Spirocyclic Acylphloroglucinol Derivatives from Hypericum beanii

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Received June 9, 2011; accepted July 1, 2011; published online July 7, 2011

Four new acylphloroglucinols with an unusual 6/6/5 spirocyclic skeleton, hyperbeanols A—D (1—4), were isolated from the methanol extract of Hypericum beanii along with 16 known compounds. Their structures were established on the basis of spectroscopic and X-ray diffraction analysis. Hyperbeanols A—C were three stereoisomers different only at the relative configuration of C-4 and C-13, which were distinguished by the nuclear Overhauser effect spectroscopy (NOESY) spectroscopic data in combination with the single X-ray analysis of hyperbeanol A (1). The cytotoxic activity of hyperbeanols A—D against the cancer cell lines SK-BR-3, HL-60, SMMC-7721, PANC-1, MCF-7, and K562 was also evaluated.

Key words Hypericum beanii; acylphloroglucinol; hyperbeanol A; hyperbeanol B; hyperbeanol C; hyperbeanol D

Polyprenylated acylphloroglucinols, featured a highly oxygenated bicyclo[3.3.1]-nonane-2,4,9-trione or bicyclo[3.2.1]octane-2,4,8-trione core decorated with C15H30 or C16H27 (prenyl, geranyl, etc.) side chains, were only isolated from the Guttiferae plants so far.1) This type of metabolites were reported to possess a wide variety of biological activities such as antimicrobial, antidepressant, antioxidative, cytotoxic, and anti-human immunodeficiency virus (HIV) effects.2—7) As an important member of the family Guttiferae, the genus Hypericum have been proved to be a prolific source of this special type metabolites.8—11) H. beanii N. Robson is a shrub of this genus and widely distributed in Guizhou and Yunnan Provinces, P.R. China.12) Two antistaphylococcal acylphloroglucinols have been isolated from this plant previously.13) Our phytochemical investigation on the methanol extract of this plant led to the isolation of four new acylphloroglucinols, hyperbeanols A—D (1—4), together with the known hypercalin B (5),14) (1S,4S,5S,10R)-4,10-guaianediol (6),15) (+)-a-linaloxime (7),16) guaia-6,10(14)-dien-4-ol (8),17) calycinoxanthone D (9),18) ueraxanthone (10),19) 2,5-dihydroxyxanthone (11),20) 1,3-dihydroxy-6-methoxyxanthone (12),21) 3,6,7-trihydroxy-1-methoxyxanthone (13),22) 1,2,5-trihydroxyxanthone (14),23) 1,5-dimethoxy-2-methoxyxanthone (15),24) 1,3,5-trihydroxy-6-methoxyxanthone (16),25) 1-hydroxyxanthone (17),26) garcinamangosone D (18),27) quercetin-3-O-β-D-galactopyranoside (19),28) and quercetin-3-O-α-L-rhamnopyranoside (20).28) The cytotoxicity of 1—5 against the cancer cell lines SK-BR-3, HL-60, SMMC-7721, PANC-1, MCF-7, and K562 were examined. Reported herein are the isolation, structure elucidation, and cytotoxic evaluation of compounds 1—5.

The MeOH extract (611 g) of the aerial parts of H. beanii (5.3 kg) was successively partitioned with petroleum ether and ethyl acetate. The petroleum ether soluble fraction (252 g) was subjected to silica gel column chromatography, Sephadex LH-20, and preparative HPLC to afford compounds 1—8. The ethyl acetate portion (167 g) was also purified by column chromatography, semipreparative, and preparative HPLC to obtain compounds 9—20.

Compound 1 was obtained as a yellow gum. Its molecular formula, C33H42O6, was established on the basis of negative high resolution-electrospray ionization-mass spectrum (HRESI-MS) analysis (m/z 533.2896, [M–H]−) and 13C-NMR spectrum (Table 1). The IR absorptions suggested the presence of hydroxyl (3431 cm−1) and carbonyl (1714 cm−1) groups. Its 1H and distortionless enhancement by polarization transfer (DEPT) spectra displayed a total of 33 signals including a nonconjugated carbonyl (δ 209.0), one 1,3-di-ketone system (δ 197.3, 113.6, 193.7), one monobenzoyl group (δ 197.0, 136.9, 127.8×2, 128.0×2, 132.2), two quaternary carbons (δ 62.4, 61.0), and 20 other carbons ascribable to two prenyl groups and another C10 unit. The 1H-NMR spectrum (Table 2) indicated the presence of two vinyl protons (δ 4.87, 2H, overlapped, m), five aromatic protons (δ 7.61, 2H,
tuated by two prenyl groups, a benzoyl, and one C10 moiety. 1
was ascribed to be an acylphloroglucinol compound substi-

1H–1H COSY (Fig. 2). Correlations of H-12/H-14
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d, J=7.0 Hz), 7.52 (1H, t, J=7.0 Hz), and 7.40 (2H, t, J=7.0 Hz), and six singlet methyls [6 1.16, 1.27, 1.53, 1.58, 1.61, 1.62 (each 3H, s)]. On comparison with the characteris-
tic data for other analogues isolated from Hypericum species, I was ascribed to be an acylphenol compound substi-
tuted by two prenyl groups, a benzoyl, and one C10 moiety. Further comparison of the NMR data of I with those of to-
toene A indicated that they were similar to one another except that the signals for the isopropyl moiety at C-27 in to-
toene A was replaced by an aromatic ring in I, which was sup-
ported by the presence of the aromatic signals in the 1D-NMR spectra of I and the heteronuclear multiple bond con-
nectivity (HMBC) correlations from H-29 and H-33 to C-27 (Fig. 2). In the HMBC spectrum, the correlations from H-7 to C-3, C-4, and C-5, H-14 to C-3, C-4, and C-5, Me-15 to C-12, C-13, and C-14, Me-16 to C-8, C-9, and C-10, H-17 to C-1, C-5, and C-6, H-22 to C-1, C-5, and C-6 all can be found. On the basis of the above HMBC correlations to-
gether with H-17/H-18, H-22/H-23, H-7/H-8/H-12/H-11/H-
10, and H-29/H-30/H-31/H-32/H-33 proton systems obtained from the 1H–1H correlation spectroscopy (COSY) (Fig. 2), the planar structure of I was determined.

The relative configuration of I was elucidated by nuclear Overhauser effect spectroscopy (NOESY) and further con-
ferred by the single X-ray diffraction analysis. The NOESY correlations of H-12/H-14α and H-14β/H-7α indicated their
axial orientations, and then the cyclonexane ring (C-4, C-7, C-8, C-12, C-13, C-14) is fixed in a chair conformation.

Me-15 and H-8 were both in the β-orientation based on the
NOESY correlations of Me-15/H-8 and H-8/H-7β. In addi-
tion, the NOESY correlations of H-29/H-7α and H-18/H-14β
observed in the NOESY spectrum indicated that the two
prenyl groups should be presented on the upper side of the
cyclonexane ring (C-4, C-7, C-8, C-12, C-13, C-14) as
to-moene B. Unfortunately, Me-16 showed no cross-peak in
the NOESY spectrum. Therefore, some solid evidence like
X-ray diffraction was necessary to validate the orientation of
Me-16. After many attempts with different solvents, a single
crystal was finally obtained in MeOH–Me2CO–H2O (90:9:1). The result of single X-ray diffraction analysis (Fig. 3) not only suggested the β-orientation of Me-16 but also further confirmed the relative configuration and planar structure of I. Accordingly, the structure of I was elucidated as shown and named hyperbeanol A.

\( \alpha \) Overlapped signals.
Hyperbeanol B (2), a yellow gum, its molecular formula, C_{33}H_{42}O_{6}, was deduced to be the same as 1 according to the HR-ESI-MS (m/z 533.2903, [M-H]^-). The 1D- and 2D-NMR spectroscopic features of 2 were very similar to those of 1 except for the resonances in the vicinity of C-13 (Tables 1, 2). Compared to those of 1, the chemical shifts of C-8, C-13, and C-14 shifted upfield 3.7, 3.7, and 2.6 ppm, respectively, while the chemical shift of C-15 shifted downfield 6.0 ppm. Moreover, the signals of H-8 and H-12 in 2 were also shifted upfield 0.35 and 0.15 ppm in the 1H-NMR spectrum, respectively. Moreover, the signals of H-8 and H-12 in 2 were also shifted upfield 0.35 and 0.15 ppm in the 1H-NMR spectrum, respectively. Additionally, the chemical shifts of C-15, C-14, C-8, and C-9, and C-12 were identical with those in 1. Thus, the structure of 2 was elucidated as shown.

Hyperbeanol C (3) had the same molecular formula as 1 based on its HR-ESI-MS (m/z 533.2919, [M-H]^ - ). Side-by-side comparison of NMR data between 3 and 1 suggested that 3 should be another stereoisomer of 1 (Tables 1, 2). The relative configuration of 3 was revealed by the analysis of NOESY spectrum. The δ-orientation of H-8, Me-15, and Me-16 was deduced to be the same with those in 1 by the NOESY correlations of H-8/H-7δ, H-8/Me-15, Me-15/H-14β, and H-8/Me-16. Different from that of 1, the NOESY correlation of Me-15/H-29 suggested that the benzoyl group should be located on the upper side of the cyclohexane ring, which indicated that the C-4 configuration in 3 was just opposite to that in 1 (Fig. 4). So, the structure of 3 was elucidated as C-4 isomeric form of 1.

Hyperbeanol D (4), a yellow gum, was assigned a molecular formula of C_{33}H_{42}O_{6}, showing an additional oxygen atom as compared to those of 1—3. Compound 4 showed identical numbers of methyl, methine, methylene, and quaternary carbon to that of 3, which indicated that 4 should be a hydroperoxyl derivative of 3. Careful comparison of their NMR data displayed that the chemical shifts of C-13 in 4 (δ 85.1) were obviously shifted downfield compared to that in 3 (δ 73.2) in their 13C-NMR spectra. So, the hydroperoxyl group was deduced to be attached at C-13 in 4, which can be further proved by the HMBC correlations of H-12, H-14, and Me-15 with C-13. As 4 showed almost identical NOESY spectrum to that of 3, the relative configuration of 4 was deduced to be the same as that of 3. Eventually, the structure of 4 was elucidated as shown.

This kind of spirocyclic acylphloroglucinols, possessing an 6/6/5 carbon skeleton, were very seldom in the natural products, and only isolated twice from Hypericum plants up to now. Biogenetically, these metabolites were derived from monocyclic polyrenylated acylphloroglucinols type precursor and often isolated as stereoisomers, such as tomoenes A—H and hyperielliptone HB. In the present study, hyperbeanols A—D should be derived from the hypercalin B (5) by an intramolecular cyclization reaction between C-4 and C-14. Since acylphloroglucinols were always isolated as gum, it is difficult to get the single crystal. So, it is also noteworthy that the X-ray analysis of 1 was the first report of X-ray analysis of this type metabolite. As for 4, it also can be seen as the first discovery of spirocyclic acylphloroglucinol substituted by a hydroperoxyl group.

The cytotoxicity of compounds 1—5 against the cancer cell lines HL-60, SMMC-7721, PANC-1, MCF-7, K562, and SK-BR-3 was tested using the methyl thiazol tetrazalium (MTT) method described in the literature with cis-platinum as the positive control. Only 2 and 4 exhibited modest cytotoxicity against K562 cells with IC_{50} 16.9 and 20.7 μM, respectively.

Experimental

General Procedures

Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A Tenor-27 spectrophotometer was employed to scan IR spectroscopy using KBr pellets; in cm⁻¹. ¹H-, ¹³C-, and 2D-NMR were recorded on Bruker AM-400 or DRX-500 spectrometers with tetramethylsilane (TMS) as internal standard. FAB-MS and HR-ESI-MS spectra were performed on a VG Autospec-3000 spectrometer and Finnigan MAT instrument, respectively. Preparative HPLC was performed on a Shimadzu LC-8A preparative liquid apparatus with Shimadzu PTC-ODS (K) column (34 mm×15 cm). Semipreparative HPLC was conducted on an Agilent 1100 equipped with UV detector and a Zorbax SB-C18 (Agilent, 9.4 mm×25 cm). Analytical HPLC was performed on an Agilent 1100 equipped with UV detector and a Zorbax SB-C18 (Agilent, 4.6 mm×25 cm). Column chromatography was performed with silica gel (200—300 mesh, Qingdao Marine Chemical Inc., Qingdao, China), silica gel H (10—40 μm, Qingdao Marine Chemical Inc., Qingdao, China), and Sephadex LH-20 (Amersham Pharmacia Biotech, Sweden).

Plant Material

The aerial parts of H. beanii were collected in Songming County, Yunnan Province, People’s Republic of China, in January 2008. The plant material was identified by Prof. Xiao Cheng, Kunming Institute of Botany, Yunnan Province, People’s Republic of China. A voucher specimen (200800115) was deposited at Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation

The dried and powdered aerial parts (5.3 kg) of H. beanii were extracted with MeOH (3×201, 2 d, each) at room temperature. The extract was evaporated to get a residue (611 g) under reduced pressure. The residue was suspended in water (1200 ml) and then partitioned...
with petroleum ether and ethyl acetate, respectively. The petroleum ether portion (252 g) was subjected to silica gel column chromatography eluted with gradient petroleum ether–Me2CO (from 1:0 to 0:1) to yield six fractions (Fr1–Fr6). Fr3 (10.3 g) was further purified by silica gel column (petroleum ether–Me2CO 50:1) to obtain 6 (35 mg), 7 (15 mg), and 8 (33 mg). Fr7 (6.7 g) was chromatographed on a silica gel column using petroleum ether–Me2CO (30:1) as eluents to get four subfractions (Fr1a–Fr1d). Subfraction Fr1a (1.1 g) was subjected to Sephadex LH-20 (CHCl3–MeOH 1:1) then followed by preparative HPLC (MeOH–H2O 80:20) to provide 3 (45 mg) and 4 (22 mg). The ethyl acetate portion (167 g) was separated on silica gel column eluted with gradient CHCl3–MeOH (from 1:0 to 0:1) and then followed by preparative HPLC (MeOH–H2O 85: 15) to get

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\text{SHELX-86,} \ \text{33}) \text{ expanded using difference Fourier techniques, and refined by the program NOMCSD[8] and full-matrix least-squares calculations. Crystallographic data for the structure I has been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 736730).}
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Acknowledgments  This work was supported by Grants from the 973 project (No. 2009CB522300), the National Natural Science Foundation of China (No. 90813004 and No. 20972167), the Innovation Foundation of Chinese Academy of Sciences (KSCX1-YWR-24), and the foundation from CAS (Xibuzhiguang to G. Xu).

References