Scropolioside-D₂ and Harpagoside-B: Two New Iridoid Glycosides from *Scrophularia deserti* and Their Antidiabetic and Antiinflammatory Activity

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Five iridoid glycosides, including the two new compounds scropolioside-D₂ (1) and harpagoside-B (2), were isolated from the aerial parts of *Scrophularia deserti* DEL (Scrophulariaceae). Their structures were elucidated on the basis of spectral data to be 6-*O*-[2'',4''-di-*O*-acetyl-3''-*O*-*trans*-cinnamoyl]- α -L-rhamnopyranosyl]-8 α -hydroxymethyl-1 α ,5 β ,6 α ,7 α ,9 β -pentahydro-7(8)-epoxy-2-oxaind-3-ene-1-*O*- β -D-glucopyranoside-6'-*O*-acetate (1) and 5-*O*- β -hydroxy-8-*O*- β -*trans*-cinnamoyl-8 α -methyl-1,6,7,9-tetrahydro-2-oxaind-3-ene-1-*O*- β -D-glucopyranoside (2), respectively. In addition, three more iridoid glycosides, scropolioside-D (3), koelzioside (4), and 8-*O*-acetyl-harpagide (5), were also isolated and characterized from this source. The biological activity and the structure activity relationship of the compounds were also studied, and scropolioside-D (3) and harpagoside-B (2) were found to possess significant antidiabetic and antiinflammatory activity, respectively.

Key words *Scrophularia deserti*; scropolioside-D₂; harpagoside-B; iridoid glycoside; antidiabetic activity; antiinflammatory activity

Scrophularia deserti DEL (Scrophulariaceae), commonly known as *afinah*, *zetaah*, *jar*, and *maseelah* in Saudi Arabia, is used as antipyretic, a remedy for kidney diseases, cardiotonic, hypoglycemic, and diuretic in typhoid fever, galactorrhoea, leucorrhoea, throat diseases, inflammation of mouth, lungs, large intestine, bladder, and heart, and as a remedy for tumors, abscesses, cancer of the lung, goiter, and aching bones.¹⁾ It has also been reported to exhibit hypoglycemic activity in normal fasting and alloxanized rats.²⁾ A literature survey revealed that no phytochemical and pharmacological work has been done so far on the plant. We report two new iridoid glycosides, scropolioside-D₂ (1) and harpagoside-B (2), along with the three other known compounds as scropolioside-D (3),³⁾ koelzioside (4),⁴⁾ and 8-*O*-acetyl-harpagide⁵⁾ from the aerial parts of the plant. The structure activity relationship (SAR) showed that scropolioside-D (3) and harpagoside-B (2) were the most active among all compounds tested for antidiabetic and antiinflammatory activity, respectively.

RESULTS AND DISCUSSION

Compound 1 called scropolioside-D₂, obtained as an amorphous powder, had the molecular composition C₃₆H₄₄O₁₈ established on the basis of high resolution (HR)-MS (*M*⁺ 764.2599), elemental analysis, ¹³C-NMR and distortionless enhancement by polarization transfer (DEPT) spectra. The IR spectrum indicated the presence of a hydroxyl group (3429 cm⁻¹), ester group (1738 cm⁻¹), double bond (1635 cm⁻¹), and oxirane ring (1203, 863, 766 cm⁻¹). The UV spectrum exhibited absorption bands at 216, 222 and 280 nm characteristic of an iridoid enol ether system and cinnamoyl chromophore.³⁾ The ¹³C-NMR and DEPT spectra⁶⁾ showed 36 carbon atoms for the molecule consisting of four methyls, two methylenes, 24 methines, and two quaternary and four carbonyl carbon atoms (in total C₃₆H₄₀). The sequential assignments of protons and carbon atoms were made with the

help of ¹H-¹H correlation spectroscopy (COSY) and ¹H-detected heteronuclear multiple quantum coherence (HMQC) experiments starting with the easily distinguishable acetal methine proton at δ 4.96 assigned to position 1 (δ _C 95.77), H-9 (δ _H 2.63, δ _C 43.66) and H-5 (δ _H 2.52, δ _C 37.56), and further correlated with the HMBC spectrum. The ¹H-NMR spectrum exhibited signals for five aromatic protons (δ _H 7.42–7.43, 7.59–7.61) and two olefinic (δ _H 6.44, d and 7.65, d, AB system, *J*_{AB} = 16.0 Hz) protons substantiating the presence of a *trans*-cinnamoyl moiety, three acetoxyl signals

- (1) Scropolioside-D₂: R₁=COCH₃, R₂=COCH₃
 (3) Scropolioside-D: R₁=H, R₂=COCH₃
 (4) Koelzioside: R₁=H, R₂=*trans*-cinnamoyl

- (2) Harpagoside-B: R₁= α H, R₂=Me, R₃=OC(=O)-C₆H₄-CH=CH₂
 (5) 8-*O*-Acetyl Harpagide: R₁= β H, R₂=H, R₃=COCH₃

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(δ_{H} 2.08, s; 2.06, s; 2.18, s; $3\times\text{Me}$; δ_{C} 173.23, 172.37, 172.12, 3 C=O) and two anomeric protons (δ_{H} 4.82, d, $J=8.0$ Hz, δ_{C} 100.29; 5.12, d, $J=1.5$ Hz, δ_{C} 98.13), indicating the presence of two sugar moieties in the molecule. The chemical shifts of proton and carbon atoms were compared with the values reported in the literature,^{7,8)} which indicated the sugars to be β -D-glucose and α -L-rhamnose, respectively. Multiplet patterns and coupling constants of anomeric protons confirmed the β -configuration of glucose and α -configuration of rhamnose.^{7,8)} The H-1 of the aglycon exhibited long-range couplings in the heteronuclear multiple bond connectivity (HMBC) spectrum with C-1' of the glucose unit; H-6 correlated with C-1'' of the rhamnose moiety, which indicated that glucose is connected with ether linkage at position 1, and rhamnose with position 6 (Fig. 1) (Table 1).

The assignments of glucosyl and rhamnosyl protons were also made with the help of ^1H - ^1H COSY experiments start-

Fig. 1. Significant Heteronuclear Multiple Bond Correlations (HMBC) for Scropolioside-D₂ (1)

Arrows point from proton to carbon.

Table 1. 1D and 2D NMR Data of Scropolioside-D₂ (1)

Positions	$^1\text{H-NMR}^a)$	$^{13}\text{C-NMR}$	DEPT ^{b)}	COSY	HMQC ^{c)}	HMBC ^{d)}		NOESY
						$^2J_{\text{CH}}$	$^3J_{\text{CH}}$	
1	4.96 d $J_{\text{ax (9)-ax (1)}}=9.5$	95.77	CH	H-9	95.77 d	C-9	C-5, C-1', C-8	H-1 α , H-10 α , H-1' α
2	—	—	—	—	—	—	—	—
3	6.43 dd (1.5, 6.5)	142.95	CH	H-4, H-5	142.95 d	C-4	C-5, C-1	H-4
4	5.13 dd (6.5, 4.5)	103.79	CH	H-3	103.79 d	C-5, C-3	C-6, C-9	H-3, H-5 β
5	2.52 m ($W_{1/2}=11.0$)	37.56	CH	H-9, H-6	37.56 d	C-6, C-4	C-1	H-9 β , H-4
6	4.03 d (8.5)	85.47	CH	H-5	85.47 d	C-7, C-5	C-1'', C-4	H-7, H-10 α
7	3.71 br s	59.80	CH	—	59.80 d	C-6	C-5, C-9	H-10 α , H-4''
8	—	66.89	C	—	66.89 s	—	—	—
9	2.63 dd $J_{\text{ax (9)-ax (1)}}=9.5$ $J_{\text{ax (9)-ax (5)}}=8.0$	43.66	CH	H-1, H-5	43.66 d	C-8, C-1, C-5	C-7, C-6	H-5 β
10 α	3.79 d (13.1)	61.96	CH ₂	H-10 β	61.96 t	C-8	C-7, C-9	H-1 α , H-6 α , H-7 α
10 β	4.18 d (13.1)	—	—	H-10 α	—	C-8	C-7	H-5''
1'	4.82 d (8.0)	100.29	CH	H-2'	100.29 d	C-2'	C-1, C-3'	H-1 α , H-3', H-4'
2'	3.33 dd (8.0, 8.5)	75.16	CH	H-1', H-3'	75.16 d	C-3', C-1'	—	H-4'
3'	3.48 dd (8.5, 9.0)	77.85	CH	H-2', H-4'	77.85 d	C-4', C-2'	—	H-2'
4'	3.41 dd (9.0, 8.5)	71.74	CH	H-3', H-5'	71.74 d	C-3', C-5'	C-6'	H-1'
5'	3.52 m	76.17	CH	H-4', H-6' α , H-6' β	76.17 d	C-4'	—	—
6' α	4.27 dd (12.0, 5.5)	64.56	CH ₂	H-5', H-6' β	64.56 t	C-7', C-5'	—	—
6' β	4.46 dd (12.0, 2.0)	—	—	H-5', H-6' α	—	C-7', C-5'	C-4'	—
7'	—	173.23	C	—	173.23 s	—	—	—
8'	2.08 s	21.25	CH ₃	—	21.25 q	C-7'	—	—
1''	5.12 d (1.5)	98.13	CH	H-2''	98.13 d	C-2''	C-3'', C-5''	—
2''	5.38 dd (1.5, 4.0)	71.12	CH	H-1'', H-3''	71.12 d	C-3''	C-4'', C-9''	—
3''	5.42 dd (4.0, 9.5)	71.74	CH	H-2'', H-4''	71.74 d	C-4''	C-9''	—
4''	5.17 dd (10.0, 9.5)	72.74	CH	H-5'', H-3''	72.74 d	C-3'', C-5''	C-6'', C-2'', C-7''	—
5''	4.06 dq (6.0, 10.0)	68.50	CH	H-6''	68.50 d	C-4'', C-6''	—	—
6''	1.25 d (6.0)	18.20	CH ₃	H-5''	18.20 q	C-5''	C-4''	—
7''	—	172.37	C	—	172.37 s	—	—	—
8''	2.06 s	21.25	CH ₃	—	21.25 q	C-7''	—	—
9''	—	172.12	C	—	172.12 s	—	—	—
10''	2.18 s	21.34	CH ₃	—	21.34 q	C-9''	—	—
1'''	—	135.89	C	—	135.89 s	—	—	—
2'''	7.59 dd (2.5, 7.5)	129.87	CH	H-3'''	129.87 d	C-3'''	C-7''', C-4'''	H-7'''
3'''	7.43 dd (8.5, 8.5)	130.55	CH	H-2'''	130.55 d	C-2'''	C-1'''	H-2'''
4'''	7.42 dd (8.5, 8.5)	132.29	CH	H-3'''	132.29 d	C-5'''	C-2'''	H-6'''
5'''	7.43 dd (8.5, 8.5)	130.55	CH	H-6'''	130.55 d	C-6'''	C-1'''	H-2'''
6'''	7.61 dd (2.5, 7.5)	129.87	CH	H-5'''	129.87 d	C-5'''	C-7'''	H-7'''
7''' (β)	7.65 d (16.0)	147.85	CH	H-8'''	147.85 d	C-8'''	C-9'''	H-8'''
8''' (α)	6.46 d (16.0)	118.34	CH	H-7'''	118.34 d	C-9'''	C-3'''	H-7'''
9'''	—	167.69	C	—	167.69 s	—	—	—

a) Assignments were based on ^1H - ^1H -COSY and HMQC experiments; coupling constants in Hertz are given in parentheses; s: singlet, d: doublet, m: multiplet, t: triplet, brs: broad singlet. b) DEPT chemical shifts are presented at $\theta=3\pi/4$ when methylene groups reach negative maximum. c) C-multiplicities were established by DEPT experiment; s=C, d=CH, t=CH₂, q=CH₃. d) The correlations in HMBC are shown from protons to carbons.

ing with their anomeric protons (δ_{H} 4.82, d, $J=8.0$ Hz, glucosyl H-1; δ_{H} 5.12, d, $J=1.5$ Hz, rhamnosyl H-1) and methylene protons (δ_{H} 4.27, dd, $J=12.0, 5.5$ Hz, H-6' H-6' α ; δ_{H} 4.46, dd, $J=12.0, 2.0$ Hz, H-6' β) in the case of glucose, and methyl protons (δ_{H} 1.25, d, $J=6.0$ Hz, Me-6'') in the case of rhamnose. The long-range couplings in the HMBC spectrum also confirmed the above assignments. H-1'' showed a correlation with C-2'', C-3'', and C-5''; H-2'' with C-3'', C-4'', and C-9''; H-3'' with C-4'' and C-9''; H-4'' with C-3'', C-5'', C-6'', C-2'', and C-7''; H-5'' with C-4'' and C-6''; whereas C-6'' (Me-6'') exhibited a correlation with C-5'' and C-4'', confirming the assigned positions of the rhamnosyl protons. The methylene protons at C-6' in the HMBC spectrum correlated with C-5' (δ_{C} 76.17) and one carbonyl carbon (δ_{C} 173.23, C-7'), indicating one acetoxyl group at the C-6' position of glucosyl; the H-2'' (δ_{H} 5.38) correlated with another carbonyl carbon (δ_{C} 172.12, C-9'') indicating another acetoxyl group at the C-2'' position of rhamnosyl; and H-4'' (δ_{H} 5.17) correlated with a third carbonyl carbon (δ_{C} 172.37), confirming the placement of the third acetoxyl group at the C-4'' position of the rhamnosyl moiety. The protons of three methyl groups of each acetoxyl unit also correlated with their respective carbonyl carbons, supporting the above assignments. The $-\text{CH}_2-$ protons at position 6' was shifted downfield to δ_{H} 4.27 and 4.46 from 3.62 and 3.92 (as appeared in scropolioside D),³⁾ which also substantiated the presence of the first acetoxyl group at the C-6' position of the glucosyl moiety in compound **1**. The H-3'' (δ_{H} 5.42) of rhamnose exhibited long-range coupling with the carbonyl carbon (167.69) at position 9'', indicating the linkage of the cinnamoyl moiety with C-3'' of the rhamnose unit (Fig. 1, Table 1). The location of two acetoxyl groups and cinnamoyl moiety were also confirmed by the downfield shift to H-2'' (δ_{H} 5.38), H-4'' (5.17), and H-3'' (5.42) of the rhamnose sugar from 4.08, 3.56, and 4.12, respectively⁹⁾ as also observed in scropolioside-A isolated from *Scrophularia scopolii*.¹⁰⁾

The spectral data of compound **1** were also compared with those of scropolioside-D, an iridoid glucoside, isolated from *Scrophularia ilwensis*,³⁾ which showed a close resemblance, with an additional acetoxyl group at position 6' of the glucose moiety. The COSY spectrum showed correlation of H-5 β (δ_{H} 2.52, m, $W_{1/2}=11.0$ Hz; δ_{C} 37.56) with H-6 α (4.03, d, $J=8.5$ Hz, 85.47) and H-9 β (2.63, dd, $J=9.5, 8.0$ Hz, 43.66), whereas H-9 β was correlated with H-6 α and H-1 α (4.96, d, $J=9.5$ Hz, 95.77). The positions of these protons were also distinguished with the help of long-range correlations in the HMBC spectrum. The nuclear Overhauser effect spectroscopy (NOESY) spectrum exhibited a correlation between H-5 β and H-9 β , and between H-1 α , H-1' α , H-10 α , H-7 α and H-6 α , but there was no correlation of H-5 β and H-9 β with H-1 α , H-1' α , H-10 α , H-7 α , and H-6 α . Other important NOESY interactions are given in Table 1. The structure of compound **1** was thus elucidated to be 6-*O*-[2'',4''-di-*O*-acetyl-3''-*O*-*trans*-cinnamoyl)- α -L-rhamnopyranosyl]-8 α -hydroxymethyl-1 α ,5 β ,6 α ,7 α ,9 β -pentahydro-7(8)-epoxy-2-oxa-3-ene-1-*O*- β -D-glucopyranoside-6'-*O*-acetate and has been designated as scropolioside-D₂.

Compound **2**, harpagoside-B, obtained as an amorphous powder, had the molecular composition $\text{C}_{25}\text{H}_{32}\text{O}_{11}$ established on the basis of HR-MS (M^+ 508.5257), elemental analysis, and ^{13}C -NMR and DEPT spectra. The IR spectrum

indicated the presence of a hydroxyl group (3435 cm^{-1}), ester group (1720 cm^{-1}), and double bond (1625 cm^{-1}). The UV spectrum exhibited absorption bands at 216, 222, and 278 nm characteristic of an iridoid enol ether system and cinnamoyl chromophore.³⁾ The ^{13}C -NMR and DEPT spectra⁶⁾ showed 25 carbon atoms for the molecule consisting of two methyls, two methylenes, 17 methines, and three quaternary and one carbonyl carbon atoms (in total $\text{C}_{25}\text{H}_{27}$). The sequential assignments of protons and carbon atoms were made with the help of ^1H - ^1H COSY and HMQC experiments starting with the easily distinguishable acetal methine proton at δ_{H} 6.21 assigned to position 1 (δ_{C} 95.10), and a methine proton assigned to H-9 (δ_{H} 2.97, δ_{C} 55.99), which were further correlated with the HMBC spectrum. The NMR spectrum exhibited signals for five aromatic protons and two olefinic (δ_{H} 6.53, d and 7.69, d, AB system, $J_{\text{AB}}=16.0$ Hz) protons of the *trans*-cinnamoyl moiety; one methoxyl singlet (δ_{H} 3.37, δ_{C} 50.36), one methyl group (1.55, 23.17), and one anomeric proton (4.66, d, $J=8.0$ Hz, δ_{C} 100.44) due to one sugar moiety in the molecule. The C-8 shifted to a lower field at δ_{C} 89.25, indicating that C₈-OH was involved in linkage formation with the cinnamoyl moiety. Had it not been linked with either the acetyl (as found in 8-acetyl harpagide)⁵⁾ or cinnamoyl moiety, it would have shifted to a higher field near δ_{C} 79.1, e.g., as observed in ajugol, where the acetylation of C₈-OH has caused a downfield shift in its acetate from δ_{C} 79.1 to 86.6.¹²⁾ Further, the C₁₀-Me was displaced downfield at δ_{H} 1.55 in compound **2** due to the presence of cinnamoyl unit at C-8, which is compatible with harpagoside, where the C₁₀-Me was shifted downfield from δ_{H} 1.27 to 1.45 on acetylation of C₈-OH.⁵⁾ It was therefore concluded that the cinnamoyl moiety in **2** was connected with the ester linkage at position 8. The chemical shift of proton and carbon atoms of the sugar moiety were compared with the values reported in the literature, which indicated it to be β -D-glucose.^{7,8)} Multiplet patterns and coupling constants of anomeric protons confirmed the β -configuration of glucose.^{7,8)} The H-1 of aglycon exhibited long-range coupling in the HMBC spectrum with C-1' of the glucose unit, indicating that glucose was connected with the ether linkage at position 1. The methoxyl protons exhibited a long-range coupling with C-6 in the HMBC spectrum and thus could be placed at position 6. C-6 appeared downfield at δ_{C} 78.13, indicating that it was connected to methyl group through an ether linkage. Had the methyl group been absent, C-6 would have appeared upfield near δ_{C} 77.4–76.4,¹²⁾ substantiating further the location of the methoxyl group at position 6. C-6 appeared downfield (δ_{C} 78.13), indicating that the methoxyl group was in the β -orientation as also seen in several related compounds containing the β -orientation of the methoxyl group. Had it been in the α -orientation, C-6 would have shifted upfield near δ_{C} 73.9–74.6 ppm.¹²⁾ C-1 appeared at δ_{C} 95.10 in **2**, indicating further that C₆-OMe was in the β -orientation (H-6 would thus be α). Had C₆-OMe been in the α -orientation, C-1 would have appeared downfield near δ_{C} 100.6–101.0 ppm as reported the literature.^{12–14)} Moreover, in harpagoside and harpagide, C₆-OH was found to be β -oriented, and hence C₆-OMe in compound **2** would also be β -oriented.¹¹⁾ The proton signals of 10-CH₃ showed correlations with C-9 and C-7 in the HMBC spectrum, which allowed placing the methyl group at position 8 (Fig. 2, Table 2). The 10-CH₃

group appeared at δ_C 23.17, indicating it to be in the α -orientation; as in oxygenated C-8 epimers, it appeared near δ_C 23–24 ppm (lower field). In contrast, β Me-10 is displayed further downfield (above δ_C 24.0 ppm) in C-8 oxygenated iridoid glycosides.¹² The NOESY spectrum showed a clear correlation of Me-10 with H-9 and H-1, indicating all to be α -oriented. There was no such correlation of Me-10 with H-9 and H-1 in NOESY spectra of harpagoside and harpagide, wherein H-9 is β -oriented. However, there was a correlation of Me-10 and H-1 (both α), but not with H-9. It was therefore concluded that H-9 in compound **2** was in α -orientation.

The spectral data of compound **2** were also compared with those of harpagoside, an iridoid glucoside isolated from *Scrophularia koraiensis*,¹¹ which showed a close resemblance with an additional methoxyl group at positions 6 and a different configuration of H-9 and H-1. The structure of compound **2** was thus elucidated to be 5-*O*- β -hydroxy-8-*O*- β -*trans*-cinnamoyl-8 α -methyl-1 α ,6 α ,7,9 α -tetrahydro-2-oxa-3-ene-1-*O*- β -D-glucopyranoside and has been designated as harpagoside-B.

Fig. 2. Significant HMBC Correlations of Harpagoside-B (**2**)

Table 2. 1D and 2D NMR Data of Harpagoside-B (**2**)

Positions	¹ H-NMR ^{a)}	¹³ C-NMR	DEPT ^{b)}	COSY	HMQC ^{c)}	HMBC ^{d)}		NOESY
						² J _{CH}	³ J _{CH}	
1	6.21 d <i>J</i> _{eq(9)-eq(1)} =1.0	95.10	CH	H-9	95.10 d	—	C-8, C-5, C-1', C-3	H-9 α , C-10 α (Me)
2	—	—	—	—	—	—	—	—
3	6.45 dd (1.5, 6.5)	144.43	CH	H-4	144.43 d	C-4	C-1, C-5	H-4
4	4.97 dd (1.5, 6.5)	107.44	CH	H-3	107.44 d	C-3	C-6, C-9	H-3
5	—	73.93	C	—	73.93 s	—	—	—
6	3.80 d (4.5)	78.13	CH	H-7a	78.13 d	C-5	C-9, C-4	H-7 α
7a	2.01 dd (4.5, 15.5)	46.67	CH ₂	H-7b, H-6	46.67 t	C-6, C-8	C-10 (Me), C-9, C-5	H-6
7b	2.28 d (15.0)	—	—	H-7a	—	—	—	—
8	—	89.25	C	—	89.25 s	—	—	—
9	2.97 d <i>J</i> _{eq(1)-eq(9)} =1.0	55.99	CH	H-1	55.99 d	C-1, C-5, C-8	C-4	H-1 α , C-10 α (Me)
10	1.55 s	23.17	CH ₃	—	23.17 q	C-8	C-9, C-7	H-1 α , H-9
11	3.37 s	50.36	CH ₃	—	50.36 q	—	C-6	—
1'	4.66 d (8.0)	100.44	CH	H-2'	100.44 d	—	C-1	H-3'
2'	3.27 t (8.5)	74.97	CH	H-1', H-3'	74.97 d	C-1'	C-3'	—
3'	3.46 t (9.0)	78.05	CH	H-2', H-4'	78.05 d	C-4', C-2'	—	H-4', H-1', H-5'
4'	3.36 dd (2.5, 1.5)	72.18	CH	H-3', H-5'	72.18 d	C-3', C-5'	C-6'	H-3'
5'	3.39 m	78.54	CH	H-4', H-6' α	78.54 d	C-6'	C-3'	H-3
6' α	3.75 dd (12.0, 5.5)	63.40	CH ₂	H-6 β , H-5'	63.40 d	C-5'	—	—
6' β	3.98 dd (12.0, 2.0)	—	—	H-6' α	—	—	—	—
1''	—	136.17	C	—	136.17 s	—	—	—
2''	7.60 dd (2.0, 7.0)	129.69	CH	H-3''	129.69 d	C-1''	C-4'', C-7'', C-6''	H-7''
3''	7.41 dd (6.5, 6.5)	130.49	CH	H-2'', H-4''	130.49 d	C-2''	C-1'', C-5''	—
4''	7.42 dd (6.5, 6.5)	132.0	CH	H-3'', H-5''	132.0 d	C-3'', C-5''	—	—
5''	7.42 dd (6.5, 6.5)	130.49	CH	H-4'', H-6''	130.49 d	C-6'', C-4''	C-1''	—
6''	7.60 dd (2.0, 7.0)	129.69	CH	H-5''	129.69 d	C-1''	C-2'', C-1''	H-7''
7'' β	7.70 d (16.0)	146.61	CH	H-8''	146.61 d	C-8'', C-1''	C-2'', C-6'', C-9''	H-8'', H-2'', H-6''
8'' α	6.54 d (16.0)	120.53	CH	H-7''	120.53 d	C-9''	C-1''	H-7'', H-2'', H-6''
9''	—	169.23	C	—	169.23 s	—	—	—

a) Assignments were based on ¹H–¹H- and ¹H–¹³C-COSY and HMQC experiments; coupling constants in Hertz are given in parentheses; s: singlet, d: doublet, m: multiplet, t: triplet. b) DEPT chemical shifts are presented at $\theta=3\pi/4$ when methylene groups reaches negative maximum. c) C-multiplicities were established by DEPT experiment; s=C, d=CH, t=CH₂, q=CH₃. d) The correlations in HMBC are shown from protons to carbons.

nated as harpagoside-B.

The other compounds were also characterized by spectral analysis and compared with the reported values, whereupon compound **3** was identified as 6-*O*-[(2'',4''-di-*O*-acetyl-3''-*O*-*trans*-cinnamoyl)- α -L-rhamnopyranosyl]-catalpol, designated as scropolioside-D isolated from *S. ilwensis*,³ **4** as 6-*O*-[2'',3''-dicinnamoyl-4''-acetyl- α -L-rhamnopyranosyl]-catalpol, called koelzioside isolated from *S. koelzii*,⁴ and **5** as 8-*O*-acetyl harpagide isolated from *Melittis melissophyllum*.⁵

Biological Activity The isolated iridoid glycosides were also screened for their antidiabetic, antiinflammatory and hypoglycemic activity. No significant hypoglycemic activity was observed. Antidiabetic and antiinflammatory effects were obtained in the test compounds and hence are described here.

Anti-diabetic Activity The results are presented in Table 3. The blood sugar was increased from its normal value [fasting: 76–110 mg/dl (4.22–6.11 mmol/l)] to 285–316 mg/dl in different groups of diabetic mice induced by alloxan. The iridoid glycosides decreased blood glucose levels in the range of 5.88 to 17.0% after 1 h, and 0.23 to 29.0% after 2 h with respect to their initial values at a dose level of 10 mg/kg *p.o.* Scropolioside-D (**3**) and 8-*O*-acetyl-harpagide (**5**) were found to be the most active exhibiting decrease of 31.47 and 17.0% after 1 h and 34.0 and 29.0% after 2 h, respectively. Koelzioside **4** (13.0, 14.0%) and scropolioside-D₂ (**1**) (5.88, 10.24%) displayed moderate activity, whereas

Table 3. Effect of Iridoid Glycosides of Aerial Parts of *Scrophularia deserti* on Blood Glucose Levels (mg/dl) of Alloxan-Induced Diabetic Mice

Group (n=6)	Blood glucose level				
	0 h (mg/dl)	1 h (mg/dl)	% Decrease	2 h (mg/dl)	% Decrease
Control (untreated)	76—110	76—110	—	76—110	—
Compound 1	305.66±6.69	287.66±12.19	5.88	274.33±12.99	10.24
Compound 2	293.33±8.82	294.33±6.98	—	290.33±7.86	1.02
Compound 3	316.66±13.01	217.0±11.79***	31.47	209.66±2.91***	34.0
Compound 4	285.33±10.65	248.33±11.66*	13.0	246.66±9.27*	14.0
Compound 5	313.0±13.84	259.5±10.37*	17.00	223.8±15.4***	29.0

a) Normal values on fasting: 76—110 mg/dl (4.22—6.11 mmol/l). Dose 10 mg/kg *p.o.* Values are expressed as mean±S.E.M.; **p*<0.05, ****p*<0.001 (Student's *t*-test).

Table 4. Effect of Iridoid Glycosides of Aerial Parts of *Scrophularia deserti* on Right Rat Paw Swelling Induced by Carrageenan

Group (n=6)	Dose	Carrageenan-induced edema	
		Mean increase in paw volume after 3 h	Inhibition (%)
Control (only carrageenan)	0.05 ml (1% w/v)	0.47±0.06	—
Compound 1	10 mg/kg <i>p.o.</i>	—	—
Compound 2	10 mg/kg <i>p.o.</i>	0.33±0.02 ^{NS}	30
Compound 3	10 mg/kg <i>p.o.</i>	—	—
Compound 4	10 mg/kg <i>p.o.</i>	0.35±0.04 ^{NS}	26
Compound 5	10 mg/kg <i>p.o.</i>	0.36±0.02*	23
Phenylbutazone (standard)	100 mg/kg <i>p.o.</i>	0.19±0.01***	60

Values are expressed as mean±S.E.M.; **p*<0.05, ****p*<0.001 (Student's *t*-test). NS: Not significant.

harpagaside-B (2) (1.02%) showed the least activity. The SAR was also established, and compounds containing one cinnamoyl moiety at C-3" as in scropolioside-D (3) had greater activity. The introduction of one more cinnamoyl moiety in place of the acetyl unit at C-4" as in koelzioside (4) resulted in a decrease in activity. The introduction of an acetyl group at C-6 as found in scropolioside-D₂ (1) further decreased the activity in an epoxy series. On the other hand, an acetyl group at C-8 as found in 8-*O*-acetyl harpagide (5) is necessary for significant antidiabetic activity, whereas the replacement of the acetyl group with a cinnamoyl unit as obtained in harpagoside-B lost the activity in a non-epoxy series of iridoid glycosides.

Antiinflammatory Activity The results are presented in Table 4. The iridoid glycosides decreased edema in the range of 23 to 30% at a dose of 10 mg/kg after 3 h with respect to the control group treated only with carrageenan, while the standard drug phenylbutazone showed a 60% decrease at a dose of 100 mg/kg, which indicated that the test drugs had moderate antiinflammatory activity. Harpagoside-B 2 (30% decrease) and koelzioside 4 (26% decrease) were most active. It may therefore be concluded that compounds containing a cinnamoyl moiety as found in harpagoside-B (2) at C-8, and in koelzioside (4) at C-4" in place of acetyl groups as present in 8-*O*-acetyl-harpagide (5), and in scropolioside-D (3) and scropolioside-D₂ (1) have significant activity. The introduction of a methyl group at C₆-OH might also enhance the activity. However, more examples are required to determine the detailed SAR.

MATERIALS AND METHODS

Melting points were determined on a Metler 9100 Electro thermal apparatus using the open capillary method. The IR

spectra were recorded as KBr pellets on a PYE UNICAM Spectrophotometer; UV spectra on a UV-160 IPC UV-Vis Spectrophotometer; optical rotation on a Perkin-Elmer 241 MC Polarimeter; mass spectra on a Finnegan MAT 300 mass spectrometer; ¹H- (500 MHz), ¹³C- and DEPT 90- and 135-NMR (125 MHz) and two dimensional (2D)-NMR (COSY, HMBC, HMQC, NOESY) on a Bruker DRX 500 spectrometer in MeOH-*d*₄ and DMSO-*d*₆ using trimethylsilane (TMS) as an internal standard reference, chemical shift in δ (ppm), and *J* values in Hz. Centrifugal preparative TLC was performed on a chromatotron (Harrison Research Inc., U.S.A.) using a 4-mm rotor and silica gel PF-254 with CaSO₄·1/2H₂O as an adsorbent.

Plant Material The aerial parts of *S. deserti* DEL were collected on March 30, 1988, from a region of 130 km from *Hafr-Al-Batun* to *Arar*, Saudi Arabia and identified by a taxonomist at our centre, Dr. Sultanul-Abidin. Voucher specimen no. 11905 has been deposited in the herbarium for future reference.

Extraction and Isolation Air-dried aerial parts (2.0 kg) were crushed to coarse powder and extracted with 95% alcohol (5×2.0 l) by cold percolation. The alcoholic extract was concentrated under reduced pressure to obtain a viscous mass (300 g), then extracted three times with petroleum ether (1.0 l) to give a petroleum ether extract (62.3 g) and a residue (210 g). The latter was dissolved in 5% MeOH in CH₂Cl₂ (2×1.0 l) to give CH₂Cl₂ extract (32 g) and a residue (172 g), which was dissolved in hot CH₃CN (0.5 l) and filtered to give a CH₃CN extract (30.5 g). Both CH₃CN and CH₂Cl₂ extracts were mixed together (62.5 g) and subjected to flash chromatography (1200 g silica gel 60) using CH₂Cl₂-MeOH gradient elution to afford the following fractions:

(1) Eluate (CH₂Cl₂-MeOH, 4:1, 600 mg) was rechromatographed on a chromatotron using 5% MeOH in CHCl₃

to give a mixture (135 mg), which was rechromatographed twice over Sephadex LH-20 using isocratic (CH_2Cl_2 -MeOH, 1:1) elution to give compound **1** (95 mg; R_f 0.51, 16% MeOH in CHCl_3).

(2) Eluate (CH_2Cl_2 -MeOH, 7:3, 4.0 g) was rechromatographed over Sephadex LH-20 (200 g) using isocratic (CH_2Cl_2 -MeOH, 1:1) elution to obtain a crude mass (142 mg), which was subjected to prep TLC on RPC_{18} (16% MeOH in CHCl_3), whereupon the lower band (R_f 0.27) was collected to yield compound **3** (15.1 mg).

(3) Eluate (CH_2Cl_2 -MeOH, 3:2, 1.8 g) was fractionated over Sephadex LH-20 using isocratic (CH_2Cl_2 -MeOH, 1:1) elution to obtain a subfraction (1337 mg), which was chromatographed on RPC_{18} (120 g) using 20% CH_3CN in H_2O to give fractions A and B. Fraction A (189 mg) was rechromatographed over Sephadex LH-20 to give compound **2** (135 mg; R_f 0.32, 17% MeOH in CHCl_3), while fraction B (95 mg) afforded compound **4** (38 mg; R_f 0.47, 15% MeOH in CHCl_3).

(4) Eluate (CH_2Cl_2 -MeOH, 1:1, 0.95 g) was fractionated over RPC_{18} (100 g) using gradient elution (CH_3CN - H_2O), whereupon the eluate obtained at (9:1, 120 mg) on chromatography over Sephadex LH-20 afforded a crude compound, which on washing with chloroform yielded compound **5** (25 mg; R_f 0.40, 20% MeOH in CHCl_3).

Scopolioside- D_2 (**1**): Obtained as an amorphous powder, mp 121–122 °C; $[\alpha]_D^{23}$ -30° ($c=0.2$, MeOH); UV (MeOH) λ_{max} 204, 216 (sh), 222 (sh), 280 nm; IR (KBr) ν_{max} 3429 (OH), 2925, 2850, 1718 (C=O), 1635 (C=C), 1231 (C–O, ester) 1203, 1076–1041 (C–O), 982, 863, 766, 668 cm^{-1} ; 1D and 2D NMR data (MeOH- d_4), see Table 1; FAB-HR-MS m/z 764.2599 $[\text{M}]^+$ (Calcd for $\text{C}_{36}\text{H}_{44}\text{O}_{18}$, 764.2528).

Harpagoside-B (**2**): Obtained as an amorphous powder, mp 108–109 °C; $[\alpha]_D^{23}$ -13.33° ($c=0.3$, MeOH); UV (MeOH) λ_{max} 203, 216 (sh), 222 (sh), 278 nm; IR (KBr) ν_{max} 3435 (OH), 2945, 2850, 1720 (C=O), 1625 (C=C), 1240 (C–O, ester), 1070–1050 (C–O), 980, 668 cm^{-1} ; 1D and 2D NMR data (MeOH- d_4), see Table 2; FAB-HR-MS m/z 508.1948 $[\text{M}]^+$ (Calcd for $\text{C}_{25}\text{H}_{32}\text{O}_{11}$, 508.1944).

Experimental Animals The animal experiments were performed in accordance with the ethical standards formulated in the Helsinki Declaration of 1964 (revised 2000), and guidelines issued by the Science and International Affairs Bureau of the Japanese Ministry of Education, Science, Sports and Culture. Male Swiss-Albino mice weighing 25–30 g, and Wistar rats of either sex weighing 200–250 g were used to determine the antidiabetic and antiinflammatory activity, respectively. The animals were maintained at $23 \pm 2^\circ\text{C}$ with a 12-h light and dark cycle, fed a Purina rat chow diet supplied by Grain Silos and Flour Mills Organization, Riyadh, Saudi Arabia, and had free access to food and water.

Determinations of Antidiabetic Activity Six animals were used in each group. The initial blood glucose levels were determined after 18-h fasting (blood withdrawn from retro orbital plexus), and then the animals were given a single intravenous injection of alloxan at a dose of 75 mg/kg with

freshly prepared physiological saline using the method reported by Lundquist and Rerup.¹⁵ The animals showing blood glucose levels >200 mg/dl 72 h after alloxan treatment were selected for the present study. The test compounds suspended in distilled water were given orally at a dose of 10 mg/kg. The fasting blood glucose levels were then monitored at time 0 (before drug), and 1 and 2 h along with untreated controls. The blood glucose was estimated by method of Nelson as described by Ashwell.¹⁶ The reduction in blood sugar level in comparison to that of untreated controls was taken as antidiabetic activity.

Determinations of Antiinflammatory Activity Six rats each were allotted to different treatment groups. Edema was induced in the rats by injecting carrageenan (0.05 ml, 1% w/v in normal saline) into the subplantar tissue of the right hind paw using the method of Winter *et al.*¹⁷ Paw volume (ml) was measured with a plethysmometer (7140, Ugo Basile) before carrageenan injection and 0, 1, 2, and 3 h thereafter. The edema was reported as the difference between the initial and final volume. The antiinflammatory effect was expressed as the percentage inhibition compared with vehicle-treated animals with respect to a reference group treated with phenylbutazone (100 mg/kg). The test compounds (10 mg/kg) with distilled water (0.1 ml/100 g rat) were administered orally 1 h before injection of the phlogistic agent.

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