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Flavonoids of *Melaleuca quinquenervia*

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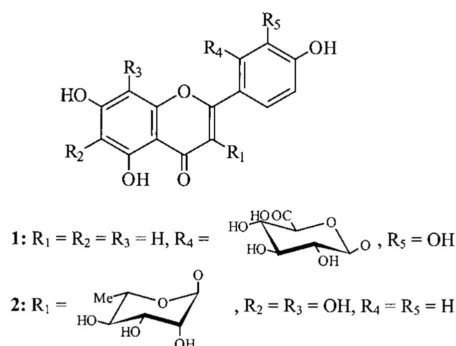
From the leaves of *Melaleuca quinquenervia* (Clav.) S. T. Blake, the new flavonoid 5,7,3′,4′-tetrahydroxyflavone 2′-*O*-β-D-glucopyranuronide, as well as eight known flavonol glycosides have been isolated and characterized. The structures were established by chromatography, chemical degradation and UV spectroscopy and confirmed by ESI-MS and NMR spectroscopy. This is the first report on the isolation and identification of these flavonoids in the genus *Melaleuca*.

1. Introduction

The genus *Melaleuca* (Myrtaceae) is indigenous to Australia and comprises about 250 species [1]. Previous studies on *M. elliptica* Smith, *M. bracteata* F. Muell, *M. quinquenervia* (Clav.) S. T. Blake and *M. styphelioides* Smith. growing in Egypt dealt with the chemical composition of their volatile oils and certain pharmacological actions [2–4]. Other phytochemical studies led to the identification of a cyclohexanoid monoterpene glucoside, melanervin and a nortriterpene from *M. quinquenervia* [5, 6] and *M. leucodendron* [7], respectively.

The present study describes the isolation of a novel flavone glucuronide (**1**) and 6,8-dihydroxykaempferol 3-*O*-α-L-rhamnopyranoside (**2**) which was isolated once before [8], in addition to quercitrin (**3**), quercetin 7-*O*-β-D-(6″-*O*-galloyl-glucopyranoside) (**4**), myricitrin (**5**), myricetin 3-*O*-β-D-glucopyranoside (**6**), myricetin 3-*O*-β-D-glucopyranuronide (**7**), myricitrin 2″-*O*-gallate (**8**) and afzelin (**9**), kaempferol (**10**), quercetin (**11**) and myricetin (**12**).



2. Investigations, results and discussion

The dry powder of the *M. quinquenervia* leaves was defatted with chloroform and extracted with hot aqueous ethanol (80%) under reflux. The extract obtained was concentrated in vacuo and examined by 2D-PC, whereby a mixture of flavonol glycosides (colours in day and UV light with and without ammonia and their responses towards different spraying reagents e.g. $FeCl_3$, $AlCl_3$ and Naturstoff). CC on polyamide using H_2O and H_2O-EtOH mixtures with decreasing polarity afforded fractions containing flavonols which were fractionated by means of consecutive microcrystalline cellulose and Sephadex LH-20 columns. Elution with aqueous ethanol and/or n-butanol saturated with water afforded the known flavonoids **2**–**12** along with the new flavone glucuronide **1**. The structures were elucidated by established methods of analysis and confirmed by negative ESI-MS with ion-source collision-induced dissociation (CID) and, in some cases, direct CoPC with respective authentic samples and NMR spectroscopy.

Compound **1** was isolated as a yellow amorphous powder which showed a single spot on PC exhibiting purple fluorescence under UV light, changing to yellow with ammonia, suggesting a flavonoid glycoside structure. The sugar moiety of **1** was identified as glucuronic acid on the basis of CoPC with an authentic sample after complete acid hydrolysis and was confirmed with negative ESI-MS analysis on using CID. The MS spectrum showed two major ion peaks at m/z 477 and 301 assigned to $[M-H]^-$ and $[M\text{-glucuronide}]^-$, respectively. The aglycone moiety of **1** was expected to have a 5,7,3′,4′-tetrahydroxy 2′-*O*-substituted flavone nucleus, on the basis of UV spectral analysis. Addition of NaOMe reagent resulted in a large bathochromic shift ($\Delta\lambda = +52$ nm) with increase in the intensity of band I, suggesting a multi-oxygenated B-ring with a free 4′-OH group [9]. Also, the presence of free *ortho* dihydroxy function in the B-ring (3′ and 4′) was interpreted from the bathochromic shift for band I (to 380 nm, $\Delta\lambda = +20$ nm) in both NaOAc and NaOAc/H_3BO_3 UV spectra relative to that of MeOH spectrum [10]. This evidence was supported by the deep orange colour on spraying with Naturstoff reagent. The small bathochromic shift ($\Delta\lambda = +7$ nm) observed in band II upon addition of NaOAc, referred to a free 7-hydroxyl group. In addition, a free 5-hydroxy function was deduced through the bathochromic shifts in both $AlCl_3$ and $AlCl_3$/HCl UV spectra. Accordingly, **1** was tentatively identified as 5,7,3′,4′-tetrahydroxyflavone, 2′-*O*-glucuronide which was confirmed by ^{1}HNMR.

The ^{1}HNMR spectrum of **1** showed two *meta* coupled protons at δ ppm 6.22 and 6.42 as doublets with J = 2.1 Hz for H-6 and H-8, respectively. Observation of H-5′ and H-6′ resonances as two *ortho* doublets at 6.85 (J = 8.5 Hz) and 7.43 (J = 8.5 Hz) and the absence of H-2′ resonance indicated a 3′,4′-dihydroxy, 2′-*O*-glucuronide in the B-ring of **1**. Finally, H-3 resonance was located as a singlet at 6.94 and a β-anomeric proton as a doublet at 5.25 (J = 7.8 Hz) for the glucuronide moiety. On the basis of all these data and the comparison of the ^{1}HNMR results with those of 5,7,2′,3′,4′-pentahydroxyflavone [11], **1** was identified as 5,7,3′,4′-tetrahydroxyflavone 2′-*O*-β-D-glucopyranuronide.

Compound **2** was isolated as a bright yellow amorphous powder and exhibited the chromatographic properties of a flavonol 3-*O*-glycoside. On acid hydrolysis, **2** gave L-rhamnose (CoPC). The structure of **2** was established as 6,8-dihydroxykaempferol, 3-*O*-α-L-rhamnopyranoside on

the basis of the comparison of its UV and ^{1}HNMR spectral data with those of the same compound, previously isolated [8].

3. Experimental

3.1. Equipment

UV-analyses were run on a Shimazu UV-240 spectrometer and 4 ml quartz cells (1 cm optical pathway). Analytically pure MeOH was used with the addition of each of the shift reagents, separately. ESI-MS spectrometry was measured on a double focussing sector field Finnigan MAT 95 mass spectrometer (Finnigan, Bremen, Germany). NMR (^1H and ^{13}C) analyses were measured on a JEOL EX-270 MHz and Varian Mercury 300 MHz spectrometers relative to TMS. PC was carried out on Whatman No. 1 sheets using solvent systems A. (15% AcOH) and B. (n-BuOH-AcOH-H_2O 4 : 1 : 5, top layer).

3.2. Plant material

M. quinquenervia (Clav.) S. T. Blake leaves were collected from the Zoological Garden, Giza, Egypt (August–September). The identity of the plant was established by Dr. B. A. Barlow, Division of Plant Industry, CSIRO, Australia. A voucher specimen is kept in the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University.

3.3. Extraction and isolation

The dry powder of *M. quinquenervia* leaves (1.5 kg) was defatted with $CHCl_3$, then extracted with EtOH (80%) which yielded a dry extract (75 g) on removal of the solvent. This extract was chromatographed on a Polyamide 6S column (Riedel-De Haen AG, Seelze Hannover, Germany), being eluted with H_2O followed by H_2O-EtOH mixtures of decreasing polarity. The obtained major flavonoid fractions were further fractionated using successive microcrystalline cellulose and Sephadex LH-20 columns to give pure **1** (10 mg) and **2** (8 mg).

3.4. *5,7,3′,4′-Tetrahydroxyflavone 2′-O-β-D-glucopyranuronide* (1)

R_f-values: 0.38 and 0.48 in solvent systems A and B, respectively. UV λ_{max} (MeOH): 256, 360; + NaOMe: 273, 442; + $AlCl_3$: 272, 300sh., 370, 415; + $AlCl_3$/HCl: 272, 300(sh)., 370, 415; + NaOAc: 263, 300(sh.), 380; + NaOAc/H_3BO_3: 261, 300sh., 380 nm. ^{1}HNMR (270 MHz, DMSO-d_6): δ ppm 7.43 (1 H, d, J = 8.5 Hz, H-6′), 6.94 (1 H, s, H-3), 6.85 (1 H, d, J = 8.5 Hz, H-5′), 6.42 (1 H, d, J = 2.1 Hz, H-8), 6.22 (1 H, d, J = 2.1 Hz, H-6), 5.25 (1 H, d, J = 7.8 Hz, H-1″), 3.0–3.8 (remaining sugar protons). ESI-MS, m/z: 477 and 301 a.m.u. Acid hydrolysis of **1**: A solution of 4 mg in 10 ml 2 N HCl (MeOH-H_2O, 1 : 1) was refluxed at 100 °C for 2 h. After cooling, the reaction mixture was extracted with EtOAc. The aqueous hydrolysate was neutralized, concentrated and then examined by CoPC to prove the presence of glucuronic acid as sugar moiety.

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References

1 Bailey, L. H.: Manual of Cultivated Plants, 4th edn., p. 2, Macmillan, New York 1958
2 Aboutabl, E. A.; DePooter, H. L.; DeBuyck, L. F.; El-Tohamy, S. F.: Flavour Fragr. J. **6**, 139 (1991)
3 DePooter, H. L.; Aboutabl, E. A.; El-Tohamy, S. F.; Abdel Alim, M. A.; Moharram, F. A.: Al-Azhar J. Nat. Prod. **6**, 154 (1991)
4 Aboutabl, E. A.; Abdel Hakim, G.; Moharram, F. A.: Phytother. Res. **10**, 345 (1996)
5 Aboutabl, E. A.; Soliman, H. S. M..; Moharram, F. A.: Al-Azhar J. Nat. Prod. **23**, 110 (1999)
6 Wagner, H.; Seligmann, O.; Horhammer, H.; Chari, V.; Morton, J. F.: Tetrahedron Lett. **17**, 1341 (1976)
7 Ching-Kuo Lee: J. Nat. Prod. **61**, 375 (1998)
8 Moharram, F. A.; Soliman, H. S. M.; Marzouk, M. S.; Mahmoud, I. I.; El-Dip, A. R. paper submitted in the XXth International Conference on Polyphenols, Freising-Weihenstephan, Germany 2000
9 Mabry, T. J.; Markham, K. R.; Thomas, M. B.: The Systematic Identification of Flavonoids, Springer, Berlin 1970
10 Nianbai Fang, S.; Mabry, T. J.: Phytochemistry **27**, 171 (1988)
11 Harborne, J. B.: The Flavonoids Advances in Research, p. 450, Chapman & Hall, London 1993

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