The constituents of Solanum angustifolium : isaolation of a new gluco-alkaloid, solangustine / by Frank Tutin and Hubert William Bentley Clewer.

LVII.—*The Constituents of Solanum Angustifolium: Isolation of a New Gluco-alkaloid, Solangustine.*

By Frank Tutin and Hubert William Bentley Clewer.

In several countries in South America, namely, Peru, Bolivia, Paraguay, and the southern portion of the province of Buenos Ayres, in the Argentine Republic, a solanaceous plant occurs which is known as "Duraznillo Blanco." This plant, which has been identified as *Solanum angustifolium*, Ruiz et Pavon, was brought to the notice of Dr. Power by Dr. E. H. Colbeck, as being a drug worthy of chemical investigation. It is employed in South America as a febrifuge, chiefly in the treatment of enteric fever. In Peru it is also used in cases of malaria, but with caution, on account of its reputed poisonous properties.

At the suggestion of Dr. Power, the present authors have therefore conducted a chemical investigation of the drug in question, which has led to the isolation of a number of compounds, including a new and interesting *gluco-alkaloid*.

Several species of the extensive genus *Solanum* have hitherto been reported to contain bases which, in addition to their alkaloidal nature, were also glucosides. The name solanine has in all cases been applied to such bases, but a perusal of the literature of this subject reveals a state of great confusion, and makes it appear doubtful whether any pure gluco-alkaloid has heretofore been isolated. Thus, the solanine from *Solanum Dulcamara* has been stated to possess the formula $C_{42}H_{87}O_{15}N$ or $C_{52}H_{92}O_{18}N$, and to yield, on hydrolysis, the base, solanidine, $C_{26}H_{41}O_2N$. Solanine from the shoots of the potato (*S. tuberosum*) has been stated to yield solanidine having the formula $C_{40}H_{61}O_2N$.

More recently, Oddo and Colombano (*Gazzetta*, 1905, **35**, i, 27) prepared solanine from *S. sodomæum*, and assigned to it the formula $(C_{23}H_{39}O_8N)_2,H_2O$. They stated that it yielded, on hydrolysis, solanidine, $C_{19}H_{29}ON$, but the sugar that was also found was not identified. At a later date, the same authors (*Atti R. Accad. Lincei*, 1906, [v], **15**, ii, 312) modified their formula for solanine to $(C_{27}H_{47}O_9N)_2,H_2O$. Solanine from the seeds of *S. tuberosum* was then investigated by Colombano (*ibid.*, 1907, [v], **16**, ii, 683), who stated that it differed from the base obtained from *S. sodomæum*, and had the formula $C_{32}H_{51}O_{11}N$. The most recent work on the subject is by Oddo and Cesaris (*Gazzetta*, 1911, **41**, i, 490), who propose to designate the bases obtained from the last-mentioned two species of *Solanum* as solanine-*t* and solanine-*s* respectively. The

latter compound they state to have the formula $(C_{27}H_{46}O_9N)_2,H_2O$, and represent its hydrolysis by the following equation:

$$(C_{27}H_{46}O_9N)_2,H_2O + H_2O + H_2 =$$

Solanine-*s*.

$$2C_{18}H_{31}ON + C_6H_{12}O_6 + C_6H_{12}O_6 + C_6H_{12}O_5.$$

Solanidine. Galactose. ? Dextrose. ?Rhamnose.

The base described in the present communication, which has been designated *solangustine*, is at once differentiated from any previously described solanines by the insolubility of its salts. It is considered that it has been definitely established that its formula is $C_{33}H_{53}O_7N$, and that, on hydrolysis, it yields *solangustidine*, $C_{27}H_{43}O_2N$, together with one molecule of dextrose.

A summary of the results of the general investigation of the plant will be found at the end of this paper.

Experimental.

The material employed in this investigation was obtained from Lima, Peru, and consisted of the leaves, twigs, and flowers of the plant which is there known as "Duraznillo Blanco." It was botanically identified by Mr. E. M. Holmes, F.L.S., as *Solanum angustifolium*, Ruiz et Pavon.

A small portion (10 grams) of the dried and ground material was digested with Prollius' fluid, and the resulting extract tested for an alkaloid. Copious precipitates were then obtained with all the usual reagents, thus indicating the presence of a considerable proportion of alkaloidal material.

Another portion (25 grams) of the dried and ground material was extracted successively in a Soxhlet apparatus with various solvents, when the following amounts of extract, dried at 100°, were obtained:

Petroleum (b. p. 35—50°)	extracted	0·60 gram	=	2·40 per cent.
Ether	,,	0·44 ,,	=	1·76 ,,
Chloroform	,,	0·36 ,,	=	1·44 ,,
Ethyl acetate	,,	0·41 ,,	=	1·64 ,,
Alcohol	,,	5·96 ,,	=	23·84 ,,
	Total	7·77 grams	=	31·08 per cent.

For the purpose of a complete examination, 30·92 kilograms of the dried material were completely extracted with hot alcohol, when, after the removal of the greater part of the solvent, 12·73 kilograms of a viscid, dark green extract were obtained.

A quantity (3 kilograms) of the above-mentioned extract was mixed with water, and steam passed through the mixture for several hours, when only a slight trace of essential oil passed over. There then remained in the distillation flask a dark brown, aqueous

liquid (A), and a quantity of a soft, dark green resin (B). The latter was separated, and repeatedly washed with boiling water, the combined washings being concentrated, and added to the main bulk of the aqueous liquid.

Examination of the Aqueous Liquid (A).

It having been ascertained that the extraction of the aqueous liquid (A) with ether could only be slowly and with difficulty effected, recourse was had to the use of chloroform. After ten extractions with the latter solvent, practically nothing more was removed, and the extracts, all of which were green, were washed and evaporated. The dark green, resinous residue was dissolved in a small amount of alcohol, and then largely diluted with ether, when a small amount of dark green, resinous material was precipitated and removed. The latter apparently contained a small amount of amorphous, alkaloidal material, but nothing definite could be obtained from it. The ethereal filtrate was shaken with eight successive portions of 1 per cent. hydrochloric acid, when the resulting extracts each yielded reactions for alkaloid. The extraction was then continued with 5 per cent. hydrochloric acid until the basic substance ceased to be removed. The extracts so obtained were separately examined, but they yielded only brown, amorphous products, possessing a strong, basic odour. The amount of these amorphous, alkaloidal products was not large, and no crystalline salt could be obtained from them.

The first liquid, which had been obtained by extraction with 1 per cent. acid, as above described, after being made alkaline and deprived of base, was re-acidified and extracted many times with ether. A small amount of a product was thus obtained which deposited some crystals and gave the colour reactions of gallic acid, but the amount was too small for further investigation.

The ethereal solution, which had been extracted with hydrochloric acid, as above described, was then shaken successively with aqueous ammonium carbonate, sodium carbonate, and potassium hydroxide. The first- and last-mentioned alkalis removed only small amounts of resin, but the sodium carbonate extract yielded a little slightly impure quercetin, very much larger amounts of which were subsequently shown to be present in the form of the gluco-rhamnoside, rutin.

The neutral portion of the ethereal extract of the aqueous liquid consisted of about 2 grams of dark green, fatty material.

Isolation of Rutin, $C_{27}H_{30}O_{16},3H_2O$.

The aqueous liquid (A), which had been extracted with chloroform, as above described, was kept for a few days, when a quantity (6 grams) of a substance was deposited in fan-shaped tufts of light yellow, microscopic crystals. This substance was very sparingly soluble in alcohol, and practically insoluble in the other usual organic solvents, with the exception of pyridine, in which it dissolved readily. It was most conveniently purified by crystallisation from water, in which it is sparingly soluble. On heating it sintered at 170°, and melted indefinitely between 185° and 220°. (Found, C = 48·9; H = 5·7. $C_{27}H_{30}O_{16},3H_2O$ requires C = 48·8; H = 5·4 per cent.)

This substance was identified as the gluco-rhamnoside, rutin; this has been shown to crystallise with 3 molecules of water, which are somewhat difficultly eliminated on heating (compare Clarke, T., 1910, **97**, 1833). It has been shown by A. G. Perkin (*ibid.*, p. 1776) that the glucosides previously known as osyritrin, myrticolorin, and violaquercetrin, respectively, are also identical with rutin.

A quantity of the rutin was hydrolysed by heating with dilute, aqueous sulphuric acid, and the resulting liquid extracted with ether. Quercetin was then obtained in small, yellow needles, melting at 314°. (Found, C = 59·4; H = 3·6. Calc., C = 59·6; H = 3·3 per cent.) It yielded penta-acetylquercetin, which formed soft, colourless needles, melting at 194°. The acid, aqueous liquid from which the quercetin had been removed was deprived of sulphuric acid and concentrated. On heating with phenylhydrazine acetate, it readily yielded an osazone, which was collected in two successive fractions. The first fraction, after recrystallisation from dilute pyridine, melted at 216°, and proved to be glucosazone. The second fraction was somewhat readily soluble in alcohol, and, after recrystallisation from this solvent, melted at 182°, and was found to consist of rhamnosazone.

Further amounts of rutin were subsequently isolated, as described below, and the total amount obtained was about 55 grams, being thus equivalent to about 0·75 per cent. of the weight of the plant employed.

The aqueous liquid (A) from which the rutin had been separated was extracted fifteen times with amyl alcohol, the resulting liquids being washed and concentrated to a small bulk under diminished pressure. The liquid obtained by the first extraction yielded only a quantity of a dark-coloured, viscid product, readily soluble in

dry amyl alcohol. On concentrating the subsequent extracts, however, a yellow, sparingly soluble solid separated. The latter was collected, and the material in the filtrate added to the above-mentioned viscid product. In this way two products were obtained: (*a*) the material sparingly soluble in dry amyl alcohol, and (*b*) that readily dissolved by the latter.

Examination of the Product (a).

The product (*a*) was mixed with water and deprived of amyl alcohol by means of steam, the resulting dark brown, aqueous solution being freed from traces of green material by extraction with ether. No crystalline substance, however, could be directly obtained from this solution of the product (*a*), and it was therefore sought to obtain some definite compound from it by hydrolysis. Such an amount of sulphuric acid was therefore added to the aqueous solution as to represent about 7·5 per cent. of the total mixture, whereupon an insoluble product separated in semi-gelatinous granules. The mixture was then warmed on a water-bath for fifteen minutes, after which the dark-coloured solid was collected on a filter. The filtrate was boiled for one hour in order to complete the hydrolysis of any glucoside it might contain. On shaking the cooled mixture with ether, and fractionally extracting the resulting ethereal liquid with various alkalis, a quantity of quercetin (m. p. 310°), together with some amorphous products, was obtained. It is evident, therefore, that either some rutin, or another glucoside of quercetin, had been extracted by means of the amyl alcohol, although the latter solvent, in a pure state, does not appear to dissolve rutin.

Isolation of 3 : 4-*Dihydroxycinnamic Acid.*

The acid, aqueous liquid which had been extracted with ether, as above described, was made strongly alkaline with potassium hydroxide, and boiled for a short time. The liquid was then quickly acidified and cooled, after which it was extracted with ether. On shaking the resulting ethereal liquid with aqueous ammonium carbonate, and subsequently acidifying the alkaline liquid, a quantity (4 grams) of a crystalline acid was obtained. The latter separated from water in pale brown prisms, melting at 216°, and was identified as 3 : 4-dihydroxycinnamic acid. (Found, C = 60·2; H = 4·6. Calc., C = 60·0; H = 4·4 per cent.) It yielded 3 : 4-dimethoxycinnamic acid, melting at 180—181°.

Isolation of a New Gluco-alkaloid, Solangustine, $C_{33}H_{53}O_7N,H_2O$.

The previously mentioned dark-coloured solid which had separated on the addition of sulphuric acid to the aqueous solution of the product (*a*), as above described, was well washed with boiling alcohol, which removed most of the colour. It was then digested for some time with slightly diluted acetic acid, when, without dissolving to an appreciable extent, it eventually assumed a crystalline form. This substance proved to be the sulphate of an alkaloid. In working up the remainder of the original extract of the plant for the sole purpose of isolating a further quantity of this sulphate, it was not found necessary to follow the above-described procedure in full. The extract was mixed with water and distilled in a current of steam for the removal of the alcohol. The resin was then separated from the aqueous liquid, and the latter extracted many times with amyl alcohol, the extracts being subsequently washed with water and concentrated to a small bulk. The resulting product, part of which consisted of solid material which separated during the concentration, was then mixed with water, and deprived of amyl alcohol by means of steam. The dark-coloured, aqueous liquid so obtained was afterwards extracted repeatedly with ether until the greater part of the material soluble in this solvent was removed. It was then treated with sulphuric acid (about 5 per cent. of the weight of the liquid), the mixture warmed gently for about fifteen minutes, cooled, and the precipitated sulphate of the alkaloid collected. The latter was finally purified in the manner already indicated.

In order to obtain the base from its sulphate, it was necessary to resort to the employment of warm amyl alcohol, since the alkaloid is insoluble, or nearly so, in all other usual solvents, and the salt insoluble, or practically so, in everything. The sulphate was therefore mixed with warm, aqueous sodium carbonate, and the mixture vigorously shaken with successive portions of warm amyl alcohol. The amyl alcohol extracts were then washed, and concentrated to a small bulk under diminished pressure, when, on cooling, the alkaloid separated in the form of hard, pale yellow crusts, which, under the microscope, were seen to consist of aggregates of small crystals. The base so obtained was found to be a new gluco-alkaloid, and has been designated *solangustine*, with reference to its botanical source. The amount of it isolated corresponded with 0·062 per cent. of the weight of the air-dried drug employed. Solangustine was recrystallised by dissolving it in a large volume of hot amyl alcohol, and then concentrating and cooling the solution. On heating, it darkens slightly at about 225°,

and melts and decomposes at 235°. It contains solvent of crystallisation, and, when dehydrated, rapidly absorbs water from the atmosphere:

0·7457,* on heating at 130°, lost 0·0233 H_2O, after which, on exposure to the air, it reabsorbed 0·0231 H_2O. $H_2O = 3·1$.

0·0991 * gave 0·2421 CO_2 and 0·0870 H_2O. $C = 66·6$; $H = 9·7$.

0·0980 * „ 0·2386 CO_2 „ 0·0832 H_2O. $C = 66·4$; $H = 9·4$.

0·3019 * „ 6·8 c.c. N_2 (moist) at 18° and 754 mm. $N = 2·6$.

$C_{33}H_{53}O_7N,H_2O$ requires $C = 66·8$; $H = 9·3$; $N = 2·4$; $H_2O = 3·0$ per cent.

It thus appears that solangustine possesses the formula $C_{33}H_{53}O_7N$, and crystallises with 1 molecule of water, and this conclusion was borne out by the analysis of its derivatives, described below. The only solvent in which solangustine will dissolve at all readily is pyridine, but it cannot be crystallised from this liquid. Solangustine contains no methoxyl group, and its *acetyl* derivative was found to be uncrystallisable.

Solangustine Sulphate, $(C_{33}H_{53}O_7N)_2,H_2SO_4,3H_2O$.—This salt was prepared in a state of purity by shaking a solution of the respective base in amyl alcohol with dilute, aqueous sulphuric acid. A precipitate then separated, which consisted of small, colourless, acicular crystals, which did not melt or decompose at 325°. Solangustine sulphate, like the corresponding base, contains water of crystallisation, and, when dehydrated, it is extremely hygroscopic:

0·2144, on heating at 140°, lost 0·0089 H_2O. $H_2O = 4·1$.

0·1583 gave 0·0277 $BaSO_4$. $''SO_4 = 7·2$.

$(C_{33}H_{53}O_7N)_2,H_2SO_4,3H_2O$ requires $H_2O = 4·1$; $''SO_4 = 7·4$ per cent.

Solangustine sulphate appears to be insoluble in all solvents with the exception of acetic acid, in which it dissolves very sparingly on boiling.

No water-soluble salt of solangustine could be obtained, and the above-described sulphate was the only salt isolated in a crystalline condition. The *hydrochloride* and *nitrate* were amorphous, insoluble products.

Hydrolysis of Solangustine.

Formation of Solangustidine, $C_{27}H_{43}O_2N$, and Dextrose.

A quantity (5 grams) of solangustine was dissolved in 500 c.c. of warm amyl alcohol, and 50 c.c. of 15 per cent. hydrochloric acid were added, together with sufficient alcohol to render the mixture homogeneous. The liquid was then boiled for six hours, after which

* Heated at 130° to expel amyl alcohol and then exposed to the air until constant.

it was cooled, shaken with a moderate volume of water, and the aqueous layer separated. The latter was found to contain sugar, but no salt of an alkaloid. As, however, the amount of sugar formed was so small as to indicate that hydrolysis had not been complete, fresh quantities of hydrochloric acid and alcohol were added to the amyl alcohol liquid, and the mixture again boiled for six hours. It was found necessary to repeat this treatment four times before sugar ceased to be formed.

The aqueous liquids containing sugar were then made exactly neutral by the cautious addition of potassium hydroxide, after which they were evaporated to a small bulk under diminished pressure. The greater part of the inorganic salt was then removed by precipitation with absolute alcohol, after which the filtrate was deprived of alcohol, and heated for two hours with aqueous phenylhydrazine acetate. The osazone which formed was collected and carefully examined for the presence of rhamnosazone by Perkin's method (T., 1910, **97**, 1777), when it was found to consist solely of dextrosazone, melting at 215°. It is thus evident that solangustine belongs to the little-known group of gluco-alkaloids.

Solangustidine Hydrochloride, $C_{27}H_{43}O_2N,HCl$.—The amyl alcohol solution from which the sugar had been removed by shaking with water, as above described, was mixed with water and deprived of amyl alcohol by means of steam. There then remained in the flask an aqueous liquid, together with a quantity of a white solid in suspension. The latter was collected, and crystallised from absolute alcohol, to which a little alcoholic hydrogen chloride had been added, when it formed lustrous plates, which did not melt at 325°. It was most readily obtained crystalline by allowing its solution in boiling alcohol to evaporate:

0·0971 gave 0·2567 CO_2 and 0·0874 H_2O. C = 72·1; H = 10·0.*
0·2047 „ 0·0658 AgCl.† Cl = 8·0.
0·1913 „ 0·0609 AgCl.‡ Cl = 7·9.

$C_{27}H_{43}O_2N,HCl$ requires C = 72·1; H = 9·8; Cl = 7·9 per cent.

This substance is thus seen to be the hydrochloride of a base which is produced, together with 1 molecule of dextrose, by the hydrolysis of solangustine. It is proposed to designate the hydrolytic base *solangustidine*, and its formation may be represented by the following equation:

$$C_{33}H_{53}O_7N + H_2O = C_{27}H_{43}O_2N + C_6H_{12}O_6.$$

Solangustidine hydrochloride is sparingly soluble in amyl alcohol and hot ethyl alcohol, but is insoluble, or practically so, in the other usual solvents. It is quite insoluble in water. When it is dis-

* Other analyses gave C = 71·7, 71·8; H = 9·9, 10·0.
† By fusion method. ‡ By Carius' method.

solved in concentrated sulphuric acid, and the solution kept for some time, a reddish-yellow, slightly fluorescent liquid is obtained.

In order to isolate solangustidine from its hydrochloride, a quantity of the latter was dissolved in alcohol, and the solution made alkaline by the addition of an alcoholic solution of sodium ethoxide. Water was then added, and the precipitated base collected. As thus obtained, solangustidine was amorphous, and had no definite melting point. When dry it formed a horn-like mass. It separated from dilute alcohol in amorphous granules, and all attempts to obtain it crystalline resulted in failure. It had evidently suffered no change by the treatment with alkali, since it readily regenerated the crystalline hydrochloride.

Solangustidine Hydrobromide, $C_{27}H_{43}O_2N,HBr$.—A quantity of solangustidine was dissolved in alcohol, and a solution of hydrogen bromide in glacial acetic acid added. The mixture was then concentrated, when a colourless, crystalline solid separated. The latter was dissolved in alcohol containing a little hydrogen bromide, and the solution concentrated, when colourless, lustrous plates, which melted and decomposed at 320°, separated from the boiling liquid. The *hydrobromide* is somewhat more soluble in alcohol than the corresponding hydrochloride, but, like the latter, is quite insoluble in water:

0·0892 gave 0·2132 CO_2 and 0·0749 H_2O. C = 65·2; H = 9·3.

$C_{27}H_{43}O_2N,HBr$ requires C = 65·6; H = 9·0 per cent.

Solangustidine Nitrate, $C_{27}H_{43}O_2N,HNO_3$.—An alcoholic solution of solangustidine was acidified with dilute nitric acid, after which the liquid was diluted with water until a turbidity was produced. The mixture was then warmed, and allowed to cool slowly, when colourless leaflets separated, which become brown at 260° and melt and decompose at 290°. The *nitrate* is practically insoluble in water, but fairly readily soluble in hot, dilute alcohol:

0·0947 gave 0·2362 CO_2 and 0·0831 H_2O. C = 68·0; H = 9·7.

$C_{27}H_{43}O_2N,HNO_3$ requires C = 68·0; H = 9·2 per cent.

Solangustidine Sulphate, $(C_{27}H_{43}O_2N)_2,H_2SO_4$.—An alcoholic solution of solangustidine was acidified with dilute sulphuric acid, when a white, amorphous powder separated. On boiling the mixture for some time the solid became crystalline, forming colourless leaflets, which do not melt at 330°, are very sparingly soluble in boiling alcohol, and insoluble in water:

0·1938 * gave 0·0508 $BaSO_4$. $''SO_4 = 10·8$.

$(C_{27}H_{43}O_2N)_2,H_2SO_4$ requires $''SO_4 = 10·4$ per cent.

* Dried at 130°.

Solangustidine picrate was prepared by adding the requisite amount of picric acid to a solution of the base in alcohol. It formed yellow needles, which melted and decomposed at 250°.

Attempts were made to prepare the aurichloride and platinichloride of solangustidine, but they resulted in failure, owing, apparently, to the fact that these derivatives are more readily soluble than the corresponding hydrochloride.

Acetylsolangustidine, $C_{27}H_{42}O_2N{\cdot}CO{\cdot}CH_3$.—A quantity (2 grams) of solangustidine hydrochloride was boiled for one hour with acetic anhydride. When cool, the mixture was poured into ether, and the ethereal solution shaken with aqueous sodium carbonate until free from acid. It was then dried and evaporated, and the residue crystallised twice from ethyl acetate. Colourless, flattened needles were then obtained, which melted at 256°:

0·1766,* on heating at 130°, lost 0·0032 H_2O, which was quantitatively reabsorbed on exposure to the air. $H_2O=1{\cdot}8$.

0·0923 * gave 0·2530 CO_2 and 0·0820 H_2O. $C=74{\cdot}8$; $H=9{\cdot}9$.

0·0854 † „ 0·2386 CO_2 „ 0·0769 H_2O. $C=76{\cdot}2$; $H=10{\cdot}0$.

0·3870 † in 21·38 of chloroform gave $\Delta t+0{\cdot}135°$. M.W. = 491.

$C_{29}H_{45}O_3N,\frac{1}{2}H_2O$ requires $C=75{\cdot}0$; $H=9{\cdot}9$; $H_2O=1{\cdot}9$ per cent.

$C_{29}H_{45}O_3N$ requires $C=76{\cdot}5$; $H=9{\cdot}9$ per cent. M.W. = 455.

The material contained in the mother liquors from the acetylsolangustidine was not homogeneous, and was found to contain unchanged solangustine. It yielded the pure acetyl derivative on more prolonged acetylation.

Attempts were made to prepare the hydrochloride of acetylsolangustidine, and, although indications were obtained of the formation of such a compound, it was not found possible to isolate it. This was owing to the fact that the salts of the acetyl base dissociated with extreme readiness, and when crystallised from alcohol, even in the presence of an excess of acid, they yield the original acetyl compound.

Acetylsolangustidine is remarkably stable towards alkalis, since, when boiled for several hours with alcoholic potassium hydroxide, it is recovered unchanged. When, however, it is heated for a very prolonged period with concentrated alcoholic potassium hydroxide it slowly undergoes some change, but the amount of material available was not sufficient to ascertain whether this resulted in the regeneration of solangustidine.

When acetylsolangustidine is boiled for some hours with glacial acetic acid and concentrated hydrochloric acid, a yellow-coloured liquid is obtained, which exhibits a remarkably strong,

* Air-dried substance. † Dried at 130°.

green fluorescence. The reaction product, however, consisted only of amorphous material.

Examination of the Product (b).

The product (*b*) (p. 563), which consisted of the material readily soluble in cold, dry amyl alcohol, was of a dark brown, viscid nature, and nothing definite could be directly separated from it. It was dissolved in water, deprived of amyl alcohol by means of steam, and then treated with sulphuric acid, when a quantity (2 grams) of rather impure solangustine sulphate was obtained, from which the pure salt could only with some difficulty be prepared.

The acid filtrate from the crude sulphate, after being boiled, yielded to ether a small amount of quercetin. It was subsequently made alkaline with potassium hydroxide, and again boiled for some time, when it yielded about 2 grams of 3 : 4-dihydroxycinnamic acid. The remainder of the product consisted only of resinous material.

The aqueous liquid (A), which had been extracted with amyl alcohol, as above described, was kept for some time, when it gradually deposited a further quantity (49 grams) of rutin. After filtration, a portion of it (200 c.c.) was examined for the presence of any further alkaloidal material. It was rendered alkaline with sodium carbonate, and extracted successively with ether, chloroform, and amyl alcohol. The first-mentioned solvent removed a very small amount of alkaloid, possessing a strong odour, somewhat resembling that of coniine, but the amount was insufficient for further examination. The chloroform and amyl alcohol removed nothing.

The remainder of the aqueous liquid (A), which amounted to 4·5 litres, was concentrated somewhat under diminished pressure, and then treated with an excess of aqueous basic lead acetate. The resulting copious, yellow precipitate was collected and washed, after which it was suspended in water and decomposed by means of hydrogen sulphide. The filtrate from the lead sulphide was then evaporated under diminished pressure, when a dark brown, viscid product was obtained, from which nothing could be directly separated. This viscid, brown material was divided into two portions, one of which was heated for an hour with dilute sulphuric acid, but the resulting products were entirely amorphous. The other portion was heated with aqueous potassium hydroxide in the manner previously described, when it yielded, in addition to amorphous products, a small amount of quercetin and a considerable quantity of 3 : 4-dihydroxycinnamic acid.

Isolation of l-*Asparagine.*

A portion (about one-fifth) of the aqueous liquid (A), which had been treated with basic lead acetate, was slightly acidified with acetic acid, and then treated with a solution of mercuric nitrate in dilute nitric acid until no further precipitate was produced. The precipitate was collected, washed, decomposed by means of hydrogen sulphide, and the liquid filtered. The filtrate was rendered slightly alkaline with ammonia, and then just acid with acetic acid, when it was concentrated under diminished pressure to a low bulk. The brown liquid so obtained was treated with animal charcoal, after which, on keeping, it deposited a crystalline substance in the form of prisms. The latter was collected and recrystallised from water, when it formed colourless prisms, melting indefinitely at 227—238°, and was identified as *l*-asparagine. The amount obtained was about 0·25 gram, being equivalent to about 0·02 per cent. of the air-dried plant. (Found,* $H_2O=12·0$. $C_4H_8O_3N_2,H_2O$ requires $H_2O=12·0$. Found,† $C=36·5$; $H=6·2$. $C_4H_8O_3N_2$ requires $C=36·4$; $H=6·1$ per cent.)

The remainder of the aqueous liquid (A) was deprived of lead by means of hydrogen sulphide, and concentrated under diminished pressure to a low bulk. The resulting syrup deposited no crystals, and no crystalline acetyl derivative could be prepared from it. It readily yielded *d*-phenylglucosazone (m. p. 212°), and therefore contained a quantity of a sugar, probably consisting chiefly of lævulose. It was carefully examined for the presence of rhamnose, but with a negative result.

Examination of the Resin (*B*)

The resin (B) was a soft, dark green mass, and amounted to 670 grams, being thus equivalent to about 9·2 per cent. of the weight of the drug employed. It was dissolved in alcohol, mixed with purified sawdust, and the thoroughly dried mixture extracted successively in a large Soxhlet apparatus with light petroleum (b. p. 35—50°), ether, chloroform, ethyl acetate, and alcohol.

Petroleum Extract of the Resin.

The petroleum extract of the resin was a dark green, fatty mass, and amounted to 540 grams. It was digested with about 2 litres of ether, and the mixture filtered, when a quantity (about 3 grams)

* Air-dried substance. † Dried at 130°.

of a solid was removed, which was examined in connexion with the unsaponifiable constituents.

The ethereal filtrate was extracted with 10 per cent. hydrochloric acid, when a small quantity of a green, amorphous, alkaloidal product was removed. It possessed a strong, basic odour, but nothing crystalline could be obtained from it.

The original ethereal solution was then extracted with aqueous potassium hydroxide, when a quantity of a flocculent solid separated. The latter was collected, when it proved to be the potassium salt of a fatty acid, and was examined in connexion with a larger amount of a similar product obtained later.

Isolation of a Phytosterolin, $C_{33}H_{56}O_6$.

The alkaline, aqueous extract, which had been separated from the ether and solid material, as above described, was acidified and extracted with ether, when a quantity of a nearly black solid separated, and was removed. Nothing crystalline could be obtained from it. The ethereal liquid was then shaken with aqueous potassium hydroxide, as before, when some neutral material, which had been occluded during the first extraction with alkali, remained in the ether. The alkaline liquid was then acidified and extracted with ether, during which operation a quantity (about 5 grams) of flocculent, green material separated, and was collected.

This product was heated with acetic anhydride in the presence of pyridine for half an hour, when, after concentration, colourless leaflets separated. The latter substance, after recrystallisation from petroleum (b. p. 90—120°) and from alcohol, melted at 168—169°, and was identified as a tetra-acetylphytosterolin. (Found, C = 69·3; H = 9·2. Calc., C = 68·7; H = 8·9 per cent.)

0·3885, made up to 20 c.c. with chloroform, gave $\alpha_D - 1°0'$ in a 2-dcm. tube, whence $[\alpha]_D - 25·7°$.

A quantity of this acetyl derivative was hydrolysed by means of alcoholic potassium hydroxide, when the resulting phytosterolin (phytosterol glucoside) was obtained. It separated from dilute pyridine in small, colourless crystals, melting at 300°. (Found, C = 72·6; H = 10·5. Calc., C = 72·3; H = 10·2 per cent.)

The benzoyl derivative was prepared by benzoylation in pyridine solution. It crystallised from a mixture of chloroform and alcohol in colourless needles, melting at 200°. (Found, C = 75·9; H = 7·8. Calc., C = 75·9; H = 7·5 per cent.)

The ethereal solution containing the free, fatty acids, which had been separated by filtration from the crude phytosterolin, as above

described, was evaporated, and the dark green residue esterified by means of methyl alcohol and sulphuric acid. The resulting ester, dissolved in ether, was shaken with aqueous potassium hydroxide, when a considerable amount of dark green, phenolic resin was removed, from which nothing crystalline could be obtained. The ethereal solution was then dried and evaporated, when, after purifying the residual esters by distillation under diminished pressure, they were examined in connexion with a similar product obtained from the combined acids, as described below.

The ethereal solution of the neutral constituents of the petroleum extract was evaporated, and the residue heated for two hours with an excess of alcoholic potassium hydroxide. Water was then added, and the mixture repeatedly extracted with ether. During this operation a quantity of a flocculent solid separated at the juncture of the aqueous and ethereal layers. This was collected, when it was found to consist of a mixture of the potassium salts of the higher fatty acids, and its examination will be described later.

Isolation of Triacontane, $C_{30}H_{62}$.

The ethereal solution of the unsaponifiable material, which had been separated from the alkaline, aqueous liquid and the potassium salt, as above described, was washed, dried, and evaporated. The residue, which amounted to 103 grams, was dissolved in alcohol, with the exception of a small amount of black, tarry material, which was discarded. On cooling the solution, a quantity (5 grams) of a solid separated, which was collected, and distilled under diminished pressure. The distillate was crystallised twice from ethyl acetate, when it formed lustrous, colourless leaflets, melting at 65·5°, and was identified as triacontane. (Found, C = 85·1; H = 14·8. Calc., C = 85·3; H = 14·7 per cent.)

Isolation of a Phytosterol, $C_{27}H_{46}O$.

The alcoholic solution of the unsaponifiable material, from which the triacontane had been separated, was concentrated, and some ethyl acetate and a little water added. On keeping this mixture for some time a quantity (about 0·5 gram) of colourless leaflets separated. This product had the properties of a phytosterol, and melted at 131°, but it did not appear to be homogeneous. It was accordingly converted into the acetyl derivative, which formed colourless leaflets, melting at 121°, and the latter crystallised repeatedly, both from alcohol and ethyl acetate. On regenerating the phytosterol and crystallising it, colourless plates were obtained, which were apparently homogeneous, and melted at 134°:

0·2760,* on heating at 120°, lost 0·0138 H_2O. $H_2O = 5·0$.
0·1029 † gave 0·3160 CO_2 and 0·1120 H_2O. $C = 83·8$; $H = 12·1$.
$C_{27}H_{46}O,H_2O$ requires $H_2O = 4·5$ per cent.
$C_{27}H_{46}O$ requires $C = 83·9$; $H = 11·9$ per cent.

The liquid from which the crude phytosterol had been removed contained a considerable quantity of a brown, sweet-smelling oil, from which no further crystalline material could be separated. It was examined for the presence of fatty alcohols by the phthalic anhydride treatment, but with a negative result.

Examination of the Fatty Acids.

The alkaline liquid from which the unsaponifiable material had been removed by means of ether was acidified and distilled with steam. This removed a small amount of a volatile acid having an odour of valeric acid. The mixture was then extracted with ether, when a small amount of phytosterolin separated, and was removed. The ethereal liquid was then dried and evaporated, and the dark green residue esterified by means of methyl alcohol and sulphuric acid. The resulting methyl esters were then freed from a little unchanged acid and much chlorophyll by shaking their ethereal solution with aqueous potassium hydroxide, and then washing it with water, after which the liquid was dried and evaporated. The residue was then purified by distillation under diminished pressure, when a quantity (94 grams) of methyl ester was obtained. This product was added to the esters of the free acids previously mentioned, which amounted to 118 grams, and the whole hydrolysed by means of alcoholic potassium hydroxide. The resulting acids were isolated, and separated into their saturated and unsaturated components by means of the lead salt, in the usual manner.

The Unsaturated Acids.—These acids were converted into the methyl ester, which amounted to 163 grams, and the latter distilled several times under diminished pressure, when the following fractions were collected: i, Below 215° (16·5 grams); ii, 215—218° (33·9 grams); iii, 218—222° (73·0 grams); iv, 222—225° (16·2 grams)/20 mm.

The iodine values of fractions i, ii, iii, and iv were respectively 182·5, 200·7, 210·9, and 207·3. Fraction iii, on analysis, gave $C = 77·5$; $H = 11·2$ per cent. (Methyl linolate requires $C = 77·5$; $H = 11·5$ per cent.; I.V. = 172·7; and methyl linolenate requires $C = 78·1$; $H = 10·8$ per cent.; I.V. = 261.)

It would therefore appear that the unsaturated acids consisted essentially of linolic and linolenic acids.

* Air-dried substance. † Dried at 110°.

The Saturated Fatty Acids.—These acids amounted to 40 grams. They were mixed with the fatty acid (6 grams) obtained from the previously mentioned sparingly soluble potassium salts, and converted into the methyl ester. The latter was fractionally distilled several times under diminished pressure, when the following fractions were collected: i, Below 200°; ii, 200—205°; iii, 205—210°; iv, 210—215°; v, 215—225°; vi, 225—235°; vii, 235°+/20 mm.

Fractions i and ii solidified on cooling, and were found to consist of methyl palmitate. They yielded palmitic acid, melting at 63°. (Found, C=74·9; H=12·5. Calc., C=75·0; H=12·5 per cent.)

Fractions iii, iv, and v all yielded impure products, which appeared to consist of mixtures of palmitic and stearic acids. (Found, C=75·6; H=12·6. Calc. for $C_{16}H_{32}O_2$, C=75·0; H=12·5; for $C_{18}H_{36}O_2$, C=76·1; H=12·7 per cent.)

Isolation of Cluytinic Acid, $C_{21}H_{42}O_2$.

The above-mentioned fraction vi of the methyl esters partly solidified. On hydrolysis it yielded an acid, which was crystallised many times from alcohol, from acetic acid, and from ethyl acetate, when it melted constantly at 69°. On being compared directly with cluytinic acid obtained from *Cluytia similis* (T., 1912, **101**, 2226) and from hops (T., 1913, **103**, 1283), it was found to be identical with both these preparations. In order further to establish its identity, however, it was converted into its methyl ester. The latter formed colourless needles, melting at 47°, and was identical with the corresponding derivative prepared from the cluytinic acid of hops (*loc. cit.*). (Found, C=77·5; H=13·1. $C_{22}H_{44}O_2$ requires C=77·6; H=12·9 per cent.)

Fraction vii of the esters, together with the residues of high boiling point which remained in the flasks after the fractional distillations, was redistilled, when it solidified on cooling. On crystallisation from ethyl acetate it yielded methyl cerotate, melting at 60°. On hydrolysis, the latter gave cerotic acid, melting at 78·5°. (Found, C=78·9; H=13·2. Calc., C=79·0; H=13·2 per cent.)

Ethereal Extract of the Resin.

This extract of the resin was dark green, and amounted to 46 grams. A portion of it (3 grams) was very sparingly soluble in ether, and formed a nearly black powder. The latter was extracted in a Soxhlet apparatus for a short time with ethyl acetate, when about 1 gram of crude phytosterolin remained undissolved. The ethyl acetate extract was evaporated, and the residue distilled under diminished pressure, when, on crystallisation from ethyl acetate, it

formed colourless leaflets, melting at 85°. This product, together with a further amount of similar material, obtained as described below, was found to consist of a higher fatty acid. It was converted into the methyl ester, which formed colourless leaflets, melting constantly at 71°. On hydrolysing the latter, the original acid was regenerated, and, on crystallisation from acetic acid, its melting point remained unchanged, at 85°:

0·1032 gave 0·3001 CO_2 and 0·1242 H_2O. C = 79·3; H = 13·4.
0·0602 „ 0·1748 CO_2 „ 0·0715 H_2O. C = 79·2; H = 13·2.
$C_{28}H_{56}O_2$ requires C = 79·2; H = 13·2 per cent.
$C_{30}H_{60}O_2$ „ C = 79·6; H = 13·2 per cent.

It would thus appear that this higher fatty acid was either melissic acid or a lower homologue of the latter. The low melting points of the acid and its ester would point to the latter conclusion being the more correct.

The ethereal solution of the more readily soluble portion of the ethereal extract of the resin yielded a small amount of green, amorphous, alkaloidal material when extracted with hydrochloric acid. On subsequently shaking it with aqueous sodium carbonate nothing was removed. When, however, the ethereal solution was shaken with aqueous potassium hydroxide, the greater portion of the dissolved material was removed from the ether, a portion of it forming an insoluble potassium salt, which was collected. The latter proved to be the salt of the above-described higher fatty acid.

The material dissolved by the alkali, which represented the greater part of the ether extract of the resin, consisted of a dark green, phenolic resin, from which nothing definite could be obtained.

Chloroform, Ethyl Acetate, and Alcohol Extracts of the Resin.

The chloroform, ethyl acetate, and alcohol extracts of the resin amounted to 36, 7, and 33 grams respectively. They all consisted of dark green, amorphous resins, from which nothing definite could be obtained, with the exception of a small amount of solangustine. The latter was isolated in the form of its sulphate from the alcohol extract of the resin.

Summary and Physiological Tests.

The material employed in this investigation consisted of the leaves, twigs, and flowers of *Solanum angustifolium*, Ruiz et Pavon, which had been obtained from Peru.

For the purpose of a complete examination, 30·92 kilograms of

the dried material were employed. This material was ground, completely extracted with hot alcohol, and the resulting extract distilled in a current of steam.

From the portion of the extract which was soluble in water there were isolated the following substances: (i) Quercetin; (ii) rutin, $C_{27}H_{30}O_{16},3H_2O$; (iii) *l*-asparagine; (iv) a new gluco-alkaloid, *solangustine*, $C_{33}H_{53}O_7N,H_2O$. On hydrolysis, solangustine yields *solangustidine*, $C_{27}H_{43}O_2N$, together with 1 molecule of dextrose. The aqueous liquid also contained small amounts of amorphous, alkaloidal material, and a considerable quantity of a sugar, which apparently was lævulose, together with viscid, amorphous products. Some of the latter yielded quercetin and 3:4-dihydroxycinnamic acid on treatment with alkalis.

The portion of the original extract which was insoluble in water yielded, in addition to much chlorophyll and resinous material, the following compounds: (i) Triacontane, $C_{30}H_{62}$; (ii) a phytosterol, $C_{27}H_{46}O$; (iii) a phytosterolin (phytosterol glucoside), $C_{33}H_{56}O_6$; (iv) palmitic, stearic, cluytinic, and cerotic acids, together with a mixture of linolic and linolenic acids. It furthermore gave a small amount of the above-mentioned new gluco-alkaloid, solangustine, and a higher fatty acid, which was either melissic acid, $C_{30}H_{60}O_2$, or a lower homologue, $C_{28}H_{56}O_2$.

The following physiological tests were conducted at the Wellcome Physiological Research Laboratories by Dr. H. H. Dale, to whom our thanks are due.

An amount of the total alcoholic extract, equivalent to 3·5 grams of the drug, and 0·48 gram of solangustine, were separately administered to a dog, but no perceptible effect of any kind resulted. The amorphous alkaloidal material, which occurred to a small extent in the plant, yielded a similarly negative result.

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