



TWO NEW ISOFLAVONES FROM *TEPHROSIA MAXIMA*

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ABSTRACT

Two new isoflavones, maxima isoflavone M (**1**), and maxima isoflavone N (**2**), were isolated from chloroform extract of the roots and aerial parts of *Tephrosia maxima*, respectively and their structure were established from spectral data.

Key words : *Tephrosia maxima*, Leguminosae, Isoflavones.

INTRODUCTION

Tephrosia PERS (Leguminosae, Papilionaceae) comprises about 400 species and representatives of this large genus are distributed throughout warm regions of both hemispheres¹. *Tephrosia maxima* is a perennial shrub found widely in Eastern Ghats of India and used as traditional medicine. Some of the isoflavones isolated from this plant were found to have antispasmodic activity². In continuation of our chemical and biological studies^{3–8} on the flavonoids of this plant, we report here the isolation and characterization of two new isoflavones named as maxima isoflavone M (**1**) and maxima isoflavone N (**2**) since maxima isoflavone A to L were reported earlier^{3–5,8}.

EXPERIMENTAL

General Procedures : Melting points were determined on an Electrothermal Digital melting point apparatus and uncorrected. IR spectra were recorded in KBr discs on Perkin–Elmer 881 instrument and UV spectra on a Shimadzu UV–1201 spectrophotometer. The ¹H–NMR and ¹³C–NMR spectra were determined on a Bruker AMX 400 MHz spectrometer and 90 MHz, Jeol JNM FT NMR spectrometer using CDCl₃ with tetramethylsilane (TMS) as internal standard. Electron impact (EI)–MS were obtained on an Autospec instrument.

Plant material : The whole plant of *T. maxima* PERS. was collected from Chippada village, Andhra Pradesh, South India in November 2002. A voucher specimen has been deposited in the herbarium of the Department of Botany, Andhra University, Visakhapatnam, India.

Extraction and Isolation

The shade dried and powdered roots (2 kg) and aerial parts (2 kg) of *T. maxima* were successively extracted with n-hexane, CHCl_3 and MeOH. The CHCl_3 extract of the roots (15 g) on purification over silica gel column using n-hexane-EtOAc step gradient (1 : 1) yielded (**1**) (30 mg). The CHCl_3 extract of the aerial parts (20 g) on purification over silica gel column using n-hexane-EtOAc step gradient (8.5 : 1.5) yielded (**2**) (28.5 mg).

RESULTS AND DISCUSSION

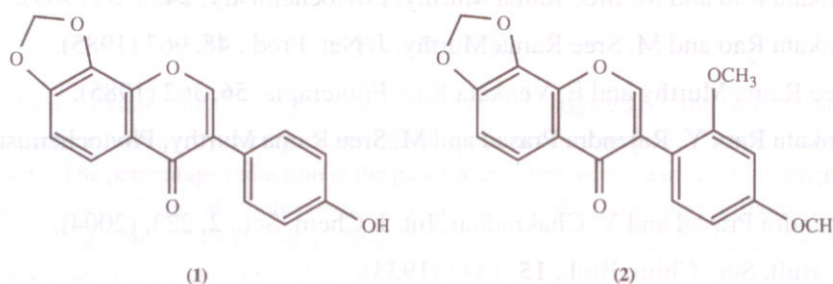
Maxima isoflavone M (**1**) obtained as colorless prisms, showed $[\text{M}]^+$ peak at m/z 282 consistent with the molecular formula $\text{C}_{16}\text{H}_{10}\text{O}_5$. The UV absorption maxima at 210, 259 and 298 nm were typical of an isoflavone skeleton. The IR spectrum of (**1**) exhibited strong absorption bands attributable to carbonyl (1622 cm^{-1}) and a methylenedioxy group ($1031, 940\text{ cm}^{-1}$).

The $^1\text{H-NMR}$ spectrum exhibited a signal attributed to the isoflavone proton at C-2 (δ 7.89, s), an orthocoupled doublet due to the proton at C-5 (δ 7.87, 1H, d, $J = 9\text{ Hz}$) and a doublet at C-6 (δ 6.89, 1H, d, $J=9\text{Hz}$). The $^1\text{H-NMR}$ spectrum indicated the presence of a methylenedioxy group (δ 6.20, 2H, s) which is further supported by a positive Labat test (green color)⁹. The remaining four protons of the B-ring were observed as two double doublets (δ 7.47, 2H, dd, $J = 9, 2\text{ Hz}$; δ 6.94, 2H, dd, $J = 9, 2\text{ Hz}$) and could be assigned to the protons at C-2', 6' and C-3', 5', respectively. The $^1\text{H-NMR}$ also showed a peak assignable to a hydroxyl group (δ 6.55, 1H, D_2O exchangeable) at C-4' of ring B. Apart from the molecular ion at m/z 282, the mass spectrum showed other important fragment ions at m/z 164, 165 and 132. The above data clearly revealed that the compound (**1**) is 4'-hydroxy-7,8-methylenedioxy isoflavone closely related to maxima isoflavone H, which is a methylether of this compound previously reported from *T. maxima*⁴. This compound was not reported earlier to which the name maxima isoflavone M is given.

Maxima isoflavone N (**2**) obtained as colorless needles, showed $[\text{M}]^+$ peak at m/z 326 consistent with the molecular formula $\text{C}_{18}\text{H}_{14}\text{O}_6$ and supported further by $^{13}\text{C-NMR}$ data. The UV absorption maxima and the absorption bands in IR spectrum are similar to that of compound (**1**) typical of an isoflavone. The isoflavone nature of this compound is confirmed by its $^1\text{H-NMR}$ spectrum in which the characteristic isoflavone proton of C-2 appeared as a singlet (δ 7.85, 1H, s). The $^1\text{H-NMR}$ also revealed the presence of a methylenedioxy group (δ 6.20, 2H, s), which is also supported by a positive Labat test (green colour). The $^1\text{H-NMR}$ also indicated a doublet due to the proton at C-5 (δ 7.92, 1H, d, $J = 9\text{ Hz}$) and another doublet assigned to the proton at C-6 (δ 7.20, 1H, d, $J = 9\text{ Hz}$) which shows that the position, 7 and 8 are substituted because even if C-8 is free, the H-6 has to appear as a double doublet ($J = 2, 9\text{Hz}$) due to the long range coupling with C-8 proton. The spectrum displayed a singlet (δ 3.91, 6H, s) attributed to the presence of two methoxyl groups. The spectrum also revealed a doublet

(δ 7.40, 1H, d, $J = 9$ Hz), which is assigned to the proton at C-6 and a double doublet (δ 7.00, 1H, dd, $J = 9$ Hz) assigned to the proton at C-5' and a doublet (δ 6.94, 1H, d, $J = 2$ Hz) attributed to the proton at C-3'. Apart from the molecular ion at m/z 326, the mass spectrum showed other important fragment ions at m/z 162 and 164. The fragment ion at m/z 164 clearly indicates the presence of methylenedioxy group on ring A and the fragment ion at m/z 162 corresponds to the B ring with two methoxy groups.

From the above data, the compound was identified as 7,8-methylenedioxy-2',4'-dimethoxyisoflavone (**2**) not reported earlier and was named as maxima isoflavone N.



Maxima isoflavone M (1) : Colorless prisms (MeOH), mp : 190–192°C, UV λ_{\max} (MeOH) nm: 210, 259, 298; IR (KBr) ν_{\max} cm^{-1} : 1622, 1031, 940; $^1\text{H-NMR}$ (CDCl_3 , 400 MHz), δ : 7.89 (1H, s, H-2), 6.20 (2H, s, $-\text{OCH}_2\text{O}-$), 7.87 (1H, d, $J = 9$ Hz, H-5), 6.89 (1H, d, $J = 9$ Hz, H-6), 6.94 (2H, dd, $J = 9, 2$ Hz, H-3', 5'), 7.47 (2H, dd, $J = 9, 2$ Hz, H-2', 6'), 6.55 (1H, s, H-4' D_2O exchangeable). El MS m/z : 282 $[\text{M}]^+$, 165, 164, 118. Anal. Calcd. for $\text{C}_{16}\text{H}_{10}\text{O}_5$: C, 68.90, H, 4.05, O, 27.05; Found: C, 69.12, H, 4.27, O, 26.61.

Maxima isoflavone N (2) : Colorless needles (CHCl_3), mp: 208–210°C, UV λ_{\max} (MeOH) nm: 256, 290; IR (KBr) ν_{\max} cm^{-1} : 1630, 1050, 923; $^1\text{H-NMR}$ (CDCl_3 , 90 MHz), δ : 7.85 (1H, s, H-2), 7.92 (1H, d, $J = 9$ Hz, H-5), 7.20 (1H, d, $J = 9$ Hz, H-6), 6.94 (1H, d, $J = 9$ Hz, H-3'), 7.00 (1H, dd, $J = 9, 2$ Hz, H-5'), 7.40 (1H, d, $J = 9$ Hz, H-6'), 6.20 (2H, s, $-\text{OCH}_2\text{O}-$), 3.91 (6H, s, 2' & 4'-OMe); $^{13}\text{C-NMR}$ (CDCl_3 , 22.5 MHz) δ : 174.19 (C-4), 164.87 (C-4'), 159.41 (C-2'), 153.24 (C-2), 151.99 (C-7), 140.34 (C-8a), 139.47 (C-6'), 134.30 (C-8), 122.99 (C-3), 119.95 (C-5), 119.68 (C-4a), 109.52 (C-1'), 107.34 (C-6), 107.03 (C-5'), 101.22 ($-\text{OCH}_2\text{O}-$), 99.62 (C-3'), 57.44 (2'-OMe), 55.06 (4'-OMe); El-MS m/z : 326 $[\text{M}]^+$, 162, 164; Anal. Calcd. for $\text{C}_{18}\text{H}_{14}\text{O}_6$: C, 65.42, H, 4.03, O, 30.55; Found: C, 66.26, H, 4.32, O, 29.42.

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