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Biyouyanagin A, an Anti-HIV Agent from *Hypericum chinense* L. var. *salicifolium*

Naonobu Tanaka,[†] Mamoru Okasaka,[†] Youko Ishimaru,[†] Yoshihisa Takaishi,*[,†] Mitsunobu Sato,[‡] Masato Okamoto,[‡] Tetsuya Oshikawa,[‡] Sharif Uddin Ahmed,[‡] L. Mark Consentino,[§] and Kuo-Hsiung Lee[||]

Faculty of Pharmaceutical Sciences, University of Tokushima, Shomachi 1-78, Tokushima, 770-8505, Japan, School of Dentistry, University of Tokushima, Kuramoto-cho, Tokushima 770-8504, Japan, BBI-Biotech Research Laboratories, Perry Parkway, Gaithersburg, Maryland 20877, and Natural Products Laboratory, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27599

takaishi@ph.tokushima-u.ac.jp

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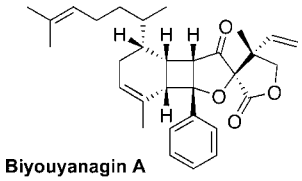
ABSTRACT



Biyouyanagin A

A structurally unique hydrophobic compound, biyouyanagin A, was isolated from the MeOH extract of the leaves of *Hypericum chinense* L. var. *salicifolium*. The structure of biyouyanagin A was elucidated on the basis of spectroscopic evidence. Biyouyanagin A showed a significant activity against HIV and inhibited cytokine production.

The recent widespread interest in the antidepressant activity of *Hypericum perforatum* (St. John's wort) has inspired the investigation of secondary metabolites from other *Hypericum* species.[1] The genus *Hypericum*, which are distributed widely in temperate regions, have been used as traditional medicines in various parts of the world. In Japan, *H. chinense* L. var. *salicifolium* (Biyouyanagi in Japanese) is used as a folk medicine for treatment of female disorders.[2]

Antibacterial acylphloroglucinols and spirolactones were also isolated from this species.[3] As a part of a program to discover new bioactive natural products from plants, we have examined the MeOH extract from the leaves of *H. chinense* and isolated a unique hydrophobic compound named biyouyanagin A, which contains sesquiterpene, cyclobutane, and spirolactone moieties. Biyouyanagin A showed a significant activity against HIV and inhibited cytokine production. In this paper, we report isolation, structural elucidation, and biological evaluation of biyouyanagin A.

Dried leaves of *H. chinense* L. var. *salicifolium* (1.48 kg) were extracted with MeOH. The MeOH extract (632.7 g) was partitioned with *n*-hexane and H_2O, and the *n*-hexane fraction (92.6 g) was subjected to repeated column chromatography to give biyouyanagin A.

Biyouyanagin A (**1**) was obtained as a colorless oil, $[\alpha]_D$ −240.0 ($CHCl_3$, *c* 0.5). The IR spectrum of **1** showed absorption bands of two carbonyl groups (1792, 1743 cm^{-1}). The ^1H NMR showed the presence of a benzene ring [δ_H 7.26−7.37 (5H, m)], a 1-substituted ethylene moiety [δ_H 5.24 (1H, dd, J = 17.6, 11.2), 4.80 (1H, d, J = 11.2), 4.62 (1H, d, J = 17.6)], two olefinic protons [δ_H 5.46 (1H, m), 5.11 (1H, brt, J = 5.6)], one oxygenated methylene group [δ_H 4.71, 3.98 (each 1H, d, J = 8.8)], five methines, three methylenes, and five methyls. The HRFABMS gave a

* To whom correspondence should be addressed. Tel: 81-88-6337275. Fax: 81-88-6339501.
[†] Faculty of Pharmaceutical Sciences, University of Tokushima.
[‡] School of Dentistry, University of Tokushima.
[§] BBI-Biotech Research Laboratories.
[||] School of Pharmacy, University of North Carolina.

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quasimolecular ion peak at *m*/*z* 475.2911 ([M + H]$^+$, calcd 475.2848) suggesting the molecular formula of $C_{31}H_{38}O_4$. The ^{13}C NMR spectral data, including DEPT spectra, were in good agreement with the above analysis (Table 1).

Table 1. NMR Data for **1**[a]

position	^{13}C (δ_C)	^{1}H (δ_H)	HMBC (^{13}C no.)
1	118.4	4.80 (1H, d, 11.2) 4.62 (1H, d, 17.6)	3
2	134.5	5.24 (1H, dd, 17.6, 11.2)	3, 4, 9, 10
3	49.0		
4	93.1		
5	209.6		
6	51.9	3.16 (1H, dd, 6.0, 1.2)	4, 5, 11, 17, 18, 22
7	89.7		
8	171.6		
9	73.7	4.71 (1H, d, 8.8) 3.98 (1H, d, 8.8)	3, 4, 8, 10
10	20.1	1.31 (3H, s)	2, 3, 4, 9
11	139.6		
12	125.9	7.37−7.26 (1H, m)	7
13	127.7	7.37−7.26 (1H, m)	
14	127.8	7.37−7.26 (1H, m)	
15	127.7	7.37−7.26 (1H, m)	
16	125.9	7.37−7.26 (1H, m)	7
17	35.9	3.01 (1H, ddd, 8.4, 6.6, 6.6)	5, 6, 7, 18, 19, 21, 22, 24
18	50.3	3.49 (1H, d, 8.4)	6, 7, 17, 19, 20, 23
19	131.4		
20	123.9	5.46 (1H, m)	
21	23.5	2.09 (1H, m) 1.99 (1H, m)	
22	38.8	1.73 (1H, m)	6,
23	21.7	1.02 (3H, d, 1.2)	18, 19, 20
24	35.1	1.46 (1H, m)	
25	16.8	0.83 (3H, d, 6.4)	22, 24, 26
26	35.0	1.45 (1H, m) 1.20 (1H, m)	24, 27, 28
27	25.9	2.02 (1H, m) 1.94 (1H, m)	
28	124.6	5.11 (1H, brt, 5.6)	27, 30, 31
29	131.4		
30	25.7	1.70 (3H, d, 1.2)	28, 29, 31
31	17.7	1.61 (3H, s)	28, 29, 30

[a] Measured in $CDCl_3$. Coupling constants given (*J*, Hz) in parentheses.

The ^{1}H−^{1}H COSY spectrum of **1** showed the following correlations: H_3-25−H-24−H_2-26−H_2-27−H-28; H-20−H_2-21−H-22−H-17−H-18. The structure of partial unit A (sesquiterpene unit, Figure 1) was indicated by the following long-range correlations in the HMBC spectrum: H_3-30 and -31 with C-28, -29; H_3-25 with C-22, -24, -26; H_3-23 with C-18, -19, -20; H-17 with C-18, -19, -21, -22, -24; and H-18 with C-17, -19, -20, -23.

The remaining ^{1}H and ^{13}C NMR signals of **1** were compared with those of hyperolactone C.[4] These data showed

(4) Aramaki, Y.; Chiba, K.; Tada, M. *Phytochemistry* **1995**, *38*, 1419−1421.

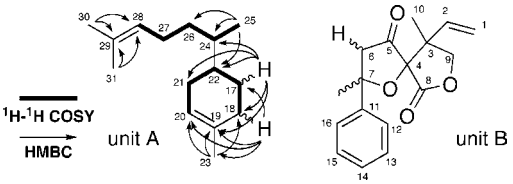


Figure 1. Partial structures of **1**.

good agreement except for the signals of H-6 [δ_H 3.16 (1H, dd, J = 6.0, 1.2) in **1** vs 5.99 (1H, s) in hyperolactone C, C-5 (δ_C 209.6 vs 196.6), C-6 (δ_C 51.9 vs 100.3), C-7 (δ_C 89.7 vs 187.3), and C-11 (δ_C 139.6 vs 127.7)]. In **1**, the long-range correlations of H-6 with C-4, -5, -11 were observed in the HMBC spectrum. These results clearly indicated that **1** has a saturated C-6/C-7 bond (methine carbon and a quaternary carbon, respectively) rather than the double bond in hyperolactone C. Thus, the structure of partial unit B (spiro-lactone unit, Figure 1) was elucidated.

The connections of units A (sesquiterpene) and B (spiro-lactone) were established on the basis of the following key correlations: H-6 with H-17 (^{1}H-^{1}H COSY); H-6 with C-17, -18, -22, H-17 with C-5, -6, -7, H-18 with C-6, -7 (HMBC). Thus, the direct connections between C-6 and C-17, C-7 and C-18 formed a cyclobutane ring.

The relative configuration was established from the following NOE correlations: H-6 with H-17, -22, and aromatic protons; H-17 with H-18, -22; H_3-10 with aromatic protons. Thus, the structure of **1** was elucidated (Figure 2).

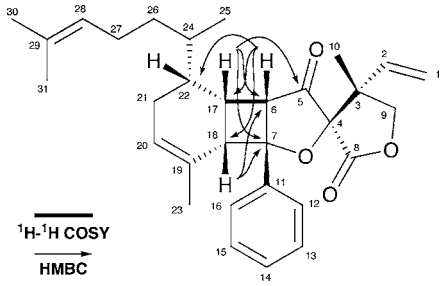
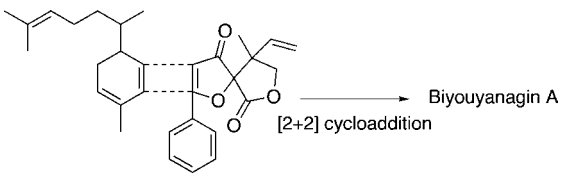


Figure 2. Biyouyanagin A (**1**).

Our postulated biosynthetic pathway of **1** from the related sesquiterpene and spirolactone is shown in Scheme 1.

Scheme 1



In the search for anti-HIV natural products, various coumarins, terpenoids, and phloroglucinols[5] have been reported to have anti-HIV activity. Accordingly, we evaluated anti-HIV activity of this novel compound. Compound **1** inhibited HIV replication in H9 lymphocytes with an EC_{50} value of 0.798 μg/mL and uninfected H9 cell growth with IC_{50} values of >25 μg/mL, giving a calculated therapeutic index (TI) value of >31.3 (Table 2). Thus, **1** can be regarded as a promising new anti-HIV agent with a unique structure and merits further evaluation and analogue design.

Table 2. Anti-HIV Activity of **1**

compd	IC_{50} (μg/mL)	EC_{50} (μg/mL)	TI
biyouyanagin A (**1**)	>25	0.798	31.3
AZT	500	0.0021	238, 738

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Table 3. Inhibitory Effects for Cytokine Release of **1**[a]

	cytokine production ratio		
compd	IL-10	IL-12	TNF-α
biyouyanagin A (**1**)	0.03	0.02	0.48
prednisolone	0.14	0.24	0.48

[a] PBMCs were treated with lipopolysaccharide (LPS) in the presence of **1** (10 μg/mL). Prednisolone (0.3 μg/mL) was used as a reference sample. Data were expressed as ratios to cytokine production induced by LPS.

Furthermore, we examined the effect of **1** in LPS-induced cytokine production, and it markedly inhibited the LPS-induced production of IL-10, IL-12, and TNF-α (Table 3). These data suggest that **1** is a strong inhibitor for cytokines and is worthy of further investigation.

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Supporting Information Available: Experimental section, plant material, extraction, isolation, and spectral data of biyouyanagin A (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

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