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Indole Alkaloids and Quassinoids from the Stems of *Brucea mollis*

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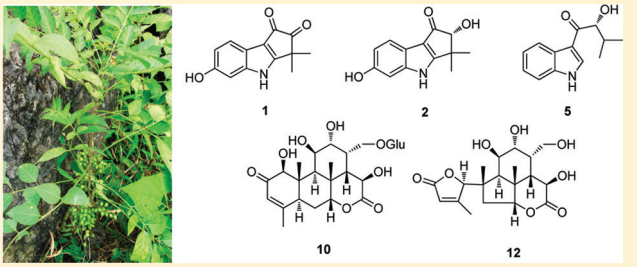
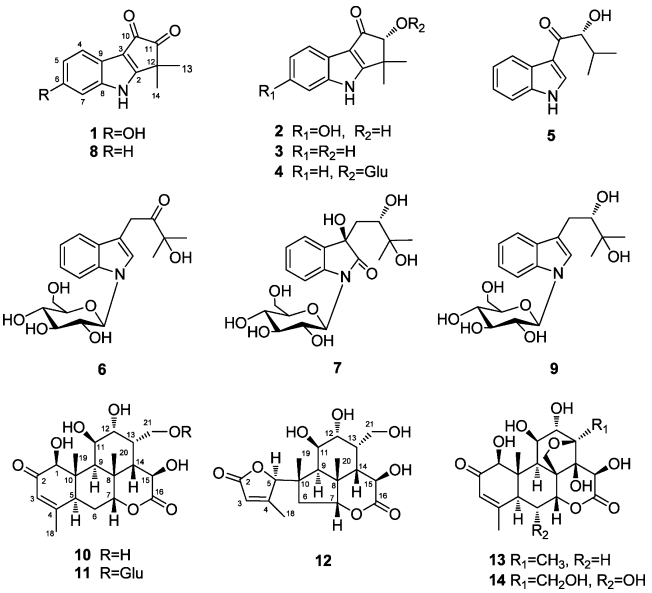
Supporting Information

ABSTRACT: Seven new indole alkaloids, bruceollines H–N (**1**–**7**), three new quassinoids, yadanziolides T–V (**10**–**12**), and four known analogues, bruceolline E (**8**), bruceolline F (**9**), bruceine D (**13**), and yadanziolide B (**14**), were isolated from an ethanol extract of the stems of *Brucea mollis*. The absolute configurations of compounds **2** and **5** were determined by comparison of their experimental and calculated ECD spectra. The absolute configuration of the known compound **9** was determined by using $Mo_2(OAc)_4$-induced CD analysis for the first time. Compounds **10**, **13**, and **14** exhibited cytotoxic activities with IC_{50} values of 3.00–5.81 μM.



The Simaroubaceae family is well known to contain structurally diverse and biologically active quassinoids with significant cytotoxic and antimalarial activities.[1,2] Plants of the genus *Brucea* (Simaroubaceae) are usually bushes or small trees mainly distributed in the tropical eastern hemisphere. This genus comprises a small group of six species, of which only two (*B. javanica* and *B. mollis*) are found in China. In particular, *B. javanica*, commonly known as "Ya dan zi", is used for the treatment of dysentery, malaria, and skin conditions such as warts and corns. *B. mollis*, distributed in southwestern China, is used as a remedy for malaria and other parasitic diseases. *B. javanica* has been intensively studied and was found to be a rich source of quassinoids;[3] however, study on the bioactive components from *B. mollis* has not been fully investigated. In previous phytochemical investigations, only 10 quassinoids, seven canthin-6-one alkaloids, and three indole alkaloids were reported from *B. mollis*;[4−7] however, the bioactivity of these compounds has not been reported. As part of an ongoing program to screen toxic herbs for cytotoxic compounds, the EtOH extract of dried stems of *B. mollis* Wall. was examined and was shown to exhibit cytotoxicity against four cultured human tumor cell lines (HCT-8, Bel-7402, BGC823, A549) with IC_{50} values of 8.81–36.81 μg/mL. In our study, 10 new compounds, **1**–**7** and **10**–**12**, and four known compounds, bruceolline E (**8**),[5] bruceolline F (**9**),[5] bruceine D (**13**),[8] and yadanziolide B (**14**),[9] were isolated from the EtOH extract and were identified by spectroscopic methods. They belong to the classes of indole alkaloids (**1**–**9**) and quassinoids (**10**–**14**). Details of the isolation, structural elucidation, and cytotoxicity of these metabolites are reported herein.



RESULTS AND DISCUSSION

Compound **1** exhibited UV absorption bands at 210, 259, 287, and 366 nm, suggesting an indole chromophore with extended conjugation. The IR absorption bands at 3445, 3131, 1752, and 1668 cm^{-1} indicated the presence of amine, hydroxy, and carbonyl groups. The molecular formula, $C_{13}H_{11}NO_3$, was established by HR-ESIMS (m/z 230.0806 $[M + H]^+$) combined with NMR data. The ^1H NMR spectrum of **1** showed the presence of two D_2O-exchangeable protons [δ_H 11.5 (brs) and 8.57 (brs)], one ABX system [δ_H 7.69 (d, J = 8.0 Hz), 7.01 (d, J = 2.0 Hz), and 6.90 (dd, J = 8.0, 2.0 Hz)], and two geminal methyls [δ_H 1.47 (6H, s)]. The ^{13}C NMR spectrum of **1** showed 13 carbon signals, including two carbonyl carbons (δ_C 206.7 and 176.0). The ^1H and ^{13}C NMR

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data of **1** indicated its structural similarity to the known compound bruceolline E (**8**),[5] except for the presence of the hydroxy group at C-6 in **1**. The C-6 signal (δ_C 157.2), together with HMBC correlations, from OH-6 (δ_H 8.57) to C-5 (δ_C 113.4), C-6 (δ_C 157.2), and C-7 (δ_C 100.0), and from H-4 (δ_H 7.69) to C-3 (δ_C 115.5), verified the location of the hydroxy group. Thus, the structure of **1** was established as shown in Figure 1 and named bruceolline H.

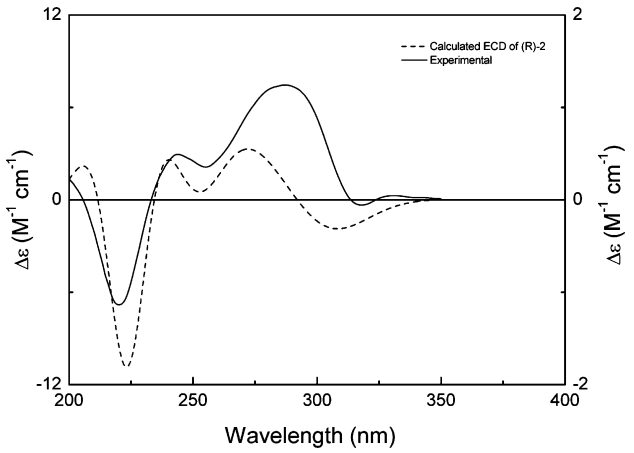


Figure 1. Calculated CDs of the 11*R*-isomer and experimental CDs of **2** in MeOH.

Compound **2** showed UV absorption bands at 218, 240, 272, and 307 nm, suggesting an indole chromophore.[10] The IR absorption bands at 3258 and 1655 cm^{-1} indicated hydroxy and conjugated carbonyl functionalities. The molecular formula, $C_{13}H_{13}NO_3$, was established by HR-ESIMS (*m*/*z* 232.0966 $[M + H]^+$) and was greater than that of **1** by two mass units. The ^1H and ^{13}C NMR data of **2** were similar to those of **1**. The differences indicated the reduction of the cyclopentane-1,2-dione moiety, culminating in the absence of one carbonyl group in the ^{13}C NMR spectrum, the presence of an oxymethine group (δ_H 4.23, δ_C 86.9), and an additional exchangeable proton (δ_H 4.62) in the ^1H NMR spectrum. The location of the hydroxy group (δ_H 4.62) at C-11 was corroborated by the HMBC correlations from H-11 (δ_H 4.23) to C-10 (δ_C 193.8), C-12 (δ_C 41.1), C-13 (δ_C 25.4), and C-14 (δ_C 24.4) and from OH-11 (δ_H 4.62) to C-10 (δ_C 193.8), C-11 (δ_C 86.9), and C-12 (δ_C 41.1). The absolute configuration of C-11 was extrapolated by comparing the experimental and calculated CD spectra, the latter performed using time-dependent density functional theory. Their optimized geometries were obtained by system conformational analysis with the MMFF94 force field, and then the ECD spectra were calculated at the B3LYP/6-31+G(d) level with the PCM model in methanol solution (Supporting Information, Figure S80 and Table S1). The results showed that experimental and calculated spectra for the 11*R*-isomer were in good agreement (Figure 1). Therefore, the absolute configuration at C-11 was deduced to be *R*. Thus, the structure of **2** was established as shown and named bruceolline I.

Compounds **3** and **4** were assigned as indole alkaloid derivatives because they possessed characteristic UV and IR spectroscopic data as mentioned for **2**. The molecular formula of **3**, $C_{13}H_{13}NO_2$, was established by HR-ESIMS (*m*/*z* 216.1014 $[M + H]^+$) and NMR data. The major difference in the ^1H NMR spectrum of **3** from that of **2** was that the set of ABX-type proton signals in **2** was replaced by four mutually coupled proton signals [δ_H 7.74 (d, *J* = 7.5 Hz), 7.47 (d, *J* = 8.0 Hz), 7.24 (td, *J* = 7.5, 1.5 Hz), and 7.17 (td, *J* = 8.0, 1.5 Hz)], which was indicative of a 1,2-disubstituted aromatic ring. This observation was supported by the HSQC and HMBC experiments (Supporting Information, Figures S16 and S17). The absolute configuration of C-11 in **3** was identified as *R* by comparison of the sign of the specific rotation and CD spectrum of **3** with that of **2** (Supporting Information, Figure S20). Hence, the structure of **3** was established as shown and named bruceolline J.

The molecular formula of **4**, $C_{13}H_{13}NO_2$, was established by HR-ESIMS (*m*/*z* 378.1561 $[M + H]^+$). The ^1H and ^{13}C NMR spectra of **4** were similar to those of **3** except for the presence of the signals for a sugar unit, which was also supported by the presence of one fragment ion peak $[M - 162 + H]^+$ at *m*/*z* 216 in the ESIMS. Acid hydrolysis of **4** gave **3** as the aglycone, along with glucose. The large coupling constant (7.5 Hz) of the anomeric proton at δ_H 4.62 (H-1′) revealed that the glucose was in the β-configuration, and the D-configuration of the moiety was established by GC analysis. The presence of a downfield methine signal at δ_C 94.6 (C-11) in the ^{13}C NMR spectrum indicated the attachment of the β-D-glucopyranosyl moiety, and this was further confirmed by the HMBC correlation from H-1′ to C-11. Thus, the structure of **4** was established as shown and named bruceolline K.

Compound **5** exhibited UV absorption bands at 210, 241, 261, and 299 nm, and IR absorption bands at 3357, 3206, and 1626 cm^{-1} indicated amine, hydroxy, and conjugated carbonyl functionalities. The molecular formula of **5**, $C_{13}H_{15}NO_2$, was established by HR-ESIMS (*m*/*z* 218.1174 $[M + H]^+$) and was two mass units greater than **3**. Comparison of the ^1H and ^{13}C NMR spectra of **5** with those of **3** revealed that both shared a similar unsubstituted indole aromatic ring, and the significant difference in **5** was the presence of an olefinic proton singlet (δ_H 8.38) and a multiplet methine (δ_H 2.20) in the ^1H NMR spectrum. These data provided evidence that **5** was a *seco*-derivative of **3**. This was confirmed by the HMBC correlations from H-2 (δ_H 8.38) to C-3 (δ_C 114.7), C-8 (δ_C 137.6), and C-9 (δ_C 127.0), from H-12 (δ_H 2.20) to C-10 (δ_C 197.5) and C-11 (δ_C 78.6), and from H_3-13 (δ_H 1.09) and H_3-14 (δ_H 0.75) to C-11 (δ_C 78.6) and C-12 (δ_C 35.0). The configuration at C-11 was unequivocally established by comparing the ECD data with those obtained through molecular modeling calculations as per the same protocol described for compound **2** (Supporting Information, Figure S82 and Table S2). Experimental and calculated spectra for the 11*R*-isomer were in good agreement (Figure 2). Therefore, the absolute configuration at C-11 was deduced to be *R*. Consequently, the structure of **5** was established as shown and named bruceolline L.

Compound **6** showed UV absorption bands at 205, 220, and 277 nm and IR absorption bands at 3388 and 1709 cm^{-1}, indicating the presence of hydroxy and unconjugated carbonyl functionalities. The molecular formula $C_{19}H_{25}NO_7$ of **6** was established by HR-ESIMS (*m*/*z* 380.1702 $[M + H]^+$). Comparison of the ^1H and ^{13}C NMR data with those of bruceolline F (**9**)[5] revealed that the oxymethine at C-11 of **9** was oxidized to a ketocarbonyl (δ_C 215.9) in **6**. This structural variation was confirmed by the HMBC correlations from H_2-10 (δ_H 4.13) to C-2 (δ_C 125.8), C-9 (δ_C 130.0), and C-11 (δ_C 215.9) and from H_3-13 (δ_H 1.40) to C-11 (δ_C 215.9), C-12 (δ_C 78.7), and C-14 (δ_C 27.0). Acid hydrolysis of **6** gave a glucose molecule. The large coupling constant (9.0 Hz) of the

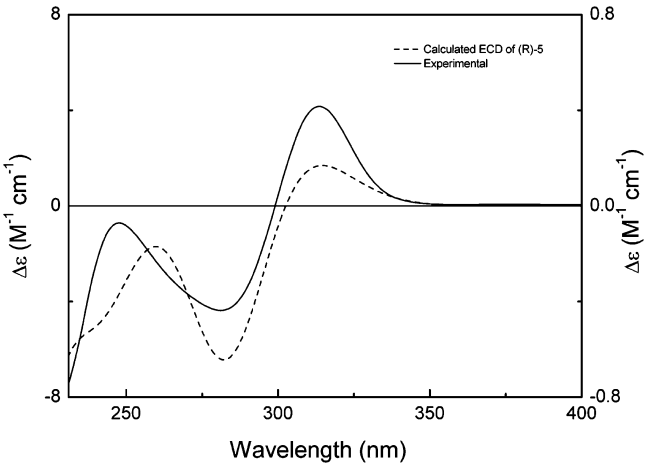


Figure 2. Calculated CDs of the 11*R*-isomer and experimental CDs of **5** in $CHCl_3$.

anomeric proton at δ_H 5.41 (H-1′) revealed that the glucose was in the β-configuration, and the D-configuration of the moiety was established by GC analysis. The presence of a significantly upfield anomeric carbon at δ_C 86.8, together with HMBC correlations from H-1′ (δ_H 5.41) to C-2 (δ_C 125.8) and C-8 (δ_C 138.3), established that the glucopyranosyl moiety was located at N-1 of the indole moiety. Thus, the structure of **6** was characterized as shown and named bruceolline M.

Compound **7** exhibited UV absorption bands at 212, 253, and 287 nm and IR absorption bands at 3388 and 1723 cm^{-1} similar to those of oxindoles.[11] The molecular formula, $C_{19}H_{27}NO_9$, was established by HR-ESIMS (m/z 414.1761 $[M + H]^+$). Comparison of ^1H and ^{13}C NMR spectra of **7** with those of **9** revealed that both shared a 2,3-dihydroxy-3-methylbutyl side chain and a sugar residue. The main difference in **7** was the absence at C-2 and C-3 of an olefinic bond and the presence of a carbonyl carbon (δ_C 180.2) and an oxygenated quaternary carbon (δ_C 76.6) in the ^{13}C NMR spectra. This revealed that **7** was a 3-substituted-3-hydroxy-2-oxindole *N*-glycoside derivative,[12] which was further confirmed by HMBC correlations from the anomeric proton (δ_H 5.27) to C-2 (δ_C 180.2) and C-8 (δ_C 142.6) and from H-4 (δ_H 7.38), H_2-10 (δ_H 2.23), and H-11 (δ_H 3.02) to C-3 (δ_C 76.6). The glycoside was deduced to be a β-glucopyranoside from ^1H and ^{13}C NMR data and confirmed to be a β-D-glucopyranoside by acid hydrolysis and GC analysis of the derivatized sugar. The absolute configuration at C-3 was elucidated to be *R*, since a positive Cotton effect in the 300−260 nm region and a negative Cotton effect in the 260−220 nm region were observed in the CD spectrum of **7** (Supporting Information, Figure S47).[13] Due to the limited quantity of this purified compound, the absolute configuration at C-11 could not be determined directly. Compound **9** possessed the same *vic*-diol unit as **7**. The absolute configuration of the 11,12-diol unit in **9** was established using the in situ dimolybdenum CD method developed by Snatzke and Frelek.[14−18] In order to avoid the interference from the hydroxy groups of the glucopyranosyl moiety, compound **9** was treated with 1 M HCl, and its aglycone **9b** was thus obtained from acid hydrolysis. The positive Cotton effects observed at 306 nm in the ICD permitted assignment of the 11*S* absolute configuration for **9b** on the basis of the empirical rule proposed by Snatzke, with the bulkier indole moiety group pointing away from the remaining portion of the complex (Figure 3). On the basis of the absolute configuration of **9** and a shared biogenesis with **7** and **9**, the C-11 stereogenic center in both metabolites presumably has the same absolute configuration. Thus, the structure of **7** was characterized as shown and named bruceolline N.

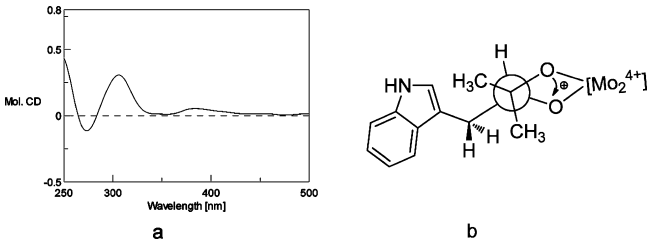
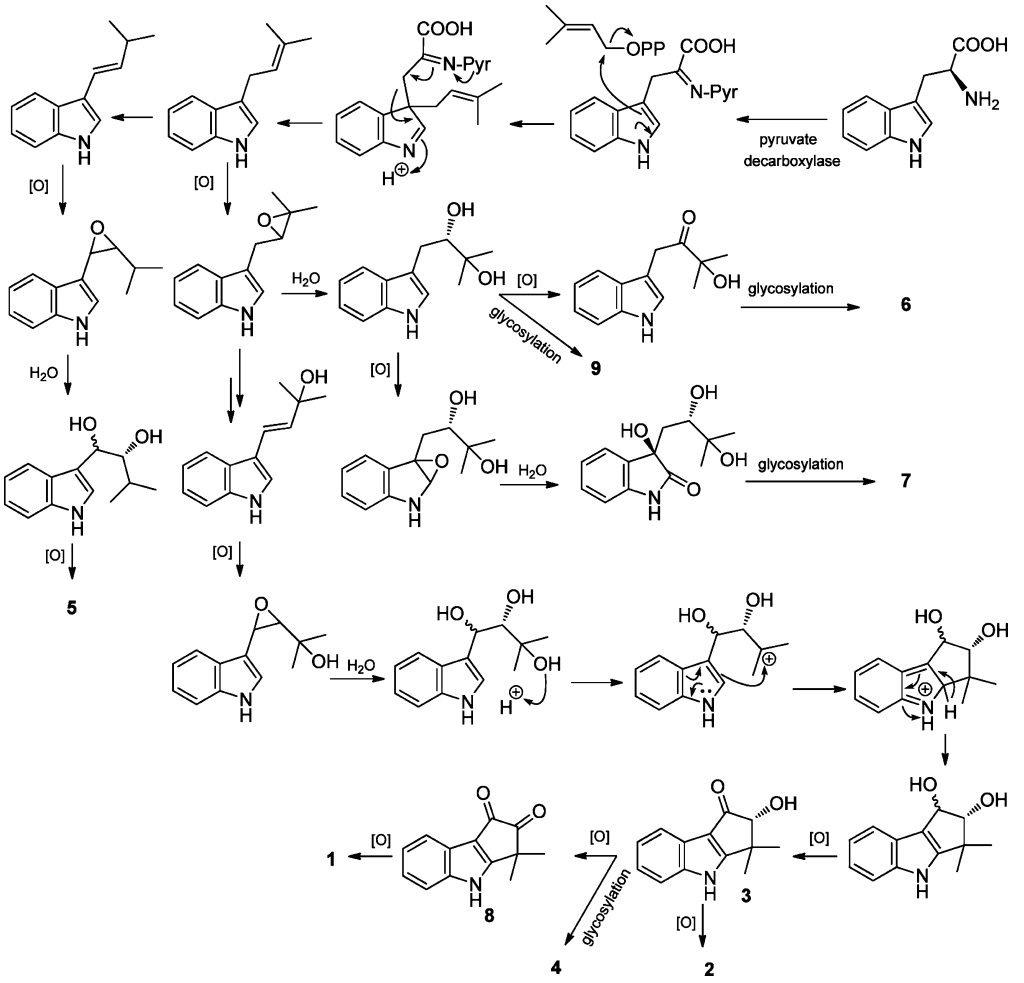


Figure 3. (a) CDs of **9b** in DMSO containing $Mo_2(OAc)_4$ with the inherent CDs subtracted. (b) Conformations of the Mo_2^{4+} complexes of **9b**.

The structures of indole alkaloids (**1**−**9**) are quite uncommon in natural products, particularly with respect to the functionalized isopentyl substituent at C-3 of the indole moiety. Biogenetically, the indole alkaloid is generally characterized as being of tryptamine origin derived from decarboxylation of tryptophan. By contrast, 3-prenylindoles markedly differ from ordinary indole alkaloids in that the side chain of tryptophan has been displaced by the prenyl unit rather than undergoing decarboxylation.[19] A plausible biogenetic route for the present alkaloids **1**−**9** is proposed in Scheme 1.

Compound **10** showed a UV absorption band at 241 nm, and the IR absorption bands at 3429, 1727, and 1661 cm^{-1} indicated hydroxy, δ-lactone, and α,β-unsaturated carbonyl functionalities. The molecular formula, $C_{20}H_{28}O_8$, was established by HR-ESIMS (m/z 397.1866 $[M + H]^+$), ^{13}C NMR, and various DEPT data. The ^1H NMR spectrum of **10** showed signals due to one olefin (δ_H 6.14), five oxymethines (δ_H 6.20, 5.89, 4.71, 4.36, and 4.16), one oxymethylene (δ_H 4.78 and 4.68), three methines (δ_H 3.25, 2.75, and 2.48), and three tertiary methyl groups (δ_H 1.82, 1.76, and 1.52). The ^{13}C NMR and DEPT spectra exhibited 20 signals, including those of a conjugated carbonyl carbon (δ_C 200.0), a pair of olefinic carbons (δ_C 165.4 and 125.2), a lactone carbonyl carbon (δ_C 175.2), five oxymethine carbons (δ_C 85.2, 84.3, 74.4, 74.3, and 68.0), and one oxymethylene carbon (δ_C 64.0). These NMR spectral data closely resembled those of yadanziolide S, which was reported as the first quassinoid isolated from *B. javanica* without a methyleneoxy bridge between C-8 and C-13.[20] By comparison of the NMR data of yadanziolide S with those of **10**, the most distinguished differences in **10** were the absence of a tertiary methyl and the presence of an oxymethylene group. The ^2J and ^3J HMBC correlations from the oxymethylene signal protons (δ_H 4.78 and 4.68) to C-12 (δ_C 74.4), C-13 (δ_C 37.3), and C-14 (δ_C 52.8) indicated that the oxymethylene group was attached to C-13, which was further confirmed by the $^1H-^1H$ COSY correlations from H-13 to H_2-21. The relative configuration of **10** was assigned on the basis of the NOE difference experiments. Correlations of H-9 with H-1, H-5, H-11, and H-15 suggested that these protons were α-orientated. On the other hand, observation of the key NOE correlations of H-7 with H-14 and H_3-20 and of H-13 with H-12, H-14, and H_3-20 revealed that these protons occupied the β-face of the molecule. On the basis of these results and

Scheme 1. Proposed Biosynthetic Pathway for Compounds 1−9



biosynthetic considerations,[1] we concluded that **10** possessed a 5*S*,10*S* configuration as in yadanziolide S. This was supported by a positive Cotton effect around 312 nm (the n → π^* transition) in the CD spectrum according to the octant rule for α,β-unsaturated ketones (Supporting Information, Figure S64).[21] Consequently, the chemical structure of **10** was characterized as shown and named yadanziolide T.

The molecular formula of compound **11** was established as $C_{26}H_{38}O_{13}$ by the HR-ESIMS (m/z 559.2390 $[M + H]^+$) combined with NMR data. The HR-ESIMS also showed a fragment ion $[M - 162 + H]^+$ at m/z 397.1864, suggesting that **11** was a glycoside. Furthermore, the NMR spectra of **11** demonstrated the presence of an additional glucose unit (δ_C 105.8, 79.0, 78.9, 75.6, 72.2, and 63.3). The ^1H and ^{13}C NMR data of the aglycone were similar to those of **10**. Acid hydrolysis of **11** gave **10** as the aglycone and glucose. The large coupling constant (7.5 Hz) of the anomeric proton at δ_H 5.10 revealed that the glucose was in the β-configuration, and the D-configuration of the moiety was established by hydrolysis and GC analysis. The presence of a downfield methylene signal at δ_C 71.8 (C-21) in the ^{13}C NMR spectrum indicated the attachment of the β-D-glucopyranosyl moiety, and this was further confirmed by the HMBC correlation from H-1′ to C-21. Accordingly, the structure of **11** was determined as shown and named yadanziolide U. This is the first naturally occurring quassinoid containing a glucosyl moiety linked at C-21.

Compound **12** showed a UV absorption band at 212 nm and IR absorption bands at 3432 and 1731 cm^{-1}, indicating the presence of hydroxy and lactone functionalities. The molecular formula $C_{19}H_{26}O_8$ was established by HR-ESIMS (m/z 383.1713 $[M + H]^+$) and NMR data. The NMR spectra of **12** were similar to those of eurylactone A,[22] except for the absence of a hydroxy group at C-14 and the presence of an oxymethylene group at C-13 in **12**. Thus, **12** was considered to have a 1,2-*seco*-1-nor-6(5→10)-*abeo*-picrasan-2,5-olide skeleton similar to eurylactone A. This conclusion was supported by the COSY, HSQC, and HMBC spectra (Supporting Information, Figures S73−S75). NOE difference experiments confirmed the relative configuration of **12** to be the same as eurylactone A (Supporting Information, Figure S76). On the other hand, the configuration at C-5 was determined by comparison of the proton chemical shift values around C-5 with those of eurylactone A and eurylactone B.[22] The ^1H NMR data of H_2-6 and H_3-18 between eurylactone A (H_2-6: δ_H 2.22, 2.10; H_3-18: δ_H 1.90) and eurylactone B (H_2-6: δ_H 3.04, 2.40; H_3-18: δ_H 2.56) revealed the characteristic difference. The signals of H_2-6 (δ_H 2.21 and 2.08) and H_3-18 (δ_H 1.92) of **12** exhibited similar chemical shifts values to those of eurylactone A, but different from those of eurylactone B. Thus, compound **12** was determined to possess the same 5*S* configuration as eurylactone A and named yadanziolide V.

Two known indole alkaloids, bruceolline E (**8**) and bruceolline F (**9**), and two known quassinoids, bruceine D

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Table 1. ^{1}H NMR Data of Compounds 1−7[a]

no.	1	2	3	4	5	6	7
2					8.38 s	7.30 s	
4	7.69 d (8.0)	7.54 d (8.5)	7.74 d (7.5)	7.81 d (7.5)	8.32 d (8.5)	7.39 d (8.0)	7.38 d (7.0)
5	6.90 dd (8.0, 2.0)	6.78 dd (8.5, 2.0)	7.24 td (7.5, 1.5)	7.28 t (8.0)	7.25 m	7.05 t (8.0)	7.13 t (7.5)
6			7.17 td (8.0, 1.5)	7.22 t (7.5)	7.24 m	7.16 t (8.0)	7.32 t (8.0)
7	7.01 d (2.0)	6.91 d (2.0)	7.47 d (8.0)	7.43 d (8.0)	7.54 d (8.5)	7.52 d (8.0)	7.22 d (8.0)
10						4.13 m	2.23 m
11		4.23 d (3.5)	4.26 d (5.0)	4.48 s	4.69 m		3.02 dd (9.5, 3.0)
12					2.20 m		
13	1.47 s	1.52 s	1.58 s	1.64 s	1.09 d (6.5)	1.40 s	1.07 s
14	1.47 s	1.29 s	1.32 s	1.38 s	0.75 d (6.5)	1.40 s	1.06 s
1′				4.62 d (7.5)		5.41 d (9.0)	5.27 d (9.5)
2′				3.32 brs		3.91 m	3.39 m
3′				3.36 overlap		3.58 m	3.50 t (9.0)
4′				3.38 overlap		3.50 m	4.16 t (9.0)
5′				3.45 m		3.50 m	3.41 m
6′				3.94 d (11.5)		3.86 dd (12.0, 2.5)	3.91 dd (12.0, 2.0)
				3.75 dd (11.5, 3.5)		3.70 dd (12.0, 5.5)	3.70 dd (12.0, 6.0)
6-OH	8.57 brs	8.41 brs					
11-OH		4.62 brs	4.57 d (5.5)		3.97 d (6.5)		
NH	11.5 brs	11.0 brs	11.1 brs		11.2 brs		

[a]^{1}H NMR data were measured at 500 MHz in acetone-d_6 for **1**−**3** and **5** and in methanol-d_4 for **4**, **6**, and **7**.

(**13**) and yadanziolide B (**14**), were identified on the basis of their spectroscopic profiles (NMR, UV, MS, and $[\alpha]_D$) and comparison to published data.

The cytotoxic activities of compounds **1**−**14** were tested in vitro against five human tumor cell lines (HCT-8, Bel-7402, BGC-823, A549, and A2780) using the MTT method. As shown in Table 4, compounds **10**, **13**, and **14** exhibited cytotoxicity against HCT-8, Bel-7402, and BGC-823 cells, with IC_{50} values of 3.00−5.81 μM. Moreover, compound **14** also displayed cytotoxicity against A549 cells, with an IC_{50} value of 3.80 μM. The other compounds were inactive (IC_{50} > 10 μM) against the cell lines tested.

Table 2. ^{13}C NMR Data of Compounds 1−7[a]

no.	1	2	3	4	5	6	7
2	171.0	171.2	171.8	174.4	134.4	125.8	180.2
3	115.5	115.1	115.1	114.5	114.7	110.0	76.6
4	122.8	121.7	121.2	121.7	122.7	119.7	125.1
5	113.4	112.2	124.1	125.1	124.1	120.8	124.2
6	157.2	155.5	122.7	123.5	122.9	122.9	130.5
7	100.0	99.4	113.4	113.6	112.8	111.5	112.7
8	142.4	144.1	142.8	143.7	137.6	138.3	142.6
9	123.6	115.8	122.6	122.7	127.0	130.0	131.6
10	176.0	193.8	194.3	194.8	197.5	33.7	40.4
11	206.7	86.9	86.9	94.6	78.6	215.9	75.6
12	42.6	41.1	41.2	42.0	35.0	78.7	73.3
13	23.3	25.4	25.3	25.3	20.3	27.0	24.8
14	23.3	24.4	24.4	24.5	15.7	27.0	25.8
1′				106.8		86.8	83.6
2′				75.5		73.8	71.6
3′				78.2		78.2	78.7
4′				71.5		71.4	70.2
5′				78.0		80.5	80.9
6′				62.8		62.7	62.9

[a]^{13}C NMR data were measured at 125 MHz in acetone-d_6 for **1**−**3** and **5** and in methanol-d_4 for **4**, **6**, and **7**.

EXPERIMENT SECTION

General Experimental Procedures. Melting points were measured on an XT5B micromelting point apparatus and were uncorrected. Optical rotations were recorded on a JASCO P-2000 automatic digital polarimeter. UV spectra were measured on a JASCO V650 spectrophotometer. CD spectra were recorded on a JASCO J-815 spectropolarimeter. IR spectra were recorded on a Nicolet 5700 FT-IR spectrometer. NMR spectra were recorded on an INOVA-500 spectrometer. ESIMS was measured on an Agilent 1100 Series LC/MSD ion trap mass spectrometer. HRESIMS data were recorded on an Agilent Technologies 6250 Accurate-Mass Q-TOF LC/MS spectrometer. Preparative HPLC was performed on a Shimadzu LC-6AD instrument with an SPD-10A detector, using a YMC-Pack ODS-A column (250 × 20 mm, 5 μm). GC data were recorded on an Agilent 7890A instrument. Sephadex LH-20 (Amersham Pharmacia Biotech AB, Sweden), ODS (45−70 μm, Merck), and silica gel (200−300 mesh, Qingdao Marine Chemical Inc. China) were used for column chromatography. TLC was carried out with glass precoated with silica gel GF_{254} (Qingdao Marine Chemical Inc. China).

Plant Material. The stems of *B. mollis* Wall. were collected from Guangxi Province, China, in July 2009, and identified by Prof. Song-Ji Wei at Guang Xi College of Traditional Chinese Medicine. A voucher specimen (ID-21977) is deposited in the herbarium of the Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, China.

Extraction and Isolation. Air-dried, powdered stems of *B. mollis* (6.5 kg) were macerated for 3 h with 20 L of 95% EtOH(aq) and refluxed for 9 h (20 L × 3). The filtrate was concentrated under reduced pressure, and the residue (320 g) was suspended in H_2O and then successively partitioned with EtOAc and *n*-BuOH. The EtOAc extract (80 g) was subjected to a silica gel column (200−300 mesh, 650 g), eluted with CH_2Cl_2/MeOH (70:1, 50:1, 30:1, 20:1, 10:1, 5:1, 1:1, = v/v), to yield seven fractions (A−G). Fraction B (10.2 g) was subjected to an ODS column (45−70 μm, 400 g), eluted with a gradient of MeOH/H_2O (from 15:85 to 90:10), to give six subfractions, B1−B6. Fraction B2 (750 mg) was chromatographed over a Sephadex LH-20 with MeOH and further purified by preparative HPLC [solvent system: CH_3CN/H_2O (30:70)] to yield **1** (32 mg) and **8** (4 mg). Fraction B3 (220 mg) was separated by preparative HPLC using the mobile phase CH_3CN/H_2O (35:65) to yield **3** (45 mg) and **10** (37 mg). Fraction B4 (1.30 g) was chromatographed over a Sephadex LH-20 column, eluted with MeOH,

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Table 3. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data of Compounds 10−12 in Pyridine-d_5

	10		11		12	
no.	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	85.2	4.16 s	85.2	4.14 s		
2	200.0		200.0		173.3	
3	125.2	6.14 s	125.2	6.13 s	120.2	5.96 s
4	165.4		165.4		168.8	
5	43.8	3.15 d (10.0)	43.8	3.15 d (10.5)	95.0	4.79 s
6α	26.6	2.08 m	26.6	2.08 m	43.5	2.21 d (15.5)
6β		2.08 m		2.08 m		2.08 dd (15.5, 4.5)
7	84.3	4.36 s	84.2	4.32 s	89.1	4.48 d (4.5)
8	39.3		39.4		45.6	
9	43.2	2.75 s	43.1	2.73 d (2.0)	46.8	3.22 s
10	49.5		49.4		44.4	
11	74.3	5.89 s	74.3	5.86 s	74.3	5.21 s
12	74.4	4.71 s	73.4	4.91 s	74.8	4.72 s
13	37.3	3.25 brs	35.4	3.40 brs	38.5	3.17 brs
14	52.8	2.48 dd (9.0, 4.0)	53.5	2.34 dd (9.0, 4.0)	49.0	2.76 dd (8.5, 3.5)
15	68.0	6.20 d (9.0)	68.0	6.16 d (9.0)	68.0	6.08 d (9.0)
16	175.2		175.2		175.6	
18	22.8	1.76 s	22.8	1.75 s	16.6	1.92 s
19	12.3	1.52 s	12.4	1.50 s	19.9	1.27 s
20	25.7	1.82 s	25.6	1.81 s	23.8	1.63 s
21a	64.0	4.78 m	71.8	5.03 overlap	63.7	4.82 m
21b		4.68 m		4.86 dd (10.5, 4.5)		4.72 m
1′			105.8	5.10 d (7.5)		
2′			75.6	4.10 m		
3′			79.0	4.21 overlap		
4′			72.2	4.23 overlap		
5′			78.9	3.92 m		
6′a			63.3	4.52 d (11.5)		
6′b				4.35 dd (11.5, 5.5)		

Table 4. Cytotoxicity of Compounds 10, 13, and 14 against Five Human Cancer Cell Lines

	IC_{50} (μM)				
compound	HCT-8	Bel-7402	BGC-823	A549	A2780
10	3.36 ± 0.08	4.40 ± 0.11	3.00 ± 0.23	>10	>10
13	5.81 ± 0.19	3.51 ± 0.05	3.80 ± 0.14	>10	>10
14	3.33 ± 0.13	4.24 ± 0.09	4.85 ± 0.37	3.80 ± 0.15	>10
adriamycin	0.42 ± 0.09	0.53 ± 0.07	0.98 ± 0.02	0.72 ± 0.08	0.33 ± 0.06

and further purified by preparative HPLC using the mobile phase CH_3CN/H_2O (28:72) to yield **5** (3 mg) and **12** (7 mg). Fraction D (3.20 g) was subjected to an ODS column (45−70 μm, 200 g), eluted with a gradient mobile phase MeOH/H_2O (from 10:90 to 80:20), to afford four fractions, D1−D4. Fraction D2 (85 mg) was separated by preparative HPLC using CH_3CN/H_2O (20:80) to yield **2** (5 mg). Fraction E (5.75 g) was subjected to an ODS column (45−70 μm, 400 g), eluted with a gradient mobile phase MeOH/H_2O (from 10:90 to 80:20), to give five subfractions, E1−E5. Fraction E2 (128 mg) was subjected to a Sephadex LH-20 column eluted with MeOH and then further purified by preparative HPLC using CH_3CN/H_2O (15:85) to yield **4** (15 mg) and **6** (8 mg). Similarly, fraction E3 (335 mg) was purified under the same conditions to yield **7** (3 mg) and **9** (23 mg).

The *n*-BuOH extract (102 g) was applied to an HP 20 macroporous adsorbent resin (1300 g) column and then eluted with H_2O, 30% EtOH, and 50% EtOH to yield three corresponding fractions. The 30% EtOH part (15.40 g) was subjected to an ODS column (45−70 μm, 400 g), eluted with a gradient of MeOH/H_2O (from 10:90 to 80:20), to give seven subfractions, N1−N7. Fraction N3 (115 mg) was separated by preparative HPLC using CH_3CN/H_2O (15:85) to yield **11** (42 mg). Fraction N4 (391 mg) was purified by preparative HPLC using CH_3CN/H_2O (25:75) to yield **13** (25 mg) and **14** (11 mg).

Bruceolline H (1): yellow needles (MeOH); mp 183−185 °C; UV (MeOH) λ_{max} (log ε) 210 (4.38), 259 (4.00), 287 (4.23), 366 (3.95) nm; IR (KBr) ν_{max} 3445, 3131, 2969, 1752, 1668, 1627, 1449, 1405, 1275, 1136, 1097, 949, 860, 840, 809 cm^{-1}; ^{1}H NMR (500 MHz, acetone-d_6) data, see Table 1; ^{13}C NMR (125 MHz, acetone-d_6) data, see Table 2; ESIMS m/z 230.1 $[M + H]^+$, 252.0 $[M + Na]^+$, 268.0 $[M + K]^+$; HR-ESIMS m/z 230.0806 $[M + H]^+$ (calcd for $C_{13}H_{11}NO_3$, 230.0812).

Bruceolline I (2): white, amorphous powder; $[\alpha]^{20}_D$ +11.3 (c 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 218 (4.44), 240 (4.20), 272 (4.22), 307 (3.92) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 220 (−1.10), 244 (0.49), 287 (1.20), 318 (−0.05) nm; IR (KBr) ν_{max} 3258, 2971, 1655, 1476, 1451, 1144, 1076, 1016, 954 cm^{-1}; ^{1}H NMR (500 MHz, acetone-d_6) data, see Table 1; ^{13}C NMR (125 MHz, acetone-d_6) data, see Table 2; ESIMS m/z 232.1 $[M + H]^+$, 254.0 $[M + Na]^+$, 270.0 $[M + K]^+$; HR-ESIMS m/z 232.0966 $[M + H]^+$ (calcd for $C_{13}H_{13}NO_3$, 232.0968).

Bruceolline J (3): white, amorphous powder; $[\alpha]^{20}_D$ +8 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 213 (4.33), 240 (3.96), 263 (3.99), 295 (3.69) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 215 (−0.24), 239 (0.28), 289 (0.24), 307 (−0.09) nm; IR (KBr) ν_{max} 3225, 2969, 1665, 1472, 1453, 1082, 923, 751 cm^{-1}; ^{1}H NMR (500 MHz, acetone-d_6)

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data, see Table 1; ^{13}C NMR (125 MHz, acetone-d_6) data, see Table 2; ESIMS m/z 216.0 [M + H]$^+$, 238.0 [M + Na]$^+$; HR-ESIMS m/z 216.1014 [M + H]$^+$ (calcd for $C_{13}H_{13}NO_2$, 216.1019).

Bruceolline K (4): white, amorphous powder; $[\alpha]^{20}_D$ +1.5 (c 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 213 (4.17), 241 (3.93), 263 (3.95), 295 (3.67) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 208 (−2.61), 262 (1.60), 306 (−0.93) nm; IR (KBr) ν_{max} 3393, 3205, 2976, 2907, 1651, 1475, 1454, 1073, 1040, 939, 833, 761 cm^{-1}; ^{1}H NMR (500 MHz, methanol-d_4) data, see Table 1; ^{13}C NMR (125 MHz, methanol-d_4) data, see Table 2; ESIMS m/z 378.1 [M + H]$^+$, 400.1 [M + Na]$^+$; HR-ESIMS m/z 378.1561 [M + H]$^+$ (calcd for $C_{19}H_{23}NO_7$, 378.1547).

Bruceolline L (5): yellowish solid; $[\alpha]^{20}_D$ +19 (c 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 210 (4.27), 241 (3.89), 261 (3.76), 299 (3.82) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 247 (−0.07), 281 (−0.43), 313 (0.41) nm; IR (KBr) ν_{max} 3357, 3206, 2965, 1626, 1520, 1422, 1082, 1117, 1023, 913, 829, 750 cm^{-1}; ^{1}H NMR (500 MHz, acetone-d_6) data, see Table 1; ^{13}C NMR (125 MHz, acetone-d_6) data, see Table 2; ESIMS m/z 218.2 [M + H]$^+$, 240.1 [M + Na]$^+$; HR-ESIMS m/z 218.1174 [M + H]$^+$ (calcd for $C_{13}H_{15}NO_2$, 218.1176).

Bruceolline M (6): white, amorphous powder; $[\alpha]^{20}_D$ +3 (c 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 205 (4.00), 220 (4.02), 277 (3.37) nm; IR (KBr) ν_{max} 3388, 2974, 2928, 1709, 1463, 1373, 1075, 745 cm^{-1}; ^{1}H NMR (500 MHz, methanol-d_4) data, see Table 1; ^{13}C NMR (125 MHz, methanol-d_4) data, see Table 2; ESIMS m/z 402.1 [M + Na]$^+$, 781.3 [2 M + Na]$^+$; HR-ESIMS m/z 380.1720 [M + H]$^+$ (calcd for $C_{19}H_{25}NO_7$, 380.1704).

Bruceolline N (7): white, amorphous powder; $[\alpha]^{20}_D$ +9.6 (c 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 212 (4.56), 253 (4.03), 287 (3.45) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 237 (−2.11), 265 (0.47) nm; IR (KBr) ν_{max} 3388, 2967, 2926, 1723, 1612, 1468, 1371, 1080, 951, 800, 755 cm^{-1}; ^{1}H NMR (500 MHz, methanol-d_4) data, see Table 1; ^{13}C NMR (125 MHz, methanol-d_4) data, see Table 3; ESIMS m/z 414.1 [M + H]$^+$; HR-ESIMS m/z 414.1761 [M + H]$^+$ (calcd for $C_{19}H_{27}NO_9$, 414.1759).

Yadanziolide T (10): white, amorphous powder; $[\alpha]^{20}_D$ +32.0 (c 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 241 (4.11) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 221 (1.75), 250 (−0.86), 312 (0.07) nm; IR (KBr) ν_{max} 3429, 2947, 1727, 1661, 1434, 1378, 1345, 1259, 1115, 1053, 1020 cm^{-1}; ^{1}H (500 MHz, pyridine-d_5) and ^{13}C NMR (125 MHz, pyridine-d_5) data, see Table 3; ESIMS m/z 397.1 [M + H]$^+$, 419.2 [M + Na]$^+$; HR-ESIMS m/z 397.1866 [M + H]$^+$ (calcd for $C_{20}H_{29}O_8$, 397.1857).

Yadanziolide U (11): white, amorphous powder; $[\alpha]^{20}_D$ +39 (c 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 241 (4.44) nm; IR (KBr) ν_{max} 3414, 2915, 1727, 1661, 1434, 1377, 1347, 1260, 1162, 1079, 1035 cm^{-1}; ^{1}H (500 MHz, pyridine-d_5) and ^{13}C NMR (125 MHz, pyridine-d_5) data, see Table 3; ESIMS m/z 581.2 [M + Na]$^+$, 597.3 [M + K]$^+$; HR-ESIMS m/z 559.2390 [M + H]$^+$ (calcd for $C_{26}H_{39}O_{13}$, 559.2385).

Yadanziolide V (12): white, amorphous powder; $[\alpha]^{20}_D$ +17 (c 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 212 (4.50) nm; IR (KBr) ν_{max} 3432, 2932, 1731, 1635, 1445, 1383, 1318, 1215, 1051, 984 cm^{-1}; ^{1}H (500 MHz, pyridine-d_5) and ^{13}C NMR (125 MHz, pyridine-d_5) data, see Table 3; ESIMS m/z 381.1 [M − H]$^-$, 405.1 [M + Na]$^+$; HR-ESIMS m/z 383.1713 [M + H]$^+$ (calcd for $C_{19}H_{27}O_8$, 383.1700).

Acid Hydrolysis of 9. Compound **9** (5 mg) was dissolved in 1 M HCl(aq) (10 mL) and heated at 60 °C for 8 h under constant stirring. The reaction mixture was diluted with H_2O and extracted with EtOAc (3 × 10 mL). The EtOAc layers were combined and evaporated to dryness and then subjected to preparative HPLC using the mobile phase CH_3CN/H_2O (25:75) to give the aglycone **9b** (1.2 mg). Compound **9b**: white, amorphous powder, $[\alpha]^{20}_D$ −15 (c 0.05, MeOH); UV (MeOH) λ_{max} 220, 280 nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 247 (−0.12) nm; $Mo_2(OAc)_4$ induced CD (MeOH) λ_{max} ($\Delta\varepsilon$) 306 (+0.30); ^{1}H NMR (500 MHz, methanol-d_4) δ_H 7.55 (1H, d, J = 8.0 Hz, H-4), 7.30 (1H, d, J = 8.0 Hz, H-7), 7.12 (1H, s, H-2), 7.06 (1H, t, J = 8.0 Hz, H-6), 6.98 (1H, t, J = 8.0 Hz, H-5), 3.65 (1H, d, J = 10.5 Hz, H-10a), 3.13 (1H, d, J = 15.0 Hz, H-10b), 2.65 (1H, m, H-11), 1.28 (3H, s, H-13), 1.27 (3H, s, H-14); ^{13}C NMR (125 MHz, methanol-d_4) δ_C 139.1 (C-8), 129.0 (C-6), 124.0 (C-2), 122.0 (C-9), 119.4 (C-4), 119.3 (C-5), 113.7 (C-3), 112.1 (C-7), 79.6 (C-11), 73.9 (C-12), 28.4 (C-10), 25.9 (C-13), 24.8 (C-14); HR-ESIMS m/z 242.1153 [M + Na]$^+$ (calcd. 242.1151 for $C_{13}H_{17}NO_2$).

Acid Hydrolysis of 4, 6, 7, and 11. Each compound (2 mg) was dissolved in 1 M HCl(aq) (5 mL) and heated at 90 °C for 2 h under constant stirring. After extraction with EtOAc (3 × 5 mL), the aqueous layer was evaporated and cryodesiccated. Each residue was dissolved in dry pyridine (1 mL), and then L-cysteine methyl ester hydrochloride (2 mg) was added. Each mixture was stirred at 60 °C for 2 h, and then 0.2 mL of *N*-trimethylsilylimidazole was added, followed by heating to dryness at 60 °C for 2 h. Each dried reactant was partitioned between *n*-hexane and H_2O (0.2 mL), and the *n*-hexane fraction was subjected to gas chromatography (GC) (column: DM-5, 0.25 mm × 30 m × 25 μM; detector: FID; temperature: 280 °C; injector temperature: 250 °C; carrier: N_2 gas). The sugars from each reactants were identified by comparison of their retention times with those for authentic standards [t_R: 19.84 min for D-glucose, 20.03 min for D-galactose].

Determination of Absolute Configuration of the 11,12-Diol Unit in 9b by Snatzke's Method. According to the published procedure,[15] a 1:1.2 mixture of diol/$Mo_2(OAc)_4$ for **9b** was subjected to CD measurements at concentration of 0.5 mg/mL. The first CD spectrum was recorded immediately after mixing, and its time evolution was monitored until stationary (about 10 min after mixing). The inherent CD was subtracted. The observed sign of the diagnostic band at around 310 nm in the induced CD spectrum was correlated to the absolute configuration of the 11,12-diol unit.

In Vitro Cytotoxicity Asssay. The EtOH extract and isolates were tested for their cytotoxic activity against HCT-8 (human colon cancer), Bel-7402 (human hepatoma cancer), BGC-823 (human gastric cancer), A549 (human lung epithelia cancer), and A2780 (human ovarian cancer) cell lines as per established colorimetric MTT assay protocols.[23] Adriamycin was used as positive control.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

IR, MS, and 1D and 2D NMR spectra for compounds **1**−**7** and **10**−**12**; 1D NMR spectra for compounds **9** and **9b**; CD spectra for compounds **2**−**5**, **7**, **9b**, and **10**; UV and CD calculations for compounds **2** and **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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