Benzoic Acid Allopyranosides and Lignan Glycosides from the Twigs of *Keteleeria evelyniana*

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Z. Naturforsch. **2011**, *66b,* 733 – 739; received February 25, 2011

Seven new compounds, including three benzoic acid allopyranosides keteleeroside A (**1**), keteleeroside B (**2**) and keteleeroside C (**3**), and four lignan glycosides 3′-demethylicariside E_3 (**4**), isocupressoside B (**5**), 3-methoxyisocupressoside B (**6**), and isomassonianoside B (**7**), along with five known compounds (**8** – **12**), were isolated from the *n*-butanol part of the methanolic extract of the twigs of *Keteleeria evelyniana*. The structures of these compounds were elucidated mainly by the analysis of their NMR and MS data. All compounds were isolated from this genus for the first time. The skeleton of lignan glycoside **4** was isolated from the Pinaceae family for the first time. All compounds were evaluated for antimicrobial activity against *Candida albicans* and *Staphylococcus aureus*, and their cytotoxic activity against K562, HT-29, B16, BGC-823, BEL-7402, SGC-7901, U251, and A549 cancer cell lines was studied. Results indicated that none of the compounds showed antimicrobial or cytotoxic activity.

Key words: Keteleeria evelyniana, Pinaceae, Benzoic Acid Allopyranosides, Lignan Glycosides

Introduction

The genus *Keteleeria* (Pinaceae) comprises 12 species, which are native to China except two from Vietnam. *Keteleeria evelyniana* Mast. is essentially native to Yunnan, Sichuan and Guizhou in China. The timber is used for construction, bridge building, furniture, and wood fiber [1]. The chemistry of *K. evelyniana* has previously been studied and non-polar constituents of the plant have been identified [2 – 6]. However, its polar constituents have not been studied. In our own program aimed at discovering new active constituents from gymnosperms, many natural compounds have been isolated, and some of them showed inhibitory activities against cancer cell lines. In continuous search for new active compounds, we investigated the polar constituents from the *n*-butanol part of the methanolic extract of the twigs of *K. evelyniana*. Herein, the isolation and structure elucidation of 12 compounds with their antimicrobial and cytotoxic activity are described.

Results and Discussion

The aqueous solution of the methanolic extract of the twigs of *K. evelyniana* was partitioned sequentially with petroleum ether, EtOAc and *n*-BuOH. Purification of the *n*-BuOH fraction produced three new benzoic acid allopyranosides, keteleeroside A (**1**), keteleeroside B (**2**) and keteleeroside C (**3**), and four new lignan glycosides, 3′-demethylicariside E_3 (**4**), isocupressoside B (**5**), 3-methoxyisocupressoside B (**6**), and isomassonianoside B (**7**) (Fig. 1). In addition to these seven new compounds, two known lignan glycosides, (2*S*,3*R*)-2,3-dihydro-2-(4′-hydroxy-3′-methoxyphenyl)-3-hydroxymethyl-7-methoxy-5-benzofuranpropanol 4′-*O*-β-D-glucopyranoside (**8**) [7] and (2*S*,3*R*)-2,3-dihydro-2-(4′-hydroxy-3′-methoxyphenyl)-3-hydroxymethyl-7-hydroxy-5-benzofuranpropanol 4′-*O*-β-D-glucopyranoside (**9**) [7], two known phenylpropanoid glycosides, 2-*O*-[4′-(α-hydroxypropyl)-2′-methoxyphenyl]-1-*O*-β-D-glucopyranosyl glycerol (**10**) [8] and 3,4′-dihydroxypropiophenone 3-*O*-β-D-glucopyranoside (**11**) [9], and one aromatic glycoside 3,4-dimethoxyphenyl 1-*O*-β-D-apiofuranosyl-(1″→6′)-β-D-glucopyranoside (**12**) [10] were also isolated. Their structures were determined by comparison of physical and spectroscopic data (^{1}H NMR, ^{13}C NMR, $[\alpha]_D$, CD and MS) with those of the corresponding authentic samples or using literature data.

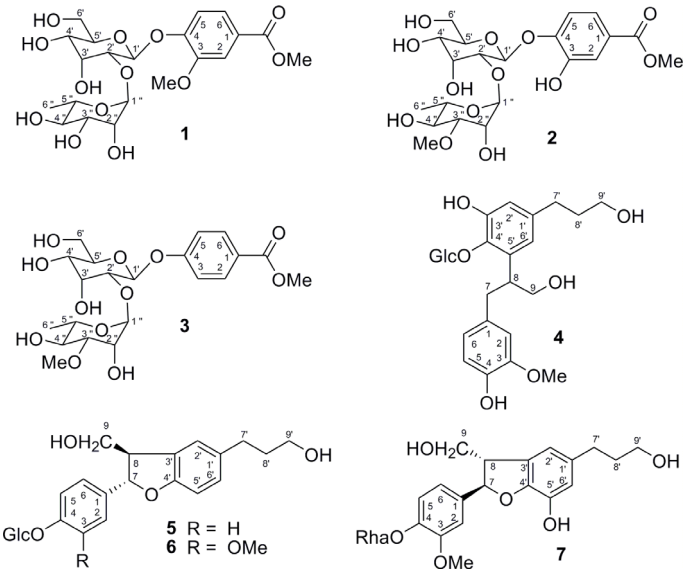

Fig. 1. Structures of compounds **1** – **7**.

All compounds were isolated from genus *Keteleeria* for the first time. The skeleton of lignan glycoside **4** was isolated from the Pinaceae family for the first time. All compounds were evaluated for antimicrobial activity against *Candida albicans* and *Staphylococcus aureus*, and their cytotoxic activity against K562, HT-29, B16, BGC-823, BEL-7402, SGC-7901, U251, and A549 cancer cell lines was investigated [11]. Results have indicated that none of the compounds shows antimicrobial or cytotoxic activity at the concentration of 10 μg mL^{-1}.

Compound **1** was isolated as a colorless solid, and its molecular formula $C_{21}H_{30}O_{13}$ was determined from the quasimolecular ion peak at *m/z* = 489.1594 ([M–H]$^-$) in the negative HRESIMS. Its UV spectrum (λ_{max} = 256, 291 nm) was similar to that of pseudolaroside B [12]. IR absorption bands at 3424, 1706 and 1602 cm^{-1} were characteristic of hydroxy, conjugated ester carbonyl and aromatic groups, respectively. The ^{1}H and ^{13}C NMR spectra of aglycone of **1** were similar to those of pseudolaroside B [12]. Two anomeric proton resonances at δ_H = 5.43 (1H, d, 7.9) and 4.95 (1H, d, 1.1) in the ^{1}H NMR spectrum indicated β- and α-glycosidic linkages. From the analysis of the 2D data of the two anomeric protons, the proton connectivities for the individual sugars of compound **1** were detected using COSY, TOCSY and HSQC-TOCSY spectra (Fig. 2), and the two sugars were found to be L-rhamnose and D-allose. The correlation between H-1′ and C-4 in the HMBC spectrum showed that the sugar moiety was located at C-4 (Fig. 2), which was confirmed by ROESY correlations of H-5 and H-1′. The correlation between H-2′ and C-1″ in the HMBC spectrum suggested that the two sugars were linked from the 2′-position of the first sugar to the 1″-position of the second. Two methoxy groups with signals at δ_H = 3.85 (3H, s) and 3.87 (3H, s) were linked to C-3 and C=O according to HMBC correlations. The configuration of the sugar units was confirmed as L-rhamnose and D-allose, after basic hydrolysis of **1** and comparing the values of R_f and specific rotation with those of authentic samples. Thus, compound **1** was elucidated as 3-methoxy-benzoic acid methyl ester 4-*O*-α-L-rhamnopyranosyl-(1″ →2′)-β-D-allopyranoside.

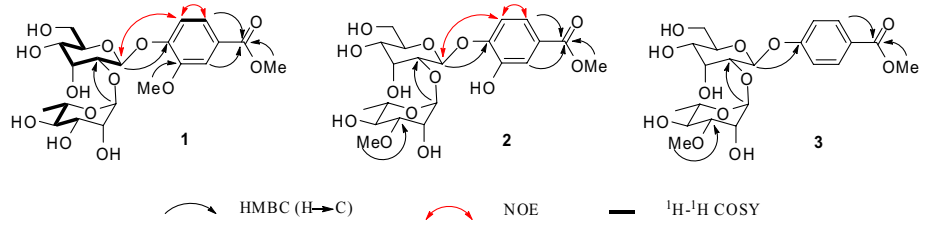

Fig. 2. Key HMBC, COSY and ROESY correlations of compounds **1** – **3**.

The molecular formula of **2**, $C_{21}H_{30}O_{13}$, was deduced to be the same as that of **1** from the HRESIMS at *m/z* = 489.1614 ([M–H]$^-$). Its UV spectrum (λ_{max} = 253, 291 nm) was nearly identical to that of **1**, indicative of a structural analog having the 3,4-dioxybenzoyl chromophore. The ^{1}H and ^{13}C NMR spectra revealed that the difference between **1** and **2** was the absence of the peak for the C-3 methoxy group [δ_H = 3.85 (3H, s), δ_C = 56.4] and the presence of a signal [δ_H = 3.41 (3H, s), δ_C = 57.3] for a C-3″ methoxy group in **2**. The sugar units isolated from the basic hydrolysis gave positive optical rotations ($[\alpha]_D^{19}$ = +13.8 (*c* = 0.20, H_2O), $[\alpha]_D^{19}$ = +9.8 (*c* = 0.11, H_2O)) indicating that they are D-allose and L-rhamnose. Therefore, compound **2** (keteleeroside B) was elucidated as 3-hydroxy-benzoic acid methyl ester 4-*O*-(3-*O*-methyl-α-L-rhamnopyranosyl)-(1″ →2′)-β-D-allopyranoside, which was confirmed by HSQC, HMBC and ROESY NMR experiments (Fig. 2).

Keteleeroside C (**3**) was assigned the molecular formula $C_{21}H_{30}O_{12}$ from the quasimolecular ion peak at *m/z* = 473.1649 ([M–H]$^-$) in the negative HRESIMS. Its UV spectrum (λ_{max} = 250 nm) was similar to that of pseudolaroside A [12]. Comparison of chemical shifts of its sugar units with those of **2** indicated that **3** had the same sugar moieties as **2**. The ^{1}H and ^{13}C NMR spectra suggested that the difference between **1** and **3** was the absence of the C-3 hydroxy group in **3** which was confirmed by comparison of the ^{1}H and ^{13}C NMR (including DEPT) spectra of the aglycone with those of pseudolarolide A [12]. Moreover, on comparison of their mass spectra, the by 16 amu lower molecular weight was also consistent with the absence of a hydroxy group in **3**. Basic hydrolysis of **3** yielded D-allose ($[\alpha]_D^{19}$ = +12.9 (*c* = 0.20, H_2O)) and L-rhamnose ($[\alpha]_D^{19}$ = +12.7 (*c* = 0.20, H_2O)) after stirring in 3 % aqueous NaOH (5 mL) at r. t. for 2 h. Hence, compound **3** (keteleeroside C) was elucidated as benzoic acid methyl ester 4-*O*-(3-*O*-methyl-α-L-rhamnopyranosyl)-(1″ →2′)-β-D-allopyranoside, which was confirmed by HSQC, HMBC NMR experiments (Fig. 2).

The molecular formula of **4**, $C_{25}H_{34}O_{11}$, was determined from the HRESIMS peak at *m/z* = 509.2031 ([M–H]$^-$). IR absorption bands at 3415 and 1599 cm^{-1} were characteristic of hydroxy and aromatic groups, respectively. The NMR spectroscopic features of **4** were similar to those of icariside E_3 [13]. The ^{1}H and ^{13}C NMR spectra revealed that the difference between **4** and icariside E_3 was the absence of the peak for a C-3′ methoxy group and the presence of a hydroxy group at C-3′ in **4**. This suggested that **4** was a demethyl derivative of icariside E_3. The glucose produced from its basic hydrolysis gave a positive optical rotation, $[\alpha]_D^{20}$ = +39.1 (*c* = 0.20, H_2O), and showed that it is D-glucose. Accordingly, compound **4** was elucidated as 3′-demethylicariside E_3, which was confirmed by HSQC, HMBC, ROESY NMR experiments.

Isocupressoside (**5**) showed the same molecular formula of $C_{24}H_{30}O_9$ as that of cupressoside B [14] by the quasimolecular ion peak at *m/z* = 461.1804 ([M–H]$^-$) in the negative HRESIMS. The ^{1}H and ^{13}C NMR spectroscopic data of **5** were almost the same as those of cupressoside B, suggesting the same planar structure and a relative *trans* stereochemistry at the two chiral centers (C-7 and C-8). The absolute configurations at C-7 and C-8 of **5** were established on the basis of CD spectroscopic evidence. The CD spectrum showed a positive Cotton effect at 236 nm and a negative Cotton effect at 222 nm, demonstrating **5** to be in the 7*S*, 8*R* configuration [14]. Therefore, the structure of **5** was established as (7*S*,8*R*)-3,3′-didemethoxy-dihydrodehydrodiconiferyl alcohol-4-*O*-β-D-glucopyranoside.

Compound **6** was assigned the molecular formula $C_{25}H_{32}O_{10}$ through the quasimolecular ion peak at *m/z* = 527.1683 ([M+Cl]$^-$) in the negative HRESIMS. Comparison of chemical shifts of the sugar unit indicated that **6** had the same sugar moiety as isocupressoside B (**5**). Basic hydrolysis of **6** yielded D-glucose, with $[\alpha]_D^{20}$ = +43.8 (*c* = 0.20, H_2O). The ^{1}H and ^{13}C NMR (including DEPT) spectra suggested that the difference between **6** and **5** was the presence of one new C-3 methoxy group in **6**. Inspection of HSQC, HMBC, ROESY spectra confirmed the structure of **6**. Compound **6** showed Cotton effects at 220 and 234 nm, which were almost identical to those of **5**. Thus, compound **6** was determined to be (7*S*,8*R*)-3′-demethoxy-dihydrodehydrodiconiferyl alcohol-4-*O*-β-D-glucopyranoside, named as 3-methoxyisocupressoside B.

Isomassonianoside B (**7**) was obtained as a colorless solid, whose molecular formula $C_{25}H_{32}O_{10}$ was deduced from the negative HRESIMS (*m/z* = 491.1920 [M–H]$^-$) as that of massonianoside B [15]. The ^{1}H and ^{13}C NMR spectroscopic data of **7** were almost identical with those of massonianoside B, suggesting that they should have the same planar structure and a relative *trans* stereochemistry at the two chiral centers (C-7 and C-8). In addition, the CD spectra of **7** gave a positive Cotton effect at 224 nm and negative Cotton effects at

245 and 284 nm. Thus, the absolute configuration of **7** was determined to be 7*R*, 8*S* configuration as shown in Fig. 1 [16].

Experimental Section

General

Optical rotations were measured with a Horbia SEAP-300 polarimeter, CD with a Chirascan spectropolarimeter (Applied Photophysics, U. K.). IR spectra were obtained on a Bio-Rad FTS-135 spectrophotometer using KBr pellets. UV spectra were measured on a Shimadzu 2401PC spectrophotometer. MS spectra were performed on a VG Autospec-3000 spectrometer and API Qstar Pulsar instrument. 1D and 2D NMR spectra were recorded either on a Bruker AM-400 or on a DRX-500 spectrometer, with chemical shifts δ given in ppm using TMS as an internal standard. Semipreparative reversed-phase HPLC was performed on an Agilent 1100 apparatus equipped with a UV detector and a YMC-Pack ODS-A (YMC, 1×15 cm) column at a flow rate of 2 mL min^{-1}. Column chromatography was performed on silica gel (100 – 200 mesh, 200 – 300 mesh, and 10 – 40 μm, Qingdao Marine Chemical, Inc., China), Sephadex LH-20 (25 – 100 μm, Pharmacia Fine Chemical Co., Ltd., Sweden), and MCI gel (75 – 150 μm, Mitsubishi Chemical Corporation, Japan). TLC was carried out on precoated silica gel GF_{254} glass plates (Qingdao Marine Chemical, Inc., China). Spots were first visualized under UV light (254 and 365 nm), followed by spraying with 10 % H_2SO_4 in 95 % EtOH and then heating.

Plant material

The twigs of *K. evelyniana* were collected in Kunming Botany Garden, Yunnan Province, China, in September 2005. The plant was identified by Prof. Zhong-Shu Yue, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. 0010482) was deposited at the herbarium of Kunming Institute of Botany, Chinese Academy of Sciences. Cancer cell lines, *i. e.* K562, HT-29, B16, BGC-823, BEL-7402, SGC-7901, U251 and A549, were purchased from the Cell Culture Center of the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, Beijing, China. Bacteria *S. aureus* and *C. albicans* were purchased from China General Microbiological Culture Collection Center, Beijing, China.

Extraction and isolation

The air-dried and powdered twigs of *K. evelyniana* (20 kg) were extracted with 95 % EtOH at r. t. three times and filtered. The filtrate was evaporated to give a residue (1.2 kg), which was suspended in H_2O and then partitioned successively with petroleum ether, EtOAc and *n*-BuOH. The *n*-BuOH-soluble fraction was evaporated to obtain a crude syrup (630 g), which was subjected to silica gel column chromatography (8×60 cm, 100 – 200 mesh), eluted with petroleum ether, $CHCl_3$, and $CHCl_3$-MeOH (95 : 5 – 1 : 1). On the basis of TLC analysis, fraction [$CHCl_3$-MeOH (4 : 1)] was then applied to a MCI column (1.5×20 cm) and eluted with a gradient of a mixture MeOH-H_2O (1 : 9→4 : 6) to afford eight fractions 1 – 8. Fraction 1 was passed through a Sephadex LH-20 column (1×90 cm, MeOH) and then subjected to semipreparative HPLC [MeOH-H_2O (3 : 7)] to give **3** (20 mg; t_R = 18.3 min). Fraction 2 was passed through a Sephadex LH-20 column (1×90 cm, MeOH) to yield compound **8** (100 mg) and three fractions 2.1 – 2.3. Fraction 2.1 was purified by semipreparative reversed-phase HPLC [MeOH-H_2O (3 : 7)] to give **1** (46 mg; t_R = 15.8 min), **2** (6 mg; t_R = 12.8 min) and **7** (6 mg; t_R = 23.4 min). Fraction 2.2 was purified by semipreparative reversed-phase HPLC [MeOH-H_2O (2 : 8)] to give **10** (6 mg; t_R = 13.6 min), **11** (21 mg) and **12** (4 mg; t_R = 17.7 min). Fraction 2.3 was purified by semipreparative reversed-phase HPLC [MeOH-H_2O (4 : 6)] to give **6** (6 mg; t_R = 15.7 min). Fraction 3 was passed through a Sephadex LH-20 column (1×90 cm, MeOH) and then subjected to semipreparative HPLC [MeOH-H_2O (3 : 7)] to give **4** (4 mg; t_R = 32.5 min) and **5** (3 mg; t_R = 39.0 min). Fraction 5 was passed through a Sephadex LH-20 column (1×90 cm, MeOH) to yield **9** (170 mg).

*Basic hydrolysis of **1** – **5** and helicide*

A solution of helicide [17] (85.4 mg) (authentic sample from our laboratory) in 3 % aqueous NaOH (5 mL) was stirred at r. t. for 2 h. The reaction mixture was neutralized to pH = 7 by 2 N HCl and then extracted with $CHCl_3$. Through TLC using $CHCl_3$-MeOH (8 : 2) as a developing system, D-allose was detected in the aqueous layer. The aqueous solution was further concentrated to dryness and subjected to silica gel chromatography (1×15 cm, 10 – 40 μm) with $CHCl_3$-MeOH (8 : 2) to give D-allose (9.8 mg), $[\alpha]_D^{19}$ = +14.3 (c = 0.50, H_2O) as an authentic sample.

A solution of compounds **1** (15.3 mg), **2** (5.2 mg), or **3** (5.4 mg) in 3 % aqueous NaOH (5 mL) was stirred at r. t. for 2 h. The reaction mixture was neutralized to pH = 7 by 2 N HCl and then extracted with $CHCl_3$. Through TLC comparison with authentic samples using $CHCl_3$-MeOH (8 : 2) as a developing system, D-allose and L-rhamnose were detected in the aqueous layer. The aqueous solution was further concentrated to dryness and subjected to silica gel chromatography (1×25 cm, 10 – 40 μm) with $CHCl_3$-MeOH (9 : 1) to give D-allose (3.0 mg, $[\alpha]_D^{20}$ = +12.5 (c = 0.30, H_2O); 0.9 mg, $[\alpha]_D^{19}$ = +13.8 (c = 0.20, H_2O); 1.0 mg, $[\alpha]_D^{19}$ = +12.9 (c = 0.20, H_2O)) and L-rhamnose (2.3 mg, $[\alpha]_D^{19}$ = +8.5 (c = 0.20, H_2O); 0.8 mg, $[\alpha]_D^{19}$ = +9.8 (c = 0.11, H_2O); 0.7 mg, $[\alpha]_D^{19}$ = +12.7 (c = 0.20, H_2O)), respectively. The

Table 1. ^{1}H NMR and ^{13}C NMR data for compounds **1**—**3** in CD_3OD.

	1		**2**		**3**	
No.	δ_C (mult)	δ_H (J in Hz)	δ_C (mult)	δ_H (J in Hz)	δ_C (mult)	δ_H (J in Hz)
1	125.0 (s)	—	125.9 (s)	—	125.0 (s)	—
2	113.4 (d)	7.55 (d, 2.0)	118.2 (d)	7.46 (d, 2.0)	132.5 (d)	7.97 (d, 8.8)
3	150.7 (s)	—	148.5 (s)	—	117.1 (d)	7.13 (d, 8.8)
4	152.4 (s)	—	151.0 (s)	—	162.9 (s)	—
5	115.9 (d)	7.20 (d, 8.5)	117.4 (d)	7.18 (d, 8.5)	117.1 (d)	7.13 (d, 8.8)
6	124.1 (d)	7.60 (dd, 8.5, 2.0)	123.0 (d)	7.49 (dd, 8.5, 2.0)	132.5 (d)	7.97 (d, 8.8)
COOMe	168.4 (s)	—	168.4 (s)	—	168.2 (s)	—
COOMe	52.6 (q)	3.87 (s)	52.5 (q)	3.85 (s)	52.5 (q)	3.87 (s)
1′	98.2 (d)	5.43 (d, 7.9)	99.2 (d)	5.44 (d, 7.9)	98.0 (d)	5.45 (d, 8.0)
2′	73.9 (d)	3.82 (overlapped)	75.2 (d)	3.81 (overlapped)	74.6 (d)	3.78 (dd, 8.0, 2.7)
3′	68.5 (d)	4.33 (t, 2.8)	69.1 (d)	4.33 (t, 2.8)	68.9 (d)	4.33 (t, 2.7)
4′	68.3 (d)	3.61 (dd, 9.8, 2.8)	68.2 (d)	3.64 (dd, 10.0, 2.8)	68.3 (d)	3.62 (dd, 9.8, 2.7)
5′	75.6 (d)	3.90 (overlapped)	75.7 (d)	3.87 (overlapped)	75.6 (d)	3.93 (overlapped)
6′a	62.7 (t)	3.67 (overlapped)	62.5 (t)	3.70 (dd, 12.2, 5.2)	62.7 (t)	3.70 (dd, 12.0, 5.5)
6′b	—	3.82 (overlapped)	—	3.83 (overlapped)	—	3.90 (overlapped)
1″	97.4 (d)	4.95 (d, 1.1)	98.3 (d)	5.02 (d, 1.6)	97.8 (d)	4.99 (d, 1.6)
2″	72.2 (d)	3.90 (overlapped)	68.2 (d)	4.14 (dd, 3.0, 1.6)	67.9 (d)	4.14 (dd, 2.8, 1.6)
3″	72.2 (d)	3.67 (overlapped)	81.8 (d)	3.35 (dd, 9.6, 3.0)	81.9 (d)	3.29 (overlapped)
4″	73.7 (d)	3.41 (t, 9.6)	72.7 (d)	3.44 (t, 9.6)	72.6 (d)	3.48 (t, 9.5)
5″	70.1 (d)	4.15 (m)	70.4 (d)	3.96 (m)	70.0 (d)	4.03 (m)
6″	18.2 (q)	1.29 (d, 6.2)	18.0 (q)	1.23 (d, 6.2)	18.0 (q)	1.32 (d, 6.2)
3-OMe	56.4 (q)	3.85 (s)	—	—	—	—
3″-OMe	—	—	57.3 (q)	3.41 (s)	57.2 (q)	3.38 (s)

D-allose and L-rhamnose were identified by comparison of their R_f and specific rotation values with those of authentic samples.

Similar treatment of **4** (2.9 mg) and **6** (3.0 mg) gave D-glucose (0.6 mg, $[\alpha]_D^{20} = +39.1$ ($c = 0.20$, H_2O) and 0.7 mg, $[\alpha]_D^{20} = +43.8$ ($c = 0.20$, H_2O), respectively).

*Keteleeroside A (= 3-methoxy-benzoic acid methyl ester 4-O-α-L-rhamnopyranosyl-(1″ → 2′)-β-D-allopyranoside; **1**):* colorless solid. – $[\alpha]_D^{26.5} = -40.0$ ($c = 0.55$, MeOH). – UV (MeOH): λ = 205 (4.05), 216 (4.01), 256 (3.68), 291 (3.37) nm. – IR (KBr): ν = 3424, 2927, 1722, 1706, 1640, 1602, 1512, 1296, 1276, 1221, 1136, 1073 cm^{-1}. – ^{1}H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) data: see Table 1. – MS ((–)-FAB): m/z = 489 ([M–H]$^-$). – HRMS ((–)-ESI): m/z = 489.1594 (calcd. 489.1608 for $C_{21}H_{29}O_{13}$, [M–H]$^-$).

*Keteleeroside B (= 3-hydroxy-benzoic acid methyl ester 4-O-(3-O-methyl-α-L-rhamnopyranosyl)-(1″ → 2′)-β-D-allopyranoside; **2**):* colorless solid. – $[\alpha]_D^{25.7} = -72.8$ (c = 0.30, MeOH). – UV (MeOH): λ = 205 (4.50), 253 (3.89), 291 (3.59) nm. – IR (KBr): ν = 3426, 2928, 1678, 1640, 1632, 1512, 1441, 1301, 1207, 1136, 1074, 1046 cm^{-1}. – ^{1}H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) data: see Table 1. – MS ((–)-FAB): m/z = 489 [M–H]$^-$. – HRMS ((–)-ESI): m/z = 489.1614 (calcd. 489.1608 for $C_{21}H_{29}O_{13}$, [M–H]$^-$).

*Keteleeroside C (= benzoic acid methyl ester 4-O-(3-O-methyl-α-L-rhamnopyranosyl)-(1″ → 2′)-β-D-allopyranoside; **3**):* colorless solid. – $[\alpha]_D^{27.7} = -101.0$ (c = 0.50, MeOH). – UV (MeOH): λ = 204 (4.27), 250 (4.24) nm. – IR (KBr): ν = 3425, 2932, 1705, 1607, 1511, 1439, 1291, 1245, 1109, 1078, 771 cm^{-1}. – ^{1}H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) data: see Table 1. – MS ((–)-FAB): m/z = 473 [M–H]$^-$. – HRMS ((–)-ESI): m/z = 473.1649 (calcd. 473.1659 for $C_{21}H_{29}O_{12}$, [M–H]$^-$).

*3′-Demethylicariside E_3 (**4**):* colorless solid. – $[\alpha]_D^{25.4} = -13.3$ (c = 0.20, MeOH). – UV (MeOH): λ = 205 (4.57), 281 (3.47) nm. – CD (c = 0.10 MeOH), $\Delta\varepsilon$ (nm) = −0.5 (213). – IR (KBr): ν = 3415, 2935, 2882, 1599, 1516, 1451, 1274, 1068, 1035 cm^{-1}. – ^{1}H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) data: see Tables 2 and 3. – MS ((–)-FAB): m/z = 509 [M–H]$^-$. – HRMS ((–)-ESI): m/z = 509.2031 (calcd. 509.2022 for $C_{25}H_{33}O_{11}$, [M–H]$^-$).

*Isocupressoside B (= (7S,8R)-3, 3′-didemethoxy-dihydrodehydrodiconiferyl alcohol-4-O-β-D-glucopyranoside; **5**):* colorless solid. – $[\alpha]_D^{27.7} = 0.00$ (c = 0.20, MeOH). – UV (MeOH): λ = 202 (4.36), 223 (3.97), 284 (3.32) nm. – CD (c = 0.10 MeOH), $\Delta\varepsilon$ (nm) = −0.6 (222), +3.0 (236). – IR (KBr): ν = 3429, 2924, 2878, 1615, 1512, 1489, 1235, 1074, 1043 cm^{-1}. – ^{1}H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) data: see Tables 2 and 3. – MS ((–)-FAB): m/z (%) = 461 (20) [M–H]$^-$. – HRMS ((–)-ESI): m/z = 461.1804 (calcd. 461.1811 for $C_{24}H_{29}O_9$, [M–H]$^-$).

*3-Methoxyisocupressoside B (= (7S,8R)-3′-demethoxy-dihydrodehydrodiconiferyl alcohol-4-O-β-D-glucopyranoside; **6**):* colorless solid. – $[\alpha]_D^{27.0} = -36.7$ (c = 0.30, MeOH). – UV (MeOH): λ = 205 (4.63), 226 (4.08), 283 (3.60) nm. – CD (c = 0.10 MeOH), $\Delta\varepsilon$ (nm) = −3.5 (220), +6.6(234). – IR (KBr): ν = 3412, 2959, 2930, 2875, 1727,

Table 2. ^{1}H NMR data for compounds **4** – **7** in CD_3OD.

No.	**4** δ_H (*J* in Hz)	**5** δ_H (*J* in Hz)	**6** δ_H (*J* in Hz)	**7** δ_H (*J* in Hz)
1	–	–	–	–
2	6.66 (s)	7.31 (d, 8.7)	7.01 (overlapped)	7.06 (d, 1.9)
3	–	7.09 (d, 8.7)	–	–
4	–	–	–	–
5	6.62 (overlapped)	7.09 (d, 8.7)	7.13 (d, 8.4)	7.07 (d, 8.3)
6	6.62 (overlapped)	7.31 (d, 8.7)	6.91 (dd, 8.4, 1.9)	6.93 (dd, 1.9, 8.3)
7a	2.92 (dd, 14.0, 6.8)	5.52 (d, 5.6)	5.51 (d, 5.7)	5.55 (d, 5.8)
7b	2.86 (dd, 14.0, 8.3)	–	–	–
8	4.00 (m)	3.44 (m)	3.42 (m)	3.44 (overlapped)
9a	3.60 (dd, 10.8, 6.7)	3.83 (overlapped)	3.84 (overlapped)	3.84 (overlapped)
9b	3.67 (overlapped)	–	3.75 (dd, 10.6, 7.9)	–
3-OMe	3.73 (s)	–	3.82 (s)	3.80 (s)
1′	–	–	–	–
2′	6.58 (d, 2.0)	7.10 (s)	7.09 (bs)	6.57 (s)
3′	–	–	–	–
4′	–	–	–	–
5′	–	6.74 (d, 8.1)	6.74 (d, 8.1)	–
6′	6.62 (overlapped)	7.03 (d, 8.1)	7.01 (overlapped)	6.59 (s)
7′	2.56 (t, 7.7)	2.64 (m)	2.62(t, 7.7)	2.56 (m)
8′	1.77 (m)	1.82 (m)	1.80 (m)	1.78 (m)
9′	3.54 (t, 6.5)	3.55 (t, 6.5)	3.55 (t, 6.5)	3.58 (t, 6.5)
3′-OMe	–	–	–	–
1″	4.53 (d, 7.8)	4.91 (d, 7.3)	4.88 (d, 7.4)	5.33 (d, 1.6)
2″	3.46 (m)	3.48 (m)	3.48 (m)	3.87 (overlapped)
3″	3.37 (t, 9.2)	3.45 (m)	3.45 (m)	3.85 (overlapped)
4″	3.46 (m)	3.38 (overlapped)	3.38 (overlapped)	3.85 (overlapped)
5″	3.17 (m)	3.39 (overlapped)	3.38 (overlapped)	3.86 (overlapped)
6″a	3.77 (dd, 11.9, 2.4)	3.84 (overlapped)	3.85 (overlapped)	1.21 (d, 6.2)
6″b	3.70 (overlapped)	–	3.67 (dd, 12.0, 4.9)	–

Table 3. ^{13}C NMR data for compounds **4** – **7** in CD_3OD.

No.	**4** δ_C (mult)	Icariside E_3 [13] δ_C (mult)	**5** δ_C (mult)	**6** δ_C (mult)	**7** δ_C (mult)
1	133.3 (s)	133.3 (s)	128.5 (s)	138.6 (s)	139.0 (s)
2	114.1 (d)	113.6 (d)	127.9 (d)	111.0 (d)	111.0 (d)
3	148.4 (s)	148.4 (s)	117.7 (d)	150.9 (s)	136.8 (s)
4	145.4 (s)	145.3 (s)	158.7 (s)	147.5 (s)	146.3 (s)
5	115.7 (d)	115.6 (d)	117.7 (d)	119.3 (d)	119.5 (d)
6	122.8 (d)	122.6 (d)	127.9 (d)	117.9 (d)	118.9 (d)
7	38.2 (t)	39.2 (t)	87.9 (d)	87.9 (d)	88.1 (d)
8	42.3 (d)	42.8 (d)	55.3 (d)	55.3 (d)	55.8 (d)
9	66.8 (t)	67.1 (t)	65.2 (t)	65.2 (t)	65.1 (t)
3-OMe	56.3 (q)	56.2 (q)	–	56.6 (q)	56.3 (q)
1′	140.6 (s)	140.3 (s)	135.7 (s)	135.8 (s)	129.4 (s)
2′	115.6 (d)	111.7 (d)	125.9 (d)	125.9 (d)	116.9 (d)
3′	150.6 (s)	153.1 (s)	137.6 (s)	128.5 (s)	136.8 (s)
4′	143.5 (s)	143.6 (s)	159.4 (s)	159.4 (s)	146.3 (s)
5′	137.8 (s)	138.6 (s)	109.8 (d)	109.8 (d)	141.8 (s)
6′	119.9 (d)	120.3 (d)	129.7 (d)	129.8 (d)	116.1 (d)
7′	32.8 (t)	33.1 (t)	32.5 (t)	32.5 (t)	32.6 (t)
8′	35.5 (t)	35.6 (t)	35.9 (t)	35.9 (t)	35.7 (t)
9′	61.9 (t)	62.2 (t)	62.2 (t)	62.2 (t)	62.2 (t)
3′-OMe	–	56.3 (q)	–	–	–
1″	107.2 (d)	105.6 (d)	102.2 (d)	102.7 (d)	101.3 (d)
2″	75.6 (d)	75.9 (d)	74.9 (d)	74.9 (d)	72.1 (d)
3″	78.0 (d)	77.9 (d)	77.9 (d)	77.8 (d)	71.9 (d)
4″	70.7 (d)	71.2 (d)	71.3 (d)	71.3 (d)	73.7 (d)
5″	78.3 (d)	78.1 (d)	78.1 (d)	78.2 (d)	70.7 (d)
6″	62.2 (t)	62.5 (t)	62.5 (t)	62.4 (t)	17.9 (q)

1681, 1515, 1490, 1467, 1454, 1286, 1277, 1206, 1135, 1074 1039, 802 cm^{-1}. – ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) data: see Tables 2 and 3. – MS ((–)-FAB): *m/z* = 491 $[M–H]^-$. – HRMS ((–)-ESI): *m/z* = 527.1683 (calcd. 527.1684 for $C_{25}H_{32}O_{10}Cl$, $[M+Cl]^-$).

Isomassonianoside B (7): colorless solid. – $[\alpha]_D^{27.0}$ = −120.00 (*c* = 0.75, MeOH). – UV (MeOH): λ = 204 (4.13), 281 (5.15) nm. – CD (*c* = 0.04 MeOH), $\Delta\varepsilon$ (nm) = +1.7 (224), −2.3 (245), −4.3 (284). – IR (KBr): λ = 3416, 2935, 2881, 1676, 1609, 1512, 1452, 1421, 1268, 1224, 1063, 1029, 822 cm^{-1}. – ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) data: see Tables 2 and 3. – MS ((–)-FAB): *m/z* (%) = 491 (20) $[M–H]^-$. – HRMS ((–)-ESI): *m/z* = 491.1920 (calcd. 491.1917 for $C_{25}H_{31}O_{10}$, $[M–H]^-$).

Acknowledgements

This work was supported by the National Natural Science Foundation of China (30725048, 91013002, U1032602, 20972168), the National New Drug Innovation Great Project of China (2011ZX09307-002-02), the National Basic Research Program of China (2009CB522300) and the Fund of Chinese Academy of Sciences (KSCX2-YW-R-177). The authors are grateful to the members of the analytical group from the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, for measuring the optical rotation data and IR, UV, CD, NMR, and MS spectra.

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