Flavonoids from *Tephrosia major*. A New Prenyl-β-hydroxychalcone[a]

Federico Gómez-Garibay, Oswaldo Téllez-Valdez, Gregorio Moreno-Torres and José S. Calderón*

Instituto de Química de la Universidad Nacional Autónoma de México Circuito Exterior, Ciudad Universitaria, Coyoacán, 04510 México, D. F. Fax: (52) (55) 56162203.
E-mail: uscalder@correo.unam.mx

* Author for correspondence and reprint requests

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The roots and aerial parts of *Tephrosia major* Micheli, afforded a new prenylated-β-hydroxychalcone, characterized as 2′,6′-dihydroxy-3′-prenyl-4′-methoxy-β-hydroxychalcone. In addition, seven prenylated flavonoids, two rotenoids, β-sitosterol, stigmasterol, lupeol and quercetin were isolated. The structure of the new β-hydroxy chalcone was established by spectroscopic methods, including 2D NMR experiments.

Introduction

β-Hydroxychalcones (dibenzoylmethanes) belong a rare group of flavonoids, which do not commonly occur in nature, to our knowledge only seven prenylated β-hydroxychalcones have been isolated. They present simple structures and usually are found as diketo-ketoenolic tautomeric mixtures (Ayabe *et al*., 1980, 1986; Demizu *et al*., 1992; Mayer, 1993; Rathore *et al*., 1987; Venkataratnam *et al*., 1987; Waterman and Mahmoud, 1985) or less commonly as a keto-enolic tautomer possessing a *Z*-configuration (Camele *et al*., 1980; Gandhidasan *et al*., 1987; Parmar *et al* 1989). Recently Stevens *et al.*, (1999) reported that 2-hydroxyflavanones exist together with dibenzoylmethanes as tautomeric pairs in solvents such as DMSO and Me_2CO. Previous phytochemical studies of the genus *Tephrosia* (Leguminosae; subfamily Papilinoideae; tribe *Tephrosieae*) have led to the isolation and identification of numerous flavonoids, rotenoids and coumestan derivatives, some of which possess insecticidal and piscicidal properties (Gomez-Garibay *et al.*, 2001). In continuation of our phytochemical studies of members of the genus *Tephrosia* we have studied *Tephrosia major*, a species endemic in northwest Mexico.

Results and Discussion

Extraction of the roots and aerial parts of the plant with petrol, ethyl acetate and methanol, followed in each case by CC and prep. TLC over silica-gel (see Experimental) gave a new flavonoid, 2′,6′-dihydroxy-3′-prenyl-4′-methoxy-β-hydroxychalcone (**1c**). In addition the known, β-sitosterol, stigmasterol, lupeol, the flavonoids, glabranin (Gómez-Garibay *et al*., 1988) 7-O-methyl-glabranin (Gómez-Garibay *et al*., 1988), tephrowatsin A (Gómez-Garibay *et al.*, 1985), quercetol B (Gómez-Garibay *et al*., 1988), obovatin (Chen *et al*., 1978), tephrobbotin (Gómez-Garibay *et al*., 1986), tephrowatsin B (Gómez-Garibay *et al.*, 1985), quercetin, the rotenoids, sumatrol, α-toxicarol were isolated. Identification of the known compounds was based on the comparison of their spectroscopic (^1H, ^{13}C, and EIMS) and physical (m.p.) data reported in the literature.

Compound **1c** was obtained as yellow crystals, m.p. 121–123°. Its molecular formula $C_{21}H_{22}O_5$ was deduced from the EIMS spectrum (M^+, *m/z* 354) and by quantification of the number of methyl, methylene, methine and quaternary carbon atoms revealed in the ^{13}C NMR and DEPT spectra (Table I). The IR spectrum of **1c** displayed bands at 1675 and 1604 cm^{-1} characteristic of an enolic β-diketone moiety (Ayabe *et al*., 1980). The ^1H NMR spectrum of **1c** showed signals for two olefinic methyl groups at δ 1.56 (3H, brs) and 1.61 (3H, brs), one allylic methylene at δ 3.23 (2H, d, J = 6.6 Hz) and one olefinic proton at δ 5.10 (1H,

[a] Part 11 in the series Flavonoids from Tephrosia species. For part 10 see Gomez-Garibay *et al.,* 2001. Contribution No. 1748 of Instituto de Química, UNAM.

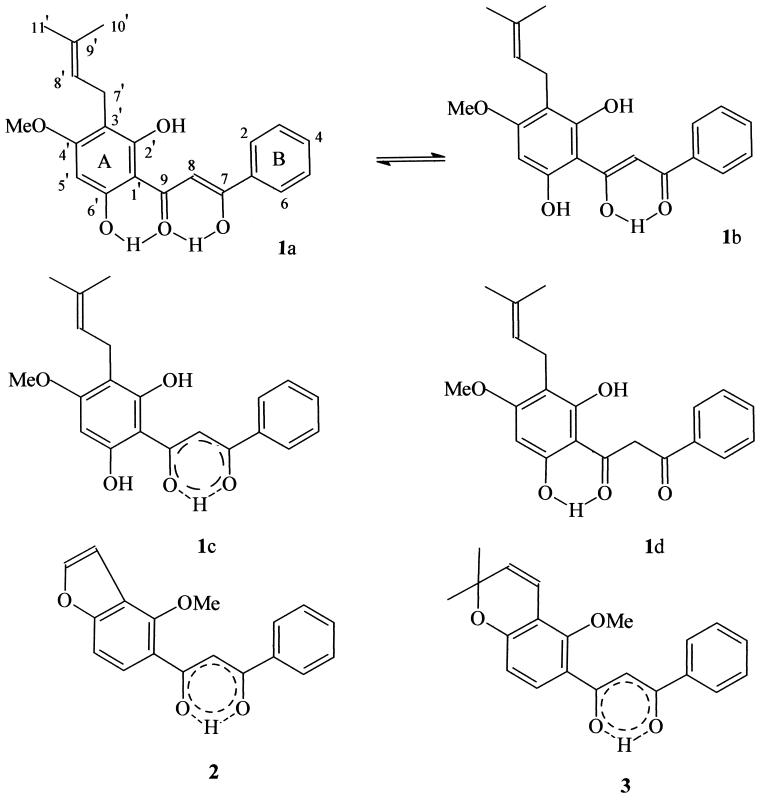

Fig. 1. Chemical structures of 2′,6′-dihydroxy-4′-methoxy-3′-prenyl-β-hydroxychalcone (**1c**), pongamol (**2**) and 2′-methoxy-3′,4′-(2,2-dimethylchromene)-β-hydroxychalcone (**3**).

brt, J = 6.6 Hz) suggested the presence of a C-3-methyl, 2-butenyl (C-prenyl) moiety (Gómez-Garibay *et al*., 1997) which was confirmed by resonances in the ^{13}C NMR spectrum of **1c** at δ 17.9 (q), 25.6 (q), 131.3 (s) 121.6 (d) and 22.3 (t), corresponding to C-11′,C-10′, C-9′,C-8′ and C-7′, respectively (Waterman and Mahmoud 1985). The ^1H NMR spectrum of **1c** also showed a three proton sharp signal at δ 3.97 (s), and one proton signal at δ 6.41 (s) assigned to an aromatic methoxyl group and, an uncoupled A ring aromatic proton, respectively. Further signals at down field (δ 10.3 and 12.5) for two phenolic protons were observed, which disappeared after D_2O exchange. All the above spectroscopical data suggested a tetrasubstituted A-ring, bearing one prenyl, one methoxy and two hydroxy groups. Further signals were observed at δ 8.22 ((2H, dd, J = 7.2 and 1.2 Hz), 7.64 (1H, brt, J = 7.2 Hz) and 7.52 (2H, brd, J = 7.2 Hz) and agree with those reported for β-hydroxychalcones possessing an unsubstituted aromatic B ring (Mayer, 1993). Fragment ions at m/z 105 $[C_7H_5O]^+$ and 77 $[C_6H_5]^+$ in the MS spectrum of **1c** confirmed the above assumption (Mayer, 1993; Parmar *et al*., 1989). The relative position of the methoxy group in the A ring was deduced from the MS spectrum of **1c**. The absence of a fragment ion at m/z M^+-31 $[M^+ - MeO]$ characteristic of 2′-OMe hydroxychalcones (Khan and Zaman, 1974; Gupta and Krishnamurti, 1977) suggested that the methoxy group was located at C-4′. Thus, the two phenolic groups, had to be placed at C-2′ and C-6′, and the prenyl group at C-3′. Identical substitution pattern was found in praecansone B, demethylpraecansone B, and 2′-hydroxy-4′,6′-dimethoxy-3′-methyl-β-hydroxychalcone (Camele *et al*., 1980; Waterman and Mahmoud, 1985; Mayer, 1993).The presence of a low field hydrogen bonded hydroxyl proton at δ 16.5 and one olefinic proton at δ 7.3 in the ^1H NMR spectrum of **1c**, characteristic of β-hydroxychalcones with Z-configuration (Gandhidasan *et al.*, 1987; Kiuchi *et al.*, 1990; Parmar *et al.*, 1989). suggested that compound **1c** exist either tautomeric structures **1a** or **1b**. The absence of signals between δ 4.4–4.8 in the ^1H NMR spectrum, excluded the presence of

Table I. ^1H and ^{13}C NMR spectral data for **1c**, **2**, **3** and dibenzoylmethane ($CDCl_3$, TMS as int. standard)[a].

Position	Compound **1c**		Compound **2**[b]	Compound **3**[c]	Dibenzoyl methane[b]
	δ_H	δ_C	δ_C	δ_C	δ_C
1		136.7 s	135.7 s	135.7 s	136.1 s
2	8.22 dd (7.2, 2.2)	128.4 d	127.1 d	127.0 d	128.0 d
3	7.52 brd (7.2)	130.3 d	128.6 d	128.6 d	129.5 d
4	7.64 brt (7.2)	133.2 d	132.1 d	132.1 d	133.4 d
5	7.52 brd (7.2)	130.3 d	128.6 d	128.6 d	129.5 d
6	8.22 dd (7.2, 2.2)	128.4 d	127.1 d	127.0 d	128.0 d
7		185.6 s	184.3 s	184.4 s[d]	186.5 s
8	7.30 s	92.5 d	97.9 d	96.7 d	93.7 d
9		186.6 s	186.1 s	185.2 s[d]	186.5 s
1′		103.5 s			
2′	10.3 s	163.6 s			
3′		110.9 s			
4′		163.6 s			
5′	6.41 s	92.5 d			
6′	12.5 s	163.6 s			
7′	3.23 d (6.6)	22.3 t			
8′	5.10 t (6.6)	121.6 d			
9′		131.3 s			
10′	1.56 brs	25.6 q			
11′	1.87 brs	17.9 q			
OMe	3.97 s	55.9 q			

[a] δ in ppm and *J* (in parentheses) in Hz.
[b] Data from Kiuchi *et al.*, 1990.
[c] Data from Magalhães *et al.*, 1996.
[d] The original assignments were revised with reference to our data. Chemical shifts were determined at 300 (^1H) and 75 (^{13}C) MHz. Carbon multiplicities were determined by DEPT experiments.

the diketo-tautomer **1d** also (Demizu *et al.*, 1992). Finally, the ^{13}C NMR spectrum of **1c** (Table I) showed resonances at δ 186.6, 92.5 and 185.6, which were assigned to C-9, C-8 and C-7, respectively, and agree closely with those reported for the enolic structure of pongamol (**2**) (Kiuchi *et al.*, 1990) and the enolic tautomer of dibenzoylmethane (Kiuchi *et al.*, 1990), whose structures were determined by X-ray diffraction (Hollander, 1973; Parmar *et al.*, 1989). All the above ^{13}C NMR data strongly supported the symmetrical structure **1c** rather than tautomeric structures **1a** or **1b**.

Experimental

General

Melting points uncorrected. ^1H NMR: TMS as int. standard. CC: silica gel (Merck, 230–400 mesh), TLC: precoated silica gel 60 F_{254} (Merck, 0.10 mm). Spots were visualized by UV (254 nm) and 10% $CeSO_4$-H_2SO_4 reagent followed by heating.

Plant material

Tephrosia major Micheli was collected in Jalisco, México in July 1992, at 15 km east of the Tuito. Identification of the material was carried out by O. Tellez. A voucher specimen has been deposited in the National Herbarium UNAM, (MEXU) of the Instituto de Biología.

Extraction and separation

The air-dried plant material, leaves and stems (1.24 kg) were extracted successively with petroleum ether (b.p. 35–60°), EtOAc, and MeOH. After evaporation of solvents green syrups A (7.3 g), B (32.6 g) and C (35.2 g), respectively, were obtained. In the same way, from the air-dried roots (582.0 g) green syrups D (23.6 g), E (20.3 g) and F (19.0 g) were obtained.

The petroleum ether (b.p. 35–60°) extract A (7.3 g) was chromatographed on a silica gel column (300 g) eluting with petroleum ether and mixtures of petroleum ether–CH_2Cl_2. From the fractions eluted with petroleum ether a mixture of β-sitosterol and stigmasterol (18.3 mg), (22 mg) and 5-*O*-methylobovatin (12.0 mg) (m.p. 159–161°) (Chen *et al*., 1978) were obtained.

The EtOAc extract B (32.6 g) was fractionated on silica gel (250 g) using petroleum ether and mixtures of petroleum ether–CH_2Cl_2 to give a mixture of β-sitosterol and stigmasterol (25.0 mg), glabranin (25 mg) (Gómez-Garibay *et al*., 1988) and 7-*O*-methylglabranin (95.0 mg) (Gómez-Garibay *et al*., 1988), were obtained.

The MeOH extract C (35.2 g) was chromatographed on a column silica gel (300 g) using mixtures of petroleum ether–EtOAc, EtOAc and mixture of EtOAc–MeOH, to give a mixture of β-sitosterol and stigmasterol (39.0 mg) lupeol, (35.0 mg) glabranin (Gómez-Garibay *et al*., 1988), (533.0 mg) 7-*O*-methylglabranin (123.0 mg) (Gómez-Garibay *et al*. 1982), quercetin (8.0 mg), sumatrol (13.0 mg) and α-toxicarol (8.0 mg).

The petroleum ether extract D (23.6 g) was chromatographed on a column silica gel (200 g) eluting with petroleum ether and mixtures of petroleum ether–CH_2Cl_2 to give a mixture of β-sitosterol and stigmasterol (35.0 mg), obovatin (Chen *et al*., 1978) 7-*O*-methylglabranin (75.0 mg) (Gómez-Garibay *et al*., 1988), sumatrol (27.0 mg) and tephrobbotin (Gómez-Garibay *et al*., 1986), (15.0 mg).

The EtOAc extract E (20.3 g) was fractionated on silica gel (200 g) using petroleum ether and mixtures of petroleum ether–EtOAc, a mixture of β-sitosterol and stigmasterol (29.0 mg), glabranin (15.0 mg) (Gómez-Garibay *et al*., 1988) and 7-*O*-methylglabranin (75.0 mg) (Gómez-Garibay *et al*., 1988), tephrowatsin A (7.8 mg) (Gómez-Garibay *et al*., 1985), tephrowatsin B (13.5 mg) (Gómez-Garibay *et al*., 1985), quercetol B (18.3 mg) (Gómez-Garibay *et al*., 1988), were obtained.

Finally, the MeOH extract F (19.0 mg) was fractionated on a silica gel column (200 g) using petroleum ether and mixtures of petroleum ether–EtOAc, glabranin (56.0 mg) (Gómez-Garibay *et al*., 1988), 7-O-methylglabranin (12.5 mg) (Gómez-Garibay *et al*., 1988), 2′,6′-dihydroxy-3′-prenyl-4′-methoxy-β-hydroxychalcone (**1e**) (24.0 mg), tephrobbotin (29.0 mg) (Gómez-Garibay *et al*., 1986), tephrowatsin A (5.8 mg) (Gómez-Garibay *et al*., 1985), tephrowatsin B (26.0 mg) (Gómez-Garibay *et al*., 1985), quercetol B (7.3 mg) (Gómez-Garibay *et al*., 1988), were obtained.

2′,6′-Dihydroxy-3′-prenyl-4′-methoxy-β-hydroxychalcone (**1e**)

Yellow crystals, m.p. 121–123°, UV λ_{max}, MeOH, nm (log ε): 202 (4.32), 229 (4.22), 273 (3.82) and 316 (3.59). IR υ_{max}, $CHCl_3$ cm^{-1}: 1675, 1604. EIMS (70 eV) *m/z* (rel. int.) 354 $[M^+]$ (0.3),336 $[M-H_2O]^+$ (0.8), 320 $[M-H_2O_2]^+$ (1.2), 249 $[C_{14}H_{17}O_4]^+$ (100), 105 $[C_7H_5O]^+$ (83), 77 $[C_6H_5]^+$ (40).

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