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Brachyanins A-C, pinene-derived meroterpenoids and phloroglucinol derivative from *Leptospermum brachyandrum*

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ABSTRACT

A pair of epimer brachyanins A (**1**) and B (**2**), along with a new phloroglucinol brachyanin C (**3**), were isolated from the leaves of *Leptospermum brachyandrum*. Brachyanins A (**1**) and B (**2**) were the first example of novel meroterpenoid with a unique skeleton that combined a synacrpic acid and a pinene units via a benzyl moiety. Their structures were elucidated through the application of extensive spectroscopic measurements and single-crystal X-ray diffraction analysis and with the absolute configurations of **1** and **2** were confirmed by the quantum chemical CD calculation. The hetero Diels-Alder as the key biotransformation was proposed to account for the biosynthesis of brachyanins A and B sheding light by the potential procursor brachyanin C.

1. Introduction

The species *Leptospermum brachyandrum* is one of the most important member in the plant family Myrtaceae, which are beautiful ever-green shrubs or small trees mainly occurred in Australia [1,2]. It was introduced to China decades ago and is now planted in the southern of China due to its ornamental and medicinal properties. Naturally occurring adduct of acylphloroglucinol and terpene from family Myrtaceae, an attractive resource for producing numerous new compounds with various drug candidates, has attracted considerable efforts from the synthetic and pharmaceutical communities [3–8]. The chemical constitutes of genus *Leptospermum* were mainly about the L. *scropraum* and revealed to be nortriketones [9], hydroxychalcone [10], beta-triketones [11], triterpenoids and flavonoids [12], whereas few efforts towards the investigation of chemical constitutes from other species in *Leptospermum* were performed. In our continuing efforts to discover new antibacterial constituents from the plants of family Myrtaceae [13–18], we have fascinated with the biological meaningful constituents of L. *brachyandrum*. The following chemical investigation led to the isolation three new compounds, designated as brachyanins A-B (**1–2**) comprising an unprecedented meroterpenoid skeleton formating with a novel benzyl substituted *β*-triketone and pinene moieties, and a new phloroglucinol brachyanin C (**3**) (Fig. 1). Herein, the isolation, structural elucidation, and antimicrobial activity of **1–3** are described.

2. Experimental

2.1. General experimental procedures

Optical rotations were recorded with an MCP-500 polarimeter (Anton Paar, Graz, Austria). UV spectra were recorded on a U-2910 spectrometer (Shimadzu, Kyoto, Japan); IR spectra were obtained on an IR Affinity-1 spectrophotometer (Shimadzu, Kyoto, Japan). High-resolution mass spectral data were obtained on a MaXis Q-TOF mass spectrometer (Bruker, Billerica, MA, USA). 1D- and 2D-NMR spectra were recorded on a Bruker Advance-500 spectrometer (Bruker BioSpin AG, Fällanden, Switzerland) using TMS as internal standard, with chemical shift (*δ*) expressed in ppm. Silica gel (80–100 mesh, 200–300 mesh, and 300–400 mesh; Qingdao Marine Chemical Inc., Qingdao, China), C_{18} reversed-phase silica gel (150–200 mesh, Merck) were used for column chromatography. All solvents were of analytical grade

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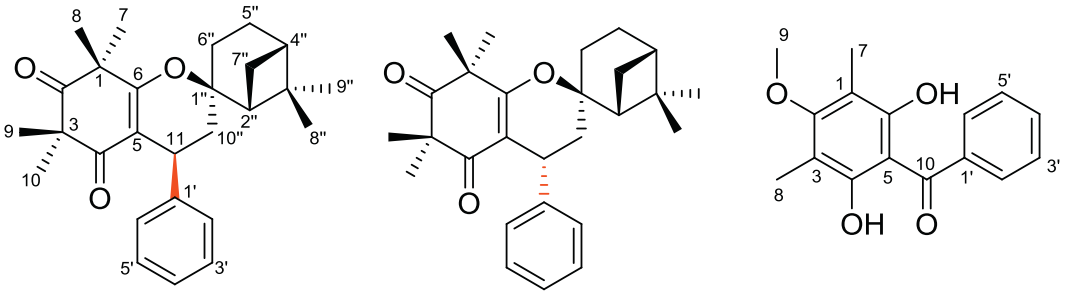

Fig. 1. Structures of compounds **1–3**.

(Shanghai Chemical Plant, Shanghai, China). Fractions were monitored by TLC using precoated silica gel GF_{254} plates (Qingdao Marine Chemical Inc., Qingdao, China), and spots were visualized by heating the silica gel plates sprayed with 5% H_2SO_4 in EtOH (5:95, *v*/v).

2.2. Plant material

L. brachyandrum leaf samples were collected from South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, Guangdong Province, China during June 2016. The voucher specimen (No# SCBG-NPL-160010) was identified by Dr. Fa-Guo Wang and has been deposited at the Laboratory of Natural Product Chemistry Biology, SCBG.

2.3. Extraction and isolation

The air-dried and powdered leaves of L. *brachyandrum* (10 kg) were successively extracted at room temperature with 95% EtOH (3 × 20 L, each 24 h). After removal of the pooled solvents by evaporation under vacuum, a crude extract (0.5 Kg) was obtained. This material was suspended in H_2O and successively partitioned with *n*-hexane (3 L × 3) and EtOAc (3 L × 3) to yield two corresponding portions. The *n*-hexane-soluble portion (109.5 g) was fractionated by silica gel CC eluting with a mixture of *n*-hexane/EtOAc (100:0, 50:1, 20:1, 8:1, 4:1, 1:1 *v*/v) to afford six fractions (A-F). Fraction B (8 g) (*n*-hexane/EtOAc, 50:1, v/v) was further chromatographed over RP-C_{18} silica gel (MeOH/H_2O, 85:15 → 100:0, *v*/v) to obtain sub-fractions B_1 and B_2. Sub-fraction B_1 (800 mg) was separated using a silica gel chromatography (*n*-hexane/EtOAc, 20:1 → 5:1, *v*/v) to afford compound **3** (5.1 mg).

The EtOAc portion (330 g) was fractionated by silica gel CC eluting with a mixture of *n*-hexane/EtOAc (100:0, 50:1, 20:1, 8:1, 4:1, 1:1, 0:1 *v*/v) to afford seven fractions (A-G). Fraction A (1.0 g) (*n*-hexane/EtOAc, 10:1, *v*/v) was further chromatographed over RP-C_{18} silica gel (MeOH/H_2O, 70:30 → 100:0, v/v) to obtain six sub-fractions A_1-A_6. Sub-fraction A_2 (150 mg) was further purified by semiprep-HPLC (MeOH/H_2O, 90:10, *v*/v, 3 mL/min) to afford compound **2** (9.0 mg, t_R = 20.9 min). Sub-fraction A_3 (80 mg) was further purified by semi-prep-HPLC (ACN/H_2O, 90:10, v/v, 3 mL/min) to afford compound **2** (5.5 mg, t_R = 25.2 min).

2.3.1. Brachyanin A (1)

colorless needles; $[\alpha]_D^{20}$ − 2.5 (*c* 2.2, MeOH); UV (MeOH) λ_{max} (log ε) 206 (4.41), 266 (4.54) nm; IR ν_{max} 2974, 2926, 1715, 1653, 1614, 1472, 1383, 1350, 1286, 1215, 1175, 1057, 969, 898, 853, 748, 700 cm^{-1}; ^1H (500 MHz) and ^{13}C (125 MHz) NMR data, see Table 1; positive ESIMS: *m/z* 407 $[M + H]^+$; HRESIMS: *m/z* 407.2584 $[M + H]^+$ (calcd for $C_{27}H_{35}O_3$, 407.2581).

2.3.2. Brachyanin B (2)

white powder; $[\alpha]_D^{20}$ − 29.2 (*c* 0.9, MeOH); UV (MeOH) λ_{max} (log ε) 204 (5.11), 266 (5.17) nm; IR ν_{max} 2976, 2928, 1717, 1651, 1456, 1381, 1348, 1169, 1055, 1005, 897, 858, 746, 700 cm^{-1}; ^1H (500 MHz) and ^{13}C (125 MHz) NMR data, see Table 1; positive ESIMS: *m/z* 429 $[M + Na]^+$; HRESIMS: *m/z* 429.2395 $[M + Na]^+$ (calcd for $C_{27}H_{34}NaO_3$, 429.2400).

Table 1
^1H (500 MHz) and ^{13}C (125 MHz) NMR Data of **1** and **2** in $CDCl_3$.

Position	1		2	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1		48.2, C		48.3, C
2		213.5, C		213.6, C
3		55.4, C		55.2, C
4		196.9, C		197.0, C
5		111.1, C		110.5, C
6		170.6, C		171.5, C
7	1.49, (s)	24.9, CH_3	1.52, (s)	24.6, CH_3
8	1.40, (s)	25.4, CH_3	1.34, (s)	26.0, CH_3
9	1.24, (s)	26.0, CH_3	1.20, (s)	25.7, CH_3
10	1.27, (s)	22.3, CH_3	1.25, (s)	22.6, CH_3
11	3.89, dd (10.5, 6.5)	34.7, CH	3.78, dd (11.5, 6.5)	36.0, CH
1'		144.6, C		144.7, C
2'	7.15, dd (7.7, 1.5)	126.9, CH	7.15, d (7.5, 1.6)	126.8, CH
3'	7.25, t (7.7)	128.4, CH	7.25, t (7.5)	128.4, CH
4'	7.17, t (7.7)	126.1, CH	7.17, t (7.5)	126.2, CH
5'	7.25, t (7.7)	128.4, CH	7.25, t (7.5)	128.4, CH
6'	7.15, dd (8.5, 1.5))	126.9, CH	7.15, dd (7.5, 1.6)	126.8, CH
1"		85.1, C		85.6, C
2"	2.05, dd (6.0, 5.0)	51.6, CH	2.30, dd (6.5, 5.0)	46.5, CH
3"		38.1, C		38.3, C
4"	1.97, overlapped	40.4, CH	1.99, overlapped	40.6, CH
5"	1.97, overlapped 1.94, m	24.9, CH_2	1.99, overlapped 1.84, m	24.6, CH_2
6"	2.01, m 1.84, m	27.8, CH_2	2.13, m 1.78, m	30.0, CH_2
7"	2.27, overlapped 1.64, d (10.0)	27.2, CH_2	2.18, m 1.55, d (10.5)	26.1, CH_2
8"	0.91, (s)	23.3, CH_3	0.97, (s)	23.2, CH_3
9"	1.14, (s)	27.2, CH_3	1.30, (s)	27.6, CH_3
10"	2.27, overlapped 1.78, dd (14.0, 10.5)	44.2, CH_2	2.43, dd (14.0, 6.5) 1.99, dd (14.0, 10.5)	43.8, CH_2

2.3.3. Brachyanin C (3)

yellow powder; UV (MeOH) λ_{max} (log ε) 292 (3.15) nm; IR ν_{max} 2922, 2850, 1716, 1597, 1448, 1325, 1273, 1117, 1076, 1022, 895, 767, 694, 657 cm^{-1}; ^1H (500 MHz) and ^{13}C (125 MHz) NMR data, see Table 2; positive ESIMS: *m/z* 273 $[M + H]^+$; HRESIMS: *m/z* 273.1121 $[M + H]^+$ (calcd for $C_{16}H_{17}O_3$, 273.1121).

2.4. X-Ray crystallographic data for brachyanin A (1)

$C_{27}H_{34}O_3$, *M* = 406.54, monoclinic, space group $P2_12_12_1$, *a* = 6.02920(10) Å, *b* = 16.9817(3) Å, *c* = 11.2850(2) (5) Å, $\alpha = \gamma = 90.00^\circ$, β = 102.928(2), *V* = 1126.14(3) $Å^3$, *T* = 100 K, *Z* = 2,