Tetrahedron Letters 50 (2009) 4747–4750

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Yezo'otogirins A–C, new tricyclic terpenoids from *Hypericum yezoense*

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ARTICLE INFO

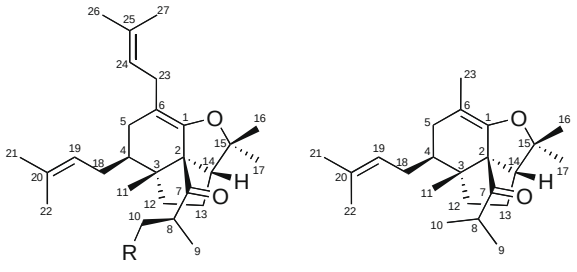
Article history:
Received 11 May 2009
Revised 2 June 2009
Accepted 5 June 2009
Available online 9 June 2009

ABSTRACT

Three new tricyclic terpenoids, yezo'otogirins A–C (**1**–**3**), were isolated from aerial parts of *Hypericum yezoense*. The structures including absolute stereochemistry of **1**–**3** were elucidated from the spectroscopic data and conformational analysis.

The genus *Hypericum* (family Clusiaceae) is known to be a traditional medicine for the treatment of burns, bruises, swelling, inflammation, and anxiety as well as bacterial and viral infections.[1] In our continuing search for structurally interesting compounds from *Hypericum* spp,[2] three new tricyclic terpenoids, yezo'otogirins A–C (**1**–**3**), were isolated from *H. yezoense*. In this Letter, we describe the isolation and structure elucidation of **1**–**3**.

The aerial parts of *H. yezoense* were extracted with MeOH, and the extracts were partitioned with *n*-hexane and H_2O. *n*-Hexane-soluble portions were subjected to a silica gel column ($CHCl_3$/MeOH) and a Sephadex LH-20 column (EtOH) chromatographies, and then purified by C_{18} HPLC (MeOH/H_2O) to yield yezo'otogirins A (**1**, 0.0003%), B (**2**, 0.0002%), and C (**3**, 0.00003%) together with a known hyperforin analogue (**4**).[3]



Yezo'otogirin A (**1**)[4] showed the molecular ion peak at *m/z* 398 $(M)^+$ in the EIMS, and the HREIMS analysis revealed the molecular formula to be $C_{27}H_{42}O_2$ (*m/z* 398.3184 $[M]^+$, Δ −0.1 mmu). The IR absorption at 1696 cm^{-1} implied the presence of carbonyl functionality. ^1H and ^{13}C NMR data (Table 1) of **1** indicated the presence of one 2-methylpropanoyl group, two prenyl groups, three sp^2 quaternary carbons, three sp^3 quaternary carbons, two sp^3 methines, three sp^3 methylenes, and three tertiary methyl groups. Analysis of the ^1H–^1H COSY spectrum revealed connections of C-8–C-9, C-8–C-10, C-23–C-24, C-12–C-14, C-4–C-5, C-4–C-18, and C-18–C-19 (Fig. 1). HMBC cross-peaks of H_2-23 to C-1, C-5, and C-6 suggested that C-1, C-5, and C-23 were connected to C-6. Connections among C-14, C-16, and C-17 through C-15 were implied by HMBC cross-peaks of H_3-17 to C-14, C-15, and C-16, while connections among C-2, C-4, C-11, and C-12 via C-3 were indicated by HMBC cross-peaks of H_3-11 to C-2, C-3, C-4, and C-12. HMBC correlations for H-14 to C-1, C-2, and C-7 suggested that C-2 was connected to C-1, C-7, and C-14. The presence of an ether linkage between C-1 and C-15 was deduced from the chemical shift of C-15 (δ_C 83.3) and unsaturated degree of **1**. Thus, the gross structure of yezo'otogirin A was elucidated to be **1**.

The relative stereochemistry of **1** is deduced from NOESY correlations as shown in Figure 2. The NOESY cross-peaks of H-14/H-12β, H-14/H-13β, H-14/H_3-16, H_3-11/H-12β, and H_3-11/H-18 indicated that these protons were β-oriented, while the α-orientation of H-4 was revealed by the correlation for H-4 to H-5α. In addition, the NOESY cross-peaks of H-8/H-5β and H-8/H_3-11 suggested that the 2-methylpropanoyl group was β-oriented. Thus, the relative stereochemistry of **1** is assigned as shown in Figure 2.

The HRESIMS of yezo'otogirin B (**2**)[5] revealed the molecular formula to be $C_{28}H_{44}O_2$ (*m/z* 435.3253 $[M+Na]^+$, Δ +1.4 mmu), which was larger by 14 mass units as compared with that of yezo'otogirin A (**1**). ^1H and ^{13}C NMR data of **2** were similar to those of **1**, except for the presence of signals due to a 2-methylbutanoyl group in place of those of a 2-methylpropanoyl group in **1**. The HMBC correlation for H-14 to C-7 indicated that the 2-methylbutanoyl group was attached to C-2 (Fig. 3). The NOESY spectrum of **2** showed correlations similar to that of **1** (Fig. 4). The relative stereochemistry of C-8 was deduced from NOESY cross-peaks of H-8/H-5β, H-8/H_3-11, and H_3-9/H_3-11. Thus, the structure and relative stereochemistry of yezo'otogirin B were elucidated to be **2**.

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doi:10.1016/j.tetlet.2009.06.021

Table 1
^{1}H and ^{13}C NMR data for yezo'otogirins A–C (**1**–**3**) in $CDCl_3$

Position	1		2		3	
	^{13}C	^{1}H[a]	^{13}C	^{1}H[a]	^{13}C	^{1}H[a]
1	149.0	—	148.8	—	149.3	—
2	73.6	—	73.6	—	73.6	—
3	48.6	—	48.6	—	48.5	—
4	47.5	1.15 (1H, m)	47.5	1.15 (1H, m)	47.0	1.22 (1H, m)
5	29.7	1.93 (1H, dd, *J* = 14.9, 3.2) 1.80 (1H, dd, *J* = 14.9, 10.9)	29.7	1.93 (1H, dd, *J* = 15.5, 3.1) 1.81 (1H, dd, *J* = 15.5, 10.8)	32.8	1.95 (1H, dd, *J* = 15.3, 11.4) 1.86 (1H, dd, *J* = 15.3, 3.0)
6	111.4	—	111.6	—	107.5	—
7	217.4	—	216.1	—	217.4	—
8	37.8	2.99 (1H, sept, *J* = 6.9)	44.5	2.74 (1H, m)	37.9	2.96 (1H, sept, *J* = 6.6)
9	18.3[b]	1.02 (3H, d, *J* = 6.9)[c]	14.2	1.00 (3H, d, *J* = 6.9)	18.3[d]	1.01 (3H, d, *J* = 6.6)[e]
10	21.6[b]	1.01 (3H, d, *J* = 6.9)[c]	27.5	1.70, 1.03 (each 1H, m)	21.5[d]	1.01 (3H, d, *J* = 6.6)[e]
11	19.5	0.73 (3H, s)	19.7	0.74 (3H, s)	19.6	0.75 (3H, s)
12	41.3	1.74, 1.34 (each 1H, m)	41.2	1.74, 1.34 (each 1H, m)	41.5	1.75, 1.46 (each 1H, m)
13	25.5	1.53 (2H, m)	25.4	1.53 (2H, m)	25.4	1.54 (2H, m)
14	54.9	3.19 (1H, t, *J* = 9.7)	54.7	3.20 (1H, t, *J* = 9.8)	54.9	3.18 (1H, t, *J* = 9.6)
15	83.3	—	83.4	—	83.4	—
16	29.4	1.13 (3H, s)	29.5	1.14 (3H, s)	29.4	1.14 (3H, s)
17	25.3	1.18 (3H, s)	25.3	1.18 (3H, s)	25.4	1.18 (3H, s)
18	29.5	1.89, 1.82 (each 1H, m)	29.5	1.88, 1.83 (each 1H, m)	29.6	1.93, 1.81 (each 1H, m)
19	124.3	5.07 (1H, t, *J* = 7.5)	124.3	5.07 (1H, m)	124.2	5.11 (1H, t, *J* = 6.9)
20	132.0	—	132.2	—	132.3	—
21	25.9	1.72 (3H, s)	25.7	1.71 (3H, s)	25.8	1.72 (3H, s)
22	17.8	1.59 (3H, s)	17.8	1.60 (3H, s)	17.8	1.60 (3H, s)
23	28.8	2.82, 2.79 (each 1H, dd, *J* = 14.2, 7.3)	28.9	2.82, 2.79 (each 1H, dd, *J* = 14.8, 6.8)	16.3	1.70 (3H, s)
24	121.8	5.06 (1H, t, *J* = 7.3)	122.0	5.07 (1H, m)		
25	132.2	—	131.9	—		
26	25.7	1.70 (3H, s)	25.9	1.70 (3H, s)		
27	17.8	1.65 (3H, s)	17.7	1.65 (3H, s)		
28			11.6	0.86 (3H, t, *J* = 7.2)		

[a] Coupling constants are given (*J*, Hz) in parentheses.
[b–e] Signals may be exchangeable.

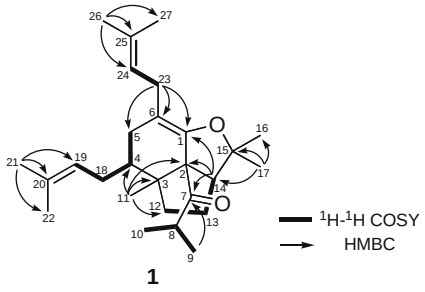


Figure 1. Selected 2D NMR correlations for yezo'otogirin A (**1**).

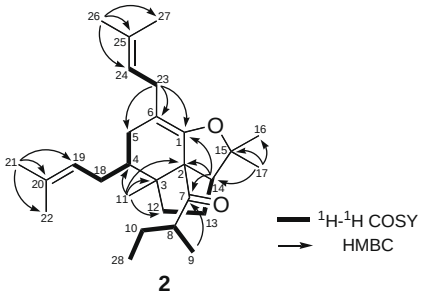


Figure 3. Selected 2D NMR correlations for yezo'otogirin B (**2**).

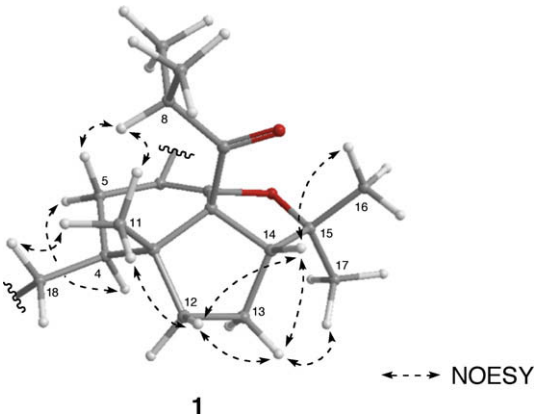


Figure 2. Selected NOESY correlations and relative stereochemistry for yezo'otogirin A (**1**) (C-19–C-27 were not shown).

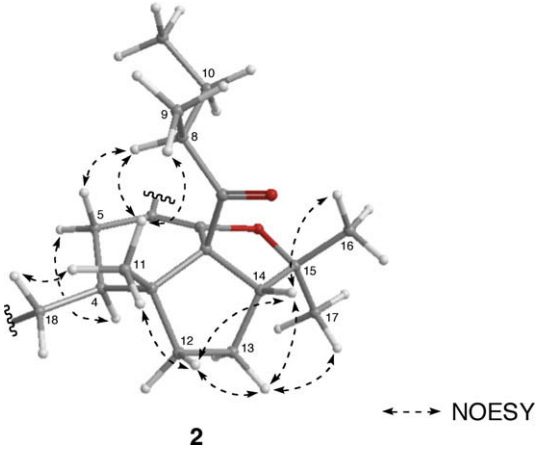


Figure 4. Selected NOESY correlations and relative stereochemistry for yezo'otogirin B (**2**) (C-19–C-27 were not shown).

The molecular formula of yezo'otogirin C (**3**),[6] $C_{23}H_{36}O_2$, was established by the HRESIMS, which was smaller by 54 mass units as compared with that of yezo'otogirin A (**1**). Except for signals of an alkyl side chain at C-6, ^1H and ^{13}C NMR data of yezo'otogirin C (**3**) were similar to those of **1** (Table 1). HMBC correlations for H_3-23 to C-1, C-5, and C-6 indicated that a methyl group was attached to C-6 (Fig. 5). The relative stereochemistry of **3** was elucidated to be the same as that of **1** from NOESY correlations for **3** (Fig. 6), which were similar to those observed for **1**. Thus, the structure of yezo'otogirin C was assigned as **3**.

Absolute stereochemistry of yezo'otogirin A (**1**) was elucidated on the basis of the pattern of CD spectrum of **1**. The most stable conformer of **1**, which was obtained by the conformational analysis

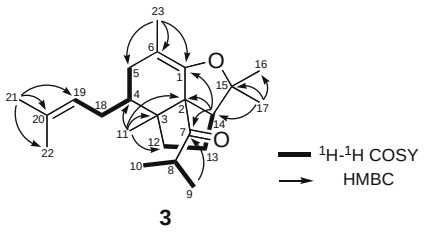


Figure 5. Selected 2D NMR correlations for yezo'otogirin C (**3**).

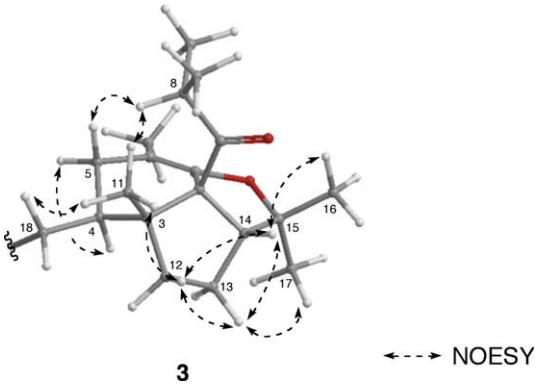


Figure 6. Selected NOESY correlations and relative stereochemistry for yezo'otogirin C (**3**) (C-19–C-22 were not shown).

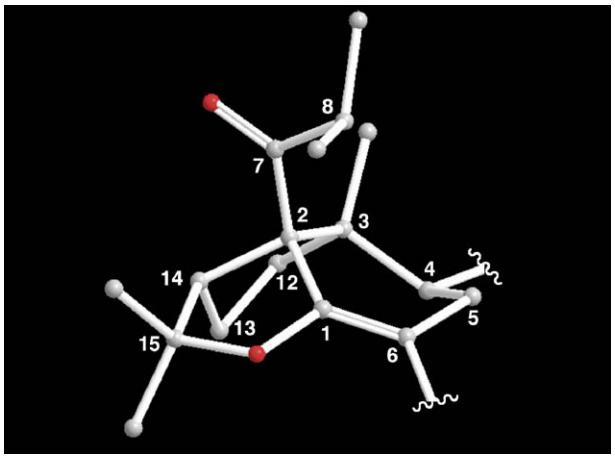


Figure 7. The most stable conformer of yezo'otogirin A (**1**) (C-18–C-27 and hydrogen atoms are not shown).

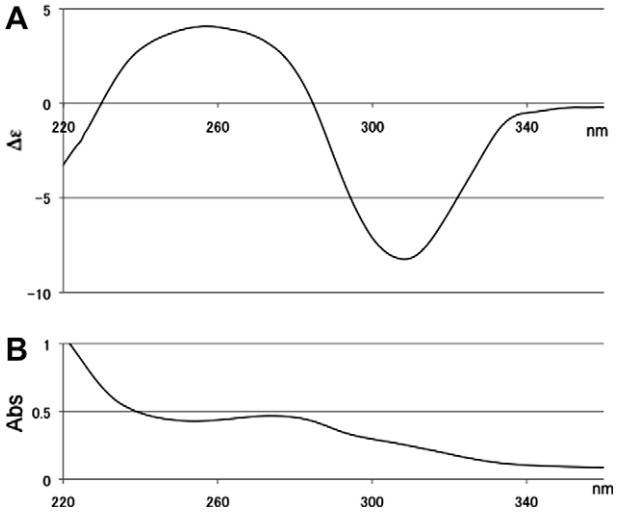
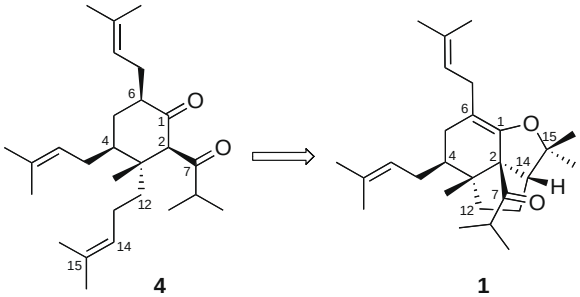


Figure 8. CD (A) and UV (B) spectra of yezo'otogirin A (**1**) in MeOH.



Scheme 1. Plausible biogenetic path of yezo'otogirin A (**1**).

(MacroModel, MM3 force-field), is shown in Figure 7. This conformer agreed with that estimated by NOESY analysis (Fig. 2). It was reported that mixing of the olefinic $\pi\rightarrow\pi^*$ transition with carbonyl $n\rightarrow\pi^*$ band of β,γ-unsaturated ketones results in the chirality of the β,γ-heterodiene π-system dominating the sign of the $n\rightarrow\pi^*$ CD band.[7] Negative Cotton effect at 308 nm ($\Delta\varepsilon$ −8.3) in the CD spectrum for **1** (Fig. 8) suggested the chirality of the β,γ-heterodiene π-system (O-7–C-7–C-2–C-1–C-6) as shown in Figure 7. Thus, the absolute configurations at C-2, C-3, C-4, and C-14 of yezo'otogirin A (**1**) were assigned to be *S*, *R*, *S*, and *R*, respectively. Absolute configurations of yezo'otogirins B (**2**) and C (**3**) were also assigned to be 2*S*, 3*R*, 4*S*, 8*S*, and 14*R* for **2**, 2*S*, 3*R*, 4*S*, and 14*R* for **3**, respectively, due to negative Cotton effects at 308 nm ($\Delta\varepsilon$ −6.6 and −2.4, respectively).

Yezo'otogirins A–C (**1**–**3**) are new tricyclic terpenoids with a rare ring system from natural sources. A plausible biogenetic path for yezo'otogirin A (**1**) is proposed as shown in Scheme 1. Yezo'otogirin A (**1**) could be derived from a known hyperforin analogue (**4**),[3] by intramolecular cyclization. Yezo'otogirins A–C (**1**–**3**) did not show cytotoxicity against L1210 murine leukemia (IC_{50} >10 μg/mL) in vitro.

Acknowledgments

We thank N. Yoshida, Health Sciences University of Hokkaido, for collection and botanical identification of the plant, S. Oka and A. Tokumitsu Center for Instrumental Analysis, Hokkaido University, for measurements of HRESIMS and HREIMS. This work was partly supported by a grant from the Kuribayashi Foundation and

a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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4. Yezo'otogirin A (**1**) colorless oil; $[\alpha]_D^{23}$ −168.2 (*c* 0.25 $CHCl_3$); IR (KBr) ν_{max} 2931, 1696, 1450, 1381, and 1086 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); EIMS *m/z* 398 $(M)^+$; HREIMS: *m/z* 398.3184 $(M)^+$ (calcd for $C_{27}H_{42}O_2$, 398.3185); CD (MeOH) $\Delta\varepsilon$ (nm) −8.3 (308) and +4.1 (257).
5. Yezo'otogirin B (**2**) colorless oil; $[\alpha]_D^{23}$ −165.7 (*c* 0.15 $CHCl_3$); IR (KBr) ν_{max} 2930, 1694, 1457, 1381, and 1090 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); ESIMS *m/z* 435 $(M+Na)^+$; HRESIMS: *m/z* 435.3253 $(M+Na)^+$ (calcd for $C_{28}H_{44}O_2Na$, 435.3239); CD (MeOH) $\Delta\varepsilon$ (nm) −6.6 (308) and +4.0 (268).
6. Yezo'otogirin C (**3**) Colorless oil; $[\alpha]_D^{23}$ −57.2 (*c* 0.05 $CHCl_3$); IR (KBr) ν_{max} 2920, 1697, 1465, 1381, and 1105 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); ESIMS *m/z* 367 $(M+Na)^+$; HRESIMS: *m/z* 367.2603 $(M+Na)^+$ (calcd for $C_{23}H_{36}O_2Na$, 367.2613); CD (MeOH) $\Delta\varepsilon$ (nm) −2.4 (308) and +1.4 (255).
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