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NOTE

Hypelodins A and B, polyprenylated benzophenones from *Hypericum elodeoides*

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Abstract Two new polyprenylated benzophenones, hypelodins A (**1**) and B (**2**), were isolated from the aerial parts of the Chinese medicinal plant *Hyperium elodeoides* (Choisy). Their structures were elucidated on the basis of spectroscopic evidence. Hypelodin A (**1**) is a polyprenylated benzophenone having a tetrahydropyrane ring with three prenyl groups and one 4-methyl-1,3-pentadiene moiety, while hypelodin B (**2**) has a cage-like structure with a 6/6/5/7/6/5 hexacyclic ring system.

Keywords Polyprenylated benzophenones · *Hypericum elodeoides* · Hypericaceae · Hypelodins A and B

Introduction

Polyprenylated benzophenones, constituents of Hypericaceae and Clusiaceae plants, have attracted much scientific interest because of their fascinating chemical structures and intriguing biological activities [1]. Plants of the genus *Hypericum* (Hypericaceae) are distributed widely in temperate regions, and have been used as traditional medicines in various parts of the world [2, 3]. During our search for structurally interesting natural products from plants, we have reported the isolation of various types of compounds such as meroterpenoids [4–7], xanthones [8, 9], benzophenone glycosides [10], chromones [11], and polyprenylated acylphloroglucinols [12, 13] from *Hypericum* plants. As a part of this program, we investigated the MeOH extract of the aerial parts of *Hypericum elodeoides* (Choisy), a Chinese medicinal plant used for the treatment of diarrhea or snake bite [14], which resulted in the isolation of two new polyprenylated benzophenones, hypelodins A (**1**) and B (**2**). In this paper, we describe the isolation and structure elucidation of **1** and **2**.

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Results and discussion

The aerial parts of *Hypericum elodeoides* (5.7 kg, dry) collected at Kunming, China, were extracted with MeOH. Chromatographic separation of the extract afforded two new polyprenylated benzophenones, hypelodins A (**1**, 4.9 mg) and B (**2**, 29.0 mg) (Chart 1), along with two known compounds, hypercohin G [15] and an analogue of hyperforin [(2*R*,3*R*,4*S*,6*R*)-6-methoxycarbonyl-3-methyl-4,6-di(3-methyl-2-butenyl)-2-(2-methyl-1-oxopropyl)-3-(4-methyl-3-pentenyl)cyclohexanone] [16]. The structures of known compounds were identified by comparison of their physicochemical data with the reported data (Chart 1).

Hypelodin A (**1**) was obtained as an optically active colorless oil, $\{[\alpha]_D^{20} + 90.3 (c 0.49,\ \text{MeOH})\}$. IR absorption

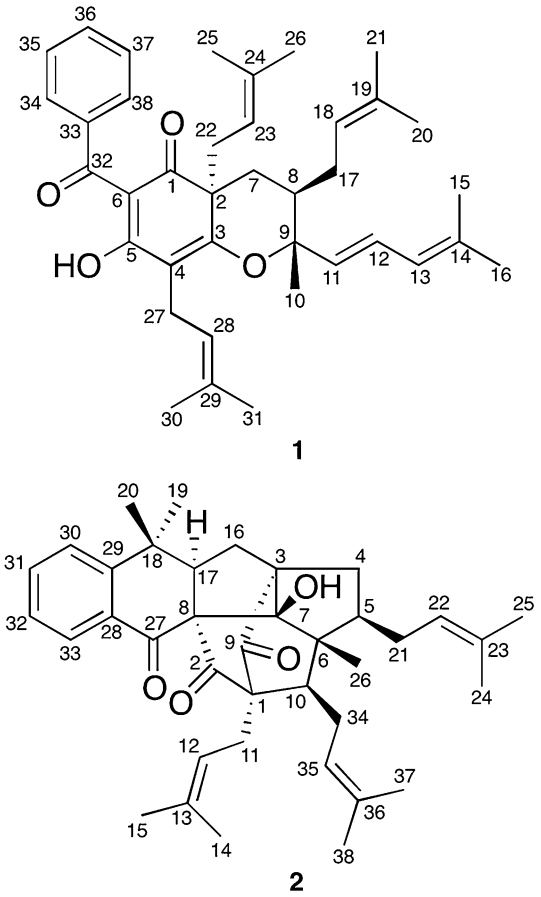


Chart 1 Structures of hypelodins A (**1**) and B (**2**)

bands at 3429, 1732, and 1668 cm^{-1} implied the presence of hydroxy and carbonyl functionalities. The molecular formula $C_{38}H_{48}O_4$ was established by the HRESIMS (*m/z* 567.3471 $[M+H]^+$). The ^{1}H- and ^{13}C-NMR spectra revealed the existence of two ketone carbonyl groups, two enols, one mono-substituted benzene ring, four tri-substituted olefins, one 1,2-di-substituted olefin, two sp^3 quaternary carbons, one sp^3 methine, four sp^3 methylenes, and nine tertiary methyls (Table 1). The ^{1}H-NMR spectrum also displayed a highly down-field shifted resonance due to a hydrogen-bonded hydroxy group (δ_H 18.33), which is the characteristic resonance of a β-diketone with enol form. These observations suggested **1** to be a polyprenylated benzophenone. The benzophenone moiety (C-1–C-6 and C-32–C-38) of **1** was deduced to be composed of two ketones, two enols, one sp^3 quaternary carbon, and one mono-substituted benzene. The ^{1}H–^{1}H COSY and HMBC spectra suggested the existence of three prenyl groups (C-17–C-21, C-22–C-26, and C-27–C-31) and a 4-methyl-1,3-pentadiene moiety (C-11–C-16) (Fig. 1).

The ^{1}H–^{1}H COSY spectrum demonstrated the connectivity of C-8 to C-7 and of C-8 to a prenyl group (C-17), while HMBC correlations for H_3-10 to C-8, C-9, and C-11

Table 1 ^{1}H- and ^{13}C-NMR data for hypelodin A (**1**) in $CDCl_3$

Position	δ_C	δ_H
1	195.9	–
2	52.3	–
3	171.2	–
4	117.0	–
5	188.8	–
6	107.4	–
7α	29.0	2.24 (1H, dd, J = 14.4, 4.0 Hz)
7β		1.32 (1H, t, J = 14.4 Hz)
8	40.2	2.00 (1H, m)
9	86.2	–
10	19.1	1.21 (3H, s)
11	133.1	5.75 (1H, d, J = 15.2 Hz)
12	126.5	6.58 (1H, dd, J = 15.2, 10.8 Hz)
13	124.2	5.90 (1H, brd, J = 10.8 Hz)
14	136.9	–
15	18.3	1.81 (3H, s)
16	25.9	1.84 (3H, s)
17	29.4	2.06, 1.64 (1H each, m)
18	121.5	4.98 (1H, t, J = 6.4 Hz)
19	133.5	–
20	17.8	1.54 (3H, s)
21	25.7	1.66 (3H, s)
22	38.5	2.65 (1H, dd, J = 14.2, 7.2 Hz)
		2.55 (1H, dd, J = 14.2, 8.0 Hz)
23	117.4	5.06 (1H, dd, J = 8.0, 7.2 Hz)
24	135.9	–
25	17.9[a]	1.62 (3H, s)
26	26.0	1.71 (3H, s)
27	21.3	3.14 (2H, brd, J = 6.8 Hz)
28	121.8	5.10 (1H, t, J = 6.8 Hz)
29	131.7	–
30	18.0[a]	1.74 (3H, s)
31	25.7	1.71 (3H, s)
32	196.2	–
33	138.8	–
34, 38	127.3	7.44 (2H, m)
35, 37	127.6	7.39 (2H, m)
36	130.6	7.46 (1H, m)
5-OH	–	18.33 (1H, s)

[a] Signals may be interchangeable

were indicative of the connectivities among C-8, C-10, and C-11 via an oxygenated sp^3 quaternary carbon (C-9). HMBC cross-peaks of H_2-22 to C-1 and H_2-7 to C-2, C-3, and C-22 suggested that C-1, C-3, C-7 and a prenyl group (C-22) were connected to C-2. A hydroxy group at C-5 and a benzoyl group at C-6 were deduced by HMBC correlations for a proton of the hydrogen-bonded hydroxy group (5-OH) to C-4, C-5, and C-6. The connectivities among

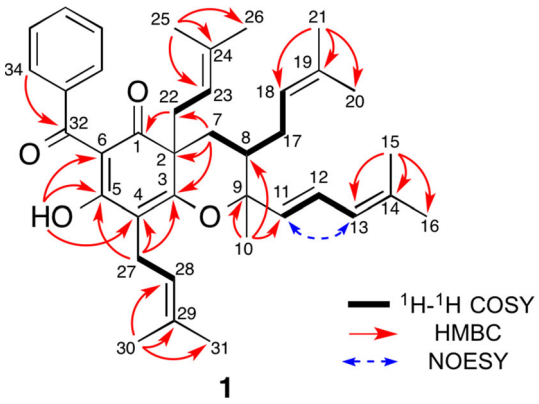


Fig. 1 Selected 2D-NMR correlations for hypelodin A (**1**)

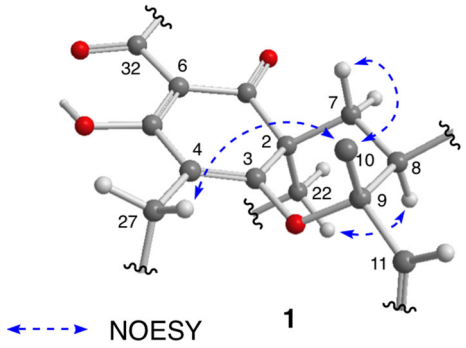


Fig. 2 Selected NOESY correlations and relative stereochemistry of hypelodin A (**1**) (protons of 10-Me are not shown)

C-3, C-5, and C-27 via C-4 were disclosed by HMBC cross-peaks of H_2-27 to C-3, C-4, and C-5. Given the unsaturation degree of **1** and the chemical shifts for C-3 (δ_C 171.2) and C-9 (δ_C 86.2), the presence of an ether linkage between C-3 and C-9 was concluded. Thus, the gross structure of hypelodin A (**1**) was elucidated as shown in Fig. 1.

A large value of $^3J_{H\text{-}7b/H\text{-}8}$ (14.4 Hz) and NOESY cross-peaks of H-8/H-22a and H-7b/H_3-10 suggested the *axial* orientations for H-8, 10-Me, and the prenyl group at C-2 (Fig. 2). Thus, the tetrahydropyran ring (C-2, C-3, and C-7–C-9) adopts a pseudo-chair conformation. The 11*E* configuration was revealed by a large $^3J_{H\text{-}11/H\text{-}12}$ value (15.2 Hz) and a NOESY cross-peak of H-11/H-13. Consequently, the relative stereochemistry of hypelodin A (**1**) was elucidated as shown in Chart 1.

Hypelodin B (**2**) was isolated as an optically active colorless oil, {$[\alpha]_D$−6.5 (*c* 2.9, MeOH)}. The IR spectrum indicated the presence of hydroxy (3440 cm^{-1}) and carbonyl (1738, 1709, and 1674 cm^{-1}) functionalities. The HRESIMS suggested the molecular formula to be $C_{38}H_{48}O_4$ {*m/z* 591.3442 $[M+Na]^+$}. The ^{13}C-NMR spectrum revealed the existence of 38 carbons including three ketones and six aromatic carbons (Table 2). In the HMBC spectrum, the down-field shifted aromatic carbon signal (δ_C 150.2, C-29) was correlated with the proton signals due to two methyl groups (H_3-19 and H_3-20),

Table 2 ^{1}H- and ^{13}C-NMR data for hypelodin B (**2**) in $CDCl_3$

Position	δ_C	δ_H
1	68.4	–
2	213.9	–
3	70.0	–
4	32.7	2.03, 1.52 (1H each, m)
5	59.4	1.86 (1H, m)
6	45.2	–
7	90.2	–
8	70.0	–
9	202.3	–
10	53.1	1.77 (1H, m)
11	23.1	2.49 (1H, dd, *J* = 15.2, 9.6 Hz)
		2.30 (1H, m)
12	118.7	4.97 (1H, m)
13	134.0	–
14	17.7	1.57 (3H, s)
15	25.8	1.68 (3H, s)
16α	28.4	2.05 (1H, m)
16β		2.15 (1H, t, *J* = 9.2 Hz)
17	56.9	2.66 (1H, t, *J* = 9.2 Hz)
18	37.1	–
19	25.8	1.35 (3H, s)
20	29.6	1.07 (3H, s)
21	32.3	2.25, 2.10 (1H each, m)
22	123.1	5.02 (1H, m)
23	132.2	–
24	17.9	1.63 (3H, s)
25	25.6	1.70 (3H, s)
26	13.4	1.44 (3H, s)
27	200.2	–
28	136.3	–
29	150.2	–
30	123.4	7.35 (1H, d, *J* = 7.2 Hz)
31	133.5	7.54 (1H, dd, *J* = 7.6, 7.2 Hz)
32	126.7	7.36 (1H, dd, *J* = 7.6, 7.2 Hz)
33	126.4	7.71 (1H, d, *J* = 7.2 Hz)
34	26.6	1.97, 1.63 (1H each, m)
35	125.1	4.87 (1H, m)
36	131.5	–
37	18.1	1.61 (3H, s)
38	25.6	1.68 (3H, s)
7-OH	–	2.74 (1H, s)

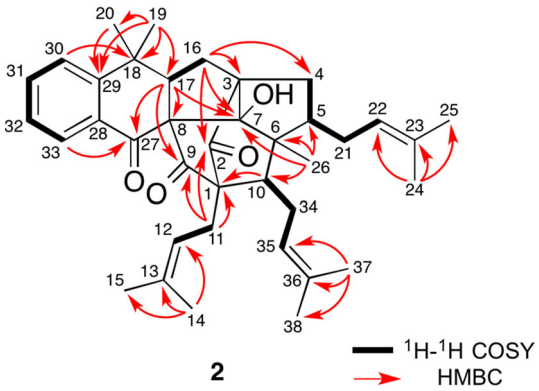


Fig. 3 Selected 2D-NMR correlations for hypelodin B (**2**)

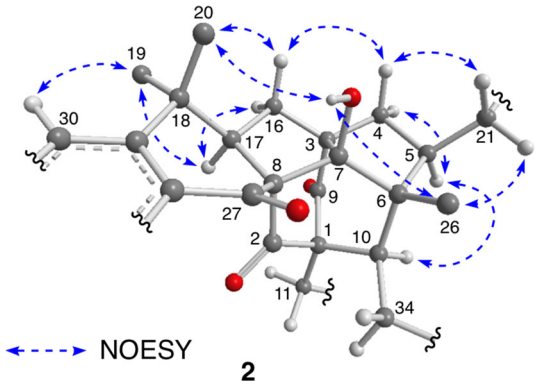


Fig. 4 Selected NOESY correlations and relative stereochemistry for hypelodin B (**2**) (protons of methyl groups are omitted)

suggesting that C-29 was not adjacent to an oxygen atom. These spectral features were similar to those of garcibracteatone, a polyprenylated benzophenone isolated from *Garcinia bracteata* (Clusiaceae) [17]. Comparison of the ^{1}H- and ^{13}C-NMR data for **2** with those for garcibracteatone implied that they have the same cage-like structure with a 6/6/5/7/6/5 hexacyclic ring system, while additional resonances of one prenyl group were observed in **2**. ^{1}H–^{1}H COSY cross-peaks of H-10/H_2-34 revealed that the prenyl group was connected to C-10 (Fig. 3). Therefore, the gross structure of **2** was elucidated as shown in Fig. 3.

NOESY cross-peaks of H_3-20/H-16b, H_3-20/7-OH, H-16b/H-4b, H-4b/H-21a, H-21b/H_3-26, and H_3-26/7-OH demonstrated that these protons are present on the same β-side (Fig. 4). This was underpinned by NOESY correlations observed among protons on the α-side in the molecule. The β-orientation of the prenyl group at C-10 was indicated by a NOESY correlation for H-5/H-10. Thus, the relative stereochemistry of **2** was elucidated as shown in Fig. 4.

The absolute stereochemisrty for **1** was not assigned, whereas a resemblance of the optical rotation value for **2** ($[\alpha]_D-6.5$) to that of garcibracteatone ($[\alpha]_D-1$) [17] might suggest that the absolute configurations of the hexacyclic structures in **2** and garcibracteatone are the same.

Hypelodin A (**1**) is a polyprenylated benzophenone having a tetrahydropyrane ring with three prenyl groups and one 4-methyl-1,3-pentadiene moiety, while hypelodin B (**2**) has a cage-like structure with a 6/6/5/7/6/5 hexacyclic ring system. A possible biogenetic pathway for **1** and **2** is shown in Scheme 1. Polyprenylated acylphloroglucinols, including polyprenylated benzophenones, are considered to be produced by prenylation of acylphloroglucinols, formed from condensation of one molecule of acyl-CoA and three molecules of malonyl-CoA, and by subsequent intramolecular cyclization [18]. Additionally, garcibracteatone was proposed to be produced from a structurally related polyprenylated benzophenone, nemorosonol [19, 20], by cycloaddition [17]. In the case of **1** and **2** (Scheme 1), polyprenylation, including introduction of a geranyl group and its prenylation, of 1,3,5-trihydroxybenzophenone might give a plausible common intermediate (**X**). Formation of an ether linkage between C-6 and C-9 in **X** would give hypelodin A (**1**), while hypelodin B (**2**) also come from the same intermediate **X** by successive intramolecular cyclization.

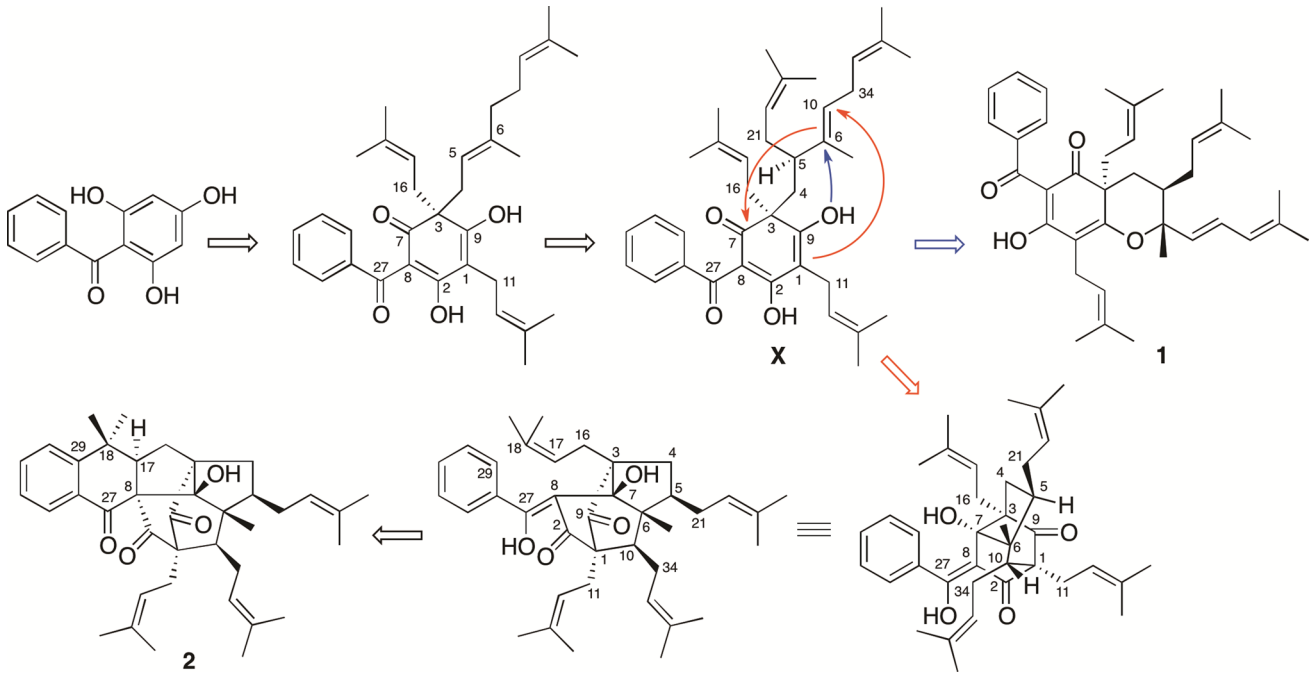
Experimental

General

Optical rotations were measured with a JASCO DIP-370 digital polarimeter. IR spectra were recorded on a 1720 infrared Fourier transform spectrometer (Perkin-Elmer). NMR spectra were run on a Bruker AVANCE instrument (^{1}H-NMR 400 MHz, ^{13}C-NMR 100 MHz) using TMS as an internal standard. MS was obtained on a Waters LCT PREMIER 2695. Column chromatography: silica gel 60 N (63–210 μm, Kanto Kagaku) and Toyopearl HW-40C (TOSOH); HPLC silica gel (YMC-Pack SIL-06, 250 × 20 mm, YMC Co., Ltd.) and ODS (Mightysil RP-18 GP Aqua, 250 × 20 mm, Kanto Kagaku and Capcell Pak C18 SG120 S5, 250 × 20 mm, Shiseido Co., Ltd.).

Plant material

The aerial parts of *Hypericum elodeoides* were collected in March 2006, in Yunnan Province, China. The plant was identified by Dr. H.-D. Sun, and a voucher specimens (UTP98012) were deposited in the herbarium of The University of Tokushima.



Scheme 1 Possible biogenetic pathway of hypelodins A (**1**) and B (**2**) [numbering system for possible biogenetic intermediates (**X**) obeys that of **2**]

Extraction and isolation

The aerial parts of *Hypericum elodeoides* (5.7 kg, dry) collected at Kunmming, China, were extracted with MeOH. After removal of the solvent, the extract (650 g) was partitioned between EtOAc and water. The organic layer was suspended in MeOH/H_2O (1:9) and then extracted with *n*-hexane. The *n*-hexane-soluble materials (64 g) were subjected to a silica gel column (*n*-hexane/EtOAc) to give eight fractions (frs. 1–8). Fr. 2 was applied to a silica gel column ($CHCl_3$/MeOH) to afford five fractions (frs. 2.1–5). Separation of fr. 2.3 on a Toyopearl HW-40 column ($CHCl_3$/MeOH) gave four fractions (frs. 2.3.1–4). Fr. 2.3.3 was loaded on silica gel HPLC (*n*-hexane/EtOAc) and then purified by ODS HPLC (MeOH/H_2O) to give hypelodins A (**1**, 4.9 mg) and B (**2**, 29.0 mg). In the purification process, two known compounds, hypercohin G [15] and an analogue of hyperforin [(2*R*,3*R*,4*S*,6*R*)-6-methoxycarbonyl-3-methyl-4,6-di(3-methyl-2-butenyl)-2-(2-methyl-1-oxopropyl)-3-(4-methyl-3-pentenyl)cyclohexanone] [16], were isolated.

Hypelodin A (**1**)

Colorless oil; $[\alpha]_D^{20}$ + 90.3(*c*0.49 MeOH); IR (KBr) ν_{max} 3429, 1732, and 1668 cm^{-1}; ^{1}H- and ^{13}C-NMR data (Table 1); HRESIMS *m/z* 567.3471 $[M–H]^-$ (calcd. for $C_{38}H_{47}O_4$, 567.3474).

Hypelodin B (**2**)

Colorless oil; $[\alpha]_D^{20}$ − 6.5(*c*2.9 MeOH); IR (KBr) ν_{max} 3440, 1738, 1709, and 1674 cm^{-1}; ^{1}H- and ^{13}C-NMR data (Table 2); HRESIMS: *m/z* 591.3442, $[M+Na]^+$ (calcd. for $C_{38}H_{48}O_4Na$, 591.3450).

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