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NOTE

Isoflavones and a Phenylethanoid from *Verbascum sinaiticum*

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Four isoflavone glycosides, orobol, orobol 7-0-β-D-glucoside and two new glycosides of known aglycones, namely, 5,3′,4′-trihydroxy-8-methyl-isoflavone 7-0-β-D-glucoside, 5-hydroxy-3′,4′-dimethoxyisoflavone 7-0-α-L-rhamnoside were isolated and identified from the aerial parts of *Verbascum sinaiticum*. In addition, the phenylethanoid glycoside, acteoside, was also detected. The structures of all compounds were established by chromatographic and spectroscopic methods. This is the first report of isoflavones from *Verbascum sinaiticum*.

Extracts of *Verbascum* species have been used as expectorants[1] as well as for their antiviral[2], aphrodiasic and narcotic[3] properties. The following compounds have been reported previously from the leaves of *V. sinaiticum*, flavonolignans, hydrocarpin, sinaiticin, chrysoeriol and luteolin, all of which exhibited does-dependent cytotoxicity when tested against cultured P-388 cells[4]. From *V. sinaiticum* Benth. Fam. *Scrophulariaceae* we report here four isoflavone glycosides, orobol (**1**), orobol 7-0-β-D-glucoside (**2**), and two new glycosides of known isoflavone aglycones, 5,3′,4′-trihydroxy-8-methylisoflavone 7-0-β-D-glucoside (**3**) and 5-hydroxy-3′,4′-dimethoxyisoflavone 7-0-α-L-rhamnoside (**4**). In addition, the phenylethanoid glycoside, acteoside (**5**), was identified.

The dried aerial parts of workup of *Verbascum sinaiticum* extracts afforded **1–5**. The purple color of **3** under UV light with no color change with NH_3 suggested an isoflavone with a free 5-hydroxyl group[5]. The ^1H NMR spectrum of **3** showed one-proton signal at δ5.1 typical for the anomeric proton of an isoflavone 7-0-glucoside, and it also exhibited a signal at δ2.43 for an aromatic methyl group; finally the remaining non-sugar signals were typical for H-6 in an A-ring and H-2′,5′ and 6′ in a B-ring in an isoflavone. The ^{13}C NMR signals for the C-8 and C-7 at δ103.6 and δ162.3 respectively, confirmed the presence of a methyl group at C-8 and 0-glucosyl moiety at C-7[6,7]. Therefore **3** is 5,3′,4′-trihydroxy-8-methylisoflavone 7-0-β-D-glucoside. Compound **4** exhibited a free 5-hydroxyl group on the basis of color UV light, and the ^{13}C NMR showed signals at δ56.2, δ56.4 which could be assigned to two methoxyl groups. The ^1H and ^{13}C NMR spectra had signals at δ5.17, δ1.1 and δ17.9 respectively, for the anomeric proton and methyl group of rhamnose. The signals for H-6, 8, 2′, 5′ and 6′ in the isoflavone were also present[8]. Therefore **4** is 5-hydroxy-3′, 4′-dimethoxyisoflavone 7-0-α-L-

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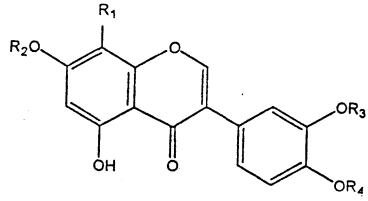
rhamnoside. The ^1H and ^{13}C NMR spectra for orobol, orobol 7-0-β-D-glucoside and acteoside were in accord with reported data[9–11].

Verbascum sinaiticum, obtained from South Sinai, Egypt, in May 1998, was



1. $R_1 = R_2 = R_3 = R_4 = H$
2. $R_1 = R_3 = R_4 = H$, R_2 = glc
3. $R_3 = R_4 = H$, $R_1 = CH_3$, R_2 = glc
4. $R_1 = H$, R_2 = rha, $R_3 = R_4 = CH_3$

identified by Dr. M. El-Gebally, Department of Taxonomy and Flora, N.R.C., Cairo, Egypt, where a voucher specimen is deposited.

^1H NMR spectra were recorded at 500 MHz, ^{13}C NMR spectra at 100 MHz and chemical shifts are given in the δ(ppm) scale with TMS as an internal standard. UV spectra were measured with a Shimadzu 1601 UV-visible spectrophotometer.

Isolation: Dried aerial parts of *Verbascum sinaiticum* were extracted with 70% aqueous ethanol. The extract was concentrated and re-extracted with ethyl acetate; next, the solvent was removed under reduced pressure. The resulting residue was chromatographed on a silica gel column with chloroform and methanol. Further isolations utilized a polyamide column eluted with water and then with an aqueous methanol mixture. Fractions were purified by TLC on silica gel plates with ethyl acetate-methanol-water (60 : 16.5 : 13.5) to yield orobol, orobol 7-0-β-D-glucoside, 5,3′,4′-trihydroxy-8-methylisoflavone 7-0-β-D-glucoside and 5-hydroxy-3′,4′-dimethoxyisoflavone 7-0-α-L-rhamnoside. Acteoside was purified by TLC on cellulose plates with ethyl acetate-methanol-water (100 : 16.5 : 13.5).

Spectral data for 1: ^1H NMR (CD_3OD): δ8.04 (1H, S, H-2), 7.01 (1H, d, J = 2 Hz, H-2′), 6.87 (1H, dd, J = 2, 8.5 Hz, H-6′), 6.67 (1H, d, J = 8.5 Hz, H-5′), 6.6 (1H, d, J = 2 Hz, H-8), 6.3 (1H, d, J = 2 Hz, H-6). ^{13}C NMR (CD_3OD) : δ180.2 (C-4), 162.1 (C-5), 160.8 (C-7), 156.8 (C-9), 153.6 (C-2), 146.6 (C-4′), 146.1 (C-3′), 122.7 (C-3), 121.8 (C-1′), 119.2 (C-6′), 116.2 (C-2′), 114.8 (C-5′), 104.2 (C-10), 98.6 (C-6), 93.8 (C-8).

Spectral data for 2: ^1H NMR (CD_3OD) : δ8.1 (1H, s, H-2), 7.03 (1H, d, J = 2 Hz, H-2′), 6.82 (1H, dd, J = 2, 8.5 Hz, H-6′), 6.79 (1H, d, J = 8. 5 Hz, H-5′), 6.75 (1H, d, J = 2 Hz, H-8), 6.51 (1H, d, J = 2 Hz, H-6), 5.08 (1H, d, J = 8Hz, H-1, glc). ^{13}C NMR (CD_3OD) : δ180.4 (C-4), 163.6 (C-7), 162.9 (C-5), 157.1 (C-9), 154 (C-2), 146.1 (C-4′), 145.7 (C-3′), 122.4 (C-3), 121.4 (C-1′), 119.9 (C-6′), 116.5 (C-2′), 115.4 (C-5′), 106 (C-10), 99.8 (C-1, glc), 99.5 (C-6), 94.6 (C-8), 77.9 (C-3, glc), 77.4 (C-5, glc), 73,5 (C-2, glc), 71.8 (C-4, glc), 62.3 (C-6, glc). Acidic hydrolysis of **2** in 7% H_2SO_4- EtOH gave compound **1** and glucose by TLC and PC.

Spectral data for 3: UV λ_{max} $MeOH_{nm}$ (log ε): 259, 288 sh, 329 sh; +$AlCl_3$: 272, 296 sh, 342 sh; +$AlCl_3$/HCl: 270, 340. ^1H NMR (CD_3OD): δ 8.09 (1H, s, H-2) 7.02 (1H, d, J = 2 Hz, H-2′), 6.99 (1H, dd, J = 2, 8.5 Hz, H-6′), 6.94

(1H, d, J = 8.5 Hz, H-5′), 6.44 (1H, s, H-6), 5.1 (1H, d, J = 8 Hz, H-1, glc), 2.43 (3H, s, Me-8). ^{13}C NMR (CD_3OD): δ 180.4 (C-4), 162.3 (C-7), 161.8 (C-5), 157.8 (C-9), 154.1 (C-2), 146.2 (C-4′), 145.9 (C-3′), 122.5 (C-3), 121.6 (C-1′). 119.6 (C-6′), 116.4 (C-5′), 106.3 (C-10), 103.6 (C-8), 99.9 (C-1, glc), 98.9 (C-6), 78.3 (C-3, glc), 78.09 (C-5, glc), 73.9 (C-2, glc), 71.8 (C-4, glc), 62.6 (C-6, glc), 21.7 (Me-8).

Spectral data for 4: UV λ_{max} $MeOH_{nm}$ (log ε): 262, 303 sh, 315 sh; +$AlCl_3$: 275, 314 sh, 332 sh; $AlCl_3$/HCl : 276, 335. ^{1}H NMR (CD_3OD) : δ 8.08 (1H, s, H-2), 7.07 (1H, d, J = 2 Hz, H-2′), 7.04 (1H, dd, J = 2, 8.5 Hz, H-6′), 6.9 (1H, d, J = 8.5 Hz, H-5′), 6.73 (1H, d, J = 2 Hz, H-8), 6.6 (1H, d, J = 2 Hz, H-6), 5.17 (1H, d, J = 2Hz, H-1, rha), 3.84 (6H, s, Me-3′, 4′), 1.1 (3H, d, J = 6 Hz, Me-rha). ^{13}C NMR (CD_3OD) : δ 180.3 (C-4), 163.5 (C-7), 162.7 (C-5), 157.2 (C-9), 153.8 (C-2), 149 (C-4′), 148.5 (C-3′), 122.7 (C-3), 121.9 (C-1′), 120 (C–6′), 112.8 (C-2′), 111.2 (C-5′), 104.9 (C-10), 100.7 (C-1, rha), 99.2 (C-6), 94.5 (C-8), 73.5 (C-4, rha), 72.3 (C-2, rha), 71 (C-3, rha), 70.09 (C-5, rha), 17.9 (Me-rha).

Spectral data for acteoside: ^{13}C NMR (CD_3OD)-aglycone moiety : δ 131.5 (C-1), 117.1 (C-2), 146.1 (C-3), 144.6 (C-4), 116.3 (C-5), 121.3 (C-6), 72.05 (C-α), 36.5 (C-β); caffeic acid moiety : δ 127.6 (C-1), 115.2 (C-2), 146.7 (C-3), 149.8 (C-4), 116.5 (C-5), 123.2 (C-6), 168.3 (C-α), 114.7 (C-β), 148.02 (C-γ); glucose moiety : δ 104.2 (C-1), 76.2 (C-2), 81.6 (C-3), 70.3 (C-4), 76 (C-5) 62.3 (C-6); rhamnose moiety: δ 103.01 (C-1), 72.05 (C-2), 72.25 (C-3), 73.8 (C-4), 70.4 (C-5), 18.4 (C-6). Acidic hydrolysis of 5 in 10% H_2SO_4-EtOH afforded caffeic acid, glucose and rhammose by TLC and PC.

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