Natural Product Sciences
12(3) : 122-124 (2006)

A New Flavonoid from *Carrichtera annua*

Abdelaaty A. Shahat[1]*, Khaled A. Abdel-Shafeek[3,1], Husseiny A. Husseiny[1], Magda Claeys[2], Sandra Apers[2], and Luc Pieters[2]

[1]Chemistry of Medicinal Plants Department, National Research Centre, 12311 Dokki, Cairo, Egypt
[2]Department of Pharmaceutical Sciences, University of Antwerp, Universiteitsplein 1, B-2610 Antwerp, Belgium
[3]University of Antwerp, B-2610, Antwerp, Belgium. [3]Chemistry Dept., Faculty of Sciences, Altahady University, Sirte, Libya

Abstract – Three flavonoid glycosides, kaempferol-3-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside or kaempferol-3-*O*-rutinoside (**1**), isorhamnetin-3-*O*-α-L-rhamnopyranosyl-(16)-β-D-glucopyranoside or isorhamnetin-3-*O*-rutinoside (**2**), and quercetin-3-*O*-β-D-glucopyranosyl-(1→2)-β-L-arabinopyranoside **3**, the latter one being a new compound, were isolated from the methanolic extract of the aerial parts of *Carrichtera annua*. Mass spectrometry and 1D and 2D NMR spectroscopy allowed establishing the structure of these compounds.
Keywords – *Carrichtera annua,* Crucifereae (Brassicaceae), flavonol glycosides, NMR

Introduction

Carrichtera annua (L.) DC. (Crucifereae or Brassicaceae) occurs in different areas in Egypt, i.e. the Nile Delta, the Mediterranean coastal area, and Sinai. Crucifereae is one of the large plant families containing ca. 3000 spp. mostly of the temperate regions (Tackholm, 1974; Boulos, 1999). It includes vegetable crops, garden and wild flowers. Many of the Crucifereae plants serve as a source of foods and condiments such as cabbage, turnip, rutabaga, and mustard green. Most the plants of this family contain flavonoids and glucosinolates. The plants of this family are used in the treatment of many diseases because of their anticancer, antirheumatic, antibacterial, antifungal, and diabetic properties (Kirtikau and Basu, 1975). We have previously isolated a new acetylated flavonol triglycoside from *Carrichtera annua*, i.e quercetin 3-*O*-[(6-feruloyl-β-glucopyranosyl)-(1→2)-β-arabinopyranoside]-7-*O*-β-glucopyranoside together with quercetin (Abdel-Shafeek *et. al.*, 2000). The flavonoid fraction of the seeds of *Carrichtera annua* was investigated using LC/ESI-MS and nano-ESI-MS/CID/MS. The flavonoid fraction was found to contain twelve flavonol-*O*-glycosides, and one of these, i. e. quercetin-3-*O*-(6-sinapoyl-β-glucopyranosyl)-(1→2)-β-arabinopyranoside was identified by NMR spectroscopy as a new compound (Cuyckens *et. al.*, 2003). We report here the isolation and structural elucidation of three flavonoid glycosides from the 80% methanolic extract of the herb of *Carrichtera annua*, one of which being a new quercetin-diglycoside.

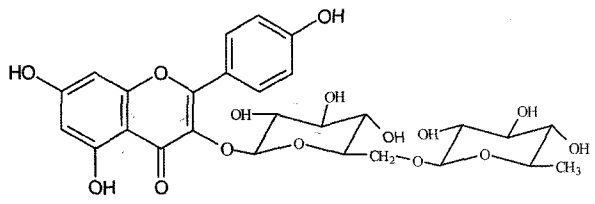
Experimental

General experimental procedures. – TLC was carried out on precoated silica gel F_{254} plates (Merck, Darmstadt, Germany) developed with EtOAc-HOAc-HCOOH-H_2O (30 : 0.8 : 1.2 : 8 v/v) (upper phase) (solvent a) and EtOAc-H_2O-MeOH-HOAc (13 : 3 : 3 : 4) (solvent b) (for sugars). Spots were detected using Neu's spray reagent (1% diphenylboric acid ethanolamine complex in MeOH) (reagent a) and thymol in H_2SO_4 (0.5 g thymol in 95 ml EtOH and 5 ml H_2SO_4) (reagent b) followed by heating the plates to 120 °C for 15 - 20 min. Column chromatography was performed on silica gel (Merck, Darmstadt, Germany) and Sephadex LH-20 (Pharmacia).

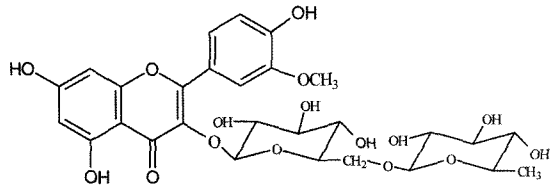
NMR spectra were recorded in CD_3OD on a Bruker DRX-400 spectrometer operating at 400.13 MHz for ^1H and at 100.61 MHz for ^{13}C. Chemical shifts are presented in ppm downfield from TMS. Mass spectra were recorded on an Autospec-oa-Tof instrument.

Plant material. – *Carrichtera annua* herb was collected in March 2000 from El-Araish, North Sinai, Egypt and identified by Prof. Dr. N. Elhadidi, Department of Plant Taxonomy and Flora, Faculty of Sciences, University of Cairo, Egypt. A voucher specimen has been deposited in

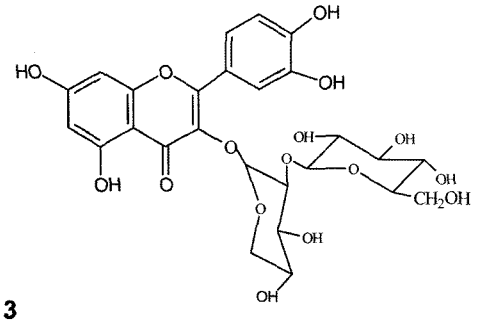
*Author for correspondence
Fax: +91-2 3694518; E-mail: aashahat@hotmail.com



1



2



3

the Herbarium of the National Research Centre, Cairo, Egypt.

Extraction and isolation. – The ground powdered herb of *Carrichtera annua* (600 g) was defatted with petroleum ether (40 - 60 °C). The defatted powder was extracted with aqueous MeOH (80%). The extract was concentrated to remove the MeOH and transferred to a silica gel column, eluted with a gradient consisting of petroleum ether/ EtOAc and EtOAc/MeOH. The fraction eluted with EtOAc/MeOH (30 : 70) was concentrated, and the residue was chromatographed with MeOH on a Sephadex LH-20 column. The first fraction (50 ml) was discarded and the second fraction (70 ml) showed three spots on TLC [solvent (a)]. Repeated column chromatography on Sephadex LH-20 with MeOH as eluant yielded compounds **1**, **2** and **3**.

Hydrolysis conditions. – For total hydrolysis, the three compounds were dissolved separatly in 5 ml 6% HCl and heated for 3 h. The hydrolysate was extracted with EtOAc. The EtOAc fraction (aglycones) and the aqueous fraction (sugars) were concentrated till dryness for identification with authentic standards on TLC.

Table 1. ^{1}H- and ^{13}C-NMR assignments of compounds **1** and **2**

C No.	1		2	
	^{13}C-NMR (δ, ppm)	^{1}H-NMR δ (ppm), mult., J (Hz)	^{13}C-NMR (δ, ppm)	^{1}H-NMR δ (ppm), mult., J (Hz)
2	158.68		158.99	
3	135.54		135.52	
4	179.38		179.30	
5	162.96		162.92	
6	100.33	6.22, br s	100.18	6.21, br s
7	166.72		166.37	
8	95.16	6.41, br s	95.09	6.41, br s
9	159.61		158.52	
10	105.56		105.66	
1'	122.79		122.98	
2'	132.42	8.06, br s	114.68	7.95, d, 1.1
3'	116.22		148.32	
4'	161.61		150.91	
5'	116.22	6.90, d, 8.7	116.15	6.92, d, 8.2
6'	132.42	8.06, d, 8.7	124.05	7.62, dd, 8.2, 1.1
		glucosyl		
1"	104.73	5.08, d, 6.8	104.56	5.19, d, 7.0
2"	75.71	3.45, m	75.86	3.50, m
3"	78.16	3.45, m	78.16	3.50, m
4"	71.51	3.26, m	72.29	3.50, m
5"	77.26	3.35, m	77.37	3.39, m
6"	68.74	3.81, d, 9.8 3.30, m	68.64	3.81, d, 9.8 3.40, m
		rhamnosyl		
1"'	102.48	4.51, br s	102.56	4.52, br s
2"'	77.11	3.65, s	72.08	3.63, s
3"'	72.32	3.53, dd, 9.3, 2.9	71.61	3.26, m
4"'	73.93	3.29, m	73.85	3.26, m
5"'	69.78	3.45, m	69.82	3.39, m
6"'	17.95	1.10, d. 6.2	17.90	1.10, d, 6.2

Results and Discussion

The ground dried powdered herb of *Carrichtera annua* was defatted with petroleum ether and then extracted with 80% MeOH. Fractionation of the resulting dried residue was achieved by repeated column chromatography on silica gel and final purification on Sephadex LH-20, yielding three flavonoids. Acid hydrolysis of these compounds yielded glucose and rhamnose for compounds **1** and **2**, and arabinose and rhamnose for compound **3**. Aglycones were kaempferol, isorhamnetin and quercetin, respectively. The sugars and the aglycones were identified by co-chromatography with authentic samples. The ^{1}H, ^{13}C NMR

Table 2. ^{1}H- and ^{13}C-NMR assignments of compound **3**

C No	^{13}C-NMR (δ, ppm)	^{1}H-NMR δ (ppm), mult., J (Hz)	C No	^{13}C-NMR (δ, ppm)	^{1}H-NMR δ (ppm), mult., J (Hz)
				Arabinosyl	
2	158.55		1"	101.54	5.34, d, 3.6
3	135.62		2"	80.39	4.20, m
4	179.66		3"	71.75	3.94, m
5	163.10		4"	66.99	3.67, m
6	100.16	6.20, br s	5"	64.01	3.80, m
7	166.80				3.30, m
8	94.92	6.39, br s		Glucosyl	
9	159.00		1"	105.49	4.56, d, 7.7
10	105.57		2"	75.23	3.35, m
1'	122.80		3"	77.94	3.35, m
2'	117.40	7.61, br s	4"	71.10	3.73, m
3'	146.40		5"	77.94	3.35, m
4'	150.20		6"	62.36	2H: 3.73, m
5'	116.41	6.91, d, 8.4			
6'	123.21	7.53, br d, 8.4			

and mass spectra of compounds **1** and **2** were in agreement with those reported for kaempferol-3-*O*-rutinoside or kaempferol-3-*O*-α-L-rhamnopyranosyl-(16)-β-D-glucopyranoside, and isorhamnetin-3-*O*-rutinoside or isorhamnetin-3-*O*-α-L-rhamnopyranosyl-(16)-β-D-glucopyranoside (Markham and Chari 1982) (Table 1).

Compound **3** was isolated as an amorphous yellow solid. The mass spectrum in ES+ mode showed a $[M+Na]^{+}$ peak at *m/z* 619, and its fragmentation pattern suggested a quercetin aglycone substituted in position 3 with a pentose (internal sugar) and a hexose (external sugar). This was supported by the presence of 26 carbon signals in its ^{13}C NMR spectrum. Its ^{1}H and ^{13}C NMR spectral data were in good agreement with those reported for quercetin-3-*O*-(6-sinapoyl-β-D-glucopyranosyl)-(1 → 2)-β-L-arabinopyranoside, which we isolated before from the seeds of the same plant, but the signals due to the sinapoyl moiety were missing (Cuyckens *et. al.*, 2003). Since H-1 of the glucosyl residue (δ 4.56, d, J = 7.7 Hz, which is in agreement with a β-configuration) showed a long-range correlation in the HMBC spectrum with C-2 of the arabinosyl moiety at δ 80.39, and since H-1 of the arabinosyl moiety (δ 5.34, d, J = 3.6 Hz) showed a long-range correlation with C-3 of the quercetin nucleus at δ 135.62 (Abdel-Shafeek *et. al.*, 2000), the structure of **3** could be unambiguously established as quercetin 3-*O*-β-D-glucopyranosyl-(1 → 2)-β-L-arbinopyranoside, being a new compound. The complete ^{1}H and ^{13}C NMR assignments, based on COSY, HSQC and HMBC experiments, are listed in Table 2.

References

Abdel-Shafeek, K.A., El-Messiry, M.M., Shahat, A.A., Seif-El Nasr, M.M., Apers, S., and Pieters, L., A new acylated flavonol triglycoside from *Carrichtera annua J. Nat. Prod.* **63**(6), 845-847 (2000).

Boulos, L., *Flora of Egypt*, Al Hadara Publishing, Cairo, Egypt, Volume one, pp. 220 (1999).

Cuyckens, F., Shahat, A.A., Van den Heuvel, H., Abdel-Shafeek, K.A., El-Messiry, M.M., Seif-El Nasr, M.M., Pieters, L., Vlietinck, A.J., and Claeys, M., The application of liquid chromatography-electrospray ionization mass spectrometry and collision-induced dissociation in the structural characterization of acylated flavonol *O*-glycosides from the seeds of *Carrichtera annua*. *Eur. J. Mass. Spectrom.* **9**, 409-420 (2003).

Kirtikau R., Basu L. Indian Medicinal Plants (2nd edn), Vol. I. Bishen Singh Mahendra Pal Singh: Dehra Dun, India, 151. (1975).

Markham, K. R., and Chari, V. M. in., *The Flavonoids: Advances in Research* (Harborne, J. B. and Mabry, T. J., eds.) Chapman and Hall, London. 19. (1982).

Täckholm, V., *Students Flora of Egypt*, second edition, pp. 43 (1974).

(Accepted August 22, 2006)