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Flavonoid galloyl glucosides from the pods of *Acacia farnesiana*

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Abstract

Three new flavonoids: naringenin 7-*O*-*β*-(4″,6″-digalloylglucopyranoside), quercetin 7-*O*-*β*-(6″-galloylglucopyranoside) and myricetin 7-*O*-*β*-(6″-galloylglucopyranoside) were identified from the pods of *Acacia farnesiana*, together with naringenin and kaempferol 7-(6″-galloylglucoside). The structures were determined by conventional methods of analysis and confirmed by ESI-MS (negative mode) and NMR spectroscopy.

Keywords: Acacia farnesiana; Leguminoseae; naringenin 7-(4″,6″-digalloylglucoside); quercetin and myricetin 7-(6″-galloylglucoside); ESI-MS; NMR

1. Introduction

As a part of our continuing search among Egyptian medicinal plants for novel phenolics, which might possess biological activity, we report here the isolation and structural elucidation of three new flavonoids (**1**, **4** and **5**) from the aqueous ethanolic pod extract of *Acacia farnesiana* Willd. Previous studies (El Sissi, El Ansari, & El Negoumy, 1973; El Negoumy & El Ansari, 1981), proved the presence of naringenin 7-(6″-galloylglucoside) in pods and kaempferol 7-(6″-galloylglucoside) in flowers of this plant. Both known compounds (**2** and **3**) were isolated during the course of the present study and subjected to ESI-MS, ^{1}H and ^{13}C NMR analysis for the first time.

2. Results and discussion

The meal of the deseeded pods of *A. farnesiana* was exhaustively extracted with aqueous ethanol (3:1). Compounds **1**–**5** were isolated and purified by polyamide column chromatography, followed by Sephadex LH-20 column chromatography and preparative paper chromatography. The known compounds **2** and **3** gave R_f similar to, and UV spectral data and hydrolytic products identical with those of naringenin 7-(6″-galloylglucoside) (**2**), (El Sissi et al., 1973) and kaempferol 7-(6″-galloylglucoside) (**3**), (El Negoumy & El Ansari, 1981), respectively. On negative ESI-MS analysis, **2** exhibited a M_r of 586 ([M-H]$^-$:585.4) and **3** exhibited a M_r of 600 amu ([M-H]$^-$:599). ^{1}H and ^{13}C NMR of **2** and **3** were recorded and assigned for the first time.

The ^{1}H NMR spectrum of **2**, (DMSO-d_6, at room temp) revealed two aliphatic resonances at δ 2.75 (*dd*, $J = 17$ and 3 Hz) and at δ 3.32 (*dd*, $J = 17$ and 13 Hz) from the axial and equatorial methylene protons at C-3 as well as a third aliphatic resonance at δ 5.48 (*dd*, $J = 13$ and 3 Hz) from the methine proton, which bears an oxygen and phenyl function at C-2 of the flavanone moiety in **2**. The presence of a 6″-*O*-galloylglucoside moiety at the 7-hydroxyl group of naringenin followed from the lowfield shift of the resonances of H-6 and H-8 protons to δ 6.18 (*d*, $J = 2.5$ Hz) and 6.20 (*d*, $J = 2.5$ Hz), respectively, as well as the downfield shift of the glucose methylenic protons H-6 and H-6′ to δ 4.22 (*dd*, $J = 12.5$ and 5 Hz) and 4.42 (*d* $J = 12.5$ Hz). The two equivalent galloyl protons, H-2 and H-6 appeared as a sharp singlet integrated to two protons at δ 6.97 ppm and the anomeric glucose proton as a doublet of $J = 8$ Hz at δ 5.10, thus proving a *β*-configuration and $^{1}C_4$ conformation for the glucose moiety of **2**. The remaining resonances in this spectrum, (Table 1), agreed well with the proposed structure of **2** as naringenin-7-*O*-*β*-(6″-galloylglucopyranoside).

The ^{13}C NMR spectrum of **2** showed the characteristic 15 distinct carbon resonances of a naringenin moiety. Glucosidation at the 7-hydroxyl of naringenin was deduced from the recognized upfield shift ($\Delta\delta$ ppm = 1.6) of the C7 carbon resonance (Table 2) as well as *β*-glucopyranose resonances in which that for the anomeric carbon appeared at δ 99.21. Galloylation of the C-6 glu-

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Table 1
^{1}H NMR spectral data for compounds **1**–**5**

Protons of	Compound 1	2	3	4	5
Flavonoid					
H-2	5.48 *dd* (12.5 & 3)	5.5 *dd* (12.5 & 3)			
H-3					
axial	3.2 *m*	3.3 *dd* (17 & 12.5)			
equatorial	2.78 *dd* (17 & 3)	2.72 *dd* (17 & 3)			
H-6	6.18 *d* (2.5)	6.18 *d* (2.5)	6.44 *d* (2.5)	6.42 *d* (2.5)	6.42 *d* (2.5)
H-8	6.20 *d* (2.5)	6.20 *d* (2.5)	6.82 *d* (2.5)	6.80 *d* (2.5)	6.72 *d* (2.5)
H-2′	7.38 *d* (7.5)	7.30 *d* (7.5)	8.03 *d* (7.5)	7.30 *d* (2)	7.28 *s*
H-3′	6.88 *d* (7.5)	6.80 *d* (7.5)	6.95 *d* (7.5)		
H-5′	6.88 *d* (7.5)	6.80 *d* (7.5)	6.95 *d* (7.5)	6.90 *d* (7.5)	
H-6′	7.38 *d* (7.5)	7.30 *d* (7.5)	8.03 *d* (7.5)	7.52 *dd* (7.5 & 2)	7.28 *s*
Glucose					
H-1	5.25 *d* (8)	5.10 *d* (8)	5.18 *d* (8)	5.20 *d* (8)	5.20 *d* (8)
H-2	3.25–3.60 *m*	3.25–3.70 *m*	3.30–3.60 *m*	3.30–3.60 *m*	3.10–3.60 *m*
H-3	3.25–3.60 *m*	3.25–3.70 *m*	3.30–3.60 *m*	3.30–3.60 *m*	3.10–3.60 *m*
H-4	5.22 *t* (8)	3.25–3.70 *m*	3.30–3.60 *m*	3.30–3.60 *m*	3.10–3.60 *m*
H-5	3.90 *m*	3.90 *m*	3.90 *m*	3.90 *m*	3.85 *m*
H-6	4.55 *dd* (12.5 & 5)	4.22 *dd* (12.5 & 5)	4.30 *dd* (12.5 & 5)	4.30 *dd* (12.5 & 5)	4.36 *m*
H-6′	4.65 *d* (12.5)	4.42 *d* (12.5)	4.46 *d* (12.5)	4.48 *d* (12.5)	4.36 *m*
Galloyl/s					
H-2 & H-6	7.18 & 7.19	6.95	6.97	6.97	6.97

Coupling constants (J in Hz) in parentheses.

cose hydroxyl group was evidenced from the downfield shift ($\Delta\delta$ ppm = 1.1) of this carbon resonance, in comparison with the resonance of the corresponding carbon in the spectrum of free β-glucopyranose (Kalinowski, Berger, & Braun, 1984). The galloyl moiety gave a characteristic pattern with five distinct resonances located at the expected chemical shifts (Table 2). Esterification of the carboxyl group of this moiety followed from the upfield shift ($\Delta\delta$ ppm = 2.22) of the resonance of the esterified carboxyl carbon. Consequently the identity of **2** is confirmed.

The ^{1}H NMR spectrum of **3** (DMSO-d_6,. room temp) showed the expected kaempferol 7-*O*-β-glucopyranoside proton pattern of signals with the exception of the recognizable downfield shift of the two methylenic glucose proton resonances to δ 4.46 (*d*, J = 12.5 Hz) and to 4.3 (*dd*, J = 12.5 and 5 Hz), which proved esterification of their geminal hydroxyl group. In addition, a sharp singlet at δ 6.97 was attributed to the H-2 and H-6 galloyl protons, thus confirming the structure of **3** as kaempferol 7-*O*-β-(6″-galloylglucopyranoside).

^{13}C NMR spectral analysis further confirmed the structure of **3**. Thus, most of the chemical shift values (Table 2) were the same as for kaempferol 7-*O*-glucopyranoside (Markham & Mohan Shari, 1982) and 6″-*O*-galloylglucose in **2**. The attachment of the galloylglucose moiety to C-7 of the kaempferol moiety followed from the upfield shift of this carbon resonance and the accompanying downfield shift of the resonances of its *ortho* related carbons (C-6 and C-8) due to the α- and β-effect, respectively (all in comparison with the chemical shifts of the corresponding carbon resonances in the ^{13}C NMR spectrum of kaempferol itself) (Nawwar, Souleman, Buddrus, & Linscheid, 1984). Galloylation at the glucopyranose carbon C-6 followed from the downfield shift of its resonance to δ 62.89 ppm (compared with 60.6 ppm in the spectrum of free β-glucopyranose (Kalinowski et al., 1984)) and from the upfield shift of the resonance of the galloyl carbonyl carbon to δ 165.81 ppm (compared with δ 167.7 in free gallic acid (Nawwar et al., 1984)).

The new compound, **1**, isolated as a light brown amorphous powder was found to possess chromatographic and colour properties (dark purple spot on PC and UV light, intense blue $FeCl_3$ colour reaction and a positive rose colour with aqueous KIO_3, specific for galloyl esters (Haddok, Gupta, Al-Shafi, & Haslam, 1982)) and UV absorption maxima consistent with galloylated naringenin 7-*O*-glucoside. It exhibited a M_r of 738 amu in negative ESI-MS, ([M-H]$^-$:737.4). On complete acid hydrolysis, **1** yielded naringenin, gallic acid (CoPC, UV and ^{1}H NMR analysis) and glucose (CoPC). On controlled acid hydrolysis it yielded naringenin 7-*O*-β-

Table 2
^{13}C NMR spectral data for compounds **1**–**5**

Carbons of	Compound 1	2	3	4	5
Flavonoid					
2	79.9	78.8	147.7	147.9	148.2
3	43.2	42.1	136.0	136.1	136.2
4	198.0	197.3	176.1	176.0	176.3
5	164.6	163.0	160.5	160.5	160.3
6	96.1	96.4	98.7	98.6	98.8
7	165.4	165.1	162.5	162.5	162.5
8	97.4	95.3	94.2	94.2	94.6
9	164.1	163.0	155.9	155.8	156.0
10	104.5	103.4	104.8	104.8	105.0
1′	130.4	128.6	121.6	121.8	121.1
2′	129.1	128.7	129.7	115.6	107.9
3′	116.0	115.3	115.5	145.2	146.1
4′	158.6	157.8	159.4	147.7	136.2
5′	116.0	115.3	115.5	115.5	146.1
6′	129.1	128.7	129.7	120.1	107.9
Glucose					
1	100.0	99.2	99.7	99.7	100.0
2	73.2	73.0	73.1	73.2	73.2
3	75.2	76.1	76.1	76.1	76.4
4	71.6	69.3	69.1	69.1	69.2
5	74.0	74.8	73.8	73.8	73.5
6	63.2	63.0	62.8	62.8	63.0
Galloyl/s					
1	121.0 & 121.4	119.4	119.3	119.4	119.4
2	109.0 & 109.4	108.7	108.6	108.7	108.8
3	145.9	145.6	145.5	145.6	145.4
4	138.8 & 138.9	138.6	138.6	138.5	138.6
5	145.9	145.6	145.5	145.5	145.5
6	109.0 & 109.4	108.7	108.6	108.7	108.8
C=O	166.4 & 166.5	165.5	165.8	165.8	166.0

(6″-galloylglucopyranoside), (**2**), among other products. Compound **2** was separated from the concentrated aqueous hydrolysate by preparative paper chromatography and fully characterized by CoPC, UV absorption, ESI-MS and ^{1}H NMR analysis.

The ^{1}H NMR spectrum of **1** exhibited the characteristic resonance pattern of naringenin 7-*O*-*β*-(6″-galloylglucopyranoside) (Table 1) with the exception of the recognized glucose proton signal appearing as a downfield shifted triblet ($J =$ 8Hz) at δ ppm = 5.22 and the additional galloyl protons which appeared as a singlet at δ ppm = 7.18 (or 7.19, see Table 1). These data indicated the presence of two galloyl moieties in the molecule of **1**. This was confirmed by the two distinct galloyl carboxylic carbon resonances at δ ppm 166.4 and 166.5 and the characteristic pattern of galloyl carbon resonances in the ^{13}C NMR spectrum. Resonances of the glucose carbons were assigned by comparison with the ^{13}C NMR data reported for similar galloyglucose (Nawwar, Hussein, & Merfort, 1994; Nawwar & Hussein, 1994), as well as by consideration of the known *α*- and *β*-effect (Nawwar, Souleman, Buddrus, & Linscheid, 1984) caused by esterification of the glucose hydroxyl groups. The *β*-anomeric carbon resonance was identified from its characteristic chemical shift value (100.02 ppm), while the further upfield glucose carbon resonance at δ 63.2 ppm was assigned to the methylenic carbon C-6 to which one of the galloyl moieties is attached. Attachment of the second galloyl moiety to C-4 of glucose was evidenced by the *β*-upfield shift recognized for the vicinal carbons (C-3 & C-5) resonances to δ ppm 75.19 and 74.03, respectively (by comparison with the chemical shift values of the corresponding carbon resonances in the spectrum of the unsubstituted *β*-glucopyranose (Nawwar et al., 1984). C-4 was found to resonate downfield at δ ppm 71.58 (*α*-effect) thus confirming galloylation of its hydroxyl group. Other resonances in this spectrum exhibited chemical shift values which were in accordance with the structure of **1** as naringenin 7-*O*-*β*-(4″,6″-digalloyl-glucopyranoside), a new natural product. This represents the first report of a flavonoid glucoside digallate.

Compound **4** was isolated as yellow crystals (mp 215°), which appeared yellow in UV light turning yellow-orange when fumed with ammonia vapour. It gave quercetin, gallic acid and glucose on complete acid hydrolysis and a M_r of 616 amu in negative ESI-MS ([M-H]$^-$:615). These data, together with R_f values and UV spectral analysis, indicated that **4** is the quercetin analogue of **3**. This was supported by controlled acid hydrolysis of **4** which gave 6-monogalloyl-(*α*/*β*)-glucopyranose as an intermediate (CoPC, UV spectral, ESI-MS and ^{1}H NMR analysis (Nawwar et al., 1994)). ^{1}H NMR (Table 1) and ^{13}C NMR (Table 2) of **4** confirmed its structure as quercetin 7-*O*-*β*-(6″-galloylglucopyranoside), which is another new natural product.

The new compound **5** (yellow crystals mp 238°), was identified as the myricetin analogue of **3** and **4** from chromatographic, UV spectral, hydrolytic, and ESI-MS ($M_r = 632$ amu, [M-H]$^-$:631) data. Its structure was confirmed by ^{1}H NMR (Table 2) and ^{13}C NMR (Table 2) as myricetin 7-*O*-*β*-(6″-galloylglucopyranoside), which is the third new natural product.

3. Experimental

^{1}H NMR spectra were measured at 400 MHz. ^{1}H resonances were measured relative to TMS and ^{13}C NMR resonances to DMSO-d_6 and converted to TMS scale by adding 39.5. Typical conditions: spectral width = 6000 Hz for ^{1}H and 22 000 Hz for ^{13}C, 32 K data points and a flip angle of 45°. ESI-MS (negative mode): the direct flow injection technique was applied, sample in MeOH was introduced (1.25 μl min^{-1}) together with MeOH sheath-liquid (5 μl min^{-1}) by a Harvard influsion pump 9 ml

min^{-1} SF6 sheath gas into the ESI-ion source of a Finnigan MAT 4600 spectrometer. PC was carried out on Whatman no. 1 paper, using solvent systems: (1) H_2O; (2) 15% HOAc; (3) BAW (*n*-BuOH–HOAc–H_2O, 4:1:5, upper layer); (4) C_6H_6–*n*-BuOH–H_2O–pyridine (1:5:3:3, upper layer). Solvents 2 and 3 were used for prep. PC on Whatman no. 3MM paper and solvents 3 and 4 for sugar analysis.

3.1. Plant material.

Fresh pods of *Acacia farnesiana* Willd, were collected from a mature tree in the Nile Delta near Tanta city, Egypt, during October 1994 and authenticated by Dr L. Boulos, Professor of Botany, NRC, Cairo, Egypt.

3.2. Isolation and identification

Powdered deseeded pods were extracted with EtOH–H_2O (3:1). The concd extract was applied to a polyamide 6S CC (Riedel-De Häen, Seelze Hanover, Germany) and eluted with H_2O followed by H_2O–EtOH mixts of decreasing polarities to yield eight major frs (I–VIII). Compounds **1** and **2** were isolated from fr. V (eluted by 40% EtOH) and **3**–**5** from fr. VI (eluted by 80% EtOH) by CC on Sephadex LH-20 using EtOH and EtOH containing 1:1 mixture of Me_2CO–H_2O (7:1). Compounds **1**–**5** were purified by prep. PC, using BAW and **1** and **2** were repurified by prep. PC, using 15% HOAc.

3.3. *Naringenin* 7-O-*β*-(4″,6″-*digalloylglucopyranoside*) (**1**)

M_r 738, −ve ESI-MS [M-H]$^-$:737. R_f-values: 0.47 (H_2O), 0.48 (HOAc), 0.74 (BAW). UV λ_{max}^{MeOH} nm: 283, 332 sh. Normal acid hydrolysis gave glucose (CoPC), naringenin [CoPC, UV spectral data [2], ^{1}H NMR: ppm 5.38 (*dd*, $J = 12.5$ & 3 Hz, H-2), 3.16 (*dd*, $J = 17.1$ and 12.5 Hz, H-3_{ax}), 2.66 (*dd*, $J = 17.1$ and 3 Hz, H-3_{eq}), 5.90 (*s*, H-6), 5.91 (*s*, H-8), 7.3 (*d*, $J = 8$ Hz, H-2′ and H-6′), 6.83 (*d*, $J = 8$ Hz, H-3′ and H-5′)] and gallic acid (CoPC, UV and ^{1}H NMR spectral data). Controlled acid hydrolysis gave **2**. ^{1}H NMR: Table 1; ^{13}C NMR: Table 2.

3.4. *Naringenin* 7-O-*β*-(6″-*galloylglucoside*) (**2**)

M_r 586, −ve ESI-MS [M-H]$^-$: 585. R_f-values: 0.51 (H_2O), 0.54 (HOAc), 0.70 (BAW). UV: λ_{max}^{MeOH} nm: 282, 330 sh. Normal acid hydrolysis gave glucose (CoPC), naringenin and gallic acid (CoPC, UV and ^{1}H NMR spectral data). ^{1}H: see Table 1; ^{13}C NMR: Table 2.

3.5. *Kaempferol* 7-O-*β*-(6″-*galloylglucoside*) (**3**)

M_r 600, −ve ESI-MS [M-H]$^-$: 599. R_f-values: 0.01 (H_2O), 0.18 (HOAc), 0.45 (BAW). UV: λ_{max}^{MeOH} nm: 267, 319, 365. Normal acid hydrolysis gave glucose (CoPC), kaempferol and gallic acid (CoPC, UV and ^{1}H NMR spectral data). ^{1}H NMR: Table 1; ^{13}C NMR: Table 2.

3.6. *Quercetin* 7-O-*β*-(6″-*galloylglucoside*) (**4**)

M_r 616, −ve ESI-MS [M-H]$^-$: 615. R_f-values: 0.01 (H_2O), 0.12 (HOAc), 0.42 (BAW). UV: λ_{max}^{MeOH} nm: 258, 268, 371. Normal acid hydrolysis gave glucose (CoPC), quercetin and gallic acid (CoPC, UV and ^{1}H NMR spectral data). ^{1}H NMR: Table 1; ^{13}C NMR Table 2.

3.7. *Myricetin* 7-O-*β*-(6″-*galloylglucoside*) (**5**)

M_r 632, −ve ESI-MS [M-H]$^-$: 631. R_f-values: 0.01 (H_2O), 0.09 (HOAc), 0.38 (BAW). UV: λ_{max}^{MeOH} nm: 252, 319, 372. Normal acid hydrolysis gave glucose (CoPC), myricetin and gallic acid (CoPC, UV and ^{1}H NMR spectral data) ^{1}H NMR: Table 1, ^{13}C NMR: Table 2.

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