NIVYASIDE — A NEW GLYCOSIDE FROM *Leucanthemum vulgare*

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From the ligulate flowers of *Leucanthemum vulgare* Lam. growing on the territory of the Georgian SSR a new glycoside has been isolated which has been called nivyaside and has the structure 8-(1-α-D-glucopyranosyl-5-deoxyquercit-5-yl)-4',5,7-trihydroxyflavone.

From an aqueous ethanolic extract of the ligulate leaves of *Leucanthemum vulgare* Lam. S., *Chrysanthemum leucanthemum* L., oxeye daisy, [Russian name: nivyanik obyknovennyi], family Asteraceae (Compositae) which grows widely in the territory of the Georgian SSR, the main constituent — the flavonoid glycoside nivyaside — has been isolated [1].

Nivyaside (I), $C_{27}H_{30}O_{14}$, mol. wt. 578.53; mp 238-240°C (decomp.) $[\alpha]_D^{20}$ −59.0° (c 1.0; 40% ethanol), yellow acicular crystals giving specific reactions for flavonoids [1, 2]. In the UV spectrum, $\lambda_{max}^{C_2H_5OH}$ 270, 335 nm (log ε 24.29,4.33). The IR spectrum shows absorption bands characteristic for hydroxy groups (3500-3100 cm^{-1}), for the carbonyl of a γ-pyrone ring (1660 cm^{-1}), for aromatic rings (1635, 1605, 1520 cm^{-1}), and others.

In the PMR spectrum of compound (I) taken in deuterodimethyl sulfoxide, there are signals corresponding to the protons of ring B. In the region of resonance of the H-6 and H-8 protons, on comparison with the spectrum of the aglycone (II), only one signal, corresponding to the H-6 proton, is observed [3, 4]. At δ 5.0 ppm there is a broadened singlet of the anomeric proton of a carbohydrate substituent. Such a signal is characteristic for the α anomer of a sugar residue of the D series having the C1 conformation [5]. In the strong field part of the spectrum, with its center at δ 3.25 ppm, is located a broadened singlet of the two protons of a methylene group.

In the carbohydrate part of an acid hydrolysate of substance (I), D-glucose was detected on PC by the aniline phthalate reagent. The results of quantitative acid hydrolysis by Kiliani's method [6], with a 38% yield of the aglycone, and of a comparison of the intensities of absorption in the UV spectrum of the aglycone (II) and of the glycoside (I) indicate a biosidic or diglycosidic nature of substance (I), but the integral intensity in the PMR spectrum of the signal at δ 3.4-4.2 ppm corresponding to a sugar residue shows only eleven protons in it, which excludes the presence of two G-glucose residues.

For the second substituent, a search for aliphatic and aromatic acids [7] gave no satisfactory results. GLC analysis showed the presence of D-glucose and of a hexitol.

Polyhydric alcohol derivatives of cyclohexane — L-viburnitol, L-quercitol, L-leucanthemitol, and L-inositol — have been detected in the leaves of *Leucanthemum vulgare* growing in Spain [8, 9]. It is known that glucosides containing viburnitol in the sugar moiety undergo enzymatic hydrolysis while glycosides containing its isomer quercitol are not hydrolyzed under these conditions.

The GLC analysis of the carbohydrate moiety of substance (I), and also qualitative reactions (see the Experimental part), an absorption band in the 840 cm^{-1} region of the IR spectrum, a two-proton signal in the PMR spectrum at δ 3.25 ppm, and the fact that glycoside (I) does not undergo enzymatic hydrolysis, shows the presence in it of a residue of the pentahydroxyhexitol quercitol [10-13].

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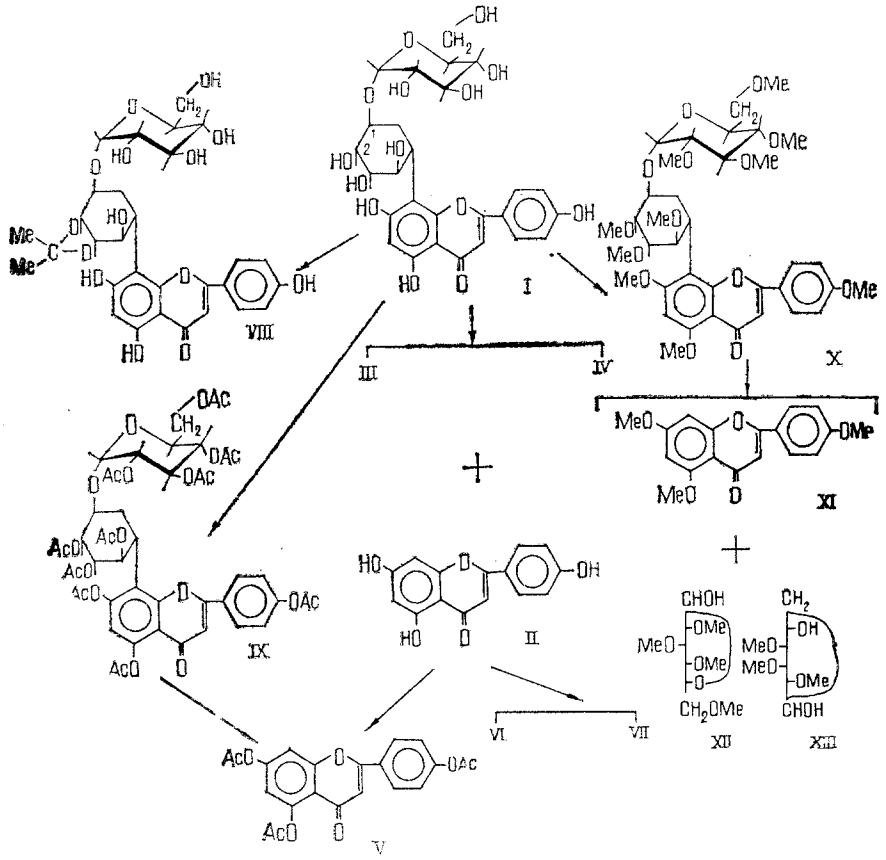
From its physicochemical properties and degradation products (p-hydroxybenzoic acid (VI) and phloroglucinol (VII)), the aglycone (II) was characterized as apigenin [14].



Chemical transformation of nivyaside

Under the usual conditions, substance (I) was not cleaved either by dilute solutions of acids or by enzyme preparations from the grape snail, or by rhamnodiastase; the action of a 0.5% aqueous solution of caustic potash did not cause structural changes. The oxidation of the glycoside (I) with perhydrol [15] in an ammoniacal medium and stepwise acid hydrolysis did not lead to the formation of a biose and an intermediate product. All this is possibly connected with the C-glycosidic nature of the glycoside (I) and the presence of a quercitol residue in its molecule.

A comparison of the UV spectra of the glycoside (I) and its aglycone (II) showed that in both cases there were three free phenolic hydroxyls — at C-5, C-7, and C-4'; this was also confirmed by a study of the products of hydrolysis (V and XI) of the methylated (X) and of the acetylated (IX) glycoside. On the basis of what has been said above, only the C-8 position of the glycoside (I) remained as the position of attachment of the sugar residues in the form of a biose.

The sequence of linkage of the carbohydrate moiety to the aglycone was elucidated by the exhaustive methylation of the glycoside under investigation. 2,3,4,6-Tetra-O-methyl-D-glucose (XII) was found in a hydrolysate of the methylated product (X), which shows the terminal position of the glucose residue in the biose molecule. In this case, the hexitol must be directly attached to the aglycone by C—C bond.

The lack of substitution of the vicinal C_2 and C_3 hydroxyls in the hexitol were shown by the formation of the acetonide (VIII) [16] and only the 5'' position, as the most suitable for nucleophilic attack, remained for attachment.

The stability of the glycoside (I) to the grape snail enzyme shows not only its α configuration but also the absence of an ordinary 1 → 6 bond between the sugar components, which is possibly caused by the presence of the sugar alcohol [17]; a well-defined signal in the IR spectrum at 840 cm^{-1} confirmed this hypothesis. The presence of three bands at 1098, 1070, and 1040 cm^{-1} shows the pyranose form of the D-glucose residue [17, 18].

On the basis of what has been said above, as the most probable structure for the glycoside (I) under investigation we propose 8-(1-α-D-glucopyranosyl-5-deoxyquercit-5-yl)-4', 5,7-trihydroxyflavone.

EXPERIMENTAL

IR spectra were taken on a UR-20 instrument in paraffin oil, UV spectra on SF-4 spectrophotometer, and PMR spectra on a JNM-4H-100 spectrometer (in DMSO) with HMDS as internal standard (δ scale). Melting points were determined on a Kofler block. The GLC analysis of the monosaccharides of the glycoside in the form of the corresponding polyol acetates was performed on a Chrom-41 chromatograph with an FID detector in a metal column (1200 mm × 3 mm) filled with Chromaton NAW impregnated with 5% of silicone XE-60. The temperature of the column was 210°C and that of the evaporator 270°C and the rate of flow of carrier gas (helium) was 50 ml/min; D-glucose and quebrachitol* were used as standards. For monitoring and for the identification of substances by paper chromatography we used the following solvent systems: 1) 5% acetic acid; 2) butanol—acetic acid—water (4:1:2); 3) ethyl acetate—acetic acid—water (18:7:8); 4) butanol—ethanol—water (5:1:4); 5) benzene—ethyl acetate—acetic acid (23:5:74.5:2); and 6) methyl ethyl ketone—chloroform (1:1). As revealing agents we used: a 1% solution of aluminum chloride (flavonoids); an ammoniacal solution of silver nitrate, a 1% solution of potassium permanganate in 1% sulfuric acid, and benzidine—potassium periodate solution (sugar alcohols); and aniline phthalate (reducing sugars).

Molecular weights were determined by the spectrophotometric method.

Isolation. The air-dry ligulate leaves of *Leucanthemum vulgare* L. (0.480 kg) were extracted with 80% methanol. The alcohol was distilled off from the extract and the aqueous residue was purified with chloroform. The substances depositing from the aqueous phase under these conditions were separated off and were recrystallized from aqueous ethanol. This gave 7.6 g of substance (I). Nivyaside forms yellow acicular crystals soluble in ethanol and dimethyl sulfoxide, slowly and sparingly soluble in 70% and 95% ethanols, and practically insoluble in chloroform, ethyl ether, and petroleum ether. $C_{27}H_{30}O_{14}$, mol. wt. 578.53; mp. 238-240°C (decomp.), $[\alpha]_D^{20}$ −59.0° (c 1.0; 40% ethanol). R_f 0.12 and 0.55 in systems 1 and 2, respectively.

UV spectrum, nm: $\lambda_{max}^{C_2H_5OH}$ 270, 335 (log ε 4.29; 4.83); (+NaOAc) 270, 340; (+$AlCl_3$) 275, 350; ($AlCl_3$ + HCl) 275, 335; (+NaOMe) 260, 395.

IR spectrum, cm^{-1}, ν_{max}: 3500-3100 (—OH stretching vibrations); 1660 (=C=O), 1635, 1605, 1520 (—C=C—); 840 (=CH_2), and others.

PMR spectrum, δ, ppm: 7.72 (d, J = 8 Hz, H-2',6'); 6.80 (d, J = 8 Hz, H-3',5'); 6.65 (s, H-3); 6.31 (s, H-6); 5.00 (broadened singlet, H'[sic]); 3.100-4.2 (sugar protons and —CH_2— group; 12.86 (broadened singlet, —OH at C-5).

Quantitative Kiliani Acid Hydrolysis [6]. An accurately weighed sample (95 mg) of (I) was boiled with 10 ml of Kiliani's mixture on the water bath under reflux, the course of the reaction being monitored by PC in systems 1 and 2. Hydrolysis was complete in 6 h. The aglycone that deposited when the reaction mixture cooled was filtered off and washed with water to neutrality. The aglycone still remaining in the hydrolysate was extracted with ethyl acetate, the solution was washed with water to neutrality and evaporated, and the residue was added to the aglycone that had deposited. This gave 36 mg of the aglycone (II).

The aglycone (II): $C_{15}H_{10}O_5$, mp 340-345°C, R_f 0.89, 0.69 in systems 2 and 5, respectively. UV spectrum, nm: $\lambda_{max}^{C_2H_5OH}$ 269, 336 (log ε 4.10; 4.13); (+NaOAc) 280, 350; (+$AlCl_3$) 275, 380; (MeONa) 275, 400.

IR spectrum, cm^{-1}, ν_{max}: 3300, 1655, 1600, 1230, 1160.

PMR spectrum (δ, ppm): 7.78 (d, J = 8 Hz, H-2',6'); 6.85 (d, J = 8 Hz, H-3',5');

*The quebrachitol was kindly provided by N. F. Kommissarenko.

6.68 (s, H-3); 6.48 (d, J = 2 Hz, H-8); 6.21 (d, J = 2 Hz, H-6).

The hydrolysate remaining after the separation of the aglycone was neutralized with EDE-10P anion-exchange resin (OH^- form), concentrated, and subjected to PC analysis in systems 2, 3, and 4 with the appropriate reagents and, as acetate derivatives, to GLC analysis. As a result, the presence of D-glucose (III) and of quercitol (IV) was detected [10-13].

Acetylation of (II). A solution of 45 mg of (II) in 2 ml of acetic anhydride was treated with five drops of concentrated H_2SO_4; after 5 min, the mixture was diluted with water and extracted with ethyl ether. The ethereal extract was concentrated, and the residue was recrystallized from ethanol. This gave 61 mg of white acicular crystals of (V) with mp 180-183°C, corresponding to 4',5,7-tri-O-acetylapigenin [19].

Alkaline Fusion of (II). A solution of 20 mg of (II) in 10 ml of 20% KOH was boiled at 130°C for 1.5 h. The pH of the cooled solution was brought to 4-5 with 10% H_2SO_4. The degradation products were extracted with ethyl ether. The extracts were evaporated and the residue was dissolved in 1 ml of ethanol and chromatographed on paper in systems 1 and 5. This showed the presence of p-hydroxybenzoic acid (VI) and of phloroglucinol (VII).

Preparation of the Acetonide (VIII). A solution of 10 mg of substance (I) in anhydrous acetone was treated with 500 mg of anhydrous copper sulfate and the mixture was boiled under reflux for 4 h. The course of the reaction was monitored by PC analysis in system 2; a less polar product than the initial glycoside, with R_f 0.28, was obtained.

Acetylation of (I). The acetylation of 50 mg of substance (I) was performed in a similar manner to that of the aglycone (II). This gave 79 mg of the acetate (IX) with mp 190-192°C. After its hydrolysis with Kiliani's mixture, the acetylated aglycone (V) was obtained, and by PC analysis and a melting point determination it proved to be identical with 4',5,7-tri-O-acetylapigenin [19].

The Kuhn Methylation of (I) [20]. Compound (I) (100 mg) was dissolved in 3 ml of dimethyl formamide that had been freshly distilled over phosphorus pentoxide and had been preheated to 40°C, 3 ml of methyl iodide was introduced and then, with stirring, 1 gram of silver oxide was added in small portions over 30 min. After 16 h, another 2 ml of methyl iodide and 0.5 g of silver oxide were added to the reaction mixture. The completeness of methylation was monitored in system 6. After the end of the reaction, the unchanged silver oxide and the silver iodide were filtered off. The precipitate was washed on the filter with chloroform and the washings were added to the filtrate. The liquid was washed with a saturated solution of sodium thiosulfate and with water, and the solvent was distilled off. The product obtained (64 mg) was purified on a column of silica gel. The column was eluted successively with benzene and mixtures of benzene and ethanol with increasing concentrations of ethanol of from 1% to 10%. The course of the separation was monitored by PC analysis in system 6. Fractions with a volume of 20 ml each were collected.

The completely methylated product (X) was eluted in fractions 27-31. After the solvent had been distilled off, the residue was hydrolyzed with Kiliani's mixture [6] which gave the methylated aglycone (XI) with mp 153-158°C, identical with 4',5,'7-tri-O-methylapigenin [19].

In the aqueous filtrate after neutralization with EDE-10P anion-exchange resin (OH^- form) and chromatography in system 2, a substance (XII) was detected the mobility of which was identical with that of the 2,3,4,6-tetra-O-methyl-D-glucose obtained from methylated astragalin [21].

SUMMARY

From the ligulate flowers of *Leucanthemum vulgare* Lam. a new flavonoid C-glycoside has been isolated; it has been called nivyaside and its probable structure has been established as 8-(1-α-D-glucopyranosyl-5-deoxyquercit-5-yl)-4',5,7-trihydroxyflavone.

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IRIDOIDS OF *Verbascum georgicum*

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From the epigeal part of *Verbascum georgicum* Benth., in addition to aucubin, as the main component of the total iridoids a new iridoid has been isolated — 6-α-L-(4'-p-methoxy-trans-cinnamoyl)rhamnopyranosylcatalpol, the structure of which has been shown by UV, PMR, and mass spectroscopy and a comparison of the ^{13}C NMR spectrum with that of 6-α-L-rhamnopyranosylcatalpol (I). The presence of catalpol and (II) in the plant has been shown by PC and TLC.

The species of mullein *Verbascum georgicum* Benth. [1], which is widespread in the mesophytic grasslands of many regions of the Armenian SSR has not been studied chemically. By a qualitative chromatographic analysis of a methanolic extract of the epigeal part of this plant we detected in it the presence of at least five substances of iridoid nature. Two of them, forming the main components of the total iridoid material, have been isolated and characterized.

The first substance was identified by PMR and mass spectroscopy and from the constants of its acetyl derivative as the known iridoid aucubin, which is characteristic for *Verbascum* species [2]. The second substance, with the composition $C_{31}H_{40}O_{16}$, proved to be new and has been called verbascoside A (I). The iridoid nature of (I) was shown by qualitative reactions [3] and by absorption at 206 nm in the UV spectrum which is characteristic for an enol ether

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