Flavonoids from Tephrosia major. A New Prenyl-\(\beta\)-hydroxychalcone\(^a\)

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Tephrosia major, Leguminosae, Prenylated-\(\beta\)-hydroxychalcone

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

Introduction

\(\beta\)-Hydroxychalcones (dibenzoylmethanes) belong a rare group of flavonoids, which do not commonly occur in nature, to our knowledge only seven prenylated-\(\beta\)-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 dibenzoylemethanes as tautomeric pairs in solvents such as DMSO and Me2CO. 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-\(\beta\)-hydroxychalcone (1c). In addition the known, \(\beta\)-sito-sterol, 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., 1985), obovatin (Chen et al., 1978), tephroobotin (Gómez-Garibay et al., 1986), tephrowatsin B (Gómez-Garibay et al., 1985), quercetin, the rotenoids, sumatrol, \(\alpha\)-toxicarol were isolated. Identification of the known compounds was based on the comparison of their spectroscopic (\(^1\)H, \(^{13}\)C, and EIMS) and physical (m.p.) data reported in the literature.

Compound 1c was obtained as yellow crystals, m.p. 121–123\(^\circ\). Its molecular formula \(\text{C}_{21}\text{H}_{22}\text{O}_{5}\) 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 \(\beta\)-diketone moiety (Ayabe et al., 1980). The \(^1\)H NMR spectrum of 1c showed signals for two olefinic methyl groups at \(\delta\) 1.56 (3H, brs) and 1.61 (3H, brs), one allylic methylene at \(\delta\) 3.23 (2H, d, \(J = 6.6\) Hz) and one olefinic proton at \(\delta\) 5.10 (1H, \(\text{C}_{21}\text{H}_{22}\text{O}_{5}\) 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 \(\beta\)-diketone moiety (Ayabe et al., 1980). The \(^1\)H NMR spectrum of 1c showed signals for two olefinic methyl groups at \(\delta\) 1.56 (3H, brs) and 1.61 (3H, brs), one allylic methylene at \(\delta\) 3.23 (2H, d, \(J = 6.6\) Hz) and one olefinic proton at \(\delta\) 5.10 (1H,
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 $\delta$ 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 $^1$H NMR spectrum of 1c also showed a three proton sharp signal at $\delta$ 3.97 (s), and one proton signal at $\delta$ 6.41 (s) assigned to an aromatic methoxyl group and, an uncoupled A ring aromatic proton, respectively. Further signals at down field ($\delta$ 10.3 and 12.5) for two phenolic protons were observed, which disappeared after D$_2$O 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 $\delta$ 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 $\beta$-hydroxychalcones possessing an unsubstituted aromatic B ring (Mayer, 1993). Fragment ions at $m/z$ 105 [C$_7$H$_5$O]$^+$ and 77 [C$_6$H$_5$]$^+$ 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- $\beta$-hydroxychalcone (Camele et al., 1980; Waterman and Mahmoud, 1985; Mayer, 1993). The presence of a low field hydrogen bonded hydroxyl proton at $\delta$ 16.5 and one olefinic proton at $\delta$ 7.3 in the $^1$H NMR spectrum of 1c, characteristic of $\beta$-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 $\delta$ 4.4–4.8 in the $^1$H NMR spectrum, excluded the presence of...
Table I. $^1$H and $^{13}$C NMR spectral data for $1c$, $2$, $3$ and dibenzoylmethane (CDCl$_3$, TMS as int. standard)$^a$.

<table>
<thead>
<tr>
<th>Position</th>
<th>Compound $1c$</th>
<th>Compound $2$</th>
<th>Compound $3$</th>
<th>Dibenzoylmethane$^b$</th>
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<tr>
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<td>$\delta_C$</td>
<td>$\delta_C$</td>
<td>$\delta_C$</td>
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<tr>
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<td>135.7 s</td>
<td>135.7 s</td>
<td>136.1 s</td>
</tr>
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<td>128.4 d</td>
<td>127.1 d</td>
<td>127.0 d</td>
</tr>
<tr>
<td>3</td>
<td>7.52 brd (7.2)</td>
<td>130.3 d</td>
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</tr>
<tr>
<td>4</td>
<td>7.64 brt (7.2)</td>
<td>133.2 d</td>
<td>132.1 d</td>
<td>132.1 d</td>
</tr>
<tr>
<td>5</td>
<td>7.52 brd (7.2)</td>
<td>130.3 d</td>
<td>128.6 d</td>
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</tr>
<tr>
<td>6</td>
<td>8.22 dd (7.2, 2.2)</td>
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</tr>
<tr>
<td>7</td>
<td>185.6 s</td>
<td>184.3 s</td>
<td>184.4 s$^d$</td>
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<td>92.5 d</td>
<td>97.9 d</td>
<td>96.7 d</td>
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<tr>
<td>9</td>
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<td>185.2 s$^d$</td>
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</tr>
<tr>
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</tr>
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<td>163.6 s</td>
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<tr>
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<td>4$'$</td>
<td></td>
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<tr>
<td>7$'$</td>
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<tr>
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<td>121.6 d</td>
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<td>25.6 q</td>
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<tr>
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</tr>
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<td>OMe</td>
<td>3.97 s</td>
<td>55.9 q</td>
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</table>

$^a$ $\delta$ 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 ($^1$H) 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 $\delta$ 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. $^1$H 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$_2$SO$_4$ 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$^\circ$), 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$_2$Cl$_2$. From the fractions eluted with petroleum ether a mixture of β-sitosterol and stigmasterol (18.3 mg), (22 mg) and 5-O-methyllobovatin (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$_2$Cl$_2$ 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$_2$Cl$_2$ 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 $\lambda_{\text{max}}$, MeOH, nm (log $\varepsilon$): 202 (4.32), 229 (4.22 ), 273 (3.82 ) and 316 (3.59). IR $\nu_{\text{max}}$, CHCl$_3$ cm$^{-1}$: 1675, 1604. EIMS (70 eV) $m/z$ (rel. int.) 354 [M$^+$] (0.3),336 [M–H$_2$O]$^+$ (0.8), 320 [M–H$_2$O$_2$]$^+$ (1.2), 249 [C$_{14}$H$_{17}$O$_4$]$^+$ (100), 105 [C$_7$H$_5$O]$^+$ (83), 77 [C$_8$H$_5$]$^+$ (40).

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