IRIDOID GLYCOSIDES OF *Verbascum laxum*

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The epigeal part of *Verbascum laxum* Filar. ét Jav. has yielded in addition to the known sinuatol, a new iridoid glycoside — 6-O-(3"-O-p-coumaroyl-α-L-rhamnopyranosyl)aucubin (I), $[\alpha]_D^{20}$ −174.2 ± 0.7° (c 1.4; MeOH). From the roots of the plant, as the main components giving an iridoid reaction, have been isolated harpagoside and the new iridoid glycoside 6-O-(2",3"-di-O-acyl[acetyl,p-methoxy-trans-cinnamoyl]-α-L-rhamnopyranosyl)aucubin (V) $[\alpha]_D^{18}$ −130 ± 2° (c 0.5; MeOH). The structures of the substances isolated were established with the aid of UV, IR, NMR, and mass spectra.

Continuing an investigation of plants of the genus *Verbascum* of the Armenian flora, we have studied the iridoid glycosides of *Verbascum laxum* Filar, et Jav. grown both in the foothill and in the subalpine zones of the republic and not previously investigated chemically.

From a methanolic extract of the epigeal part of samples of the plant collected in the incipient flowering stage we have isolated iridoid glycosides (I) and (II) and have also detected by paper and thin-layer chromatography the presence of aucubin (III) and of catalpol (IV) in the extract.

According to its IR spectrum, glycoside (I) contained an ester grouping. Its acid hydrolysis led to the formation of glucose and rhamnose and the black polymeric product that is usual for iridoids. On alkaline hydrolysis (I) formed glycoside (II) and p-coumaric acid. Analysis of the high-resolution PMR spectrum of glycoside (II) permitted the assumption of the identity of the latter with sinuatol (II) — an iridoid glycoside of the aucubin series isolated previously from *Verbascum sinuatum* L. [1]. The mass spectrum of the acetyl derivative of glycoside (I) contained strong peaks of ions with m/z 419, 377, 231, and 189, completely expectable if a p-coumaroylrhamnosyl fragment were present in the structure of the initial glycoside. The presence in the spectrum of a set of fragments with m/z 331, 289, 271, 229, 211, 169, 127, and 109 (with the peak of the ion with m/z 331 having the maximum intensity) and also of the peaks of ions with m/z 191 and 131, which are typical for C-10—O acetyl derivatives of aucubin glycosides [2] in the spectrum also showed the presence of a p-coumaroyl residue in the rhamnosyl moiety of the molecule.

A study of the ^{13}C NMR spectrum of glycoside (I) (Table 1) enabled the fact that the glycoside belonged to the sinuatol derivatives to be confirmed and the position of the p-coumaroyl fragment to be determined more accurately. The downfield shift (by 3.10 ppm) of the C-3" signal of the rhamnosyl skeleton and the upfield shifts (by −4.27 and −3.98 ppm, respectively) of the C-2" and C-4" signals as compared with those for methyl α-L-rhamnopyranoside [3] showed that the p-coumaroyl fragment was attached to oxygen at C-3".

Thus, glycoside (I) had the structure of 6-O-(3"-O-p-coumaroyl-α-L-rhamnopyranosyl)aucubin (I) and was a new iridoid glycoside not previously described.

Two other glycosides — (V) and (VI) — were isolated as the main components of the iridoid fraction of a methanolic extract of the roots of the plant.

Glycoside (V) was also an ester (according to its IR spectrum). Its PMR spectrum showed the signals of the protons of one acetyl group and of a p-methoxy-trans-cinnamic acid group. The alkaline hydrolysis of glycoside (V) gave p-methoxy-trans-cinnamic acid and sinuatol. When glycoside (V) was acetylated, a peracetate was formed, the PMR spectrum of which showed

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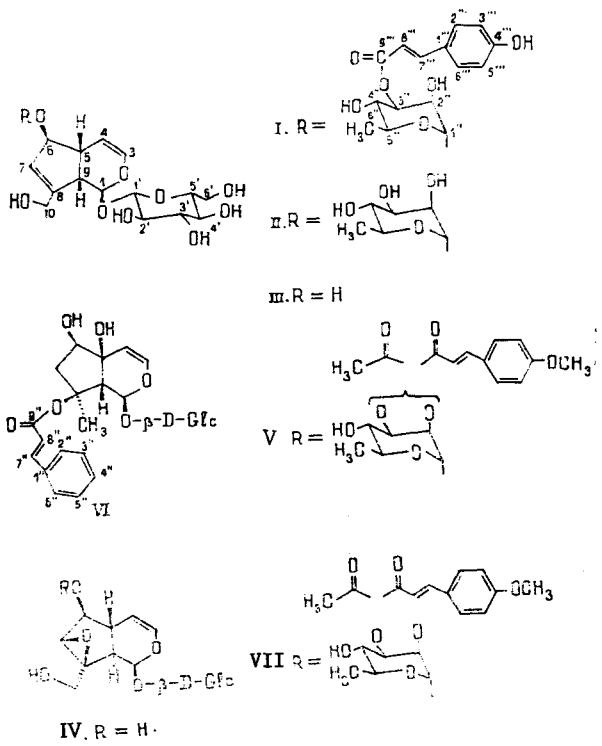

in the 2.00 ppm region the signals of seven acetoxy groups. The mass spectrum of the peracetate had the peaks of ions with m/z 408 and 391 corresponding to the fragmentation of a diacetylmethoxycinnamoylrhamnopyranose. The mass spectrum of glycoside (V) itself contained the peaks of ions with m/z 349, 331, and 289, corresponding to the ion of an acetylmethoxycinnamoyldeoxyrhamnopyranose. These facts show that the acetic and p-methoxy-trans-cinnamic acids esterified two hydroxy groups of the rhamnosyl moiety. The ^{13}C NMR spectrum of glycoside (V) (see Table 1), confirmed the probability of these assumptions. The good agreement of the chemical shifts of the signals of the carbon atoms of the rhamnosyl moiety of the molecule with those of a known glycoside — a 6-O-2',3"-di-O-acyl-α-L-rhamnopyranosyl)catalpol isolated from *Verbascum sinuatum* L. [4] — showed quite definitely that in glycoside (V) the hydroxy groups at C-2" and C-3" were acylated. Thus glycoside (V) has the structure of a 6-O-(2",3"-di-O-acyl[acetyl, p-methoxy-trans-cinnamoyl]rhamnopyranosyl)aucubin (V) and is a new iridoid glycoside.

On the basis of an analysis of its UV, IR, and PMR and ^{13}C NMR spectra, and also of the mass spectrum of its acetyl derivative and a direct chromatographic comparison of the product of alkaline hydrolysis with the known iridoid glycoside harpagide, glycoside (VI) was identified as harpagoside. Harpagoside has been detected previously in *Verbascum nigrum* L. [5] and *V. sinuatum* L. [4].

EXPERIMENTAL

UV spectra were taken on a Specord UV-VIS instrument in methanolic solution, IR spectra on a UR-20 instrument (in paraffin oil), NMR spectra (δ scale) on Varian A-60 and XL-200 and Bruker WM-250 spectrometers (with TMS as internal standard), and mass spectra on a MKh 1320 instrument (70 eV); optical activities were determined on a Polamat A instrument. Chromatography was performed on type S paper (PC) in the butan-1-ol—acetic acid—water (4:1:5) system and on Silufol plates (TLC) in the following systems: 1) chloroform—methanol (3:1), and 2) ethyl acetate—methanol—chloroform—water (7:2:1:1). The iridoids were detected on chromatograms in UV light and with the benzidine reagent (0.5 g of benzidine, 20 ml of acetic acid, and 80 ml of methanol) followed by heating at 100°C. Sugars were revealed with the aniline phthalate reagent.

Isolation of the Iridoid Fractions from the Epigeal Part. The dry comminuted epigeal part of the plant gathered in the incipient flowering phase (2.5 kg) was steeped with methanol (8 × 10 liters). The combined methanolic extracts were concentrated to a volume of 0.5 liter

TABLE 1. Comparison of the ^{13}C NMR Spectra of Glycoside (I), Methyl α-L-Rhamnopyranoside (VIII), Glycoside (V), and the 6-O-(2",3"-Di-O-Cyl-α-L-rhamnopyranosyl)catalpol (VII) [4]

C atom	Glycoside (I)*	VIII*	Glycoside (V)†	VII**
C-1	95,13	—	98,05	95,2
C-3	139,86	—	141,97	142,3
C-4	103,82	—	105,39	103,4
C-5	42,26	—	44,08	37,2
C-6	86,44	—	89,40	84,4
C-7	132,15	—	127,07	50,4
C-8	148,62	—	149,93	6,6
C-9	46,63	—	48,21	43,3
C-10	59,38	—	61,37	61,5
C-1′	97,69	—	100,13	99,8
C-2′	73,01	—	74,88	74,8
C-3′	76,98	—	78,16	78,6
C-4′	69,85	—	71,56	71,7
C-5′	76,17	—	77,88	77,7
C-6′	60,86	—	62,68	63,0
C-1″	99,16	101,09	98,45	97,8
C-2″	66,28	70,55	71,56	71,4
C-3″	73,38	70,28	73,34	73,2
C-4″	67,87	71,85	71,56	71,5
C-5″	68,66	68,07	70,34	70,2
C-6″	17,71	17,69	18,08	18,0
C-1‴	124,65	—	128,19	128,2
C-2‴,6‴	129,98	—	131,11	131,2
C-3‴,5‴	115,37	—	115,51	115,5
C-4‴	159,64	—	163,31	163,4
C-7‴	143,89	—	147,04	147,2
C-8‴	114,38	—	115,47	115,5
C=O	166,19	—	167,80	167,9
OCH_3	—	—	55,9	56,0
CH_3CO	—	—	20,88;172,22	20,9;172,3

*Spectrum taken in DMSO-d_6 solution.
†In CD_3OD solution.

and were mixed with 1 liter of water, and the mixture was washed successively with benzene (4 × 0.5 liter), chloroform (3 × 0.5 liter), and ether (3 × 0.5 liter). The purified aqueous solution was passed through a layer of inactive neutral alumina. The adsorbent was washed with water until the reaction for iridoids was negative, and the eluates were combined and evaporated to a volume of 0.4 liter. Part of the resulting solution (100 ml) was transferred to a column containing Woelm polyamide. The column was eluted with water, and 100-ml fractions were collected and were analyzed for PC and TLC. Fractions 1 and 2 together contained 14.2 g of a mixture of substances appearing in the form of spots with R_f 0.50 (gray), 0.43 (orange), and 0.33 (green) (PC) or 0.40 (gray), 0.37 (orange), and 0.27 (green) (TLC, system 2). Fraction 3 contained 0.48 g of a mixture of substances with R_f 0.87 (green), 0.85 (green), and 0.33 (green) (PC) or 0.46 (green), 0.45 (green), and 0.27 (green) (TLC, system 2). Fractions 4-8 together contained 2.5 g of a mixture of substances with R_f 0.87 and 0.85 (PC) or 0.46 and 0.45 (TLC). A mixture of fractions 9-11 contained 1.5 g of a substance appearing in the form of a single main spot with R_f 0.87 (PC) or 0.45 (TLC).

Identification of Aucubin (III) and Catalpol (IV). The substances with R_f 0.50 (PC), 0.40 (TLC), and 0.43 (PC), 0.37 (TLC, system 2), on the chromatographic analysis of fractions 1-2 with markers corresponded in their chromatographic mobilities and colorations of the spots to aucubin and catalpol, respectively.

Isolation of Sinuatol (II). Fraction 3 (0.48 g) was dissolved in 20 ml of water and the solution was chromatographed on a column of Sephadex LH-20. The substances were eluted with water and 10-ml fractions were collected. Fractions 3-5, after evaporation, yielded 146 mg of an amorphous glycoside with R_f 0.33 (green) (PC) or 0.27 (Green) (TLC); $[\alpha]_D^{20}$ −156 ± 2° (c 0.5; water). PMR spectrum (250 mHz, DMSO-d_6, δ, ppm): 1.16 (3H, d, J = 6 Hz, 3H-6"), 2.70 (1H, m, H-5) 272-2.78 (1H, m, H-9), 2.94-3.70 (18H, m, methylene groups), 3.98 (1H, d, J = 16 Hz, H_a-10), 4.17 (1H, d, J = 16 Hz, H_b-10), 4.33 (1H, br.s H-6), 4.50 (1H, d, J = 7.5 Hz, H-1'), 4.67 (1H, br.s H-1"), 4.81 (1H, d, J = 7.5 Hz, H-1), 5.08 (1H, d.d, J = 6 and 3.5 Hz, H-4), 6.41 (1H, d.d, J = 6 Hz and 1.5 Hz, H-3), 5.78 (1H, br.s, H-7). In its chromatographic behavior, its $[\alpha]_D$ value, and PMR spectrum, the glycoside corresponded to sinuatol [1].

6-O-(3"-O-p-Coumaroyl-α-L-rhamnopyranosyl)aucubin (I). Combined fractions 9-11 obtained by means of the polyamide column were evaporated, and the residue (1.5 g) was chromatographed on a column of silica gel (L 40/100). The column was washed with mixtures of chloroform and methanol. The fractions eluted by chloroform–methanol (100:15) contained predominantly a single component, with R_f 0.85 (PC) or 0.45 (TLC, system 2). They were combined and evaporated, and the residue was chromatographed again on a column of silica gel with elution by chloroform–methanol (100:15). This gave 220 mg of a white amorphous powder of glycoside (I) $[\alpha]_D^{20}$ −174.2 ± 0.7° (c 1.4; methanol), $\lambda_{max}^{CH_3OH}$: 205, 213 sh., 225, 312 nm; ν_{max}^{Nujol}: 1695, 1690, 1620, 1600, 1580, 1500 cm^{-1}. The octaacetyl derivative of glycoside (I) obtained by the usual method [6] was amorphous, with R_f 0.54 (TLC, system 1). Mass spectrum of the octaacetyl derivative m/z (%): 539 (0.7), 523 (0.4), 477 (0.3), 449 (0.3), 436 (0.7), 419 (30), 407 (0.8), 394 (1.8), 377 (14), 376 (2), 368 (1.5), 361 (4.6), 331 (100), 313 (1.1), 299 (2.0), 289 (5.8), 273 (10), 271 (13), 231 (22), 229 (10), 221 (5), 211 (8), 200 (2.8), 191 (28), 189 (56), 169 (100), 164 (14), 157 (11), 153 (15), 147 (80), 145 (19), 139 (11), 131 (22), 127 (22), 116 (18), 111 (20), 109 (73), 91 (8), 83 (15), 71 (15), 69 (18).

Alkaline Hydrlysis of 6-O-(3"-O-p-Coumaroyl-α-L-rhamnopyranosyl)aucubin (I). A solution of 0.1 g of glycoside (I) in 10 ml of 0.05 N caustic soda solution was heated in the water bath for 3 h. Sinuatol (II) was identified in the reaction mixture by PC and TLC. After acidification with a 0.1% solution of hydrochloric acid, the reaction mixture was extracted with ether. The ethereal extract yielded 4 mg of an acid with MP 213°C, M^+ 164. A mixture with a sample of p-hydroxy-trans-cinnamic acid melted without depression.

Acid Hydrolysis of 6-O-(3"-O-p-Coumaroyl-α-L-rhamnopyranosyl)aucubin (I). A solution of 20 mg of glycoside (I) in 5 ml of 5% sulfuric acid was heated in the water bath for 1 h. The black-violet precipitate that had formed was filtered off. After the usual working-up procedure, glucose and rhamnose were detected in the filtrate with the aid of PC.

Isolation of the Iridoid Fractions from the Roots. The air-dry comminuted roots (2.65 g) of *V. laxum* collected in the fruit-bearing period were steeped in methanol (10 × 5 liters). The combined methanolic extracts were evaporated to a volume of 1 liter, mixed with 1 liter of water, and filtered, and the filtrate was washed with chloroform (3 × 200 ml) and was then extracted (20 × 0.2 liter) with chloroform–ethanol (3:1). The extracts were combined, the solvent was distilled off, and 251.3 g (9.51%) of resinous residue was obtained. Part of this substance (10 g) was dissolved in 30 ml of chloroform–ethanol (20:1) and was chromatographed on a column of silica gel. The column was eluted with the same solvent system with a gradually increasing concentration of ethanol. The fractions eluted by the 20:1 mixture contained 4.62 g of a mixture of substances appearing in the form of two spots on TLC: R_f 0.57 (green) and 0.54 (brown, system 2). The fractions eluted by the 100:6 mixture contained 1.2 g of the substance with R_f 0.57, and the fractions eluted by (100:7)-(100:10) mixtures contained 1.82 g of a mixture of substances with R_f 0.57, 0.54, 0.46 (green), 0.45 (green), and 0.27 (green) (TLC, system 2).

6-O -(2",3"-Di-O-acyl[acetyl, p-methoxy-trans-cinnamoyl]-α-L-rhamnopyranosyl)aucubin (V). The fraction containing 1.2 g of the substance with R_f 0.57 (green) (TLC, system 2) was rechromatographed on a column of silica gel. Chloroform–methanol (25:1) eluted 1.12 g of a chromatographically pure substance with R_f 0.57, $[\alpha]_{546}^{18}$ −130 ± 2 (c 0.5; methanol); $\lambda_{max}^{CH_3OH}$: 209, 225, 288, 320 nm; ν_{max}^{Nujol}: 1710, 1695, 1635, 1600, 1560, 1500 cm^{-1}. PMR spectrum (250 MHz, CD_3OD, δ, ppm, differentiated signals): 7.45 (1H, d, J = 15.8 Hz, H-7'"), 7.35 (2H, d, J = 9 Hz, H-3'", H-5'"), 6.77 (2H, d, J = 9 Hz, H-2'", H-6'"), 6.22 (1H, d, J = 15.8 Hz, H-8'"), 6.20 (1H, d, J = 6 Hz, H 3), 5.75 (2H, br.s, H-7), 3.65 (3H, br.s. CH_3O–), 2.7-2.9 (2H, m, H-5 and H-9), 1.97 (3H, s, CH_3CO), 1.37 (3H, d, J = 6 Hz, 3H-6"). Mass spectrum, m/z (%): 349 (0.1), 331 (0.6), 289 (0.4), 231 (1.5), 205 (2.4), 189 (4.4), 178 (8.4), 171 (3.0), 161 (11), 154 (9), 148 (9), 147 (10), 134 (50), 133 (6), 120 (17), 119 (20), 106 (20), 105 (30), 97 (36). 71 (60), 57 (100), 43 (100).

The peracetate of glycoside (V), obtained by the usual method [6], was an amorphous substance with R_f 0.89 (tlc, system 1). Mass spectrum, m/z (%): 408 (4), 391 (9), 331 (42), 271 (5), 202 (8), 200 (8), 191 (8), 178 (8), 177 (8), 169 (95), 161 (100), 157 (10), 149 (8), 148 (28), 147 (30), 133 (16).

Alkaline Hydrolysis of Glycoside (V). A solution of 0.2 g of glycoside (V) in 10 ml of 0.05 N caustic soda solution was heated at 50°C for 3 h and was then cooled and was transferred to a column of Sephadex LH-20. The substances were eluted with water, 30-ml fractions being collected. Fractions 1-3 yielded 45 mg of sinuatol (II), and from fractions 5 6, after acidi-

fication with 0.5% hydrochloric acid and extraction with ether, was obtained 16 mg of p-methoxy-trans-cinnamic acid with mp 186-187°C (from methanol), giving no depression of the melting point with an authentic sample of the acid.

Harpagoside. Part of the fraction (4.0 g) containing the two substances with R_f 0.57 and 0.54 (TLC, system 2) was chromatographed on a column of silica gel in the chloroform–ethanol (25:1) system, 40-ml fractions being collected. Fractions 8-10 yielded 1.32 g of a chromatographically individual substance with R_f 0.54 (brown), $[\alpha]_{546}^{20}$ − 49 ± 1° (c 1.1; methanol). PMR spectrum (250 MHz, CD_3OD, δ, ppm): 7.62 (1H, d, J = 16.5 Hz, H-7"), 7.55 (2H, m, Ar), 7.35 (3H, m, Ar), 6.46 (1H, d, J = 16.5 Hz, H-8"), 6.37 (1H, d, J = 6.2 Hz, H-3), 6.12 (1H, br.s H-1), 4.88 (1H, d.d J = 6.5 and 1 Hz, H-4), 4.57 (1H, d, J = 7.5 Hz, H-1'), 3.90 (1H, d.d, J = 12 and 2 Hz), 3.70 (1H, m, H-6), 3.80-3.62 (1H, m), 3.40-3.10 (4H, m), 2.88 (1H, br.s, H-9), 2.23 (1H, d, J = 16 Hz, H-7a), 1.97 (1H, d.d, J = 16 Hz and 4.2 Hz, H-7b), 1.48 (3H, s, 3H-10). In its optical activity and its UV, IR and ^{13}C NMR spectra, the substance was identical with harpagoside (VI).

The acetyl derivative of glycoside (VI), obtained by the usual method [6], had mp 205-206°C (from ethanol–water). Mass spectrum, m/z (%): 339 (0.9), 331 (56), 271 (3.5), 242 (4.4), 229 (3.5), 191 (5.2), 169 (86), 157 (7), 149 (18), 148 (30), 147 (26), 131 (16), 109 (34), 103 (14), 43 (100).

Alkaline Hydrolysis of Harpagoside. A solution of 42 mg of glycoside (VI) in 20 ml of 0.5% caustic soda was heated in the water bath for 2 h. Harpagide was identified in the reaction mixture by TLC (system 2) and PC.

SUMMARY

From the epigeal part of *Verbascum laxum* Filar. et Jav., in addition to sinuatol, the new iridoid glycoside 6-O-(3"-O-p-coumaroyl-α-L-rhamnopyranosyl)aucubin has been isolated. The roots of the plant have yielded as the main components of the iridoid fraction harpagoside and the new iridoid glycoside 6-O-(2",3"-di-O-acyl[acetyl, p-methoxy-trans-cinnamoyl]-α-L-rhamnopyranosyl)aucubin. The presence of aucubin and catalpol in the plant, as well, has been shown chromatographically.

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