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Isolation and identification of a new flavonoid glycoside from *Carrichtera annua* L. seeds

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ABSTRACT

Background: Flavonoids are a major group of constituents and are assumed to be among the beneficial components. Recently, they have also received considerable interest as components of foodstuffs and nutraceuticals because of their antioxidant and anticancer properties. **Materials and Methods:** About 500 g of air-dried powdered seeds of *C. annua* were defatted seeds and extracted with 70% methanol. The combined methanol extract was partitioned with chloroform and *n*-butanol. The butanol extract was concentrated and subjected to column chromatography on polyamide. **Results:** The fraction eluted with aqueous methanol (40% and 50%) was found to contain three main flavonoids (1, 2, and 3). Repeated column chromatography on polyamide and Sephadex LH-20 gave compound 1. Compounds 2 and 3 were further purified using preparative paper chromatography with 20% HOAc and Sephadex LH-20 column. **Conclusions:** Reinvestigation of the flavonoidal constituents of the butanol fraction of the aqueous methanolic extract of *Carrichteraannua* seeds led to isolation and identification of a new flavonoidal glycosidenamed as quercetin 3-*O*-[(6-sinapoyl-β-glucopyranosyl)-(1→2)-β-arabinopyranosyl]-7-*O*-β-glucopyranoside 1, in addition to, quarecetin-3-*O*-glucoside 2, isorhamnetin-3-*O*-β–runtinoside3, and isorhamnetin4. Structures of the isolated compounds were established by UV, MS, and ^{1}H and ^{13}C NMR.

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INTRODUCTION

The family *Brassicaceae* (= *Cruciferae*) consists of 350 genera and about 3500 species, and includes several genera such as *Camelina, Crambe, Sinapis, Thlaspi*, and *Brassica*. The genus *Brassica* is the most important one within the tribe Brassiceae, which includes some crops and species of great worldwide economic importance such as *Brassica oleracea* L., *Brassica napus* L., and *Brassica rapa* L. The same species can be utilized for several uses according to different forms or types. The genus is categorized into oilseed, forage, condiment, and vegetable crops by using their buds, inflorescences, leaves, roots, seeds, and stems. Brassicaceae vegetables represent an important part of the human diet worldwide, and are consumed by people all over the world.[1] The largest genera are *Draba* (365 species), *Cardamine* (200 species), *Erysimum* (225 species), *Lepidium* (230 species), and *Alyssum* (195 species). Numerous species have food economic importance such as cabbage, cauliflower, turnip, and rape.[2] The species are utilized as salad plants due to their content of anti-scorbutic and low content of erucic acids. Many Cruciferous species are known for their use in folk medicine for the treatment of snake bites. Moreover, they are used as an antimicrobial agent for relief of biliary colic and wound sores. They have enhancing the detoxification effect of chemical carcinogen and some species exhibit hypoglycemic and hypotension effects.[3,4] *Carrichtera annua* is an endemic annual herb belonging to a family belonging to the Cruciferae (or Brassicaceae), growing up to 40 cm specially in north Sinai at Elarrish region, Egypt. *C. annua* in particular is known to be used by the native Bedouins as an antidiabetic and antispasmodic. Flavonoids are a major group of constituents and are assumed to be among the beneficial components. Recently, they have also received considerable interest as components of foodstuffs and nutraceuticals because of their antioxidant and anticancer

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properties.[5-7] The flavonoidal fraction of the seeds of *C. annua*, was investigated using LC/ESI-MS and nano-ESI-MS/CID/MS. The flavonoidal fraction was found to contain 12 flavonol *O*-glycosides, which were structurally related and of which 11 were acylated with one or more benzoyl, feruloyl, or sinapoyl groups.[8] In a previous study different known and new flavonoids compounds were isolated from the seeds and the herb and investigated by LC/MS and or NMR.[8-10] In this study, we report the isolation and structure elucidation of a new acylated flavonol triglycoside.

MATERIALS AND METHODS

Plant material

The plant was collected from Al-Arish region, Sinai, Egypt in April 2008 during the seedling stage. The plant was collected and identified as described previously.[8]

Apparatus and techniques

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. TLC was carried out on precoated Silica gel 60 F_{245} plates (Merck), developed with EtOAc–HOAc–HCOOH–H_2O (30:0.8:1.2:8, v/v, upper phase (solvent 1), and 100:10:10:20) (solvent 2) and Neu's spray reagent (1% diphenylboric acid ethanolamine complex). Column chromatography was performed on Silica gel (Merck), polyamide 6S (Riedel, De Haën), and Sephadex LH-20 (Pharmacia).

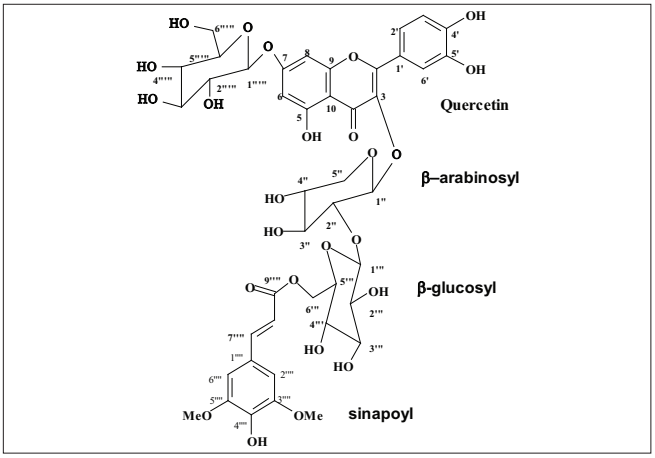
Extraction and isolation of flavonoids

About 500 g of air-dried powdered seeds of *C. annua* were extracted with petroleum ether (br. 40–60°C) by maceration (1.2 L × 4). The defatted seeds were extracted with methanol (70%) for 3 days (1.5L × 3). The combined methanol extract was evaporated under reduced pressure at 45°C and the residue dissolved in hot distilled water (400 ml) and left in the refrigerator overnight and filtered the precipitated matters. The filtrate was partitioned with chloroform (500 ml × 2) and *n*-butanol (600 ml × 4). The butanol extract was concentrated and subjected to column chromatography (CC) on polyamide. Elution was started with distilled water and decreasing the polarity in 10% with methanol to 100% methanol. The fractions were collected by monitoring on thin layer chromatography (TLC) using solvent 1 and/or solvent 2 as developing solvents. The fraction eluted with aqueous methanol (40% and 50%) was found to contain three main flavonoids (1, 2, and 3), this fraction was subjected again to CC using 30% methanol for elution and increasing methanol to 70% as mentioned above. Compound 1 was isolated and purified by using Sephadex LH-20 column eluted with methanol to give 22 mg of compound 1. Compounds 2 and 3 were further purified using preparative paper chromatography with 20% HOAc and passing over a small Sephadex LH-20 column using methanol as eluent (compound 2 was 18 mg and compound 3 was 16 mg, respectively). Compound 4 was isolated from the fraction eluted with 70% methanol (polyamide column) in the same manner to afford (10 mg).

RESULTS AND DISCUSSION

The flavonoidal compounds were obtained from the *n*-butanol soluble fraction of the defatted aqueous methanol of *C. annua* powdered seeds. Acid hydrolysis of these compounds yielded quercetin and isorhamnetin as aglycones and arabinose and glucose as sugars. Compound **1** was isolated as an amorphous yellow substance, and its ^{1}H and ^{13}C NMR spectra showed the presence of a quercetine moiety, three sugar residues, sinapoyl moiety and two methoxyl groups. The chemical shifts of C-2 and C-3 (*δ* 156.9 and 135.2, respectively) indicated C-3 substitution of the quercetin moiety.[11]

The ^{1}H NMR spectrum showed, in the aromatic region, a pair of *meta* coupled doublets at δ 6.28 and δ 6.21 (J = 1.6 Hz), which were attributed to H-6 and H-8 of quercetin (A-ring), respectively, and the typical three-spin system of the 1,3,4-trisubstituted B-ring: a *meta* coupled doublet at δ 7.50 (J = 2 Hz) (H-2′), an *ortho*-coupled doublet at δ 6.79 (J = 8.8 Hz) (H-5′), and an *ortho* and *meta* coupled doublet of doublets at δ 7.55 (J = 8.8, 2 Hz) (H-6′). Substitution of quercetin in the position C-3 was evident from the chemical shift of C-2 (δ 156.9), whereas in flavonols with an unsubstituted hydroxyl functionality at this position C-2 is expected around δ 147.[12]



Compound 1

The signal at δ 161.9 was assigned to C-5 and the assignment of C-6 and C-8 was confirmed through the long range coupling observed in the HMBC experiment with the ^{1}H NMR signal at δ 6.28 (H-6) while the signal at δ 164.8 showed correlations to both H-6 and H-8 (δ 6.1) so the signal at 164.8 was assigned for C-7, compound **1** was a 3,7 di-substituted quercetin. The 2D NMR spectra allowed the assignment of all signals of the glucose moiety at C-7 which could be identified as a glucopyranoside[13] and the β configuration of the anomeric carbon was cleared from the coupling constant of H-1′′′′′′ (J = 7.5 Hz) observed in the ^{1}H NMR spectrum.[14] The presence of a sugar moiety at C-7 was further confirmed through the UV spectra of both the compound and it's aglycone after acid hydrolysis where band-II showed bathochromic shift (10 nm) in the sodium acetate spectrum of the aglycone and does not occur in the compound itself[13]. In fact, the UV spectra with different shift reagents proved the presence of quercetin moiety with free hydroxyl groups at C-3′, C-4′, C-5, and substituted at C-3 where band-I (MeOH) appeared at 335 nm.[9]. Two methoxy groups appeared in ^{1}H NMR at δ 3.7 which correlated to C-3′′′′ and C-5′′′′. ^{13}C NMR at δ 148.5. The C-7′′′′–C-8′′′′ double bond showed a large coupling constant (J = 14.9 Hz) which prove the *trans*-configuration of the double bond. The carbonyl group of the sinapoyl moiety occurring at δ 168.3 exhibited a long range ^{13}C–^{1}H correlation with a ^{1}H NMR signal at δ 4.11 assigned to one of the H-6′′′′ proton of a hexose unit representing the second glucose moiety. The anomeric proton at δ 4.55 showed a long range correlation with a ^{13}C NMR signal at δ 81.9 correspond to the proton at δ 4.19 in the HSQC spectrum. The anomeric proton H-1′′ of arabinose was assigned at δ 5.52, so we can conclude that the glucose moiety is attached at C-2′′ in arabinose. All data of ^{1}H and ^{13}C were presented in Table 1. The FAB mass spectrum of the compound displayed a molecular ion peak at m/z 987 [M+ Na]$^+$ which is compatible with the molecular formula $C_{43}H_{48}O_{25}$, this can confirm the presence of two hexose moieties, pentose moiety, and sinapoyl moiety attached to quercetin nucleus. The presence of the sinapoyl moiety was confirmed through the peak at m/z 207 and its fragments (at m/z = 192, 175) also the ion peak at m/z 523 attributed to [Na$^+$sinapoyl-hexose–pentose] part which attached to quercetin at m/z 303. The terminal hexose can be deduced from the ion at m/z 803 which matched with [M$^+$ + H-162]$^+$. Since the loss of sinapoyl-hexose-pent part (m/z 465) is much more pronounced than the loss of terminal hexose (at m/z 803) so, we can conclude that these parts are at C-3 and C-7.[8] These spectral data were in good agreement with those reported for quercetin 3-*O*-[(6-feruloyl-β-glucopyranosyl)-(1→2)-β-arabinopyranoside] -7-*O*-β-glucopyranoside which was isolated before from the herb of this plant.[9]

Table 1: 1H and 13C NMR data of compound 1 in DMSO

C no.	13C (ppm)	1H (ppm), multi., J (Hz)	Carbon no.	13C (ppm)	1H (ppm), multi., J (Hz)
2	156.9			**Sinapoyl**	
3	135.2		1′′′′	125.86	
4	179.2		2′′′′	105.9	6.2, s
5	161.9		3′′′′	148.5j	
6	100.0	6.28, d, 1.6 (meta)	4′′′′	138.95	
7	164.8		5′′′′	148.5	
8	94.2	6.1, d, 1.6 (meta)	6′′′′	105.9	6.2, s
9	157.3		7′′′′	146.4	7.1, d, 15.2
10	104.9		8′′′′	114.8	5.65, d, 15.5
1′	121.8		9′′′′	168.0	
2′	116.7	7.52, d, 2 (meta)	2CH3O–	56.12	Sharp 3.8, s
3	145.3		**7-O-glucosyl**		
4′	149.1		1′′′′′	101.1	5.1, d, 6.9
5′	115.8	6.95, d, 8.8 (ortho)	2′′′′′	74.1	3.23, m
6′	122.58	7.32, dd 8.8, 2 (ortho, meta)	3′′′′′	77.23	3.1, m
3-O-arabinosyl			4′′′′′	70.3	3.3, m
1′′	100.35	5.52, d, 3.5	5′′′′′	78.05	3.45, m
2′′	81.9	4.19, dd, 5.5, 3.5	6′′′′′	61.4	3.6, dd,10.5, 7.9,
3′′	71.5	3.9, d, 5.4			
4′′	66.92	3.77, m			
5′′	63.2	3.9, m, 3.25, m			
3-O-glucosyl					
1′′′	105.95	4.55, d, 8			
2′′′	75.2	3.19, m			
3′′′	77.52	3.35, dd, 8.5, 8.5			
4′′′	71.9	3.18, m			
5′′′	74.98	3.45, dd, 7.6, 7.4			
6′′′	63.85	4.5, m			

From all the previous data we can conclude the structure of compound **1** as quarecetin-3-*O* [(6-sinapoyl-β-glucopyranosyl (1→2)-β-arabinopyroside]-7-*O*-glucopyranoside,which is considered as a new compound. The other three compounds were identified as quarecetin-3-*O*-glucoside, isorhamnetin-3-*O*-runtinoside, and isorhamnetin by comparison with the literature data.[10-12] It is evident to note that the compound isorhamnetin -3-*O*-runtinoside was isolated before from the herb of this plant.[10]

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