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# Phenolic compounds from *Eucalyptus maculata*

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From the chloroformic extract of the resinous exudate of the stems of *Eucalyptus maculata,* four phenolic constituents have been isolated. A new di-cinnamic acid glucose ester was isolated, in addition to cinnamic acid, 7-methyl aromadendrin and sakuranetin. The isolated compounds were elucidated using UV, IR, 1D and 2D NMR and MS.

## 1. Introduction

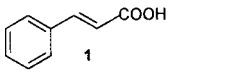
*Eucalyptus maculata* Hook (Family Myrtaceae ) is one of several species of genus *Eucalyptus* indigenous to Australia and introduced to and cultivated in Egypt [1]. From a medicinal point of view, it is used for treatment of asthma and chronic bronchitis [2]. In an earlier work, Gell et al. (1958) reported the presence of ellagic acid, *p*-hydroxycinnamic acid and 7-methyl aromadendrin [3]. Also Mishra and Misra (1980) isolated isosakuranetin and leucopelargonidin-3-*O*-α-L-rhamno-β-glucopyranoside from the stem bark of the same plant [4]. Nothing was reported concerning the constituents of the resin obtained from the stem of *E. maculata,* growing in Egypt. This study was carried out to isolate and identify its major constituents.

## 2. Investigations, results and discussion

The chloroform extract of the resinous material obtained from the stem of *E. maculata*, afforded four phenolic compounds. Compound **1** was identified as cinnamic acid by comparison of its spectral data (MS, NMR) with data reported in the literature [5, 6] and by comparison with authentic sample (TLC, mp and IR).

Compound **2** had a molecular formula $C_{16}H_{14}O_6$ and its IR spectrum showed bands at 3450 (OH) and 1620 (C=O) $cm^{-1}$. UV spectral analysis of compound **2** in methanol (330, 289 nm) and after addition of the different shift reagents suggested a dihydroflavonol skeleton with free hydroxyl groups at 3′ or 4′, 3 and 5 positions [7]. This was also confirmed from the $^1$H NMR spectrum by the presence of two doublets at $\delta_H$ 4.56 and 5.03 (J = 11.5 Hz), characteristic of *trans* H-2/H-3 protons. The $^1$H- and $^{13}$C NMR spectral data of compound **2** (see Experimental and Table), were indistinguishable from the data reported for 7-*O*-methyl aromadendrin [(2*R*, 3*R*)-3,5,4′-trihydroxy-7-methoxyflavanone] [8, 9], which was previously isolated from the stem bark of the same plant [3].

Compound **3** had a molecular formula $C_{16}H_{14}O_5$, and its IR spectrum showed peaks at 3450 (OH) and 1620 (C=O) $cm^{-1}$. UV spectral analysis of compound **3** in methanol (331, 285 nm) and after addition of the different shift reagents suggested the presence of a flavanone skeleton with free hydroxyl groups at 3′ or 4′ and 5 positions [7]. The $^1$H NMR spectrum showed an AA′BB′ system, characteristic of 1,4-disubstituted aromatic rings (B ring), in addition to one OMe group located at C-7, which was easily deduced from the UV spectrum after addition of NaOAc [7]. From the $^1$H- and $^{13}$C NMR spectral data and by comparison with literature data [9–11], compound **3** could be identified as sakuranetin [(2*S*)-5,4′-dihydroxy-7-methoxyflavanone].

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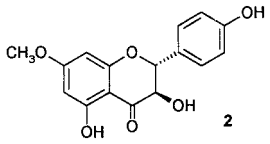


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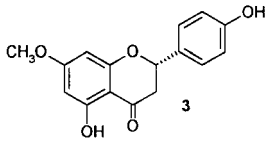


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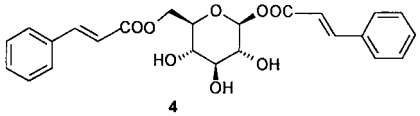


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Compound **4** had a molecular formula $C_{24}H_{24}O_8$, and gave a positive Molish's test for carbohydrates and/or glycosides, indicating its glycosidic nature. Alkaline hydrolysis with 1 N Sodium hydroxide revealed the presence of cinnamic acid (TLC) as well as glucose as the sugar moiety (TLC). $^1$H- and $^{13}$C NMR spectral data of compound **4** revealed the presence of two cinnamoyl moieties. This was confirmed by the presence of signals at $\delta_c$ 165.5 and

**Table: $^{13}$C NMR (125 MHz, $CD_3OD$) chemical shift values of compounds 2 and 3**

| Carbon No. | $^{13}$C NMR ($\delta_C$) | |
|---|---|---|
| | 2 | 3 |
| 2 | 85.1 | 80.9 |
| 3 | 73.9 | 44.6 |
| 4 | 198.6 | 198.1 |
| 5 | 164.9 | 165.3 |
| 6 | 96.5 | 96.3 |
| 7 | 169.7 | 169.6 |
| 8 | 95.5 | 95.5 |
| 9 | 164.2 | 164.7 |
| 10 | 102.8 | 104.4 |
| OMe | 56.9 | 56.9 |
| 1′ | 129.0 | 129.3 |
| 2′ | 130.4 | 130.9 |
| 3′ | 116.6 | 116.8 |
| 4′ | 159.1 | 159.1 |
| 5′ | 116.6 | 116.8 |
| 6′ | 130.4 | 129.3 |

167.2 (two carbonyl groups), $\delta_c$ 7.80 and 7.71 (2 H, d, J = 16) and $\delta_c$ 6.49 (2 H, d, J = 16 Hz), corresponding to two conjugated double bonds of the two cinnamoyl moieties. On the other hand, two sets of protons at $\delta_c$ 7.53 and 7.38 integrated for 4 and 6 protons, respectively, and corresponding to two monosubstituted aromatic rings of the two cinnamoyl moieties. These moieties were positioned at C-6 and C-1 of the glucose moiety as indicated by a downfield shift of the respective carbons (+1.4 and +1 ppm, respectively) relative to that of glucopyranose [12]. The configuration of H-1 at C-1 of the glucose moiety was deduced as α-, as indicated by the small coupling constant (J = 3.9 Hz) of the anomeric proton [12]. The α-configuration was also confirmed from NOESY spectrum. The NOESY spectrum of the sugar moiety showed spatial relations between H-1 and H-5, H-3 and H-5, and between H-2 and H-4 [12], which confirm the α-configuration of H-1. The option β-mannose was excluded by a different colour reaction on TLC. The assignment of all sugar protons was unambiguously established from the HOHAHA spectrum. From these findings, compound **4** could be identified as 1,6-dicinnamoyl-*O*-α-D-glucopyranoside.

To our knowledge, compound **4** is a new natural product, while compounds **1** and **3** were isolated for the first time from *E. maculata*.

## 3. Experimental

### 3.1. General

M.p.s were recorded on a Gallenkamp apparatus, and were uncorrected. Optical rotations were measured on a Perkin-Elmer 241 MC polarimeter at room temperature. IR spectra were recorded on a Pye Unicum Sp3-300. FAB-MS (positive ion mode) spectra were measured on a JEOL-JMS700 spectrometer with glycerol or NBA as a matrix and $EI^+$-MS spectra were on JEOL JMS-AX 505 (70 eV). UV spectra were recorded on a Schimadzu 265 spectrophotometer in methanol and after addition of different shift reagents. $^1$H- and $^{13}$C NMR spectra were recorded on a Varian 500 MHz spectrometer, using $CDCl_3$ as solvent and TMS as int. standard. TLC screening of the fractions was carried out using precoated silica gel $GF_{254}$ plates (Merck), developed by 5% MeOH/$CHCl_3$ and detected by *p*-anisaldehyde as spray reagent or by visualization under UV light (254 nm).

### 3.2. Plant material

The resinous material was collected in Jan.1998 from the stems of the plant cultivated in Zoo garden, Giza, Egypt). The plant was kindly identified by Dr. M. El-Gebaley (Plant Taxonomy and Egyptian Flora Department, National Research Centre, Giza, Egypt)

### 3.4. Extraction and isolation

The air-dried powdered resin of *E. maculata* (180 g) was extracted by percolation with methanol. The residue left after distillation of the solvent was partitioned between water and chloroform. The combined chloroformic extract gave a brown residue (15 g) after distillation of the solvent. A portion of the chloroformic extract (10 g) was chromatographed on Si gel column (7 × 25 cm) using chloroform with increasing amounts of methanol (1–8%). Fractions of 100 ml each were collected. Three main fractions were collected: fractions A, B and C were eluted between 5500–8800 ml (620 mg, 4% MeOH/$CHCl_3$), 9200–12300 ml (2 g, 6% MeOH/$CHCl_3$) and 12400–12800 ml (2.5 g, 8% MeOH/$CHCl_3$), respectively. Fraction A (300 mg) was further purified on a Chromatotron plate (2 mm, Sigel and 5 ml fractions) using 3% MeOH/$CHCl_3$ eluting system to afford compound **1** (38 mg), in fraction between 100–150 ml. Compound **2** (1.4 g) was obtained from fraction B upon concentration. Fraction C was fractionated on another Si gel column (4 × 14 cm) using 3% MeOH/$CHCl_3$ as eluting system, and fractions 50 ml each were collected, which gave 3 groups of fractions. Fraction B-1 (50–200 ml) afforded additional amount of compound **2** (1.5 g), while fraction B-2 (250–300 ml) afforded compound **3** (200 mg). Fractions B-3 (300 mg) eluted between 350–450 ml afforded compound **4** (90 mg).

### 3.5. Alkaline hydrolysis of 4

The sample (5 mg) was heated with 2 ml of 1 N sodium hydroxide at 60 °C for 40 min and the solution was neutralized by passing through small column packed with Dowex 50-WX8 ($H^+$). The column was eluted with water and the combined eluate was extracted with ether.

### 3.6. 7-O-Methyl aromadendrin [(2R, 3R)-3,5,4′-trihydroxy-7-methoxyflavanone] (2)

White needle crystals, m.p. 184–185 °C ($CHCl_3$/ether), IR $\upsilon_{max}$ (KBr) $cm^{-1}$: 3450 (OH), 1620, 1560, 1520, 830. $^1$H-NMR ($CD_3OD$): δ 3.81 (3 H, s, OMe), 4.56 (1 H, d, J = 11.5 Hz, H-3 ), 5.03 (1 H, d, J = 11.5 Hz, H-2) , 6.03 (1 H, d, J = 2.2 Hz , H-6), 6.08 (1 H, d, J = 2.2 Hz, H-8), 6.87 (2 H, d, J = 8.8 Hz, H-3′, H−5′), 7.37 (2 H, d, J = 8.8 Hz, H−2′, H−6′). $^{13}$C NMR: see Table 1. EI-MS m/z (rel. int.): 302 $[M]^+$ (100) , 273 (100), 179 (50), 167 (100), 134 (100), 107 (65).

### 3.7. Sakuranetin [(2S)-5,4′-dihydroxy-7-methoxyflavanone] (3)

White needle crystals, m.p. 145–146 °C ($CHCl_3$/ether); IR $\upsilon_{max}$ (KBr) $cm^{-1}$: 3450, 1620, 1560, 1520, 830. $^1$H-NMR ($CD_3OD$): δ 2.74 (1 H, dd, J = 3, 17 Hz, H-$3_a$) 3.12 (1 H, dd, J = 13.2, 17 Hz, H-$3_b$), 3.81 (3 H, s, OMe), 5.35 (1 H, dd, J = 3, 13.2 Hz, H-2 ), 6.0 (2 H, brs, H-6 & H-8), 6.84 (2 H, d , J = 8.8 Hz , H-3′, H-5′), 7.31 (2 H, d, J = 8.2 Hz, H-2′, H-6′). $^{13}$C NMR: see Table 1. EI-MS: m/z (rel. int.): 286 (100), 269 (20), 243 (20), 193 (90), 180 (100), 167(100), 149 (52), 120 (82).

### 3.8. 1,6-Dicinnamoyl-O-α-D-glucopyranoside (4)

Molecular formula $C_{16}H_{14}O_5$, $[\alpha]_D^{25}$ + 9.4° (c. 0.011, MeOH), IR $\upsilon_{max}$ (KBr) $cm^{-1}$ 3450, 1620, 1560, 1520, 830. UV $\lambda_{max}$ (MeOH) nm (ε): 267 (93,200). $^1$H-NMR ($CD_3OD$): cinnamoyl moieties 7.80, 7.71 (2 H, d, J = 16, 2 × H-β), 7.53 (4 H, m, 2 × H-2, and 2 × H-6′), 7.38 (6 H, m 2 × H-3, H-4, H-5), 6.49 (2 H, d, J = 16, 2 × H-α), α-glucose 5.69 (1 H, d, J = 3.9, H-1), 4.54 (1 H, d, J = 12, H-6A), 4.43 (1 H, dd, J = 12, 4.5, H-6B), 3.74 (1 H, m, H-5), 3.57 (2 H, m, H-2, H-3), 3.5 (1 H, m, H-4). $^{13}$C NMR ($CD_3OD$), cinnamoyl: 165.47, 167.23 (2 × C = O), 146.46, 145.37 (2 × C-α), 133.37, 133.87 (2 X C-1), 130.39, 130.15, 2 X 128.59, 2 X 128.54 ( 2 X C-3, 2 X C-4, 2 X C-5), 2 X 127.89, 2 X 127.82 ( 2 X C-2, 2 X C-6), 117.05, 116.56 (2 X C-β), α-glucose 94.03 (C-1), 76.2 (C-3), 74.57 (C-5), 72.14 (C-2), 69.43 (C-4), 63.16 (C-6). $FAB^+$-MS m/z (rel. int.): 463 $[M + Na]^+$ (30), 423 $[M + H\text{-}H_2O]^+$ (15), 293 [M-cinnamoyl]$^+$ (20), 276 $[M + H$ - cinnamoyl - $H_2O]^+$ (20), 131 $[Ph\text{-}C = C\text{-}CO]^+$ (100). EI-MS (m/z ): 440 $[M]^+$ (0.4 ).

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**References**

1 Assem, K. A.: A contribution to the pharmacognostical study of the leaves of certain *Eucalyptus* species cultivated in Egypt, M. Pharm. Thesis (1964)
2 Kirtiker, K. R.; Baju, B. D.: Indian Medicinal Plants, Vol. II, p. 1043 Shastri, B. N. (1952)
3 Gell, R. J.; Pinkey, J. T.; Ritchie, E.: Australian J. Chem. **11**, 372 (1958)
4 Mishra, C. S.; Misra, K.: Planta Med. **38**, 169 (1980)
5 Bhacca, N. S.; Hollis, D. P.; Johnson, L. F.; Pier, E. F.; Shoolery, J. N.: High Resolution NMR Spectra Catalog, Varian Associates, National press, U.S.A. (1963).
6 Agrawal, P. K.; Bansal, M. C.: in: Agrawal, P. K. (ed.): Carbon 13 NMR of Flavonoids, p. 345, Elsevier Science Publishers B.V., Amsterdam 1989
7 Mabry, T. J.; Markham, K. R.; Thomas, M. B.: The Systematic Identification of Flavonoids. Springer, Berlin 1970
8 Chiappini, G.; Fardella, A.; Menghini, A.; Rossi, C.: Planta Med. **44**, 159 (1982)
9 Grande, M.; Piera, F.; Cuenca, A.; Torres, P.; Bellido, I. S.: Planta Med. 414 (1985)
10 Liu, Y.-L.; Ho, D. K.; Cassady, J. M.; Cook, v. M.; Baird, W. M.: J. Nat. Prod. **55**, 357 (1992)
11 Niwa, M.; Tatematsu, H., Liu; G. Q.; Hirata, Y.: Chemistry Letters, 539 (1984)
12 Agrawal, P. K.: Phytochemistry **31**, 3307 (1992)

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