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Methylated Ellagic Acid Glycosides from *Eucalyptus rostrata*

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Leaves of *Eucalyptus rostrata* contained two new natural glycosides: 3,3′,4-tri-O-methylellagic acid-4′-O-β-D-galactoside (1) and 3′,3′,4-tri-O-methylellagic acid-4′-O-mannosylglucoside (2). The known glycoside 3′,3′,4-tri-O-methylellagic acid-4′-O-β-D-glucoside (3) in addition to the polyphenolic constituents, namely, 3,3′,4-tri-O-methylellagic acid (4); 3-3′-di-O-methylellagic acid (5); ellagic (6) and gallic (7) acids have also been isolated. The structures of all the isolated components were determined by both chemical and spectral analyses.

INTRODUCTION

Eucalyptus rostrata Schl. (Myrtaceae) is the most widely planted tree. It has several important economic medicinal values[1] and is a source of vegetable tanning material. A high content of flavandiol was detected in the leaves of some *Eucalyptus* species[2, 3] as well as ellagic acid and its esterified hydroxydiphenic acid or ellagitannins which are fungitoxic.[4] Some of them can be confused chromatographically with hydroxystilbenes with which they sometimes co-occur. Hillis and Hart[5] were able to isolate and identify ellagic and methylated ellagic acids and their glycosides from the wood of *E. sideroxylon* which they isolated from extracts of *E. globulus*[6] as well. *E. rostrata* leaves also proved to contain ellagitannins, flavonols and their glycosides.[7, 8]

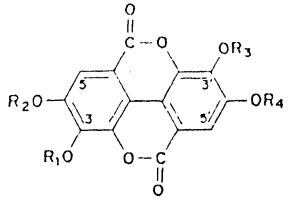
The present work includes the isolation and identification of some additional derivatives including two new glycosides.

RESULTS AND DISCUSSION

The leaves of *E. rostrata* were extracted with 50% aqueous acetone. The dried extract was fractionally extracted with ethyl acetate which in turn was extracted with chloroform. The new glycoside compound (I), 3,3′,4-tri-O-methylellagic-4′-O-β-D-galactoside, was detected by its mauve fluorescent colour under UV light (254 nm), which changed to faint mauve on exposure to ammonia vapours. On complete acid hydrolysis, it yielded galactose (co-paper chromatography) and the aglycone (1a) which was also released from its enzymatic hydrolysis using β-galactosidase and gave R_f-values and UV spectral data identical to those reported for 3, 3′, 4-tri-O-methylellagic acid.[9] The hypsochromic shift of the UV spectral maxima of (I) compared with that of (1a) and with the R_f-values in different solvent systems showed that compound (1) is 3,3′,4-tri-O-methylellagic acid-4′-O-β-D-galactoside and its structure was further confirmed through ^{1}H-NMR spectroscopy where the δ-value of 3′-methoxyl group (4.05 ppm) was shifted downfield than that of the

methoxyl groups at 3 and 4 positions (3.96 and 3.89 ppm, resectively), indicating glycosylation at 4′-position with the galactose moiety[9], which the signal of its anomeric proton appeared at δ 5.2 ppm.

Compound (2) appeared similar to (1) but with a slight difference in its R_f-values specially in aqueous acetic acid. Its UV spectral data were similar to those of compound (1). Its complete acid hydrolysis yielded 3,3′,4-tri-O-methyl-ellagic acid as the aglycone moiety (identified as before) and the sugars glucose and mannose which were identified by co-paper chromatography, *i.e.*, the two sugars are in position 4′. Enzymatic hydrolysis using β-glucosidase failed to give any intermediate, *i.e.* glucose was attached directly to the aglycone and mannose was terminal. Partial acid hydrolysis of (2) failed to give any intermediate suggested that the link between the two sugars is (1→6). This was confirmed through ^{1}H-NMR spectroscopy where the presence of a doublet signal at δ 5.75 ppm (J = 7 Hz) and at δ 4.70 ppm (J = 1.3 Hz) of the two anomeric protons of glucose and mannose, respectively, suggested the (1→6) linkage.[10]



(1) R_1, R_2, R_3 = Me, R_4 = Galactoside
(2) R_1, R_2, R_3 = Me, R_4 = Mannosylglucoside

EXPERIMENTAL

Plant material: *Eucalyptus rostrata* Schl (Myrtaceae) leaves were collected from the Orman Botanical Garden, Giza (Egypt). Voucher specimen is deposited at the National Research Centre Herbarium (CAIRC). It was identified by Prof. Dr. Lotfy Boulos (NRC).

Extraction, Isolation and Identification

Eucalyptus rostrata leaves were air dried, ground and extracted with 50% aqueous acetone. The aqueous acetone extract was subjected to fractional extraction using ehyl acetate followed by chloroform. The two new compounds (1) and (2) and the known compound (3) were isolated from the chloroform extract which was subjected to preparative paper chromatography using 30% AcOH as the solvent system and Whatman 3 MM paper (46 × 57 cm). Column chromatography, using Sphadex LH-20 as adsorbent, was used for the purification of all the isolated compounds prior to spectral and chemical methods of analyses. Complete and controlled acid hydrolyses (2N HCl, 1 h and 0.1 N HCl, respectively) of the glycosides yielded the sugar residues and the aglycones, all of which were co-chromatographed with authentic samples. Enzymatic hydrolysis[10] was done using β-glucosidase or β-galactosidase. All UV data were

recorded using the standard procedures.[10] ^{1}H-NMR spectra were taken on a Jeol Fx 270 spectrometer. Known compounds were identified by comparison of their UV, ^{1}H-NMR and R_f-values with those of authentic samples.

3,3′,4-Tri-O-methylellagic acid-4′-O-β-D-galactoside (1)

R_f-values × 100 on paper chromatography (Whatman No.1): (a) [BuOH : AcOH : H_2O (6 : 1 : 2)] 54; (b) [BuOH : AcOH : H_2O (4 : 1 : 5)] 76; (c) [6% AcOH] 42 and (d) [30% AcOH] 51. UV spectral data λ_{max}nm: (MeOH) 272,282*, 356, 371; (MeOH/NaOAc) 272, 283*, 356, 371; (MeOH/NaOMe) 272, 282*, 356, 371 nm (* shoulder). ^{1}H-NMR data δ (ppm) at 7.75 (s, H-5); 7.6 (s, H-5′); 4.05 (s, 3′-OMe); 3.96 (s, 3-OMe); 3.89 (s, 4-OMe); 5.2 (d, J = 7 Hz, H-1″ of galactose); 2.8–3.75 (m, galactosyl moiety).

3,3′,4-Tri-O-methylellagic acid (1a)

Its colour under UV light was mauve changing to yellow on exposure to ammonia vapours. R_f-values × 100 (Whatman No.1): (a) 82; (b) 93; (c) 6 and (d) 38. UV spectral data λ_{max} nm: (MeOH) 247, 287*, 357*, 370; (MeOH/-NaOAc) 253, 279*, 408; (MeOH/NaOMe) 255, 276*, 404 (* shoulder). ^{1}H-NMR data δ (ppm) at 7.44 (s, H-5); 7.38 (s, H-5′); 398 (s, 3′-OMe); 3.96 (s, 3-OMe); 3.91 (s, 4-OMe).

3,3′,4-Tri-O-methylellagic acid-4′-O-mannosyl (1→6) β-D-glucoside (2)

It has the same colour as (1) under UV light and NH_3/UV. R_f-values × 100 on paper chromatography (Whatman No.1): (a) 51; (b) 74; (c) 46 and (d) 58. UV spectral data λ_{max} nm (s, MeOH) 271, 362, 371*; (MeOH/NaOAc) 271, 395; (MeOH/NaOMe) 272, 310*, 405 (* shoulder). ^{1}H-NMR data δ (ppm) at 7.64 (s, H-5); 7.57 (s, H-5′); 4.15 (s, 3′-OMe); 4.03 (s, 3-OMe); 4.00 (s, 4-OMe); 5.75 (d, J = 7 Hz, H-1″ of glucose); 4.70 (d, J = 3 Hz, H-1‴ of mannose); 2.9–3.7 (m, protons of glucose and mannose).

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