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Soladulcamarine, the Alkaloidal Glycoside of *Solanum dulcamara*

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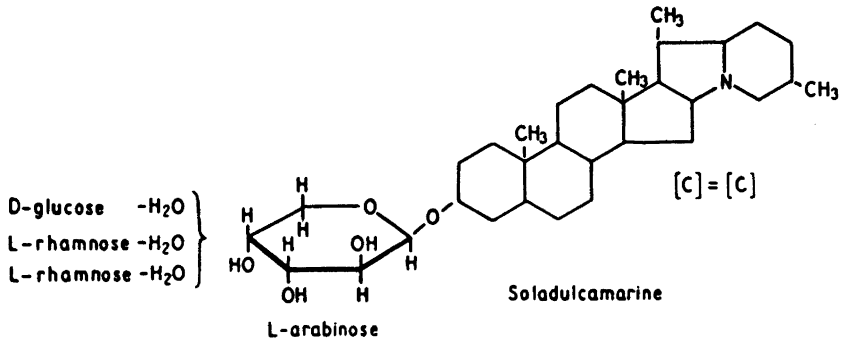
Extraction of the fresh leaves and stems of *Solanum dulcamara* led to the iolation of an alkaloidal glycoside m. p. 193—197°C (decomp.), which on hydrolysis yielded 1 mole of D-glucose, 1 mole of L-arabinose, 2 moles of L-rhamnose and an unsaturated alkaloidal aglycone m. p. 220—222°C. The names soladulcamarine and soladulcamaridine are suggested for the glycoside and its aglycone, respectively. Soladulcamaridine is tentatively assigned a solanidane structure.

Recently J. Tuzson [1], P. Tuzson and Kiss [2], and McKee [3] by paper chromatography have shown that the alkaloids in *Solanum dulcamara* and *Solanum turberosum* are not identical. P. Tuzson and Kiss[2] isolated from the latter plant, after acid hydrolysis, a steroidal saturated alkaloidal aglycone. They proposed the name soladulcidine for the aglycone, a name already in use to identify a bitter principle occurring in the same plant [4].

Schreiber [4] by paper chromatography found three alkaloidal glycosides and has shown all three glycosides to have soladulcidine as aglycone. Schreiber gives the empirical formula as $C_{27}H_{43}O_2N$ (m.p. 206.5° and $[\alpha]_D = -52.6°$ ($CHCl_3$)). Soladulcidine was found to be identical with 5α-solasodanol-(3β).

Our investigation has by paper chromatography indicated two alkaloidal glycosides to be present in *S. dulcamara*. The one of them is considered as the main alkaloid and has been isolated. It is neither solanine nor another known alkaloid, but a new alkaloidal glycoside for which we suggest the name *soladulcamarine*. The aglycone, also a new compound, has been designated *soladulcamaridine*.

The plant material used in this investigation consisted of naturally occurring plants. Only the tops were used. After crushing, the tops were extracted with 5 % acetic acid and the alkaloid together with inorganic salts were precipitated with ammonia-water. The yield was 0.3 g of crude alkaloid per kg of fresh material.

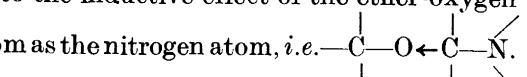


The alkaloid mixture was to some extent purified by column chromatography. Unfortunately, recrystallization of the alkaloid from various solvents was not possible as the alkaloid separated out as a gel in every attempt, but a chromatographically pure semi-crystalline alkaloidal glycoside was obtained through fractional crystallization.

By acid hydrolysis the glycoside was found to consist of an aglycone portion, soladulcamaridine, and a tetrasaccharide moiety composed of D-glucose, L-arabinose and two D-rhamnose units. The isolation from the plant genus *Solanum* of an alkaloidal glycoside containing L-arabinose has not been reported before. Soladulcamarine does not reduce Fehling's solution or react with phenylhydrazin. These results indicate that no free aldehyde groups are present and that the monosaccharides are united through their potential aldehyde groups. Oxidation of soladulcamarine with periodic acid revealed after hydrolysis and paper chromatography only L-arabinose. Since periodic acid oxidizes vicinal hydroxyl groups and the L-arabinose after oxidation still is attached to the alkaloidal aglycone, it can be concluded that the glycoside besides being an arabinoside has the hydroxyl groups of arabinose linked through glycosidic linkages to the monosaccharides in such a manner that arabinose has no vicinal hydroxyl groups. Soladulcamarine is not hydrolyzed when treated with 0.01 N hydrochloric acid for 10 h as shown by the lack of reducing power towards Fehling's solution. From this it is assumed that all the sugars are pyranose [5]. All *Solanum* alkaloids so far isolated have been shown to have the hydroxyl group of the aglycone connected to the sugar unit through a β-linkage. From biogenetic reasons it is assumed that an identical linkage also is present in soladulcamarine. The glycoside was found to have the empirical formula $C_{50}H_{81}O_{18}N$, 5 H_2O (M 1 074) and the equivalent weight was determined to 1 087 (titration in glacial acetic acid with perchloric acid) and 1 165 (acid/base titration). The aglycone has the empirical formula $C_{27}H_{43}ON$, 1 1/2 H_2O (M 425) and the equivalent weight was determined to 438.

The basicity of soladulcamarine and soladulcamaridine was determined in term of their pK_b-values and found to be 5.57 and 5.78, respectively. Bloom and Briggs [6] have determined the basicity of solanidine and solasodine and their derivatives. They found that the pK_b of solasodine and solasonine were

6.30 and 6.26, respectively, and of solanidine 5.38. They concluded that the weak basicity of solasodine was due to the inductive effect of the ether-oxygen atom attached to the same carbon atom as the nitrogen atom, *i.e.*



The pK_b values for soladulcamarine and soladulcamaridine seem to be closer to the values for solanidine.

All alkaloids isolated from the plant genus *Solanum* have either been solanidane or solasodane derivatives. The results discussed above indicate that soladulcamaridine probably is a solanidane derivative. The aglycone gave positive Alberti and Liebermann-Burchard [8] reactions for a steroid-double bond, but a negative reaction for conjugated double bonds or α,β-unsaturated alcohols [9]. The aglycone gave no precipitate with digitonin and can, therefore, not have a 3β-hydroxyl group. The colour reactions given by the aglycone were not similar to the colours given by solanidine, but closer to the colours given by solasodine.

It was not possible to prepare a picrate of the aglycone, which is in accordance with the difficulty often met in preparing picrates of tertiary amines.

Stipes dulcamara which is an old drug and consists of the stems of *Solanum dulcamara* was examined for alkaloids. The stems gave an extremely small yield of an alkaloidal glycoside. By paper chromatography it was found identical with soladulcamarine. The small yield is in conformity with the fact that the stems mostly consist of xylem and very little green bark.

EXPERIMENTAL

The melting points are corrected and have been determined with the hot stage microscope essentially according to Kofler.

Isolation of the glycoside. The plant material was collected at Lyngby Aamose near Copenhagen. 7 kg of the fresh tops were crushed in a mill and immediately extracted with 5 % acetic acid using the isolation procedure described by Kuhn and Löw [10]. The yield of crude alkaloidal glycoside was 2.2 g. Paper chromatography with following developing solvents: The upper phase of ethyl acetate — glacial acetic acid — water (3:1:3) to which is added 15 ml of 85 % ethanol per 160 ml of upper phase [10], and ethanol — N hydrochloric acid — water (50:2:48) [11] revealed two alkaloids when the spots were developed with Dragendorff reagent. The alkaloid with highest R_F-value was only present to a small extent.

The glycoside mixture formed a gel when attempted to dissolve it in water-saturated 1-butanol. Therefore, a solution in methanol was chromatographed on neutral aluminium oxide (Alcoa) and eluted with the same solvent. In this manner a certain degree of purification was obtained.

It was not possible to recrystallize the glycoside from various solvents due to formation of a gel. The solvents used both as pure solvents and in aqueous solution to attempt recrystallization were: 1-butanol, 1-propanol, ethanol, dioxan, acetone and ethyl ether.

Fractional recrystallization, on the other hand, was more successful. Addition of acetone to a concentrated methanol solution of the glycoside mixture produced an amorphous precipitate which adsorbed the green coloured impurities. In this manner 1.55 g of material was obtained. Addition of petroleum ether to the mother liquor gave 115 mg of faint yellow coloured material. This latter material was redissolved in a small volume of methanol and gave by repeated fractional recrystallization 20 mg of semi-crystalline glycoside. Repeated fractionation of crude glycoside gave some more semi-crystalline glycoside. M. p. 193—197°C (decomp.) (Found: C 55.80, 55.95; H 8.52, 8.79; H_2O 8.73. Calc. for $C_{50}H_{81}O_{18}N$, 5 H_2O: C 55.91; H 8.54; H_2O 8.40).

1 kg of commercially available *Stipes dulcamara* was extracted in the same manner as above. About 2 mg of crude glycoside mixture was obtained. By paper chromatography it was found to be identical with soladulcamarine.

The equivalent weight of the pure glycoside was determined by potentiometric titration of the glycoside dissolved in glacial acetic acid with 0.02 N perchloric acid (Found 1 101, 1 087. Calc. 1 074). The equivalent weight determined by dissolving the glycoside in a known excess of 0.01 N hydrochloric acid and back-titrating with 0.01 N sodium hydroxide was found to be 1 165 and 1 180. The glycoside gave a positive α-naphthol reaction for carbohydrates and a positive orcinol test (Bial reaction) for pentoses [7].

Isolation of the aglycone, soladulcamaridine. 0.5 g of soladulcamarine was refluxed with 25 ml N hydrochloric acid for 2 h. During the hydrolysis the hydrochloride of the aglycone began to precipitate. The hydrochloride was collected by filtration, dissolved in 80 % methanol and concentrated ammonia-water was added to precipitate the free alkaloid. 255 mg alkaloid was obtained. For further purification the alkaloid was dissolved in a small volume of benzene and chromatographed on a column of neutral aluminium oxide (13 × 1.6 cm). Benzene-methanol (9:1) was used as eluant and 30 ml fractions were collected. Fraction III contained the alkaloid and none of the other fractions gave any residue after evaporation of the solvent. The alkaloid was recrystallized from acetone and gave needle-shaped crystals melting at 220–222°C, $[\alpha]_D^{23}$ −78.4°, $[\alpha]_{Hg}^{23}$ −111° (methanol, c = 0.428). Before analysis the compound was dried at 105°C for 8 h. (Found: C 76.19, 76.24; H 10.34, 10.12. Calc. for $C_{27}H_{43}ON$, 1 1/2 H_2O: C 76.36; H 10.92.)

The equivalent weight was determined to 438 and 442 by dissolving the aglycone in a known excess of 0.01 N hydrochloric acid and backtitrating with 0.01 N sodium hydroxide. Soladulcamaridine dissolved in methanol does not give a precipitate with an ethanolic solution of digitonin. The compound gives positive Alberti [7] and Liebermann-Burchard reactions[9] for steroid double bonds, but no Rosenheim reaction [8] for conjugated double bonds or α,β-unsaturated alcohols.

Identification of the carbohydrates. The acidic mother-liquor remaining after precipitation of the aglycone from the hydrolysate as described above was treated with Amberlite IR-4B. Aliquots were chromatographed on Whatman No. 1 paper at 23°C. Aniline hydrogen phthalate was used as colour-developing reagent.

The solvent 1-butanol — ethanol — water (8:1:2) revealed spots with the following R_F-values: 0.43 L-rhamnose, 0.21 L-arabinose, 0.15 D-glucose.

The solvent 2,6-lutidine — water (65:35) revealed spots with R_F-values: 0.68 L-rhamnose, 0.54 L-arabinose, 0.51 D-glucose.

The results were controlled by chromatographing pure monosaccharides alone and in mixtures alongside the unknown monosaccharides.

Quantitative determination of the carbohydrates. Furthermore, the amounts of individual monosaccharides were determined quantitatively utilizing the ability of the sugars to reduce 2,3,5-triphenyltetrazolium chloride to red formazan. 2.4 mg of glycoside was refluxed in 0.5 ml N hydrochloric acid for 2 h (sealed tube).* Aliquots containing 98 μg hydrolysed glycoside were chromatographed and the amount of monosaccharide in each spot determined by the method of Fischer and Dörfel [12].

The results are given in Table 1.

Table 1. Quantitative determination of monosaccharides in 98 μg aliquots of hydrolysed soladulcamarine.

	μg	mole × 10^8	μg	mole × 10^8
L-Glucose	16	8.9	15.5	8.6
D-Rhamnose	28	17	30.5	19
L-Arabinose	12	8.0	9.0	6.0

* It was found unnecessary to neutralise the hydrolysate with Amberlite IR-4B before chromatographing. If the paper was well dried at room temparature after application of the solution to the paper no change in the R_F-values was observed.

The optical rotation of the carbohydrate mixture. 0.32 g of soladulcamarine was hydrolysed by refluxing with 20 ml N sulfuric acid for 2 h. The hydrolyzate was neutralized with barium carbonate and allowed to stand overnight. The precipitate was removed by filtration and carefully washed with water. The filtrate was concentrated to 10.0 ml *in vacuo*. The rotation found was $[\alpha]_D^{23}$ + 41.8° on the assumption that 1 074 g of glycoside gave 654 g of carbohydrate. For a mixture of 1 mole of D-glucose, 1 mole of L-arabinose and 2 moles of L-rhamnose the optical rotation can be calculated to $[\alpha]_{Hg}^{25}$ +43.4. The carbohydrate content of the hydrolysate was determined after the method of Rupp and Lehmann [13]. 585 g of carbohydrate was found, calculated as glucose per mole of glycoside. The theoretical value is 654 g of "glucose".

Periodate-oxidation of the glycoside (Kuhn and Löw [10]). 20 mg of soladulcamarine was allowed to stand for three days at room temperature with 4 ml 0.05 N acetic acid and 2 ml 0.25 N potassium periodate. After addition of 1 ml ethylene glycol the solution was heated shortly, made alkaline with solid sodium carbonate and then heated again to 50—60°C. The precipitate was collected by centrifugation, washed with water and then boiled with 2 ml N sulfuric acid. The sulfuric acid was neutralized with barium carbonate and the sugar solution separated from barium sulfate by filtration. After evaporation of the water the residue was chromatographed. Only L-arabinose was detected.

Colour reactions of soladulcamaridine [14]. 2—3 mg of soladulcamaridine and 2—3 mg of the following aldehydes were dissolved in about 1 ml of acetic acid to which 1 ml of conc. sulphuric acid was then carefully added. By mixing and heating following colour changes did develop:

p-*hydroxybenzaldehyde:* red/violet dichroic-purple-blue-violet/brown dichroic.
anisaldehyde: red/green dichroic — violet/green dichroic — blueviolet.
vanillin: green — blue — violet.
resorcinol: yellow — brown — olive — blue — violet — red/violet dichroic — fuchsin — cherry red — cherry red / chocolate fluorescent.

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