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Triterpene and Sterol Derivatives from the Roots of *Breynia fruticosa*

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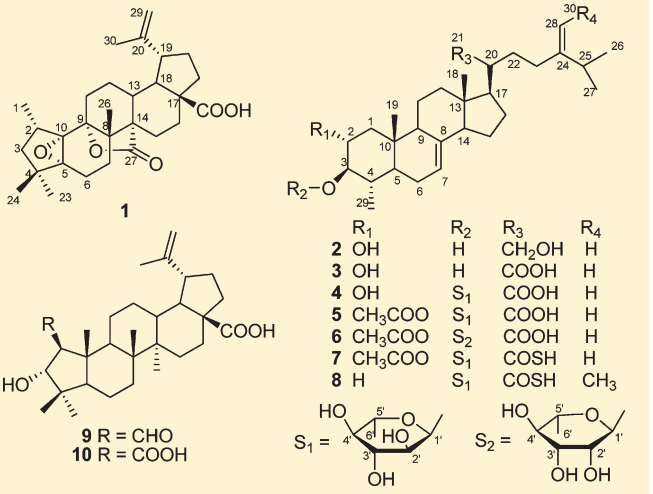
Supporting Information

ABSTRACT: A new nor-ceanothane-type triterpenoid, breynceanothanolic acid (**1**), and seven novel 4α-methyl sterols, fruticosides A−G (**2**−**8**), were obtained from the roots of *Breynia fruticosa*. The new compound structures were established by means of extensive spectroscopic and chemical methods. Compounds **7** and **8** are sulfur-containing derivatives of the 4α-methyl sterols, and the sugar moiety of compounds **4**, **5**, **7**, and **8** (L-quinovose) is uncommon in plants. Compounds **1** and **2** exhibited moderate cytotoxicity against five human cancer cell lines.



4-Methyl sterols such as dinosterol[1] often occur in marine phytoplankton, principally dinoflagellates and diatoms.[2] They are of particular importance because they are considered to be unambiguous biomarkers for organic matter derived from dinoflagellates in sediments and crude oils.[3] Although many 4-methyl sterols have been identified from the marine dinoflagellates[4] and soft corals,[5] the presence of 4-methyl sterols is rare in plants.[6] *Breynia fruticosa* (L.) Hook. f. (Euphorbiaceae) has been used as a folk medicine for the treatment of chronic bronchitis and inflammation by the "Dai" ethnic minority in southern China.[7] A novel nor-ceanothane-type triterpenoid, breynceanothanolic acid (**1**), seven new 4α-methyl steroids, fruticosides A−G (**2**−**8**), and 16 known compounds were isolated from the roots of *B. fruticosa*. Compounds **1**−**8**, zizyberanalic acid (**9**),[8] and isoceanothic acid (**10**)[9] were evaluated for their cytotoxic activity against five human cancer cell lines: human myeloid leukemia HL-60, hepatocellular carcinoma SMMC-7721, lung cancer A-549, breast cancer MCF-7, and colon cancer SW480.

RESULTS AND DISCUSSION

Compound **1**, obtained as an amorphous powder, possessed the molecular formula $C_{29}H_{40}O_5$ on the basis of the HRESIMS molecular ion at *m*/*z* 491.2777 $[M + Na]^+$ (calcd for $C_{29}H_{40}O_5Na$ at *m*/*z* 491.2773). IR absorption bands at 3432, 1759, 1686, 1641, and 894 cm^{-1} suggested hydroxy, carboxy, and double-bond functional groups. The ^1H NMR spectrum of **1** (Table 1) was highly informative and contained signals at δ_H 0.93 (3H, s), 1.01 (3H, s), 1.06 (3H, s), 1.12 (3H, d, *J* = 7.2 Hz), 1.66 (3H, s, vinylic methyl), 2.97 (1H, dt, *J* = 5.5, 13.8 Hz, allylic proton), and 4.61 and 4.77 (2H, s, =CH_2). The ^{13}C NMR spectrum displayed 29 carbon resonances ascribable to five methyls, 10 methylenes, four methines, and 10 quaternary carbons (Table 3). The above data indicated that **1** possessed a ceanothane triterpenoid skeleton, characteristic of a five-membered ring A with a methyl at C-2, similar to those of zizyberanal acid,[10] zizyberanalic acid (**9**), and isoceanothic acid (**10**). However, 29 carbon resonances and the lack of the signal of a methyl in the ^{13}C NMR spectrum suggested that a methyl group was absent in **1**.

The HMBC correlations of δ_H 1.12 (3H, d, *J* = 7.2 Hz, Me-1) with δ_C 34.2 (d, C-2) and of δ_H 2.48 (1H, br t, *J* = 9.5 Hz, H-2) with δ_C 46.9 (t, C-3) and 69.0 (s, C-10) suggested that the secondary methyl was located at C-2. The methyl group at C-10

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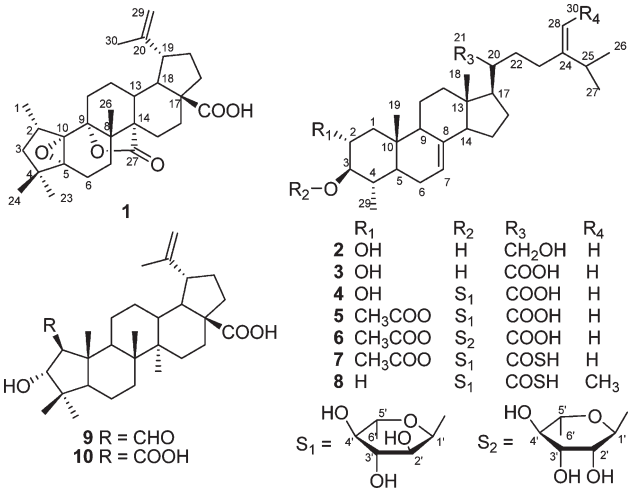

was absent, which was supported by the HMBC spectrum. Instead, a three-membered epoxy link appeared between C-5 and C-10, which was indicated by HMBC correlations of H-2, H-3, H-6, H-23, and H-24 with δ_C 71.6 (s, C-5), and H-1, H-2, H-3, and H-6 with δ_C 69.0 (s, C-10). Furthermore, a five-membered lactone including C-8, C-9, C-14, and C-27 was constructed on the basis of HMBC correlations as shown in Figure 1. In addition, a quaternary carbon at δ_C 180.3 was assigned to a carboxyl group at C-28 on the basis of HMBC correlations. The above data revealed the planar structure of **1**, which possessed a novel carbon skeleton.

The ROESY correlations of Me-1/Me-23, Me-24/Me-26, H-13/Me-26, and H-13/H-19 indicated the α-orientation of Me-1 and the β-orientation of H-13 and H-19. Biogenetically, **1** might be derived from the 5α-OH precursor, in which −OH could attack C-10 and undergo S_N2-type nucleophilic substitution, then form an α-oriented epoxide together with loss of a methyl group. The structure was supported by comparing the 1D NMR spectra of **1** with those of 5α,10α,19β,28-diepoxy-25-(10→2β)abeo-A(1)-nor-18α-oleanan-3-one.[11] Other parts of the structure were identical to zizyberanal acid[10] by detailed analysis of 1D and 2D NMR data of **1**. Therefore, compound **1** was elucidated as 5α,10α-epoxy-9α,27α-lactone-25(10→2α)abeo-A(1)-norlup-20(29)-en-28-oic acid and named breynceanothanolic acid (**1**).

Fruticoside A (**2**), a white, amorphous powder, was positive in the Liebermann−Burchard assay. The molecular formula $C_{29}H_{48}O_3$ was determined by the positive HRESIMS at m/z 445.3678 $[M + H]^+$ in combination with 1D NMR spectra. The IR spectrum indicated the presence of OH (3439 cm^{-1}) and terminal methylene (1639, 892 cm^{-1}) groups.[12] The ^1H NMR spectrum (Table 1) exhibited signals for five methyl groups (two singlets at δ_H 0.84, 0.54 and three doublets at δ_H 1.02, 1.02, 1.00) and three olefinic protons [δ_H 5.19 (1H, d, J = 4.0 Hz), 4.68 (1H, s), and 4.73 (1H, s)]. The ^{13}C NMR spectrum displayed 29 carbon resonances (Table 3), including five methyl groups, 10 methylenes, 10 methines, and four quaternary carbons.

The $^1H-^1H$ COSY spectrum of **2** revealed three partial fragments, **a**−**c** (Figure 2). The above evidence, as well as the prominent fragment ion in the EIMS at m/z 301 $[M - C_9H_{17}O]^+$ indicating a nine-carbon side chain, suggested that **2** could be a 4-methyl ergosterol-type steroid with two double bonds and three OH groups.[13] From fragment **a**, OH groups at C-2 and C-3, a methyl at C-4, and a double bond at C-7═C-8 were readily established. The third OH at C-21 was deduced from fragment **c** as well as from HMBC correlations of δ_H 3.62 (1H, dd, J = 2.8, 9.6 Hz) and 3.72 (1H, dd, J = 2.8, 9.6 Hz) with δ_C 50.1 (d, C-17), 42.4 (d, C-20), and 27.1 (t, C-22). Analyses of other HMBC correlations connected fragments **a**−**c** to those quaternary carbons, which finally established the structure of **2** to be a 4-methyl ergosterol derivative similar to 4α-methyl-3β,14β-dihydroxy-5α-ergost-24(28)-en-23-one.[12] In the ROESY spectrum, correlations of Me-19 with H-2 and H-4 and of H-5α with H-3 and Me-29 indicated the α-orientation for both OH-2 and Me-29 and the β-orientation for OH-3. The latter was also supported by the coupling constant of H-3 (J = 10.0 Hz).[14] Thus, fruticoside A (**2**) was elucidated as 4α-methyl-2α,3β,21-trihydroxy-5α-ergost-7,24(28)-diene.

Fruticoside B (**3**) was obtained as an amorphous powder. The molecular formula $C_{29}H_{46}O_4$ was established by the negative HRESIMS (found $[M - H]^-$ at m/z 457.3322, calcd for $C_{29}H_{45}O_4$ at m/z 457.3317), corresponding to seven degrees of unsaturation. The IR spectrum revealed the presence of OH (3365 cm^{-1}), double bonds (1643, 890 cm^{-1}), and a carboxylic group (1716 cm^{-1}). The 1D NMR data (Tables 1 and 3) were similar to those of **2**, except that the oxygenated methylene carbon at C-21 (δ_C 62.1, t) in **2** was oxidized into a carboxylic carbon (δ_C 179.7, s) in **3**, as supported by the HMBC correlations of δ_H 2.10 (1H, H-20) and 1.56 (2H, H-22) with δ_C 179.7 (s, C-21). ROESY correlations of Me-19/H-2, Me-19/H-4, and H-3/Me-29 suggested that the relative configuration of **3** was also the same as that of **2**. Detailed analysis of 2D NMR data established fruticoside B (**3**) to be 4α-methyl-2α,3β-dihydroxy-5α-ergost-7,24(28)-dien-21-oic acid.

Fruticoside C (**4**) had the molecular formula $C_{35}H_{56}O_8$, established by the negative HRESIMS, corresponding to eight degrees of unsaturation. The IR spectrum indicated the presence of OH (3418 cm^{-1}), double-bond (1641, 890 cm^{-1}), and carboxylic groups (1701 cm^{-1}). The 1D NMR spectra of **4** displayed similarities to those of **3**, except for an additional sugar unit. An anomeric proton signal at δ_H 4.82 (1H, d, J = 4.0 Hz, H-1′), a secondary methyl group at δ_H 1.08 (3H, d, J = 6.0 Hz, H-6′), and four additional protons between δ_H 2.80 and δ_H 3.71 in the ^1H NMR spectrum suggested that **4** contained a 6-deoxyhexose unit.[15] Furthermore, the coupling constants of H-1′ with H-2′ (J = 4.0 Hz), H-2′ with H-3′ (J = 9.0 Hz), H-3′ with H-4′ (J = 9.0 Hz), and H-4′ with H-5′ (J = 9.5 Hz) in the ^1H NMR spectrum was consistent with an α-L-quinovosyl unit in **4**. Acidic hydrolysis of **4** liberated L-quinovose, which was determined by comparison of the optical rotation value ($[\alpha]^{18}_D$ −9.3; H_2O) with literature[16] and by comparing the ^{13}C NMR data for the sugar moiety of **4** with those reported for the L-quinovosyl group.[17] HMBC correlation of H-1′ with C-3 (δ_C 90.2, d) demonstrated the linkage of C-3/C-1′. Therefore, the structure of fruticoside C (**4**) was elucidated as 4α-methyl-2α-hydroxy-5α-ergost-7,24(28)-dien-21-oic acid-3β-*O*-α-L-quinovopyranoside.

Fruticoside D (**5**) had the molecular formula $C_{37}H_{58}O_9$, corresponding to nine degrees of unsaturation. The IR data showed the presence of OH (3433 cm^{-1}), double bonds (1641, 891 cm^{-1}), and carbonyl groups (1736, 1711 cm^{-1}). 1D NMR spectra of **5** were similar to those of **4**, except for an additional acetyl group. The HMBC correlation of the downfield shifted H-2 [δ_H 4.77 (1H, m,)] with the acetyl carbon at δ_C 170.0

Table 1. ^{1}H NMR Data of Compounds 1−4$^{\alpha}$ at 400 MHz

position	1	2	3	4
1	1.12, d (7.2)	1.08, m	1.05, m	0.93, m
		2.05, m	1.96, m	1.95, m
2	2.48, br t (9.5)	3.51, m	3.43, m	3.52, m
3	0.98, m	2.88, br t (10.0)	2.78, br t (9.5)	2.85, br t (9.8)
	1.73, m			
4		1.38, m	1.29, m	1.45, m
5		1.05, m	0.98, m	0.95, m
6	1.85, m	1.57, m	1.48, m	1.50, m
		2.07, m	2.03, m	2.03, m
7	1.19, m	5.19, d (4.0)	5.09, d (4.0)	5.12, d (4.0)
	1.41, m			
9		1.72, m	1.60, m	1.65, m
11	1.90, m	1.48, m	1.49, m	1.40, m
	1.95, m	1.59, m		1.52, m
12	1.76, m	1.28, m	1.08, m	1.04, m
	2.09, m	1.94, m	1.64, m	1.70, m
13	2.60, dt (6.8, 9.0)			
14		1.81, br s	1.75, br s	1.86, br s
15	1.20, m	1.54, m	1.34, m	1.42, m
		1.42, m		1.49, m
16	2.16, m	1.56, m	1.31, m	1.32, m
	2.25, m	1.35, m	1.88, m	1.81, m
17		1.56, m	1.64, m	1.63, m
18	1.74, m	0.54, s	0.45, s	0.48, s
19	2.97, dt (5.5, 13.8)	0.84, s	0.74, s	0.74, s
20		1.47, m	2.10, m	2.03, m
21	1.53, m	3.62, dd (2.8, 9.6)		
	1.98, m	3.72, dd (2.8, 9.6)		
22	1.55, m	1.34, m	1.56, m	1.49, m
	2.05, m	1.90, m		1.51, m
23	1.01, s	1.96, m	1.88, m	1.88, m
		2.12, m	1.92, m	
24	1.06, s			
25		2.24, m	2.10, m	2.15, m
26	0.93, s	1.02, d (7.2)	0.93, d (7.0)	0.94, d (7.2)
27		1.02, d (7.2)	0.93, d (7.0)	0.94, d (7.2)
28		4.68, s	4.57, s	4.62, s
		4.73, s	4.65, s	4.71, s
29	4.61, s	1.00, d (6.0)	0.92, d (6.3)	0.96, d (6.8)
	4.77, s			
30	1.66, s			
1′				4.82, d (4.0)
2′				3.35, overlapped with DMSO
3′				3.27, br t (9.0)
4′				2.80, dd (9.0, 9.5)
5′				3.71, dq (6.0, 9.5)
6′				1.08, d (6.0)

$^{\alpha}$ Compound **1** was measured in $CDCl_3$, **2** and **3** in $CDCl_3$ + CD_3OD, **4** in DMSO; δ in ppm and J in Hz.

suggested that the 2-OH was acetylated. The remaining structure was identical to that of **4** by detailed analysis of 2D NMR and acid hydrolysis of **5**. Consequently, fruticoside D (**5**) was determined to be 4α-methyl-2α-acetoxy-5α-ergost-7,24(28)-dien-21-oic acid-3β-*O*-α-L-quinovopyranoside.

The other closely related product, **6**, with a lower R_f value on silica plates than that of **5**, showed identical physical data in the HRESIMS and IR spectra, indicating the existence of the same molecular formula and functional groups as in **5**. Detailed comparison of the 1D NMR data of **6** with those of **5** suggested

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Table 2. ^{1}H NMR data of Compounds 5−8$^{\alpha}$ at 500 MHz

position	5	6	7	8
1	1.05, m	1.09, m	0.91, m	1.66, m
	1.90, m	1.88, m	1.94, m	0.94, m
2	4.77, m	4.74, m	4.74, m	1.72, m
3	3.15, br t (9.5)	3.13, br t (9.5)	3.11, br t (9.8)	2.88, m
4	1.50, m	1.47, m	1.48, m	1.31, m
5	1.03, m	1.04, m	0.94, m	0.86, m
6	1.49, m	1.50, m	1.42, m	1.43, m
	2.05, m	2.02, m	1.95, m	1.94, m
7	5.13, br s	5.12, br s	5.03, d (4.0)	5.02, d (4.0)
9	1.65, m	1.65, m	1.50, m	1.48, m
11	1.28, m	1.24, m	1.25, m	1.24, m
	1.47, m	1.45, m	1.35, m	1.36, m
12	1.04, m	1.03, m	1.03, m	1.05, m
	1.70, m	1.72, m	1.62, m	1.63, m
14	1.77, br s	1.73, br s	1.62, m	1.63, m
15	1.33, m	1.31, m	1.30, m	1.30, m
	1.49, m	1.47, m	1.42, m	1.43, m
16	1.22, m	1.21, m	1.22, m	1.40, m
	1.85, m	1.83, m	1.85, m	1.72, m
17	1.62, m	1.62, m	1.73, m	1.73, m
18	0.49, s	0.47, s	0.47, s	0.48, s
19	0.79, s	0.77, s	0.72, s	0.64, s
20	2.05, m	2.02, m	1.48, m	1.61, m
22	1.48, m	1.47, m	1.63, m	1.61, m
	1.52, m	1.50, m		
23	1.88, m	1.87, m	1.60, m	1.50, m
			2.00, m	1.88, m
25	2.16, m	2.15, m	2.04, m	2.66, m
26	0.95, d (7.2)	0.94, d (6.5)	0.83, d (6.7)	0.78, d (7.0)
27	0.95, d (7.2)	0.94, d (6.5)	0.83, d (6.7)	0.78, d (7.0)
28	4.62, s	4.60, s	4.48, s	4.94, q (7.0)
	4.71, s	4.69, s	4.55, s	
29	1.08, d (6.5)	0.91, d (6.5)	0.96, d (6.3)	0.83, d (6.2)
30				1.48, d (7.0)
1′	4.71, d 4.0)	4.59, br s	4.80, d (3.5)	4.70, d (4.0)
2′	3.32, m	3.64, br s	3.28, dd (3.5, 9.5)	3.27, dd (4.0, 9.5)
3′	3.19, br t (9.2)	3.37, dd (3.0, 9.5)	3.41, br t (9.5)	3.43, br t (9.5)
4′	2.81, br t (9.3)	3.13, m	2.84, br t (9.2)	2.88, br t (9.3)
5′	3.50, dq, (6.0, 9.3)	3.43, dq (6.0, 9.5)	3.57, dq (6.3, 9.2)	3.61, dq (6.5, 9.3)
6′	1.04, d (6.0)	1.06, d (6.0)	1.04, d (6.3)	1.07, d, (6.5)
CH_3CO_2	1.93, s	1.94, s	1.87, s	

$^{\alpha}$ Compounds **5** and **6** were measured in DMSO, **7** and **8** in $CDCl_3$ + CD_3OD; δ in ppm and J in Hz.

that a different 6-deoxy sugar was substituted at C-3 in **6**. After acid hydrolysis of **6** with 10% HCl−MeOH, an L-rhamnosyl unit was established by comparison of its optical rotation data ($[\alpha]^{18}_{D}$ +11.4; H_2O) and R_f value with an authentic sample.[18] The α-configuration of the L-rhamnose was determined by the coupling constant of the anomeric proton at δ_H 4.59 (1H, br s, H-1′). Thus, compound **6** was assigned as 4α-methyl-2α-acetoxy-5α-ergost-7,24(28)-dien-21-oic acid-3β-*O*-α-L-rhamnopyranoside and was named fruticoside E.

Fruticoside F (**7**) gave a positive Liebermann−Burchard test. The negative FABMS of **7** showed a quasimolecular ion at m/z 661 $[M - H]^-$, along with two isotope peaks at m/z 662 (33.8%, relative intensity) and 663 (7.7%, relative intensity), suggesting a sulfur atom in **7**.[19] The molecular formula was established unequivocally to be $C_{37}H_{57}O_8S$ by the negative HRESIMS (m/z 661.3772 $[M - H]^-$). The HRESIMS and ^{13}C NMR spectra of **7** compared with those of **5** and **7** displayed similarities to **5**, except for a carbothioic moiety at δ_C 179.6 (s) instead of a carboxylic group in **5**. In addition, sulfhydrylation of **5** with NaSH and carbonyl diimidazole/DMF reagent afforded the product **7**.[20] Other parts of the structure were identical to those of **5**, by detailed analyses of 2D NMR and acid hydrolysis of **7**. Thus, fruticoside F (**7**) was 4α-methyl-2α-acetoxy-5α-ergost-7,24(28)-diene-21-carbothioic acid-3β-*O*-α-L-quinovopyranoside.

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Table 3. ^{13}C NMR Data of Compounds 1−8[α] at 100 MHz (1−4) or 125 MHz (5−8)

position	1	2	3	4	5	6	7	8
1	21.3, CH_3	44.4, CH_2	44.8, CH_2	44.5, CH_2	42.3, CH_2	42.3, CH_2	42.3, CH_2	36.8, CH_2
2	34.2, CH	71.7, CH	71.2, CH	67.1, CH	72.8, CH	72.3, CH	73.3, CH	21.9, CH_2
3	46.9, CH_2	80.7, CH	80.7, CH	90.2, CH	84.8, CH	84.8, CH	84.9, CH	85.7, CH
4	38.7, C	37.9, CH	37.9, CH	35.2, CH	38.1, CH	38.2, CH	38.1, CH	38.6, CH
5	71.6, C	46.3, CH	46.4, CH	46.6, CH	45.6, CH	45.4, CH	45.9, CH	46.8, CH
6	16.7, CH_2	26.4, CH_2	26.4, CH_2	26.5, CH_2	26.0, CH_2	26.3, CH_2	26.3, CH_2	26.5, CH_2
7	25.7, CH_2	117.5, CH	117.6, CH	117.3, CH	117.3, CH	117.1, CH	117.7, CH	117.9, CH
8	44.5, C	138.3, C	138.1, C	138.1, C	137.9, C	137.1, C	137.6, C	138.2, C
9	85.6, C	49.3, CH	48.9, CH	48.8, CH	48.4, CH	48.6, CH	47.8, CH	48.5, CH
10	69.0, C	36.0, C	36.0, C	35.2, C	35.4, C	35.6, C	35.5, C	34.3, C
11	28.3, CH_2	21.2, CH_2	21.1, CH_2	20.5, CH_2	20.8, CH_2	20.9, CH_2	20.8, CH_2	20.6, CH_2
12	24.0, CH_2	38.7, CH_2	36.9, CH_2	37.5, CH_2	37.0, CH_2	36.7, CH_2	37.5, CH_2	37.5, CH_2
13	34.7, CH	42.9, C	42.8, C	42.7, C	42.6, C	42.6, C	42.4, C	42.9, C
14	52.6, C	54.6, CH	54.1, CH	53.7, CH	53.4, CH	53.8, CH	54.4, CH	54.6, CH
15	23.9, CH_2	22.6, CH_2	22.1, CH_2	21.8, CH_2	21.7, CH_2	22.0, CH_2	21.5, CH_2	21.7, CH_2
16	36.8, CH_2	27.6, CH_2	26.7, CH_2	26.5, CH_2	26.0, CH_2	26.5, CH_2	21.6, CH_2	29.0, CH_2
17	54.8, C	50.1, CH	52.3, CH	52.0, CH	52.0, CH	52.0, CH	56.0, CH	56.3, CH
18	49.6, CH	11.9, CH_3	11.7, CH_3	11.7, CH_3	11.7, CH_3	11.7, CH_3	12.5, CH_3	12.8, CH_3
19	47.7, CH	14.8, CH_3	14.8, CH_3	14.8, CH_3	14.3, CH_3	14.3, CH_3	14.0, CH_3	13.7, CH_3
20	149.5, C	42.4, CH	47.4, CH	47.0, CH	47.2, CH	47.7, CH	38.1, CH	38.6, CH
21	29.9, CH_2	62.1, CH_2	179.7, C	177.0, C	177.0, C	177.3, C	179.6, C	178.9, C
22	31.1, CH_2	27.1, CH_2	30.4, CH_2	30.1, CH_2	30.2, CH_2	30.4, CH_2	38.0, CH_2	39.1, CH_2
23	24.1, CH_3	30.9, CH_2	31.8, CH_2	31.5, CH_2	31.5, CH_2	31.7, CH_2	27.3, CH_2	24.5, CH_2
24	26.0, CH_3	156.4, C	155.2, C	154.8, C	154.7, C	155.1, C	155.4, C	144.5, C
25		33.6, CH	33.6, CH	33.3, CH	33.3, CH	33.3, CH	33.6, CH	28.4, CH
26	13.1, CH_3	21.7, CH_3	21.6, CH_3	21.7, CH_3	21.6, CH_3	21.6, CH_3	21.3, CH_3	20.6, CH_3
27	178.2, C	21.7, CH_3	21.6, CH_3	21.7, CH_3	21.6, CH_3	21.6, CH_3	21.3, CH_3	20.6, CH_3
28	180.3, C	106.0, CH_2	106.5, CH_2	106.9, CH_2	106.9, CH_2	106.6, CH_2	106.0, CH_2	117.0, CH
29	111.1, CH_2	14.8, CH_3	14.8, CH_3	15.3, CH_3	15.2, CH_3	15.1, CH_3	15.0, CH_3	15.1, CH_3
30	18.4, CH_3							12.5, CH_3
1′				97.0, CH	100.3, CH	102.1, CH	99.0, CH	100.3, CH
2′				72.3, CH	72.6, CH	70.7, CH	72.6, CH	72.8, CH
3′				72.8, CH	72.7, CH	70.4, CH	73.3, CH	74.1, CH
4′				75.5, CH	75.9, CH	72.0, CH	75.6, CH	75.5, CH
5′				68.1, CH	67.9, CH	69.1, CH	67.7, CH	67.3, CH
6′				17.8, CH_3	17.9, CH_3	17.9, CH_3	17.1, CH_3	17.0, CH_3
CH_3CO_2					21.5, CH_3	21.4, CH_3	21.3, CH_3	
CH_3CO_2					170.0, C	170.0, C	171.3, C	

[α] Compound **1** was measured in $CDCl_3$, **2**, **3**, **7**, and **8** in $CDCl_3$ + CD_3OD, **4**, **5**, and **6** in DMSO; δ in ppm and J in Hz.

Fruticoside G (**8**) had the molecular formula $C_{36}H_{58}O_6S$ as determined by negative HRESIMS (found $[M - H]^-$ 617.3868, calcd for $C_{36}H_{57}O_6S$ at m/z 617.3875), corresponding to eight degrees of unsaturation. In view of the negative FABMS data of **8**, the relative intensities of two isotope peaks to the $[M - H]^-$ peak were 7.5% and 32.8%, respectively, which also suggested a sulfur atom in **8**.[19] A carbothioic group at C-21 was also confirmed by the HMBC correlations of H-20 with C-21, C-22, and C-17. Comparison of 1D NMR data of **8** with those of **7** showed that the two compounds were similar, with the exception of an additional secondary methyl signal at δ_C 12.5 and the absence of an acetyl and an OH group in **8**. HMBC correlations from the additional methyl protons at δ_H 1.48 (3H, d, J = 7.0 Hz) to C-28 and C-24, as well as the same coupling constant as H-28 (δ_H 4.94, q, J = 7.0 Hz), suggested that the additional secondary methyl was linked at C-28. The HMBC correlations of H-3 with δ_C 100.3 (d, C-1′), 21.9 (t, C-2), 38.6 (d, C-4), and 15.1 (q, C-29) suggested that the sugar moiety was connected to 3-OH by an ether linkage. Other parts of the structure were identical to those of **7**, by detailed analysis of 2D NMR and acid hydrolysis of **8**. Thus, fruticoside G (**8**) was determined to be 4α-methyl-5α-stigmast-7,24(28)-diene-21-carbothioic acid-3β-*O*-α-L-quinovopyranoside.

Compound **1** is an unprecedented 25(10→2)abeo-A(1)-nortriterpenoid. Compounds **7** and **8** are sulfur-containing derivatives of the 4α-methyl sterols, and compounds **4**, **5**, **7**, and **8** possess the uncommon 6-deoxy sugar L-quinovose. The known compounds were identified as zizyberanalic acid (**9**), isoceanothic acid (**10**), stigmastane-3β,5α,6β-triol,[21] stigmast-5-ene-3β,7α-diol,[22] 3β-hydroxy-5α,8α-epidioxyergosta-6,22-diene,[23] β-stiosterol,[24] daucosterol,[24]

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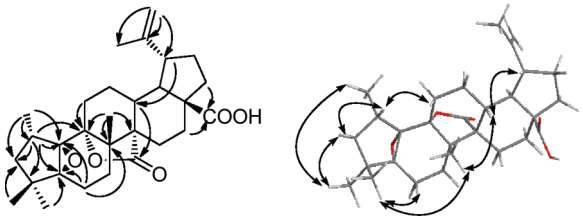

Figure 1. Key HMBC (→) and ROESY (↔) correlations of **1**.

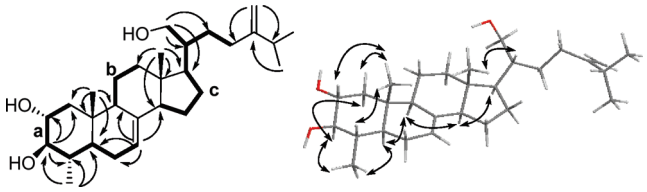

Figure 2. Key ^{1}H−^{1}H COSY (—), HMBC (→), and ROESY (↔) correlations of **2**.

Table 4. Cytotoxicity of Compounds 1 and 2

	IC$_{50}$ (μM)		
cells	**1**	**2**	cisplatin
HL-60	10.2	3.0	21.7
SMMC-7721	14.3	18.5	18.1
A-549	14.5	15.7	2.6
MCF-7	12.7	15.4	24.8
SW480	20.0	18.4	15.8

sitoindoside I,[25] glochidone,[26] glochidonol,[26] 20(29)-lupene-1β,3α-diol,[26] 20(29)-lupen-3β-ol,[27] 20(29)-lupene-1β,3β-diol,[28] betulinic acid,[29] 2α,3β-dihydroxy-20(29)-lupen-28-oic acid,[30] and platanic acid,[31] by comparison with spectroscopic data in the literature.

Compounds **1**−**10** were evaluated for their cytotoxicity against five human cancer cell lines using the MTT method as reported previously.[32] Cisplatin (Sigma, USA) was used as the positive control. The results showed that compounds **1** and **2** exhibited moderate cytotoxicity compared to cisplatin (Table 4). The other compounds were inactive (IC$_{50}$ values >40 μM).

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were obtained on an X-4 micro melting point apparatus. Optical rotations were measured with a Horiba SEPA-300 polarimeter. IR spectra were obtained with a Tenor 27 spectrophotometer using KBr pellets. 1D and 2D NMR spectra were run on a Bruker DRX-500 spectrometer or on an AV-400 spectrometer with TMS as an internal standard. Chemical shifts (δ) were expressed in ppm with reference to the solvent signals. EIMS were taken on a Finnigan Trace DSQ. FABMS were recorded with a VG Autospec-3000 spectrometer. ESIMS and HRESIMS spectroscopic data were obtained on an API QSTAR Pulsar I spectrometer. Column chromatography (CC) was performed on silica gel (200−300 mesh, Qingdao Marine Chemical Ltd., Qingdao, China), RP-18 gel (20−45 μm, Fuji Silysia Chemical Ltd., Japan), and Sephadex LH-20 (Pharmacia Fine Chemical Co., Ltd., Sweden). Fractions were monitored by TLC (GF 254, Qingdao Marine Chemical Ltd., Qingdao, China), and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

Plant Material. The roots of *Breynia fruticosa* were collected in Xishuangbanna of Yunnan Province, People's Republic of China, and identified by Mr. Jing-Yun Cui of Xishuangbanna Tropical Plant Garden. A voucher specimen (Cui200811-25) has been deposited at the Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. Air-dried roots of *B. fruticosa* (8.5 kg) were crushed and extracted with 90% MeOH at room temperature (48 h × 4). The MeOH extracts were evaporated in vacuo to give a viscous residue, which was partitioned with EtOAc and H_2O. The EtOAc fraction (120 g) was subjected to silica gel CC ($CHCl_3$−Me_2CO, 1:0 to 0:1) to produce fractions I−VIII. Fraction II (9.0 g) yielded glochidone (458 mg), glochidonol (207 mg), and 20(29)-lupen-3β-ol (56 mg) after repeated silica gel CC. Fraction III (12.0 g) was separated by silica gel CC (petroleum ether−Me_2CO, 8:1 to 3:1), then by Sephadex LH-20 ($CHCl_3$−MeOH, 1:1), to yield β-stiosterol (890 mg), 2α,3β-dihydroxy-20(29)-lupen-28-oic acid (32 mg), **9** (17 mg), and a mixture. The mixture was chromatographed on a silica gel column (petroleum ether−EtOAc, 6:1 to 2:1) to afford **1** (38 mg), 20(29)-lupene-1β,3α-diol (26 mg), and stigmast-5-ene-3β,7α-diol (29 mg). Fraction IV (8.0 g) was subjected to MPLC with RP-18 CC (MeOH−H_2O, 7:3−10:0), followed by silica gel CC (petroleum ether−Me_2CO, 4:1 to 1:1), to yield **10** (721 mg), 3β-hydroxy-5α,8α-epidioxyergosta-6,22-diene (7 mg), and platanic acid (15 mg). Separation of fraction V by RP-18 CC, eluted with MeOH−H_2O (3:7−10:0), and then by silica gel CC ($CHCl_3$−Me_2CO, 8:1) provided **2** (50 mg), 20(29)-lupene-1β,3β-diol (21 mg), and a mixture. The latter was submitted to silica gel CC ($CHCl_3$−Me_2CO, 8:1 to 4:1) and then Sephadex LH-20 CC ($CHCl_3$−MeOH, 1:1) to give **3** (26 mg) and betulinic acid (12 mg). Fraction VI (12.0 g) was separated by RP-18 CC (MeOH−H_2O, 3:7−10:0), followed by silica gel CC ($CHCl_3$−MeOH, 15:1, 10:1, 8:1) and RP-18 CC (MeOH−H_2O, 80:20, 85:15, 90:10), to yield **4** (962 mg), **8** (38 mg), and sitoindoside I (104 mg). Fraction VII (18.0 g) was subjected to MPLC with RP-18 (MeOH−H_2O, 2:8−10:0) to give stigmastane-3β,5α,6β-triol (11 mg), daucosterol (108 mg), and subfractions VII-a and VII-b. Subfraction VII-a was separated by silica gel CC ($CHCl_3$−MeOH, 6:1) followed by RP-18 CC (MeOH−H_2O, 80:20 to 85:15) to yield **5** (254 mg) and **7** (225 mg). Subfraction VII-b was subjected to RP-18 CC using MeOH−H_2O (80:20) to afford **6** (54 mg).

Breynceanothanolic Acid (***1***): white, amorphous powder; mp 244−246 °C; $[\alpha]^{18}_D$ −39.0 (*c* 0.82, CH_3Cl); IR (KBr) ν_{max} 3432, 2956, 1759, 1686, 1641, 894 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; EIMS *m*/*z* 468 [M]$^+$ (1.8), 450 (10.6), 424 (20.1), 409 (51.5), 307 (52.7), 187 (70.4), 173 (100), 105 (58.8); HRESIMS *m*/*z* 491.2777 (calcd for $C_{29}H_{40}O_5Na$, 491.2773).

Fruticoside A (***2***): white, amorphous powder; mp 147−148 °C; $[\alpha]^{18}_D$ −33.3 (*c* 0.15, MeOH); IR (KBr) ν_{max} 3439, 2961, 2872, 1639, 892 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; EIMS *m*/*z* 444 [M]$^+$ (10.2), 429 (8.4), 411 (9.6), 342 (14.4), 301 [M − $C_9H_{17}O$]$^+$ (100), 261 (14.4); HRESIMS *m*/*z* 445.3678 (calcd for $C_{29}H_{49}O_3$, 445.3681).

Fruticoside B (***3***): white, amorphous powder; mp 235−238 °C; $[\alpha]^{18}_D$ −44.4 (*c* 0.06, MeOH); IR (KBr) ν_{max} 3365, 2962, 2671, 1716, 1643, 890 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; negative ESIMS *m*/*z* 457 [M − H]$^-$; HRESIMS *m*/*z* 457.3322 (calcd for $C_{29}H_{45}O_4$, 457.3317).

Fruticoside C (***4***): white, amorphous powder; mp 272−274 °C; $[\alpha]^{18}_D$ −51.3 (*c* 0.15, MeOH); IR (KBr) ν_{max} 3418, 2963, 2814, 1701, 1641, 890 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; negative ESIMS *m*/*z* 603 [M − H]$^-$; HRESIMS *m*/*z* 603.3884 (calcd for $C_{35}H_{55}O_8$, 603.3896).

Fruticoside D (***5***): white, amorphous powder; mp 219−220 °C; $[\alpha]^{18}_D$ −59.1 (*c* 0.11, MeOH); IR (KBr) ν_{max} 3433, 2960, 2874, 1736, 1711, 1641, 891 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; negative

ESIMS m/z 645 $[M - H]^-$; HRESIMS m/z 645.4013 (calcd for $C_{37}H_{57}O_9$, 645.4002).

Fruticoside E (**6**): white, amorphous powder; mp 241−242 °C; $[\alpha]^{18}_D$ −30.4 (*c* 0.09, MeOH); IR (KBr) ν_{max} 3432, 2958, 1710, 1638, 893 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; negative ESIMS m/z 645 $[M - H]^-$; HRESIMS m/z 669.3977 (calcd for $C_{37}H_{58}O_9Na$, 669.3978).

Fruticoside F (**7**): white, amorphous powder; mp 210−211 °C; $[\alpha]^{18}_D$ −71.1 (*c* 0.16, MeOH); IR (KBr) ν_{max} 3445, 2962, 2937, 1734, 1641, 888 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; negative FABMS m/z 661 $[M - H]^-$, 662 $[M + 1 - H]^-$, 663 $[M + 2 - H]^-$; HRESIMS m/z 661.3772 (calcd for $C_{37}H_{57}O_8S$, 661.3774).

Fruticoside G (**8**): white, amorphous powder; mp 247−251 °C; $[\alpha]^{18}_D$ −72.7 (*c* 0.16, MeOH); IR (KBr) ν_{max} 3440, 2934, 1710, 1631 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; negative FABMS m/z 617 $[M - H]^-$, 618 $[M + 1 - H]^-$, 619 $[M + 2 - H]^-$; HRESIMS m/z 617.3868 (calcd for $C_{36}H_{57}O_6S$, 617.3875).

Acid Hydrolysis of 4−8. Compounds **4**−**8** (15 mg each) were refluxed with 10% HCl−MeOH (20 mL) on a water bath at 60 °C for 8 h, respectively. The reaction mixtures were evaporated to dryness and redissolved in H_2O, then partitioned with EtOAc, to afford EtOAc and H_2O layers. The sugars were compared with authentic samples (L-rhamnose and D-quinovose) and identified by TLC using $CHCl_3$−MeOH (6:4) as rhamnose (R_f 0.49) in **6** and quinovose (R_f 0.52) in **4**, **5**, **7**, and **8**. Purifications of the H_2O layers were performed by preparative TLC, eluted four times with $CHCl_3$−MeOH−H_2O (70:30:1), to afford L-rhamnose (R_f 0.56, $[\alpha]^{18}_D$ +11.4; H_2O) in **6** and L-quinovose (R_f 0.62, $[\alpha]^{18}_D$ −9.3, −10.5, −8.9, −6.9; H_2O) in **4**, **5**, **7**, and **8**, respectively. The EtOAc layers, monitored by HPTLC on silica gel GF_{254} plates using $CHCl_3$−MeOH (10:1) and $CHCl_3$−Me_2CO (5:1), showed several decomposition products.

Fruticoside D (5) Converted to Fruticoside F (7). To a solution of **5** (32.3 mg, 0.05 mmol) in DMF (1 mL) was added carbonyl diimidazole (16.3 mg, 0.1 mmol), and the reaction mixtures were stirred at 25 °C for 6 h. NaSH (13.5 mg, 0.24 mmol) was then added and stirring continued at 25 °C for 20 h. The reaction mixtures were poured into aqueous 2 M HCl (20 mL) cooled in an ice bath. The resulting precipitate was filtered and dried in vacuo to give **7** (8.7 mg, 26.9%).

Cytotoxicity Assay. Five human cancer cell lines, human myeloid leukemia HL-60, hepatocellular carcinoma SMMC-7721, lung cancer A-549, breast cancer MCF-7, and colon cancer SW480, were used in the cytotoxic assay. Cells were cultured in DMEM medium (Hyclone, USA), supplemented with 10% fetal bovine serum (Hyclone, USA), in 5% CO_2 at 37 °C. The cytotoxicity assay was performed according to the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) method in 96-well microplates.[33] Briefly, 100 μL of adherent cells was seeded into each well of 96-well cell culture plates and allowed to adhere for 12 h before addition of test compounds, while suspended cells were seeded just before drug addition with initial density of 1×10^5 cells/mL. Each tumor cell line was exposed to the test compound at concentrations of 0.064, 0.32, 1.6, 8, and 40 μM in triplicates for 48 h, with cisplatin (Sigma, USA) as a positive control. After compound treatment, cell viability was detected and a cell growth curve was graphed. IC_{50} values were calculated by Reed and Muench's method.[34]

■ ASSOCIATED CONTENT

Supporting Information. 1D and 2D NMR, MS, and IR spectra of breynceanothanolic acid (**1**) and fruticosides A−G (**2**−**8**). These materials are available free of charge via the Internet at http://pubs.acs.org.

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