IRIDOID GLYCOSIDES OF *Verbascum saccatum*

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An aqueous solution of a methanolic extract of the epigeal part of *Verbascum saccatum* C. Koch (1.4 kg) was washed with organic solvents and chromatographed on columns of alumina, polyamide, and silica gel, leading to the isolation of 3.6 g of a new iridoid glycoside — saccatoside (I) — in the form of an amorphous substance with $[\alpha]_D^{20} - 200 \pm 2.5°$ (c 0.2; methanol). The structure of (I), corresponding to 6-O-(2"-p-coumaroyl-α-L-rhamnopyranosyl)catalpol, was shown on the basis of the spectral characteristics of (I) and of its octaacetyl derivative (II) (mp 99°C, $[\alpha]_D^{20} - 150 \pm 2.5°$) and alkaline hydrolysis to 6-O-α-L-rhamnopyranosylcatalpol (III). In addition to (I), aucubin and (III) were detected chromatographically in the iridoid fraction. Details of the IR, NMR, and ^{13}C spectra of (I) and (II) and of the mass spectrum of (II) are given.

Continuing an investigation of the iridoids of species of *Verbascum* (mullein, family Scrophulariaceae) [1], we have studied a methanolic extract of the epigeal part of the plant *Verbascum saccatum* C. Koch., growing on the territory of the Armenian SSR.

Successive chromatography on columns with polyamide and silica gel of an aqueous solution of the methanolic extract that had been washed with organic solvents and freed from flavonoids with alumina yielded a new iridoid glycoside which we have called saccatoside (I). In the remainder of the iridoid fraction we identified aucubin and 6-O-α-L-rhamnosylcatalpol (II) chromatographically [1, 2].

Saccatoside has the composition $C_{30}H_{38}O_{16}$. Its UV spectrum shows absorption bands characteristic for an enol ether fragment of an iridoid and a cinnamic acid chromophore. The alkaline hydrolysis of saccatoside formed 6-O-α-L-rhamnopyranosylcatalpol (II, composition $C_{21}H_{32}O_{14}$), and p-coumaric acid ($C_9H_8O_3$). Thus, saccatoside is the 6-O-α-L-rhamnopyranosylcatalpol ester of p-coumaric acid. The position of the acyl fragment and, consequently, the structure of saccatoside was deduced on the basis of an analysis of the ^{13}C NMR spectra of this glycoside and of the mass spectrum of its octaacetyl derivative (III).

The mass spectrum of the octaacetate (III) lacked the peaks of high-mass ions from the fragmentation of triacetylrhamnose but showed the presence of ions with m/z 436, 429, and 376, which can be ascribed to triacetyl-p-coumaroylrhamnose and its fragments. At the same time, together with peaks of fragments from the decomposition of p-acetoxycinnamic acid (m/z 189, 161, 147) and of tetraacetyl glucose (m/z 331, 289, 271, 229, 211, 169, 109) and characteristic catalpol ions (m/z 200, 149), the peak of an ion with m/z 223 was observed, which showed the presence of an acetoxy group at C-10 [2-3].

The ^{13}C spectrum of saccatoside (Table 1) confirmed the position of the acyl residue in the rhamnosyl moiety of the molecule and, on the basis of the observation of the α- and β-effects of acylation ($\Delta\alpha$ + 1.63 ppm; $\Delta\beta$ − 3.33 and −1.91 ppm) in comparison with the spectrum of 6-O-α-L-rhamnosylcatalpol [2], permitted the determination of the position of the p-coumaroyl residue at C-2". The structure of saccatoside therefore corresponds to 6-O-(2"-p-coumaroyl-α-L-rhamnopyranosyl)catalpol (I).

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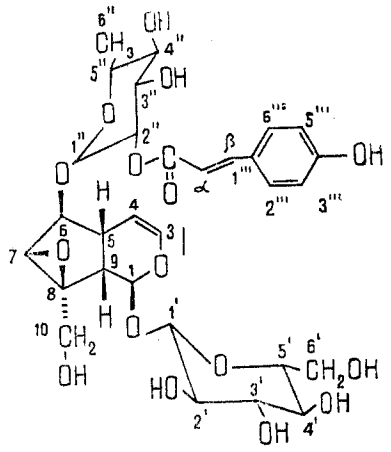
TABLE 1. ^{13}C NMR Spectra of Saccatoside (I) and of 6-O-α-L-Rhamnopyranosylcatalpol (II) [2]

Carbon atom	Chemical shifts, δ, ppm		Carbon atom	Chemical shifts, δ, ppm	
	I	II		I	II
C-1	92,57	93,22	C-1″	95,36	98,99
C-3	139,95	140,39	C-2″	72,27	70,64
C-4	104,16	102,50	C-3″	68,41	70,33
C-5	35,42	35,66	C-4″	72,00	71,87
C-6	81,45	81,49	C-5″	68,71	68,85
C-7	57,11	57,49	C-6″	17,69	17,85
C-8	65,33	65,30	C-1‴	124,94	
C-9	41,73	41,22	C-2‴and6‴	130,34	
C-10	58,58	58,91	C-3‴and 5‴	115,07	
C-1′	97,71	97,91	C-4‴	159,07	
C-2′	73,26	73,45	C-α	145,21	
C-3′	77,30	77,43	C-β	115,69	
C-4′	70,05	70,33	C=O	166,12	
C-5′	76,22	76,45			
C-6′	61,17	61,41			



I

EXPERIMENTAL

UV spectra were taken on a Specord UV-Vis instrument in methanolic solution, IR spectra on a UR-20 instrument (tablets with KBr), the ^{13}C NMR spectrum on a Varian XL-200 instrument (in DMSO), and the mass spectrum on a MKh-1320 spectrometer, and optical activities were determined on a SM-1 instrument. Chromatography was performed on type S [medium] paper (PC, in the butan-1-ol—acetic acid—water (4 : 1 : 5) system) and on Silufol plates (TLC) with chloroform—methanol (8 : 2) and (30 : 1) (systems 1 and 2, respectively). The iridoids were detected on the chromatograms with the benzidine reagent (0.5 g of benzidine, 20 ml of acetic acid, and 80 ml of ethanol) followed by heating at 100°C.

Isolation of the Iridoid Fractions. The dry comminuted leaves and inflorescences of *Verbascum saccatum* (1.4 kg) were steeped with methanol in a percolator (8 × 10 liters). The combined extracts were evaporated in a vacuum evaporation apparatus to a volume of 0.3 liter and were then diluted with water to 0.5 liter and washed successively (4 × 0.25 liter) with benzene, ether, and chloroform. The washed aqueous solution showed (PC) the presence of three substances of iridoid nature. The aqueous solution was passed through a layer of alumina [7.5 cm in diameter and 30 cm high; neutral, activity grade (III)], which was then washed with water until the reaction for iridoids was negative. The combined eluates were evaporated under reduced pressure to a volume of 100 ml, and transferred to a column of polyamide (4.5 × 65 cm), which was then washed with water. Fractions with a volume of 100 ml were analyzed by PC and TLC. The first two fractions contained 69.3 g of a mixture of sugars, glucose, and iridoids with R_f 0.38 (orange spot) and 0.50 (gray spot) (PC) which corresponded in their chromatographic mobilities and coloration to the spots of samples of (II) and aucubin.

Saccatoside (I). Fractions 3-4, containing a single substance with R_f 0.77, were combined and evaporated to dryness, and the residue was chromatographed on a column containing 150 g of silica gel (KSK, 70/230 mesh) in system 1. This yielded 3.6 g of white amorphous saccatoside, $[\alpha]_D^{20} -200 \pm 2.5°$ (c 0.2; methanol); $\lambda_{max}^{C_2H_5OH}$, nm: 206, 222, 312; ν_{max}, cm^{-1}; 3200-3600 (OH), 1700 (C—O), 1655, 1540, 1630 (C—C), 1515 (ArH). Found, %: C 54.98; H 6.00. $C_{39}H_{38}O_{16}$. Calculated, %: C 55.04; H 5.95.

Saccatoside Octaacetate (III). A mixture of 57.4 mg of (I), 1.5 ml of anhydrous pyridine, and 1.5 ml of freshly distilled acetic anhydride was kept at room temperature for 24 h, and then 50 ml of water was added, and after the mixture had been allowed to stand for an hour the resulting amorphous precipitate was filtered off, washed on the filter with water, and dried in a desiccator over phosphorus pentoxide. The dried substance was dissolved in 5 ml of system 2 and chromatographed on a column of silica gel (2 cm × 60 cm) in system 2. This gave 63 mg of (III) with mp 99°C (from ethanol), R_f 0.80 (PC), 0.48 (TLC, system 2); $\lambda_{max}^{CHCl_3}$ 1730 cm^{-1} (broad band), $[\alpha]_D^{21} -150 \pm 2.5°$ (c 0.2; $CHCl_3$). Mass spectrum, m/z (%): 436(6), 419 (3), 376 (18), 331 (30), 289 (5), 279 (9), 271 (3), 242 (20), 239 (6), 229 (5), 223 (9), 215 (8), 211 (7), 200 (35), 195 (7), 189 (25), 169 (55), 163 (20), 161 (10), 157 (75), 153 (14), 149 (100), 147 (50), 146 (28), 145 (25), 140 (32), 131 (36), 129 (20), 121 (24), 115 (80), 111 (30), 109 (30).

Alkaline Hydrolysis of Saccatoside. A solution of 0.98 g of saccatoside in 4 ml of a 0.05 N solution of potassium hydroxide was heated at 30°C for 4 h and was then cooled and was transferred to a column of Sephadex LH-20 (2 × 30 cm), and the column was washed with water, 30-ml fractions being collected. Fractions 1-3 yielded 0.26 g of 6-O-α-L-rhamnopyranosylcatalpol with R_f 0.38 (PC), 0.30 (TLC; ethanol—chloroform (1 : 1) system), $[\alpha]_D^{20} -134 \pm 2.5°$ (c 0.5; ethanol); identified by a direct PC and TLC comparison with a sample of (II) and by a comparison of UV, PMR ^{13}C NMR, and mass spectra.

By acidification with 0.1% hydrochloric acid and extraction with ether, fractions 4-6 yielded 10 mg of p-coumaric acid with mp 210-213°C, melting without depression in admixture with an authentic sample.

The mass and PMR spectra were taken by R. Grigoryan, A. P. Engoyan, and K. S. Lusaranyan.

SUMMARY

It has been established that the epigeal part of *Verbascum saccatum* C. Koch. contains aucubin, 6-O-α-L-rhamnopyranosylcatalpol, and a new iridoid glycoside — 6-O-(2"-p-coumaroyl-α-L-rhamnopyranosyl)catalpol.

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