Yezo’otogirins D–H, Acylyphloroglucinols and Meroterpenes from Hypericum yezoense

Naonobu Tanaka,*a,b Eri Tsuji,a Yoshiki Kashiwada,b and Jun’ichi Kobayashi*a

*aGraduate School of Pharmaceutical Sciences, Hokkaido University; Sapporo 060–0812, Japan; and
bGraduate School of Pharmaceutical Sciences, Tokushima University; Tokushima 770–8505, Japan.

Received March 10, 2016; accepted April 3, 2016

Investigation of the methanolic extract from the aerial parts of Hypericum yezoense resulted in the isolation of three new acylyphloroglucinols, yezo’otogirins D–F (1–3), and two new meroterpenes, yezo’otogirins G (4) and H (5). The structures of 1–5 were assigned on the basis of spectroscopic data. Yezo’otogirin D (1) is an acylyphloroglucinol with a monoterpene moiety linked through an ether bond, while yezo’otogirins E (2) and F (3) are polyprenylated acylyphloroglucinols possessing a tricyclic core. Yezo’otogirins G (4) and H (5) are linear meroterpenes with an enolized β-diketone moiety. Yezo’otogirin E (2) exhibited antimicrobial activity against Escherichia coli and Staphylococcus aureus.

Key words acylyphloroglucinol; meroterpene; Hypericum yezoense; yezo’otogirin

The plants belonging to the genus Hypericum (Hypericaceae) are distributed widely in temperate regions and have been used as traditional remedies in various parts of the world.1,2 Structurally interesting acylyphloroglucinol and meroterpenes possessing diverse biological activities such as antidepressant, antimicrobial, antiviral, and cytotoxic activities have been isolated from Hypericum plants.3,4 In our continuing search for structurally unique natural products from Hypericum plants,5–8 we have reported the isolation of tricyclic meroterpenes, yezo’otogirins A–C,9 from the aerial parts of H. yezoense. Further investigation on the constituents of this species afforded three new acylyphloroglucinols, yezo’otogirins D–F (1–3), and two new meroterpenes, yezo’otogirins G (4) and H (5) (Chart 1). In this paper, the isolation and structure elucidation of 1–5 are described.

The methanolic extract of the aerial parts of H. yezoense was partitioned with n-hexane and water. Repeated chromatographic separations of the n-hexane-soluble fraction with a silica gel column, an octadecylsilyl (ODS) column, and a Sephadex LH-20 column gave the fractions containing acylyphloroglucinols and meroterpenes, which were purified by ODS HPLC to give yezo’otogirins D (1, 0.00012%), E (2, 0.00042%), F (3, 0.00082%), G (4, 0.00062%), and H (5, 0.00012%).

Yezo’otogirin D (1) was obtained as an optically active pale yellow oil \(\{[\alpha]_D +10.8\ (c\ 0.06, \text{MeOH})\}\), and its molecular formula was elucidated to be \(\text{C}_{22}\text{H}_{34}\text{O}_{12}\) by the high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) \((m/z\ 401.19327 \ [M+Na]^+\), \(\Delta -0.19\text{mmu})\). The IR spectrum displayed absorptions at 3303 and 1731 cm\(^{-1}\), indicating the existence of hydroxy and carbonyl functionalities, respectively. The \(^1\text{H}-\text{NMR}\) spectrum showed the resonances due to one vinyl group, one isopropyl group, one oxygenated \(sp^3\) methine, two \(sp^3\) methylenes, and four tertiary methyls as well as the signals of three \(\text{D}_2\text{O}\)-exchangeable protons (Table 1). The \(^{13}\text{C}-\text{NMR}\) and distortionless enhancement by polarization transfer (DEPT) spectra suggested the presence of one fully substituted benzene ring, one carbonyl carbon, and two quaternary carbons. These observations implied 1 to be an acylyphloroglucinol derivative with a \(C_{10}\) unit.

The acyl group of 1 was assigned as a 2-methylpropanoyl group by an heteronuclear multiple bond coherence (HMBC) cross-peak of \(H_2-11\) to C-8 (Fig. 1). The downfield-shifted chemical shift of \(1\text{-OH}\) implied the presence of a hydrogen bond between 1-OH and the carbonyl group (C-8), which allowed the assignment of the 2-methylpropanoyl group at C-6. HMBC correlations for 3-OH with C-2, 5-OH with C-6, and H-7 with C-1, C-2, and C-3 suggested the existence of a methyl group at C-7 and hydroxy groups at C-3 and C-5. The chemical shift of C-4 (\(\delta_c 122.0\)) implied the presence of an oxygen function at C-4. In the C\(_{10}\) unit (C-1’–C-10’), \(^1\text{H}–^1\text{H}\) correlation spectroscopy (COSY) analysis revealed the presence of the spin system for C-4’ to C-6’, while HMBC analysis disclosed the connectivities among C-4’, the vinyl group (C-2’), and the methyl group (C-10’) via C-3’, and among C-6’ and the methyl groups (C-8’ and C-9’) via C-7’. The chemical shifts of C-3’ (\(\delta_c 84.8\)), C-6’ (\(\delta_c 85.2\)), and C-7’ (\(\delta_c 84.1\)) indicated that these carbons were oxygenated. A nuclear Overhauser effect spectroscopy (NOESY) correlation for H-6’ with H-1’ suggested the presence of an ether linkage between C-3’ and C-6’, forming a tetrahydrofuran ring, as well as the anti relationship of the vinyl group at C-3’ and the substituent at C-6’. Given the molecular formula of 1, the connectivity of C-4 and C-7’ via an oxygen atom was deduced. Thus, the structure of yezo’otogirin D (1) was elucidated as shown in Chart 1.

Yezo’otogirin E (2) was isolated as an optically active pale yellow oil \(\{[\alpha]_D +21.4\ (c\ 1.7, \text{MeOH})\}\). The molecular...

* To whom correspondence should be addressed. e-mail: ntanak@tokushima-u.ac.jp; jkobay@pharm.hokudai.ac.jp

© 2016 The Pharmaceutical Society of Japan
formula of 2 was assigned as C_{26}H_{35}O_{4} by the HR-ESI-MS (m/z 411.25470 [M+Na]^+, Δ +0.68 mmu). In the 1H-NMR spectrum of 2, a pair of downfield-shifted hydroxy signals due to keto–enol tautomerism were observed at δ_H 18.76 and 18.44 in the ratio of ca. 2:1, characteristic of a diketo enolic moiety of polyprenylated acylphloroglucinols found in Hypericum plants. Comparison of the one-dimensional (1D)-NMR data (for major tautomer 2a in Table 2; minor tautomer 2b in Experimental) with the data in the literature suggested that 2 is a tricyclic acylphloroglucinol derivative similar to ialibinone A^{10} but with a different substituent at C-3. The substituent was assigned as 6-methylhepta-1,5-diene (C-15–C-22) by interpretation of the 1H–1H COSY and HMBC spectra (Fig. 2). The connectivity of C-3 to C-15 was elucidated by an HMBC correlation for H_2-16 with C-3. The relative stereochemistry of the tricyclic moiety of 2, including the relative configuration of C-3, was concluded to be the same as ialibinone A by resemblance of the chemical shifts and NOESY analysis. Similarly, the structure of yezo’otogirin F (3), which also showed a pair of the signals due to major (3a) and minor (3b) tautomers in the ratio of ca. 5:4 in the 1H-NMR spectrum (CDCl_3), was assigned as a 3-epimer of 2 by 2D-NMR analysis and comparison of the spectral data with the spectral data for ialibinone B^{10} (3-epimer of ialibinone A).

Yezo’otogirin G (4) was isolated as an optically active pale yellow oil ([α]_D^{20}−10.8 (c 1.27, MeOH)). The HR-ESI-MS revealed the molecular formula of 4 to be C_{15}H_{24}O_{4} (m/z 291.15668 [M+Na]^+, Δ 0.00 mmu). The 1H- and 13C-NMR spectra showed the signals of one carboxy, one methoxy, one prenyl, one isopropyl, and one methyl groups as well as the characteristic resonances due to one enolized β-diketone moiety (C-3–C-5)\textsuperscript{11} (Table 3). Interpretation of the HMBC spectrum revealed the connectivities between the isopropyl group (C-6) and the β-diketone moiety (C-5), and among the β-diketone moiety (C-3), the methyl group, the prenyl group, and the methoxy carbonyl group via a quaternary carbon (C-2) (Fig. 3). Therefore, the structure of yezo’otogirin G (4) was assigned as shown in Chart 1.

The molecular formula of yezo’otogirin H (5) was assigned as C_{20}H_{32}O_{4} in light of the HR-ESI-MS. Though the 1D NMR spectral feature of 5 was similar to that of 4, the signals due to the prenyl group for 4 were replaced by those of the geranyl group for 5 (Table 3). The geranyl group at C-2 for 5 was confirmed by an HMBC correlation for H_3-9 with C-10, while the geometry of the double bond at C-11 was elucidated to be E by a NOESY cross-peak of H-11 with H_2-14. Thus, the structure...
of yezo’otogirin H (5) was assigned as shown in Chart 1. The absolute configurations of C-2 for 4 and 5 remained unsolved. The structures of yezo’otogirins G (4) and H (5) are partially correlated with that of yojironin A,11) a meroterpene isolated from Hypericum yojiroanum. As in the case of yojironin A, a plausible biogenetic precursor (X) composed of valine and two acetate units might be generated followed by methylation and prenylation or geranylation to generate 4 and 5 (Chart 2).

Yezo’otogirin E (2) exhibited moderate antimicrobial activity against Escherichia coli (MIC 4.0 µg/mL) and Staphylococcus aureus (MIC 8.0 µg/mL), while yezo’otogirins G (4) and H (5) showed weak antimicrobial activity against Bacillus subtilis (MIC 16 µg/mL) and Trichophyton mentagrophytes (IFM62679, IC50 16 µg/mL).

Experimental

General Optical rotations and IR spectra were recorded on a JASCO P-1030 digital polarimeter and a JASCO FT/IR-230 spectrophotometer, respectively. NMR spectra were measured by a Bruker AMX-600 spectrometer and a JEOL ECA 500 spectrometer. The resonances of residual CHCl3 (7.26 and 77.0 ppm) were used as internal references for 1H- and 13C-NMR chemical shifts, respectively. HR-ESI-MS spectra were recorded on a Thermo Scientific Exactive spectrometer.

Plant Material Hypericum yezoense was cultivated at the Experimental Station for Medicinal Plant Studies, Hokkaido University, and the aerial parts were collected in August 2012. Herbarium specimen (specimen number: HYJ201208) was deposited in Graduate School of Pharmaceutical Sciences, Tokushima University.

Extraction and Isolation The dried aerial parts of H. yezoense (850 g) were extracted with MeOH to afford the extract (88.5 g), which was partitioned with n-hexane and water. The n-hexane-soluble fraction (36.7 g) was loaded on a silica gel column chromatography (MeOH:CH3OH, 70:30 to 100:0) and purified by ODS HPLC (COSMOSIL 5C18-MS-II, Nacalai Tesque, 20×250mm, MeOH:H2O, 90:10) to give yezo’otogirin H (5, 1.0mg). Fraction 4 was separated by an ODS column chromatography (MeOH:H2O, 50:50 to 100:0) and a silica gel column chromatography (n-hexane:EtOAc, 95:5 to 0:100), and

---

### Table 2. 1H- and 13C-NMR Data for Major Tautomers (2a and 3a) of Yezo’otogirins E (2) and F (3) in CDCl3

| Position | δC | δH (J in Hz) | δC | δH (J in Hz) |
|----------|----|--------------|----|--------------|
| 1        | 72.0 | — | 72.3 | — |
| 2        | 25.9 | 2.51 (1H, t, 13.3) | 24.6 | 2.52 (1H, dd, 13.7, 7.0) |
| 3        | 53.6 | 2.16 (1H, dd, 13.3, 5.9) | 57.6 | 2.50 (1H, m) |
| 4        | 42.7 | — | 44.8 | — |
| 5        | 55.4 | 2.08 (1H, m) | 55.8 | 2.29 (1H, dd, 9.8, 5.8) |
| 6        | 34.4 | 1.74 (1H, dd, 13.0, 9.4) | 32.7 | 2.22 (1H, m) |
| 7        | 61.3 | — | 62.1 | — |
| 8        | 201.6 | — | 201.7 | — |
| 9        | 109.6 | — | 108.0 | — |
| 10       | 191.0 | — | 191.0 | — |
| 11       | 206.1 | — | 206.7 | — |
| 12       | 24.4 | 0.97 (3H, s) | 16.4 | 0.58 (3H, s) |
| 13       | 25.8 | 0.82 (3H, s) | 27.2 | 0.94 (3H, s) |
| 14       | 12.4 | 1.40 (3H, s) | 12.4 | 1.38 (3H, s) |
| 15       | 147.0 | — | 146.8 | — |
| 16       | 111.9 | 4.95, 4.88 (each 1H, s) | 112.2 | 5.00, 4.88 (each 1H, s) |
| 17       | 36.6 | 2.07, 1.97 (each 1H, m) | 36.8 | 2.09, 2.00 (each 1H, m) |
| 18       | 27.0 | 2.12 (2H, m) | 27.1 | 2.13 (2H, m) |
| 19       | 124.0 | 5.11 (1H, t, 6.7) | 124.0 | 5.12 (1H, m) |
| 20       | 131.7 | — | 131.8 | — |
| 21       | 17.7 | 1.61 (3H, s) | 17.7 | 1.62 (3H, s) |
| 22       | 25.6 | 1.69 (3H, s) | 25.7 | 1.69 (3H, s) |
| 23       | 208.6 | — | 209.6 | — |
| 24       | 34.8 | 4.01 (1H, sept, 6.7) | 34.9 | 4.04 (1H, sept, 6.7) |
| 25       | 18.4 | 1.15 (3H, d, 6.7) | 18.6 | 1.18 (3H, d, 6.7) |
| 26       | 19.0 | 1.15 (3H, d, 6.7) | 18.9 | 1.14 (3H, d, 6.7) |
| 8-OH     | 18.76 (1H, s) | — | 18.85 (1H, s) | — |

*a) The data for minor tautomers (2b and 3b) of yezo’otogirins E (2) and F (3) are listed in Experimental.
Table 3. 1H- and 13C-NMR Data for Yezo’otogirins G (4) and H (5) in CDCl₃

| Position | δH (J in Hz) | δC (J in Hz) |
|----------|--------------|--------------|
| 1        | 173.6        | —            |
| 2        | 56.2         | —            |
| 3        | 196.9        | —            |
| 4        | 54.2         | 5.52 (1H, s) |
| 5        | 195.4        | —            |
| 6        | 35.9         | 2.46 (1H, sept, 6.7) |
| 7        | 19.5         | 1.14 (3H, d, 6.7) |
| 8        | 19.6         | 1.14 (3H, d, 6.7) |
| 9        | 19.5         | 1.34 (3H, s) |
| 10       | 34.1         | 2.61, 2.50 (1H, dd, 14.3, 7.6) |
| 11       | 118.2        | 4.98 (1H, t, 7.6) |
| 12       | 135.6        | —            |
| 13       | 17.9         | 1.60 (3H, s) |
| 14       | 26.0         | 1.69 (3H, s) |
| 15       | —            | —            |
| 16       | —            | 26.6         |
| 17       | —            | 124.1        |
| 18       | —            | 131.5        |
| 19       | 15.0         | 25.7         |
| 5-OH     | —            | 15.33 (1H, s) |
| 1-OMe    | 52.4         | 3.70 (3H, s) |

Chart 2. Possible Biogenetic Pathway of Yezo’otogirins G (4) and H (5)

then purified by ODS HPLC (YMC-Pack ODS-AQ, YMC Co., Ltd., 10×250 mm, MeOH:H₂O, 90:10) to isolate yezo’otogirin G (4, 5.3 mg). A column chromatography of fr. 5 on silica gel (n-hexane:EtOAc, 95:5 to 0:100) gave five fractions (frs. 5.1–5). The MeOH-soluble part of fr. 5.2 was separated by an ODS column chromatography (MeOH:H₂O, 50:50 to 0:100) and ODS HPLC (YMC-Pack ODS-AQ, 20×250 mm, MeOH:H₂O, 85:15) to give yezo’otogirin E (2, 3.5 mg) and F (3, 7.0 mg). An ODS column chromatography (MeOH:H₂O, 50:50 to 0:100) of the MeOH-soluble portion of fr. 6 afforded eight fractions (frs. 6.1–8). Fraction 6.2 was subjected to a silica gel column chromatography (n-hexane:EtOAc 100:0 to 0:100) and a Sephadex LH-20 column chromatography (MeOH:H₂O, 80:20 to 100:0) to afford seven fractions (frs. 6.2.1–7). Yezo’otogirin D (1, 1.0 mg) was isolated from fr. 6.2.5 using ODS HPLC (COSMOSIL 5C₁₈-AR-II, 10×250 mm, MeOH:H₂O, 75:25).

Yezo’otogirin D (1)

Colorless amorphous solid, [α]D=+10.8 (c 0.06, MeOH), IR (KBr) νmax 3303, 1731, and 1627 cm⁻¹, HR-ESI-MS: m/z 401.19327 [M+Na]⁺ (calcd for C₂₁H₂₃O₄Na, 401.19346), 1H- and 13C-NMR data (Table 1).

Yezo’otogirin E (2)

Pale yellow oil, [α]D=+21.4 (c 1.7, MeOH), IR (KBr) νmax 3308 and 1763 cm⁻¹, HR-ESI-MS: m/z 411.25470 [M–H]⁻ (calcd for C₂₃H₃₃O₄Na, 411.25480), 1H- and 13C-NMR for major tautomer (2a) (Table 2), 1H-NMR for minor tautomer (2b) δH

Yezo’otogirin F (3)

Pale yellow oil, [α]D=+75.9 (c 1.7, MeOH), IR (KBr) νmax 3315 and 1759 cm⁻¹, HR-ESI-MS: m/z 411.25453 [M–H]⁻ (calcd for C₂₃H₃₃O₄Na, 411.25480), 1H- and 13C-NMR for major tautomer (3a) (Table 2), 1H-NMR for minor tautomer (3b) δH
Chem. Pharm. Bull. 

(C-11), 200.3 (C-10), 193.6 (C-8), 146.6 (C-15), 131.7 (C-20),
123.9 (C-19), 112.4 (C-16), 107.9 (C-9), 68.2 (C-1), 66.2 (C-7),
58.2 (C-3), 57.7 (C-5), 45.1 (C-4), 36.8 (C-17), 34.6 (C-24), 31.1
(C-6), 27.2 (C-13), 27.1 (C-18), 25.7 (C-22), 25.2 (C-2), 18.8
(C-26), 18.7 (C-25), 17.7 (C-21), 16.9 (C-12), and 13.1 (C-14).

Yezo'otogirin G (4)
Pale yellow oil, $\alpha$D −10.8 (c 1.27, MeOH), IR (KBr) $\nu_{\text{max}}$
1739 and 1602 cm$^{-1}$, HR-ESI-MS: $m/z$ 291.15668 [M+Na]$^+$
(called for C$_{15}$H$_{24}$O$_4$Na, 291.15668), $^1$H- and $^{13}$C-NMR (Table
3).

Yezo'otogirin H (5)
Pale yellow oil, $\alpha$D −16.8 (c 0.19, MeOH), IR (KBr) $\nu_{\text{max}}$
1733 and 1616 cm$^{-1}$, HR-ESI-MS: $m/z$ 359.21921 [M+Na]$^+$
(called for C$_{20}$H$_{32}$O$_4$Na, 359.21928), $^1$H- and $^{13}$C-NMR (Table
3).

Antimicrobial Assay

Antimicrobial assay was carried out
as previously described.12

Acknowledgments
We thank Prof. T. Gonoi, Dr. A.
Takahashi-Nakaguchi, and Dr. K. Sakai, Mycology Research
Center, Chiba University, for evaluation of antimicrobial ac-
tivity. This work was partly supported by a Grant-in-Aid for
Scientific Research from the Ministry of Education, Culture,
Sports, Science and Technology of Japan.

Conflict of Interest
The authors declare no conflict of interest.

References
1) Cuesta-Rubio O., Piccinelli A. L., Rastrelli L., “Studies in natural
products chemistry,” Vol. 32, ed. by Atta-ur-Rahman A., Elsevier
Science B. V., Amsterdam, 2005, pp. 671–720.
2) Avato P., “Studies in natural products chemistry,” Vol. 30, ed. by
Atta-ur-Rahman A., Elsevier Science B. V., Amsterdam, 2005, pp.
603–634.
3) Zhao J., Liu W., Wang J.-C., Chem. Biodivers., 12, 309–349 (2015).
4) Tanaka N., Kobayashi J., Heterocycles, 90, 23–40 (2015).
5) Hashida C., Tanaka N., Kawazoe K., Murakami K., Sun H.-D.,
Takaishi Y., Kashiwada Y., J. Nat. Med., 68, 737–742 (2014).
6) Abe S., Tanaka N., Kobayashi J., J. Nat. Prod., 75, 484–488 (2012).
7) Tanaka N., Abe S., Kobayashi J., Tetrahedron Lett., 53, 1507–1510
(2012).
8) Tanaka N., Abe S., Hasegawa K., Shiro M., Kobayashi J., Org. Lett.,
13, 5488–5491 (2011).
9) Tanaka N., Kakuguchi Y., Ishiyama H., Kubota T., Kobayashi J.,
Tetrahedron Lett., 50, 4747–4750 (2009).
10) Winkelmann K., Heilmann J., Zerbe O., Rali T., Sticher O., J. Nat.
Prod., 63, 104–108 (2000).
11) Mamemura T., Tanaka N., Shibazaki A., Gonoi T., Kobayashi J.,
Tetrahedron Lett., 52, 3575–3578 (2011).
12) Nagai H., Mikami Y., Yazawa K., Gonoi T., Yasumoto T., J. Anti-
biot., 46, 520–522 (1993).