529. Solanum *Alkaloids. Part XVI.*[1] *The Sugar Moiety of Solasonine.*

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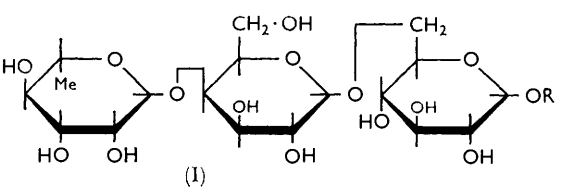
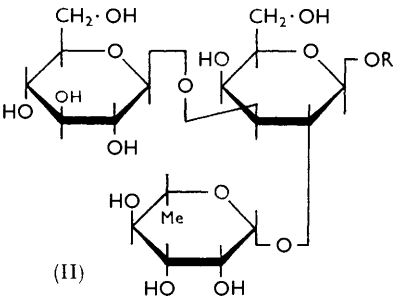
The sugar moiety of solasonine (II; R = solasodine) has been shown to be solatriose, *O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→3)]-β-D-galactopyranose (II; R = H).

On hydrolysis, both solanine and solasonine yield one mole each of rhamnose, glucose, and galactose.[2] Both alkaloids have no reducing properties showing that the sugars are joined through their potential aldehyde groups.[2a] Zemplén and Gerecs [3] produced evidence from the reactions of the acetylated glycoside suggesting that solanine was constituted in the order of components, rhamnose–galactose–glucose–solanidine. From a re-interpretation of Oddo and Caronna's work [4] and on analogy with solanine it was suggested [5] that the order of components in solasonine was the same, *i.e.*, rhamnose–galactose–glucose–solasodine. As a result of experiments involving, in turn, oxidation with periodate and with bromine and hydrolysis, and on assuming the above order of components the structure (I; R = solasodine) was earlier proposed for solasonine.[2a] Since solanine gave an almost identical oxidation pattern with periodate, it was also suggested that the sugar portions of the two alkaloids were the same. Subsequently, however, Kuhn, Löw, and Trischmann [6] showed from examination of the sugars derived by hydrolysis of the base and its permethylated derivative that solanine has the structure (II; R = solanidine), with the galactose unit of the trisaccharide, solatriose (II; R = H), directly linked to the alkamine. In an earlier experiment, Kuhn and his co-workers [7] subjected solasonine to periodate oxidation for 72 hours and, after hydrolysis of the product, detected galactose chromatographically as the only surviving sugar, a result consistent with structure (II; R = solasodine) for solasonine but not in agreement with (I; R = solasodine) and three other structures previously considered.[2a] The sugar portion of solasonine has now been reinvestigated and shown to be solatriose.



(I)



(II)

When solasonine was hydrolysed with 0·01M-sulphuric acid for 75 minutes at 100° rhamnose was the only sugar which could be detected by paper chromatography; rhamnose is thus in a terminal position. Hydrolysis with 0·2M-sulphuric acid for 40 minutes at 100° afforded three oligosaccharides, A, B, and C, as well as rhamnose, glucose, and galactose, all detected chromatographically. Although oligosaccharides A and B were not obtained crystalline their homogeneous solutions had R_{lactose} values in agreement with

[1] Part XV, Briggs, Cambie, and Hoare, *J.*, 1961, 4645.
[2] (*a*) Briggs and Vining, *J.*, 1953, 2809; (*b*) Schreiber, *Chem. Tech.* (*Berlin*), 1955, **7**, 271.
[3] Zemplén and Gerecs, *Ber.*, 1928, **61**, 2294.
[4] Oddo and Caronna, *Ber.*, 1934, **67**, 446.
[5] Briggs, Newbold, and Stace, *J.*, 1942, 3.
[6] Kuhn, Löw, and Trischmann, *Chem. Ber.*, 1955, **88**, 1492.
[7] Kuhn, Löw, and Trischmann, *Chem. Ber.*, 1955, **88**, 289.

those of solatriose[6] (II; R = H) and solabiose (3-*O*-β-D-glucopyranosyl-D-galactopyranose),[6] respectively, when co-chromatographed with authentic specimens for long periods and over large migration distances in three different solvent systems. Oligosaccharide C had an $R_{lactose}$ value in agreement with that of 2-*O*-α-L-rhamnopyranosyl-D-galactopyranose,[6] but was not obtained in sufficient quantity for rigid purification and more detailed investigation.

These chromatographic results alone could be taken as an identification of solatriose in the anomeric form described by Kuhn and co-workers,[6] and all subsequent results are in agreement. Thus, as expected, oligosaccharide A gave approximately equal amounts of rhamnose, glucose, and galactose on hydrolysis and failed to give a colour with triphenyltetrazolium hydroxide,[8] behaviour which is typical of reducing sugars substituted in the 2 position.[9] There was insufficient material for a measurement of rotation. The identity of oligosaccharide B with solabiose was confirmed by means of its osazone and rotation. The free sugar afforded glucose and galactose on hydrolysis and gave a positive test with triphenyltetrazolium hydroxide; the osazone yielded glucose as the only reducing sugar on hydrolysis.

Oligosaccharide A can only be built from solabiose with attachment of the rhamnose unit in position 2, to account for its failure to react with triphenyltetrazolium hydroxide, in further support of its identification with solatriose.

The structure of solasonine was also confirmed by following Kuhn's procedure[6] for solanine. Methylation of solasonine in dimethylformamide with silver oxide and methyl iodide,[6] followed by hydrolysis, gave a mixture of methylated sugars which was partly separated by fractionation between chloroform and water, whence the more highly methylated sugars passed into the organic phase. Separation of the components from the chloroform phase on a charcoal–Celite column gave 2,3,4,6-tetra-*O*-methyl-α-D-glucose and 2,3,4-tri-*O*-methyl-L-rhamnose, and chromatography of the methylated sugars from the aqueous phase on a cellulose column yielded 4,6-di-*O*-methyl-α-D-galactose, these being the methylated sugars also obtained from solanine.[6]

During preliminary hydrolyses with various strengths of acid, further results in harmony with the above conclusions were obtained by examination of the free sugars produced and of the fragments of partially hydrolysed glycosidic alkaloid.

As already stated, rhamnose alone was removed when solasonine was treated with 0·01M-sulphuric acid for 75 minutes at 100°. After its treatment with 0·1M-acid for 10 minutes at 100° considerable amounts of rhamnose and moderate amounts of glucose, but only traces of galactose, as well as small amounts of oligosaccharides A, B, and C were detected chromatographically. Between hydrolysis times of 4 minutes, when it was first detected, and 12·5 minutes, when its concentration became low, the yield of oligosaccharide C was always very much less than that of glucose; up to a hydrolysis time of 40 minutes, galactose could barely be detected. Only a structure with galactose directly linked to the alkamine fits these results.

After hydrolysis with 0·1M-acid for 30 minutes at 100°, paper chromatography showed the presence of unchanged solasonine and three partially hydrolysed glycosidic alkaloid fractions corresponding in R_F value[1] to solasodine galactoside, solasodine rhamnosylgalactoside, and solasodine glucosylgalactoside, a result only in harmony with a branched-chain triose.

With the revised structures of solanine and solasonine the earlier results of periodate oxidation[2a] are now anomalous. Over-oxidation[10] cannot be the full explanation since

[8] (*a*) Mattson, Jenson, and Dutcher, *Science*, 1947, **106**, 294; (*b*) Trevelyan, Proctor, and Harrison, *Nature*, 1950, **166**, 444; (*c*) Wallenfels, *Naturwiss.*, 1950, **37**, 491.

[9] Bell and Dedonder, *J.*, 1954, 2866; Gardiner and Percival, *J.*, 1958, 1414; McLennan, Randall, and Smith, *Biochem. J.*, 1961, **80**, 309.

[10] Head and Hughes, *J.*, 1952, 2046; Dyer, in "Methods of Biochemical Analysis," Interscience Publ., Inc., New York, 1956, Vol. III, p. 111; Barker and Shaw, *J.*, 1959, 584; Marder and Schuerch, *J. Org. Chem.*, 1959, **24**, 1977; Lee, *J.*, 1960, 1474.

it does not account for the relatively slow formation of two mol. of formic acid. However, repetition of the oxidations, with five mol. of periodate but in the dark at 20°, afforded the expected results, both solanine and solasonine consuming 4 mol. of periodate and liberating 2 mol. of formic acid in 25 hours; there was very slight further oxidation (not exceeding 0·2 mol.) in 200 hours.

Kuhn and his co-workers[7] have shown that the galactose unit is not attacked in this reaction. The consumption of periodate and formation of formic acid must, therefore, be due only to the glucose and rhamnose units in which, also, furanose forms are excluded.

Mainly on the basis of periodate oxidation a linear structure for solamargine, the dirhamnosylglucoside of solasodine, was suggested.[11] As the experimental procedure was similar to that used in earlier experiments on solanine and solasonine the structure proposed is now unlikely. On biogenetic grounds it appears more probable that solamargine, which often accompanies solasonine,[1] has the same sugar portion as α-chaconine (solanidine dirhamnosylglucoside),[12] which often accompanies solanine.

Experimental

Analyses are by Dr. A. D. Campbell and his associates, University of Otago, New Zealand. Infrared spectra were determined as KBr discs with a Beckman IR2 instrument. Unless stated otherwise, descending paper chromatography was carried out on Whatman's No. 1 paper, equilibrated for 12 hr., with the following solvent systems: (for sugars) A, butan-1-ol–pyridine–water (3 : 1 : 1·5, upper phase with 1 vol. of pyridine); B, ethyl acetate–acetic acid–water (3 : 1 : 3); C, butan-1-ol–ethanol–water (4 : 1 : 5); (for glycosides) D, ethyl acetate–acetic acid–water (11 : 2 : 1·85, to give a homogeneous mixture).[1]

Partial Hydrolyses of Solasonine.—In general a sample of solasonine (*ca.* 20 mg.) was added portionwise to boiling dilute sulphuric acid (*ca.* 5 c.c.) and heated under reflux for the appropriate time. The rapidly cooled solution was neutralised with barium carbonate, then filtered, and the filtrate plus washings were concentrated to a small volume under an air stream. Aqueous ammonia (*d* 0·88; 1 drop) was added, the precipitated alkaloids were removed, and the combined filtrate and washings were evaporated to dryness. The minimum volume of water for dissolution was added, and the solution was filtered and concentrated to suitable volume. Paper chromatography of the sugars was then carried out in solvent A or B with authentic L-rhamnose, D-glucose, and D-galactose as controls.

The following is a summary of repeated experiments:

0·01M-acid; 75 min. Small amounts of rhamnose only were produced.

0·1M-acid; 4, 6, 8, and 10 min. In all cases, considerable amounts of rhamnose, moderate amounts of glucose, traces of galactose, and small quantities of three slow-moving oligosaccharides, A, B, and C (increasing order of R_F value), were produced, the relative concentration of A decreasing and of B increasing with time, that of C being small and approximately constant.

0·1M-acid; 20, 30, and 40 min. In all cases, considerable amounts of rhamnose, moderate amounts of glucose, and moderate to small quantities of oligosaccharide B were formed. Paper chromatography of the partially hydrolysed alkaloids in solvent D showed four spots with R_S values, 1·3 (strong), 2·5 (strong), 5·4 (weak), and 10·7 (weak) (cf. ref. 1). ($R_S = R_F$ value relative to that of solanine.)

0·1M-acid; 80 min. Results similar to those in the last paragraph were obtained except that the quantity of galactose was appreciable.

Chromatographic migration distances (cm.) (mean of three determinations).

Solvent	Time	Oligosaccharide A	Solatriose	Oligosaccharide B	Solabiose
A	60 hr.	26·9	27·2	31·0	31·5
B	246 hr.	11·1	11·2	18·4	18·5
C	270 hr.	20·6	20·6	27·7	27·6

Purification of Oligosaccharides A, B, and C.—The brown sugar syrup obtained after hydrolysis of solasonine [20 g., containing a little solamargine (paper chromatography)] with 0·2M-sulphuric acid (3 l.) for 40 min. at 100° and purification as above, was shown chromatographically

[11] Briggs and Brooker, *J.*, 1953, 2833.
[12] Kuhn, Löw, and Trischmann, *Chem. Ber.*, 1955, **88**, 1690.

to consist mainly of rhamnose and glucose with a small amount of oligosaccharide B, a smaller amount of oligosaccharide A, and traces of galactose and oligosaccharide C. The oligosaccharides were purified by repeated chromatography on Whatman's No. 3 MM paper with solvent B in the usual manner.

Oligosaccharide A (*Solatriose*).—After several purifications oligosaccharide A was obtained as a brown hygroscopic gum which gave a pale brown spot with aniline hydrogen phthalate. It had $R_{lactose}$ value in solvent A, 1·04 (lit.,[6] 1·08 for solatriose; for co-chromatography see the Table).

By Wallenfels's procedure [8c] oligosaccharide A gave no colour with triphenyltetrazolium hydroxide. When hydrolysis was with 0·1M-sulphuric acid for 2 hr., glucose, galactose, and rhamnose, in approximately equal amounts, were detected by paper chromatography.

Oligosaccharide B (*Solabiose*).—Repeated paper chromatography gave oligosaccharide B as a brown hygroscopic gum which, even after four purifications, contained traces of oligosaccharide A, as indicated by prolonged paper chromatography. It had $R_{lactose}$ value in solvent A, 1·22 (lit.,[6] 1·26 for solabiose; for co-chromatography see Table) and $[\alpha]_D^{20}$ +35° (*c* 0·2 in H_2O) with no mutarotation (lit.,[6] $[\alpha]_D^{22}$ +40·7°, no mutarotation). It gave a dark brown spot with aniline hydrogen phthalate.

Oligosaccharide B gave a red colour with triphenyltetrazolium hydroxide. When hydrolysed with 0·2M-sulphuric acid for 2 hr. it gave equal spots of glucose and galactose and a small spot of rhamnose on paper chromatography.

A mixture of dried oligosaccharide B (50 mg.), phenylhydrazine (0·06 c.c.), acetic acid (0·06 c.c.), and water (0·5 c.c.) was heated in a sealed flask at 100° for 1·5 hr. After cooling, ether (2 c.c.) was added and the mixture shaken and stored at 0° for some weeks. The product (29 mg.) was collected, washed with water and ether, and dried. Purification from methanol and two crystallisations from ethanol gave solabiose osazone as yellow needles, m. p. 216—218° (decomp.; evacuated tube) (lit.,[6] m. p. 225°). (When a comparison of this material with an authentic specimen was made the m. p.s had dropped to 204—205° and 206°, respectively, but there was no depression on admixture.) Acid hydrolysis and paper chromatography led to the detection of glucose alone.

Oligosaccharide C.—Oligosaccharide C was produced only in traces under any of the conditions employed. In spite of chromatographic purification it afforded small amounts of glucose (cf. Kuhn *et al.*[6]) as well as galactose and rhamnose after acid hydrolysis. The glucose probably arises from the presence of a rhamnosylglucose from solamargine in the starting material. This oligosaccharide had $R_{lactose}$ in solvent A 1·8, with considerable streaking (lit.,[6] 1·81 for 2-*O*-α-L-rhamnopyranosyl-D-galactopyranose).

Permethylsolasonine Methiodide.—Solasonine (7 g.; dried at 110° over $MgClO_4$ *in vacuo* for 5 days) and methyl iodide (45 c.c.) in dimethylformamide (125 c.c.) were treated with portions of silver oxide (45 g.; dried at room temperature over P_2O_5 *in vacuo*) while nitrogen was bubbled through the cooled (0—5°) solution. The mixture was then shaken in an atmosphere of nitrogen for 70 hr. with periods of cooling in ice. The solids were removed and washed with dimethylformamide (80 c.c.) and chloroform (100 c.c.), which caused formation of a precipitate in the filtrate. This was dissolved by shaking it with a solution of potassium cyanide (35 g.) in water (750 c.c.), after which the chloroform layer was separated. The aqueous layer was then extracted with chloroform (5 × 200 c.c.), and the combined chloroform extracts were washed with water (2 × 100 c.c.). Removal of solvent from the dried organic extracts gave a brown gum (9·85 g.).

For analysis a portion was again treated with potassium cyanide, and a methanolic solution of the gum so obtained was treated with charcoal and evaporated to dryness. The residue was dissolved in a little benzene and precipitated with light petroleum (b. p. 50—70°). A sample purified in this way was obtained as a pale brown gum which after drying at 80° over $MgClO_4$ *in vacuo* for 12 hr. coalesced at 140—145° (Found: OMe, 22·75. $C_{56}H_{96}INO_{16}$ requires 9OMe, 23·95%).

"Permethylsolasonine."—*Permethylsolasonine methiodide* (9·5 g.), dissolved in methanol (300 c.c.) and water (60 c.c.), was dehalogenated by shaking it with silver carbonate (45 g.) for 4 hr. The silver salts were removed and washed with methanol. Concentration of the combined filtrates *in vacuo* gave a pale brown gum (7·8 g.) which slowly softened and coalesced between 130° and 150°. For analysis the substance was dried at 100° over P_2O_5 *in vacuo* to constant weight (Found: C, 59·8; H, 8·6; O, 28·3; OMe, 21·0%). The material is not pure

since the analytical figures do not agree with those required either by permethylsolasonine or its carbonate (cf. " permethyltomatine carbonate " [13]).

Hydrolysis of " Permethylsolasonine " and Purification of the Methylated Sugars.—" Permethylsolasonine " (7·5 g.) and 5% methanolic hydrochloric acid (250 c.c.) were heated under reflux for 6 hr. The mixture was cooled, water (50 c.c.) added, and the solution concentrated *in vacuo* to 90 c.c. Further water (50 c.c.) was added and precipitated solids were removed from the cooled mixture. The methyl glycosides were then hydrolysed by adding concentrated hydrochloric acid (10 c.c.) to the gently boiling solution and heating the mixture under reflux for 2½ hr. Chloroform (50 c.c.) was added to the cooled solution, a black tar which separated was removed, and the aqueous layer was extracted with chloroform (6 × 50 c.c.). The combined extracts were washed with water until neutral and then evaporated to a brown gum (1·67 g., after drying) which showed two spots (reddish-brown and dark-brown) on treatment of paper chromatograms with aniline hydrogen phthalate (cf. the behaviour of a mixture of 2,3,4,6-tetra-*O*-methyl-α-D-glucose and 2,3,4-tri-*O*-methyl-L-rhamnose [6]). The sugars were separated by chromatography of the gum (1·0 g.) on a charcoal–Celite (1 : 1) column [14] with 2·5% aqueous ethyl methyl ketone as eluant. After 22 fractions (each 50 c.c.) had been collected, further fractions (200 c.c.) were eluted with 5% aqueous ethyl methyl ketone–5% aqueous ethanol (1 : 1).

2,3,4-*Tri*-O-*methyl*-L-*rhamnose*. Fractions 11—24 were combined and concentrated, and the residue was freed from inorganic impurities by dissolution in methanol. Removal of solvent from the filtrate gave a pale brown viscous oil (207 mg.), homogeneous by paper chromatography in solvent C, and having the same *R* value (relative to 2,3,4,6-tetra-*O*-methylglucose), 1·03, as 2,3,4-tri-*O*-methyl-L-rhamnose (lit.,[15] 1·01) when co-chromatographed with an authentic sample, and $[\alpha]_D^{22}$ +24° (*c* 2·1 in H_2O) with no mutarotation (lit.,[6] $[\alpha]_D^{24}$ +27·5°, no mutarotation). It gave a dark brown spot with aniline hydrogen phthalate and a pink colour with triphenyltetrazolium hydroxide. The anilide, prepared by Kuhn and his co-workers' procedure,[6] sublimed at 200—210°/0·01 mm. to give flakes with m. p. 120—124° (lit.,[6] m. p. 124—125°).

2,3,4,6-*Tetra*-O-*methyl*-α-D-*glucose*. Fractions above no. 26, containing only the substance giving a reddish-brown colour with aniline hydrogen phthalate, were combined and evaporated to dryness *in vacuo* at room temperature to give brown crystals (370 mg.). After repeated crystallisation from ligroin and hexane they formed colourless needles with variable m. p. 90—96°, undepressed by authentic 2,3,4,6-tetra-*O*-methyl-α-D-glucose (lit.,[16] m. p. 88—89°,[17] 96°) (Found: C, 51·0; H, 8·3; OMe, 51·9. Calc. for $C_{10}H_{20}O_6$: C, 50·8; H, 8·5; OMe, 52·5%), $[\alpha]_D^{22}$ +91·5° (*c* 1·0 in H_2O) ⟶ $[\alpha]_D^{22}$ +84° (24 hr.) {lit.,[17] $[\alpha]_D$ +92·2° ⟶ 83·8° (final rotation in H_2O)}. It had the same R_F value (0·75, in butan-1-ol saturated with water) as 2,3,4,6-tetra-*O*-methyl-α-D-glucose (lit.,[18] 0·74) when co-chromatographed with an authentic specimen, and the infrared spectra were identical. It gave no colour with triphenyltetrazolium hydroxide. The anilide, prepared by Kuhn and his co-workers' method [6] and sublimed at 200—230°/0·01 mm., had m. p. 136°, $[\alpha]_D^{23}$ +230° (*c* 0·2 in Me_2CO) (lit.,[6] m. p. 136—137°, $[\alpha]_D^{23}$ +237°).

Demethylation with hydrobromic acid by Hough, Jones, and Wadman's method [18] followed by paper chromatography led to the detection of glucose.

4,6-*Di*-O-*methyl*-α-D-*galactose*. The aqueous layer obtained from the hydrolysis of " permethylsolasonine " was neutralised with silver carbonate, and the silver salts were removed and washed well with methanol. Further separation of impurities from the concentrated filtrate and washings finally gave a brown gum which was purified by chromatography on a cellulose column with moist butan-1-ol as eluant.[19] Fractions containing the pure sugar, as determined by paper chromatography, gave a brown oil (102 mg.) on removal of the solvent *in vacuo*. Repeated crystallisation from dry acetone and twice from ethyl acetate–methanol gave 4,6-di-*O*-methyl-α-D-galactose as needles, m. p. and mixed m. p. 140·5° (Found: C, 46·4; H, 7·9; OMe, 29·3. Calc. for $C_8H_{16}O_6$: C, 46·15; H, 7·75; OMe, 29·9%), $[\alpha]_D^{20}$ +135° (*c* 0·5 in H_2O)

[13] Kuhn, Löw, and Trischmann, *Chem. Ber.*, 1957, **90**, 203.
[14] Lindberg and Wickberg, *Acta Chem. Scand.*, 1954, **8**, 569.
[15] Hirst, Hough, and Jones, *J.*, 1949, 928.
[16] Bishop, Blank, and Gardner, *Canad. J. Chem.*, 1960, **38**, 869.
[17] Irvine and Oldham, *J.*, 1921, **119**, 1744.
[18] Hough, Jones, and Wadman, *J.*, 1950, 1702.
[19] Hough, Jones, and Wadman, *J.*, 1949, 2511.

$\longrightarrow$ $[\alpha]_D^{20}$ +76° (16 hr.) {lit.,[6] $[\alpha]_D^{23}$ +133° $\longrightarrow$ $[\alpha]_D^{23}$ +75·1° (17 hr.)}. The chromatographic behaviour in solvent C was identical with that of the authentic material and the infrared spectra were identical. The osazone crystallised from aqueous methanol as yellow needles, m. p. 160—161°, $[\alpha]_D^{20}$ +50° (*c* 0·5 in $CHCl_3$) $\longrightarrow$ $[\alpha]_D^{20}$ +20° (24 hr.) $\longrightarrow$ $[\alpha]_D^{20}$ −21° (150 hr.) {lit.,[20] m. p. 160—162°, $[\alpha]_D^{20}$ +50° $\longrightarrow$ $[\alpha]_D^{20}$ −21° (143 hr.)}.

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[20] Bacon, Bell, and Lorber, *J.*, 1940, 1147.