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Novel biflavonoids from the leaves of *Leucaena diversifolia* and *Albizia procera* and their protein binding efficiency

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The leaves of *Leucaena diversifolia* and *Albizia procera* have been found to contain two novel biflavonoids, leucaediflavone (1) and albiproflavone (2) which were characterized as (5,2′,3′-trihydroxy-5′,6′-furano-7,8-dimethylallyloxy)-(5,3′-dihydroxy-5′,6′-furano-7,8-dimethylallyloxy)-6*C*-2′-biflavonyl and bis[5,3′,4′-trihydroxy-2′-hydroxymethyl-5′,6′-furano-8,7-(3-hydroxyphenyl]-6*C*-6-biflavonyl, respectively on the basis of spectral (UV, IR, MS, 1D and 2D NMR) evidences. Both showed protein binding efficiency 201.81and 253.05 μg BSA/mg respectively.

Flavonoidal oligomers are well known for their astringent nature causing feed deterrency for the herbivores[1]. In our programme on feed deterrent principles from tree leaves, we herein report for the first time the presence of novel biflavonoids, leucaediflavone and albiproflavone from *Leucaena diversifolia* and *Albizia procera* (Fam. : Leguminosae) leaves, respectively, and their protein binding efficiency.

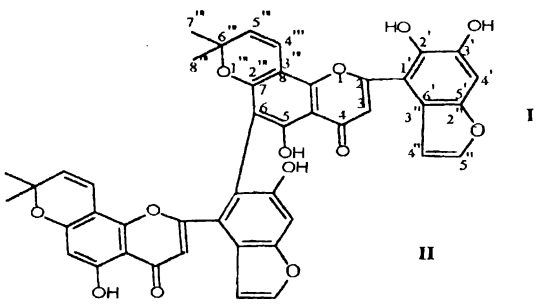
Results and discussion

Leucaediflavone **1**, was obtained as light brown amorphous solid m.p. 190°. It. responded positive test for flavonoid[2] and negative test for sugar[3]. The UV spectrum gave an indication of flavonoid skeleton by showing absorption at 258, 270 and 348 nm[4]. The positive FABMS of **1** showed high molecular ion peak at *m/z* 767 $[M + H]^+$ corresponding to formula $C_{44}H_{30}O_{13}$ in accordance with NMR data indicated it to be biflavonoid. The IR spectrum of **1** exhibited absorption bands at 3460 (OH), 1650 (chelated carbonyl), 1363 (gem dimethyl group), 1510 and 1166 cm^{-1} (fused furan ring). The UV spectral observations with diagnostic shift reagents (experimental) indicated that there were hydroxylation on 5,2′,3′ positions[4]. On the basis of UV data the location of OH group could be assigned on C-5, C-3′ in both the monomers of biflavonoid as well as an OH at C-2′ in I unit. The pentahydroxy nature of **1** was also supported by the ^1H NMR, exhibiting a downfield chemical shift as a sharp singlet at δ 12.5 integrated for two protons of OH groups (D_2O exchangeable)[5] at C-5 in both monomeric units and broad hump between δ 8.1–9.0 suggested the presence of another three phenolic hydroxyl groups on B rings at C-2′, C-3′of unit I and C-3′ of unit II (all D_2O exchangeable)[5]. Diagnostic C-3 protons for biflavonoid structure were confirmed by the presence of singlet at δ 6.64 (2H). Location of three aromatic protons were assigned by the chemical shift appearing as singlet δ 7.37 for C-6 of unit II, and C-4′ of both units. Gem dimethyl grouping adjacent to oxygen with olefinic *cis* protons were attributed due to proton signals at δ 1.21 (6H, s, 2 × Me), 0.83 (6H, s, 2 × Me), 7.4 (2H, d, *J* 9 Hz) and 6.8 (2H, d, *J* 9 Hz)[6]. Presence of two equally shielded furan rings was confirmed by the presence of signals at δ 6.42 (2H, d, *J* 2 Hz) and 6.16 (2H, d, *J* 2 Hz)[6].

In FABMS the molecular ion peak at *m/z* 349 due to double RDA and release of radical CH=C=O indicated linking of ring A of I unit to the ring B of II, and *m/z* 307 followed by appearance of mass ion at *m/z* 217 due to breaking interflavonyl linkage also supported the said structure. The substitution pattern of ring A and B as well as interflavonoid linkage were also established by HMBC experiment (Fig. 1).

All the available features, with the absence of AB system in molecule and biosynthetic consideration[7] led to conclude that the two chromene rings were attached on A rings of biflavonoids on C-7 and C-8 in both the units, and interflavonoid linkage was between C-6 and C-2′. Thus leucaediflavone **1**, could be characterized as (5,2′,3′-trihydroxy-5′,6′-furano-7,8-dimethylallyloxy)-(5,3′-dihydroxy-5′,6′-furano-7,8-dimethylallyloxy)-6*C*-2′-biflavonyl (Fig. 1).

Albiproflavone **2** was obtained as a dark yellow powder



I

II

Leucaediflavone (**1**)

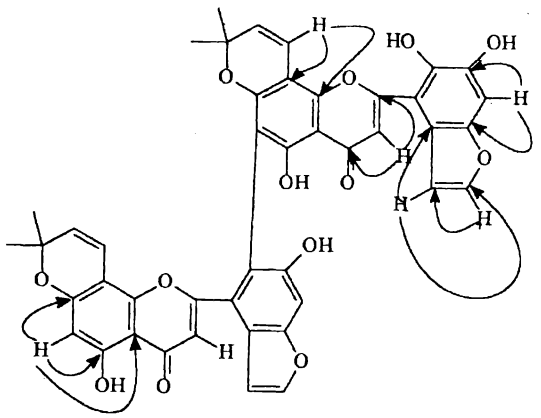
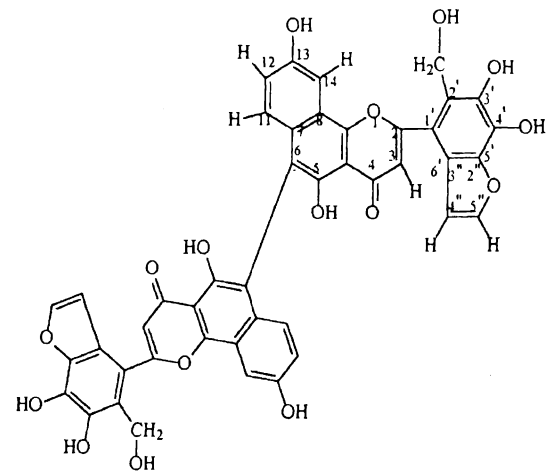


Fig. 1. HMBC correlationship of leucaediflavone.

from leaves of *Albizia procera*, m.p. 216° with molecular formula $C_{44}H_{26}O_{16}$ (FABMS). It also answered test for flavonoid[2] and showed IR absorption at 3419 (hydroxyl), 1656 cm^{-1} (chelated carbonyl). The UV spectrum exhibited characteristic bands of a flavone[4] skeleton at λ_{max} (MeOH) 254, 266 and 348 nm and with shift reagents it was found to be with 5,3′ and 4′ hydroxyl groups[4]. The ^{13}C NMR showed 22 signals for 44 carbons indicating symmetric nature of biflavone molecule. ^{1}H NMR of **2** also displayed the highly shielded chemical shifts as singlet at δ 13.1 and a broad hump from 8.2–9.7 integrating for two proton and six protons (D_2O exchangeable) respectively for OH at C-5, C-3′ and 4′ and C-13 on both the units. The C-3 protons on I and II monomer were inferred by the observation of broad singlet at δ 6.57 (2H). Appearance of two doublets at δ 6.24 (2H, *J* 1.2 Hz) and 6.5 (2H, *J* 1.2 Hz) were indicative of furan rings in molecule[6]. The ^{1}H-^{13}C long range coupling between proton of C-4″ to C-6′ and C-5″ confirmed the fusion of 2″, 3″ of furan to C-5′ and C-6′. Two doublet at δ 7.4 (2H, *J* 8.4 Hz) and 6.98 (2H, *J* 8.4 Hz) were suggestive of *ortho* coupled protons of additional aromatic D rings and a broad singlet at δ 7.49 supported for *meta* coupled proton of C-14. The fusion of D ring to 7 and 8 position of A ring was evident from observed cross peak in HMBC (Fig. 2). FABMS of **2** exhibited the fragment at *m/z* 149 due to attachment of furan at 5′ and 6′ positions of B ring and peak at *m/z* 287 was due to presence of D-I, D-II ring fused with A-I, A-II at 7 and 8 positions. In the aliphatic region the presence of singlet at δ 2.04 (D_2O exchangeable), with a multiple at δ 3.7 led for assignment of CH_2OH. It was further supported by existence of ^{2}J correlation ship with C-2′ and ^{3}J with C-1′ (Fig. 2). Thus albiproflavone **2** could be characterized as bis[5-3′,4′-trihydroxy-2′-hydroxymethyl-5′,6′-furano-8,7-(3-hydroxyphenyl)]-6*C*-6-biflavonyl (Fig. 2).



Albiproflavone (**2**)

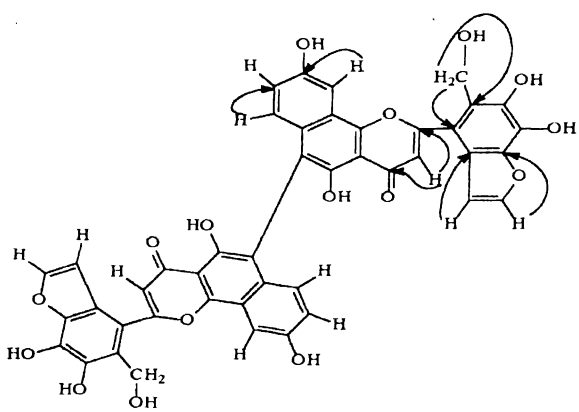


Fig. 2. HMBC correlationship of albiproflavone.

Protein binding efficiency : It was determined by the reported method[9] leucaediflavone **1** and albiproflavone **2** showed the optimum protein binding efficiency as 201.81 μg BSA/mg and 253.05 μg BSA/mg, respectively thus show-

ing the involvement of the dimeric flavone in protein binding similar to condensed tannin in woody plants.

Experimental

M.ps. were determined on Bock Monoscope and are uncorrected. UV spectra were recorded on a Unichem UV-2 spectrophotometer, and IR spectra on a Perkin-Elmer infra cord 157 spectrophotometer, NMR spectra (DMSO-d_6) on a Brucker 300 spectrometer (300 MHz) with TMS as internal standard and FAB mass spectra on a Jeol SX 102/DA-6000 spectrophotometer data system using argon/xenon (6 KV, 10 MA) as the FAB gas.

The leaves of *L. diversifolia* and *A. procera* were collected from the Central Research Farm of the Indian Grassland and Fodder Research Institute, Jhansi.

Extraction and isolation of leucaediflavone (**1**) : Fresh leaves (4 kg) of *L.diversifolia* were homogenized with acetone water (7 : 3) containing 0.1% ascorbic acid 2–3 times. The whole extract was left overnight and filtered. The total acetone extract was reduced to aqueous phase under vacuum. The resultant after washing with each Et_2O and EtOAc was diluted with methanol (1 : 1) to charge on Sephadex LH-20 column (equilibrated with 50% aq. methanol) and eluted with 50% methanol. The pooled eluants (50% methanol) were rechromatographed over Sephadex LH-20 column and eluted with methanol : water in different ratio. The fractions from methanol : water (3 : 7), were concentrated and lyophilized as leucaediflavone (**1**) (R_f = 0.72 PC, BAW); λ_{max} (MeOH) (log ε) 258 (6.39), 270 (6.3), 348 (6.45) nm; (NaOMe) 275 (6.32), 402 (6.47) nm; ($AlCl_3$), 272 (6.21), 300 (6.06), 416 (6.47) nm; ($AlCl_3$ + HCl) 262 (6.36), 276 (6.36), 294 (6.36), 360 (6.2), 382 (6.2) nm; (NaOAc) 268 (6.37), 414 (6.31) nm; ($NaOAc/H_3BO_3$), 258 (6.32), 366 (6.32) nm; ν_{max} (KBr) 3460 (OH), 1650 (chelated >C=O), 1363 (gem dimethyl), 1513, 1166 (furan) cm^{-1}; FABMS (+ve mode) *m/z* 767 $[M + H^+]$ ($C_{44}H_{30}O_{13}$); δ_H (DMSO-d_6) 6.64 (2H, s, 2 × H-3), 7.34 (3H, s, H-6, 2 × H-4′), 7.4 (2H, d, *J* 9, 2 × H-4‴), 6.8 (2H, d, *J* 9, 2 × H-5‴), 1.21 (6H, s, 2Me), 0.83 (6H, s, 2Me), 6.42 (2H, s, 2 × H-4″), 6.16 (2H, s, 2× H-5″), 12.5 (2H, s, OH, D_2O exchangeable) and 8.9–9.0 (3H, s, OH, D_2O exchangeable); δ_c (DMSO-d_6) 163.5 (C-2 I, II), 106.8 (C-3 I,II), 180.2 (C-4 I,II), 162.3 (C-5 I,II), 110.3 (C-6 I), 94.3 (C-6 II), 145.3 (C-7,2‴ I), 145.8, 107.3 (C-8,3‴ I,II), 157.2 (C-9 I,II), 105.3 (C-10 I,II), 125.5 (C-1′ I), 127.5, 149.5 (C-2′ I,II), 112.3 (C-2′ II), 152.3 (C-3′ I), 151.4 (C-3′ II), 113.5 (C-4′ I,II), 144.3 (C-5′,2″ I,II), 104.3 (C-6′,3″ I,II), 110.9 (C-4″ I,II), 119.4 (C-5″ I,II), 114.9 (C-4‴ I,II), 129.4 (C-5‴ I,II), 78.5 (C-6‴ I,II), 28.4 (C-7″,8‴ I,II).

Extraction and isolation of albiproflavone (**2**) : Fresh leaves (4.5 kg) of *A. procera* were processed as described earlier and after usual work up the aqueous phase in methanol : water (1 : 1) was chromatographed over Sephadex LH-20 column and eluates collected with 50% methanol were subjected for preparative TLC (Si-gel, EtOAc : MeOH : H_2O, 12 : 1 : 1) to yield the product as dark yellow solid (R_f 0.85); λ_{max} (MeOH) (log ∈) 254 (6.49), 266 (6.3), 348 (6.55) nm; (NaOMe) 266 (6.5), 402 (6.62) nm; ($AlCl_3$) 272 (6.79), 302 (6.52), 326 (6.0), 426 (6.75) nm; ($AlCl_3$/HCl) 274 (6.6), 294 (6.71), 362 (6.63), 384 (6.65) nm; (NaOAc) 268 (6.79), 400 (6.52) nm; ($NaOAc/H_3BO_3$) 258 (6.7), 366 (6.7), 376 (6.75) nm; υ_{max} (KBr) 3419 (OH), 1656 (chelated CO), 1610, 1124 (furan) cm^{-1}; *m/z* 811 $[M + H^+]$ ($C_{44}H_{26}O_{16}$); δ_H (DMSO-d_6) 6.57 (2H, s, 2 × H-3), 7.49 (2H, s, 2 × H-14), 7.4 (2H, d, *J* 8.4, 2 × H-12), 6.9 (2H, d, *J* 8.4, 2 × H-11), 3.7 (4H, m, 2 × H-1‴), 6.24 (2H, d, *J* 1.2, 2 × H-4″), 6.5 (2H, d, *J* 1.2, 2 × H-5″), 13.01 (2H, s, 2 × H-5, D_2O exchangeable), 8.2–9.7 (6H, b hump, 2 × H-3′,4′, 13D_2O exchangeable), 2.04 (2H, s, 2 × H-1‴, D_2O exchangeable); δ_c (DMSO-d_6) 162.9 (C-2 I,II), 102.8 (C-3 I,II), 181.3 (C-4 I,II), 157.2 (C-5 I,II), 111.2 (C-6 I,II), 113.4 (C-7 I,II), 114.3 (C-8 I,II), 149.3 (C-9 I,II), 102.8 (C-10 I,II), 110.6 (C-11 I,II), 107.3 (C-12 I,II), 142.3 (C-13 I,II), 109.4 (C-14 I,II), 122.9 (C-1′ I,II), 128.8 (C-2′ I,II), 158.3 (C-3′ I,II), 162.1 (C-4′ I,II), 148.9 (C-5′,2″ I,II), 128.8 (C-6′, 3″ I,II), 110.3 (C-4′ I,II), 119.4 (C-5″ I,II), 42.30 (C-1‴ I,II).

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