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CHEMICAL CONSTITUENT FROM THE PODS OF *CASSIA FISTULA*

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ABSTRACT

The present paper shows the isolation of glycoside of flavonoid from the pods of medicinal plant *Cassia fistula*. The structure determination based on chemical evidences, chromatographic and spectroscopic method and identified as 3, 5, 3', 4' -tetrahydroxyflavone-7-*O*-[-β-D-arabinopyranosyl (1→2)] -β-D-arabinopyranoside.

KEYWORDS: Flavone, Chromatographic, *Cassia Fistula,* Phytochemical Investigation

INTRODUCTION

Cassia fistula (Amaltas) *is a* tree of medium sized with bright yellow flowers generally growing at roadside and gardens having long cylindrical pods containing 20-25 seeds. *Cassia fistula* has anti-inflammatory, antibacterial, antifungal, and wound healing properties. Most of the *Cassia* species are rich source of tannins, flavonoids glycosides, stearic acids, oleic, oxalic, linoleic, lupeol, sitosterol and hexacosanol, oxyanthraquinones and anthraquinones derivatives.

In continuation of our research on the phytochemical investigation of medicinal plants, flavonoid glycoside was isolated from pods of *Cassia fistula*. The water insoluble portion of the cold ethanolic extract of the air-dried and crushed pods of *Cassia fistula* on sintered column chromatography yield compound **1**.

Compound **1**, $C_{25}H_{26}O_{14}$ (M^+ 566), mp 202°C, was a non-reducing glycoside. On acid, hydrolysis with 7% sulphuric acid it gave an aglycone **1a** and a sugar moiety. The sugar was identified by co-chromatography as D-arabinose.

The aglycone **1a**, $C_{15}H_{10}O_7$ (M^+ 302) gave characteristic colour reactions of flavonol. The flavonol nature of the compound was identifed by its positive Shinoda test and pew,s test. These reactions suggested that it may be a 3-hyroxyflavone i.e. flavonol. The compound also gave yellow colour with zirconium oxychloride indicating the presence of free hydroxyl group at C-5, also confirmed by its UV spectrum showing a bathochromic shift of 49nm in band I in the presence of $AlCl_3$ and HCl and shift of 18nm in the band II with NaOAc and of 37nm in band I with $AlCl_3$ shows the presence of four hydroxy groups at 5, 7 and 3' 4' positions in flavonol respectively.

Acetylation of aglycone gave pentaacsetate due to the presence of five hydroxyl groups in the compound. The ^{1}H-NMR and ^{13}C-NMR spectrum of flavonol also confirmed the presence of five protons in aromatic region, confirmed that compound is tetra substituted flavonol.

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The ^{13}C NMR spectrum of the compound showed singlet at 108.5, 151.2, 167.5, 154.6 and 148.2 which indicated the presence of hydroxyl group at C-3, C-5, C-7, C-3' and C-4' respectively. (**Table 1**). Thus aglycone is 5,7,3',4'-tetrahydroxy flavonol. The site of glycosidation were found to be at C-7 on the basis of bathochromic shift with NaOAc in UV and ^{1}H-NMR and ^{13}C NMR spectral data of compound **1**.

Quantitative estimation and molecular weight difference of compound **1** and aglycone **1a** suggested the presence of two moles of sugar per mole of aglycone and signals of anomeric protons in ^{1}H-NMR at δ 4.99 (1H, d, J=5.5 Hz) 5.79 (1H, d, J=7.5 Hz) and 3.25-3.68 (m, 10H, sugar protons) confirmed that glycoside was disaccharide. The glycoside on permethylation by Hakamori method followed by acid hydrolysis yielded two compounds namely 3, 4 -di-*O*-methyl-D-arabinose and 2,3,4-tri- *O* -methyl -D-arabinose. Above result confirmed that interglycosidic linkage was (1→2) between sugars.

From the above evidences, the structure of compound 1 was determined as 3, 5, 3', 4' -tetrahydroxyflavone-7-*O*-[-β-D-arabinopyranosyl (1→2)] -β-D-arabinopyranoside.

EXPERIMENTAL SECTION

The pods of *Cassia fistula* were collected from the roadside area of a highway, near Allahabad, U.P., India. TLC were carried out on silica gel G with increasing solvent polarity. IR and UV spectra (KBr) were recorded on Perkin-Elmer and Beckman-DK2 spectrophotometer respectively. ^{1}H NMR spectra of **1** were recorded at 300 MHz in C_6D_6 and ^{13}C NMR spectra at 100 MHz in C_6D_6. Mass spectra were recorded at 70 eV.

EXTRACTION AND ISOLATION

The air-dried and finely crushed pods (6kg) of *Cassia fistula* were repeatedly extracted with ethanol (4x10*l*), concentrated under reduce pressure in a rotatory evaporator and were extracted successively with different solvents of increasing polarity over a sintered column. Elution with solvent by pods hexane: EA (9:1, v/v) compound **1**.

Compound **1**, mp 202°C, yield 455mg, homogeneous on TLC, R_f 0.59 solvent hexane: EA (9:1, v/v); Anal. Found: C, 53.01%; H, 4.62%; Calcd. For $C_{25}H_{26}O_{15}$: C, 53.25%; H, 4.59%. UV(MeOH) nm: 275, 374; +$AlCl_3$: 255, 392; +$AlCl_3$/HCl : 263, 408; +NaOAc : 269, 344. ^{1}H NMR ($CDCl_3$, 300MHz): 5.89 (d, 1H, J=2.2Hz, H-6), 5.90 (d, 1H, J=2.2Hz, H-8), 6.52 (d, 1H, J=2.5Hz, H-2'), 6.83 (d, 1H, J=7.5Hz, H-5'), 7.62 (dd, 1H, J=7.6 and 2.5Hz, H-6'), 11.92 (s, 1H), 4.99(d, 1H, J=5.5Hz), 5.79 (d, 1H, J=7.5Hz), 3.25-3.68 (m, 10H, sugar protons). MS: m/z 566 $[M^+]$ 302, 301, 274, 153, 152, 137, 134; ^{13}C NMR data are given in **Table-I**.

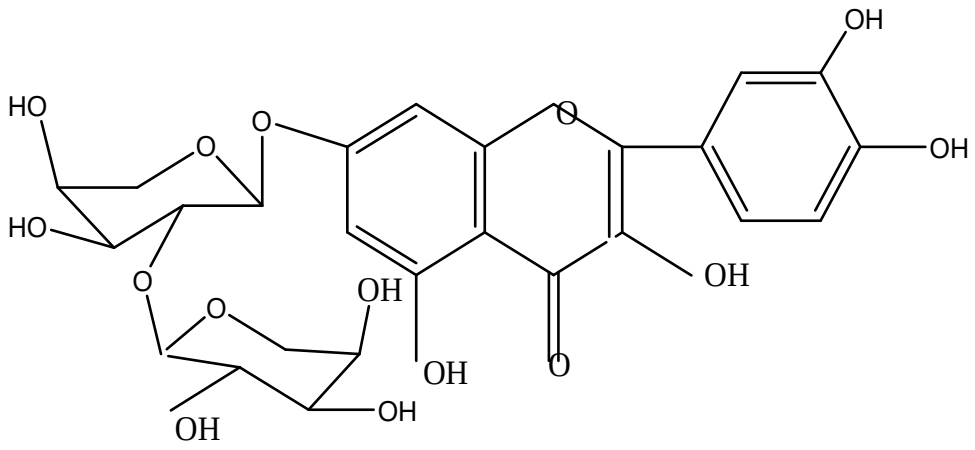

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Table I.^{13}C NMR spectra of compounds 1, 1a,

C	1	1a		
2	165.3(s)	164.3(s)	arabinose 1	88.3(d)
3	108.5(s)	107.4(s)	2	79.5(d)
4	210.4(s)	210.4(s)	3	73.6(d)
5	151.2(s)	151.2(s)	4	76.3(d)
6	99.8(d)	99.4(d)	5	36.7(t)
7	167.5(s)	160.3(s)		
8	104.4(d)	102.4(d)	arabinose 1	99.29(d) 2. 71.5(d)
9	118.8(s)	116.8(s)		
10	116.9(s)	114.2(s)	3	69.6(d)
1'	137.2(s)	136.2(s)	4	68.4(d)
2'	115.5(d)	115.5(d)	5	37.1(t)
3'	154.6(s)	154.5(s)		
4'	148.2(s)	148.3(s)		
5'	115.9(d)	115.9(d)		
6'	120.8(d)	120.6d)		

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