# New Triterpenoid Saponins, Asterbatanoside D and E, from *Aster batangensis*

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**Abstract**

Two new triterpenoid saponins named asterbatanoside D and E have been isolated from *Aster batangensis* and their structures elucidated as 3-*O*-*β*-D-glucopyranosyl-bayogenin-28-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside and 3-*O*-6′-acetyl-*β*-D-glucopyranosyl-bayogenin-28-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside by means of MS, 1D and 2D NMR techniques (COSY, TOCSY, ROESY, HMQC, and HMBC), and chemical reactions.

**Key words**

*Aster batangensis*, Compositae, asterbatanoside D and E, triterpenoid saponins, bayogenin, 2D NMR.

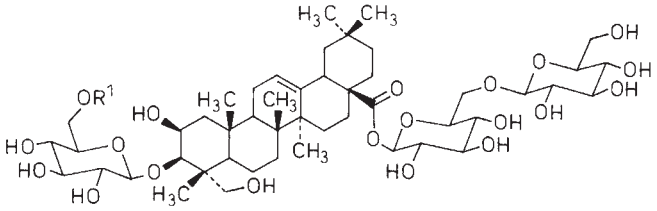
## Introduction

*Aster batangensis* Bur. et Franch (Compositae) is used as drugs for the treatment of snake bite in Chinese folk medicine (1). During a search for new bioactive compounds from medicinal plants, we found that the *n*-butanol extract from *A. batangensis* showed inhibitory activity (20%) against spontaneous activity of mice at a dose of 25 mg/kg *s.c.* This encouraged us to study the glycosides of this plant, which has not been chemically investigated before. In this paper, we wish to report the isolation and structural elucidation of two new oleanane-type saponins (**1** and **2**) from the roots of this plants, collected from Li-Jiang County, Yunnan Province, southwestern China, by a combination of DQF COSY (2), HMQC (3), TOCSY (4) and ROESY (5–7) techniques.

## Materials and Methods

### *General experimental procedures*

Melting points were determined on a Kofler apparatus and uncorr.; $[\alpha]_D$ were measured at 28 °C on a JASCO DIP-181 polarimeter. IR spectra were obtained on a Perkin-Elmer 599B infrared spectrometer. FAB-MS were recorded by the direct-inlet on a VG ZAB-HS mass spectrometer using glycerol as matrix. EIMS were obtained on a MAT-95 mass spectrometer. $^1$H-, $^{13}$C-, and DEPT NMR spectra were obtained on a Bruker AM-400 spectrometer operating at 400 MHz for $\delta_H$ and 100 MHz for $\delta_C$. COSY, TOCSY, ROESY, HMQC and HMBC spectra were obtained on a Bruker AMX-600 spectrometer operating at 600 MHz for $\delta_H$ and 125 MHz for $\delta_C$. The 2D TOCSY spectrum with a data matrix 512 × 1024 had 32 scans (with 2 dummy scans) per $t_1$ value and a delay time between scans of 0.9 sec. The 2D phase-sensitive ROESY spectrum with a data matrix 256 × 1024 had 48 scans (with 4 dummy scans) per $t_1$ value, a mixing time of 0.8 sec with a 2 % random variation, and a delay time between scans of 2 sec. The 2D HMQC (and HMBC) spectrum with a 128 × 2048 (128 × 1024) data matrix had 296 scans and 2 dummy scans (400 + 4) per $t_1$ value and a delay between scans of 1 sec (1 sec). The value of the delay to optimize one-bond correlations in the HMQC spectrum and supress them in the HMBC spectrum was 3.45 msec, and the evolution delay for long range couplings in the latter was set to 70 msec. All 1D and 2D NMR spectra were recorded using the standard Bruker software package, and data manipulation of the 2D spectra were performed on a Bruker Aspect × 32 data station. PC of sugars were run on Whatman No. 1 using the solvent systems *n*-BuOH-pyridine-$H_2O$ (6 : 4 : 3) and *n*-BuOH-AcOH-$H_2O$ (4 : 1 : 5, upper layer), respectively, and detected with aniline phthalate. MPLC was carried out on a Lichroprep RP-8 (40 – 60 μm) Lobar column (31 cm × 25 mm i.d.) with MeOH-$H_2O$ (5 : 5 – 6 : 4) as eluent (flow rate 2 ml/min). $SiO_2$, 140–200 mesh (Qingdao Marine Chemical Factory) was used for column chromatography and $SiO_2GF_{254}$ for TLC.



**1** $R^1$ = H, asterbatanoside D
**2** $R^1$ = Ac, asterbatanoside E

### *Plant material*

The roots of *A. batangensis* were collected in August 1992 from Li-Jiang County, Yunnan Province, southwestern China. A voucher specimen was identified by Prof. Z. W. Lu and deposited in the Herbarium of Kunming Institute of Botany, Academia Sinica, China.

### *Extraction and separation*

The dried roots (14 kg) of *A. batangensis* were extracted with 70% ethanol for five times at room temperature. After concentration in vacuo, the residue (2496 g) was suspended in $H_2O$ and then extracted with petroleum ether, EtOAc, and *n*-

BuOH, successively. The *n*-BuOH extract (543 g) was subjected to CC over highly porous resin (SIP-1300, 1.5 kg) eluting initially with $H_2O$ (5 l), followed by EtOH (5 l). The EtOH eluent (368.9 g) was chromatographed on a column of silica gel (1.8 kg, 200–300 mesh) eluted with $CHCl_3$-MeOH-$H_2O$ (8 : 1 : 0.1 – 1 : 1 : 0.1) gradient (500 ml each eluent) to separate into five crude fractions (fraction 1–5) monitoring by TLC. Fraction 3 was subjected to further CC over silica gel (200–300 mesh, 400 g) with $CHCl_3$-MeOH-$H_2O$ (4 : 1 : 0.1) (50 ml each eluent) to separate into 2 fractions monitoring by TLC, each fraction was further purified by Lichroprep RP-8 (40–63 μm, 31 × 2.5 cm) eluted with MeOH-$H_2O$ (6 : 4) (50 ml each eluent) to afford 55 mg of asterbatanoside E (**2**) and 2.2 g of asterbatanoside D (**1**).

### *Asterbatanoside D* (**1**)

Colorless needles. m.p. 220–222 °C; $[\alpha]_D$: +11.49 (MeOH, *c* 0.47). IR $\nu_{KBr}$: 3400, 1730, 1640, 890, 1000–1100 $cm^{-1}$; $C_{48}H_{78}O_{20}$; FAB-MS *m/z*: 997 $[M + Na]^+$ and 981 $[M + Li]^+$; $^1$H-NMR (pyridine-$d_5$): aglycone moiety: $\delta$ = 0.81, 0.82, 1.13, 1.16, 1.31, 1.55 (each 3H, each s, tert-Me × 6), 3.13 (1H, dd, *J* = 12.0, 4.0 Hz, 18-H), 3.64 (1H, d, *J* = 10.5 Hz, 23-Ha), 4.78 (1H, br. s, 2-H), 5.38 (1H, br. s, 12-H); sugar moiety: $\delta$ = 5.00 (1H, d, *J* = 7.7 Hz, outer glc-1-H of 28-O-sugar moiety), 5.15 (1H, d, *J* = 7.6 Hz, glc-1-H of 3-O-sugar moiety), 6.22 (1H, d, *J* = 8.0 Hz, inner glc-1-H of 28-O-sugar moiety), 4.68 (1H, d, *J* = 10.3 Hz, inner glc-6-H of 28-O-sugar moiety); $^{13}$C NMR: shown in Tables **1** and **2**.

### *Alkaline hydrolysis of* **1**

A solution of **1** (15 mg) in 5% KOH-MeOH was heated at 100 °C for 4 h. The reaction mixture was cooled to room temperature and neutralized to pH 6 with dilute HCl. After removal of MeOH, the remaining mixture was passed through a column of highly porous resin (SIP-1300, 50 g) eluted with $H_2O$ (500 ml) and then MeOH (500 ml). The MeOH eluent was treated with $CH_2N_2$ and evaporated to dryness. The residue was subjected to CC over silica gel (200–300 mesh, 10 g) eluted with $CHCl_3$-MeOH (6 : 1) (5 ml each eluent) to afford the methyl ester of prosapogenin (**1a**) which was identified as the methyl ester of 3-*O*-β-D-glucopyranosyl-bayogenin by comparison of physical, $^1$H- and $^{13}$C-NMR data with an authentic sample. **1a**: Colourless needles from MeOH; m.p. 249–251 °C; $[\alpha]_D$: +49.51° (MeOH, *c* 0.48); IR $\nu_{KBr}$: 3400, 1740, 1640, 1000 $cm^{-1}$; FAB-MS *m/z*: 687 $[M + Na]^+$ and 671 $[M + Li]^+$; $^1$H-NMR (pyridine-$d_5$): aglycone moiety: $\delta$ = 0.87, 0.88, 0.90, 1.18, 1.36, 1.57 (each 3H, each s, tert-Me × 6), 3.08 (1H, d, d, *J* = 13.7, 4.0 Hz, 18-H), 3.69 (3H, s, OMe), 3.69 and 4.15 (each 1H, each d, *J* = 10.0 Hz, 23-H), 4.34 (1H, overlap, 3α-H), 4.82 (1H, m, 2α-H), 5.37 (1H, br. s, 12-H), sugar moiety: Table **2**; $^{13}$C-NMR: Tables **1** and **2**.

**Table 1** $^{13}$C-NMR data of **1b** and aglycone moieties of **1**, **1a**, and **2** (pyridine-$d_5$, 100 MHz for $\delta_C$, ppm).

| carbon | 1 | 2 | 1a | 1b | DEPT |
|---|---|---|---|---|---|
| 1 | 43.99 | 44.17 | 44.13 | 44.88 | $CH_2$ |
| 2 | 70.46 | 70.51 | 70.54 | 71.57 | CH |
| 3 | 82.68 | 83.39 | 83.19 | 73.21 | CH |
| 4 | 42.11 | 42.61 | 42.25 | 42.26 | C |
| 5 | 48.35 | 48.41 | 48.54 | 48.52 | CH |
| 6 | 17.84 | 18.62 | 18.15 | 18.34 | $CH_2$ |
| 7 | 32.66 | 32.72 | 32.95 | 32.87 | $CH_2$ |
| 8 | 39.85 | 39.89 | 39.96 | 39.91 | C |
| 9 | 47.51 | 47.57 | 47.88 | 48.26 | CH |
| 10 | 36.80 | 36.84 | 37.04 | 37.21 | C |
| 11 | 23.81 | 23.85 | 24.05 | 24.01 | $CH_2$ |
| 12 | 122.90 | 122.92 | 123.25 | 123.24 | CH |
| 13 | 144.00 | 144.00 | 144.22 | 144.31 | C |
| 14 | 42.63 | 42.14 | 42.84 | 42.52 | C |
| 15 | 28.07 | 28.09 | 28.15 | 28.17 | $CH_2$ |
| 16 | 23.17 | 23.20 | 23.56 | 23.68 | $CH_2$ |
| 17 | 46.84 | 46.86 | 47.06 | 46.99 | C |
| 18 | 41.53 | 41.56 | 41.96 | 41.94 | CH |
| 19 | 45.98 | 46.00 | 46.18 | 46.19 | $CH_2$ |
| 20 | 30.57 | 30.58 | 30.87 | 30.82 | C |
| 21 | 33.78 | 33.79 | 34.10 | 34.03 | $CH_2$ |
| 22 | 32.34 | 32.37 | 32.95 | 32.93 | $CH_2$ |
| 23 | 65.22 | 65.25 | 65.82 | 67.89 | $CH_2$ |
| 24 | 14.91 | 14.92 | 15.09 | 14.50 | $CH_3$ |
| 25 | 17.15 | 17.15 | 17.34 | 17.33 | $CH_3$ |
| 26 | 17.44 | 17.86 | 17.34 | 17.41 | $CH_3$ |
| 27 | 26.00 | 25.97 | 26.30 | 26.23 | $CH_3$ |
| 28 | 176.36 | 176.36 | 177.99 | 180.02 | C |
| 29 | 32.95 | 32.95 | 33.18 | 33.14 | $CH_3$ |
| 30 | 23.49 | 23.51 | 23.76 | 23.79 | $CH_3$ |
| OMe | | | 51.57 | | $CH_3$ |

**Table 2** NMR data of sugar moieties of **1**, **2**, and **1a** (pyridine-$d_5$, 400 MHz for $\delta_H$, 100 MHz for $\delta_C$, ppm, *J* = Hz).

| position | 1 | | 2 | | 1a | |
|---|---|---|---|---|---|---|
| | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ |
| 3-O-sugar | | | | | | |
| glc | | | | | | |
| 1′ | 105.62 | 5.15 (d, 7.6) | 105.81 | 5.06 (d, 7.8) | 105.66 | 5.19 (d, 7.8) |
| 2′ | 75.29 | | 75.00 | 3.95 | 75.54 | 4.03 |
| 3′ | 78.20 | | 78.10 | 4.09 | 78.64 | 4.34 |
| 4′ | 70.68 | | 70.74 | 4.12 | 71.76 | 4.21 |
| 5′ | 78.09 | | 75.12 | 3.93 | 78.26 | 3.95 |
| 6′a | 62.38 | | 64.51 | 4.68 | 62.83 | 4.44 |
| 6′b | | | | 4.79 | | 4.47 |
| $CH_3CO$ | | | 20.62 | 1.94 | | |
| | | | 170.68 | | | |
| 28-O-sugar | | | | | | |
| inner glc | | | | | | |
| 1″ | 95.50 | 6.22 (d, 8.0) | 95.52 | 6.24 (d, 8.1) | | |
| 2″ | 73.67 | | 73.72 | 4.09 | | |
| 3″ | 78.55 | | 78.58 | 4.20 | | |
| 4″ | 71.33 | | 71.51 | 4.28 | | |
| 5″ | 77.80 | | 77.84 | 4.07 | | |
| 6″a | 69.17 | | 69.21 | 4.28 | | |
| 6″b | | | | 4.68 | | |
| outer glc | | | | | | |
| 1‴ | 105.11 | 5.00 (d, 7.7) | 105.15 | 5.01 (d, 7.7) | | |
| 2‴ | 74.97 | | 74.84 | 3.99 | | |
| 3‴ | 78.28 | | 78.23 | 4.16 | | |
| 4‴ | 71.24 | | 71.27 | 4.18 | | |
| 5‴ | 78.37 | | 78.31 | 3.86 | | |
| 6‴a | 62.38 | | 62.40 | 4.27 | | |
| 6‴b | | | | 4.44 | | |

### *Acid hydrolysis of* **1**

A solution of **1** (25 mg) in 2N HCl-MeOH (5 ml) was allowed to stand at 100 °C for 4 h, and the aglycone (**1b**) was obtained which was identified as bayogenin by comparison of physical and spectral data with an authentic sample. $^{13}$C-NMR data of **1b** are shown in Table **1**. The sugar part was examined by PC and TLC in direct comparison with an authentic sample, which showed the presence of glucose.

### *Asterbatanoside E* (**2**)

An amorphous powder; m.p. 217–219 °C; $[\alpha]_D$: +13.45° (MeOH, *c* 0.76); FAB-MS *m/z*: 1039 $[M + Na]^+$ and 1023 $[M + Li]^+$; $C_{50}H_{80}O_{21}$; $^1$H-NMR (pyridine-$d_5$): aglycone moiety: $\delta$ = 0.82, 0.83, 1.05, 1.22, 1.31 and 1.58 (each 1H, each s, tert-Me × 6), 1.93 (3H, s, $CH_3CO$), 3.13 (1H, dd, *J* = 12.0, 4.0 Hz, 18-H), 3.65 and 4.33 (each 1H, each d, *J* = 10.5 Hz, 23-H), 5.41 (1H, br. s, 12-H); sugar moiety: Table **2**; $^{13}$C-NMR data are shown in Tables **1** and **2**.

*Conversion of* **2** *to* **1**

A solution of **2** (11 mg) in 1 % $NaHCO_3$ in EtOH (1 : 1, 5 ml) was heated at 100 °C for 40 min. The reaction mixture was neutralized with Dowex 50W-X8 ($H^+$ form) and evaporated to dryness. The residue was subjected to CC over silica gel (200 – 300 mesh, 5 g) with $CHCl_3$-MeOH-$H_2O$ (9 : 3 : 0.5) to give the deacyl glycoside which was identified as having the same structure as **1** by comparison of $^{13}$C-NMR data with those of **1**.

*Acid hydrolysis of* **2**

A solution of **2** (5 mg) in 2 N HCl-MeOH (2 ml) was heated at 100 °C for 4 h, and the precipitates formed were collected. The filtrate was evaporated repeatedly at 40 °C until the solution showed a neutral reaction. The residue was identified as glucose by PC and TLC in direct comparison with an authentic sample.

## Results and Discussion

The *n*-butanol-soluble fraction of the 70 % ethanol extract from the roots of *A. batabgensis* was subjected to CC on highly porous resin (SIP-1300) and rechromatographed over silica gel and reverse-phase C-8 columns to afford asterbatanoside D (**1**) and E (**2**).

Asterbatanoside D (**1**) was obtained as colorless needles, m.p. 220–222 °C, $[\alpha]_D$: +11.49° (MeOH, *c* 0.47). The FAB-MS showed two quasimolecular ion peaks at *m/z* 997 $[M + Na]^+$ and 981 $[M + Li]^+$ which, together with $^{13}$C data (Tables **1** and **2**), suggested the molecular formula as $C_{48}H_{78}O_{20}$. It showed absorptions in its IR spectrum at 3400 $cm^{-1}$ for the hydroxy group, at 1730 $cm^{-1}$ for the ester carbonyl, and at 1640 and 890 $cm^{-1}$ for a trisubstituted double band. The structure of aglycone of **1** was confirmed by the result of acidic hydrolysis of **1** which afforded bayogenin (**1b**) identified by comparison of its co-TLC, $^1$H- and $^{13}$C-NMR data with an authentic sample and literature data (8).

Comparison of the $^{13}$C-NMR data due to aglycone moieties of **1** with those of bayogenin (**1b**) indicated that the C-3 signal in **1** appeared at lower field (+9.47 ppm), C-2 at higher field (−1.11 ppm), and C-28 at higher field (−3.66 ppm) than those of bayogenin, indicating that glycosylations took place at the C-3 and C-28 positions. In its $^1$H-NMR spectrum, there were signals of three anomeric protons at $\delta$ = 6.22 (d, $J$ = 8.0 Hz), 5.15 (d, $J$ = 7.6 Hz), and 5.00 (d, $J$ = 7.7 Hz). The $^{13}$C-NMR spectrum showed the presence of three anomeric carbon signals at $\delta$ = 95.50, 105.11, and 105.62. These indicated that **1** consisted of 1 mol of aglycone and 3 mol of D-glucose. The $\beta$ configurations at the anomeric centers of three D-glucopyranosyl moieties were suggested by the chemical shifts, the large $J_{1,2}$ couplings of anomeric protons (8.0, 7.7, and 7.6 Hz, respectively), and their $^{13}$C-NMR data (see Table **2**).

Alkaline hydrolysis of **1** afforded a prosapogenin. The methyl ester of the prosapogenin (**1a**) was determined as 3-*O*-$\beta$-D-glucopyranosyl-bayogenin methyl ester by comparison of co-TLC, and $^1$H- and $^{13}$C-NMR data with an authentic sample which has been obtained in our laboratory. Thus, the remaining two glucose units must be attached to C-28. Examination of $^{13}$C-NMR data in the sugar carbon region showed that the 28-O-inner glucose C-6″ resonated at $\delta$ = 69.17, which was shifted more downfield by +6.67 ppm than that of methyl $\beta$-D-glucopyranoside (9), suggesting the presence of a terminal glucose unit at this position. Consequently, the structure of asterbatanoside D (**1**) was established as 3-*O*-$\beta$-D-glucopyranosyl-bayogenin-28-*O*-$\beta$-D-glucopyranosyl-(1→6)-$\beta$-D-glucopyranoside.

Asterbatanoside E (**2**) was obtained as an amorphous powder, m.p. 217–219 °C, $[\alpha]_D$: +13.45° (MeOH, *c* 0.76). In the FAB-MS, two intense ions at *m/z* 1039 and 1023, compatible with $[M + Na]^+$ and $[M + Li]^+$ quasimolecular ions, as well as $^{13}$C-NMR data (Tables **1** and **2**) suggested that the molecular weight of **2** was 1016 for $C_{50}H_{80}O_{21}$.

The NMR parameters of **2** showed that the chemical shifts of $^1$H and $^{13}$C in aglycone moiety were superimposable with those of **1**. In addition, a characteristic acetyl group signals were observed at $\delta$ = 1.93 (3H, s) in the $^1$H-NMR spectrum and $\delta$ = 20.62 (q) and 170.68 (s) in the $^{13}$C-NMR spectrum. These findings suggested that **2** was 3, 28-O-bisdesmoside of bayogenin and that an acetyl group was linked to the sugar units.

The molecular weight of **2** was 42 mass units larger than that of **1**, which suggested that **2** might be monoacetyl glycoside of **1**. This suggestion was unambiguously confirmed by the conversion of **2** to **1** by basic hydrolysis with 0.5 % NaH $CO_3$.

The $^{13}$C-NMR data in sugar region of **2** indicated that the C-6 signal of one glucose unit was deshielded by +2.0 ppm and C-5 signal was shielded by −2.98 ppm due to the acetlyation effect. These acylation-induced shifts are reasonable when acylation took place at the 6-position of the glucose moiety (10–13). Thus, the site

**Table 3** Summary of 2D NMR data of **2**.

| proton | COSY ($^1$H) | HMQC ($^{13}$C) | TOCSY ($^1$H) | ROESY ($^1$H) |
|---|---|---|---|---|
| 3-O-sugar | | | | |
| glc-1′ | 2′ | 1′ | 2′,3′,4′,5′,6′a,6′b | 3′,5′,3 |
| 2′ | 1′,3′ | 2′ | 1′,3′,4′,5′,6′a,6′b | |
| 3′ | 2′,4′ | 3′ | 1′,2′,4′,5′,6′a,6′b | 1′,5′ |
| 4′ | 3′,5′ | 4′ | 1′,2′,3′,5′,6′a,6′b | |
| 5′ | 4′,6′a | 5′ | 1′,2′,3′,4′,6′a,6′b | 3′,1′,6′b |
| 6′a | 5′,6′b | 6′ | 1′,2′,3′,4′,5′,6′b | 6′b |
| 6′b | 6′a | 6′ | 1′,2′,3′,4′,5′,6′a | 6′a,5′ |
| $CH_3CO$ | | δ 20.62 | | |
| 28-O-sugar | | | | |
| inner glc | | | | |
| 1″ | 2″ | 1″ | 2″,3″,4″,5″,6″a | 3″,5″ |
| 2″ | 1″,3″ | 2″ | 1″,3″,4″,5″,6″a,6″b | |
| 3″ | 2″,4″ | 3″ | 1″,2″,4″,5″,6″a,6″b | 1″,5″ |
| 4″ | 3″,5″ | 4″ | 1″,2″,3″,5″,6″a,6″b | |
| 5″ | 4″,6″a | 5″ | 1″,2″,3″,4″,6″a,6″b | 1″,3″ |
| 6″a | 5″,6″b | 6″ | 1″,2″,3″,4″,5″,6″b | 6″b |
| 6″b | 6″a | 6″ | 1″,2″,3″,4″,5″,6″a | 6″a |
| outer glc | | | | |
| 1‴ | 2‴ | 1‴ | 2‴,3‴,4‴,5‴,6‴a | 3‴,5‴,6‴a |
| 2‴ | 1‴,3‴ | 2‴ | 1‴,3‴,4‴,5‴,6‴a,6‴b | |
| 3‴ | 2‴,4‴ | 3‴ | 1‴,2‴,4‴,5‴,6‴a,6‴b | 1‴,5‴ |
| 4‴ | 3‴,5‴ | 4‴ | 1‴,2‴,3‴,5‴,6‴a,6‴b | |
| 5‴ | 4‴,6‴a | 5‴ | 1‴,2‴,3‴,4‴,5‴,6‴b | 1‴,3‴,6‴b |
| 6‴a | 6‴b,5‴ | 6‴ | 1‴,2‴,3‴,4‴,5‴,6‴b | 6‴b |
| 6‴b | 6‴a | 6‴ | 1‴,2‴,3‴,4‴,5‴,6‴a | 5‴,6‴a |

of acetyl group was deduced to be one of the following two possibilities: the acetyl group is joined to the C-6 position of the 28-O-terminal glucose unit or the C-6 position of the 3-O-glucose unit. In order to solve this problem, a series of 2D NMR experiments were made including COSY, HMQC, TOCSY, HMBC, and ROESY spectra. The 2D NMR data are summarized in Table **3**.

The $^1$H-NMR spectrum showed three anomeric proton signals at $\delta$ = 6.01 (d, $J$ = 8.0 Hz), 5.06 (d, $J$ = 7.8 Hz), and 5.01 (d, $J$ = 7.7 Hz). The spin systems corresponding to those protons were assigned unambiguously by DQF COSY, and TOCSY spectra, and thereby $^{13}$C-NMR data of each monosaccharide of **2** were determined by HMQC spectrum. The NMR data of the sugar corresponding to the second anomeric proton ($\delta$ = 5.06) showed that its H-6′a ($\delta$ = 4.68) and H-6′b ($\delta$ = 4.79) were deshielded, C-6′ was deshielded at $\delta$ = 64.41, and C-5′ was shielded at $\delta$ = 75.12. This suggested that the acetyl groyp was located at the C-6 position of this sugar. In the ROESY spectrum, one significant contour between aglycone-H-3 and the anomeric proton signal at $\delta$ = 5.06 ppm was observed; thus, the 6′-acetylglucopyranosyl unit was located at the C-3 position of the aglycone. This result was further confirmed by the HMBC spectrum which showed one cross peak between the aglycone H-3 and C-1′ signals at $\delta$ = 105.81 of the 6′-acetyl glucopyranosyl unit.

The glucose unit corresponding to the anomeric proton at $\delta$ = 6.01 was easily assigned as the sugar with direct connection at the C-28 position. The anomeric proton at $\delta$ = 5.01 showed a contour with H-6″a of inner glucose in the 28-O-sugar moieties in the ROESY spectrum; thus, the glucose unit corresponding to this anomeric proton was located at the C-6″ position of the inner glucose unit.

Consequently, the structure of asterbatanoside E (**2**) was established as 3-*O*-6′-acetyl-$\beta$-D-glucopyranosyl-bayogenin-28-*O*-$\beta$-D-glucopyranosyl-(1→6)-$\beta$-D-glucopyranoside.

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