683. Solanum *Alkaloids. Part VIII.** *Solamargine, a New Alkaloid from* Solanum marginatum.

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From the green fruit of *S. marginatum* a new alkaloid, solamargine, $C_{45}H_{73}O_{15}N$, has been isolated. On hydrolysis with aqueous-alcoholic hydrochloric acid, conditions where there is complete solution throughout, it affords, solasodine, glucose, and rhamnose. With 2—3% aqueous hydrochloric acid partial hydrolysis occurs with liberation of rhamnose and formation of solasodine glucoside as an insoluble hydrochloride, producing solasodine and glucose on further hydrolysis. The molecular formula corresponds with the union of solasodine with one molecule of glucose and two of rhamnose, so that the partial structure, rhamnose–rhamnose–glucose–solasodine, is indicated for solmargine.

Solanum marginatum, a shrub endemic to the Nile delta, Abyssinia, and Italy, has been introduced into New Zealand where it now grows wild round Auckland, Wellington, and in the Marlborough Sounds of the South Island. The green fruits are large, up to 5 cm. in diameter, and extremely bitter.

From the green fruit a new glycosidic alkaloid, $C_{45}H_{73}O_{15}N$, has been islated and characterised as its picrate and picrolonate; the name solamargine is proposed for it. It forms a crystalline benzoate, which is unsuitable for characterisation since the benzoic acid sublimes when the salt is heated.

Hydrolysis of solamargine with aqueous-alcoholic hydrochloric acid at 100°, conditions where there is complete solution throughout, afforded, on cooling, a crystalline hydrochloride, which on basification yielded solasodine, identified by mixed melting points of the free base and its picrate. The remaining solution, after deionisation, was examined for sugars by several variations of paper chromatography: only glucose and rhamnose were detected. When 2—3% aqueous hydrochloric acid, which hydrolyses solanine, solasonine, and solauricine completely, was used, a crystalline hydrochloride separated. The free base, m. p. 251—253° (decomp.), obtained from it, could only be crystallised from a mixture of methyl alcohol and concentrated ammonia and was shown to be solasodine β-glucoside, $C_{33}H_{53}O_7N$. After removal of ionic material from the solution arising from this hydrolysis, crystalline rhamnose was obtained and identified by its mixed melting point and its osazone.

Hydrolysis of solasodine glucoside with aqueous-alcoholic hydrochloric acid afforded

* Part VII, *J.*, 1952, 1654.

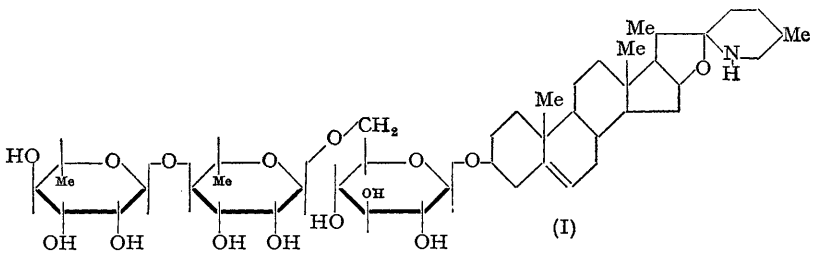
solasodine hydrochloride and from the resulting deionised sugar solution only one spot could be obtained by paper chromatography. This corresponded to glucose from a control solution, and was confirmed by the preparation of glucosazone. Solasodine glucoside also affords solasodine when sublimed in a high vacuum.

Solasodine glucoside has a more negative rotation ($[\alpha]_D$ $-122° \pm 4°$) than solasodine ($[\alpha]_D$ $-113{\cdot}5° \pm 2°$). Since α-glucopyranosides tend to have a positive rotation and the corresponding β-glucopyranosides a negative rotation it appears that glucose is joined to solasodine by a β-linkage. Solasodine glucoside, however, resisted hydrolysis for six months when dissolved with a preparation of emulsin in a sodium acetate–acetic acid buffer solution of pH 4·6, capable of immediately hydrolysing amygdalin. Glucose was however readily detected thereafter by paper chromatography on hydrolysis with aqueous-alcoholic hydrochloric acid.

An attempt was made to prepare solasodine β-glucoside by condensation of solasodine with acetobromoglucose in the presence of silver oxide under anhydrous conditions. It gave, however, solasodine hydrobromide.

The molecular formula of solamargine agrees with the union of solasodine with a hexose and two methyl pentose units. Since glucose and rhamnose were the only sugars formed on hydrolysis the above evidence indicates that solamargine is constituted as rhamnose–rhamnose–glucose–solasodine.

Solamargine does not reduce Fehling's and Tollens's reagents, showing that the sugars are joined through their potential aldehyde groups. The glucose linkage has already been shown to be β and from the great ease of hydrolysis of the rhamnose units it is probable that this sugar also is joined through β-linkages. The above evidence, together with further results on the position of the linkages in the sugar moiety to be submitted later, suggests that solamargine is probably (I).



(I)

That solasodine β-glucoside was a derivative of solasodine was early shown by colour reactions given with *p*-substituted aldehydes and resorcinol (Part II, *J.*, 1942, 3). Dehydrogenation with selenium yielded 2-ethyl-5-methylpyridine, identified as its picrate and styphnate. The significance of this for the structure of solasodine was referred to in Part V (*J.*, 1950, 3013). Solasodine glucoside, in contrast with the free base, readily forms easily crystallisable salts with inorganic and organic acids. With nitrous acid it forms a *N*-nitroso-derivative, but some hydrolysis occurs at the same time since *N*-nitrososolasodine (Part V, *J.*, 1950, 3013) was recovered from the mother-liquors.

Part of the solamargine is completely hydrolysed when treated with 2—3% aqueous hydrochloric acid since, besides solasodine glucoside and rhamnose, solasodine can also be isolated after chromatography of the basic fraction and glucose can be identified as glucosazone in the sugar fraction.

Experimental

M. p.s marked * were taken in evacuated tubes. The analyses are by Drs. Weiler and Strauss, Oxford, Mr. J. Mills, Adelaide University, or Dr. T. S. Ma and Mr. A. D. Campbell, University of Otago, Dunedin.

Green berries of *S. marginatum*, collected in June at Mt. Wellington near Auckland, were passed through a juice extractor, and the resulting juice was filtered through calico bags, boiled for a few minutes, and stored in sterile bottles under toluene. The berries were worked up in small batches to avoid undue exposure to the air since this results in darkening of

the juice and makes the isolation of the pure alkaloid much more difficult. The residual pulp was extracted three times by storage in aqueous acetic acid (3%), and the extracts were treated in the same manner as the juice. After settling, the black supernatant liquor was carefully siphoned off, boiled, and filtered through steam-jacketed, gravity filters. From the filtered solution crude solamargine was precipitated by slow passage of ammonia which was continued until the mixture was cool. The glycoside was taken up in boiling 3% acetic acid solution, then reprecipitated by ammonia, and the whole process repeated. The crude glycoside was then extracted (Soxhlet) to free it from inorganic material, shown to be magnesium ammonium phosphate which behaves like an alkaloid in being soluble in acids and insoluble in ammoniacal solutions. Final purification was brought about by repeated crystallisation from 50% alcohol, with charcoal at first, the yield being 0·3—0·5%. A sample, crystallised ten times, formed thin, colourless, pointed plates, m. p. 301° (decomp.) * after sintering at 270°, $[\alpha]_D^{20}$ −105°±4° (*l*, 0·25; *c*, 0·986 in methyl alcohol) (Found: C, 62·3; H, 8·45; N, 1·7. $C_{45}H_{73}O_{15}N$ requires C, 62·3; H, 8·5; N, 1·6%). *Solamargine* is very soluble in methyl and ethyl alcohol, and from aqueous-alcoholic solutions containing more than 50% alcohol it separates in gelatinous form.

Solamargine picrate, formed from its components in boiling 50% alcohol, after repeated crystallisation from 20% alcohol containing a trace of picric acid, yielded yellow needles, m. p. 188—189° (decomp.)* (Found: C, 55·6; H, 7·2. $C_{45}H_{73}O_{15}N,C_6H_3O_7N_3$ requires C, 55·8; H, 6·9%). That no change had occurred in the formation of this derivative was shown by its reconversion into solamargine, m. p. and mixed m. p. 301° (decomp.)*, by lithium hydroxide solution at 100°.

Solamargine picrolonate was formed overnight from the components in hot 50% alcohol. After four crystallisations from 80% alcohol it yielded long, yellow needles, m. p. 204—205° (decomp.) (Found: C, 57·7; H, 7·1; N, 6·7. $C_{45}H_{73}O_{15}N,C_{10}H_8O_5N_4$ requires C, 58·3; H, 7·2; N, 6·2%).

Solmargine benzoate separated overnight in needles from a hot solution of the components in 60% alcohol. No definite m. p. was observed but long pointed plates sublimed with m. p. 119—120° (benzoic acid has m. p. 121°). The loss on heating overnight at 100°/20 mm. was 11·4% ($C_{45}H_{73}O_{15}N,C_7H_6O_2$ requires $C_7H_6O_2$, 12·3%).

Complete Hydrolysis of Solamargine.—Solamargine (250 mg.) was heated at 100° for ½ hour in 10% hydrochloric acid (10 c.c.). Sufficient alcohol (12 c.c.) to effect complete solution was then added and the heating continued for 1½ hours. Needles formed on cooling (yield 93 mg.), with m. p. 307·5—308° (decomp.)* after recrystallisation from 80% alcohol. The free base was recovered from this hydrochloride by treatment of a suspension with excess of ammonia at 100° for 1 hour. Repeated crystallisation from 80% alcohol, methyl alcohol, and acetone, successively, afforded colourless, hexagonal plates, m. p. and mixed m. p. with authentic solasodine, 199·5—200°.* The picrate crystallised from 80% alcohol in rods, m. p. and mixed m. p. with solasodine picrate, 142—143° (decomp.).

Identification of the Sugars Obtained on Complete Hydrolysis of Solamargine.—In a further experiment where hydrolysis was complete, the solasodine hydrochloride was filtered off and the filtrate heated for a further half hour at 100° to remove alcohol. After removal of a second crop of solasodine hydrochloride the residue was neutralised with silver carbonate, the silver residues were filtered off and washed with water, and the combined filtrates saturated with hydrogen sulphide. After filtration, hydrogen sulphide was removed by aeration and the sugar solution concentrated in a vacuum at 45—50°. Aliquots of this solution were examined for sugars by paper chromatography at 18°, on Whatman No. 1 filter paper strips, 10 × 48 cm. The unknown mixture of sugars was chromatographed according to the general procedure of Jermyn and Isherwood (*Biochem. J.*, 1949, **44**, 402) with various solvents, and a control spot of solution containing known sugars was placed alongside or superimposed on an unknown spot placed alongside a second unknown spot.

(*a*) Development of the unknown sugar spot with the water-poor phase of the system ethyl acetate–acetic acid–water (3 : 1 : 3), freshly prepared, for 30 hours followed by spraying with ammoniacal silver nitrate solution (Partridge, *Nature*, 1946, **158**, 270) afforded two spots corresponding to glucose and rhamnose from control solutions.

(*b*) Development of the unknown sugar spot with the water-poor phase of the solvent system ethyl acetate–pyridine–water (2 : 1 : 2), and spraying with ammoniacal silver nitrate solution also afforded two spots corresponding to glucose and rhamnose from control solutions.

(*c*) The solvent system *n*-butanol–pyridine–water (3 : 1 : 1) (Hough, Jones, and Wadman, *J.*, 1950, 1702) was used as developing solvent, and aniline hydrogen phthalate as spraying reagent (Partridge, *Nature*, 1949, **164**, 443).

In all cases only two spots were obtained, corresponding to glucose and rhamnose.

Partial Hydrolysis of Solamargine to Solasodine β-Glucoside.—Solamargine (10 g.), dissolved in 2% aqueous hydrochloric acid (100 c.c.), was heated at 100° for 1 hour. A precipitate formed in a few minutes and was voluminous and gelatinous after 15 minutes. After some time, the hydrochloride was filtered off, suspended in water, and heated with excess of ammonia for 1 hour. The product could not be crystallised from any of twenty common solvents but crystallised from methyl alcohol–ammonia (*d* 0·880) and, after repeated crystallisation, formed long, colourless needles, m. p. 251—253° (decomp.),* $[\alpha]_D^{20}$ −122°±4° (*l*, 0·25; *c*, 0·954 in methyl alcohol) (Found: C, 69·3, 69·2; H, 9·4, 9·5; N, 2·8. $C_{33}H_{53}O_7N$ requires C, 68·8; H, 9·3; N, 2·4%). In a determination of active hydrogen atoms (Zerewitinoff) a solution of methylmagnesium iodide in anisole gave an evolution of methane from cholesterol in good agreement with the theoretical value but with *solasodine β-glucoside* the reagents gelatinised on mixing and no gas was evolved after several hours at 25°.

It was not possible to obtain a crystalline sugar when the filtrate from the above hydrochloride was treated with silver carbonate and worked up in the usual way. This was achieved, however, in the following manner. Solamargine (20 g.) was hydrolysed by water (200 c.c.) and concentrated sulphuric acid (2 c.c.) at 100° for 2 hours. The final clear solution was decolourised with charcoal, treated with excess of barium carbonate at 100° for 1 hour, and filtered. The free base was recovered from the residue by extraction with methyl alcohol. The filtrate was concentrated at 100° to a syrup and placed in a vacuum-desiccator. When rubbed, the product solidified and, on trituration with absolute alcohol, small prisms were formed, m. p. and mixed m. p. with rhamnose, 91—93°. An osazone prepared from this material crystallised from 80% alcohol in yellow needles, m. p. and mixed m. p. with rhamnose phenylosazone, 184·5—185°.

In a preliminary test for rhamnose in the above experiment by Dehn, Jackson, and Ballard's ammonium molybdate method (*Ind. Eng. Chem., Anal.*, 1932, **4**, 413) we found that the concentration of the rhamnose solution affects the colour produced. When the reagent is added to a few mg. of rhamnose the solution changes from yellowish to green as recorded, but in more dilute solution, comparable with the above hydrolysis solution, a bluish-green colour forms, becoming green overnight. The hydrolysis solution behaved similarly.

In additional hydrolyses of solamargine under approximately the same experimental conditions as above, the basic reaction product was chromatographed on alumina from chloroform solution. The material obtained from the first fraction of the eluate, after repeated crystallisation from methanol and acetone, separated in hexagonal plates, m. p. and mixed m. p. with solasodine, 196—198°. The soluble portion from the hydrolysis yielded glucosazone, m. p. and mixed m. p. 205·5—206° (decomp.).

Salts of solasodine β-glucoside crystallised from alcohol to which a little water was added for clarification but only a few were isolated. The *picrate* formed needles, m. p. 191° (Found: C, 58·4, 58·1; H, 7·0, 6·9; N, 7·4. $C_{33}H_{53}O_7N,C_6H_3O_7N_3$ requires C, 58·2; H, 7·0; N, 7·0%). The tartrate (needles), citrate (needles), and oxalate (plates) had m. p. 216°, 165°, 308°, respectively, all with decomposition. Formation of a hydrochloride (see above), hydrobromide, hydriodide, sulphate, nitrate, chloroplatinate, chloroaurate (all needles) and benzoate (plates) was also observed.

To solasodine β-glucoside (2 g.) in alcohol (15 c.c.) and glacial acetic acid (1 c.c.) a solution of sodium nitrite (600 mg. in 3 c.c.) was added. The crystalline *nitroso*-derivative, which formed overnight, recrystallised from 80% alcohol as needles, m. p. 233·5—234° (decomp.) (Found: C, 65·5, 65·8, 66·0; H, 9·0, 8·6, 9·0; N, 5·9, 5·2, 5·4. $C_{33}H_{52}O_8N_2$ requires C, 65·5; H, 8·7; N, 4·6%). A fraction of the main product was not readily soluble in 80% alcohol and after repeated crystallisation from alcohol formed rectangular prisms, m. p. and mixed m. p. with *N*-nitrososolasodine, 260—261° (decomp.) (Found: C, 73·0; H, 9·3; N, 6·6, 6·55. Calc. for $C_{27}H_{42}O_3N_2$: C, 73·3; H, 9·6; N, 6·3%). *N*-Nitrososolasodine β-glucoside is unchanged by aqueous or alcoholic ammonia at 100° (1 hour).

Hydrolysis of Solasodine β-Glucoside and Identification of Glucose.—(a) *By paper chromatography.* Solasodine β-glucoside (5 mg.) was hydrolysed by a hot mixture of 10% hydrochloric acid (0·25 c.c.) and alcohol (1 c.c.). After removal of the solasodine hydrochloride (needles) the filtrate was treated with silver carbonate, and the sugar solution worked up as previously described. Development of a spot of the resultant solution with the water-poor phase of the system ethyl acetate–acetic acid–water (3 : 1 : 3) as developing solvent and ammoniacal silver nitrate as spraying reagent afforded only one spot, corresponding to glucose from control experiments.

(b) *By formation of glucosazone.* Solasodine β-glucoside (100 mg.) was hydrolysed as above and the sugar solution, worked up as before, concentrated in a vacuum over sulphuric acid at room temperature to *ca.* 2 c.c. Treatment with phenylhydrazine in the usual way followed by recrystallisation of the product from acetone afforded yellow needles, m. p. and mixed m. p. with glucosazone, 205—206° (decomp.).

Sublimation of Solasodine β-Glucoside.—When heated at *ca.* 190—200° in a high vacuum solasodine β-glucoside decomposed and solasodine sublimed. The product separated from alcohol as hexagonal plates, m. p. and mixed m. p. with solasodine 201—202·5°.

Dehydrogenation of Solasodine β-Glucoside.—Solasodine β-glucoside (5 g.) and selenium (15 g.), intimately mixed, were heated under nitrogen at 280°. A greenish liquid distilled, and at 290—300° severe frothing occurred. Heating was continued for 4 hours at 320°. The dark distillate was acidified with hydrochloric acid and extracted with ether. The aqueous fraction was made alkaline with solid sodium hydroxide and then yielded to ether a small amount of dark oil. On distillation in a micro-column (Craig, *Ind. Eng. Chem., Anal.*, 1936, **8**, 219) three fractions were collected: (*a*) b. p. up to 100° (23·5 mg.), slightly yellow; (*b*) b. p. 100—150° (24·0 mg.), pale brown; and (*c*) b. p. 150—200° (31·5 mg.), brown, partly solid. Fractions (*a*) and (*b*) gave identical picrates when treated with a saturated solution of picric acid in alcohol and, after repeated crystallisation from alcohol, had m. p. 141·5—142·5°, undepressed by authentic 2-ethyl-5-methylpyridine picrate, kindly presented by Dr. Lyman C. Craig (Found: C, 48·2; H, 3·9; N, 16·0. Calc. for $C_8H_{11}N,C_6H_3O_7N_3$: C, 48·0; H, 4·0; N, 16·0%). The mother-liquors from the picrate were decomposed with lithium hydroxide solution (cf. Burger, *J. Amer. Chem. Soc.*, 1945, **67**, 1615) and then yielded to ether an oil which was converted into the styphnate in methyl alcoholic solution. The yellow needles formed on cooling had m. p. 169·5°. Prelog and Szpilfogel (*Helv. Chim. Acta*, 1942, **25**, 1306) record m. p. 170° for the styphnate of 2-ethyl-5-methylpyridine.

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