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NEW LACTOYL GLYCOSIDE QUERCETIN FROM *Melia azedarach* LEAVES

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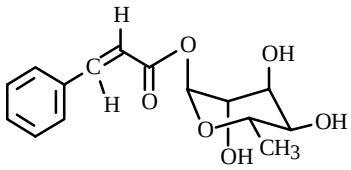
In the course of phytochemical investigations of Melia azedarach *leaves, a novel cinnamoyl glycoside, cinnamoyl-1-α-L-rhamnoside (**1**), and a novel acylated quercetin triglycoside, quercetin-3-*O*-[rhamnosyl 1→6(4″-lactoyl glucoside)]-4′-*O*-glucoside (**2**), have been isolated and characterized on the basis of spectroscopic methods, together with the six known flavonoid compounds kaempferol-3-*O*-rutinoside, 3-*O*-rhamnoside, quercetin-3-*O*-rutinoside, 3-*O*-rhamnoside, and the aglycones quercetin and kaempferol. All isolated compounds have been evaluated for their structures by chromatographic methods, chemical degradation, and UV and NMR spectroscopy. The antioxidant activity of the extract was studied as well.*

Key words: *Melia azedarach,* Meliaceae, leaves, novel acylated quercetin.

Melia is a fast growing deciduous tree, native to southwestern Asia, and is cultivated and naturalized in many warm and temperate countries of the world. Different parts of the tree, such as the bark and the leaves, are used in folk medicine [1], where the leaf juice is anthelmintic, antilithic, and diuretic; the flowers and leaves are applied as a poultice in the treatment of neuralgia and nervous headache; the stem bark is anthelmintic, astringent, and a bitter tonic, and the root is highly effective against ringworm and other parasitic skin diseases [2–4]. Chemical studies on this species resulted in kaempferol 3-rutinoside, rutin, and isoprenylated flavanone from the leaves [5, 6], apigenin 5-galactoside from the roots [7], and apigenin 7-rhamnopyranosyl(1→4)glucopyranoside from the stem bark [8]. Based on this information we here investigated the aerial parts of *Melia azedarach* and report the isolation of the two new natural compounds: cinnamoyl-1-α-L-rhamnoside (**1**) and quercetin 3-*O*-[rhamnosyl 1→6(4″-lactoyl glucoside)]-4′-*O*-glucoside (**2**), with the other six known compounds kaempferol 3-*O*-rutinoside (**3**), 3-*O*-rhamnoside (**4**), quercetin-3-*O*-rutinoside (**5**), 3-*O*-rhamnoside (**6**), quercetin (**7**), and kaempferol (**8**) from the aqueous ethanolic extract of this plant. The structure elucidation was based on ^{1}H and ^{13}C NMR together with the different physical and chemical investigations.



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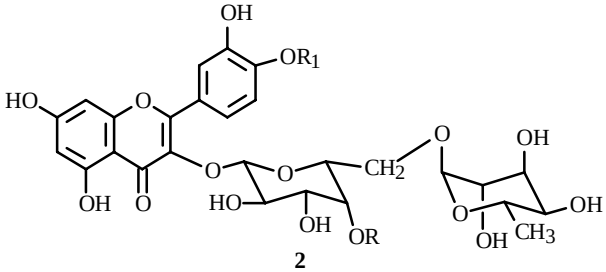

2

2: R = lactoyl; R_1 = glucose

Among all flavonoids so far tested, quercetin and its derivatives showed pharmaceutical activities, e.g., cytotoxic activity *in vitro* or *in vivo* [9], strong spasmolytic activity [10], and influence on the metabolism of blood vessel walls, while the cinnamic acid derivatives play a multiplicity of roles in the environment [9]. For the above reasons the extract was investigated and found to have moderate antioxidant activity.

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Preliminary study of the *Melia* leaves cultivated in Egypt showed the presence of a number of flavonoid compounds; thus the *Melia* leaves were collected, dried, ground, and extracted several times by aqueous ethanol. The ethanol extract was concentrated under vacuum, applied on a Sephadex LH-20 column, and eluted by water and water/ethanol mixtures to afford five fractions (I–V). Fraction I and II were further purified to give the two new natural cinnamoyl 1-α-L-rhamnoside (**1**) and the new natural quercetin 3-*O*-[rhamnosyl 1→6(4″-lactoyl glucoside)]-4′-*O*-glucoside (**2**) respectively. Fractions III, IV, and V were applied on small Sephadex LH-20 columns to give the other known compounds.

The chromatographic data of **1** showed that it possesses cinnamoyl-like characteristics (dark absorbing on paper chromatochraphy under UV light, unchanged on fuming with ammonia, and a dark spot with $FeCl_3$ with high R_f values in both aqueous and organic solvents). The UV spectral maxima in MeOH are dominated by an intense absorption peak at 302–320 nm, which is commonly associated with cinnamic acid acylation [11]. This view was supported by acid hydrolysis of **1**, which gave aglycone cinnamic acid and the sugar rhamnose (CoPC), suggesting **1** to be cinnamoyl rhamnoside. ^{1}H NMR spectral analysis confirmed the proposed structure whereby the signals of the olefinic group were appearing at δ 7.6 and 6.58 ppm (d, J = 16 Hz, H-β and H-α) with the signals of the aromatic cinnamoyl ring at δ 7.54 (d, J = 7.3 Hz, H-2, 6), 7.35 (br t, J = 7.3 Hz, H-3, 5), 7.28 (br d, J = 7.2 Hz, H-4), the anomeric proton of the rhamnose at δ 4.5 ppm (d, J = 2 Hz) with H-2′ at δ 4.21 (dd, J = 1.5, 4 Hz), and the methyl group at δ 0.87 (d, J = 6 Hz) revealed the rhamnosyl moiety to be α-linked and confirming **1** as cinnamoyl-1-α-L-rhamnoside.

With the use of common diagnostic reagents, the UV spectral data suggested that **2** is a disubstituted 3,4′-flavonol whereby the addition of NaOMe led to a bathochromic shift (52nm) in band I with a decrease in intensity; it was stable, indicating substitution in position 4′; however, no shift was produced on addition of $NaOAc/H_3BO_3$, indicating the absence of the 3′,4′-dihydroxyl group in ring (B). The shift (41nm) produced in band (I) after addition of $AlCl_3$/HCl corresponding to the methanol curve indicates the presence of a free hydroxyl group in ring A and a substituted one at position-3 [12]. Acid hydrolysis of compound **2** gave the aglycone quercetin, lactic acid, and the sugar glucose and rhamnose, which were identified by Co-PC, while enzymatic hydrolysis of **2** with β-glucosidase gave rise to the intermediate **2a**, whose R_f values and UV spectral data using different diagnostic reagent showed its identity with those reported for quercetin 3-glycoside; its complete acid hydrolysis gave rise to quercetin as the aglycone with lactic acid and the sugar glucose and rhamnose, so we can suggest that **2a** is quercetin 3-lactoyl rutinoside, which was confirmed by its ^{1}H NMR spectra which gave signals similar to those of quercetin 3-rutinoside with the appearance of additional signals at δ 4.18 (q, J = 7 Hz) and δ 1.18 (d, J = 7 Hz) of the lactoyl moiety. From these data we suggest that **2** is quercetin-3-lactoylrutinoside-4′-glucoside. The structure was finally confirmed by ^{1}H and ^{13}C NMR spectroscopy whereby the two glucose anomeric proton signals (H-1″ and H-1‴) at δ 5.34 ppm (d, J = 7.5 Hz) and 5.03 ppm (d, J = 7.5 Hz) and the anomeric carbons at δ 101.7, 101.3 ppm indicated their attachment directly to the 3 and 4′ positions of the quercetin, respectively. The presence of the rhamnose anomeric protons (H-1″) at δ 4.38 ppm (d, J = 2 Hz) with its methyl doublet signal at δ 0.98 ppm indicated the 1→6 attachment and was confirmed by the carbon signals at δ 101.2 and 18.1 ppm. The signals at δ 4.18 (q, J = 7 Hz) and δ 1.18 (d, J = 7 Hz), which were assigned to the methyl and methine protons of the lactoyl moiety, respectively, were found attached to C-4″ of the glucose, whose signal appeared at δ 71.0 ppm more downfield than the unsubstituted one (at δ 70 ppm) with an upfield shift of the C-5″ and C-3″ signal, besides the presence of the other well known H- and C- signals of the quercetin, the sugar, and the lactoyl moieties.

The antioxidant activity of the aqueous ethanolic extract of *Melia azedarach* leaves was tested using ESR measurements [13], which it showed that it has slightly moderate antioxidant activity as the addition of the ethanolic extract decreases the intensity of the characteristic absorbance signal of the reactive radical DPPH, whose ESR signal with the g-value appears at 2.0023 with maximum absorption.

EXPERIMENTAL

General Methods. ^{1}H (200 MHz) and ^{13}C (50 MHz) – NMR: Varian GEMINI-200 spectrometer in DMSO-d_6, values given in ppm; UV: Shimadzu spectrophotometer model UV-240; CC: Polyamide 6S and Sephadex LH-20; PC: carried out on Whatman No.1 and 3MM using solvent systems (1) BAW (*n*-BuOH: HOAc: H_2O, 6:1:2); (2) H_2O; (3) 15 % AcOH (AcOH : H_2O).

Complete acid hydrolysis: carried out for 60 min at 100°C using 2 N HCl. The hydrolysate was then extracted with diethyl ether and ethyl acetate to detect aglycone, while the mother liquor was carefully purified with 10%

N-methyldioctylamine in chloroform to detect the sugar; Enzymatic hydrolysis: 0.5 mL of 0.05% of the enzyme (BDH chemicals ltd., England) in 0.05 N acetate buffer (pH 5.0) was added to 10 mg of the compound in 10 mL water.The mixture was incubated to 37–40°C for 24 h. Examination was carried out by paper chromatography.

Plant Material. The plant material was collected from El-Sharkaia. A voucher specimen has been deposited in the National Research Centre Herbarium (NRCH).

Antioxidant Activity Measurements. The recording of ESR spectra was performed with a Varian 109 ESR spectrometer; the ESR parameters were set at 100 KHz, X-band microwave frequency 9.77 GHz, microwave power 20 mW.

This technique is used to detect the free electrons in the samples either in organic compounds (free radical) or transition elements (paramagnetic centers) whereby a free electron (on its own) has a characteristic signal at the g-value of 2.0023 while the decrease of this signal characterizes the antioxidant efficacy of the sample according to the percentage of the decrease.

Characteristics of the New Compound Cinnamoyl-1-α-L-rhamnoside (1). R_f values ×100: 95(1), 76(2), 47(3); UV (MeOH, λ_{max}, nm): 240, 295sh, 318 +NaOMe: decomposition; ^{1}H NMR (δ, ppm, J/Hz): 7.6 (d, J = 16, H-β), 7.54 (d, J = 7.3, H-2, 6), 7.35 (br t, J = 7.3, H-3, 5), 7.28 (br d, J = 7.2, H-4), 6.58 (d, J = 16, H-α), 4.5 (d, J = 1.2, H-1′), 4.21 (dd, J = 1.5, 4, H-2′), 3.07 – 3.5 (m, sugar protons), 0.87 (d, J = 6, CH_3 of rhamnose).

Characteristics of the New Compound Quercetin 3-*O*-[rhamnosyl 1→6(4″-lactoyl glucoside)]-4′-*O*-glucoside (2). R_f values ×100: 43(1), 85(2), 65(3); UV (MeOH, λ_{max}, nm): 257, 268sh, 295sh, 358 +NaOMe: 272, 328, 410 + NaOAc: 270, 315sh, 378 +NaOAc/H_3BO_3: 265, 310sh, 378 + $AlCl_3$: 282, 305sh, 408 +$AlCl_3$/HCl: 279, 300sh, 358, 399; ^{1}H NMR (δ, ppm, J/Hz): 7.5 (dd, J = 2, 8, H-6′), 7.48 (d, J = 2, H-2′), 6.8 (d, J = 8, H-5′), 6.24 (d, J = 2.5, H-8), 6.02 (d, J = 2.5, H-6), 5.34 (d, J = 7.5, H-1″ of glucose), 5.3 (d, J = 7.5, H-1‴ of glucose), 4.38 (d, J = 1.2, H-1‴ of rhamnose), 4.18 (q, J = 7, methyl of lactoyl), 3.07 – 3.8 (m, sugar protons), 1.18 (d, J = 7, methine of lactoyl), 0.98 (d, J = 6, CH_3 of rhamnose); ^{13}C NMR (δ, ppm): 157.17 (C-2), 133.63 (C-3), 177.74 (C-4), 161.56 (C-5), 98.7 (C-6), 164.72 (C-7), 94.2 (C-8), 157.93 (C-9), 105.29 (C-10), 123.48 (C-1′), 115.7 (C-2′), 146.9 (C-3′), 146.3 (C-4′), 117.3 (C-5′), 120.4 (C-6′), 3-glucose moiety: 101.7 (C-1″), 73.2 (C-2″), 76.2 (C-3″), 71.0 (C-4″), 74.0 (C-5″), 66.9 (C-6″), 174.0 (C=O of the lactoyl group), 65.9 (methine of the lactoyl group), 21.0 (CH_3 of the lactoyl group); rhamnose moiety: 101.2 (C-1‴), 70.3 (C-2‴), 70.7 (C-3‴), 72.0 (C-4‴), 68.1 (C-5‴), 18.1 (C-6‴), 4′- glucose moiety: 101.3 (C-1⁗), 73.7 (C-2⁗), 76.5 (C-3⁗), 70.5 (C-4⁗), 77.4 (C-5⁗), 61.4 (C-6⁗).

REFERENCES

1. M. N. El-Hadidi and L.Boulos, In: *The Street Trees of Egypt*, The American University in Cairo Press, 1988.
2. J. A. Duke and E. S. Ayensu, In: *Medicinal Plants of China*, Reference Publications Inc., 1985.
3. R. N.Chopra, S. L. Nayar, and I. C. Chopra, in: *Glossary of Indian Medicinal Plants (Including the Supplement)*, Council of Scientific and Industrial Research, New Delhi, 1986.
4. J. Lust, In: *The Herb Book*, Bantam books, 1983.
5. J. A. Marco, *J. Nat. Prod.*, **49,** 170 (1986).
6. H. S. Garg and D. S. Bhakuni, *Phytochemistry*, **23**, 2115 (1984).
7. H. O. Gupta and S. K. Srivastava, C*urr. Sci.*, **54**, 570 (1985).
8. A. M. Mishra and S. K. Srivastava, C*urr. Sci.*, **53**, 694 (1984).
9. H. Wagner, T. Swain, J.B. Harborne, and C. F Van Sumere (Eds), in*: Biochemistry of Plant Phenolics*, Plenum Press, New York and London, 1977, 589 pp.
10. K. Bohm, in: *Die Flavonoid*, K. G. Cantor, Aulendorf/Wurtt, 1967.
11. K. R. Markham, in: *Techniques of Flavonoid Identification*, Academic Press, London, 1982.
12. T. J. Mabry, in: *The Systematic Identification of Flavonoids*, Springer Verlag, Berlin, 1970.
13. C. B. Ahn, Y. J. Jeon, D. S. Kang, T. S. Shin, and B. M. Jung, *Food Res. Int.*, **37**, 253 (2004).