New Rearranged Clerodane Diterpenes from *Tinospora baenzigeri*

Pittaya TUNTIWACHWUTTIKUL*,[a] and Walter Charles TAYLOR[b]

Department of Chemistry, Faculty of Science, Silpakorn University,[a] Nakorn Pathom 73000, Thailand and Department of Organic Chemistry, University of Sydney,[b] NSW 2006, Australia. Received January 31, 2001; accepted April 17, 2001

Baenzigeroside B, a new rearranged clerodane diterpene glucoside, was isolated from the stems of *Tinospora baenzigeri*. The aglycone of baenzigeroside B, baenzigeride B (isolated as its acetate), was found together with baenzigeride A, baenzigerosides A and B in the leaves of the same plant. Baenzigeride B and baenzigeroside B are the first examples of a new class of rearranged clerodane diterpenes. The possible biogenesis of the compounds is discussed.

Key words *Tinospora baenzigeri*; Menispermaceae; rearranged clerodane diterpene; baenzigeroside

The stems of *Tinospora 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.[1)] The stems of *T. baenzigeri* were found to contain several isoquinoline alkaloids.[2,3)] Recently we reported the presence of a rearranged clerodane diterpene, baenzigeride A (**1**) and its glucoside, baenzigeroside A (**2**), in the stems of *T. baenzigeri*.[4)] In this paper, we report the isolation and structural elucidation of a new rearranged clerodane diterpene glucoside, baenzigeroside B (**4**) from the the stems of *T. baenzigeri.* We have also isolated and identified the aglycone of glucoside **4**, baenzigeride B (**3**), which was isolated as its acetate derivative (**3a**), together with baenzigeride A (**1**), glucosides **2** and **4** in the leaves of *T. baenzigeri*. Compound **3** was not detected in the extract of the stems of the plant.

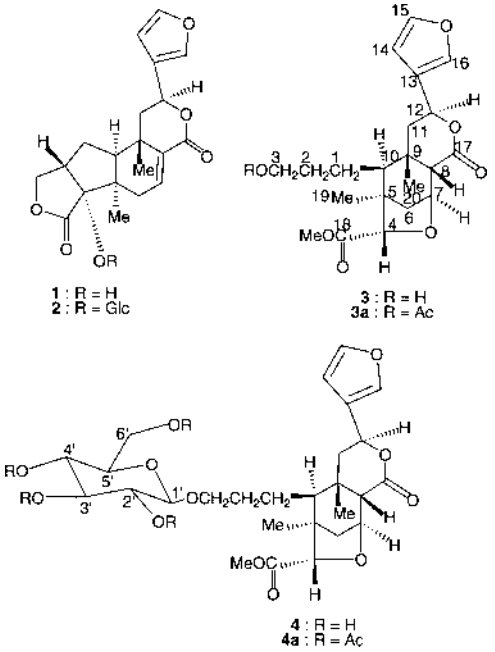
Baenzigeroside B (**4**) was obtained as a colorless powder, $C_{27}H_{38}O_{12}$, $[\alpha]_D^{25}$ +20.3°. The UV spectrum showed bands at 206 and 268 nm. Compound **4** had IR bands at 3450 (broad), 1730 and 1590 cm^{-1} indicating the presence of hydroxyl, ester carbonyl and olefinic groups, respectively.

The ^{13}C-NMR of **4** (Table 2) exhibited signals for two carbonyl groups (δ 170.4, 172.5) and two quaternary carbons at δ 47.8 (C-5) and 36.2 (C-9). Four olefinic carbon signals appeared at δ 108.6, 124.7, 139.8 and 143.5. There were also bands arising from six methylene carbons (δ 24.0, 31.8, 42.0, 42.0, 62.1, 69.2), 10 methine groups (δ 49.0, 53.8, 70.2, 70.7, 73.7, 76.1, 77.0, 78.9, 80.1, 102.9), two methyl carbons (δ 21.0, 27.3) and a methoxyl group (δ 51.6).

The ^{1}H-NMR spectrum of **4** (Table 1) showed signals at δ 7.46 (t, J=1.7 Hz, 1H), 7.56 (dt, J=1.7, 0.9 Hz, 1H) and 6.49 (dd, J=1.7, 0.9 Hz, 1H) which were assigned to two α-(H-15, H-16) and a β-proton (H-14), respectively, of a β-substituted furan ring. This was consistent with the ^{13}C-NMR data of **4** which contained signals from three olefinic carbons (δ 143.5, 139.8, 108.6) and an olefinic quaternary carbon (δ 124.7) of the furan moiety. A doublet of doublets at δ 5.58 (J=12.2, 2.4 Hz) and two sets of doublet of doublets at δ 2.19 (J=15.0, 2.4 Hz) and δ 1.67 (J=15.0, 12.2 Hz) were assigned to an ABX system of H-12 and $H_\alpha H_\beta$-11, respectively. The COSY experiments were useful in the assignment of $H_\alpha H_\beta$-6, H-7, H-8, H-10 and HaHb-1, HaHb-2 and HaHb-3. A doublet of doublet of doublets at δ 2.12 (J=12.2, 6.4, 1.4 Hz), a doublet at δ 1.44 (J=12.2 Hz) and two doublet of doublets at δ 5.02 (J=6.4, 4.6 Hz) and 2.85 (J=4.6, 1.4 Hz) arose from $H_\beta H_\alpha$-6, H-7 and H-8, respectively. The signals of H-10 and HaHb-3 appeared at δ 1.81 (dd, J=6.2, 2.6 Hz), 3.90 (dt, J=10.0, 6.0 Hz) and 3.56 (dt, J=10.0, 6.0 Hz), respectively. Four singlets at δ 4.43, 3.75, 1.27 and 1.12 were assigned to H-4, a methoxyl group and two tertiary methyl groups, CH_3-20 and CH_3-19, respectively. Signals for two non-equivalent methylene groups, HaHb-1 and HaHb-2, appeared at δ 1.72, 1.57, 1.87 and 1.70, all m, respectively. There was also a doublet at δ 4.27 (J=7.8 Hz) of the anomeric proton of a glucose moiety. The other glucose protons were assigned from 1D-TOCSY experiments.

The ^{13}C-NMR was assigned by a combination of DEPT, HMQC and HMBC experiments. The long-range correlations observed between C-1′ and HaHb-3 and between H-1′ and C-3 are especially indicative of structure **4** (Fig. 2.).

The relative stereochemistry of compound **4** was derived from the ^{1}H NOE correlations (Fig. 1.). The important NOE's observed were between CH_3-19 (δ 1.12) and H-10 (δ 1.81), H_β-6 (δ 2.12), H_α-6 (δ 1.44), Ha-1 (δ 1.72), Hb-1 (δ 1.57); H-4 (δ 4.43) and OCH_3 (δ 3.75); CH_3-20 (δ 1.27) and Ha-1 (δ 1.72), H-4 (δ 4.43), H-8 (δ 2.85), H_α-11 (δ 2.19) and H_β-11 (δ 1.67). Thus, the equatorial C-19 methyl and the axial



* To whom correspondence should be addressed. e-mail: pittayat@su.ac.th

Table 1. ^{1}H-NMR Spectral Data of **3a** ($CDCl_3$), **4** ($CDCl_3$/DMSO-d_6) and **4a** ($CDCl_3$)

Position	**3a**	**4**	**4a**
1a	1.71 (m)	1.72 (m)	1.62 (m)
1b	1.60 (m)	1.57 (m)	1.51 (m)
2a	1.73 (m)	1.87 (m)	1.82 (m)
2b	1.62 (m)	1.70 (m)	1.60 (m)
3ab	4.07 (m)		
3a		3.90 (dt, 10.0, 6.0)	3.91 (ddd, 10.0, 6.0, 4.8)
3b		3.56 (dt, 10.0, 6.0)	3.47 (ddd, 10.0, 7.2, 4.8)
4	4.46 (s)	4.43 (s)	4.43 (s)
6α	1.42 (d, 12.2)	1.44 (d, 12.2)	1.41 (d, 12.6)
6β	2.22 (ddd, 12.2, 6.4,1.6)	2.12 (ddd, 12.2, 6.4, 1.4)	2.20 (ddd, 12.6, 6.4, 1.4)
7	5.125 (dd, 6.4, 4.8)	5.02 (dd, 6.4, 4.6)	5.11 (dd, 6.4, 4.8)
8	2.91 (dd, 4.8, 1.6)	2.85 (dd, 4.6, 1.4)	2.88 (dd, 4.8, 1.4)
10	1.72 (m)	1.81 (dd, 6.2, 2.6)	1.68 (dd, 6.3, 2.4)
11α	2.07 (dd, 15.0, 3.0)	2.19 (dd, 15.0, 2.4)	2.14 (dd, 15.2, 2.4)
11β	1.698 (dd, 15.0, 12.0)	1.67 (dd, 15.0, 12.2)	1.65 (dd, 15.2, 12.1)
12	5.49 (dd, 12.0, 3.0)	5.58 (dd, 12.2, 2.4)	5.47 (dd, 12.1, 2.4)
14	6.426 (dd, 1.7, 0.9)	6.49 (dd, 1.7, 0.9)	6.46 (dd, 1.8, 0.9)
15	7.438 (t, 1.7)	7.46 (t, 1.7)	7.44 (t, 1.8)
16	7.498 (dt, 1.7, 0.9)	7.56 (dt, 1.7, 0.9)	7.52 (dt, 1.8, 0.9)
19	1.12 (s)	1.12 (s)	1.10 (s)
20	1.29 (s)	1.27 (s)	1.27 (s)
OCH_3	3.75 (s)	3.75 (s)	3.76 (s)
OAc	2.05 (s)		
1′		4.27 (d, 7.8)	4.48 (d, 8.0)
2′		3.23 (dd, 8.4, 7.8)	4.94 (dd, 9.6, 8.0)
3′		3.38 (t, 8.4)	5.20 (t, 9.6)
4′		3.42 (t, 8.4)	5.04 (dd, 10.0, 9.6)
5′		3.24 (ddd, 8.4, 4.8, 2.4)	3.68 (ddd,10.0, 4.6, 2.4)
6a′		3.72 (dd, 12.0, 4.8)	4.26 (dd, 12.6, 4.6)
6b′		3.80 (dd, 12.0, 3.0)	4.11 (dd, 12.6, 2.4)
4×OAc			2.02, 2.03 (2×), 2.09 (all s)

H-10 have the α-configuration while the axial C-20 methyl and the equatorial H-8 occupy the β-face (relatively). These results are consistent with the carbocyclic ring having a chair conformation with the 5,7-epoxymethano group bridging 1,3-diaxially on the β-side. The methoxycarbonyl group at C-4 is α. The lactone ring is attached in a *cis* manner as shown in Fig. 1 and can adopt a chair conformation also, so that H-12 is axial and can give a mutual NOE to H-10. H-8 and H_β-6 are equatorial and show a w-coupling (J=1.4 Hz).

The NMR data for the acetate derivative (**4a**) also supported the proposed structure.

Baenzigeride B (**3**) was isolated as its acetate derivative (**3a**). Compound **3a** showed in its ^{1}H-NMR spectrum signals similar to those present in the spectrum of **4** and **4a** with the absence of the signals from the glucose moiety. In similar fashion, the spectrum was assigned by decoupling, COSY and NOE experiments. The ^{13}C-NMR spectrum was assigned by DEPT, HMQC and HMBC experiments.

The novel skeleton of baenzigeride B (**3**) and baenzigeroside B (**4**) can arise from the *cis-ent*-neoclerodane epoxide (A) which has been considered to be the precursor of baenzigeride A (**1**) and baenzigeroside A (**2**)[4] (Chart 1). Rearrangement with breaking the 3–4 bond of ring A can give the ketoaldehyde (B). Reduction of B could give the alcohol (C) which could undergo Michael addition to C-7 to give the novel skeleton of **3** and glucosidation of **3** could yield baenzigeroside B. As mentioned previously[4] epoxide like (A) are not yet known to be naturally occurring; the known clerodane 2,3-epoxides, such as jateorin, are also oxygenated at C-1 and usually have a 1,18-lactone group.[5] In the case of baenzigeride B and baenzigeroside B simple oxidative cleavage of a $\Delta^{3,4}$-unsaturated ester derivative, *i.e.* D, could be involved. A number of compounds containing this system, including the borapetosides, have been isolated from *Tinospora* spp.[6] Intriguingly, they all contain an oxygen function at C-6 as well. Unfortunately baenzigeroside B (**4**) (EC_{50}>20 mg/ml) did not show anti-malarial activity in an *in vitro* screening against *Plasmodium falciparum*.

Experimental

Microanalyses were carried out by the Scientific Technological Research Equipment Center, Chulalongkorn University, Bangkok, Thailand. Melting points are uncorrected. Optical rotations were determined with a Jasco digital polarimeter. UV spectra were recorded with a Shimadzu UV-240 spectrophotometer. IR spectra were recorded with a Jasco A-302 spectrophotometer. ^{1}H- and ^{13}C-NMR were measured in $CDCl_3$ or $CDCl_3$/DMSO-d_6 on a Bruker Avance 400 (400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR) spectrometer. Chemical shifts are given in δ (ppm) with tetramethylsilane as an internal standard. MS were recorded on a VG 7070 mass spectrometer operating at 70 ev or with VG Quattro triple quadrupole mass spectrometer for the electrospray mass spectra. Column chromatrography was carried out on Kieselgel 60 (Merck; 70—230 mesh). TLC was performed on precoated PF_{254} plates (Merck); spots were detected by spraying with 1% $CeSO_4$ in 10% aq. H_2SO_4 followed by heating. 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, Thailand.

Extraction and Isolation a) The fresh stems of *T. baenzigeri* (13.2 kg) were extracted with 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 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 fraction 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 gradient of MeOH–CH_2Cl_2–H_2O (lower phase) (30 : 3 : 1,

Table 2. ^{13}C-NMR Spectral Data of **3a** ($CDCl_3$), **4** ($CDCl_3$/DMSO-d_6) and **4a** ($CDCl_3$)

Carbon	**3a**	**4**	**4a**	DEPT
1	24.0	24.0	24.0	CH_2
2	31.2	31.8	32.2	CH_2
3	64.1	69.2	69.3	CH_2
4	80.2	80.1	80.3	CH
5	47.8	47.8	47.8	C
6	42.3	42.0	42.3	CH_2
7	79.0	78.9	79.1	CH
8	53.7	53.8	53.7	CH
9	36.3	36.2	36.4	C
10	49.7	49.0	49.7	CH
11	42.5	42.0	42.0	CH_2
12	70.2	70.2	70.4	CH
13	124.5	124.7	124.6	C
14	108.3	108.6	108.5	CH
15	143.9	143.5	143.8	CH
16	139.7	139.8	139.7	CH
17	170.2	170.4	170.6	C
18	172.4	172.5	172.5	C
19	21.2	21.0	21.2	CH_3
20	27.5	27.3	27.4	CH_3
18-OCH_3	51.9	51.6	51.8	CH_3
1′		102.9	100.9	CH
2′		73.7	71.3	CH
3′		77.0	72.7	CH
4′		70.7	68.4	CH
5′		76.1	71.9	CH
6′		62.1	61.9	CH_2
OAc	20.9		20.7	CH_3
			20.6	CH_3
			20.5 (2×)	CH_3
OAc	171.0		170.4	C
			170.2	C
			169.4	C
			169.1	C

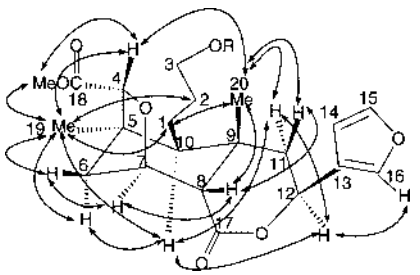


Fig. 1. NOE Correlations of **4**

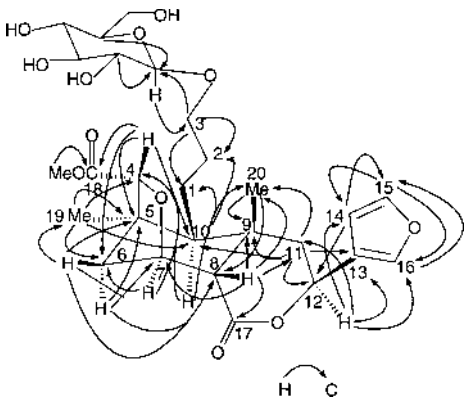


Fig. 2. Selected HMBC Correlations of **4**

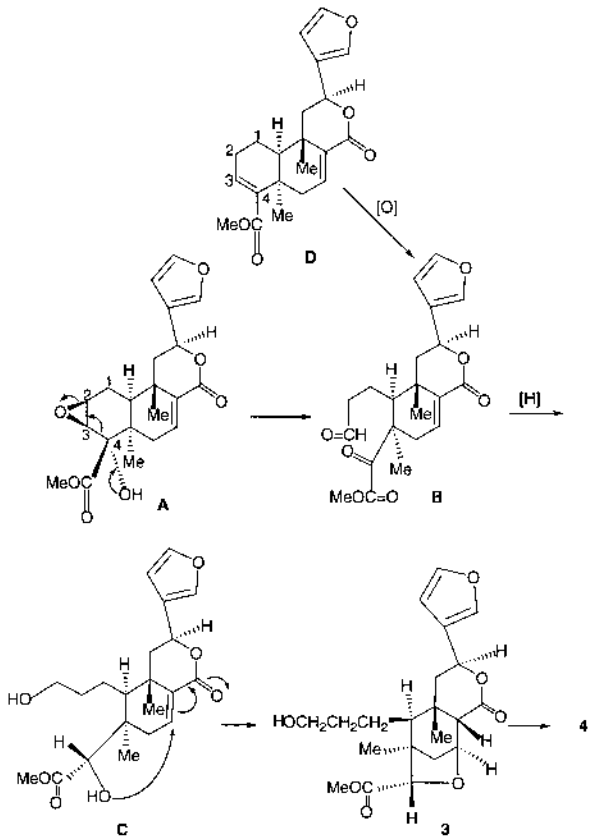


Chart 1. Possible Biogenesis of Baenzigeride B (**3**) and Baenzigeroside B (**4**)

20 : 3 : 1, 15 : 3 : 1, 10 : 3 : 1, 7 : 3 : 1, 6 : 4 : 1) and MeOH. Successive fractions were combined on the basis of their behaviour on TLC and evaporated to give 21 fractions. Purification of fraction 6 (1.53 g) on a silica gel column using a gradient of EtOAc–MeOH as the eluent gave baenzigeroside B (**4**) (554 mg).

b) The fresh leaves of *T. baenzigeri* (962.0 g) were extracted with EtOH at room temperature. The ethanolic extract was evaporated to give a dark green thick oil (53.3 g). The oil was partitioned between H_2O (200 ml) and EtOAc (3×200 ml) and then *n*-BuOH (3×200 ml). The EtOAc and *n*-BuOH fractions were evaporated to give a dark green thick oil (9.2 g) and a brown solid (7.4 g), respectively. The EtOAc fraction was chromatographed on a column of silica gel (300 g) and was eluted with a gradient of hexane/EtOAc. Successive fractions were combined on the basis of their behaviour on TLC and evaporated to give 18 fractions. Crystallization of fraction 10 (769 mg) in EtOH give baenzigeride A (**1**) as colorless rhombics (362 mg). Purification of fraction 11 (365 mg) on a column of silica gel (37 g) using a gradient of hexane/EtOAc gave compound **1** as a pale yellow solid (18 mg) and compound **3** (impure) as a pale yellow solid (32 mg). The *n*-BuOH fraction (7.4 g) was chromatographed on a column of silica gel (250 g) and eluted with a gradient of CH_2Cl_2/MeOH/H_2O (30 : 3 : 1, 20 : 3 : 1, 10 : 3 : 1, 7 : 3 : 1, 6.5 : 3.5 : 1.5, 6 : 4 : 1). Successive fractions were combined on the basis of their behaviour on TLC and evaporated to give 13 fractions. Purification of fraction 3 (441 mg) on a silica gel column using a gradient of EtOAc/MeOH give baenzigeroside B (**4**) which was further purified by column chromatography using silica gel with Et_2O/MeOH to give **4** (264 mg). Purification of fraction 5 (421 mg) on a silica gel column using a gradient of EtOAc/MeOH as the eluent gave baenzigeroside A (**2**) as a colorless solid (195 mg).

Baenzigeroside B (**4**): A colorless powder (482 mg), mp 136—137 °C. $[\alpha]_D^{25}$ +23.3° (c=0.33, MeOH). UV λ_{max}^{MeOH} (log ε) nm: 206 (3.81), 268 (3.33). IR ν_{max}^{Nujol} cm^{-1}: 3450, 1730, 1590, 1440, 1370, 1280, 1230, 1180, 1090, 1040. FAB-MS *m/z* (rel. int. %): 577 ($M+Na^+$) (100), 482 (10), 393 (50), 286 (50), 273 (54), 242 (48), 229 (40), 227 (40), 195 (37). HR-MS *m/z*: 577.2270 (Calcd for $C_{27}H_{38}O_{12}Na$: 577.2259). ^{1}H- and ^{13}C-NMR data: Tables 1 and 2.

Acetylation of Baenzigeroside B (4) A mixture of **4** (96 mg), dry pyri-

dine (1 ml) and acetic anhydride (1 ml) was stirred at room temperature for 10 h. After the usual workup, the crude acetate derivative (**4a**) was obtained as a slightly yellow solid (101 mg). This solid was purified by column chromatography using silica gel with hexane/EtOAc (2 : 1) as the eluent to give acetate **4a** which was crystallized from MeOH as colorless needles (91 mg), mp 145—146 °C. $[\alpha]_D^{25}$ +210.2° (c=0.09, MeOH). UV λ_{max}^{MeOH} (log ε) nm: 206 (3.79), 268 (3.25). IR ν_{max}^{Nujol} cm^{-1}: 1730, 1240, 1220, 1110, 1070, 1040. MS *m*/*z* (rel. int. %): 723 ($M+H^+$) (46), 443 (4), 391 (11), 331 (100), 273 (18). *Anal.* Calcd for $C_{35}H_{46}O_{16}$: C, 58.20; H, 6.40. Found: C, 58.20; H, 6.20. ^{1}H- and ^{13}C- NMR data: Tables 1 and 2.

Acetylation of Baenzigeride B (3) A mixture of compound **3** (impure) (17 mg), acetic anhydride (0.5 ml) and pyridine (0.5 ml) was refluxed for 2 h. After the usual workup, the crude acetate derivative was purified by column chromatography using silica gel and hexane/EtOAc (2 : 1) to give the acetate derivative of compound **3** (**3a**) as a pale yellow solid (2.8 mg). $[\alpha]_D^{25}$ +62.0° (c=0.44, MeOH). IR ν_{max}^{Nujol} cm^{-1}: 1740, 1608, 1280, 1240, 1080, 1020. MS *m*/*z* (rel. int. %): 434 (M^+) (100), 406 (5), 392 (12), 375 (45), 357 (20), 315 (30), 95 (100), 81 (82). HR-MS *m*/*z*: 434.1950 (Calcd for $C_{23}H_{30}O_8$: 434.1941). ^{1}H- and ^{13}C-NMR data: Tables 1 and 2.

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