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Immunosuppressive chalcone-isoflavonoid dimers from *Campylotropis hirtella*

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ABSTRACT

Twelve new chalcone-isoflavonoid dimers were isolated from the roots of *Campylotropis hirtella*. The structures of these compounds were elucidated by NMR analysis, while the absolute configurations were determined from the CD spectra. We measured immunosuppressive activities of these compounds on splenic lymphocytes and found that many of the compounds displayed immunosuppressive activities. Compound **8** was the most active, with IC_{50} values of 3.68 μM for T lymphocyte suppression and 1.79 μM for B lymphocyte suppression.

1. Introduction

Flavonoids are a class of plant metabolites often studied for their pharmacological activities, such as antiviral, anti-inflammatory and antitumor effects. These natural polyphenolic compounds, with a carbon skeleton of C6–C3–C6, can easily undergo phenol oxidative coupling reactions and aggregate into bioflavonoids (C6–C3–C6)$_2$. Due to diversity of flavonoids, its coupling product, biflavonoids can also be classified into various classes.[1] Within biflavonoid classes, the natural occurring arylbenzofuran-isoflavonoid dimers was rarely reported; only few papers described its presence in *Lespedeza cyrtobotrya* Miq. and *Lespedeza floribunda*.[2] Our group has previously reported that many novel isoflavonoids separated from the roots of Radix *Campylotropis hirtellae* show strong immunosuppressive activity.[3] However, biflavonoids from this plant have never been studied. To further explore naturally occurring immunosuppressive agents, 12 new chalcone-isoflavonoid dimers were isolated from *C. hirtellae*. The structures of these compounds were elucidated by NMR analysis, while the absolute configurations were determined from the CD spectra. The carbonyl bridge between 2-arylbenzofuran and isoflavonoid subunit, crucial in initiating phenol oxidative coupling reaction, plays a key role in determining interflavonoid bond location. By measuring immunosuppressive activities on splenic lymphocytes, we found that many compounds were immunosuppressive and that compound **8** was the most active, with IC_{50} values of 3.68 μM for T lymphocyte suppression and 1.79 μM for B lymphocyte suppression.

2. Results and discussion

Hirtellanone A-E (**1**–**5**) were assumed to be dimeric flavonoids composed of an isoflavan and a 2-arylbenzofuran from their molecular formula and NMR data. The HR-ESI-MS of compound **1** (Hirtellanone A) displayed a molecular ion peak at 651.1878, suggesting a molecular formula of $C_{37}H_{32}O_{11}$. The ^{13}C NMR spectra showed a total of 37 signals for **1** (Table 1). The ^1H NMR spectrum and HMQC experiments showed 29 sp^2 carbons, including 11 carbons bearing an oxygen atom and one carbonyl, and eight sp^3 carbons corresponding to two aromatic methoxyl groups, two geminated methyl groups, two methylene, one methine and a quaternary carbon. The ^1H NMR spectrum of Hirtellatone A showed five aliphatic proton signals at δ_H 2.51 (1H, d, 15.6 Hz), δ_H 2.73 (1H, dd, 15.6 Hz, 12.4 Hz), δ_H 3.12 (1H, brs), δ_H 3.69 (1H, d, 10.2 Hz) and δ_H 4.03 (1H, d, 10.2 Hz), which were characteristic isoflavan signals.[4] The ^1H NMR also showed a set of 2, 2-dimethylpyrene ring protons at δ_H 1.35, δ_H 1.38 (each 3H, s), δ_H 6.48 and δ_H 5.58 (each 1H, 10 Hz), In addition, the olefinic signal at δ_H 6.48 coupled with methoxyl signal at δ_H 3.65 in NOE

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Table 1
^{1}H and ^{13}C NMR Spectroscopic data of compound **1**–**4** in Acetone-d_6

Position	Compound **1**		Compound **2**		Compound **3**		Compound **4**	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
2		151.8		150.9		150.8		156.3
3		117.2		117.0		116.9		118.8
4		195.1		194.5		194.2		195.1
5	7.58 (1H, d, 8.5 Hz)	121.3	7.29 (1H, s)	101.7	7.20 (1H, s)	104.7	7.55 (1H, d, 8.5 Hz)	121.5
6	6.90 (1H, dd, 8.5 Hz, 1.9 Hz)	112.7		145.1		142.4	6.93 (1H, dd, 8.5 Hz, 1.8 Hz)	112.9
7		156.0		145.3		144.1		156.8
8	6.99 (1H, d, 1.7 Hz)	97.7	7.05 (1H, s)	97.1	7.08 (1H, s)	97.1	7.04 (1H, d, 1.7 Hz)	97.8
9		155.6		148.8		148.4		155.8
10		120.5		118.8		119.3		120.3
1′		99.5		98.9		103.4		104.1
2′		159.6		157.1		157.9		156.8
3′	6.14 (1H, s)	96.1	6.14 (1H, d, 2.0 Hz)	95.5		113.4		107.8
4′		162.1		160.4		158.0		159.5
5′	5.91(1H, s)	91.4	5.93 (1H, d, 2.0 Hz)	90.7	6.34 (1H,s)	98.7	6.21 (1H, s)	99.2
6′		157.9		158.8		154.5		157.4
2″	3.69 (1H, d, 10.2 Hz) 4.03 (1H, d, 10.3 Hz)	69.4	3.74 (1H, m) 4.04 (1H, m)	68.7	3.62 (1H,m), 4.04 (1H, m)	68.7	3.69 (1H, m) 4.05 (1H, dt, 10.3 Hz, 3.0 Hz)	69.4
3″	3.12 (1H, brs)	32.1	3.15 (1H, brs)	31.4	3.16 (1H, m)	31.2	3.18 (1H, m)	32.1
4″	2.51 (1H, t, 15.6 Hz) 2.73 (1H, dd, 15.6 Hz, 12.4 Hz)	25.1	2.54 (1H, t, 15.6 Hz) 2.75 (1H, t, 15.6 Hz)	24.4	2.53 (1H, m) 2.74 (1H,m)	24.0	2.56 (1H, m) 2.73 (1H, m)	24.9
5″		152.9		152.3		152.2		152.9
6″		107.9		107.2		107.2		107.2
7″		154.9		154.2		154.2		154.9
8″	5.99 (1H, s)	100.1	6.02 (1H, s)	99.4	6.00 (1H, s)	99.4	5.99 (1H, s)	100.2
9″		155.7		155.0		155.1		155.8
10″		108.7		102.0		107.9		108.7
1‴		119.2		118.5		118.3		119.4
2‴		162.1		161.6		161.6		164.1
3‴	6.44 (1H, s)	102.6	6.48 (1H, s)	101.8	6.47 (1H, s)	102.1	6.41 (1H, s)	102.8
4‴		164.1		163.3		163.8		162.8
5‴		113.8		113.1		113.0		113.7
6‴	7.44 (1H, s)	132.2.	7.46 (1H, s)	131.5	7.43 (1H, s)	131.4	7.43 (1H, s)	132.4
α	6.48 (1H, d, 10.0 Hz)	117.2	6.51 (1H, d, 10.0 Hz)	116.5	6.50 (1H, d, 10.0 Hz)	116.4	6.49 (1H, d, 9.9 Hz)	117.8
β	5.58 (1H, d, 10.0 Hz)	127.8	5.59 (1H, d, 10.0 Hz)	127.1	5.58 (1H, d, 10.0 Hz)	127.0	5.58 (1H, d, 9.9 Hz)	127.7
γ		75.8		75.1		75.0		75.7
δ	1.38 (3H, s)	27.5	1.40 (3H, s)	26.7	1.40 (3H, s)	26.7	1.35 (3H, s)	27.2
ε	1.35 (3H, s)	27.1	1.37 (3H, s)	26.4	1.40 (3H, s)	26.7	1.36 (3H, s)	27.2
α′					3.11 (1H, t, 5.8 Hz)	21.8	6.33 (1H, d, 10.0 Hz)	116.7
β′					4.96(1H, t, 6.8 Hz)	123.2	5.53 (1H, d, 10.0 Hz)	127.6
γ′						129.2		76.7
δ′					1.65 (3H, s)	24.5	1.38 (3H, s)	28.8
ε′					1.63 (3H, s)	16.5	1.37 (3H, s)	28.7
2′-OMe	3.52 (3H, s)	61.1	3.51 (3H, s)	60.3	3.41 (3H, s)	60.2	3.52 (3H, s)	61.9
5″-OMe	3.65 (3H, s)	54.9	3.68 (3H, s)	54.1	3.67 (3H, s)	54.10	3.65 (3H, s)	60.9
5-OMe			3.94 (3H, s)	55.5				
OH	12.66 8.80, 8.72		12.70 8.85		12.82 8.6–8.9		12.64 9.93, 9.14	

experiment.[1a] In HMBC, a chelated hydroxyl signal at δ_H 12.8 coupled with C-4‴ (δ_C 164.1), C-5‴ (δ_C 113.8) and C-3‴ (δ_C 102.6). A singlet signal at δ_H 7.44 (δ_C 132.2) was long-range coupled with C-4 (δ_C 195.1), C-4‴ (δ_C 164.1), C-2‴ (δ_C 162.1) and C-3″ (δ_C 32.1). The singlet aromatic proton signal at δ_H 6.44 (δ_C 102.6) coupled with C-4‴ (δ_C 164.1), C-2‴ (δ_C 162.1), C-1‴ (δ_C 119.2) and C-5‴ (δ_C 113.8), These data led to the assignment of the substructure A (Fig. 1).

In ^{1}H NMR, three aromatic protons δ_H 7.58 (1H, d, 8.5 Hz), δ_H 6.90 (1H, dd, 8.5 Hz, 1.9 Hz) and δ_H 6.99 (1H, d, 1.9 Hz) form a typical ABX pattern. In HSQC, δ_H 6.99 corresponding to δ_C 97.7 and in HMBC, this proton was long-range coupled to C-9 (δ_C 155.3), C-7 (δ_C 156.3), C-6 (δ_C 112.7) and C-10 (δ_C 120.5). The aromatic proton signal at δ_H 6.14 was coupled to C-4′ (δ_C 161.1), C-1′ (δ_C 99.5) and C-3′ (δ_C 91.4). The signal at δ_H 5.91 was coupled to C-4′ (δ_C 161.1), C-2′ (δ_C 159.6), C-1′ (δ_C 99.5) and C-5′ (δ_C 96.1). A methoxyl signal at δ_H 3.52 correlated to C-2′ (δ_C 159.6). These data led to establish the substructure B and C (Fig. 1).[5] Thus, the two reminiscent tetra-substituted sp^2 carbons (δ_C 151.8 and δ_C 117.5) should be involved in the linkage of these three substructures. Therefore, four alternative arylbenzofuran structures (**1a**–**1d**, Fig. 2) could be proposed for **1**.

Only five chalcone-flavone dimers[6] and eight pterocarpan-benzofuran dimers[2] with the carbon skeletons **1a** and **1c** have already been described in the literature, and the distinct chemical

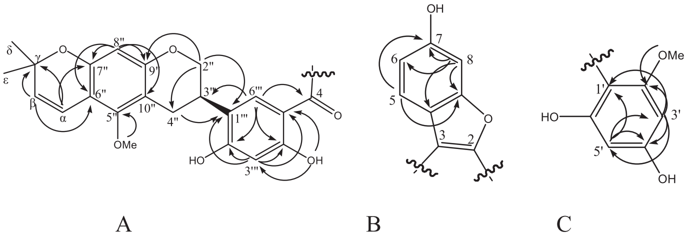

Fig. 1. Key HMBC (H→C) correlations of compound **1**.