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Isolation and Characterization of Anthraquinone Derivatives from the Heartwood of *Cassia glauca* Lam.

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Phytochemical examination of the heartwood of *Cassia glauca* resulted in the isolation of two new anthraquinone glycosides, 8-hydroxy-6-methoxy-3-methyl anthraquinone-1-O-α-L-rhamnosyl(1→2)-β-D-glucoside (**I**) and 3-hydroxy-6,8-dimethoxy-2 methyl anthraquinone-1-O-α-L-rhamnosyl(1→6)-β-D-glucoside (**II**). The known anthraquinone derivatives, chrysophanol (**III**), physcion (**IV**) and rubiadin (**V**) have also been isolated. The structural elucidations of all the isolated compounds were established on the basis of spectroscopic and chemical studies.

Key Words: *Cassia glauca*, Heartwood, Anthraquinone glycosides.

INTRODUCTION

Cassia glauca Lam. (Fern. Leguminosae, subfam. Caesalpiniacease) is a small tree and almost all parts of the plant are well known for their medicinal values[1]. The plant is a source of various anthraquinone derivatives and flavonoids[2-5]. Since there is no report of extensive chemical investigation of the heartwood of the plant, we, therefore, examined this part of the plant for their polyphenolic anthraquinones. Consequently, the present paper deals with the isolation and structure elucidation of two new anthraquinone glycosides along with some known anthraquinones.

EXPERIMENTAL

Melting points were determined in open capillaries and are uncorrected. UV spectra (MeOH) were recorded on a Beckman DU-6 spectrometer, 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 and TLC using plates coated with silica gel G.

Extraction, isolation and identification: The air dried and defatted crushed heartwood (4 kg) was extracted in a Soxhlet extractor successively with chloroform and ethanol. The ethanol extract after concentration under reduced pressure was resolved into water-soluble and insoluble parts.

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The water soluble part after concentration under reduced pressure was subjected to column chromatography on silica gel and eluted with EtOAc-MeOH in varying proportion. Elution of the column with EtOAc-MeOH (9:1 v/v) and (8:2 v/v) yielded two new compounds: **I** (0.8 g) and **II** (0.75 g), respectively.

The chloroform extract after concentration under reduced pressure was also chromatographed over silica gel and eluted with petroleum ether + $CHCl_3$ + EtOAc gradient. Elution with solvents of increasing polarity afforded **III** [0.40 g, petroleum ether-$CHCl_3$ (9:1 v/v)], **IV** [0.55 g, petroleum ether-$CHCl_3$ (7:3 v/v) and **V** [0.45 g, petroleum ether-$CHCl_3$ (6:4 v/v)].

The complete and partial hydrolysis (2 N H_2SO_4, 2 h and 7 % formic acid in cyclohexanol, respectively) of the glycoside under investigation furnished 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 ºC. The known compounds were identified by comparison of their m.m.p., UV, IR and R_f values with those of authentic samples. The elemental and spectral analysis of the two new anthraquinone glycosides are as follows:

Compound I: The compound was crystallized from methanol-ether mixture as a light brown amorphous compound, m.p. 240-242 °C, Anal (%), Found: C, 57.60; H,5.65; calcd. for $C_{28}H_{32}O_{14}$: C, 56.75; H, 5.40; UV(MeOH)λ_{max}: 255, 266, 288, 415 nm; IR (KBr, ν_{max}, cm^{-1}): 3360 (OH), 2940 (OMe), 1700 and 1650 (unchelated and chelated >C=O), 1600 and 1510 (aromatic), 1460, 1370, 1120 and 1030 (O-gly), 900, 840, 720; PMR (DMSO-d_6) δ: 1.20 (br, J = 6 Hz, rhamnose methyl), 2.39 (3H, s, CH_3), 3.92 (3H, s, OCH_3), 3.42-3.90 (Br, sugar protons), 5.00 (1H, d, J = 9 Hz, C-l' rhamnosyl), 5.40 (1H, d, J = 9 Hz, C-1' glucosyl), 6.82 (1B, d, J = 2.5 Hz, C-7), 7:15 (1H, s, C-2), 7.65 (1H, d, J = 2.5 Hz, C-5), 7.90 (1B, s, C-4); MS (m/z): 592 (M^+, 100 %) The compound also gave positive colour tests for a glycoside.

The glycoside (0.5 g) was hydrolyzed with H_2SO_4 (2 N) for 2 h. The aglycone was recovered as usual sand crystallized from methanol-ether mixture as a brown coloured compound (0.20 g), m.p. 205 °C, Anal. (%), Found: C, 66.20; H, 3.98; calcd. for $C_{16}H_{12}O_5$: C, 67.60 ; H, 4.22 ; UV (MeOH)λ_{max}: 256, 268, 285, 436 nm; IR (KBr, ν_{max}, cm^{-1}): 3380 (OH), 2935 (OCH), 1678 and 1625 (unchelated and doubly chelated >C=O), 1570 and 1520 (aromatic), 1470, 1375, 1260, 1160, 1060, 900, 720: PMR (DMSO-d_6) δ: 2.40 (3H, s, CH), 3.90 (3H, s, OCH_3), 6.86 (1H, d, J = 2.5 Hz, C-7), 7.10 (1H, s, C-2), 7.60 (1H, d, J = 2.5Hz, C-5), 7.88 (1H, s, C-4); MS (m/z): 284 (M^+, 100 %) ; acetate (Ac_2O/Py), m.p. 183 °C, (Found (%): -$COCH_3$, 22.84, Calcd. for $C_{16}H_{10}O_5(COCH)_2$; -$COCH_3$, 23.74]; methyl ether (Me_2SO_4/K_2SO_4), m.p. 220-222 °C, [Found (%):-OCH_3, 28.88, Calcd. for $C_{15}H_7O_2(OCH_3)_3$; OCH_3, 29.80].

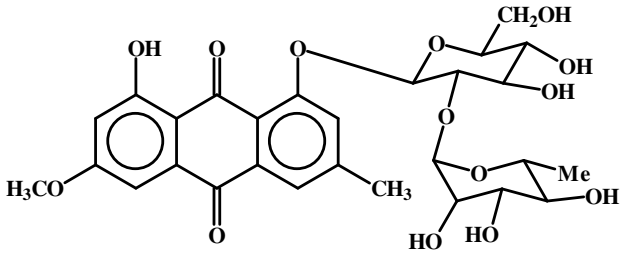
Compound II: The compound was crystallized from methanol-ether mixture as a light brown amorphous solid, m.p. 230-232 °C; Anal. (%), Found: C, 57.10; H, 5.66; Calcd for $C_{29}H_{34}O_{15}$: C, 55.94; H, 5.46; UV (MeOH)λ_{max}: 256, 266, 286, 417 nm; IR (KBr, ν_{max}, cm^{-1}): 3385 (OH), 2940 and 2880 (OCH_3), 1720, 1640, 1570, 1460, 1250, 1180, 1075, 960, 840, 810, 730; PMR (DMSO-d_6) δ: 0.90 (br, J = 6 Hz, rhamnose methyl), 2.38 (3H, s, CH), 3.30-3.75 (br, sugar protons); 3.94 (6H, s, OCH_3), 5.20 (1H, d, J = 9 Hz, C-1" rhamnosyl), 5.45 (1H, d, J = 9 Hz, C-1' glucosyl), 6.85 (1H, d, J = 2.5 Hz, C-7), 7.68 (1H, d, J = 2.5 Hz, C-5), 7.85 (1H, s, C-4) and a phenolic hydroxyl at 7.55 (1H, s, D_2O exchangable, C_3-OH); MS (m/z) ; 622 (M^+, 100 %). The compound also showed positive colour tests for a glycoside.

The glycoside (0.5 g) was hydrolyzed with 2 N H_2SO_4 for 2 h. The aglycone was recovered as usual and crystallized from methanol-ether mixture as a brown coloured compound (0.25 g), m.p. 272-274 °C Anal. (%), Found: C, 57.32; H = 4.75; Calcd. for $C_{17}H_{14}O_6$: C, 58.00; H, 5.04; UV(MeOH)λ_{max}: 254, 266, 296, 419 nm; IR (KBr, ν_{max}, cm^{-1}): 3390 (OH), 2940 (OCH_3), 1664 and 1621 (unchelated and chelated >C-O), 1588, 1450, 1375, 1270, 1225, 1160, 1130, 856, 730; PMR (DMSO-d_6) δ: 2.40 (3H, s, CH_3), 3.90 (6H, s, OCH_3), 6.80 (1H, d, J = 2.5 Hz, C-7), 7.64 (1H, d, J = 2.5 Hz, C-5) 7.80 (1H, s, C-4); MS (m/z): 314 (M^+, 100 %); acetate (Ac_2O/Py), m.p. 230-232 °C, Anal. (%), Found: $COCH_3$, 27.28, calcd. for $C_{17}H_{12}O_6(COCH_3)_2$, 26.63; methyl ether ($Me_2SO_4/K_2SO_4$), m.p. 193-194 °C, Found: -OCH_3, 37.08, Calcd. for $C_{15}H_6O_2(OCH_3)_4$, 36.25.

RESULTS AND DISCUSSION

The compound **1**, m.p. 240-242 °C, was analyzed for $C_{28}H_{32}O_{14}$ (elemental analysis and M^+). The conclusive colour reactions and spectral data suggested it to be an anthraquinone glycoside[3,6-8]. The glycoside on acid hydrolysis yielded a brown coloured aglycone and two sugars identified as glucose and rhamnone by R_f values 0.18 and 0.34, respectively (PC; solvent-BAW, 4:1:5 v/v, spray-AHP) and co-chromatography with authentic samples.

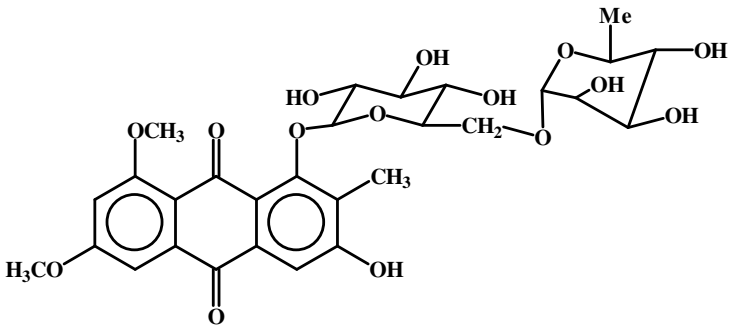
The aglycone, $C_{16}H_{12}O_5$ (M^+ at m/z 284), m.p. 205 ºC, responded to all characteristic tests and spectral data of anthraquinone[3,8] and the structure was established as 1,8-dihydroxy-6-methoxy-3-methyl anthraquinone (physcion) which was finally confirmed by m.m.p. and super-imposable IR-spectra of the authentic sample. Acid hydrolysis of the methylated glycoside yielded a compound, m.p. 175 ºC, which was identified as 1-hydroxy-6,8-dimethoxy-3-methyl anthraquinone by comparison with an authentic sample[9]. This indicated that the two sugars, D-glucose and L-rhamnose are present in the form of bioside and linked at position-1 of the aglycone. The presence of two sugars in the form of bioside was confirmed by periodate oxidation of the glycoside (1 mol of glycoside consumed 3.03 mol of periodate and liberated 1.02 mol of formic acid). The partial hydrolysis of the glycoside with 7 % formic acid in cyclohexanol showed that rhamnose is the terminal sugar.



Compound **I**

The glycoside was permethylated, hydrolyzed and the resulting partially methylated sugars were identified as 2,3,4-tri-O-methyl-L-rhamnose and 3,4,6-tri-O-methyl-D-glucose by the reported method using 2,3,4,6-tetra-O-methyl glycose as standard[10,11] and co-chromatography with authentic samples. This indicated that the inter-sugar linkage is rhamnosyl(1→2)glucose. In the PMR spectrum of the glycoside, a signal at δ 1.20 was observed which is typical of rhamnose methyl group. This confirmed the inter-sugar linkage in the bioside to be in the neohesperidoside (1→2) type[12,13]. Enzymetic hydrolysis showed that L-rhamnose is linked to D-glucose through α-linkage and D-glucose to anthraquinone through β-linkage. Thus, the structure of compound **I** was characterized as 8-hydroxy-6-methoxy-3-methyl anthraquinone-1-O-α-L-rhamnosyl(1→2)-β-D-glucoside.

Compound II: m.p. 230-232 °C was analyzed for $C_{29}H_{34}O_{15}$. The conclusive colour reactions and spectral data indicated it to be an anthraquinone glycoside[3,6-8]. The glycoside, on acid hydrolysis (2 N H_2SO_4, 2 h) gave two sugars identified as D-glucose and L-rhamnose (paper chromatography and co-chromatography with authentic samples) and an aglycone which was characterized as 1,3-dihydroxy-6,8-dimethoxy-2-methyl anthraquinone by comparison with UV, IR, PMR and mass spectral data of literature[3,8,14].



Compound **II**

The position of attachment of sugar moiety to the aglycone and nature of intersugar linkage was established by colour reactions, degradation of methylated glycoside and PMR spectra. The aglycone gave a positive reaction for 1,3-dihydroxy

system[15,16] whereas the original glycoside did not, indicating the attachment of sugar moiety at position-1 of the aglycone in the glycoside. Further, the PMR spectrum of the glycoside displayed a signal at δ 7.55 ppm for C_3-OH group which confirmed that OH group at position-1 of the aglycone is involved in the glycoside formation. This also indicated that the two sugars, glucose and rhamnose are present in the form of bioside which was confirmed by periodate oxidation of the glycoside. The partial hydrolysis of the glycoside showed that rhamnose is the terminal sugar in the bioside. The glycoside, on methylation followed by acid hydrolysis, gave 2,3,4-tri-O-methyl-L-rhamnose and 2,3,4-tri-O-methyl-D-glucose using TMG as standard[10,11]. This indicated that the inter-sugar linkage is rhamnosyl (1→6) glucose. A broad signal at δ 0.91 in PMR spectrum of the glycoside further confirmed the inter-sugar linkage in the bioside to be in the form of rutinoside (1→6) type[12,13]. Enzymatic hydrolysis with diastase and then with emulsion of the glycoside indicated that L-rhamnose is linked with D-glucose through α-linkage and D-glucose to aglycone through β-linkage. Thus, the structure of compound **II** was established as 3-hydroxy-6,8-dimethoxy-2-methyl anthraquinone-1-O-α-L-rhamnosyl (1→6)β-D-glucoside.

The known compounds **III**, **IV** and **V** were characterized as chrysophanol, physcion and rubiadin, respectively. The identities were confirmed by the corresponding known samples.

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