

## 285. *Solanum Alkaloids. Part XIII.\* The Examination of the Alkaloids from Seven Solanum Species.*

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The glycosidic alkaloids of the fruit of *S. laciniatum* Ait., *S. nodiflorum* Jacq. (forms A and B), *S. miniatum* Bernh., *S. "simile," S. gracile* Otto, *S. douglasii* Dun., and *S. nigrum* L. have been examined. Solasonine has been isolated from the first four species and solamargine from the others.

THE morellae-verae species, *Solanum nodiflorum* Jacq. (forms A and B), *S. gracile* Otto, *S. douglasii* Dun., *S. miniatum* Bernh., and *S. nigrum* L. have often been placed together under the name *S. nigrum* L. and the dulcamara species, *S. aviculare* Forst., *S. "simile,"* and *S. laciniatum* Ait., under the name *S. aviculare* Forst. It has been shown, however, that each is a separate species and, with the exception of *S. nodiflorum* (Forms A and B), they do not interbreed freely.<sup>1</sup>

The first member of the *Solanum* genus to be examined chemically appears to have been *S. nigrum*.<sup>2</sup> Desfosses<sup>3</sup> obtained an alkaloid from this plant which he named "solanine" and later Schutte<sup>4</sup> stated that this alkaloid appeared to be identical with the base contained in *S. tuberosum*. This latter alkaloid is now known to be solanine.<sup>5</sup> Various reports mention the "solanine" content of *S. nigrum*<sup>6,7,8</sup> in connection with its use in pharmacology or agriculture. In a comprehensive survey of the species of the

Species	Somatic chromosome no.	Natural distribn.	Status in N.Z.	Seed source of Dunedin crop	Remarks	Yield (%) of alkaloid
<i>Section: morellae-verae</i>						
<i>S. nodiflorum</i> Jacq. form A.	24	U.S.A.	Weed of cultivation	Berkeley, California	Form with purplish leaves but white flowers	0-30
„ form B.	24	Tropics and subtropics	Possibly indigenous	Fanal Is., Hauraki Gulf	The type form of the species; no purple pigment except at nodes; interbreeds freely with form A.	0-45
<i>S. gracile</i> Otto	24	Unknown	Naturalised in Westland	F1 hybrids between Westland race and a race from C. E. Jorgensen, Copenhagen	Close to the Californian <i>S. douglasii</i> in appearance but genetically isolated from it	Solamargine 0-23
<i>S. douglasii</i> Dun.	24	California	Not present	Carpinteria, California	—	0-20
<i>S. miniatum</i> Bernh.	48	Eurasia	Not present	Portugal	—	0-10
<i>S. nigrum</i> L.	72	Eurasia	Extensively naturalised	F1 hybrids between a Portuguese race and one from Three Kings Is.	Species most likely to be used by chemical workers in temperate countries except U.S.A. where it is said to be still rare	0-07
<i>Section: dulcamara</i>						
<i>S. aviculare</i> Forst.	46	N.Z., Australia	Indigenous	Narromine, N.S.W.	—	Solasonine 0-74
" <i>S. simile</i> "	92	W. Australia	Not present	Garden Is., Freemantle	Will have to be re-named	0-41
<i>S. laciniatum</i> Ait. Bernh.	92	N.Z., Australia	Indigenous	Dunedin	—	0-88

family Solanaceae which have been reported to contain glycosidic alkaloids,<sup>2</sup> Schreiber has stated that the occurrence of solanine, which is often mentioned in the earlier literature, is doubtful and its presence should be verified by further investigation. This is the case with

\* Part XII, preceding paper.

<sup>1</sup> Professor G. T. S. Baylis (University of Otago, Dunedin, N.Z.), *Trans. Roy. Soc., N.Z.*, 1954, **82**, 639 and personal communication. His remarks appear in Table 1.

<sup>2</sup> Schreiber, *Chem. Tech. (Berlin)*, 1954, **6**, 648.

<sup>3</sup> Desfosses, *Jahresb.*, 1820, **2**, 114; *Pharm. J.*, 1821, **7**, 414.

<sup>4</sup> Schutte, *Arch. Pharm.*, 1891, **229**, 527.

<sup>5</sup> Prelog and Jeger, "The Chemistry of Solanum and Veratrum Alkaloids," in Manske and Holmes, "The Alkaloids," Academic Press Inc., New York, 1953, Vol. III, p. 248.

<sup>6</sup> Pollacci and Galotti, *Boll. Soc. ital. Biol. sper.*, 1940, **15**, 328; *Chim. et Ind.*, 1940, **44**, 528; *Chem. Abs.*, 1943, **37**, 2875.

<sup>7</sup> Leclerc, *Presse med.*, 1938, **46**, 480; *Chem. Abs.*, 1938, **32**, 5924.

<sup>8</sup> Brieg, *Suddeut. Apoth.-Zig.*, 1940, **80**, 501; *Chem. Abs.*, 1941, **35**, 2273.

*S. nigrum*, the alkaloid in which has now been shown to be solamargine, and not solanine as previously believed. Solamargine was also isolated from *S. gracile* and *S. douglasii*, which differ morphologically only in two minor features<sup>1</sup> but are maintained as distinct species because they interbreed only with difficulty.

The fruit of *S. aviculare* has been examined by Bell and Briggs<sup>9</sup> and found to contain solasonine and more recently Kuhn and his co-workers<sup>10</sup> have shown that the leaves of the plant contain the same alkaloid and a further alkaloid corresponding to solamargine. When extracting the berries of *S. auriculatum*, *S. sodomaeum*, and *S. marginatum* by an improved method, Briggs and Brooker<sup>11</sup> isolated solasodamine, a glycosidic alkaloid containing a tetrasaccharide moiety, while *S. aviculare* under the same conditions gave only solasonine.

The berries \* of the species now examined were extracted by the improved method of Briggs and Brooker<sup>11</sup> in which the possibility of enzymic hydrolysis has been minimised. Solasonine was isolated from *S. laciniatum*, *S. nodiflorum* forms A and B, *S. miniatum*, *S. "simile"*, and again from *S. aviculare*.

In their examination of the leaves of *S. aviculare*, Kuhn and his co-workers<sup>10</sup> were able to separate solasonine and solamargine by chromatography, on alumina, of the total alkaloid mixture with water-saturated butan-1-ol. For good recovery from the columns it was necessary to stir and store the alumina with moist butanol before packing it. Elution with the same solvent gave solasonine and, then in higher yield, solamargine. In the present investigation, chromatography of the partially purified alkaloid material, under these conditions, gave only a single alkaloid in each of two cases tried (*viz.*, *S. laciniatum* and *S. gracile*). The compounds gave good analyses for solasonine and solamargine respectively. Kuhn and his co-workers were also able to separate solasonine (shown to be homogeneous by paper chromatography) from solamargine by purification from methanol without the addition of water. Solasonine is soluble with difficulty in absolute methanol but is soluble when water is present, while solamargine is freely soluble in absolute or aqueous methanol. In each case in the present investigation the total alkaloid content was either completely insoluble or completely soluble in absolute methanol and in the case of solamargine could not be crystallised from concentrations greater than 50% alcohol (*cf.* Briggs *et al.*<sup>12</sup>). From these results it appears that only a single alkaloid was present as the major component in the fruits of the present species.

In an attempt to identify possible mixtures of glycosidic alkaloids present in the fruits by paper chromatography the system of Kuhn and his co-workers<sup>10,13</sup> [ethyl acetate-acetic acid-water (3 : 1 : 3 v/v), upper phase with 15 vol. % of 85% ethanol] was used. Attempts, however, to separate authentic samples of solanine, solasonine, solamargine, and solasodamine failed. Moreover it was not even possible to obtain reproducible  $R_{as}$  values. Numerous modifications designed to standardise conditions met with no success and in view of the fluctuations of  $R_{as}$  values reported by Kuhn *et al.*<sup>10</sup> we regard the system as unsatisfactory for characterisation.

Satisfactory identification of the glycosidic alkaloids was achieved by normal analytical and degradative methods. The purified compounds were analysed and crystalline picrates and picrolonates were prepared and analysed in all cases. With one exception, *viz.*, *S. miniatum*, from which the glycosidic alkaloid was isolated in only small yield, the identity was confirmed, after complete hydrolysis, by identification of the alkamine by mixed melting points and infrared spectra and by paper chromatography of the liberated sugars under identical conditions.

\* Forwarded by Professor G. T. S. Baylis (University of Otago, Dunedin, N.Z.).

<sup>9</sup> Bell and Briggs, *J.*, 1942, 1.

<sup>10</sup> Kuhn, Löw, and Trischmann, *Chem. Ber.*, 1955, **88**, 289.

<sup>11</sup> Briggs and Brooker, preceding paper.

<sup>12</sup> Briggs, Brooker, Harvey, and Odell, *J.*, 1952, 3587.

<sup>13</sup> Kuhn, Löw, and Trischmann, *Chem. Ber.*, 1955, **88**, 1492; 1957, **90**, 202; Kuhn and Löw, *Ann. Acad. Sci. Fennicae, Ser. A*, 1955, **2**, 488.

## EXPERIMENTAL

M. p.s were taken in evacuated tubes. Microanalyses are by Dr. A. D. Campbell, University of Otago, N.Z., samples being dried at 100° to constant weight. Infrared spectra were measured as KBr discs with a Beckman IR2 instrument.

*S. laciniatum Ait.—Isolation of solasonine.* Fresh green berries (466 g.) were coarsely minced and immediately extracted under reflux with boiling methanol for 2 hr. The pulp was separated from the mixture by using Celite as filter-aid, and washed well with hot methanol, and the filtrate was concentrated under reduced pressure to smaller volume under an anti-foaming device. After addition of an equal volume of water, ammonia was passed into the boiling solution, and the coagulated precipitate collected after cooling. The grey glycosidic alkaloid was reprecipitated twice, in a crystalline state, from 3% acetic acid solution with ammonia. Repeated crystallisation (charcoal) from aqueous methanol (75%) gave colourless, flat needles (4.10 g.) of solasonine, m. p. 301—302° (decomp.), with sintering at 296°. Chromatography of the partially purified material (m. p. 297—301°) in water-saturated butan-1-ol on alumina (previously stirred and kept for 1 hr. with moist butan-1-ol), and development with butan-1-ol-methanol (1 : 1), gave a single product (needles from 75% methanol), m. p. 301—302° (decomp.), identical with that purified by crystallisation alone (Found: C, 61.2; H, 8.1; N, 1.5. Calc. for  $C_{45}H_{73}O_{16}N$ : C, 61.1; H, 8.3; N, 1.6%).

The picrate, prepared from solasonine (100 mg.), saturated alcoholic picric acid solution (1 c.c.), and 30% alcohol (3 c.c.), after repeated crystallisation from 30% alcohol, gave yellow needles m. p. 197—198° (decomp.) (lit.,<sup>9</sup> m. p. 199—199.5°) (Found: C, 55.7; H, 7.0. Calc. for  $C_{45}H_{73}O_{16}N, C_6H_3O_7N_3$ : C, 55.0; H, 6.9; N, 5.0%).

The picrolonate, prepared from a solution of solasonine (100 mg.) and picrolonic acid (40 mg.) in 80% alcohol (3 c.c.), after repeated crystallisation from 80% alcohol, gave pale yellow needles, m. p. 233—234° (decomp.) (lit.,<sup>9</sup> m. p. 231°) (Found: C, 57.7; H, 7.25; N, 5.6. Calc. for  $C_{45}H_{73}O_{16}N, C_{10}H_8O_5N_4$ : C, 57.5; H, 7.1; N, 6.1%).

*Hydrolysis of Solasonine.*—Solasonine (500 mg.) in ethanol (10 c.c.) was hydrolysed by refluxing concentrated hydrochloric acid (2 c.c.) for 3 hr. The crystalline hydrochloride (slender needles) deposited after hydrolysis for 30 min. was collected after cooling overnight and was washed with water (10 c.c.). Treatment of a suspension of the hydrochloride with concentrated ammonia (25 c.c.) for 1 hr. at 100° gave the crude aglycone. Three crystallisations from methanol yielded large colourless hexagonal plates of solasodine, m. p. and mixed m. p. 196—198°. The infrared spectrum was identical with that of authentic solasodine.

Concentration of the hydrolysis filtrate and washings, *in vacuo*, to suitable volume and examination by paper strip chromatography with butan-1-ol-pyridine-water (3 : 1 : 1) on Whatmann No. 1 paper showed the presence of galactose, glucose, and rhamnose (aniline hydrogen phthalate spray reagent).

*S. nodiflorum Jacq. Form A.—Isolation of solasonine.* Coarsely minced fresh green berries (500 g.), on extraction, gave colourless flat needles of solasonine (1.48 g.), m. p. 297—298° (decomp.), with sintering at 296° (Found: C, 61.7; H, 8.4; N, 1.5%). The picrate formed fine yellow needles, m. p. 199—200° (decomp.) (Found: C, 55.2; H, 6.8%). The picrolonate formed pale yellow needles, m. p. 231—232° (decomp.) (Found: C, 57.9; H, 6.9; N, 6.0%).

Hydrolysis of the purified glycoside gave solasodine, m. p. and mixed m. p. 197.5—198°. The infrared spectrum was identical with that of authentic solasodine. Paper chromatography of the concentrated sugar solution showed the presence of galactose, glucose, and rhamnose.

*S. nodiflorum Jacq. Form B.—Isolation of solasonine.* Fresh green berries (178 g.), on extraction, gave solasonine (0.80 g.), m. p. 301—302° (decomp.) (Found: C, 61.6; H, 8.25; N, 1.6%) [picrate, needles, m. p. 197—199° (decomp.) (Found: C, 54.9; H, 6.75%); picrolonate, needles, m. p. 233—234° (decomp.) (Found: C, 57.4; H, 7.0; N, 5.5%)].

Hydrolysis and spectrum were as for form A.

"*S. simile.*"—*Isolation of solasonine.* Fresh green berries (467 g.), on extraction, gave solasonine (1.93 g.), m. p. 302—303° (decomp.), with sintering at 297° (Found: C, 61.1; H, 8.0; N, 1.5%) [picrate, needles, m. p. 199—200° (decomp.) (Found: C, 54.85; H, 6.6; N, 5.2%); picrolonate, needles, m. p. 234—235° (decomp.) (Found: C, 57.2; H, 6.8; N, 5.8%)]. Further identification was as above.

*S. miniatum Bernh.—Isolation of solasonine.* Fresh green berries (144 g.) collected in March, gave on extraction solasonine (142 mg.), m. p. 302—303° (decomp.) with sintering

at 296° (Found: C, 61.2; H, 8.2; N, 1.7%) [picrate, needles, m. p. 198—199° (decomp.) (Found: C, 55.4; H, 7.1; N, 5.0%); picrolonate, needles, m. p. 234° (decomp.) (Found: C, 57.4; H, 7.1; N, 5.2%)]. Insufficient glycosidic alkaloid was isolated for hydrolysis. A second sample of *S. miniatum* berries (73 g.), collected in April, on extraction gave only a low yield (27 mg.) of alkaloid which still contained traces of inorganic material.

*S. aviculare* Forst.—*Isolation of solasonine*. Fresh green berries (367 g.), on extraction, gave solasonine (2.71 g.), m. p. 302—303° (decomp.), with sintering at 297° (Found: C, 61.6; H, 8.1; N, 1.6%).

*S. gracile* Otto.—*Isolation of solamargine*. Fresh green berries (576 g.) were extracted and worked up in the usual manner. Repeated crystallisation from aqueous methanol (50%) gave rectangular colourless plates of solamargine (1.31 g.), m. p. 301—303° (decomp.) with sintering at 296°. Chromatography of the partially purified material (m. p. 301—302°) in water-saturated butan-1-ol on alumina (as above) and development with butan-1-ol-methanol (1:1) gave a single product (plates from 50% methanol), m. p. 301—302° (decomp.), identical with that purified by crystallisation alone (Found: C, 62.0; H, 8.3; N, 1.45. Calc. for  $C_{45}H_{73}O_{15}N$ : C, 62.3; H, 8.5; N, 1.6%).

The picrate, formed from its components in boiling 50% alcohol, on repeated crystallisation from 20% ethanol containing a trace of picric acid, gave yellow needles, m. p. 186—187° (decomp.) (lit.,<sup>12</sup> m. p. 188—189°) (Found: C, 56.3, 56.6; H, 7.4, 7.1. Calc. for  $C_{45}H_{73}O_{15}N, C_8H_3O_7N_3$ : C, 55.8; H, 6.9; N, 5.1%). The picrolonate, formed from its components in hot 80% ethanol, after four recrystallisations from the same solvent gave pale yellow needles, m. p. 202—203° (decomp.) (lit.,<sup>12</sup> m. p. 204—205°) (Found: C, 58.5; H, 7.0; N, 5.1. Calc. for  $C_{45}H_{73}O_{15}N, C_{10}H_8O_5N_4$ : C, 58.3; H, 7.2; N, 6.2%).

*Hydrolysis of Solamargine*.—Hydrolysis of the purified glycoside (500 mg.) under the usual conditions gave solasodine, m. p. and mixed m. p. 197—198°, having the authentic infrared spectrum. Paper chromatography of the concentrated sugar solution showed the presence of glucose and rhamnose.

*S. douglasii* Dun.—*Isolation of solamargine*. Fresh green berries (279 g.), on extraction, gave solamargine (548 mg.), m. p. 304—305° (decomp.) with sintering at 296° (Found: C, 62.4; H, 8.6; N, 1.7%) [picrate, needles, m. p. 186—187° (decomp.) (Found: C, 56.4; H, 6.85; N, 4.4%); picrolonate, needles, m. p. 200—201° (decomp.) (Found: C, 58.8; H, 7.5; N, 5.6%)].

Hydrolysis of the purified glycoside (100 mg.) gave solasodine, m. p. and mixed m. p. 199—200°, having the authentic infrared spectrum. Paper chromatography of the concentrated sugar solution showed the presence of glucose and rhamnose.

*S. nigrum* L.—*Isolation of solamargine* (in part B. R. DAVIS). Fresh green berries (320 g.), on extraction, gave solamargine (220 mg.), m. p. 313° (decomp.), with sintering at 298° (Found: C, 62.1; H, 8.5; N, 1.5%) [picrate, needles, m. p. 187—188° (decomp.) (Found: C, 55.9; H, 6.9%); picrolonate, needles, m. p. 203—204° (decomp.) (Found: C, 58.1; H, 7.0%)], further identified as in the preceding case.

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