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New lignan glycosides from *Cupressus duclouxian* (Cupessaceae)

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From the branches and leaves of *Cupressus duclouxiana* two new lignan glycosides named cupressoside A (1) and cupressoside B (2), together with matairesinoside (3), dihydrodehydrodiconiferyl alcohol (4), dihydrodehydrodiconiferyl alcohol-9-*O*-α-L-rhamnopyranoside (5), dihydrodehydrodiconiferyl alcohol-4-*O*-α-L-rhamnopyranoside (6), (−)-isolariciresinol (7) and (−)-isolariciresinol-9-*O*-β-D-xylopyranoside (8), were isolated. The structures of these compounds were determined on the basis of their HR-FAB-MS, IR, UV, ^{1}H and ^{13}C NMR (DEPT), and 2D NMR (HMQC, HMBC, COSY, NOESY) spectral data.

Keywords: *Cupressus duclouxiana*; Cupessaceae; Lignan glycosides; Cupressoside A; Cupressoside B

1. Introduction

Cupressus duclouxiana (Cupessaceae) is a common ornamental tree distributed in Yunnan and Sichuan Province of Southwest China [1], on which no chemical studies have thus far been reported. As part of an investigation on Gymnosperm, the chemical constituents of *C. duclouxiana* collected at Kunming Institute of Botany were investigated. Eight lignans were isolated, i.e. cupressoside A (1) and cupressoside B (2), together with matairesinoside (3), dihydrodehydrodiconiferyl alcohol (4), dihydrodehydrodiconiferyl alcohol-9-*O*-α-L-rhamnopyranoside (5), dihydrodehydrodiconiferyl alcohol-4-*O*-α-L-rhamnopyranoside (6), (−)-isolariciresinol (7) and (−)-isolariciresinol-9-*O*-β-D-xylopyranoside (8), among which compounds 1 and 2 are new compounds. In this paper we describe the isolation and structural elucidation of compounds1–8.

2. Results and discussion

Cupressoside A (1) was isolated as white amorphous powder; high-resolution FAB^{-}-MS of 1 indicated a molecular formula of $C_{25}H_{32}O_{10}$ with 10 degrees of unsaturation. IR absorption

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bands at 3431 and 1614 cm^{-1} were characteristics of OH and aromatic groups, respectively. The ^{13}C NMR spectrum (DEPT) showed six quaternary C-atoms, 13 CH, four CH_2 and two Me. The ^1H NMR spectrum showed six aromatic-proton signals at δ 6.99 (d, $J = 2.0$ Hz), 6.83 (d, $J = 8.1$ Hz), 6.87 (dd, $J = 2.0, 8.1$ Hz), 6.74 (d, $J = 2.1$ Hz), 6.86 (d, $J = 8.3$ Hz) and 6.69 (dd, $J = 2.1, 8.3$ Hz), which were assigned to two 1,2,4-substituted benzene rings.

In the $^1H-^1H$ COSY spectrum of 1 the proton signal at δ 3.66 (t, $J = 6.5$ Hz) was coupled with two protons at δ 1.85 (m), which was further coupled with benzyl methylene protons at δ 2.60 (t, $J = 7.1$ Hz), suggesting the presence of an *n*-propanol moiety. The anomeric proton at δ 4.63 (brs) and the ^{13}C NMR spectrum (DEPT) signals at δ 101.7, 72.4, 72.6, 74.1, 69.8 and 18.0 suggested the presence of an α-L-rhamnopyranosyl group. Therefore, according to the above data, compound 1 was established to be a lignan glycoside. HMBC data allowed to correlate the proton signal at δ 3.86 (OMe) with the C-signal at δ 149.2 (C(3)), suggesting OMe at C(3).This was father confirmed by the NOESY spectrum. The proton signal at δ 2.6 (H-C(7′)) was correlated with the C-signals at δ 117.7 (C(2′)), 136.1 (C(1′)) and 122.4 (C(6′)), in accord with *n*-propanol substitution at C(1′). The anomeric proton signal at δ 4.63 was correlated with the C-signal at δ 67.7 (C(9′)), suggesting that the rhamnopyranosyl group was located at C(9′). In the ^1H NMR spectrum the coupling constants and chemical shifts of H–C(7) and H–C(8) at δ 4.83 (d, $J = 10.4$ Hz) and 4.00 (ddd, $J = 2.5, 4.8, 10.4$ Hz) showed that H–C(7) and H–C(8) were in a *trans*-arrangement. Acid hydrolysis of 1 gave the aglycone 1a, its ^1H NMR and ^{13}C NMR spectral data were identical to those of (7*S*,8*S*)-3-methoxy-3′,7-epoxy-8,4′-oxyneolignan-4,9,9′-triol [2].The optical rotation of 1a ($[\alpha]_D^{24.7}$ + 0.39) suggested that it has the same stereochemistry as the aglycone ($[\alpha]_D^{25}$ + 0.34) obtained by Fang *et al.* [2]. This provided evidence that 1 has the same 7*S*,8*S* configuration. Finally the structure of compound 1 was determined to be (7*S*,8*S*)-3-methoxy-3′,7-epoxy-8,4′-oxyneolignan-4,9,9′-triol-9′-*O*-α-L-rhamnopyranoside (figure 1).

Cupressoside B (2) was obtained as white amorphous powder. The FAB^+-MS exhibited the $[M + 1]^+$ at 463.The molecular formula was established as $C_{24}H_{30}O_9$ by positive mode TOF-MS. IR absorption bands at 3416, 1614 and 1511 cm^{-1} were characteristics of OH and aromatic groups, respectively.

The ^1H NMR spectrum of compound 2 showed three aromatic proton signals at δ 6.71 (d, $J = 8.1$ Hz), 6.99 (dd, $J = 1.6, 8.1$ Hz) and 7.05 (d, $J = 1.6$ Hz), which were assigned to three protons in a 1,2,4-substituted benzene ring. Another four aromatic proton signals at δ

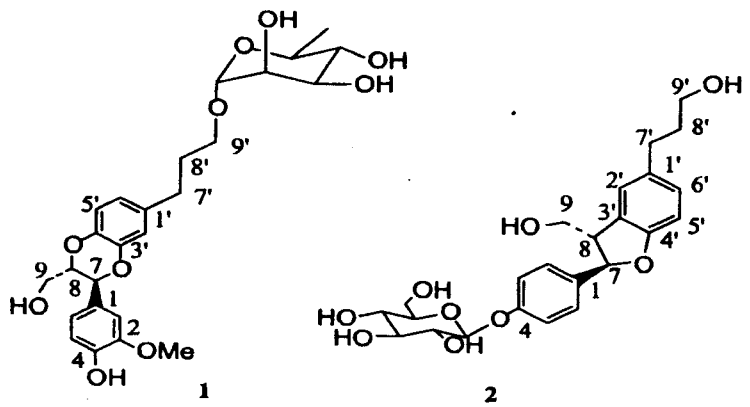

Figure 1. Structures of isolated compounds 1 and 2.

7.28 (2H, d, $J = 8.6$ Hz) and 7.06 (2H, d, $J = 8.6$ Hz) formed AA′BB′ systems arising from the protons of a 1,4-disubstituted benzene ring.

In the ^{13}C NMR (DEPT) spectrum, the presence of a dihydrobenzofuran skeleton and a glucose were suggested. One anomeric proton signal at δ 4.88 (d, $J = 7.6$ Hz) and the ^{13}C NMR (DEPT) signals at δ 102.3, 74.9, 78.0, 71.4, 78.1 and 62.5 suggested the presence of a β-D-glucopyranosyl group. This was also confirmed by a fragment *m/z* 300 ($[M + H\text{-}163]^+$) in the FAB^+-MS. Three methylene proton signals at δ 1.79 (m), 2.61 (t, $J = 7.7$ Hz) and 3.55 (t, $J = 6.6$ Hz) were coupled reciprocally, indicating the presence of a *n*-propanol side-chain. 2D-NMR data including HMBC and NOESY of compound 2 established the connectivity of partial structures and substituents. HMBC spectrum allowed to correlate the signal at δ 2.61 (H–C(7′)) with the C-signals at δ 125.9 (C(2′)), 135.7 (C(1′)) and 129.7 (C(6′)), suggesting *n*-propanol side-chain substitution at C(1′).The anomeric proton signal at δ 4.88 was correlated with the C-signal at δ 158.7 (C(4)), which suggested that the β-D-glucopyranosyl group was located at C(4). Comparison of the ^1H NMR and ^{13}C NMR data of 2 with (7*R*,8*S*)-dihydrodehydrodiconiferyl alcohol 4-*O*-β-D-glucopyranoside [3] showed that the absence of the two methoxy signals at δ 3.83 (MeO–C(3)) and 3.86 (MeO–C(3′)), and the presence of further two aromatic proton signals at δ 6.70 and 7.06 in the ^1H NMR of 2 were the main difference. Since the coupling constant between H–C(7) and H–C(8) was 5.6 Hz, the relative configuration at C-7 and C-8 was decided to be *trans*. The absolute configuration was assigned on the basis of circular dichroism (CD) spectrum. A negative Cotton effect at 238 nm and a positive one at 221 nm allowed the assignment of 7*R*,8*S* configuration for compound 2. Therefore, the structure of 2 was established as (7*R*,8*S*)-3,3′-didemethoxy-dihydrodehydrodiconiferyl alcohol-4-*O*-β-D-glucopyranoside (figure 1).

Comparison of the chemical properties with reported data allowed us to identify compounds 3–8 as matairesinoside (3) [4], dihydrodehydrodiconiferyl alcohol(4) [2], dihydrodehydrodiconiferyl alcohol-9-*O*-α-L-rhamnopyranoside (5) [7], dihydrodehydrodiconiferyl alcohol-4-*O*-α-L-rhamnopyranoside (6) [7], (−)-isolariciresinol (7) and (−)-isolariciresinol-9-*O*-β-D-xylopyranoside (8) [6], respectively.

3. Experimental

3.1 General experimental procedures

Optical rotations were recorded on a Horiba SEPA-300 polarimeter (Horiba, Tokyo, Japan). UV spectra were recorded on a UV 210A spectrometer. IR spectra were recorded on a Bio-Rad FTS135 spectrophotometer, KBr pellets. 1D- and 2D-NMR spectra were measured with a Bruker AM-400 and Buker AM-500 spectrometer, respectively, using TMS as internal standard. MS data were measured with a VG-Auto-Spec-3000 mass spectrometer. CD spectra were determined with a J-20C automatic spectropolarimeter. Silica gel (200–300 mesh) for column chromatography (CC) and GF_{254} for TLC were obtained from the Qingdao Marine Chemical Factory, Qingdao, Peoople's Republic of China.

3.2 Plant material

The branches and leaves of *Cupressus duclouxiana* were collected in Kunming Institute of Botany, Kunming, Yunnan Province of P.R. China in May 2002. It was identified by Professor

Ning-hua Tan, and a voucher specimen is deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

3.3 *Extraction and isolation*

Dried branches and leaves of *Cupressus duclouxiana* (21.0 kg) were extracted three times with 95% EtOH under reflux (3 × 50 l) for 4, 2 and 1 h, respectively. After evaporation of the combined extracts, the residue was suspended in H_2O and then extracted with petroleum ether (60–90°C), EtOAc and *n*-BuOH. The *n*-BuOH extract (600.0 g) was decoloured on Dian HP 20 with a gradient H_2O/EtOH 1:0 → 0:1. The 70% EtOH eluate (170.0 g) was subjected to column chromatography (silica gel, $CHCl_3$/MeOH 95:5 → 25:75; RP-18, MeOH/H_2O 3:7 → 7:3), and resubmitted to column chromatography (silica gel, $CHCl_3$/MeOH 95:5 → 20:80) to afford compounds 1 (10 mg), 2 (9 mg), 3 (14 mg), 4 (19 mg), 5 (65 mg), 6 (8 mg), 7 (21 mg) and 8 (13 mg).

3.3.1 Acid hydrolysis. Compound 1 (5 mg) was dissolved in MeOH (1.0 ml) and 2 N HCl (1.0 ml), and hydrolysed by refluxing in a boiling water bath for 2 h. The hydrolysate was allowed to cool, diluted twofold with H_2O and neutralized by 0.5 N NaOH. The aglycone was extracted with $CHCl_3$ and purified on a silica gel column with solvent ($CHCl_3$/MeOH, 95:5) to give the aglycone 1a (1 mg). The aqua layer was evaporated to give a residue. A rhamnose was identified in the residue by paper chromatography (BuOH/AcOH/H_2O 5:1:5, upper layer) comparison with an authentic sample.

Table 1. ^1H NMR and ^{13}C NMR data (CD_3OD) of 1.

	δ (H)	*δ (C)*	*^1H–^1H COSY*	*HMBC (H → C)*
C(1)		129.7		
H–C(2)	6.99 (d, J = 1.9)	112.2	H-6	C-7, C-4, C-1
C(3)		149.2		
C(4)		148.3		
H–C(5)	6.83 (d, J = 8.1)	116.3	H-6	C-1, C-3
H–C(6)	6.87 (dd, J = 1.9, 8.1)	121.7	H-2, H-5	C-7, C-2, C-4
H–C(7)	4.83 (d, J = 10)	77.7	H-8	C-2, C-6, C-1
H–C(8)	4.00 (ddd, J = 2.5, 4.8, 10.4)	79.8	H-7, H-9	
CH_2(9)	3.37 (dd, J = 13.9)	62.2	H-8	
C(1′)		136.1		
H–C(2′)	6.74 (d, J = 2.1)	117.7	H-6′	C-7′, C-6′, C-3′, C-1′
C(3′)		145.1		
C(4′)		143.0		
H–C(5′)	6.86 (d, J = 8.3)	117.7	H-6′	C-1′, C-3′, C-4′
H–C(6′)	6.69 (dd, J = 2.1, 8.3)	122.4	H-5′, H-2′	C-7′, C-2′, C-4′
CH_2(7′)	2.60 (m)	32.4	H-8′	C-8′, C-9′, C-1′, C-2′
CH_2(8′)	1.85 (m)	32.6	H-7′, H-9′	C-7′, C-9′, C-1′
CH_2(9′)	3.36 (m), 3.66 (m)	67.7	H-8′	
H–C(1″)	4.63 (brs)	101.7	H-2″	C-9′, C-5″, C-3″
H–C(2″)	3.79 (m)	72.4	H-1″, H-3″	
H–C(3″)	3.66 (m)	72.6	H-2″, H-4″	
H–C(4″)	3.36 (m)	74.1	H-3″, H-5″	
H–C(5″)	3.55 (m)	69.8	H-4″, H-6″	
Me(6″)	1.23 (d, J = 6.2)	18.0	H-5″	
MeO–C(3)	3.86 (s)	56.6		C-3

Table 2. ^{1}H NMR and ^{13}C NMR data (CD_3OD) of 2.

	δ (H)	*δ (C)*	*^{1}H–^{1}H COSY*	*HMBC (H → C)*
C(1)		128.6		
H–C (2, 6)	7.28 (d, J = 8.6)	127.9	H-3, H-5	C-3, C-4, C-5, C-7
H–C(3, 5)	7.06 (d, J = 8.6)	117.8	H-2, H-6	C-4
C(4)		158.7		
H–C(7)	5.49 (d, J = 5.6)	87.9	H-8	C-8, C-9, C-2, C-6
H–C(8)	3.39 (m)	55.2	H-7, H-9	C-1, C-3′, C-4′
CH_2(9)	3.83 (m)	65.3	H-8	C-7, C-8
C(1′)		135.7		
H–C(2′)	7.05 (d, J = 1.6)	125.9	H-6	C-3′, C-4′, C-6′
C-(3′)		137.7		
C-(4′)		159.4		
H–C(5′)	6.71 (d, J = 8.1)	109.8	H-6′	C-1′, C-4′
H–C(6′)	6.99 (dd, J = 1.6, 8.1)	129.7	H-2′, H-5′	C-2′, C-4′, C-7′
CH_2(7′)	2..61 (t, J = 7.1)	32.5	H-8′	C-1′, C-2′, C-6′, C-8′, C-9′
CH_2(8′)	1.79 (m)	35.9	H-7′, H-9′	C-1′, C-7′, C-9′
CH_2(9′)	3.55 (t, J = 6.5)	62.3	H-8′	C-7′, C-8′
H–C(1″)	4.88 (d, J = 7.6)	102.3	H-2″	C-4, C-3″
H–C(2″)	3.44 (t, J = 3.7)	74.9	H-1″	
H–C(3″)	3.39 (m)	78.0	H-2″, H-4″	
H–C(4″)	3.36 (m)	71.4	H-3″, H-5″	
H–C(5″)	3.42 (m)	78.1	H-4″, H-6″	
CH_2(6″)	3.85 (m)	62.5	H-5″	

3.3.2 Cupressoside A (1). White amorphous powder. $[\alpha]_D^{23.1}$ −22.8 (c 0.373, MeOH). IR: 3431, 2926, 1614, 1507, 1276, 1128, 1093, 1046. UV: 205, 226, 282. ^{1}H NMR and ^{13}C NMR: see table 1. FAB$^-$-MS: 491 ([M − H]$^-$). HR-FAB$^-$-MS: 491.1921 [$C_{25}H_{32}O_{10}$-H]$^-$ (calcd for $C_{25}H_{31}O_{10}$, 491.1927).

1a: $[\alpha]_D^{24.7}$ + 0.39 (c 0.502, MeOH). ^{1}H NMR (CD_3OD, 400 Hz): δ 1.79 (2H, m), 2.58 (2H, t, J = 7.3 Hz), 3.54 (2H, t, J = 6.5 Hz), 3.46 (1H, dd, J = 4.6, 12.3 Hz), 3.66 (1H, d, J = 12.3 Hz), 3.86 (3H, s), 4.00 (1H, J = 2.0, 3.6, 10.1 Hz), 4.86 (1H, d, J = 9.3 Hz), 6.69 (1H, d, J = 8.2, 1.9 Hz), 6.74 (1H, d, J = 1.9 Hz), 6.83 (1H, d, J = 8.2 Hz), 6.85 (1H, dd, J = 8.2 Hz), 6.89 (1H, dd, J = 8.2, 1.8 Hz), 6.99 (1H, d, J = 1.8 Hz).

3.3.3 Cupressoside B (2). White amorphous powder. $[\alpha]_D^{23.1}$ −7.35 (c 0.408, MeOH). IR: 3416, 2929, 2365, 1614, 1511, 1489, 1235, 1074. UV: 202, 223, 228. ^{1}H NMR and ^{13}C NMR: see table 2. CD (c 0.11 mg/ml, MeOH) Δε (nm): − 1.5 (238), + 1.2 (221). FAB$^+$-MS: 463 ([M + 1]$^+$). TOF$^+$-MS: 485.1789 [$C_{24}H_{30}O_9$ + Na]$^+$ (calcd for $C_{24}H_{30}O_9$ + Na, 485.1787).

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