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Flavonoids from *Acacia tortilis*†

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A novel isoflavone, 5,7-dihydroxy-4′- *p*-methyl benzyl isoflavone **1a**, and three known flavonoids apigenin, luteolin and quercetin have been isolated from the leaves of *Acacia tortilis*. Their structures were elucidated by chemical and physical data (IR, UV, ^{1}H-NMR, ^{13}C-NMR and MS spectra).

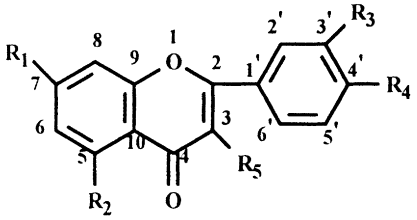
Keywords: flavonoids, *Acacia tortilis*

The genus *Acacia* comprising over 500 species, found in the warmer, drier parts of the World, chiefly in Arabia, Australia and Africa.[1] In India, there are about 22 indigenous species, distributed throughout the plains. Some of the *Acacia* species are of considerable value for reafforestation and reclamation of waste land. They are the good sources of tannin, gum and timber.[1] *Acacia tortilis* (Syn: A. raddiana Savi) (Leguminosae) was found to be a very useful source of protein.[2] The acid digest of cell wall constituents fibres and cellulose found in the leaves provide nutrients for the animals as fodder.[3] It is also used for the relaxation of smooth muscle.[4] Earlier investigations of this plant described the isolation of apigenin glycoside,[5] quercetin glycoside and isorhaminetin glycoside from leaves,[6] and *n*-hexacosanol, betulin, α-, β-amyrin and β-sitosterol from the stem bark.[7] Medicinal properties and scanty work on this plant stimulated our interest to carry out its comprehensive investigation. We now report here the isolation and characterisation of a novel flavonoid 5,7-dihydroxy-4′-*p*-methyl benzyl isoflavone **4a**, along with the three known flavonoids, apigenin, luteolin and quercetin from the leaves of *Acacia tortilis*.

The dried and powdered leaves of *Acacia tortilis* (3 kg) procured from Yeman, were exhaustively extracted with light petroleum ether (60–80), benzene and finally with methanol respectively. The methanol extract was concentrated by heating over a boiling water bath under reduced pressure, and a brown gummy mass was obtained. It gave a positive colour test for flavonoids.[8] TLC examination in benzene–pyridine–formic acid (BPF, 36:9:5) and toluene–ethylformate-formic acid (TEF, 5:4:1) systems showed it to be a mixture of several compounds. The brown gummy mass was purified by refluxing it with petroleum ether, benzene and chloroform. The semi-solid mass left behind was chromatographed over silica gel column. Fractional elution with benzene–ethylacetate (1:1) and ethylacetate yielded four compounds. They were purified by repeated crystallisation and labeled as **At-1**, **At-2**, **At-3** and **At-4**.

The compound **At-1**, **At-2** and **At-3** was characterised as apigenin, luteolin and quercetin by comparison of the IR, UV, ^{1}H NMR, ^{13}C-NMR and MS spectra with these of authentic sample.[9-12]

At-4 was eluted from the column by ethylacetate and was crystallised from methanol as pale yellow granular crystals, m.p. 168°C analysing for $C_{23}H_{18}O_4$. **At-4** gave a greenish brown colour with alcoholic ferric chloride, and a pink colour with sodium amalgam / HCl and a yellow colour with conc. H_2SO_4[13]. The colour test and UV absorption, λ_{max}262 and inflection at 339 nm indicated an isoflavone nucleus. This was further supported by a singlet in its ^{1}H-NMR spectrum at δ 7.86 ascribed to H-2 proton of isoflavone. A red shift of 10 nm with $AlCl_3$ and 11 nm with NaOAc showed the presence of hydroxyl group at the 5 and 7 positions which was further confirmed by the appearance of the singlets at δ_H 12.46 and δ_H 9.27 in the ^{1}H-NMR spectrum. The ^{1}H-NMR spectrum displayed a singlet integrating for three protons at δ 2.50 corresponding to methyl group and a pair of meta-coupled doublets of one proton each at δ_H 6.17 (*J*=2.5 Hz) and 6.40 (*J*=2.5 Hz), attributed to H-6 and H-8 protons of ring-A respectively. Another pair of ortho coupled doublets of four protons each at δ_H 6.89 (*J*=9 Hz) and 7.56 (*J*=9 Hz) were assigned to H-3′,5′,3″,5″ and H-2′, 6′,2″,6″ respectively. A solitary one proton singlet at δ_H 7.86 was ascribed to the H-2 proton of isoflavone. The CH_2 protons appeared at δ_H 2.59. On acetylation, **4a** afforded a diacetate **4b**, which revealed the presence of two acetoxyl groups by the two independent singlets of three protons each at δ_H 2.45 (5-OH) and 2.35 (7-OH) in its ^{1}H-NMR spectrum. Its ^{13}C-NMR revealed the presence of a carbonyl group at δ 180.0 and four oxygenated carbons at 153.5 (C-2), 161.5 (C-5), 163.7 (C-7) and 158.1 (C-9). The CH_2 carbon appears at δ 31.8 and CH_3 carbon at 19.1 while the assignments of other carbons are given in (Table 1). The assigned structure was further supported by the MS spectrum (Scheme 1) which showed the molecular ion peak at *m*/*z* 358. The retro-Diels-Aider fragments appeared at *m*/*z* 152, 206 and the base peak was observed at *m*/*z* 91 corresponded to a tropylium ion. On the basis of above studies it was characterized as the novel isoflavone named as 5,7-dihydroxy-4′-p-methylbenzyl isoflavone **4a**.



	R_1	R_2	R_3	R_4	R_5
1a	OH	OH	H	OH	H
1b	OAc	OAc	H	OAc	H
2a	OH	OH	OH	OH	H
2b	OAc	OAc	OAc	OAc	H
3a	OH	OH	OH	OH	OH
3b	OAc	OAc	OAc	OAc	OAc

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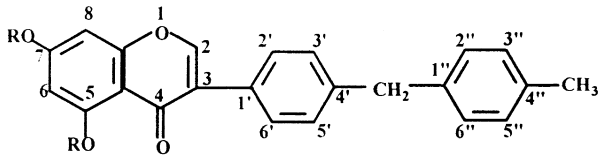
† This is a Short Paper, there is therefore no corresponding material in *J Chem. Research (M)*.



4a R=H
4b R=Ac

Experimental

The melting points were taken on a Kofler block and are uncorrected. All UV spectra were measured on Beckmann Model DU and Pye Unicam PU-8800 spectrophotometers in methanol / ethanol. IR spectra were taken on Shimadzu IR-408 Perkin Elmer 1800 (FTIR). The MS and ^{1}H NMR spectra were obtained from different institutes. The MS spectra were mostly measured in E.I. mode on Jeol D-300 while, the ^{1}H NMR spectra were usually recorded on JEOL 4H-100 MHz, Brucker DPX 200 MHz and 270 MHz and in $CDCl_3$ / DMSO-d_6 using TMS as internal standard. The silica gel used for different chromatographic purposes, was obtained from E. Merck (India), E. Merck (Germany) and SRL (India). TLC solvent systems used were benzene-pyridine-formic acid (BPF, 36:9:5) and toluene-ethylformate-formic acid (TEF, 5:4:1). Alcoholic ferric chloride solution, were used as spray reagents for visualization of spots on TLC.

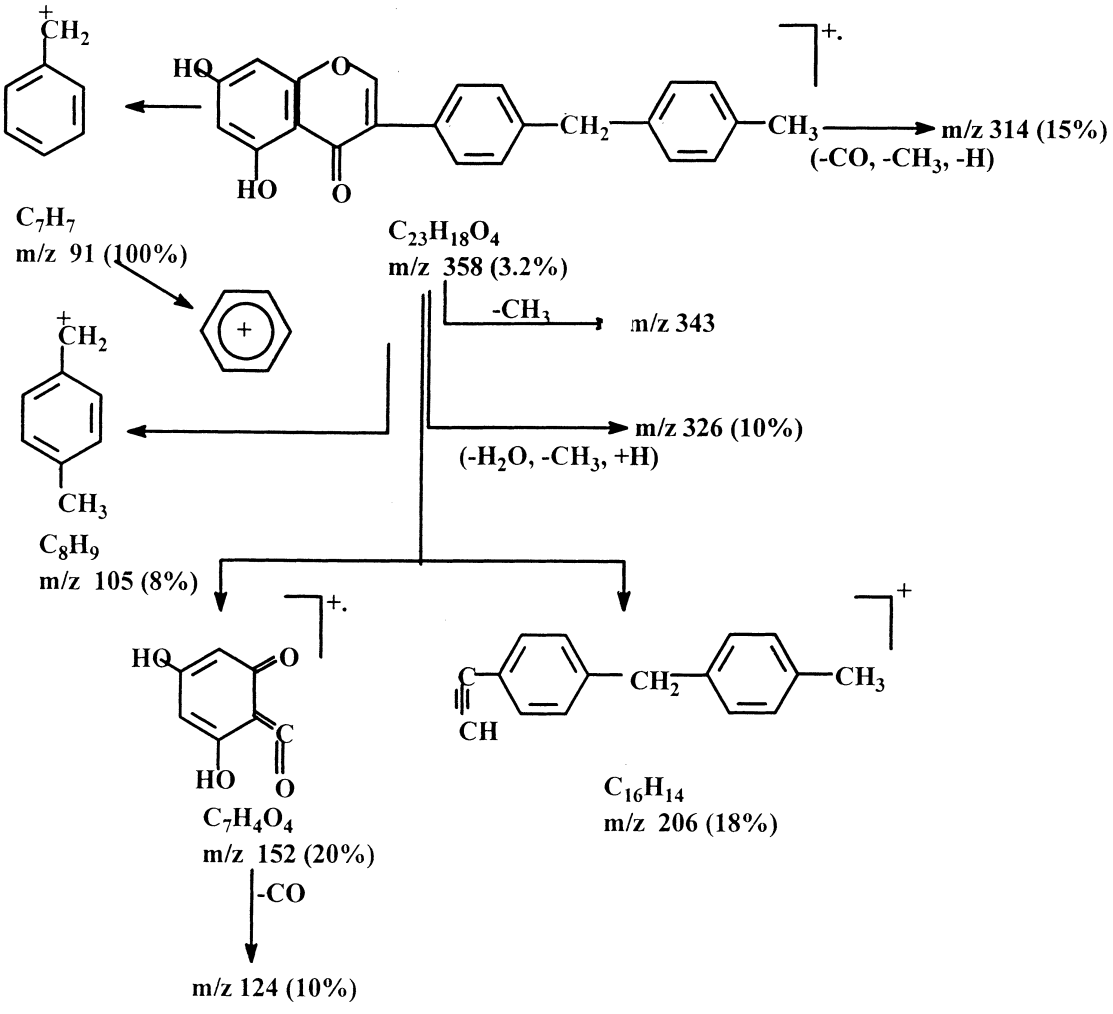
Extraction and isolation: Leaves of *Acacia tortilis* were collected from Yemen in the month of March. The dried and powdered leaves of *Acacia tortilis* (3 kg) were exhaustively extracted with light petroleum ether (60–80), benzene and finally with methanol. The methanol extract was concentrated by heating over a boiling water bath under reduced pressure, a brown gummy mass was obtained. It gave a positive colour test for flavonoids[8]. TLC examination in TEF and BPF systems showed it to be mixture of several compounds. The brown gummy mass was purified by refluxing it with petroleum ether, benzene and chloroform. The semi-solid mass left behind was chromatographed over silica gel column.

Fraction eluted with benzene–ethylacetate (1:1) followed by crystallisation with benzene–acetone yielded *apigenin* (**At-1**) **1a** obtained as yellow shining crystals. m.p. 352°C. (Found: C, 66.78; H, 3.74. $C_{15}H_{10}O_5$ requires: C, 66.66; H, 3.70%). UV λ_{max}/nm (MeOH) 265,297 sh, 338; + $AlCl_3$ 279, 300, 340, 390;+ $AlCl_3$/HCl 279, 299, 340, 389; + NaOAc 279, 304, 376; + NaOAc/H_3BO_3 266, 300 sh, 338; ^{13}C-NMR (200 MHz, DMSO-d_6) Table 1.

Acetylation of ***At-1***: Crystalline (**At-1**) (25 mg) was treated with acetic anhydride (2 ml) and pyridine (1 ml) and allowed to stand overnight at room temperature and then heated on a water bath for 2 hours. After usual work up as described earlier, the solid obtained on several crystallization from chloroform-methanol, gave colourless crystals **1b** m.p. 183–84°C. ^{1}H-NMR (100 MHz, $CDCl_3$) δ 7.85 (2H, d, *J*=9 Hz, H-2′,6′), 7.04 (2H, d, *J*=9 Hz, H-3′,5′), 6.60 (1H, s, H-3), 6.66 (1H, d, *J*=2.5 Hz, H-8), 6.51 (1H, d, *J*=2.5 Hz, H-6), 2.42 (3H, s, OAc-5), 2.35 (6H, s, OAc-4′,7).

Further elution of the column with benzene-ethylacetate (1:1) followed by crystalisation by ethylacetate–acetone yielded *Luteolin* (**At-2**) **2a** obtained as yellow fine crystal, m.p. >315°C. (Found: C, 62.94; H, 3.50. $C_{15}H_{10}O_6$ requires: C, 62.93; H, 3.49%). IR υ_{max}/cm^{-1} (KBr) 3400 (OH), 1640 (>C=O), 800, 840; UV λ_{max}/nm (MeOH) 258, 265, 292 sh, 346; + NaOMe 296, 328 sh, 396; + $AlCl_3$/HCl 267 sh, 295 sh, 355, 384; + NaOAc 291, 326 sh, 377; + NaOAc/H_3BO_3 277, 291 sh, 360, 432 sh; ^{13}C-NMR (200 MHz, DMSO-d_6) Table 1.

Acetylation of **At-2**: Crystalline (**At-2**) was treated with acetic anhydride (2 ml) and pyridine (1 ml). It was allowed to stand



Scheme 1

Table 1 ^{13}C-NMR spectral data of **At-1**, **At-2**, **At-3** and **At-4**

Carbon	At-1(1a)	At-2 (2a)	At-3 (3a)	At-4 (4a)
C_2	164.0	164.4	146.8	153.5
C_3	162.7	103.2	135.7	124.1
C_4	181.7	182.1	175.8	180.0
C_5	157.2	157.8	156.1	161.5
C_6	98.7	99.1	98.2	99.6
C_7	163.6	164.6	163.9	163.7
C_8	93.9	94.1	93.4	94.5
C_9	161.4	162.0	160.7	158.1
C_{10}	103.6	104.1	103.0	105.4
C1′	121.2	119.2	122.0	121.4
C2′	128.3	113.7	115.1	132.3
C3′	116.0	146.2	145.1	118.1
C4′	161.1	150.1	147.7	131.5
C5′	116.0	116.4	115.7	118.1
C6′	128.3	122.0	120.0	132.3
C1″	–	–	–	131.9
C2″	–	–	–	139.7
C3″	–	–	–	123.1
C4″	–	–	–	138.8
C5″	–	–	–	123.1
C6″	–	–	–	139.7
CH_2	–	–	–	31.8
CH_3	–	–	–	19.1

Spectrum run at 200 MHz in DMSO-d_6.

overnight at room temperature and then heated on a water bath for 2 hours. After usual work up, it was crystallised with chloroform–methanol as colourless needles **2b** m.p. 200–201°C. ^{1}H-NMR (270 MHz, $CDCl_3$) δ 6.45 (1H, d, *J*=2.5 Hz, H-6), 6.59 (1H, s, H-3), 6.95 (1H, d, *J*=2.5 Hz, H-8), 7.25 (1H, d, *J*=9 Hz, H-5′), 7.75 (1H, dd, J_1=9 Hz and J_2=2.20 Hz, H-6′), 7.80 (1H, d, *J*=2.20 Hz, H-2′), 2.43 (3H, s, OAc-5), 2.35 (3H, s, OAc-7), 2.33 (6H, s, OAc-3′,4′). EI-MS (70 eV) *m/z* 286 $[M]^{+\bullet}$, 153 $[A_1+H]^{+\bullet}$, 134 $[B_1]^{+\bullet}$.

Further elution of the column with benzene–ethylacetate (1:1) followed by crystallisation by methanol yielded *quercetin* (**At-3**) **3a** obtained as yellow crystals, m.p. 311–12°C. (Found: C, 59.70; H, 3.33. $C_{15}H_{10}O_7$ requires: C, 59.62; H, 3.31%). UV λ_{max}/nm(MeOH) 256, 270 sh, 301 sh, 372; + NaOMe 247 sh, 321 (Dec);+$AlCl_3$ 274, 304 sh, 334, 458;+$AlCl_3$/HCl264, 358, 427;+ NaOAc 257 sh, 274, 329, 390;+NaOAc/H_3BO_3 264, 303 sh, 389; ^{13}C-NMR (200 MHz, DMSO-d_6) Table 1.

Acetylation of **At-3**: Crystalline (**At-3**) (20 mg) was acetylated by heating it with acetic anhydride (2 ml). It was pyridine (1 ml). It was allowed to stand overnight at room temperature and then heated on a water bath for 2 hours. The reaction mixture was cooled to room temperature and poured onto crushed ice. The solid was collected, washed with water and dried. On crystallisation from methanol it gave cream coloured crystals of **3b** m.p. 194–95°C (10 mg). ^{1}H-NMR (100 MHz, $CDCl_3$) δ 7.74 (1H, d, *J*=2.5 Hz, H-2′), 7.63 (q, J_1=2.5 Hz, J_2=8.5 Hz, H-6′), 6.92 (1H, d, *J*=8.5 Hz, H-5′), 6.87 (1H, d, *J*=2.5 Hz, H-8), 6.65 (1H, d, *J*=2.5 Hz, H-6), 2.35–2.40 (15H, m, 5 × OAc).

Finally, elution of the column with ethylacetate gave a fraction which on crystallisation with methanol afforded *5,7-dihydroxy-4′-p-methyl benzyl isoflavone* (**At-4**) **4a**, which was obtained as pale yellow crystals m.p168°C yield (30 mg). It was analysed for $C_{23}H_{18}O_4$; IR υ_{max}/cm^{-1}(KBr) 2980 (br, OH), 1670, 1480 (C=C); UV λ_{max} /nm (MeOH) 262, 295, 339; +NaOMe 267, 338 (Dec);+ $AlCl_3$ 272, 299, 367;+ $AlCl_3$/HCl 273, 369;+ NaOAc 273, 324;+ NaOAc/H_3BO_3 269, 296 (Dec); ^{1}H-NMR (200 MHz, DMSO-d_6) δ 2.50 (3H, s, CH_3), 2.59 (2H, s, CH_2), 6.17 (1H, d, *J*=2.5 Hz, H-6), 6.40 (1H, d, *J*=2.5 Hz, H-8), 6.89 (4H, d, *J*=9 Hz and 2.5 Hz, H-3′,5′,3″,5″), 7.56 (4H, d, *J*=9 Hz and 2.5 Hz, H-2′,6′,2″,6″), 7.86 (1H, s, H-2), 2.59 (2H, s, CH_2), 9.27 (1H, brs, 7-OH), 12.46 (1H, s, 5-OH). EI-MS (70 eV) *m/z* 358 ($M^{+\bullet}$) (3.2%), 314 ($M^{+\cdot}$-CO-CH_3-H, 15%), 326 ($M^{+\cdot}$-H_2O-CH_3+H, 10%). RDA fragment 152 (2%), 124 (152-CO, 10%), 91 (100%), 206 (18%). ^{13}C-NMR (200 MHz, DMSO-d_6) Table 1.

Acetylation of **At-4**: Crystalline (**At-4**) (10 mg) was treated with acetic anhydride (2 ml) and pyridine (1 ml). It was allowed to stand overnight at room temperature and then heated on a water bath for 2 hours. After usual work up as described earlier, the solid obtained on several crystallisations from chloroform-methanol gave white crystals, **4b** m.p. 130°C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 2.45 (3H, s, OAc-5), 2.35 (3H, s, OAc-7), 2.52 (3H, s, CH_3), 2.60 (2H, s, CH_2), 6.2 (1H, d,*J*=2.5 Hz, H-6), 6.44 (1H, d, *J*=2.5 Hz, H-8), 6.92 (4H, d, *J*-9 Hz and 2.5 Hz, H-3′,5′,3″,5″), 7.5 (4H, d, *J*=9 Hz and 2.5 Hz, H-2′,6′,2″,6″), 8.02 (1H, s, H-2).

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References

1 Council of Scientific and Industrial Research, Delhi, *The Wealth of India*, Raw materials, 1948, vol. **I**, p. 4.
2 Harsh and H.C. Bohra, *Botany Study Center*, ICAR Publication, 1985, **25**, 1.
3 R.W. Wrangham and P.G. Waterman, *J. Animal Ecol*, 1981, **50**, 715.
4 M. Hagos, G. Samuelsson, L. Kennel and B.M. Modawi, *Plant Medica*, 1987, **53**, 27.
5 H. Thieme and A. Khogali, *Pharmazie*, 1974, **29**, 352.
6 R.P. Rastogi and B.N. Mehrotra, *Compend. Ind. Med. Plants*, 1998, **5**, 6.
7 L. Prakash and M. Singh, *J. Ind. Chem. Soc*., 1986, LXIIII.
8 J. Shinoda, *J. Chem. Pharm. Soc*., Japan, 1928, **48**, 214.
9 J.B. Harbone, *Comparative Biochemistry of the flavonoids*, Academic Press, 1967.
10 T.J. Mabry, K.R. Markham and M.B. Thomas, *The systematic identification of the Flavonoids*, Springer, New York, 1970.
11 V.M. Chari, R.J. Grayer-Barkmerjer, J.B. Harbone and G. Osterdahi, *Phytochemistry*, 1981, **20**, 1977.
12 H. Wagner and V.M. Chari. *Tetrahedron Lett*., 1976, **21**, 1799.
13 T.A. Geissman, *The Chemistry of Flavonoid Compounds*, Pergamon Press, Oxford, London, 1964, p. 72.