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Two new flavonol glycosides from leaves of *Koelreuteria paniculata*

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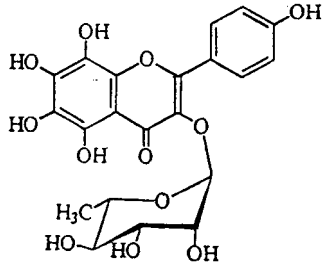
Two new flavonol glycosides were isolated from dried leaves of *Koelreuteria paniculata* Laxm. (Sapindaceae) and characterized as 6,8-dihydroxy-afzelin and afzelin 3″-*O*-gallate, based on chemical and spectral evidences, in addition to nine known polyphenolic metabolites, including eight isolated for the first time from this species.

1. Introduction

The Sapindaceae family contains 150 genera (about 2000 species) [1]. Many members of this family e.g *Cardiospermum*, *Dodonaea* and *Sapindus* have attracted the attention of various authors from phytochemical and biological points of view. Some compounds isolated from *Koelreuteria* species have been found to have bacteriostatic [2], insecticidal [3], antitumor [4] and antiinflammatory [5] activities. Kaempferol, quercetin, afzelin and kaempferol 3-*O*-arabinoside were identified from *K. henryi* [4]. However, gallic acid, methyl gallate, ethyl gallate and quercetrin 2″-*O*-gallate as well as two saponin glycosides have been isolated from *K. paniculata* [2, 5, 6].
This species is known as pinyin and lua'n hua' in China, where it is an important source for yellow and black dyes [7, 8] and is apparently used for eye ailments [9]. This study deals with the isolation and identification of the constituents of a 70% ethanol extract of the leaves of *K. paniculata* growing in Egypt in addition to their biological activities.

2. Investigations, results and discussion

The air dried leaves of *K. paniculata* Laxm. were successively extracted with petroleum ether, then the marc was exhaustively extracted by percolation with 70% EtOH. The prepared aqueous ethanol extract (see Experimental) is considered to be non-toxic ($LD_{50} = 2$g/kg b.w). It showed significant analgesic, anti-inflammatory, molluscicidal and antimicrobial activities, but also produced a mild ulcerogenic effect. In addition, it showed no antiepileptic activity. Most of these biological activities were attributed to the presence of a high content of flavonoids and saponins [10, 11] which were detected using 2DPC and TLC, respectively. Thus, the extract was fractionated on a cellulose column and the fractions obtained were purified by consecutive column chromatography, using cellulose and Sephadex LH-20. By means of chromatographic fractionation, a new flavonol glycoside, 6,8-dihydroxy-afzelin (**1**) and a new flavonol glycoside gallate ester, Kaempferol 3-*O*-(3″-*O*-galloyl)-α-L-rhamnopyranoside (**2**), four known flavonols, quercetrin (**3**) [12], afzelin (**4**) [13], quercetin (**5**) and kaempferol (**6**) together with six polyphenols: gallic acid (**7**) [14], methyl gallate (**8**), ferulic acid (**9**), ellagic acid (**10**), 3,5-di-*O*-galloyl-quinic acid (**11**) and 3,4,5-tri-*O*-galloyl-quinic acid (**12**) [15] were obtained. Among these known metabolites, which were identical with published data, **3–6** and **9–12** were isolated for the first time from *K. paniculata*.
The structures of the known compounds were elucidated on the basis of chemical degradation, UV, negative ESI-MS, 1D NMR spectroscopy and 2D NMR in some cases.



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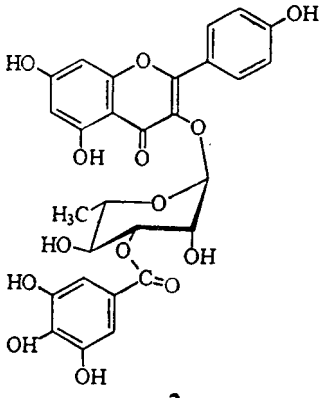

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Direct flow injection negative ESI-MS was used to obtain more information about the structures of the flavonol glycosides **2–4** and quinic acid gallate esters **11, 12**. Generally, not only is the molecular ion peak formed, but also the technically simple induction of fragmentation by the variation of in-source collision-induced dissociation (CID) voltage from −50 to −120 V allows the aglycone, acyl moieties and the content of sugar units to be determined very efficiently. During the analysis, $[M-H]^-$ ions were detected as base peaks for all measured metabolites often without any further fragments. However, adducts with chloride $[M+Cl]^-$, dimers $[2\,M-H]^-$ and doubly charged $[M-2\,H]^{2-}$ ions were also observed to detect M_r of these metabolites. Which of these ions appear together with the common $[M-H]^-$ ion depends on the concentrations, the desolvation potential and the concentration of Cl^- in the system [16, 17]. Under CID, the ion fragments [M-gallyl]$^-$, [M-galloyldeoxyrhamnoside]$^-$ = [kaempferol-H]$^-$ and its oxidized ion [kaempferol-2 H]$^-$ were recorded in the case of **2**. However, in case of the galloylquinic acid derivative **11**, the fragment ions [M-gallyl]$^-$,

Table 1: Negative ESI-MS data with in-source CID data for 2–4, 11 and 12[a]

Compd..	M_r	I	II	III	IV	Fragments on CID
2	584	583	619	291	–	$[M\text{-gallyl}]^-$ = 431, $[kaempferol\text{-}H]^-$ = 285, $[kaempferol\text{-}2\,H]^-$, $[gallate]^-$ = 169 and $[gallate\text{-}CO_2]^-$ = 125
3	448	447	483	–	895	$[M\text{-deoxyrhamnosyl}]^-$ = 301 = $[Qu-H]^-$, $[Qu-2\,H]^-$ = 300 and $[Qu-3\,H-CO]^-$ = 271
4	432	431	467	–	863	$[M\text{-deoxyrhamnosyl}]^-$ = 285 = $[K-H]^-$, $[K-2\,H]^-$ = 284 and $[K-3\,H-CO]^-$ = 255
11	496	495	531	247	991	$[M\text{-gallyl}]^-$ = 343, $[M\text{-gallyl-}H_2O]^-$ = 325, $[quinate]^-$ = 191, $[gallate]^-$ = 169 and $[gallate\text{-}CO_2]^-$ = 125
12	648	647	–	323	–	$[M\text{-gallyl}]^-$ = 495, $[M\text{-gallyl-}H_2O]^-$ = 477 together with the fragments of **11**

[a] I = $[M-H]^-$, II = $[M+Cl]^-$, III = $[M-2\,H]^{2-}$, IV = $[2\,M-H]^-$, K = Kaempferol, Qu = Quercetin

$[M\text{-gallyl-}H_2O]^-$, $[M\text{-}2\text{ gallyl} + 2\,H]^-$ = $[quinic\ acid\text{-}H]^-$, $[gallate]^-$ and $[gallate\text{-}CO_2]^-$ were detected. As example, ESI-MS results are given in Table 1 for the flavonol glycosides **2–4** and quinic acid derivatives **11, 12**.

Compound **1** was obtained as a pale yellow amorphous powder. It showed the chromatographic properties of a flavonol 3-*O*-glycoside and this was supported by its positive reaction with Naturstoff-polyethylene glycol reagent (its purple fluorescence turned to deep orange in UV-365 nm on PC) [18, 19]. The UV methanol spectrum and bathochromic shifts on addition of NaOAc, $AlCl_3$, and $AlCl_3$/HCl indicated the presence of a 5,7-dihydroxy-function [20]. The disappearance of the bathochromic shift of band I in the NaOAc spectrum on addition of H_3BO_3 together with a bathochromic shift with increase in intensity in the NaOMe spectrum revealed the presence of free 4′-OH. Total acid hydrolysis led to the identification of L-rhamnose in the aqueous hydrolysate and a flavonol aglycone [21].

On the basis of the data discussed above and CI-MS analysis which established the molecular formula as $C_{21}H_{20}O_{12}$, **1** was tentatively identified as dihydroxy kaempferol 3-*O*-rhamnoside.

In the ^{1}H NMR spectrum, absence of the two *meta* doublets H-6 and H-8 indicated a 5,6,7,8-tetrahydroxy A-ring. Ring B was assigned as a 1,4-disubstituted benzene due to the two *ortho* coupled signals each integrated to two protons at δ ppm 7.66 (J = 8.4 Hz) and 6.87 (J = 8.4 Hz) assigned to H-2′/6′ and H-3′/5′, respectively. The sugar functionality was identified as α-rhamnopyranose by the anomeric proton signal at 5.29 (d, J = 1.5 Hz), dd signal of H-2″ at 3.97 (J = 3.4 & 1.5 Hz) and d resonance of the CH_3-rhamnosyl protons at 0.8 (J = 5.4 Hz). Thus, **1** was identified as 6,8-dihydroxy-kaempferol 3-*O*-α-L-rhamnopyranoside (6,8-dihydroxy-afzelin).

Table 2: ^{1}H NMR spectral data of compounds 1[a] and 2[b], J (Hz) in parentheses

H	1[a]	2[b]
6	–	6.28, 1 H, d (2.1)
8	–	6.48, 1 H, d (2.1)
3′,5′	6.87, 2 H, d (8.4)	7.03, 2 H, d (8.7)
2′,6′	7.66, 2 H, d (8.4)	7.93, 2 H, d (8.7)
1″	5.29, 1 H, d (1.5)	5.62, 1 H, d (1.7)
2″	3.97, 1 H, dd (3.4, 1.5)	4.52, 1 H, dd (1.7, 3.4)
3″	–	5.21, 1 H, dd (9.8, 3.4)
3″,4″,5″	3.1–3.8, 3 H, m	–
4″,5″	–	3–3.7, 2 H, m
CH_3-6″	0.8, 3 H, d (5.4)	0.95, 3 H, d (6)
2‴/6‴	–	7.16, 2 H, s

[a] 300 MHz, DMSO-d_6 and [b] 270 MHz, $(CD_3)_2CO$

Compound **2** was isolated as a bright yellow amorphous powder and fluoresced dark purple under UV-light. After being sprayed with Naturstoff-polyethylene glycol reagent, it gave an intense greenish yellow fluorescence in UV-365 nm on PC, which is characteristic of kaempferol derivatives. The UV MeOH spectrum suggested that **2** was a kaempferol 3-*O*-glycoside derivative and shifts on addition of NaOMe, NaOAc and H_3BO_3 indicated that the 5,7 and 4′ hydroxyls were unsubstituted [20]. The presence of galloyl ester in the structure of **2** was assumed due to the extra absorption band at 272 nm in the MeOH spectrum [22].

Compound **2** gave kaempferol, gallic acid and L-rhamnose on complete acid hydrolysis. The structure of compound **2** was therefore assumed to be kaempferol 3-*O*-(galloyl-rhamnoside). This evidence was supported by negative ESI-MS (−50 V), which gave the $[M-H]^-$ ion at *m/z* 583 as the base peak corresponding to a MF $C_{28}H_{24}O_{14}$. The presence of the galloylrhamnoside moiety was confirmed by the fragment ions of $[M\text{-gallyl}]^-$, $[M\text{-galloyldeoxy-rhamnoside}]^-$ = $[kaempferol-H]^-$, its oxidized form $[kaempferol-2\,H]^-$, $[gallate]^-$ and $[gallate\text{-}CO_2]^-$ (Table 1).

The complete structural elucidation of compound **2** was obtained by ^{1}H NMR analysis [400 MHz, $(CD_3)_2CO$]. The ^{1}H NMR spectrum (**Table 2**) showed signals for two *meta*-coupled protons at δ ppm 6.28 (1 H, d, J = 2.1 Hz, H-6) and δ 6.48 (1 H, d, J = 2.1 Hz, H-8) and also for an *ortho*-coupled system at 7.93 (2 H, d, J = 8.7 Hz, H-2′/6′) and 7.03 (2 H, d, J = 8.7 Hz, H-3′/5′), confirming a kaempferol derivative. The identity of the glycosidic moiety as 3-*O*-α-rhamnopyranoside was established from the splitting patterns and J-values of H-1″, H-2″ and CH_3-6″ as δ 5.62 (1 H, d, J = 1.7 Hz), 4.52 (1 H, dd, J = 3.4 & 1.7 Hz) and 0.95 (3 H, d, J = 6.0 Hz), respectively. The *O*-galloylation at 3″-OH was deduced from the downfield shift dd-resonance of H-3″ at δ 5.21 (1 H, J = 9.8 & 3.4 Hz) and singlet resonance of H-2‴/6‴ (galloyl equivalent 2H) in the aromatic region at δ 7.16.

On the basis of the foregoing structural evidence and ^{1}H NMR data of compound **2**, which were compared with those of kaempferol 3-*O*-(3″-acetyl)-α-L-rhamnoside [23], the structure of compound **2** was completely assigned as kaempferol 3-*O*-(3″-galloyl)-α-L-rhamnopyranoside.

3. Experimental

3.1. Equipment

UV-spectra were taken with a Shimazu UV 240 UV-vis recording spectrophotometer, with the sample solutions prepared in a 4 ml quartz cell (1 cm optical pathway). In case of flavonoids, all shift reagents were added separately to the methanolic solution of each substance investigated [24]. Nega-

tive ESI-MS spectrometric analyses were measured on a MAT 95 double focusing sector field mass spectrometer (Finnigan, Bremen, Germany) equipped with a Finnigan ESI-II ion source. Complete conditions of MS measurements have been given in the literature [16, 17]. CI-MS were measured on a SSQ 7000 mass spectrometer (Finnigan, Bremen, Germany). All NMR spectra were recorded in $(CD_3)_2CO$ or DMSO-d_6 on a Jeol Ex-270 MHz, Varian Mercury 300 MHz relative to TMS or on Joel GX 400 NMR spectrometers, operated at 400 MHz for ^1H NMR and 100 MHz for ^{13}C. PC-chromatography was performed on Whatman No. 1 sheets using solvent systems A. (15% AcOH) and B. (n-BuOH-AcOH-H_2O 4 : 1:5, top layer). The chromatograms were sprayed with Naturstoff-PEG reagent and visualized under UV light (365 nm) in the case of flavonoids, while the galloyl esters were detected on PC with cold 1% aqueous $NaNO_2$-glacial AcOH (10 : 1 v/v) or with saturated aqueous KIO_3 solution. CC analyses were carried out on microcrystalline cellulose for CC (Merck) or Sephadex LH-20 (particle size 25–100 μm, ICN Biomedicals Inc.), using aqueous alcoholic and/or n-BuOH saturated with H_2O solvent systems.

3.2. Plant material

K. paniculata Laxm. leaves were collected from the Zoological Garden, Giza, Egypt during June–August 1996. The sample was identified by Dr. M. El-Gebaly, Researcher of Plant Flora Taxonomy, National Research Centre, El-Dokki, Cairo, Egypt. A voucher specimen has been kept in the Department of Pharmacognosy, Faculty of Pharmacy, Helwan University, Cairo, Egypt.

3.3. Extraction and isolation

The powder of air-dried leaves of *K. paniculata* (2 kg) was successively extracted with petroleum ether, then the marc was percolated successively with 70% aqueous EtOH. This extract was concentrated under reduced pressure and low temperature (40 °C) in water followed by precipitation with EtOH. The precipitate was discarded and the soluble portion was dried under reduced pressure to yield 9.8 g of residue. The residue was divided into six fractions (I–VI) by CC on cellulose using saturated n-BuOH with water (organic layer) – EtOH gradient system, followed by EtOH/H_2O. The obtained fractions were further successively fractionated on Sephadex LH-20 and/or cellulose columns to give pure samples of the known compounds **3–12**. Fraction IV was partitioned twice on Sephadex LH-20 with saturated n-BuOH with H_2O (organic layer), then with EtOH as the mobile phase, giving compounds **1** (12 mg) and **2** (15 mg).

3.4. 6,8-Dihydroxy-afzelin (1)

UV λ_{max} (MeOH): 263, 340; + NaOMe: 270, 325, 385; + NaOAc: 268, 350; + H_3BO_3: 263, 340; + $AlCl_3$: 272, 305(sh.), 345, 390; + HCl: 270, 301, 340, 390. Acid hydrolysis of compound **1**: A solution of 3 mg in 10 ml 2M HCl (50% aqueous MeOH v/v) was heated (∼100 °C) under reflux for 2 h. The solution was allowed to cool and extracted with EtOAc, then the aqueous phase was neutralized, concentrated and examined by CoPC to prove the presence of L-rhamnose. ^1H NMR (300 MHz, DMSO-d_6, room temperature): Table 2.

3.5. Kaempferol 3-O-(3″-galloyl)-α-L-rhamnopyranoside (2)

UV λ_{max} (MeOH): 268, 272, 345; + NaOMe: 272, 322, 390; + NaOAc: 272, 345; + H_3BO_3: 268, 305 (sh.), 345.
Acid hydrolysis of compound **2** was carried out as for **1** to prove the presence of L-rhamnose in the aqueous phase (CoPC). Gallic acid and kaempferol were identified in the organic phase (CoPC). Negative ESI-MS: Table 1. ^1H NMR (270 MHz, $(CD_3)_2CO$, room temperature): Table 2.

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