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New flavonoid from the stem of *Pongamia pinnata*

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Abstract : Flavonoid glycoside 3,7,4'-trihydroxy-3'-methoxyflavone-5-*O*-[-β-D-xylopyranosyl (1→2)]-β-D-galactopyranoside 1 have been isolated from the stem of *Pongamia pinnata*. The structures have been established on the basis of chemical evidences and spectroscopic method.

(**Keywords:** *Pongamia pinnata*, 3,7,4'-trihydroxy-3'-methoxyflavone-5-*O*-[−β-D-xylopyranosyl (1→2)]-β-D-galactopyranoside)

Introduction

Pongamia pinnata L. a species of tree belonging to the family Leguminosae, subfamily Papilionaceae, is distributed from India to Northern Australia and Fiji in the east[1]. Various parts of this plant are used medicinally to treat eye diseases, lucoderma, piles, wounds, ulcer, skin diseases and painful rheumatic joints[2]. Earlier studies of *Pongamia pinnata* have afforded a number of flavones[3-7]. This paper describes the isolation and structure elucidation of new flavone glycosides from the stem of the *Pongamia pinnata*.

Experimental

The plant material was collected from Gwalior, M.P., India in June 2000. All mp's are uncorrected. TLC was carried out on silica gel G (Merck 7734) with solvent system benzene : ethyl acetate (1 : 9 v/v)

IR and UV spectra (KBr) were recorded on Perkin-Elmer and Beckman-DK2 spectrophotometer respectively. ^{1}H NMR spectra of **1** were recorded at 400 MHz in $CDCl_3$ and ^{13}C NMR spectra at 100 MHz in $CDCl_3$ in FT mode. Mass spectra were recorded on a JEOL SX 102/ DA-6000 mass spectrometer.

Results and Discussion

Compound **1**, $C_{27}H_{30}O_{16}$ $(M^+ 610)$, mp 265°C, was a flavonoe glycoside which on hydrolysis gave aglycone **1a** and two sugar moieties. Both sugars were identified as D-xylose and D-galactose on the basis of co-paper chromatography with authentic samples.

The aglycone **1a**, $C_{16}H_{12}O_7$ $(M^+ 316)$, gave the characteristic colour reactions of flavonoid. This was confirmed by red colour with magnesium and hydrochloric acid (Shinoda test)[8]. It gave positive pew's test[9,10] and a bright yellow colour with zirconium oxychloride and citric acid[11] suggested that it to be a 3-hydroxyflavone or flavonol, which was further supported by its UV spectrum. The aglycone showed the presence of four hydroxyl and one methoxy groups as revealed by IR, ^{13}C and ^{1}H NMR spectral studies.

IR spectrum of the aglycone showed an absorption peak of OH group at 3465 cm^{-1} and two absorption peak at 1670 and 1595 cm^{-1} for an a, b-unsaturated ketone (C_4- of flavone).

The absorption peak at 2870 and 1200 cm^{-1} showed the presence of methoxy group. In ^{1}H NMR spectrum one singlet at d 3.96 corresponding to three protons, showed the presence of one methoxy group in the aglycone. The presence of methoxy group was further confirmed by ^{13}C NMR signals at d 61.3 ppm.

The relative positions of the one methoxy group and four hydroxy groups were determined by specific colour reactions and spectral data.

The compound was soluble in aqueous sodium carbonate it provided evidence for the presence of phenolic group at C-7 and C-4' positon[12], which was also evidenced by its UV spectrum showing a bathochromic shift of 20 nm in band II with sodium acetate[13] and 48nm in band I with sodium methoxide in methanol[14] respectively.

The aglycone gave a positive colour test with vanillin hydrochloric acid reagent (reagent for 1,3 dihydroxy group) confirms the presence of 5,7-hydroxy group[15]. The presence of 5-hydroxyl group was confirmed by bathochromic shift of 59nm in band I with $AlCl_3$/HCl.

^{1}H NMR spectrum of the aglycone exhibited signals at d 6.34 (s, 1H, *J*=2.5 Hz), d 6.57 (s, 1H, *J*=2.5 Hz), 7.93 (d, 1H, *J*=2.5 Hz), 7.05 (d, 1H, *J*=9Hz) and 7.91 (dd, 1H, *J*=2.5 and 9Hz) corresponding to five aromatic protons. The lower d value (6.34) showed the presence of proton at position 6 in the ring-A.

As no vacant position was available in the ring-A, so all the three remaining protons were present in the ring-B. Coupling constant (*J*) values of these protons indicated that H-2' is meta coupled, H-5' is ortho coupled and H-6' is both ortho and meta coupled. This clearly showed that all the three protons are present in the ring-B. Thus, the position of protons are at C-6, C-8, C-2', C-5' and C-6'. Such appearance is indicative of a 3'4'- disubstituted B-ring in **1a**.

In ^{13}C NMR spectrum the absorption at 148.02(s) and 61.3(q) also confirms that methoxy group must be present at C-3'. This was also confirmed by comparing NMR spectrum of the compound with 5,7,4'-trihydroxy-3'-methoxy flavonol[16, 21] (isorhamnetin). Methylation of **1** with dimethyl sulphate followed by acid hydrolysis gave 5-hydroxy-3,7,3',4'-tetramethoxy flavone[17,18] showing that the site of bioside attachment at C-5. This attachment was also confirmed by ^{13}C NMR and UV spectral shift of glycosides and aglycone after addition of conventional shift reagent[19].

Hydrolysis with 7% H_2SO_4 gave an aglycone and two sugar moieties. The sugars were identified as D-galactose and D-xylose. ^{1}H NMR spectrum of **1** also showed two anomeric proton signals of D-galactose at d 5.27 (d, 1H, *J*=5.2Hz) and D-xylose at 4.89 (d, 1H, *J*=6.5Hz) which indicated b-linkage with the aglycone[20].

Hydrolysis of **1** with b-glycosidase gave D-xylose but not the galactose. This confirmed that xylose was a terminal sugar unit and galactose was directly linked to aglycone. Thus, glycoside **1** was identified as 3,7,4'-trihydroxy-3'-methoxyflavone-5-*O*-[-β-D-xylopyranosyl (1→2)]-β- D-galactopyranoside.

Extraction and Isolation

The air-dried and crushed stem (4.5kg) was exhaustively extracted with boiling ethanol. The concentrated extract (6.0g) was chromatographed over a silica gel column by eluting with organic solvents of increasing polarity. Elution with benzene : ethyl acetate (3 : 7, v/v)) yielded compound **1**.

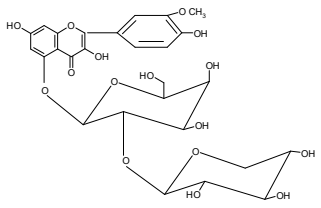
Compound **1**, mp 265°C, yield 400mg, homogeneous on TLC, R_f 0.53 solvent C_6H_6 : $CHCl_3$ (4 : 6, v/v); Anal. Found: C, 56.90; H, 5.62; Calcd. for $C_{33}H_{40}O_{17}$: C, 56.93; H, 5.69%. UV (MeOH)nm: 268, 360; + $AlCl_3$/HCl : 268, 420; + NaOAc: 283, 360; + NaOMe: 268, 415. IR (KBr) : 3450, 2870, 1650, 1590, 1190 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): d 6.34 (s, 1H, *J*=2.5 Hz), d 6.57 (s, 1H, *J*=2.5 Hz), d 7.93 (d, 1H, *J*=2.5 Hz), 7.05 (d, 1H, *J*=9Hz) and d7.91 (dd, 1H, *J*=2.5 and 9Hz), d 5.75 (d, 1H, *J*=7.5Hz), d 4.88 (d, 1H, *J*=5.2Hz) and 3.25-3.89 (m, 11H, sugar protons); MS : m/z 610[M$^+$] 595, 582, 316, 301, 288, 261, 164, 151, 123, 121, 55;

^{13}C NMR data are given in Table-I.

Table I- ^{13}C NMR spectra of compounds **1, 1a,**

C	1	1a
2	164.2(s)	164.3(s)
3	133.2(s)	133.2(s)
4	181.6(s)	181.5(s)
5	162.2(s)	156.6(s)
6	99.8(d)	99.8(d)
7	148.9(s)	148.9(s)
8	166.3(s)	166.0(s)
9	160.4(s)	160.2(s)
10	108.7(s)	108.7(s)
1'	122.2(s)	122.2(s)
2'	112.4(d)	112.4(d)
3'	147.0(s)	147.1(s)
4'	149.0(s)	149.8(s)
5'	115.2(d)	115.2(d)
6'	122.8(d)	122.7(d)
1"	29.0(t)	29.0(t)
2"	27.0(d)	27.2(d)
3"	29.5(s)	29.5(s)
4"	24.4(q)	24.4(q)
5"	24.5(q)	24.5(q)
–OCH_3	60.2(q)	60.2(q)
galactose 1	110.2(d)	
2	83.8(d)	
3	71.9(d)	
4	67.4(d)	
5	74.6(d)	
6	60.8(t)	
xylose 1	105.2(d)	
2	75.2(d)	
3	73.6(d)	
4	70.4(d)	
5	67.1(t)	

▪



3, 7 4'-trihydroxy-3'-methoxyflavone-5-O{-*b*-D-xylopyranosyl (1→2)}-β-D-galactopyranoside **1**

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