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Hypatulins A and B, Meroterpenes from *Hypericum patulum*

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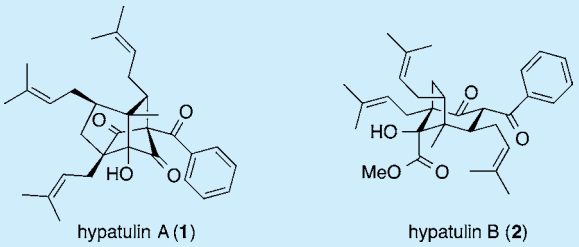
Supporting Information

ABSTRACT: Two novel prenylated benzophenone related meroterpenes, hypatulins A (**1**) and B (**2**), were isolated from the leaves of *Hypericum patulum*. The structures of **1** and **2** were assigned by spectroscopic analysis, chemical conversion, and calculations of the ECD (electron circular dichroism) spectra. Hypatulin A (**1**) had a unique densely substituted tricyclic octahydro-1,5-methanopentalene core, while hypatulin B (**2**) possessed a bicyclo[3.2.1]octane moiety. Hypatulin A (**1**) exhibited antimicrobacterial activity against *Bacillus subtilis*. A possible biogenetic pathway of the new meroterpenes **1** and **2** from a prenylated benzophenone was presented.



The genus *Hypericum* consists of more than 500 species, which accounts for more than 80% of the Hypericaceous plants.[1] Most of them are found in temperate regions around the world. Prenylated acylphloroglucinols (PAPs) and meroterpenes are recognized as constituents of the Hypericaceous and related plants, and these compounds possess a large variety of chemical structures.[2] Some PAPs exhibit interesting biological activity such as antidepressant, antitumor, antiviral, and antimicrobial activities.[2a] Prenylated benzophenones are a class of PAPs having a benzoyl group as the acyl moiety. Recently, several prenylated benzophenones with structurally and biogenetically interesting cage-like architectures have been isolated from the *Hypericum* plants.[3]

In the course of our search for structurally unique metabolites from Hypericaceous plants, we have recently reported the isolation of some meroterpenes from *H. chinense*,[4] *H. yojiroanum*,[5] and *H. yezoense*[6] and prenylated benzophenones from *H. elodeoides*[7] and *Triadenum japonicum*.[8] As part of this research program, constituents of *Hypericum patulum* were investigated, which resulted in the isolation of two novel prenylated benzophenone related meroterpenes, hypatulins A (**1**) and B (**2**). We describe herein the isolation and structure elucidation of **1** and **2**.

The dried leaves of *Hypericum patulum* were extracted with MeOH to give the extract, which was partitioned with *n*-hexane and water. The *n*-hexane-soluble materials were separated by column chromatographies to furnish a fraction containing meroterpenes, which was purified by ODS HPLC to isolate hypatulins A (**1**, 36.9 mg) and B (**2**, 3.6 mg).

Hypatulin A (**1**)[9] was obtained as an optically active colorless amorphous solid {$[\alpha]^{25}_{D}$ +40.4 (*c* 0.046, MeOH)}. HRESIMS analysis returned the molecular formula of **1** to be $C_{32}H_{40}O_4$ (*m*/*z* 511.2807 $[M + Na]^+$ Δ−1.7 mmu). The IR spectrum suggested the presence of hydroxy (3455 cm^{-1}) and carbonyl (1786 and 1738 cm^{-1}) functionalities. The ^1H NMR spectrum displayed the resonances due to one phenyl group, three trisubstituted olefins, two sp^3 methines, four sp^3 methylenes, and seven tertiary methyls (Table 1), while the ^{13}C NMR spectrum showed 32 signals including three ketone carbonyl, 12 olefinic or aromatic, one oxygenated tertiary, and three quaternary carbon signals. The planar structure of **1** was assigned by 2D NMR analysis. Interpretation of the $^1H-^1H$ COSY and HMBC spectra revealed the presence of three prenyl groups (C-11−C-15, C-16−C-20, and C-21−C-25) and one benzoyl group (C-26−C-32) as well as the existence of a bicyclo[3.2.1]octan-2-one moiety (C-1 and C-3−C-9) with an angular methyl group (10-Me) at C-7 (Figure 1). $^1H-^1H$ COSY cross-peaks of H_2-16/H-6 and H_2-21/H-8 indicated the connectivities of the prenyl groups to C-6 and to C-8. HMBC correlations for H_2-5/C-11 and H-8/C-26 suggested the existence of the prenyl group at C-4 and the benzoyl group at C-1. The chemical shift of C-3 (δ_C 91.1) implied the

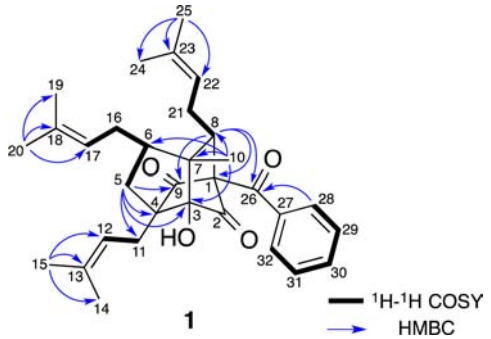


Figure 1. Selected 2D NMR correlations for hypatulin A (**1**).

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Table 1. ^{1}H and ^{13}C NMR Data for Hypatulins A (1) and B (2) in CD_3OD

	1		2	
position	^{13}C	^{1}H (J in Hz)	^{13}C	^{1}H (J in Hz)
1	75.8	–	57.6	4.56 (1H, d, 11.7)
2	207.1	–	173.9	–
3	91.1	–	89.4	–
4	70.2	–	64.5	–
5	43.4	2.09 (1H, dd, 13.7, 10.4)	41.3	2.02 (1H, dd, 13.8, 10.8)
		1.88 (1H, dd, 13.7, 9.9)		1.54 (1H, dd, 13.8, 7.3)
6	49.8	2.29 (1H, m)	47.4	2.49 (1H, m)
7	53.2	–	54.6	–
8	49.2	2.92 (1H, dd, 8.8, 5.9)	45.8	2.50 (1H, m)
9	208.2	–	209.5	–
10	23.1	1.21 (3H, s)	17.6	1.20 (3H, s)
11	32.3	2.82 (1H, dd, 14.1, 10.3), 2.27 (1H, m)	28.8	2.37 (1H, dd, 14.3, 7.5), 2.30 (1H, m)
12	120.9	5.32 (1H, dd, 10.3, 5.0)	122.8	5.09 (1H, t, 7.5)
13	137.0	–	133.4	–
14	18.0	1.66 (3H, s)	17.9	1.59 (3H, s)
15	26.3	1.76 (3H, s)	26.2	1.59 (3H, s)
16	29.4	2.23 (2H, m)	32.4	2.50, 2.32 (each 1H, m)
17	125.1	5.09 (1H, t, 6.9)	124.9	5.17 (1H, t, 7.4)
18	133.0	–	133.4	–
19	18.0	1.60 (3H, s)	18.2	1.70 (3H, s)
20	25.9	1.69 (3H, s)	25.9	1.73 (3H, s)
21	29.8	2.13 (2H, m)	31.4	2.30 (1H, m), 2.19 (1H, dt, 14.3, 9.9)
22	123.8	5.06 (1H, t, 6.4)	127.3	4.62 (1H, m)
23	134.3	–	133.0	–
24	18.2	1.52 (3H, s)	17.7	1.17 (3H, s)
25	26.1	1.68 (3H, s)	25.8	1.17 (3H, s)
26	196.1	–	199.4	–
27	138.6	–	140.4	–
28, 32	129.7	7.50 (2H, d, 7.3)	129.7	7.87 (2H, d, 7.4)
29, 31	129.2	7.39 (2H, t, 7.3)	129.5	7.51 (2H, t, 7.4)
30	134.0	7.55 (1H, t, 7.3)	133.8	7.58 (1H, t, 7.4)
2-OMe			52.5	3.73 (3H, s)

presence of a hydroxy group at C-3, which was confirmed by the deuterium induced isotope shift ($\Delta\delta$ +0.07) for C-3 measured in CD_3OH (Table S1). Though an HMBC correlation was not observed for a ketone carbonyl group (C-2), the connectivity of C-1 to C-3 via C-2 was deduced by the 13 degrees of unsaturation from the molecular formula of **1**, forming an octahydro-1,5-methanopentalene core. Thus, the planar structure of hypatulin A (**1**) was elucidated as shown in Figure 1.

The octahydro-1,5-methanopentalene skeleton restricted the relative configurations for C-1, C-3, C-4, and C-7 as shown in Figure 2. The 6*R** and 8*S** configurations were elucidated by NOESY correlations for H-5a/H-6, H-5a/H-21a, and H_3-10/H-8. Therefore, the relative stereochemistry of **1** was assigned as shown in Figure 2.

The absolute stereochemistry of hypatulin A (**1**) was elucidated by comparison of the experimental ECD spectrum and those calculated spectra. The TDDFT calculated ECD spectra of two possible enantiomers **1a** (1*S*,3*R*,4*R*,6*R*,7*R*,8*S*) and **1b** (1*R*,3*S*,4*S*,6*S*,7*S*,8*R*) are shown in Figure 3, and the former was similar to the experimental spectrum, indicating the

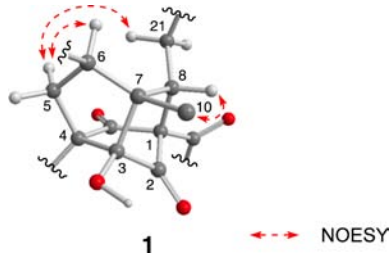

Figure 2. Selected NOESY correlations and the relative stereochemistry for the octahydro-1,5-methanopentalene core of hypatulin A (**1**) (protons of 10-Me are omitted).

1*S*, 3*R*, 4*R*, 6*R*, 7*R*, and 8*S* configurations of **1**. Thus, the structure of hypatulin A was assigned as **1**.

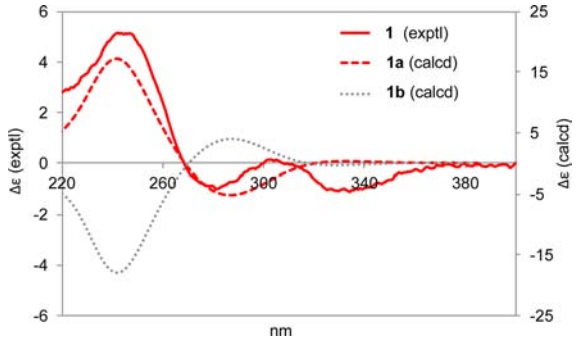

Figure 3. Experimental and calculated ECD spectra of hypatulin A (**1**) (calculated spectra are blue-shifted by 15 nm).

Hypatulin B (**2**)[10] was isolated as an optically active colorless amorphous solid {$[\alpha]^{25}_{D}$ +27.0 (c 0.17, MeOH)}, and the molecular formula, $C_{33}H_{44}O_5$, was obtained by the HRESIMS (m/z 543.3064 $[M + Na]^+$ Δ −2.2 mmu). Analysis of the 1D NMR spectra (Table 1) implied **2** to be a meroterpene structurally related to **1**. The 2D NMR spectra disclosed the presence of a bicyclo[3.2.1]octan-2-one moiety (C-1 and C-3−C-9) as well as the connectivities of prenyl groups to C-4, C-6, and C-8, a methyl group (10-Me) to C-7, and a benzoyl group to C-1 (Figure 4). The existence of a hydroxy group at C-3 was

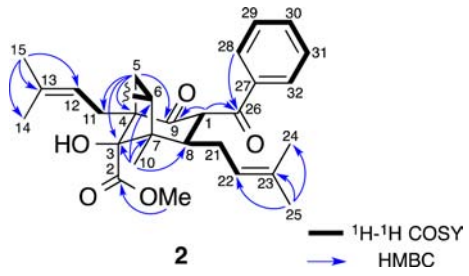

Figure 4. Selected 2D NMR correlations of hypatulin B (**2**) {the prenyl group (C-16−C-20) at C-6 is omitted}.

assigned by the chemical shift (δ_C 89.4) for C-3 together with an observation of the deuterium induced isotope shift ($\Delta\delta$ + 0.09) for C-3. An HMBC cross-peak of the methoxy proton signal to C-2 suggested the presence of a methoxycarbonyl group. Given the molecular formula of **2**, the connectivity of C-3 to the methoxycarbonyl group was deduced. Therefore, the gross structure of **2** was assigned as shown in Figure 4.

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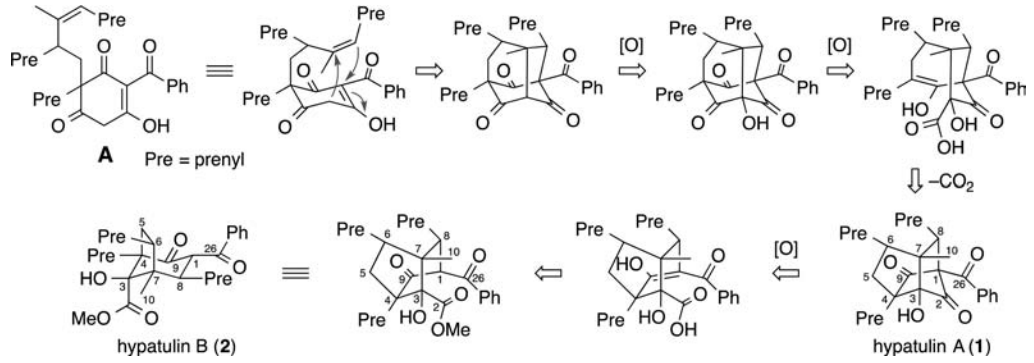
Scheme 1. Possible Biogenetic Pathway of Hypatulins A (1) and B (2)



NOESY correlations for H-1/H-5b, H-1/H-6, and H-5b/H-6 in **2** indicated these protons to be located on the same β-side, and thus the pseudochair conformation of the cyclohexanone ring (C-1, C-3, C-4, and C-7−C-9) was suggested (Figure 5). A

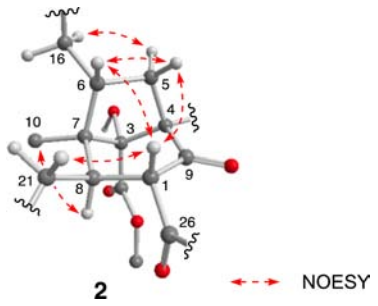


Figure 5. Selected NOESY correlations and the relative stereochemistry for the core unit of hypatulin B (**2**) (protons of methyl groups are omitted).

large coupling constant of H-1 (J = 11.7 Hz) and NOESY cross-peaks of H-1/H-21a and H_3-10/H-8 disclosed the H-8α orientation. The relative configuration of C-3 could not be assigned by NOESY analysis.

The ^{1}H NMR of hypatulin A (**1**) measured in pyridine-d_5 displayed new signals due to a mixture of two compounds in a ratio of ca. 1:1, of which signals arising from one compound were identical to those from **1**. The signals due to the other compound were similar to those of **2** except for the absence of the methoxy signal, suggesting that **1** was converted into a demethyl analogue of **2** under basic condition. These observations prompted us to convert **1** into **2** with MeOH under basic condition. Treatment of **1** with 4-dimethylaminopyridine in MeOH gave **1c**. The ^{1}H NMR spectrum and specific rotation of **1c** were identical to those of natural hypatulin B (**2**). Therefore, the absolute configurations of C-3, C-4, C-6, C-7, and C-8 for **2** were indicated to be the same as those of **1**. Consequently, the structure of hypatulin B elucidated to be **2**. Hypatulin B (**2**) might be produced from **1** during the extraction and isolation process.

A possible biosynthetic pathway of hypatulins A (**1**) and B (**2**) is shown in Scheme 1. Hypatulin A (**1**) might be generated by intramolecular cyclization, oxidation, oxidative ring cleavage, and cyclization associated with decarboxylation of a plausible biogenetic precursor (**A**), a prenylated benzophenone with four isoprene units. Oxidative cleavage of **1** followed by methylation will give hypatulin B (**2**). This was confirmed by chemical conversion of hypatulin A (**1**) into hypatulin B (**2**).

The leaves of *Hypericum patulum* were investigated to give two novel meroterpenes, hypatulins A (**1**) and B (**2**). Hypatulin A (**1**) has a unique octahydro-1,5-methanopentalene core with three prenyl groups, one hydroxy group, and one benzoyl group, while some natural products having the octahydro-1,5-methanopentalene moiety have been reported to date.[11] Hypatulins A (**1**) and B (**2**) were evaluated for their antimicrobial activities on strains of *Staphylococcus aureus* (MRSA and MSSA), *Bacillus subtilis*, and *Escherichia coli*. Hypatulin A (**1**) exhibited antimicrobial activity against *B. subtilis* (MIC 16 μg/mL), while hypatulin B (**2**) did not show such activity.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b02725.

Experimental section, and 1D and 2D NMR spectra of hypatulins A and B (PDF)

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Notes

The authors declare no competing financial interest.

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(9) Hypatulin A (**1**): colorless amorphous solid; $[\alpha]^{25}_{D}$ +40.4 (*c* 0.046, MeOH); IR (KBr) ν_{max} 3455, 1786, 1738, and 1672 cm^{-1}; UV (MeOH) λ_{max} 245 (ε 9200) nm; ECD (MeOH) $\Delta\varepsilon$ (nm) −1.1 (334), −1.0 (281), and +5.1 (243); ^{1}H and ^{13}C NMR (Table 1); HRESIMS: *m*/*z* 511.2807 $[M + Na]^+$ (calcd for $C_{32}H_{40}O_4Na$, 511.2824).

(10) Hypatulin B (**2**): colorless amorphous solid; $[\alpha]^{25}_{D}$ +27.0 (*c* 0.17, MeOH); IR (KBr) ν_{max} 3507, 1738, and 1672 cm^{-1}; UV (MeOH) λ_{max} 243 (ε 10 500); ^{1}H and ^{13}C NMR (Table 1); HRESIMS: *m*/*z* 543.3064 $[M + Na]^+$ (calcd for $C_{33}H_{44}O_5Na$, 543.3086).

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