

Alkaloidal Glycosides from *Solanum dulcamara*

II*. Three New Alkaloidal Glycosides and a Reassessment of Soladulcamaridine

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Three new alkaloidal glycosides, named α -, β - and γ -solamarine, are isolated from *Solanum dulcamara* L. The aglycone is Δ^6 -tomatidinol-(3 β) and the carbohydrate component of α -solamarine is formed from glucose (1 mole), galactose (1 mole) and rhamnose (1 mole), of β -solamarine from glucose (1 mole) and rhamnose (2 moles), and of γ -solamarine from glucose (1 mole) and rhamnose (1 mole). Furthermore, solasodine and yamogenin are isolated from the same plant.

The previously reported soladulcamaridine is found to be inhomogeneous, separable on thin-layer chromatography into Δ^6 -tomatidinol-(3 β) in addition to traces of $\Delta^{8,9}$ -tomatidiane and solasodine. The name, hence, should be discontinued.

In a continuation of our investigation of the alkaloidal glycoside constituents of *Solanum dulcamara* L., three substances with $R_{\alpha\text{-solanine}}$ -values 1.18, 2.65 and 3.80 have been detected paperchromatographically in plant material collected in July 1961 and August 1960. The plant material used earlier for the isolation of soladulcamaridine¹ was collected in June 1957 and contained two substances with $R_{\alpha\text{-solanine}}$ -values 0.80 and 1.18. The $R_{\alpha\text{-solanine}}$ -value for soladulcamaridine was 0.80. Now the alkaloids corresponding to $R_{\alpha\text{-solanine}}$ 1.18, 2.65 and 3.80 have been isolated and are given the names α -, β - and γ -solamarine (Table 1).

Upon acid hydrolysis the aglycone part from all three glycosides was shown by thin-layer chromatography in chloroform-methanol (19:1) to consist of three compounds with R_F -values 0.80, 0.51 and 0.34. The two components with the highest R_F -values represented the main constituents, whereas the one with R_F 0.34 is to be considered as a minor component.

Solasodine and tomatidine, chromatographed alone and in mixtures alongside the unknown aglycones, revealed that solasodine and tomatidine were

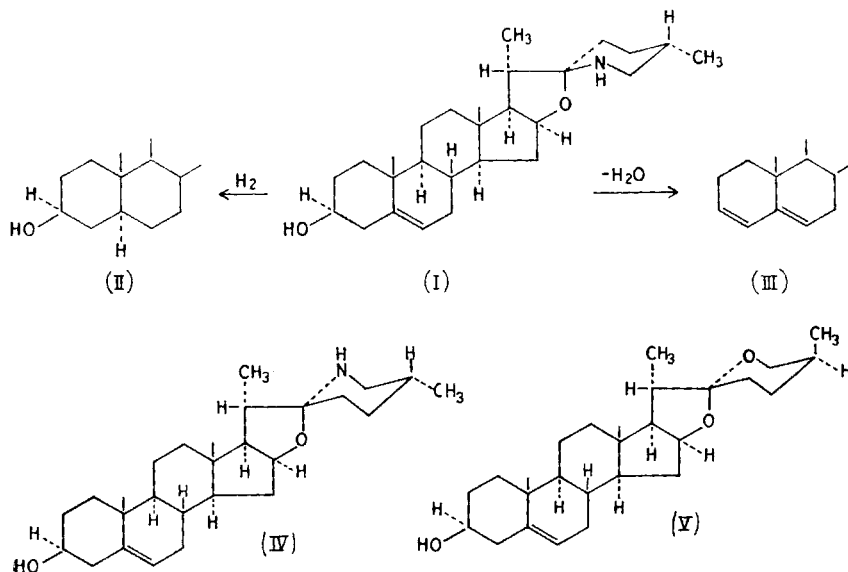
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Table 1. Alkaloidal glycosides isolated from *Solanum dulcamara* L. (Lyngby Aamose).

Isolated glycoside	Formula	Aglycone	Carbohydrate components	R_{α} -solanine
Soladulcamarine ¹	$C_{50}H_{81}NO_{18}$	Δ^5 -tomatidenol-(3 β)	1 mole glucose 2 moles rhamnose 1 mole arabinose	0.80
α -Solamarine	$C_{45}H_{73}NO_{18}$	Δ^5 -tomatidenol-(3 β)	1 mole galactose 1 mole glucose 1 mole rhamnose	1.18
β -Solamarine	$C_{45}H_{73}NO_{15}$	Δ^5 -tomatidenol-(3 β)	1 mole glucose 2 moles rhamnose	2.65
γ -Solamarine	$C_{39}H_{63}NO_{11}$	Δ^5 -tomatidenol-(3 β)	1 mole glucose 1 mole rhamnose	3.80

chromatographically undistinguishable from the two unknown aglycones with the lowest R_F -values. The third aglycone with the higher R_F -value could perhaps be a dehydrated aglycone. Furthermore, on chromatoplates sprayed with antimony trichloride all the unknown aglycones gave a reddish-violet colour similar to the colour produced by solasodine indicating unsaturated sapogenins. Saturated sapogenins, as tomatidine, give a greyish-violet colour with antimony trichloride.

By chromatography on alumina it was proved possible to isolate two of the aglycones. The first of these (R_F 0.80) was an anhydrogenin with the empirical formula $C_{27}H_{41}NO$, devoid of hydroxyl stretching vibration in the



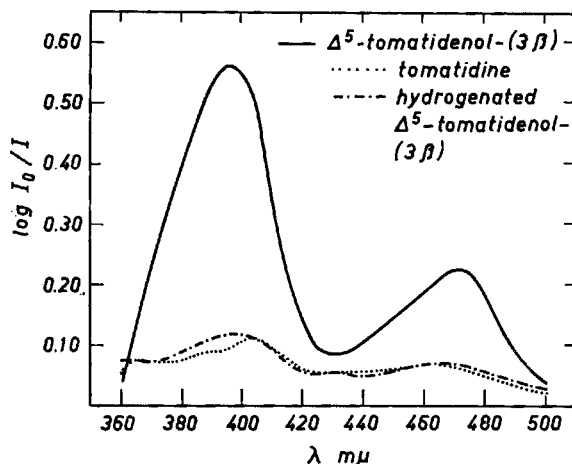


Fig. 1. Absorption spectra in ethanol-concentrated sulfuric acid¹⁵.

IR-spectrum and exhibiting ultraviolet absorption maxima at 228, 234 and 242 (shoulder) $\mu\mu$, strongly indicating a heteroannular diene. The Rosenheim reaction for dienes was also positive.

The second compound (R_F 0.51), $C_{27}H_{43}NO_2$, isomeric with solasodine, could be converted into an O,N-diacetyl derivative. The digitonin precipitation assay suggested the presence of a 3β -hydroxyl grouping. One double bond was indicated through a yellow colour with tetranitromethane and the absorption of one mole of hydrogen in the presence of palladium on carbon. Furthermore, the colour reaction for 3β -hydroxy- Δ^5 en-steroids according to Witter and Stone² was positive and the absorption spectrum in concentrated sulfuric acid-ethanol in accordance with a 3-hydroxy- Δ^5 en-steroid (Fig. 1). Upon catalytic hydrogenation tomatidine (II) was obtained in good yield, identified on critical comparison with an authentic specimen of the latter. Hence, the second aglycone is considered to be Δ^5 -tomatidenol-(3β) (I). On dehydration, the aforementioned anhydrogenin (R_F 0.81) is produced. Consequently, the latter may be an artefact, most likely $\Delta^{3,5}$ -tomatidiene (III).

Table 2. Steroidal aglycones isolated from *Solanum dulcamara* L. (Lyngby Aamose).

Steroidal aglycone	Formula	M.p.	$[\alpha]_D^{20}$	R_F
$\Delta^{3,5}$ -Tomatidiene	$C_{27}H_{41}NO$	136–138°	– 88.6°	0.80
Δ^5 -Tomatidenol-(3β)	$C_{27}H_{43}NO_2$	238–240°	– 37.9°	0.51
Solasodine	$C_{27}H_{43}NO_2$	198–199°	– 91.8°	0.34
Yamogenin	$C_{27}H_{42}O_3$	189–191°	– 121.3°	

The third aglycone isolated by fractional crystallization was found to be identical with solasodine (IV).

Schreiber and Rönsch³ as well as Sander⁴ recently reported the isolation of two aglycones from a geographical form of *S. dulcamara*. Both groups suggest that these are ^{3,5} Δ -tomatidiene and Δ^5 -tomatidenol-(3 β)^{*}.

The isolation of Δ^5 -tomatidenol-(3 β) and small amounts of solasodine after hydrolysis of the three alkaloidal glycosides clearly indicated that the latter were not homogeneous. Attempts to find chromatographic solvent systems for separating them were unsuccessful, whereas fractional precipitation gave glycosides which on hydrolysis gave solely Δ^5 -tomatidenol-(3 β) and its dehydration product.

Upon complete hydrolysis with 1 N hydrochloric acid α -solamarine gave equimolecular amounts of glucose, galactose and rhamnose, in addition to the aglycone. β -Solamarine, on the other hand gave one mole of glucose and two moles of rhamnose, whereas γ -solamarine afforded one mole of glucose and 1 mole of rhamnose.

In addition, yamogenin (V) was isolated, a constituent previously reported by Schreiber and Rönsch³.

Soladulcamaridine, the aglycone of soladulcamarine was tentatively assigned a solanidane structure¹. This assignment was based only on its basicity, whereas its colour reactions were similar to those of solasodine. Soladulcamaridine has now been found to be inhomogeneous, separable on thin-layer chromatography into Δ^5 -tomatidenol-(3 β), in addition to traces of $\Delta^{3,5}$ -tomatidiene and solasodine. The name soladulcamaridine, therefore, should be abandoned. The pK_B -value of pure Δ^5 -tomatidenol-(3 β) has now been determined to 6.93, quite different from the value earlier presented¹.

DISCUSSION

Biochemically it is of interest that the carbohydrate components of α - and β -solamarine are the same as those found in leaves of the potato plant, *S. tuberosum*, as well as *S. chacoense* and *S. aviculare*:

Solanum tuberosum and *Solanum chacoense*

α -solanine: solanidine + galactose + glucose + rhamnose

α -chaconine: solanidine + glucose + rhamnose + rhamnose

Solanum aviculare

solasonine: solasodine + galactose + glucose + rhamnose

solamargine: solasodine + glucose + rhamnose + rhamnose

Solanum dulcamara

α -solamarine: Δ^5 -tomatidenol-(3 β) + galactose + glucose + rhamnose

β -solamarine: Δ^5 -tomatidenol-(3 β) + glucose + rhamnose + rhamnose.

Neither the four first mentioned alkaloidal glycosides, nor the plants containing these alkaloids have any effect on the potato beetle (*Leptinotarsa decemli-*

* According to a personal communication from Dr. K. Schreiber the structure of Δ^5 -tomatidenol-(3 β) has now been confirmed by synthesis.

neata) and its larvae. It is well-known that the carbohydrate moiety is responsible for the effect of some *Solanum* alkaloids on the potato beetle. Hence, it is not surprising if α - and β -solamarine would have no or only a very slight effect as "Frassgift", *i.e.* resistance factor, compared to the highly active tomatine and demissine both containing tetrasaccharide moieties. On the other hand, it is possible that soladulcamarine as a tetrasaccharide would exhibit some resistance towards attack of the potato beetle. In fact, the occurrence of this alkaloidal tetrasaccharide and its possible seasonal degradation to β -solamarine may be responsible for the fact that *S. dulcamara* often can be seen as a host plant for the potato beetle⁵, although Schreiber⁶ finds that some forms of the plant are not attacked by the beetle and that a crude glycoside mixture containing soladulcidine as aglycone is resistant towards attack.

In this connection it should be noticed that the $R_{\alpha\text{-solanine}}$ -values for soladulcamarine and α -solamarine are not very different and that it is quite difficult to exclude that soladulcamarine is present in the plant material collected in July and August. On hydrolysis of the crude glycoside mixture from this plant material arabinose was chromatographically observed. The latter probably arises from soladulcamarine, although its formation as an artefact can hardly be excluded, since it has been pointed out by Harborne and Sherratt⁷ that paper chromatography with solvents containing hydrochloric acid can produce arabinose as an artefact.

Schreiber and Rönisch³ did not observe L-arabinose as part of alkaloidal glycosides on chromatography of several geographical forms of *S. dulcamara*, but favour the view that uronic acids take part in the biosynthesis of this type of glycosides and that L-arabinose may eventually arise from enzymic decarboxylation of D-galacturonic acid. Alkaloidal glycosides with uronic acid as part of the glycoside moiety will behave as zwitter ions. In this connection it is interesting that the yield of glycosides isolated from plant material collected in June is quite low (Table 3).

Soladulcamarine, solely formed in plant material collected in June, seems to be more rapidly converted than α -solamarine because plant material collected later in the year do not contain any soladulcamarine, but two new alkaloids, β - and γ -solamarine, differing from soladulcamarine only by being devoid of

Table 3. Yields of alkaloidal glycosides obtained from plant material collected at different dates.

Kg of fresh plant material	Data collected	G of crude precipitate	G ethanol soluble alkaloid	Yield in %
7.0	June 14, 1957	22.1	2.2	0.03
6.9	July 17, 1961	13.9	6.7	0.10
14.7	Aug. 7, 1960	49.7	37.7	0.26
1.0 (stems)	commercially available	0.8	0.002	0.00

arabinose and arabinose combined with rhamnose, respectively. α -Solamarine, on the other hand, appears to be present in all the plant materials investigated.

The presence of solasodine as aglycone together with Δ^5 -tomatidenol-(3β) together with the fact that upon hydrolysis only the sugars characteristic for solasonine and solamargine are detected, strongly suggests the presence of the two latter glycosides in the glycoside mixture. Chromatographically solasonine and solamargine are difficult to differentiate from α - and β -solamarine, although one solvent system gave a clear indication of solasonine being present.

Schreiber and Rönsch³ as well as Sander⁴ have shown that *Solanum dulcamara* L., which with respect to morphology and anatomy is homogeneous, is quite heterogeneous in a phytochemical sense. Still, it is interesting that Δ^5 -tomatidenol-(3β) and solasodine can be isolated, representing sapogenins of the 25L and 25D series, respectively, from the same plant material. The employed material was collected from an area of about 25 \times 25 m, but even so it is difficult to ascertain that the plant material is homogeneous in genetic respect.

As to the stereochemistry of the sapogenins of the 25D and 25L series it is noteworthy that the alkaloidal sapogenins of the two series, e.g. solasodine and Δ^5 -tomatidenol-(3β), can be separated by thin-layer chromatography, whereas the sapogenins of the spirostane type cannot⁸. This is in accordance with the earlier proposal^{9,10} that alkaloidal sapogenins of the two series differ not only at C(25), but also at C(22) giving rise to compounds probably differing more with respect to chemical and physical properties than the ordinary neo- and iso-sapogenins which deviate only in the orientation of the methyl group at C(25).

EXPERIMENTAL

The melting points are uncorrected and have been determined with the Kofler hot stage microscope (manufacture C. Weygand).

Paper chromatography. Whatman's paper No. 1, equilibrated for 12 h, was used with the top phase of ethyl acetate-pyridine-water(3:1:3). In this solvent system, the following R_F -solanine-values were recorded: solanine 1.00; solasonine 0.88; solamargine 2.92; tomatine 0.85; soladulcamarine 0.80; α -solamarine 1.18; β -solamarine 2.65; γ -solamarine 3.80.

A 25 % solution of antimony trichloride in chloroform was used as spray. When heated for 5 min at 110° the saturated compound tomatine gave a greyish-violet colour, whereas the other unsaturated compounds gave a reddish-violet colour.

In this system the compounds all give somewhat elongated spots. Therefore, mixtures of glycosides are difficult to differentiate.

The sugars from the hydrolysis were examined as previously described^{1,11}.

Thin-layer chromatography. The method described by Tschesche, Freytag and Snatzke¹² was used. Over 30 different solvent systems were tried on alkaloidal glycosides and it was found that the two systems given in Table 4 were far superior to the system described above as well as that utilized by Paseshnichenko and Guseva¹³.

The alkaloidal sapogenins obtained by hydrolysis on Silica Gel G were examined by thin-layer chromatography in the system chloroform-methanol(19:1). The R_F -values as average of several chromatograms were: $\Delta^{3,5}$ -tomatidiene 0.81; Δ^5 -tomatidenol-(3β) 0.51; tomatidine 0.51; solasodine 0.36; 5 α -solasodanol-(3β) 0.36; solanidine 0.32; 5 α -solanidanol-(3β) 0.32.

Dragendorff's reagent and the above mentioned antimony trichloride reagent were used as sprays.

Table 4. R_F -values (average of several chromatograms) of alkaloidal glycosides using thin-layer chromatography on silicagel G.

Alkaloidal glycoside	Ethyl acetate 30 pyridine 10 (top layer) water 30		Chloroform 20 (bottom layer) ethanol 20 (bottom layer) 1 % NH_3 -water 10	
	R_F	R_{α} -solanine	R_F	R_{α} -solanine
Solanine	0.22	1.00	0.26	1.00
α -Chaconine	0.35	1.59	0.36	1.38
Solasonine	0.24	1.09	0.33	1.27
Solamargine	0.45	2.06	0.55	2.12
Tomatine	0.29	1.32	0.29	1.12
Soladulcamarine	0.22	1.00	0.25	0.96
α -Solamarine	0.32	1.46	0.33	1.27
β -Solamarine	0.46	2.09	0.55	2.12
γ -Solamarine	0.60	2.73	0.78	3.00

Table 5. Fractionation of the glycoside mixture on neutral alumina.

Fraction	Alkaloidal glycoside (mg)	Colour	R_F (Chloroform-ethanol 1 % NH_3 -water 2:2:1)
1— 23	0		
24— 59	233	brown oil	1.00
60— 98	461	yellow colored	0.78
99—105	116	colorless	0.78 + 0.55
106—183	4981	—	0.55
184—201	433	—	0.55 + 0.33
202—238 + 2 liter	1945	—	0.33

α -Solanine $\sim R_F$ 0.33; β -Solanine $\sim R_F$ 0.55; γ -Solanine $\sim R_F$ 0.78;
 Δ^5 -Tomatidenol-(3 β) $\sim R_F$ 1.00.

Isolation of the glycoside mixture. The plant material was collected at Lyngby Aamose near Copenhagen. The fresh tops (14.7 kg) were crushed in a mill and immediately extracted with 5% acetic acid using the isolation procedure described by Kuhn and Löw¹⁴. The yield of crude ethanol-soluble alkaloidal glycoside was 37.7 g.

Fractionation of the glycoside mixture. Attempts to isolate the individual glycosides on columns of silica gel using the two solvent systems mentioned under thin-layer chromatography were not successful, just as precipitation of the glycoside mixture with cholesterol gave a gel unsuited for further fractionation. Therefore, it was attempted to use water-saturated 1-butanol on alumina, which has been successfully used by Kuhn and Löw¹⁴, even when it was noted that only poor separation was achieved on thin-layer chromatography in this solvent system. Furthermore, the glycoside mixture tended to form a gel when dissolved in aqueous butanol.

1.5 kg of alumina (Woelm neutral, Act. I) was suspended in water-saturated 1-butanol and allowed to stand for 12 h before packing. To assure complete equilibration 1.5 l of the aqueous butanol was allowed to run through the column. The size of the column was 5.5 × 61 cm. The glycoside mixture (9.2 g) was dissolved in 200 ml of water-saturated 1-butanol by gentle heating and added warm to the column to avoid gel formation. 32-ml fractions were collected, individually examined by thin-layer chromatography and evaporated *in vacuo*. The results are presented in Table 5.

The component with R_F 1.00 was an oil which by thin-layer chromatography was shown to contain the aglycone, Δ^8 -tomatidenol-(3 β), described below. It was not possible to detect the dehydration product of the latter. γ -Solamarine was contaminated by a brown-coloured component which gave a positive Dragendorff reaction. It was possible partly to remove this impurity by extracting an ammoniacal solution of the glycoside with ethyl acetate. Upon acid hydrolysis all three glycosides on thin-layer chromatography indicated the presence of two aglycones and one anhydro-aglycone (R_F 0.34, 0.51, 0.80).

The glycosides were purified by fractional precipitation. Addition of acetone to concentrated methanolic solutions of the glycosides produced amorphous precipitates. Repeated fractionation gave colourless, semicrystalline glycosides, which after hydrolysis gave two spots on thin-layer chromatography, one representing an aglycone and one an anhydro-aglycone (R_F 0.51, 0.80).

α -Solamarine. M.p. 278–281° (decomp.), with sintering at 236°, $[\alpha]_D^{20}$ –45.0° (pyridine, c 0.65) (Found: C 57.34; H 8.41. Calc. for $C_{45}H_{73}NO_{18}$, 3 H₂O: 57.62; H 8.49. After drying to constant weight at elevated temperature: C 61.29; H 8.39. Calc. for $C_{45}H_{73}NO_{16}$: C 61.14; H 8.32). The equivalent weight was determined by titration (Found: 895, 909. Calc.: 884).

β -Solamarine. M.p. 275–277° (decomp.), with sintering at 270°, $[\alpha]_D^{20}$ –85.6° (pyridine, c 0.40) (Found: C 59.10; H 8.87. Calc. for $C_{45}H_{73}NO_{15}$, 3 H₂O: C 58.62; H 8.64. After drying to constant weight at elevated temperature: C 62.71; H 8.33. Calc. for $C_{45}H_{73}NO_{15}$: C 62.27; H 8.48). The equivalent weight was determined by titration (Found: 902, 873. Calc.: 877).

γ -Solamarine. M.p. 243–248° (decomp.), with sintering at 195°, $[\alpha]_D^{20}$ –86.1° (pyridine, c 0.36) (Found: after drying to constant weight at elevated temperature: C 64.77; H 8.83. Calc. for $C_{39}H_{63}NO_{11}$: C 64.88; H 8.80). The equivalent weight was determined by titration (Found: 756, 749. Calc.: 721).

Dihydro- β -solamarine. β -Solamarine (249 mg) was dissolved in 20 ml of a mixture of dioxan-glacial acetic acid-water (1:1:1) and hydrogenated at room temperature using palladium on carbon (10%) as a catalyst. Uptake: 1.01 mole of hydrogen. The solution was filtered, evaporated *in vacuo*, dissolved in a small volume of 50% methanol, and the glycoside was precipitated by adding concentrated ammonia. The glycoside would not crystallize, but was precipitated from a concentrated methanolic solution with acetone. M.p. 277–280° (decomp.), with sintering at 269°, $[\alpha]_D^{20}$ –74.8° (pyridine, c 1.03). For analysis the compound was dried *in vacuo* over P₂O₅. (Found: C 61.38; H 8.56. Calc. for $C_{45}H_{73}NO_{15}$, 1/2 H₂O: C 61.49; H 8.71).

Picolonate of β -solamarine. The derivative was prepared in and recrystallized from 80% ethanol. It formed yellow, rectangular plates, m.p. 193–195° (decomp.) (Found: C 56.91; H 7.26; H₂O 3.12. Calc. for $C_{45}H_{73}NO_{15}$, C₁₀H₈N₄O₅, 2 H₂O: C 56.54; H 7.33; H₂O 3.08).

Table 6. Fractionation of the aglycones on neutral alumina.

Fraction	MI	Eluent	Aglycone (mg)	R_F
1	100	benzene	72	0.80
2	50	benzene-chloroform (9:1)	8	0.80
3	50	» » (7:3)	0	
4	50	» » (5:5)	23	0.51
5	50	chloroform	102	0.51

Hydrolysis. After refluxing 500 mg of pure β -solamarine with 20 ml of methanolic 1 N hydrochloric acid for 2 h, 241 mg (calc. 258 mg) of the aglycone-hydrochloride was obtained. The free base was liberated in 80 % methanol by adding concentrated ammonia. The aglycone was shown by thin-layer chromatography to consist of two substances, a major component with R_F 0.51 and a minor constituent with R_F 0.80. Subsequent to hydrolysis in aqueous solution, the two components were present in about equal amounts. α -Solamarine and γ -solamarine afforded the same products on hydrolysis. The above aglycone mixture (212 mg) was dissolved in 15 ml of benzene and chromatographed on 15 g of alumina (Woelm neutral, Act. III) (1.2 \times 16 cm) as explained in Table 6.

$\Delta^3,5$ -Tomatidiene (III). The residue obtained by evaporation of fraction 1 was recrystallized twice from methanol giving needles, m.p. 136–138°, $[\alpha]_D^{20} - 88.6^\circ$ (methanol, c 0.53). For analysis the compound was dried *in vacuo* over P_2O_5 (Found: C 81.92; H 10.20. Calc. for $C_{27}H_{41}NO$: C 81.99; H 10.44). The ultraviolet spectrum in methanol had λ_{max} 228 $m\mu$ (ϵ 21 100), λ_{max} 234 $m\mu$ (ϵ 22 500) and an inflection at 243 $m\mu$ (ϵ 13 900). The Rosenheim reaction for dienes was positive and no epimerization was possible: After treatment of a solution of the diene in chloroform with gaseous hydrogen chloride, thin-layer chromatography revealed only one spot, identical with the original material (R_F 0.80).

Δ^3 -Tomatidenol-(3 β) (I). Fractions 4 and 5 were evaporated and twice recrystallized from acetone to give needles, m.p. 238–240°, $[\alpha]_D^{20} - 37.9^\circ$ (methanol, c 0.37). (After drying *in vacuo* over P_2O_5 : C 77.86; H 10.29. Calc. for $C_{27}H_{43}NO_2$: C 77.95; H 10.40). The equivalent weight 423 was determined by titration. After standing overnight an ethanolic solution of the aglycone and an 1 % solution of digitonin in 90 % ethanol had deposited a precipitate. The aglycone gave a positive pink colour in the Witter and Stone² reaction for 3 β -hydroxy- Δ^5 en-steroids and an absorption spectrum in ethanol-concentrated sulfuric acid characteristic for 3-hydroxy- Δ^5 en-steroids (Fig. 1)¹⁵.

Tomatidine (II). 1) Δ^5 -Tomatidenol-(3 β) (100 mg) dissolved in 10 ml of glacial acetic acid was hydrogenated at room temperature with palladium on carbon (10 %) as a catalyst. Uptake: 1.03 moles of hydrogen. The solution was filtered and evaporated *in vacuo* at 30°, concentrated ammonia was added and the aqueous phase was extracted with chloroform. The dried chloroform-solution was evaporated and the product recrystallized twice from acetone to give 39 mg of needles, m.p. 209–210°, alone or in admixture with authentic tomatidine, $[\alpha]_D^{20} + 5.6$ (methanol, c 0.43), R_F 0.51.

2) Dihydro- β -solamarine (207 mg) was refluxed for 2 h in a mixture of 20 ml methanol and 2 ml concentrated hydrochloric acid. After concentration to 10 ml *in vacuo*, the aglycone was precipitated with concentrated ammonia. Yield 194 mg. On chromatography in benzene on 15 g of alumina (Woelm neutral, Act. III) no Δ^3 -tomatidene was obtained. Elution with benzene-methanol (9:1) gave tomatidine, crystallizing from acetone in needles, m.p. and mixed m.p. 208–10°.

Table 7. Paper chromatography of the monosaccharides obtained by hydrolysis of the glycosides.

Compound	R_F -values in 1-butanol-ethanol-water (8:1:2)			R_{GM} -values in ethyl acetate-pyridine-water (3.6:1:1.15)		
	α -Solamarine	0.15	0.17	0.44	1.00	0.85
β -Solamarine	0.16		0.43	1.00		4.22
γ -Solamarine	0.17		0.46	1.01		4.23
D-Glucose	0.16			1.00		
D-Galactose		0.18			0.80	
L-Rhamnose			0.44			4.20

O,N-Diacetyl-tomatidine. 10 mg of the hydrogenated Δ^5 -tomatidenol-(3 β) was dissolved in 0.5 ml of pyridine and allowed to stand with 0.5 ml of acetic anhydride for 24 h. The solution was poured onto ice, the precipitate was collected and recrystallized from ethanol-water to give crystals, m.p. 193–195°, undepressed on admixture with authentic *O,N*-diacetyl-tomatidine.

O,N-Diacetyl- Δ^5 -tomatidenol-(3 β). Δ^5 -Tomatidenol-(3 β) (10 mg) was acetylated as described above. M.p. 160–2°, $[\alpha]_D^{20}$ –39.6° (methanol, *c* 0.36). (Found: C 74.73; H 9.52). Cal.c. for $C_{31}H_{47}NO_4$: C 74.81; H 9.52).

Dehydration of Δ^5 -tomatidenol-(3 β). Δ^5 -Tomatidenol-(3 β) (10 mg) was dissolved in 1 ml of methanol containing 20 % of dry hydrogen chloride (prepared by passing hydrogen chloride in methanol until the mixture had increased in weight by 20 %). When heated to 100° the solution became clear and needles of $\Delta^{3,5}$ -tomatidiene hydrochloride started separating. This suspension was treated with hot dilute ammonia. In this way $\Delta^{3,5}$ -tomatidiene was obtained. After crystallization from methanol the m.p. was 135–137°, alone and in admixture with the anhydro-aglycone obtained by hydrolysis of the glycosides. R_F 0.80.

Identification of the carbohydrates: The acidic mother-liquour from the hydrolysate of the three glycosides (described under "Hydrolysis") was treated with Amberlite IR-4B. Aliquots were chromatographed in 1-butanol-ethanol-water (8:1:2) and ethyl acetate-pyridine-water (3:6:1:1.15)¹⁶. Authentic D-glucose, D-galactose and L-rhamnose

Table 8. Quantitative determination of monosaccharides in 20 μ l aliquots of α -, β - and γ -solamarine.

Compound	Glucose		Rhamnose		Galactose	
	μ g	mole $\times 10^8$	μ g	mole $\times 10^8$	μ g	mole $\times 10^8$
α -Solamarine	24.5	13.6	21.4	13.0	21.0	11.7
β -Solamarine	20.5	11.4	37.6	23.0		
γ -Solamarine	26.6	14.8	22.1	13.5		

were chromatographed alongside the unknown monosaccharides. The results are given in Table 7.

Quantitative determination of the carbohydrates. The amounts of individual monosaccharides were determined quantitatively utilizing the ability of the sugars to reduce 2,3,5-triphenyltetrazolium chloride to red formazan. 2.9, 2.5 and 2.6 mg of α -, β - and γ -solamarine, respectively, were refluxed in 0.5 ml N hydrochloric acid for 2 h (sealed tube). 20 μ l aliquots of the three solutions were chromatographed and the amount of monosaccharide in each spot determined by the method of Fischer and Dörfel¹⁷. The results are given in Table 8.

Solasodine (IV). 2.00 g of the crude glycoside mixture was hydrolyzed in methanolic hydrochloric acid and worked up as described under "Hydrolysis". Thin-layer chromatography indicated the presence of three aglycones. Chromatography on alumina (Woelm neutral) separated the diene and gave 386 mg of a mixture of Δ^6 -tomatidenol-(3 β) and solasodine. Two crops of Δ^6 -tomatidenol-(3 β) were obtained by crystallization from acetone. The mother-liquor was evaporated and crystallization from methanol gave a mixture of the two components. The mother-liquor remaining from the last crystallization on standing deposited long needles of crude solasodine, m.p. 186–191°. Recrystallization from methanol gave a few mg of chromatographically pure solasodine, m.p. 198–199°, alone and in admixture with authentic solasodine.

Yamogenin (V). 10 l of the ammoniacal filtrate from the alkaloidal glycoside precipitation (cf. Ref.¹⁴) was concentrated to 500 ml and extracted three times with 250 ml of 1-butanol. The combined butanol extracts were mixed with an equal volume of water and the organic solvent largely removed by distillation. The water solution was made 1.5 N with respect to hydrochloric acid and refluxed for 5 h to effect hydrolysis. The crude precipitate was filtered off, washed with water and dried. Yield 360 mg. The precipitate was extracted with benzene in a Soxhlet extractor and chromatographed on alumina (Woelm neutral). Elution with benzene-chloroform (9:1) gave 57 mg of a compound which, recrystallized from methanol, melted at 189–191° (lit. value¹⁸ 201°), $[\alpha]_D^{20} - 121.3^\circ$ (chloroform, $c = 0.35$). Acetylation at room temperature with acetic anhydride and pyridine and recrystallization from acetone gave the acetate, m.p. 181–182° (lit. value¹⁸ 182°). The infrared spectrum showed the bands characteristic for a neo-sapogenin (a band at 920 cm^{-1} with about four times the intensity of the band at 897 cm^{-1}).

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