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A NEW KAEMPFEROL TRIOSIDE FROM *Farsetia aegyptia*

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*A new kaempferol trioside, kaempferol-3-*O*-(2″-α-L-arabinopyranosyl)-α-L-rhamnopyranoside-7-*O*-α-L-rhamnopyranoside, along with eight known flavonoid compounds were isolated from the methanolic extract of* Farsetia aegyptia *Turra. growing in Egypt. The structures were established on the basis of detailed spectral analysis (UV, ^{1}H NMR, ^{13}C NMR, APT, HMBC, FABMS, and EIMS).*

Key words: *Farsetia aegyptia* Turra., Brassicaceae (= Cruciferae), new kaempferol trioside.

Brassicaceae is one of the largest angiosperm families, comprising approximately 338 genera and more than 3709 species distributed worldwide [1]. It includes many economically important vegetable salad plants and crop species. Some of Cruciferae are grown domestically in gardens as ornamentals. The seeds of Cruciferae are largely used as condiment and fertilizer [2]. The plants of this family are used in the treatment of many diseases because of their anticancer, antibacterial, antifungal, antirheumatic, and antidiabetic properties [3]. The genus *Farsetia* is represented by three species that grow in Egypt. The most common one is *Farsetia aegyptia* Turra. [4, 5]. The plant is known to be used by native Bedouins as antidiabetic and antispasmodic. Moreover, it is used for the relief of rheumatic pains and taken internally as cooling medicine after pounding [3].

The powdered aerial parts of *Farsetia aegyptia* Turra. was defatted with petroleum ether (40–60°C) and extracted three times at room temperature with 70% methanol–water. The methanolic extract was fractionated and chromatographed [6, 7]. A new naturally occurring flavonol triglycoside [8], kaempferol-3-*O*-(2″-α-L-arabinopyranosyl)-α-L-rhamnopyranoside-7-*O*-α-L-rhamnopyranoside (**1**), was isolated, together with eight known flavonoids: kaempferol-3-*O*-(2″-β-D-glucopyranosyl)-α-L-rhamnopyranoside-7-*O*-α-L-rhamnopyranoside (**2**), kaempferol-3,7-di-*O*-α-L-rhamnopyranoside (**3**), kaempferol (**4**), isorhamnetin-3-*O*-β-L-arabinopyranoside-7-*O*-(2‴-β-D-glucopyranosyl)-α-L-rhamnopyranoside (**5**), isorhamnetin-3-*O*-α-L-rhamnopyranosyl-7-*O*-β-D-glucopyranoside (**6**), isorhamnetin (**7**), apigenin-7-*O*-β-D-glucopyranoside (**8**), and apigenin (**9**).

Compound **1** was isolated as a pale yellow amorphous powder. The color reaction on paper chromatography with ammonia vapor changed from brown to yellow, indicating that the compound has a flavonoid chromophore [6]. UV spectral data with diagnostic shift reagents suggested the presence of a 3,7-disubstituted flavonol glycoside with free hydroxyl groups at the 5 and 4′ positions [6, 7]. Two intermediate spots were detected upon mild acid hydrolysis with 0.1 N HCl, before yielding the aglycone. Complete acid hydrolysis (2 N HCl, 1 h, 100°C) yielded rhamnose and arabinose (Co-PC) and kaempferol (3,5,7,4′-tetrahydroxyflavone) (Co-PC, UV, EIMS, and ^{1}H NMR). The negative-ion FABMS showed a molecular ion peak $[M-H]^-$ at *m/z* 709, corresponding to $C_{32}H_{38}O_{18}$.

The ^{1}H NMR spectrum showed two pairs of doublets at δ 7.8 (J = 9.0 Hz) and δ 6.95 (J = 9.0 Hz) assigned to H-2′,6′ and H-3′,5′, respectively. The two meta coupled doublets at δ 6.7 (J = 2.0 Hz) and δ 6.4 (J = 2.0 Hz) are assigned to H-8 and H-6, respectively. This downfield chemical shift confirmed that C-7 was substituted in ring A [6]. The ^{1}H NMR spectrum also revealed three distinct anomeric proton resonances at δ 5.54 (J = 2 Hz), δ 5.38 (J =2 Hz), and δ 4.2 (J = 6.9 Hz), attributed to H-1‴ of the α-rhamnopyranose unit at position 7, H-1″ of the α-rhamnopyranose at position 3, and H-1⁗ of the α-arabinopyranose unit, respectively [9]. These data also confirmed the presence of two signals at δ 0.9 and δ 1.1. The chemically shifted signal at δ 0.9 was assigned to the rhamnose unit linked at C-3, while the signal at 1.1 was assigned to the rhamnose at C-7 of the aglycone [9].

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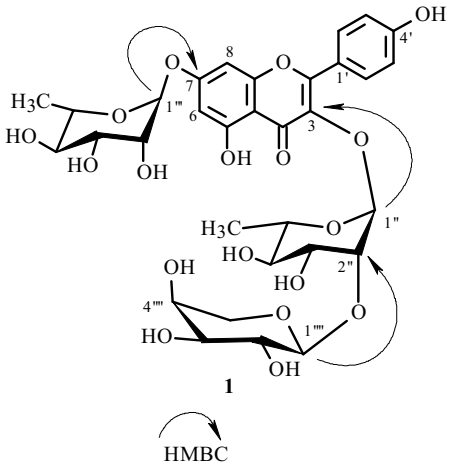

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HMBC

The ^{13}C NMR spectrum of compound **1** displayed 32 carbon resonances; 15 of which were assigned to kaempferol as the aglycone moiety, 12 to two rhamnose moieties, and five to the arabinose moiety [10]; the anomeric carbon atoms of the two rhamnose units resonate at δ 101.3 and 99.7. The chemically shifted signal at δ 101.3 was assigned to the rhamnose unit linked at C-3, while the signal at 99.7 was assigned to the rhamnose at C-7 of the aglycone [11].

The APT experiment showed the presence of two methyl groups at δ 18.6 and δ 18.1 assigned to C-6″ and C-6‴ of two rhamnose units, one methylene at δ 66.4 assigned to C-5⁗ of the arabinose unit, and 20 methine groups. The remaining carbon resonances were attributed to nine quaternary carbon atoms.

In the HMBC spectrum the anomeric proton of one rhamnopyranosyl unit (H-1″, δ 5.37) showed a correlation with C-3 (δ 134), and the second rhamnopyranosyl unit (H-1‴, δ 5.54) showed a correlation with C-7 (δ 162.4). The arabinose H-1⁗ was cross-correlated with C-2″ of the rhamnose moiety at position 3 of the aglycone; this was confirmed from the shift of the C-2″ signal at δ 81.2 [9]. Based on the above evidence, compound **1** was identified as kaempferol-3-*O*-(2″-α-L-arabinopyranosyl)-α-L-rhamnopyranoside-7-*O*-α-L-rhamnopyranoside.

Compounds **2**, **3**, **4**, and **8** were isolated from *Farsetia aegyptia* for the first time [8]. In addition to compounds **6** and **7**, compound **5** was isolated previously as a new flavonoid from the same species [12].

EXPERIMENTAL

1D and 2D NMR experiments (^1H, ^{13}C, APT, and HMBC) were recorded on a Jeol EX-500 spectrometer: 500 MHz (^1H NMR), 125 MHz (^{13}C NMR); and on an INOVA Mercury 300 spectrometer: 300 MHz (^1H NMR), 75 MHz (^{13}C NMR). UV spectrophotometer: Shimadzu UV-240. FABMS: Jeol JMS-AX 500. EIMS: Finnigan-Mat SSQ 7000. Polyamide S6: Riedel-De-Haen AG, Seelze Haen AG, Seelze Hanver, Germany. PC (descending) Whatman No. 1 and 3 MM papers, using solvent systems 1) H_2O, 2) 15% HOAc (H_2O–HOAc 85:15), 3) CAW ($CHCl_3$–HOAc–H_2O 90:45:6), 4) BAW (*n*-BuOH–HOAc–H_2O 4:1:5, upper layer), 5) (C_6H_6–*n*-BuOH–H_2O–pyridine 1:5:3:3, upper layer). Solvents 4 and 5 were used for sugar analysis, Sephadex LH-20 (Pharmazia).

Plant Material. A fresh sample of *Farsetia aegyptia* Turra. was collected on Suez–Cairo desert road (Egypt) in April 2005. The sample was identified by Prof. Dr. Abdel Salam M. Al-Nowaihi and Dr. Salwa A. Kawashty. A voucher specimen was deposited in the Herbarium of the National Research Centre (CAIRC).

Extraction and Isolation of Flavonoid Constituents. Air-dried ground aerial parts of *Farsetia aegyptia* (2.5 kg) were defatted with petroleum ether (40–60°C) and extracted three times at room temperature with 70% methanol–water. The methanolic extract was evaporated under reduced pressure and temperature, affording 300 g residue. It was subjected to a polyamide column (125 cm × 5 cm) starting with water as eluent and decreasing the polarity by increasing the concentration of methanol. A total of 52 fractions was collected, each 250 mL; these were combined according to PC using H_2O, 15% HOAc, and BAW as solvents to give eight fractions (A-H). Fraction A was chromatographed on PC using CAW (double solvent) to yield two subfractions. They were purified on a Sephadex LH-20 column (30 cm × 1.5 cm) using methanol–water (1:1) and repeated by using methanol to yield the pure compounds **1** (20 mg) and **2** (8 mg). Fraction B was chromatographed on PC using 15% HOAc and then BAW to yield compound **5** (10 mg). Fractions C, D, E, F, G, and H yielded compounds **3** (35 mg),

6 (10 mg), **8** (14 mg), **4** (17 mg), **7** (8 mg), and **9** (10 mg), respectively. Purification of the isolated compounds was carried out on a Sephadex LH-20 column (25 cm × 1 cm) using methanol as eluent. The structure elucidation and identification of the pure isolated flavonoid compounds were carried out through chemical investigation (complete and mild acid hydrolysis) and physical investigation (UV, ^{1}H NMR, ^{13}C NMR, APT, HMBC, FABMS, and EIMS) as well as a comparison with previously reported data [6, 12, 13].

Kaempferol-3-*O*-(2′′-α-L-arabinopyranosyl)-α-L-rhamnopyranoside-7-*O*-α-L-rhamnopyranoside (1). Pale yellow crystals, mp 230–233°C, R_f 0.47 (BAW). UV/Vis (λ_{max}, MeOH, nm, log ε): (MeOH) 267 (2.3), 346 (1.8); (+NaOMe) 268 (2.3), 394 (2.4); (+$AlCl_3$) 273 (2.3), 302 (1.1), 348 (1.3), 399 (1.2); (+$AlCl_3$/HCl) 274 (2.3), 300 (1.1), 345 (1.4), 398 (1.3); (+NaOAc) 266 (2.5), 350 (1.7); (+NaOAc/H_3BO_3) 264 (2.6), 314 (1.3), 348 (1.9). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 7.8 (2H, d, J = 9.0, H-2′,6′); 6.95 (2H,d, J = 9.0, H-3′,5′); 6.7 (1H, d, J = 2.0, H-8); 6.4 (1H,d, J = 2.0, H-6); 5.54 (1H, d, J = 2.0, H-1‴); 5.38 (1H, d, J = 2.0, H-1′′); 4.2 (1H, d, J = 6.9, H-1⁗), 3.1–4.0 (m, sugar protons overlapped with -OH proton signals), 1.1 (3H, d, J = 6, CH_3-rhamnose at 7-position), 0.9 (3H, d, J = 6, CH_3-rhamnose at 3-position). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 178.8 (C-4) (C), 162.4 (C-7) (C), 162.3 (C-5) (C), 161.8 (C-4′) (C), 158.2 (C-2) (C), 156.8 (C-9) (C), 134 (C-3) (C), 131 (C-2′) (CH), 131 (C-6′) (CH), 120.2 (C-1′) (C), 116.3 (C-3′) (CH), 116.3 (C-5′) (CH), 106.9 (C-1⁗) (CH), 106.5 (C-10) (C), 101.5 (C-1′′) (CH), 100.1 (C-6) (CH), 99.1 (C-1‴) (CH), 94.5 (C-8) (CH), 81.2 (C-2′′) (CH), 79.6 (C-3⁗) (CH), 77.5 (C-4‴) (CH), 76.9 (C-2⁗) (CH), 72.3 (C-2‴) (CH), 71 (C-3′′) (CH), 70.9 (C-5′′) (CH), 70.7 (C-4⁗) (CH), 70.6 (C-4′′) (CH), 70.4 (C-3‴) (CH), 70.0 (C-5‴) (CH), 66.4 (C-5⁗) (CH_2), 18.6 (C-6′′) (CH_3), 18.1 (C-6‴) (CH_3). Negative FABMS, *m/z* 709 $(M-1)^-$ corresponding to molecular formula $C_{32}H_{38}O_{18}$.

Kaempferol-3-*O*-(2′′-β-D-glucopyranosyl)-α-L-rhamnopyranoside-7-*O*-α-L-rhamnopyranoside (2) [13]. Pale yellow crystals, mp 242–247°C, R_f 0.43 (BAW). UV/Vis (λ_{max}, MeOH, nm, log ε): (MeOH): 265 (2.4), 344 (1.6); (+NaOMe) 266 (2.3), 389 (2.4); (+$AlCl_3$) 275 (0.6), 299 (1.1), 344 (1.5), 394 (1.3); (+$AlCl_3$ HCl) 275 (2.3), 300 (1.1), 344 (1.4), 393(1.3); (+NaOAc) 265 (2.5), 380 (1.6); (NaOAc/H_3BO_3) 265 (2.5), 345 (1.8). ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 7.7 (2H, d, J = 8.5, H-2′,6′); 6.8 (2H, d, J = 8.5, H-3′,5′); 6.65 (1H, d, J = 2.0, H-8); 6.35 (1H, d, J = 2.0, H-6); 5.48 (1H, d, J = 2.0, H-1′′); 5.33 (1H, d, J = 2.0, H-1‴); 4.16 (1H, d, J = 7.00, H-1⁗); 3.4 (m, sugar protons overlapped with -OH proton signals); 1.1 (3H, d, J = 6, CH_3-rhamnose at position 7); 0.85 (3H, d, J = 6, CH_3-rhamnose at position 3). ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm): 176.8 (C-4), 162.2 (C-7), 162.11 (C-5), 161.1 (C-4′), 156.5 (C-9), 156.7 (C-2), 134.9 (C-3), 131.1 (C-2′, C-6′), 120.9 (C-1′), 116.2 (C-3′, C-5′), 106.8 (C-1⁗), 106.75 (C-10), 101.3 (C-1′′), 100.1 (C-6), 99.9 (C-1‴), 95.1 (C-8), 81.1 (C-2′′), 79 (C-3⁗), 76.7(C-5⁗), 76.2 (C-2⁗), 74.2 (C-4′′), 72.1 (C-4‴), 71.1 (C-3′′), 70.9 (C-5′′), 70.7 (C-4‴), 70.5 (C-3‴), 70.3 (C-2‴), 69.8 (C-5‴), 62.1 (C-6⁗), 18.4 (C-6′′), 17.95 (C-6⁗). Negative FABMS, *m/z* 739 $(M-1)^-$ corresponding to molecular formula $C_{33}H_{41}O_{19}$.

Kaempferol-3,7-di-*O*-α-*L*-rhamnopyranoside (3) [6]. Whitish yellow crystals, mp 201–205°C, R_f 0.62 (BAW). UV/Vis (λ_{max}, MeOH, nm, log ε): (MeOH) 266 (1.07), 345 (0.8); (+NaOMe) 266 (0.9), 387 (1.2); (+$AlCl_3$) 274 (1.1), 300 (1.1), 345 (0.8), 399 (0.4); (+$AlCl_3$/HC1) 274 (1.1), 299 (0.5), 341 (0.7), 399 (0.6); (+NaOAc) 265 (1.1), 354 (0.7), 389 (0.6); (+NaOAc/H_3BO_3) 266 (1.1), 314 (0.7), 243 (0.8). ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 7.76 (2H, d, J = 9.0, H-2′,6′); 6.87 (2H, d, J = 9.0, H-3′,5′); 6.66 (1H, d, J =1.8, H-8); 6.35 (1H, d, J = 1.8, H-6); 5.5 (1H, d, J =2, H-1′′); 5.3 (1H, d, J = 2, H-1‴); 3–4 (m, sugar protons overlapped with -OH proton signals); 1.1 (3H, d, J = 6.0, CH_3-rhamnose at position 7); 0.8 (3H, J = 6.0, CH_3-rhamnose at position 3). ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm): 178 (C-4), 162.3 (C-7); 161.5 (C-5); 160.7 (C-4′); 158.3 (C-2), 156.6 (C-9), 135 (C-3), 131.2 (C-2′, C-6′), 121 (C-1′), 116 (C-3′, C-5′), 106.3 (C-10), 102.4 (C-1′′) 100 (C-6), 99.03 (C-1‴), 95 (C-8), 72.2 (C-4′′), 71.7 (C-5′′), 71.2 (C-4‴), 70.9 (C-2′′), 70.8 (C-2‴), 70.6 (C-3′′), 70.6 (C-3‴), 70.3 (C-5‴), 18.00 (C-6′′), 18.5 (C-6‴).

Kaempferol (4) [6]. Yellow crystals, mp 276–280°C, R_f 0.72 (BAW). EIMS, *m/z* 286.

Isorhamnetin-3-*O*-β-L-arabinopyranoside-7-*O*-(2‴-β-D-glucopyranosyl)-α-L-rhamnopyranoside (5) [12]. Pale yellow crystals, mp 248–253°C, R_f 0.42 (BAW). UV/Vis (λ_{max}, MeOH, nm, log ε): (MeOH) 256 (0.9), 358 (0.7); (+NaOMe) 265 (0.9), 415 (0.7); (+$AlCl_3$) 263 (0.9), 299 (0.4), 362 (0.4), 406 (0.5); (+$AlCl_3$/HCl) 262 (0.9), 301 (0.4), 361 (0.5), 401 (0.5); (+NaOAc) 259 (1.1), 365 (0.6), 417 (0.3); (NaOAc/H_3BO_3) 256, 293, 357. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 7.8 (1H, d, J = 2.0, H-2′); 7.4 (1H, dd, J = 8.5, 2.0, H-6′); 6.7 (1H, d, J = 8.5, H-5′); 6.6 (1H, d, J = 2.0, H-8); 6.37 (1H, d, J = 2.0, H-6); 5.4 (1H, d, J = 6.0, H-1′′), 5.6 (1H, d, J = 1.6, H-1‴), 4.46 (1H, d, J = 7.5, H-1⁗), 3.81 (3H, s, OCH_3); 3–4 (m, sugar protons overlapped with -OH proton signals); 1.08 (3H, d, J = 6.0, CH_3 of rhamnose). ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm): 178 (C-4), 161.7 (C-7), 161.3 (C-5), 157.2 (C-2), 156.3 (C-9), 150.5 (C-4′), 147.6 (C-3′), 134.3 (C-3), 123.2 (C-6′), 121.1 (C-1′), 115.8 (C-5′), 113.5 (C-2′), 106.1 (C-10), 105.1 (C-1⁗), 102.1 (C-1′′), 99.9 (C-6), 98.6 (C-1‴), 95.3 (C-8), 81.2 (C-2‴), 77.2 (C-3⁗), 76.7 (C-5⁗), 74.5 (C-2⁗), 72.4 (C-3′′), 71.4 (C-2′′), 70.8 (C-4‴), 70.3 (C-4⁗), 70.1(C-5⁗), 69.3 (C-3‴), 67.08 (C-4′′), 65.4 (C-5′′), 61.5 (C-6⁗), 56.2 (OCH_3), 18.3 (C-6‴).

Isorhamentin-3-*O*-α-L-rhamnopyranosyl-7-*O*-β-D-glucopyranoside (6) [12]. Pale yellow crystals, mp 234–240°C, R_f 0.59 (BAW). UV/Vis (λ_{max}, MeOH, nm, log ε): (MeOH) 262 (0.9), 352 (0.6); (+NaOMe) 262 (1.0), 406 (0.7); (+$AlCl_3$) 258 (0.7), 292 (0.3), 350 (0.4), 400 (0.3); (+$AlCl_3$/HCl) 259 (0.6), 295 (0.3), 247 (0.4), 400 (0.3); (+NaOAc) 263 (0.4), 350 (0.2); (+NaOAc/H_3BO_3) 263 (0.5), 352 (0.3), 404 (0.4). ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 7.8 (1H, d, J = 2.0, H-2′); 7.67 (1H, dd, J = 8.5, 2.0, H-6′); 6.9 (1H, d, J = 8.5, H-5′); 6.75 (1H, d, J = 2.0, H-8); 6.34 (1H, d, J =2.0, H-6); 5.25 (1H, d, J = 2.0, H-1″); 5.05 (1H, d, J = 7.2, H-1‴); 3.72 (3H, s, OCH_3); 3–4 (m, sugar protons overlapped with -OH proton signals); 0.85 (3H, J = 6.0, CH_3 of rhamnose).

Isorhamnetin (7) [12]. Pale yellow crystals, mp 246–248°C, R_f 0.77 (BAW). UV/Vis (λ_{max}, MeOH, nm, log ε): (MeOH) 257 (0.4), 267 (0.2), 299 sh (0.4) 355 sh (0.2), 377 (0.3); (+NaOMe) 282 (0.3), 323 sh (0.4), 446 (0.4); (+$AlCl_3$) 264 (0.4), 299 (0.2), 425(0.3); (+$AlCl_3$/HCl) 262 (0.2), 298 (0.3), 347 (0.5), 427 (0.3); (+NaOAc) 256 (0.6), 277 (0.2), 333 (0.3), 387 (0.4); (+NaOAc/H_3BO_3) 257 (0.3), 268 (0.2), 297 (0.2), 370 (0.3). EIMS, *m/z* 316.

Apigenin-7-*O*-β-D-glucopyranoside (8) [6]. Yellow crystals, mp 178–180°C, R_f 0.72 (BAW).

Apigenin (9) [6]. Yellow crystals, mp 280–286°C, R_f 0.72 (BAW). EIMS, *m/z* 270.

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