

Notolutesins A–J, Dolabrane-Type Diterpenoids from the Chinese Liverwort *Notoscyphus lutescens*

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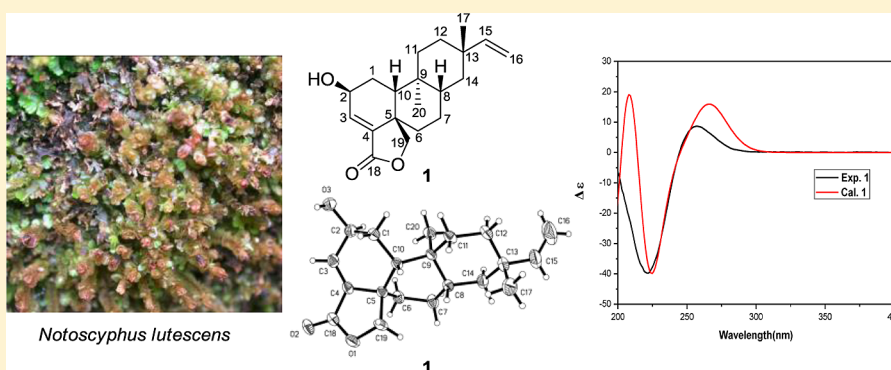
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Supporting Information



ABSTRACT: Ten new dolabrane-type diterpenoids, notolutesins A–J (1–10), were isolated from the Chinese liverwort *Notoscyphus lutescens*, along with four known compounds. The structures of the new compounds were established on the basis of extensive spectroscopic data, and that of 1 was confirmed by single-crystal X-ray crystallography. The absolute configuration of 1 was determined by comparing its experimental and calculated electronic circular dichroism spectra. All of the isolates were evaluated for their cytotoxicity against a small panel of human cancer cell lines, and compound 1 exhibited an IC₅₀ value of 6.2 μM against the PC3 human prostate cancer cell line.

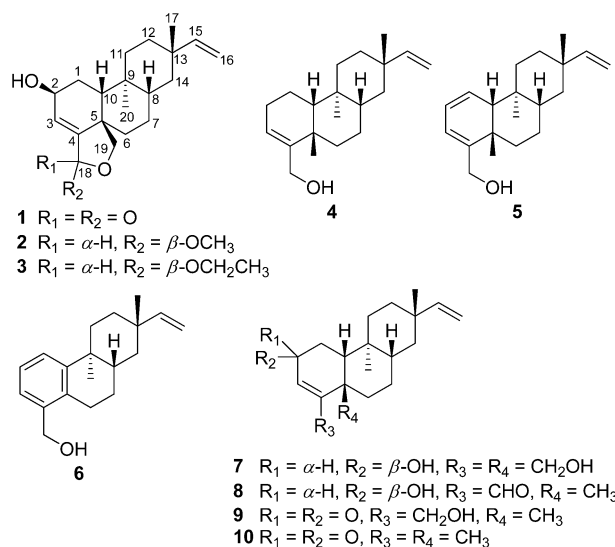
Liverworts (Hepaticae) are well known to produce lipophilic mono-, sesqui-, and diterpenoids, as well as aromatic compounds, of which many exhibit a variety of biological activities.¹ Species belonging to the family Jungermanniaceae are rich sources of labdane-, kaurane-, and clerodane-type diterpenoids.² Over the past few decades, chemical studies on the family Jungermanniaceae have focused mainly on the genus *Jungermannia*,^{3,4} while no phytochemical investigation has been reported on species of the genus *Notoscyphus*, due to difficulties concerning their collection and taxonomy.

In the course of an ongoing search for new bioactive substances from the Chinese liverworts,^{5–9} the autochthonal liverwort *Notoscyphus lutescens*, collected from Mount Dawei, Yunnan Province, People's Republic of China, in April 2012, was investigated phytochemically. Ten new dolabrane-type

diterpenoids, notolutesins A–J (1–10), together with four known diterpenoids, *ent-trans*-communic acid,¹⁰ *ent-trans*-communol,¹¹ 15,16-bis-*nor*-13-oxo-8(17),11(*E*)-labdadien-18-ol,¹² and (–)-pimara-9(11),15-dien-19-ol,¹³ were obtained. This is the first isolation of dolabrane-type diterpenoids from the family Jungermanniaceae. All of the structures were elucidated based on extensive spectroscopic data analyses, with single-crystal X-ray diffraction analysis performed to confirm the structure of 1. The cytotoxic activity of the isolated compounds was tested against five human cancer cell lines. Details of the isolation, structure elucidation, and cytotoxic activities of the compounds obtained are described herein.

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RESULTS AND DISCUSSION

Notolutesin A (**1**) was obtained as colorless needles (CHCl_3). It gave a molecular formula of $\text{C}_{20}\text{H}_{28}\text{O}_3$, as deduced from the pseudomolecular ion peak at m/z 334.2380 $[\text{M} + \text{NH}_4]^+$ (calcd 334.2377), requiring seven indices of hydrogen deficiency. The IR spectrum of **1** exhibited absorption bands at 3483 and 1758 cm^{-1} , accounting for the presence of OH and C=O groups, respectively. The ^1H NMR spectrum (Table 1) of **1** displayed resonances for two tertiary methyls [δ_{H} 1.01 (s, H₃-17) and 0.88 (s, H₃-20)], an oxygenated methylene [δ_{H} 4.49 (d, $J = 8.4$

Hz, Ha-19) and 3.83 (d, $J = 8.4$ Hz, Hb-19)], an oxygenated methine [δ_{H} 4.45 (br t, H-2)], a monosubstituted double bond [δ_{H} 5.80 (dd, $J = 17.4, 10.8$ Hz, H-15), 4.93 (d, $J = 17.4$ Hz, Ha-16), and 4.87 (d, $J = 10.8$ Hz, Hb-16)], and an olefinic proton at δ_{H} 6.85 (br s, H-3). The ^{13}C NMR data (Table 3) and HSQC spectrum denoted the presence of 20 resonances for two methyls, seven sp^3 methylenes, of which one was oxygenated [δ_{C} 78.8 (C-19)], three sp^3 methines, of which one was oxygenated [δ_{C} 65.0 (C-2)], three sp^3 quaternary carbons, four olefinic carbons [δ_{C} 109.3 (C-16), 135.6 (C-4), 138.0 (C-3), and 150.7 (C-15)], and one lactone carbonyl carbon [δ_{C} 170.2 (C-18)]. The aforementioned data indicated the characteristic of a dolabrane-type diterpenoid skeleton and a structure related to dolabradene^{14,15} for **1**. The ^1H – ^1H COSY data (Figure 1) of **1** revealed the presence of four isolated spin systems: C-10(H)–C-1(H₂)–C-2(H)–C-3(H), C-6(H₂)–C-7(H₂)–C-8(H), C-11(H₂)–C-12(H₂), and C-15(H)–C-16(H₂). On the basis of the HMBC correlations (Figure 1) from H₂-19 to C-4, C-5, C-6, and C-10, from H₃-20 to C-8, C-9, C-10, and C-11, and from H₃-17 to C-12, C-13, C-14, and C-15, the 6/6/6 tricyclic core of the diterpenoid with 9,13-dimethyl and 2-oxygenated substitutions could be elucidated. The γ -lactone ring was established from the HMBC correlations of H₂-19/C-18 and H-3/C-18. Thus, the gross structure of **1** with a four-ring system was established.

The relative configurations of **1** were determined by analyzing its NOESY data (Figure 2). NOE correlations of H-19b/H-10/H-8/H₃-17 suggested that all of these are cofacial, and they were assigned arbitrarily as β -oriented. In addition, H-

Table 1. ^1H NMR Spectroscopic Data for Compounds 1–5^a

position	1	2	3	4	5
1a	1.95 (ddd, 12.6, 6.6, 4.8)	2.04 (ddd, 13.2, 9.6, 4.8)	2.03 (ddd, 13.2, 9.6, 4.2)	1.70 (dd, 13.2, 7.8)	5.78 (m)
1b	1.69 (m)	1.15 (m)	1.15 (m)	1.46 (m)	
2a	4.45 (br t)	4.52 (t, 4.8)	4.51 (t, 4.8)	2.18 (dt, 18.6, 4.8)	6.02 (dd, 15.0, 5.4)
2b				2.06 (m)	
3	6.85 (br s)	6.39 (d, 5.4)	6.39 (d, 6.0)	5.54 (br s)	5.81 (d, 5.4)
6a	1.42 (m, H- α)	1.32 (m, H- α)	1.32 (m, H- α)	1.43 (m, H- α)	1.47 (td, 13.2, 4.2)
6b	1.68 (m, H- β)	2.48 (d, 7.8, H- β)	2.52 (d, 11.4, H- β)	1.80 (m, H- β)	2.14 (dt, 14.4, 3.0)
7a	1.67 (m)	1.28 (m)	1.34 (m)	1.43 (m)	1.27 (m)
7b	1.24 (m)	1.18 (m)	1.20 (m)	1.18 (m)	1.16 (m)
8	1.32 (m)	1.37 (m)	1.37 (m)	1.34 (m)	1.33 (m)
10	1.60 (t, 7.2)	1.25 (m)	1.25 (m)	1.19 (m)	1.61 (d, 6.0)
11a	1.65 (m)	1.35 (m)	1.35 (m)	1.60 (dd, 12.6, 3.0)	1.53 (dt, 13.2, 3.0, H- α)
11b	1.27 (m)	1.27 (m)	1.27 (m)	1.06 (dd, 12.6, 4.2)	1.24 (m, H- β)
12a	1.51 (td, 13.8, 4.2)	1.46 (td, 14.4, 4.2)	1.46 (td, 15.0, 4.2)	1.58 (dd, 15.0, 4.2, H- α)	1.20 (m)
12b	1.22 (m)	1.17 (m)	1.17 (m)	1.21 (m, H- β)	1.14 (m)
14a	1.29 (m)	1.31 (m)	1.30 (m)	1.33 (m)	1.31 (m)
14b	1.20 (m)	1.05 (td, 13.2, 2.4)	1.05 (d, 13.2)	0.97 (dd, 9.6, 1.8)	0.99 (dd, 12.0, 2.0)
15	5.80 (dd, 17.4, 10.8)	5.78 (dd, 17.4, 10.8)	5.78 (dd, 17.4, 10.8)	5.81 (dd, 17.4, 10.8)	5.78 (m)
16a	4.93 (d, 17.4)	4.90 (d, 17.4)	4.90 (d, 17.4)	4.91 (dd, 17.4, 1.2)	4.89 (dd, 17.4, 1.2)
16b	4.87 (d, 10.8)	4.84 (d, 10.8)	4.84 (d, 10.8)	4.84 (dd, 10.8, 1.2)	4.82 (dd, 10.8, 1.2)
17	1.01 (s)	1.03 (s)	1.03 (s)	1.02 (s)	1.03 (s)
18		4.26 (s)	4.36 (s)	4.09 (s)	4.25 (s)
19a	4.49 (d, 8.4)	4.25 (d, 11.4)	4.25 (d, 10.8)	1.09 (s)	1.06 (s)
19b	3.83 (d, 8.4)	4.09 (d, 11.4)	4.08 (d, 10.8)		
20	0.88 (s)	0.42 (s)	0.42 (s)	0.75 (s)	0.73 (s)
21a		3.29 (s)	3.71 (quintet, 7.2)		
21b			3.40 (quintet, 7.2)		
22			1.12 (t, 7.2)		

^aRecorded at 600 MHz in CDCl_3 . Chemical shifts (δ) are expressed in ppm, and J values are presented in Hz.

Table 2. ^1H NMR Spectroscopic Data for Compounds 6–10^a

position	6	7	8	9	10
1a	7.31 (d, 6.6)	2.15 (ddd, 13.2, 9.6, 4.2)	2.43 (dd, 14.4, 8.4)	2.65 (d, 18.6, H- α)	2.62 (d, 18.6, H- α)
1b		1.17 (m)	1.92 (ddd, 15.0, 9.6, 6.6)	2.75 (dd, 18.6, 6.6, H- β)	2.72 (dd, 18.6, 6.6, H- β)
2a	7.20 (t, 7.8)	4.45 (t, 4.8)	4.72 (t, 6.6)		
2b					
3	7.24 (d, 6.6)	6.37 (d, 5.4)	6.54 (br s)	6.19 (s)	5.84 (s)
6a	2.88 (m)	2.04 (dt, 13.8, 3.0)	1.23 (m, H- α)	2.04 (d, 15.0)	2.19 (dt, 14.4, 3.0)
6b		1.22 (m)	3.16 (d, 14.4, H- β)	1.34 (m)	1.31 (m)
7a	1.70 (m)	1.32 (m)	1.07 (d, 14.4)	1.16 (m)	1.16 (m)
7b	1.60 (m)	1.18 (m)	0.97 (m)	1.00 (m)	1.13 (m)
8	1.82 (tt, 12.6, 3.0)	1.38 (m)	1.33 (m)	1.33 (m)	1.38 (m)
10		1.37 (m)	1.40 (m)	1.67 (m)	1.67 (m)
11a	2.12 (dt, 12.6, 3.0, H- α)	1.28 (m, H- α)	1.70 (d, 12.6)	1.69 (m)	1.69 (m)
11b	1.64 (m, H- β)	1.40 (m, H- β)	1.17 (d, 13.8)	1.09 (td, 13.2, 3.0)	1.09 (td, 13.2, 4.2)
12a	1.72 (m)	1.47 (td, 14.4, 3.6)	1.49 (td, 13.8, 3.0)	1.52 (td, 14.4, 3.0)	1.52 (td, 14.4, 4.2)
12b	1.42 (dq, 13.2, 3.0)	1.18 (m)	1.22 (m)	1.22 (m)	1.22 (m)
14a	1.48 (t, 13.2)	1.30 (m)	1.36 (m)	1.40 (m)	1.33 (m)
14b	1.23 (dt, 13.8, 3.0)	1.02 (m)	0.99 (m)	1.18 (m)	1.00 (d, 12.6)
15	5.88 (dd, 17.4, 10.8)	5.79 (dd, 17.4, 10.8)	5.78 (dd, 17.4, 10.8)	5.78 (dd, 17.4, 10.8)	5.78 (dd, 17.4, 10.8)
16a	4.98 (dd, 17.4, 1.2)	4.90 (d, 17.4)	4.90 (d, 17.4)	4.90 (d, 17.4)	4.90 (d, 17.4)
16b	4.90 (dd, 10.8, 1.2)	4.84 (d, 10.8)	4.83 (d, 10.8)	4.84 (d, 10.8)	4.84 (d, 10.8)
17	1.03 (s)	1.03 (s)	1.01 (s)	1.02 (s)	1.02 (s)
18	4.68 (br s)	4.39 (s)	9.64 (s)	4.39 (s)	1.96 (s)
19a		3.35 (d, 6.6)	1.28 (s)	1.32 (s)	1.25 (s)
19b		2.90 (d, 6.6)			
20	1.06 (s)	0.45 (s)	0.74 (s)	0.63 (s)	0.63 (s)

^aRecorded at 600 MHz in CDCl_3 . Chemical shifts (δ) are expressed in ppm, and J values are presented in Hz.

Table 3. ^{13}C NMR Spectroscopic Data (δ) for Compounds 1–10^a

position	1	2	3	4	5	6	7	8	9	10
1	32.1 t	29.1 t	29.1 t	17.7 t	126.7 d	124.5 d	29.6 t	28.5 t	35.2 t	35.3 t
2	65.0 d	67.8 d	67.8 d	26.8 t	124.7 d	125.8 d	67.3 d	65.7 d	199.0 s	199.2 s
3	138.0 d	130.5 d	131.1 d	122.2 d	120.4 d	124.9 d	126.2 d	152.9 d	124.3 d	128.6 d
4	135.6 s	143.2 s	143.2 s	147.8 s	146.1 s	133.8 s	146.6 s	149.1 s	169.0 s	168.7 s
5	43.3 s	44.0 s	44.0 s	38.3 s	36.6 s	138.8 s	39.4 s	37.4 s	38.4 s	39.7 s
6	25.3 t	28.8 t	28.9 t	36.9 t	35.1 t	25.4 t	28.3 t	35.5 t	36.3 t	37.4 t
7	24.3 t	27.2 t	27.3 t	25.8 t	27.5 t	25.6 t	27.5 t	27.1 t	27.3 t	27.1 t
8	38.0 d	41.3 d	41.3 d	42.4 d	41.2 d	36.3 d	41.6 d	41.9 d	41.7 d	41.7 d
9	35.9 s	36.5 s	36.5 s	37.0 s	38.9 s	37.1 s	36.7 s	37.7 s	38.6 s	38.4 s
10	45.5 d	45.2 d	45.2 d	54.0 d	55.2 d	148.6 s	48.4 d	56.4 d	55.7 d	55.0 d
11	34.3 t	33.9 t	33.9 t	35.1 t	33.7 t	33.9 t	33.6 t	35.8 t	34.2 t	34.2 t
12	31.9 t	31.5 t	31.6 t	32.1 t	31.6 t	32.9 t	31.6 t	31.8 t	31.7 t	31.8 t
13	36.4 s	36.5 s	36.6 s	36.6 s	36.8 s	36.5 s	36.8 s	36.4 s	36.4 s	36.3 s
14	39.7 t	39.1 t	39.2 t	39.1 t	39.5 t	39.7 t	39.2 t	39.0 t	39.0 t	39.0 t
15	150.7 d	151.1 d	151.2 d	151.5 d	151.5 d	151.8 d	151.2 d	151.2 d	150.9 d	151.0 d
16	109.3 t	109.0 t	109.1 t	108.7 t	108.7 t	109.1 t	108.9 t	108.9 t	109.1 t	109.1 t
17	22.7 q	23.1 q	23.1 q	23.2 q	23.0 q	22.9 q	23.1 q	23.1 q	23.2 q	23.2 q
18	170.2 s	106.6 d	105.0 d	63.1 t	63.7 t	63.7 t	61.7 t	194.9 d	61.8 t	21.0 q
19	78.8 t	62.0 t	62.0 t	22.2 q	31.6 q		75.5 t	34.5 q	33.5 q	32.4 q
20	14.2 q	12.0 q	12.1 q	12.5 q	10.7 q	21.4 q	11.2 q	14.3 q	13.9 q	13.8 q
21		55.0 q	63.1 t							
22			15.1 q							

^aRecorded at 150 MHz in CDCl_3 . Chemical shifts (δ) are expressed in ppm.

6 α and H-6 β were determined by NOE correlations with H₃-20 and H-19a, respectively. NOEs observed between H₃-20 and H-2 supported the α -orientation of H-2. The conclusions stated above were confirmed by a single-crystal X-ray crystallography study (Figure 3). Thus, the structure and relative configuration of **1** were elucidated as shown.

Notolutesin B (**2**) was assigned the molecular formula $\text{C}_{21}\text{H}_{32}\text{O}_3$, based on its ^{13}C NMR data and HRESIMS, indicating the presence of one more carbon atom than **1**, which was consistent with the presence of an additional oxygenated methyl [δ_{H} 3.29 (s, H₃-21); δ_{C} 55.0 (C-21)] in **2**. Furthermore, the ^1H and ^{13}C NMR data (Tables 1 and 3)

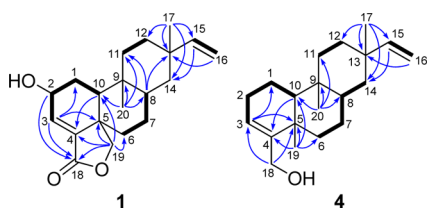


Figure 1. ^1H – ^1H COSY (bold) and selected HMBC (arrow) correlations of 1 and 4.

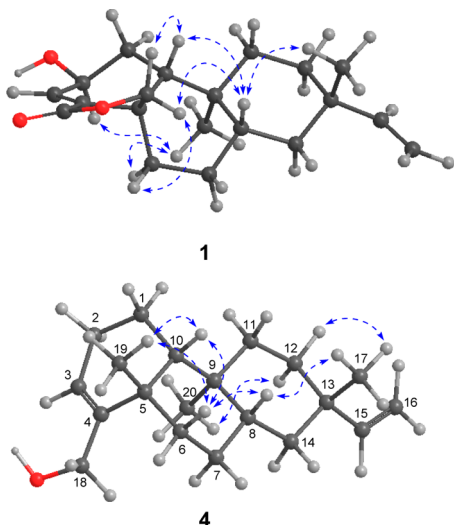


Figure 2. Key NOESY correlations of 1 and 4.

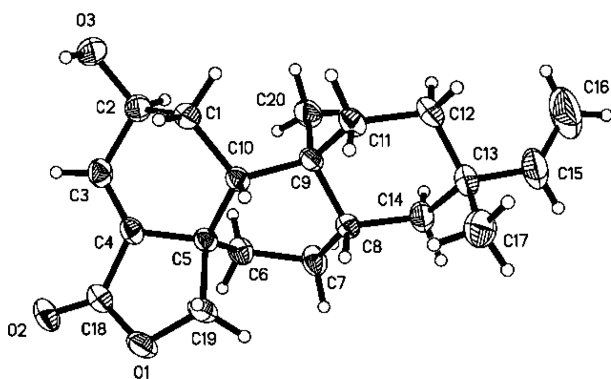


Figure 3. X-ray crystal structure of compound 1.

provided evidence that the 18,19-lactone carbonyl carbon in 1 was reduced to an acetal methine [δ_{H} 4.26 (s, H-18); δ_{C} 106.6 (C-18)] in 2, which was confirmed by the key HMBC correlations (Figure S10, Supporting Information) of H_3 -21/C-18, H_2 -19/C-18, and H-18/C-3. NOE correlations (Figure S11, Supporting Information) of Hb-19/H-10, Ha-19/H-8, and H-8/H $_3$ -17 suggested that H-8, H-10, and Me-17 are β -oriented. H-6 α and H-6 β were designated from NOE correlations with H_3 -20 and Ha-9, respectively, while the α -orientation of H-18 was elucidated by a NOE correlation of H-18/H β -6. The ^{13}C NMR data and HRESIMS of notolutesin C (3) revealed a molecular formula of $\text{C}_{22}\text{H}_{34}\text{O}_3$, which showed 14 mass units more than 2. The ^1H and ^{13}C NMR data (Tables 1 and 3) of 3 closely matched those of 2, apart from the absence of the oxygenated methyl and the appearance of an oxygenated methylene [δ_{H} 3.71 (quintet, $J = 7.2$ Hz, Ha-21) and 3.40 (quintet, $J = 7.2$ Hz, Hb-21); δ_{C} 63.1 (C-21)] and a methyl [δ_{H}

1.12 (t, $J = 7.2$ Hz, H_3 -22); δ_{C} 15.1 (C-22)]. Key ^1H – ^1H COSY correlations (Figure S21, Supporting Information) of H_3 -22/ H_2 -21 and HMBC correlations (Figure S21, Supporting Information) from H_2 -21 to C-18 suggested that the methoxy group at C-18 in 2 is replaced by an ethoxy group in 3. Compounds 3 and 2 might be artifacts arising from compound 1 during the extraction and isolation in the presence of EtOH and MeOH, respectively. Thus, the structures of 2 and 3 were determined as depicted.

Notolutesin D (4) was obtained as colorless needles (MeOH). A molecular formula of $\text{C}_{20}\text{H}_{32}\text{O}$ was established on the basis of the ^{13}C NMR data and by HRESIMS, accounting for five indices of hydrogen deficiency. An examination of the ^1H and ^{13}C NMR data (Tables 1 and 3) showed the structure of 4 to be similar to that of 1. Further analysis of the NMR data of 4 indicated that the 18,19-lactone ring of 1 was absent. In contrast, an oxygenated methylene [δ_{H} 4.10 (s, H_2 -18); δ_{C} 63.1 (C-18)] and a tertiary methyl [δ_{H} 1.09 (s, H_3 -19); δ_{C} 22.2 (C-19)] could be located at C-4 and C-5, respectively, which were supported by HMBC correlations (Figure 1) from H_2 -18 to C-3 and C-5 and from H_3 -19 to C-4, C-5, C-6, and C-10, respectively. Key ^1H – ^1H COSY correlations (Figure 1) of H-3/ H_2 -2/ H_2 -1/H-10 established the absence of an OH group at C-2 in 4. NOE correlations (Figure 2) of H-10/ H_3 -19, H_3 -19/H β -6, H β -6/H-8, and H-8/ H_3 -17 were used to determine that these groups are all β -oriented, while H-12 α and H-12 β were determined by NOE correlations with H_3 -20 and H_3 -17, respectively. Thus, the structure of 4 was determined to be as shown.

The HRESIMS of notolutesin E (5) showed a $[\text{M} + \text{NH}_4]^+$ ion peak at m/z 304.2638 (calcd 304.2635, $\text{C}_{20}\text{H}_{34}\text{ON}$), indicating an additional index of hydrogen deficiency when compared to 4. The ^1H and ^{13}C NMR data (Tables 1 and 3) of 5 revealed its structure to be similar to that of 4, with a noticeable difference being the observation of disubstituted double-bond resonances [δ_{H} 5.78 (m, H-1) and 6.02 (dd, $J = 15.0$, 5.4 Hz, H-2); δ_{C} 126.7 (C-1) and 124.7 (C-2)], which were supported by ^1H – ^1H COSY correlations of H-10/H-1/H-2/H-3 (Figure S41, Supporting Information). Thus, the structure of 5 was determined to be as depicted.

Compound 6 was assigned a molecular formula of $\text{C}_{19}\text{H}_{26}\text{O}$, based on its ^{13}C NMR data and HRESIMS, suggesting one additional index of hydrogen deficiency when compared to 5. In the ^1H NMR spectrum, the olefinic protons at δ_{H} 7.31 (d, $J = 6.6$ Hz, H-1), 7.20 (d, $J = 7.8$ Hz, H-2), and 7.24 (d, $J = 6.6$ Hz, H-3) underwent a large downfield shift, indicating the presence of a conjugated system. HMBC correlations (Figure S52, Supporting Information) from H_2 -18 and H-3 to C-5 (δ_{C} 138.8) and from H_3 -20 and H_2 -11 to C-10 (δ_{C} 148.6) established that ring A formed an aromatic ring and the methyl group at C-19 was absent. Thus, 6 was assigned as a 19-*nor*-dolabrane diterpenoid, and its structure was determined as shown.

Notolutesin G (7) was isolated as a colorless oil. Its molecular formula was determined to be $\text{C}_{20}\text{H}_{32}\text{O}_3$ from the ^{13}C NMR and HRESIMS data, indicating the presence of two additional oxygen atoms when compared to 4. Comparison of the ^1H and ^{13}C NMR data (Tables 2 and 3) with those of 4 indicated that both compounds have an identical skeleton. However, their substituents were found to differ only in that C-2 (δ_{C} 67.3) and C-19 (δ_{C} 75.5) in 7 are oxygenated. The HMBC correlations (Figure S63, Supporting Information) from H-2 [δ_{H} 4.45 (t, $J = 4.8$ Hz)] to C-4 and C-10 and from

H₂-19 [δ_{H} 3.35 (d, J = 6.6 Hz) and 2.90 (d, J = 6.6 Hz)] to C-4, C-5, C-6, and C-10 indicated the presence of OH groups at C-2 and C-19, respectively. NOE correlations of H₂-19/H-10, H-10/H-8, and H-8/H₃-17 supported these groups being β -oriented. H-11 α and H-11 β were determined by NOEs with H₃-20 and H₃-17, respectively. Key NOE correlations of H-2 and H₃-20 supported the β -orientation of OH-2 (Figure S64, Supporting Information). Thus, compound 7 was determined to be as shown.

Notolutesin H (8) was obtained as colorless crystals (CHCl₃), and its molecular formula, C₂₀H₃₀O₂, was established from the HRESIMS and ¹³C NMR data, suggesting one more index of hydrogen deficiency than 7. The ¹H and ¹³C NMR data (Tables 2 and 3) of these two compounds were closely comparable, except that the oxymethylenes at C-4 and C-5 in 7 were replaced by an aldehyde [δ_{H} 9.64 (s, H-18); δ_{C} 194.9 (C-18)] and a tertiary methyl [δ_{H} 1.28 (s, H₃-19); δ_{C} 34.5 (C-19)] in 8, respectively. The aldehyde group was assigned at C-4 due to the HMBC correlations (Figure S74, Supporting Information) from H-18 to C-3, C-4, and C-5. Similarly, HMBC correlations of H₃-19/C-4, C-5, C-6, and C-10 confirmed the location of the methyl group (Me-19) at C-5. Thus, the structure of 8 was determined as depicted.

Notolutesin I (9) was assigned the molecular formula C₂₀H₃₀O₂ by its ¹³C NMR data and from the HRESIMS. The ¹H and ¹³C NMR data (Tables 2 and 3) were similar to those of 7, with the exception of the replacement of the hydroxy group at C-2 in 9 by a keto group [δ_{C} 199.0 (C-2)] in 7. In addition, the presence of a hydroxy group at C-19 in 7 was methylated in 9. HMBC correlations (Figure S85, Supporting Information) were observed from H-10 and H₂-18 to C-2 and from H₃-19 to C-4, C-5, C-6, and C-10, respectively. Thus, the structure of compound 9 was determined as shown.

The molecular formula of notolutesin J (10) was determined to be C₂₀H₃₀O from the ¹³C NMR data and by HRESIMS, suggesting one less oxygen atom present than in 9. Accordingly, the ¹H and ¹³C NMR data (Tables 2 and 3) of these compounds were closely comparable, except that the oxymethylene [δ_{H} 4.39 (s, H₂-18) and δ_{C} 61.8 (C-18)] in 9 was found to be replaced by a tertiary methyl [δ_{H} 1.06 (s, H₃-18) and δ_{C} 32.4 (C-18)] in 10, as confirmed by HMBC correlations (Figure S96, Supporting Information) from H₃-18 to C-3, C-4, and C-5. Thus, the structure of compound 10 was determined as shown.

The absolute configuration of compound 1 was established by comparing its experimental and calculated ECD spectra predicted by TDDFT (Experimental Section; Figure 4). The results showed that the experimental and calculated ECD spectra were in good agreement. From the biosynthetic standpoint, the absolute configurations of compounds 2–10 were inferred as being the same as that of 1.

Four known diterpenoids, *ent-trans*-communal acid,¹⁰ *ent-trans*-communal,¹¹ 15,16-bis-*nor*-13-oxo-8(17),11(*E*)-labdadien-18-ol,¹² and (–)-pimara-9(11),15-dien-19-ol,¹³ were also isolated. Their structures were identified by comparison with reported spectroscopic data.

The cytotoxicities of the isolated compounds were evaluated against PC3 and DU145 human prostate and H1688 and A549 human lung carcinoma cell lines, using the MTT method;¹⁶ taxol and adriamycin were used as the positive controls. Only compound 1 showed cytotoxic activity against the PC3 cell line (IC₅₀ 6.2 μ M), but was inactive against the other three tested cell lines (IC₅₀ > 10 μ M).

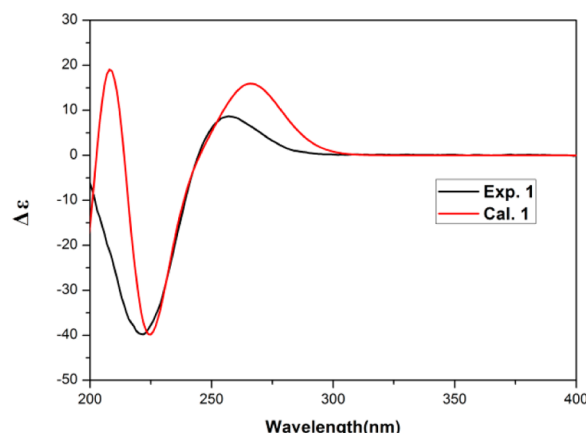


Figure 4. Experimental and calculated ECD spectra for compound 1.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were measured using an X-6 micromelting point apparatus (Tech Instrument Co., Ltd., Beijing, People's Republic of China) and are uncorrected. Optical rotations were obtained using a Gyromat-Hp polarimeter. UV spectra were acquired with an Agilent 8453E UV–visible spectroscopy system, with ECD spectra being obtained on a Chirascan spectropolarimeter (Applied Photophysics Ltd., Leatherhead, UK). IR spectra were recorded using a Nicolet iN 10 Micro FTIR spectrometer. NMR spectra were recorded on a Bruker Avance DRX-600 spectrometer operating at 600 (¹H) and 150 (¹³C) MHz, with TMS as the internal standard. HRESIMS was performed on an LTQ-Orbitrap XL instrument. HPLC separations were performed on an Agilent 1200 G1311A quaternary pump equipped with an Agilent 1200 G1322A degasser, an Agilent 1200 G1329B 1260ALS, an Agilent 1200 G1315D DAD detector, and ZORBAX SB-C₁₈ 5 μ m columns (9.4 mm \times 250 mm). Silica gel (200–300 mesh; Qingdao Haiyang Chemical Co. Ltd., Qingdao, People's Republic of China), RP C₁₈ silica gel (40–63 μ m, Fuji), Sephadex LH-20 (25–100 μ m; Pharmacia Biotech, Denmark), and MCI gel (CHP20P, 75–150 μ m, Mitsubishi Chemical Industries Ltd.) were used for CC. TLC was carried out with glass precoated silica gel GF₂₅₄ plates (Qingdao Haiyang Chemical Co. Ltd.). The compounds were visualized under UV light and by spraying with H₂SO₄–EtOH (1:9, v/v) followed by heating. Implementation of the MM2 force field in ChemBio3D Ultra software from CambridgeSoft Corporation (Cambridge, MA, USA, version. 11.0) was used to calculate the molecular models.

Plant Material. *Notoscyphus lutescens* was collected in April 2012 from Mount Dawei (1500 m), Yunnan Province, People's Republic of China, and authenticated by one of the authors (J.-C.Z.). A voucher specimen (No. 20120404-42) was deposited at the Department of Natural Products Chemistry, School of Pharmaceutical Sciences, Shandong University.

Extraction and Isolation. The air-dried powdered plant material of *N. lutescens* (170 g) was extracted with 95% EtOH at room temperature (3 \times 1.5 L, each for 2 weeks). Evaporation of the solvent provided a dark residue (14.0 g). The EtOH extract was separated by MCI gel column chromatography (MeOH–H₂O, 3:7 to 9:1) to give four fractions (Frs 1–4). Fr 2 (3.2 g) was chromatographed on a silica gel column (petroleum ether–acetone, 200:1 to 10:1) to give six subfractions (Frs 2.a–2.f). Fr 2.a (521 mg) was separated over RP C₁₈ (MeOH–H₂O, 6:4 to 9:1) to obtain six fractions (Frs 2.a.1–2.a.6). Purification of Fr 2.a.3 by preparative HPLC (MeCN–H₂O, 78:22, 1.5 mL/min) yielded 6 (5.2 mg, t_{R} 12.3 min) and 10 (4.4 mg, t_{R} 18.2 min). Preparative HPLC (MeCN–H₂O, 80:20, 1.5 mL/min) of Fr 2.a.4 yielded *ent-trans*-communal acid (1.9 mg, t_{R} = 12.9 min), *ent-trans*-communal (3.5 mg, t_{R} 18.8 min), and 5 (2.6 mg, t_{R} 22.7 min). Purification of Fr 2.a.6 by preparative HPLC (MeCN–H₂O, 83:17, 1.5 mL/min) yielded 4 (5.7 mg, t_{R} 20.3 min) and (–)-pimara-9(11),15-dien-19-ol (36.4 mg, t_{R} 21.5 min). Seven fractions (Frs 2.b.1–2.b.7)

were obtained from Fr. 2.b (747 mg) by using RP C₁₈ (MeOH–H₂O, 5:5 to 9:1). Fr 2.b.2 was purified by HPLC (MeCN–H₂O, 53:47, 1.5 mL/min) to give 15,16-bis-*nor*-13-oxo-8(17),11(*E*)-labdadien-18-ol (1.3 mg, *t_R* 11.4 min). Preparative HPLC (MeCN–H₂O, 67:33, 1.5 mL/min) on Fr 2.b.4 afforded **2** (8.2 mg, *t_R* 17.8 min), **8** (11.7 mg, *t_R* 19.1 min), and **3** (7.3 mg, *t_R* 27.3 min). Fr. 2.c (476 mg) was separated by RP C₁₈ (MeOH–H₂O, 5:5 to 9:1) to obtain a major portion, which was purified by preparative HPLC (MeCN–H₂O, 58:42, 1.5 mL/min) to afford **1** (1.7 mg, *t_R* 13.8 min), **7** (2.4 mg, *t_R* 15.1 min), and **9** (5.0 mg, *t_R* 19.8 min).

Notolutesin A (1): colorless needles (CHCl₃); mp 195–197 °C; [α]_D²⁰ –78.1 (*c* 0.46, MeOH); UV (MeOH) λ_{\max} (log ϵ) 214 (3.96) nm; ECD (MeOH) λ_{\max} ($\Delta\epsilon$) 222 (–0.83), 257 (+0.18) nm; IR ν_{\max} 3483, 2926, 1758, 1461, 1408, and 1290 cm^{–1}; ¹H NMR (600 MHz) and ¹³C NMR (150 MHz) data (CDCl₃), see Tables 1 and 3; HRESIMS *m/z* 334.2380 [M + NH₄]⁺ (calcd for C₂₀H₃₂O₃N, 334.2377).

Notolutesin B (2): colorless oil (CHCl₃); [α]_D²⁰ –33.7 (*c* 0.09, MeOH); UV (MeOH) λ_{\max} (log ϵ) 193 (4.82) nm; ECD (MeOH) λ_{\max} ($\Delta\epsilon$) 204 (–5.32) nm; IR ν_{\max} 3440, 2923, 2859, 1450, 1106, and 1005 cm^{–1}; ¹H NMR (600 MHz) and ¹³C NMR (150 MHz) data (CDCl₃), see Tables 1 and 3; HRESIMS *m/z* 355.2249 [M + Na]⁺ (calcd for C₂₁H₃₂O₃Na, 355.2244).

Notolutesin C (3): colorless oil (CHCl₃); [α]_D²⁰ –32.0 (*c* 0.13, MeOH); UV (MeOH) λ_{\max} (log ϵ) 193 (4.75) nm; ECD (MeOH) λ_{\max} ($\Delta\epsilon$) 206 (–3.33) nm; IR ν_{\max} 3442, 2923, 2860, 1105, and 1002 cm^{–1}; ¹H NMR (600 MHz) and ¹³C NMR (150 MHz) data (CDCl₃), see Tables 1 and 3; HRESIMS *m/z* 369.2401 [M + Na]⁺ (calcd for C₂₂H₃₄O₃Na, 369.2400).

Notolutesin D (4): colorless needles (MeOH); mp 187–190 °C; [α]_D²⁰ +16.7 (*c* 0.06, MeOH); UV (MeOH) λ_{\max} (log ϵ) 193 (4.95) nm; ECD (MeOH) λ_{\max} ($\Delta\epsilon$) 203 (–0.70) nm; IR ν_{\max} 3242, 2917, 2855, 1460, 1377, and 997 cm^{–1}; ¹H NMR (600 MHz) and ¹³C NMR (150 MHz) data (CDCl₃), see Tables 1 and 3; HRESIMS *m/z* 306.2793 [M + NH₄]⁺ (calcd for C₂₀H₃₆ON, 306.2791).

Notolutesin E (5): colorless oil (CHCl₃); [α]_D²⁰ +329.4 (*c* 0.09, MeOH); UV (MeOH) λ_{\max} (log ϵ) 271 (4.58) nm; ECD (MeOH) λ_{\max} ($\Delta\epsilon$) 270 (+19.41) nm; IR ν_{\max} 3184, 2973, 2924, 2857, 1462, and 1132 cm^{–1}; ¹H NMR (600 MHz) and ¹³C NMR (150 MHz) data (CDCl₃), see Tables 1 and 3; HRESIMS *m/z* 304.2638 [M + NH₄]⁺ (calcd for C₂₀H₃₄ON, 304.2635).

Notolutesin F (6): colorless oil (CHCl₃); [α]_D²⁰ +61.2 (*c* 0.10, MeOH); UV (MeOH) λ_{\max} (log ϵ) 206 (4.94), 260 (3.66) nm; ECD (MeOH) λ_{\max} ($\Delta\epsilon$) 207 (+1.33), 225 (–0.63) nm; IR ν_{\max} 3317, 2968, 2927, 1467, and 1377 cm^{–1}; ¹H NMR (600 MHz) and ¹³C NMR (150 MHz) data (CDCl₃), see Tables 2 and 3; HRESIMS *m/z* 271.2057 [M + H]⁺ (calcd for C₁₉H₂₇O, 271.2056).

Notolutesin G (7): colorless oil (CHCl₃); [α]_D²⁰ +20.2 (*c* 0.10, MeOH); UV (MeOH) λ_{\max} (log ϵ) 194 (4.81) nm; ECD (MeOH) λ_{\max} ($\Delta\epsilon$) 211 (–2.50) nm; IR ν_{\max} 3424, 2925, 2861, 1638, and 1058 cm^{–1}; ¹H NMR (600 MHz) and ¹³C NMR (150 MHz) data (CDCl₃), see Tables 2 and 3; HRESIMS *m/z* 338.2692 [M + NH₄]⁺ (calcd for C₂₀H₃₆O₃N, 338.2690).

Notolutesin H (8): colorless needles (CHCl₃); mp 200–202 °C; [α]_D²⁰ –43.5 (*c* 0.09, MeOH); UV (MeOH) λ_{\max} (log ϵ) 237 (4.88) nm; ECD (MeOH) λ_{\max} ($\Delta\epsilon$) 211 (+10.00), 242 (–10.92) nm; IR ν_{\max} 3268, 2968, 2919, 2865, 1696, and 1452 cm^{–1}; ¹H NMR (600 MHz) and ¹³C NMR (150 MHz) data (CDCl₃), see Tables 2 and 3; HRESIMS *m/z* 303.2321 [M + H]⁺ (calcd for C₂₀H₃₁O₂, 303.2319).

Notolutesin I (9): colorless oil (CHCl₃); [α]_D²⁰ +11.0 (*c* 0.09, MeOH); UV (MeOH) λ_{\max} (log ϵ) 246 (4.61) nm; ECD (MeOH) λ_{\max} ($\Delta\epsilon$) 210 (+2.11), 251 (–2.20), 329 (+0.58) nm; IR ν_{\max} 3414, 2922, 1719, 1653, and 1464 cm^{–1}; ¹H NMR (600 MHz) and ¹³C NMR (150 MHz) data (CDCl₃), see Tables 2 and 3; HRESIMS *m/z* 303.2323 [M + H]⁺ (calcd for C₂₀H₃₁O₂, 303.2319).

Notolutesin J (10): colorless oil (CHCl₃); [α]_D²⁰ +18.5 (*c* 0.11, MeOH); UV (MeOH) λ_{\max} (log ϵ) 248 (4.79) nm; ECD (MeOH) λ_{\max} ($\Delta\epsilon$) 205 (+3.36), 249 (–2.99), 327 (+0.94) nm; IR ν_{\max} 2921, 2859, 1723, 1663, and 1466 cm^{–1}; ¹H NMR (600 MHz) and ¹³C

NMR (150 MHz) data (CDCl₃), see Tables 2 and 3; HRESIMS *m/z* 287.2371 [M + H]⁺ (calcd for C₂₀H₃₁O, 287.2369).

X-ray Crystallographic Analysis of Compound 1. C₂₀H₂₈O₃, *M* = 316.42, orthorhombic system, space group *P*2₁2₁2₁, *a* = 6.2545(4) Å, *b* = 8.1557(6) Å, *c* = 34.847(2) Å, *V* = 1777.5(2) Å³, *Z* = 4, *D*_{calcd} = 1.182 Mg/m³, μ (Mo *K* α) = 0.078 mm^{–1}, *F*(000) = 688, and *T* = 293(2) K. A crystal of dimensions 0.48 × 0.42 × 0.13 mm³ was selected for measurements on a Bruker APEX2 CCD area-detector diffractometer with a graphite monochromator (φ – ω scans), Mo *K* α radiation (λ = 0.710 69 Å). APEX2 Software Suite¹⁷ was used for cell refinement and data reduction. The structure was refined with full-matrix least-squares calculations on *F*² using SHELXL-97.¹⁸ A total of 4122 reflections, collected in the θ range 2.34° to 27.63°, yielded 3572 unique reflections (*R*_{int} = 0.0285). The final stage converged to *R*₁ = 0.0553 (*wR*₂ = 0.1496) for 4122 observed reflections [with *I* > 2 σ (*I*)] and 211 variable parameters, *R*₁ = 0.0472 (*wR*₂ = 0.1409) for all unique reflections, and goodness-of-fit = 1.008.

Crystallographic data for the structure of **1** have been deposited with the Cambridge Crystallographic Data Centre as CCDC 996127. A copy of the data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

Theory and Calculation Details for Compound 1. Calculations were performed by the Materials Studio software and the Gaussian 09 program package. The conformational search was performed by Molecular Dynamical simulations based on the COMPASS force field¹⁵ and by scanning the potential energy surface on the main chain dihedral angles using the semiempirical AM1. The starting conformer of compound **1** came from the corresponding X-ray structure (Figure 3). All ground-state geometries were optimized at the B3LYP/6-31G* level at 298 K, and harmonic frequency analysis was computed to confirm the minima and, hence, calculation of room-temperature free energy. Multiple low-energy conformations of compound **1** were found, and four lower energy conformations were selected to predict the ECD spectrum of the substance. Electronic excitation energies and rotational strengths in the gas phase and in methanol were calculated using TDDFT^{19,20} at the same level in the velocity formalism for the first 60 states. The solvent effect of MeOH has been modeled by a conductor-like screening model for real solvents (COSMO).^{21,22} The ECD curves were simulated by using the Gaussian function:²³

$$\Delta\epsilon(E) = \frac{2}{2.296 \times 10^{-39}} \times \frac{1}{\sqrt{\pi} w} \sum_i \Delta E_{0i} R_{0i} e^{-[2(E - \Delta E_{0i})/w]^2}$$

where σ is half the bandwidth at 1/*e* height and ΔE_i and *R_i* are the excitation energies and rotatory strengths for transition *i*, respectively. Here σ = 0.4 eV.

Cytotoxicity Assays. The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide, Sigma] assay was used for the test compounds for cytotoxicity determination in 96-well plates.¹⁶ The cells were plated in 96-well plates for 24 h prior to treatment and were exposed continuously to different concentrations of the compounds for 24 h. All of the samples for the cytotoxicity assays were dissolved in DMSO. Subsequently, 10 μ L of MTT (5 mg/mL in phosphate-buffered saline) was added to each well, and the plates were incubated at 37 °C under 5% CO₂ for 4 h. The absorbance of the solution was measured at 570 nm on a plate reader (Bio-Rad). IC₅₀ values (the concentration resulting in 50% inhibition of cell growth) for the test compounds were calculated from the plotted results, with untreated cells being set at 100%. The experiments were conducted a minimum of three times. Taxol and adriamycin were used as the positive controls, with IC₅₀ values of (0.94, 1.79, 0.85, and 1.04) and (11.74, 21.45, 4.22, and 23.71) against the PC3, DU145, H1688, and A549 cell lines, respectively.

■ ASSOCIATED CONTENT

Supporting Information

1D-NMR, 2D-NMR, HRESIMS, IR, UV, and ECD spectra of the new compounds **1–10**; key HMBC (solid arrows), ¹H–¹H

COSY (bold lines), and NOESY (dashed arrows) correlations for compounds **2**, **3**, and **5–10**; and X-ray data of compound **1** are available free of charge via the Internet at <http://pubs.acs.org>.

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Author Contributions

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Notes

The authors declare no competing financial interest.

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