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FLAVONOID CONSTITUENTS OF *EPHEDRA ALATA*

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Key Word Index—*Ephedra alata*; Ephedraceae; herbacetin 8-methyl ether 3-*O*-glucoside-7-*O*-rutinoside; herbacetin 7-*O*-(6″-quinyl glucoside); flavonol *O*-glycosides and *C*-glycosylflavones.

Abstract—Two new flavonol glucosides have been identified in *Ephedra alata*, namely, herbacetin 8-methyl ether 3-*O*-glucoside-7-*O*-rutinoside and herbacetin 7-*O*-(6″-quinylglucoside). The known flavonoids vicenin II, lucenin III, kaempferol 3-rhamnoside, quercetin 3-rhamnoside and herbacetin 7-glucoside were also found. The structure of the isolated compounds was determined mostly by FABMS and ^{1}H NMR spectroscopy. The final structure of the new compounds and of herbacetin 7-glucoside was confirmed by ^{13}C NMR spectroscopy.

INTRODUCTION

Ephedra species are characterized by alkaloids of the ephedrine series [1]. Their flavonoid constituents include di-*C*-glycosylflavones [2], flavonol-3-*O*-glycosides [2, 3] and proanthocyanidins [4]. Among these species, *Ephedra alata* grows wild in the Egyptian desert and provides extracts used in folk medicine [5] as depurative, hypotensive, antiasthmatic, sympathomimetic and astringent agents. The branches are chewed for cephalalgia, used in miscarriage and as a bronchodilator. *E. alata* has not been studied previously for its phenolic constituents. The present work reports the isolation and identification of two new flavonoids from the whole plant, namely, herbacetin 8-methyl ether 3-*O*-glucoside-7-*O*-rutinoside (**2**) and herbacetin 7-*O*-(6″-quinylglucoside) (**7**). In addition, vicenin II (**1**), lucenin III (**3**), kaempferol 3-rhamnoside (**4**), quercetin 3-rhamnoside (**5**) and herbacetin 7-glucoside (**6**) were isolated and identified. Compound **6** was recently reported from *E. lomatolepis* [6] and herbacetin 8-methyl ether 3-glucoside from *E. equisetina* [3].

RESULTS AND DISCUSSION

The ground whole plant of *E. alata* was successively extracted with petrol, chloroform, ethanol and water. TDPC revealed the presence of several flavonoids in the ethanol extract, from which compounds **1**–**7** were isolated and purified by applying a combination of CC on polyamide and prep. PC. Two of these compounds (**2** and **7**) are new flavonol glycosides. Other compounds (**1**, **3**–**6**) are known and gave chromatographic, UV absorption and hydrolytic data identical with those reported for vicenin II [7], lucenin III [8], kaempferol 3-*O*-rhamnoside [9], quercetin 3-*O*-rhamnoside [9] and herbacetin 7-*O*-glucoside [10], respectively. The structures of compounds **1** and **3**–**6** were confirmed by positive FABMS (see Experimental). ^{1}H NMR of **1** and **3** are in accordance with the proposed structure for each. Thus, 6,8-di-*C*-*β*-glycosylation was confirmed by the absence of the 6- and 8-flavone proton signals in each case and by the 9 Hz coupling constants measured from the signals (doublet) of the anomeric sugar protons (see Experimental), confirming the structure of **1** as vicenin II and that of **3** as lucenin III. The ^{1}H NMR spectral data of **4** and **5** confirmed their structures to be kaempferol 3-*O*-rhamnoside and quercetin 3-*O*-rhamnoside, respectively. In the ^{1}H NMR spectrum of **6**, the 8-hydroxylation was confirmed by the absence of an 8-proton signal and the 7-*O*-*β*-glucosidation by the downfield shift ($\Delta\delta$ = 0.36 ppm) of the 6-flavonol proton signal when compared with the same signal of the aglucone itself (see Experimental) and by the 9 Hz coupling constant measured from the signal of the anomeric sugar proton. The ^{13}C NMR spectrum of **6** was recorded and assigned for the first time. For unambiguous assignment, the 7-*O*-glucosidation substituent rules were applied to the ^{13}C NMR data of gossypetin [11]. These calculated data were used as the basis for assigning the signals of the carbons from C-2 to C-10 in the spectrum of **6** (see Experimental). Other carbon signals in this spectrum (C-1′ to C-6′ and C-1″ to C-6″) were assigned by comparison with the ^{13}C NMR data of kaempferol-7-*O*-glucoside [12]. The NMR spectral data, given above, confirmed the structure of **6** to be herbacetin 7-*O*-*β*-glucopyranoside.

Compound **2** was isolated as an intense yellow amorphous powder with a molecular weight of 786 as shown by FABMS. Chromatographic properties (dull black colour, unchanged by ammonia vapour on PC under UV light, high R_f-values in aqueous solvents and low R_f-values in organic solvents) suggested a polyglycosidic flavonoid structure. Compound **2** on acid hydrolysis yielded herbacetin 8-methyl ether (R_f-values, CIMS, UV and ^{1}H NMR spectral analysis), glucose and rhamnose (co-PC). On controlled acid hydrolysis or *β*-glucosidase treatment of **2**, an intermediate **2a** was formed. The molecular weight 624 (FABMS), chromatographic and UV analysis beside acid hydrolysis showed the structure of **2a** to be herbacetin 8-methyl ether 7-*O*-rutinoside. The UV spectral analysis of **2** in methanol and in the presence of diagnostic reagents [9] suggested that the sugar moieties are bound to the 3 and 7 positions of the herbacetin 8-methyl ether moiety (negative shift with NaOAc and with NaOAc–H_3BO_3 and a stable NaOMe

spectrum). These analytical data showed **2** to be herbacetin 8-methyl ether 3-*O*-glucoside-7-*O*-rutinoside. The ^{1}H NMR spectrum of **2** (see Experimental) revealed three anomeric sugar proton signals at δ5.56 (*d*, J = 8 Hz), 4.8 (*d*, J = 8 Hz) and 4.48 (*d*, J = 2.5 Hz) assigned to the 3β glucoside H-1, the 7β-glucoside H-1 and the 7α-rhamnosyl H-1, respectively. Other signals in this spectrum agree well with the suggested structure of **2**. In the ^{13}C NMR spectrum (see Experimental), the presence of two β-glucopyranosides and one α-rhamnopyranosyl moieties in the molecule of **2** follows from the three anomeric carbon signals at δ101.6, 101.2 and 101.1, the methyl signal at 17.7 and the carbon signals in the sugar region between δ60 and 80. Signals of the carbons of the flavonoid moiety were assigned on the basis of the calculated chemical shift values obtained by applying the 8-*O*-methylation-3-*O*-glucosidation substituent rules on the ^{13}C NMR data of herbacetin 7-*O*-glucoside. Both recorded and calculated chemical shift values were found to be in close agreement and confirm the final structure of **2** as herbacetin 8-methyl ether 3-*O*-β-glucopyranoside-7-*O*-rutinoside.

Compound **7** was isolated as a dark yellow amorphous powder which exhibited a molecular weight of 638 as shown by FABMS. It showed chromatographic colour properties and UV data similar to those of herbacetin 7-glucoside. Acid hydrolysis of **7** afforded herbacetin, glucose and quinic acid (co-PC). The R_f-values, the absence of a sodium acetate UV shift and the result of acid hydrolysis suggested a herbacetin 7-*O*-(quinylglucoside) structure for **7**. In the ^{1}H NMR spectrum of **7**, acylation with quinic acid is evidenced by the presence of a methylene proton signal at δ1.8 (*m*, 2‴-H$_2$ and 6‴-H$_2$) and a methine proton signal at δ4.88 (*m*, 4‴-H). Signals of the two methine protons at 3‴ and 5‴ in the quinyl moiety are hidden by the six glucose proton signals which appeared as a multiplet between δ3.3 and 3.85. Other signals, in the same spectrum are closely similar to those present in the ^{1}H NMR spectrum of herbacetin 7-*O*-β-glucoside. In the ^{13}C NMR spectrum of **7** (see Experimental), the presence of the quinyl moiety follows from the carbon signals at δ172.9, 75.7, 74.8 and 69.3, assignable to C-7‴, C-4‴, C-1‴ and C-3‴ together with C-5‴, respectively. Signals of C-2‴ and C-6‴ are hidden by the signals of the solvent (DMSO-d_6) centred at 39.5. The spectrum also shows a downfield shift of $\Delta\delta$2.2 ppm for the signal of C-6″ accompanied by an upfield shift of $\Delta\delta$2.9 ppm for the signal of C-5″ of the glucose moiety (all in comparison with the corresponding signals in the ^{13}C NMR spectrum of herbacetin 7-glucoside), thus indicating that the quinyl moiety in **7** is attached to carbon 6 of the glucose moiety. The remaining signals in the spectrum of **7** possess chemical shift values which agree well with the deduced structure as herbacetin 7-*O*-β-(6″-quinylglucopyranoside).

EXPERIMENTAL

NMR spectra were recorded on a Jeol FX-100 Spectrometer. ^{1}H NMR chemical shifts were measured relative to TMS and ^{13}C NMR chemical shifts relative to DMSO-d_6 and converted into the TMS scale by adding 39.5. Typical conditions: spectral width 5000 Hz 8K data points and a flip angle of 45°. FABMS were recorded on a MM7070 E instrument (VG Analytical). PC was carried out on Whatman paper No. 1 using solvent systems: 1, H_2O; 2, HOAc (HOAc–H_2O, 3:17); 3, BAW (*n*-BuOH–HOAc–H_2O, 4:1:5, top layer); 4, HOAc* (HOAc–H_2O, 3:2); 5, BPOH (C_6H_6–*n*-BuOH–C_5H_5N–H_2O, 1:5:3:3, top layer). Solvent system 2 was used in prep. PC on Whatman paper No. 3MM. Solvent systems 3 and 5 were used for sugar analysis.

Plant material. Shrubs of *E. alata* were collected from the Suez desert in Egypt during Feb. 1982 and classified by Dr. Loutfy Boulos Professor of Botany, National Research Centre, Cairo.

Isolation and identification. Fresh plant material was extracted with petrol 40–60°, $CHCl_3$, EtOH and H_2O, respectively. The EtOH extract was dried under vacuum, applied to a polyamide column and eluted with H_2O followed by H_2O–MeOH mixtures of decreasing polarities. Pure **1** was isolated from the 80:20 fraction by crystallization. Compounds **2** and **3** were isolated from the 40:60 fraction by prep. PC. Compounds **4** and **5** were separated from the 60:40 fraction on a cellulose column using H_2O as eluent. Pure **6** and **7** were isolated by prep. PC from the 70:30 fraction and the 90:10 fraction, respectively.

Vicenin II (**1**). R_f-values: 0.23 (H_2O), 0.55 (HOAc), 0.71 (HOAc), 0.33 (BAW). MW = 594, pos. FABMS (MH^+: 595). UV λ_{max}^{MeOH} nm: 272, 333; + NaOAc 282, 393; + NaOAc–H_3BO_3 283, 390; + $AlCl_3$ 280, 305, 345, 383; + NaOMe 275, 361, 402. Compound **1** was recovered unchanged (co-PC) after alcoholic acid hydrolysis (1.5 N HCl, methanolic, 100°, 7 hr). ^{1}H NMR of **1**, aglucone moiety: δ6.76 (*s*, H-3), 6.92 (*d*, J = 8 Hz, H-3′ and H-5′), 8 (*d*, J = 8 Hz, H-2′ and H-6′); sugar moieties δ4.84 (*br s*, $W_{1/2}$ = 16 Hz, H-1″ and H-1‴), 3.08–3.88 (*m*, 12 sugar protons).

Herbacetin 8-methyl ether 3-O-glucoside-7-O-rutinoside (**2**). R_f-values: 0.76 (H_2O), 0.74 (HOAc), 0.77 (HOAc*), 0.24 (BAW). MW = 786, FABMS (MH^+: 787). UV λ_{max}^{MeOH} nm: 272, 330, 360; + NaOAc 272, 328, 360; + NaOAc–H_3BO_3 274, 328, 358; + $AlCl_3$ 280, 310, 355, 412; + NaOMe 275, 365*, 412 (stable). Acid hydrolysis (1.5 N aq. HCl, 100°, 2 hr) of **2** gave glucose, rhamnose (co-PC) and herbacetin 8-methyl ether: R_f-values: 0.00 (H_2O), 0.46 (HOAc*), 0.72 (BAW), 0.48 (Forestal, conc. HCl–H_2O–HOAc, 3:10:30). CIMS *m/z*: 316 $[M]^+$, 315 $[M-1]^+$, 301 $[M-15]^+$, 286 (base peak) $[M-OCH_2]$. UV λ_{max}^{MeOH} nm: 256*, 276, 330, 377; + NaOAc 278, 330*, 373; + NaOAc–H_3BO_3 280, 325*, 375; + $AlCl_3$ 278, 364, 442; + NaOMe 286, 338, 430 (decomp). ^{1}H NMR of herbacetin 8-methyl ether: δ3.92 (*s*, 8 × OMe), 6.2 (*s*, H-6), 6.9 (*d*, J = 8 Hz, H-3′ and H-5′), 8.1 (*d*, J = 8 Hz, H-2′ and H-6′). Hydrolysis of **2** by dil. HCl (0.1 N, aq., 100°, 15 min) or by β-glucosidase yielded herbacetin 8-methyl ether 7-*O*-rutinoside **2a**. R_f-values: 0.15 (H_2O), 0.24 (HOAc), 0.40 (BAW). MW = 624, pos. FABMS (MH^+: 625). UV λ_{max}^{MeOH} nm: 277, 332, 378; + NaOAc 277, 330, 377; + NaOAc–H_3BO_3 280, 330, 375; + $AlCl_3$ 280, 365, 443; + NaOMe 285, 335, 428 (decomp.). Acid hydrolysis of **2a** yielded glucose, rhamnose and herbacetin 8-methyl ether (co-PC). ^{1}H NMR of **2**, aglycone moiety: δ3.92 (*s*, 8-OMe), 6.54 (*s*, H-6), 6.88 (*d*, J = 8 Hz, H-3′ and H-5′), 7.88 (*d*, J = 8 Hz, H-2′ and H-6′); sugar moieties: δ4.48 (*d*, J = 2.5 Hz, rhamnosyl H-1), 4.8 (*d*, J = 8 Hz, 7-*O*-glucoside H-1), 5.56 (*d*, J = 8 Hz, 3-*O*-glucoside H-1), 3.2–3.8 (*m*, 16 sugar protons), 0.92 (*d*, J = 6 Hz, rhamnosyl-Me). ^{13}C NMR of **2**, aglycone: δ157.4 (C-2), 133.2 (C-3), 177.0 (C-4), 152.2 (C-5), 102.1 (C-6), 150.0 (C-7), 129.6 (C-8), 144.5 (C-9), 104.8 (C-10), 121.6 (C-1′), 129.6 (C-2′), 129.6 (C-6′), 115.6 (C-3′), 115.6 (C-5′), 160.1 (C-4′), 55.9 (OMe); 3-*O*-β-glucopyranoside: δ101.1* (C-1), 74.4** (C-2), 76.6 (C-3), 70.1*** (C-4), 77.6 (C-5), 61.3 (C-6); β-glucopyranoside in 7-*O*-rutinoside: δ101.2* (C-1), 74.2** (C-2), 76.6 (C-3), 70.1*** (C-4), 76.6 (C-5), 66.1 (C-6); α-rhamnopyranosyl in 7-*O*-rutinoside: δ101.6* (C-1), 70.6*** (C-2), 70.8*** (C-3), 72.3 (C-4), 69.1 (C-5), 17.7 (C-6). Assignments bearing the same subscript may be reversed.

Lueenin III (**3**). R_f-values: 0.12 (H_2O), 0.44 (HOAc), 0.61 (HOAc*), 0.17 (BAW). MW = 580, FABMS ($[M]^+$ 580). UV λ_{max}^{MeOH} nm: 258*, 272, 349; + NaOAc 265*, 275, 370;

$NaOAc–H_3BO_3$ 265, 375; $+AlCl_3$ 278, 305*, 330, 420; +NaOMe 270, 285*, 330, 410. **3** on reflux with 1.5 N HCl methanolic, (100°, 7 hr) yielded its isomer lucenin I: R_f-values: 0.08 (H_2O), 0.40 (HOAc), 0.58 (HOAc), 0.13 (BAW). ^1H NMR of **3**: aglycone moiety: δ6.48 (*s*, H-3), 6.88 (*d*, J = 8 Hz, H-5'), 7.52–7.64 (*m*, H-2' and H-6'); sugar moieties: δ4.96 (*d*, J = 9 Hz, 6-glucosyl H-1), 5.04 (*d*, J = 8 Hz, 8-xylosyl H-1), 3.44–3.64 (*m*, ten sugar protons).

Kaempferol 3-rhamnoside (**4**) and quercetin 3-rhamnoside (**5**) were identified by standard procedures (R_f-values, UV, acid hydrolysis and ^1H NMR). MW of **4** = 432, FABMS (MH^+: 433) and of **5** = 448, FABMS (MH^+: 449), respectively.

Herbacetin 7-glucoside (**6**). R_f-values: 0.15 (H_2O), 0.12 (HOAc), 0.48 (HOAc*), 0.45 (BAW). MW = 464, FABMS (MH^+: 465). UV λ_{max}^{MeOH} nm: 278, 332, 382; +NaOAc 278, 330, 380; $+NaOAc–H_3BO_3$ 278, 330, 380; $+AlCl_3$ 265, 285*, 370, 445; +NaOMe 255*, 295*, 370 (decomp.). Acid hydrolysis (1.5 N HCl, aq., 100°, 2 hr) of **6** afforded glucose (co-PC) and herbacetin: R_f-values: 0.00 (H_2O), 0.40 (HOAc*), 0.62 (BAW), 0.44 (Forestal). UV λ_{max}^{MeOH} nm: 255*, 276, 300*, 310*, 335*, 380; +NaOAc 256*, 278, 300*, 330*, 375; $+NaOAc–H_3BO_3$ 255*, 285, 320*, 375; $+AlCl_3$ 265*, 280, 370, 420*, 448; +NaOMe (immediate decomp.). ^1H NMR of herbacetin: δ6.28 (*s*, H-6), 7 (*d*, J = 8 Hz, H-3' and H-5'), 8.2 (*d*, J = 8 Hz, H-2' and H-6'). ^1H NMR of **6**, aglucone moiety: δ6.64 (*s*, H-6), 7 (*d*, J = 8 Hz, H-3' and H-5'), 8.2 (*d*, J = 8 Hz, H-2'and H-6'); sugar moiety: δ4.96 (*d*, J = 9 Hz, glucose H-1), 3.4–4.0 (*m*, six glucose protons overlapped with a water signal). ^{13}C NMR of **6**, aglucone: δ147.8 (C-2), 136.2 (C-3), 176.7 (C-4), 151.8 (C-5), 99.0 (C-6), 150.7 (C-7), 127.9 (C-8), 144.1 (C-9), 105.1 (C-10), 122.3 (C-1'), 130.1 (C-2'), 130.1 (C-6'), 115.9 (C-3', and C-5'), 159.7 (C-4'); 7-*O*-β-glucopyranoside: δ101.7 (C-1), 73.5 (C-2), 76.0 (C-3), 70.1 (C-4), 76.9 (C-5), 61.1 (C-6).

Herbacetin 7-O-(6''-quinylglucoside) (**7**). R_f-values: 0.16 (H_2O), 0.24 (HOAc), 0.64 (HOAc*), 0.58 (BAW). MW = 638, FABMS (MH^+: 639). UV λ_{max}^{MeOH} nm: 277, 305*, 332, 382; +NaOAc 277, 330, 380; $+NaOAc–H_3BO_3$ 280, 315, 375; $+AlCl_3$ 282, 318, 368, 445; +NaOMe (immediate decomp.). Compound **7** on acid hydrolysis yielded glucose, herbacetin and quinic acid (co-PC). ^1H NMR of **7**, aglycone moiety: δ6.56 (*s*, H-6), 6.92 (*d*, J = 8 Hz, H-3' and H-5'), 8.08 (*d*, J = 8 Hz, H-2' and H-6'); sugar moiety: δ4.76 (*d*, J = 9 Hz, glucose H-1), 3.3–3.85 (*m*, six glucose and two quinyl protons); quinyl moiety: δ4.88 (*m*, H-4'''), 1.8 (*m*, 2'''-H_2 and 6'''-H_2). ^{13}C NMR of **7**, aglycone: δ147.7 (C-2), 135.9 (C-3), 176.6 (C-4), 151.8 (C-5), 98.0 (C-6), 150.4 (C-7), 127.0 (C-8), 143.9 (C-9), 104.9 (C-10), 122.0 (C-1'), 130.0 (C-2'), 130.0 (C-6'), 115.8 (C-3'), 115.8 (C-5'), 159.6 (C-4'); glucose moiety: δ101.2 (C-1), 74.0 (C-2), 76.3 (C-3), 70.2 (C-4), 74.0 (C-5), 63.3 (C-6); quinyl moiety: δ74.8 (C-1), 69.3 (C-3), 69.3 (C-5), 75.7 (C-4). The signals of C-2 and C-6 are hidden by the DMSO-d_6 signals.

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