carcinoma KB cells (IC_{50} > 10 *μ*g/mL, each) in vitro and did not exhibit antimicrobial activity.

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Supporting Information Available. Experimental section and 1D and 2D NMR spectra for biyoulactones A−C. This material is available free of charge via the Internet at http://pubs.acs.org.