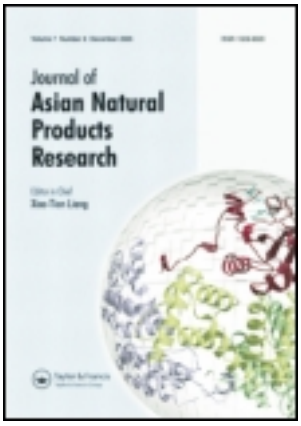

Journal of Asian Natural Products Research

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ganp20

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Available online: 10 Aug 2011

To cite this article: Zhen Zhang, Xin Fang, Yue-Hu Wang, Guang-Ming Liu, Huai Xiao, Xiao-Jiang Hao & Hong-Ping He (2011): Puberosides C-E, triterpenoid saponins from Glochidion puberum , Journal of Asian Natural Products Research, 13:9, 838-844

To link to this article: http://dx.doi.org/10.1080/10286020.2011.598148

Journal of Asian Natural Products Research
Vol. 13, No. 9, September 2011, 838–844

Puberosides C–E, triterpenoid saponins from *Glochidion puberum*

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(*Received 21 March 2011; final version received 14 June 2011*)

Three new triterpenoid glycosides, named puberosides C–E (**1**–**3**), were isolated from the water-soluble fraction of *Glochidion puberum* (Linn.) Hutch. Their structures were determined as 3α-[(*O*-β-D-glucopyranosyl-(1 → 3)-*O*-α-L-arabinopyranosyl)oxy]-22α-*trans*-cinnamoyl-olean-12-ene-16α,28-diol, 3α-[(*O*-β-D-glucopyranosyl-(1 → 3)-*O*-α-L-arabinopyranosyl)oxy]-22α-*cis*-cinnamoyl-olean-12-ene-16α,28-diol, and 3α-[(*O*-β-D-glucopyranosyl-(1 → 3)-*O*-β-D-glucopyranosyl)oxy]-22α-benzoyloxy-olean-12-ene-16α,28-diol by the combination of 1D, 2D NMR, and MS spectral analyses.

Keywords: *Glochidion puberum*; triterpenoid glycosides; puberoside C; puberoside D; puberoside E

1. Introduction

The genus *Glochidion* of the family Euphorbiaceae comprises approximately 300 species that are mainly distributed in tropical Asia. *G. puberum* (Linn.) Hutch, a shrub belonging to the genus *Glochidion*, is widely distributed in China [1]. The roots, stems, leaves, and fruits of *G. puberum* are used in traditional Chinese medicine to treat dysentery, diarrhea, influenza, fever, cough, impaludism, rheumatoid arthritis, and dyspepsia. It was reported that some triterpenes from *Glochidion* had been found to possess antitumor-promoting and cytotoxic activities [2,3]. Previous studies also revealed that the EtOH extracts of genus *Glochidion* were shown to exhibit significant DPPH-radical-scavenging activity [4]. To find potentially bioactive secondary metabolites from this genus, we investigated the chemical constituents of this species, which led to the isolation of three triterpenoid glycosides, puberosides C–E (**1**–**3**) (see Figure 1). This paper deals with the isolation and structural determination of these compounds from the dried aerial parts of *G. puberum*.

2. Results and discussion

Puberoside C (**1**) was isolated as a white powder. Its molecular formula was established as $C_{50}H_{74}O_{14}$ by negative HR-ESI-MS (*m/z* 933.4750 $[M + Cl]^-$, calcd 933.4767). The IR absorption spectrum showed the presence of hydroxyl group (3423 cm^{-1}), carbonyl group (1705 cm^{-1}), and aromatic ring (1580 and 1451 cm^{-1}). Comparison of the ^1H and ^{13}C NMR spectral data of **1** with those of puberoside

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ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020.2011.598148
http://www.informaworld.com

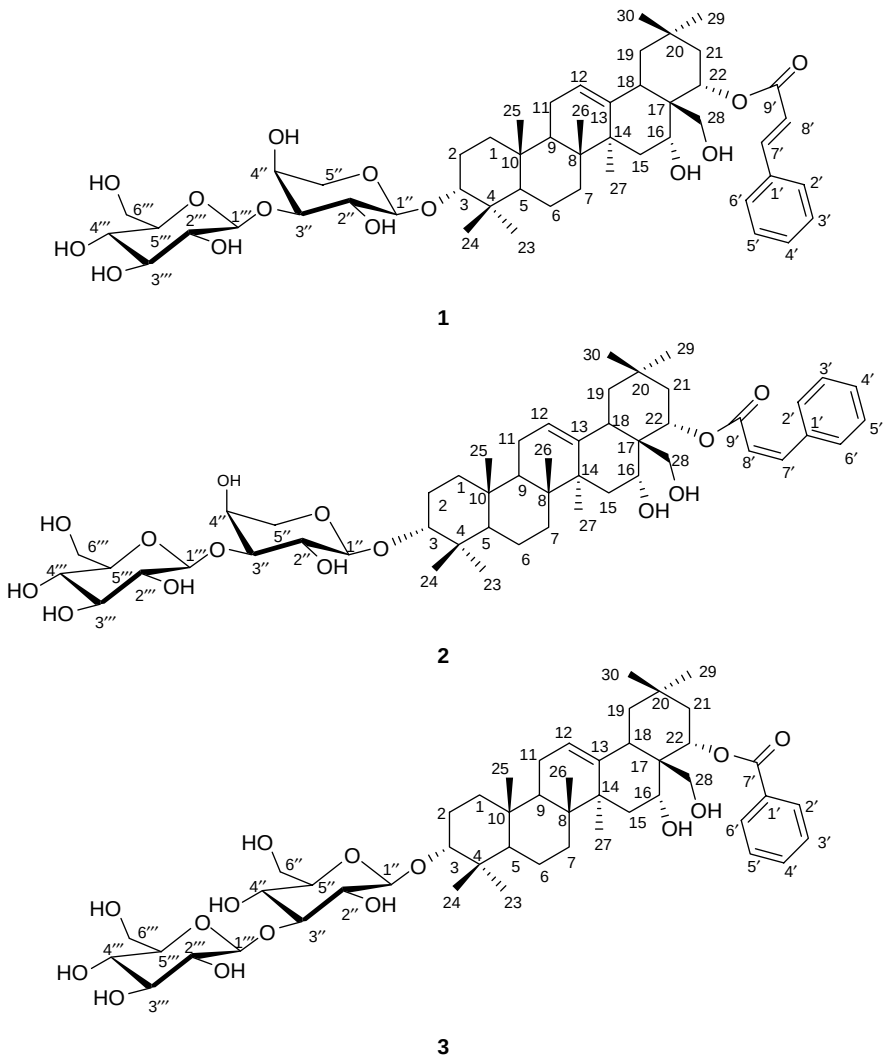

Figure 1. Structures of compounds **1**–**3**.

A [5] indicated that both compounds have two monosaccharides and a substituted triterpenoid moiety. The similarity of chemical shifts of two monosaccharides in the two compounds suggested that both compounds have the same sugar moiety, as further confirmed by 2D NMR studies. Though comparison with known compound puberoside A implied that **1** might have a structure similar to that of puberoside A, **1** had more than one degree of unsaturation than puberoside A, according to its molecular formula. Analysis of DEPT and HSQC spectra of **1** also showed that **1** had two additional methines compared with puberoside A. Moreover, analysis of the HMBC spectrum of **1** showed that **1** had a cinnamic substituted triterpenoid moiety. The *trans*-isomer of cinnamoyl in **1** was reasonably deduced by comparing the coupling constant ($J = 16.0$ Hz) with that in the literature [6]. The HMBC correlations of H-22/C-16, C-18, C-20, and C-9′, together with H-7′/C-2′, C-6′, C-9′ and

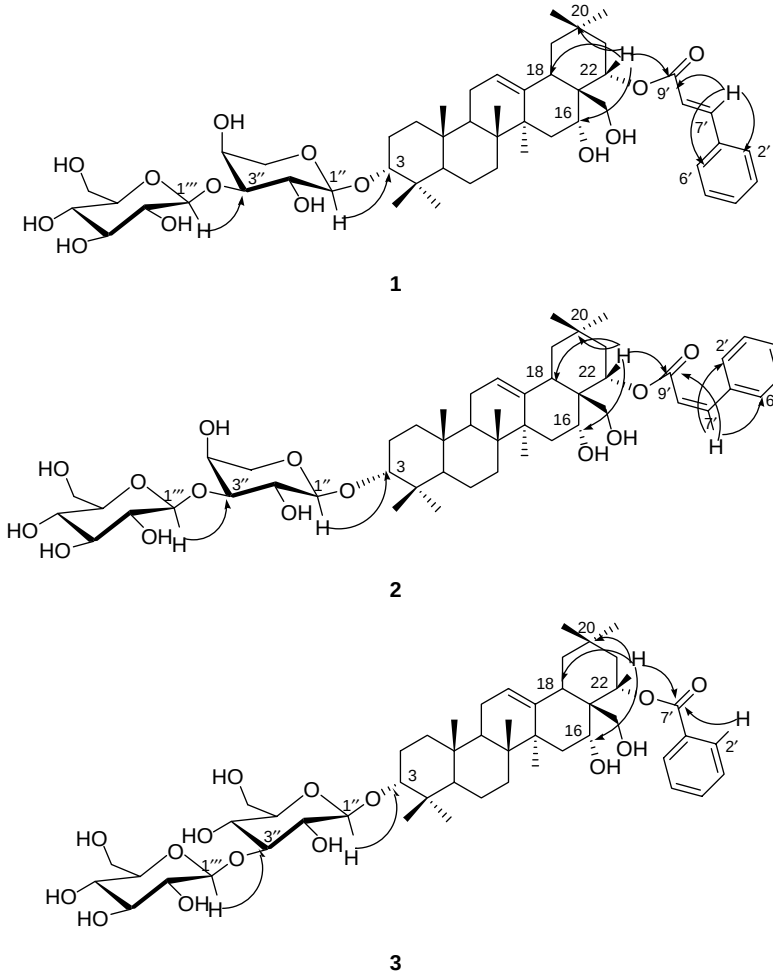

Figure 2. Key HMBC correlations of **1**–**3**.

H-8′/C-1′, C-9′, showed that the triterpenoid moiety and cinnamoyl were linked through ester bond as shown in Figure 2.

The relative configuration of triterpenoid moiety in **1** was demonstrated by the ROESY spectrum. ROESY correlations of H-3/H-24 and H-16/H-28 suggested that hydroxyl groups at C-3 and C-16 were α-oriented. The correlations between H-16 and H-22 in the ROESY spectrum revealed H-22 to be β-configuration, which indicated α-configuration of hydroxyl group at C-22. Thus, the structure of **1** was *established* as 3α-[(*O*-β-D-glucopyranosyl-(1 → 3)-*O*-α-L-arabinopyranosyl)oxy]-22α-*trans*-cinnamoyl-olean-12-ene-16α,28-diol.

Puberoside D (**2**) was obtained as a white powder, and its molecular formula, established as $C_{50}H_{74}O_{14}$, was determined by the $[M + Cl]^-$ ion peak at *m/z* 933.4766 (calcd 933.4767) in the HR-ESI-MS. The IR absorption spectrum showed the presence of hydroxyl group (3418 cm^{-1}), carbonyl group (1720 cm^{-1}), and aromatic ring (1452, 771, and 698 cm^{-1}). The ^1H and ^{13}C NMR spectral data for **2** were similar to those of **1**, with the only

exception being that the coupling constant between H-7′ and H-8′ of **1** and **2** was not the same. The *cis*-isomer of alkene in **2** is reasonably deduced by comparing the coupling constant ($J = 12.9$ Hz) with that in the literature [7]. The relative configuration of **2** was suggested to be the same as that of **1**, on the basis of ROESY data. Thus, the structure of **2** was determined as 3α-[(*O*-β-D-glucopyranosyl-(1 → 3)-*O*-α-L-arabinopyranosyl)oxy]-22α-*cis*-cinnamoyl-olean-12-ene-16α,28-diol.

The molecular formula $C_{49}H_{74}O_{15}$ was assigned to puberoside E (**3**) from its HR-ESI-MS peak at *m/z* 937.4702 $[M + Cl]^-$. The IR spectrum showed strong absorption bands at 3419, 1700, 1603, 1585, and 1452 cm^{-1}, suggesting the presence of hydroxyl, carbonyl groups, and aromatic ring, respectively. Comparison of ^1H and ^{13}C NMR spectral data of **3** with those of puberoside A [5] suggested that their structures are closely similar. The only difference between them was that the sugar moieties of **3** possessed one more CH_2O unit when compared with that of puberoside A [5]. Analysis of ^{13}C and DEPT NMR spectra indicated that two methylenes (δ_C 62.7 and 62.6) could be found in **3**, whereas only one methylene (δ_C 62.3) could be found in puberoside A. Furthermore, the ^1H and ^{13}C NMR spectra of **3** (Table 1) supported the presence of two glucopyranose moieties. The β-configuration of anomeric protons of two glucopyranoses was determined by the coupling constants (7.9 and 7.7 Hz) of anomeric protons [8]. The HMBC correlation between H-1‴ and C-3″ confirmed the 1 → 3 linkage of the two sugars. The linkage of glycosidation at C-3 was confirmed by an HMBC experiment, which showed a long-range correlation between C-3 (δ 90.7) and the anomeric proton H-1″ (δ 4.39) of glucose. The relative configuration of **3** was suggested to be the same as that of **1** by the ROESY spectrum. Thus, the structure of **3** was elucidated as 3α-[(*O*-β-D-glucopyranosyl-(1 → 3)-*O*-β-D-glucopyranosyl)oxy]-22α-benzoyloxy-olean-12-ene-16α,28-diol.

3. Experimental

3.1 General experimental procedures

Optical rotations were recorded on a Horiba SEPA-300 high-sensitive polarimeter. IR spectra were measured on a Bio-Rad FTS-135 spectrometer with KBr pellets. UV spectra were measured on a Shimadzu 2401 PC. NMR spectra were obtained on a Bruker AM-400 or DPX-500 NMR spectrometer using TMS as an internal standard. ESI-MS and HR-ESI-MS spectra were recorded using a VG Auto Spec-3000 spectrometer. Column chromatography was carried out on MCI GEL CHP20P (Mitsubishi Chemical Corporation, Tokyo, Japan) and silica gel (200–300 mesh; Qingdao Marine Chemical Plant, Qingdao, China); semipreparative HPLC was carried out using an Agilent 1100 liquid chromatograph equipped with a Zorbax SB-C_{18} column (10 μm, i.d. 9.4 × 250 mm, Agilent Co., Ltd, Wilmington, DE, USA), eluted with a liner gradient of MeOH:H_2O (10–30% MeOH, 17 min; flow rate, 2.0 ml/min; detection, UV 254 nm) at 30°C. TLC was carried out with glass precoated with silica gel GF254.

3.2 Plant material

The aerial parts of *G. puberum* were collected in Xishuangbanna, Yunnan Province of China, in March 2004. The plant was identified by Prof. De-Ding Tao (Kunming Institute of Botany, Chinese Academy of Sciences), and a voucher specimen (Kun No. 0615419) is deposited at the State Key Laboratory of Phytochemistry and Plant Resources in west China, Kunming Institute of Botany, Chinese Academy of Sciences.

3.3 Extraction and isolation

The dried aerial parts of *G. puberum* (6 kg) were extracted with hot 95% EtOH. After

Table 1. ^{1}H (400 Hz) and ^{13}C (100 Hz) NMR spectral data of compounds **1**–**3** (CD_3OD, ppm, J in Hz).

	1		**2**		**3**	
	δ_C	δ_H (J in Hz)	δ_C	δ_H[a]	δ_C	δ_H
1	39.9 (t)	1.77 (m)	39.9 (t)	1.89 (m)	38.3 (t)	1.89 (m)
		0.99 (m)		0.98 (m)		0.99 (br s)
2	27.2 (t)	1.89 (m)	27.3 (t)	1.83 (m)	27.5 (t)	1.89 (m)
		1.73 (m)		1.69 (m)		1.77 (m)
3	89.1 (d)	3.16 (dd, J = 9.2, 3.6)	90.4 (d)	3.17 (dd, J = 9.6, 3.2)	90.7 (d)	3.21 (dd, J = 9.6, 3.6)
4	39.9 (s)		40.3 (s)		40.2 (s)	
5	55.7 (d)	0.81 (br s)	56.9 (d)	0.84 (br s)	56.9 (d)	0.82 (br s)
6	18.0 (t)	1.62 (m)	19.3 (t)	1.65 (m)	19.3 (t)	1.60 (m)
		1.59 (m)		1.50 (m)		1.51 (m)
7	33.0 (t)	1.41 (m)	34.3 (t)	1.40 (m)	33.6 (t)	1.42 (m)
		1.64 (m)		1.67 (m)	33.6 (t)	1.65 (m)
8	42.0 (s)		41.2 (s)		39.8 (s)	
9	48.1 (d)	1.59 (m)	48.1 (d)	1.58 (m)	48.2 (d)	1.64 (m)
10	36.4 (s)		37.7 (s)		37.6 (s)	
11	23.4 (t)	1.42 (m)	24.6 (t)	1.61 (m)	24.7 (t)	1.98 (m)
				1.42 (m)		
12	122.9 (d)	5.32 (br s)	124.1 (d)	5.32 (br s)	124.3 (d)	5.36 (br s)
13	142.2 (s)		136.4 (s)		143.5 (s)	
14	43.3 (s)		44.2 (s)		44.2 (s)	
15	36.0 (t)	1.97 (m)	37.6 (t)	1.99 (m)	37.6 (t)	1.99 (m)
		1.54 (m)		1.51 (m)		1.50 (m)
16	69.9 (d)	4.27(br s)	69.4 (d)	4.25 (br s)	69.4 (d)	4.30 (br s)
17	42.9 (s)		44.4 (s)		43.5 (s)	
18	41.9 (d)	2.41 (m)	43.1(d)	2.40 (br s)	43.4 (d)	2.45 (dd, J = 11.2, 3.6)
19	45.9 (t)	1.84 (m)	47.1 (t)	1.86 (m)	47.2 (t)	1.94 (m)
		1.18 (m)		1.18 (br s)		1.24 (m)
20	29.8 (s)		31.1 (s)		31.0 (s)	
21	39.9 (t)	1.57 (m)	37.7 (t)	1.50 (m)	39.9 (t)	1.95 (m)
		1.01 (br s)		1.00 (m)		1.02 (m)
22	70.8 (d)	5.77 (br s)	72.1 (d)	5.77 (br s)	72.1 (d)	5.91 (br s)
23	29.8 (q)	1.06 (3H, s)	28.5 (q)	1.08 (3H, s)	28.5 (q)	1.07 (3H, s)
24	16.0 (q)	0.86 (3H, s)	17.0 (q)	0.85 (3H, s)	17.0 (q)	0.87 (3H, s)

Table 1 – *continued*

	1		**2**		**3**	
	δ_C	δ_H (J in Hz)	δ_C	δ_H[a]	δ_C	δ_H
25	14.9 (q)	0.98 (3H, s)	16.1 (q)	0.98 (3H, s)	16.2 (q)	0.99 (3H, s)
26	15.7 (q)	1.05 (3H, s)	17.3 (q)	1.05 (3H, s)	17.3 (q)	1.07 (3H, s)
27	27.2 (q)	1.28 (3H, s)	27.9 (q)	1.32 (3H, s)	27.9 (q)	1.30 (3H, s)
28	65.3 (t)	3.54 (m)	64.4 (t)	3.54 (br s)	64.7 (t)	3.67 (br s)
		3.85 (m)		3.89 (d, $J = 11.0$)		4.02 (d, $J = 8.8$)
29	33.0 (q)	0.93 (3H, s)	33.6 (q)	0.93 (3H, s)	34.3 (q)	0.94 (3H, s)
30	27.2 (q)	1.06 (3H, s)	27.3 (q)	1.04 (3H, s)	27.5 (q)	1.03 (3H, s)
1′	134.5 (s)		136.4 (s)		132.2 (s)	
2′, 6′	127.9 (d)	7.61 (m)	130.9 (d)	7.68 (br s)	130.5 (d)	8.05 (d, $J = 7.3$)
3′, 5′	128.8 (d)	7.41 (m)	129.1 (d)	7.39 (br s)	129.6 (d)	7.49 (dd, $J = 7.3, 7.6$)
4′	130.2 (d)	7.41 (m)	130.0 (d)	7.39 (br s)	134.1 (d)	7.60 (dd, $J = 7.3, 7.6$)
7′	144.7 (d)	7.67 (d, $J = 16.0$)	143.9 (d)	7.05 (d, $J = 12.7$)	167.2 (s)	
8′	118.4 (d)	6.66 (d, $J = 16.0$)	121.2 (d)	6.06 (d, $J = 12.7$)		
9′	166.7 (s)		167.3 (s)			
1″	105.8 (d)	4.35 (d, $J = 7.3$)	107.1 (d)	4.35 (d, $J = 7.7$)	106.3 (d)	4.39 (d, $J = 7.9$)
2″	74.1 (d)	3.30 (br s)	72.1 (d)	3.35 (m)	75.4 (d)	3.37 (br s)
3″	82.6 (d)	3.63 (m)	83.8 (d)	3.64 (m)	88.1 (d)	3.55 (br s)
4″	69.9 (d)	4.02 (br s)	69.5 (d)	4.03 (br s)	71.5 (d)	3.27 (m)
5″	65.4 (t)	3.82 (m)	66.7 (t)	3.87 (m)	78.2 (d)	3.42 (br s)
		3.52 (m)		3.53 (m)		
6″					62.7 (t)	3.89 (m)
						3.69 (m)
1‴	104.1 (d)	4.55 (d, $J = 7.6$)	105.4 (d)	4.61 (d, $J = 7.5$)	105.3 (d)	4.58 (d, $J = 7.7$)
2‴	74.1 (d)	3.35 (br s)	75.3 (d)	3.30 (br s)	75 (d)	3.37 (br s)
3‴	77.4 (d)	3.31 (br s)	77.7 (d)	3.28 (br s)	75.5 (d)	3.31 (br s)
4‴	70.8 (d)	3.33 (m)	71.2 (d)	3.31 (m)	70.4 (d)	3.31 (m)
5‴	77.6 (d)	3.29 (br s)	77.9 (d)	3.27 (m)	77.8 (d)	3.33 (br s)
6‴	61.1 (t)	3.83 (m)	62.4 (t)	3.80 (m)	62.6 (t)	3.86 (m)
		3.68 (m)		3.62 (m)		3.70 (m)

Note: [a] Recorded at 500 MHz.

the removal of EtOH *in vacuo*, the visco extract was suspended in H_2O, and then partitioned with petroleum ether and EtOAc successively. The water-soluble fraction (78 g) was subjected to MCI GEL, eluting stepwise with MeOH:H_2O (from 3:7 to 1:0), to give four parts. The second part (17 g) was further subjected to a silica gel column, eluted with $CHCl_3$: MeOH (from 9:1 to 7:3), to give three fractions (I–III). Then fraction II (0.13 g) was finally purified by HPLC (see Section 3.1) to afford compounds **1** (7 mg), **2** (5 mg), and **3** (9 mg), respectively.

3.3.1 *Puberoside C (**1**)*

A white powder. $[\alpha]_D^{26.0}$ + 14.29 (c = 0.14, CH_3OH). UV (CH_3OH) λ_{max} (log ε) 388 (2.51), 277 (4.84), 222 (4.74), 216 (4.79), 205 (4.85), and 192 (4.64) nm. IR (KBr) ν_{max}: 3423, 1705, 1580, 1451 cm^{-1}. For ^1H and ^{13}C NMR spectral data see Table 1. HR-ESI-MS: *m/z* 933.4750 $[M + Cl]^-$ (calcd for $C_{50}H_{74}O_{14}Cl$, 933.4767).

3.3.2 *Puberoside D (**2**)*

A white powder. $[\alpha]_D^{25.9}$ + 50.00 (c = 0.06, CH_3OH). UV (CH_3OH) λ_{max} (log ε) 394 (2.61), 271 (4.57), and 204 (4.93) nm. IR (KBr) ν_{max}: 3418, 1720, 1452, 771, 698 cm^{-1}. For ^1H and ^{13}C NMR spectral data, see Table 1. HR-ESI-MS: *m/z* 933.4766 $[M + Cl]^-$ (calcd for $C_{50}H_{74}O_{14}Cl$, 933.4767).

3.3.3 *Puberoside E (**3**)*

A white powder. $[\alpha]_D^{25.9}$ + 18.57 (c = 0.35, CH_3OH). UV (CH_3OH) λ_{max} (log ε) 272 (3.85), 227 (4.76), 202 (4.88), and 194 (4.52) nm. IR (KBr) ν_{max}: 3419, 1700, 1603, 1585, 1452 cm^{-1}. For ^1H and ^{13}C NMR spectral data, see Table 1. HR-ESI-MS: *m/z* 937.4702 $[M + Cl]^-$ (calcd for $C_{49}H_{74}O_{15}Cl$, 937.4716).

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