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Acylated flavonoid glycosides from *Bassia muricata*

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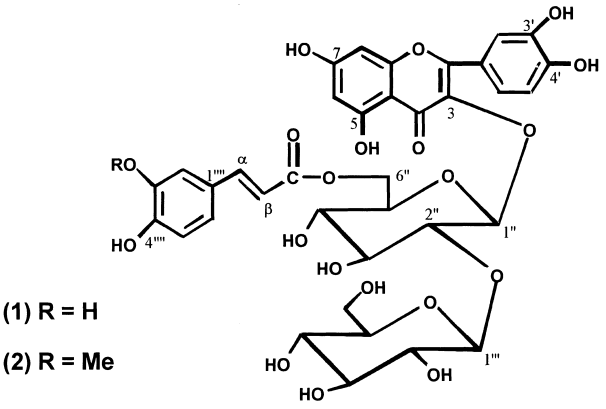
Abstract

From the arial parts of *Bassia muricata*, two acylated flavonoid glycosides quercetin-3-*O*-(6″-caffeoyl)-sophoroside and quercetin-3-*O*-(6″-feruloyl)-sophoroside have been isolated together with two known flavonoid glycosides quercetin-3-*O*-sophoroside and quercetin-3,7-*O*-β-diglucopyranoside, as well as four known triterpenoidal saponins, oleanolic acid-3-*O*-β-glucopyranoside, chikusetsusaponin IVa, chikusetsusaponin IVa methyl ester and oleanolic acid-3,28-β-diglucopyranoside. The structures of the isolated compounds were verified by means of MS and NMR spectral analyses.

Keywords: *Bassia muricata*; Chenopodiaceae; Acylated flavonoid glycosides

1. Introduction

Bassia muricata (L.) Murr. (Chenopodiaceae) is a common sandy herb growing in Egyptian deserts (Tackholm, 1974). The plant is used in folk medicine to treat renal and rheumatic diseases (Al-Yahya et al., 1990). The chloroform and ethanolic extracts of the plant produced pilo-erection, significant hypotension, a remarkable decrease in WBCs counts, and a significant increase in prothrombin time besides their significant contractions of isolated guinea pig ileum. Meanwhile, the ether and benzene extracts showed antimicrobial activities (Al-Yahya et al., 1990). Reviewing current literature showed that only a few species of *Bassia* have been investigated and were found to contain triterpenoidal saponins identified as bassic acid glycosides (Rizk, 1986). This work describes the isolation and structural elucidation of two new acylated flavonoid glycosides (**1**) and (**2**) together with two known flavonoid glycosides and four known triterpenoidal saponins.



2. Results and discussion

The ethanolic extract of the aerial parts of *B. muricata* was defatted with diethylether and the aqueous layer was subjected to column chromatography on Diaion HP-20. The 50% methanol eluate was repeatedly chromatographed on columns of silica gel and then by MPLC and HPLC to afford eight glycosides.

The molecular formula of compound **1** was deduced as $C_{36}H_{36}O_{20}$ from HR FAB-MS spectrometry (see Experimental). Inspection of the ^{13}C NMR and DEPT spectra of **1** (Table 1) displayed the presence of two β-

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glucopyranosyl units from the anomeric signals at δ 101.5 and 105.6 as well as the other signals characteristic for glucopyranosyl units as shown in Table 1 (Bradbury and Jenkins, 1984). Besides, the spectra showed 15 carbon signals for 3-*O*-glycosylated quercetin (Harborne and Mabry, 1982) and 9 carbon signals for a caffeoyl moiety (Bloor et al., 1998). From ^{1}H NMR spectral data of **1** (Experimental), the doublet at δ 5.11 (J = 7.6 Hz) of the anomeric proton of one of the two glucopyranosyl units indicated its β configuration. The anomeric proton of the second glucosyl unit was obscured by the H_2O signal. However, the β configuration of the glucosyl residues were very obvious from the downfield shifts of their anomeric carbons when compared with those reported for α-anomers in the ^{13}C NMR spectral analysis (Bradbury and Jenkins, 1984). Moreover the coupling constant (15.6 Hz) of the two doublets at δ 5.59 and 7.30 (each 1H) indicated the *trans* configuration of the caffeoyl moiety (Fiasson et al., 1997). In the ^{13}C NMR spectrum of **1**, the downfield shift of C-2″ (δ 82.2) together with the upfield shift of C-1″ (δ 101.5) proved the attachment of the terminal glucosyl unit to this position (Bradbury and Jenkins, 1984). Furthermore, the signal at δ 64.6 was assigned to the C-6 of one of the two glucosyl units to which the caffeoyl moiety is attached (Miyase et al., 1992). To determine the attachment site of the caffeoyl moiety, compound **1** was acetylated and subjected to EI–MS spectral analysis according to the procedure established by Komori et al. (1975) which revealed a characteristic peak at m/z 331 (18%) corresponding to tetraacetylated glucose as a cationic fragment with a molecular formula $(C_{14}H_{19}O_9)^+$ as shown in Fig. 1. This significant peak proved the presence of an unsubstituted terminal glucosyl unit (Komori et al, 1975). Consequently, the attachment site of the caffeoyl moiety must be at C-6 of the internal β-glucosyl unit (C-6) and the structure of compound **1** was assigned as shown.

Table 1
^{13}C NMR spectral data of compounds **1** and **2** (100 MHz, CD_3OD)

C	1	2	C	1	2
2	158.1	158.1	*Terminal*	*sugar*	
3	135.1	135.2	**1‴**	105.6	105.6
4	179.9	179.9	**2‴**	74.7	74.7
5	162.8	162.8	**3‴**	76.7	76.7
6	99.8	99.8	**4‴**	71.7	71.8
7	165.6	165.5	**5‴**	77.7	77.7
8	94.7	94.7	**6‴**	61.7	61.7
9	158.1	158.1			
10	105.9	106.0		*Caffeoyl*	*Feruloyl*
1′	122.7	122.7	α	114.5	114.8
2′	116.3	116.3	β	146.4	146.7
3′	145.8	145.9	**1⁗**	127.4	127.3
4′	149.7	150.3	**2⁗**	115.0	111.1
5′	117.4	117.4	**3⁗**	149.3	149.7
6′	123.8	123.8	**4⁗**	146.9	149.0
Internal	*sugar*		**5⁗**	116.3	116.2
1″	101.5	101.5	**6⁗**	122.8	123.9
2″	82.2	82.4	−COO	169.1	168.9
3″	75.7	75.6	−OMe		56.2
4″	70.0	70.1			
5″	75.8	76.0			
6″	64.6	64.5			

The molecular formula of compound **2** was determined as $C_{37}H_{38}O_{20}$ from HR FAB–MS spectrometry (see Experimental). ^{13}C and DEPT NMR spectra of 2 were almost similar to those of **1**. However, the signal at δ 56.2 in 2 together with the downfield shift of C-4‴ (+2.1 ppm) indicated the presence of a feruloyl moiety instead of the caffeoyl moiety (Miyase et al., 1992). EI–MS of the acetylated derivative of **2** exhibited the same results as **1**. Consequently, the site of attachment of the feruloyl moiety was proved to be at C-6″ of the internal glucosyl unit and the structure of **2** was determined as shown.

On the basis of ^{1}H and ^{13}C NMR, compounds **3**–**8** were identified as follows: compound (**3**) quercetin-3-*O*-sophoroside (Veit et al, 1990), compound (**4**) quercetin-3,7-*O*-β-diglucopyranoside (Harborne et al., 1982), compound (**5**) oleanolic acid-3-*O*-β-glucopyranoside (Mahato et al., 1988), compound (**6**) chikusetsusaponin IVa (Mohamed et al., 1998), compound (**7**) chikusetsusaponin IVa methyl ester, compound (**8**) oleanolic acid-3,28-β-diglucopyranoside (Mahato et al., 1988).

3. Experimental

^{1}H and ^{13}C NMR (TMS as int. standard): 400 MHz and 100 MHz respectively were recorded on a Jeol JNM

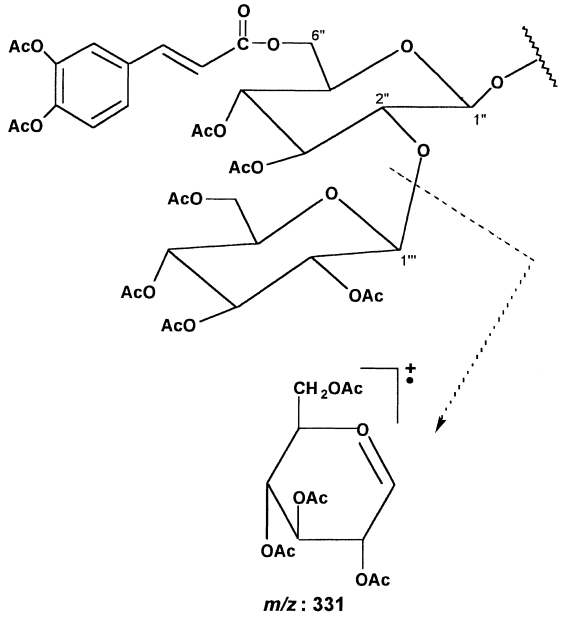

Fig. 1. Results of EI–MS analysis of the acetate derivative of compound **1**.

α-400 spectrometer. MS spectra were recorded on a Jeol JMS-SX 102 spectrometer by direct inlet method at an ionizing voltage of 70 eV. MPLC: RP-18 column (20 mm i.d. ×40 cm); flow rate of mobile phase 3 ml/min. HPLC: D-ODS-5 and polyamine columns (each 20 mm i.d. ×25 cm, YMC) with a Toyo Soda high speed chromatograph HLC-803 D pump and a Tosoh refraction index (RI-8) detector; flow rate of mobile phase 6 ml/min, injection vol. 0.8–1.0 ml. CC: Kieselgel 60 (70-230 mesh, Merck) and Diaion HP 20 (Mitsubishi). TLC: silica gel 60 precoated plates, F-254 and HPTLC using RP-18 precoated plates, F-254 s (Merck).

3.1. Plant material

Aerial parts of *B. muricata* (L.) Murr. were collected from Assiut Valley, Assiut, Egypt, in June 1997. The plant was identified by Professor A. Fayed, Department of Botany, Faculty of Science, Assiut University, Assiut, Egypt. A voucher specimen is deposited at the Herbarium of Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut, Egypt.

3.2. Extraction and isolation of compounds (*1*–*8*)

The air dried powdered aerial parts of *B. muricata* (2 kg) were extracted with 70% EtOH. The dried ethanolic extract (220 g) was suspended in H_2O and defatted with diethylether. The aq. fr. (115 g) was applied to a column of Diaion HP-20 and eluted with H_2O, 50% MeOH, MeOH and acetone successively. The 50% MeOH eluate (30 g) was chromatographed on silica gel CC using EtOAc–MeOH–H_2O (40:10:1 and 70:30:3) to give 6 fractions. Fraction 4 (4.5 g) eluted with EtOAc–MeOH–H_2O (70:30:3) was subjected to MPLC using ODS column and 40–60% MeOH as a solvent system followed by HPLC using polyamine column and 90% MeCN as solvent to afford compounds **1** (yellow powder, 58 mg), **2** (yellow powder, 42 mg), **3** (yellow powder, 134 mg) and **4** (yellow powder, 97 mg). Fraction 5 (6.7 g) eluted with EtOAc–MeOH–H_2O (70 : 30 : 3) was subjected to MPLC using ODS column and 40–60% MeOH as solvent followed by HPLC using an ODS column and 58% MeOH as solvent to afford compounds **5** (white powder, 137 mg), **6** (white powder, 228 mg), **7** (white powder, 26 mg) and **8** (white powder, 21 mg).

3.2.1. Compound (*1*)

Quercetin-3-*O*-(6″-caffeoyl)-sophoroside. R_t 23 min (polyamine, 90% MeCN). UV λ_{max} MeOH nm: 251, 329 and 295 (sh). HR FAB–MS (negative) *m*/*z*: 787.1736 $[M–H]^-$ $C_{36}H_{35}O_{20}$ (req. 787.1721). ^{13}C NMR (CD_3OD, Table 1). ^1H NMR (CD_3OD): δ 7.65 (1H, *dd*, *J* = 2.0, 8.8 Hz, H-6′), 7.30 (1H, *d*, *J* = 15.6 Hz, H-β), 6.86 (1H, *d*, *J* = 8.8 Hz, H-5′), 6.79 (1H, *bs*, H-2′ or 2‴″), 6.50-6.78 (3H, *m*, H-2‴″, 5‴″, 6‴″), 6.21 (1H, *bs*, H-8), 6.13 (1H, *bs*, H-6), 5.59 (1H, *d*, *J* = 15.6 Hz, H-α), 5.11 (1H, *d*, *J* = 7.6 Hz, H-1″) and H-1″ was obscured by H_2O signal.

3.2.2. Compound (*2*)

Quercetin-3-*O*-(6″-feruloyl)-sophoroside. R_t 21 min (polyamine, 90% MeCN). UV λ_{max} MeOH nm: 253, 330 and 297 (sh). HR FAB–MS (negative) *m*/*z*: 801.1890 $[M–H]^-$ $C_{37}H_{37}O_{20}$ (req. 801.1878). ^{13}C NMR (CD_3OD, Table 1). ^1H NMR (CD_3OD): δ 7.65 (1H, *dd*, *J* = 2.0, 8.5 Hz, H-6′), 7.31 (1H, *d*, *J* = 16.1 Hz, H-β), 6.86 (1H, *d*, *J* = 8.5 Hz, H-5′), 6.79 (1H, *bs*, H-2″), 6.69 (1H, *dd*, *J* = 1.8, 8.1 Hz, H-6‴″), 6.63 (1H, *bs*, H-2‴″), 6.62 (1H, *d*, *J* = 8.1 Hz, H-5‴″), 6.14 (1H, *bs*, H-8), 6.10 (1H, *bs*, H-6), 6.03 (1H, *d*, *J* = 16.1 Hz, H-α), 5.04 (1H, *d*, *J* = 7.6 Hz, H-1″), 4.75 (1H, *d*, *J* = 7.8 Hz, H-1‴) and 3.70 (3H, *s*, −OMe).

3.3. Acetylation of compounds *1* and *2*.

A sample of 10 mg of compounds **1** and **2** was separately dissolved in 2 ml acetic anhydride-dried pyridine (1:1) and left overnight at room temp. The solvents were removed by evaporation under reduced pressure and the residues were subjected to EI–MS spectral analysis.

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