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Isolation of Polyphenolic Constituents from Pods of *Cassia marginata* Roxb. Hort and Their Biological Activity

K.N. RAI* and SARITA KUMARI
Department of Chemistry, Shershah College, Sasaram-821 115, India

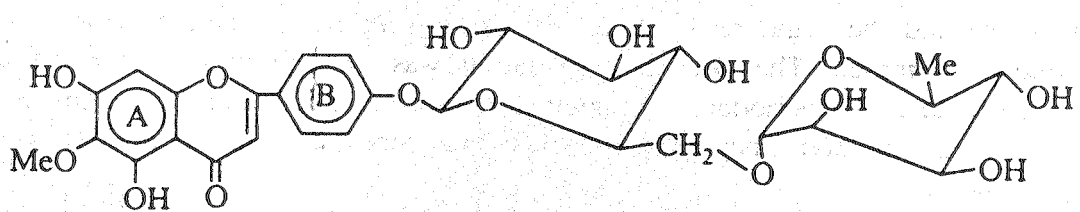
Phytochemical studies on the pods of *Cassia marginata* resulted in the isolation of two new polyphenolic glycosides: 5,7-dihydroxy-6-methoxy flavone-4′-*o*-α-L-rhamnosyl-(1 → 6)-β-D-glucoside (I) and 5,7-dihydroxy-4′-methoxy flavonol-3-*o*-α- L-rhamnosyl-(1→2)-*o*-β-D-glucoside (II). The known glycoside kaempferol-*o*-β-D-glucoside (III) in addition to kaempferol (IV), kaempferide (V), leucocyanidin (VI) and an anthraquinone derivative, rhein (VII) have also been isolated. The structures of all the isolated compounds were established on the basis of chemical and spectral analysis. The acetone extract has been screened for its antibacterial activity.

Key Words: *Cassia marginata*, Polyphenolic Constituents, Antibacterial activity.

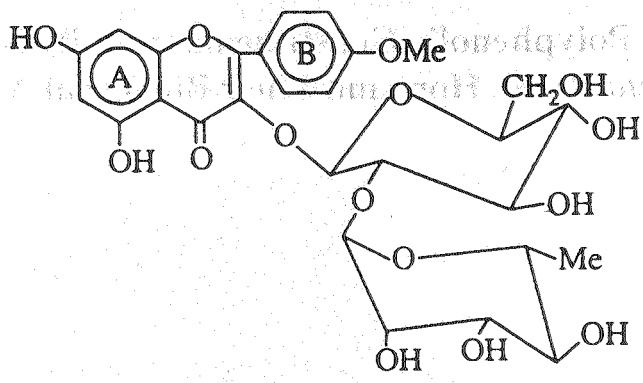
INTRODUCTION

Cassia marginata, Roxb. Hort (Fam. Leguminosae, Subfam. Caeselpiniaceae) is a low tree with long spreading and weeping branches, tomentose branchlets and bifarious leaves. It has beautiful deep rose red flower and highly reputed for its medicinal value[1]. The plant is a source of various novel bio-active compounds[2–4].

The present paper deals with the isolation and characterization of two new flavonoid glycosides, four known components of phenolic and flovonoid nature from the pods of the plant. Incidentally, both glycosides are of the same molar mass, although both are of different kinds of flavonoids obtained at quite different polarity.



(I)



(II)

EXPERIMENTAL

Melting points were determined inopen capillaries and are uncorrected. UV spectra (MeOH) were recorded on a Beckman DU-6 spectrophotometer, IR spectra (KBr) on a Perkin-Elmer 577 spectrometer, PMR spectra (DMSO-d_6) on a Perkin-Elmer R-32 spectrometer (300 MHz) using TMS as internal standard and mass spectra on a JEOL JMS 300 spectrometer at 70 eV and 300°C. Purity of the compounds was checked by paper chromatography (PC) and thin layer chromatography (TLC) using plates coated with silica gel G.

Extraction, Isolation and Identification

The air dried and defatted powdered pods (2 kg) were extracted with hot ethanol. The acetone extract after concentration under reduced pressure was resolved into water-soluble and insoluble parts.

The water-soluble part after concentration under reduced pressure was subjected to column chromatography on silica gel and eluted with increasing proportion of ethyl acetate and methanol. Elution of the column with EtOAc-MeOH (6 : 4 v/v) and (8 : 2 v/v) afforded two new compounds: **I** (0.5 g) and **II** (0.55 g) respectively. The known compound **III** (0.45 g) was isolated with EtOAc-MeOH (9 : 1 v/v) eluates.

The water-insoluble part (coloured residue) of acetone was dissolved in minimum quantity of methanol, chromatographed over silica gel and eluted with petrol → C_6H_6 → EtOAc → MeOH gradient. Elution with solvents of increasing polarity yielded **IV** [0.40 g, EtOAc-MeOH (9 : 1 v/v)], **V** [0.35 g, EtOAc-MeOH (8 : 2 v/v)], **VI** [0.22 g, EtOAc-MeOH (7 : 3 v/v)] and an anthraquinone derivative, **VIII** [0.47 g, C_6H_6-EtOAc (8 : 2 v/v)].

The complete and partial acid hydrolysis (2N HCl, 2 h and 7% formic acid in cyclohexanol, respectively) of the glycoside under investigation yielded the aglycone and the sugar residues, all of which were co-chromatographed with authentic samples. The periodate oxidation was done with 0.1 M sodium metaperiodate. The periodate consumed and formic acid liberated were estimated by titrimetric method. Enzymatic hydrolysis was done using diastase or emulsion at 30–40°C.

The known compounds were identified by comparison of their UV, IR and R_f values with those of authentic samples. The elemental and spectral analyses of the two new compounds are as follows.

Compound I: The compound was crystallized from methanol as colourless plates, m.p. 196–198°C, $R_f = 0.42$, (PC, BuOH: AcOH : $H_2O = 4 : 1 : 5$ v/v). Anal. (%), (Found C, 56.12; H, 5.40; Calcd. for $C_{28}H_{32}O_{15}$: C, 55.26; H, 5.26. UV (MeOH) λ_{max} : 278, 331, nm; + $AlCl_3$: 306, 378; + $AlCl_3$/HCl: 307, 378; + NaOAc; 294, 331; + NaOAc/H_3BO_3: 276, 337, + NaOMe: 282, 388 nm; IR (KBr, cm^{-1}) ν_{max}: 3440 ν(OH), 2950 ν(OCH_3), 1650 ν(chelated >C═O), 1570 and 1520, ν(aromatic); EIMS (m/z): 608 (M^+); of the acetate PMR (DMSO-d_6) δ: 0.90 (br, J = 6 Hz, rhamnose methyl), 2.40 (3H, s, OAc at C-7), 3.85 (3H, s, OCH_3), 4.00–5.50 (br, sugar protons), 6.36 (1H, s, C-3), 7.25 (1H, d, J = 2.5 Hz, C-8), 7.35 (2H, d, J = 9 Hz, C-3′5′) and 7.90 (2H, d, J = 9 Hz, C-2′6′).

The glycoside (0.2 g) was hydrolyzed with HCl (2 N) for 2 h. The aglycone was recovered as usual[5] and crystallized from methanol as pale yellow needles (0.09 g) m.p. 265–268°C. Anal. (%), Found: C, 64.66; H, 4.20; Calcd. for $C_{16}H_{12}O_6$: C, 64.00; H, 4.00. $R_f = 0.85$ (PC, BAW, 4 : 1 : 5) and 0.70 (PC, 30% AcOH); UV (MeOH) λ_{max}: 275, 340 nm; + $AlCl_3$: 307, 386; + $AlCl_3$/HCl: 307, 386; + NaOAc: 294, 343; + NaOAc/H_3BO_3: 274, 346; + NaOMe: 280, 398 nm. IR (KBr, cm^{-1}) ν_{max}: 3450 ν(OH), 2960 ν(OCH_3), 1650 ν(chelated >C═O), 1570 and 1520 ν(aromatic); EIMS (m/z): 300 (M^+, 32%), 285 (M^+, —CH_3, 28%), 282 (26), 257 (49), 167 (28), 159 (29), 139 (100), 119 (55), 89 (23) and 70 (90). Acetylation of the aglycone gave a triacetate, m.p. 168–169°C; PMR (DMSO-d_6) δ : 2.30 (3H, s, OAC at C-4′), 2.35 (3H, s, OAC at C-5), 2.45 (3H, s, OAc at C-7), 3.88 (3H, s, OCH_3), 6.33 (1H, s, C-3), 7.20 (1H, d, J = 9 Hz, C-8), 7.26 and 8.86 (A_2B_2 system, 4H, d, J = 9 Hz, C-3′5′ and C-2′6′).

Compound II: The compound was crystallised from methanol as light yellow coloured plates, m.p. 144–145°C, Found: C, 55.90; H, 5.52; Calcd. for $C_{28}H_{32}O_{15}$: C, 55.26; H, 5.26%); $R_f = 0.72$ (PC, BAW, 4 : 1 : 5) and 0.60 (PC, 20%, Anal. (%), AcOH); UV (MeOH) λ_{max}: 268, 355 nm; + $AlCl_3$: 271, 401; + $AlCl_3$/HCl: 272, 401; + NaOAc: 280, 358: + NaOAc/H_3BO_3: 267, 355 nm; IR (KBr, cm^{-1}) λ_{max}; 3420 ν(OH), 2930 ν(OCH_3), 1640 ν(chelated >C═O), 1600 and 1520 ν(aromatic), 1120 and 1025 ν(*o*-gly); EIMS (m/z): 608 (M^+); of the acetate PMR (DMSO-d_6) δ: 1.18 (br, J = 6 Hz, rhamnose methyl), 1.95–2.20 (18H, s, OAc rhamnoglucoside), 2.38 (3H, s, OAc at C-5), 2.40 (3H, s, OAc at C-7), 3.86 (3H, s, OCH_3 at C-4′), 3.90–5.60 (br, sugar protons), 6.80 (1H, J = 2.5 Hz, C-6), 7.30 (1H, d, J = 2.5 Hz, C-8), 7.40 (2H, d, J = 9 Hz, C-3′5′) and 7.98 (2H, d, J = 9 Hz, C-2′6′).

The glycoside (0.2 g) was hydrolyzed with HCl (2 N) for 3 h. The aglycone was recovered as usual[5] and crystallized from methanol as yellow needles (0.095 g); m.p. 228–229°C; Anal. (%), Found; C, 4.40; H, 4.25; Calcd. for $C_{16}H_{12}O_6$: C, 64.00, H = 4.00. $R_f = 0.90$ (PC, BAW, 4 : 1 : 5); UV (MeOH) λ_{max}: 267, 367; + $AlCl_3$: 271, 423; + $AlCl_3$/HCl: 270, 422; + NaOAc: 279, 368; IR (KBr, cm^{-1}) ν_{max}: 3430 ν(OH), 2950 ν(OCH_3), 1660 ν(chelated >C═O), 1620 and 1520 ν(aromatic); EIMS (M/z): 300 (M^+, 32%), 285 (M^+, —CH_3, 28%). Acetylation of the aglycone gave a triacetate, m.p. 194°; PMR (DMSO-d_6) δ: 2.34 (3H, s, OAc at C-3), 2.37 (3H, s, OAc at C-5), 2.40 (3H, s, OAc at C-7), 3.89 (3H, s, OCH_3 at C-4′), 6.82 (1H, d, J = 2.5 Hz C-6), 7.25 (1H, d, J = 2.5 Hz, C-8), 7.37 (2H, d, J = 9 Hz, C-3′5′) and 7.87 (2H, d, J = 9 Hz, C-2′6′).

RESULTS AND DISCUSSION

Compound I, m.p. 196–98°C, was analyzed for $C_{28}H_{32}O_{15}$ (elemental analysis and M^+). The conclusive colour reactions and UV spectrum with different chemical shifts indicated it to be a flavone glycoside[6]. The glycoside on acid hydrolysis gave a pale yellow aglycone and two sugars identified as glucose and rhamnose by R_f values 0.18 and 0.34 respectively by PC and co-chromatography with authentic samples.

The aglycone $C_{16}H_{12}O_6$ (M^+ at m/z 300), m.p. 265–68°C, responded to all positive tests of flavone[6] and the structure was established as 5,7,4′-trihydroxy-6-methoxy flavone by comparison with the UV, IR, PMR and mass spectral data of the literature[2].

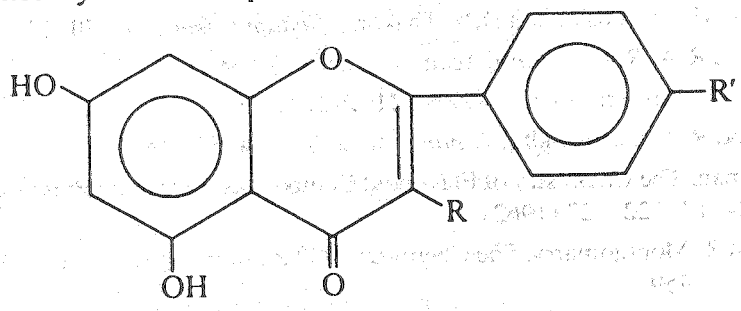
The attachment of sugar moiety to the flavone (aglycone) was established by comparison of UV data of the aglycone (λ_{max} 275, 340 nm) with that of the glycoside (278, 331 nm) which showed a hypsochromic shift of 9 nm in band I and a bathochromic shift of 3 nm in band II; This indicated the glycosidation at position 4′ of the flavone[6]. The aromatic acetate methyl signal at δ 2.30 in the aglycone acetate showed the presence of OAc at C-4′ which was absent in the glycoside acetate confirming the glycosidation at the same position. This also indicates that the two sugars, glucose and rhamnose, are present in the form of bioside which was confirmed by periodate oxidation of the glycoside with 7% formic acid in cyclohexanol indicated that rhamnose is the terminal sugar in the biocide.

The glycoside was methylated, hydrolyzed and the resulting partially methylated sugars were identified as 2,3,4-tri-*o*-methyl-L-rhamnose and 2,3,4-tri-*o*-methyl-D-glucose by the reported method using 2,3,4,6-tetra-*o*-methyl glucose as standard[7, 8] and co-chromatography with authentic samples. This indicated that the inter-sugar linkage is rhamnosyl (1 → 6) glucose. In the PMR spectrum of the glycoside acetate a broad signal at δ 0.90 was observed which is typical of rhamnose methyl group. This confirmed the inter-sugar linkage in the bioside to be in the form of rutinoside (1 → 6) type[9, 10]. The enzymatic hydrolysis with diastase and subsequent hydrolysis with emulsin indicated that L-rhamnose is linked to D-glucose through α-linkage and D-glucose to flavone through β-linkage. Thus, the structure of the compound I was established as 5,7-dihydroxy-6-methoxy flavone-4′-*o*-α-L-rhamnosyl-(1 → 6)-β-D-glucoside. Compound II, m.p. 144–45°C was analyzed as $C_{28}H_{32}O_{15}$. The colour reaction and UV spectrum with different diagonostic shift reagents[11] suggested it to be a flavonol glycoside. On hydolysis with HCl (2 N), the glycoside gave two sugars identified as D-glucose and L-rhamnose (PC and co-chromatography with authentic samples) and an aglycone which was characterized as 3,5,7-trihydroxy-4′-methoxy flavonol by the comparison with the UV, IR, PMR and mass spectral data of literature[12].

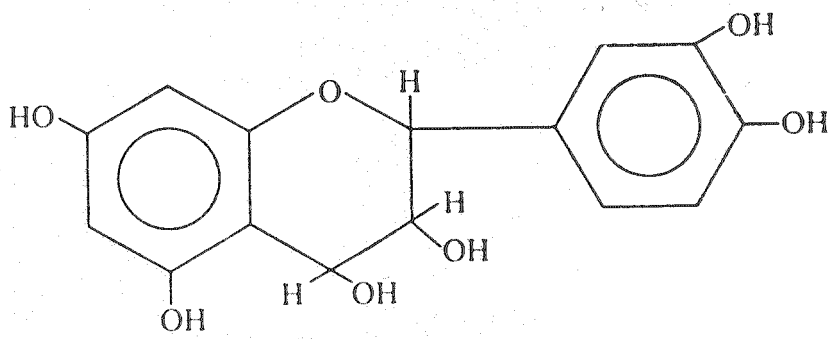
The attachment of sugar moiety at position-3 of the flavonol was confirmed by the aromatic acetate methyl signal at δ 2.34 in the flavonol, which was found absent in the glycosidic acetate. This also indicates that the two sugars are present as biocide which are confirmed by periodate oxidation of the glycoside. On permethylation followed by acid hydrolysis, it gave 2,3,4-tri-*o*-methyl-L-rhamnose and 2,4,6-tri-*o*-methyl-D-glucose using standard method[7, 8]. This established that inter-sugar linkage is rhamnosyl (1 → 2) glucose. In the PMR spectrum of

the glycoside a signal at δ 1.18 was observed, which confirmed the inter-sugar linkage in the biocide to be in the neohesperidoside (1 → 2) type[9, 10]. Enzymatic hydrolysis showed that L-rhamnose is linked to D-glucose through α-linkage and D-glucose to flavonol through β-linkage. Thus, the structure of compound **II** was assigned as 5,7-dihydroxy-4′-methoxy flavonol-3-*o*-α-L-rhomnosyl-(1 → 2)-*o*-β-D-glucoside.

The known compounds **III, IV, V, VI** and **VII** were characterized as kaempferol, kaempferide, leucocyanidin and rhein, respectively. The identities were confirmed by direct comparison with the corresponding known samples.



(III) R = O-glucoside; R′ = OH; (IV) R = R′ = OH; (V) R = OH, R′ = OCH_3



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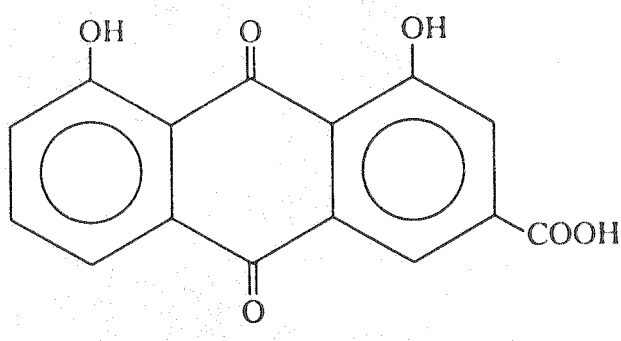

(VII)

Screening for Antibacterial Activity

The antibacterial effect of the acetone extract (10 mg of acetone extract in 2 mL DMSO + 8 mL water) was studied on five strains of bacteria namely *Staphylococcus aureus, Sarcina lutea, Bacillus subtilis* (all gram positive) and *Escherichia coli, Brodetella brochiseptica* (both gram negative). The agar diffusion method[13] was applied using nutrient agar medium[14]. The zone of inhibition showed very slight antibacterial activity against *B. subtilis, S. lutea* and *E. coli* .

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