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New Phenylpropanoid Glucosides from *Eucalyptus maculata*

Omar A. Rashwan*

Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt. Tel: +20-2-3624105, Fax: +20-2-3635140

* To whom correspondence should be addressed; e-mail omarrashwan@hotmail.com

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Abstract: Three compounds were isolated from the butanol soluble fraction of the resinous exudate from the stem of *Eucalyptus maculata*. In addition to *p*-coumaric acid two new compounds were identified. They were identified as 1-*O*-cinnamoyl 6-*O*-*p*-coumaroylglucose and 7-methyl-aromadendrin-4′-*O*-(6″-*trans*-*p*-coumaroyl)-*β*-D-gluco-pyranoside by spectroscopic and chemical means.

Keywords: *Eucalyptus maculata*, Myrtaceae, diacylglucose, acyl flavanonol glucoside, coumaric acid, NMR, MS.

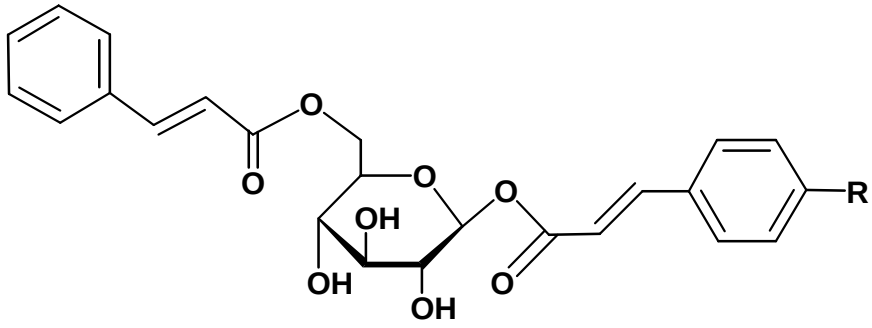
Introduction

Eucalyptus maculata Hook (Myrtaceae) is a medicinal plant traditionally used for the treatment of asthma and chronic bronchitis [1]. In a recent communication, Abdel-Sattar *et al*. reported the isolation of 1,6-*O*-dicinnamoyl glucose, 7-methylaromadendrin and sakuranetin from the resinous exudate of the stem of this plant [2]. The isolation of a phenylpropanoid from the stem exudate of *E. maculata* [2] was encouraging enough to pursue further investigation of the extracts of this plant. Accordingly, we carried out chemical examination of the butanol soluble fraction of the resin exudate, from which three compounds were isolated.

Results and Discussion

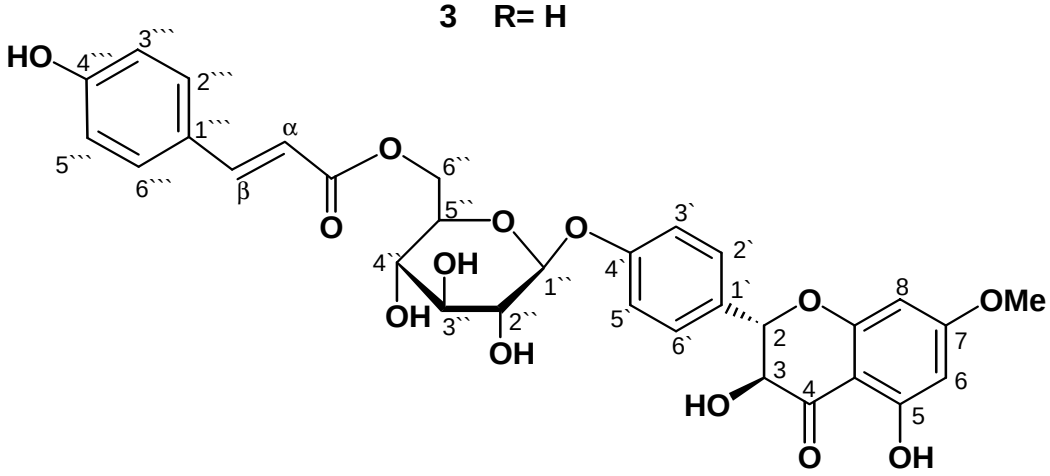
Three phenolic compounds (**1**-**3**) were isolated from the butanol soluble fraction of the resinous material obtained from the stem of *E. maculata*. Compound **1** was identified as *p*-hydroxycinnamic acid (*p*-coumaric acid) by comparison of its spectral data (MS, NMR) with data reported in the literature [3, 4] and by comparison with an authentic sample (TLC, mp and IR).

Compound **2** gave a positive test for sugars and/or glycosides. HR-FABMS gave $[M-H]^-$ at 455.1331, corresponding to the molecular formula $C_{24}H_{24}O_9$ (calculated 455.1342) and this was further confirmed from the ion peak at *m/z* 479 $[M+Na]^+$ in the electrospray ionization (ESI) mass spectrum (positive mode). TLC examination of the acid and alkaline hydrolysates revealed the presence of cinnamic acid, *p*-coumaric acid, as well as glucose as the sugar moiety. The *β*-configuration of the glucose moiety was indicated from the large coupling constant of its anomeric proton (cf. Agrawal, [5]), and by the downfield shift (δ 5.53) of this proton [6]. The previous finding was also supported by the similarity of ^{1}H- and ^{13}C-NMR spectral data of compound **2** with those of 1,6-dicinnamoyl-*O*-α-D-glucopyranoside (**3**), previously isolated from the same plant [2].



2 R= OH

3 R= H



4 (2,3-*trans*)

The presence of a cinammoyl moiety in **2** was confirmed by comparison of its NMR data with those of **3**. However, one of the cinnamoyl moieties in **3** was replaced by a *p*-coumaroyl moiety in **2**, as was ascertained from the observation of proton signals due to a *p*-disubstituted aromatic ring system [δ_H 7.55 (2H, d, *J* = 8.5 Hz, H-2, H-6) and 6.80 (2H,d, *J* = 8.5 Hz, H-3, H-5)]. Acylation at C-1 and C-6 of the glucose unit was confirmed by comparison of the ^{1}H-and ^{13}C-NMR chemical shifts of the glucose unit compared with those reported in **3** [1], which suggested acylation, as in **3**. The acylation at C-6 of the glucose moiety was determined from the downfield shift of the C-6 (+ 2.4) relative to the respective carbon in β-glucopyranose [5]. TLC of the partial acid hydrolysate of **2** using 5% HCl [7] indicated that *p*-coumaroyl moiety was located at C-1 (*p*-coumaric acid was first identified on TLC) and cinnamoyl moiety was located at C-6 (cinnamic acid was later identified). This finding was further supported from the HMBC spectrum, which showed long-range correlation between the carbonyl of the coumaric acid at δ_C 166.8 to the anomeric proton (δ_H 5.53) and to the protons at δ_H 6.65 and 7.65 (H_α and H_β, respectively). From the foregoing findings, compound **2** was thus identified as 1-*O*-cinnamoyl 6-*O*-*p*-coumaroyl-*β*-D-glucopyranoside, which represents a new natural product described here for the first time.

Compound **4** was obtained as white amorphous powder, $[\propto]_D^{25}$ + 29.4° (*c*. 0.2, MeOH). HR-FABMS of compound **4** gave $[M-H]^-$ at 609.1658, assigned to the molecular formula $C_{31}H_{30}O_{13}$ (calculated 609.1667). UV spectral analysis of **4** in MeOH (220, 292, 310_{sh} nm) and after addition of different shift reagents suggested the presence of a flavanonol skeleton with free hydroxyl groups at 3 and 5 positions [8]. The ^{1}H-NMR spectrum of **4** showed the presence of two doublets at δ_H 4.18 and 5.86 (*J*=11.6 Hz), characteristic of the *trans* H-2/H-3 protons, and supported the above finding. By acid hydrolysis, the flavonoid aglycone part was identified as 7-*O*-methyl aromadendrin, previously isolated from the same plant [2]. The results of acid and alkaline hydrolysis revealed in addition to 7-*O*-methyl aromadendrin, the presence of *p*-coumaric acid and glucose (TLC). The coupling constant of the anomeric proton indicated the *β*-configuration of the glucose moiety [5]. The glycosylation position was determined to be at C-4′ hydroxyl group from the observation of the downfield shift of C-4′ (δ 157.6) and the long-range correlation between H-1″ and C-4′ in the HMBC spectrum of **4**. Acylation of glucose moiety at C-6″ was established from the downfield shift of C-6″ (+ 1.4 ppm) and the upfield shift of C-5″ (- 2.7 ppm) relative to the respective carbons in glucopyranose [5]. From the aforementioned data, compound **4** was elucidated as 7-methylaromadendrin-4′-*O*-(6″-*trans*-*p*-coumaroyl)-*β*-D-glucopyranoside, which was isolated here for the first time from the title plant and from nature.

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Experimental

General

IR spectra were recorded on a JASCO FT/IR-230 IR spectrometer. UV spectra were measured on a Shimadzu UV-2200 UV-VIS spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were measured with a JNM-LA400WB Lambda (JEOL) NMR (^{1}H-, 400 MHz; ^{13}C-, 100 MHz) with the chemical shifts (δ ppm) expressed relative to TMS as internal standard or with a Jeol EX 270 NMR spectrometer at 270 MHz (^{1}H-) and 67.5 MHz (^{13}C-). Electrospray ionization mass (ESI-MS) spectra were measured with a Perkin-Elmer SCIEX APT *III* biomolecular mass analyzer. FABMS and HR-FABMS spectra were measured using JEOL JSM-700T spectrophotometer and glycerol as matrix. TLC was carried out using precoated silica gel F_{254} plates (Merck) and detection was made by visualization under UV light (254 nm) and after spraying with *p*-anisaldehyde spray reagent followed by heating.

Plant Material

The resinous material was collected in February 2001, from the stem of the plant cultivated in Zoo garden, Giza, Egypt. The plant was kindly identified by Dr. M. Gibali (Plant Taxonomy and Egyptian Flora Department, National Research Centre, Giza, Egypt).

Extraction and Isolation

The air-dried powdered resin of *E. maculata* (90 g) was extracted by percolation with methanol at room temperature. The methanol extract was evaporated to dryness under reduced pressure to give a solid residue (80 g). This residue was suspended in water (200 mL) and shaken with chloroform followed by *n*-butanol. The combined *n*-butanol extract was evaporated to dryness to give a brown residue (40 g). A portion of this material (20 g) was chromatographed on a Si gel column (6.5 x 24 cm), using chloroform-methanol-water (80:20:0.5), while 100 mL fractions were collected. The Fractions that eluted between 3500-5600 mL were pooled and evaporated to give 3.7 g of dry residue, designated as Fr. A. Further chromatography of Fr. A on a Si gel column (3.5 x 18 cm) using chloroform-methanol-water (80:20:0.1) gave three subfractions (B-1, B-2 and B-3). Fraction B-1 (800 mg) was rechromatographed on a Si gel column using chloroform-methanol (95:5) to give compound **1** (120 mg, 500-700 mL), compound **2** (100 mg, 1000-1150 mL) and 220 mg of an impure aliquot of **4** (1600-1750 mL). Further purification of compound **4** (20 mg) was achieved by chromatography on a Si gel column using 3 % MeOH/$CHCl_3$ as eluant.

Alkaline Hydrolysis of **2** *and* **4**

This was performed by heating 5 mg of the sample with 1 N NaOH (2 mL) at 60 ^{o}C for 40 min, followed by neutralization of the solution by passage through a small column of Dowex 50-WX 8 (H^{+}). The column was eluted with water and the combined eluate was extracted with ether.

Acid hydrolysis of **4.**

The hydrolysis was performed according to ref. [7, 8].

1-O-Cinnamoyl 6-O-coumaroyl-β-D-glucopyranoside (**2**).

Molecular formula $C_{24}H_{24}O_9$, $[\propto]_D^{25}$ - 24.6 ° (*c.* 0.35, MeOH), IR υ_{max} (KBr) cm^{-1} : 3500, 1720, 1660, 1605, 830. UV (MeOH) λ_{max} (ε): 286 (2.8 x10^3), 312 (2.3 X 10^3) nm. ^{1}H-NMR (270 MHz, CD_3OD) δ: **cinnamoyl**: 7.75 (2H, m, H-2, H-6), 7.65 (1H, d, *J* = 16 Hz, H_β), , 7.40 (3H, m, H-3, H-4, H-5), 6.40 (H,d, *J* = 16 Hz, H_α), ***p*-coumaroyl**: 7.65 (1H, d, *J*=16, H_β), 7.55 (2H, *J* = 8.5 Hz, H-2, H-6), 6.80 (2H,d, *J* = 8.5 Hz, H-3, H-5), 6.65 (1H, d, *J* = 16 Hz, H_α), **β-D-glucose**: 5.53 (1H, d, *J*= 7 Hz, H-1), 4.45 (1H, d, *J*=11 Hz, H-6A), 4.20 (1H,dd, *J*=11, 4.5 Hz, H-6B), 3.60 (1H, m, H-5), 3.40-3.55 (3H, m, H-2, H-3, H-4). ^{13}C NMR (67.5 MHz, CD_3OD) δ: **cinnamoyl**: 167.0 (C=O), 145.6 (C_β), 135.2 (C-1), 129.7 (C-2, C-4, C-6), 129.1 (C-3, C-5), 118.6 (C_α), ***p*-coumaroyl**: 166.0 (C=O), 160.8 (C-4), 146.8 (C_β), 131.1 (C-2, C-6), 126.7 (C-1), 116.6 (C-3, C-5), 114.7 (C_α), **β-D-glucose**: 95.1 (C-1), 77.6 (C-3), 75.7 (C-5), 73.8 (C-2), 70.8 (C-4), 64.2 (C-6). HR-MS $[M-H]^-$ (negative mode) *m/z* 455.1331 (calculated 455.1342). ESI-MS (positive mode) *m/z* (rel. int.): 479 $[M + Na]^+$ (100), 293 [M- *p*-coumaroyl $]^+$ (31), 131 $[Ph-C{=}C-CO]^+$ (55).

7-Methylaromadendrin-4′-O-(6″-trans-p-coumaroyl)-β-D-glucopyranoside (**4**)

Obtained as a white amorphous powder. $[\propto]_D^{25}$ + 29.4 ° (*c.* 0.2, MeOH), IR υ_{max} (KBr) cm^{-1}: 3448, 2922, 1686, 1637, 1510, 1075 and 833 cm^{-1}; UV (MeOH) λ_{max} (log ε): 220 (4.35), 293 (4.26), 315 (4.20); (+ NaOMe): 220, 245_{sh}, 292, 362, $AlCl_3$ 222, 314, 370; (+ $AlCl_3$/HCl): 222, 314, 370; (+ NaOAc): 222, 293, 315_{sh}; (+ NaOAc/H_3BO_3): 224, 292, 315_{sh}. ^{1}H-NMR (400 MHz, CD_3OD): **flavanonol** : δ 3.74 (3H, *s*, OMe), 4.18 (1H, *d*, *J*=11.6 Hz, H-3), 5.86 (1H, *d*, *J*= 11.6 Hz, H-2), 5.93 (1H, *br d*, H-6), 6.02 (1H, *br d*, H-8), 7.03 (2H, *d*, *J*= 8.8 Hz, H-3′, H-5′), 7.30 (2H, *d*, *J*= 8.8 Hz, H-2′, H-6′), ***p*-coumaroyl**: δ 7.56 (1H, *d*, *J*=16, H_β), 7.32 (2H, *d*, *J* = 8 Hz, H-2‴, H-6‴), 6.67 (2H,*d*, *J* = 8 Hz, H-3‴, H-5‴), 6.62 (1H, *d*, *J* = 16 Hz, H_α), **β-D-glucose**: δ 4.83 (1H, *d*, *J*= 6.5, H-1″), 4.46 (1H, *d*, *J*=11, H-6_A″), 4.20 (1H, *dd*, *J*=11, 4.5, H-6_B″), 3.60 (1H, m, H-5″), 3.40-3.55 (3H, m, H-2″, H-3″, H-4″). ^{13}C NMR (100 MHz, CD_3OD) **flavanonol**: δ 196.3 (C-4), 168.3 (C-7), 163.3 (C-9), 162.5 (C-5), 157.6 (C-4′), 130.4 (C-1′), 128.6 (C-2′, C-6′), 116.6 (C-3′,C-5′), 101.0 (C-10), 95.8 (C-8), 94.1 (C-6), 82.9 (C-2), 72.2 (C-3), **β-D-glucose**: δ 100.6 (C-1″), 76.3 (C-3″), 74.0 (C-5″), 73.0 (C-2″), 70.1 (C-4″), 63.2 (C-6″), ***p*-coumaroyl**: δ 167.6 (C=O), 159.4 (C-4‴), 145.5 (C_β), 129.8 (C-2‴, C-6‴), 125.5 (C-1‴), 115.6 (C-3‴, C-5‴), 113.7 (C_α). HR-MS $[M-H]^-$ (negative mode) *m/z* 609.1658 (calculated 609.1667). ESI -MS *m /z* (rel. int.): 461 [M- *p*-coumaroyl]$^+$ (28), 325 [M-methylaromadendrinoyl+Na]$^+$ (10), 259 (75), 223 (44), 149 (100).

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Sample Availability: Available from the authors.