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Phytochemistry 64 (2003) 645–647
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Cissampeloflavone, a chalcone-flavone dimer from *Cissampelos pareira*

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Received 6 December 2002; received in revised form 17 February 2003

Dedicated to the memory of Professor Jeffrey B. Harborne

Abstract

From the aerial parts of *Cissampelos pareira* L. (Menispermaceae), a chalcone-flavone dimer has been isolated which, mainly from NMR spectroscopic and MS data, was proved to be 2-(4-hydroxy-3-methoxyphenyl)-7-(4-methoxyphenyl)-6-(2-hydroxy-4,6-dimethoxybenzoyl)-furano[3,2-*g*]benzopyran-4-one. This has been assigned the trivial name cissampeloflavone. The compound has good activity against *Trypanosoma cruzi* and *T. brucei rhodesiense* and has a low toxicity to the human KB cell line.

Keywords: *Cissampelos pareira*; Menispermaceae; Phytochemistry; Chalcone-flavone dimer; Cissampeloflavone; Antiprotozoal; Antitrypanosomal

1. Introduction

Plants collected from the Orinoco jungle area of Venezuela have been screened for a range of biological activities. An acetone extract of *Cissampelos pareira* was shown to be active in antiprotozoal tests and a novel chalcone-flavone dimer (**1**) has been isolated from the extract, the structure of which is reported in this communication. Compound 1 was shown to have good activity in the antiprotozoal assays against *Trypanosoma cruzi* (intracellular form) and *T. brucei rhodesiense* (extracellular form).

2. Results and discussion

Column chromatographic separation of an acetone extract of the dried aerial parts of the plant, using *n*-hexane-ethyl acetate mixtures yielded a yellow solid, which after crystallization from ethyl acetate gave crystals (**1**) with a melting point of 218–220 °C.

The ^{1}H NMR spectrum of **1** (Table 1) showed three distinct sets of aromatic ring proton absorptions: an AA′BB′ system for four protons, two at δ 6.90 and two at δ 7.66 ($J = 8.8$ Hz), indicating a *para*-substituted benzene ring; two doublets (2H) at δ 5.79 and δ 6.17 ($J = 2.2$ Hz), characteristic of a 1,2,3,5-tetrasubstituted aromatic ring; and an ABX system at δ 7.05 ($J = 8.4$ Hz), δ 7.40 ($J = 2.0$ Hz) and δ 7.52 ($J = 2.0$, 8.4 Hz), indicative of a 1,3,4-trisubstituted aromatic ring. In addition, there remained a singlet aromatic proton absorption at δ 7.14. With the exception of four methoxyl group signals (δ 55.4, 55.5, 55.7 and 56.2), absorptions due to aliphatic type carbons were absent in the ^{13}C NMR spectrum. Two hydrogen-bonded hydroxyl group absorptions (δ 13.35 and 13.69) were correlated with two carbonyl groups (^{13}C NMR spectrum: δ 183.8 and 193.3, respectively) by ^{1}H-^{13}C long range HMBC connectivity spectra. The HR FAB mass spectrum of **1** gave a molecular ion $(M+1)^+$ at m/z 611.1570 (calculated for $C_{34}H_{26}O_{11}+1 = 611.1556$),

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doi:10.1016/S0031-9422(03)00241-3

Table 1
^{1}H and ^{13}C NMR chemical shift connectivities (δ) in ppm of compound **1** and corresponding ^{13}C NMR chemical shifts of compound **2**

Carbon number	Carbon chemical shift (δ)		Proton connectivity and chemical shift (δ)		Coupling constant J (Hz)
	2	**1**			
2	164.9	164.5			
3	102.8	103.7	H_3	6.58	
4	183.7	183.8			
5	153.7	155.0	C_5–OH	13.35	
6	156.6	113.8			
7	113.0	154.0			
8	91.0	90.2	H_8	7.14	
9	154.0	157.1			
10	105.6	106.0			
1′	121.3	123.4			
2′	110.6	108.4	$H_{2'}$	7.40	2.0
3′	148.3	146.9			
4′	150.9	149.4	$C_{4'}$–OH	6.03	
5′	116.1	115.1	$H_{5'}$	7.05	8.4
6′	121.6	120.9	$H_{6'}$	7.52	2.0,8.4
α	151.3	117.8			
β	160.0	152.2			
1″	192.2	193.3			
2″	106.5	107.7			
3″	163.9	163.3			
4″	92.0	91.2	$H_{4''}$	5.79	2.2
5″	166.8	167.5			
6″	96.0	93.6	$H_{6''}$	6.17	2.2
7″	167.0	168.0	$C_{7''}$–OH	13.69	
1‴	120.2	122.0			
2‴	127.8	128.2	$H_{2'''}$	7.66	8.8
3‴	114.9	114.3	$H_{3'''}$	6.90	8.8
4‴	160.4	160.4			
5‴	114.9	114.3	$H_{5'''}$	6.90	8.8
6‴	127.8	128.2	$H_{6'''}$	7.66	8.8
OMe-3′	56.0	56.2	Ome	4.02	
OMe-3″	56.3	55.5	Ome	3.27	
OMe-5″		55.7	Ome	3.86	
OMe-4‴	55.5	55.4	Ome	3.82	

showing that the basic structure of **1** was composed of a thirty carbon skeleton.

The assignments of all the proton and carbon chemical shifts were undertaken using various two dimensional techniques. The HMBC connectivity spectrum in some cases was able to show coupling through four carbons from a very weak methine proton signal. For example, δ 91.2 (proton δ 5.79) to δ 163.3 to δ 107.7 to δ 193.3 and then to δ 117.8 verified the connection from one ring system to another. The structural features were found to be very similar to those of flavone-chalcone dimers isolated from *Aristolochia ridicula*, in particular of 4′,5,5″,7″-tetrahydroxy-3′,3″,4‴-trimethoxy-6-*O*-β, 7α-flavone-chalcone (**2**) (Carneiro et al., 2000), although 1 has a methoxyl group substituent at 5″ instead of an hydroxyl group. However, the chemical shifts of the α- and β-carbon atoms of the furan ring differed significantly between **1** and **2**. HMBC connectivities were shown from C-5-O*H* (δ 13.35) to C-5 (δ 155.0) to C-6 (δ 113.8) and then weakly to the α-carbon (δ 117.8), and from C-5-O*H* (δ 13.35) to C-10 (δ 106.0). Further connectivities were observed from C-8-H (δ 7.14) to C-7 (δ 154.0) to C-6 (δ 113.8), and from C-8-H (δ 7.14) to C-9 (δ 157.7) to C-10 (δ 106.0) to C-4 (δ 183.7). These data show that the orientation of the furan ring of 1 relative to the flavone moiety is reversed in comparison with 2. Thus the chemical shift values for C-6 and C-7 for 2 are δ 156.6 and δ113.0, respectively, and for **1**, δ 113.8 and δ154.0, respectively. The chemical shift of the β-carbon of **1** (δ 152.2) is similar to that of 2 (δ 160.0), but the signal for the α-carbon of **1** (δ 117.8), differs markedly from that of 2 (δ 151.3). These values for **1** are consistent with the chemical shifts of methyl 2-methyl-3-furancarboxylate (δ 113.3; α-C and δ 159.3; β-C) (Pouchet and Behnke, 1993).

From all the above information, the complete chemical shift assignments for **1** were deduced and these are given in Table 1. Compound **1** was thus shown to be 2-(4-hydroxy-3-methoxyphenyl)-7-(4-methoxyphenyl)-6-(2-hydroxy - 4,6 - dimethoxybenzoyl) - furano[3,2 - *g*]benzopyran-4-one, which appears to be novel and has been assigned the trivial name cissampeloflavone.

Table 2
In vitro (%) inhibition of protozoa by cissampeloflavone (**1**)

Protozoan	% Inhibition produced by cissampeloflavone (μg/ml)									ED_{50} (μg/ml)
	30	10	3	1	0.3	0.1	0.04	0.01	0.005	
Leishmania donovani	23.0	13.1	0							>30
Trypanosoma cruzi	99.3	72.7	67.5	32.7						2.09
T. brucei rhodesiense STIB900	100	82.3	80.4	49.9	47.3		37.4	27.5	0	0.61
Plasmodium falciparum 3D7	29.4	28.4	12.5	14.7	16.7	0.64				>30

When tested in antiprotozoal assays, cissampeloflavone (**1**) was found to have good activity against *Trypanosoma cruzi* (intracellular form) and *T. brucei rhodesiense* (extracellular form), but poor activity against *Plasmodium falciparum* and *Leishmania donovani* (Table 2). Encouragingly, the compound had a low cytotoxicity to the human KB cell line (106 μg/ml). Other compounds in the original acetone extract of the plant material may also have antiprotozoal activity, but **1** was the only compound characterized which was isolated in sufficient amount to allow testing.

3. Experimental

Mp: uncorr. ^1H and ^{13}C NMR spectra, in $CDCl_3$, were obtained at 600 MHz and 150 MHz, respectively, with TMS and $CDCl_3$ (δ 77.02) as int. standards for proton and carbon nuclei, respectively. ^1H and ^{13}C assignments were made by recording 1 D ^1H and ^{13}C spectra fully coupled and decoupled, plus INEPT. Connectivity experiments included 2 D ^1H–^1H COSY, TOCSY (proton–proton), ^1H–^{13}C HMQC (proton–carbon) and ^1H-^{13}C HMBC (proton–carbon–carbon). All spectra were processed using Varian Vnmr software on a Sun Ultrasparc 5 workstation.

For comparative purposes, compound **1** has been numbered using the same system as that employed by Carneiro et al. (2000).

3.1. Plant material

Samples of *Cissampelos pareira* L. (Menispermaceae) were collected in November, 1998 from Isla Babilla in the river Orinoco, Amazonas State, Venezuela. Voucher specimens have been deposited in the herbarium of the Faculty of Pharmacy University of los Andes (Index Herbariorum MERF; accession number 2490). The aerial parts of the plant were dried at 40°.

3.2. Extraction and fractionation of extract

Powdered *C. pareira* (2781 g) was extracted with Me_2CO at room temperature. After conc to dryness, part of the extract (53 g) was fractionated by vacuum liquid chromatography using the method of Coll and Bowden (1986). The extract was mixed with 159 g silica gel (2–25 μm; Aldrich), which was placed over a further 320 g of the adsorbent. Elution was initially with *n*-hexane (500 ml), followed by *n*-hexane-EtOAc mixts (9:1, 4:1, 7:3, 3:2, 1:1, 2:3, 3:7, 1:4, 1:9; 500 ml of each), EtOAc (100 ml) and MeOH (100 ml); fractions of 100 ml were collected. The *n*-hexane-EtOAc (3:2) eluate (0.98 g) was applied to a silica gel column (80×3 cm, 0.063–0.200 mm; Merck) and eluted first with *n*-hexane (150 ml), then with mixtures of *n*-hexane-EtOAc (9:1, 4:1, 7:3, 3:2, 1:1, 2:3, 3:7, 1:4, 1:9; 150 ml of each) and finally with EtOAc (150 ml); fractions of 50 ml were collected. From the *n*-hexane-EtOAc (1:1) fraction, a yellow compound was obtained, which after crystallization from EtOAc gave yellow crystals of **1** (112 mg).

3.3. Parasites, in vitro assays and cytotoxicity tests

The parasites employed for the antiprotozoal studies and the methods used for the in vitro assays and the cytotoxicity tests have been described in detail by Asres et al. (2001).

Acknowledgements

The NMR Facility of the University of Portsmouth is supported by a Wellcome Trust/JREI grant. We thank Dr. Amala Raman, Department of Pharmacy, King's College London and Dr. Mike Cocksedge, The School of Pharmacy, University of London for the mass spectra. We also thank the Consejo de Desarrollo Cientifico Humanistico y Tecnologico de la Universidad de los Andes for their financial support.

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