Diterpenoids from *Diplopterygium rufopilosum*

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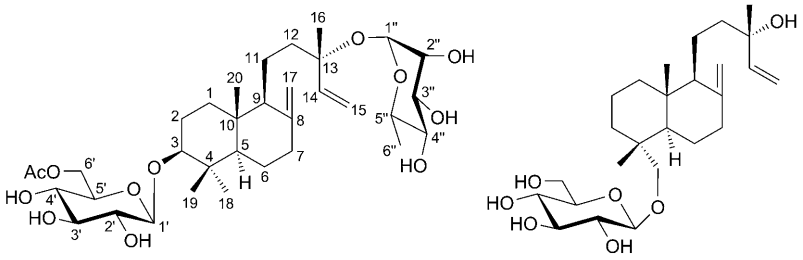
Two new diterpenoids, (3*β*,13*S*)-3-*O*-[6-*O*-acetyl-*β*-D-glucopyranosyl]-13-*O*-*α*-L-rhamnopyranosyl-labda-8(17),14-diene (**1**) and (4*R*,13*S*)-18-*O*-*β*-D-glucopyranosyllabda-8(17),14-dien-13-ol (**2**) have been isolated from the 95% EtOH extract of the dry fronds of *Diplopterygium rufopilosum*. Their structures were characterized by spectroscopic methods, including 1D-NMR, 2D-NMR, and HR-ESI-MS.

Introduction. – The genus *Diplopterygium*, which belong to the family of Gleicheniaceae, comprises more than 40 species widely distributed in the tropical and subtropical regions of Africa, Asia, and northwest part of Australia. Nine species of *Diplopterygium* occur in China [1]. To the best of our knowledge, the phytochemistry of *Diplopterygium rufopilosum* has not been investigated previously. The previous studies on the chemistry of *Diplopterygium* include the isolation of two new glycosides, hymenoside X and hexanoside A, from *Diplopterygium laevissimum* [2], and a triterpenoid, diplopterol [3], together with nonacosan-10-one from *Diplopterygium glaucum* [4]. In our investigation, *D. rufopilosum* from Caojian town, Yunnan province, was studied. This article deals with the isolation and structure elucidation of two new diterpenoids on the basis of the spectroscopic analyses.



1 **2**

Fig. 1. *The structures of compounds* **1** *and* **2**

Results and Discussion. – *Chemistry.* The AcOEt fraction of the 95% EtOH extract of *D. rufopilosum* was purified by repeated column chromatography to afford compounds **1** and **2**. They were identified as two new diterpenoids, (3*β*,13*S*)-3-*O*-[6-*O*-

acetyl-β-D-glucopyranosyl]-13-*O*-α-L-rhamnopyranosyllabda-8(17),14-diene (**1**) and (4*R*,13*S*)-18-*O*-β-D-glucopyranosyllabda-8(17),14-dien-13-ol (**2**)[1]) on the basis of the spectroscopic analyses, including 2D-NMR techniques (HMQC, HMBC, NOESY and ^{1}H,^{1}H-COSY) (*Fig. 1*).

Compound **1** was obtained as colorless oil, and its molecular formula was deduced as $C_{34}H_{56}O_{12}$ by HR-ESI-MS ($[M-H]^-$ at *m*/*z* 655.3596; calc. 655.3594), corresponding to seven degrees of unsaturation. The ^{1}H- and ^{13}C-NMR spectra of **1** showed resonances characteristic of a diterpene, two hexoses, and an Ac group (*Tables 1* and *2*). Assignment of each glycosidic H-atom system was achieved by ^{1}H,^{1}H-COSY and HMQC experiments. The diterpene moiety of **1** contained four Me groups (δ(C) 28.6,

Table 1. *^{13}C-NMR Data of Compounds* **1** *and* **2** *in CD_3OD* (δ in ppm)

C-Atom	**1**	**2**
1	38.4 (*t*)	36.5 (*t*)
2	25.0 (*t*)	18.5 (*t*)
3	90.8 (*d*)	38.8 (*t*)
4	40.5 (*s*)	38.2 (*s*)
5	49.4 (*d*)	49.3 (*d*)
6	27.7 (*t*)	24.8 (*t*)
7	39.3 (*t*)	39.2 (*t*)
8	149.5 (*s*)	149.6 (*s*)
9	59.3 (*d*)	58.0 (*d*)
10	40.3 (*s*)	40.3 (*s*)
11	18.9 (*t*)	19.4 (*t*)
12	42.0 (*t*)	42.4 (*t*)
13	80.8 (*s*)	73.1 (*s*)
14	143.8 (*d*)	147.1 (*d*)
15	115.7 (*t*)	111.2 (*t*)
16	23.3 (*q*)	28.3 (*q*)
17	107.5 (*t*)	106.9 (*t*)
18	28.6 (*q*)	79.3 (*t*)
19	16.8 (*q*)	18.0 (*q*)
20	15.0 (*q*)	15.3 (*q*)
1′	106.7 (*d*)	104.8 (*d*)
2′	75.5 (*d*)	74.8 (*d*)
3′	78.1 (*d*)	77.3 (*d*)
4′	73.3 (*d*)	71.8 (*d*)
5′	75.0 (*d*)	77.9 (*d*)
6′	64.7 (*t*)	63.1 (*t*)
1″	90.8 (*d*)	–
2″	72.5 (*d*)	–
3″	71.8 (*d*)	–
4″	74.1 (*d*)	–
5″	69.8 (*d*)	–
6″	18.9 (*d*)	–
C=O	172.8 (*s*)	–
Me	20.8 (*q*)	–

[1]) For the systematic names, see the *Exper. Part*.

Table 2. *^{1}H-NMR Data of Compounds* **1** *and* **2** *in CD_3OD* (δ in ppm and J in Hz)

	1	**2**
H_{ax}–C(1)	1.16 (*ddd*, J = 13.2, 13.0, 4.0)	1.33 (*ddd*, J = 13.6, 13.2, 4.1)
H_{eq}–C(1)	1.76 (*ddd*, J = 13.0, 4.0, 3.4)	1.66 (*ddd*, J = 13.6, 4.1, 3.4)
H_{ax}–C(2)	1.65 – 1.67 (overlapped)	1.39 – 1.41 (*m*)
H_{eq}–C(2)	1.91 – 1.93 (*m*)	1.52 – 1.55 (*m*)
H_{ax}–C(3)	3.15 – 3.19 (*dd*, J = 13.2, 4.5)	1.05 (*ddd*, J = 13.6, 13.2, 4.1)
H_{eq}–C(3)	–	1.73 (*ddd*, J = 13.6, 4.1, 3.4)
H–C(5)	1.13 (*dd*, J = 13.5, 4.0)	1.58 (*dd*, J = 13.6, 4.0)
H_{ax}–C(6)	1.38 – 1.40 (*m*)	1.27 – 1.29 (*m*)
H_{eq}–C(6)	1.64 – 1.68 (overlapped)	1.68 – 1.71 (overlapped)
H_{ax}–C(7)	2.01 (*ddd*, J = 13.5, 13.0, 4.0)	1.96 (*ddd*, J = 13.6, 13.2, 4.0)
H_{eq}–C(7)	2.39 (*ddd*, J = 13.0, 4.0, 3.4)	2.33 (*ddd*, J = 13.6, 4.0, 3.4)
H–C(9)	1.53 (*t*, J = 7.0)	1.59 – 1.62 (overlapped)
H_a–C(11)	1.37 – 1.39 (*m*)	1.48 – 1.50 (*m*)
H_b–C(11)	1.56 – 1.60 (*m*)	1.59 – 1.62 (overlapped)
H_a–C(12)	1.28 (*td*, J = 13.5, 7.0)	1.23 (*td*, J = 13.5, 7.0)
H_b–C(12)	1.70 (*td*, J = 13.5, 7.0)	1.69 – 1.71 (overlapped)
H–C(14)	5.77 (*dd*, J = 17.0, 10.5)	5.93 (*dd*, J = 17.0, 10.5)
H_a–C(15)	5.17 (*dd*, J = 17.0, 1.5)	4.95 (*dd*, J = 17.0, 1.5)
H_b–C(15)	5.22 (*dd*, J = 10.5, 1.5)	5.18 (*dd*, J = 10.5, 1.5)
Me(16)	1.33 (*s*)	1.19 (*s*)
H_a–C(17)	4.56 (*d*, J = 1.5)	4.56 (*d*, J = 1.5)
H_b–C(17)	4.83 (*d*, J = 1.5)	4.78 (*d*, J = 1.5)
Me(18)	1.04 (*s*)	0.76 (*s*)
Me(19) or CH_2(19)	0.82 (*s*)	2.94 (*d*, J = 13.0), 3.69 (*d*, J = 13.0)
Me(20)	0.70 (*s*)	0.70 (*s*)
H–C(1′)	4.31 (*d*, J = 8.0)	4.21 (*d*, J = 8.0)
H–C(2′)	3.18 – 3.20 (*m*)	3.18 – 3.20 (*m*)
H–C(3′)	3.31 – 3.33 (*m*)	3.34 – 3.38 (*m*)
H–C(4′)	3.24 – 3.28 (*m*)	3.27 – 3.31 (*m*)
H–C(5′)	3.42 – 3.44 (*m*)	3.68 – 3.70 (*m*)
CH_2(6′)	4.25 (*dd*, J = 13.0, 6.6), 4.32 (*dd*, J = 13.0, 6.6)	3.63 (*dd*, J = 13.0, 7.0), 3.82 (*dd*, J = 13.0, 7.0)
H–C(1″)	4.83 (*d*, J = 1.3)	–
H–C(2″)	3.68 – 3.70 (*m*)	–
H–C(3″)	3.28 – 3.32 (*m*)	–
H–C(4″)	3.32 – 3.34 (*m*)	–
H–C(5″)	3.73 – 3.75 (*m*)	–
H–C(6″)	1.21 (*d*, J = 6.0)	–
Me	2.07 (*s*)	–

23.3, 16.8, and 15.0), six CH_2 groups (δ(C) 42.0, 39.3, 38.4, 27.7, 25.0, and 18.9), four olefinic C-atoms (δ(C) 149.5, 143.8, 115.7, and 107.5), two CH groups (δ(C) 59.3 and 49.4), two quarternary C-atoms (δ(C) 40.5 and 40.3), an O-bearing CH group (δ(C) 90.8), and an O-bearing quaternary C-atom (δ(C) 80.8) according to the ^{1}H- and ^{13}C-NMR spectra, which suggested that **1** was similar to (13*S*)-labda-8(17),14-diene-3,13-diol, a common aglycone of the labdane type glycosides of *Diplopterygium* [5]. To the best of our knowledge, the absolute configuration at C(13) of labdane- and

clerodane-type diterpenes from *Diplopterygium glaucum*, which belongs to the genus *Diplopterygium* [6], and even from other genera of the family Gleicheniacea, such as *Dicranopteris linearis*, *Dicranopteris ampla* [7], *Dicranopteris dichotoma* [8], and *Gleichenia quadripartita* (POIRET) T. MOORE [9] was always (*S*), which indicated that compounds isolated from *D. rufopilosum* should be (13*S*)-configured from a biogenetic perspective. The sugar moieties were identified as a α-rhamnopyranosyl and a β-glucopyranosyl unit by the coupling constants of their anomeric H-atoms (δ(H) 4.31 (*d*, $J = 8.0$, H–C(1′)), 4.83 (*d*, $J = 1.3$, H–C(1″)) and similarity of their NMR data (*Table 1* and *2*) with those in the literature [8], and considering that all rhamnose and glucose units in structures of compounds isolated from fern species have L- and D-configuration, respectively, confirming these assignments [6]. The HMBC correlation between the anomeric H-atoms (δ(H) 4.31 and 4.83) of the glucopyranosyl and rhamnopyranosyl unit, and C(3) (δ(C) 90.8) and C(13) (δ(C) 80.8) of the aglycone, respectively. The 6′-location of the AcO group in the glucopyranosyl unit was confirmed by HMBC of H–C(6′) (δ(H) 4.32 and 4.25) with the CO C-atom (δ(C) 172.8) of the AcO group (*Fig. 2*).

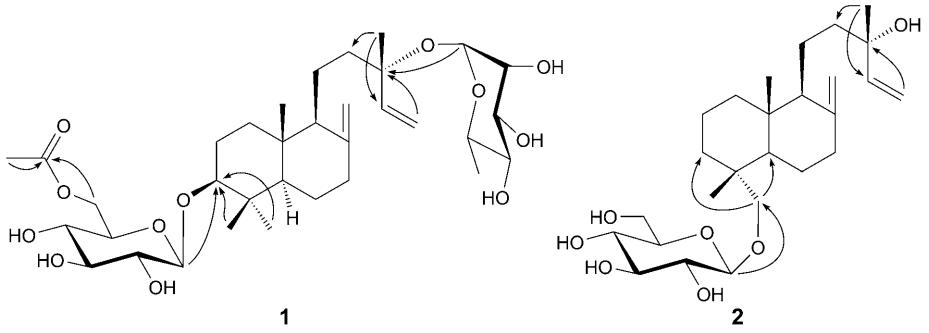


Fig. 2. *Key HMBCs* (H → C) *of compounds* **1** *and* **2**

The relative configuration was determined by a ROESY experiment. The ROESY correlations between H–C(3), H_α–C(1), and H–C(5) confirmed that H–C(3) was α-oriented. Thus, the structure of **1** was determined as (3β,13*S*)-3-*O*-[6-*O*-acetyl-β-D-glucopyranosyl]-13-*O*-α-L-rhamnopyranosyllabda-8(17),14-diene[1]).

Compound **2**, colorless oil, showed a molecular-ion peak at *m*/*z* 467 ($[M-H]^-$), in its mass spectrum and the molecular formula $C_{26}H_{44}O_7$ was established by HR-ESI-MS (*m*/*z* 467.3006 ($[M-H]^-$); calc. 467.3008). The ^{1}H- and ^{13}C-NMR spectra of **2** (*Tables 1* and *2*) indicated the presence of a diterpene and a hexose. The ^{1}H- and ^{13}C-NMR resonances of the aglycone of **2** were similar to those of **1**, except for the chemical shift of C(13) (δ(C) 73.1) and the location of the glucopyranosyl unit in the aglycone (*Tables 1* and *2*), which suggested that **2** was also a diterpene glycoside of (13*S*)-labda-8(17),14-diene-3,13-diol. The sugar moiety was identified as a β-glucopyranosyl unit by the coupling constants of the anomeric H-atom (δ(H) 4.21 (*d*, $J = 8.0$, H–C(1′))) and its NMR data (*Tables 1* and *2*) [6]. The HMBC correlation of the anomeric H-atom (δ(H) 4.21) to C(18) (δ(C) 79.3), together with the ROESY

correlations of H–C(19) with H_{ax}–C(2), H_{ax}–C(6), and H–C(20), indicated that the glucopyranosyl unit was at C(18) of the aglycone (*Fig. 2*), indicating (4*R*)-configuration. From a biogenetic perspective, the absolute configuration at C(13) of **2** should be (*S*), as compound **1** [6–9]. Thus, the structure of **2** was determined as (4*R*,13*S*)-18-*O*-*β*-D-glucopyranosyllabda-8(17),14-dien-13-ol.

Experimental Part

1. *General.* All solvents were distilled before use. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh, 10–40 μm; *Qingdao Marine Chemical Factory*, Qingdao, P. R. China), and *Sephadex LH-20* (*Amersham Pharmacia Biotech*, Sweden). TLC: silica gel GF_{254} (10–40 μm; *Qingdao Marine Chemical Factory*, Qingdao, P. R. China). Optical rotations: *JASCO-20C* digital polarimeter. IR Spectra: *Perkin–Elmer 577* spectrometer; KBr disc; $\tilde{\nu}$ in cm^{-1}. ^1H- and ^{13}C-NMR spectra: *Bruker AM-400* spectrometer δ in ppm, *J* in Hz. MS: *VG AutoSpec-3000* mass spectrometer; in *m*/*z*. HR-ESI-MS: *API QSTAR Pulsar-1* mass spectrometer.

2. *Plant Material.* The fronds of *D. rufopilosum* were collected in the Caojian town, Yunnan province, P. R. China, in July 2008, and identified by Prof. *Cheng Xiao* of Kunming institution of botany, Kunming, P. R. China. A voucher specimen (No. 2008101201) was deposited with the Kunming Institute of Botany.

3. *Extraction and Isolation.* The dry fronds of *D. rufopilosum* (6.6 kg) were extracted with 95% EtOH (3 × 20 l) for 24 h at r.t. After removal of EtOH under reduced pressure, the aq. brownish syrup (4 l) suspended in H_2O (500 ml) and then partitioned with AcOEt to afford AcOEt extract (88 g). AcOEt Soluble extract was subjected to chromatography over SiO_2 column, eluting with gradient $CHCl_3$/MeOH to afford six fractions, *Frs. 1–6*. *Fr. 3* (16.1 g) and *Fr. 4* (18.3 g) repeatedly purified on SiO_2 CC, *Sephadex LH-20*, *RP-18*, and semi-prep. HPLC to yield compounds **1** (11.0 mg) and **2** (8.7 mg).

4. *Compound Characterization.* 4.1. *(2S,4aR,5S,8aR)-Decahydro-1,1,4a-trimethyl-6-methylidene-5-{(3S)-3-methyl-3-[(α-L-rhamnopyranosyl)oxy]pent-4-en-1-yl}naphthalen-2-yl 6-O-Acetyl-β-D-glucopyranoside* (**1**). Colorless oil. $[\alpha]_D^{23.3} = -24.30$ ($c = 0.032$, MeOH). UV (MeOH): 203 (3.75). IR (KBr): 3423, 2937, 1720, 1641, 1385, 1040. ^1H-NMR (CD_3OD, 400 MHz): see *Table 2*. ^{13}C-NMR (CD_3OD, 100 MHz): see *Table 1*. FAB-MS (neg.): 655 ($[M-H]^-$). HR-ESI-MS: 655.3596 ($[M-H]^-$, $C_{34}H_{55}O_{12}^-$; calc. 655.3594).

4.2. *{(1R,4aR,5S,8aR)-Decahydro-1,4a-dimethyl-6-methylide-5-[(3S)-3-hydroxy-3-methylpent-4-en-1-yl]naphthalen-1-yl}methyl β-D-Glucopyranoside* (**2**). Colorless oil. $[\alpha]_D^{23.3} = +12.16$ ($c = 0.039$, MeOH). UV (MeOH): 204 (3.56). IR (KBr): 3435, 2929, 2853, 1740, 1629, 1070. ^1H-NMR (CD_3OD, 400 MHz): see *Table 2*. ^{13}C-NMR (CD_3OD, 100 MHz): see *Table 1*. FAB-MS (neg.): 467 ($[M-H]^-$). HR-ESI-MS: 467.3006 ($[M-H]^-$, $C_{26}H_{43}O_7^-$; calc. 467.3008).

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