Diterpenes from *Coleus forskohlii* (WILLD.) BRIQ. (Labiatae)

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Three new minor labdane diterpene glycosides, forskoditerpenoside C, D, and E (1—3), and a novel labdane diterpene forskoditerpene A (4) were isolated from the ethanol extract of the whole plant of *Coleus forskohlii*. Their structures and relative stereochemistry were elucidated on the basis of extensive spectroscopic analyses including 1D-, 2D-NMR, and HR-ESI-MS experiments. Compounds 1—3 showed an unusual 8,13-epoxy-labd-14-en-11-one glycoside pattern. They showed relaxative effects on isolated guinea pig tracheal spirals *in vitro*. Compound 4 with a cyclopropyl, confirmed by a single-crystal X-ray diffraction determination, is the first known labdane derivative with a spiro element.

Key words *Coleus forskohlii*; Labiatae; forskoditerpenoside C; forskoditerpenoside D; forskoditerpenoside E; forskoditerpene A

Coleus forskohlii (WILLD.) BRIQ. (Labiatae) has been used for medical treatment in Hindu and Ayurvedic traditional medicine from ancient times.[1)] Since its major constituent forskolin was discovered to have positive inotropic, antihypertensive, and adenylatecyclase-stimulating activities, it has ever aroused a great deal of scientific interest, and many 8,13-epoxy-labd-14-en-11-one diterpenes have been obtained.[1—11)] The plant was subsequently found in Yunnan Province of China, while the major diterpene was determined to be coleonol B.[10,11)] So as to discover more novel and active compounds, further examination of the polar fraction of the whole plant extract has been carried out and yielded two new diterpene glycosides and a sesquiterpene in our earlier research.[12)] Continuing work yielded three other new diterpene glycosides forskoditerpenosides C, D, and E (**1**—**3**) and a diterpene forskoditerpene A (**4**). Complete NMR data studies on each compound were performed unambiguously to determine the structures of the compounds and to assign all the proton and carbon resonances. The isolation and structural elucidation of them are reported below. Forskoditerpenosides C, D, and E (**1**—**3**) were tested for their effects on isolated guinea pig tracheal spirals *in vitro*.

Results and Discussion

Air-dried whole plants of *C. forskohlii* were extracted with EtOH three times. The concentrated extract was dissolved in water and successively extracted with petroleum ether (60—90 °C) and *n*-BuOH. The *n*-BuOH soluble fraction was extracted with H_2O in reflux and the combined solution was separated by means of various chromatographic procedures to afford four labdane diterpenes (**1**—**4**).

Forskoditerpenoside C (**1**), an amorphous white powder, possessed the molecular formula $C_{28}H_{44}O_{11}$ by HR-ESI-MS *m/z* 579.2776 $[M+Na]^+$ (Calcd for $C_{28}H_{44}O_{11}Na$, 579.2775). The DEPT spectra showed six methyls, five methylenes, eleven methines, and six quaternary carbons. The IR spectrum indicated the presence of hydroxy (3437 cm^{-1}) and ester carbonyl (1736 cm^{-1}) groups. The NMR spectra of **1** (Tables 1, 2) displayed one carbonyl (δ_C 208.8), one acetoxyl [δ_H 1.99 (s)/δ_C 171.1 and 22.3], a terminal vinylic group [ABX system, δ_H 6.02 (1H, dd, J=10.7, 17.2 Hz), 4.94 (1H, dd, J=10.7, 1.5 Hz), 5.25 (1H, dd, J=17.2, 1.5 Hz), along with δ_C 148.7, 112.5], two geminal protons adjacent to carbonyl [AB system, δ_H 2.37 and 2.90 (each 1H, d, J=16.4 Hz)], and five tertiary methyls [δ_H 0.92 (s), 0.96 (s), 1.26 (s), 1.42 (s) and 1.50 (s)]. According to these spectral data, **1** was considered to be a 8,13-epoxy-labd-14-en-11-one diterpene.[1—5,8—11)] The ^{1}H-NMR signal at δ 5.71 (1H, dd, J=4.0, 2.4 Hz) was assigned to H-6 by its coupling constants.[13,14)] The chemical shifts of H-6 and C-6 suggested that

Table 1. ^{1}H-NMR Spectroscopic Data for Compounds **1**—**3**

H	**1**[a)]	**2**[b)]	**3**[b)]
1β	4.10 br s	4.23 br s	4.49 br s
2α	1.93 m	1.83 br d, 12.5	1.82 dd, 14.9, 3.0
2β	1.93 m	1.96 br t, 12.5	2.00 br t, 14.9
3β	1.01 m	1.06 br d, 12.7	1.08 br t, 13.2
3α	1.74 dt, 5.0, 12.8	1.71 br t, 12.7	1.70 br d, 13.2
5α	1.61 d, 2.4	1.65 br s	1.53 d, 2.2
6α	5.71 dd, 4.0, 2.4	5.73 br s	5.60 br d, 2.5
7α	3.84 d, 4.0	5.13 br d, 3.9	2.28 dd, 2.6, 14.0
7β			1.95 dd, 3.0, 14.0
9α	3.41 s	3.47 s	3.32 s
12β	2.37 d, 16.4	2.52 d, 17.6	2.68 d, 18.0
12α	2.90 d, 16.4	2.81 d, 17.6	2.72 d, 18.0
14	6.02 dd, 10.7, 17.2	5.97 dd, 10.7, 17.1	5.96 dd, 10.5, 17.1
15-*cis*	4.94 dd, 1.5, 10.7	5.11 br d, 10.7	5.16 br d, 10.5
15-*trans*	5.25 dd, 1.5, 17.2	5.40 br d, 17.1	5.38 br d, 17.1
16	1.26 s	1.27 s	1.32 s
17	1.50 s	1.54 s	1.52 s
18	0.96 s	0.97 s	0.99 s
19	0.92 s	0.94 s	1.00 s
20	1.42 s	1.43 s	1.40 s
C-6-acetate	1.99 s	2.10 s	2.08 s
C-7-acetate		2.06 s	
1′	4.08 d, 7.6	4.19 d, 7.4	4.24 d, 7.7
2′	3.11—3.31 m	3.28 m	3.27 br t, 8.3
3′	3.11—3.31 m	3.75 m	3.51 br t, 9.0
4′	3.11—3.31 m	3.75 m	3.56 br t, 9.1
5′	3.11—3.31 m	3.28 m	3.37 m
6′	3.77 dd, 11.3, 2.5	3.81 br s	3.87 dd, 11.7, 3.3
	3.60 dd, 11.3, 5.2	3.81 br s	3.79 dd, 11.7, 4.6

a) Measured in Me_2CO-d_6, 600 MHz. *b*) Measured in $CDCl_3$, 600 MHz.

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Table 2. ^{13}C-NMR Spectroscopic Data for Compounds **1**—**3**

C	1	2	3	C	1	2	3
1	82.4	81.5	82.5	16	30.8	31.2	31.6
2	24.8	22.8	22.9	17	23.9	23.6	29.4
3	38.8	36.4	36.6	18	33.8	32.4	32.8
4	35.0	33.6	33.8	19	24.2	23.0	23.2
5	48.7	46.7	48.7	20	18.8	17.6	18.1
6	73.5	69.4	69.8	C-6-acetate	171.1	169.8	169.9
7	79.9	78.0	45.9		22.3	21.2	21.8
8	82.5	78.0	75.1	C-7-acetate		170.2	
9	60.0	58.1	59.4			20.8	
10	43.4	41.5	41.9	1′	105.6	103.2	103.4
11	208.8	208.2	209.1	2′	75.8	73.4	73.8
12	52.9	50.6	50.2	3′	79.0	76.5	76.6
13	77.3	75.0	73.8	4′	72.6	69.9	70.4
14	148.7	146.0	147.1	5′	78.0	75.0	75.9
15	112.5	113.0	113.0	6′	63.8	61.9	62.6

Measured in the same solvents as those in ^{1}H-NMR data, 150 MHz.

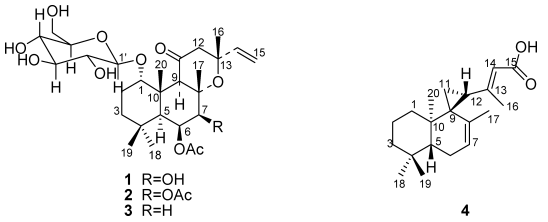

Fig. 1. The Structures of Compounds **1**—**4**

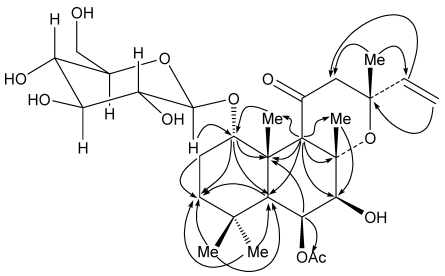

Fig. 2. Key HMBC Correlations from H to C for Compound **1**

an acetyl group was attached to C-6, as confirmed by the cross-peak between δ_H 5.71 and δ_C 171.1 in the HMBC spectrum. Then, the signals at δ 1.61 (1H, d, J=2.4 Hz) and 3.84 (1H, d, J=4.0 Hz) were assigned to H-5 and H-7, respectively, interpreted from their coupling constants as well as HMQC and HMBC correlations. [13] The signal at δ 4.10 (1H, br s), which showed long-range correlations with C-3, C-5, C-10, and C-20 in the HMBC spectrum, was attributed to H-1, correlated to C-1 at δ 82.4 in HMQC spectrum. The singlet methine [δ 3.41 (1H, s)] located at C-9 was determined by its correlations with C-5, C-7, C-8, C-10, C-17, and C-20 in HMBC spectrum. Besides the above spectral data, there were a proton signal at δ 4.08 (1H, d, J=7.6 Hz), linked to the carbon at δ 105.6 in HMQC spectrum, and six proton signals between δ 3.11 and 3.77 and five oxygenated carbons between δ 63.8 and 79.0. Combining the Molish reaction, a sugar moiety was considered to exist in **1**. After acid hydrolysis, the sugar was identified as D-glucose by TLC comparison with an authentic sample and the optical rotation. The coupling constant (J=7.6 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 the cross-peak in the HMBC spectrum between C-1 and H-1′. The relative stereochemistry of **1** was deduced mainly by analysis of the correlations in the NOESY experiment. The orientations of 1β-H, 6α-H, 7α-H, and 9α-H were confirmed by the coupling constants in ^{1}H-NMR and the correlations in NOEs of H-1/H_3-20, H-6/H-5, H-6/H-7, H-7/H-5, H-9/H-5, H-9/H-7, and H-9/H-12α. [13,14] The presence of NOE correlations between H-5/H-7, H-5/H-9, H-7/H-12α, H-9/H-12α, H_3-19/H_3-20, H_3-19/H_3-Ac, H_3-20/H_3-17, H_3-17/H_3-16, H_3-17/H_3-Ac, and H_3-20/H_3-16, which were in good agreement with the naturally occurring labdane derivatives reported in the literature, allowed the assignment of the ring conjunction as *trans*-form for both A/B and B/C. Therefore, the chemical structure of **1** was elucidated as 6β-acetoxy-7β-hydroxy-8,13-epoxy-labd-14-en-11-one-1α-*O*-β-D-glucopyranoside. [14,15] The aglycon of **1**, 9-deoxy coleonol B, was synthesized many years ago without any reported NMR data. [16]

Forskoditerpenoside D (**2**), an amorphous white powder, possessed the molecular formula $C_{30}H_{46}O_{12}$ on the basis of HR-ESI-MS m/z 621.2883 $[M+Na]^+$. Its IR spectrum showed absorption bands at 3423, 1745, and 1707 cm^{-1}, suggesting the presence of hydroxyl, carbonyl, and ester carbonyl functions. The NMR spectra showed very close resemblances to those of **1** (Tables 1, 2), which suggested that **2** might possess the same labdane diterpene glycoside structure. In the spectra, there were one carbonyl (δ_C 208.2), two acetoxyls [δ_H 2.10 (s), 2.06 (s)/δ_C 170.2, 169.8, 21.2 and 20.8], a terminal vinylic group [ABX system, δ_H 5.97 (1H, dd, J=10.7, 17.1 Hz), 5.11 (1H, br d, J=10.7 Hz), 5.40 (1H, br d, J=17.1 Hz), along with δ_C 146.0, 113.0], two geminal protons adjacent to carbonyl [AB system, δ_H 2.52 and 2.81 (each 1H, d, J=17.6 Hz)], five tertiary methyls [δ_H 0.94 (s), 0.97 (s), 1.27 (s), 1.43 (s) and 1.54 (s)]. The ^{1}H-NMR signals at δ 4.23 (1H, br s), 1.65 (1H, br s), 5.73 (1H, br s) and 3.47 (1H, s) were assigned to be H-1, H-5, H-6, and H-9, respectively, by their HMQC and HMBC correlations and comparing with those of **1**. Then, the proton at δ 5.13 (1H, br d, J=3.9 Hz) was linked to C-7, suggesting that the other acetyl group was attached to C-7, as confirmed by the cross-peak between δ_H 5.13 and δ_C 170.2 in the HMBC spectrum. Accordingly, the aglycon was deduced as forskolin G and the spectral data were consistent with literature values. [17] The monosacchride, β-D-glucose, detected by the same manner as compound **1**, was located at C-1 by HMBC correlation and glycosylation shift of C-1 (δ 81.5). 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-5/H-9, H-9/H-12α, H_3-16/H_3-17, H_3-16/H_3-20, H_3-17/H_3-20 and H_3-19/H_3-20). According to the above evidence, compound **2** was elucidated as 6β,7β-diacetoxy-8,13-epoxy-labd-14-en-11-one-1α-*O*-β-D-glucopyranoside.

Forskoditerpenoside E (**3**), $C_{28}H_{44}O_{10}$, by the HR-ESI-MS m/z 563.2827 $[M+Na]^+$ (Calcd for $C_{28}H_{44}O_{10}Na$, 563.2826), one oxygen less than that of **1**, was deduced to be the 7-deoxyl derivative of **1**. The IR spectrum showed absorption bands at 3447, 1740, and 1701 cm^{-1} for the existence of hydroxyl, carbonyl, and ester carbonyl functions. Inspection of the NMR spectra (Tables 1, 2) along with the two-dimensional data confirmed the presence of a carbonyl, an ace-

toxyl, a terminal vinylic group, two geminal protons adjacent to carbonyl, and five tertiary methyls, and other shared structural features of **2** with **1**. Comparison of the above spectra with those of **1** revealed the conspicuous loss of a hydroxyl group. Its ^{1}H-NMR spectrum showed an AB system at δ 2.28 (1H, dd, J=2.6, 14.0 Hz) and 1.95 (1H, dd, J=3.0, 14.0 Hz), replacing the signal [δ 3.84 (1H, d, J=4.0 Hz), H-7] in **1**, corresponding to the upfield shift of C-7 (δ 45.9) in the ^{13}C-NMR spectrum, due to the absence of a hydroxyl group on C-7, which was confirmed by the correlations from H_2-7 to C-5, C-6, C-8, C-9, and C-17 in the HMBC spectrum. Thus, the aglycon of **3** was determined as plectrornatin B.[18] The sugar moiety was identified as β-D-glucose by acid hydrolysis and the coupling constant (J=7.7 Hz) of H-1′. The HMBC correlation between C-1 and H-1′ indicated that the glucose was attached to C-1. As the same with compounds **1** and **2**, the structure of **3** was established as 6β-acetoxy-8,13-epoxy-labd-14-en-11-one-1α-*O*-β-D-glucopyranoside.

Forskoditerpene A (**4**) was obtained as colorless needles. Its molecular composition, $C_{20}H_{30}O_2$, was obtained from HR-ESI-MS at *m/z* 325.2142 $[M+Na]^+$ (Calcd for $C_{20}H_{30}O_2Na$, 325.2143). The IR spectrum of **4** displayed absorption bands at 3430 and 1629 cm^{-1} due to hydroxyl and unsaturated carbonyl groups. The ^{13}C-NMR and DEPT spectra showed 20 carbon signals for five methyls, five methylens, four methines, and six quaternary carbons, including one carboxylic and four olefinic carbons. The ^{1}H-NMR spectrum (Table 3) of **4** revealed five methyl singlets at δ 0.88, 0.89, 0.95, 1.40, and 2.18 and two broad singlets at δ 5.54 and 5.75, without any oxygenated proton. Most of the carbon resonances were in good agreement with labdane derivatives, which suggested **4** as a labdanoid.[19,20] The correlations in HMBC spectrum between δ_H 1.86 (H-12), 1.40 (H-17) with δ_C 132.5, and δ_H 1.40 (H-17) with δ_C 125.0 suggested that a double bond was present at C-7 (8). The carbons at δ 20.2, 116.3, 162.5, and 171.7 suggested the presence of an α,β-unsaturated carboxylic group with β-methyl substituted, which was confirmed by the ^{1}H-NMR signal at δ 2.18 (3H, s), 5.75 (1H, br s) and the fragment ion peak in ESI-MS at 257 $[M-H-CO_2]^-$. The proton signal at δ 1.86 (br t, 7.6) was assigned to H-12 for its HMBC correlations with C-13 and C-14. The HMBC correlations from H-12 and H-11 to C-8, C-9, and C-10 suggested that both C-11 and C-12 might be connected with C-9 directly, which was confirmed by the abnormal upshift of C-11 at δ 11.2 for the formation of the cyclopropyl.

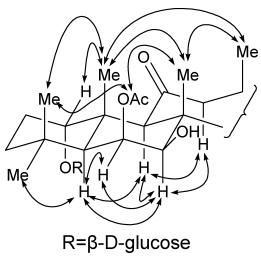

Fig. 3. Key NOESY Correlations for Compound **1**

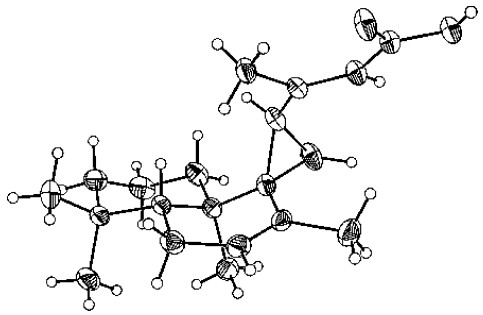

Fig. 4. ORTEP Diagram of Compound **4**

Table 3. ^{1}H- and ^{13}C-NMR Spectroscopic Data for Compound **4**

	H[a]	C[b]
1α	1.06 bd, 12.4	32.3
1β	0.89 m	
2α	1.59 qt, 10.2, 3.2	18.7
2β	1.45 m	
3α	1.44 m	42.6
3β	1.13 m	
4		33.6
5	1.52 dd, 11.6, 6.4	48.1
6α	2.00 m	25.2
6β	2.17 m	
7	5.54 br s	125.0
8		132.5
9		41.5
10		36.1
11	1.11 dd, 7.6, 3.4, 0.94 m	11.2
12	1.86 br t, 7.6	31.4
13		162.5
14	5.75 br s	116.3
15		171.7
16	2.18 s	20.2
17	1.40 s	24.0
18	0.89 s	32.9
19	0.88 s	21.5
20	0.95 s	19.0

a) Measured in $CDCl_3$, 600 MHz. *b*) Measured in $CDCl_3$, 150 MHz.

Table 4. Crystal Data and Structure Refinement for **4**

Identification code	Compound name
Empirical formula	$C_{20}H_{30}O_2$
Formula weight	302.44
Temperature	295 K
Wavelength	0.71073 Å
Crystal system, space group	Monoclinic, *C*2
Unit cell dimensions	a=29.215(2) Å
	b=7.667(1) Å
	c=16.656(2) Å
	β=91.63(4)°
Volume	3729.3(11) Å^3
Z, Calculated density	8, 1.077 g/cm^3
Absorption coefficient	0.067 mm^{-1}
F(000)	1328
Crystal size	1.0×0.15×0.08 mm
Theta range for data collection	2.45 to 24.98°
Limiting indices	$-8\le h\le 34$, $-8\le k\le 8$, $-19\le l\le 19$
Reflections collected/unique	4222/4067 [R(int)=0.0405]
Completeness to θ=25.00	83.7%
Absorption correction	None
Max. and min. transmission	0.9935 and 0.9601
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	4067/11/400
Goodness-of-fit on F^2	1.085
Final *R* indices [$I>2\sigma(I)$]	R_1=0.042, ωR_2=0.112
R indices (all data)	R_1=0.042, ωR_2=0.112
Largest diff. peak and hole	0.146 and −0.101 e·Å^{-3}

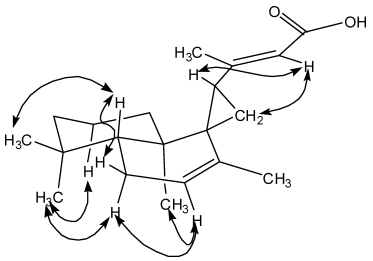

Fig. 5. Key NOESY Correlations for Compound **4**

The above deduction was confirmed by a single-crystal X-ray diffraction (Fig. 4, Table 4) study of **4**, from which its relative stereochemistry was also determined. Figure 4 clearly indicates the unique spiro structure of **4**. H-5 and the C-20 (methyl) were on different sides of an A/B *trans*-decalin moiety with β- and α-oriented configurations, respectively. The key correlations in the NOESY spectrum of **4** (Fig. 5) were in agreement with that relative stereochemistry. Therefore, compound **4** was elucidated as 5β, 9β, 10α, 12β-9,12-cyclo-7,13*E*-labdadien-15-oic acid.

In this paper, we have reported four new diterpenes, forskoditerpenoside C, D, and E (**1**—**3**) and forskoditerpene A (**4**). Compounds **1**—**3** possessed a characteristic 8,13-epoxy-labd-14-en-11-one in their structures, which are the main constituents isolated from the plant *Coleus forskohlii*, but the glycosides of the kind of diterpene were only found in our earlier work. Compound **4** had a unique spiro-type cyclopropyl element in its structure, which was not previously found in the labdane diterpenoids. The structures of them were unambiguously determined and all the proton and carbon resonances were definitely assigned as in Tables 1 and 2 by the aid of HMQC, HMBC, and NOESY spectra and X-ray diffraction.

The assay of relaxative effects of compounds **1**—**3** was performed according to the published method.[21] They were tested for their effects on isolated guinea pig tracheal spirals *in vitro*. They relaxed guinea pig tracheal spirals that were constricted by histamine (2 μg/ml). They produced a concentration-related effect on tracheal spirals with IC_{50} values of 7.3, 10.3, and 32.8 μM, respectively, while that of the diterpene coleonol B was 0.091 μM.

Experimental

Optical rotations were obtained using a JASCO P-1020 digital polarimeter (cell length: 1.0 dm). IR spectra were measured on a Shimadzu ftir-8400s spectrophotometer. 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. Tetramethylsilane was used as internal standard for ^{1}H- and ^{13}C-NMR spectra, and chemical shifts were recorded in δ values. X-ray diffraction was detected by MAC DIP-2030K Area-detector. 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. Preparative HPLC was conducted on an Agilent 1100 instrument with a UV detector at 210 nm under a shim-pack prep-ODS column (20×250 mm). 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). Silica gel GF_{254} for TLC was made by Qingdao Marine Chemistry Ltd., and TLC plates were visualized by dipping with 10% sulfuric acid in EtOH followed by heating.

Plant Material The whole plant of *C. forskohlii* (Labiatae) was collected in Yunnan Province, People's Republic of China, in May 2004, and authenticated by Senior Engineer Gonghua 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 plants (110 kg) were air-dried, powdered, and extracted with 95% EtOH (3×700 l) at 80 °C for 4 h. 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 50 l of H_2O in reflux for three times and the combined solution was concentrated to give 400 g extract, which was loaded onto a neutral aluminum oxide (100—200 mesh, 2.4 kg) column eluting with petroleum ether–Me_2CO (100 : 25), then with MeOH to obtain methanol fraction (35 g). Then, the methanol fraction was chromatographed over a column of silica gel (200—300 mesh, 280 g) utilizing a gradient from 100% $CHCl_3$ to $CHCl_3$–MeOH (100 : 15) to afford six fractions A—F, pooled by common TLC characteristics. Fraction A (3.5 g) was applied to a silica gel column with elution of petroleum ether–EtOAc (100 : 10) and purified by a Sephadex LH-20 column ($CHCl_3$–MeOH, 1 : 1), to yield compound **4** (25 mg). Fraction D (1.0 g), purified by a Sephadex LH-20 column ($CHCl_3$–MeOH, 1 : 1), was then separated by preparative HPLC on an ODS column with CH_3CN–H_2O (4 : 6) as mobile phase (10 ml/min), to give compounds **2** (8 mg) and **3** (25 mg), respectively. 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 **1** (15 mg).

Forskoditerpenoside C (**1**): Amorphous white powder, $[\alpha]_D^{28}$ −25.4° (c=0.09, MeOH), IR (KBr) λ_{max} 3437, 2943, 1736, 1686, 1641, 1391, 1242 and 1026 cm^{-1}, ^{1}H-NMR (Me_2CO-d_6, 600 MHz) and ^{13}C-NMR (Me_2CO-d_6, 150 MHz), see Tables 1 and 2, ESI-MS *m/z* 555 $[M-H]^-$, HR-ESI-MS *m/z* 579.2776 $[M+Na]^+$ (Calcd for $C_{28}H_{44}O_{11}Na$, 579.2775).

Forskoditerpenoside D (**2**): Amorphous white powder, $[\alpha]_D^{28}$ −127.3° (c=0.69, MeOH), IR (KBr) λ_{max} 3423, 2939, 1745, 1707, 1645, 1393, 1248 and 1043 cm^{-1}, ^{1}H-NMR ($CDCl_3$, 600 MHz) and ^{13}C-NMR ($CDCl_3$, 150 MHz), see Tables 1 and 2, ESI-MS *m/z* 597 $[M-H]^-$, HR-ESI-MS *m/z* 621.2883 $[M+Na]^+$ (Calcd for $C_{30}H_{46}O_{12}Na$, 621.2881).

Forskoditerpenoside E (**3**): Amorphous white powder, $[\alpha]_D^{28}$ −11.8° (c=0.18, MeOH), IR (KBr) λ_{max} 3447, 2937, 1740, 1701, 1645, 1389, 1250 and 1036 cm^{-1}, ^{1}H-NMR ($CDCl_3$, 600 MHz) and ^{13}C-NMR ($CDCl_3$, 150 MHz), see Tables 1 and 2, ESI-MS *m/z* 539 $[M-H]^-$, HR-ESI-MS *m/z* 563.2827 $[M+Na]^+$ (Calcd for $C_{28}H_{44}O_{10}Na$, 563.2826).

Forskoditerpene A (**4**): Colorless needles, mp 148.0—150.0 °C ($CHCl_3$–MeOH, 4 : 1), $[\alpha]_D^{28}$ −101.5° (c=0.12, $CHCl_3$), IR (KBr) λ_{max} 3430, 2965, 2930, 2900, 1629, 1440, 1251 and 1179 cm^{-1}, ^{1}H-NMR ($CDCl_3$, 600 MHz) and ^{13}C-NMR ($CDCl_3$, 150 MHz), see Table 3, ESI-MS *m/z* 301 $[M-H]^-$, HR-ESI-MS at *m/z* 325.2142 $[M+Na]^+$ (Calcd for $C_{20}H_{30}O_2Na$, 325.2143).

Acid Hydrolysis of 1—3 Compound **1** (3.8 mg) in 2 N 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), 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 the solvent showed a neutral reaction, the residue was purified through Sephadex LH-20 column (1 : 1 $CHCl_3$/MeOH) affording 0.82 mg sugar which was identified as glucose by TLC (4 : 5 : 1 *n*-BtOH/Me_2CO/H_2O 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 $[\alpha]_D^{23}$ +46.6° (c=0.085, H_2O). By the same method, compounds **2** and **3** were hydrolyzed respectively and the products identified as decomposed aglycon mixture and D-glucose, respectively.

Bioassay The assay of relaxed effects on isolated guinea pig tracheal spirals was performed according to the published method.[21]

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