IRIDOID GLYCOSIDES OF Verbascum lumn

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The epigeal part of Verbascum lumn 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), [α]D20 = -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-0-acyl[acetyl, p-methoxy-trans-cinnamoyl]-α-L-rhamnopyranosyl) aucubin (V) [α]D20 = -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 lumn Filar. ét 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-10-0 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 13C 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-coumaryl 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...
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 diacetyl methoxycinnamoyl rhamnopyranose. 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 acetyl methoxycinnamoyl deoxy rhamnopyranose. 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 6-0-2',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 6-0-(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 harpagide. Harpagide 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 butanol-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 x 10 liters). The combined methanolic extracts were concentrated to a volume of 0.5 liter.