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A pair of new atropisomeric cupressuflavone glucosides isolated from *Juniperus communis* var. *depressa*

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Abstract—A pair of new atropisomers, (*M*)- and (*P*)-cupressuflavone 4′-*O*-β-D-glucoside, were isolated from *Juniperus communis* var. *depressa*, and their absolute structures and axial configurations were determined using 2D NMR and circular dichroism. These are the first reported stable (*M*)- and (*P*)-isomers of cupressuflavone glucosides isolated from natural sources.

Biflavones such as cupressuflavone and amentoflavone are widely distributed in the plant genus *Juniperus* (Cupressaceae).[1] Although monomeric flavones lack a chiral center and thus are optically inactive, some biflavones are chirally active. The first optically active natural biflavone, 4′,4‴,7,7″-tetra-*O*-methylcupressuflavone, was isolated in 1968,[2] but it was not until 1992 that its absolute axial configuration was assigned as the (*M*)-configuration.[3] Biflavone glycosides are rare in nature, and to date only amentoflavone glucosides have been reported.[4]

Previous phytochemical studies on North American plants by our laboratory have led to the identification of a number of glycosides of flavonoids,[5] neolignans,[5,6] and phenylpropanoids[6] isolated from the leaves and stems of *Juniperus communis* var. *depressa* collected in Oregon; here we report the isolation and structural characterization of a pair of new atropisomeric biflavone glucosides (**1** and **2**).

Dried leaves and stems (2.4 kg) were used for extraction with MeOH at room temperature and filtered. The filtrate was evaporated in vacuo, yielding a dark greenish extract (488 g). An aliquot (202 g) was extracted with an *n*-hexane/MeOH mixture; the MeOH-soluble fraction was further extracted with an *n*-BuOH/water mixture. The resulting *n*-BuOH extract (76 g) was fractionated on a silica gel column eluted with $CHCl_3$/MeOH/H_2O (7:3:1, lower phase). Ten fractions (numbered A to J) were collected; fraction H was subjected to ODS column chromatography (eluted with 50% MeOH) and Sephadex LH-20 column chromatography (eluted with MeOH), followed by HPLC separation (JAIGEL-GS column; eluted with 50% MeOH). Two compounds were isolated: **1** (30.7 mg) and **2** (4.1 mg). Fraction E was purified by Sephadex LH-20 (eluted with 70% acetone) and ODS column chromatography (eluted with 70% acetone) to afford cupressuflavone (810 mg), which was identified by direct comparison with an authentic sample.[7]

Compound **1**, a pale yellow powder, $[\alpha]_D$ −132.6 (*c* 0.50, MeOH) and compound **2**, also a pale yellow powder, $[\alpha]_D$ −84.5 (*c* 0.41, MeOH), were analyzed by negative ion HR FAB-MS. Both compounds provided a $[M-H]^-$ ion at *m*/*z* 699.1352, consistent with the molecular formula $C_{36}H_{28}O_{15}$. FAB-MS of both **1** and **2** also afforded a significant fragment at *m*/*z* 537 [M−H−162 (hexose

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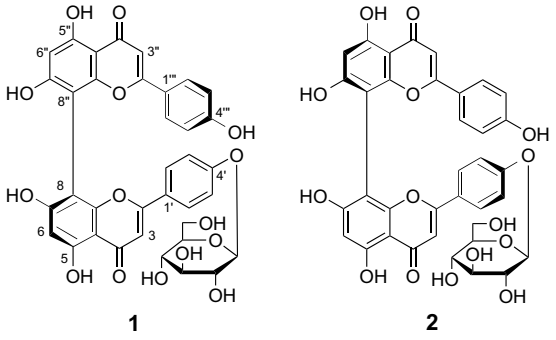

Figure 1. Structures of (*M*)- and (*P*)-cupressuflavone 4′-*O*-β-D-glucopyranosides (**1**) and (**2**).

unit)]$^-$. UV spectral data [$\lambda_{max}(\varepsilon)$ 230 nm (25,100), 275 nm (25,820), and 325 nm (24,890) in **1**; 236 nm (21,280), 273 nm (21,970), and 321 nm (22,040) in **2**] suggest these compounds are flavones. Taken together, the MS and UV data suggest that both **1** and **2** are biflavone monoglycosides containing a hexosyl residue. In order to further characterize **1** and **2**, ^{1}H and ^{13}C NMR analyses (COSY, NOESY, HMQC, and HMBC) were performed, and all protons and carbons were assigned (Table 1). The assignments (chemical shifts, multiplicities, and coupling constants) ascribed to each glucosyl part of **1** and **2** substantiated the presence of a β-D-glucopyranosyl moiety as the common monosaccharide in both compounds. In contrast, the aglycone moieties of **1** and **2** gave the following proton signals: two sets of *ortho*-coupled protons assignable to two *p*-substituted phenyl moieties [δ 6.74 (1H, d, $J = 8.8$ Hz, H-3‴, 5‴), 7.02 (1H, d, $J = 8.8$ Hz, H-3′, 5′), 7.48 (1H, d, $J = 8.8$ Hz, H-2‴, 6‴), 7.60 (1H, d, $J = 8.8$ Hz, H-2′, 6′) in **1**; δ 6.74 (1H, d, $J = 8.8$ Hz, H-3‴, 5‴), 7.02 (1H, d, $J = 8.8$ Hz, H-3′, 5′), 7.49 (1H, d, $J = 8.8$ Hz, H-2‴, 6‴), 7.60 (1H, d, $J = 8.8$ Hz, H-2′, 6′) in **2**], four aromatic singlets (δ 6.445, 6.454, 6.76, 6.87 in **1**; δ 6.440, 6.456, 6.78, 6.90 in **2**), and two chelated phenolic hydroxyls [δ 13.10 (1H, s, OH-5), 13.14 (1H, s, OH-5″) in **1**; δ 13.12 (1H, s, OH-5), 13.16 (1H, s, OH-5″) in **2**].

Table 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectral data of **1** and **2** in DMSO-d_6

No.	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
2		163.0[a]		162.9[a]
3	6.87 (s)	103.7	6.90 (s)	103.6
4		182.1		182.0
5	13.10 (s, OH)	160.9	13.12 (s, OH)	160.8
6	6.454 (s)[a]	98.8	6.456 (s)[a]	98.8
7		163.0[a]		162.9[a]
8		98.8[b]		98.8[b]
9		154.9[c]		154.9[c]
10		103.8		103.6
1′		124.1		124.0
2′ (6′)	7.60 (d, 8.8)	127.7	7.60 (d, 8.8)	127.6
3′ (5′)	7.02 (d, 8.8)	116.6	7.02 (d, 8.8)	116.6
4′		160.2		160.2
2″		163.5[a]		163.5[a]
3″	6.76 (s)	102.6	6.78 (s)	102.6
4″		182.1		182.0
5″	13.14 (s, OH)	160.9	13.16 (s, OH)	160.8
6″	6.445 (s)[a]	98.9	6.440 (s)[a]	98.9
7″		163.5[a]		163.5[a]
8″		98.6[b]		98.6[b]
9″		154.8[c]		154.8[c]
10″		103.7		103.6
1‴		121.3		121.1
2‴ (6‴)	7.48 (d, 8.8)	128.0	7.49 (d, 8.8)	127.9
3‴ (5‴)	6.74 (d, 8.8)	115.8	6.74 (d, 8.8)	115.8
4‴		161.1		161.0
Glc 1	4.99 (d, 7.3)	99.8	4.98 (d, 7.3)	99.8
2	3.21 (ddd, 7.3, 9.0, 4.4) 5.29 (d, 4.4, OH)	73.2	3.22 (ddd, 7.3, 9.0, 4.4) 5.32 (d, 4.4, OH)	73.2
3	3.27 (ddd, 9.0, 9.0, 4.4) 5.05 (d, 4.4, OH)	76.4	3.27 (ddd, 9.0, 9.0, 4.4) 5.09 (d, 4.4, OH)	76.4
4	3.15 (ddd, 9.0, 9.0, 5.0) 5.00* (OH)	69.6	3.15 (ddd, 9.0, 9.0, 5.1) 5.03 (d, 5.1, OH)	69.5
5	3.37*	77.0	3.37*	77.0
6	3.44* 3.66 (br d, 10.8) 4.53 (br s, OH)	60.6	3.44* 3.66 (br dd, 10.3, 5.1) 4.55 (br t, 5.1, OH)	60.6

Assignments with the same superscript letters (a, b, c) may be interchanged in each column.
* Denotes overlapping with other signals.

In the HMBC spectra of **1** and **2**, the chelated proton of OH-5 gave cross-peaks with the C-5, C-6, and C-10 carbons, and the OH-5″ proton gave cross-peaks with the C-5″, C-6″, and C-10″ carbons. Additional HMBC cross-peaks were observed in both **1** and **2** between H-3 and C-4 and C-1′, and between H-3″ and C-4″ and C-1‴. The HMBC data are consistent with 8,8″-coupled dimers of apigenin (5,7,4′-trihydroxyflavone), that is, cupressuflavone as the common aglycone in **1** and **2**. Furthermore, **1** and **2** exhibit NOESY cross-peaks between the anomeric H and H-3′ and H-5′ and HMBC correlations between the anomeric H and C-4′; these assignments are consistent with a β-D-glucopyranosyl moiety linked at the OH-4′ of cupressuflavone. Based on the above spectral evidence, **1** and **2** were both assigned to cupressuflavone 4′-*O*-β-D-glucopyranoside, and are believed to be isomers arising from atropisomerism around the 8–8″ linkage (Fig. 1).

To date, the absolute axial configuration of only one biflavonoid has been determined, 4′,4‴,7,7″-tetra-*O*-methylcupressuflavone.[3,8] The CD spectrum of natural 4′,4‴,7,7″-tetra-*O*-methylcupressuflavone exhibits intense positive and negative Cotton signals at 362.0 and 326.2 nm, respectively. However, the exciton chirality method could not be applied to this biflavone with a complex π-electron chromophore. Therefore, natural 4′,4‴,7,7″-tetra-*O*-methylcupressuflavone exhibiting a positive CD Cotton signal at 362.0 nm was substantiated to be in the (*M*)-axial configuration (a*R*) by quantum chemical calculation of the CD spectrum,[3] and by enantioselective total synthesis.[8]

The CD spectra of **1** and **2** (Fig. 2) support the conclusion that these compounds are atropisomers. Compound **1** exhibits Cotton signals at 364.9 nm (Δε +5.3), 326.2 nm (−23.9), and 269.1 nm (+9.1); these sign and wavelength maxima are consistent with 4′,4‴,7,7″-tetra-*O*-methylcupressuflavone, described above, suggesting that **1** corresponds to the (*M*)-axial configuration of cupressuflavone 4′-*O*-β-D-glucopyranoside. The reversed CD signals of **2** [364.3 nm (Δε −6.6), 325.7 nm (+24.9), and 271.7 nm (−12.5)] support the conclusion that **2** exhibits (*P*) chirality at the 8–8″ axis.

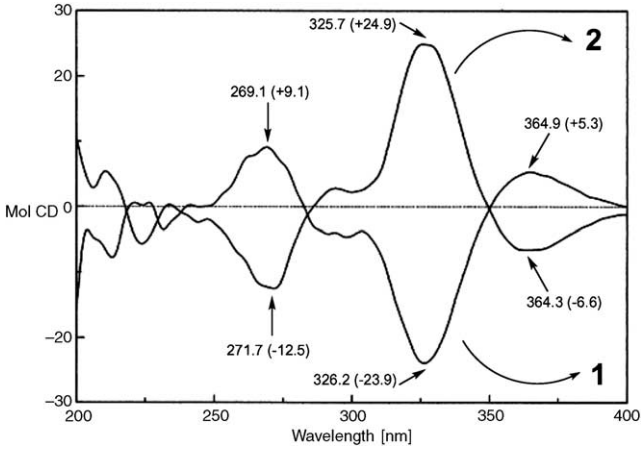

Figure 2. CD spectra of **1** and **2** in MeOH.

Compounds **1** and **2**, corresponding to the (*M*)- and (*P*)-cupressuflavone 4′-*O*-β-D-glucopyranoside, are the first pair of biflavone atropisomers to be isolated from natural sources. In addition, although biflavones such as cupressuflavone have been documented in gymnospermous plants, this is the first report of cupressuflavone glycosides being found in nature. Cupressuflavone, the common aglycone of (**1**) and (**2**), is optically inactive, and previously only one optically active natural biflavone, 4′,4‴,7,7″-tetra-*O*-methylcupressuflavone with the (*M*)-configuration, had been reported. This letter provides the first example of natural biflavone with the (*P*)-configuration. In axially chiral biphenyls, occurrence of atropisomerism may be attributed to the presence of bulky *ortho* substituents but in the present case, the (*M*)- and (*P*)-isomers of cupressuflavone 4′-*O*-β-D-glucopyranoside, as well as their optically inactive aglycone, have the hydroxy groups at 7- and 7″-positions *ortho* to the 8–8″ axis. Accordingly, the bulky glucosyl moiety attached at 4′-OH restricts free rotation around the 8–8″ linkage, resulting in the formation of the stable (*M*)- and (*P*)-isomers. Furthermore, these isomers were also stable in DMSO-d_6 solution warmed to 50 °C during the ^{1}H NMR measurements and were not converted from each other. In conclusion, a pair of chiral atropisomers (**1** and **2**) has been isolated from natural sources, where glucosidation of the achiral biflavone cupressuflavone produced a chiral center. Although this is the first such pair reported, the discovery of further natural biflavone glycoside atropisomers can be anticipated.

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