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A NEW TIGLIANE-TYPE DITERPENE ESTER FROM *Wikstroemia scytophylla*[a]

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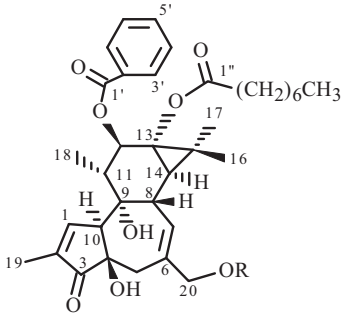
*A new tigliane-type diterpene ester, wiksphyllamin B (**1**), and one known compound, 12-*O*-benzoylphorbol 13-octanoate (**2**), were isolated from the stems of* Wikstroemia scytophylla *Diels. Their structures were identified using spectroscopic methods. The incorrect assignment of ^{13}C NMR data of compound **2** in the literature was revised according to the 2D NMR spectra.*

Keywords: tigliane-type diterpene, *Wikstroemia scytophylla* Diels, wiksphyllamin B.

Wikstroemia scytophylla Diels, a shrub in the family Thymelaeaceae, is regionally distributed in the Provinces of Yunnan, Sichuan, and Tibet in China. There is no report on the chemical constituents and bioactivity of *Wikstroemia scytophylla*, whereas *Wikstroemia indica*, of the same genus as *Wikstroemia scytophylla*, has long been used as a traditional crude drug for the treatment of pneumonia, rheumatism, and bronchitis in China [1]. *Wikstroemia indica* has many functional constituents, including flavonoids, coumarins and lignans [1–3].

Investigation of the stems of *Wikstroemia scytophylla* led to the isolation of a new tigliane-type diterpene ester named wiksphyllamin B (**1**) and one known compound, 12-*O*-benzoylphorbol 13-octanoate (**2**) [4, 5]. Their structures were determined by spectroscopic methods, mainly 1D and 2D NMR. Additionally, the incorrect assignment of ^{13}C NMR data of compound **2** in the literature was revised according to the 2D NMR spectra.

Compound **1** was obtained as a yellow oil. The molecular formula was deduced as $C_{51}H_{76}O_9$ through the analysis of HR-ESI-MS (*m/z* 855.5398 $[M + Na]^+$, calcd for $C_{51}H_{76}O_9Na$, 855.5387). The IR spectrum showed absorption bands at 3417, 1721, and 1629 cm^{-1}, which were indicative of hydroxy groups, carbonyls, and double bonds. The ^{13}C NMR (DEPT) spectrum of **1** showed 6 methyls, 22 methylenes, 12 methines, and 11 quaternary carbons, as assigned in Table 1.



1, 2

1: R = $CO(CH_2)_{14}CH_3$

2: R = H

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TABLE 1. ^{1}H NMR and ^{13}C NMR Data of **1** (CD_3OD) and **2** ($CDCl_3$, δ, ppm, J/Hz)

C atom	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1	7.57 (1H, br.s)	160.3 d	7.63 (1H, br.s)	160.8 d
2		134.6 s		133.0 s
3		209.9 s		209.0 s
4		74.5 s		73.7 s
5	2.58 (1H, d, J = 11.8)	38.9 t	2.55 (1H, d, J = 11.0)	38.6 t
	2.49 (1H, d, J = 11.8)		2.51 (1H, d, J = 11.0)	
6		138.2 s		140.6 s
7	5.75 (1H, d, J = 4.6)	133.1 d	5.74 (1H, d, J = 4.3)	129.2 d
8	3.39 (1H, dd, J = 5.2, 4.6)	40.4 d	3.36 (1H, dd, J = 5.2, 4.3)	39.2 d
9		79.7 s		78.4 s
10	3.18 (1H, s)	57.3 d	3.31 (1H, d, J = 2.4)	56.2 d
11	2.41 (1H, m)	44.6 d	2.34 (1H, m)	43.3 d
12	5.70 (1H, d, J = 10.4)	79.1 d	5.69 (1H, d, J = 10.2)	77.7 d
13		66.7 s		65.5 s
14	1.20 (1H, d, J = 5.2)	37.2 d	1.15 (1H, d, J = 5.2)	36.7 d
15		27.5 s		26.0 s
16	1.23 (3H, s)	24.2 q	1.23 (3H, s)	23.8 q
17	1.41 (3H, s)	17.6 q	1.41 (3H, s)	17.1 q
18	1.94 (3H, d, J = 6.2)	14.9 q	1.97 (3H, d, J = 6.4)	14.5 q
19	1.75 (3H, s)	10.3 q	1.78 (3H, d, J = 1.5)	10.1 q
20	4.52 (1H, d, J = 12.8)	70.1 t	4.09 (1H, d, J = 12.8)	68.1 t
	4.50 (1H, d, J = 12.8)		4.03 (1H, d, J = 12.8)	
1′		167.9 s		166.3 s
2′		131.3 s		130.2 s
3′, 7′	8.02 (1H, d, J = 7.4)	130.6 d	8.05 (1H, d, J = 7.2)	129.7 d
4′, 6′	7.50 (1H, t, J = 7.4)	129.7 d	7.47 (1H, t, J = 7.7)	128.4 d
5′	7.60 (1H, t, J = 7.4)	134.5 d	7.60 (1H, t, J = 7.5)	132.9 d
1″		175.1 s		176.5 s
2″	2.31 (2H, m)	35.1 t	2.40 (2H, m)	34.4 t
3″	1.63 (2H, m)	25.8 t	1.67 (2H, m)	24.6 t
4″	1.29 (2H, m)	30.8–30.1 t	1.32–1.25 (2H, m)	29.0 t
5″	1.29 (2H, m)	30.8–30.1 t	1.32–1.25 (2H, m)	28.9 t
6″	1.29 (2H, m)	32.9 t	1.32–1.25 (2H, m)	31.6 t
7″	1.29 (2H, m)	23.7 t	1.32–1.25 (2H, m)	22.6 t
8″	0.90 (3H, t, J = 6.0)	14.5 q	0.90 (3H, t, J = 7.0)	14.0 q
1‴		177.9 s		
2‴	2.38 (2H, m)	35.2 t		
3‴	1.59 (2H, m)	26.1 t		
4‴–13‴	1.29 (2H, m)	30.8–30.1 t		
14‴	1.29 (2H, m)	33.1 t		
15‴	1.29 (2H, m)	23.8 t		
16‴	0.89 (3H, t, J = 5.6)	14.5 q		

The ^{1}H NMR spectrum (see Table 1) exhibited five aromatic protons at δ 7.50 (2H, t, J = 7.4 Hz), 7.60 (1H, t, J = 7.4 Hz), and 8.02 (2H, d, J = 7.4 Hz), indicative of the presence of a monosubstituted aromatic ring, two olefinic protons at δ 5.75 (1H, d, J = 4.6 Hz) and 7.57 (1H, br.s), and six methyls at δ 0.89 (3H, t, J = 5.6 Hz, H-16‴), 0.90 (3H, t, J = 4.8 Hz, H-8″), 1.94 (3H, d, J = 6.2 Hz, H-18), 1.23 (3H, s, H-16), 1.41 (3H, s, H-17), and 1.75 (3H, s, H-19). A series of protons centered at 1.29 (m) implied the presence of straight-chain alkane moieties. The ^{13}C NMR data of **1** displayed carbon resonances due to a ketone carbonyl (δ 209.9) and three ester carbonyls (δ 167.9, 175.1 and 177.9). The NMR spectra were very similar to those of 12-*O*-benzoylphorbol 13-nonanoate [5] and 12-*O*-benzoylphorbol 13-octanoate [4], except for an additional straight-chain alkane moiety and an ester carbonyl (δ_C 177.9), suggesting one more straight-chain ester group.

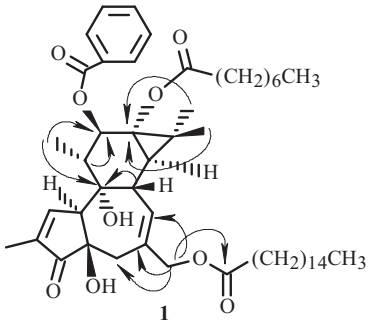

Fig. 1. The key HMBC correlations of **1.**

The EI-MS spectrum showed the fragment peak *m/z* 127 (-$CO(CH_2)_6CH_3$), indicating that compound **1** had the same ester group as 12-*O*-benzoylphorbol 13-octanoate (**2**). The fragment peak *m/z* 239 exhibited the presence of -$CO(CH_2)_{14}CH_3$, which was further confirmed by HR-EI-MS (*m/z* 239.2373 $[C_{16}H_{31}O]^+$, calcd for $C_{16}H_{31}O$, 239.2375).

The HMBC (Fig. 1) correlations of **1** from H-20 [δ_H 4.52 (1H, d, J = 12.8 Hz)] to C-1′′′ (δ_C 177.9), C-5 (δ_C 38.9), C-6 (δ_C 138.2), and C-7 (δ_C 133.1) suggested this fragment -$CO(CH_2)_{14}CH_3$ is located at the C-20 forming ester group. The HMBC correlations of **1** from H-18 [δ_H 1.94 (3H, d, J = 6.2 Hz)] to C-9 (δ_C 79.7), C-12 (δ_C 79.1), and from H-10 [δ_H 3.18 (1H, s)] to C-9 (δ_C 79.7), indicated the incorrect assignment of C-9 (δ_C 67.0) in the literature [4, 5]. The incorrect assignment of C-13 (δ_C 79.9) in the literature [4, 5] could also be proved through the HMBC correlations of **1** from H-16 [δ_H 1.23 (3H, s)] to C-13 (δ_C 66.7), C-14 (δ_C 37.2), and from H-17 [δ_H 1.41 (3H, s)] to C-13 (δ_C 66.7), C-14 (δ_C 37.2). The other correlations in the HMBC spectrum further confirmed the connections of compound **1**. Thus, the structure of compound **1** was assigned as shown, and this compound was named wiksphyllamin B.

12-*O*-Benzoylphorbol 13-octanoate (**2**) was obtained as a yellow oil. It has been reported previously [4]. Here, the ^{13}C NMR data of C-9 (δ 78.4) and C-13 (δ 65.5) in **2** as **1** can also be assigned through the HMBC (Fig. 1) correlations, whereas these two carbon shift were assigned oppositely in the literature.

EXPERIMENTAL

General Experimental Procedures. Optical rotations were measured on a Horiba SEPA-300 polarimeter. UV spectra were obtained on a Shimadzu double-beam 210A spectrometer. IR spectra were obtained on a Tensor 27 spectrometer with KBr pellets. NMR spectra were recorded on a Bruker AV-400, a DRX-500, or AVANCE III-600 spectrometer with TMS as an internal standard. ESI-MS and HR-ESI-MS were recorded with an API QSTAR Pulsar 1 spectrometer. EI-MS and HR-EI-MS were recorded with a Waters Autospec Premier instrument. Silica gel (200–300 mesh, Qingdao Marine Chemical Inc., People′s Republic of China), RP-18 (40–70 μm, Fuji Silysia Chemical Ltd., Japan) and Sephadex LH-20 (Amersham Biosciences, Sweden) were used for column chromatography. Fractions were monitored by TLC, and spots were visualized by heating after spraying with 10% H_2SO_4 in ethanol.

Plant Material. The stems of *Wikstroemia scytophylla* were collected in Diqing Tibetan Autonomous Prefecture, Yunnan Province, People′s Republic of China, and identified by Dr. L. L. Yue. A voucher specimen (JHZ0002) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, People's Republic of China.

Extraction and Isolation. The dried and powdered stems of *Wikstroemia scytophylla* D. (3.0 kg) were extracted with 95% EtOH under reflux for three times (3 × 10 L). The extract was concentrated and suspended in water followed by successive partition with petroleum ether (3 × 5 L), EtOAc (3 × 5 L), and *n*-BuOH (3 × 5 L). The EtOAc extract (320 g) was separated on a silica gel column (4.9 kg, 200–300 mesh) using gradient solvent petroleum ether–acetone (100:1, 98:2, 95:5, 90:10, 80:20, 70:30, 60:40, 10 L) to afford six fractions. Fraction 2 (35 g) was separated on a silica gel column using gradient solvent petroleum ether–acetone (4:1–1:1) to afford fractions 2-1–2-7. Fraction 2-2 (9 g) was gel filtrated on Sephadex LH-20 ($CHCl_3$–MeOH 1:1) to give four subfractions (2-2-1–2-2-4). Fraction 2-2-1 (2.2 g) was subjected to repeated RP-18 (MeOH–H_2O, 70%, 80%, 90%, and 100%) and Sephadex LH-20 ($CHCl_3$–MeOH 1:1) column chromatography to yield **1** (35 mg) and **2** (8 mg).

Wiksphyllamin B (1). $C_{51}H_{76}O_9$, yellow oil; $[\alpha]_D^{21.5}$ +2.5313° (*c* 1.280, CH_3OH). UV (CH_3OH, λ_{max}, nm) (log ε): 202 (4.254), 230 (4.226). IR (KBr, ν_{max}, cm^{-1}): 3418, 2926, 2854, 1721, 1629, 1270. ^1H NMR (400 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD), see Table 1. HR-ESI-MS (+) *m/z* 855.5398 $[M + Na]^+$ (calcd for $C_{51}H_{76}O_9Na$, 855.5387); HR-EI-MS *m/z* 239.2373 $[C_{16}H_{31}O]^+$ (calcd for $C_{16}H_{31}O$, 239.2375). ESI-MS (+) (*m/z*, I_{rel}, %): 855 $[M + Na]^+$ (100), 711 (55). EI-MS (*m/z*, I_{rel}, %): 832 $[M]^+$ (6), 711 (4), 566 (9), 432 (12), 328 (14), 310 (42), 256 (17), 239 (12), 213 (15), 173 (11), 127 (37), 105 (100), 85 (32), 77 (33), 69 (42), 57 (72).

12-*O*-Benzoylphorbol 13-Octanoate (2). $C_{35}H_{46}O_8$, yellow oil; $[\alpha]_D^{21.2}$ +8.1695° (*c* 0.590, CH_3OH). UV (CH_3OH, λ_{max}, nm) (log ε): 202 (4.151), 230 (4.151). IR (KBr, ν_{max}, cm^{-1}): 3426, 2927, 1718, 1629, 1271. ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1. HR-ESI-MS (–) *m/z* 629.2874 $[M + Cl]^-$ (calcd for $C_{35}H_{46}O_8Cl$, 629.2881). ESI-MS (–) (*m/z*, I_{rel}, %): 629 $[M + Cl]^-$ (100), 594 $[M]^-$ (10). EI-MS (*m/z*, I_{rel}, %): 594 $[M]^+$ (4), 473 (16), 328 (45), 310 (48), 173 (25), 127 (48), 113 (44), 105 (100), 95 (26), 85 (39), 83 (75), 69 (57), 57 (88).

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