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Novel biflavonoids from *Cephalotaxus oliveri* Mast.

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ABSTRACT

Three new biflavonoids, umcephabiflovin A (**1**), umcephabiflovin B (**2**), and *S*-taiwanhomoflavone-B (**3**), together with seven known flavonoids (**4–10**) and seven known alkaloids (**11–17**), were isolated from the twigs of *Cephalotaxus oliveri* Mast. The flavonoids were found to inhibit α-glucosidase activity.

1. Introduction

Plants in the *Cephalotaxus* genus have attracted significant interest because of their bioactive constituents. For example, alkaloids, such as homoharringtonine, show antitumor effects, and homoharringtonine has been approved by the FDA for the treatment of chronic myeloid leukemia. Previous studies described the isolation of alkaloids, terpenes, lignans and flavonoids from *Cephalotaxus* plants (Abdelkafi and Nay, 2012; Chang et al., 2017; Lee et al., 1998). However, phytochemistry investigations on *C. oliveri* Mast. are rarely reported. In this study, three new biflavonoids, including umcephabiflovin A (**1**) and B (**2**) as well as *S*-taiwanhomoflavone-B (**3**) (Fig. 1), together with seven known flavonoids including amentoflavone (**4**), ginkgetin (**5**), putraflavone (**6**), apigenin (**7**), naringenin (**8**), poriol (**9**) and apigenin-7-*O*-*β*-glucoside (**10**), and seven alkaloids including 3-epischelhammericine (**11**), deoxyharringtonine (**12**), isoharringtonine (**13**), harringtonine (**14**), homoharringtonine (**15**), cephalotaxine (**16**) and drupacine (**17**), were isolated from the chloroform fraction of the EtOH extract of *C. oliveri* via extraction followed by silica gel column chromatography, Sephadex LH-20 column chromatography and prep-HPLC purification. Chemical structures were determined by HRMS and 1D and 2D NMR experiments (^{1}H, ^{13}C, DEPT, COSY, HSQC and HMBC). Absolute configurations were elucidated through CD spectra.

2. Results and discussion

Compound **1** was obtained as a pale yellow amorphous solid. The HR-ESI–MS spectrum exhibited quasi-molecular ions at *m/z* 585.1744 $[M+H]^+$ and 607.1558 $[M+Na]^+$ and a dimer ion at *m/z* 1191.3240 $[2\ M+Na]^+$, which indicated the molecular formula $C_{33}H_{28}O_{10}$. Maximum absorptions in the UV spectrum were observed at 288 nm and 337 nm, which are characteristic of flavonoids (Mabry et al., 1970).

The ^{1}H NMR spectrum (Table 1) of compound **1** showed a couple of ABX coupling systems at δ_H 3.30 (1H, *dd*, *J* = 17.2, 12.9 Hz), 2.77 (1H, *dd*, *J* = 17.2, 3.0 Hz), 5.53 (1H, *dd*, *J* = 12.9, 3.0 Hz) and 3.39 (1H, *dd*, *J* = 17.2, 13.4 Hz), 2.74 (1H, *dd*, *J* = 17.2, 2.8 Hz), 5.55 (1H, *dd*, *J* = 13.4, 2.8 Hz). A pair of A_2B_2 coupling systems were observed at δ_H 7.43 (2H, *d*, *J* = 8.8 Hz), 6.85 (2H, *d*, *J* = 8.8 Hz) and δ_H 7.35 (2H, *d*, *J* = 8.6 Hz), 6.80 (2H, *d*, *J* = 8.6 Hz) suggesting the existence of two *para*-substituted benzene rings. The resonances at δ_H 1.89 (3H, *s*), 3.77 (3H, *s*) and 3.81 (3H, *s*) indicated the presence of one methyl and two methoxy groups. Two isolated protons at δ_H 6.21(1H, *s*) and 6.41(1H, *s*) were also observed. Three hydroxy signals appeared at δ_H 9.62, 12.00 and 12.20. In the ^{13}C NMR spectrum, 33 total carbon atoms were observed corresponding to two carbonyl carbons (δ_C197.7 and 196.9), 14 aromatic quaternary carbons (δ_C 165.2, 161.1, 160.4, 160.1, 159.4, 158.0, 157.9, 154.2, 131.8, 128.6, 123.0, 104.2, 102.6 and 102.2), 12 tertiary carbons (δ_C 128.5, 128.5, 128.3, 128.3, 115.2, 115.2, 114.3, 114.3, 92.4, 91.2, 79.1 and 78.3), two secondary carbons (δ_C 42.0 and 42.0), two methoxy carbons (δ_C 56.6 and 56.2), and one methyl carbon (δ_C 6.9). These data are consistent with compound **1** being a biflavonoid. Analysis of the ^{1}H and ^{13}C data showed seven oxygenic carbons (C-5, 8, 4′, 5″, 6″, 7″ and 4‴) in addition to three pairs of carbons assigned to C-2, 4, 8a and C-2″, 4″, 8″a (Table 1) which are characteristic of the flavanone skeleton. However, only two methoxy and three hydroxy groups were observed in the ^{1}H NMR spectrum.

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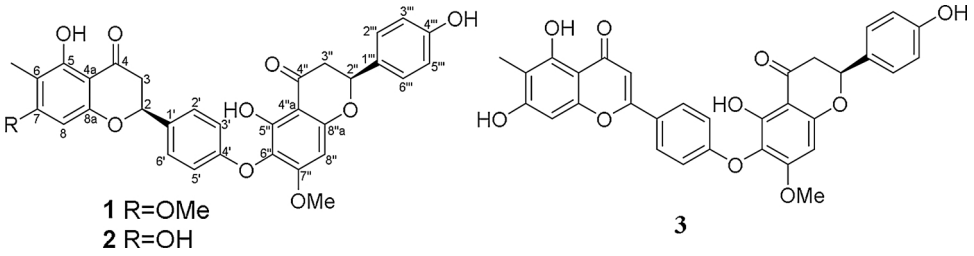

Fig. 1. Structures of the new biflavonoids from *C. oliveri*.

Table 1
^{1}H NMR and ^{13}C NMR data of **1**, **2** and **3** (600 MHz, DMSO-*d6* for 1 and 3, Methanol-*d4* for 2, δ in ppm, *J* in Hz).

No.	1		2		3	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
2	78.3	5.53(dd, 12.9, 3.0)	80.1	5.37(dd, 13.0, 3.0)	164.4	–
3	42.0	3.30(dd, 17.2, 12.9) 2.77(dd, 17.2, 3.0)	44.2	3.10(dd, 17.2, 13.0) 2.73(dd, 17.2, 3.0)	102.9	–
4	196.9	–	197.3	–	182.3	–
4a	102.2	–	102.9	–	101.3	–
5	159.4	–	162.6	–	160.8	–
6	104.2	–	105.6	–	103.3	–
7	165.2	–	167.3	–	164.6	–
8	91.2	6.21(s)	95.6	5.96(s)	94.2	5.98(s)
8a	161.1	–	162.3	–	160.2	–
1′	131.8	–	133.8	–	121.0	–
2′	128.3	7.43(d, 8.8)	128.7	7.39(d, 8.8)	128.4	7.42(d, 8.8)
3′	114.3	6.85(d, 8.8)	115.8	6.87(d, 8.8)	114.4	6.89(d, 8.8)
4′	158.0	–	160.0	–	157.8	–
5′	114.3	6.85(d, 8.8)	115.8	6.87(d, 8.8)	114.4	6.89(d, 8.8)
6′	128.3	7.43(d, 8.8)	128.7	7.39(d, 8.8)	128.4	7.42(d, 8.8)
2″	79.1	5.55(dd, 13.4, 2.8)	81.0	5.47(dd, 13.1, 3.0)	78.1	5.47(dd, 12.8, 3.0)
3″	42.0	3.39(dd, 17.2, 13.4) 2.74(dd, 17.2, 2.8)	44.0	3.23(dd, 17.2, 13.1) 2.79(dd, 17.2, 3.0)	42.1	2.73(dd, 3.0, 17.2) 3.26(dd, 12.8, 17.2)
4″	197.7	–	198.9	–	196.3	–
4″a	102.6	–	104.1	–	105.3	–
5″	154.2	–	162.0	–	152.5	–
6″	123.0	–	125.4	–	125.2	–
7″	160.4	–	162.3	–	158.4	–
8″	92.4	6.41(s)	93.1	6.32(s)	92.1	7.11(s)
8″a	161.1	–	156.1	–	154.0	–
1‴	128.6	–	130.8	–	132.2	–
2‴	128.5	7.35(d, 8.6)	129.1	7.37(d, 8.6)	128.7	8.02(d, 8.8)
3‴	115.2	6.80(d, 8.6)	116.4	6.84(d, 8.6)	116.1	6.95(d, 8.8)
4‴	157.9	–	159.2	–	161.5	–
5‴	115.2	6.80(d, 8.6)	116.4	6.84(d, 8.6)	116.1	6.95(d, 8.8)
6‴	128.5	7.35(d, 8.6)	129.1	7.37(d, 8.6)	128.7	8.02(d, 8.8)
5-OH	–	12.20(s)	–	–	–	12.41(s)
6-Me	6.9	1.89(s)	7.0	1.95(s)	7.0	1.87(s)
7-R[a]	56.2	3.81(s)	–	–	–	10.79(s)
5″-OH	–	12.00(s)	–	–	–	13.04(s)
7″-O-Me	56.6	3.77(s)	56.9	3.82(s)	56.8	3.89(s)
4‴-OH	–	9.62(s)	–	–	–	10.45(s)

[a] For compound 1: R = OMe; for compounds 2 and 3, R = OH.

Thus, it was concluded that compound **1** was a biflavonoid connected by a C—O—C bond.

The interflavonoid ether linkage in compound **1** was further confirmed by analysis of its ^{1}H NMR and HMBC spectra (Fig. 2). Two chelated hydroxy groups at δ_H 12.00 and 12.20 were attributed to the two hydroxy groups located at C-5 and C-5″. In the HMBC spectrum, the methyl group was defined at C-6 as it showed a ^{2}J correlation with C-6 (δ_C 104.2) and ^{3}J correlations with C-7 (δ_C 165.2) and C-5 (δ_C 159.4). The two methoxy groups were located at C-7 and C-7″ based on ^{3}J

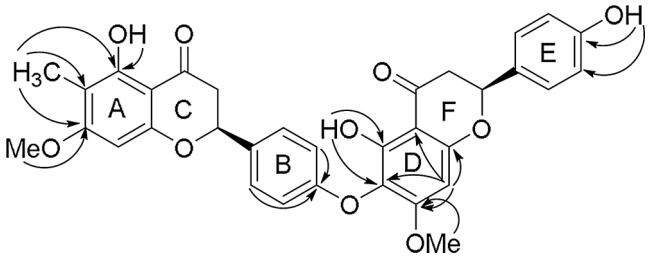

Fig. 2. Key HMBC correlations of **1**.

correlations. For the C-7 methoxy group, ^{3}J correlations were observed between δ_H 3.81 (7-O-Me) and C-7 (δ_C 165.2). For the C-7″ methoxy group, ^{3}J correlations were observed between δ_H 3.77 (7″-O-Me) and C-7″ (δ_C 160.4). The position of the 4‴-OH (δ_H 9.62) was determined due to its correlations with C-3‴ (δ_C 115.2), C-4‴ (δ_C 157.9) and C-5‴ (δ_C 115.2). These spectral observations accounted for eleven out of the thirteen oxygenated carbon atoms in compound **1**. This suggests that the remaining oxygenated carbon atoms (C-4′ and 6″) in compound **1** form an interflavonoid ether linkage between the flavanones. The CD spectrum of compound **1** exhibited a positive maximum at 334 nm and negative minimum at 288 nm, indicating the *S* configurations at both C-2 and C-2″ (Gaffield, 1970; Slade et al., 2005). Thus, the structure of compound **1** was established as [(2*S*)-5-hydroxy-6-methyl-7-methoxyflavanone]-(4′-*O*-6″)-[(2″*S*)5″,4‴-dihydroxy-7″-methoxyflavanone] and **1** was tentatively named umcephabiflovin A.

Compound **2** was obtained as a yellow amorphous solid. The HR-ESI–MS spectrum exhibited major ion peaks at *m/z* 571.1586 $[M+H]^+$, 593.1401 $[M+Na]^+$, and 1163.2931 $[2\,M+Na]^+$ indicating the molecular formula $C_{32}H_{26}O_{10}$. In comparison to compound **1**, the NMR spectral data for compound **2** was strikingly similar with the exception of a hydroxy group at C-7 in compound **2** instead of a methoxy group at C-7 for compound **1**. The position of the functional groups was also confirmed by analysis of the HMBC spectrum in which the C-6-methyl protons [δ_H 1.95 (3H, *s*)] showed a correlation with C-7 (δ_C 167.3) and the C-7″-methoxy protons [δ_H 3.82 (3H, *s*)] was correlated with C-7″(δ_C 162.3). The absolute configurations at C-2 and C-2″ were again determined to be *S* as the CD spectrum of compound **2** exhibited a positive maximum at 334 nm and a negative minimum at 288 nm (Gaffield, 1970; Slade et al., 2005). Thus, compound **2** was determined to be the 7-demethyl derivative of compound **1**, [(2*S*)-5,7-dihydroxy-6-methylflavanone]-(4′-*O*-6″)-[(2″*S*)5″,4‴-dihydroxy-7″-methoxyflavanone] and was named umcephabiflovin B.

Compound **3** was obtained as a pale yellow amorphous solid. The molecular formula $C_{32}H_{24}O_{10}$ was determined from the HR-ESI–MS spectrum, which showed *m/z* 569.1352 $[M+H]^+$ (Calc. 569.1448). The distinctive maximum absorptions at 291 nm and 333 nm in the UV spectrum together with the NMR data and IR absorptions at 1609 (aromatic), 1645 (conjugated CO) and 3400 (OH) cm^{-1} indicate that **3** should be a biflavonoid composed of a flavone and a flavanone. The ^{1}H NMR spectrum showed three aliphatic protons at δ_H 5.47 (1H, *dd*, *J* = 3.0, 12.8 Hz), 3.26 (1H, *dd*, *J* = 12.8, 17.2 Hz) and 2.73 (1H, *dd*, *J* = 3.0, 17.2 Hz) in an ABX coupling system, indicating the presence of a flavanone unit. The presence of the flavone unit was determined from