# TRITERPENE GLYCOSIDES OF GYPSOPHILLA TRICHOTOMA

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Khimiya Prirodnykh Soedinenii, Vol. 6, No. 4, pp. 434–438, 1970

UDC 547.918+544.597

We studied the triterpene glycoside content in various vegetative organs of Gypsophilla trichotoma Wend. (threefork gypsophila), family Caryophyllaceae, collected in the Tashkent experimental section of the Botanical Institute of AS UzSSR.

Eight substances were found in a methanolic extract of the roots by thin-layer chromatography (TLC) on silica gel. Four of them were apparently oligosaccharides, since on hydrolysis only glucose was found. The other four, less polar substances, proved to be glycosides, and we have called them "trichosides" A, B, C, and D (in order of increasing polarity). The results of a study of the various organs of the plant are given below.

| Plant organ | Total extractive substances (% on the air-dried raw material | Glycoside |
|---|---|---|
| Roots (seven-year) | 65 | A, B, C, D |
| Roots (four-year) | 42 | A, B, C, D |
| Stems | 13 | Not detected |
| Leaves | 8 | A, D |
| Flowers | 9 | A |
| Fruit | 2 | D |

Trichosides A, B, and C are found in very small amounts in all the organs of the plant. The amount of trichoside D is the highest, and this was isolated in the individual state by the chromatography of a methanolic extract of the roots on silica gel. Trichoside D, and also its acetate, did not rotate the plane of polarization of light within the limits of error of the determination.

The acetylation of trichoside D was difficult. Even after repeated treatment of the substance with acetylating agents under various conditions an acetate showing a weak hydroxyl band in the IR spectrum was obtained.

To identify the aglycone and elucidate the qualitative composition of the sugars, we hydrolyzed trichoside D with acids of different concentrations. Hydrolysis also took place with some difficulty. The most satisfactory results were given by the treatment of the glycoside with Kiliani's mixture. In this way it was established that the carbohydrate moiety of trichoside D contains D-galactose, D-glucose, D-xylose, L-arabinose, L-rhamnose, D-fucose, and D-glucuronic acid.

As far as the aglycone was concerned, the precipitate that deposited was found to contain a group of substances whose number varied according to the hydrolysis conditions. The chromatography of this mixture yielded gypsogenin.

The formation of a number of substances in place of a single aglycone on acid hydrolysis, although this is characteristic for gypsogenin because of its lability, nevertheless cast doubt on the individuality of the glycoside. In order to elucidate this, we reduced trichoside D with sodium borohydride, making an attempt to reduce the aldehyde group, whose presence is one of the main reasons for the lability of gypsogenin, to an alcohol group.

In fact, the hydrolysis of trichosidol D (the product of the reduction of trichoside D) led to hederagenin contaminated with an extremely small amount of its lactone. Therefore, the aglycone of trichoside is gypsogenin.

In order to determine the degree of branching of the carbohydrate chains, trichoside D was oxidized with sodium periodate. The hydrolysate of the oxidized product was found to contain D-fucose, L-rhamnose, and D-glucuronic acid. Consequently, these sugars are either centers of branching or have substituents on the hydroxyls at $C_3$.

The localization of the carbohydrate chains of trichoside D and their qualitative monosaccharide composition were determined by alkaline hydrolysis. A saponified glycoside (the progenin of trichoside D) without an acyloside chain was obtained and in its acid hydrolysate D-galactose, D-xylose, L-arabinose, and D-glucuronic acid were detected. The hydrolysate of the acyloside oligosaccharide that had been split off gave D-glucose, D-galactose, D-xylose, and L-rhamnose. The absence of D-fucose from the hydrolysate can be explained by the assumption that it was attached directly to the carboxyl of the gypsogenin and underwent degradation on alkaline hydrolysis.

We carried out acid and alkaline hydrolysis and also periodate oxidation with trichosidol D and obtained similar results.

Thus, trichoside D is a new gypso genin glycoside; it has two carbohydrate chains. The chain attached to the hydroxyl at $C_3$ has at least four sugars, and the acyloside chain has at least five sugars, i.e., trichoside D contains at least nine sugars.

In the structure of the carbohydrate chains, trichoside D differs from other gypsogenin glycosides, gypsoside [1] and acanthophylloside B [2], in which the carbohydrate moiety has the same qualitative composition. At the same time, both trichoside D and the product of its reduction, trichosidol D, migrate on TLC (silica gel) in various solvent systems at the same level as gypsoside and acanthophylloside B. Consequently, the chromatographic comparison of triterpene glycosides with large sugar chains is inadequate for their identification and such results must be treated with great caution.

## EXPERIMENTAL

Chromatography was carried out on "M" paper of the "Goznak" Leningrad Mill and silica gel of types KSK and ShSK and the following solvent systems: 1) butan-1-ol–acetic acid–water (4 : 1 : 5); 2) butan-1-ol–acetic acid–water (4 : 1 : 1), 3) butan-1-ol–ethanol–25% ammonia (7 : 2 : 5), 4) butan-1-ol–ethanol–25% ammonia (10 : 2 : 5), 5) chloroform–ethanol (25 : 1), 6) benzene–ethanol (10 : 1), and 7) chloroform–methanol (25 : 0.5). The sugars were revealed with aniline phthalate, and the glycosides and aglycones with phosphomolybdic acid.

**Isolation of the saponins.** The comminuted roots were exhaustively extracted, first with cold and then with hot methanol. In the investigation of the epigeal organs of the plant, they were preextracted with ether. The combined extracts were dried at 40° C in vacuo and precipitated with acetone. The residue was dissolved in methanol and again precipitated with ether. The residue was found by TLC on KSK silica gel in systems 1 and 3 to contain eight substances: A, B, C, D, E, F, G, and H (in order of increasing polarity). The yields of total extractive substances and information on the presence of the glycosides are tabulated at the beginning of this paper.

**Separation of the total glycosides.** The dry material (16 g) was chromatographed on a column of KSK silica gel (1400 g) in system 4. The amount of glycosides in the fractions were monitored by TLC in systems 2 and 3. Fractions containing substances AB, ABC, BCD, DE, DEF, EF, FG, and GH were isolated.

Fractions EF and GH were evaporated to dryness (each separately) and hydrolyzed with 5% $H_2SO_4$ with heating for 3 hr. No precipitate deposited and glucose was found in the hydrolysates by paper chromatography in systems 1 and 2.

**Isolation of trichoside D.** The combined fractions with substances DE and DEF were rechromatographed on a column of KSK silica gel in system 4. Monitoring was carried out by TLC in systems 2 and 3. Fractions containing the individual glycoside were evaporated to dryness and the residue was dissolved with heating in water-saturated butanol. On cooling, a white precipitate of trichoside D was formed with mp 231–235° C, $[\alpha]_D^{20}$ 0° (water), readily soluble in water, less readily in ethanol.

**Trichoside D acetate.** The glycoside (0.4 g) was acetylated in a mixture of 5 ml of pyridine and 5 ml of acetic anhydride at room temperature for 2 days. After the reaction mixture had been decomposed with water, the dried residue was reacetylated under the same conditions. In a third stage, the precipitate was heated to 90° C in acetic anhydride containing sodium acetate for 2 hr and was left for a day at room temperature. The mixture of acetates was chromatographed on a column of ShSK silica gel (1 : 100), first with chloroform and then with the addition of ethanol (from 1–50%). The fractions were monitored by TLC on KSK silica gel in system 5. Several substances were isolated. The least polar of them had an ill-defined peak at 3400 $cm^{-1}$ (IR spectrum) and mp 138–144° C $[\alpha]_D^{20}$ 0° (chloroform).

**Acid hydrolysis of trichoside D.** One gram of the glycoside was heated in 40 ml of Kiliani's mixture [acetic acid–

water (35 : 10 : 55)] at 100° C for 6 hr. The completeness of hydrolysis was checked by TLC in silica gel in systems 2 and 3. The reaction mixture was diluted with a twofold amount of water. The precipitate was washed, dried, and chromatographed on a column of ShSK silica gel (1 : 100), first with chloroform and then with the addition of methanol (from 1–50%). A substance with mp 268–270° C was isolated whose physicochemical properties and chromatographic behavior (systems 5–7) were identical with those of an authentic sample of gypsogenin.

By paper chromatography in systems 1 in the presence of markers the aqueous fraction was found to contain D-glucose, D-galactose, D-xylose, L-arabinose, D-fucose, L-rhamnose, and D-glucuronic acid.

**Reduction of trichoside D to trichosidol D.** A solution of 0.8 g of the glycoside in 200 ml of water was gradually treated with 1.6 g of sodium borohydride and the mixture was heated at 60–70° C for 6 hr. Then it was cooled, acidified with acetic acid, evaporated, and chromatographed on a column containing 80 g of cellulose in system 1. The fractions containing the glycoside were freed from mineral impurities on Sephadex G-25 and evaporated. After recrystallization from aqueous butanol, the product of the reduction of trichoside D had mp 226–228° C $[\alpha]_D^{20}$ + 27.4° (c 1.01, water).

**Acid hydrolysis of trichosidol D.** The glycoside (0.1 g) was hydrolyzed under the conditions described for trichoside D. TLC on silica gel in systems 5, 6, and 7 showed the presence of hederagenin and traces of hederagenin lactone. Paper chromatography in system 1 showed the presence of the sugars found for trichoside D.

**Alkaline hydrolysis of trichoside D.** One gram of the glycoside was heated in 30 ml of 10% of NaOH in 70% ethanol at 90° C for 6 hr. Then the reaction mixture was neutralized with dil $H_2SO_4$ and the ethanol was evaporated under vacuum. The residue was made up to 30 ml with water and was extracted with butanol. The butanol extracts were washed with water and evaporated to dryness, and the residue was chromatographed on ShSK silica gel in system 4. The fractions were monitored by TLC in the same system. The progenin of trichoside D with mp 192–195° C (from ethanol), $[\alpha]_D^{20}$ + 39.8° (c 1.0, ethanol) was isolated.

Acid hydrolysis of the progenin yielded gypsogenin and, as identified by paper chromatography in system 1, L-arabinose, D-xylose, D-galactose, and D-glucuronic acid.

The aqueous layer of the alkaline hydrolysate containing the split-off oligosaccharide was evaporated to dryness and chromatographed on a column of ShSK silica gel in system 4. The fractions with the oligosaccharide were evaporated to dryness and the residue was hydrolyzed with 3% $H_2SO_4$ with heating. Paper chromatography in system 1 showed the hydrolysate to contain D-glucose, D-galactose, D-xylose, and L-rhamnose.

**Alkaline hydrolysis of trichosidol D.** The glycoside (0.2 g) was hydrolyzed under the conditions described for trichoside D. A saponified glycoside (the progenin of trichosidol D) was isolated with mp 257–260° C (from ethanol), $[\alpha]_D^0$ + 20.7° (c 1.2, ethanol). The composition of the sugars in the progenin and in the oligosaccharide were the same as for the progenin of trichoside D and its acyloside moiety.

**Determination of the residual sugars after the periodate oxidation of trichoside D.** The glycoside (0.25 g) was oxidized with a 1% solution of sodium periodate (250 ml) at 5° C for a day. The excess periodate was decomposed by the addition of ethylene glycol and the reaction mixture was evaporated in vacuo. Then the residue was treated with 40 ml of 5% $H_2SO_4$ and heated for 5 hr. The hydrolysate was neutralized and purified with barium carbonate, and then paper chromatographed in system 1 to show the presence of D-fucose, L-rhamnose, and D-glucuronic acid.

## CONCLUSIONS

The plant Gypsophilla trichotoma Wend. has been found to contain four triterpene glycosides, which have been called "trichosides" A, B, C, and D.

Trichoside D has been isolated in the individual state. It has been shown that its aglycone is gypsogenin. The carbohydrate chain attached to the hydroxyl at $C_3$ contains D-galactose, L-arabinose, D-xylose, and D-glucuronic acid, and the acyloside chain contains D-glucose, D-galactose, D-xylose, L-rhamnose, and D-fucose.

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15 May 1970

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