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Aromadendrin-4′-O-β-D-glucopyranosyl (1→6)-O-β-D-xylopyranoside, A New Dihydroflavonolglycoside from the Aerial Part of *Dalbergia latifolia* Roxb.

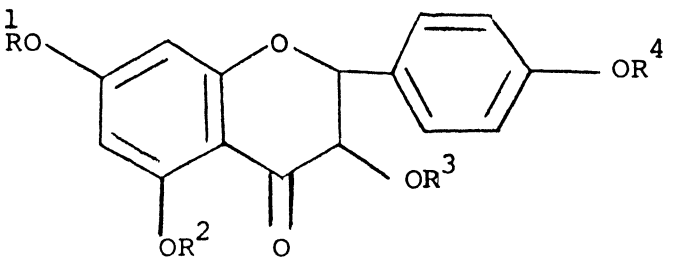
R.N. YADAVA*, ANILA ROY and NEERU JAIN
Natural Product Laboratory
Department of Chemistry
Dr. H.S. Gour University, Sagar-470 003, India

The isolation and structural elucidation of a new dihydroflavonol glycoside, aromadendrin-4′-O-β-D-glucopyranosyl (1→6)-O-β-D-xylopyranoside is reported.

INTRODUCTION

Dalbergia latifolia[1–3] natural order leguminosae is commonly known as Sheesham in Hindi and found in East Bengal, Bihar, Sikkim, Bundelkhand, Madhya Pradesh and Western Peninsula. The medicinal value[3] of the plant prompted us to investigate it further phytochemically.

The present paper deals with the isolation and structural elucidation of a new dihydroflavonol glycoside, aromadendrin-4′-O-β-D-glucopyranosyl (1→6)-O-β-D-xylopyranoside, from the methanolic extract of the aerial part of *Dalbergia latifolia* Roxb.



1. $R^1 = R^2 = r^3 = H$, R^4 = Glucosyl (1→6)-O-β-D-xylopyranoside
2. $R^1, R^2, R^3, R^4 = H$
3. $R^1, R^2, R^3 = CH_3$, $R^4 = H$

RESULTS AND DISCUSSION

The plant material was collected from United Chemicals and Allied Products, Calcutta and identified in the department of botany of this university. The methanolic extract of the aerial part of *Dalbergia latifolia* on solvent treatment followed by column chromatography over silica gel (B.D.H.) gave compound 1, which was crystallized from MeOH-ether as light yellow needles, m.p.

277°C, $[M]^+$ 582, m.f. $C_{26}H_{30}O_{15}$. Its IR spectrum showed strong absorption bands at 3420 cm^{-1}, ν(OH), 2945 cm^{-1}, ν(C—H), 1645 cm^{-1}, ν(C=O), 1510, 1205, 1150, 1025, 800 cm^{-1} and a broad band at 1120–1010 cm^{-1} indicating its glycosidic nature. It responded to Shinoda test[4], Molisch test, $FeCl_3$ test. Its UV spectrum showed an intense band II at 284, and a weak band I at 329 mm as shoulder, which is characteristic of dihydroflavonol. The UV spectral data with diagnostic shifts reagents[5] suggested that it is a 5,7,3,4′-tetrasubstituted flavanone glycoside with free hydroxyl group at 5, 7 and 3-position.

On complete acid hydrolysis with 10% H_2SO_4, 1 gave an aglycone (2), m.p. 212°C and D-glucose and D-xylose as sugar moieties (Pc and GLC). The aglycone showed a bathochromic shift of 50 nm in band I and 20 nm in band II with NaOMe relative to MeOH (absent in glycoside) suggesting that C-4′ of compound 1 must be involved in glycosylation. The aglycone was identified as aromadendrin (spectral and chromatographic comparison with authentic sample).[6]

The glycoside 1 yielded a nona-acetate derivative, molecular formula $C_{44}H_{48}O_{24}$, m.p. 187–88°. ^{1}H-NMR spectrum of the acetate derivative of 1 showed the expected signals in the aromatic region. Two orthocoupled doublets at δ 7.97 and 7.24 (J = 8.5 Hz) which corresponded to AA′BB′ pattern were assigned to C-2′,6′ and C-3′,5′ protons of the B-ring. Two metacoupled doublets at δ 6.79 and 7.02 (J = 2.5 Hz) were attributed to C-6 and C-8 protons respectively.

The doublets at δ 4.25 (J = 17 Hz) and 4.96 (J = 17 Hz) were ascribed to C-3 and C-2 protons respectively, confirming the dihydroflavonol skeleton of compound 1. The anomeric protons of two sugars appeared at δ 5.19 (J = 9.0 Hz, H-1 xylose β-configuration) and 5.63 (J = 9.0 Hz, H-1 glucose β-configuration). The remaining sugar protons appeared in the range of δ 4.82–5.67 as multiplet.

The mass spectrum of the acetate derivative of 1 showed a fragment ion at m/z 414 which corresponded to the loss of acetylated sugar moiety from the molecular ion. The mass spectrum showed the presence of acetylated pentopyranoside at m/z 259 and acetylated hexopyranoside at m/z 273. The aglycone fragment was observed at m/z 288. A retro-Diels-Alder fragmentation pattern was observed at m/z 153 and m/z 136 leading to fragments $[A_1 + H^+]^{+\cdot}$ and $B_3^{+\cdot}$. The results supported the presence of two hydroxyl groups in ring-A and one hydroxyl group in ring-B. One of the most diagnostic fragment ions, $B_4^{+\cdot}$, was observed at m/z 121 which is characteristic of the dihydroflavonol.

On enzymatic hydrolysis of 1 with β-xylosidase, xylose was liberated indicating the β-nature of the inter-sugar linkage. The liberation of glucose on complete hydrolysis with almond emulsion showed the β-nature of the linkage between glucose and the aglycone.

Methylation of 1 followed by acid hydrolysis gave a partially methylated aglycone 3 which showed a bathochromic shift of 46 nm in band I with NaOMe confirming that C-4′ hydroxyl which was glycosylated in 1 had become free and both the sugar moieties were present at the C-4′ position. The methylated sugars were identified as 2, 3, 4-tri-O-methyl-D-glucose and 2,3,4-tri-O-methyl-D-xylose by silica gel co-TLC (toluene-MeOH, 4 : 1) with authentic sugars, as accorded to Petek[7] and Hudson[8], confirming the inter-sugar linkage as (1→6).

On the basis of these findings, compound 1 has been identified as Aromadendrin-4′-O-β-D-glucopyranosyl (1 → 6)-O-β-D-xylopyranoside.

EXPERIMENTAL

Melting points were determined on a Reichert microscope hot-stage apparatus and are uncorrected. IR spectra (KBr) were taken on a Shimadzu IR-408 spectrometer. ^{1}H-NMR spectra were taken on JEOL-GX (270 MHz) spectrometer using TMS as an internal standard. MS were obtained by electron impact at 70 eV.

Air dried and powdered aerial part of *Dalbergia latifoluim* Roxb. (4 kg) were extracted with 95% EtOH. The combined concentrated ethanolic extracts were successively extracted with petroleum-ether, chloroform, ethyl acetate, acetone and methanol.

The ethyl acetate fraction showed the presence of flavonoid compounds. It was subjected to column chromatography over silica gel. The fraction eluted with benzene-EtOAc (2 : 8) afforded compound 1, which on crystallization with methanol-ether gave light yellow crystals of compound 1, m.p. 277°C, molecular formula $C_{26}H_{30}O_{15}$, $[M]^+$ 582, Found: C, 53.61; H, 5.17%; Calcd.: C, 53.60; H, 5.15%. UVλ_{max} (MeOH) 250 (sh), 284, 329 (sh), (MeOH + NaOMe) 246, 325, ($AlCl_3$) 270, 361, ($AlCl_3$ + HCl) 272, 312, 370 (sh), (NaOAc) 264, 296, 327 nm (sh). IR ν_{max} (KBr) 3420, 2945, 1645, 1510, 1205, 1150, 1025, 1120–1010, 800 cm^{-1}

Acetylation of 1

Compound 1 on acetylation with Ac_2O/Py (1 : 2, 48 h, 25°C) and on usual work up afforded a nona-acetate derivative, m.p. 187–88°C, molecular formula $C_{44}H_{48}O_{24}$ (Found: C, 57.09; H, 5.04%, Calcd.: C, 57.08; H, 5.00%. ^{1}H-NMR (90 MHz, $CDCl_3$, ppm). δ 7.97 (2H, d, J = 8.5 Hz, H-2′,6′), 7.24 (2H, d, J = 8.5 Hz, H-3′,5′), 6.79 (1H, d, J = 2.5 Hz, H-6), 7.02 (1H, d, J = 2.5 Hz, H-8), 4.25 (1H, d, J = 17 Hz, H-3), 4.96 (1H, d, J = 17 Hz, H-2), 5.19 (1H, d, J = 9.0 Hz, H-1‴ of xylose), 5.63 (1H, d, J = 9.0 Hz, H-1″ of glucose), 4.82–5.67 (3H, m), 2.07–2.12 (m 18H, sugar acetoxyls), 2.47 (3H, s, OAc-5), 2.37 (3H, s, OAc-7), 2.32 (3H, s, OAc-3). EIMS: m/z $[M]^+$ absent, 414 [M^+-acetylated sugar moiety]$^{+\cdot}$, 288 [414–3Ac·]$^+$ [aglycone fragment]$^+$, 271 [aglycone fragment OH·]$^{+\cdot}$, 153 $[A_1 + H]^{+\cdot}$, 136 $[B_3]^{+\cdot}$, 121 [136-HCO·]$^+$ or $[B_4]^+$, 122 $[B_3^+]$-CO, 124 $[A_1$-28]$^{+\cdot}$, 137 $[[A_1 + H]^{+\cdot}$-16]$^+$, 259 [xyl $(Ac)_3]^{+\cdot}$, 273 [glu $(Ac)_3]^{+\cdot}$

Acid Hydrolysis of 1

A solution of the glycoside 1 in 10% H_2SO_4 was heated on a water bath for 2 h at 100°C to ensure complete hydrolysis. The light yellow aglycone was separated out and dried. The curde product was cyrstallised from methanol as yellow needles, m.p. 212°C and $[M]^+$ 288. Compound 2 was identified as aromadendrin by spectral and chromatographic comparison with authentic sample[6]. Found: C, 62.53; H, 4.16%; Calcd.: C, 62.53; H, 4.16%. UV$_{max}$ (MeOH) 267, 324 (sh), (MeOH + NaOMe) 287, 303 (sh), 374 nm. IR$_{max}$ (KBr) 3430, 2945, 1680, 1200, 1020, 820 cm^{-1}.

The aqueous hydrolysate after neutralization with $BaCO_3$ and $BaSO_4$ was filtered off. The filtrate was then concentrated under reduced pressure and was found to contain glucose and xylose (by mp : mmp, Co-PC and Co-TLC).

GLC of sugars

The neutral aqueous hydrolysate of compound 1 was silylated with trimethylchlorodisilazane (TMCS) and hexamethyl disilazane (HMDS) in pyridine and subjected to GLC (2% OV-1, column. temp. 150–250°C, 10 min, dect. temp. 300°C, N_2 flow rate 50 mL/min) along with the silyl derivatives of standard sugars (R_t 3.9, 4.5 min for xylose and 3.5, 4.2 min for glucose). The R_t (min) values observed for the TMS ether derivatives of investigated sugars corresponded to the R_t values of glucose and xylose.

Methylation of compound 1 followed by acid hydrolysis

The glycoside was methylated by Me_2SO_4-K_2CO_3 for 3 h. The methyl ether was hydrolysed with 7% HCl and the aglycone extracted with EtOAc. The partial methyl ether was characterised as 4′-hydroxy-3,5,7-trimethoxyflavanone by spectral studies. ^{1}H-NMR data (90 MHz, DMSO-d_6, δ ppm). 3.82 (3H, s, OCH_3-7), 3.85 (3H, s, OCH_3-3), 3.97 (3H, s, OCH_3-5), 7.05 (1H, d, J = 2.5 Hz, H-6), 7.28 (1H, d, J = 2.5 Hz, H-8), 7.82 (2H, d, J = 8.5 Hz, H-2′,6′), 7.45 (2H, d, J = 8.5 Hz, H-3′,5′), 4.98 (1H, d, J = 17 Hz, H-2), 4.18 (1H, d, J = 17 Hz, H-3), 11.25 (1H, s, 4′OH-H). Found: C, 65.48; H, 5.46; $C_{18}H_{18}O_6$ requires C, 65.45; H, 5.45%.

The methylated sugars were identified as 2,3,4-tri-O-methyl-D-glucose and 2,3,4-tri-O-methyl-D-xylose by comparison with R_f.[7,8]

Enzymatic hydrolysis of 1

Compound 1 (75 mg) was treated with β-xylosidase (10 mg) and was incubated in $(NH_4)_2SO_4$-NaOAc buffer (pH 5.0) at 25°C for 30 h and after addition of H_2O, it was extracted with *n*-BuOH. The *n*-BuOH extract was chromatographed on a silica gel column to give a partial glycoside (3), m.p. 192–93°C identified as aromadendrin-4′-O-glucoside. From the H_2O layer K-xylose was identified by Pc.

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