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Note

Flavonoid triglycosides from the seeds of *Syzygium aromaticum*

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Abstract—Two new apigenin triglycosides, apigenin 6-*C*-[β-D-xylopyranosyl-(1‴→2″)-β-D-galactopyranoside]-7-*O*-β-D-glucopyranoside and apigenin 6-*C*-[β-D-xylopyranosyl-(1‴→2″)-β-D-galactopyranoside]-7-*O*-β-D-(6⁗-*O*-*p*-coumarylglucopyranoside) were isolated from the ethanol extract of the seeds of *Syzygium aromaticum*. Their structures were elucidated by chemical and spectral analysis (UV, FABMS, ^{1}H, ^{13}C NMR, HMQC, HMBC, NOESY and DEPT).

Keywords: *Syzygium aromaticum*; Myrtaceae; Flavonoid triglycosides

Syzygium aromaticum (L.) Merr. & Perry belongs to the family Myrtaceae, members of which are well known for their medicinal properties. *S. aromaticum* buds (clove) are used in folk medicine as diuretic, odontalgic, stomachic, tonicardiac, aromatic condiment and condiment with carminative and stimulant properties.[1] Volatile oils from species of *Syzygium* exhibit antibacterial activity.[2,3] Compounds from *S. aromaticum* possess growth inhibitory activity against oral pathogens and these active compounds have been identified as 5,7-dihydroxy-2-methylchromone-8-*C*-β-D-glucopyranoside, biflorin, kaempferol, rhamnocitrin, myricetin, gallic acid, ellagic acid and oleanolic acid.[4] Oleanolic acid has been identified as anti-HIV principle from *S. clavflorum*.[5] Also, from *S. aromaticum*, triterpene acids and orsellinic acid glycosides have been isolated.[6,7] The present study deals with the isolation and structure elucidation of two new flavonoids, apigenin trioside **1** and its *p*-coumaryl ester **2** from *S. aromaticum* seeds.

From the aqueous ethanol extract of *S. aromaticum* seeds, two flavone triglycosides were identified (Fig. 1). Under UV light, compounds **1** and **2** showed brown spots which changed to yellow with ammonia vapor and gave a yellow color with *Naturstoff reagenz* A.

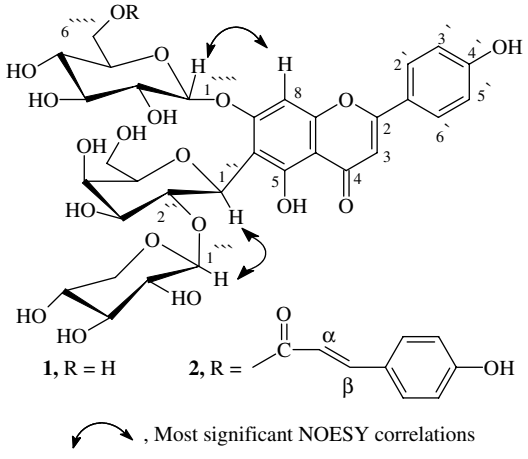

Figure 1.

Compounds **1** and **2** were UV active in methanol and with diagnostic reagents showed properties in accord with those of a flavone 7-O-glycoside.[8] Acid hydrolysis of both **1** and **2** failed to produce an aglycone, indicating the presence of a *C*-glycosidic linkage. It gave glucose and xylose, in addition to 6-*C*-galactosylapigenin and *p*-coumaric acid, as indicated by paper co-chromatography with authentic samples. The UV spectral data and acid hydrolysis showed that the locations of the sugars were at two different positions of the aglycone.

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The positive ion FABMS spectrum of compound **1** showed a molecular ion peak $[M+H]^+$ at *m/z* 727, corresponding to $C_{32}H_{38}O_{19}$. The ^1H NMR spectrum of **1** exhibited an AA′ BB′-type system at δ 7.79 and δ 6.88 for a *p*-disubstituted benzene ring, together with two singlets at δ 6.84 and δ 6.59, assigned to H-8 and H-3, respectively, and referring to 6,7-disubstituted apigenin. The spectrum showed also three doublets for the three anomeric protons of three sugar moieties. The δ 5.11 (*J* 7.06 Hz) resonance was due to a 7-*O*-glucopyranosyl moiety, the δ 4.97 (*J* 9.89 Hz) signal was attributed to 6-*C*-galactopyranosyl unit, and the third upfield δ 4.26 (*J* 6.64 Hz) resonance was assigned to an *O*-β-D-terminal xylosyl unit. The ^{13}C NMR spectrum of **1** displayed 32 carbon resonances (Table 1), 15 of which were assigned to the apigenin aglycone moiety, six signals for each of the *O*-glucosyl and *C*-galactosyl units, and five for the terminal xylosyl moiety.[9] DEPT experiments showed the presence of three methylene groups, twenty methine groups and the remaining nine carbons were quaternary. The resonances of the protonated carbons were assigned using HMQC experiments. The anomeric protons of the glucosyl, *C*-galactosyl and xylosyl units showed correlation with the corresponding anomeric carbons at δ 102.4, δ 72.9 and δ 106.5, respectively. The assignment of most signals was in accordance with the literature and in particular, the carbon resonances at δ 161.5 (C-5), δ 110.8 (C-6), δ 164.2 (C-7) and δ 94.9 (C-8) were similar to those reported for apigenin 6-*C*-glycosyl-7-*O*-glycoside.[9] Consequently, the signal appeared at δ 94.9 was assigned to C-8 and the proton at δ 6.84, which showed a distinct cross peak with C-8 in HMQC experiment must be H-8. The appearance of the anomeric carbon of galactose (C-1″) at higher field at δ 72.9 referred to the presence of a C-galactoside bond.[9] HMBC correlations between δ 4.97 (H-1″) with δ 110.8 (C-6) and δ 4.26 (H-1‴) with δ 82.5 (C-2″) and δ 5.11 (H-1⁗) with δ 164.2 (C-7) indicated the presence of 6-*C*-[β-D-xylopyranosyl-(1‴→2″)-β-D-galactopyranoside] and 7-*O*-β-D-glucopyranoside moieties. Also, the appearance of the carbonyl carbon at δ 183.9 in compound **1** confirmed that the 5-OH is free, because glycosidation at C-5 leads to a higher field shift of the carbonyl carbon of flavones.[10] Finally, the structure of **1** was further confirmed on the basis of NOESY (Fig. 1) showing that the anomeric proton of glucose (δ 5.11) correlated with H-8 (δ 6.84) and the anomeric proton of galactose (δ 4.97) correlated with the anomeric proton of xylose (δ 4.26). This confirmed the locations of glucose at 7-position and xylose at position 2 of galactose, which located to 6-position. Thus, **1** was unambiguously identified as apigenin 6-*C*-[β-D-xylopyranosyl-(1‴→2″)-β-D-galactopyranoside]-7-*O*-β-D-glucopyranoside.

The positive ion FABMS spectrum of compound **2** showed a molecular ion peak $[M+H]^+$ at *m/z* 873, compatible with the molecular formula $C_{41}H_{44}O_{21}$. The ^1H NMR spectrum of compound **2** was very close to that of **1** and differs only by the presence of additional signals at δ 7.01 (d, 2H, *J* 8.66 Hz) and δ 6.50 (d, 2H, *J* 8.66 Hz), together with two signals of two *trans* olefinic protons at δ 7.52 (d, 1H, *J* 16 Hz) and δ 6.22 (d, 1H, *J* 16 Hz), assigned to a *p*-coumaryl moiety. It exhibited also a signal pattern of 6,7-disubstituted apigenin and also three doublets for three anomeric protons, at δ 5.16 (d, 1H, *J* 7.42 Hz) for 7-β-*O*-glucopyranosyl, at δ 4.97 (d, 1H, *J* 10.0 Hz) due to 6-*C*-galactopyranosyl and at δ 4.14 (d, 1H, *J* 7.22 Hz) for *O*-β-D-xylosyl units. The location of *p*-coumaryl moiety was deduced to be at C-6⁗ OH of the glucose moiety from the ^1H NMR spectrum, which signals due to CH_2 protons of glucose

Table 1. ^{13}C NMR data of compounds **1** and **2** in MeOH-d_4 at 125 MHz

C-No.	1	2	DEPT
Aglycone			
2	166.4	166.4	C
3	104.2	104.1	CH
4	183.9	184.0	C
5	161.5	161.6	C
6	110.8	110.8	C
7	164.2	164.0	C
8	94.9	94.4	CH
9	158.7	158.7	C
10	106.7	106.7	C
1′	122.7	122.8	C
2′,6′	129.5	129.5	CH
3′,5′	117.0	117.0	CH
4′	162.7	162.9	C
Galactosyl			
1″	72.9	72.6	CH
2″	82.5	83.0	CH
3″	73.2	73.5	CH
4″	71.2	70.9	CH
5″	82.0	81.7	CH
6″	62.9	61.9	CH_2
Xylosyl			
1‴	106.5	107.5	CH
2‴	74.0	74.5	CH
3‴	79.7	79.7	CH
4‴	71.1	72.1	CH
5‴	66.5	67.3	CH_2
Glucosyl			
1⁗	102.4	101.2	CH
2⁗	75.1	75.0	CH
3⁗	78.4	77.6	CH
4⁗	69.2	69.5	CH
5⁗	77.4	75.6	CH
6⁗	62.1	65.0	CH_2
p-Coumaryl			
1	—	126.6	C
2,6	—	130.9	CH
3,5	—	116.7	CH
4	—	161.1	C
α	—	114.4	CH
β	—	147.1	CH
COO	—	168.8	C

appeared at δ 4.48 and δ 4.50 at lower field than those observed in **1**.[11,12] The ^{13}C NMR spectrum of **2** displayed 41 carbon resonances (Table 1), 32 of which were similar to those of **1**, in addition nine carbon resonances were due to the *p*-coumaryl moiety, one of which at δ 168.8 was due to the carbonyl carbon and two at δ 147.1 and δ 114.4 for the two olefinic carbons. The relatively downfield shift of C-6‴‴of glucose of **2** to δ 65 confirmed the location of *p*-coumaryl unit at C-6‴‴. The assignment of the protonated carbons of **2** could be achieved by HMQC experiments. HMBC correlations between δ 4.48 and δ 4.50 of the two 6‴‴ protons with the carbonyl signal of *p*-coumaric at δ 168.8 showed the location of *p*-coumaryl at C-6‴‴. Furthermore, the structure of **2** was confirmed on the basis of NOESY (Fig. 1), which was similar to that of **1**. Thus, the structure of **2** was established as apigenin 6-*C*-[β-D-xylopyranosyl-(1‴→2″)-β-D-galactopyranoside]-7-*O*-β-D-(6‴‴-*O*-*p*-coumarylglucopyranoside). To our knowledge, **1** and **2** are reported here for the first time from *S. aromaticum* seeds as new naturally occurring compounds.

1. Experimental

1.1. General methods

TLC was performed on precoated E. Merck 60 F 254 Silica Gel and cellulose plates and visualized with UV light or *Naturstoff reagenz* A. The ^{1}H and ^{13}C NMR were recorded on a Bruker AMX and/or a Varian Unity Inova instruments at 500 MHz (1H) and 125 MHz (13C) with TMSi as an internal reference and MeOH-d_4 as solvent at room temperature. FABMS was recorded on a Finnigan MAT TSQ 700 spectrometer. UV in methanol were determined using Shimadzu UV 240 spectrophotometer.

1.2. Plant material, extraction and isolation

The seeds of *S. aromaticum* were obtained from the local market (Harraz Company, Cairo) and identified by Dr. I. A. Mashaly, Department of Botany, Mansoura University, Mansoura, Egypt. A voucher specimen has been deposited at the Herbarium, NRC, Cairo, Egypt. The seeds of *S. aromaticum* (600 g) were crushed and extracted with *n*-hexane, then with dichloromethane to remove oils and fats. The residue was extracted with 70% EtOH by soaking at room temperature and the ethanol extract was evaporated under diminished pressure, affording a dry extract (21 g) which was chromatographed on a cellulose CC. The column was eluted with water and with water–MeOH step gradient and 10 fractions (500 ml, each) were collected and further separated on Sephadex LH-20 CC, using MeOH–water 1:1 to give the apigenin trioside **1** (8 mg) and its *p*-coumaryl ester **2** (7 mg).

1.3. Apigenin 6-*C*-[β-D-xylopyranosyl-(1→2)-β-D-galactopyranoside]-7-*O*-β-D-glucopyranoside (1)

Yellow amorphous powder; R_f 0.24 (silica gel TLC) in BAW and 0.88 (cellulose TLC) in 15% HOAc; UV (MeOH) λ_{max}: 270, 332; +NaOMe 272, 306 *sh*, 352 *sh*, 396; +NaOAc 270, 345, 390; +NaOAc–H_3BO_3 270, 334 nm; FABMS: *m*/*z* 727 $[M+H]^+$; HRMS: *m*/*z* 727.4412 (Calcld *m*/*z* 727.4428 for $C_{32}H_{38}O_{19}$); ^{1}H NMR (CD_3OD): δ 7.79 (d, 2H, *J* 8.62 Hz, H-2′, H-6′), 6.88 (d, 2H, *J* 8.62 Hz, H-3′, H-5′), 6.84 (s, 1H, 8-H), 6.59 (s, 1H, H-3), 5.11 (d, *J* 7.06 Hz, H-1‴‴), 4.97 (d, *J* 9.89 Hz, 1H, H-1″), 4.40 (dd, *J* 9.0, 6.0 Hz, 1H, H-2″), 4.26 (d, *J* = 6.6 Hz, 1H, H-1‴), 3.12–3.88 (m, 16H, remaining sugar protons overlapped by OH protons); for ^{13}C NMR data: See Table 1.

1.4. Apigenin 6-*C*-[β-D-xylopyranosyl-(1→2)-β-D-galactopyranoside]-7-*O*-β-D-(6-*O*-*p*-coumarylglucopyranoside) (2)

Yellow amorphous powder; R_f 0.26 (silica gel TLC) in BAW and 0.81 (cellulose TLC) in 15% HOAc. UV (MeOH) λ_{max}: 272, 316; +NaOMe 274, 306 sh, 396; +NaOAc 272, 316, 390; +NaOAc-H_3BO_3 270, 298 sh, 316, 382 sh; $AlCl_3$ 280, 302, 338, 380 sh; $AlCl_3$ + HCl 280, 300, 335, 380 nm; FABMS: *m*/*z* 873 $[M+H]^+$; HRMS: *m*/*z* 873.2446 (Calcd *m*/*z* 873.2488 for $C_{41}H_{44}O_{21}$); ^{1}H NMR (CD_3OD): 7.82 (d, 2H, *J* 8.84 Hz, 2′-H, 6′-H), 7.52 (d, 1H, *J* 16, H-β), 7.01 (d, 2H, *J* 8.66 Hz, H-2coum, H-6coum), 6.89 (d, 2H, *J* 8.84 Hz, H-3′, H-5′), 6.79 (s, 1H, H-8), 6.59 (s, 1H, H-3), 6.50 (d, 2H, *J* 8.66 Hz, H-3coum, H-5coum), 6.22 (d, 1H, *J* 16 Hz, H-α), 5.16 (d, 1H, *J* 7.42 Hz, H-1‴‴), 4.97 (d, 1H, *J* 10.0 Hz, H-1″), 4.50 (dd, 1H, *J* 12.0, 3.0 Hz, H-6‴‴a), 4.48 (dd, 1H, *J* 12.0, 5.0 Hz, H-6‴‴b), 4.40 (dd, 1H, *J* 9.0, 6.0 Hz, H-2″), 4.14 (d, 1H, *J* 7.22 Hz, H-1‴), 3.14–3.88 (m, 14H, remaining sugar protons overlapped by OH protons); for ^{13}C NMR data: See Table 1.

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