Beesiosides A−F, Six New Cycloartane Triterpene Glycosides from *Beesia calthaefolia*

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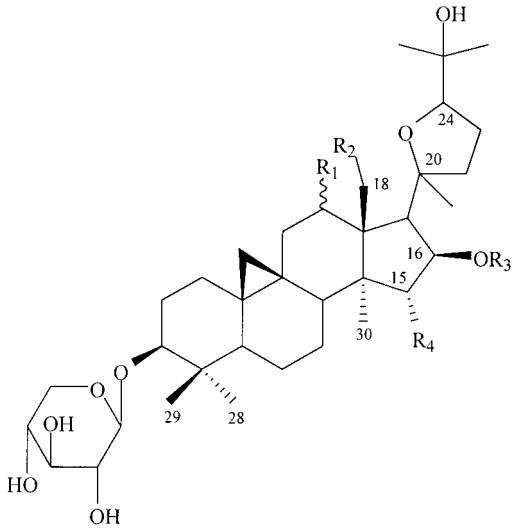
Six new cycloartane triterpene glycosides (**1**−**6**), beesiosides A−F, were isolated from whole plants of *Beesia calthaefolia*, and their structures were elucidated on the basis of extensive NMR experiments and chemical methods. Beesiosides A−F were assigned as (20*S**,24*R**)-epoxy-9,19-cyclolanostane-3*β*,16*β*,18,-25-tetraol-3-*O*-*β*-D-xylopyranoside (**1**), (20*S**,24*R**)-epoxy-9,19-cyclolanostane-3*β*,12*β*,16*β*,18,25-pentaol-3-*O*-*β*-D-xylopyranoside (**2**), (20*S**,24*R**)-epoxy-9,19-cyclolanostane-3*β*,12α,16*β*,18,25-pentaol-3-*O*-*β*-D-xylopyranoside (**3**), (20*S**,24*R**)-16*β*-acetoxy-20,24-epoxy-9,19-cyclolanostane-3*β*,12α,18,25-tetraol-3-*O*-*β*-D-xylopyranoside (**4**), (20*S**,24*R**)-epoxy-9,19-cyclolanostane-3*β*,15α,16*β*,18,25-pentaol-3-*O*-*β*-D-xylopyranoside (**5**), and (20*S**,24*R**)-16*β*-acetoxy-20,24-epoxy-9,19-cyclolanostane-3*β*,12*β*,25-triol-3-*O*-*β*-D-xylopyranoside (**6**), respectively.

Beesia calthaefolia (Maxim.) Ulbr. (Ranunculaceae) is widely distributed in the southwest and northwest of the People's Republic of China. As a well-known Chinese folk herb medicine, it possesses antiinflammatory, antipyretic, analgesic, and detoxifying functions and can "invigorate blood circulation". Its rhizomes or the whole plant are used to treat colds, rheumatic arthritis, dysentery, sore throats, and headaches.[1] Previous phytochemical investigations have resulted in the isolation of 9,19-cyclolanostane triterpene glycosides[2−5] and triterpenoids and sterols from this plant.[6,7] In the present work, we have isolated six new cycloartane triterpene glycosides (**1**−**6**) from the ethanol extract of the whole plant of *B. calthaefolia*. Also obtained in this investigation was (20*S**,24*R**)-epoxy-9,19-cyclolanostane-3*β*,12*β*,16*β*,25-tetraol-3-*O*-*β*-D-xylopyranoside (**7**), whose structure except for the absolute configuration of C-20 and C-24 was in accordance with the known compound cycloalpioside C.[9] This paper reports the isolation and structural elucidation of **1**−**6** and the identification of the previously known compound **7**.



	R_1	R_2	R_3	R_4	
1	H	OH	H	H	(20*S**,24*R**)
2	β-OH	OH	H	H	(20*S**,24*R**)
3	α-OH	OH	H	H	(20*S**,24*R**)
4	α-OH	OH	Ac	H	(20*S**,24*R**)
5	H	OH	H	OH	(20*S**,24*R**)
6	β-OH	H	Ac	H	(20*S**,24*R**)
7	β-OH	H	H	H	(20*S**,24*R**)

Results and Discussion

Beesioside A (**1**) was obtained as colorless needles, and its IR spectrum showed strong hydroxy (3450, 1090, 1050 cm^{-1}) absorption peaks. The positive-ion FABMS and HRFABMS showed an ion peak at *m*/*z* 623 $[M + H]^+$ and *m*/*z* 623.417323 (calcd 623.415909), indicating the molecular formula $C_{35}H_{58}O_9$ and corresponding to seven degrees of unsaturation. The base peak at *m*/*z* 143 in the EIMS of **1**, resulting from the cleavage between C-17 and C-20, suggested the presence of the same side chain (a 25 hydroxy-20,24-epoxy residue) as present in beesiosides II and III.[3,4] The ^{1}H NMR spectrum of **1** showed a double doublet signal at *δ* 3.97 (*J* = 8.6, 5.2 Hz) which was assignable to H-24 in the side chain. The ^{1}H NMR spectrum of **1** also exhibited two characteristic cyclopropane protons at *δ* 0.51 and 0.20 (each d, *J* = 3.6 Hz), six singlet methyl signals at *δ* 0.95, 0.97, 1.20 1.29, 1.36, and 1.51, and a doublet signal at *δ* 4.85 (d, *J* = 7.5 Hz) for an anomeric proton, indicating that **1** is a cycloartane-type glycoside.[8] The sugar was identified as xylose by acid hydrolysis followed by comparison with an authentic sample by TLC. Xylose was assigned in the D-configuration, since this is the only naturally occurring isomer of this sugar.[10] With the aid of ^{1}H−^{1}H COSY NMR data, the ^{1}H NMR spectrum of **1** additionally showed hydroxymethylene proton signals at *δ* 4.51 (1H, brd) and 4.35 (m), a hydroxymethine proton signal at *δ* 4.84 (m), an oxymethine proton signal at *δ* 3.48 (dd, *J* = 11.7, 4.3 Hz), and other signals for a five-

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Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) Spectral Data of **1**−**3** in Pyridine-d_5

	1[a]		**2**[a]		**3**[a]	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	1.18 m, 1.55 m	32.2 t	1.25 m, 1.55 m	32.4 t	1.15 m, 1.55 m	32.3 t
2	1.92 m, 2.34 m	30.1 t	1.86 m, 2.27 m	30.0 t	1.83 m, 2.25 m	30.1 t
3	3.48 dd (11.7, 4.3)	88.5 d	3.45 dd (11.6, 4.2)	88.5 d	3.44 dd (11.6, 4.3)	88.5 d
4		41.4 s		41.3 s		41.4 s
5	1.30 m	47.9 d	1.30 m	47.8 d	1.30 m	48.0 d
6	0.59 q (12.5)	20.9 t	0.64 q (12.6)	20.9 t	0.55 q (12.5)	21.1 t
	1.47 m		1.50 m		1.50 m	
7	1.05 m, 1.28 m	26.5[b] t	1.06 q (12.2), 1.35 m	26.3 t	1.15 m, 1.30 m	26.4 t
8	1.95 m	47.6 d	2.04 dd (12.8, 4.5)	46.4 d	1.90 m	47.4[b] d
9		20.2 s		21.5 s		20.3 s
10		26.8 s		27.0 s		26.6 s
11α	2.03 m,	26.7 t	2.69 dd (15.5, 9.3)	39.0 t	2.25 m	37.8 t
11β	1.52 m		1.35 m		1.62 m	
12	2.04 m, 1.55 m	29.2 t	4.24 brd (8.2)	73.8 d	4.17[b] m	69.0 d
13		51.8 s		55.6 s		55.7 s
14		47.0 s		49.0 s		47.4[b] s
15α	2.07 dd (13.0, 7.9)	49.1 t	2.12 m	50.1 t	2.12 dd (12.8, 8.1)	50.7 t
15β	2.13 dd (13.0, 4.1)		2.18 dd (14.7, 4.1)		2.20 m	
16	4.84 m	72.8 d	4.70[b]m	72.8 d	4.77 m	72.5 d
17	2.30 d (7.1)	55.7 d	2.84 d (9.3)	58.6 d	3.20 d (7.5)	51.1 d
18	4.35[b] m	65.8 t	4.72 d (12.4)	61.5 t	4.17[b] m	63.9 t
	4.51 br.d		4.70[b] m		4.31[b] m	
19	0.20 d (3.6)	30.4 t	0.36 d (3.7)	30.9 t	0.08 d (3.6)	29.4 t
	0.51 d (3.6)		0.53 d (3.7)		0.32 d (3.6)	
20		86.4 s		86.1 s		86.5 s
21	1.36 s	26.0 q	1.68 s	27.6 q	1.61 s	28.0 q
22α	1.68 m	36.8 t	2.13 m	34.0 t	2.00 m	34.7 t
22β	2.49 m		2.88 m		2.85 m	
23α	1.98 m	24.6 t	1.95 m	25.7 t	2.00 m	26.2 t
23β	2.25 m		2.30 m		2.20 m	
24	3.97 dd (8.6, 5.2)	85.3 d	3.95[b] m	82.7s	3.94 t (7.4)	83.3 s
25		70.8 s		70.1 s		70.4 s
26	1.51 s	28.3 q	1.57 s	28.1 q	1.52 s	27.7 q
27	1.20 s	26.5[b] q	1.33 s	27.3 q	1.31[b] s	27.5 q
28	1.29 s	25.8 q	1.28 s	25.8 q	1.30 s	25.8 q
29	0.97 s	15.4 q	0.94 s	15.4 q	0.94 s	15.5 q
30	0.95 s	22.6 q	0.97 s	22.5 q	1.31[b] s	23.6 q
1′	4.85 d (7.5)	107.5 d	4.81 d (7.4)	107.4 d	4.81 d (7.6)	107.5 d
2′	4.01 t (8.4)	75.5 d	3.95[b] m	75.5 d	3.97 t (8.2)	75.6 d
3′	4.14 t (8.8)	78.5 d	4.09 t (8.6)	78.5 d	4.11 t (8.8)	78.5 d
4′	4.21 td (9.0, 5.2)	71.3 d	4.15 m	71.2 d	4.20 m	71.3 d
5′	3.72 t (10.7)	67.1 t	3.69 t (10.4)	67.0 t	3.69 t (10.9)	67.1 t
	4.35[b] m		4.31 dd (11.3, 5.0)		4.31[b] m	

[a] Signals were assigned by ^{1}H−^{1}H COSY, ^{13}C−^{1}H COSY, NOESY, and HMBC spectra. [b]Signals overlapped in each vertical column.

membered sugar at δ 4.01 (t, J = 8.4 Hz), 4.14 (t, J = 8.8 Hz), 4.21 (td, J = 9.0, 5.2 Hz), 3.72 (t, J = 10.7 Hz), and 4.35 (m).

The ^{13}C NMR spectrum of **1** displayed a total of 35 carbon signals, including 11 oxygen-bearing carbon signals. On the basis of its ^{1}H−^{1}H COSY, ^{13}C−^{1}H COSY, NOESY, and HMBC NMR spectra, all ^{1}H and ^{13}C NMR signals of **1** could be assigned as shown in Table 1. In the ^{13}C−^{1}H COSY spectrum, an oxygenated carbon signal at δ 72.8 correlated with the proton signal at δ 4.84, suggesting the presence of a hydroxy group. In turn, the oxygenated carbon signal at δ 65.8 correlated with both the proton signals at δ 4.51 and 4.35, indicating the presence of a hydroxymethyl group. The hydroxy group was assigned to C-16 due to the presence of a doublet proton signal (H-17) at δ 2.30 (J = 7.1 Hz). Furthermore, the HMBC spectrum of **1** showed long-range correlations between H-17 and C-16 (δ 72.8), and H-16 and C-13 (δ 51.8), which supported the above conclusion. The coupling constants between H-16 and H-17 (J = 7.1 Hz) suggested a *cis*-relationship of the hydroxy group and the side chain.[8] Consequently, a 16β-hydroxy configuration was indicated. The hydroxymethyl group was placed at C-18 due to the observation of long-range correlations in the HMBC spectrum between H-18 (δ 4.35) and C-14 (δ 47.0), H-18 and C-17 (δ 55.7), and H-17 and C-18 (δ 65.8).

The coupling constant (J = 7.5 Hz) of the anomeric proton in the ^{1}H NMR spectrum of **1** indicated the D-xylose was in the β configuration. The β-D-xylpyranose unit was shown to be attached at C-3 by the observation of a long-range cross-peak between H-1′ and C-3 (δ 88.5) in the HMBC spectrum. The H-3α signal was assigned from its chemical shift and coupling patterns (Table 1). The 20*S**,-24*R** configuration was established by the significant correlations detected in the NOESY spectrum of **1** between H-16α/H-17α/Me-21, Me-21/H-22α, H-22α/H-22β, H-23α/H-23β, and H-24α/H-23α/Me-26/Me-27. Accordingly, the structure of beesioside A (**1**) was established as (20*S**,-24*R**)-epoxy-9,19-cyclolanostane-3β,16β,18,25-tetraol-3-*O*-β-D-xylopyranoside.

Beesioside B (**2**) was determined to have the molecular formula $C_{35}H_{58}O_{10}$, 16 mass units greater than that of **1**, by the observation of the ion peak at *m*/*z* 639.410072 (calcd 639.410824) $[M + H]^+$ in the HRFABMS. A ^{13}C NMR spectral comparison showed that **2** differs structurally from **1** only at the C-12 position. In the ^{13}C NMR spectrum of **2**, the C-12 (δ 73.8), C-11 (δ 39.0), and C-13 (δ 55.6) signals were shifted downfield by 44.6, 12.3, and 3.8 ppm, respec-

tively, compared to those of **1**, indicating the presence of a hydroxy group at C-12. The structure of **2** was further established by analysis of its 2D NMR spectra. Based on its ^{1}H−^{1}H COSY, ^{13}C−^{1}H COSY, and HMBC spectra, all ^{1}H and ^{13}C NMR signals could be assigned as shown in Table 1. The long-range correlations between H-12 and C-9, H-12 and C-17, H-12 and C-18, and H-17 and C-12 confirmed the presence of a hydroxy group at the C-12 position in **2**.

The configuration of the C-12 hydroxy group was confirmed by the coupling constants between H-12 and H_2-11. In the ^{1}H NMR spectrum of **2**, the H-12 proton appeared as a broad doublet at δ 4.24 (J = 8.2 Hz). Thus, the C-12 hydroxy group in **2** was assigned with a β configuration.[5] Comparison of the ^{13}C NMR spectral data with that of **1** showed the C-18 (δ 61.5) signal was shifted upfield by 4.3 ppm, also suggesting that the hydroxy group at C-12 should be β due to the γ-gauche effect.

To examine the exact stereochemistry of **2**, we investigated it in more detail by means of NOESY NMR experiments. The cross-peaks observed between H-12/H_3-30 and H-12/H-17 in the NOESY spectrum of **2** substantiated the 12β-hydroxy configuration proposed unambiguously. Likewise, the cross-peaks between H-16/H-17 and H-17/H_3-30 also supported a 16β-hydroxy configuration. Furthermore, in the NOESY spectrum of **2**, significant correlations were detected between Me-21/H-22α/H-23α/H-24α, H-22α/H-22β, H-23α/H-23β, and H-24α/Me-26/Me-27, which enabled the establishment of the 20*S**,24*R**configuration. Thus, the structure of beesioside B (**2**) was determined as (20*S**,24*R**)-epoxy-9,19-cyclolanostane-3β,12β,16β,18,25-pentaol-3-*O*-β-D-xylopyranoside.

Beesioside C (**3**) had the same molecular formula by HRFABMS (*m*/*z* 639.413928, calcd 639.410824 [M + H]$^+$) as **2**. On the basis of its ^{1}H−^{1}H COSY, ^{13}C−^{1}H COSY, and HMBC NMR spectra, all signals were assigned as shown in Table 1. A ^{13}C NMR spectral comparison of **3** with **2** suggested that **3** is the C-12 epimer of **2**. In the ^{13}C NMR spectrum of **3**, the C-17 (δ 51.1) signal was shifted upfield by 7.5 ppm due to a γ-gauche effect, suggesting an α-OH group occurring at C-12. Moreover, a downfield shift of H_3-30 (δ 1.31), owing to the *syn*-parallel disposition of the C-30 methyl and the C-12OH, compared with the analogous signal (δ 0.97) of **2**, further supported the proposed α-OH configuration at OH-12. In the NOESY spectrum, no cross-peak was observed between H-12 and H_3-30. From these data, the structure of beesioside C (**3**) was determined as (20*S**,24*R**)-epoxy-9,19-cyclolanostane-3β,12α,16β,18,25-pentaol-3-*O*-β-D-xylopyranoside.

Beesioside D (**4**) had the molecular formula $C_{37}H_{60}O_{11}$ by HRFABMS (*m*/*z* 681.420810, calcd 681.421388 [M + H]$^+$), 42 mass units greater than that of **3**, corresponding to an acetyl derivative of **3**. The signal at δ 2.10 (3H, s) in the ^{1}H NMR spectrum and the signal at δ 170.3 in the ^{13}C NMR spectrum showed the presence of an acetoxy group in **4** (Table 2). The ^{1}H and ^{13}C NMR spectra of **4** were very similar to those of **3**, except for the presence of signals due to the acetoxy group. ^{1}H and ^{13}C NMR spectral data comparison of **4** with **3** showed significant acetylation shifts for the signals of H-16 (+1.01 ppm), C-16 (+2.9 ppm), C-15 (−3.7 ppm), and C-17 (−0.7 ppm), demonstrating the position of the acetoxy group to be at C-16. On alkaline hydrolysis, compound **4** afforded **4a**, which was shown to be identical with **3** by mp, TLC, and IR comparison. The structure of **4** was further supported by analysis of its 2D NMR (^{1}H−^{1}H COSY, ^{13}C−^{1}H COSY, HMBC, and NOESY) spectra. Accordingly, the structure of beesioside D (**4**) was formulated as (20*S**,24*R**)-16β-acetoxy-20,24-epoxy-9,19-cyclolanostane-3β,12α,18,25-tetraol-3-*O*-β-D-xylopyranoside.

Beesioside E (**5**) gave a HRFABMS sodiated molecular ion at *m*/*z* 661.394448 (calcd 661.392768 [M + Na]$^+$), corresponding to the molecular formula of $C_{35}H_{58}O_{10}$ and requiring seven sites of unsaturation. Since a base peak was observed at *m*/*z* 143, the same as that of **1**, compound **5** was also deduced to have a 25-hydroxy-20,24-epoxy residue in the side-chain structure. The ^{1}H NMR spectrum showed that **5** possesses a cyclopropane ring, six methyl groups, an AB-type hydroxymethyl group (H_2-18), and an ABX-type system of three methine proton signals (H-15, 16, and 17). The ^{1}H−^{1}H COSY spectrum of **5** showed cross-peaks between the signal at δ 4.72 (dd, J = 8.4, 3.5 Hz, H-16) and two methine signals at δ 4.91 (d, J = 3.5 Hz, H-15) and 2.53 (d, J = 8.4, Hz, H-17). In the HMQC spectrum of **5**, the proton signals due to H-15, H-16, and H-17 were correlated with the carbon signals at δ 87.0, 82.6, and 53.4, respectively. Thus, two hydroxyl groups in **5** were located at C-15 and C-16. On the basis of ^{1}H−^{1}H COSY, HMQC, and HMBC spectra, all ^{1}H and ^{13}C NMR signals were assigned as shown in Table 2.

A ^{13}C NMR spectral comparison of **5** with **1** showed the two compounds differed structurally at the C-15 position. In the ^{13}C NMR spectrum of **5**, the C-15, C-14, and C-16 signals were shifted downfield by 37.9, 2.7, and 9.8 ppm, respectively, compared to those of **1**, supporting the presence of a hydroxy group at C-15. Moreover, the C-30 (δ 13.6) signal was shifted upfield by 9.0 ppm, suggesting that the hydroxy group at C-15 should have an α-configuration due to a γ-gauche effect. The stereochemistry of the two hydroxy groups at C-15 and C-16 could also be deduced from their coupling constants. The coupling constant between H-16 and H-17 was 8.4 Hz, suggesting a *cis*-relationship of the OH-16 group and the side chain, so a OH-16β substituent was substantiated. The coupling constant between H-15 and H-16 was 3.5 Hz, comparable with the data that Sakurai et al. obtained during the structure elucidation of beesioside II,[3] indicating an OH-15α configuration. In the NOESY spectrum of **5**, significant cross-peaks between H-17/H_3-30, H-16/H_3-30, H-8β/H-15, and H-24α/Me-21/Me-26/Me-27 were observed, which enabled the establishment of OH-15α, OH-16β, and (20*S**,24*R**) configurations. Thus, the structure of beesioside E (**5**) was determined as (20*S**,24*R**)-epoxy-9,19-cyclolanostane-3β,-15α,16β,18,25-pentaol-3-*O*-β-D-xylopyranoside.

Beesioside F (**6**) was assigned the molecular formula $C_{37}H_{60}O_{10}$, as established from the [M + H]$^+$ peak at *m*/*z* 665.430021 (calcd 665.426474) in the positive-ion HR-FABMS. From the fragment peak observed at *m*/*z* 143 in the EIMS of **6**, compound **6** was also deduced to have a 25-hydroxy-20,24-epoxy residue in the side chain. The signal at δ 2.10 (3H, s) in the ^{1}H NMR spectrum and the signal at δ 170.2 in the ^{13}C NMR spectrum suggested the presence of an acetoxy group in **6**. Comparison of the ^{1}H and ^{13}C NMR spectral data with those of **2** revealed that **6** also possesses a β-D-xylopyranosyl unit attached at the C-3β position and a OH-12β group. Based on its ^{1}H−^{1}H COSY, ^{13}C−^{1}H COSY, and HMBC NMR spectra, all ^{1}H and ^{13}C NMR signals could be assigned for **6** as shown in Table 2. In the HMBC spectrum of **6**, long-range correlations were observed between H-12 (δ 4.13) and C-18 (δ 13.7), and H-12 and C-11 (δ 38.1), which confirmed the presence of the OH-12 group. The ^{1}H NMR spectrum of **6** clearly showed a doublet proton signal assignable to H-17 at δ 2.82 (d, J = 8.3 Hz), suggesting the presence of a OAc-16 group. ^{1}H and

Table 2. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) Spectral Data of **4**−**6** in Pyridine-d_5

	4[a]		**5**[b]		**6**[a]	
position	δ_H (J in Hz)	δ_C	δ^1H (J in Hz)	δ_C	δ^1H (J in Hz)	δ_C
1	1.15 m, 1.55 m	32.2 t	1.21 m, 1.57 m	32.4 t	1.25 m, 1.51 m	32.2 t
2	1.85 m, 2.24 m	30.0 t	1.88 m, 2.31 m	30.1 t	1.88 m, 2.26 m	30.0 t
3	3.45 dd (11.6, 4.1)	88.4 d	3.47 dd (11.5, 4.2)	88.5 d	3.46 dd (11.6, 4.3)	88.4 d
4		41.3 s		41.3 s		41.3 s
5	1.33 m	47.8 d	1.34 m	47.8 d	1.30 m	47.5 d
6	0.64 q (12.4)	21.1 t	0.63 q (12.5),	21.1 t	0.74 q (12.5)	20.6 t
	1.51 m		1.50 m		1.52 m	
7	1.08 q (12.1), 1.28 m	26.6 t	1.28 m, 1.50 m	26.8 t	0.95 m, 1.20 m	26.0 t
8	1.71 m	47.4 d	2.08 m	48.5 d	1.52 m	46.4 d
9		20.0 s		20.5 s		20.3 s
10		26.2 s		26.7 s		26.6 s
11α	2.25 m	36.9 t	2.12 m	26.6 t	2.52 dd (15.5, 9.0)	38.1 t
11β	1.62 dd (15.1, 9.7)		1.03 m		1.40 m	
12	4.49 t-like (J>4.0)	67.9 d	2.05 m, 1.72 m	29.7 t	4.13[c] m	72.4 d
13		55.8 s		53.1 s		53.0 s
14		47.4 s		49.7 s		48.3 s
15	2.05 m	47.0 t	4.91 d (3.5)	87.0 t	2.17 dd (13.1, 8.0)	45.7 t
	2.25 m				1.48 m	
16	5.78 q-like	75.4 d	4.72 dd (8.4, 3.5)	82.6 d	5.62 q-like (5.7)	75.3 d
17	3.41 d (8.6)	50.4 d	2.53 d (8.4)	53.4 d	2.82 d (8.3)	57.8 d
18	4.20[c] m	63.3 t	4.30 d (13.0)	65.9 t	1.49 s	13.7 q
	4.33[c] m		4.54 d (13.0)			
19	0.10 d (3.6)	29.2 t	0.25 d (3.7)	30.5 t	0.40 d (3.9)	30.4 t
	0.35 d (3.6)		0.53 d (3.7)		0.56 d (3.9)	
20		85.4 s		86.4 s		85.0 s
21	1.40 s	28.7 q	1.37 s	26.4 q	1.34 s	28.1 q
22α	1.78 m	34.2 t	1.67 m	37.0 t	1.74 m	35.0 t
22β	3.09 dt		2.48 m		2.65 dt	
23α	1.97 m	26.0 t	1.94 m	24.7 t	1.88 m	25.7 t
23β	2.28 m		2.26 m		2.26 m	
24	3.93 t (7.8)	83.3 s	3.97 t (6.6)	85.4 s	3.82 dd (9.2, 6.5)	82.7 s
25		70.2 s		70.6 s		69.7 s
26	1.50 s	28.2 s	1.20 s	26.5 q	1.56 s	28.8 q
27	1.28[c] s	27.3 q	1.50 s	28.4 q	1.33 s	27.7 q
28	1.28[c] s	25.8 q	1.25 s	25.7 q	1.31 s	25.8 q
29	0.95 s	15.4 q	0.96 s	15.4 q	1.00 s	15.4 q
30	1.28[c] s	22.8 q	1.32 s	13.6 q	0.86 s	20.1 q
CO*CH*$_3$	2.10 s	21.6 q			2.10 s	21.5 q
*CO*CH$_3$		170.3 s				170.2 s
1′	4.82 d (7.5)	107.4 d	4.82 d (7.4)	107.4 d	4.82 d (7.5)	107.5 d
2′	3.99 t (8.1)	75.5 d	3.98 t (8.2)	75.5 d	3.99 t (8.6)	75.5 d
3′	4.13 t (8.7)	78.5 d	4.12 t (8.7)	78.4 d	4.13[c] m	78.5 d
4′	4.20[c] m	71.2 d	4.18 m	71.2 d	4.19 dt (9.7, 5.2)	71.2 d
5′	3.70 t (10.6)	67.0 t	3.71 t (10.6)	67.0 t	3.71 t (11.3)	67.0 t
	4.33[c] m		4.33 m		4.33 dd (11.3, 5.2)	

[a] Signals were assigned by ^{1}H−^{1}H COSY, ^{13}C−^{1}H COSY, NOESY, and HMBC spectra. [b]Signals were assigned by ^{1}H−^{1}H COSY, HMQC, NOESY, and HMBC spectra. [c]Overlapped signal in every vertical column.

^{13}C NMR spectral data comparison of **6** with **2** showed significant acetylation shifts for the signals of H-16 (+0.92 ppm) and C-16 (+2.5 ppm), demonstrating the position of the acetoxy group at C-16. The coupling constant between H-16 and H-17 indicated that the C-16 group has a β configuration.

Acidic hydrolysis of **6** afforded its aglycon **6a**. In the ^{1}H NMR spectrum of **6a**, the H-12 proton appeared as a broad doublet signal at δ 4.15 (J = 7.9 Hz). Thus, the OH-12 group in **6** was located in the β configuration as discussed for **2**. The presence of a cross-peak between H-12 and H$_3$-30 in the NOESY spectrum of **6** substantiated unambiguously the 12β configuration. Moreover, in the NOESY spectrum, correlations were also detected between Me-21/H-22α/H-23α/H-24α, H-22α/H-22β, H-23α/H-23β, H-22β/H-23β, and H-24α/Me-26/Me-27. These findings enabled a determination of a 20*S**,24*R** configuration. Accordingly, the structure and stereochemistry of beesioside F (**6**) were determined as (20*S**,24*R**)-16β-acetoxy-20,24-epoxy-9,19-cyclolanostane-3β,12β,25-triol-3-*O*-β-D-xylopyranoside.

Compound **7** was identified as (20*S**,24*R**)-epoxy-9,19-cyclolanostane-3β,12β,16β,25-tetraol-3-*O*-β-D-xylopyranoside by analyzing its 1D and 2D NMR (^{1}H−^{1}H COSY, ^{13}C−^{1}H COSY, NOESY, and HMBC) spectra. This structure was in accordance with the known compound cycloalpioside C except for the absolute configuration of C-20 and C-24.[9]

Experimental Section

General Experimental Procedures. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. IR spectra were recorded on a Perkin-Elmer 983G spectrometer. NMR spectra were measured in pyridine-d_5 on a Bruker AM-500 spectrometer, using TMS as internal standard. NMR experiments included the ^{1}H−^{1}H COSY, ^{13}C−^{1}H COSY, HMQC, HMBC, and NOESY pulse sequences. Coupling constants (J values) are given in Hz. A VG ZAB-2F mass spectrometer was used to record the EIMS, and an Autospec-Ultima ETOF spectrometer was used to record the FABMS and HRFABMS. Si gel 60H (400−500 mesh) and Si gel GF$_{254}$ sheets (0.20−0.25 mm) (both from Qingdao Haiyang Chemical Group Co., Qingdao, Shandong Province, People's Republic of China) were used for column chromatography and TLC, respectively.

Plant Material. The whole plant of *B. calthaefolia* was collected at Fanjing Moutain, Guizhou Province, People's Republic of China, in August 1998, and identified by Dr. Sibao Chen, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College. A voucher specimen (HB-98-0324) is deposited at the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College.

Extraction and Isolation. The air-dried and pulverized whole plant of *B. calthaefolia* was extracted two times with 95% EtOH for 2 h under reflux and then extracted two times with 50% EtOH for 2 h under reflux. After combination and removal of solvent, the residue (1.5 kg) was suspended in water (3000 mL) and partitioned successively with petroleum ether (3000 mL × 3), $CHCl_3$ (3000 mL × 3), and *n*-BuOH (3000 mL × 3). The $CHCl_3$-soluble fraction (600 g) was subjected to low-pressure column chromatography on Si gel 60H (400−500 mesh). Gradient elution with petroleum ether−EtOAc (10:0−1:9), EtOAc, and EtOAc−MeOH (9:1−1:9) gave four fractions, A (42 g), B (120 g), C (180 g), and D (160 g).

Fraction C was isolated by repeated LPLC over Si gel 60H, eluting with petroleum ether−EtOAc−MeOH (9:1:0−8:2:0−7:2:0.5−6:3:1) and $CHCl_3$−MeOH (10:0−9:1) to afford beesioside A (**1**, 1.20 g), beesioside B (**2**, 2.50 g), beesioside C (**3**, 0.32 g), beesioside D (**4**, 0.20 g), beesioside E (**5**, 30 mg), beesioside F (**6**, 35 mg), and compound **7** (2.20 g).

Beesioside A (1): colorless needles; mp 261−263 °C ($CHCl_3$−MeOH); $[\alpha]^{20}_D$ +21.1° (*c* 0.18, $CHCl_3$−MeOH, 1:1); IR (KBr) ν_{max} 3450, 3330, 2965, 2930, 2860, 1465, 1380, 1375, 1340, 1210, 1160, 1090, 1050, 990, 950 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; EIMS *m/z* 604 (M^+ − 18, 1), 490 (3), 472 (3), 454 (4), 436 (4), 395 (4), 373 (25), 355 (12), 337 (11), 143 (100), 125 (21), 107 (18), 73 (25), 57 (10), 43 (19); positive-ion FABMS *m/z* 623 $[M + H]^+$, 491, 473, 455, 437, 419, 143 (100), 125; positive-ion HRFABMS *m/z* 623.417323 $[M + H]^+$ (calcd for $C_{35}H_{59}O_9$, 623.415909).

Beesioside B (2): amorphous powder; mp 280−282 °C ($CHCl_3$−MeOH); $[\alpha]^{20}_D$ +9.1° (*c* 0.06, MeOH); IR (KBr) ν_{max} 3435, 2960, 2940, 2880, 1460, 1440, 1385, 1350, 1215, 1160, 1110, 1080, 1060, 1045, 1015, 970 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; EIMS *m/z* 602 ($M^+ - 2H_2O$, 1), 584 (0.1), 488 (1), 470 (2), 452 (3), 393 (3), 351 (4), 143 (100), 125 (20), 73 (20), 43 (10); positive-ion FABMS *m/z* 639 $[M + H]^+$, 489, 453, 435, 143 (100), 125; positive-ion HRFABMS *m/z* 639.410072 $[M + H]^+$ (calcd for $C_{35}H_{59}O_{10}$, 639.410824).

Beesioside C (3): amorphous powder; mp 284−286 °C ($CHCl_3$−MeOH), $[\alpha]^{20}_D$ +15.9° (*c* 0.15, $CHCl_3$−MeOH, 1:1); IR (KBr) ν_{max} 3445, 2960, 2925, 2885, 1460, 1440, 1385, 1345, 1210, 1150, 1110, 1090, 1045, 1010, 975 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; positive-ion FABMS *m/z* 639 $[M + H]^+$, 507, 489, 471, 453, 371, 143 (100), 125; positive-ion HRFABMS *m/z* 639.413928 $[M + H]^+$ (calcd for $C_{35}H_{59}O_{10}$, 639.410824).

Beesioside D (4): amorphous powder; mp 289−291 °C ($CHCl_3$−MeOH), $[\alpha]^{20}_D$ +23.9° (*c* 0.13, $CHCl_3$−MeOH, 1:1); IR (KBr) ν_{max} 3420, 2965, 2940, 2880, 1720, 1700, 1460, 1390, 1380, 1350, 1250, 1160, 1110, 1065, 1040, 985 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 2; EIMS *m/z* 602 (0.2), 470 (1), 452 (2), 353 (2), 143 (100), 125 (22), 73 (12), 43 (10); positive-ion FABMS *m/z* 681 $[M + H]^+$, 585, 503, 471, 453, 435, 353, 143 (100), 125; positive-ion HRFABMS *m/z* 681.420810 $[M + H]^+$ (calcd for $C_{37}H_{61}O_{11}$, 681.421388).

Alkaline Hydrolysis of 4. Compound **4** (21 mg) was treated with 2.5% KOH−MeOH solution (15 mL) at 80 °C for 2.5 h. After neutralization with 0.4 N HCl, 25 mL water was added to the mixture, and the whole was extracted with aqueous saturated *n*-BuOH. Removal of the solvent under reduced pressure yielded a product, which was purified by low-pressure column chromatography [Si gel 60H, $CHCl_3$−MeOH (10:0−9:1)] to furnish **4a** (11 mg). Compound **4a** was shown to be identical with compound **3** by mixed mp determination and by TLC [$CHCl_3$−MeOH (9:1), petroleum−EtOAc−MeOH (6:3:1), benzene−EtOH (8.5:1.5)] and IR (KBr).

Beesioside E (5): amorphous powder; mp 178−180 °C ($CHCl_3$−MeOH); $[\alpha]^{20}_D$ +13.2° (*c* 0.11, MeOH); IR (KBr) ν_{max} 3450, 2965, 2940, 2885, 1460, 1445, 1385, 1350, 1165, 1110, 1050, 1060, 1045, 1010 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 2; positive-ion FABMS *m/z* 639 $[M + H]^+$, 489, 472, 453, 435, 143 (100), 125; positive-ion HRFABMS *m/z* 661.394448 $[M + Na]^+$ (calcd for $C_{35}H_{58}O_{10}Na$, 661.392768).

Beesioside F (6): amorphous powder; mp 254−256 °C ($CHCl_3$−MeOH); $[\alpha]^{20}_D$ +17.9° (*c* 0.09, $CHCl_3$−MeOH, 1:1); IR (KBr) ν_{max} 3450, 2960, 2940, 2885, 1725, 1460, 1385, 1380, 1340, 1245, 1150, 1115, 1060, 1045, 985 cm^{-1}; ^1H NMR and ^{13}C NMR data see Table 2; positive-ion FABMS *m/z* 665 $[M + H]^+$, 605, 587, 507, 455, 437, 419, 371, 143 (100), 125; positive-ion HRFABMS *m/z* 665.430021 $[M + H]^+$ (calcd for $C_{37}H_{61}O_{10}$, 665.426474).

Acidic Hydrolysis of 6. A solution of **6** (16 mg) in EtOH (40 mL) was mixed with 10% aqueous HCl (5 mL) and benzene (10 mL). The heterogeneous mixture obtained was then heated under reflux for 24 h. The reaction mixture was poured into ice water, and the whole was extracted with EtOAc. The EtOAc extract was washed successively with saturated aqueous $NaHCO_3$ and water and dried over Na_2SO_4. Removal of the solvent under reduced pressure yielded a product, which was purified by low-pressure column chromatography [Si gel 60H, $CHCl_3$−MeOH (10:0−9.5:0.5)] to furnish **6a** (8.5 mg). Compound **6a**: IR (KBr) ν_{max} 3410, 2960, 2920, 2860, 1735, 1450, 1380, 1360, 1240, 1040, 990 cm^{-1}; ^1H NMR (500 MHz, pyridine-d_5) δ 0.44 (1H, d, J = 3.9 Hz, H-19), 0.64 (1H, d, J = 3.9 Hz, H-19), 0.82 (1H, q, J = 12.4 Hz, H-6), 0.90, 1.05, 1.21, 1.34, 1.37, 1.53, 1.56 (each 3H, s, 7 × CH_3), 2.11 (3H, s, $COCH_3$), 2.57 (1H, dd, J = 15.5, 8.9 Hz, H-11α), 2.85 (1H, d, J = 8.3 Hz, H-17), 2.67(1H, dt, H-22), 3.50 (1H, dd, J = 11.4, 4.3 Hz, H-3α), 3.85 (1H, dd, J = 8.9, 6.7 Hz, H-24), 4.15 (1H, brd, J = 7.9 Hz, H-12), 4.82 (1H, q-like, J = 7.7 Hz, H-16); ^{13}C NMR (500 MHz, pyridine-d_5) δ 13.8 (q, C-18), 14.8 (q, C-29), 20.3 (t, C-6), 20.3 (s, C-9), 21.1 (q, C-30), 21.5 (q, $COCH_3$), 25.7 (t, C-23), 26.2 (q, C-28), 26.2 (t, C-7), 26.9 (s, C-10), 27.7 (q, C-27), 28.0 (q, C-21), 28.8 (q, C-26), 30.5 (t, C-19), 31.2 (t, C-2), 32.2 (t, C-1), 35.1 (t, C-22), 38.2 (t, C-11), 41.1 (s, C-4), 45.7 (t, C-15), 46.4 (d, C-8), 47.4 (d, C-5), 48.3 (s, C-14), 53.1 (s, C-13), 57.8 (d, C-17), 69.9 (s, C-25), 72.4 (d, C-12), 75.3 (d, C-16), 78.0 (d, C-3), 82.9 (s, C-24), 85.1 (s, C-20), 170.1 (s, $COCH_3$); EIMS *m/z* 514 (M^+ − 18), 439, 353, 143 (85), 125 (20), 59 (32), 43 (100).

Alkaline Treatment of 6. Compound **6** (12 mg) was treated with 2.5% KOH−MeOH solution (10 mL) at 80 °C for 2.5 h. After neutralization with 0.4 N HCl, 15 mL water was added to the mixture, and the whole was extracted with aqueous saturated *n*-BuOH. Removal of the solvent under reduced pressure yielded a product, which was purified by low-pressure column chromatography [Si gel 60H, $CHCl_3$−MeOH (10:0−9.2:0.8)] to furnish **6b** (5 mg). Compound **6b** was shown to be identical with compound **7** by mixed mp determination and by TLC [$CHCl_3$−MeOH (9:1), petroleum−EtOAc−MeOH (6:3:1), benzene−EtOH (8.5:1.5)] and IR (KBr).

Sugar Unit of 1−6. Compounds **1**−**6** (each 2 mg) were refluxed with 10% HCl in 75% EtOH (3 mL) for 6 h. Each reaction mixture was diluted with H_2O, neutralized with Ag_2CO_3. The neutral hydrolysate revealed the presence of D-xylose by co-TLC [*n*-BuOH−AcOH−H_2O (4:1:1), R_f = 0.48] when compared with an authentic sample.

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