84. Ilwensisaponins A, B, C, and D: Triterpene Saponins from *Scrophularia ilwensis* [1])

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From the aerial parts of *Scrophularia ilwensis*, four new triterpene saponins, ilwensisaponins A–D (**1–4**) were isolated. The structures of the compounds were elucidated using chemical and spectral data as 13β,28-epoxy-3-β-{{[β-D-glucopyranosyl-(1→2)]-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)]-β-D-fucopyranosyl}-oxy}olean-11-en-23-ol (**1**), 3-β-{{[β-D-glucopyranosyl-(1→2)]-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)]-β-D-fucopyranosyl}oxy}oleana-11,13(18)-diene-23,28-diol (**2**), 3-β-{{[β-D-glucopyranosyl-(1→2)]-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)]-β-D-fucopyranosyl}oxy}-11α-methoxyolean-12-ene-23,28-diol (**3**), and 3-β-{{[β-D-glucopyranosyl-(1→2)]-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)]-β-D-fucopyranosyl}oxy}olean-12-ene-11α,23,28-triol (**4**).

1. Introduction. – In previous papers, we reported the isolation and structure elucidation of phenylpropanoid glycosides [2] and iridoid glycosides [3] from *Scrophularia scopolii*. As a continuation of our investigations of the glycosidic constituents of plants belonging to the genus *Scrophularia*, we recently reported two new iridoid glycosides, karsoside (6′-*O*-[β-D-xylopyranosyl]methylcatalpol) and scropolioside D (6-*O*-{(2″,4″-di-*O*-acetyl-3″-*O*-[(*E*)-cinnamoyl]-α-L-rhamnopyranosyl}catalpol) [4]. We now report the isolation and structure elucidation of four new triterpene saponins, called ilwensisaponins A–D (**1–4**), from the aerial parts of *Scrophularia ilwensis* C. KOCH.

2. Results and Discussion. – The H_2O-soluble part of the MeOH extract of the aerial parts of *S. ilwensis*, on fractionation over polyamide, yielded eight fractions. Fractions rich in saponins were further subjected to MPLC (see *Exper. Part*) and yielded ilwensisaponins A–D (**1–4**).

The ^{1}H-NMR data of **1–4** suggested that they all had similar structures, containing the same sugar moiety but differing in their aglycones. All yielded glucose, rhamnose, and fucose upon acid hydrolysis. The molar ratio of the sugars from each compound was 2:1:1, as determined by ^{1}H- and ^{13}C-NMR analysis (see *Exper. Part*, *Tables 1* and *2*).

Ilwensisaponin A (**1**) was obtained as an amorphous compound with the molecular weight 1072 (FAB-MS: *m/z* 1095 ([*M* + Na]$^+$)), as calculated for $C_{54}H_{88}O_{21}$. Upon acetylation, **1** yielded a dodecaacetate **1a** (FAB-MS: *m/z* 1576 ([*M*]$^+$), 1599 ([*M* + Na]$^+$), calc.

[1]) This study was first partly presented at the '39th Annual Congress on Medicinal Plant Research', Saarbrücken, Germany, September 3–7, 1991; see [1].

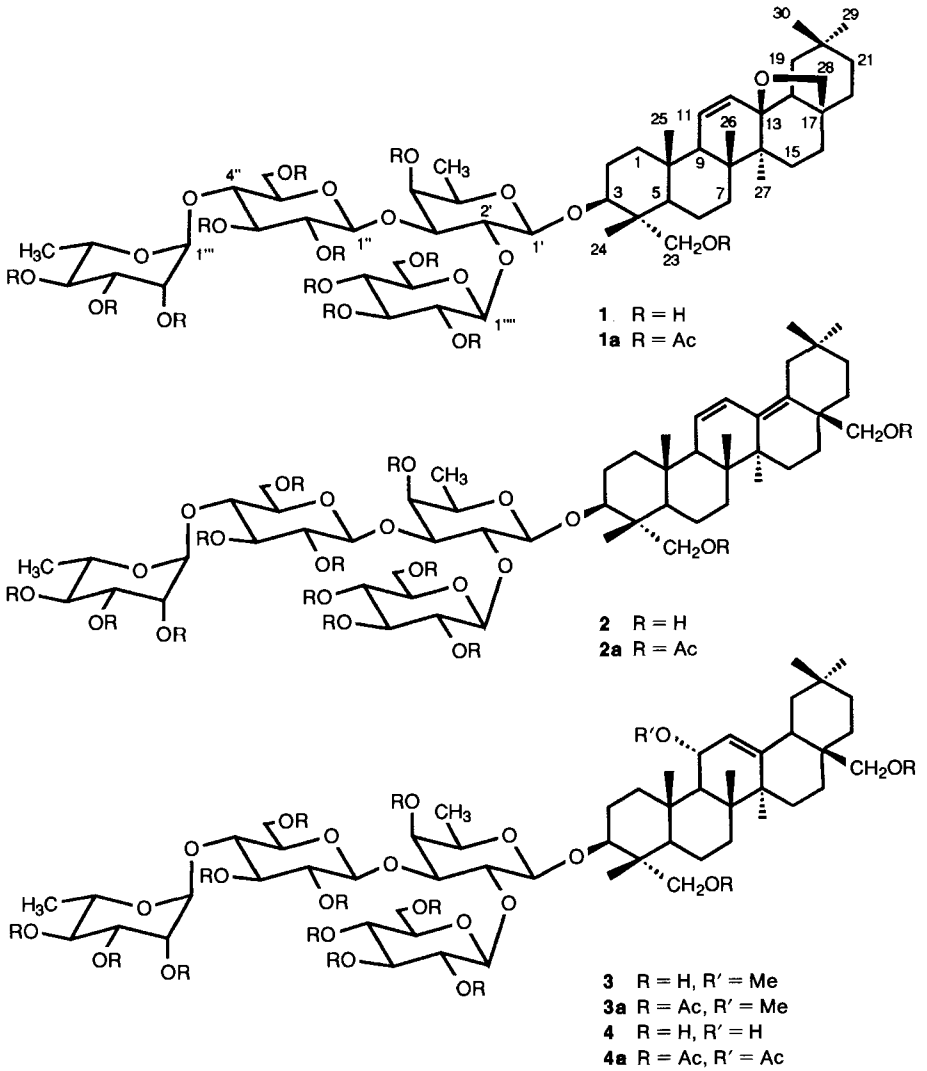


Table 1. *^{13}C-NMR Data* (75.5 MHz) *of Ilwensisaponins A* (**1**), *B* (**2**), *C* (**3**), *and D* (**4**) (CD_3OD) *and of Their Acetylated Derivatives* **1a**, **2a**, *and* **3a** ($CDCl_3$)

	1	1a	2	2a	3	3a	4
C(1)	39.3 (*t*)	37.8	39.1 (*t*)	37.6	40.8 (*t*)	39.1	41.3 (*t*)
C(2)	26.3 (*t*)	25.2	24.8 (*t*)	24.1	26.8 (*t*)	25.5	26.4 (*t*)
C(3)	85.6 (*d*)	83.7	85.7 (*d*)	83.7	85.6 (*d*)	83.6	85.4 (*d*)
C(4)	42.7 (*s*)	41.5	43.4 (*s*)	41.8	44.5 (*s*)	37.6	44.6 (*s*)
C(5)	48.0 (*d*)	47.5	48.2 (*d*)	47.5	48.8 (*d*)	47.6	48.1 (*d*)
C(6)	18.2 (*t*)	17.2	18.9 (*t*)	17.9	18.9 (*t*)	17.9	18.7 (*t*)
C(7)	32.0 (*t*)	31.1	33.0 (*t*)	32.1	33.8 (*t*)	32.8	33.7 (*t*)
C(8)	42.8 (*s*)	41.8	40.8 (*s*)	37.9	42.9 (*s*)	41.6	42.9 (*s*)
C(9)	54.6 (*d*)	53.3	55.7 (*d*)	54.3	53.4 (*d*)	52.2	55.9 (*d*)

Table 1 (cont.)

	1	1a	2	2a	3	3a	4
C(10)	37.1 (*s*)	35.9	37.3 (*s*)	36.2	38.0 (*s*)	35.6	38.0 (*s*)
C(11)	131.4 (*d*)	130.8	127.2 (*d*)	126.5	77.6 (*d*)	75.9	68.0 (*d*)
C(12)	134.0 (*d*)	132.0	126.5 (*d*)	125.4	122.9 (*d*)	122.3	127.4 (*d*)
C(13)	86.8 (*s*)	84.7	137.8 (*s*)	137.0	150.6 (*s*)	148.1	148.0 (*s*)
C(14)	44.4 (*s*)	41.9	41.4 (*s*)	40.3	44.4 (*s*)	43.0	44.5 (*s*)
C(15)	35.8 (*t*)	34.8	33.0 (*t*)	33.0	26.7 (*t*)	25.4	26.7 (*t*)
C(16)	26.5 (*t*)	25.6	26.3 (*t*)	25.1	22.8 (*t*)	22.8	22.6 (*t*)
C(17)	45.1 (*s*)	43.7	44.4 (*s*)	42.1	38.8 (*s*)	42.06	38.8 (*s*)
C(18)	52.5 (*d*)	51.0	135.9 (*s*)	133.2	43.4 (*d*)	42.1	42.9 (*d*)
C(19)	38.2 (*t*)	37.1	39.1 (*t*)	38.2	47.7 (*t*)	45.9	47.0 (*t*)
C(20)	32.8 (*s*)	31.6	33.7 (*s*)	32.9	31.9 (*s*)	30.9	31.8 (*s*)
C(21)	35.8 (*t*)	34.8	36.0 (*t*)	34.9	35.3 (*t*)	33.9	35.0 (*t*)
C(22)	31.8 (*t*)	30.8	29.7 (*t*)	30.0	32.2 (*t*)	31.2	32.0 (*t*)
C(23)	64.4 (*t*)	65.3	63.7 (*t*)	65.4	64.7 (*t*)	65.2	64.5 (*t*)
C(24)	12.6 (*q*)	12.3	12.7 (*q*)	12.4	13.2 (*q*)	12.8	12.9 (*q*)
C(25)	18.8 (*q*)	18.1	18.9 (*q*)	18.3	16.9 (*q*)	17.2	16.6 (*q*)
C(26)	20.1 (*q*)	19.3	21.0 (*q*)	20.2	18.8 (*q*)	18.1	18.3 (*q*)
C(27)	16.9 (*q*)	19.5	16.9 (*q*)	16.6	25.7 (*q*)	25.1	25.8 (*q*)
C(28)	77.9 (*t*)	76.9	64.5 (*t*)	65.8	69.8 (*t*)	70.6	69.5 (*t*)
C(29)	34.0 (*q*)	33.6	32.8 (*q*)	32.2	33.7 (*q*)	33.0	33.4 (*q*)
C(30)	23.9 (*q*)	23.5	24.8 (*q*)	24.3	24.0 (*q*)	23.5	23.7 (*q*)
MeO	–	–	–	–	54.3 (*q*)	54.2	–
Fucose							
C(1′)	105.1 (*d*)	103.3	105.1 (*d*)	103.3	105.1 (*d*)	103.3	104.8 (*d*)
C(2′)	78.3 (*d*)	74.8	78.3 (*d*)	74.8	78.3 (*d*)	74.8	78.0 (*d*)
C(3′)	84.3 (*d*)	79.3	84.4 (*d*)	79.3	84.3 (*d*)	79.3	84.3 (*d*)
C(4′)	72.4 (*d*)	73.0	72.4 (*d*)	73.0	72.4 (*d*)	73.0	72.1 (*d*)
C(5′)	71.3 (*d*)	69.0	71.3 (*d*)	69.0	71.3 (*d*)	68.9	71.0 (*d*)
C(6′)	17.8 (*q*)	16.5	17.8 (*q*)	16.5	17.8 (*q*)	16.5	17.7 (*q*)
Glucose							
C(1″)	104.7 (*d*)	100.1	104.7 (*d*)	100.1	104.7 (*d*)	100.1	104.4 (*d*)
C(2″)	75.4 (*d*)	72.8	75.4 (*d*)	72.9	75.4 (*d*)	72.8	75.2 (*d*)
C(3″)	76.8 (*d*)	72.8	76.8 (*d*)	72.9	76.8 (*d*)	72.8	76.5 (*d*)
C(4″)	79.3 (*d*)	76.0	79.3 (*d*)	76.1	79.3 (*d*)	76.0	79.0 (*d*)
C(5″)	76.4 (*d*)	72.7	76.4 (*d*)	72.7	76.4 (*d*)	72.7	76.1 (*d*)
C(6″)	63.6 (*t*)	60.5	63.5 (*t*)	60.5	63.6 (*t*)	60.5	63.3 (*t*)
Rhamnose							
C(1‴)	102.9 (*d*)	99.3	102.9 (*d*)	99.3	102.9 (*d*)	99.3	102.6 (*d*)
C(2‴)	72.7 (*d*)	68.4	72.7 (*d*)	68.4	72.7 (*d*)	68.4	72.4 (*d*)
C(3‴)	72.4 (*d*)	68.5	72.3 (*d*)	68.6	72.3 (*d*)	68.5	72.2 (*d*)
C(4‴)	73.7 (*d*)	70.5	73.7 (*d*)	70.6	73.7 (*d*)	70.6	73.5 (*d*)
C(5‴)	70.7 (*d*)	67.7	70.7 (*d*)	67.8	70.7 (*d*)	67.7	70.4 (*d*)
C(6‴)	17.8 (*q*)	17.2	17.8 (*q*)	17.2	17.9 (*q*)	17.2	17.8 (*q*)
Glucose (term.)							
C(1⁗)	103.5 (*d*)	100.0	103.5 (*d*)	100.0	103.5 (*d*)	100.0	103.2 (*d*)
C(2⁗)	76.1 (*d*)	71.9	76.1 (*d*)	71.9	76.0 (*d*)	71.9	75.8 (*d*)
C(3⁗)	78.3 (*d*)	74.1	78.3 (*d*)	74.1	78.3 (*d*)	74.1	78.0 (*d*)
C(4⁗)	72.2 (*d*)	69.8	72.2 (*d*)	69.9	72.2 (*d*)	69.8	71.9 (*d*)
C(5⁗)	76.8 (*d*)	71.7	76.8 (*d*)	71.8	76.8 (*d*)	71.7	76.5 (*d*)
C(6⁗)	61.7 (*t*)	62.0	61.7 (*t*)	62.0	61.7 (*t*)	62.0	61.4 (*t*)
MeCO		168.7–170.7 (*s*)		168.7–171.3 (*s*)		168.7–171.2 (*s*)	
*Me*CO		20.6–21.0 (*q*)		20.7–21.0 (*q*)		20.6–21.0 (*q*)	

Table 2. *^{1}H-NMR Data* ($CDCl_3$, 300 MHz) *of Ilwensisaponin A Dodecaacetate* (**1a**), *Ilwensisaponin B Tridecaacetate* (**2a**), *Ilwensisaponin C Tridecaacetate* (**3a**), *and Ilwensisaponin D Tetradecaacetate* (**4a**). δ in ppm, J in Hz.

	1a	**2a**	**3a**	**4a**
Aglycone				
H–C(3)	3.48[a])	3.49[a])	3.50[a])	3.50[a])
H–C(11)	5.37 (*dd*, J = 10.6, 3.3)	5.58 (*d*, J = 10.8)	3.78[a])	5.38 (*dd*, J = 3.9, 8.5)
H–C(12)	5.84 (*d*, J = 10.6)	6.40 (*dd*, J = 10.8, 3)	5.36 (*d*, J = 3.5)	5.22 (*d*, J = 3.9)
H_a–C(23)	4.08 (*d*, J = 11.9)	4.18 (*d*, J = 11.1)	4.14 (*d*, J = 11.6)	4.14 (*d*, J = 11.8)
H_b–C(23)	4.24 (*d*, J = 11.9)	4.26 (*d*, J = 11.1)	4.22 (*d*, J = 11.6)	4.24 (*d*, J = 11.8)
H_a–C(28)	3.25 (*d*, J = 6.9)	3.98 (*d*, J = 11.3)	3.66 (*d*, J = 10)	3.65 (*d*, J = 10)
H_b–C(28)	3.70 (*d*, J = 6.9)	4.25 (*d*, J = 11.3)	3.97 (*d*, J = 10)	3.97 (*d*, J = 10)
Me–C(24)	0.78 (*s*)	0.71 (*s*)	0.80 (*s*)	0.80 (*s*)
Me–C(25)	0.91 (*s*)	0.79 (*s*)	1.05 (*s*)	1.05 (*s*)
Me–C(26)	1.07 (*s*)	0.79 (*s*)	0.96 (*s*)	1.00 (*s*)
Me–C(27)	0.92 (*s*)	0.91 (*s*)	1.20 (*s*)	1.23 (*s*)
Me–C(29)	0.95 (*s*)	0.96 (*s*)	0.89 (*s*)	0.90 (*s*)
Me–C(30)	0.87 (*s*)	0.96 (*s*)	0.89 (*s*)	0.88 (*s*)
MeO	–	–	3.24 (*s*)	–
Ac	1.98 (× 2), 1.99, 2.03, 2.06, 2.07, 2.10, 2.11, 2.12, 2.13, 2.14, 2.18 (11*s*)	1.99 (× 2), 2.00, 2.03, 2.06, 2.064, 2.08, 2.105, 2.11, 2.13, 2.14 (× 2), 2.18 (11*s*)	1.98 (× 2), 1.99, 2.03, 2.05, 2.06, 2.08, 2.10, 2.104, 2.11, 2.12, 2.14, 2.20 (12*s*)	1.99 (× 2), 2.00, 2.02, 2.03, 2.05, 2.07, 2.08, 2.10, 2.11, 2.13, 2.138, 2.14, 2.20 (13*s*)
Fucose[b])				
H–C(1′)	4.26 (*d*)	4.27 (*d*)	4.28 (*d*)	4.27 (*d*, J = 7.4)
H–C(2′)	3.80 (*dd*)	3.83 (*dd*)	3.83 (*dd*)	3.83 (*dd*, J = 7.4, 9.8)
H–C(3′)	3.74 (*dd*)	3.75 (*dd*)	3.80 (*dd*)	3.79 (*dd*, J = 9.8, 3.5)
H–C(4′)	5.18 (*d*)	5.19 (*d*)	5.19 (*d*)	5.19 (*d*, J = 3.2)
H–C(5′)	3.63 (br. *d*)	3.64 (*d*)	3.63 (br. *d*)	3.62 (br. *d*)
H–C(6′)	1.14 (*d*)	1.15 (*d*)	1.14 (*d*)	1.14 (*d*, J = 6.2)
Glucose[b])				
H–C(1″)	4.71 (*d*)	4.71 (*d*)	4.71 (*d*)	4.72 (*d*, J = 7.1)
H–C(2″)	4.84 (*dd*)	4.85 (*dd*)	4.85 (*dd*)	4.84 (*dd*, J = 7.1, 8.6)
H–C(3″)	5.10[a])	5.04–5.13[a])	5.12[a])	5.10[a])
H–C(4″)	3.92 (*t*)	3.92 (*t*)	3.91 (*t*)	3.92 (*t*, J = 9.4)
H–C(5″)	3.49 (*m*)	3.49 (*m*)	3.50 (*m*)	3.48 (*m*)
Ha–C(6″)	4.10 (*dd*)	4.10 (*dd*)	4.10 (*dd*)	4.10 (*dd*, J = 12, 2)
Hb–C(6″)	4.75 (*dd*)	4.76 (*dd*)	4.74 (*dd*)	4.74 (*dd*, J = 12, 4)
Rhamnose[b])				
H–C(1‴)	4.86 (br. *s*)	4.86 (br. *s*)	4.86 (br. *s*)	4.87 (br. *s*)
H–C(2‴)	5.08–5.13[a])	5.04–5.13[a])	5.08–5.12[a])	5.08–5.13[a])
H–C(3‴)	5.17 (*dd*)	5.14 (*dd*)	5.17 (*dd*)	5.16 (*dd*, J = 3.2, 10)
H–C(4‴)	5.04 (*t*)	5.04 (*t*)	5.04 (*t*)	5.04 (*t*, J = 10)
H–C(5‴)	3.83 (*m*)	3.83 (*m*)	3.82 (*m*)	3.82 (*m*)
Me(6‴)	1.15 (*d*)	1.15 (*d*)	1.14 (*d*)	1.15 (*d*, J = 6.2)
Glucose (term.)[b])				
H–C(1⁗)	4.64 (*d*)	4.64 (*d*)	4.64 (*d*)	4.64 (*d*, J = 7.9)
H–C(2⁗)	4.91 (*dd*)	4.92 (*dd*)	4.91 (*dd*)	4.91 (*dd*, J = 7.9, 9.7)
H–C(3⁗)	5.08–5.13[a])	5.04–5.13[a])	5.08–5.12[a])	5.08–5.13[a])
H–C(4⁗)	5.12[a])	5.04–5.13[a])	5.08–5.12[a])	5.08–5.13[a])
H–C(5⁗)	3.60 (*m*)	3.63 (*m*)	3.61 (*m*)	3.62 (*m*)
Ha–C(6⁗)	4.04 (*dd*)	4.06 (*dd*)	4.04 (*dd*)	4.04 (*dd*, J = 12.3, 2.3)
Hb–C(6⁗)	4.34 (*dd*)	4.34 (*dd*)	4.33 (*dd*)	4.34 (*dd*, J = 12.3, 4.4)

[a]) Signal patterns are unclear due to overlapping.

[b]) The same coupling constants as for **4a** were observed for **1a**, **2a**, and **3a**.

for $C_{78}H_{112}O_{33}$). The spectral data established the structure of **1** to be 13β,28-epoxy-3-{{[β-D-glucopyranosyl-(1→2)]-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)]-β-D-fucopyranosyl}oxy}olean-11-en-23-ol.

The ^{1}H-NMR of **1** revealed signals for 6 tertiary Me groups arising from the aglycone moiety. Resonances for the enantiomeric protons of the sugar moiety were observed at δ 4.92 (br. *s*, H–C(1‴), α-L-rhamnose), 4.66 (*d*, J = 7.7 Hz, 2 H, H–C(1″) and H–C(1⁗), 2 β-D-glucose), and 4.52 (*d*, J = 7.9 Hz, H–C(1′), β-D-fucose). The signal at δ 1.31 (*d*, J = 6.2 Hz, 6 H) was assigned to the secondary Me groups of the rhamnose and fucose units. Additionally, 2 olefinic protons were observed at δ 5.37 (*dd*, J = 11.1 and 2.3 Hz, H–C(11)) and 5.98 (*d*, J = 11.1 Hz, H–C(12)). The ^{13}C-NMR data of **1** (*Table 1*) also supported a tetraglycosidic structure. When the sugar unit was subtracted from the molecular formula, the aglycone was found to have the formula $C_{30}H_{48}O_3$, implying 7 degrees of unsaturation. Only one was present as a multiple bond, **1** was thus hexacyclic. Functionalities present within the aglycone were a primary alcohol (δ 64.6 (*t*, C(23)), an ether (δ 86.0 and 77.9 (*s* and *t*, resp., C(13) and C(28))), and a C=C bond (δ 131.4 and 134.0 (2*d*, C(11) and C(12), resp.)), as well as the sugar moiety.

The complete structure of **1** was established from the results of extensive 2D-NMR measurements made with its acetylated derivative **1a**, all resonances (*Tables 1* and *2*) being assigned by homonuclear and heteronuclear COSY experiments (see below). Of the 12 AcO groups in the ^{1}H-NMR, 11 were attributed to the sugar moiety; only one belonged to the aglycone confirming the presence of the primary alcohol in **1**. Upon acetylation, no downfield shifts were observed for the signals of H–C(2′), H–C(3′), and H–C(4″) of **1** (δ 3.8 (*dd*, J = 7.4, 9.8 Hz), 3.74 (*dd*, J = 9.8, 3.5 Hz), and 3.92 (*t*, J = 9.4 Hz), resp.), indicating C(2′), C(3′), and C(4″) to be the sites of interglycosidic linkages.

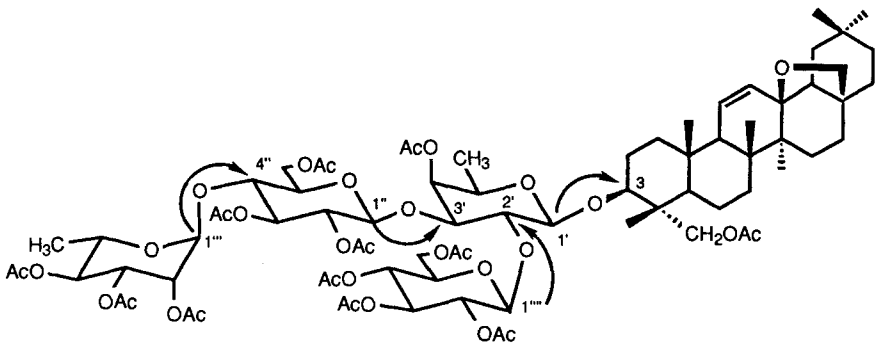
The ^{13}C-NMR spectra of **1** and **1a** showed 30 distinct resonances which were assigned to the aglycone moiety, *i.e.* 6 Me, 11 CH_2, 6 CH, and 7 C (from DEPT experiments with **1** and **1a** and ^{1}H,^{13}C-heteronuclear COSY and HMBC experiments with **1a**).

The sapogenin of **1a** was found to have a structure related to saikogenins, which contain a 13β,28-epoxy group and a C(11)=C(12) bond [5–7]. Indeed, the ^{1}H-NMR of the sapogenin of **1a** showed 2 *AB* systems at δ 4.08 and 4.24 (*d*, J_{AB} = 11.9 Hz, each 1 H, CH_2(23)) and 3.25 and 3.7 (*d*, J_{AB} = 6.9 Hz, each 1 H, CH_2(28)) as well as for a double bond at δ 5.37 (*dd*, J = 10.6, 3.3 Hz, H–C(11)) and 5.84 (*d*, J = 10.6 Hz, H–C(12)). Furthermore, the corresponding ^{13}C-resonances at δ 65.3 (*t*, C(23)), 76.9 (*t*, C(28)), 130.8 (*d*, C(11)), and 132 (*d*, C(12)) supported this proposal. The ^{13}C-NMR data for the aglycone of **1** were in good agreement with those published for 13β,28-epoxyolean-11-ene-3β,23-diol [5]. Finally, a ^{1}H,^{13}C-heteronuclear long-range COSY experiment performed with **1a** (*Fig.*) established correlations between *H*–C(1‴) (δ 4.86) of rhamnose and C(4″) (δ 76) of glucose (inner), *H*–C(1″) (δ 4.71) of glucose (inner) and C(3′) (δ 79.3) of fucose, *H*–C(1⁗) (δ 4.64) of glucose (terminal) and C(2′) (δ 74.8) of fucose, and *H*–C(1′) (δ 4.26) of fucose and C(3) (δ 83.7) of sapogenin. Signals in the FAB-MS of **1a** arising from the sugar moiety at m/z 1079, 561, 331, and 273 were evaluated as tetraglycosidic sugar moiety (undecaacetyltetraosyloxonium ion), diglycosidic side chain (hexaacetyl-rhamnopyranosyl-glucoseoxonium ion), terminal glucose (tetraacetyl-glucoseoxonium ion), and terminal rhamnose units (triacetyl-rhamnoseoxonium ion), respectively.

Ilwensisaponin B (**2**) by mass spectrometry had the molecular formula $C_{54}H_{88}O_{21}$. The FAB-MS of **2** indicated it to have the same molecular weight as **1** (m/z 1095 ([M + Na]$^+$)). Upon acetylation, **2** yielded a tridecaacetate **2a** (FAB-MS: m/z 1618 ([M]$^+$), 1641 ([M + Na]$^+$), calc. for $C_{80}H_{114}O_{35}$). According to the spectral data, **2** is 3-β-{{[β-D-glucopyranosyl-(1→2)]-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)]-β-D-fucopyranosyl}oxy}oleana-11,13(18)-diene-23,28-diol.

The aglycone moiety of **2** of formula $C_{30}H_{48}O_3$ implied 7 degrees of unsaturation of which 2 were present as multiple bonds, indicating **2** to be pentacyclic. The two C=C bonds were conjugated (UV: 244, 252, and 261 nm; ^{13}C-NMR: δ 127.2 (*d*, C(11)), 126.5 (*d*, C(12)), 137.8 (*s*, C(13)), and 135.9 (*s*, C(14))), with proton signals at δ 5.6 and 6.43 for H–C(11) and H–C(12), respectively. Furthermore, 2 *AB* systems (δ 63.7 (*t*) and 64.5 (*t*)) were observed and assigned to two primary alcohols located at C(23) and C(28).

The ^{1}H-NMR of **2a** exhibited 13 AcO signals (*Table 2*). The ^{1}H- and ^{13}C-NMR resonances observed for the sugar moiety of **2** and **2a** were in good agreement with those of **1** and **1a**, indicating the presence of same sugars with the same sequence. This deduction was also supported by the FAB-MS of **2a**, which showed the same fragmenta-



1a ilwensisaponin A dodecaacetate

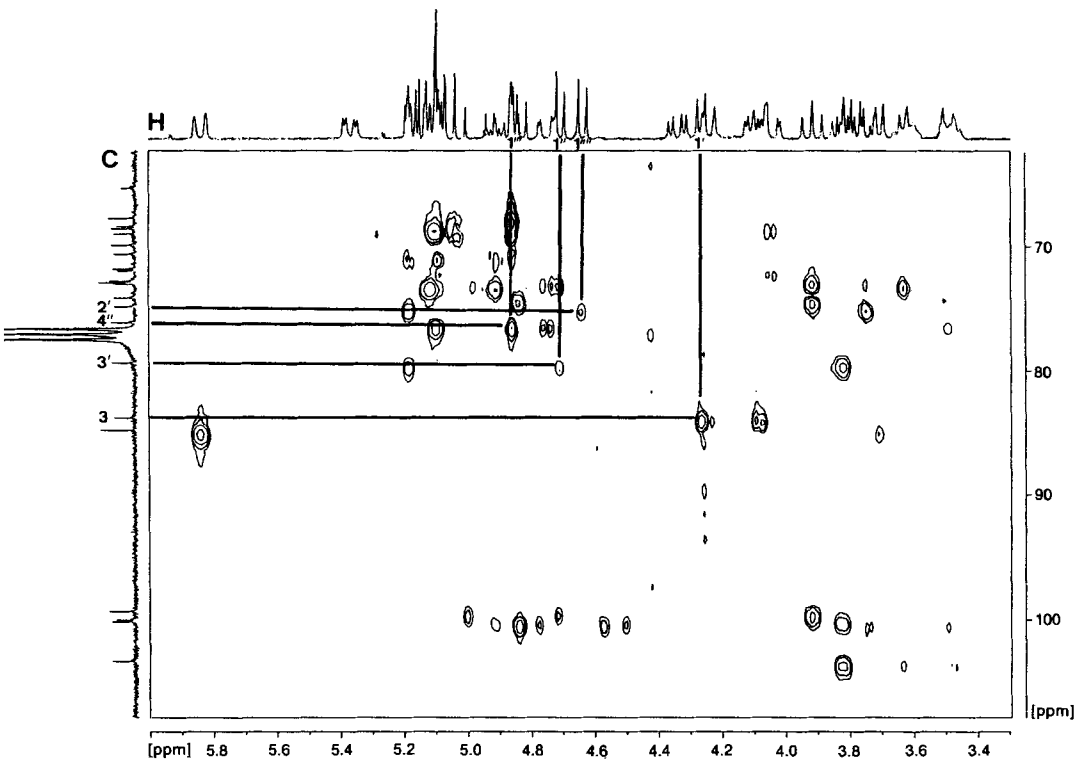


Figure. *Proton-detected 2D $^{1}H,^{13}C$-heteronuclear long-range COSY of* **1a** (600 MHz, $J = 10$ Hz)

tion peaks for its sugar moiety (m/z 1079, 561, 331, and 273). Two of the AcO signals were thus assigned to the aglycone moiety confirming the presence of two primary OH groups at C(23) and C(28) of **2**. Comparison of the ^{1}H- and ^{13}C-NMR data of **1a** with that of **2a** revealed that the differences between the aglycones could be rationalized in terms of an isomerization of **1** or **1a** to **2** or **2a**.

Ilwensisaponin C (**3**) by mass spectrometry (FAB-MS: m/z 1127 ($[M + Na]^+$)) was found to have the molecular formula $C_{55}H_{92}O_{22}$. Acetylation of **3** yielded a tridecaacetate

3a (FAB-MS: *m/z* 1650 ($[M]^+$), 1673 ($[M + Na]^+$), calc. for $C_{81}H_{118}O_{35}$). Compound **3** is 3-*β*-{{[*β*-D-glucopyranosyl-(1→2)]-[*α*-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→3)]-*β*-D-fucopyranosyl}oxy}-11*α*-methoxyolean-12-ene-23,28-diol.

The ^{1}H-NMR spectrum of **3** showed signals for 6 tertiary Me (*δ* 1.32, 1.15, 1.06, 0.96, 0.94, 0.79), an olefinic H (*δ* 5.42 (*d*)), MeO (*δ* 3.29 (*s*)) as well as signals arising from the sugar moieties (4 anomeric protons (*δ* 4.92, 4.66 (×2), 4.52) and 2 secondary Me groups (*δ* 1.31, 6 H)). The similarity of the sugar moiety of **3** with those of **1** and **2** was also revealed by the ^{13}C-NMR data of **3** (*Table 1*). After assignment of the ^{13}C-NMR signals of the sugar moiety, the resonances remaining for the aglycone were 6 Me (all tertiary), 11 CH_2, 6 CH, 7 C, and 1 MeO (*δ* 54.3 (*q*)), the functionalities being 2 primary alcohols (*δ* 64.7 (*t*) and 69.8 (*t*)), two CH attached to the oxygenated groups (*δ* 77.6 (*d*) and 85.6 (*d*)), and a C=C bond (*δ* 122.9 (*d*) and 150.6 (*s*)). These results clearly supported the presence of a pentacyclic olean-12-ene skeleton [8].

Fragment peaks in the MS (*m/z* 1079, 561, 331, and 273) and ^{1}H- and ^{13}C-NMR resonances (*Tables 1* and *2*) arising from the sugar moiety of **3a** were all in accordance with those of **1a** and **2a**. The sequence within the sugar chain of **3** was thus as in **1** and **2**. The location of the MeO group was determined from homo- and heteronuclear COSY of **3a**, establishing the assignments of *H*–C(11) (*δ* 3.78) and C(11) (*δ* 75.9). The former showed correlations with *H*–C(12) (*δ* 5.36) and *H*–C(9) (*δ* 1.70) indicating the MeO group to be at C(11). From the chemical shift of C(11) in **3** (*δ* 77.6) and **3a** (*δ* 75.9) it can be deduced that the MeO group has an *α*-configuration as reported for saikosaponin-b_4 [7].

Ilwensisaponin D (**4**), the most polar isolate, had the molecular formula $C_{54}H_{90}O_{22}$ as determined by mass spectrometry (FAB-MS: *m/z* 1113 ($[M + Na]^+$)). Acetylation of **4** yielded the tetradecaacetate **4a** whose FAB-MS (*m/z* 1701 ($[M + Na]^+$), calc. for $C_{82}H_{118}O_{36}$), showed the same fragmentation pattern (*m/z* 1079, 561, 331, and 273) for the sugar moiety as previously observed for **1a**, **2a**, and **3a**. Compound **4** is 3-*β*-{{[*β*-D-glucopyranosyl-(1→2)]-[*α*-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→3)]-*β*-D-fucopyranosyl}oxy}olean-12-ene-11*α*,23,28-triol.

The ^{1}H-NMR spectrum of **4** showed resonances for 4 anomeric protons and 2 secondary Me groups, similar to those of **1–3**. Structural diagnostic signals arising from the aglycone moiety were 6 tertiary Me groups, an olefinic proton (*δ* 5.21 (*d*, *J* = 3.3 Hz, H–C(12)), and a methine proton (*δ* 4.21 (*dd*, *J* = 3.3 and 8.5 Hz)), all other resonances being virtually identical to those found for **3**. The *δ* 4.21 resonance indicated the presence of a secondary OH group on the aglycone moiety.

The ^{1}H-NMR data of **4a** (*Table 2*) confirmed the sugar moieties and their attachments to be the same as in **1–3**. Signals arising from the aglycone moiety were for 6 tertiary Me groups, 2 *AB* systems (*δ* 4.14 and 4.24 (J_{AB} = 11.8 Hz, CH_2(23)) and 3.65 and 3.97 (J_{AB} = 10 Hz, CH_2(28))), an olefinic proton (*δ* 5.22 (*d*, *J* = 3.9 Hz, H–C(12)), and a methine proton that was both allylic and on a O-bearing C-atom (*δ* 5.38 (*dd*, *J* = 3.9 and 8.5 Hz)). The latter proton was coupled with H–C(12) and was, therefore, assigned to H–C(11). Upon acetylation, a 1.18 ppm downfield shift of H–C(11) was observed, confirming C(11) to be the position of the secondary OH group. The resonance of C(11) was at *δ* 68.0 (*d*), *i.e.* shifted by 9.6 ppm to higher field when compared to **3**. These results clearly indicate **4** to be the 11-hydroxy derivative of **3**. The *α*-configuration of the OH group at C(11) of **4** was deduced from the *trans*-coupling constant *J*(9,11) of 8.5 Hz and also from *J*(11,12) of 3.9 Hz in **4a**.

The sugar moieties of ilwensisaponins A–D (**1–4**), except for the interglycosidic linkages, are similar to those found in verbascosaponin isolated from *Verbascum phlomoides* [9] and *V. nigrum* [10]. Recently, songarosaponins A–C were reported from *V. songaricum* [11], and ilwensisaponins A and C from *V. nigrum* [12], songarosaponin A being identical to ilwensisaponin B (**2**).

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Experimental Part

General. Isolations were carried out using medium-pressure liquid chromatography (MPLC): *Lewa-5* pump, *Rheodyne* injector, *Büchi* glass column (18.5 mm × 322 mm); stationary phase: *Sepralyte* 40 μm; fraction collector: *LKB 1700* minirac. TLC: silica gel *60* F_{254} plates (*Merck*) and silica gel *G* plates, 0.25 mm thick; detection by spraying 30% H_2SO_4 in H_2O and 1% vanillin in conc. H_2SO_4 (saponins) and by aniline phthalate reagent and heating at 100–110° for 5–10 min (sugars). IR Spectra (ν_{max} cm^{-1}): *Perkin-Elmer-257* spectrophotometer; KBr pellets. ^{1}H- and ^{13}C-NMR Spectra: *Bruker-AMX-300* and *Bruker-AMX-600* instruments; δ in ppm, J in Hz. FAB-MS: *ZAB2-SEQ* instrument using NOBA (= 3-nitrobenzyl alcohol) as matrix.

Plant Material. Scrophularia ilwensis was collected from Kars (Eastern Anatolia, Turkey), Selim, Karahamza territories in May 1989. Voucher specimens were deposited at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University.

Extraction and Isolation. Air-dried aerial parts of the plant (480 g) were extracted twice with MeOH at 50°. After evaporation, the residue was suspended in H_2O and defatted with petroleum ether. The aq. 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: Frs. *A* (22 g), *B* (1.46 g), *C* (1.18 g), *D* (953 mg), *E* (618 mg), *F* (1.5 g), *G* (342 mg), *H* (263 mg). Frs. *F*, *G*, and *H* were rich in saponins and subjected separately to MPLC (50→80% MeOH/H_2O, flow rate 5 ml/min), resulting in the isolation of **4** (ilwensisaponin D, totally 57 mg), **3** (ilwensisaponin C, totally 107 mg), **2** (ilwensisaponin B, totally 55 mg), and **1** (ilwensisaponin A, totally 188 mg).

Ilwensisaponin A (**1**). Amorphous, colourless powder. IR (KBr): 3400, 1630, 1055. ^{1}H-NMR (300 MHz, CD_3OD): aglycone moiety: 5.98 (*d*, J = 11.1, H–C(12)); 5.37 (*dd*, J = 11.1, 2.3, H–C(11)); 1.13, 1.01, 0.99, 0.98, 0.94, 0.76 (6*s*, each 3 H, 6 Me); sugar moiety: 4.92 (br. *s*, H–C(1‴)); 4.66 (*d*, J = 7.7, H–C(1″), H–C(1⁗)); 4.52 (*d*, J = 7.9, H–C(1′)); 1.31 (*d*, J = 6.2, 2 Me). ^{13}C-NMR: *Table 1*. FAB-MS (NOBA): 1095 ($[M + Na]^+$, calc. for $C_{54}H_{88}O_{21}$).

Ilwensisaponin B (**2**). Amorphous, colourless powder. IR (KBr): 3400, 1640, 1060. ^{1}H-NMR (300 MHz, CD_3OD): aglycone moiety: 6.43 (*d*, J = 10.5, H–C(12)); 5.60 (*d*, J = 10.5, H–C(11)); 0.99, 0.97, 0.95, 0.80, 0.74, 0.73 (6*s*, each 3 H, 6 Me); sugar moiety: 4.9–4.8 (overlapping signal, H–C(1‴)); 4.62 (*d*, J = 7.7, H–C(1″), H–C(1⁗)); 4.49 (*d*, J = 7.4, H–C(1′)); 1.27 (*d*, J = 6.2, 2 Me). ^{13}C-NMR: *Table 1*. FAB-MS (NOBA): 1095 ($[M + Na]^+$, calc. for $C_{54}H_{88}O_{21}$).

Ilwensisaponin C (**3**). Amorphous, colourless powder. IR (KBr): 3400, 1630, 1055. ^{1}H-NMR (300 MHz, CD_3OD): aglycone moiety: 5.42 (*d*, J = 3.3, H–C(12)); 4–3.5 (H–C(11)); 3.29 (*s*, MeO); 1.32, 1.15, 1.06, 0.96, 0.94, 0.79 (6*s*, each 3 H, 6 Me); sugar moiety: 4.92 (H–C(1‴)); 4.66 (*d*, J = 7.8, H–C(1″), H–C(1⁗)); 4.52 (*d*, J = 7.4, H–C(1′)); 1.31 (*d*, J = 6.2, 2 Me). ^{13}C-NMR: *Table 1*. FAB-MS (NOBA): 1127 ($[M + Na]^+$, calc. for $C_{55}H_{92}O_{22}$).

Ilwensisaponin D (**4**). Amorphous, colourless powder. IR (KBr): 3400, 1630, 1055. ^{1}H-NMR (300 MHz, CD_3OD): aglycone moiety: 5.21 (*d*, J = 3.3, H–C(12)); 4.21 (*dd*, J = 3.3, 8.5, H–C(11)); 1.33, 1.16, 1.07, 0.94, 0.935, 0.79 (6*s*, each 3 H, 6 Me); sugar moiety: 4.91 (H–C(1‴)); 4.66 (*d*, J = 7.7, H–C(1″), H–C(1⁗)); 4.52 (*d*, J = 7.7, H–C(1′)); 1.31, 1.30 (2*d*, J = 6.2, each 3 H, 2 Me). ^{13}C-NMR: *Table 1*. FAB-MS (NOBA): 1113 ($[M + Na]^+$, calc. for $C_{54}H_{90}O_{22}$).

Acid Hydrolysis of **1**–**4**. Saponins **1**–**4** (each 10 mg) were separately dissolved in 5 ml of 5% HCl soln. and heated at 100° for 5 h, cooled, and filtered. The filtrates were neutralized by passing them through *Dowex* (Cl form) and evaporated. The residues were examined for sugars by paper chromatography (descending method) using 1-BuOH/pyridine/H_2O 9:5:4.

Acetylation of **1**–**4**. Treatment of **1**–**4** (each *ca.* 20 mg), separately, with Ac_2O (1 ml), pyridine (1 ml), and 4-(dimethylamino)pyridine (20 mg) at r.t. overnight, followed by column chromatography (silica gel, benzene/acetone 4:1) gave **1a**–**4a**, resp. ^{1}H-NMR: *Table 2*. ^{13}C-NMR: *Table 1*. FAB-MS: see *General Part*.

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