Alkaloidal Glycosides from *Solanum dulcamara*

IV *. The Constitution of *β*- and *γ*-Solamarine **

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Acid hydrolysis of the alkaloidal glycoside *β*-solamarine gives rise to one mole of *Δ⁵*-tomatidenol-(3*β*), one mole of D-glucose, and two moles of L-rhamnose. Permethylation of the glycoside followed by acid hydrolysis results in the isolation of 3,6-di-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-L-rhamnose. Analyses of the molecular rotations of products of partial hydrolysis of *β*-solamarine indicates the configuration of the glycosidic linkages. Thus *β*-solamarine has the structure (I).

The other glycoside, *γ*-solamarine, also has *Δ⁵*-tomatidenol-(3*β*) as the aglycone, and the carbohydrate component is formed from one mole of D-glucose and one mole of *β*-rhamnose. *γ*-Solamarine is, together with a new glucoside, *Δ⁵*-tomatidenol-(3*β*)-D-glucoside, isolated as one of the products of partial hydrolysis of *β*-solamarine. Paper chromatography and electrophoresis of permethylated, hydrolyzed *γ*-solamarine identifies 2,3,4-tri-*O*-methyl-L-rhamnose and 2,3,6-tri-*O*-methyl-D-glucose. Hence the constitution of *γ*-solamarine is considered to be (II) (R = $C_{27}H_{42}NO-$).
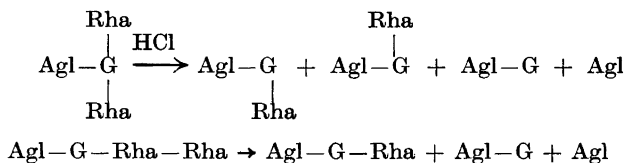
In a previous communication [1] the isolation of three new alkaloidal glycosides from *Solanum dulcamara* L. was described. It is purpose of the present paper to describe the structure elucidation of two of these glucosides, *β*- and *γ*-solamarine. The aglucone of both glycosides has already been identified as *Δ⁵*-tomatidenol-(3*β*)[1] and it has also been established that the carbohydrate moiety of *β*-solamarine was formed from one mole of glucose and two moles of rhamnose and that of *γ*-solamarine from one mole of glucose and one mole of rhamnose.

β-Solamarine. At an early stage of this investigation it became clear that the sugar moiety of *β*-solamarine was branched: oxidition with periodate, followed by hydrolysis and paper chromatography, revealed glucose as the sole monosaccharide. Again, partially hydrolyzed *β*-solamarine was shown by thin-layer chromatography (*cf.* Fig. 1) to consist of five products (incl. unhydrolyzed

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β-solamarine), all giving positive Dragendorff reactions. Partial hydrolysis of an alkaloidal glycoside with a linear trisaccharide should only give three alkaloidal products of hydrolysis in addition to the remaining unhydrolyzed glycoside e.g.:

$$
\begin{array}{c}
\text{Rha} \qquad\qquad\qquad \text{Rha} \\
| \quad \text{HCl} \qquad\qquad\quad | \\
\text{Agl}-\text{G} \longrightarrow \text{Agl}-\text{G} + \text{Agl}-\text{G} + \text{Agl}-\text{G} + \text{Agl} \\
| \qquad\qquad\qquad | \\
\text{Rha} \qquad\qquad\quad \text{Rha}
\end{array}
$$

$$\text{Agl}-\text{G}-\text{Rha}-\text{Rha} \rightarrow \text{Agl}-\text{G}-\text{Rha} + \text{Agl}-\text{G} + \text{Agl}$$

It has not been possible to isolate the trisaccharide moiety as an entity, but permethylation of β-solamarine proved how the monosaccharide units are linked together. Subsequent to acid hydrolysis of the permethylated glycoside, paper chromatography indicated the presence of two methylated sugars, one of which was a dimethyl glucose and the other a trimethyl rhamnose; isolation gave 2,3,4-tri-O-methyl-L-rhamnose and 3,6-di-O-methyl-D-glucose. The first of these was identified through its anilide and compared with authentic material synthesized by direct methylation of L-rhamnose instead of using methyl L-rhamnoside for the permethylation. The second sugar was proved identical

Table 1. Molecular rotation differences of β-solamarine and its products of hydrolysis.

Compound	$[\alpha]_D$	$[M]_D$	$\Delta[M]_D$
Δ^5-Tomatidenol-(3β)	-37.9	-157	$\}-102$
Δ^5-Tomatidenol-(3β)-D-glucoside	-45.0	-259	$\}-362$
γ-Solamarine	-86.1	-621	$\}-130$
β-Solamarine	-85.6	-751	
a-Methyl-D-glucopyranoside [3]	$+158.9$	$+309$	
β-Methyl-D-glucopyranoside [3]	-34.2	-66	
a-Methyl-L-rhamnopyranoside [3]	-62.5	-111	
β-Methyl-L-rhamnopyranoside [3]	$+95.4$	$+170$	

(I)   (II)
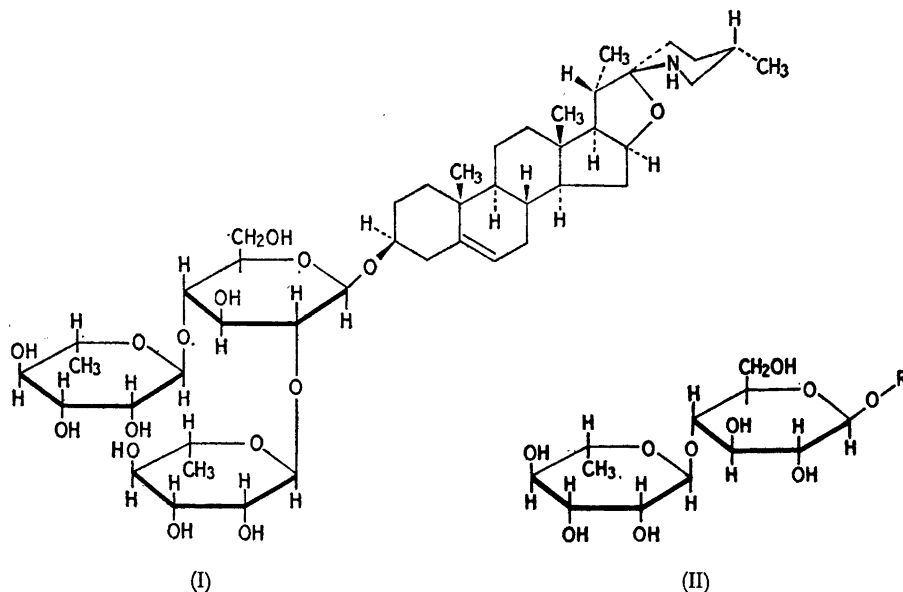
(m.p., mixed m.p., and elementary analysis) with an authentic specimen kindly supplied by professor Richard Kuhn, Heidelberg.

The isolation of these two sugars provides an unequivocal proof that the trisaccharide is branched. The two rhamnose units are linked to C_2 and C_4- of the D-glucose residue and this again to the hydroxyl group of Δ^5-tomatidenol-(3β).
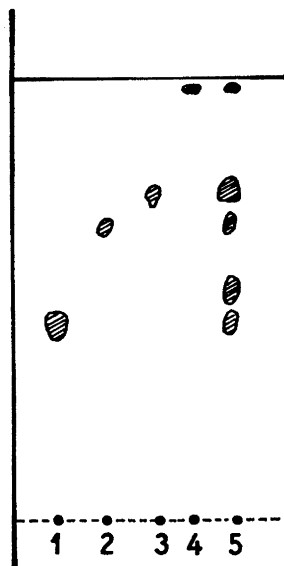
Partial hydrolysis of β-solamarine results in the production of two isomeric rhamnoglucosides, one of these being identical with γ-solamarine, and of Δ^5-tomatidenol-(3β)-D-glucoside. The isolation of these compounds made it possible, through analysis of molecular rotation differences (cf. Table 1), to conclude that in β-solamarine the two L-rhamnose residues are α-glycosidically attached to the glucose residue and the latter β-glycosidically bound to the aglycone.

The last conclusion is confirmed by the facts that emulsin is able to hydrolyze Δ^5-tomatidenol-(3β)-D-glucoside, and that the infrared spectrum of the glucoside is in agreement with that of a β-D-glucoside.[2]

The constitution of β-solamarine is therefore considered to be O-α-L-rhamnopyranosyl-(1→2)-O-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranosyl-Δ^5-tomatidenol-(3β) (I).

γ-Solamarine. The isolation of γ-solamarine as one of the products of partial hydrolysis of β-solamarine leaves only two possibilities for the attachment of rhamnose to the glucose part, either at C_2 or C_4. After permethylation and hydrolysis of γ-solamarine, paper chromatography revealed the presence of 2,3,4-tri-O-methyl-L-rhamnose and a trimethyl glucose. The trimethyl glucose must be either 2,3,6-tri-O-methyl-D-glucose or 3,4,6-tri-O-methyl-

Fig. 1. Thin-layer chromatogram of partially hydrolyzed β-solamarine. (1)β-Solamarine. (2)γ-Solamarine. (3)Δ^5-Tomatidenol-(3)β-D-glucoside.   (4)Δ^5-Tomatidenol-(3β) (and $\Delta^{3,5}$-tomatidadiene). (5) Partially hydrolyzed β-solamarine.

D-glucose. Paper electrophoresis in borate buffer gave an M_G-value of O just as a periodate-permanganate reagent [4] used for spraying did not produce any colour with the trimethyl glucose. Hence, the methylated derivative is considered to be 2,3,6-tri-O-methyl-D-glucose, and the constitution of γ-solamarine therefore O-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-Δ^5-tomatidenol-(3β) (II; R = —$C_{27}H_{42}NO$).

The isolation of γ-solamarine as one of the products of partial hydrolysis of β-solamarine raises the question as to whether γ-solamarine is a naturally occurring substance or an artefact produced during extraction of the plant material with dilute acetic acid. In the opinion of the author γ-solamarine is definitely to be considered as a genuine plant constituent on the following grounds: thin-layer chromatography performed on the crude alkaloid mixture isolated from the plant material only revealed three alkaloidal glycosides, α-, β-, and γ-solamarine (*cf.* Ref.[1]). Among those γ-solamarine is the only glycoside likely to be a product of partial hydrolysis of β-solamarine. If partial hydrolysis of β-solamarine really had occurred during the isolation procedure, Δ^5-tomatidenol-(3β)-D-glucoside ought to have been present and that in a concentration greater than that of γ-solamarine (*cf.* Fig. 1).

Finally, Kuhn *et al.*[5] have determined the structure of α- and β-chaconine and these alkaloidal glycosides have the same sugar moiety as β- and γ-solamarine, respectively, the former differing only by containing solanidine as the aglycone.

EXPERIMENTAL

Melting points are uncorrected and have been determined on the Kofler hot stage microscope (manufacture C. Weygand).

Paper chromatography. Paper: Whatman No. 1 and 3. Solvent: 1-butanol-ethanol-water (5:1:4).[6] Reagent: aniline hydrogen phthalate.

Thin-layer chromatography. Plates made manually according to Tschesche, Freytag and Snatzke.[7] Solvent: bottom layer of chloroform-ethanol-1 % aqueous ammonia (2:2:1).[1] Reagent: Dragendorff.

Permethylation of β-solamarine. A solution of β-solamarine (5 g) in dimethyl formamide (100 ml) was methylated by shaking at room temperature with 20 g of barium oxide and 0.8 g of barium hydroxide. After addition of 500 ml of chloroform, the suspension was filtered and worked up as described by Kuhn.[8] 5.7 g of β-solamarine permethylate was obtained as a colourless amorphous powder, not possessing the composition of an homogeneous compound (Found: OCH_3 20.3. Calc. for $C_{55}H_{94}NO_{15}I$: 8 OCH_3 21.8). The IR-spectrum indicated no hydroxyl group absorption.

Hydrolysis. The permethylate (5 g) was hydrolyzed, without dehalogenation, by refluxing with 100 ml of 2N methanolic hydrochloric acid for 6 h. Upon dilution with 100 ml of water, the hydrolysis mixture was concentrated to 50 ml and the precipitated aglycone filtered off. The acidic filtrate was refluxed once more for 3 h, clarified with active carbon, filtered, and extracted 8 times with chloroform. The chloroform extracts were neutralized with sodium hydrogen carbonate and dried with anhydrous sodium sulfate. Evaporation of the chloroform gave 1.9 g of a brown syrup (A). On paper chromatography, only one spot was detected (R_G* 1.00), indistinguishable from that given by an authentic specimen of 2,3,4-tri-O-methyl-L-rhamnose.

The brown coloured solution remaining after the chloroform extraction was decolourized once more with active carbon, deionized with Dowex 3, and evaporated *in vacuo* to give 1.05 g of a syrup (B). Paper chromatography indicated the presence of a dimethyl glucose and trimethyl rhamnose (R_G 0.51 and 1.01). Reference samples of 3.6-di-O-methyl-D-glucose and 4.6-di-O-methyl-D-glucose run simultaneously gave spots not clearly distinguishable from the unknown dimethyl glucose.

2,3,4-Tri-O-methyl-L-rhamnose. The syrup (A) was purified by distillation *in vacuo* to give a highly viscous colourless oil (bath.temp. 140—150°/1 mm), $[α]_D^{26}$ + 25.3° (c 0.4,H_2O), n_D^{23} 1.4565. (Found: C 51.86; H 8.86. Calc. for $C_9H_{18}O_5$: C 52.41; H 8.80).

2,3,4-Tri-O-methyl-L-rhamnose anilide. Tri-O-methyl-L-rhamnose (300 mg) was converted into the anilide following the procedure of Kuhn *et al.*[5] Sublimation *in vacuo* afforded needles with m.p. 122—124° alone and in admixture with authentic 2,3,4-tri-O-methyl-L-rhamnose anilide, $[α]_D^{26}$ + 139.8° (c 0.70, abs. C_2H_5OH). (Found: C 64.13; H 8.17. Calc. for $C_{15}H_{23}NO_4$: C 64.03; H 8.24).

3,6-Di-O-methyl-D-glucose. The syrup (B) dissolved in water was fractionated on a column (2.5 × 25 cm) of active carbon-Celite (1:1). Elution with 500 ml of water gave no sugars. Elution with an aqueous 2 % ethanolic solution gave the following 100-ml fractions: Nos. 1—2: no sugar; Nos. 3—7: traces of a monomethyl hexose (R_G 0.29); Nos. 8—10: 15 mg (R_G 0.28. 0.50); Nos. 11—15: 260 mg (R_G 0.50); Nos. 16—30: 690 mg (R_G 0.51, 1.01).

The syrup obtained from fractions Nos. 11—15 could not be induced to crystallize, but was streaked onto two sheets of prewashed Whatman No. 3 paper. After chromatography and elution a small yield of 3,6-di-O-methyl-D-glucose was obtained. Recrystallized from ethyl acetate the compound melted at 112—116°. Admixed with authentic recrystallized material of m.p. 117—119°, the m.p. was 113—117.5°. The analytical data were concordant with $C_8H_{16}O_6$ (Found: C 46.37; H 7.84. Calc: C 46.15; H 7.75).

Synthesis of 2,3,4-Tri-O-methyl-L-rhamnose. (*cf.* Ref.[5]). L-Rhamnose monohydrate (10 g) was dehydrated and directly permethylated in dimethyl formamide (200 ml) with 40 g of barium oxide and 1.6 g of barium hydroxide according to Kuhn.[8] A dark-coloured oil was obtained in a yield of 11 g. Distillation *in vacuo*, bath temp. 110°/1 mm, did not result in any purification, and the distillate was still highly coloured. The oil

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* *i. e.* the ratio between the distances travelled by the compound and tetra-O-methyl-D-glucose.[6]

Table 2. Second fractionation of partially hydrolyzed β-solamarine on alumina.

Fraction	Glycosides of Δ^5-tomatidenol-(3β)	Weight (mg)
8	glucoside	49
9	γ-solamarine	13
10 — 16	γ-solamarine + isomeric rhamnoglucoside	87
17 — 18	γ-solamarine + isomeric rhamnoglucoside + β-solamarine	24
19	isomeric rhamnoglucoside + β-solamarine	11
20 — 22	β-solamarine	26

was allowed to pass through a column of active carbon (1 × 10 cm). A colourless eluate was obtained, proved by gas chromatography to be homogenous. Hydrolysis and distillation as described by Kuhn *et al.*[5] gave 2,3,4-tri-*O*-methyl-L-rhamnose, $[a]_D^{23}$ + 24.6° (c 0.84, H_2O), n_D^{23} 1.4553.

Partial hydrolysis of β-solamarine. After refluxing 1 g of β-solamarine with 50 ml of 0.1 N hydrochloric acid for 1 h, the hydrolyzate was made alkaline with ammonia, centrifuged, washed with water, and dried. The hydrolyzate, shown by thin-layer chromatography to consist of five substances (*cf.* Fig. 1), was chromatographed on alumina (Fluka "basisch") with water-saturated 1-butanol-ethyl acetate (1:1) as eluant. A total of 10 25-ml fractions were collected. Fraction 1 contained 188 mg of Δ^5-tomatidenol-(3β) and $\Delta^{3,5}$-tomatadadiene, fractions 7—10 83 mg of unhydrolyzed β-solamarine, whereas the remaining fractions contained only partly fractionated products of hydrolysis. These last fractions were pooled, evaporated to dryness *in vacuo* and rechromatographed using the same conditions as above. A total of 22 5-ml fractions were collected as described in Table 2.

Δ^5-*Tomatidenol-(3β)-D-glucoside.* Fraction 8 was evaporated to dryness and recrystallized twice from methanol to give needle-shaped crystals, m.p. 249—251° (decomp.), $[a]_D^{25}$ —45.0° (c 1.01, CH_3OH). (Found after drying over P_2O_5 at 105° for 2 h: C 65.65; H 9.22. Cal. for $C_{33}H_{35}NO_7$, $1\frac{1}{2}H_2O$*: C 65.75; H 9.36). On hydrolysis in 1 N hydrochloric acid only glucose could be detected by paper chromatography. The IR-spectrum (in KBr) exhibited two of the bands characteristic for β-D-glucosides:[2] 888 cm^{-1}, 782 cm^{-1}.

γ-*Solamarine.* On thin-layer chromatograms the spot given by fraction 9 was indistinguishable from that given by an authentic specimen of γ-solamarine. Acid hydrolysis produced glucose and rhamnose identified by paper chromatography.

Emulsin hydrolysis. Δ^5-Tomatidenol-(3β)-D-glucoside (3 mg) and 3 mg of emulsin (Fluka) were suspended in 0.2 ml of water at room temperature. After standing for 48 h paper chromatography clearly revealed the presence of glucose. Treated in the same manner β-solamarine as well as γ-solamarine were not hydrolyzed by emulsin.

Permethylation of γ-solamarine. γ-Solamarine (300 mg) was permethylated and hydrolyzed as above. Paper chromatography indicated the presence of two compounds, tri-*O*: methyl-L-rhamnose and a trimethyl glucose (R_G 0.81); 2,3,6-tri-*O*-methyl-D-glucose gave an R_G-value of 0.81. The spot given by the unknown trimethyl glucose could not be developed with the periodate-permanganate reagent,[4] but only with aniline hydrogen phthalate reagent; again the same compound upon electrophoresis in 0.1 M borate buffer at pH 10 gave an M_G of 0.

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* Solasodine-β-D-glucoside, dried for 2 h in the same manner, has also been found to be a hydrate with $1\frac{1}{2}$ moles of water (Kuhn and Löw [9]).

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