3,3′-DI-O-METHYLELLAGIC ACID 4-O-β-D-XYLOPYRANOSYL-(1→4)-β-D-GLUCOPYRANOSIDE FROM THE STEM BARK OF *DIOSPYROS DISCOLOR*.

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ABSTRACT

From the stem bark of *Diospyros discolour* a new glycoside, 3,3′-di-O-methylellagic acid-4-O-β-D-xylopyranosyl-(1→4)-β-D-glucopyranoside (I) has been isolated and characterized by spectroscopic and chemical methods.

INTRODUCTION

DIOSPYROS DISCOLOR (Syn. *Diospyros mabola*) is called as 'Bilayati gab'[1] in Hindi in India. In our previous communication[2] we have reported the presence of betulin, betulinic acid and lupeol as the triterpenoidal constituents from the stem bark of *D. discolour*. We now report the isolation and characterization of a new glycoside, 3,3′-di-O-methylellagic acid-4-O-β-D-xylopyranosyl-(1→4)-β-D-glucopyranoside (I) from the stem bark of this plant.

MATERIALS AND METHODS

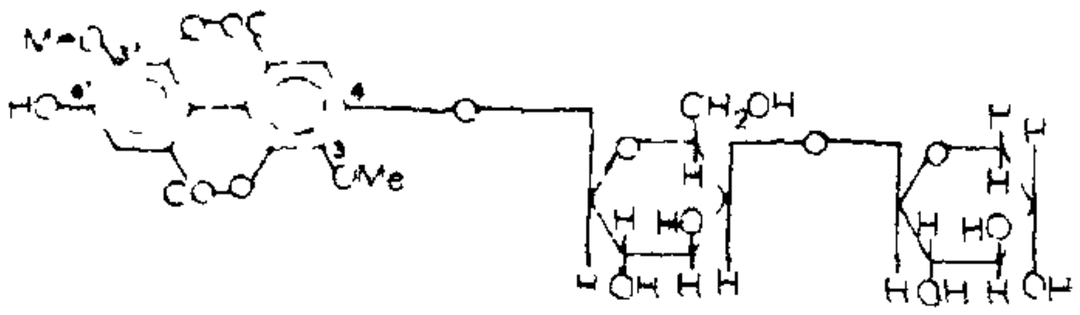
Compound (I) was shown to be a non-reducing glycoside by a positive Molisch's test and a negative aniline hydrogen phthalate test. An yellow colour with alkali and dark bluish green precipitate with ferric chloride indicate its phenolic nature[3]. Preliminary diagnostic tests and a positive Greissmeyer reaction[4], suggested that it was an ellagic acid derivative[3]. IR spectrum of (I) exhibited strong absorptions at 3440 (br, OH), 1730, 1720 (lactone), 1170 (methoxyl), 1595, 1560 and 1470 (aromatic) and 820 (glycoside) which were similar to other ellagic acid derivatives[5–8]. This was further substantiated by its UV, λ_{max}^{EtOH} 249, 355 (sh) and 370 nm.

On acid hydrolysis the glycoside gave an aglycone and a mixture of sugars identified as D-xylose and D-glucose by Co-paper chromatographies. The aglycone, $C_{16}H_{10}O_8$, m.p. 273–74°, λ_{max}^{EtOH} 248, 359 (sh), 372 nm, analysed for two methoxyl groups (Zeisel). A bathochromic shift of 33 nm with sodium ethylate suggested the presence of atleast one free phenolic hydroxyl either at the 3,3′ or the 4,4′ position of the ellagic acid molecule. The absence of a bathochromic shift with sodium acetate indicated that the position 3-3′ are substituted with two methoxyl groups[9]. The aglycone was therefore identified as 3,3′-di-O-methylellagic acid (lit. m.p. 274°, mmp and Co-TLC)[5]. Quantitative sugar estimation suggested the presence of two mol reducing sugar/mol of the aglycone and was further substantiated by elemental analysis of glycoside and its derivative.

The glycoside (I) was methylated with CH_2N_2 and the resulting methyl ether on acid hydrolysis yielded an aglycone identified as 3,3′, 4′-tri-O-methylellagic acid by its m.p. 287–88° (lit. 288–89°) and its monoacetate, m.p. 260–62° (lit. 264°)[7–10]. This confirmed that both the sugar units are linked at position-4 of the aglycone in the form of a disaccharide. In the disaccharide unit, xylose and glucose were present in molar ratio of 1:1 as indicated by Co-PC. Xylose was found to be the terminal sugar as it appeared first during the acid hydrolysis of the glycoside, followed later by glucose.

As the glycoside was non-reducing, the reducing group of both the sugars must be involved in linkage, thus C-1 of the glucose unit must be linked at position-4 of the aglycone and C-1 of the xylose must be involved in an intersugar linkage with a hydroxyl group of the glucose. The structure was finally established by premethylation (Hakomori's method)[11] of (I) followed by acid hydrolysis which gave 2,3,4-tri-O-methyl-D-xylose and 2,3,6-tri-O-methyl-D-glucose identified by their RG values[12,13]. This confirmed the (1→4) inter-sugar linkage. Further structural information was provided by periodate oxidation of the glycoside. The liberation of one mol of formic acid with consumption of three mol of sodium meta periodate supported the pyranose form of both the sugars.

The stereochemical nature of inter-sugar as well as the glycoside linkage were established by enzymatic hydrolysis of (I) which afforded 3,3′-di-O-methylellagic acid (mmp and Co-TLC), D-xylose (Co-PC) and D-glucose (Co-PC), respectively. This confirmed the inter-sugar linkage and glycosidic linkage as β in nature. This led to the formation of glycoside as 3,3′-



(I)

di-O-methylellagic acid-4-O-β-D-xylopyranosyl-(1→4) β-D-glucopyranoside. The evidences cited above were confirmed by PMR data of (I) which showed the absorptions (d_6-DMSO, 60 MHz) at δ, 7.20 (br, s, 2H, H-5 and 5'), 5.00 (m, 1H, C-1, gluco), 4.90 (m, 1H, C-1, xylo), 4.06 (s, 6H, 2 × OMe, C-3 and 3') and 3.50–3.80 (m, 9H, most of the sugar protons). This is first report of (I) in nature, although its 4-O-glucoside[6], 4-O-rhamnoside[14] and 4-O-glucuranosyl-arabinosyl-arabinosyl-glucoside[15] have been reported earlier.

RESULTS

Plant material: Plant material was precured from United Chemicals and Allied Products, Calcutta, India.

Chromatography: R_f values are for descending paper chromatography, and the solvents being (a) n-BuOH:AcOH:H_2O (4:1:5), (b) 10% glacial AcOH and (c) n-BuOH:EtOH:H_2O (5:1:4) using Whatman No. 1 chromatostrips.

Extraction: The air dried and powdered stem bark (3 kg) of *D. discolor* was exhaustively extracted with rectified spirit under reflux for 160 hr. The total spirit extract (30 lit) was concentrated (500 ml) and poured into distilled water (1 lit). The water soluble fraction concentrated to a syrupy mass and successively extracted with pet. ether, C_6H_6, $CHCl_3$ and EtOAc. The EtOAc extract was passed through a column of silica-gel and eluted with EtOAc: Me_2CO (5:5) which after crystallization from Me_2CO–Et_2O mixture afforded a brown crystals (yield 1.400 g).

3,3'-di-O-methylellagic acid-4-O-β-D-xylopyranosyl-(1→4)-β-D-glucopyranoside (*I*). m.p. 250–52° (d); R_f 0.23 and 0.53 in solvents (a) and (b) respectively; (spray $FeCl_3$); TLC: R_f, 0.74 (MeOH: Me_2CO, 6:4) and 0.39 ($CHCl_3$: MeOH, 7:3) (iodine as a developer). [Found; C, 51.90; H, 4.46; Calculated for $C_{27}H_{28}O_{17}$: C, 51.92; H, 4.48%]; IR ν_{max}^{KBr} cm^{-1}, 3440, 1730, 1720, 1170, 1495, 1560, 1530, 1470, 1445, 1355, 1340, 1210, 1020, 935 and 820.

Acid hydrolysis: I (800 mg) was refluxed with aqueous H_2SO_4 (7%, 40 ml) for 3 hr, the solution cooled and extracted with Et_2O. The Et_2O extract was evaporated and the residue crystallized from Me_2CO:Et_2O as pale yellow crystals, m.p. 273–74° (lit. 274°). [Found; C, 58.11; H, 3.30; OMe; 18.70; calculated for $C_{16}H_{10}O_8$: C, 58.18; H, 3.03, 2 × OMe, 18.78%]; The remaining water layer was neutralized with $BaCO_3$, concentrated and chromatographed (PC), R_f, 0.28 (D-xylose) and 0.18 (D-glucose) [solvent (a), spray aniline hydrogen phthalate and confirmed by Co-PC].

Quantitative acid hydrolysis: I (100 mg) was refluxed with aqueous H_2SO_4 (7%, 15 ml) for 3 hr. The solution was extracted with Et_2O, dried, concentrated and the solid isolated and weighed. The filtrate and washing were collected and neutralized with $BaCO_3$, made up to 25 ml and sugars were estimated by colorimetric method of Folin and Wu[16]. Found dimethyl-ether of ellagic acid 51.80; reducing sugars 52.00, calculated for $C_{27}H_{28}O_{17}$; dimethyl ether of ellagic acid: 52.88, reducing sugars 52.8%.

Methylation and hydrolysis: I (200 mg) was methylated with CH_2N_2 by standard procedure and methylated glycoside (150 mg) was hydrolysed with acid (7% H_2SO_4, 15 ml) to give 3,3', 4'-tri-O-methylellagic acid, m.p. 287–88° (lit. 288–89°). [Found; OMe; 26.9; calculated for $C_{17}H_{12}O_8$; OMe, 27.0%].

Methyl ether acetate: To the methylated aglycone obtained above, Ac_2O (2 ml) and C_5H_5N (3 ml) were added and kept at 20° for 48 hr. The methyl ether acetate was obtained as pale yellow crystals from Me_2CO:Et_2O, m.p. 260–62° (lit. 264–65°).

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