Sulfur-Containing Spiroketal Glycosides from *Breynia fruticosa*

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Phytochemical investigation of the chemical constituents of the aerial parts of *Breynia fruticosa* afforded 10 sulfur-containing spiroketal glycosides, including eight new compounds (**1**−**8**) and two known compounds, epibreynin B (**9**) and breynin B (**10**). Epibreynin B (**9**) was previously reported as a semisynthetic oxidation product of breynin A and is now reported from a natural source for the first time. The structures of **1**−**10** were determined on the basis of spectroscopic and chemical methods.

The genus *Breynia* (Euphorbiaceae) is comprised of 25 species of plants, seven of which are found in China. *Breynia fruticosa* (L.) Hook. f. is the one distributed abundantly in the south of China and has been used as a Chinese folk medicine for the treatment of chronic bronchitis and wounds. Previous phytochemical studies on *Breynia* plants led to the identification of sulfur-containing spiroketal glycosides,[1−4] alkaloids,[5,6] and terpenoid and phenolic glycosides,[7] along with several other components.[8,9] Among those identified components, breynins A and B, the two sulfur-containing spiroketal glycosides, exhibited significant oral hypocholesterolemic activity.[1−4] As a part of our ongoing program on the discovery of new bioactive components from terrestrial plants, the aerial parts of *B. fruticosa* were studied systematically. This paper describes the isolation and structural elucidation of eight new sulfur-containing spiroketal glycosides (**1**−**8**), along with two known compounds, epibreynin B (**9**) and breynin B (**10**). Epibreynin B (**9**) was previously reported as a semisynthetic oxidation product of breynin A[4] and is now reported from a natural source. The structural identification of these compounds is based on the known skeleton, breynolide, whose structure was secured via single-crystal X-ray analysis[4,10] and also on the basis of spectroscopic and chemical methods.

Results and Discussion

Powdered, air-dried aerial parts of *Breynia fruticosa* (30.0 kg) were percolated with 95% EtOH (×3) at room temperature. The filtrate was concentrated to dryness *in vacuo* and then suspended in 20% EtOH. After filtration of the precipitated chlorophyll and evaporation of EtOH from the filtrate, the aqueous residue was extracted with $CHCl_3$ and *n*-BuOH, successively. Ten sulfur-containing compounds were afforded from the *n*-BuOH fraction through a series of column chromatographies including silica gel and Sephadex LH-20 columns, HPLC, and PTLC steps.

Compound **1** showed a pseudomolecular ion at *m/z* 921.3094 $[M + H]^+$ in the positive-ion mode HRESIMS and at *m/z* 943.6 $[M + Na]^+$ in the positive ESIMS. When considered in conjunction with its ^{13}C NMR data, it indicated a molecular formula of $C_{40}H_{56}O_{22}S$. The assignment of ^1H and ^{13}C NMR data (Tables 1 and 2) was based on HSQC, HMBC, $^1H-^1H$ COSY (Figure 1), and TOCSY spectra. In the ^{13}C NMR spectrum of **1**, the presence of three sugar units was indicated, along with 22 carbons for the aglycone, breynogenin (**11**), which was identified by comparison of spectroscopic data with literature values.[4,11] The identities of the sugar moieties were elucidated from extensive analysis of the NMR data (^{13}C, ^1H, $^1H-^1H$ COSY, TOCSY, HMBC, ROESY, and HSQC) of the carbohydrate chain. From the three anomeric carbons (δ_C 106.9, 104.1, and 102.9) and three anomeric protons [δ_H 4.08 (1H, d, $J = 7.4$ Hz), 4.42 (1H, d, $J = 7.7$ Hz), and 5.10 (1H, d, $J = 1.5$ Hz)] and other characteristic NMR resonances, the sugar units were identified as β-quinovopyranose, β-glucopyranose, and α-rhamnopyranose, which was further confirmed by strong NOEs between H-1_{qui} and H-3_{qui}, H-5_{qui}; H-1_{glc} and H-3_{glc}, H-5_{glc}; and H-1_{rha} and H-2_{rha} in its ROESY spectrum.[12] The ^{13}C NMR chemical shift of C-3 (δ_C 91.8) suggested that **1** had a glycosyl linkage at C-3. The sugar sequence and their linkage sites were derived from the HMBC experiment, correlating H-1_{qui} (δ_H 4.42)/C-3 (δ_C 91.8), H-1_{glc} (δ_H 4.08)/C-2_{qui} (δ_C 86.2), and H-1_{rha} (δ_H 5.10)/C-3_{glc} (84.6). The remaining HMBC correlations are shown in Figure 1. Other COSY and HMBC correlations (Figure 1) were consistent with the aglycone of **1** being breynogenin (**11**). Thus, compound **1** was determined as breynogenin 3-*O*-α-rhamnopyranosyl-(1→3)-β-glucopyranosyl-(1→2)-β-quinovopyranoside, trivial name breynin C.

Compound **2** showed a quasimolecular ion $[M + Na]^+$ at *m/z* 959.2845 in the positive HRESIMS, indicative of a molecular formula of $C_{40}H_{56}O_{23}S$. When its ^1H and ^{13}C NMR data (Tables 1 and 2) were compared with those of **1**, the deshielding of C-2 (from δ_C 36.3 to 62.1) and C-17 (from δ_C 47.3 to 62.8) of the aglycone was observed. Comparison of their NMR data with those in the literature showed that the aglycone of **2** was β-sulfoxidebreynogenin.[4,13] In order to further confirm its structure, acetylation of **2** was performed to give the undecaacetate **2a**. Its ^1H NMR spectrum displayed more clearly separated sugar proton resonances. Analysis of its $^1H-^1H$ COSY, TOCSY, and ROESY spectra enabled assignment of all sugar protons of **2a**. The chemical shifts of the shielded H-2_{qui} (δ_H 3.58) and H-3_{glc} (δ_H 3.65) in the ^1H NMR spectrum of **2a**, and also the NOE correlations between H-1_{qui} (δ_H 4.36) and H-3 (δ_H 3.89), between H-1_{glc} (δ_H 4.49) and H-2_{qui} (δ_H 3.58), and between H-1_{rha} (δ_H 4.72) and H-3_{glc} (δ_H 3.65), indicated identical glycosyl moieties in **1** and **2**. Analysis of the $^1H-^1H$ COSY, TOCSY, and ROESY spectra of **2a** also confirmed the structure of its aglycone. Accordingly, compound **2** was characterized as β-sulfoxidebreynogenin 3-*O*-α-rhamnopyranosyl-(1→3)-β-glucopyranosyl-(1→2)-β-quinovopyranoside (epibreynin D).

Compounds **3**, **4**, and **5** were assigned the elemental compositions $C_{34}H_{46}O_{19}S$, $C_{39}H_{54}O_{23}S$, and $C_{46}H_{66}O_{27}S$, respectively, from analysis of HRESIMS and NMR data. On the basis of HSQC, TOCSY, $^1H-^1H$ COSY, and HMBC spectra, all ^1H and ^{13}C resonances of **3**, **4**, and **5** were assigned as shown in Tables 1 and 2. Comparison of the NMR data of **3**, **4**, and **5** with **2** showed that these compounds possessed the same aglycone but differed glycosyl moieties. The anomeric resonances at δ_H 4.12 (1H, d, $J = 7.2$ Hz) and 4.44 (1H, d, $J = 7.8$ Hz) in the ^1H NMR spectrum suggested that **3** had two sugar units, which were determined to be β-glucopyranose and β-quinovopyranose by a combination of $^1H-^1H$ COSY, HSQC, and ROESY experiments and a detailed comparison

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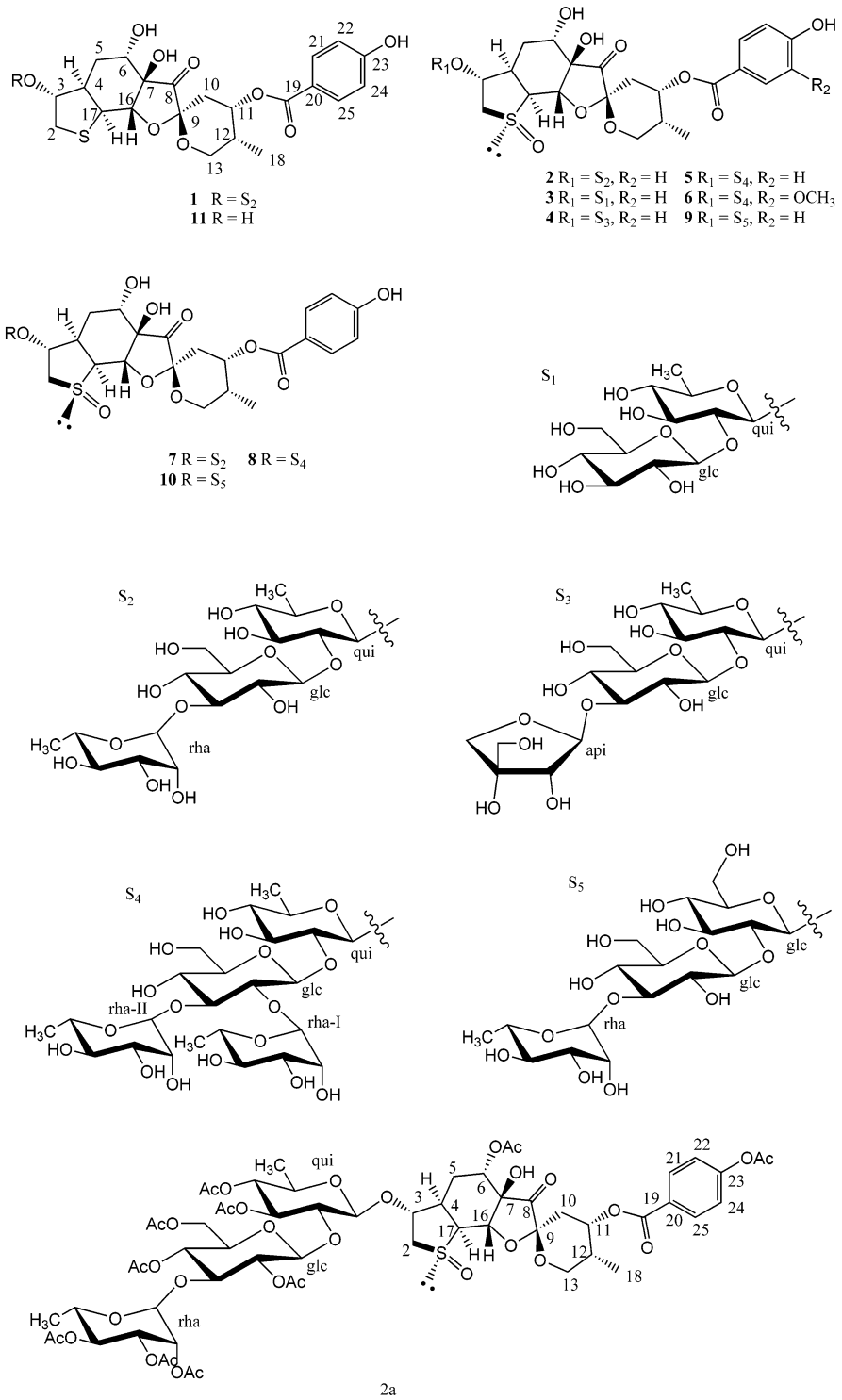
Chart 1



of NMR data with those of **2**. The HMBC spectrum showed cross-peaks between the anomeric protons H-1$_{qui}$ with C-3, C-2$_{qui}$, C-3$_{qui}$, and C-5$_{qui}$ and H-1$_{glc}$ with C-2$_{qui}$, C-2$_{glc}$, C-3$_{glc}$, and C-5$_{glc}$, respectively. These interactions supported the presence of a sugar moiety, glc-(1→2)-qui, and its linkage to C-3 of *β*-sulfoxidebreynogenin. From the above evidence, the structure of **3** was established as *β*-sulfoxidebreynogenin 3-*O*-*β*-glucopyranosyl-(1→2)-*β*-quinovopyranoside.

The NMR data of **4** were similar to those of **3**, except for resonances of one additional sugar unit. On the basis of $^{1}H-^{1}H$ COSY, HSQC, and TOCSY spectra, all sugar protons and carbons could be clearly assigned (Tables 1 and 2), from which the extra sugar unit was concluded to be *β*-apiofuranose.[12,14] The interglycosidic linkages were determined from the following HMBC correlations: H-1$_{qui}$ (δ_H 4.42)/C-3 (δ_C 88.2); H-1$_{glc}$ (δ_H 4.12)/C-2 $_{qui}$ (δ_C 86.1); and H-1$_{api}$ (δ_H 5.22)/C-3$_{glc}$ (δ_C 86.4). On the basis of the above evidence, compound **4** was identified as *β*-sulfoxidebreynogenin 3-*O*-*β*-apiofuranosyl-(1→3)-*β*-glucopyranosyl-(1→2)-*β*-quinovopyranoside.

The NMR data of compound **5** were similar to those of **2** (Tables 1 and 2), except for the presence of one additional sugar unit, which was concluded to be α-rhamnopyranose from the characteristic NMR spin patterns. The monosaccharide sequence was determined by analysis of HMBC correlations. Cross-peaks were observed

Table 1. ^{13}C NMR Data of **1**−**8** (100 MHz, CD_3OD)

position	1	2	3	4	5	6	7	8
2	36.3 (t)	62.1 (t)	62.1 (t)	61.9 (t)	61.7 (t)	61.7 (t)	57.7 (t)	57.9 (t)
3	91.8 (d)	88.2 (d)	88.1 (d)	88.2 (d)	86.2 (d)	86.7 (d)	87.9 (d)	86.1 (d)
4	39.7 (d)	40.3 (d)	40.2 (d)	40.2 (d)	40.1 (d)	40.1 (d)	38.8 (d)	38.8 (d)
5	27.7 (t)	29.0 (t)	29.0 (t)	29.0 (t)	29.0 (t)	28.9 (t)	29.6 (t)	29.8 (t)
6	71.2 (d)	71.8 (d)	71.9 (d)	71.9 (d)	71.0 (d)	71.2 (d)	70.6 (d)	70.5 (d)
7	76.0 (s)	73.8 (s)	73.7 (s)	73.7 (s)	73.8 (s)	73.7 (s)	75.5 (s)	75.6 (s)
8	213.7 (s)	210.3 (s)	210.9 (s)	210.5 (s)	210.4 (s)	210.3 (s)	212.7 (s)	212.6 (s)
9	100.1 (s)	101.4 (s)	101.5 (s)	101.5 (s)	101.2 (s)	101.3 (s)	100.9(s)	100.7 (s)
10	32.9 (t)	33.0 (t)	32.9 (t)	33.0 (t)	32.3 (t)	32.8 (t)	32.7 (t)	32.2 (t)
11	70.2 (d)	70.3 (d)	70.2 (d)	70.2 (d)	70.2 (d)	70.2 (d)	70.2 (d)	70.2 (d)
12	34.7 (d)	34.6 (d)	34.6 (d)	34.6 (d)	34.4 (d)	34.5 (d)	34.6 (d)	34.4 (d)
13	64.1 (t)	64.3 (t)	64.0 (t)	64.4 (t)	64.6 (t)	64.4 (t)	64.7 (t)	65.2 (t)
16	77.4 (d)	74.7 (d)	74.4 (d)	74.7 (d)	74.7 (d)	74.7 (d)	75.4 (d)	75.6 (d)
17	47.3 (d)	62.8 (d)	62.7 (d)	62.7 (d)	62.2 (d)	62.5 (d)	71.0 (d)	70.0 (d)
18	13.1 (q)	13.2 (q)	13.2 (q)	13.2 (q)	13.0 (q)	13.0 (q)	13.0 (q)	12.7 (q)
19	168.0 (s)	168.0 (s)	168.0 (s)	168.0 (s)	168.0 (s)	168.0 (s)	168.0 (s)	168.0 (s)
20	123.0 (s)	123.2(s)	122.9 (s)	122.8 (s)	123.0 (s)	116.9 (s)	123.0(s)	122.9 (s)
21	133.7 (d)	133.6 (d)	133.6 (d)	133.6 (d)	133.5 (d)	126.2 (d)	133.6 (d)	133.5 (d)
22	117.0 (d)	116.9(d)	116.9 (d)	117.0 (d)	116.7 (d)	116.8 (d)	117.0 (d)	116.8 (d)
23	163.8 (s)	163.6 (s)	164.1 (s)	164.2 (s)	163.6 (s)	149.0 (s)	163.8 (s)	163.8 (s)
24	117.0 (d)	116.9 (d)	116.9 (d)	117.0 (d)	116.7 (d)	149.0 (s)	117.0 (d)	116.8 (d)
25	133.7 (d)	133.6 (d)	133.6 (d)	133.6 (d)	133.5 (d)	113.8 (d)	133.7 (d)	133.5 (d)
24-OCH_3						57.0 (q)		
	qui	qui	qui	qui	qui	qui	qui	qui
1	104.1 (d)	103.4 (d)	103.3 (d)	103.4 (d)	102.2 (d)	102.5 (d)	103.9 (d)	102.7 (d)
2	86.2 (d)	85.8 (d)	85.8 (d)	86.1 (d)	82.2 (d)	83.3 (d)	85.0 (d)	81.2 (d)
3	77.9 (d)	77.7 (d)	77.9 (d)	77.8 (d)	78.1 (d)	78.3 (d)	78.0 (d)	78.4 (d)
4	76.5 (d)	76.4 (d)	76.3 (d)	76.4 (d)	76.7 (d)	76.7 (d)	76.5 (d)	77.0 (d)
5	73.7 (d)	73.6 (d)	74.0 (d)	73.8 (d)	73.6 (d)	73.6 (d)	73.7 (d)	73.7 (d)
6	18.4 (q)	18.4 (q)	18.4 (q)	18.4 (q)	18.1 (q)	18.1 (q)	18.3 (q)	18.1 (q)
	glc	glc	glc	glc	glc	glc	glc	glc
1	106.9 (d)	106.9 (d)	107.0 (d)	106.9 (d)	104.0 (d)	104.5 (d)	106.5 (d)	103.7 (d)
2	77.0 (d)	77.0 (d)	76.7 (d)	76.0 (d)	79.8 (d)	79.5 (d)	77.0 (d)	79.8 (d)
3	84.6 (d)	84.3 (d)	78.0 (d)	86.4 (d)	87.6 (d)	88.0 (d)	84.2 (d)	87.6 (d)
4	69.4 (d)	69.4 (d)	70.9 (d)	69.3 (d)	70.1 (d)	69.9 (d)	69.7 (d)	70.5 (d)
5	77.5 (d)	77.7 (d)	77.7 (d)	77.4 (d)	77.3 (d)	77.1 (d)	77.8 (d)	77.7 (d)
6	61.9 (t)	62.0 (t)	62.0 (t)	61.9 (t)	62.1 (t)	61.8 (t)	62.2 (t)	62.5 (t)
	rha	rha		api	rha-I	rha-I	rha	rha-I
1	102.9 (d)	102.8 (d)		111.7 (d)	103.0 (d)	103.0 (d)	102.8 (d)	102.9 (d)
2	72.5 (d)	72.5 (d)		78.2 (d)	72.1 (d)	72.2 (d)	72.5 (d)	72.2 (d)
3	72.6 (d)	72.6 (d)		80.7 (s)	72.3 (d)	72.3 (d)	72.6 (d)	72.4 (d)
4	74.3 (d)	74.3 (d)		75.3 (t)	73.6 (d)	74.1 (d)	74.3 (d)	74.0 (d)
5	70.5 (d)	70.4 (d)		65.3 (t)	70.8 (d)	70.8 (d)	70.4 (d)	70.6 (d)
6	18.2 (q)	18.2 (q)			18.4 (q)	18.4 (q)	18.2 (q)	18.5 (q)
					rha-II	rha-II		rha-II
1					103.9 (d)	104.0 (d)		104.0 (d)
2					72.6 (d)	72.6 (d)		72.7 (d)
3					72.4 (d)	72.4 (d)		72.5 (d)
4					73.8 (d)	73.8 (d)		74.3 (d)
5					71.7 (d)	71.8 (d)		71.0 (d)
6					18.4 (q)	18.5 (q)		18.4 (q)

between H-1_{qui} (δ_H 4.42)/C-3 (δ_C 86.2); H-1_{glc} (δ_H 4.58)/C-2 $_{qui}$ (δ_C 82.2); H-1_{rha-I} (δ_H 5.05)/C-2_{glc} (δ_C 79.8); and H-1_{rha-II} (δ_H 4.93)/C-3_{glc} (δ_C 87.6). Therefore, the structure of compound **5** was established as *β*-sulfoxidebreynogenin 3-*O*-[α-rhamnopyranosyl-(1→3)]-α-rhamnopyranosyl-(1→2)-*β*-glucopyranosyl-(1→2)-*β*-quinovopyranoside. Compounds **3**−**5** are new and have been assigned the trivial names epibreynins E (**3**), F (**4**), and G (**5**), respectively.

The HRESIMS of compound **6** showed a sodiated molecular ion at *m*/*z* 1135.3535 $[M + Na]^+$, which, in conjunction with ^{13}C NMR data, established the molecular formula of $C_{47}H_{68}O_{28}S$. On the basis of its HSQC, $^1H-^1H$ COSY, and TOCSY NMR spectra, all resonances of compound **6** were assigned as shown in Tables 1 and 2. Comparison of the NMR data for compounds **5** and **6** suggested the presence of the same sugar moieties. The ^{13}C NMR spectrum showed 47 resonances, of which 23 were attributed to the aglycone moiety, including a methoxy group at δ_C 57.0. In the ^1H NMR spectrum, resonances confirming the substitution pattern of the benzoyl moiety [δ_H 7.72 (1H, dd, $J = 8.3$, 1.5 Hz); δ_H 7.56 (1H, d, $J = 1.5$ Hz); δ_H 6.88 (1H, d, $J = 8.3$ Hz)] were observed. The correlation of a methoxy resonance at δ_H 3.89 with a carbon at δ_C 149.0 in the HMBC spectrum indicated a methoxy group at C-24, which was further confirmed by correlations between H-21 (δ_H 7.72) and C-19 (δ_C 168.0), C-23 (δ_C 149.0), C-25 (δ_C 113.8); H-22 (δ_H 6.88) and C-20 (δ_C 116.9), C-24 (δ_C 149.0); and H-25 (δ_H 7.56) and C-19 (δ_C 168.0), C-21 (δ_C 126.2), C-23 (δ_C 149.0) in the HMBC spectrum (Figure 1). The assignments of the sugar moieties and linkages were also confirmed from the HMBC and COSY spectra. Accordingly, compound **6** was determined as 24-methoxy-*β*-sulfoxidebreynogenin 3-*O*-[α-rhamnopyranosyl-(1→3)]-α-rhamnopyranosyl-(1→2)-*β*-glucopyranosyl-(1→2)-*β*-quinovopyranoside and named epibreynin H.

Compound **7** exhibited the same elemental formula ($C_{40}H_{56}O_{23}S$) as **2**. The ^1H and ^{13}C NMR spectra of **7** were similar to those of **2**, except for the significant differences of the chemical shifts of C-2 [δ_C 57.7 (**7**), 62.1 (**2**)], C-17 [δ_C 71.0 (**7**), 62.8 (**2**)], and C-7 [δ_C 75.5 (**7**), 73.8 (**2**)]. Detailed comparison of ^{13}C NMR data of **7** and **2** with those reported revealed the aglycones of **2** and **7** to be isomers at the sulfoxide position.[4] That is, compound **7** possessed α-sulfoxidebreynogenin as its aglycone. Analysis of NMR data (^{13}C, ^1H, $^1H-^1H$ COSY, HSQC, TOCSY, HMBC, and ROESY) showed

Table 2. ^{1}H NMR Data of **1**−**8** (400 MHz, CD_3OD)

position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
2α	3.20 (m)	4.20 (d, 14.9)	4.26 (d, 14.9)	4.26 (d, 15.0)	4.16 (d, 15.0)	4.26 (d, 14.7)	3.78 (dd, 15.4, 5.7)	3.85 (dd, 14.0, 5.7)
2*β*	3.04 (dd, 11.7, 3.3)	3.12 (m)	3.14 (m)	3.13 (m)	3.15 (m)	3.12 (m)	3.25 (m)	3.10 (m)
3	4.32 (br d, 3.3)	4.67 (br d, 4.2)	4.71 (br d, 4.8)	4.60 (br d, 3.7)	4.72 (br s)	4.71 (br s)	4.44 (br d, 4.9)	4.50 (br s)
4	2.85 (dt, 13.3, 4.3)	3.16 (m)	3.18 (m)	3.21 (m)	3.17 (m)	3.20 (m)	3.14 (m)	3.20 (m)
5α	1.65 (dt, 13.7, 3.7)	1.81 (m)	1.64 (m)	1.80 (m)	1.82 (m)	1.84 (m)	1.25 (m)	1.24 (br d, 14.2)
5*β*	1.50 (dt, 2.3, 13.7)	1.81 (m)	1.56 (m)	1.76 (m)	1.82 (m)	1.80 (m)	1.70 (dt, 14.2, 3.6)	1.70 (dt, 14.2, 3.5)
6	3.92 (m)	3.92 (m)	3.84 (m)	3.83 (m)	3.92 (m)	3.90 (m)	3.98 (m)	3.93 (m)
10	2.05 (br d, 3.8)	2.10 (m)	2.04 (m)	2.15 (m)	2.06 (br d, 3.5)	2.14 (m)	2.12 (br d, 4.1)	2.15 (m)
11	5.48 (m)	5.38 (m)	5.40 (m)	5.40 (m)	5.42 (m)	5.46 (m)	5.38 (m)	5.50 (m)
12	2.15 (m)	2.18 (m)	2.18 (m)	2.20 (m)	2.22 (m)	2.23 (m)	2.17 (m)	2.18 (m)
13α	3.58 (m)	4.05 (m)	4.06 (t, 11.0)	4.02 (t, 11.2)	4.04 (m)	4.06 (m)	3.98 (m)	3.90 (m)
13*β*	3.98 (m)	3.65 (m)	3.65 (dd, 11.0, 4.5)	3.65 (dd, 11.2, 4.4)	3.73 (m)	3.69 (m)	3.68 (m)	3.80 (m)
16	4.37 (br s)	4.90 (br s)	4.86 (br s)	4.89 (br s)	4.93 (br s)	4.91 (br s)	4.87 (br s)	4.75 (br s)
17	4.19 (br d, 5.0)	3.94 (m)	3.90 (m)	3.92 (m)	3.98 (m)	3.96 (m)	3.95 (m)	4.08 (m)
18	0.88 (d, 6.9)	0.88 (d, 6.9)	0.89 (d, 6.8)	0.90 (d, 6.8)	0.92 (d, 6.7)	0.93 (d, 7.0)	0.96 (d, 6.9)	0.98 (d, 6.9)
21	8.05 (d, 8.8)	8.05 (d, 8.4)	8.05 (d, 8.6)	8.03 (d, 8.7)	8.00 (d, 8.3)	7.72 (dd, 8.3, 1.5)	8.05 (d, 8.7)	8.00 (d, 8.7)
22	6.90 (d, 8.8)	6.88 (d, 8.4)	6.90 (d, 8.6)	6.88 (d,8.7)	6.86 (d, 8.3)	6.88 (d, 8.3)	6.90 (d, 8.7)	6.90 (d, 8.7)
24	6.90 (d, 8.8)	6.88 (d, 8.4)	6.90 (d, 8.6)	6.88 (8.7)	6.86 (d, 8.3)		6.90 (d, 8.7)	6.90 (d, 8.7)
25	8.05 (d, 8.8)	8.05 (d, 8.4)	8.05 (d, 8.6)	8.03 (8.7)	8.00 (d, 8.3)	7.56 (d, 1.5)	8.05 (d, 8.7)	8.00 (d, 8.7)
24-OCH_3						3.89 (s)		
	qui	qui	qui	qui	qui	qui	qui	qui
1	4.42 (d, 7.7)	4.42 (d, 7.7)	4.44 (d, 7.8)	4.42 (d, 7.7)	4.42 (d, 7.1)	4.42 (d, 7.5)	4.46 (d, 7.5)	4.46 (d, 7.5)
2	3.36 (m)	3.28 (m)	3.30 (m)	3.30 (m)	3.47 (m)	3.41 (m)	3.45 (m)	3.58 (m)
3	3.45 (m)	3.45 (t, 9.2)	3.46 (m)	3.47 (m)	3.50 (m)	3.48 (m)	3.48 (m)	3.52 (m)
4	3.14 (t, 8.2)	3.14 (m)	3.15 (m)	3.13 (m)	3.06 (m)	3.1 (m)	3.14 (m)	3.04 (m)
5	3.31 (m)	3.33 (m)	3.34 (m)	3.38 (m)	3.31 (m)	3.33 (m)	3.32 (m)	3.25 (m)
6	1.35 (d, 6.1)	1.30 (d, 6.1)	1.31 (d, 6.1)	1.25 (d, 6.4)	1.24 (d, 6.0)	1.29 (d, 6.5)	1.28 (d, 6.0)	1.24 (d, 6.6)
	glc	glc	glc	glc	glc	glc	glc	glc
1	4.08 (d, 7.4)	4.05 (d, 7.6)	4.12 (d, 7.2)	4.12 (d, 7.8)	4.58 (d, 7.6)	4.48 (d, 6.9)	4.18 (d, 7.7)	4.70 (d, 7.2)
2	3.23 (m)	3.24 (m)	3.16 (m)	3.30 (m)	3.38 (m)	3.39 (m)	3.23 (m)	3.48 (m)
3	3.25 (m)	3.31 (m)	3.22 (m)	3.32 (m)	3.44 (m)	3.40 (m)	3.32 (m)	3.48 (m)
4	3.30 (m)	3.30 (m)	3.28 (m)	3.33 (m)	3.44 (m)	3.41 (m)	3.34 (m)	3.42 (m)
5	2.38 (dt, 9.2, 2.6)	2.48 (m)	2.46 (dt, 9.2, 2.6)	2.43 (dt, 9.6, 2.4)	2.76 (br d, 9.6)	2.52 (br s)	2.62 (m)	2.95 (m)
6	3.46 (m); 3.62 (m)	3.52 (m); 3.57 (m)	3.50 (m); 3.57 (m)	3.52 (m); 3.56 (m)	3.52 (2H, m)	3.46 (2H, m)	3.56 (m); 3.66 (m)	3.58 (m); 3.66 (m)
	rha	rha		api	rha-I	rha-I	rha	rha-I
1	5.10 (d, 1.5)	5.15 (d, 1.5)		5.22 (d, 3.4)	5.05 (br s)	5.05 (br s)	5.15 (d, 1.3)	5.05 (br s)
2	3.92 (m)	3.93 (m)		4.00 (m)	3.88 (m)	3.85 (m)	3.96 (m)	3.86 (m)
3	3.68 (dd, 9.5, 3.3)	3.67 (m)			3.70 (m)	3.71 (m)	3.72 (m)	3.72 (m)
4	3.40 (t, 9.6)	3.38 (t, 9.5)		3.82 (d, 10.0), 4.11 (m)	3.42 (m)	3.42 (m)	3.40 (m)	3.40 (m)
5	3.94 (m)	3.98 (m)		3.60 (m)	4.03 (m)	4.04 (m)	3.94 (m)	4.05 (m)
6	1.25 (d, 6.3)	1.27 (d, 6.2)			1.27 (d, 6.2)	1.31 (d, 6.1)	1.26 (d, 6.0)	1.30 (d, 6.4)
					rha-II	rha-II		rha-II
1					4.93 (br s)	4.88 (br s)		4.90 (br s)
2					3.90 (m)	3.90 (m)		3.90 (m)
3					3.67 (m)	3.65 (m)		3.65 (m)
4					3.43 (m)	3.42 (m)		3.42 (m)
5					3.96 (m)	3.95 (m)		3.98 (m)
6					1.25 (d, 6.0)	1.25 (d, 6.2)		1.26 (d, 6.2)

that compound **7** had the same saccharide chain as those of **1** and **2**. The correlations of an anomeric proton at H-1_{qui} (δ_H 4.46, ^3J = 7.5 Hz) with C-3 (δ_C 87.9) and that of H-3 (δ_H 4.44) with C-1_{qui} (δ_C 103.9) located the sugar moiety at C-3. The structure of **7** was defined as α-sulfoxidebreynogenin 3-*O*-α-rhamnopyranosyl-(1→3)-*β*-glucopyranosyl-(1→2)-*β*-quinovopyranoside (breynin D).

The molecular formula of compound **8** was determined as $C_{46}H_{66}O_{27}S$ from a pseudomolecular ion at *m*/*z* 1105.3359 $[M + Na]^+$ in the positive-ion mode HRESIMS and at *m*/*z* 1081.3 $[M - H]^-$ in negative-ion mode ESIMS. It exhibited the same elemental formula as **5**. On the basis of HSQC, TOCSY, $^1H-^1H$ COSY, and HMBC spectra, all ^1H and ^{13}C resonances of **8** were assigned as shown in Tables 1 and 2. A detailed NMR comparison of **8**, **7**, and **5** indicated that compound **8** had the same aglycone as **7** and the same saccharide chain as **5**, which was further confirmed by NMR experiments. In the ^{13}C NMR spectra, the most critical differences between compounds **8** and **5** were at C-2 (δ_C 57.9 and 61.7), C-17 (δ_C 70.0 and 62.2), and C-7 (δ_C 75.6 and 73.8), which indicated that **8** and **5** were also diastereomers.[4] Compound **8** was thus established as α-sulfoxidebreynogenin 3-*O*-[α-rhamnopyranosyl-(1→3)]-α-rhamnopyranosyl-(1→2)-*β*-glucopyranosyl-(1→2)-*β*-quinovopyranoside (breynin G).

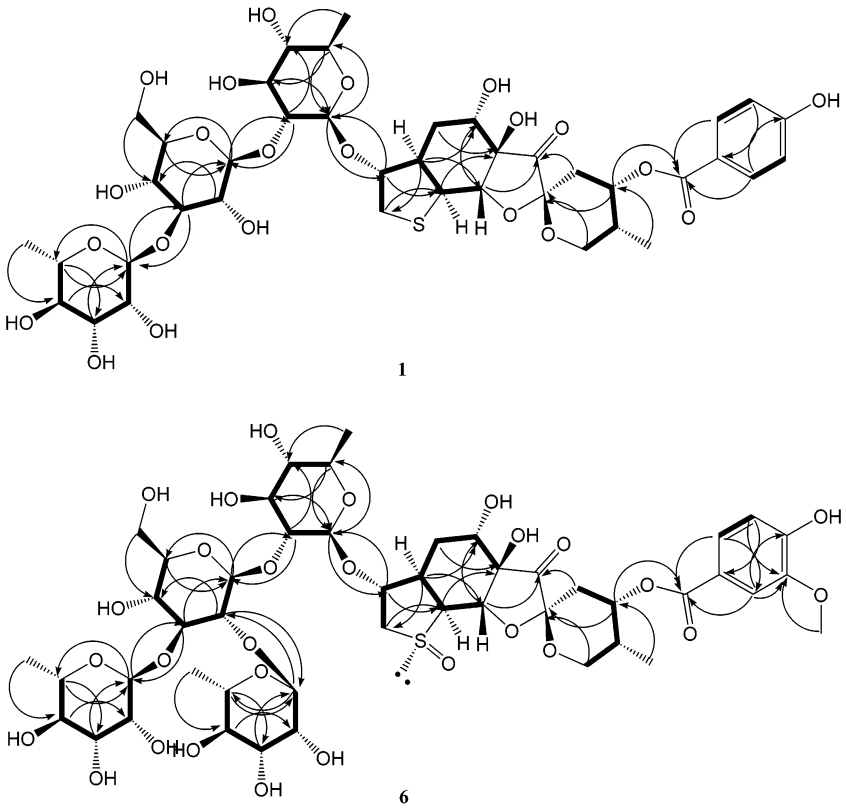


Figure 1. $^1H-^1H$ COSY (—) and key HMBC correlations ($^1H \curvearrowright {}^{13}C$) of compounds **1** and **6**.

Two known sulfur-containing spiroketal glycosides, **9** (epibreynin B) and **10** (breynin B), were also isolated from *B. fruticosa*. Although compound **9** was previously reported as a semisynthetic oxidation product,[4] this is the first time that it has been isolated from a natural source. Compounds **9** and **10** could be obtained by oxidation of breynin A with Davis' phenyloxaziridine in a 1:7 ratio.[4] Although compounds **2**−**10** containing sulfoxide functions may be oxidative artifacts during the isolation process, they may also be oxidized by the oxidase in the plant. Natural products with sulfoxide and sulfone groups have also been reported from *Allium* species.[15−18] The skeleton of the aglycones of **1**−**10** could be classified into the sesquiterpenoid category; however, sulfur-containing sesquiterpenes from terrestrial resources are relatively rare.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 241MC polarimeter. IR spectra were recorded using a Perkin-Elmer 577 spectrometer. LR-ESIMS were measured using a Finnigan LCQ-DECA instrument, and HR-ESIMS data were obtained on a Mariner spectrometer. NMR spectra were recorded on a Bruker AM 400 or INOVA-600 spectrometer with TMS as internal standard, and chemical shifts are expressed in δ ppm. Preparative HPLC was carried out using a Varian SD-1 instrument, equipped with Merck NW25 C_{18} column (10 μm, 20 mm × 250 mm), and Prostar 320 UV/vis detector. Column chromatographic separations were carried out by using polyporous resin D-101 (Huazhen Corporation of Science and Technology, Shanghai, China), silica gel H60 (300−400 mesh) (Qingdao Haiyang Chemical Group Corporation, Qingdao, China), and Sephadex LH-20 (Pharmcia Biotech AB, Uppsala, Sweden) as packing materials. HSGF254 silica gel TLC plates (Yantai Chemical Industrial Institute, Yantai, China) and RP-18 WF_{254} TLC plates (Merck) were used for analytical TLC.

Plant Material. The aerial parts of *B. fruticosa* were collected in the suburb of Nanning, Guangxi Province, People's Republic of China, in June 2005 and identified by Prof. Ding Fang of Guangxi Institute of Traditional Chinese Medicine. A voucher specimen (No. SIMMS0506) is deposited in the Herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Extraction and Isolation. The aerial parts of *B. fruticosa* (30.0 kg) were powdered and percolated at room temperature with 95% EtOH (40 L × 3). The filtrate was concentrated to dryness *in vacuo*, suspended in 20% EtOH, and filtered. After evaporation of EtOH from the filtrate, the aqueous residue (10 L) was extracted with $CHCl_3$ and *n*-BuOH (10 L × 3 each), successively. The *n*-BuOH fraction (800 g) was subjected to a column of Diaion D-101 and eluted with H_2O and 25, 50, 75, and 100% MeOH. The 75% MeOH fraction (40 g) was further separated by VLC over a silica gel column using $CHCl_3$/MeOH (10:1, 8:1, 6:1, 4:1, 2:1, and 1:1) to give five subfractions (A−E). Fraction D (4.96 g) was chromatographed on a silica gel column eluted with a gradient of $CHCl_3$/MeOH (3:1and 1:1) to give four subfractions (D_1−D_4). Fraction D_3 (432 mg) was further subjected to HPLC eluted with MeOH/H_2O (10% to 70% MeOH within 90 min) and then over Sephadex LH-20 CC with 95% EtOH to yield **1** (18 mg). Further purification of subfraction D_4 (170 mg), by HPLC using MeOH/H_2O (10% to 70% MeOH within 90 min) as eluant, afforded four subfractions, D_{41}−D_{42}. Fraction D_{41} was further purified by PTLC (developed with *n*-BuOH/HOAc/H_2O, 6:1:1) and Sephadex LH-20 CC using 95% EtOH as eluant to afford **10** (10 mg). Fraction E (11.6 g) was subjected to Sephadex LH-20 CC with 95% EtOH to give two subfractions, E_1 and E_2. Fraction E_1 (1.20 g) was chromatographed on a silica gel column eluting with a $CHCl_3$/MeOH/H_2O (3:1:0.1) gradient to yield **2** (137 mg) and another two subfractions, E_{11} and E_{12}. Compounds **3** (7.0 mg) and **4** (9.0 mg) were obtained from fraction E_{11} (67 mg) by PTLC (developed with *n*-BuOH/HOAc/H_2O, 6:1:1) and Sephadex LH-20 CC with 95% EtOH. Fraction E_{12} (320 mg) was further subjected to HPLC eluted with MeOH/H_2O (10% to 30% MeOH within 20 min and 30% to 70% MeOH within 70 min) to yield **5** (85 mg), **6** (14 mg), **7** (20 mg), **8** (16 mg), and fraction E_{121}. From fraction E_{121} (17 mg), **9** (11 mg) was obtained using PTLC (developed with *n*-BuOH/HOAc/H_2O, 6:1:1) and was purified by Sephadex LH-20 CC with 95% EtOH.

Breynin C (1): amorphous powder; $[\alpha]^{24}_D$ +7.3 (*c* 0.60, MeOH); IR (KBr) ν_{max} 3420, 2933, 1782, 1691, 1608, 1516, 1281, 1167, 1072, 773 cm^{-1}; ^{13}C and ^1H NMR data, see Tables 1 and 2; ESIMS (positive-ion mode) *m*/*z* 943.6 $[M + Na]^+$; HRESIMS *m*/*z* 921.3094 $[M + H]^+$ (calcd for $C_{40}H_{57}O_{22}S$, 921.3062).

Epibreynin D (2): amorphous powder; $[\alpha]^{24}_D$ −1.4 (*c* 0.57, MeOH); IR (KBr) ν_{max} 3415, 2933, 1784, 1697, 1608, 1516, 1279, 1165, 1070,

773 cm^{-1}; ^{13}C and ^{1}H NMR data, see Tables 1 and 2; ESIMS (positive-ion mode) *m/z* 959.2 $[M + Na]^+$; HRESIMS *m/z* 959.2845 $[M + Na]^+$ (calcd for $C_{40}H_{56}O_{23}SNa$, 959.2831).

Epibreynin E (3): colorless gum; $[\alpha]^{24}_D$ +4.4 (*c* 0.34, MeOH); IR (film) ν_{max} 3410, 2921, 1784, 1693, 1610, 1516, 1279, 1165, 1072, 760 cm^{-1}; ^{13}C and ^{1}H NMR data, see Tables 1 and 2; ESIMS (positive-ion mode) *m/z* 813.1 $[M + Na]^+$; HRESIMS *m/z* 813.2257 $[M + Na]^+$ (calcd for $C_{34}H_{46}O_{19}SNa$, 813.2252).

Epibreynin F (4): colorless gum; $[\alpha]^{24}_D$ −1.3 (*c* 0.48, MeOH); IR (film) ν_{max} 3410, 2920, 1784, 1692, 1610, 1516, 1279, 1165, 1072, 760 cm^{-1}; ^{13}C and ^{1}H NMR data, see Tables 1 and 2; ESIMS (positive-ion mode) *m/z* 945.1 $[M + Na]^+$; HRESIMS *m/z* 945.2706 $[M + Na]^+$ (calcd for $C_{39}H_{54}O_{23}SNa$, 945.2674).

Epibreynin G (5): amorphous powder; $[\alpha]^{24}_D$ −7.7 (*c* 0.76, MeOH); IR (KBr) ν_{max} 3406, 2933, 1784, 1694, 1608, 1514, 1279, 1167, 1070, 774 cm^{-1}; ^{13}C and ^{1}H NMR data, see Tables 1 and 2; ESIMS (positive-ion mode) *m/z* 1105.2 $[M + Na]^+$; HRESIMS *m/z* 1105.3418 $[M + Na]^+$ (calcd for $C_{46}H_{66}O_{27}SNa$, 1105.3410).

Epibreynin H (6): colorless gum; $[\alpha]^{24}_D$ −9.6 (*c* 0.80, MeOH); IR (KBr) ν_{max} 3419, 2933, 1784, 1697, 1599, 1512, 1284, 1168, 1074, 1043, 770 cm^{-1}; ^{13}C and ^{1}H NMR data, see Tables 1 and 2; ESIMS (positive-ion mode) *m/z* 1135.2 $[M + Na]^+$; HRESIMS *m/z* 1135.3535 $[M + Na]^+$ (calcd for $C_{47}H_{68}O_{28}SNa$, 1135.3516).

Breynin D (7): colorless gum; $[\alpha]^{24}_D$ −10.0 (*c* 0.62, MeOH); IR (film) ν_{max} 3385, 2933, 1782, 1691, 1610, 1516, 1277, 1167, 1072, 773 cm^{-1}; ^{13}C and ^{1}H NMR data, see Tables 1 and 2; ESIMS (positive-ion mode) *m/z* 959.2 $[M + Na]^+$; HRESIMS *m/z* 959.2850 $[M + Na]^+$ (calcd for $C_{40}H_{56}O_{23}SNa$, 959.2831).

Breynin G (8): colorless gum; $[\alpha]^{24}_D$ −17.5 (*c* 0.87, MeOH); IR (KBr) ν_{max} 3415, 2933, 1782, 1691, 1608, 1514, 1279, 1167, 1074, 772 cm^{-1}; ^{13}C and ^{1}H NMR data, see Tables 1 and 2; ESIMS (positive-ion mode) *m/z* 1105.2 $[M + Na]^+$; HRESIMS *m/z* 1105.3359 $[M + Na]^+$ (calcd for $C_{46}H_{66}O_{27}SNa$, 1105.3410).

Acetylation of Epibreynin D (2). Ac_2O (1.0 mL) was added to a solution of epibreynin D (**2**) (4.0 mg) in pyridine (1.0 mL). The mixture was allowed to stir at room temperature for 48 h. Following concentration *in vacuo* and purification by PTLC (developed with $CHCl_3$), the undecaacetate **2a** was obtained (2.3 mg).

Compound 2a: amorphous powder; $[\alpha]^{24}_D$ −4.0 (*c* 0.10, MeOH); ESIMS (positive-ion mode) *m/z* 1379.2 $[M + Na]^+$; ^{1}H NMR (600 MHz, $CDCl_3$) 8.10 (2H, d, J = 8.8 Hz, H-21/25), 7.15 (2H, d, J = 8.8 Hz, H-22/24), 5.44 (1H, m), 5.04 (1H, t, J = 9.3 Hz, H-3_{qui}), 4.70−5.04 (6H, m, H-6, H-2_{glc}, H-4_{glc}, H-2_{rha}, H-3_{rha}, H-4_{rha}), 4.72 (1H, br s, H-1_{rha}), 4.61 (1H, br d, J = 4.4 Hz, H-16), 4.57 (1H, t, J = 9.8 Hz, H-4_{qui}), 4.49 (1H, d, J = 7.8 Hz, H-1_{glc}), 4.36 (1H, d, J = 7.8 Hz, H-1_{qui}), 3.98−4.8 (4H, m, H-2, H-6_{glc}), 3.93 (1H, dd, J = 11.3, 7.3 Hz, H-13α), 3.89 (1H, br d, J = 5.8 Hz, H-3), 3.78−3.85 (2H, m, H-13β, H-5_{rha}), 3.65 (1H, t, J = 9.2 Hz, H-3_{glc}), 3.58 (1H, t, J = 9.3 Hz, H-2_{qui}), 3.40−3.46 (2H, m, H-5_{qui}, H-5_{glc}), 3.10 (1H, dd, J = 9.3, 5.4 Hz, H-17), 2.80 (1H, m, H-4), 2.34 (3H, s, CH_3CO), 2.19−2.30 (3H, m, H-10, H-12), 2.15 (3H, s, CH_3CO), 2.10 (3H, s, CH_3CO), 2.09 (3H, s, CH_3CO), 2.08 (3H, s, CH_3CO), 2.05 (3H, s, CH_3CO), 2.01(3H, s, CH_3CO), 2.00 (3H, s, CH_3CO), 1.97 (3H, s, CH_3CO), 1.95 (1H, m, H-5β), 1.91 (3H, s, CH_3CO), 1.85 (1H, m, H-5α), 1.09 (3H, d, J = 6.4 Hz, H-6_{rha}), 1.06 (3H, d, J = 6.3 Hz, H-6_{qui}), 1.02 (3H, d, J = 6.8 Hz, H-18).

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Supporting Information Available: 1D and 2D NMR spectra of compound **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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