

## TWO NEW ISOFLAVONES FROM *TEPHROSIA MAXIMA*

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### ABSTRACT

Two new isoflavones, maxima isoflavone K (**1**) and maxima isoflavone L (**2**) were isolated from the chloroform extract of the roots of *Tephrosia maxima* 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 hemispheres<sup>1</sup>. *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 be having antispasmodic activity<sup>2</sup>. In continuation of our chemical and biological studies<sup>3–7</sup> on the flavonoids of this plant, we report here the isolation and characterization of two new isoflavones, named maxima isoflavone K (**1**) and maxima isoflavone L (**2**), since maxima isoflavones A to J were reported earlier<sup>3–5</sup>.

### EXPERIMENTAL

**General procedures :** Melting points were determined on an Electrothermal Digital melting point apparatus and are uncorrected. IR spectra were recorded in KBr discs on a Perkin–Elmer 881 instrument and UV spectra on a Shimadzu UV–1201 spectrophotometer. The <sup>1</sup>H–NMR and <sup>13</sup>C–NMR spectra were determined on a Bruker AMX 400 MHz spectrometer and 90 MHz, Jeol JNM FT NMR spectrometer using CDCl<sub>3</sub> and DMSO–d<sub>6</sub> with tetramethylsilane (TMS) as an 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 of *T. maxima* (2 kg) were successively extracted with n–hexane, CHCl<sub>3</sub> and MeOH. The n–hexane extract (20 g) on purification over a silica gel column using n–hexane–EtOAc step gradient (9:1) yielded (**2**) (50

mg). The  $\text{CHCl}_3$  extract on purification over silica gel column using n-hexane-EtOAc step gradient (0.5:9.5) yielded (**1**) (20 mg).

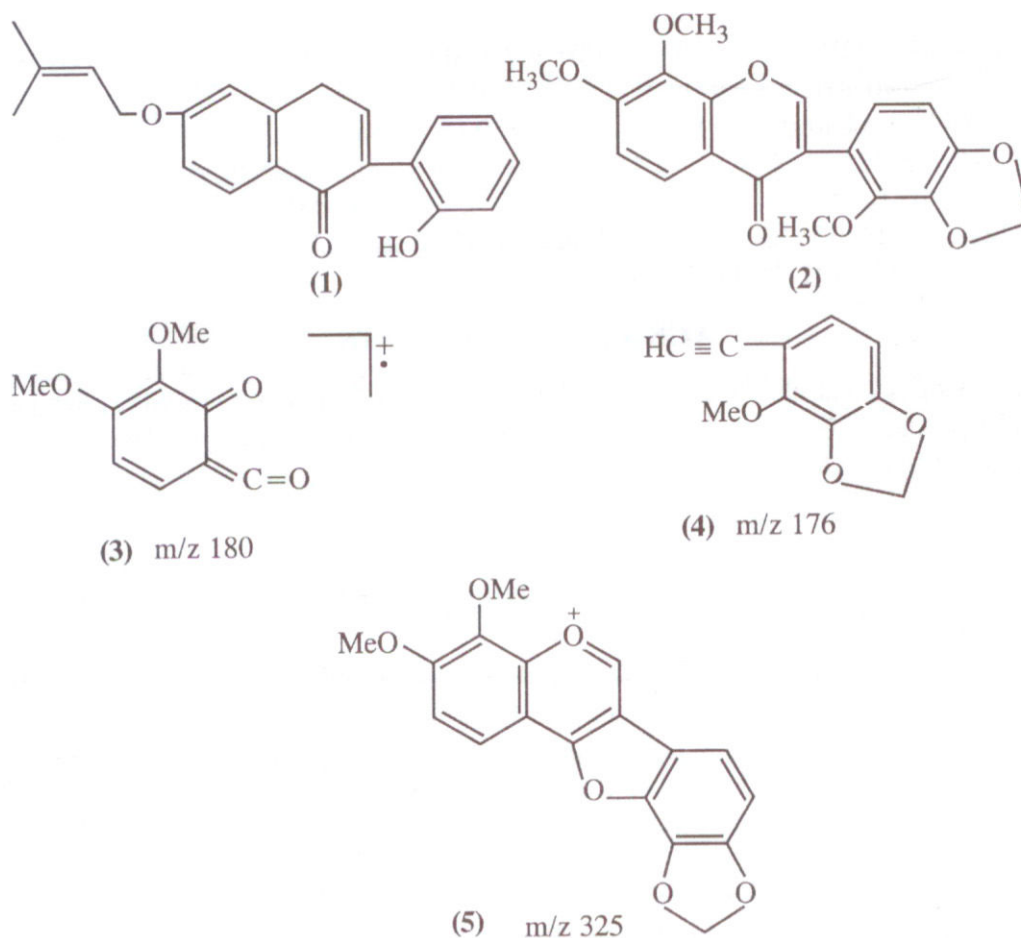
## RESULTS AND DISCUSSION

Maxima isoflavone K (**1**), obtained as yellow needles, showed  $[\text{M}]^+$  peak at  $m/z$  366 consistent with the molecular formula  $\text{C}_{21}\text{H}_{18}\text{O}_6$ . It was supported by  $^{13}\text{C}$ -NMR spectra, which showed 21 carbon resonances. The UV absorption maxima at 248, 252 and 302 nm were typical of an isoflavone skeleton. The IR spectrum of (**1**) exhibited strong absorption bands attributable to carbonyl ( $1660\text{ cm}^{-1}$ ) and a methylenedioxy group ( $1035, 933\text{ cm}^{-1}$ )

The  $^1\text{H}$ -NMR spectrum exhibited a signal attributed to the isoflavone proton at C-2 ( $\delta$  8.01, s), an ortho coupled doublet due to the proton at C-5 ( $\delta$  8.30, 1H, d,  $J = 9$  Hz) and a double doublet attributed to the proton at C-6 ( $\delta$  7.15, 1H, dd,  $J = 9, 2$  Hz). The spectrum also revealed a doublet characteristic of the proton at C-8 ( $\delta$  6.95, 1H, d,  $J = 2$  Hz). The  $^1\text{H}$ -NMR spectrum indicated the presence of methylenedioxy group ( $\delta$  6.02, s), which is further supported by a positive Labat test (green colour)<sup>8</sup>. The  $^1\text{H}$ -NMR also showed peaks assignable to  $\gamma,\gamma$ -dimethylallyloxy group at C-7 and a hydroxyl group at C-2' ( $\text{D}_2\text{O}$  exchangeable). The two singlets at  $\delta$  6.61 and 6.64 each integrating for one proton are assigned to the protons at C-3' and C-6', respectively. The mass spectrum showed apart from the molecular ion at  $m/z$  366, other important fragment ions at  $m/z$  298, 162 and 136. The fragment ion at  $m/z$  298 is a result of loss of prenyl unit ( $M - 68$ ) and the fragment ion at  $m/z$  162 clearly indicated the presence of methylenedioxy group and hydroxyl group in ring B. The fragment ion at  $m/z$  136 is due to the ring A, after loss of prenyl group from  $\gamma,\gamma$ -dimethylallyloxy group at C-7. This data along with the  $^{13}\text{C}$ -NMR data clearly revealed that the compound (**1**) is 7- $\gamma,\gamma$ -dimethylallyloxy-2'-hydroxy-4',5'-methylenedioxyisoflavone not reported earlier to which the name maxima isoflavone K is given.

Maxima isoflavone L (**2**), obtained as colourless needles showed  $[\text{M}]^+$  peak at  $m/z$  356 consistent with the molecule formula  $\text{C}_{19}\text{H}_{16}\text{O}_7$  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 ( $\delta$  8.25, 1H, s). The spectrum also revealed a doublet due to the proton at C-5 ( $\delta$  7.81, 1H, d,  $J = 9$  Hz) and another doublet assigned to the proton at C-6 ( $\delta$  7.27, 1H, d,  $J = 9$  Hz). This clearly indicated that the positions 7 and 8 were substituted. The compound possessed a methylenedioxy group ( $\delta$  6.15, 2H, s) and also gave a positive Labat test for methylenedioxy group. The  $^1\text{H}$ -NMR spectrum displayed three signals ( $\delta$  3.68, 3H, s; 3.96, 3H, s; 3.90, 3H, s) attributed to the presence of three methoxyls. The spectrum revealed two ortho coupled doublets ( $\delta$  7.40, 1H, d,  $J = 9\text{ Hz}$ ;  $\delta$  6.90, 1H, d,  $J = 9\text{ Hz}$ ) assigned to the protons at C-6' and 5'. The fragment ion (**3**), at  $m/z$  180 indicated that the ring A contains two methoxy groups at 7 and

8 positions and the fragment ion (4), at  $m/z$  176 revealed the presence of methylenedioxy group and a methoxy group adjacent to each other in ring B. However, the mass spectrum of this compound confirmed that C-2' has a methoxyl substituent by the characteristic fragment ion (5), at  $m/z$  325 formed by the elimination of methoxy group. So the methylenedioxy group is placed at 3' and 4' of B ring. Moreover methylenedioxy group in B ring was found to be present only at 3' and 4' in the isoflavones earlier isolated from *T. maxima*<sup>3</sup>. The structure is further confirmed by its <sup>13</sup>C-NMR data. Thus the compound is identified as 7,8,2'-trimethoxy-3', 4'-methylenedioxyisoflavone, named as maxima isoflavone L.



Maxima isoflavone K (1): Yellow needles ( $\text{CHCl}_3$ ), mp 143–144°C, UV  $\lambda_{\text{max}}$  (MeOH) nm: 248, 252, 302. IR (KBr)  $\nu_{\text{max}}$   $\text{cm}^{-1}$ : 1660, 1035, 933. <sup>1</sup>H-NMR ( $\text{CDCl}_3$ , 400MHz)  $\delta$ : 8.01 (1H, s, H-2), 6.02 (2H, s,  $-\text{OCH}_2\text{O}-$ ), 8.30 (1H, d,  $J = 9\text{Hz}$ , H-5), 7.15 (1H, dd,  $J = 9, 2\text{Hz}$ , H-6), 6.95 (1H, d,  $J = 2\text{Hz}$ , H-8), 6.61 (1H, s, H-3'), 6.64 (1H, s, H-6'), 4.53 (2H, d,  $J = 2\text{Hz}$ , H-1''), 5.38 (1H, m, H-2''), 1.75 (3H, s, H-4''), 1.82 (3H, s, H-5'') 8.81 (1H, s, OH-5',  $\text{D}_2\text{O}$

exchangeable).  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ , 100MHz)  $\delta$ : 178.37 (C-4), 164.16 (C-7), 157.86 (C-8a), 154.92 (C-2), 100.48 (C-3'), 141.96 (C-5'), 141.53 (C-4'), 139.64 (C-3''), 127.85 (C-5), 125.08 (C-2''), 112.14 (C-1'), 117.40 (C-3), 118.36 (C-4a), 116.22 (C-6), 108.16 (C-6'), 152.26 (C-2'), 101.46 (-OCH<sub>2</sub>O-), 101.48 (C-8), 65.74 (C-1''), 25.83 (C-5''), 18.38 (C-4''). EI-MS  $m/z$ : 366  $[\text{M}]^+$ , 298, 162, 136. Anal. Calcd for  $\text{C}_{21}\text{H}_{18}\text{O}_6$ : C, 68.85; H, 4.95; O, 26.20; Found: C, 68.96; H, 4.87; O, 26.17.

Maxima Isoflavone L (**2**): Colourless needles ( $\text{CHCl}_3$ -MeOH), mp 215–216°C, UV  $\lambda_{\text{max}}$  (MeOH) nm: 248, 256, 305. IR (KBr)  $\nu_{\text{max}}$   $\text{cm}^{-1}$ : 1625, 1060, 920.  $^1\text{H}$ -NMR (DMSO- $d_6$ , 90MHz)  $\delta$ : 8.25 (1H, s, C-2), 7.81 (1H, d,  $J = 9$  Hz, C-5), 7.27 (1H, d,  $J = 9$  Hz, C-6), (2H, s, -OCH<sub>2</sub>O-), 3.68 (3H, s, 2'-OMe), 3.90 (3H, s, 7-OMe), 3.96 (3H, s, 8-OMe), 7.40 (1H, d,  $J = 9$  Hz, H-6'), 6.90 (1H, d,  $J = 9$  Hz, H-5').  $^{13}\text{C}$ -NMR (DMSO- $d_6$ , 22.5MHz)  $\delta$ : 174.63 (C-4), 156.21 (C-7), 154.50 (C-2), 151.82 (C-2'), 150.15 (C-8a), 146.34 (C-4'), 136.78 (C-8), 132.94 (C-3'), 122.37 (C-6'), 122.44 (C-3), 120.69 (C-5), 118.85 (C-4a), 112.53 (C-1'), 111.35 (C-6), 102.52 (C-5'), 101.26 (-OCH<sub>2</sub>O-), 61.02 (8-OMe), 56.97 (7-OMe), 56.76 (2'-OMe). EI-MS  $m/z$ : 356  $[\text{M}]^+$ , 180, 176. Anal. Calcd for  $\text{C}_{19}\text{H}_{16}\text{O}_7$ : C, 64.04; H, 4.53; O, 31.43; Found: C, 65.12; H, 4.65; O, 30.23.

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