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Hypoxia-inducible factor-1 and nuclear factor-κB inhibitory meroterpene analogues of bakuchiol, a constituent of the seeds of *Psoralea corylifolia*

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Abstract—Two new meroterpenoids, 12,13-dihydro-12,13-dihydroxybakuchiol (**2**) and (12′*S*)-bisbakuchiol C (**3**), were isolated from the seeds of *Psoralea corylifolia* L. (Fabaceae). The structures of **2** and **3** were elucidated by spectroscopic and chemical methods. Six meroterpenoids isolated from *P. corylifolia* and three semi-synthetic analogues were evaluated for HIF-1 and NF-κB inhibition, and *O*-methyl and *O*-ethylbakuchiols (**6** and **7**) inhibited HIF-1 and NF-κB activation without significantly decreasing the viability of the AGS and HeLa cells, respectively.

Bakuchiol (**1**), a meroterpenoid isolated from the seeds of *Psoralea corylifolia* L. (Fabaceae), has been shown to exhibit the various biological properties including antidiabetic,[1,2] antiinflammatory,[3] antimicrobial,[4] antioxidant,[5] cytotoxic,[6] and liver protective activities.[7,8] In our search for biologically active agents of natural origin, a methanol extract of the seeds of *P. corylifolia* potently inhibited hypoxia-inducible factor-1 (HIF-1) activation induced by hypoxia (100% inhibition at 20 μg/mL) in a HIF-1-mediated reporter gene assay in AGS human gastric cancer cells. HIF-1 controls a number of cellular events required for the adaptation of cancer cells to hypoxia. Therefore, HIF-1 inhibitors are considered as potential therapeutic agents for cancer.[9,10] In a preliminary communication, we reported the initial phytochemical and biological investigation of this plant, with the isolation of bakuchiol (**1**) as a HIF-1 inhibitory constituent, as well as two novel dimeric meroterpenoids, bisbakuchiols A and B (**8** and **9**), which were not active in the HIF-1 mediated reporter gene assay.[11] Here, we report the isolation of two new meroterpenes (**2** and **3**) and a known meroterpene, 12,13-dihydro-12,13-epoxybakuchiol (**4**),[12] and the preparation of bakuchiol analogues (**5**–**7**) (Fig. 1), in addition to the biological evaluation of these compounds obtained.

Compound **2** was obtained as yellow oil and shown to possess a molecular formula of $C_{18}H_{26}O_3$ by positive HRFABMS (*m*/*z* $[M+Na]^+$, 313.1776). The ^1H and ^{13}C NMR spectra of **2** were comparable to those of bakuchiol (**1**), suggesting that **2** is a modified bakuchiol and a meroterpene (Table 1). Thus, AA'XX'-type proton signals at δ_H 6.71 (2H, d, J = 8.8 Hz, H-3 and H-5) and 7.19 (2H, d, J = 8.8 Hz, H-2 and H-6), *trans* double bond signals at δ_H 6.04 (1H, d, J = 16.4 Hz, H-8) and 6.25 (1H, d, J = 16.4 Hz, H-7), vinyl group signals at δ_H 5.02 (overlap, H-18) and 5.91 (1H, m, H-17), and three methyl signals at δ_H 1.11 (3H, s, H-14), 1.15 (3H, s, H-15), and 1.20 (3H, s, H-16) were apparent. However, the absence of the proton signal of an olefinic methine (H-12) of bakuchiol (**1**) and the presence of signals at δ_H 3.21 (1H, dd, J = 2.0 and 10.8 Hz, H-12), δ_C 74.0 (s, C-13), and δ_C 80.6 (d, C-12) led to the inference that oxidative modification had occurred at the C-12 and C-13 double bond of bakuchiol

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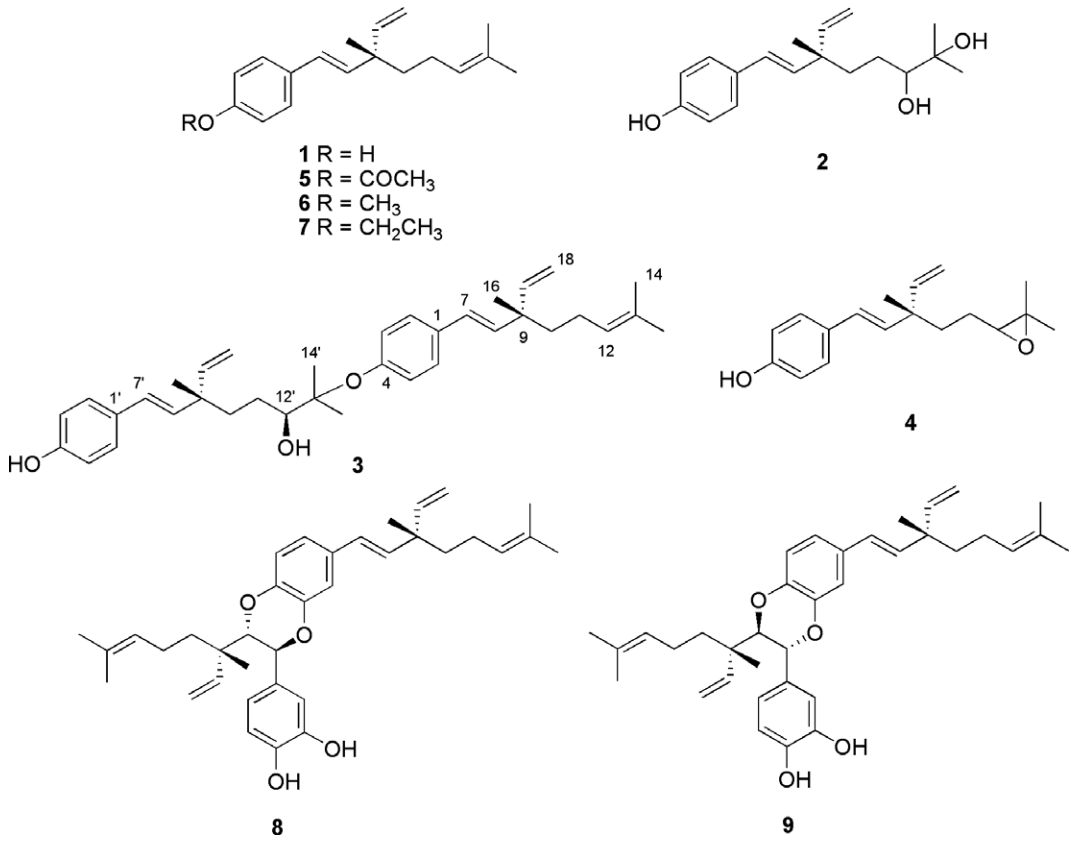

Figure 1. Structures of compounds **1**–**9**.

(**1**).[12] Based on these observations and by comparison of its spectroscopic data with those of bakuchiol (**1**),[12] compound **2** was suggested to be a 12,13-dihydroxylated bakuchiol, confirmed by HMQC and HMBC NMR experiments. Therefore, the structure of the new compound **2** was determined to be 12,13-dihydro-12,13-dihydroxybakuchiol.[13] Mosher's methodology was applied to determine the absolute stereochemistry of the C-12 position according to established procedures.[14–16] However, after several attempts, Mosher's esters of **2** could not be prepared, so the absolute configuration of the C-12 position remains unknown.

Compound **3** was obtained as yellow oil and shown to possess a molecular formula of $C_{36}H_{48}O_3$ by positive HRFABMS (*m*/*z* $[M+Na]^+$, 551.3498). The ^1H and ^{13}C NMR spectra of **3** exhibited two characteristic sets of signals corresponding to bakuchiol (**1**) and compound **2**, respectively (Table 1). Briefly, two sets of AA'XX'-type proton signals at δ_H 6.91 (2H, d, J = 8.4 Hz, H-3 and H-5) and 7.28 (2H, d, J = 8.4 Hz, H-2 and H-6); 6.78 (2H, d, J = 8.4 Hz, H-3′ and H-5′) and 7.24 (2H, d, J = 8.4 Hz, H-2′ and H-6′), proton signals of two *trans* double bonds at δ_H 6.13 (1H, d, J = 16.0 Hz, H-8) and 6.30 (1H, d, J = 16.0 Hz, H-7); 6.06 (1H, d, J = 16.0 Hz, H-8′) and 6.28 (1H, d, J = 16.0 Hz, H-7′), proton signals of two vinyl groups at δ_H 5.90 (1H, dd, J = 10.4 and 17.2 Hz, H-17) and 5.04 (overlap, H-18); 5.89 (1H, dd, J = 10.4 and 17.2 Hz, H-17′) and 5.04 (overlap, H-18′) were observed in the ^1H NMR spectrum of **3**. In the aliphatic region, an oxygenated methine signal at δ_H 3.61 (1H, dd, J = 1.6 and 9.2 Hz, H-12′) and six methyl signals at δ_H 1.20 (3H, s, H-15′), 1.21 (3H, s, H-14′), 1.22 (6 H, s, H-16 and H-16′), 1.60 (3H, s, H-14), and 1.69 (3H, s, H-15) were apparent. Therefore, it was inferred that compound **3** is a dimeric meroterpene, in which bakuchiol (**1**) and compound **2** are connected by ether linkage, which was supported by mass fragmentations (Fig. 2). This inference was supported by 2D NMR experiments and was consistent with the molecular formula obtained. The connectivity between the two meroterpene units was determined by the analysis of ^{13}C NMR data. Thus, the downfield shift of the C-13′ (α carbon) signal of **3** and the upfield shifts of the C-12′, 14′, and 15′ (β carbons) compared to the corresponding ^{13}C NMR data of **2** clearly indicated the linkage of the two units (C-4–C-13′).[17] The absolute configurations of the C-12′ and C-9 (C-9′) positions were clarified by Mosher's methodology and biogenetic consideration, respectively. Mosher's esters (**3s** and **3r**) indicated the *S* configuration at C-12′, because of the positive values ($\Delta\delta_{S-R}$) obtained for H-14′ and H-15′ and the negative differences for H-10′ and H-11′ (Fig. 2).[18] Based on biogenetic consideration (given the occurrence of 9*S*-bakuchiol only from nature),[12,19] the stereochemistry of the stereogenic centers at C-9 and C-9′ could be assumed. Accordingly, the structure of the new dimeric meroterpene, (12′*S*)-bisbakuchiol C (**3**), was elucidated.[20] To the best of our knowledge, (12′*S*)-bisbakuchiol C (**3**) is the first example

Table 1. NMR spectroscopic data ($CDCl_3$) for compounds **1**–**4**[a]

Position	δ_H (*J* in Hz)		δ_C			
	2[b]	**3**	**1**	**2**[b]	**3**	**4**
1			130.9 s	131.1 s	134.0 s	130.3 s
2, 6	7.19 d (8.8)	7.28 d (8.4)	127.4 d	128.4 d	126.9 d	127.3 d
3, 5	6.71 d (8.8)	6.91 d (8.4)	115.4 d	116.4 d	124.5 d	115.4 d
4			154.6 s	157.9 s	153.4 s	155.1 s
7	6.25 d (16.4)	6.30 d (16.0)	126.4 d	128.4 d	126.7 d	127.0 d
8	6.04 brd (16.4)	6.13 d (16.0)	135.9 d	136.1 d	137.4 d	135.0 d
9			42.5 s	43.5 s	42.8 s	42.2 s
10	1.45 m, 1.87 m	1.51[c] m	41.3 t	40.2 t	41.5 t	37.5 t
11	1.27 m, 1.63 m	1.98[c] m	23.2 t	27.4 t	23.4 t	24.1 t
12	3.21 dd (10.8, 2.0)	5.12 brt	124.8 d	80.6 d	125.0 d	65.1 d
13			131.3 s	74.0 s	131.6 s	59.2 s
14	1.11 s	1.60 s	17.6 q	25.0 q	17.9 q	18.6 q
15	1.15 s	1.69 s	25.7 q	26.0 q	25.9 q	24.8 q
16	1.20 s	1.22 s	23.3 q	24.2 q	23.5 q	23.3 q
17	5.91 m	5.90 dd (17.2, 10.4)	145.9 d	147.8 d	146.0 d	145.4 d
18	5.02[c]	5.04[b]	111.9 t	112.7 t	112.4[d] t	112.4 t
1′					130.8 s	
2′, 6′		7.24 d (8.4)			127.6 d	
3′, 5′		6.78 d (8.4)			115.6 d	
4′					155.1 s	
7′		6.28 d (16.0)			127.0 d	
8′		6.06 d (16.0)			135.8 d	
9′					42.6 s	
10′		1.50 m, 1.98[c] m			38.6 t	
11′		1.44 m, 1.50[c] m			26.4 t	
12′		3.61 dd (9.2, 1.6)			78.9 d	
13′					83.8 s	
14′		1.21 s			20.8 q	
15′		1.20 s			23.3 q	
16′		1.22 s			23.8 q	
17′		5.89 dd (17.2, 10.4)			146.0 d	
18′		5.04[c]			112.2[d] t	

[a] The assignments were based on the DEPT, HMQC, and HMBC experiments.
[b] Measured for CD_3OD solution.
[c] Overlapping signals.
[d] Signals may be interchangeable.

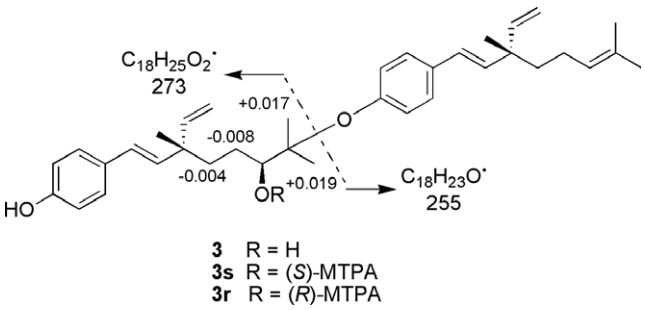

Figure 2. Mass fragmentation of compound **3** and $\Delta\delta_{S\text{-}R}$ values of MTPA esters (**3s** and **3r**) of compound **3** by Mosher's methodology.

of a dimeric meroterpene in which two meroterpenes are linked by ether linkage.

Compounds **2**–**4** were evaluated for their potential to inhibit the HIF-1 activation induced by hypoxia using a HIF-1-mediated reporter gene assay in AGS human gastric cancer cells,[21–23] but were inactive (IC_{50} values >20 μg/ml) in the assay (Table 2). Therefore, as reported in our preliminary communication, bakuchiol (**1**) is the only HIF-1 inhibitory principle (IC_{50} value 6.1 μM) isolated from *P. corylifolia*.[11] Previously, bakuchiol (**1**) was reported to suppress the activation of NF-κB,[3] which regulates a number of genes involved in inflammation and cancer responses.[24] This led us to evaluate the meroterpenes obtained from *P. corylifolia* for NF-κB inhibition. Bakuchiol (**1**) and bisbakuchiol C (**3**) inhib-

Table 2. Biological activities of compounds **1**–**7** (IC_{50} values, μM)

	AGS[a]		HeLa[b]	
	HIF-1	MTT	NF-κB	MTT
1	6.1	15.3	6.9	11.0
2	—[c]	—	—	—
3	—	—	12.2	—
4	—	—	—	—
5	5.7	26.8	5.7	18.1
6	8.7	55.8	5.7	—
7	26.3	—	12.2	—

[a] AGS, human gastric cancer cell.
[b] HeLa, human cervical adenocarcinoma cell.
[c] IC_{50} value >50 μM; 17-desmethoxy-17-*N*,*N*-dimethylaminoethylaminogeldanamycin (HIF-1, IC_{50} 0.0036 μM; MTT, IC_{50} 16.0 μM) and celastrol (NF-κB, IC_{50} 0.15 μM; MTT, IC_{50} 2.06 μM) were used as positive controls.

ited the NF-κB activation induced by TNF-α in HeLa human cervical adenocarcinoma cells with IC_{50} values of 6.9 and 12.2 μM, respectively, while compounds **2** and **4** and bisbakuchiols A and B were inactive (IC_{50} values >50 μM) in the assay (Table 2).[25–28]

However, bakuchiol (**1**) exhibited significant cytotoxicity to the AGS and HeLa cells as measured by MTT assays (IC_{50} values 15.3 and 11.0 μM, respectively) (Table 2), suggesting that bakuchiol (**1**) may regulate the expression of HIF-1 and NF-κB, and/or the stability of the AGS and HeLa cells. These results prompted us to prepare simple bakuchiol analogues in an attempt to increase HIF-1 and NF-κB inhibitory efficacy, to reduce the cytotoxicity, and to obtain a preliminary notion of structure–activity relationship of bakuchiol (**1**). Acetylbakuchiol (**5**),[29,30] and *O*-methyl[5,31] and *O*-ethylbakuchiols[32] (**6** and **7**) were prepared as described in the Supplementary data. These analogues were tested in both assay systems and acetylbakuchiol (**5**) displayed almost the same biological activities as those of bakuchiol (**1**). However, *O*-methyl and *O*-ethylbakuchiols (**6** and **7**) retained the inhibitory effects against HIF-1 (IC_{50} values, 8.7 and 26.3 μM, respectively) and NF-κB (IC_{50} values, 5.7 and 12.2 μM, respectively) activation without significantly affecting the viability of AGS and HeLa cells, respectively, at the concentration of 50 μM.

According to these results, it was inferred that the nature of phenolic hydroxyl group and 12,13-double bond of bakuchiol (**1**) play important roles in the biological profiles of bakuchiol (**1**) in HIF-1 and NF-κB inhibition. Thus, a bulky substitution on the hydroxyl group of bakuchiol (**1**) reduces the inhibitory activity against HIF-1 and NF-κB activation [bisbakuchiols A–C (**8**, **9**, and **3**)].[11] It was also noted that 12,13-double bond of bakuchiol (**1**) is required for the HIF-1 and NF-κB inhibitory activity (**2** and **4**).

In summary, we evaluated the six meroterpenoids isolated from *P. corylifolia* and three semi-synthetic analogues for HIF-1 and NF-κB inhibition, and found out that *O*-methyl and *O*-ethylbakuchiols (**6** and **7**) inhibited HIF-1 and NF-κB activation without significantly decreasing the viability of AGS and HeLa cells, respectively. Further studies are needed to optimize the biological profiles of the meroterpenoids and to elucidate how the meroterpenoids inhibit HIF-1 and NF-κB activation.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.03.028.

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12′, C-13′, C-14′. UV λ_{max} (MeOH) nm (log ε): 262 (4.58), 207 (4.65). ESIMS *m/z*: 255.4, 273.4, $[M+Na]^+$ 551.6, $[M-H]^-$ 527.7. HRFABMS *m/z* $[M+Na]^+$ 551.3498 (Calcd for $C_{36}H_{48}O_3Na$: 551.3501).

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