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Rearranged clerodane diterpenes from *Tinospora baenzigeri*

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Abstract

Baenzigeride A, a new rearranged clerodane diterpene and baenzigeride A glucoside, baenzigeroside A, have been isolated from the stems of *Tinospora baenzigeri*. The structures were established on the basis of the spectroscopic data. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Tinospora baenzigeri*; Menispermaceae; Clerodane diterpene

1. Introduction

Species of the genus *Tinospora* are widely employed as medicinal plants throughout a large part of Asia and Africa (Maurya, Wazir & Kapil, 1994). The stems of *T. baenzigeri* (Thai name: chingcha chalee) are used as anti-pyretic and anti-malarial in Thailand. The extracts of the roots, leaves and stems of *T. baenzigeri* have been shown to have good in vitro anti-malarial activity (Meeting on Medicinal Plants and Malaria, 1989). Previous studies on other species of *Tinospora* have revealed the presence of several clerodane diterpenes, clerodane diterpene glucosides, steroids, flavonoids, lignans and alkaloids (Maurya Wazir & Kapil, 1994). Chemical investigations of the stems of *T. baenzigeri* have indicated the presence of several isoquinoline alkaloids (Bisset & Nwaiwa, 1983; Pachaly & Schneider, 1981). We now report the isolation and structural determination of novel clerodane-derived diterpenes, baenzigeride A (**1**) and baenzigeroside A (**2**). Baenzigeride A was isolated from the chloroform extract of the stem of *T. baenzigeri* and the glucoside **2** was obtained from the *n*-butanol-soluble fraction of the ethanolic extract of the stems of *T. baenzigeri*.

2. Results and discussion

Baenzigeride A (**1**) was obtained as colorless rhombs, $C_{20}H_{22}O_6$, $[\alpha]_D^{25} + 23.79°$. The UV spectrum showed a band at 282 nm. Compound **1** had IR bands at 3500, 1750, 1740 and 1660 (weak) cm^{-1} indicating the presence of hydroxyl, γ-lactone, δ-lactone and olefinic groups, respectively.

In the ^{1}H-NMR spectrum of **1** (Table 1), the signals at δ7.47 (*dd*, J = 0.8, 1.8 Hz, 1H), 7.43 (*t*, J = 1.8 Hz, 1H) and 6.42 (*dd*, J = 0.8, 1.8 Hz, 1H) were assigned to two α-(H-16 and H-15) and a β-proton (H-14), respectively, of a β-substituted furan ring. This was consistent with the ^{13}C-NMR of **1** which contained resonances from three olefinic methine carbons (δ 143.4, 139.4 and 108.4) and an olefinic quarternary carbon (δ 123.7) of the furan moiety. An olefinic proton signal appeared as a doublet of doublets at δ 6.67 (J = 2.2, 6.6 Hz) and two methylene protons resonated at δ 2.17, doublet of doublet of doublets (J = 1.2, 6.6, 18.0 Hz) and 2.41, doublet of doublet (J = 2.2, 18.0 Hz); these were assigned to an ABX system of H-7 and HaHb-6, respectively. This was supported by the presence of signals from an olefinic quarternary carbon (C-8) (δ 134.0), an olefinic methine carbon (C-7) (δ 133.5) and a methylene carbon (C-6) (δ 30.0) in the ^{13}C-NMR spectrum. A second ABX system at δ

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Table 1
^{1}H-NMR spectral data of baenzigeride A (**1**) and baenzigeride A acetate (**1a**) in $CDCl_3$

Proton	**1**	COSY	NOED	**1a**
H_β-1	2.06, *ddd* (13.2, 11.8, 10.2)	H_α-1, H-2, H-10	H_α-1, H_β-6, CH_3-20	2.07, *ddd* (8.8, 13.0, 13.0)
H_α-1	1.71, *ddd* (2.4, 7.0, 13.2)	H_β-1, H-2, H-10	H_β-1, H_α-3, H-10 CH_3-20	1.80, *dd* (6.0, 13.0)
H-2	2.78, *ddt* (10.2, 7.8, 2.4)	$H_\alpha H_\beta$-1, $H_\alpha H_\beta$-3	H_β-1, H_β-3, H_β-6	2.81, *dt* (2.2, 8.8)
H_β-3	4.58, *dd* (7.8, 9.4)	H-2, H_α-3	H-2, H_α-3	4.85, *t* (8.8)
H_α-3	4.00, *dd* (2.4, 9.4)	H-2, H_β-3	H_α-1, H_β-3, H-10	4.04, *dd* (2.2, 8.8)
H_β-6	2.41, *dd* (2.2, 18.0)	H-2, H_α-6, H-7	H-2, H_α-6, H-7	2.35, *dd* (2.6, 19.0)
H_α-6	2.17, *ddd* (1.2, 6.6, 18.0)	H_β-6, H-7, H-10		2.28 *ddd* (1.0, 6.0, 19.0)
H-7	6.67, *dd* (2.2, 6.6)	$H_\alpha H_\beta$-6	$H_\alpha H_\beta$-6	6.58, *dd* (2.6, 6.0)
H-10	1.89, *ddd* (1.2, 7.0, 11.8)	$H_\alpha H_\beta$-1, H_α-6	H_α-3, H_α-11, H-12 CH_3-19, CH_3-20	1.91, *dd* (6.0, 13.0)
H_α-11	2.30, *dd* (5.3, 14.0)	H_β-11	H-10, H_β-11, H-12, CH_3-19	2.33, *dd* (6.0, 14.0)
H_β-11	1.98, *dd* (11.3, 14.0)	H_α-11		2.03, *dd* (11.2, 14.0)
H-12	5.12, *dd* (5.3, 11.3)	$H_\alpha H_\beta$-11	H_α11, H-16, CH_3-19	5.14, *dd* (6.0, 11.2)
H-14	6.42, *dd* (0.8, 1.8)	H-15, H-16		6.42, *dd* (0.9, 1.9)
H-15	7.43, *t* (1.8)	H-14, H-16		7.43, *t* (1.9)
H-16	7.47, *dd* (0.8, 1.8)	H-14, H-15		7.46, *dd* (0.9, 1.9)
CH_3-19	1.27, *s*		H_α-6, H-7, H-10, H_α-11, H-12	1.30, *s*
CH_3-20	1.17, *s*		$H_\alpha H_\beta$-1, H_β-6, H-10, H_β-11	1.20, *s*
OAc				2.18, *s*

5.12 (*dd*, J = 5.3, 11.3 Hz), 2.30 (*dd*, J = 5.3 and 14.0 Hz) and 1.98 (*dd*, J = 11.3, 14.0 Hz) was assigned to H-12 and HaHb-11, respectively, with H-12 being axial. A COSY experiment was useful in the assignment of HaHb-3, H-2, HaHb-1 and H-10. The COSY cross-peaks are shown in Table 1. Two doublet of doublets at δ 4.58 (J = 7.8, 9.4 Hz) and 4.00 (J = 2.4, 9.4 Hz) and a doublet of doublet of triplets (*ddt*) at δ 2.78 (J = 10.2, 7.8, 2.4 Hz) arose from HaHb-3 and H-2, respectively. The signals of HaHb-1 and H-10 appeared at δ 2.06 (*ddd*, J = 13.2, 11.8, 10.2 Hz), 1.71 (*ddd*, J = 2.4, 7.0, 13.2 Hz) and 1.89 (*ddd*, J = 1.2, 7.0, 11.8 Hz), respectively. Two singlets at δ 1.17 and 1.27 were the protons of two tertiary methyl groups, CH_3-20 and CH_3-19, respectively.

The ^{13}C-NMR spectrum was assigned by a combi-

Table 2
^{13}C-NMR data of baenzigeride A (**1**) and baenzigeride A acetate (**1a**) in $CDCl_3$

Carbon	**1**	HMBC	**1a**
1	33.3	H-2, HaHb-3, H-10	33.5
2	43.0	HaHb-1, HaHb-3	39.8
3	72.7	HaHb-1	74.9
4	85.6	HaHb-1, H-2, HaHb-3, Ha-6, H-10	89.4
5	46.7	CH_3-19, Hb-1, HaHb-6, H-7, H-10	46.6
6	30.0	CH_3-19, H-7, H-10	30.4
7	133.5	HaHb-6	131.4
8	134.0	CH_3-20, HaHb-6, H-7, H-10, HaHb-11	134.1
9	34.2	CH_3-20, Ha-1, H-7, H-10, HaHb-11, H-12	35.8
10	50.8	CH_3-19, CH_3-20, HaHb-1, H-2, 49.1 HaHb-6, HaHb-11	
11	43.4	CH_3-20, H-10, H-12	43.1
12	70.9	HaHb-11, H-14	71.5
13	123.7	HaHb-11, H-12, H-14, H-15	123.5
14	108.4	H-12, H-15	108.3
15	143.4	H-14, H-16	143.6
16	139.4	H-12, H-14, H-15	139.6
17	169.7	H-7, H-12, Ha-6	170.8
18	177.4	H-2, HaHb-3	173.3
19	21.2	HaHb-6, H-10	20.6
20	27.5	HaHb-11	28.4
*CH_3*CO			20.0
CH_3 *CO*			170.3

Table 3
^{1}H-NMR spectral data of baenzigeroside A (**2**) in $CDCl_3$/DMSO-d_6

Proton	**2**	COSY	NOED
H_β-1	2.10, *dt* (10.2, 12.6)	H_α-1, H-2, H-10	H_α-1, H_β-6, CH_3-20
H_α-1	1.73, *ddd* (1.8, 6.9, 12.6)	H_β-1, H-2, H-10	H_β-1, H_α-3, CH_3-20
H-2	3.08, *ddt* (10.2, 7.5, 1.8)	$H_\alpha H_\beta$-1, $H_\alpha H_\beta$-3	H_β-1, H_β-3, H_β-6
H_β-3	4.74, *dd* (7.5, 9.3)	H-2, H_α-3	H-2, H_α-3
H_α-3	4.03, *dd* (1.8, 9.3)	H-2, H_β-3	H_α-1, H_β-3
H_α-6	2.30, *ddd* (1.1, 6.2, 19.2)	H_β-6, H-7, H-10	H_β-6, H-7, CH_3-19
H_β-6	2.39, *dd* (2.4, 19.2)	H_α-6, H-7	H-2, H_α-6, H-7
H-7	6.57, *dd* (2.4, 6.2)	$H_\alpha H_\beta$-6	$H_\alpha H_\beta$-6
H-10	1.83, *ddd* (1.1, 6.9, 12.6)	$H_\alpha H_\beta$-1	H_α11, CH_3-19, CH_3-20
H_α-11	2.33, *dd* (5.7, 13.8)	H_β-11, H-12	H_β-11, H-12 CH_3-19
H_β-11	1.99, *dd* (11.2, 13.8)	H_α-11, H-12	H_α-11, CH_3-20
H-12	5.14, *dd* (5.7, 11.2)	$H_\alpha H_\beta$-11	H_α-11, H-16, CH_3-19
H-14	6.44, *dd* (0.9, 1.6)	H-15	H-15
H-15	7.44, *t* (1.6)	H-14	H-14
H-16	7.48, *dd* (0.9, 1.6)		
CH_3-19	1.30, *s*		H_α-6, H-7, H-10, H_α-11, H-12, H-1′
CH_3-20	1.15, *s*		$H_\alpha H_\beta$-1, H-10, H_β-11
H-1′	4.49, *d* (7.5)	H-2′	H-3′, H-5′
H-2′	3.32, *dd* (7.5, 8.0)	H-1′, H-3′	
H-3′	3.44, *t* (8.0)	H-2′, H-4′	
H-4′	3.42, *t* (8.0)	H-3′, H-4′	
H-5′	3.23, *ddd* (3.0, 5.1, 8.0)	H-4′, HaHb-6′	
Ha-6′	3.72, *dd* (5.1, 11.7)	Hb-6′, H-5′	
Hb-6′	3.82, *dd* (3.0, 11.7)	Ha-6′, H-5′	

Table 4
^{13}C-NMR chemical shifts of baenzigeroside A (**2**) in $CDCl_3$/DMSO-d_6

Carbon	**2**	HMBC
1	35.2	HaHb-3, H-10
2	41.3	HaHb-1, HaHb-3
3	74.4	HaHb-1
4	91.1	Hb-1, H-2, HaHb-3, H-10, CH_3-19, H-1′
5	47.9	Hb-1, HaHb-6, H-7, H-10, CH_3-19
6	30.7	H-7, H-10, CH_3-19
7	132.5	HaHb-6
8	134.2	HaHb-6, H-10, Hb-11, CH_3-20
9	34.0	Ha-1, H-7, H-10, HaHb-11, CH_3-20
10	49.5	HaHb-1, H-2, Hb-6, Ha-11, CH_3-19, CH_3-20
11	43.6	H-10, H-12, CH_3-20
12	71.4	Hb-11
13	124.1	Hb-11, H-12, H-14, H-15
14	108.7	H-12, H-15, H-16
15	143.7	H-14, H-16
16	139.7	H-12, H-14, H-15
17	170.2	H-7, H-12
18	174.4	H-2, Hb-3
19	20.6	HaHb-6, H-10
20	28.4	HaHb-11
1′	99.8	H-2′, H-3′, H-5′
2′	73.9	H-1′, H-3′, H-4′
3′	76.6	H-1′, H-2′, H-4′, H-5′
4′	70.4	H-3′, H-5′, HaHb-6′
5′	76.4	H-1′, H-4′, HaHb-6′
6′	62.0	H-4′, H-5′

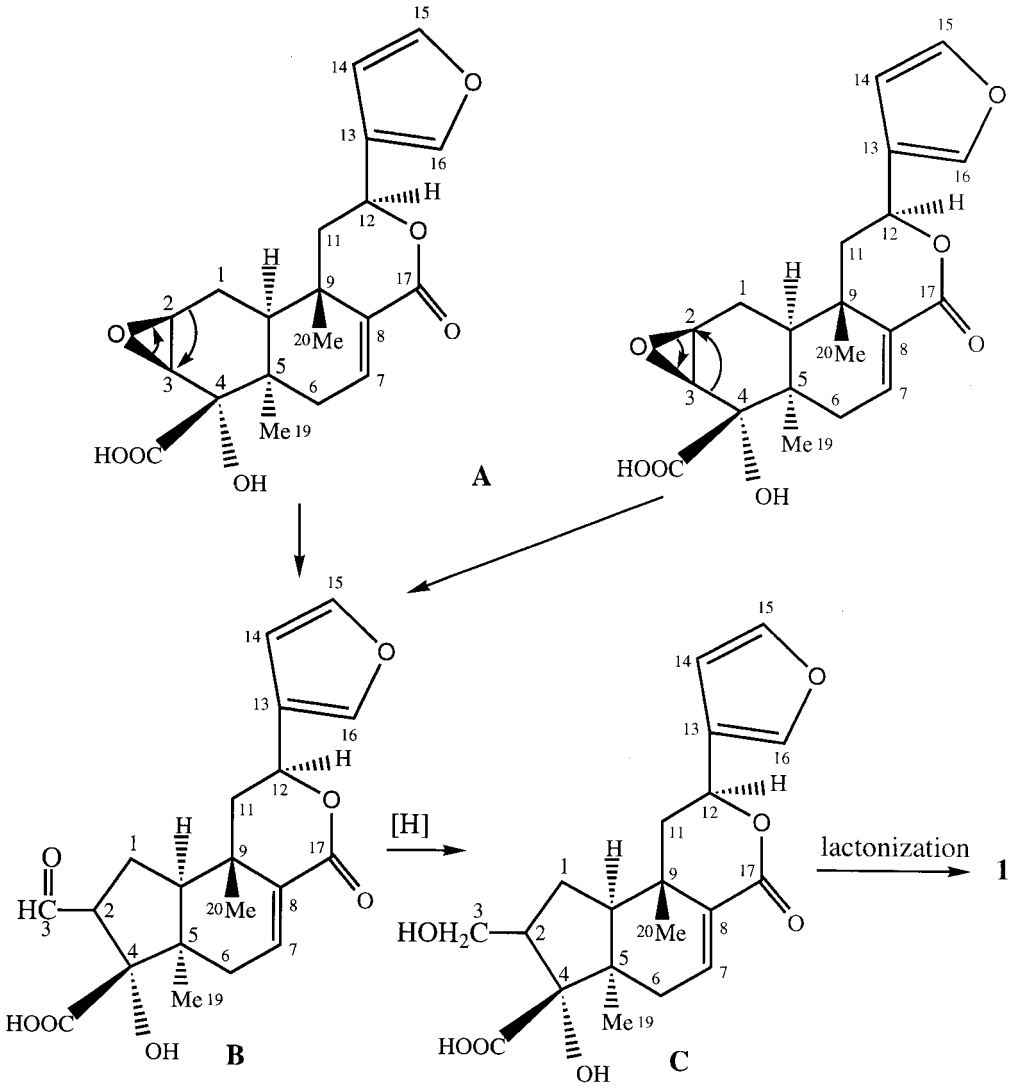
nation of DEPT, 2D HMQC and 2D HMBC experiments (Table 2).

The relative stereochemistry of **1** was derived from the ^{1}H NOED spectra. Important NOE's were observed between H-2 and H_β-1, H_β-3, H_β-6; H_α-3 and H_α-1; CH_3-20 and H_β-1, H_α-1, H_β-6, H-10, H_β-11; CH_3-19 and H_α-6, H-7, H-10, H_α-11, H-12. Thus H-10, H-12 and CH_3-19 are on the one side of the molecule and CH_3-20 and H-2 on the other side, with ring A/B *trans* and B/C *cis* fused as shown. This is consistent with the *cis-ent*-neoclerodane stereochemistry usually found in diterpenoids from *Tinospora* spp. (Maurya et al., 1994; Merrit & Ley, 1992). The NOE's observed from CH_3-19 and CH_3-20 can be explained if ring C adopts a flattened boat conformation, with CH_3-20 quasi-equatorial; ring D is a boat, with H_α-11 and H_β-11 quasi-axial and H-12 axial.

The α-configuration of the C_4-OH is assigned from the NOED observed between CH_3-19 and H-1′ in baenzigeroside A (see below).

Other NMR data for the acetate derivative (**1a**) (Tables 1 and 2) also supported the proposed structure.

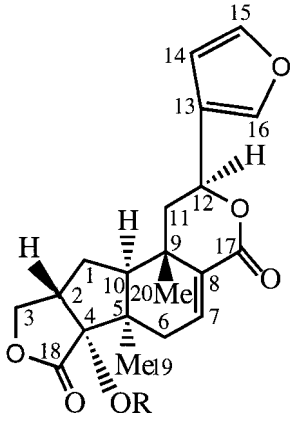
Baenzigeroside A (**2**) showed in its ^{1}H-NMR spectrum signals similar to those present in the spectrum of **1** and **1a**, with additional signals from the glucose moiety. In similar fashion, the spectrum was assigned by decoupling, COSY and NOED experiments (Table



Scheme 1. Possible biogenesis of baenzigeride A (**1**).

3). The ^{13}C-NMR spectrum was assigned by DEPT, 2D HMQC and 2D HMBC experiments (Table 4). The HMBC spectrum had correlations similar to those in the spectum of **1**. In addition, the glucose anomeric proton H-1′ had a ^{3}J correlation with C4 which confirmed the position of the glucosidic group. A NOED from CH_3-19 to H-1′ confirmed the relative stereochemistry shown in **2**.

The novel skeleton of baenzigeride A can arise from the *cis-ent*-neoclerodane epoxide (**A**) (Scheme 1). Rearrangement with ring-A contraction through migration of either the 1–2 or 3–4 bond can give the aldehyde (**B**). Reduction of **B** could give the primary alcohol (**C**) which could lactonize to **1**. It is worth noting that epoxides like **A** are not yet known to be naturally occurring; the known clerodane 2,3-epoxides, such as jateorin, also have a 1,18-lactone group



1 : R = H
1a : R = Ac
2 : R = Glc

(Merrit & Ley, 1992). Unfortunately baenzigeride A (**1**) (EC_{50} > 20 μg/ml) and its glucoside (**2**) (EC_{50} > 20 μg/ml) did not show anti-malarial activity in an in vitro screening against *Plasmodium falciparum*.

3. Experimental

Unless otherwise stated, microanalyses were carried out by the Scientific and Technological Research Equipment Center, Chulalongkorn University, Bangkok, Thailand. Mps: uncorr. UV: MeOH. IR spectra: Jasco A-302 Spectrophotometer. ^{1}H-NMR: $CDCl_3$ and DMSO-d_6, Bruker DRX 400 (400 MHz), TMS as international standard. ^{13}C-NMR: $CDCl_3$ and DMSO-d_6, 100.62 MHz, TMS as int. standard. MS: VG 7070 mass spectrometer operating at 70 eV or with VG Quattro triple quadrupole mass spectrometer for the electrospray mass spectrometer. Optical rotation: MeOH or CH_2Cl_2. TLC: precoated PF_{254} plate (Merck); spots were detected by spraying with 1% $CeSO_4$ in 10% aq. H_2SO_4 followed by heating. CC: silica gel 70–230 mesh (Merck). A voucher specimen (Bansiddhi 97435) of the plant material has been deposited at the Herbarium, the Division of Medicinal Plant Research and Development, Department of Medical Science, Nonthaburi 11000, Thailand.

3.1. Extraction and isolation

The dried and ground stems of *T. baenzigeri* (978 g) were extracted in a Soxhlet extractor with $CHCl_3$. The extract was evaporated in vacuo to give a brown viscous product (33.5 g). A portion (6.7 g) of the product was fractionated by flash column chromatography on silica gel with an increasing amount of CH_2Cl_2 in light petroleum, CH_2Cl_2 and an increasing amount of MeOH in CH_2Cl_2 as the eluent. Fractions of similar behaviour on TLC were combined to give baenzigeride A (**1**) as a slightly green solid (1.47 g).

Milled fresh stems of *T. baenzigeri* (13.2 kg) were extracted with 95% EtOH at room temperature. After filtration, the filtrate was evaporated in vacuo to give a dark green thick oil (419.4 g). A portion of the ethanolic extract (210.0 g) was suspended in H_2O (600 ml) and extracted with Et_2O (3 × 300 ml) and then *n*-BuOH (3 × 300 ml). The *n*-BuOH fr. was concentrated under reduced pressure to yield a brown thick oil (39.7 g). The oil (35.1 g) was chromatographed on a column of silica gel (750 g) and was eluted with a gradient of CH_2Cl_2–MeOH–H_2O (lower phase) (30:3:1, 20:3:1, 15:3:1, 10:3:1, 7:3:1, 6:4:1) and MeOH. Successive frs. were combined on the basis of their behaviour on TLC and evaporated to give 21 frs. Purification of the 7th fr. (1.81 g) on a silica gel column using EtOAc–MeOH (99:1, 98:2, 95:5, 9:1, 8:2 and 6:4) gave baenzigeroside A (**2**) as a light brown solid (264 mg).

3.2. Baenzigeride A (*1*)

The slightly green solid (1.47 g) of baenzigeride A (**1**) was crystallized from Et_2O–MeOH to give a pale yellow solid (368 mg). A portion of the later solid (66 mg) was then recrystallized from EtOH to give **1** as colorless rhombs, mp 184–185° (Found: $[M]^+$ 358.1421. Calc. for $C_{20}H_{22}O_6$ 358.1414). $[\alpha]_D^{25}$ + 23.79° (*c*, 0.14, MeOH). λ_{max}^{MeOH} nm: 282 (log ε 3.90). ν_{max}^{Nujol}: 3500, 3150, 1750, 1740, 1660, 1600, 1250, 1230, 1200, 1180, 1120, 1070, 1040, 1010, 980, 890, 790 cm^{-1}. MS *m/z* (rel. int.): 358$[M]^+$(10), 312(100), 246(13), 212(15), 202(15), 164(50), 151(49), 146(85), 133(60), 119(50), 115(18), 105(49), 101(43), 95(90), 94(75), 81(58), 77(38), 65(28), 56(64), 41(70). For ^{1}H-NMR and ^{13}C-NMR data see Tables 1 and 2, respectively.

3.3. Acetylation of compound *1*

A mixture of compound **1** (50 mg), dry pyridine (1 ml) and acetic anhydride (1 ml) was refluxed for 3 h. After the usual workup, the crude acetate derivative (**1a**) was obtained as a solid (55 mg). This solid was purified by column chromatography using silica gel with $CHCl_3$–EtOAc as the eluent to give compound **1** (12.2 mg) and the acetate derivative (**1a**) as a colorless solid (36.7 mg). This solid was crystallized from MeOH–Et_2O to afford pure **1a** as colorless needles, mp 169–171°. (Found: $[M+H]^+$ 401.1610. Calc. for $C_{22}H_{25}O_7$ 401.1599). $[\alpha]_D^{25}$ + 7.44° (*c*, 0.07, CH_2Cl_2). λ_{max}^{MeOH} nm: 282(log ε 3.93). ν_{max}^{Nujol}: 1770, 1740, 1720, 1660, 1260, 1205, 1070, 1050 cm^{-1}. MS *m/z* (rel. int.): 401$[M+H]^+$(25), 273(12), 247(13), 226(7), 208(13), 195(7). For ^{1}H-NMR and ^{13}C-NMR data see Table 1 and 2, respectively.

3.4. Baenzigeroside A (*2*)

The light brown solid (264 mg) of the glucoside **2** was crystallized from acetone as a colorless powder (52 mg), mp 210–212°. (Found: 57.7; H, 6.0. $C_{26}H_{32}O_{11}\cdot H_2O$ requires: C, 58.0; H, 6.4%). $[\alpha]_D^{25}$ - 7.92°(*c*, 0.27, MeOH). λ_{max}^{MeOH} nm: 221(log ε 3.13). ν_{max}^{Nujol} cm^{-1}: 3400, 1760, 1730, 1662, 1270, 1100, 1050. MS *m/z* (rel. int.): 521$[M+H]^+$(< 1), 358[M-Glu + H]$^+$(20), 340[M-Glu- H_2O + H]$^+$(10), 325[M-Glu-H_2O-CH_3 + H] + (8), 312[M-Glu-CO_2-H]$^+$(20), 295[M-Glu-H_2O-CO_2 + H]$^+$(4), 204$[C_{12}H_{12}O_3]^+$(10), 189[204-CH_3]$^+$ (20), 146[204-CH_3-CO_2]$^+$(40), 94$[C_6H_6O]^+$(90), 81 [94-CH]$^+$(100). For ^{1}H-NMR and ^{13}C-NMR data see Tables 3 and 4, respectively.

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References

Maurya, R., Wazir, V., & Kapil, R. S. (1994). *J. Indian Chem. Soc*, *71*, 361.

Meeting on Medicinal Plants and Malaria, Ministry of University Affairs, Bangkok, Thailand, 10 November, 1989.

Bisset, F. G., & Nwaiwa, J. (1983). *Planta Med*, *48*, 275.

Pachaly, P., Schneider, (1981) *C. Arch. Pharm 314*. 251. (Chem. Abstr. 95 (1981) 3352 p).

Merrit, A. T., & Ley, S. V. (1992). *Nat. Prod. Rep*, *9*, 243.