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# LEAF FLAVONOIDS OF *ALBIZIA LEBBECK*

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**Key Word Index**—*Albizia lebbeck*; Leguminosae; quercetin and kaempferol 3-rhamnosyl (1 → 6)glucosyl(1 → 6)galactoside; flavonol trisaccharides; NMR spectral analysis.

**Abstract**—Two new tri-*O*-glycoside flavonols: kaempferol and quercetin 3-*O*-α-rhamnopyranosyl(1 → 6)-β-glucopyranosyl(1 → 6)-β-galactopyranosides, were identified from the leaves of *Albizia lebbeck*. Structures were established by conventional methods of analysis and confirmed by ESI-MS, $^1$H and $^{13}$C-NMR spectral analysis.


## INTRODUCTION

In a continuing search among Egyptian Leguminosae for novel phenolics with possible biological activity [1, 2], the aqueous ethanolic leaf extract of *Albizia lebbeck*, Benth. was found to contain a complex mixture of flavonol glycosides. The present study describes the isolation of two new compounds **1** and **2** from this extract.

## RESULTS AND DISCUSSION

Compound **1** was isolated as a light brown amorphous powder which gave chromatographic and UV spectral data characteristic of a quercetin 3-*O*-oligosaccharide [3, 4]. Normal acid hydrolysis of **1** afforded quercetin (CoPC, UV, $^1$H- NMR analysis), rhamnose, glucose and galactose (CoPC). Controlled acid hydrolysis of **1** failed to yield any glycosidic intermediate but gave quercetin directly. The compound was unaffected by β-glucosidase after 48 h at 37°C, but was hydrolysed by α-rhamnosidase using the same conditions to produce an intermediate **1a**. UV spectral data, *Mr* = 626 (negative ESI-MS, $[M-H]^-$: *m/z*: 625) and $^1$H and $^{13}$C-NMR data for **1a** were found to be identical to those of quercetin 3-*O*-β-glucosyl(1 → 6)-β-galactopyranoside [3]. Compound **1** showed on negative ESI-MS a molecular ion peak $[M-H]^-$ at *m/z*: 771, corresponding to a *Mr* of 772. $^1$H-NMR spectral analysis of **1** gave three distinct anomeric protons resonances at *d* 5.26 (*d*, *J* = 8 Hz), 4.45 (*d*, *J* = 2.5 Hz) and at 4.3 (*d*, *J* = 8 Hz), assignable to the galactoside H-1, rhamnosyl H-1 and glucosyl H-1 anomeric protons, respectively. These chemical shift values indicated the attachment of the galactoside anomeric carbon to the quercetin hydroxyl group, and the attachment of each of the remaining sugar moieties to an alcoholic sugar hydroxyl. The recorded coupling constants proved that α-configuration of the anomeric rhamnosyl carbon and the β-configuration at the anomeric carbons in the glucosyl and galactoside moieties. Consequently, the conformation of the three sugar moieties is $^1C_4$ for the rhamnosyl and $^4C_1$ for both the glycosyl and galactoside moieties. The remaining proton resonances in this spectrum possessed chemical shift values and mode of splitting which were in accordance with the structure of **1** as an α-rhamnopyranosyl quercetin 3-*O*-glucopyranosyl(1 → 6)-β-galactopyranoside. The attachment of the rhamnose to C-6 of the glucosyl moiety was evidenced by the downfield shift of the glycosyl C-6 carbon resonance to δ 67.0 ppm and the accompanying upfield shift of the resonances of the adjacent carbons C-5 to 75.71 ppm. The chemical shift values of all of the recorded sugar carbon resonances (Table 1) confirmed the pyranose form of the three sugar moieties in the molecule of **1** [5]. These data finally confirmed the structure of **1** to be quercetin 3-*O*-α-rhamnopyranosyl(1 → 6)-β-glucopyranosyl(1 →6)-β-galactopyranoside, which is a new natural product.

Compound **2** was isolated as a pale brown amorphous powder. ESI-MS, chromatographic, UV spectral analysis and enzymic hydrolysis and $^1$H NMR spectral data proved it to be the kaempferol analogue of **1**. $^{13}$C NMR spectral analysis finally confirmed the structure of **2** as kaempferol 3-*O*-α-rhamnopyranosyl-(1 → 6)-β-glucopyranosyl(1 → 6)-β-galactopyranoside, which has not been previously reported as a natural product.

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Table 1. $^{13}$C-NMR chemical shifts (ppm) of compounds **1**, **1a** and **2**

| | Compounds | | |
|---|---|---|---|
| Carbon No. | **1** | **1a** | **2** |
| Aglycone moiety | | | |
| 1 | 156.8 | 156.3 | 156.9 |
| 3 | 133.5 | 133.5 | 133.1 |
| 4 | 177.1 | 177.4 | 176.6 |
| 5 | 161.2 | 161.2 | 161.1 |
| 6 | 99.6 | 98.6 | 99.2 |
| 7 | 164.5 | 164.1 | 164.7 |
| 8 | 94.2 | 93.5 | 94.3 |
| 9 | 156.5 | 156.3 | 156.4 |
| 10 | 103.3 | 104.1 | 103.5 |
| 1′ | 121.0 | 121.0 | 120.7 |
| 2′ | 115.5 | 115.2 | 130.7 |
| 3′ | 145.1 | 144.8 | 115.3 |
| 4′ | 149.1 | 148.4 | 160.3 |
| 5′ | 116.3 | 115.9 | 115.3 |
| 6′ | 121.7 | 121.9 | 130.7 |
| Rhamnose moiety | | | |
| 1 | 100.7 | | 100.8 |
| 2 | 70.2 | | 70.4 |
| 3 | 70.3 | | 70.7 |
| 4 | 71.3 | | 72.0 |
| 5 | 68.4 | | 68.3 |
| Me | 17.8 | | 17.7 |
| Glucose Moiety | | | |
| 1 | 102.2 | 103.2 | 102.1 |
| 2 | 74.5 | 74.2 | 74.2 |
| 3 | 76.6 | 76.8 | 76.6 |
| 4 | 70.5 | 70.0 | 70.7 |
| 5 | 75.9 | 76.8 | 75.7 |
| 6 | 67.2 | 61.1 | 67.0 |
| Galactose moiety | | | |
| 1 | 101.7 | 102.2 | 101.0 |
| 2 | 72.0 | 71.3 | 72.0 |
| 3 | 73.3 | 73.5 | 72.0 |
| 4 | 68.4 | 68.3 | 68.3 |
| 5 | 73.7 | 73.9 | 74.1 |
| 6 | 68.3 | 67.3 | 68.1 |

## EXPERIMENTAL

$^1$H-NMR spectra were measured on a JEOL 270 MHz, and the chemical shifts were measured relative to TMS. $^{13}$C-NMR resonances were measured relative to DMSO-$d_6$ and converted to the TMS scale by adding 39.5. Typical conditions: spectral width = 6000 Hz for $^1$H and 22,000 Hz for $^{13}$C-NMR, 32 K data point and a flip angle of 45°. ESI-MS (negative mode) by the direct flow injection technique i.e. the sample in MeOH was introduced (1.25 ml/min) together with MeOH sheath liquid (5 ml/min) by a Harvard infusion pump 9 ml/min $SF_6$ 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) *n*-BuOH–HOAc–$H_2O$ (4:1:5, upper layer) (BAW); (3) HOAc–$H_2O$ (3:17); (4) $C_6H_6$–*n*-BuOH–$H_2O$–pyridine (1:5:3:3, upper layer). Solvents **2** and **4** were used for sugar analysis.

### *Plant material*

Fresh leaves of *A. lebbeck* were collected from a mature tree of ca 10 m height, growing in the zoological garden at Cairo, during March 1995 and identified by Dr L. Boulos, Professor of Botany, National Research Centre, Cairo. A voucher specimen has been deposited in the Herbarium of the National Research Centre.

### *Isolation and identification*

The dried powdered leaves of *A. lebbeck* were exhaustively extracted with EtOH–$H_2O$ (3:1). The concentrated extract was applied to a polyamide 6S CC (Riedel-De Häen AG, Seelze Hanover, Germany) and eluted with $H_2O$–EtOH mixtures of decreasing polarities. The successive eluates were individually collected, dried *in vacuo* and subjected to 2D-PC. Pure **1** and **2** were isolated from the 20% fraction through Sephadex LH-20 column fractionation using $H_2O$ as an eluent.

### *General hydrolytic procedures*

Normal acid hydrolysis: 2 N aq. HCl, 100°, 3 h for flavonol glycosides. Controlled acid hydrolysis: 0.5 N aq. HCl, 100°, 30 min. Enzymic hydrolysis: 0.5 ml of β-glucosidase or α-rhamnosidase (pectinase) in 0.05 M acetate buffer, pH 5.1, 37°.

*Quercetin* 3-O-*α-rhamnopyranosyl*(1 → 6)-*β-glucopyranosyl*(1 → 6)-*β-galactopyranoside* (**1**). $R_f$-values: 75 ($H_2O$), 79 (HOAc–$H_2O$), 45 (BAW). UV spectral data $\lambda_{max}^{MeOH}$: 256, 265 sh, 364; +NaOAc, 256 sh, 271, 380; +NaOAc–$H_3PO_3$, 264, 380; +$AlCl_3$, 265, 300 sh, 363 sh, 420; +NaOMe, 272, 325, 409 sh nm. *Mr* 772, −Ve ESI-MS, *m/z*: $[M-H]^-$ 771. Normal acid hydrolysis gave rhamnose, glucose, galactose (CoPC) and quercetin (CoPC, UV absorption and $^1$H-NMR).

Controlled acid hydrolysis gave quercetin. Hydrolysis with α-rhamnosidase gave quercetin 3-*O*-*β*-glucosyl(1 → 6)-*β*-galactoside (**1a**), $R_f$-values of **1a**: 64 ($H_2O$), 69 (HOAc–$H_2O$), 58 (BAW). UV spectral data of **1a** $\lambda_{max}^{MeOH}$: 257, 267 sh, 368 sh, 426; +NaOAc, 256 sh, 270, 377; +NaOAc–$H_3PO_3$, 262, 378; +$AlCl_3$, 267, 302 sh, 426; +NaOMe, 270, 322, 412 nm. −Ve ESI-MS of **1a**: *Mr* 626, *m/z*; $[M-H]^-$ 625. $^1$H-NMR of **1a**: aglycone moiety; δ 6.2 (*d*, *J* = 2.5 Hz, H-6), 6.4 (*d*, *J* = 2.5 Hz, H-8), 6.82 (*d*, *J* = 7.5 Hz, H-5′), 7.52 (*d*, *J* = 2.5 Hz, H-2′), 7.68 (*dd*, *J* = 2.5 Hz and 7.5 Hz, H-6′); sugar moieties: 5.32 (*d*, *J* = 8 Hz, H-1 galactoside), 4.08 (*d*, *J* = 7.5 Hz, H-1 glucosyl), 3.3–3.75 (*m*, sugar protons hidden by hydroxyl protons). $^{13}$C-NMR of **1a**: Table 1. $^1$H-NMR of **1**: aglycone moiety:

$\delta$ 6.12 (*d*, $J = 2$ Hz, H-6), 6.34 (*d*, $J = 2$ Hz, H-8), 6.83 (*d*, $J = 8$ Hz, H-5′), 7.52 (*m*, H-2′ and H-6′); sugar moieties: 4.45 (*d*, $J = 2.5$ Hz, rhamnosyl H-1), 4.3 (*d*, $J = 8$ Hz, glycosyl H-1), 5.26 (*d*, $J = 8$ Hz, galactoside H-1), 3–3.8 (*m*, sugar protons), 0.85 (*d*, $J = 6$ Hz, methyl rhamnosyl protons). For $^{13}$C-NMR of **1** see Table 1.

*Kaempferol 3-O-α-rhamnopyranosyl*(1 → 6)-*β-glucopyranosyl*(1 → 6)-*β-galactopyranosides* (**2**). $R_f$-values (× 100): 77 ($H_2O$), 82 (HOAc–$H_2O$), 47 (BAW). UV spectral data $\lambda_{max}^{MeOH}$: 266, 300 sh, 352; +NaOAc, 272, 308, 385; +NaOAc–$H_3PO_3$, 267, 302 sh, 357; +$AlCl_3$, 273, 305, 348, 395; +NaOMe, 272, 325, 398 nm. *Mr* 756, −Ve ESI-MS, *m/z*: $[M-H]^-$ 755. Normal acid hydrolysis gave rhamnose, glucose, galactose (CoPC) and kaempferol (CoPC, UV absorption and $^1$H-NMR). Controlled acid hydrolysis gave kaempferol. Hydrolysis with α-rhamosidase gave kaempferol 3-*O*-*β*-glucosyl(1 → 6)-*β*-galactoside (**2a**) [6], $R_f$-values: 67 ($H_2O$), 72 (HOAc–$H_2O$), 60 (BAW). UV spectral data $\lambda_{max}^{MeOH}$: 267, 298 sh, 349; +NaOAc, 273, 303, 375; +NaOAc–$H_3PO_3$, 266, 303, 355; +$AlCl_3$, 272, 305, 346, 400; +NaOMe, 272, 328, 400 nm. −Ve ESI-MS of **2a**: *Mr* 610, *m/z*; $[M-H]^-$ 609. $^1$H-NMR of **2**: aglycone moiety; $\delta$ 6.16 (*d*, $J = 2$ Hz, H-6), 6.4 (*d*, $J = 2$ Hz, H-8), 6.87 (*d*, $J = 7.5$ Hz, H-3′ and H-5′), 7.97 (*d*, $J = 7.5$ Hz, H-2′ and H-6′); sugar moieties: $\delta$ 4.42 (*br s*, $\Delta\gamma_{1/2} = 4$ Hz, rhamnosyl H-1), 4.36 (*d*, $J = 8$ Hz, H-1 glycosyl), 5.23 (*d*, $J = 8$ Hz, H-1 galactoside), 3.15–3.75 (*m*, sugar protons), 0.85 (*d*, $J = 6$ Hz, methyl rhamnosyl proton). For $^{13}$C-NMR of **2** see: Table 1.

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