Two Minor Diterpene Glycosides and an Eudesman Sesquiterpene from *Coleus forskohlii*

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Two new labdane diterpene glycosides, forskoditerpenosides A, B (1, 2) and a new eudesmane sesquiterpene, 4β,7β,11-enantioeudesmantriol (3), were isolated from the ethanol extract of the whole plant of *Coleus forskohlii*. Their structures and stereochemistry were elucidated by extensive spectroscopic analysis and chemical methods. The structure of compound 3 was confirmed by X-ray diffraction. This is the first report about the occurrence of glycosides derived from the kind of labdane diterpene, 8,13-epoxy-labd-14-en-11-one, in the nature. Compounds 1 and 2 showed relaxative effects on isolated guinea pig tracheal spirals *in vitro*.

Key words *Coleus forskohlii*; Labiatae; diterpene glycoside; eudesmane sesquiterpene

Coleus forskohlii (WILLD.) BRIQ. (Labiatae) is largely distributed over the tropical and subtropical regions of India, Pakistan, Sri Lanka, tropical East Africa and Brazil, and it has been used since ancient times for medical treatment in Hindu and Ayurvedic traditional medicine.[1,2] Its characteristic constituents, 8,13-epoxy-labd-14-en-11-one diterpenes, especially the main constituent forskolin, showed positive inotropic, antihypertensive and adenylatecyclase stimulating activities.[2—12] The plant was later found in Yunnan Province of China and its aqueous extract has been used as a natural medicine to cure asthma, cough, acute and chronic bronchitis.[10—12] Up to now, almost the whole studies have been concentrated on these lipophilic labdane diterpene aglycones, and only two previous studies reported the polar fraction of the whole plant extract, from which only caffeic acid and two monoterpene glycosides were obtained.[13,14] In order to discover more novel and active compounds, further examination of the polar fraction has yielded two new diterpene glycosides, forskoditerpenosides A, B (**1**, **2**) and a new eudesmane sesquiterpene, 4β,7β,11-enantioeudesmantriol (**3**), whose structures were established on the basis of 1D, 2D NMR and HR-ESI-MS. The relative configuration of **3** was further confirmed by single-crystal X-ray diffraction. Their isolation and structural elucidation are reported below. Compounds **1** and **2** were tested for effect on isolated guinea pig tracheal spirals *in vitro*.

Results and Discussion

The 95% EtOH extract of the whole plant of *C. forskohlii* afforded compounds **1**—**3**, which were identified as 6β-acetoxy-7β,9α-dihydroxy-8,13-epoxy-labd-14-en-11-one-1α-*O*-β-D-glucopyranoside (**1**), 6β,7β-diacetoxy-9α-hydroxy-8,13-epoxy-labd-14-en-11-one-1α-*O*-β-D-glucopyranoside (**2**), and 4β,7β,11-enantioeudesmantriol (**3**).

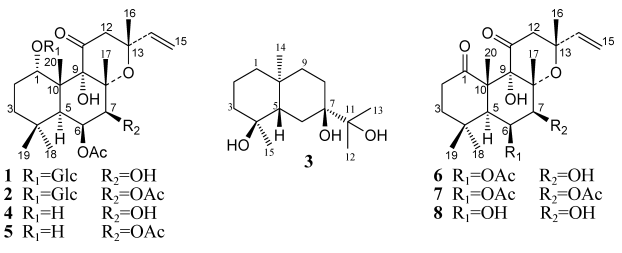

Fig. 1. The Structures of **1**—**8**

Forskoditerpenoside A (**1**) was obtained as white amorphous powder. It gave an $[M+Na]^+$ ion with *m*/*z* 595.2723 in the HR-ESI-MS, appropriate for the molecular formula $C_{28}H_{44}O_{12}Na$. Inspection of the IR spectrum revealed absorbances indicative of a hydroxyl functionality (3414 cm^{-1}) and a carbonyl (1717 cm^{-1}). The NMR spectra of **1** (Table 1) displayed one carbonyl (δ_C 210.1), one acetoxyl [δ_H 1.99 (s)/δ_C 171.2 and 22.4], one terminal vinylic group [ABX system, δ_H 6.06 (1H, dd, J=10.8, 17.4 Hz), 5.09 (1H, dd, J=1.3, 17.4 Hz) and 4.79 (1H, dd, J=1.3, 10.8 Hz), along with δ_C 149.1, 110.4], two geminal protons adjacent to carbonyl [AB system, δ_H 2.16 and 3.44 (each 1H, d, J=15.3 Hz)], five tertiary methyls [δ_H 0.95 (s), 1.02 (s), 1.31 (s), 1.46 (s) and 1.54 (s)]. These spectral data were consistent with the characteristics for 8,13-epoxy-labd-14-en-11-one type diterpenes.[8—12] The ^{1}H-NMR signal at δ 5.74 (1H, dd, J=2.8, 4.8 Hz) was assigned to H-6 by its coupling constants.[15,16] The chemical shifts of H-6 and C-6 suggested that an acetyl group was attached to C-6, as confirmed by the cross-peak between δ_H 5.74 and δ_C 171.2 in the HMBC spectrum (Fig. 2). Then, the signals at δ 2.39 (1H, d, J=2.8 Hz) and 4.28 (1H, d, J=4.8 Hz) were assigned to H-5 and H-7, respectively, interpreted from their coupling constants as well as HMQC and HMBC correlations.[15] The signal at δ 4.33 (1H, br s), which showed long-range correlations with C-2, C-3, C-5, C-10 and C-20 in the HMBC spectrum, was attributed to H-1, correlated to C-1 at δ 86.5 in HMQC spectrum. According to the above analysis, the structure of **1** was very similar to that of coleonol B isolated from this plant earlier, in exception of a signal for an anomeric proton at δ 4.21 (1H, d, J=7.8 Hz), linked to the anomeric carbon at δ 106.0 in its HMQC spectrum, as well as five oxygenated carbons between δ 63.7 and 79.0 and six proton signals between δ 3.17 and 3.81. Combining the Molish reaction, one sugar moiety was considered to exist in **1**.[12,15] After acid hydrolysis, the sugar was identified as D-glucose by TLC comparison with an authentic sample and its optical rotation. The coupling constant (J=7.8 Hz) of the anomeric proton H-1′ indicated that the glucose was connected to the aglycon *via* a β-linkage. The sugar was fixed at C-1 due to its downfield shift of +12.9,

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Table 1. NMR Data for Forskoditerpenosides A and B (**1**, **2**) (Multi, *J*)

No.	**1**[a]		**2**[b]	
	δ_H[c]	δ_C[d]	δ_H[c]	δ_C[d]
1β	4.33 br s	86.5	4.42 br s	85.5
2α	2.05 m	25.8	1.95 br d, 12.6	24.5
2β	2.05 m		2.06[e]	
3α	1.69 dt, 5.3, 12.5	39.1	1.72 br d, 13.3	37.2
3β	1.03 m		1.10 br d, 13.3	
4		34.9		33.8
5α	2.39 d, 2.8	44.9	2.42 d, 2.5	43.2
6α	5.74 dd, 4.8, 2.8	73.5	5.77 dd, 4.7, 2.5	69.7
7α	4.28 d, 4.8	74.6	5.56 d, 4.7	74.5
8		85.0		82.2
9α		85.0		81.7
10		45.9		44.3
11		210.1		208.7
12α	3.44 d, 15.3	51.3	3.41 d, 15.7	49.5
12β	2.16 d, 15.3		2.32 d, 15.7	
13		77.5		76.3
14	6.06 dd, 10.8, 17.4	149.1	5.92 dd, 10.7, 17.2	146.3
15-*cis*	4.79 dd, 1.3, 10.8	110.4	4.92 br d, 10.7	110.4
15-*trans*	5.09 dd, 1.3, 17.4		5.19 br d, 17.2	
16	1.31 3H, s	30.2	1.35 3H, s	30.6
17	1.54 3H, s	24.3	1.62 3H, s	23.5
18	1.02 3H, s	33.8	1.02 3H, s	32.7
19	0.95 3H, s	24.3	0.97 3H, s	23.4
20	1.46 3H, s	21.1	1.49 3H, s	19.9
C-6-Acetate	1.99 3H, s	171.2, 22.4	2.08 3H, s[e]	170.0, 21.5
C-7-Acetate			2.05 3H, s[e]	170.3, 20.9
1′	4.21 d, 7.8	106.0	4.27 d, 7.7	104.1
2′	3.17 m	75.3	3.10—3.52 m	73.2
3′	3.28 br s	79.0	3.10—3.52 m	76.9
4′	3.28 br s	72.5	3.10—3.52 m	70.3
5′	3.28 br s	78.6	3.10—3.52 m	75.5
6′	3.81 m	63.7	3.82 2H, br s	62.3
	3.63 m			

a) Measured in Me_2CO-d_6. *b*) Measured in $CDCl_3$. *c*) Measured at 600 MHz. *d*) Measured at 150 MHz. *e*) Overlapped signals. OH signals were observed in data of **1**, typically as broad signals, but varied in appearance, and are not listed here.

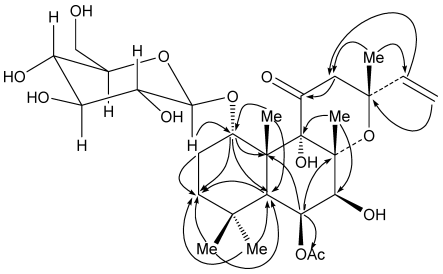

Fig. 2. Key HMBC Correlations from H to C for Compound **1**

compared with that of coleonol B, and the cross-peak in the HMBC spectrum between C-1 and H-1′.[12] The relative stereochemistry of **1** was deduced mainly by analysis of the correlations in the NOESY experiment (Fig. 3). The orientations of 1β-H, 6α-H and 7α-H were confirmed by the coupling constants in ^{1}H-NMR and the NOEs of H-1/H_3-20, H-5/H-6, H-5/H-7 and H-6/H-7.[15,16] The presence of NOE correlations between H-5/H_3-18, H-5/H-7, H_3-19/H_3-20, H_3-19/H_3-Ac, H_3-20/H_3-17, H_3-20/H_3-Ac, H_3-17/H_3-Ac and H_3-17/H_3-16, were in good agreement with the naturally occurring labdane derivatives reported in the literature,[16,17] allowing the assignment of the ring conjunction as *trans*-form for

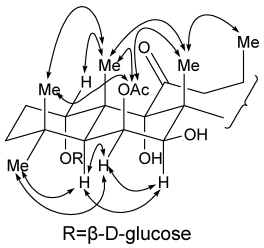

Fig. 3. Selected NOESY Correlations of Compound **1**

both A/B and B/C. Therefore, the chemical structure of **1** was elucidated as 6β-acetoxy-7β,9α-dihydroxy-8,13-epoxy-labd-14-en-11-one-1α-*O*-β-D-glucopyranoside.

The absolute stereochemistry of **1** was determined by the correlation with known compound, 8,13-epoxy-6β,7β,9α-trihydroxylabd-14-ene-1,11-dione (**6**). The enzymatic hydrolysis of **1** gave the aglycon (**4**), the selective oxidation of **4** produced 8,13-epoxy-6β-acetoxy-7β,9α-dihydroxylabd-14-ene-1,11-dione (**6**), which was hydrolyzed to give 8,13-epoxy-6β,7β,9α-trihydroxylabd-14-ene-1,11-dione (**8**) identified by optical activity and spectral data. The absolute stereochemistry of **8** was established undoubtedly by the use of the exciton chirality circular dichroism (CD) method on the 6,7-dibenzoate derivative in the literature.[18] Accordingly, the absolute configurations at C-1, 5, 6, 7, 8, 9, 10 and 13 of **1** were confirmed as *S*, *S*, *S*, *S*, *R*, *S*, *R* and *R*, respectively, which were identical with the labdane diterpenes such as forskolin[2] isolated from the same plant.

Forskoditerpenoside B (**2**) was isolated as white amorphous powder with positive Molish reaction. Its molecular composition, $C_{30}H_{46}O_{13}Na$, was obtained from HR-ESI-MS at *m*/*z* 637.2836 $[M+Na]^+$. The IR spectrum showed absorption bands at 3425, 1742 and 1709 cm^{-1}, suggesting the presence of hydroxyl, ester, and ketone functions. Inspection of the NMR spectra (Tables 1, 2) along with the two-dimensional data confirmed the presence of one carbonyl, two acetoxyls, a terminal vinylic group, two geminal protons adjacent to carbonyl, and five tertiary methyls, and other shared structural feature of **2** with **1**. Comparison of the above spectra with those of **1** revealed the conspicuous addition of an acetyl group. The downfield shift of H-7 [δ 5.56 (1H, d, J=4.7 Hz)] in **2**, indicated that the extra acetoxyl was connected to C-7, which was reinforced by an HMBC correlation from H-7 to the carbonyl signal at δ 170.3. The ^{1}H-NMR signal at δ 5.77 (1H, dd, J=4.7, 2.5 Hz), 4.42 (1H, br s) and 2.42 (1H, d, J=2.5 Hz) were assigned to H-6, H-1 and H-5, respectively, interpreted from the comparison with **1** and their coupling constants as well as HMQC and HMBC correlations. Thus, the aglycon of **2** was determined as 6-acetylforskolin.[19,20] The sugar moiety was identified as β-D-glucopyranoside by acid hydrolysis and the coupling constant (J=7.7 Hz) of H-1′. As listed in Table 2, the downfield shift of +10.9 (δ 85.5) observed for C-1, compared with that in 6-acetylforskolin, and correlation in the HMBC spectrum between C-1 and H-1′, indicated that the glucose was attached to C-1.[20] The relative stereochemistry of **2** was in agreement with that of **1** due to their similar NOESY correlations (H-5/H_3-18, H-5/H-7, H_3-19/H_3-20, H_3-20/H_3-17 and H_3-17/H_3-16). Therefore, the structure of **2** was established as 6β,7β-diacetoxy-9α-hydroxy-8,13-epoxy-labd-14-en-11-one-1α-*O*-

Table 2. NMR and HMBC Data for 4β,7β,11-Enantioeudesmantriol (**3**) (Multi, *J*, $CDCl_3$)

No.	δ_H [b] (HMQC)	δ_C [c]	HMBC (C–H)
1	1.40 m, 1α	40.8	H-3, H-9, H-14
	1.16 td, 12.7, 4.3, 1β		
2	1.50 m, 2α	20.2	H-1, H-3
	1.59 m, 2β		
3	1.81 m, 3α	43.7	H-1, H-2, H-15
	1.44 m, 3β		
4		72.3	H-2, H-3, H-5, H-6, H-15
5	1.67 dd, 13.0, 2.7	48.6	H-1, H-3, H-6, H-9, H-14, H-15
6	1.40 m, 6α	26.2	H-5
	1.81 m, 6β		
7		76.6	H-5, H-6, H-8, H-9, H-12, H-13
8	1.58 m, 8α	26.7	H-6, H-9
	1.47 m, 8β		
9	1.25 m, 9α	40.0	H-1, H-5, H-8, H-14
	1.56 m, 9β		
10		34.4	H-1, H-2, H-5, H-6, H-8, H-9, H-14
11		75.7	H-12, H-13
12[a]	1.24 3H, s	24.6	
13[a]	1.26 3H, s	24.7	
14	0.85 3H, s	17.6	H-1, H-5, H-9
15	1.10 3H, s	22.3	H-3, H-5

a) Exchangeable signals. *b*) Measured at 600 MHz. *c*) Measured at 150 MHz.

β-D-glucopyranoside.

By the same process as described in compound **1**, the absolute configurations at C-1, 5, 6, 7, 8, 9, 10 and 13 of **2** were also evaluated as *S*, *S*, *S*, *S*, *R*, *S*, *R* and *R*, respectively.

The HR-ESI-MS of **3** revealed a quasi-molecular ion peak at *m/z* 279.1920 $[M+Na]^+$, consistent with the molecular formula $C_{15}H_{28}O_3Na$. The IR spectrum of **3** displayed absorption band at 3370 cm^{-1} due to hydroxyl group. The ^{1}H-NMR spectrum (Table 2) of **3** revealed four methyl singlet at δ 0.85, 1.10, 1.24, 1.26 and a double doublet at δ 1.67, and no olefinic proton. The ^{13}C-NMR and DEPT spectra showed 15 carbon signals due to four methyls, six methylens, one methine and four quaternary carbons. Only three oxygenated carbons (δ 72.3, 76.6, 75.7) were observed in the spectra, suggesting that there were three hydroxyl groups in the structure by the molecular formula, and all of them were quaternary carbons. These results established a hydroxylated eudesmol skeleton for **3**.[21—23]

Further NMR studies including HMQC and HMBC (Table 2) experiments allowed the determination of the planar structure of **3**. The presence of an isopropyl group was indicated by the fact that the methyl protons resonated at δ 1.24 and 1.26 (H-12, H-13) correlating with the oxygenated quaternary carbons at δ 76.6 (C-7) and 75.7 (C-11) in HMBC spectrum. The other oxygenated carbon (δ 72.3) was considered to be C-4 for its HMBC correlations with H-2, H-3, H-5, H-6 and H-15. The methyl protons at δ 0.85 and δ 1.10 were attributed to H-14 and H-15, respectively, interpreted from their HMQC and HMBC spectra. The ^{1}H-NMR signals at δ 1.67 (dd, J=13.0, 2.7 Hz) was assigned to H-5 by its cross-peaks with C-4, C-6, C-7, C-9, C-10, C-14 and C-15 in HMBC, while the signal at δ 1.16 (td, J=12.7, 4.3 Hz) was attributed to H-1. Then, the structure of **3** was considered to be 4,7,11-eudesmantriol.

A single-crystal X-ray diffraction (Fig. 4, Tables 3—6) study of **3** was carried out, from which its relative stereochemistry was determined. The ORTEP perspective drawing depicted in Fig. 4 supported the deduction concerning this structure made by both NMR and mass spectrometry. The configurations at H-5, C-11 (isopropyl), C-14 (methyl), and C-15 (methyl) were β-, α-, α- and α-oriented in **3**, which was consistent with those of enantio-eudesmantriol.[21,24] The Fig. 4 clearly indicated that H-5 and the C-14 (methyl) were on the different sides of an A/B *trans*-decalin moiety. The key correlations in the NOESY spectrum of **3** (Fig. 5) were in agreement with above relative stereochemistry. Therefore, compound **3** was elucidated as 4β,7β,11-enantioeudesmantriol.

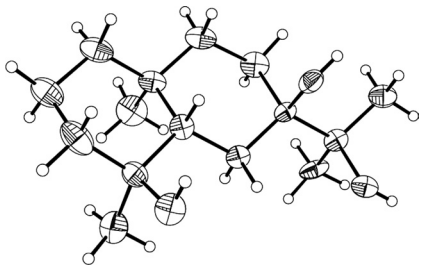

Fig. 4. ORTEP Diagram of Compound **3**

X-ray crystal structure of **3** showing the *trans*-A/B fusion and the relative stereochemistry of the hydroxyl and isopropyl groups.

Table 3. Crystal Data and Structure Refinement for **3**

Identification code	Compound name
Empirical formula	$C_{30}H_{62}O_9$
Formula weight	566.8
Temperature	298(2) K
Wavelength	0.71073 Å
Crystal system, space group	Monoclinic, $P2_1$
Unit cell dimensions	a=12.641(7) Å
	b=7.443(4) Å
	c=17.612(10) Å
	β=95.633(10)°
Volume	1648.9(16) $Å^3$
Z, Calculated density	2, 1.142 Mg/m^3
Absorption coefficient	0.082 mm^{-1}
F(000)	628
Crystal size	0.50×0.21×0.08 mm
Theta range for data collection	1.62 to 25.00°
Limiting indices	$-14\leq h\leq 15$, $-8\leq k\leq 8$, $-16\leq l\leq 20$
Reflections collected/unique	8529/3112 [R_{int}=0.0998]
Completeness to θ=25.00	98.80%
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9935 and 0.9601
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	3112/1/352
Goodness-of-fit on F^2	1.007
Final R indices [$I\geqslant 2\sigma(I)$]	R_1=0.0790, ωR_2=0.1862
R indices (all data)	R_1=0.1753, ωR_2=0.2563
Absolute structure parameter	−10(10)
Largest diff. peak and hole	0.265 and −0.293 e·$Å^{-3}$

In this paper, we reported the structures of two new diterpenes, forskoditerpenosides A and B (**1**, **2**), together with a new sesquiterpene 4β,7β,11-enantioeudesmantriol (**3**). Compounds **1** and **2** are 8,13-epoxy-labd-14-en-11-one type diterpene glycosides which were discovered for the first time. The structures of them were unambiguously determined and all the proton and carbon resonances were definitely assigned as Tables 1 and 2 by the aid of HMQC, HMBC, NOESY and X-ray diffraction.

Table 4. Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Parameters ($Å^2 \times 10^3$) for **3**

	x	*y*	*z*	*U*(eq)		*x*	*y*	*z*	*U*(eq)
O(1)	4065(4)	7722(9)	1710(3)	57(2)	C(12)	7718(7)	8370(17)	1888(6)	74(3)
O(2)	5607(4)	8579(8)	3928(3)	48(2)	C(13)	6533(6)	5780(12)	4141(5)	40(2)
O(3)	5519(4)	4841(8)	4131(3)	45(2)	C(14)	7302(7)	4447(14)	3856(6)	60(3)
O(4)	3332(4)	4258(10)	1627(3)	60(2)	C(15)	6878(7)	6319(15)	4966(5)	57(3)
O(5)	1784(5)	3740(9)	3679(4)	61(2)	C(16)	2227(7)	4106(17)	1314(5)	56(3)
O(6)	2335(4)	7286(9)	3986(3)	58(2)	C(17)	2190(8)	2374(18)	871(6)	79(4)
O(7)	4040(4)	6337(9)	5042(3)	53(2)	C(18)	1032(8)	1740(20)	652(7)	90(4)
O(8)	3632(4)	9255(10)	3134(4)	60(2)	C(19)	421(8)	1607(19)	1339(6)	81(4)
O(9)	3903(5)	3020(10)	3134(4)	68(2)	C(20)	407(7)	3304(13)	1805(5)	52(2)
C(1)	5123(7)	8248(14)	1527(5)	47(2)	C(21)	−5(7)	2865(16)	2575(6)	63(3)
C(2)	5006(8)	9969(18)	1078(6)	72(3)	C(22)	57(6)	4411(15)	3126(5)	55(3)
C(3)	6058(8)	10889(18)	973(6)	69(3)	C(23)	1181(6)	5151(13)	3276(5)	47(2)
C(4)	6691(8)	11191(15)	1732(6)	64(3)	C(24)	1638(6)	5528(12)	2536(5)	40(2)
C(5)	6884(6)	9536(13)	2230(5)	44(2)	C(25)	1565(6)	3936(13)	2007(5)	45(2)
C(6)	7271(7)	10043(13)	3035(5)	50(2)	C(26)	1986(8)	5735(19)	810(6)	77(4)
C(7)	7390(6)	8522(14)	3586(5)	51(3)	C(27)	−327(7)	4709(19)	1383(6)	79(4)
C(8)	6369(6)	7412(11)	3616(5)	34(2)	C(28)	1237(6)	6773(15)	3827(5)	51(2)
C(9)	5918(6)	6956(12)	2813(4)	39(2)	C(29)	697(8)	8462(16)	3480(7)	76(3)
C(10)	5807(6)	8582(13)	2293(5)	43(2)	C(30)	838(8)	6340(20)	4585(6)	79(4)
C(11)	5455(8)	6680(17)	1049(6)	68(3)					

U(eq) is defined as one third of the trace of the orthogonalized U_{ij} tensor.

Table 5. Bond Lengths [Å] for **3**

O(1)–C(1)	1.459(10)	C(5)–C(12)	1.534(13)	C(18)–C(19)	1.501(14)
O(2)–C(8)	1.445(10)	C(5)–C(10)	1.549(11)	C(19)–C(20)	1.507(16)
O(3)–C(13)	1.458(9)	C(6)–C(7)	1.489(13)	C(20)–C(21)	1.535(12)
O(4)–C(16)	1.456(10)	C(7)–C(8)	1.538(10)	C(20)–C(27)	1.539(14)
O(5)–C(23)	1.442(10)	C(8)–C(9)	1.511(11)	C(20)–C(25)	1.546(11)
O(6)–C(28)	1.440(10)	C(8)–C(13)	1.528(11)	C(21)–C(22)	1.503(14)
C(1)–C(2)	1.505(15)	C(9)–C(10)	1.516(12)	C(22)–C(23)	1.523(12)
C(1)–C(11)	1.522(14)	C(13)–C(14)	1.509(12)	C(23)–C(24)	1.503(11)
C(1)–C(10)	1.549(11)	C(13)–C(15)	1.530(12)	C(23)–C(28)	1.546(14)
C(2)–C(3)	1.523(14)	C(16)–C(17)	1.504(15)	C(24)–C(25)	1.505(12)
C(3)–C(4)	1.507(13)	C(16)–C(26)	1.515(16)	C(28)–C(30)	1.509(13)
C(4)–C(5)	1.517(13)	C(16)–C(25)	1.551(12)	C(28)–C(29)	1.529(15)
C(5)–C(6)	1.503(12)	C(17)–C(18)	1.551(13)		

Using a previously published protocol,[25] compounds **1** and **2** were tested for effect on isolated guinea pig tracheal spirals *in vitro*. They relaxed guinea pig tracheal spirals that were constricted by histamine (2 μg/ml). They all produced a concentration-related effect on tracheal spirals with the IC_{50} values of 8.7 and 7.9 μM, respectively, while that of the diterpene coleonol B was 0.088 μM.

Experimental

Optical rotations were obtained using a JASCO P-1020 digital polarimeter (cell length: 1.0 dm). IR spectra (KBr discs) were measured on a Shimadzu FTIR-8400s spectrophotometer. ESI-MS and HR-ESI-MS experiments were performed on an Agilent 1100 Series LC/MSD Trap mass spectrometer and an Agilent TOF MSD 1946D spectrometer, respectively. NMR spectra were recorded on Bruker-DRX-600 spectrometers (^{1}H-NMR, HMQC, HMBC and NOESY at 600 MHz; ^{13}C-NMR at 150 MHz), using $(CD_3)_2CO$ or $CDCl_3$ as solvent. TMS was used as internal standard for ^{1}H- and ^{13}C-NMR spectra, and chemical shifts were recorded in δ values. Single-crystal X-ray diffraction was detected by Bruker SMART CCD Area-detector. Silica gel GF_{254} for TLC was the product of Qingdao Marine Chemistry Ltd., and TLC plates were visualized by 10% H_2SO_4–EtOH for diterpenes, and phenylamine-*o*-benzenedicarboxylic acid for sugars. Absorbents for column chromatography were silica gel (200—300 mesh, Qingdao Marine Chemistry Ltd., People's Republic of China), neutral aluminum oxide (Shanghai Ludu Chemistry Ltd., People's Republic of China) and Sephadex LH-20 (20—100 μ, Pharmacia). Preparative HPLC was performed on an Agilent 1100 instrument with a UV detector at 210 nm.

Plant Material The whole plant of *C. forskohlii* (Willd.) Briq. (Labiatae) was collected in Yunnan Province, People's Republic of China, in May 2004, and authenticated by Senior Engineer Gong-Hua Wang, Yunnan Xingzhong Pharmaceutical Company. A voucher specimen (No. 040502) was deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

Extraction and Isolation The whole plant (110 kg) were air-dried, powdered and extracted with 95% EtOH (700 l×3) for 4 h at 80 °C. The EtOH extract (12.9 kg) was dissolved in H_2O, and successively extracted with petroleum ether (60—90 °C) and *n*-BuOH. The concentrated *n*-BuOH fraction (2.6 kg) was extracted with H_2O (50 l) in reflux for three times and the combined solution was concentrated to give 400 g extract. The extract was loaded onto a neutral aluminum oxide column eluting with petroleum ether–Me_2CO (100 : 25) and MeOH, respectively, to obtain methanol fraction (35 g). Then, the methanol fraction was chromatographed over a column of silica gel utilizing a gradient from 100% $CHCl_3$ to $CHCl_3$–MeOH (100 : 15) to afford six fractions (Fraction A—F), pooled by common TLC characteristics. Fraction C (2.0 g), purified by a Sephadex LH-20 column ($CHCl_3$–MeOH, 1 : 1) and then by a silica gel column with a gradient of petroleum ether–EtOAc (100 : 40—100 : 100), gave compound **3** (32 mg). Fraction E (2.6 g), purified by a Sephadex LH-20 column ($CHCl_3$–MeOH, 1 : 1), was further subjected on a silica gel column with elution of petroleum ether–EtOAc (100 : 40) to yield compound **2** (8 mg). Fraction F (1.2 g) was purified by Sephadex LH-20 column ($CHCl_3$–MeOH, 1 : 1) affording compound **1** (15 mg).

Forskoditerpenoside A (**1**): White amorphous powder; $[\alpha]_D^{28}$ +9.1° (c=0.15, MeOH); IR (KBr) cm^{-1}: 3414, 2928, 1717, 1645, 1392, 1258 and

Table 6. Bond Angles [°] for **3**

O(1)–C(1)–C(2)	107.4(7)	O(4)–C(16)–C(17)	104.0(8)
O(1)–C(1)–C(11)	102.9(7)	O(4)–C(16)–C(26)	106.9(8)
C(2)–C(1)–C(11)	112.3(8)	C(17)–C(16)–C(26)	112.8(8)
O(1)–C(1)–C(10)	107.2(7)	O(4)–C(16)–C(25)	106.2(6)
C(2)–C(1)–C(10)	109.8(8)	C(17)–C(16)–C(25)	110.3(9)
C(11)–C(1)–C(10)	116.3(8)	C(26)–C(16)–C(25)	115.6(9)
C(1)–C(2)–C(3)	113.8(9)	C(16)–C(17)–C(18)	111.7(9)
C(4)–C(3)–C(2)	110.7(8)	C(19)–C(18)–C(17)	111.5(9)
C(3)–C(4)–C(5)	115.6(9)	C(18)–C(19)–C(20)	114.7(10)
C(6)–C(5)–C(4)	111.1(8)	C(19)–C(20)–C(21)	109.0(9)
C(6)–C(5)–C(12)	109.9(7)	C(19)–C(20)–C(27)	110.2(8)
C(4)–C(5)–C(12)	108.1(8)	C(21)–C(20)–C(27)	109.3(8)
C(6)–C(5)–C(10)	104.7(6)	C(19)–C(20)–C(25)	108.5(8)
C(4)–C(5)–C(10)	108.8(7)	C(21)–C(20)–C(25)	104.9(7)
C(12)–C(5)–C(10)	114.3(8)	C(27)–C(20)–C(25)	114.6(9)
C(7)–C(6)–C(5)	115.3(8)	C(22)–C(21)–C(20)	114.0(8)
C(6)–C(7)–C(8)	113.6(7)	C(21)–C(22)–C(23)	112.2(7)
O(2)–C(8)–C(9)	106.7(6)	O(5)–C(23)–C(24)	109.4(7)
O(2)–C(8)–C(13)	107.7(6)	O(5)–C(23)–C(22)	105.2(7)
C(9)–C(8)–C(13)	113.9(7)	C(24)–C(23)–C(22)	110.4(7)
O(2)–C(8)–C(7)	106.4(7)	O(5)–C(23)–C(28)	105.7(7)
C(9)–C(8)–C(7)	109.2(7)	C(24)–C(23)–C(28)	113.6(8)
C(13)–C(8)–C(7)	112.5(6)	C(22)–C(23)–C(28)	112.1(7)
C(8)–C(9)–C(10)	113.0(7)	C(23)–C(24)–C(25)	112.6(8)
C(9)–C(10)–C(5)	112.4(6)	C(24)–C(25)–C(20)	112.4(7)
C(9)–C(10)–C(1)	114.1(7)	C(24)–C(25)–C(16)	114.7(8)
C(5)–C(10)–C(1)	115.5(7)	C(20)–C(25)–C(16)	114.5(7)
O(3)–C(13)–C(14)	105.9(7)	O(6)–C(28)–C(30)	106.5(7)
O(3)–C(13)–C(8)	107.9(6)	O(6)–C(28)–C(29)	104.1(8)
C(14)–C(13)–C(8)	112.0(7)	C(30)–C(28)–C(29)	110.8(9)
O(3)–C(13)–C(15)	107.8(7)	O(6)–C(28)–C(23)	108.3(7)
C(14)–C(13)–C(15)	110.9(7)	C(30)–C(28)–C(23)	112.8(9)
C(8)–C(13)–C(15)	112.0(7)	C(29)–C(28)–C(23)	113.6(8)

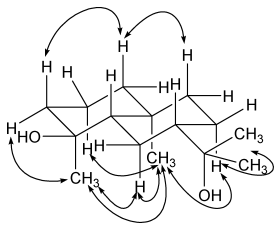

Fig. 5. Key NOESY Correlations of Compound **3**

1072; ^{1}H-NMR (Me_2CO-d_6, 600 MHz) and ^{13}C-NMR (Me_2CO-d_6, 150 MHz): Table 1; ESI-MS *m/z*: 571 $[M-H]^-$; HR-ESI-MS *m/z*: 595.2723 $[M+Na]^+$ (Calcd for $C_{28}H_{44}O_{12}Na$: 595.2724).

Forskoditerpenoside B (**2**): White amorphous powder; $[\alpha]_D^{28}$ +10.4° (c=0.15, MeOH); IR (KBr) cm^{-1}: 3425, 2928, 1742, 1709, 1645, 1389, 1248 and 1063; ^{1}H-NMR ($CDCl_3$, 600 MHz) and ^{13}C-NMR ($CDCl_3$, 150 MHz): Table 1; ESI-MS *m/z*: 613 $[M-H]^-$; HR-ESI-MS *m/z*: 637.2836 $[M+Na]^+$ (Calcd for $C_{30}H_{46}O_{13}Na$: 637.2830).

4β,7β,11-Enantioeudesmantriol (**3**): Colorless needle, mp 61—62 °C, $[\alpha]_D^{25}$ +33.5° (c=0.16, MeOH); IR (KBr) cm^{-1}: 3370, 2977, 2868, 1454, 1383, 1362, 1165 and 1070; ^{1}H-NMR ($CDCl_3$, 600 MHz) and ^{13}C-NMR ($CDCl_3$, 150 MHz): Table 2; ESI-MS *m/z*: 279 $[M+Na]^+$; HR-ESI-MS *m/z*: 279.1920 $[M+Na]^+$ (Calcd for $C_{15}H_{28}O_3Na$: 279.1930).

Acid Hydrolysis of 1 and 2 Compound **1** (4 mg) in 2 M CF_3COOH–MeOH (2 ml) was heated at 100 °C on an oil bath for 4 h. The reaction mixture was diluted with H_2O (6 ml), and then extracted with $CHCl_3$. The combined $CHCl_3$ extracts were washed with H_2O and evaporated to afford decomposed aglycon mixture. After repeated evaporation to dryness of the aqueous layer with MeOH until neutral,[26] the residue was purified through Sephadex LH-20 column ($CHCl_3$–MeOH, 1 : 1) affording 0.9 mg sugar which was identified as glucose by TLC (*n*-BtOH–Me_2CO–H_2O, 4 : 5 : 1) and visualized with phenylamine-*o*-benzenedicarboxylic acid) in direct comparison with an authentic sugar. The glucose was determined as D-form for its optical rotation $[\alpha]_D^{23}$ +47.5° (c=0.03, H_2O). By the same method, compound **2** was hydrolyzed and the products were identified as decomposed aglycon mixture and D-glucose.

Enzymatic Hydrolysis of 1 and 2 A solution of **1** (6.0 mg) and **2** (3.5 mg) in 0.1 M acetate buffer (pH 5.0, 2.0 ml) was treated with β-glucosidase (Fluka EC 2325897) (15 mg) and the reaction mixture was stirred at 45 °C for 4 h, respectively. The mixture was then poured into EtOH and the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (800 mg) eluted with *n*-hexane–EtOAC (5 : 1) to give **4** (4.1 mg) and **5** (2.8 mg), respectively. Compound **4**: White powder, $[\alpha]_D^{28}$ −7.7° (c=0.10, $CHCl_3$); ESI-MS *m/z*: 409 $[M-H]^-$; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 4.66 (1H, br s, 1β-H), 1.99 (1H, m, 2α-H), 2.03 (1H, m, 2β-H), 1.00 (1H, m, 3α-H), 1.06 (1H, m, 3β-H), 2.31 (1H, d, J=3.0 Hz, 5α-H), 5.85 (1H, dd, J=3.0, 3.9 Hz, 6α-H), 4.29 (1H, d, J=3.9 Hz, 7α-H), 2.53 (1H, d, J=18.0 Hz, 12α-H), 3.19 (1H, d, J=18.0 Hz, 12β-H), 6.12 (1H, dd, J=12.0, 17.4 Hz, 14-H), 5.00 (1H, dd, J=1.2, 12.0 Hz, 15-H), 5.19 (1H, dd, J=1.2, 17.4 Hz, 15-H), 1.40 (3H, s, 16-H), 1.61 (3H, s, 17-H), 1.07 (3H, s, 18-H), 0.98 (3H, s, 19-H), 1.42 (3H, s, 20-H), 2.10 (3H, s, 6-OAc). Compound **5**: White powder, $[\alpha]_D^{28}$ −7.6° (c=0.10, $CHCl_3$); ESI-MS *m/z*: 451 $[M-H]^-$; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 4.80 (1H, br s, 1β-H), 2.03 (1H, m, 2α-H), 2.05 (1H, m, 2β-H), 1.01 (1H, m, 3α-H), 1.06 (1H, m, 3β-H), 2.52 (1H, d, J=2.8 Hz, 5α-H), 5.99 (1H, dd, J=2.8, 4.5 Hz, 6α-H), 5.93 (1H, d, J=4.5 Hz, 7α-H), 2.39 (1H, d, J=16.4 Hz, 12α-H), 3.26 (1H, d, J=16.4 Hz, 12β-H), 6.07 (1H, dd, J=10.7, 17.4 Hz, 14-H), 4.70 (1H, dd, J=1.4, 10.7 Hz, 15-H), 5.14 (1H, dd, J=1.4, 17.4 Hz, 15-H), 1.21 (3H, s, 16-H), 1.64 (3H, s, 17-H), 0.78 (3H, s, 18-H), 0.81 (3H, s, 19-H), 1.43 (3H, s, 20-H), 1.79 (3H, s, 6-OAc), 1.93 (3H, s, 7-OAc).

Oxidation of 4 and 5 The oxidation of **4** and **5** was performed following the procedure described by Bhat *et al.*[19] To a stirred suspension of Collins reagents (8.0 mg) in methylene chloride (3 ml) was added a solution of **4** (4.0 mg) and **5** (2.7 mg) in methylene chloride (2 ml), respectively. The mixture was stirred at room temperature for 2.5 h and filtered. The residue was washed with methylene chloride (3 ml) and 1 N hydrochloride acid, saturated aqueous sodium hydrogencarbonate, and water, dried with anhydrous sodium sulfate, then evaporated to dryness. The residue gave **6** (3.7 mg) and **7** (2.5 mg), respectively.

Hydrolysis of 6 and 7 The hydrolysis of **6** and **7** was performed according to the method described by Valdes *et al.*[18] To 3.5 mg of **6** and 2.4 mg of **7** in 2 ml MeOH was added 14 mg of K_2CO_3 at 0 °C, respectively. After stirring at room temperature for 3 h, the reaction mixture was partitioned between 10 ml EtOAc and water (1 : 1). The organic layer was washed with 10 ml water and dried *in vacuo*. The isolation of the product by preparative HPLC using Shim-Pack Prep-ODS column (20×2.5 cm) eluted with CH_3CN–H_2O (4 : 6) yielded the same compound **8** (2.6 mg) and (1.9 mg), respectively. Compound **8**: White powder, $[\alpha]_D^{28}$ −11.0° (c=0.08, $CHCl_3$); IR (KBr) cm^{-1}: 3510, 3460, 1725, 1701, 1690, 988, 919; ESI-MS *m/z* 365 $[M-H]^-$; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 3.22 (1H, ddd, J=13.5, 8.1, 5.8 Hz, 2α-H), 2.20 (1H, ddd, J=13.5, 3.6, 4.0 Hz, 2β-H), 1.58 (1H, m, 3α-H), 1.76 (1H, m, 3β-H), 2.12 (1H, d, J=2.4 Hz, 5α-H), 4.42 (1H, dd, J=2.4, 4.0 Hz, 6α-H), 2.14 (1H, br s, 6β-OH), 4.09 (1H, d, J=4.0 Hz, 7α-H), 2.28 (1H, br s, 7β-OH), 4.11 (1H, s, 9α-OH), 3.45 (1H, d, J=15.3 Hz, 12α-H), 2.39 (1H, d, J=15.3 Hz, 12β-H), 6.07 (1H, dd, J=17.3, 10.6 Hz, 14-H), 5.00 (1H, dd, J=10.6, 0.8 Hz, 15-H), 5.21 (1H, dd, J=17.3, 0.8 Hz, 15-H), 1.08 (3H, s, 16-H), 1.42 (3H, s, 17-H), 1.50 (3H, s, 18-H), 1.62 (3H, s, 19-H), 1.88 (3H, s, 20-H). The optical rotation and spectral data were identical with 8,13-epoxy-6β,7β,9α-trihydroxylabd-14-ene-1,11-dione in the literature.[18]

Bioassay The assay of relaxed effects on isolated guinea pig tracheal spirals was performed according to the published method.[24]

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