Int. J. Chem. Sci.: 7(3), 2009, 2135-2140

5, 7, 3′, 4′-TETRAHYDROXY – 3 - METHOXY-FLAVONE – 7 – O - β - D – GLUCOPYRANOSYL - (1→4) – O - β - D - GALACTOPYRANOSIDE FROM THE SEEDS OF *COCHLOSPERMUM RELIGIOSUM*

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ABSTRACT

The methanol soluble part and the concentrated ethanol extract of the seeds of the plant *Cochlospermum religiosum,* were subjected to various colour reactions and chemical degradations, UV, IR, ^{1}H NMR and mass spectroscopic analyses identified the constituent as 5, 7, 3′, 4′-tetrahydroxy-3-methoxy-flavone-7-O-β-D-glucopyranosyl-(1→4)-O-β-D-galactopyranoside.

Key words : *Cochlospermum religiosum*, Flavon glycoside

INTRODUCTION

Cochlospermum religiosum[1-3] belongs to natural order cochlospermaceae and is commonly known as “Kumbi” in Hindi, “Katira” in Arabic and “Yellow silk” cotton” in English. It is distributed in the Garhwal, Bundelkhand, Bihar, Orissa, Bengal, Madhya Bharat and Madras state. The plant is a small medium deciduous tree, with thick fibrous light coloured bark. It has strong gereral resemblance to the silk cotton trees. The gum of the plant is sweetish, cooling and sedative, stomachic, good in gonorrhoea, syphilis, asthma, eye troubles and trachoma. It softens the skin and is used in cough. In Punjab, the leaves and flowers are given as stimulants. Bookbinder and shoemakers use it in their manufacture. In cosmetics and icecreams, it finds use as thickener during manufacture.

EXPERIMENTAL

Material and methods

Air-dried defatted, powdered and defalted seeds of the plant *Cochlospermum*

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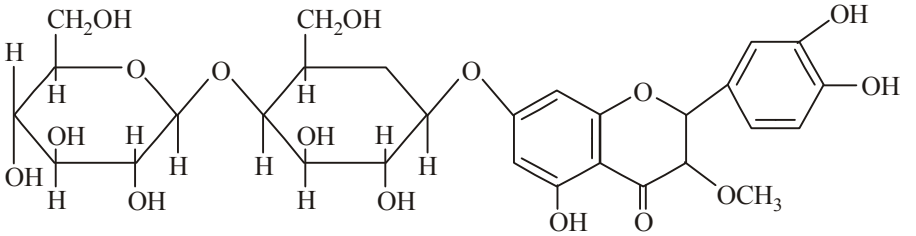
religiosum were extrated with 95% ethanol and the extract was concentrated under reduced pressure to yield brown viscous mass, which was successively extracted with petroleum ether (60-80°C), C_6H_6, EtOAc and MeOH. The methanol soluble part was chromatographed on Silica-gel G column using $CHCl_3$-MeOH in various proportions. The fractions 6-14, on evaporation of solvent gave amorphous compound glycoside, which was purified by preparative TLC and column chromatography. The glycoside, which was crystallized from Et_2O gave light yellow crystalline needles (2.87 g) $C_{28}H_{32}O_{17}$, mp 281-283ºC, M^+ 640 (found : C, 52.53 H, 4.87.calcd : C, 52.58; H, 4.85). It gave single spot on TLC (C_6H_6 : AcOH : H_2O, 5 : 3 : 2) on silica gel-G; IR (KBr) ν_{max} 3355 (OH), 2985 (C-H stretching) and 2872 (OMe), 1648 (α, β-unsaturated C = O), 1552 (arornatic ring system), 1270 (C-O-C stretching) and 1075 (O-gly) cm^{-1}; UV (MeOH) λ_{max} 256-355; (+ NaOMe) 258, 270; (+ $AlCl_3$) 256, 280; ($AlCl_3$/HCl) 257, 375; (+NaOAc) 259, 260, 356 nm, (+NaOAc/H_3BO_3) 258, 384 nm. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.34 (1H, s, H-6), 6.51 (1H, s, H-8), 3.86 (3H, s, OMe), 7.22 (1H, d, J = 2.5 Hz, H-2', 6.98 (1H, d, J = 8.4 Hz, H-5'). 7.45 (1H, d, J = 2.6 Hz, H-6'); 2.41 (3H, s, OAc-3'), 2.40 (3H, s, OAc-4'), 1.86-2.17 (21 H, m, sugar 7 x OAc), 4.81-5.50 (10H, m, sugar H's), 4.56 (1H, d, J = 7.6 Hz H-1', galactose) and 6.09 (1H, d, J = 8.1 Hz, H-1''', glucose)[1].



Flavonoidal glycoside

Acid hydrolysis of the flavonoidal glycoside

50 mg of the glycoside was refluxed with 10% H_2S_4O (10 mL) for 5 hrs at 100°C and on cooling, a precipitate was obtained, which was treated with diethyl ether. The ethereal layer was washed with water to dryness and the residue was chromatographed over silica-gel G using $CHCl_3$: MeOH (2 : 1) to gave aglycone $C_{16}H_{12}O_7$, mp 214-215°C, [M^+] m/z 316 (found : C, 60.84 H, 3, 80; calcd C, 60.75; H, 3.79). The aglycone was identified as 5, 7, 3', 4'-tetrahydroxy-3-methoxy flavone by comparison of its spectral data. The aqueous hydrolysate after neutralization with $BaCO_3$, was subjected to PC using solvent n-BuOH-AcOH-H_2O (4 : 1 : 5) and aniline hydrogen phthalate as spraying reagent. The sugars were identified as galactose and glucose (R_f 0.16 and R_f 0.18), respectively (by

Co-PC and Co-TLC will authentic samples.

Alkaline degradation of the aglycone

The alkaline degradation of the glycoside was done by refluxing the aglycone (80 mg) with 50% KOH and EtOH (5 mL) for 24 h. The reaction mixture was cooled and neutralized with HCl (10%) and extracted with diethyl ether. The ethereal layer was treated with 50% $NaHCO_3$ and the aqueous portion on acidification yielded a compound $C_7H_6O_4$, m.p. 201-202°C, M^+ 154 (found : C, 54.38; H, 3.73; calcd : C, 54.46; H, 3.89) and it was identified as 3, 4-dihydroxy benzoic acid. The aqueous phase was treated with 10% NaOH and on acidification gave the compound m. f. $C_8H_8O_4$, mp 156-157°C, M^+ 168 (found : C, 56.05; H, 4.64 calcd : C, 57.14; H, 4.76), which was identified as 2, 4, 6-trihydroxy acetophenone.

The permethylation of the glycoside

60 mg of the glycoside was treated with MeI (4 mL) and Ag_2O (40 mg) in dimethyl formamide (10 mL) in a 150 mL conical flask and left for 40 hours, at room temperature. The contents were filtered, washed with DMF and then hydrolysed with 10% ethanolic H_2SO_4 to give permethylated aglycone $C_{19}H_{21}O_{10}$ (46 mg), mp 246-247°C, M^+ m/z 409, UV (MeOH) λ_{max} 258, 355 nm (+NaOMe), 260, 301, 355, (+NaOAc); 258, 302, 396, (+$AlCl_3$), 259, 356 ($AlCl_3$/HCl); 259, 354 nm. IR (KBr) ν_{max} 3536, 2910, 2872, 1656, 1625, 1276, 1150 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) : δ 6.30 (1H, s, H-6), 6.50 (1H, s, H-8), 7.25 (1H, d, J = 2.2 Hz, H-2'), 7.22 (1H, d, J = 8.8 Hz, H-5'), 7.48 (1H, d, J = 9.2 Hz, H-6') 3.88 (3H, s, OMe), 3.90 (3H, s, OMe), 3.82 (3H, s, OMe), 3.75 (3H, s, OMe). The permethylated aglycone, was identified as 3, 5, 3', 4'-tetramethoxy-7-hydroxy flavone and methylated sugars were identified as 2, 3, 6, -tri-O-methyl-D-galactose and 2, 3, 4, 6-tetra-O-methyl-D-glucose.

RESULTS AND DISCUSSION

The methanol soluble fraction of the ethanolic extract of the seeds *Cochlospermum religiosum* yielded a flavone glycoside m. f. $C_{28}H_{32}O_{17}$, mp 281-283°C, M^+ 640. It was crystallized from methanol in yellow crystalline needles. It responded positive to Molisch[5] and Shinoda[6] tests indicating it to be a flavonoidal glycoside. It also showed characteristic colour reactions of flavonoids. It's UV spectrum showed two peaks at 256 and 355 nm with MeOH, which are characteristic of flavonoids[7]. A bathochromic shift of 16 nm in band I with NaOMe and 24 nm in band 1 with $AlCl_3$/HCl suggested free hydroxyl groups

at C-4' and C-5 positions, in the glycoside. The presence of o-dihydroxy groups in ring B was confirmed due to the bathochromic shift of 16 nm in band I with $NaOAc/H_3BO_3$ on addition of MeOH[8]. The absence of any characteristic shift with NaOAc indicated the hydroxyl group at C-7 and absence of bathochromic shift in band II on addition of $AlCl_3$ with MeOH confirmed the presence of $–OCH_3$ at C-3 in the flavone glycoside[9].

The acid hydrolysis of the flavone glycoside yielded an aglycone m. f. $C_{16}H_{12}O_7$, mp 214-215°C, $[M^+]$ m/z 316 and D-glucose (R_f 0.17) and D-galactose (R_f 0.15) as sugar moieties. The aglycone was identified as 5, 7, 3', 4'-tetrahydroxy-3-methoxy flavone, by comparison of it mp, UV IR, ^{1}H- NMR and MS data with authentic sample. The sugars were identified as D-glucose and D-galactose by Co-PC and Co-TLC with authentic sample.

The flavone glycoside, on acetylation with Ac_2O/pyridine gave a deca acetate derivative m. f, $C_{38}H_{62}O_{37}$, mp 203-204°C. It's ^{1}H NMR showed a singlet of three proton intensity at δ 3.84, which indicated the presence of a methoxy group. In the glycoside, the protons of ring B showed ABX coupling pattern of, 3', 4' dioxygenation and showed meta coupled of one proton intensity at δ 7.29 (J = 2.6 Hz) for H-2' proton and orthocoupled doublet of one proton intensity at δ 6.99 (J = 8.6 Hz) for H-5' proton. A doublet at δ 7.48 showed both ortho (J = 8.6 Hz) and meta (J = 2.7 Hz) coupling. Two singlets at δ 6.33 and δ 6.52, each of one proton intensity were assigned to H-6 and H-8 protons, respectively and doublets at δ 4.56 (J = 7.6 Hz) and δ 6.05 (J = 8.0 Hz), each of one proton intensity, were assigned for the anomeric proton of D-glucose and D-galactose. Two sharp singlets at δ 2.30 and δ 2.40, each of three proton intensity, were assigned to phenolic acetoxyl at C-3' and C-4' position, respectively. A multiplet of 12 hydrogen intensity, in the range of δ 4.51-5.50 was obtained for the remaining sugar protons and a multiplet of 21 proton intensity in the range of δ 1.80-2.16 was assigned to the remaining sugar acetoxyls. The ^{1}H NMR of the acetylated derivative conformed the presence of free OH group at C-5, which was not acetylated because of presence of strong intra molecular hydrogen bonding with 4-keto group in the glycoside[10].

The species formed in the mass spectrum of the glycoside were in full agreement with the proposed structure. The mass spectrum showed the base peak at m/z 316 (M^+-Me), which is characteristic of 3-methoxy flavone. The RDA fragment at m/z 152 showed the presence of two hydroxyl groups in ring A, while another fragmentation at m/z 110 indicated the presence of two hydroxyl groups in the ring B of the aglycone. The structure of aglycone was confirmed by alkaline degradation, when it yielded two products

identified as 2, 4, 6-trihydroxy acetophenone[11], $C_8H_8O_4$, [M^+] 157, mp 158°C and 3, 4-dihydroxy benzoic acid $C_7H_6O_4$, M^+ 154, mp 200-201°C[12].

The permethylation of the flavone glycoside with (Mel/Ag_2O/DMF) followed by it's hydrolyes with 10% HCl gave the compound $C_{19}H_{21}O_{10}$, mp 246-247ºC identified as 3, 5, 3', 4'-tetramethoxy-7-hydroxy flavone by study of its ^1H NMR, UV and IR spectral data and the methylated sugars 2, 3, 6, -tri-O-methyl-D-galactose and 2, 3, 4, 6, -tetra-O-methyl-D-glucose, which also revealed that Cl"-OH of the galactose was linked to the C_7-OH of the aglycone and C4"-OH of galactose linked to C1"-OH of glucose, showing inter-sugar glycosidic linkage as (1→4).

Enzymatic hydrolysis of the flavone glycoside

About 10 mg of the flavone glycoside was mixed with enzyme almond emulsion (120 mL) and treated in a round-bottomed flask (50 mL) at 25°C for 30 Hrs and the liberated D-glucose (R_f 0.18) and D-galactose (R_f 0.16), were identified by Co-PC and Co-TLC, using BAW (4 : 1 : 5) as solvent system and aniline hydrogen phthalate phthalate as spraying reagent.

The quantitative estimation of sugar in the glycoside was done according to the procedure of Mishra and Rao[13] which revealed that both the sugars were present in the equimolar ratio (1 : 1). The periodate oxidation[4] of the glycoside showed that both the sugars were present in the pyranose form.

The enzymatic hydrolysis libeated aglycone, D-glucose and D-galactose, further confirming the presence of β-linkage between aglycone and galactose as well as between galactose and glucose.

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Accepted : 20.05.2009