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KARSOSIDE AND SCROPOLIOSIDE D, TWO NEW IRIDOID GLYCOSIDES FROM *SCROPHULARIA ILWENSIS*

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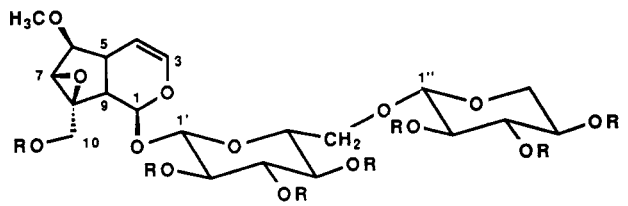
ABSTRACT.—Two new iridoid glycosides, karsoside [**1**] and scropolioside D [**2**], were isolated from the aerial parts of *Scrophularia ilwensis*. Their structures were elucidated on the basis of chemical and spectral data as 6′-*O*-(β-D-xylopyranosyl)-methylcatalpol and 6-*O*-[(2″,4″-di-*O*-acetyl-3″-*O*-*trans*-cinnamoyl)-α-L-rhamnopyranosyl]-catalpol, respectively. Additionally, four known iridoids (aucubin, harpagide, 8-*O*-acetylharpagide, and ajugol), a phenylpropanoid glycoside (angoroside C), and two flavonoids (quercetin-3-*O*-rutinoside and kaempferol-3-*O*-rutinoside) were isolated and identified.

The genus *Scrophularia* is represented by 57 species in the flora of Turkey (1). As a continuation of our systematic phytochemical studies on the aerial parts of the genus *Scrophularia* (2–5), we have investigated *Scrophularia ilwensis* C. Koch (Scrophulariaceae), which is widespread in Central and Eastern Anatolia. In a previous study (6), we reported three new oleanane-type triterpenic saponins. In this study, two new iridoid glycosides, karsoside [**1**] and scropolioside D [**2**], were isolated together with the four known iridoids aucubin, harpagide, 8-*O*-acetylharpagide, and ajugol. In additional to the iridoids, a phenylpronanoid glycoside angoroside C and two flavonoids, quercetin-3-*O*-rutinoside and kaempferol-3-*O*-rutinoside, were isolated. All the compounds isolated were identified by means of spectral (uv, ir, nmr, fabms) and chemical (acetylation, alkaline hydrolysis) evidence.



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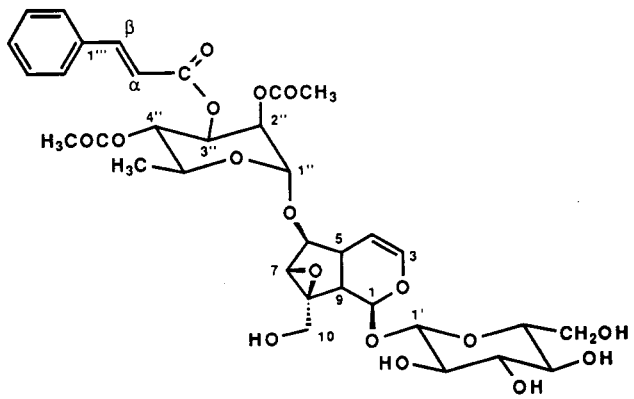
3 R=Ac



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RESULTS AND DISCUSSION

Compound **1** was obtained as an amorphous powder with the molecular formula $C_{21}H_{32}O_{14}$ (fabms *m/z* $[M + Na]^+$ 531). Its uv and ir spectra showed absorption bands characteristic of a nonconjugated iridoid enol-ether system. The ^{1}H-nmr spectrum of **1** showed a signal pattern similar to that of 6-*O*-methylcatalpol (5) except for the signals of sugar moieties (Table 1). The signals at δ 4.38 (d, $J = 6.3$ Hz) and at δ 4.83 (d, $J = 8.1$ Hz), were assigned as the anomeric protons of β-D-glucose and β-D-xylose, respectively. The ^{13}C-nmr spectral data of **1** were in good accordance with those of β-D-xylose (7) and 6-*O*-methylcatalpol (5). The resonance for C-6′ observed at δ 70.8 indicated the site of glycosidation to be on the primary hydroxyl group of glucose.

TABLE 1. ^{1}H-nmr Spectral Data of Karsoside [**1**], Heptaacetylkarsoside [**3**], and Scropolioside D [**2**].

Proton	Compound					
	1 (CD_3OD, 300 MHz)		**3**[a] ($CDCl_3$, 300 MHz)		**2** (CD_3OD, 500 MHz)	
	δ	*J* (Hz)	δ	*J* (Hz)	δ	*J* (Hz)
H-1	5.09 d	9.6	4.97–4.92[b]		5.10 d	9.7
H-3	6.37 dd	5.8/1.7	6.29 dd	6.0/1.7	6.39 dd	6.0/1.7
H-4	5.05 dd	5.8/4.7	5.05 dd	6.0/4.7	5.08 dd	6.0/4.6
H-5	2.33 m		2.43 m		2.49 m	
H-6	3.83–3.57[b]		3.71 br s		4.06 dd	8.2/1.0
H-7	3.83–3.57[b]		3.65 br s		3.67 br s	
H-9	2.63 dd	7.7/9.6	2.59 dd	7.7/9.6	2.59 dd	7.6/9.7
H_A-10	3.93–3.83[b]		4.06 d	12.6	4.15 d	13.2
H_B-10	4.25 d	12.8	4.80 d	12.6	3.80 d	13.2
OMe	3.50 s		3.50 s		—	
H-1′	4.83 d	8.1	4.80 d	8.0	4.76 d	7.9
H-2′	3.93–3.26[b]		4.97–4.92[b]		3.25 dd	7.9/9.1
H-3′	3.93–3.26[b]		5.22 t	9.4	3.40 t	9.1
H-4′	3.93–3.26[b]		4.97–4.92[b]		3.24 t	9.0
H-5′	3.93–3.26[b]		3.72 m		3.31 m	
H_A-6′	4.11 dd	11.6/1.6	3.62 br d	11.5	3.62 dd	11.9/6.7
H_B-6′	3.93–3.26[b]		3.80 br d	11.5	3.92 dd	11.9/2.1
H-1″	4.38 d	6.3	4.60 d	6.3		
H-2″	3.93–3.26[b]		4.86 dd	6.3/8.0		
H-3″	3.93–3.26[b]		5.10 t	8.0		
H-4″	3.93–3.26[b]		4.97–4.92[b]			
H_A-5″	3.93–3.26[b]		3.41 dd	12.0/8.2		
H_B-5″	3.93–3.26[b]		4.14 dd	12.0/5.0		
H-1‴					5.09 d	1.7
H-2‴					5.35 dd	1.7/3.5
H-3‴					5.38 dd	3.5/9.9
H-4‴					5.14 t	9.9
H-5‴					4.04 dq	9.9/6.3
H-6‴					1.21 d	6.3
H-2‴–H-5‴					7.39–7.78[b]	
H-α					6.43 d	16.1
H-β					7.65 d	16.1
Ac					2.03 s, 2.15 s	

[a]Compound **3** has additional acetyl signals at δ 2.12, 2.06, 2.05, 2.049, 2.034, 2.026, and 1.998 (aliphatic × 7).

[b]Signal patterns unclear due to overlapping.

Acetylation of **1** gave the heptaacetyl derivative **3**. The ^{1}H-nmr spectrum of **3** revealed the presence of seven aliphatic acetyl groups. According to the spectral data obtained, no downfield shifts occurred upon acetylation for H-6′ (δ 3.62, 3.80), which confirmed the interglycosidic linkage to be as 1″ to 6′ between xylose and glucose (Table 1). The fabms of **3** showed the mol wt at 802 (*m/z* $[M+H]^+$ 803, $[M+Na]^+$ 825). Fragmentation peaks observed at *m/z* 547 and 259 were assigned to a biglycosidic sugar moiety [hexaacetyl-xylopyranosyl-glucoseoxonium ion]$^+$ and a terminal sugar [triacetyl-xyloseoxonium ion]$^+$. In conclusion, the structure of **1** was determined to be 6′-*O*-(β-D-xylopyranosyl)-methylcatalpol, for which we propose the trivial name karsoside.

Compound **2** was obtained as an amorphous powder with the molecular formula $C_{34}H_{42}O_{17}$ (fabms *m/z* $[M+H]^+$ 723). Its uv spectrum showed absorption bands that are characteristic of an iridoid enol ether system and a cinnamoyl chromophore (205, 224, and 311 nm). The ir absorptions were in accordance with an ester as well as with a nonconjugated enol ether system. The ^{1}H-nmr spectrum of **2** (Table 1) showed a signal pattern very similar to that of scropolioside A isolated from *Scrophularia scopolii* (4,5), except for the lack of a methoxyl signal in the acyl moieties. In addition to five aromatic (δ 7.39–7.78) and two olefinic (δ 6.43 and 7.65, AB system, J_{AB} = 16.1 Hz) protons arising from the *trans*-cinnamoyl moiety, two acetoxyl signals were observed at 2.03 and 2.15 ppm, indicating the presence of two acetyl and a *trans*-cinnamoyl as acyl moieties. Two signals for anomeric protons appeared at δ 5.09 (d, J = 1.7 Hz) and 4.76 (d, J = 7.9 Hz), indicating α-L-rhamnose and β-D-glucose to be sugar moieties. The locations of the three acyl groups were deduced from the fact that the ^{1}H-nmr signals of H-2″, H-3″, and H-4″ of rhamnose were shifted downfield as in scropolioside A (δ 5.35, 5.38, and 5.14, respectively). Comparison of the ^{13}C-nmr shift values of the C atoms of the sugar moieties of **2** and scropolioside A indicated that the compounds had similar glycosidation patterns.

These results were confirmed by alkaline hydrolysis of **2**, which yielded 6-*O*-(α-L-rhamnopyranosyl)-catalpol, isolated in the same manner from scropoliosides A, B, and C (4,5). The exact distribution of the three acyl residues on the rhamnose moiety were confirmed from the results of a long range 2D-^{1}H, ^{13}C heteronuclear correlation (HMBC) measurement made with **2** (8). From this spectrum it was clear that the protons at δ 5.38 (H-3″), 5.35 (H-2″), and 5.14 (H-4″) long range coupled to the carbon signals at δ 167.0 (carbonyl carbon of *E*-cinnamoyl moiety), 171.5, and 171.3 (carbonyl carbons of acetyl groups), respectively. Based on these data, the structure of **2** was determined to be 6-*O*-[(2″,4″-di-*O*-acetyl-3″-*O*-*trans*-cinnamoyl)-α-L-rhamnopyranosyl]-catalpol.

All the spectral data (uv, ir, ^{1}H-nmr, and fabms) obtained for other compounds isolated were in good agreement with the reported data for aucubin (5), harpagide (9), 8-*O*-acetyl harpagide (5), ajugol (5), angoroside C (3), quercetin-3-*O*-rutinoside, and kaempferol-3-*O*-rutinoside (10,11).

EXPERIMENTAL

PLANT MATERIAL.—Plant material was collected from Kars, Eastern Anatolia, in the vicinity of Karahamza village on May 15, 1989. A voucher specimen is deposited in the Herbarium of Hacettepe University, Faculty of Pharmacy (HUEF 89002).

EXTRACTION AND ISOLATION.—Air-dried aerial parts of the plant (480 g) were extracted twice with MeOH at 50°. After evaporation of solvent under vacuum, the residue was suspended in H_2O and defatted with petroleum ether, and the aqueous phase was lyophilized to yield 60 g of extract. An aliquot of the extract (35 g) was chromatographed on polyamide, eluting with H_2O followed by increasing concentrations of MeOH to yield eight main fractions: A (22 g), B (1.46 g), C (1.18 g), D (0.95 g), E (0.62 g), F (1.5 g), G (0.34 g), and H (0.26 g). Fraction A (22 g), which was rich in iridoids, was subjected to Si gel cc

using $CHCl_3$-MeOH-H_2O (80:20:2) to yield six main fractions, A1–A6. Fraction A4 (360 mg) was applied to mplc with Sepralyte C-18 using an H_2O/MeOH gradient solvent system (50–80%) at a flow rate 5 ml/min. Five fractions, A4a–A4e, were obtained. Fraction A4c was pure karsoside [**1**] (42 mg). Scropolioside D [**2**] (50 mg) was obtained as an amorphous powder by rechromatographing fraction C (1.18 g) on a Si gel column using $CHCl_3$-MeOH (8:2) as eluent. Aucubin, harpagide, 8-*O*-acetyl harpagide, ajugol, angoroside C, and the two flavonoids, quercetin-3-*O*-rutinoside and kaempferol-3-*O*-rutinoside, were isolated from fractions A4a, A4b, A4d, A4e, E, and F, after a series of chromatographic procedures.

Karsoside [**1**].—Uv λ max (MeOH) 209, 237 (sh), 273, 327 nm; ir ν max (KBr) 3400 (OH), 2900 (C-H), 1630 (C=C) cm^{-1}; ^1H nmr (300 MHz, CD_3OD) see Table 1; ^{13}C nmr (75.5 MHz, CD_3OD) δ 96.3 (d, C-1), 142.2 (d, C-3), 104.8 (d, C-4), 37.5 (d, C-5), 88.1 (d, C-6), 60.8 (d, C-7), 67.7 (s, C-8), 43.1 (d, C-9), 61.7 (t, C-10), 59.0 (q, OMe), 100.1 (d, C-1′), 74.5 (d, C-2′), 77.4 (d, C-3′), 71.2 (d, C-4′), 76.8 (d, C-5′), 70.8 (t, C-6′), 105.6 (d, C-1″), 74.3 (d, C-2″), 77.2 (d, C-3″), 70.8 (d, C-4″), 66.7 (t, C-5″); fabms (noba) *m/z* $[M+Na]^+$ 531 (calcd for $C_{21}H_{32}O_{14}$, 508).

Acetylation of **1**.—Treatment of **1** (5 mg) with Ac_2O (0.5 ml) and pyridine (1.0 ml) at room temperature overnight followed by cc over Si gel C_6H_6-Me_2CO (4:1) gave the heptaacetate derivative **3**. Uv λ max (MeOH) 207 nm; ir ν max (KBr) 1715 (C=C), 1640 (C=C) cm^{-1}; ^1H nmr (300 MHz, CD_3OD) see Table 1; fabms (noba) *m/z* $[M+H]^+$ 803 (calcd for $C_{35}H_{53}O_{21}$, 802), $[M+Na]^+$ 825, [hexaacetyl-xylopyranosyl-glucose-oxonium ion]$^+$ 547, [triacetyl-xyloseoxonium ion]$^+$ 259.

Scropolioside D [**2**].—Uv λ max (MeOH) 205, 224, 311 nm; ir ν max (KBr) 3400 (O-H), 1715 (C=O), 1640 (C=C) cm^{-1}; ^1H nmr (500 MHz, CD_3OD), see Table 1; ^{13}C nmr (125 MHz, CD_3OD) δ 94.5 (d, C-1), 142.2 (d, C-3), 102.9 (d, C-4), 36.8 (d, C-5), 84.4 (d, C-6), 59.1 (d, C-7), 66.3 (s, C-8), 42.9 (d, C-9), 61.1 (t, C-10), 99.4 (d, C-1′), 74.3 (d, C-2′), 77.2 (d, C-3′), 72.0 (d, C-4′), 78.1 (d, C-5′), 62.5 (t, C-6′), 97.2 (d, C-1″), 71.0 (d, C-2″), 70.3 (d, C-3″), 71.3 (d, C-4″), 69.0 (d, C-5″), 17.7 (q, C-6″), 135.0 (s, C-1‴), 129.7 (d, C-2‴ and C-6‴), 129.1 (d, C-3‴ and C-5‴), 131.5 (d, C-4‴), 117.5 (d, C-α), 147.2 (d, C-β), 167.0 (s, CO), 171.5 and 171.3 (each s, *C*OMe), 20.9 (q, CO*Me* × 2); fabms *m/z* $[M+Na]^+$ 745 (calcd for $C_{34}H_{42}O_{17}$, 722), [diacetyl-cinnamoyl-rhamnoseoxonium ion]$^+$ 361, [cinnamoyl]$^+$ 131, [cinnamic acid]$^+$ 149.

Alkaline hydrolysis of **2**.—A solution of **2** (10 mg) in 5% methanolic KOH (2 ml) was kept at room temperature for 2 h. The mixture was neutralized with 1M HCl and filtered. The filtrate was evaporated to dryness in vacuo, and the residue, 6-*O*-(α-L-rhamnopyranosyl)-catalpol, was identified by comparing with authentic samples in tlc.

LITERATURE CITED

1. P.H. Davis, "Flora of Turkey and East Aegean Islands," University Press, Cambridge, 1978, Vol. 6, p. 458.
2. I. Çalış, G.-A. Gross, and O. Sticher, *Phytochemistry*, **26**, 2057 (1987).
3. I. Çalış, G.-A. Gross, and O. Sticher, *Phytochemistry*, **27**, 1465 (1988).
4. I. Çalış, G.-A. Gross, T. Winkler, and O. Sticher, *Planta Med.*, **54**, 168 (1987).
5. I. Çalış and M. Özipek, *Doğa-Turkish J. Pharmacy*, **2**, 25 (1992).
6. I. Çalış, M. Zor, A.D. Wright, and O. Sticher, *Planta Med.*, **57** (Suppl. 2), A68 (1992).
7. I. Çalış, A.A. Başaran, I. Saracoğlu, O. Sticher, and P. Rüedi, *Phytochemistry*, **30**, 3073 (1991).
8. M.F. Summers, L.G. Marzilli, and A. Bax, *J. Am. Chem. Soc.*, **108**, 4285 (1985).
9. A. Lenherr, "Biosystematische und Chemotaxonomische Untersuchungen In Der Artengruppe *Stachys recta* L." Ph.D. Thesis, Eidgenössischen Technischen Hochschule, Zürich, ETH, No. 7453, 1983, p. 76.
10. T.J. Mabry, K.K. Markham, and M.B. Thomas, "The Systematic Identification of Flavonoids," Springer Verlag, Heidelberg, 1970, p. 296.
11. J.B. Harborne and T.J. Mabry, "The Flavonoids: Advances in Research," Chapman and Hall, London, 1982, pp. 83, 90.

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