Beesiosides G, H, and J−N, Seven New Cycloartane Triterpene Glycosides from *Beesia calthifolia*

Jian-hua Ju,*,† Dong Liu,† Geng Lin,† Yu-mei Zhang,† Jun-shan Yang,*,† Yang Lu,‡ Ning-bo Gong,‡ and Qi-tai Zheng‡

Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100094, People's Republic of China, and Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, People's Republic of China

Received July 13, 2001

Seven new cycloartane glycosides (**1**−**7**), beesiosides G, H, and J−N, together with beesioside I (**8**) and beesioside A, were isolated from the rhizomes of *Beesia calthifolia*, and their structures were established by spectroscopic and chemical methods. Beesiosides G, H, and J−N were assigned as 20ξ_1,24ξ_2-epoxy-9,19-cyclolanostane-3β,16β,18,25-tetraol-3-*O*-β-D-glucopyranoside (**1**), 20ξ_1,24ξ_2-epoxy-9,19-cyclolanostane-3β,16β,18,25-tetraol-3-*O*-[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside (**2**), (20*S*,24*R*)-15α,16β-diacetoxy-20,24-epoxy-9,19-cyclolanostane-3β,18,25-triol-3-*O*-β-D-xylopyranoside (**3**), (20*S*,24*S*)-16β-acetoxy-18,24;20,24-diepoxy-9,19-cyclanostane-3β,15β,25-triol-3-*O*-β-D-xylopyranoside (**4**), (20*S*,24*S*)-16β-acetoxy-18,24;20,24-diepoxy-9,19-cyclanostane-3β,25-diol-3-*O*-β-D-xylopyranoside (**5**), 20ξ_1,24ξ_2-epoxy-15α-acetoxy-9,19-cyclolanostane-3β,16β,25-triol-3-*O*-β-D-xylopyranoside (**6**), and 20ξ_1,24ξ_2-epoxy-9,19-cyclolanostane-3β,12α,15α,16β,25-pentaol-3-*O*-β-D-xylopyranoside (**7**), respectively.

Beesia calthifolia (Maxim.) Ulbr. (Ranunculaceae) is widely distributed northwest and southwest in the People's Republic of China. As a well-known Chinese folk herb medicine, its rhizomes or the whole plant is used to treat colds, rheumatic arthritis, dysentery, sore throats, and headaches.[1] Previous phytochemical research of *B. calthifolia* native to Yunnan Province resulted in the isolation of beesiosides I−IV.[2−5] In a preceding paper, we reported six new cycloartane triterpene glycosides, beesiosides A−F, from the whole plant of *B. calthifolia* native to Guizhou Province of China.[6] Our chemical investigation of the rhizomes of *B. calthifolia* native to Gansu Province has led to another seven new cycloartane glycosides, namely, beesiosides G, H, and J−M (**1**−**7**), together with beesioside I (**8**) and beesioside A. This paper describes the experimental evidence that led to the structural and stereochemical assignments of **1**−**7** (Chart 1).

Results and Discussion

Beesioside G (**1**) showed strong hydroxyl (3400, 1090, 1040 cm^{-1}) absorptions in its IR spectrum, and its molecular formula was determined to be $C_{36}H_{60}O_{10}$ by positive high-resolution FABMS. The base peak at *m*/*z* 143 in the positive FABMS of **1** resulted from cleavage of the cycloartane skeleton between C-17 and C-20, suggesting the presence of a 25-hydroxy-20,24-epoxy residue, as in beesiosides A−F.[6] The ^{1}H and ^{13}C NMR spectral patterns of **1** were very similar to those of beesioside A[6] except for the ^{1}H and ^{13}C NMR signals due to xylose (Table 1). The ^{1}H NMR spectrum of **1** showed only one anomeric doublet proton signal at δ 4.93 (J = 7.5 Hz) in the downfield region, indicative of the presence of a β-linked sugar. The sugar was identified as D-glucose by acid hydrolysis and TLC analysis with an authentic sample. The ^{13}C NMR spectrum of **1** displayed a total of 36 carbon signals, including six oxygenated carbon signals ascribable to a β-D-glucopyranose moiety. All signals were assigned on the basis of an HMQC spectrum and by comparison with beesioside A (Table 1). Thus, beesioside J (**1**) was determined to be 20ξ_1,24ξ_2-epoxy-9,19-cyclolanostane-3β,16β,18,25-tetraol-3-*O*-β-D-glucopyranoside.

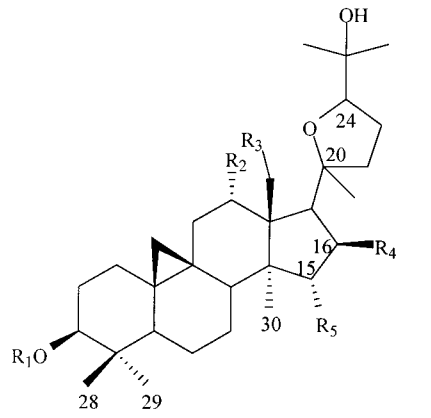
Beesioside H (**2**) had a molecular formula $C_{42}H_{70}O_{15}$, as deduced from its positive HRFABMS, corresponding to a hexosyl derivative of **1**. Compound **2** had the same aglycon moiety as **1**, as evidenced by the close similarity of the ^{1}H and ^{13}C NMR spectral data (Table 1). The ^{1}H NMR spectrum of **2** showed two anomeric protons at δ 4.86 (d, J = 7.7 Hz) and 5.11 (d, J = 7.8 Hz), indicative of the presence of two β-linked sugars. Both sugars was identified as D-glucose by acid hydrolysis. On the basis of HMQC and HMBC spectra and by comparison with **1**, all ^{1}H and ^{13}C NMR signals were assigned as shown in Table 1. In the ^{13}C NMR spectrum of **2**, the C-6′ (δ 70.3) signal was shifted downfield by 7.2 ppm compared with that of **1**, indicating a second β-glucosyl moiety to be attached at the C-6′ position. The site of attachment of each sugar moiety was confirmed by the means of the HMBC spectrum. In the HMBC spectrum of **2**, the anomeric proton signal at δ 4.86 (H-1′) showed a long-range correlation with the carbon signal at δ 88.6 (C-3); likewise, another anomeric proton signal at δ 5.11 (H-1″) showed a long-range correlation with the carbon signal at δ 70.3 (C-6′). Thus, beesioside H (2) was formulated as 20ξ_1,24ξ_2-epoxy-9,19-cyclolanostane-3β,16β,18,25-tetraol-3-*O*-[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside.

Beesioside J (**3**) had a molecular formula $C_{39}H_{62}O_{12}$, as deduced from its positive HRFABMS. Its IR spectrum showed strong hydroxyl (3400, 1090, 1045 cm^{-1}) and carboxyl (1725, 1270, 1230 cm^{-1}) absorptions. The base peak at *m*/*z* 143 in the positive FABMS of **3** resulted from cleavage between C-17 and C-20, suggesting the presence of a 25-hydroxy-20,24-epoxy residue. The ^{1}H and ^{13}C NMR spectra indicated that **3** had two acetoxyl groups. Detailed ^{1}H and ^{13}C NMR spectral analysis revealed that **3** possessed a cyclopropane ring, six methyl groups, a hydroxymethyl group at C-18, and a β-D-xylosyl unit at C-3. Additionally, the ^{1}H NMR spectrum of **3** exhibited ABX

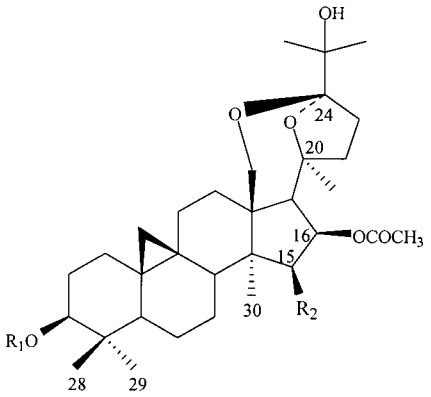
* To whom correspondence should be addressed. (J-h.J.) Tel: +86-10-62899739. Fax: +86-10-62898425. E-mail: jianhuaju@hotmail.com. (J.-s.Y.) E-mail: junshanyang@hotmail.com.
† Institute of Medicinal Plant Development.
‡ Institute of Materia Medica.

10.1021/np010294h CCC: $22.00
Published on Web 01/23/2002

Chart 1



	R_1	R_2	R_3	R_4	R_5
1	Glc	H	OH	OH	H
2	Glc (1→6) glc	H	OH	OH	H
3	Xyl	H	OH	OAc	OAc
6	Xyl	H	H	OH	OAc
7	Xyl	OH	H	OH	OH



	R_1	R_2
4	Xyl	OH
5	Xyl	H
8	Xyl	OAc

type signals of three methine protons ascribable to H-15, H-16, and H-17. Compared with beesioside E, [6] H-15 and H-16 were shifted downfield by 1.42 and 1.24 ppm, indicating the two acetoxyl groups in **3** were located at C-15 and C-16. The coupling constants suggested the 15α and 16*β* configurations. To determine the exact structure of **3** and its stereochemistry, an X-ray structure analysis of **3** was undertaken. The results are illustrated in Figure 1. The torsion angles $H_{17}-C_{17}-C_{16}-H_{16}$ (18°) and $H_{16}-C_{16}-C_{15}-H_{15}$ (−143.8°) obtained are in good agreement with the values calculated from the coupling constants in the ^{1}H NMR of **3**, which confirmed the 15α,16*β* configurations. The absolute sterochemistries at C-20 and C-24 were established as *S* and *R*, respectively. Accordingly, beesioside J (**3**) was unambiguously determined to be (20*S*,24*R*)-15α,16*β*-diacetoxy-20,24-epoxy-9,19-cyclolanostane-3*β*,18,25-triol-3-*O*-*β*-D-xylopyranoside.

Beesioside K (**4**) gave a HRFABMS quasimolecular ion at *m*/*z* 679.40223 (calcd 679.40333) $[M + H]^+$, 42 mass units less than that of beesioside I (**8**), corresponding to formula $C_{37}H_{58}O_{11}$. Its IR spectrum showed strong hydroxyl (3450, 1070, 1040 cm^{-1}) and carboxyl (1710, 1250 cm^{-1}) absorptions. Detailed ^{1}H and ^{13}C NMR comparisons revealed that **4** differed structurally from **8** only at the C-15*β* position, which has a hydroxyl group in place of the acetoxyl group in **8**. In the ^{1}H NMR spectrum of **4**, ABX type signals of three methine protons appeared at *δ* 4.40 (d, *J* = 8.7 Hz), 5.87 (dd, *J* = 11.2, 8.7 Hz), and 2.67 (d, *J* = 11.2 Hz) and were assigned to H-15, H-16, and H-17, respectively. Compared with the spectrum of **8**, H-15 was shifted upfield by 1.23 ppm, indicating a hydroxyl group in **4** to be located at C-15 in place of the acetoxyl group in **8**. Furthermore, in the ^{13}C NMR spectrum of **4**, the signal due to C-15 (*δ* 81.6) was shifted upfield by 0.5 ppm, while the signal due to C-16 (*δ* 78.5) was shifted downfield by 3.3 ppm, which also supported the above conclusion. On alkaline hydrolysis, compound **4** afforded **4a**, compound **8** afforded **8a**, and **4a** and **8a** were shown to be identical by IR (KBr), mixed mp determination, and TLC comparisons. Accordingly, beesioside K (**4**) was determined as (20*S*,24*S*)-16*β*-acetoxy-18,24;20,24-diepoxy-9,19-cyclanostane-3*β*,15*β*,25-triol-3-*O*-*β*-D-xylopyranoside.

Beesioside L (**5**) had molecular formula $C_{37}H_{58}O_{10}$, as deduced from its positive HRFABMS. Its IR spectrum showed strong hydroxyl (3500, 1090, 1040 cm^{-1}) and carboxyl (1735, 1235 cm^{-1}) absorptions. The ^{1}H and ^{13}C NMR spectra of **5** showed close similarity to those of **4** (Table 2). Analysis of the ^{1}H and ^{13}C NMR spectra of **5**, with the aid of ^{1}H−^{1}H COSY, HMQC, and NOESY spectra, demonstrated that **5** lacked the 15α-OH in comparison with **4**. In the ^{13}C NMR spectrum of **5**, the signals due to C-15 (*δ* 43.0) and C-16 (*δ* 73.2) were shifted upfield by 38.6 and 5.3 ppm, respectively, while the signal due to C-30 (*δ* 22.2) was shifted downfield by 7.6 ppm due to the lack of a *γ*-gauche effect, which supported the above conclusion. On a Dreiding model of **5**, if the configuration of C-20 is *S*, C-24 should also be *S*, and if C-20 is *R*, C-24 should be *R*. In the ^{1}H NMR spectrum of **5**, H-17 appeared at *δ* 2.61 (d, *J* = 10.5 Hz). The coupling constant of $J_{16,17}$ suggested that 16-OAc was in *β* configuration, which was also supported by a cross-peak between H-16 and H-17 in the NOESY spectrum. The NOE detected between H-17/Me-21 and OAc-16/H-22*β*/H-23*β* also supported the 20*S*,24*S* configurations. On the basis of these findings, beesioside L (**5**) was determined to be (20*S*,24*S*)-16*β*-acetoxy-18,24;20,24-diepoxy-9,19-cyclanostane-3*β*,25-diol-3-*O*-*β*-D-xylopyranoside.

Beesioside M (**6**) had molecular formula $C_{37}H_{60}O_{10}$, as deduced by its positive HRFABMS. Its IR spectrum showed strong hydroxyl (3500, 1090, 1045 cm^{-1}) and carboxyl (1705, 1260 cm^{-1}) absorptions. The base peak at *m*/*z* 143 from cleavage between C-17 and C-20 suggested the side chain to be a 25-hydroxy-20,24-epoxy residue. The ^{1}H and ^{13}C NMR spectra showed that **6** possesses a cyclopropane ring, seven methyl groups, a *β*-D-xylopranosyl unit attached at C-3, and an acetoxyl group (δ_H 2.08 (3H, s), δ_C 171.2). The ^{1}H NMR of **6** exhibited ABX signals ascribable to H-15 (*δ* 5.62, d, *J* = 4.5 Hz), H-16 (*δ* 4.53, dd, *J* = 9.0, 4.5 Hz), and H-17 (*δ* 2.41, d, *J* = 9.0 Hz). With the aid of ^{1}H−^{1}H COSY, HMQC, and HMBC spectra, all signals were assigned as shown in Table 2. Compared with beesioside E,[6] H-15 was shifted downfield by 0.71 ppm, indicating the presence of 15-OAc and 16-OH groups. The coupling constants ($J_{15,16}$ = 4.5 Hz and $J_{16,17}$ = 9.0 Hz) suggested the 15α and 16*β* configurations. Thus, beesioside M (**6**) was determined to be 20ξ_1,24ξ_2-epoxy-15α-acetoxy-9,19-cyclolanostane-3*β*,16*β*,25-triol-3-*O*-*β*-D-xylopyranoside.

Beesioside N (**7**) had molecular formula $C_{35}H_{58}O_{10}$, as deduced by its positive HRFABMS. Its IR spectrum showed strong hydroxyl (3450, 1080, 1040 cm^{-1}) absorptions. The base peak at *m*/*z* 143, from cleavage between C-17 and C-20, suggested the presence of a 25-hydroxy-20,24-epoxy side chain. The ^{1}H and ^{13}C NMR spectra (Table 2) showed that **7** possesses the side chain, a cyclopropane ring, seven

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

	1[a]		**2**[b]		**3**[c]	
position	δ ^{1}H (*J* in Hz)	δ ^{13}C	δ ^{1}H (*J* in Hz)	δ ^{13}C	δ ^{1}H (*J* in Hz)	δ ^{13}C
1	1.07 m; 1.46 m	32.2	1.18 m; 1.62 m	32.2		32.2
2	1.84 m; 2.36 m	29.9	1.88 m; 2.48 m	30.0		30.3
3	3.50 dd (11.8, 4.5)	88.7	3.50 dd (11.6, 4.3)	88.6	3.42 dd (11.5, 4.1)	88.3
4		41.3		41.3		41.2
5	1.27 m	47.9	1.21 m	47.8		46.8
6	0.61 q (12.5) 1.50 m	20.9	0.58 q (12.4) 1.43 m	20.9	0.37 q (12.0)	20.5
7	1.04 m, *f*	26.5 [d]	0.98 m, *f*	26.4[d]		26.6[e]
8	1.95 m	47.5	1.90 m	47.6		47.4
9		20.1		20.1		19.8
10		26.7		26.5		26.2[e]
11	2.02 m; *f*	26.6	1.96 m; *f*	26.7		26.0[e]
12	2.04 m, 1.59 m	29.1	1.96 m, 1.45 m	29.1		29.6
13		51.8		51.7		52.7
14		46.9		46.9		48.6
15α	2.10[d] m	49.1	2.05[d] m	49.0		84.8
15*β*	2.10[d] m		2.05[d] m		6.33 d (4.7)	
16	4.84 m	72.7	4.80 m	72.7	5.96 dd (10.0, 4.7)	79.2
17	2.30 d (7.0)	55.7	2.27 d (7.0)	55.6	2.76 d (10.0)	53.5
18	4.34 dd (13.5) 4.51 dd (13.5)	65.7	4.46 m 4.28 m	65.7	4.15 m[d] 4.39 dd (11.8, 7.8)	64.9
19	0.18 d (4.0) 0.51 d (4.0)	30.4	0.17 d (3.8) 0.49 d (3.8)	30.4	0.16 d (3.5) 0.42 d (3.5)	29.9
20		86.4		86.4		84.3
21	1.37 s	26.0	1.36 s	26.0	1.36 s	27.2[ee]
22	2.48 m; 1.68 m	36.8	2.45 m; 1.65 m	36.8		36.6
23	2.24 m; 1.98 m	24.6	2.23 m; 1.96 m	24.5		25.4
24	3.97 dd (8.5, 5.0)	85.3	3.95 m	85.2	3.84 t (7.2)	84.2
25		70.8		70.8		70.0
26	1.50 s	28.2	1.49 s	28.2	1.17 s	27.6[ee]
27	1.21 s	26.5[d]	1.20 s	26.4[d]	1.49 s	28.0[ee]
28	1.31 s	25.8	1.25 s	25.7	1.23 s	25.7
29	0.98 s	15.4	0.96 s	15.4	0.89 s	15.3
30	0.96 s	22.6	0.89 s	22.6	1.27 s	14.3
CO*CH*$_3$					2.06 s	21.7
CO*CH*$_3$					2.09 s	21.4
*C*OCH$_3$						171.5
*C*OCH$_3$						170.8
1′	4.93 d (7.5)	106.8	4.86 d (7.7)	106.7	4.77 d (7.4)	107.3
2′	4.02 t (8.5)	75.8	3.94 m	75.6	3.94 t (8.0)	75.3
3′	3.94 m	78.2	4.17 m	78.3[dd]	4.08 t (8.7)	78.4
4′	4.19 m	71.9	4.02 m	71.7[ddd]	4.15 m[d]	71.1
5′	4.21 m	78.8	4.06 m	77.1	3.67 t (10.7) 4.29 dd (11.2, 5.0)	66.9
6′	4.54 dd (11.8, 2.5) 4.38 m	63.1	4.77 m 4.28 m	70.3		
1″			5.11 d (7.8)	105.3		
2″			4.01 m	75.2		
3″			4.13 m	78.5		
4″			4.18 m	71.7[ddd]		
5″			3.90 m	78.3[dd]		
6″			4.45 m; 4.29 m	62.8		

[a] Signals were assigned by HMQC and compared with beesioside A. [b] Signals were assigned by HMQC and HMBC and compared with beesiosides A and G. [c] Signals were assigned by comparison with beesioside E and F. [d,dd,ddd] Overlapped signals. [e,ee] Values in any vertical column may be reversed, although those given here are preferred. [f] Not assigned signals.

methyl groups, a *β*-D-xylopranosyl unit attached at C-3, and hydroxyl groups at C-15α and C-16*β*. Additionally, the ^{1}H NMR spectrum of **7** exhibited a proton signal at δ 4.23 (t, J = 8.0 Hz), indicating the presence of another hydroxyl group. This hydroxyl group was speculated to be at C-12α by consideration of the coupling patterns as discussed in beesioside B.[6] On the basis of ^{1}H−^{1}H COSY and HMQC and comparison with related beesioside E, all signals were assigned as shown in Table 2. In the ^{1}H NMR spectrum of **7**, a downfield shift of H_3-30 (δ 1.60), owing to the syn-parallel disposition of the C-30 methyl and the C-12 OH, compared with that (δ 1.32) of beesioside E,[6] further supported the α-OH group at C-12. More convincing, no cross-peak was observed between the H-12 and H_3-30 in the NOESY spectrum of **7**. All the above findings proved that the hydroxyl group at C-12 should be α. From these data, beesioside N (**7**) was determined to be 20ξ_1,24ξ_2-epoxy-9,19-cyclolanostane-3*β*,12α,15α,16*β*,25-pentaol-3-*O*-*β*-D-xylopyranoside.

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 Bruker AM-500 spectrometers, 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. An Autospec-Ultima ETOF spectrometer was used to record FABMS and HR-FABMS spectra. 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. Sephadex LH-20 (Pharmacia, 40 μm) was purchased from Sigma Chemicals.

Plant Material. Rhizomes of *B. calthifolia* were collected in Wen County, Gansu Province, People's Republic of China, in August 1998 and identified by Dr. Si-bao Chen, Institute

Table 2. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) Spectral Data of **5**−**7** in Pyridine-d_5

	5[a]		**6**[b]		**7**[c]	
position	δ ^{1}H (*J* in Hz)	δ_C	δ ^{1}H (*J* in Hz)	δ ^{13}C	δ ^{1}H (*J* in Hz)	δ ^{13}C
1	1.18 m; 1.55 m	32.1	1.19 m; 1.53 m	32.4	1.22 m; 1.60 m	32.6
2	1.89 m; 2.35 m	30.9	1.92 m; 2.34 m	30.1	1.89 m; 2.31 m	30.2
3	3.48 dd (11.5, 3.0)	88.3	3.49 dd (11.5, 4.5)	88.5	3.46 dd (11.8, 4.0)	88.6
4		41.3		41.3		41.4
5	1.25 m	47.4	1.31 m	47.5	1.30 m	47.8
6	0.67 q-like (12.5), *f*	20.5	0.61 q (12.0) 1.42 m	21.1	0.73 q (12.5) 1.55 m	21.5
7	*f*, *f*	26.5[d]	1.05 m; 1.34 m	26.1	1.15 m; 1.28 m	26.1
8	1.38 m	46.9	1.77 dd (10.5, 4.0)	48.0	1.72 m	49.5
9		18.9		19.6		20.2
10		27.5		26.8		27.0
11	*f*, *f*	26.5[d]	2.02 m; 1.10 m	26.0	2.41 dd (15.0, 7.0) 1.73 m	37.3
12	1.48 m; 2.84 m	28.3	2.44 m; 1.71 m	37.5	4.23 t (8.0)	73.7
13		44.3		48.0[e]		48.9
14		52.6		47.6[e]		51.7
15	1.42 m; 2.02 m	43.0	5.62 d (4.5)	90.0	4.50 d (4.0)	89.3
16	5.64 m	73.2	4.53 dd (9.0, 4.5)	79.2	4.70 dd (10.5, 4.0)	80.9
17	2.61 d (10.5)	59.3	2.41 d (9.0)	54.3	3.39 d (10.5)	48.5
18	4.50 d (13.0) 4.30 d (13.0)	66.7	1.65 s	21.7	1.62 s	20.7
19	0.13 d (2.5) 0.47 d (2.5)	31.1	0.25 d (4.0) 0.48 d (4.0)	30.5	0.25 d (4.0) 0.43 d (4.0)	29.7
20		87.2		86.1		86.5
21	1.34 s	32.6	1.51 s	28.3	1.63 s	28.6
22	1.96 m; 2.89 m	37.8	2.49 m; 1.80 m	34.1	2.93 dt; 1.98 m	35.8
23	2.12 m; 2.78 m	30.0	2.23 m, 1.88 m	24.3	2.18 m, 1.92 m	26.1
24		114.1	3.90 t (8.0)	84.8	3.93 t (7.5)	83.5
25		72.8		70.1		70.2
26	1.55 s	25.7[d,e]	1.26 s	26.5	1.52 s	27.7
27	1.67 s	25.7[d,e]	1.32 s	26.4	1.38 s	27.6
28	1.31 s	25.8[ee]	1.30 s	25.7	1.01 s	13.8
29	1.00 s	15.4	1.01 s	15.4	1.28 s	25.8
30	0.89 s	22.2	1.08 s	13.5	1.60 s	15.6
CO*CH*$_3$	2.09 s	21.4	2.08 s	21.5		
*CO*CH$_3$		170.3		171.2		
1′	4.86 d (7.0)	107.6	4.85 d (7.5)	107.7	4.82 d (7.5)	107.6
2′	4.04 t (8.0)	75.6	4.03 t (8.0)	75.6	4.00 t (8.0)	75.6
3′	4.17 t (9.0)	78.6	4.16 t (8.5)	78.6	4.14 t (8.5)	78.6
4′	4.21 m	71.2	4.22 m	71.2	4.20 m	71.3
5′	3.73 t (7.5) 4.35 dd (11.3, 4.5)	67.1	3.73 t (11.3) 4.36 dd (11.3, 5.0)	67.1	3.71 t (10.5) 4.33 dd (11.5, 5.0)	67.2

[a] Signals were assigned by ^{1}H−^{1}H COSY, HMQC, and NOESY and compared with beesioside I. [b] Signals were assigned by ^{1}H−^{1}H COSY and HMQC and compared with related compound beesioside E. [c] Signals were assigned by ^{1}H−^{1}H COSY, HMQC, and NOESY and compared with related compound beesioside E. [d] Overlapped signals. [e] Values in any vertical column may be reversed, although those given here are preferred. [f] Not assigned signals.

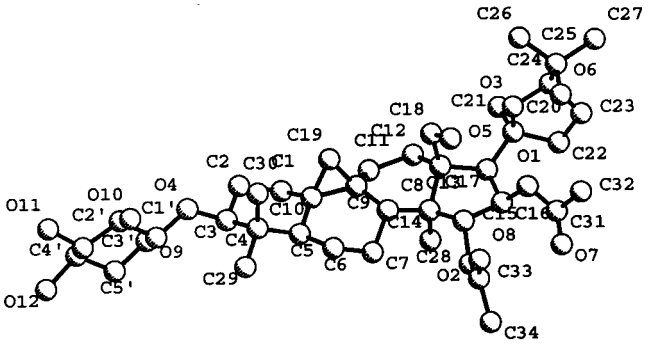


Figure 1. X-ray crystal structure for **3**.

of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College. A voucher specimen (HB-98-0325) is deposited in the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College.

Extraction and Isolation. Air-dried and pulverized rhizomes of *B. calthifolia* (3.5 kg) were extracted twice with 95% EtOH for 2 h under reflux and then extracted twice with 50% EtOH for 2 h under reflux. After combination and removal of solvent, the residue (850 g) was suspended in H_2O (1700 mL) and fractionated by successive extraction with $CHCl_3$ (1700 mL × 3) and *n*-BuOH (1700 mL × 3). The $CHCl_3$-soluble fraction (292 g) was subjected to low-pressure column chromatography (LPLC) on Si gel 60H (400−500 mesh). Gradient elution with petroleum ether−EtOAc−MeOH (9:1:0−8:2:0.2−7:2.5:0.5−6:3:1) gave four fractions, A (25 g), B (38 g), C (70 g), and D (75 g). Fraction C was subjected to LPLC on Si gel 60H with $CHCl_3$−MeOH (10:0−8:2) to gave four fractions (I−IV). Fraction I was further fractionated by repeated LPLC on Si gel 60H with petroleum ether−EtOAc−MeOH (6:3:1) and preparative TLC [petroleum ether−EtOAc−MeOH (5:4:0.4)] to give beesioside J (**3**, 1.42 g) and beesioside M (**6**, 1.15 g). Fraction II was further fractionated to give beesioside L (**5**, 20 mg), beesioside I (**8**, 4.5 g), and beesioside A (15 mg). Fraction D was subjected to repeated LPLC on Si gel 60H by gradient elution with $CHCl_3$−MeOH (9.5:0.5−7:3) to give three fractions (I−III). Fraction I was suspended in Me_2CO, and the insoluble part was subjected to LPLC on Si gel 60H by gradient elution with $CHCl_3$−MeOH (9.7:0.3−9:1) to give beesioside K (**4**, 0.15 g). Fractions II and III were subjected to repeated LPLC on Si gel 60H by gradient elution with $CHCl_3$−MeOH (9.5:0.5−8:2) and purified by Sephadex LH-20 ($CHCl_3$−MeOH 8:2) to give beesioside N (**7**, 0.15 g) and beesioside G (**1**, 25 mg), respectively. The *n*-BuOH-soluble part was chromatographed on Si gel 60H by gradient elution with $CHCl_3$−MeOH−H_2O (10:0:0−9:1:0.1−7:3:0.3) to give five fractions (I−V). Fraction II was chromatographed on Si gel 60H by gradient elution with $CHCl_3$−MeOH−H_2O (9:1:0.1−8:2:0.2) and on Sephadex LH-20 (MeOH−H_2O, 1:0.1) to give beesioside H (**2**, 25 mg).

Beesioside G (1): amorphous powder; mp 200−204 °C ($CHCl_3$−MeOH); $[\alpha]^{20}_D$ +18.3° (*c* 0.11, $CHCl_3$−MeOH, 1:1); IR (KBr) ν_{max} 3400, 2960, 2920, 1460, 1380, 1090, 1040; ^{1}H NMR and ^{13}C NMR data, see Table 1; positive FABMS *m*/*z* 675

$[M + Na]^+$, 653 $[M + H]^+$, 495, 473, 455, 437, 419, 143 (100), 125, 93, 71; positive HRFABMS *m/z* 653.42387 (calcd 653.42647) $[M + H]^+$.

Beesioside H (2): amorphous powder; mp 190−194 °C ($CHCl_3$−MeOH); $[\alpha]^{20}_D$ +23.8° (*c* 0.08, MeOH); IR (KBr) ν_{max} 3440, 2960, 2925, 1460, 1380, 1360, 1160, 1090, 1040; ^{1}H NMR and ^{13}C NMR data, see Table 1; positive FABMS *m/z* 853 $[M + K]^+$, 837 $[M + Na]^+$, 815 $[M + H]^+$, 797, 779, 761, 653, 635, 617, 599, 455, 437, 419, 143 (100), 125, 93, 71; positive HRFABMS *m/z* 815.47992 (calcd 815.47929) $[M + H]^+$.

Beesioside J (3): colorless prism; mp 198−202 °C (EtOAc−MeOH); $[\alpha]^{20}_D$ +15.1° (*c* 0.16, EtOAc−MeOH, 3:7); IR (KBr) ν_{max} 3530, 3400, 2965, 2935, 1725, 1465, 1370, 1270, 1230, 1090, 1045, 990, 980; ^{1}H NMR and ^{13}C NMR data, see Table 1; positive FABMS *m/z* 723 $[M + H]^+$, 705, 681, 663, 591, 573, 453, 435, 417, 308, 143 (100), 125; positive HRFABMS *m/z* 723.43199 (calcd 723.43195) $[M + H]^+$.

X-ray Crystallography of Beesioside J (3). Beesioside J (**3**) was crystallized from a EtOAc−MeOH (3:7) mixed solution as colorless transparent plates. A crystal of dimensions 0.15 × 0.15 × 0.20 mm was selected for X-ray diffraction analysis. The space group is $P22_12_1$ (Standant type $P2_12_12$). The crystal belongs to the orthorhombic system. The unit-cell parameters are $a = 9.970(1)$ Å, $b = 15.514(1)$ Å, $c = 26.265(1)$ Å. The volume of the unit cell is $V = 4062.5(5)$ Å^3. Four molecules are conained in one unit cell, $Z = 4$.

The diffraction intensities of the crystal were collected on a MAC DIP-2030K diffractometer, using graphite-monochromatized Mo Kα radiation. The distance between the crystal and IP is 100 mm, ω scan range is 0−180°, $\Delta\phi = 5°$, stationary count for 5°, 36 pictures were taken for 8.5 min each. A total of 3876 unique data were measured, 2974 were observable ($|F|^2 \geq 8\sigma|F|^2$).

All computations were performed on a computer with the direct method (SHELXS-86). Fifty non-hydrogen atom positions were obtained directly in the E map. The types of atoms were determined and corrected using the full-matrix least-squares method. The locations of all hydrogen atoms were determined by geometry calculation methods and difference Fourier synthesis. The final reliable factor: $R_f = 0.075$, $R_w = 0.077$ ($w = 1/\sigma|F|^2$), $(\Delta/\sigma)_{max} = 0.085$, $(\Delta\rho)_{min} = -0.330$ e/Å^3, $(\Delta\rho)_{max} = 0.370$ e/Å^3, $S = 2.667$.[7]

Beesioside K (4): amorphous powder; mp 278−282 °C (EtOAc−MeOH); $[\alpha]^{20}_D$ −12.0° (*c* 0.5, $CHCl_3$−MeOH, 1:1); IR (KBr) ν_{max} 3450 (br, OH), 2970, 2930, 2860, 1710, 1460, 1380, 1365, 1250,1160, 1100, 1070, 1040, 990, 970; ^{1}H NMR (C_5D_5N) δ 0.22 (1H, d, $J = 3.0$ Hz, H-19), 0.54 (1H, d, $J = 3.0$ Hz, H-19), 0.72 (1H, q, $J = 12.5$ Hz, H-6), 1.02, 1.23, 1.29, 1.31, 1.54, 1.65 (each 3H, s, 6 × CH_3), 1.98 (3H, s, 16-COC*H*$_3$), 2.67 (1H, d, $J = 11.2$ Hz, H-17), 3.50 (1H, dd, $J = 11.5$, 3.9 Hz, H-3α), 4.45 (1H, d, $J = 13.0$ Hz, H-18), 4.65 (1H, d, $J = 13.0$ Hz, H-18), 4.40 (1H, d, $J = 8.7$ Hz, H-15), 5.87 (1H, dd, $J = 11.2$, 8.7 Hz, H-16), 3.71 (1H, t, $J = 10.6$ Hz, H-5′), 4.00 (1H, t, $J = 8.2$ Hz, H-2′), 4.12 (1H, t, $J = 8.6$ Hz, H-3′), 4.19 (1H, m, H-4′), 4.33 (1H, dd, $J = 11.2$, 5.0 Hz, H-5′), 4.84 (1H, d, $J = 7.4$ Hz, H-1′); ^{13}C NMR (C_5D_5N) δ 14.6 (q, C-30), 15.4 (q, C-29), 19.7 (s, C-9), 20.8 (t, C-6), 21.4 (s, COC*H*$_3$), 25.7 (q, C-26, C-27, C-28), 26.5 (t, C-11), 26.6 (t, C-7), 27.7 (s, C-10), 28.3 (t, C-12), 30.1 (t, C-23), 30.9 (t, C-2), 31.9 (t, C-19), 32.5 (t, C-1), 32.6, (q, C-21), 38.0 (t, C-22), 41.4 (s, C-4), 46.3 (s, C-13), 47.5 (d, C-5), 48.6 (d, C-8), 51.4 (s, C-14), 56.9 (d, C-17), 66.9 (t, C-18), 67.1 (t, C-5′), 71.3 (d, C-4′), 72.8 (s, C-25), 75.6 (d, C-2′), 78.5 (d, C-16, C-3′), 81.6 (d, C-15), 87.0 (s, C-20), 88.5 (d, C-3), 107.5 (d, C-1′), 114.1 (s, C-24), 171.0 (s, *C*OCH$_3$); positive FABMS *m/z* 679 $[M + H]^+$, 547 $[M - 132 + H]^+$, 529 $[M - 150 + H]^+$; positive HRFABMS *m/z* 679.40223 (calcd 679.40333) $[M + H]^+$.

Alkaline Treatment of 4 and 8. Compound **8** (1.2 g) was treated with 2.5% KOH−MeOH solution (100 mL) at 80 °C for 2.5 h. Usual workup afforded **8a** (0.8 g). Compound **4** (20 mg) was treated with 2.5% KOH−MeOH solution (10 mL) at 80 °C for 2.5 h. Usual workup afforded **4a** (11 mg). **8a** and **4a** were shown to be identical by mixed mp determination and TLC and IR comparisons. Compound **4a** or **8a**: ^{1}H NMR (C_5D_5N) δ 0.23 (1H, d, $J = 3.8$ Hz, H-19), 0.57 (1H, d, $J = 3.8$ Hz, H-19), 0.76 (1H, q, $J = 12.5$ Hz, H-6), 1.03, 1.24, 1.29, 1.55, 1.68, 1.73 (each 3H, s, 6 × CH_3), 2.63 (1H, d, $J = 11.1$ Hz, H-17), 3.50 (1H, dd, $J = 11.6$, 4.3 Hz, H-3α), 4.50 (1H, d, $J = 12.9$ Hz, H-18), 4.66 (1H, d, $J = 12.9$ Hz, H-18), 4.29 (1H, d, $J = 8.6$ Hz, H-15), 4.56 (1H, dd, $J = 10.8$, 8.8 Hz, H-16), 3.71 (1H, t, $J = 10.9$ Hz, H-5′), 4.01 (1H, t, $J = 8.6$ Hz, H-2′), 4.12 (1H, t, $J = 8.8$ Hz, H-3′), 4.21 (1H, m, H-4′), 4.33 (1H, dd, $J = 11.3$, 5.2 Hz, H-5′), 4.84 (1H, d, $J = 7.5$ Hz, H-1′); ^{13}C NMR (C_5D_5N) δ 14.8 (q, C-30), 15.4 (q, C-29), 19.8 (s, C-9), 20.9 (t, C-6), 25.7 (q, C-27 and C-28), 25.8 (q, C-26), 26.6 (t, C-11), 26.8 (t, C-7), 27.7 (s, C-10), 28.5 (t, C-12), 30.1 (t, C-23), 30.9 (t, C-2), 31.9 (t, C-19), 33.2 (q, C-21), 32.5 (t, C-1), 38.5 (t, C-22), 41.3 (s, C-4), 47.6 (d, C-5), 48.8 (d, C-8), 50.8 (s, C-14), 58.2 (d, C-17), 67.0 (t, C-5′ and C-18), 71.2 (d, C-4′), 72.9, (s, C-25), 75.5 (d, C-2′), 76.8 (d, C-16), 78.5 (d, C-3′), 84.2 (d, C-15), 88.1 (s, C-20), 88.5 (d, C-3), 107.5 (d, C-1′), 113.7 (s, C-24).

Beesioside L (5): amorphous powder; mp 250−254 °C (EtOAc−MeOH); $[\alpha]^{20}_D$ −2.1° (*c* 0.09, $CHCl_3$−MeOH, 1:1); IR (KBr) ν_{max} 3500 (br, OH), 2970, 2920, 2840, 1735, 1460, 1380, 1360, 1235, 1160, 1090, 1040; ^{1}H and ^{13}C NMR data, see Table 2; positive FABMS: 685 $[M + Na]^+$, 115 (100), 59, 43; positive HRFABMS *m/z* 685.39256 (calcd 685.39276) $[M + Na]^+$.

Beesioside M (6): amorphous powder; mp 158−164 °C ($CHCl_3$−MeOH); $[\alpha]^{20}_D$ −3.3° (*c* 0.06, $CHCl_3$−MeOH, 1:1); IR (KBr) ν_{max} 3500, 2965, 2940, 2870, 1705, 1460, 1380, 1360, 1260, 1090, 1045, 960; ^{1}H NMR and ^{13}C NMR data, see Table 2; positive FABMS *m/z* 687 $[M + Na]^+$, 665 $[M + H]^+$, 455, 437, 143 (100), 125, 115, 71, 43; positive HRFABMS *m/z* 665.42761 (calcd 665.42647) $[M + H]^+$.

Beesioside N (7): amorphous powder; mp 252−256 °C ($CHCl_3$−MeOH); $[\alpha]^{20}_D$ +14.2° (*c* 0.19, $CHCl_3$−MeOH, 1:1); IR (KBr) ν_{max} 3450, 2965, 2940, 2870, 1450, 1380, 1360, 1160, 1040, 990; ^{1}H NMR and ^{13}C NMR data, see Table 2; positive FABMS *m/z* 661 $[M + Na]^+$, 639 $[M + H]^+$, 621, 603, 585, 489, 471, 453, 435, 143 (100), 125, 71, 43; positive HRFABMS *m/z* 661.39076 (calcd 661.39276) $[M + Na]^+$.

Beesioside I (8): amorphous powder; mp 260−262 °C (EtOAc−MeOH); $[\alpha]^{20}_D$ −7.9° (*c* 0.14, $CHCl_3$−MeOH, 1:1); IR (KBr) ν_{max} 3400 (br, OH), 2970, 2930, 2860, 1740, 1460, 1380, 1365, 1240,1160, 1100, 1040, 990, 970; ^{1}H NMR (C_5D_5N) δ 0.15 (1H, d, $J = 3.5$ Hz, H-19), 0.48 (1H, d, $J = 3.5$ Hz, H-19), 0.58 (1H, q, $J = 12.5$ Hz, H-6), 0.98, 1.17, 1.25, 1.29, 1.51, 1.60 (each 3H, s, 6 × CH_3), 2.08, 2.11(each 3H, s, 2 × COC*H*$_3$), 2.68 (1H, d, $J = 11.5$ Hz, H-17), 3.48 (1H, dd, $J = 11.5$, 4.0 Hz, H-3α), 4.45 (1H, d, $J = 13.0$ Hz, H-18), 4.56 (1H, d, $J = 13.0$ Hz, H-18), 5.63 (1H, d, $J = 8.8$ Hz, H-15), 5.90 (1H, dd, $J = 11.5$, 8.8 Hz, H-16), 3.70 (1H, t, $J = 10.6$ Hz, H-5′), 3.99 (1H, t, $J = 8.2$ Hz, H-2′), 4.11 (1H, t, $J = 8.7$ Hz, H-3′), 4.19 (1H, m, H-4′), 4.32 (1H, dd, $J = 11.2$, 5.1 Hz, H-5′), 4.83 (1H, d, $J = 7.5$ Hz, H-1′); ^{13}C NMR δ 15.3 (q, C-30), 15.4 (q, C-29), 19.2 (s, C-9), 20.5 (t, C-6), 21.2 (s, 2 × COC*H*$_3$), 25.6 (q, C-26), 25.7 (q, C-27 and C-28), 26.0 (t, C-7), 26.3 (t, C-11), 27.7 (s, C-10), 28.0 (t, C-12), 30.0 (t, C-23), 30.9 (t, C-2), 31.5 (t, C-19), 32.3 (t, C-1), 32.4 (q, C-21), 38.2 (t, C-22), 41.3 (s, C-4), 45.8 (s, C-13), 47.1 (d, C-5), 47.2 (d, C-8), 51.5 (s, C-14), 56.3 (d, C-17), 66.4 (t, C-18), 67.0 (t, C-5′), 71.2 (d, C-4′),72.8 (s, C-25), 75.2 (d, C-16), 75.5 (d, C-2′), 78.5 (d, C-3′), 82.1 (d, C-15), 86.8 (s, C-20), 88.3 (d, C-3), 107.5 (d, C-1′), 114.3 (s, C-24), 170.5 (s, *C*OCH$_3$), 170.8 (s, *C*OCH$_3$); positive FABMS 721 $[M + H]^+$, 589 $[M - 132 + H]^+$, 571 $[M - 150 + H]^+$.

Acid Hydrolysis of 1−7. Compounds **1**−**7** (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-glucose ($R_f = 0.39$) for **1**−**2** and D-xylose ($R_f = 0.48$) for **3**−**7** by co-TLC [*n*-BuOH−AcOH−H_2O (4:1:1)] when compared with authentic samples.

Acknowledgment. This work was supported by the National Natural Science Foundation of the People's Republic of China, under Grant No. 29732040.

References and Notes

(1) *Florae Reipublicae Popularis Sinincae*, Tomus 27; Science Press: Beijing, 1979; pp 88−90.
(2) Sakurai, N.; Nagai, M.; Goto, T.; Inoue, T.; Xiao, P. G. *Chem. Pharm. Bull.* **1995**, *41*, 272−275.
(3) Sakurai, N.; Nagai, M.; Nagase, M.; Kawai, K.; Inoue, T.; Xiao, P. G. *Chem. Pharm. Bull.* **1986**, *34*, 582−589.
(4) Inoue, T.; Sakurai, T.; Nagaim, M.; Xiao, P. G. *Phytochemistry* **1985**, *24*, 1329−1331.
(5) Sakurai, N.; Goto, T.; Nagai, M.; Inoue, T.; Xiao, P. G. *Heterocycles* **1990**, *30*, 897−904.
(6) Ju, J. H.; Liu, D.; Lin, G.; Xu, X. D.; Han, B.; Yang, J. S.; Tu, G. Z.; Ma, L. B. *J. Nat. Prod.* **2002**, *65*, 42−47.
(7) Crystallographic data for beesioside J (**3**) reported in this paper have been deposited with the Cambridge Crystallographic Data Center. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44-(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

NP010294H