The constituents of Gloriosa superba / by Hubert William Bentley Clewer, Stanley Jospeh Green and Frak Tutin.

XCI.—*The Constituents of* Gloriosa superba.

By Hubert William Bentley Clewer, Stanley Joseph Green and Frank Tutin.

The climbing, liliaceous plant, *Gloriosa superba*, Linn., occurs throughout India and Ceylon, and is there common on hedges during the rainy season. It is known by a large number of vernacular names, and in English as the "Superb Lily." The flowers are used by the Hindus in the worship of Siva and the Lingam, whilst the roots, which form elongated tubers, are reputed to possess medicinal properties, and were included by the Sanskrit writers in the seven minor poisons of India (*Pharmacographia Indica*, 1893, III, 480). The tubers are stated to be used for a variety of medicinal purposes, but chiefly for promoting labour pains and procuring abortion.

The only examination of the drug in question which appears hitherto to have been made was conducted by Warden (*Ind. Med. Gaz.*, 1880, **15**, 253; 1881, **16**, 138), who states that the root contains a neutral, bitter principle, named "superbine," three resins, a fluorescent principle, and salicylic acid. "Superbine" was considered to be either identical with, or closely allied to, the bitter principle of squill, and as little as 0·0107 gram of it proved fatal to a full-grown cat. Single determinations of the carbon, hydrogen, and nitrogen content gave results indicating the formula $C_{52}H_{66}O_{17}N_2$, which, however, was put forward with reserve.

The present investigation has shown that the bitterness of *Gloriosa* tubers is due to the alkaloid, colchicine, which has hitherto been known to occur only in Colchicum. Among other compounds which have now been isolated from *Gloriosa*, *2-hydroxy-6-methoxybenzoic acid* may be mentioned, as this substance has not hitherto been known. A summary of the results obtained will be found at the end of this paper.

Experimental.

The material employed for this investigation consisted of the tubers of *Gloriosa superba*, Linn., which had been specially collected in Ceylon, through the kindness of Mr. T. Petch, of the Botanical Gardens, Paradeniya.

Ten grams of the dried and ground material were digested with Prollius' fluid, and the resulting extract was tested with the usual alkaloid reagents, when reactions were obtained indicating the presence of a considerable amount of alkaloid.

In order to ascertain if an enzyme were present, 400 grams of the dried and ground tubers were macerated with water for eighteen hours, after which the liquid was expressed from the marc, and filtered. The filtrate was then treated with about twice its volume of alcohol, and the resulting brownish precipitate collected. When dry, the latter amounted to 4·3 grams. It contained a considerable proportion of inorganic matter, but had marked enzymic activity, since it readily hydrolysed amygdalin.

Twenty-five grams of the ground material were 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	0·135 gram	= 0·54 per cent.
Ether.....	,,	0·24 ,,	= 0·96 ,, ,,
Chloroform	,,	0·11 ,,	= 0·44 ,, ,,
Ethyl acetate	,,	1·02 ,,	= 4·08 ,, ,,
Alcohol	,,	1·03 ,,	= 4·12 ,, ,,
Total.....		2·535 grams	= 10·14 per cent.

For the purpose of a complete examination, 42·52 kilograms of the dried and ground tubers were thoroughly extracted by percolation with hot alcohol. After removal of the greater part of the alcohol, 10·35 kilograms of a dark brown extract were obtained. The whole of this extract was mixed with water and subjected to distillation in a current of steam, when 3·8 grams of a pale-coloured essential oil were obtained. This essential oil gave a colour reaction indicating the presence of furfuraldehyde, and distilled at 150—250°/15 mm. On keeping, it darkened in colour, and deposited some colourless crystals. The latter melted at 60°, and appeared to consist essentially of palmitic acid.

After the distillation of the extract with steam there remained in the distillation flask a dark brown, aqueous liquid (*A*), together with a quantity of dark-coloured resinous material (*B*). The latter was separated by filtration and well washed with hot water, the concentrated washings being added to the main bulk of the aqueous liquid.

Examination of the Aqueous Liquid (A).—The total quantity of the aqueous liquid, after being concentrated somewhat under diminished pressure, was extracted many times with ether, until the ethereal extracts were found no longer to contain alkaloid. The combined ethereal extracts were then concentrated, and completely extracted successively with aqueous hydrochloric acid, ammonium carbonate, sodium carbonate, and potassium hydroxide.

Isolation of an Alkaloid.—The acid extracts were rendered alkaline by means of sodium carbonate, and thoroughly extracted with ether, after which the ethereal extracts were concentrated

somewhat, and deprived of some phenolic material by means of aqueous potassium hydroxide. On evaporating the dried ethereal liquid, a residue was obtained, which slowly crystallised. The solid so obtained crystallised from ethyl acetate in small, very pale yellow leaflets, melting constantly at 177—178°, which were anhydrous. A further small amount of this base was obtained from the petroleum extract of the resin, as subsequently mentioned, the total amount isolated being about 0·75 gram:

0·1014 gave 0·2429 CO_2 and 0·0591 H_2O. C = 65·3; H = 6·5.
0·0890 „ 0·2141 CO_2 „ 0·0540 H_2O. C = 65·6; H = 6·7.
0·2776 „ 12·3 c.c. N_2 (moist) at 19° and 768 mm. N = 5·2.
0·1198 „ 0·2816 AgI. MeO = 31·0.

$C_{23}H_{38}O_9N_2$ requires C = 65·4; H = 6·3; N = 4·8; 6MeO = 31·7 per cent.

$C_{15}H_{17}O_4N$ requires C = 65·4; H = 6·3; N = 5·1; 3MeO = 33·8 per cent.

The results of the analyses given above agree best with the higher formula, but, owing to the small amount of material available, the possibility of the lower formula being the correct one could not be excluded. No gold salt could be obtained from this base, since it readily reduced auric chloride to the metallic state.

Isolation of Salicylic, 2-Hydroxy-6-methoxybenzoic and Benzoic Acids.—The ammonium carbonate extracts of the ethereal extract of the aqueous liquid were acidified, and extracted with ether. The crystalline residue obtained on evaporating the ether was dissolved in methyl alcohol, some sulphuric acid added, and the mixture heated for some time, after which the resulting ester was isolated. Although this process of esterification was repeated several times, the greater portion of the acid remained in the free state. The ester which was obtained, after being deprived of free acid, was separated into phenolic and non-phenolic portions by means of aqueous sodium hydroxide. The non-phenolic ester, which had the odour of methyl benzoate, yielded, on hydrolysis, a small amount of an acid, which crystallised from water in colourless leaflets, melting at 121°, and was identified as benzoic acid.

The phenolic ester, when hydrolysed, gave a mixture of acids which was found to be similar to the portion of the original acid mixture which had resisted esterification. The total quantity was therefore fractionally crystallised many times from a mixture of chloroform and benzene, when it was eventually separated into two compounds, melting at 155° and 135° respectively. The former of these products, which was the smaller in amount, proved to be salicylic acid. (Found, C = 60·7; H = 4·5. Calc., C = 60·9; H = 4·4 per cent.)

The acid melting at 135° was similar in appearance to salicylic acid, and yielded the same colour with ferric chloride. A mixture of it with the latter acid, however, melted at about 120°:

0·1018 gave 0·2132 CO_2 and 0·0446 H_2O. C = 57·1; H = 4·8.

0·2393 „ 0·3269 AgI. MeO = 18·2.

$C_8H_8O_4$ requires C = 57·1; H = 4·8; MeO = 18·4 per cent.

It thus appeared that the acid melting at 135° was a methoxysalicylic acid. The only known acid of this character with which the compound from *Gloriosa* agrees at all in its properties is 2-hydroxy-5-methoxybenzoic acid, which melts at 141°. A quantity of the latter was therefore prepared from quinol monomethyl ether by Körner and Bertoni's method (*Ann. di chim. medicin*, 1881, 65), when the synthetic acid was found to differ from the naturally occurring one. Thus, a mixture of the two preparations melted at 118°, and the colour produced on treating the synthetic acid with ferric chloride was blue, whereas the natural acid yielded a violet colour identical with that furnished by salicylic acid. It therefore appeared that the acid melting at 135° occurring in *Gloriosa* must be *2-hydroxy-6-methoxybenzoic acid*, a compound which has not hitherto been known.

In order to confirm this assumption, resorcinol was heated at 120—130° with water and ammonium carbonate, when a mixture of 2 : 6- and 2 : 4-dihydroxybenzoic acids resulted (Senhoffer and Brunner, *Wien. Akad.*, 1879, 80). This crude mixture was then vigorously methylated by means of methyl sulphate and potassium hydroxide, and the product was heated for some time in alcoholic solution with an excess of the alkali. Water was then added, and the mixture extracted with ether, when a quantity of a crude ester which had resisted hydrolysis by the alkali was obtained. This product was distilled under the ordinary pressure, when the portion of higher boiling point solidified on cooling. On crystallising this product from petroleum, long, colourless needles, melting at 88°, were obtained. This compound must be *methyl 2 : 6-dimethoxybenzoate*, a substance not hitherto known, the fact of its not being hydrolysed by alcoholic potassium hydroxide being evidently due to steric hindrance caused by the two methoxy-groups in the ortho-position with respect to the carboxyl group. An identical ester was obtained by the methylation of the 2-hydroxy-6-methoxybenzoic acid occurring in *Gloriosa*:

0·1169 gave 0·2619 CO_2 and 0·0657 H_2O. C = 61·1; H = 6·2.

$C_{10}H_{12}O_4$ requires C = 61·2; H = 6·1 per cent.

The materials removed from the original ethereal extract of the aqueous liquid by the extractions with sodium carbonate and

potassium hydroxide previously mentioned were small in amount, and amorphous. After the extraction with these alkalis, the ether only contained a small amount of indefinite, fatty matter.

A portion of the original aqueous liquid (*A*) which had been extracted with ether, as previously described, was extracted many times with amyl alcohol, but it was found that the product so removed was chiefly of an alkaloidal nature, and was slowly extracted from the amyl alcohol by means of aqueous hydrochloric acid. The portion of the amyl alcohol extract which was not basic was small in amount, and amorphous. Nothing definite could be obtained from it by either acid or alkaline hydrolysis. It was subsequently found that the alkaloid remaining in the aqueous liquid after extraction with ether could be more conveniently removed by means of chloroform, and this method was therefore resorted to.

Isolation of Colchicine, $C_{22}H_{25}O_6N$.—The chloroform extract of the aqueous liquid gave a crystalline substance containing chloroform of crystallisation. When deprived of the solvent, the substance was amorphous. It could not conveniently be recrystallised from chloroform owing to its great solubility, but was eventually obtained crystalline from ethyl acetate. The product so obtained was then readily recrystallised from the last-mentioned solvent when dry, when it formed aggregates of small, soft needles, having a very pale yellow colour, and melting at 155—157°:

0·5813* on drying in a vacuum lost 0·0086 H_2O. $H_2O = 1·5$.
0·1008† gave 0·2428 CO_2 and 0·0620 H_2O. $C = 65·7$; $H = 6·8$.
0·3024† ,, 9·7 c.c. N_2 (moist) at 19° and 772 mm. $N = 3·7$.
0·1967† ,, 0·4561 AgI. $MeO = 30·6$.

$C_{22}H_{25}O_6N$ requires $C = 66·2$; $H = 6·3$; $N = 3·5$; $4OMe = 31·1$ per cent.

The properties and composition of this alkaloid suggested that it was colchicine, and, apart from the differences mentioned below, it was found to yield the reactions characteristic of the latter, as described in the United States Pharmacopœia. The colchicine, however, which is obtained from the corm or seeds of *Colchicum autumnale*, Linn., is described as an amorphous powder, melting at 142°, and has only been obtained crystalline in the form of a chloroform compound, containing three molecules of chloroform of crystallisation. For the sake of comparison, therefore, colchicine isolated from *Colchicum autumnale* was also investigated, and found to crystallise from ethyl acetate in soft, pale yellow needles, melting at 155—157°, alone or when mixed with the base derived from *Gloriosa superba*. With the object of further confirming the

* Air-dried. † Dried in a vacuum.

identity of the two preparations, their specific rotatory powers were compared with the following results:

0·1759 * of the base from *Gloriosa*, made up to 20 c.c. with chloroform, gave α_D −2°8′ in a 2-dcm. tube, whence $[\alpha]_D^{16·5}$ −121·3°.

0·1759 * of the base from *Colchicum*, made up to 20 c.c. with chloroform, gave α_D 2°7′ in a 2-dcm. tube, whence $[\alpha]_D^{16·5}$ −120·3°.

In both these determinations, and also in several others carried out in chloroform solution, it was noticed that the observed rotation dropped from 5—8′ during the first thirty minutes after solution, when it remained constant. The rotation of colchicine was also observed to vary very appreciably with the concentration, the specific rotation being less in more concentrated solutions when employing chloroform. A determination in aqueous solution yielded the following results:

0·3543,* made up to 20 c.c. with water, gave α −15°12′ in a 2-dcm. tube, whence $[\alpha]_D$ −429·0°.

The colchicine from *Gloriosa* yielded an aurichloride, which formed soft, yellow needles, and melted at 209° after drying over sulphuric acid:

0·0800 gave 0·0214 Au. Au = 26·7.

$C_{22}H_{25}O_6N,HAuCl_4$ requires Au = 26·7 per cent.

The above-recorded analysis of the colchicine seems to indicate that the latter still retained a small amount of water of crystallisation after drying in a vacuum, and this is probably the case, since the water which was lost was very rapidly reabsorbed on exposure to the air. On drying colchicine at 105°, however, some slight decomposition appeared to occur.

The amount of colchicine isolated in the pure state from *Gloriosa* was 16·5 grams, which is equivalent to approximately 0·04 per cent. of the weight of the drug employed. When, however, the dried and ground *Gloriosa* tubers were assayed for colchicine by the method described in the United States Pharmacopœia, the amount of alkaloid found was equivalent to 0·3 per cent. of the weight of the dried tubers.

A portion of the aqueous liquid (*A*) which had been extracted with ether and amyl alcohol was treated with a slight excess of basic lead acetate. A voluminous, yellow precipitate was thus produced, which was collected, washed, suspended in water, and decomposed by means of hydrogen sulphide. After the removal of

* Air-dried

the lead sulphide, the filtrate was evaporated under diminished pressure. The product so obtained was dark brown, and amorphous. Nothing crystalline could be separated from it, and it gave only amorphous products when hydrolysed by means of acids or alkalis.

The filtrate from the precipitate produced by basic lead acetate was treated with hydrogen sulphide for the removal of the lead, and the filtered liquid concentrated under diminished pressure to the consistency of a syrup. The latter evidently contained a considerable amount of dextrose, since it readily yielded *d*-phenylglucosazone (m. p. 217°), and, on acetylation, yielded β-pentaacetyldextrose (m. p. 130—131°). (Found, C = 49·3; H = 5·8. Calc., C = 49·2; H = 5·6 per cent.)

Isolation of Choline, $C_5H_{15}O_2N$.—A quantity of the original, aqueous liquid (*A*) which had been extracted with ether and chloroform was concentrated. The resulting syrupy liquid was extracted with alcohol, the alcoholic liquid evaporated, and the residue again treated with alcohol, this operation being repeated until a product was obtained which was soluble in nearly absolute alcohol. To the alcoholic liquid so obtained, a saturated alcoholic solution of mercuric chloride was added, and the mixture kept for several days. The precipitate which had formed was collected, washed with a little alcohol, dissolved as completely as possible in warm water, and the mercury removed from the filtrate by hydrogen sulphide. To the filtered, aqueous liquid, slightly acidified with sulphuric acid, a solution of phosphotungstic acid was added, the precipitate thus produced being treated with barium hydroxide, the mixture filtered, and the excess of barium removed by carbon dioxide. The clear solution was then neutralised with hydrochloric acid and evaporated, first under diminished pressure and finally in a vacuum desiccator, to dryness. The residue was extracted with cold, absolute alcohol, the solution decolorised by means of animal charcoal, and the filtered liquid evaporated. The residue was dissolved in a little water and treated with auric chloride, when a pale yellow precipitate was produced. The latter was collected and dried, when it proved to be choline aurichloride. (Found, Au = 44·3. Calc., Au = 44·5 per cent.) The amount of choline present in *Gloriosa* is, however, extremely small. Attempts to isolate asparagine from the aqueous liquid yielded a negative result.

Examination of the Resin (B).—The resin (*B*) was a hard, dark brown mass, and amounted to 436·4 grams, being thus equivalent to nearly 1·03 per cent. of the weight of the dried tubers 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 amounted to 194·7 grams. It was dissolved in ether, and the ethereal solution shaken with 10 per cent. aqueous hydrochloric acid, which rapidly removed some alkaloidal material. The acid extracts were rendered alkaline by means of sodium carbonate, and extracted with ether, when the alkaloid was removed. On evaporating the ether, a small, dark-coloured residue was obtained, which slowly crystallised. This solid, when purified by crystallisation from ethyl acetate, yielded about 0·15 gram of the previously described alkaloid, $C_{33}H_{38}O_9N_2$, melting at 178°.

The ethereal solution which had been extracted with acid was then shaken with 30 per cent. aqueous potassium hydroxide, which removed the more strongly acid constituents, whilst the salts of the fatty acids remained in the ether. On acidifying the alkaline liquid so obtained and extracting it with ether, a product was obtained which was found to consist solely of benzoic, salicylic, and 2-hydroxy-6-methoxybenzoic acid. The original ethereal liquid was then washed with very dilute aqueous potassium hydroxide, when a dark-coloured, soapy liquid was obtained, the pale yellow, neutral constituents remaining dissolved in the ether. The alkaline liquid was then acidified, and extracted with ether, when a small amount of pale-coloured, flocculent solid remained undissolved. After removing the latter, the ethereal solution was again extracted with dilute, aqueous potassium hydroxide, when a further quantity of neutral material, which had been occluded during the first extraction with dilute alkali, remained dissolved in the ether. The final extract with dilute alkali was then acidified, and the acids extracted by means of ether, when an additional amount of the above-mentioned, flocculent solid was obtained.

Isolation of a Phytosterolin.—The above-mentioned sparingly soluble solid material which had been collected by filtration from the ethereal liquids, as above described, was heated with several successive portions of ethyl acetate to remove adhering fatty matter, after which it was extracted in a Soxhlet apparatus for several days with alcohol. A colourless, sparingly soluble substance was then slowly removed, which was collected, and recrystallised from dilute pyridine. It then formed colourless, microscopic crystals, which melted at 293°, and had the properties of a phytosterolin (phytosterol glucoside):

0·1339 gave 0·3548 CO_2 and 0·1260 H_2O. C = 72·3; H = 10·5.

$C_{33}H_{56}O_6$ requires C = 72·3; H = 10·2 per cent.

On heating the phytosterolin with acetic anhydride in the presence of pyridine, it yielded an acetyl derivative, which crystallised from alcohol in colourless leaflets, melting at 163°.

A quantity of the phytosterolin was hydrolysed by prolonged boiling with alcohol containing some hydrochloric acid, when it yielded a phytosterol. The latter, like the corresponding product occurring in the free state in the plant, described below, proved to be a mixture of stigmasterol and another alcohol, which was probably sitosterol. It is evident, therefore, that stigmasterol glucoside, and probably also sitosterol glucoside, occur in *Gloriosa*.

Examination of the Fatty Acids.—The ethereal solution of the fatty acids which had been separated by filtration from the crude phytosterolin, as above described, was evaporated, and the residual acids converted into their methyl esters by means of methyl alhohol and sulphuric acid. The resulting esters, together with the corresponding esters of the combined fatty acids, obtained as subsequently described, were purified by distillation under diminished pressure, when they passed over at 208–270°/15 mm. The purified esters were then hydrolysed, and the resulting acids separated into their saturated and unsaturated components by means of the lead salts in the usual manner. The saturated fatty acid, after purification by the distillation of its methyl ester, melted at 62°, and was found to consist of palmitic acid. (Found, C = 75·0; H = 12·5. Calc., C = 75·0; H = 12·5 per cent.)

The unsaturated fatty acids were small in amount, and distilled at 235—243°/15 mm. They gave C = 76·7; H = 11·7 per cent. Iodine value = 127. A mixture of one part of linolic acid and two parts of oleic acid requires C = 76·8; H = 11·8 per cent. Iodine value = 121.

The ethereal solutions of the neutral portions of the petroleum extract of the resin were evaporated, and the residue heated with an excess of alcoholic potassium hydroxide. The mixture was then diluted with water, and extracted many times with ether for the removal of the unsaponifiable material. The fatty acids were then isolated from the alkaline, aqueous liquid, and examined in connexion with the free fatty acids, as already described. The ethereal solution of the unsaponifiable material was evaporated, and the resulting pale yellow, crystalline residue crystallised from a considerable volume of absolute alcohol. The product thus obtained, which did not appear homogeneous, was recrystallised from alcohol until it no longer gave a reaction indicating the presence of phytosterol, when about 2·5 grams of a product of low melting point were obtained. Since the latter did not appear homogeneous, it was treated with phthalic anhydride in a manner previously

described (T., 1914, **105**, 1835), when it was ultimately separated into a fatty alcohol, melting at 77°, and a hydrocarbon, melting at 63—65°; both of these products, however, were insufficient for analysis.

Isolation of Stigmasterol.—The alcoholic mother liquors from the crude mixture of fatty alcohol and hydrocarbon were concentrated, and some ethyl acetate and a little water added. A considerable quantity of long, colourless plates then separated, which gave the colour reaction characteristic of the phytosterols. The entire product was heated with acetic anhydride, and the resulting acetyl derivative crystallised from alcohol until it melted constantly at 133°:

0·1007 gave 0·3003 CO_2 and 0·1020 H_2O. C = 81·3; H = 11·3.

0·2095, made up to 20 c.c. with chloroform, gave α_D −1·23° in a 2-dcm. tube, whence $[\alpha]_D$ −58·7°.

These results indicated the probability of this phytosterol being a mixture containing some stigmasterol. The above-described acetyl derivative was therefore treated with bromine in the manner described by Windaus and Hauth (*Ber.*, 1906, **39**, 4378; 1907, **40**, 3681), when a crystalline bromo-derivative rapidly separated. On recrystallising the latter from a mixture of chloroform and alcohol, small, glistening crystals, melting at 210°, were obtained, which were identified as tetrabromoacetylstigmasterol. When debrominated by means of zinc dust, the above compound yielded acetylstigmasterol (m. p. 140°), and the latter, on hydrolysis, was converted into stigmasterol, melting at 167°. The mother liquors from the crude tetrabromoacetylstigmasterol, when debrominated in a similar manner, and subsequently hydrolysed, yielded a small amount of a phytosterol, which, after recrystallisation, melted at 133°, and was probably sitosterol, but the amount obtained was not sufficient for its identity to be fully confirmed.

Ethereal Extract of the Resin.—The ethereal extract of the resin was dark brown, and amounted to 87·4 grams. It was digested with a litre of ether, when a portion of it, which remained undissolved, was removed by filtration. The ethereal solution was then shaken with 10 per cent. hydrochloric acid, when a quantity of an alkaloid was removed. The acid extracts were then rendered alkaline by means of sodium carbonate, and extracted, first with ether and subsequently with chloroform, since a portion of the alkaloid was only with great difficulty removed by the former solvent. On evaporating the chloroform extract, an amorphous, basic product was obtained, which will be referred to later, but the ethereal extracts, when evaporated to a moderately small volume, deposited a quantity (0·15 gram) of light, feathery needles.

The latter were collected, and recrystallised from ethyl acetate, when long, colourless needles, melting at 267°, were obtained:

0·0845 gave 0·2058 CO_2 and 0·0498 H_2O. C = 66·4; H = 6·7.

This substance contained nitrogen, and was a weak base, but the amount was too small to permit of the percentage of nitrogen being estimated. The percentage of carbon and hydrogen found, however, are in good agreement with those required for a methyl-colchicine, $C_{23}H_{27}O_6N$, namely, C = 66·8; H = 6·5 per cent.

The ethereal filtrate from the above-described crystalline base yielded, on evaporation, some amorphous alkaloidal material, which was added to the amorphous base obtained by extraction with chloroform mentioned above.

The original ethereal solution of the resin which had been extracted with acid, as above described, was thoroughly examined, when it was found to consist essentially of phenolic, resinous material, together with a minute quantity of a crystalline phenolic substance.

The above-mentioned portion of the ether extract of the resin which was not dissolved by digestion with ether was heated with chloroform, when a pale-coloured product remained undissolved. The latter, on examination, was found to consist of a further quantity of phytosterolin, identical with that previously described. The chloroform solution, when extracted with acid, yielded a further quantity of amorphous, basic product, but the remainder of the material contained in it was of a resinous nature.

The total amount of the various amorphous, basic products from the ether extract of the resin were united, dissolved in alcohol, and mixed with purified sawdust, and the thoroughly dried mixture was extracted in a Soxhlet apparatus for a long time with ether. The material thus removed was dissolved in chloroform, and deprived of some dark-coloured, phenolic material by means of aqueous potassium hydroxide. On then evaporating the dried chloroform solution almost to dryness, a residue was obtained which slowly crystallised. The latter was digested with ethyl acetate, and the solution filtered, when some brown, amorphous material, part of which was basic, was removed. On concentrating the ethyl acetate solution, a quantity of crystalline colchicine was obtained.

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

The chloroform extract of the resin was dark brown, and amounted to 87 grams. It yielded a further amount of colchicine, together with amorphous basic products. The portion of the

extract which was non-basic was thoroughly examined, but it yielded only resinous products, besides a small amount of the previously described 2-hydroxy-6-methoxybenzoic acid.

The ethyl acetate and alcohol extracts of the resin were dark brown, resinous masses, and amounted to 18·3 and 49 grams respectively. Nothing definite could be obtained from them, and they contained nothing of a glucosidic nature.

Summary and Physiological Tests.—The results of the foregoing investigation may be summarised as follows:

The material employed consisted of the dried tubers of *Gloriosa superba*, Linn., which had been specially collected in Ceylon. Preliminary tests showed the presence of an enzyme, which readily hydrolysed amygdalin, and a considerable amount of an alkaloid. An alcoholic extract of the ground material yielded, in addition to amorphous products, the following definite compounds: (1) Benzoic, 2-*hydroxy*-6-*methoxybenzoic*, and salicylic acids; (2) choline; (3) dextrose; (4) palmitic and a mixture of unsaturated acids; (5) small amounts of a hydrocarbon (m. p. 63—65°) and a fatty alcohol (m. p. 77°); (6) a mixture of phytosterols which contained stigmasterol; (7) a mixture of phytosterolins, containing stigmasterol glucoside; (8) a mixture of alkaloids which consisted chiefly of colchicine, $C_{22}H_{25}O_6N$, together with small amounts of two other crystalline bases. The amount of colchicine present in the drug, when estimated according to the method of the United States Pharmacopœia, was found to be 0·3 per cent.

The physiological action of the colchicine from *Gloriosa* was compared with that of the base from *Colchicum autumnale* at the Wellcome Physiological Research Laboratories by Dr. J. H. Burn, to whom our thanks are due, when the two bases were found to be identical in their effects.

It is evident that the toxic properties of *Gloriosa* tubers are due, essentially, to the colchicine present.

THE WELLCOME CHEMICAL RESEARCH LABORATORIES,
LONDON, E.C. [*Received, May 13th*, 1915.]

PRINTED IN GREAT BRITAIN BY R. CLAY AND SONS, LTD.,
BRUNSWICK STREET, STAMFORD STREET, S.E., AND BUNGAY, SUFFOLK.