A New Acylated Flavonol Triglycoside from *Carrichtera annua*

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A new acylated flavonol triglycoside, quercetin 3-*O*-[(6-feruloyl-*β*-glucopyranosyl)-(1→2)-*β*-arabinopyranoside]-7-*O*-*β*-glucopyranoside (**1**), as well as the known flavonoid quercetin, were isolated from the whole plants of *Carrichtera annua*. The structure of **1** was established by UV, MS, and 1D and 2D NMR spectroscopy, including DEPT, DQF−COSY, TOCSY, HSQC, HSQC−TOCSY, and HMBC experiments.

Carrichtera annua (L.) DC. is small hispid herb belonging to the Cruciferae (or Brassicaceae), representative of herbs and shrubs in mainly temperate regions. It occurs in different areas in Egypt, such as the Nile delta, the Mediterranean coastal area, and Sinai.[1] Although plants of the Cruciferae are used in traditional medicine for the treatment of many diseases, such as cancer, rheumatism, diabetes, and bacterial and fungal infections, no phytochemical investigations on *C. annua* have been reported yet.[2−4] As part of a phytochemical study on Egyptian medicinal plants, we report here the isolation and structure elucidation of a new acylated flavonol triglycoside (**1**).

Compound **1** was obtained from the *n*-BuOH-soluble part of the methanolic extract of *C. annua.* Its ^{1}H and ^{13}C NMR spectra showed the presence of a quercetin moiety, three sugar residues, a caffeic acid moiety, and a methoxyl group. The chemical shifts of C-2 and C-3 (δ 156.7 and 134.5, respectively) indicated C-3 substitution of the quercetin moiety.[5] A ^{13}C NMR signal at δ 160.9 was assigned to C-5, based on its long-range ^{13}C−^{1}H correlation observed in a HMBC experiment with the ^{1}H NMR signal at δ 6.40 (H-6), whereas the ^{13}C NMR signal at δ 162.8 showed correlations to both H-6 and H-8 (δ 6.60); hence the latter ^{13}C NMR signal was assigned to C-7. The assignment of H-8 was confimed by its long-range correlation to the ^{13}C NMR signal at δ 155.8 (C-9). C-7 showed a three-bond correlation with an anomeric proton at δ 5.05. Hence, compound **1** was a 3,7-disubstituted quercetin derivative. Indeed, the UV spectrum and its changes after addition of shift reagents indicated the presence of free hydroxyl groups at C-5, C-3′, and C-4′; the absorption maximum at 332 nm (in MeOH) confirmed that the C-3 hydroxyl was substituted.[6] TLC after acid hydrolysis with appropriate reference compounds indicated the presence of quercetin as aglycon, and glucose and arabinose. The 2D NMR spectra allowed the assignment of all ^{1}H and ^{13}C NMR signals of the 7-glycosyl residue, which could be identified as a glucopyranoside.[5] The *β*-configuration of the anomeric carbon was evident from the coupling constant of H-1′′′′′ (J = 7.5 Hz) observed in the ^{1}H NMR spectrum.[7]

A methoxyl singlet, observed at δ 3.72 in the ^{1}H NMR spectrum of **1**, was correlated with a quaternary carbon at δ 147.8 in the HMBC spectrum. Based on the long-range ^{13}C−^{1}H correlations observed for the caffeic acid moiety, the latter signal could be assigned to C-3′′′′ of this acyl residue, which, therefore, was identified as a feruloyl group. The trans-configuration of the C-7′′′′−C-8′′′′ double bond was evident from the large coupling constant (J = 15.8 Hz) between H-7′′′′ and H-8′′′′. The carbonyl group of the feruloyl moiety, occurring at δ 166.4, showed a long-range ^{13}C−^{1}H correlation with a ^{1}H NMR signal at δ 4.17, assigned to one of the H-6′′′ hydrogens of a hexose unit, representing the second glucose residue. All ^{1}H and ^{13}C NMR signals of this glucosyl moiety could be assigned based on the 2D NMR spectral data. A TOCSY experiment showed a correlation between one of the H-6′′′ signals at δ 4.17 and the anomeric proton at δ 4.44, demonstrating that they belonged to the same spin system. Characteristic correlations observed in the HMBC, DQF−COSY, and TOCSY experiments are shown in Figure 1. The *β*-configuration of the anomeric carbon was evident from the coupling constant of H-1′′′ (J = 7.8 Hz) observed in the ^{1}H NMR spectrum. In this way the 6-*O*-*trans*-feruloyl-*β*-glucopyranosyl residue could be characterized unambiguously.

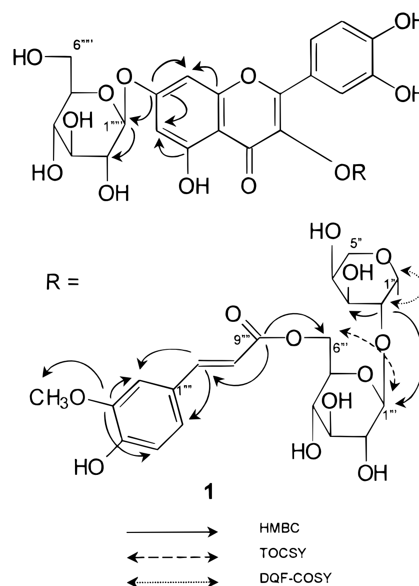

Figure 1. Structure of **1** and characteristic correlations observed in HMBC (C→H), DQF−COSY, and TOCSY NMR experiments.

The anomeric proton of this residue, observed at δ 4.44, showed a long-range correlation with a ^{13}C NMR signal at δ 79.9, corresponding to a proton at δ 4.10 in the HSQC spectrum. The latter signal showed a ^{1}H−^{1}H correlation, observed in the DQF−COSY experiment, with the third

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anomeric proton at δ 5.58, assigned to H-1″ of the arabinosyl moiety. Therefore, glycosylation of arabinose at the C-2″ position became evident. Because compound **1** is a 3,7-disubstituted quercetin derivative, the arabinosyl residue was attached to C-3 of quercetin. 2D NMR allowed the assignment of all ^{1}H and ^{13}C NMR signals of the arabinopyranosyl moiety. Determination of the anomeric configuration of 2-substituted xylose or arabinose units is hampered because of the equilibrium between $^1C_4$ and $^4C_1$ conformations of the sugar, due to steric hindrance. Equilibrium between $^1C_4$ and $^4C_1$ conformers with significant alteration of $^3J_{H-1,H-2}$ and $^1J_{C-1,H-1}$ values has been observed in various 2-*O*-glucosylated arabinopyranosides of bulky aglycons.[8] For compound **1**, the large coupling constant $^1J_{C-1'',H-1''}$ (180 Hz), measured as the residual C−H coupling in the HMBC spectrum, and the small coupling constant $^3J_{H-1'',H-2''}$ (1.8 Hz) observed for H-1″ suggested a β-configuration of the anomeric carbon. This was evident from the 2-*O*-glycosylation shifts and the coupling constants reported by Mizutani et al. for a series of 2-*O*-glycosylated α- and β-L-arabinopyranosides, with $^1J_{C-1,H-1}$ being larger and $^3J_{H-1,H-2}$ being smaller for 2-*O*-glucopyranosyl-β-arabinopyransosides than for 2-*O*-glucopyranosyl-α-arabinopyranosides.[9,10] In contrast to the case of 2-*O*-glycosylation of α-L-arabinopyranoside, no significant shielding of C-3, C-4, or C-5 and no change in the values of $^3J_{H-1,H-2}$ and $^1J_{C-1,H-1}$ were observed for the 2-*O*-glycosylated β-L-arabinopyranoside series, indicating the predominance of the usual $^4C_1$ conformation of the β-L-arabinopyranoside portion. The β-configuration of the arabinopyranosyl moiety in compound **1** is also in agreement with the $^3J_{H-1,H-2}$ coupling constants for the anomeric protons for a series of pyranosides and furanosides in flavonoid glycosides reported by Markham and Geiger.[7] Therefore, compound **1** was identified as quercetin 3-*O*-[(6-*O*-*trans*-feruloyl-β-glucopyranosyl)-(1→2)-β-arabinopyranoside]-7-*O*-β-glucopyranoside, which is a new compound. The structure was confirmed by a $[M - H]^-$ peak at *m*/*z* 933 in the negative FABMS, consistent with a molecular formula $C_{42}H_{46}O_{24}$. In addition, quercetin was obtained from the EtOAc-soluble part of the methanolic extract of *C. annua* and identified by comparison with literature data.[5−7]

Table 1. ^{1}H and ^{13}C NMR Assignments for **1** in DMSO-d_6

carbon	^{13}C NMR[a] δ (ppm)	mult.	^{1}H NMR[b] δ (ppm), mult., *J* (Hz)
2	156.7	s	
3	134.5	s	
4	177.7	s	
5	160.9	s	
6	99.2	d	6.40, br s
7	162.8	s	
8	94.4	d	6.60, br s
9	155.8	s	
10	105.6	s	
1′	120.4	s	
2′	115.9	d	7.50, d, 2.2
3′	145.2	s	
4′	149.3	s	
5′	115.5	d	6.87, d, 8.5
6′	122.3	d	7.60, dd, 8.5, 2.2
1″	99.0	d	5.58, d, 1.8
2″	79.9	d	4.10, br t
3″	68.9	d	3.89, br t
4″	64.2	d	3.70, m
5″	61.4	t	3.15, m
			3.60, m
1‴	104.4	d	4.44, d, 7.8
2‴	73.7	d	3.04, dd, 8.2, 8.2
3‴	76.5[c]	d	3.25, m
4‴	69.6[d]	d	3.15, m
5‴	73.8	d	3.40, m
6‴	63.1	t	4.27, m
			4.17, dd, 11.8, 5.5
1⁗	125.4	s	
2⁗	111.0	d	7.11, d, 1.6
3⁗	147.8	s	
4⁗	149.3	s	
5⁗	115.5	d	6.71, d, 8.1
6⁗	122.8	d	6.90, dd, 8.1, 1.6
7⁗	145.0	d	7.40, d, 15.8
8⁗	114.1	d	6.24, d, 15.8
9⁗	166.4	s	
1‴‴	99.9	d	5.05, d, 7.5
2‴‴	73.2	d	3.25, m
3‴‴	76.4[c]	d	3.25, m
4‴‴	69.8[d]	d	3.15, m
5‴‴	77.1	d	3.45, m
6‴‴	60.6	t	3.50, m
			3.70, m
OCH$_3$	55.6	q	3.72, s

[a] Recorded in DMSO-d_6 at 100 MHz. [b] Recorded in DMSO-d_6 at 400 MHz. [c,d] Assignments bearing the same superscript may be reversed.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer model 241 polarimeter. UV spectra were recorded on a Uvikon 931 UV−vis spectrophotometer. All NMR spectra were run on a Bruker DRX-400 instrument operating at 400 MHz for ^{1}H and at 100 MHz for ^{13}C, using standard pulse sequences. Chemical shifts are reported on the δ scale in parts per million downfield from TMS. Carbon multiplicities were determined in DEPT-135 and DEPT-90 experiments. All 2D NMR spectra were recorded using pulsed field gradients. ^{1}H−^{1}H correlations were observed in double quantum filtered (DQF) COSY and TOCSY experiments. One-bond ^{13}C−^{1}H correlations were observed in a HSQC experiment.[11] Long-range ^{13}C−^{1}H correlations were observed in HMBC and HSQC−TOCSY experiments. FABMS were recorded on a VG 70 SEQ instrument using glycerol as the liquid matrix. TLC was carried out on precoated Si gel 60 F_{245} plates (Merck), developed with EtOAc−HOAc−HCOOH−H_2O (30:0.8:1.2:8, v/v, upper phase, and 100:10:10:20), *n*-BuOH−HOAc−H_2O (4:1:5), and for sugars EtOAc−H_2O−MeOH−HOAc (13:3:3:4). Neu's spray reagent (1% diphenylboric acid−ethanolamine complex) and 1% methanolic $AlCl_3$ were used to visualize flavonoids; thymol in H_2SO_4 (0.5 g thymol in 95 mL EtOH and 5 mL 97% H_2SO_4), followed by heating the plates to 120 °C for 15−20 min, to visualize sugars. Preparative paper chromatography was carried out on Whatman 3MM paper. Column chromatography was performed on Si gel (Merck), polyamide 6S (Riedel, De Haën) and Sephadex LH-20 (Pharmacia).

Plant Material. Whole plants of *C. annua* (L.) DC. (Cruciferae) were collected from El-Araish, North Sinai, Egypt, in March 1998, and identified by Prof. Dr. N. Elhadidi, Department of Plant Taxonomy and Flora, Faculty of Sciences, University of Cairo, Cairo, Egypt. A voucher specimen has been deposited at the Herbarium of the National Research Centre, Cairo, Egypt.

Extraction and Isolation. Dried and powdered whole plants (1.5 kg) of *C. annua* were defatted in a Soxhlet extractor with petroleum ether (40−60 °C). The defatted powder was extracted with 70% MeOH, the solvent removed under reduced pressure, and the residue dissolved in hot water. This residue was left in the refrigerator overnight and filtered. The filtrate was partitioned against $CHCl_3$, EtOAc, and *n*-BuOH. From the EtOAc fraction, quercetin (16 mg) was obtained by column chromatography on Si gel with a $CHCl_3$−MeOH gradient (increasing polarity). The *n*-BuOH-soluble fraction was concentrated and subjected to column chromatography on polyamide. Elution was initiated with distilled water gradually

increasing in 10% increments to 100% MeOH. Fractions were collected by monitoring on TLC. The fractions showing the same spots were combined, again subjected to column chromatography using the same system as mentioned above, and further separated by paper chromatography using *n*-BuOH−HOAc−H_2O (4:1:5). In this way compound **1** (29 mg) was obtained. Final purification was performed by passage over Sephadex LH-20 with MeOH.

For acid hydrolysis, compound **1** was dissolved in 5 mL 6% HCl and heated for 3 h. This solution was extracted with EtOAc. The EtOAc fraction (aglycon) and the aqueous fraction (sugars) were concentrated until dryness for identification.

Quercetin 3-*O*-[(6-*O*-*trans*-feruloyl-β-glucopyranosyl)-(1→2)-β-arabinopyranoside]-7-*O*-β-glucopyranoside (1): brown amorphous powder; $[\alpha]_D$ −15.8° (*c* 0.64, MeOH); UV (MeOH) λ_{max} 254, 270 (sh), 332 nm, (+ NaOMe) 265, 382 nm, (+ $AlCl_3$) 276, 331, 418 nm, (+ $AlCl_3$/HCl) 272, 334 nm, (+ NaOAc) 272, 334 nm, (+ NaOAc/H_3BO_3) 260, 334 nm; ^1H (DMSO-d_6) and ^{13}C NMR (DMSO-d_6), see Table 1; FABMS (negative ion mode) *m*/*z* 933 $[M - H]^-$.

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