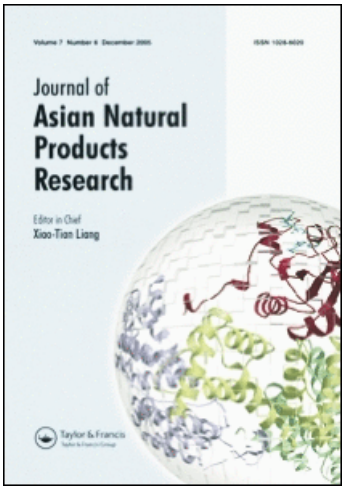

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A new flavone glycoside: 5,7,3',4'-tetrahydroxy-3-methoxy flavone-7- O -β- d -galactopyranosyl-(1→4)- O -β- d -glucopyranoside from the stem of Acacia catechu willd

R. N. Yadava[a]; Sheetal Sodhi[a]
[a] Natural Products Laboratory, Department of Chemistry, Dr. H.S. Gour University, Sagar, M.P., India

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A NEW FLAVONE GLYCOSIDE: 5,7,3′,4′-TETRAHYDROXY-3-METHOXY FLAVONE-7-*O*-β-D-GALACTOPYRANOSYL-(1 → 4)-*O*-β-D-GLUCOPYRANOSIDE FROM THE STEM OF *ACACIA CATECHU* WILLD

R.N. YADAVA* and SHEETAL SODHI

Natural Products Laboratory, Department of Chemistry, Dr. H.S. Gour University, Sagar, M.P., 470003, India

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A new bio-active flavone glycoside, m.p. $C_{28}H_{32}O_{17}$, mp 283–284°C, M^+640 [EIMS] was isolated from the ethylacetate soluble fraction of the ethanolic extract of the stems of *Acacia catechu* and its structure was characterised as 5,7,3′,4′-tetrahydroxy-3-methoxy flavone-7-*O*-β-D-galactopyranosyl-(1 → 4)-*O*-β-D-glucopyranoside by various chemical degradations and spectral analyses.

Keywords: *Acacia catechu*; Leguminosae; Flavone glycoside; 5,7,3′,4′-Tetrahydroxy-3-methoxyflavone-7-*O*-β-D-galactopyranosyl-(1 → 4)-*O*-β-D-glucopyranoside

INTRODUCTION

Acacia catechu (*Leguminosae*) [1–3] is commonly known as "khair" in Hindi. It is widely distributed in India, especially in the Deccan. The plant extract is used medicinally as an astringent in fevers. The bark is bitter and acrid and is used as antipyretic and anthelmintic; cures sore throat, itching, indigestion, inflammations, anaemia, ulcers, boils, and snake bite. Earlier workers [4,5] have reported about the spectral and stereochemical studies on protein biological values of seeds of this plant.

RESULTS AND DISCUSSION

The EtOAc soluble fraction of the ethanolic extract of the stems of *A. catechu* yielded a new compound **1**, $C_{28}H_{32}O_{17}$, mp 283–284°C, M^+640. It was crystallised from ethanol as yellow crystalline needles. It gave positive response to Molisch [6] and Shinoda [7] tests

*Corresponding author. Tel. +91-07582-26465. E-mail: rnyadava@yahoo.com

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indicating **1** to be a flavonoidal glycoside. It also showed characteristic colour reactions of flavonoids [8,9]. The UV spectrum of **1** showed two peaks at 257 and 356 nm with MeOH that are characteristic of flavonoids [8]. A bathochromic shift of 15 nm in band I with NaOMe, and 22 nm in band **1** with $AlCl_3$/HCl suggested free hydroxyl groups at C-4′ and C-5 positions, respectively. The presence of *o*-dihydroxy groups in ring B was confirmed by the bathochromic shift of 15 nm in band I with NaOAc/H_3BO_3 with MeOH. Absence of any characteristic shift with NaOAc indicated a blocked hydroxyl at C-7 [9] and absence of bathochromic shift in band II on addition of $AlCl_3$ with MeOH confirmed the presence of $-OCH_3$ at C-3 [10].

Acid hydrolysis of compound **1** yielded an aglycone **2**, $C_{16}H_{12}O_7$, mp 214–216°C, [M^+] *m/z* 316 and D-glucose (R_f 0.17) and D-galactose (R_f 0.15) as sugar moieties. The aglycone **2** was identified as 5,7,3′,4′-tetrahydroxy-3-methoxy flavone, by comparison of its mp, UV, IR, ^{1}H-NMR, and MS data with literature values [11]. The sugars were identified as D-glucose and D-galactose by Co-PC and Co-TLC.

The compound **1**, on acetylation with Ac_2O/pyridine gave a deca acetate derivative **3**, $C_{38}H_{62}O_{37}$, mp 204–205°C. ^{1}H NMR of **3** showed a singlet of three proton intensity at δ 3.85, which indicated the presence of a methoxy group. Protons of ring B showed ABX coupling pattern, fixing 3′,4′ dioxygenation and showed meta coupled doublet 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 double 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 6.08 ($J = 8.1$ Hz), each of one proton intensity, were assigned for the anomeric proton of D-glucose and D-galactose. Two sharp singlets at δ 2.31 and δ 2.42, each of three proton intensity, were assigned to phenolic acetoxyl at C-3′ and C-4′ positions, respectively. A multiplet of 12 hydrogen intensity, in the range of δ 4.52–5.52, was obtained for the remaining sugar protons and a multiplet of 21 proton intensity in the range of δ 1.82–2.17 was assigned to the remaining sugar acetoxyls. The ^{1}H NMR of **3** indicated the presence of free OH group at C-5, which was not acetylated because of presence of strong intramolecular hydrogen bonding with 4-keto group [11].

The MS data of **3** was in full agreement with the proposed structure **1**. Molecular ion peak as expected was not observed. The MS 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 hydroxy groups in ring A, while a fragmentation at *m/z* 110 indicated the presence of two hydroxy groups in the ring B of the aglycone. The ^{13}C-NMR of **2** is in accord with the proposed structure (**1**). In ^{13}C NMR spectrum, the chemical shifts at δ 156.3 for C-2, δ 138.5 for C-3 and δ 178.1 for C-4, were indicative of 3-*O*-methyl etherification [12] and a shift at δ 178.22 revealed the presence of carbonyl group at C-4. The structure of aglycone **2** was confirmed by alkaline degradation which yielded two products identified as 2,4,6-trihydroxy acetophenone [13], $C_8H_8O_4$, [M^+] 157, mp 158°C and 3,4-dihydroxy benzoic acid [14] $C_7H_6O_4$, M^+154, mp 200–201°C.

Permethylation of glycoside **1** (MeI/Ag_2O/DMF) followed by acid hydrolysis with 10% HCl afforded compound **4**, $C_{19}H_{21}O_{10}$, mp 247–248°C identified as 3,5,3′,4′-tetramethoxy-7-hydroxy flavone by study of its ^{1}H-NMR, UV and IR spectral data (see Experimental) and the methylated sugars 2,3,6-tri-*O*-methyl-D-glucose and 2,3,4,6-tetra-*O*-methyl-D-galactose were identified according to Petek [15], which also revealed that C_1''–OH of the glucose was linked to the C_7–OH of the aglycone **2** and C4″–OH of glucose linked to C_1'''–OH of galactose, showing inter-sugar glycosidic linkage (1 → 4).

Quantitative estimation of sugar according to the procedure of Mishra and Rao [16] revealed that both the sugars were present in the equimolar ratio (1:1). Periodate oxidation [17] of **1** showed that both the sugars were present in the pyranose form.

Enzymatic hydrolysis of **1** with almond emulsin liberated aglycone, D-glucose and D-galactose, confirming the presence of β-linkage between aglycone and glucose as well as between glucose and galactose.

EXPERIMENTAL SECTION

General Experimental Procedure

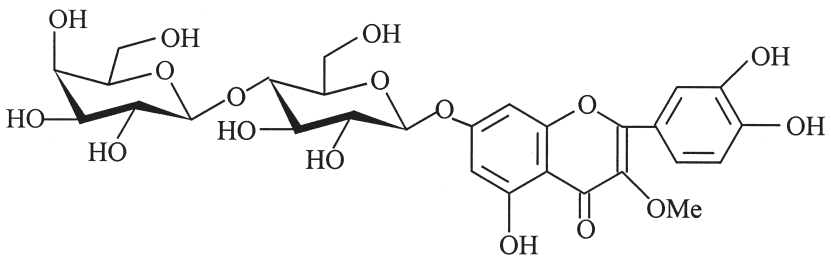
UV spectra were taken on Hitachi 320, IR spectra were run on a Perkin Elmer 1800(FTIR) spectrometer, Mass spectra were recorded on a Jeol-D-300 spectrometer. ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR (DMSO-d_6) spectra were taken on a Bruker DRX-300 using TMS as internal standard. Melting points were determined in capillaries and are uncorrected.

Plant Material

The stem of *A. catechu* was collected from the "Dhamoni forest" in Sagar (M.P.), India and was indentified by the Taxonomist, Department of Botany, Dr H.S. Gour University, Sagar (M.P.), India. A voucher specimen has been deposited in the Natural products Laboratory, Department of Chemistry, Dr H.S. Gour University, Sagar (M.P.), India.

Extraction and Isolation

Air-dried and powdered stems (3 kg) of plant *A. catechu* were extracted 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, Ac_2 O and MeOH. The EtOAc soluble part was chromatographed on Si-gel G column using $CHCl_3$–MeOH in various proportions. Fractions 10–25, on evaporation of solvent gave amorphous compound **1**, which was purified by preparative TLC and column chromatography. Compound **1** was crystallised from Et_2O as light yellow crystalline needles (3.512 g) $C_{28}H_{32}O_{17}$, mp 283–284°C, 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, 40 : 20 : 10) on silica gel-G; IR (KBr) ν_{max} 3350 (OH), 2986 (C–H stretching), 2870(OMe), 1650 (α,β-unsaturated C=O), 1550 (aromatic ring system), 1270 (C–O–C stretching), 1075 (O-gly) cm^{-1}; UV (MeOH) λ_{max} 257, 356; (+ NaOMe) 259, 371; (+ $AlCl_3$) 257, 358; (+ $AlCl_3$/HCl) 258, 378; (+ NaOAc) 259, 357 nm, (+ NaOAc/H_3BO_3) 259, 383 nm. ^{1}H NMR (300 MHz, $CDCl_3$), of **3** at δ 5.33 (1H, s, H-6), 6.52 (1H, s, H-8), 3.85 (3H,s, OMe), 7.23 (1H, d, J = 2.6 Hz, H-2′), 6.97 (1H, d,



The structure of compound 1

$J = 8.6$ Hz, H-5′), 7.48 (1H, d, $J = 2.7$ Hz, 8.6 Hz, H-6′); 2.31 (3H, s, OAc − 3″), 2.42 (3H, s, OAc − 4′), 1.86–2.17 (21H, m, sugar 7 × OAc), 4.82–5.52 (10H, m, sugar H's), 4.57 (lH, d, $J = 7.6$ Hz H-1′, glucose), 6.09 (1H, d, $J = 8.1$ Hz, H-1‴, galactose);^{13}C NMR (300 MHz, DMSO-d_6) δ 156.3 (C-2), 138.5(C-3), 178.22 (C-4), 163.0 (C-5), 98.4 (C-6), 159.3 (C-7), 94.8 (C-8), 154.1 (C-9), 103.6 (C-10), 130.8 (C-2′), 158.8 (C-3′), 114.9 (C-4′), 113.0 (C-5′), 132.4 (C-6′), 100.5 (C-1″), 74.6 (C-2″), 76.6 (C-3″), 75.8 (C-4″), 76.7 (C-5″), 61.8 (C-6″), 100.2 (C-1‴), 73.3 (C-2‴), 74.2 (C-3‴), 70.5 (C-4‴), 76.4 (C-5‴), 63.1 (C-6‴); EIMS *m/z* 640 [M^+] (absent), 316 [M^+-acetylated sugar moieties] (17), 314(13), 300(100), 297(60), 284(38), 272(8.2), 152(5).

Acid Hydrolysis of Compound 1

Compound **1** (40 mg) was refluxed with 10% H_2SO_4 (10 ml) for 4 h at 100°C and on cooling a precipitate was obtained, which was treated with Et_2O. The ethereal layer was washed with water to dryness and the residue was chromatographed over silica-gel G using $CHCl_3$: MeOH (5 : 3) to yield aglycone **2** (2.450 g) $C_{16}H_{12}O_7$, mp 214–216°C, [M^+] *m/z* 316 (found: C, 60.95, H, 3.98; 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 neutralisation 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 glucose and galactose (R_f 0.18 and R_f 0.16), respectively (by Co-PC and Co-TLC).

Alkaline Degradation of the Aglycone 2

Alkaline degradation was carried out by refluxing the aglycone (100 mg) with 40% KOH and EtOH (5 ml) for 24 h. The reaction mixture was cooled, neutralised with HCl (10%) and extracted with Et_2O. The ethereal layer was treated with 50% $NaHCO_3$ and the aqueous portion on acidification yielded a compound **2b**, m.f. $C_7H_6O_4$, mp 200–201°C, M^+ 154 (found: C, 54.43; H, 3.86; calcd: C, 54.46; H, 3.89) and identified as 3,4-dihydroxy benzoic acid. The aqueous phase was treated with 10% NaOH and on acidification afforded compound **2a**, m.f. $C_8H_8O_4$, mp 157–158°C, M^+ 168 (found: C, 57.05; H, 4.63 calcd: C, 57.14; H, 4.76) and identified as 2,4,6-trihydroxy acetophenone.

Permethylation of Compound 1

The compound **1** (50 mg) was treated with MeI (5 ml) and Ag_2O (50 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 **4**, $C_{19}H_{21}O_{10}$ (16 mg), mp 247–248°C, M^+ *m/z* 409, UV (MeOH) λ_{max} 259, 354 nm (+ NaOMe), 260, 302, 356 (+ NaOAc); 258, 302, 396 (+ $AlCl_3$), 259, 356 (+ $AlCl_3$/HCl); 259, 354 nm. IR (KBr) ν_{max} 3535, 2912, 2872, 1655, 1624, 1278, 1151 cm^{-1} ^{1}H-NMR (300 MHz, $CDCl_3$): δ 6.30 (1H, s, H-6), 6.51 (1H, s, H-8), 7.26 (1H, d, $J = 2.1$ Hz, H-2′), 7.21 (1H, d, $J = 8.8$ Hz, H-5′), 7.48 (1H, d, $J = 9.2$ Hz, H-6′), 3.88 (3H, s, OMe), 3.91 (3H, s, OMe), 3.82 (3H, s, OMe), 3.76 (3H, s, OMe). The permethylated aglycone **4** was identified as 3,5,3′,4′-tetramethoxy-7-hydroxy flavone and methylated sugars were identified as 2,3,6,-tri-*O*-methyl-D-glucose and 2,3,4,6-tetra-*O*-methyl-D-galactose.

Enzymatic Hydrolysis of Compound 1

A mixture of compound **1** (8 mg) and enzyme almond emulsin (12 ml) were treated in a round-bottomed flask (50 ml) at 25°C for 30 h and liberated D-glucose (R_f 0.18) and D-galactose (R_f 0.16), identified by Co-PC and Co-TLC, using BAW (4:1:5) as solvent system and aniline hydrogen phthalate as detecting agent.

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