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Bisbakuchiols A and B, novel dimeric meroterpenoids from *Psoralea corylifolia*

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Abstract—Two novel dimeric meroterpenoids, bisbakuchiols A and B, along with (*S*)-bakuchiol were isolated from the seeds of *Psoralea corylifolia* L. (Fabaceae). Bisbakuchiols A and B contain an unprecedented dimeric meroterpenoid skeleton in which two meroterpenes are linked through a dioxane bridge. All compounds were evaluated for their potential to inhibit hypoxia-inducible factor-1 (HIF-1) activation induced by hypoxia in a HIF-1-mediated reporter gene assay in AGS human gastric cancer cells. (*S*)-Bakuchiol inhibited hypoxic activation of HIF-1 with an IC_{50} value of 6.1 μM.

Hypoxic regions within tumor tissues trigger angiogenesis and thus enable tumors to develop their own blood supply. The cellular response to hypoxia is controlled by hypoxia-inducible factor-1 (HIF-1), which has been shown to orchestrate a large number of molecular events required for the adaptation of tumor cells to hypoxia. Therefore, HIF-1 has become an attractive target for the development of anti-cancer drugs.[1–3]

In our search for biologically active agents of natural origin, a methanol extract of the seeds of *Psoralea corylifolia* L. (Fabaceae) potently inhibited HIF-1 activation induced by hypoxia (100% inhibition at 20 μg/mL) in a HIF-1-mediated reporter gene assay.[4] The seeds of *P. corylifolia* have been used to treat dermatological disorders, diarrhea, impotence, and knee pain.[5] Several flavonoids and meroterpenoids from this plant have been shown to exhibit antibacterial, anti-inflammatory, antimutagenic, antioxidant, and antiplatelet effects.[6,7]

Bioassay-guided phytochemical investigation of the methanol extract of *P. corylifolia* using a HIF-1-mediated reporter gene assay led to the isolation of two novel dimeric meroterpenoids, bisbakuchiols A and B (**1** and **2**), as well as the known meroterpenoid, (*S*)-bakuchiol (**3**).[8] Bisbakuchiols A and B (**1** and **2**) feature a novel dimeric meroterpenoid skeleton in which two meroterpenes are linked through a dioxane bridge (Fig. 1). This Letter deals with the isolation and structural characterization of **1** and **2** and the biological evaluation of the three compounds isolated.

Successive Si gel and Sephadex LH-20 column chromatography and HPLC steps yielded a mixture of compounds from which **1** and **2** were purified by recycling HPLC. The UV spectra of compounds **1** and **2**, which were obtained by analytical HPLC using a PDA detector during the purification, exhibited the same profiles as that of (*S*)-bakuchiol (**3**)[8] and the molecular formula of **1** and **2** were both determined to be $C_{36}H_{46}O_4$ by HRMS. These observations led to the inference that compounds **1** and **2** have a meroterpenoid skeleton and are closely related isomers.

In the ^1H, ^{13}C, and DEPT NMR spectra of compound **1** (Table 1), characteristic signals for two phenyls, two

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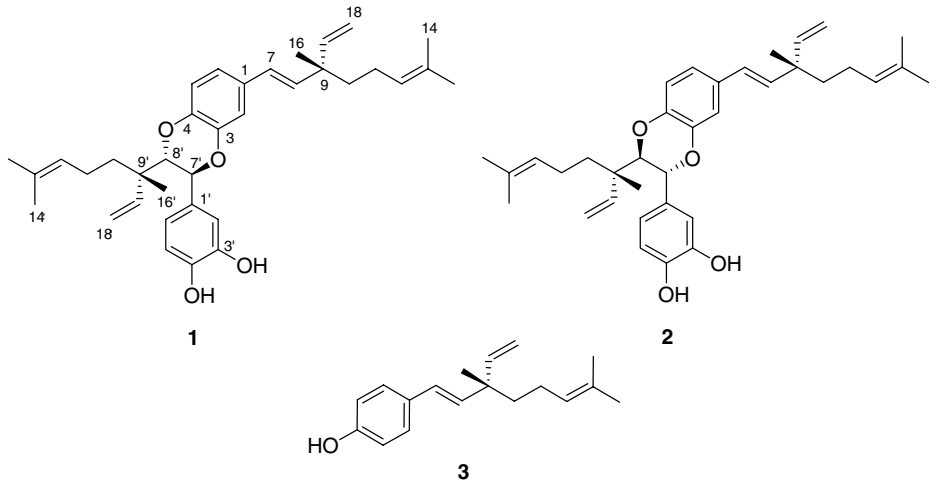

Figure 1. Structures of compounds **1**–**3**.

Table 1. NMR data for bisbakuchiols A and B (**1** and **2**) in $CDCl_3$

Position	δ_H		δ_C	
	1	**2**	**1**	**2**
1			131.6	131.6
2	6.94 br s	6.93 br s	113.9	114.1
3			143.8	143.4
4			143.7	143.1
5	6.86[a]	6.85 d (8.5)	116.7	116.7
6	6.86[a]	6.85 d (8.5)	119.7	119.8
7	6.20 d (16.0)	6.20 d (16.0)	126.7	126.7
8	6.04 d (16.0)	6.05 d (16.0)	136.4	136.4
9			42.7	42.7
10	1.48 m	1.47 m	41.5	41.5
11	1.95 m	1.93 m	23.4	23.4
12	5.08	5.10 t (7.5)	125.0	125.0
13			131.5	131.5
14	1.59 s	1.58 s	17.8	17.8
15	1.68 s	1.68 s	25.9	25.9
16	1.18 s	1.18 s	23.6	23.6
17	5.88 dd (10.5, 17.0)	5.87 dd (11.0, 17.5)	146.1	146.1
18	5.06[a]	5.01	112.1	112.1
1′			131.3	131.4
2′	6.86[a]	6.81[a]	115.5	115.3
3′			143.1	143.7
4′			144.4	144.2
5′	6.86[a]	6.81[a]	115.5	115.4
6′	6.80[a]	6.76[a]	121.7	121.4
7′	4.75 d (7.0)	4.90 d (6.0)	77.5	76.4
8′	3.96 d (7.0)	4.03 d (6.0)	82.0	81.5
9′			43.3	44.0
10′	1.34 m, 1.75 m	1.47 m, 1.54 m	38.5	38.9
11′	1.83 m, 1.95 m	1.83 m, 1.93 m	22.2	22.4
12′	5.06[a]	5.05[a]	124.7	124.8
13′			131.7	131.7
14′	1.58 s	1.58 s	17.9	17.9
15′	1.68 s	1.68 s	25.9	25.9
16′	0.68 s	1.08 s	20.1	17.6
17′	5.84 dd (11.0, 17.0)	5.54 dd (10.5, 17.0)	142.1	142.1
18′	4.90 d (17.5), 5.06[a]	4.74 d (16.5), 4.76 d (9.0)	114.2	113.0

[a] Overlapped signal.

vinyls, two tertiary methyls, and two double-oxygenated phenyls were observed and these signals were suggestive of the presence of two meroterpenoid moieties, which was substantiated by 2D NMR experiments. However, NMR signals were observed for only one *trans* double bond at δ_H 6.20 (1H, d, $J = 16.0$ Hz, H-7)/δ_C 126.7

(C-7) and δ_H 6.04 (1H, d, $J = 16.0$ Hz, H-8)/δ_C 136.4 (C-8), and two oxygenated methines coupled to each other at δ_H 4.75 (1H, d, $J = 7.0$ Hz, H-7′)/δ_C 77.5 (C-7′) and δ_H 3.96 (1H, d, $J = 7.0$ Hz, H-8′)/ δ_C 82.0 (C-8′), and the proton signal at δ_H 4.75 (H-7′) displayed correlations with three aromatic carbon signals at δ_C 131.3 (C-1′), 115.5 (C-2′), and 121.7 (C-6′) in the HMBC spectrum. These results clearly indicated that compound **1** is a dimeric molecule of two meroterpenoid units with a 1,4-dioxane ring. The connectivity between the two meroterpenoid units through a 1,4-dioxane bridge was verified from the HMBC correlations. Thus, the methine proton signals at δ_H 4.75 (H-7′) and 3.96 (H-8′) were correlated with oxygenated aromatic carbon signals at δ_C 143.8 (C-3) and 143.7 (C-4), respectively, in the HMBC NMR experiment with a long-range coupling constant [$^{2,3}J$(C,H)] of 4 Hz (Fig. 2).[9,10] These correlations confirmed the connectivities of the dioxane bridge (C-7′–C-3 and C-8′–C-4).

The stereochemistry of **1** was inferred from the coupling constant and NOESY NMR data, and molecular modeling. The coupling constant ($J = 7.0$ Hz) between H-7′ and H-8′ suggested a *trans* configuration of the two methine protons of the dioxane ring, which is in good agreement with the calculated dihedral angle (177°) of an energy-minimized structure of **1** by MM3 molecular modeling (CAChe™ 5.0). Also, a NOE correlation between H-8′ and H-16′ was observed, supported by a calculated interatomic distance of 2.6 Å. Moreover, H-16′ methyl proton signal was relatively shifted to lower field (δ_H 0.68) due to diamagnetic anisotropy of the benzene ring, which was confirmed by the energy-minimized structure, wherein this methyl group was located under the benzene ring (Fig. 2). These results indicated 7′*S*, 8′*S*, and 9′*S* configurations, which is also assumed from the occurrence of (9*S*)-bakuchiol (**3**) only from nature.[11,12] Therefore, the structure of this novel dimeric meroterpenoid, bisbakuchiol A (**1**),[13] was elucidated as shown.

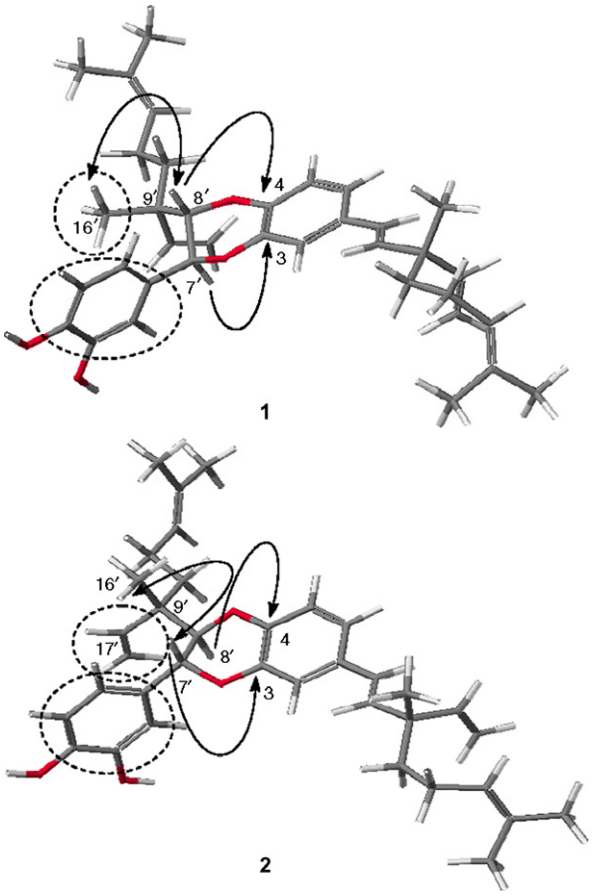

Figure 2. Energy-minimized structures of **1** and **2** with selected HMBC (→) and NOE (↔) correlations, and diamagnetic anisotropic effects of the benzene rings.

Compound **2** showed similar chemical shifts, coupling constants, and 2D NMR correlations to those of bisbakuchiol A (**1**) in the NMR spectra (Table 1). Thus, the NMR signals at δ_H 4.90 (1H, d, $J = 6.0$ Hz, H-7′)/ δ_C 76.4 (C-7′) and δ_H 4.03 (1H, d, $J = 6.0$ Hz, H-8′)/ δ_C 81.5 (C-8′) and the HMBC correlations H-7′/C-3 (δ_C 143.4) and H-8′/C-4 (δ_C 143.1) clearly indicated the presence and the position of a *trans* configured 1,4-dioxane ring, which is the same skeleton as that of **1**. However, unlike compound **1**, the proton of H-7′ displayed a NOE correlation with the methyl proton of H-16′, which was also supported by a calculated interatomic distance of 2.3 Å. Furthermore, the diamagnetic anisotropy of the benzene ring shielded the vinyl proton signals to δ_H 5.54 (H-17′), 4.74 (H-18′), and 4.76 (H-18′) (Fig. 2). Incorporating these data, compound **2** is a diastereomer of **1**, in which the relative configuration of 7′ and 8′ of **2** would be *R* and *R*, respectively. Accordingly, the structure of compound **2**, bisbakuchiol B,[14] was assigned as shown. There are several reports of natural compounds which have a dioxane moiety.[9,10,15–17] To the best of our knowledge, bisbakuchiols A and B (**1** and **2**) represent the first examples of dimeric meroterpenoids in which two meroterpenes are linked through a dioxane bridge.

Using a HIF-1-mediated reporter gene assay in human gastric cancer cells (AGS), compounds **1**–**3** were evaluated for their potential to inhibit the HIF-1 activation induced by hypoxia. The assay was performed according to the established protocols.[4,18,19] (*S*)-Bakuchiol (**3**) inhibited hypoxic activation of HIF-1 with an IC_{50} value of 6.1 μM, while bisbakuchiols A and B (**1** and **2**) were inactive (IC_{50} values >20 μg/mL) in the assay. Further studies are required to characterize the mechanism involved in the inhibition of hypoxic activation of HIF-1 by (*S*)-bakuchiol (**3**).

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Supplementary data

Supplementary data (experimental details, 1D and 2D NMR spectra of bisbakuchiols A and B) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.10.059.

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13. *Bisbakuchiol A* (**1**): opaque resin; $[\alpha]_D^{25} + 32.0°$ (c 0.1, $CHCl_3$); UV (MeOH) λ_{max} ($\log\varepsilon$) 271 (4.15), 206 (4.65) nm; ESIMS m/z 565.7 $[M+Na]^+$, 541.7 $[M-H]^-$, 1083.9 $[2M-H]^-$; HRFABMS m/z 565.3298, calcd for $C_{36}H_{46}O_4Na$, 565.3294.
14. *Bisbakuchiol B* (**2**): opaque resin; $[\alpha]_D^{25} - 3.1°$ (c 0.1, $CHCl_3$); UV (MeOH) λ_{max} ($\log\varepsilon$) 271 (4.13), 206 (4.65) nm; ESIMS m/z 565.7 $[M+Na]^+$, 541.7 $[M-H]^-$, 1083.8 $[2M-H]^-$; HRESIMS m/z 543.3492, calcd for $C_{36}H_{47}O_4$, 543.3474.
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