Chemical examination of sarsparilla root / by Frederick Belding Power and Arthur Henry Salway.

XXV.—*Chemical Examination of Sarsaparilla Root.*

By Frederick Belding Power and Arthur Henry Salway.

Sarsaparilla root is obtained from different species of *Smilax* which are indigenous to tropical America, from Mexico to Brazil. It has been used medicinally for several centuries, and still maintains a place in the various national Pharmacopœias.

The above-mentioned root has been the subject of numerous chemical investigations, which appear to have been conducted chiefly with the object of ascertaining the nature of its so-called active principles, or the constituents to which the assumed therapeutic virtues of the root are due. The earliest of these investigations was

apparently that of Pallotta (*J. Pharm. Chim.*, 1824, **10**, 543), who obtained a substance in the form of a white powder, possessing a peculiar odour and a bitter taste. This evidently impure substance was considered to be a new organic base, and was designated pariglina or parillin. Berzelius, in 1826, suggested for it the name smilacin. Products were subsequently obtained by Thubeuf (*J. Pharm. Chim.*, 1832, **18**, 734; 1834, **20**, 162, 679) and by Batka (*Annalen*, 1834, **11**, 313; *J. Pharm. Chim.*, 1834, **20**, 43), which were designated respectively salseparin and parillinic acid, but it was shown by Poggiale (*J. Pharm. Chim.*, 1834, **20**, 553) that these substances, although obtained by different methods, were practically identical in composition and properties with the pariglina (parillin) of Pallotta. The investigations mentioned were followed by various others, which need not be here enumerated, until Flückiger (*Arch. Pharm.*, 1877, **210**, 535) isolated and described a substance, for which he retained the original name of parillin. This was definitely shown to be a glucoside, and to belong to the class of saponins, the glucosidal nature of "smilacin" having previously been observed by O. Gmelin (*Annalen*, 1859, **110**, 174). Flückiger proposed for parillin the alternative formulæ, $C_{40}H_{70}O_{18}$ or $C_{48}H_{86}O_{18}$, and considered that the saponins as a class might be represented by the general formula $C_nH_{2n-10}O_{18}$.

The most recent extended investigation of sarsaparilla root was conducted by W. v. Schulz (*Pharm. J.*, 1892, **52**, 6; *Arb. des pharmakol. Inst. zu Dorpat*, 1896, **14**, 14), who has stated it to contain three distinct saponin glucosides, to which the following names, formulæ, and characters were assigned:

I. Parillin, of Pallotta and of Flückiger ("Smilacin" of Berzelius), $C_{26}H_{44}O_{10},2\frac{1}{2}H_2O$. M. p. 176·14°; $[\alpha]_D$ −42·33°. Crystalline, and almost insoluble in cold water.

II. Smilasaponin ("Smilacin" of Merck), $(C_{20}H_{32}O_{10})_5,12H_2O$. $[\alpha]_D$ −26·25°. Amorphous, and soluble in water.

III. Sarsasaponin, $(C_{22}H_{36}O_{10})_{12},24H_2O$. M. p. 220·26°; $[\alpha]_D$ −16·25°. Crystalline, and readily soluble in water.

It was noted by v. Schulz that although the analysis of the last-mentioned compound gave somewhat higher figures for the hydrogen than the formula $C_{22}H_{36}O_{10}$ requires, and the formula $C_{37}H_{62}O_{17}$ would therefore appear at first sight to be more correct, he has adopted the former expression in order to bring the substance into the series having the general formula $C_nH_{2n-8}O_{10}$. This general formula has been considered by Kobert to represent the composition of all the above-mentioned compounds, as well as that of a number of other substances belonging to the class of saponins (compare van Ryn, *Die Glykoside*, p. 217). It has, furthermore, been stated by

v. Schulz that the hydrolysis of the sarsaparilla glucosides is not easily effected by heating under ordinary conditions with dilute acids, and that it is most completely accomplished when the operation is conducted in a sealed tube. The ultimate products of the hydrolysis, besides sugar, have been designated respectively as parigenin, smilasapogenin, and sarsasapogenin, and to all of these the formula $C_{14}H_{23}O_2$ or $C_{28}H_{46}O_4$ has been assigned.

It has been assumed by v. Schulz (*loc. cit.*) that the above-mentioned behaviour of the sarsaparilla glucosides on heating with dilute acids is due to the presence of several sugar complexes in the molecule, which become successively eliminated, and that the first products of hydrolysis therefore still retain the character of glucosides. This view has also been entertained by Rosenthaler and Ström (*Arch. Pharm.*, 1912, **250**, 290) with respect to the saponin obtained from the white or Levant soaproot (from a species of *Gypsophila*). The last-mentioned authors propose to designate the first product of hydrolysis as "pro-sapogenin," restricting the term sapogenin to the final product of hydrolysis, which is obtained by heating the glucoside with acid under pressure. The so-called pro-sapogenin, although crystalline, did not yield satisfactory results on analysis, which it was thought might be due to the presence of some impurity, such as sapogenin.

Although some glucosides are known which, by suitable methods of hydrolysis, are capable of yielding intermediate glucosidal products, such, for example, as amygdalin and apiin (*Ber.*, 1895, **28**, 1508; *Annalen*, 1901, **318**, 121), it appears very doubtful whether the saponins in a pure state actually possess this character. The present investigation of sarsaparilla root has shown it to contain but one definite saponin glucoside, which agrees fairly well in its percentage composition and characters with the sarsasaponin of v. Schulz (*loc. cit.*), and this name has therefore been retained, although a different formula has now been assigned to it. The sarsasaponin of the present authors is, however, readily hydrolysed by heating with dilute acids, without the formation of an intermediate product, but it has now been ascertained that it is accompanied in sarsaparilla root by a phytosterolin (phytosterol glucoside), which, as has recently been shown (T., 1913, **103**, 399, 1022), is not changed by the ordinary methods of hydrolysis. There can be little doubt that the glucosidic products other than sarsasaponin which have hitherto been obtained from sarsaparilla root were not pure substances. The composition and characters assigned to the so-called parillin would appear to indicate that it consisted essentially of a mixture of the substance now designated as sarsasaponin with varying proportions of a phytosterolin, whilst in the course of

the present investigation it has been definitely ascertained that smilasaponin ("smilacin") is not a homogeneous compound.

With consideration of the confusion which has existed respecting the glucosides of sarsaparilla root, and the fact that nothing has been known of its other constituents, apart from the recorded presence of starch, traces of an essential oil, a little fatty oil, resin, and inorganic salts, it has seemed desirable to subject this root to a more systematic and complete chemical examination. The results of the present research, together with the deductions from them, are summarised at the end of this paper.

Experimental.

The material employed for this investigation consisted of a good quality of commercial grey Jamaica sarsaparilla, such as is recognised by the British Pharmacopœia, and which is there described as the dried root of *Smilax ornata*, Hooker, fil.

In order to ascertain whether an enzyme were present, a quantity (500 grams) of the powdered material was mixed with water and kept for two days at the ordinary temperature. The mixture was then filtered under pressure, and alcohol added to the filtrate. A flocculent precipitate was thus produced, which, when dried in a vacuum over sulphuric acid, amounted to 2·6 grams, or 0·52 per cent. of the weight of root employed. This substance slowly hydrolysed amygdalin, thus indicating its enzymic activity.

Another portion (10 grams) of the powdered root was tested for the presence of an alkaloid, but with a negative result.

Twenty-five grams of the powdered root were next 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	0·11	gram	= 0·44	per cent.
Ether	,,	0·11	,,	= 0·44	,,
Chloroform	,,	0·07	,,	= 0·28	,,
Ethyl acetate	,,	0·20	,,	= 0·80	,,
Alcohol	,,	1·30	,,	= 5·20	,,
Total		1·79	grams	= 7·16	per cent.

For the purpose of a complete chemical examination 22·1 kilograms of the ground material were extracted by continuous percolation with hot alcohol. After the removal of the greater portion of the alcohol, a viscid, dark-coloured extract was obtained, amounting to 2·95 kilograms.

Distillation of the Extract with Steam. Separation of an Essential Oil.

The whole of the above extract was mixed with water, and a vigorous current of steam passed through the mixture for several hours. The distillate, on extraction with ether, yielded 2·6 grams of an essential oil, being thus equivalent to about 0·01 per cent. of the weight of root employed. This essential oil, when distilled under diminished pressure, passed over between 70 and 200°/15 mm. as a pale yellow liquid, which possessed a pleasant, somewhat aromatic odour, a density of 0·977 at 15°/15°, and was not completely soluble in 70 per cent. alcohol. It was found to contain furfuraldehyde, and also gave a bluish-black coloration with ferric chloride, thus indicating the presence of a phenolic substance.

After the above operation the steam distillation flask contained a considerable quantity of a brown resin, which formed with the aqueous liquid a viscid emulsion. Since the resin did not separate on keeping, the mixture was agitated with hot amyl alcohol. By this means a very dark-coloured aqueous liquid (*A*) was obtained, whilst the amyl-alcoholic extract contained the resin (*B*), partly in solution and partly in suspension. This extract was filtered, the filtrate well washed with water, the solvent then removed, and the residual semi-solid resin, together with that portion of the resin which was insoluble in amyl alcohol, put aside for subsequent examination.

Examination of the Aqueous Liquid (A).

Isolation of a new Dicarboxylic Acid, Sarsapic Acid, $C_6H_4O_6$.

The aqueous liquid from which the resin had been removed, as above described, was repeatedly extracted with ether. The combined ethereal extracts were then concentrated to a convenient volume and shaken with an aqueous solution of ammonium carbonate. On acidifying the ammonium carbonate extract it yielded about 6 grams of a semi-solid substance, which was dissolved in ether, the ethereal solution being washed, dried, and the solvent removed. The residue, which gradually solidified, was first freed from a little adhering oil by drying on a porous tile, and then crystallised from hot water containing a little alcohol. A substance was thus obtained which separated in slender, colourless needles, melting at 305°:

0·0850 gave 0·1307 CO_2 and 0·0200 H_2O. C = 41·9; H = 2·6.

$C_6H_4O_6$ requires C = 41·9; H = 2·3 per cent.

0·1728 required for neutralisation 20·0 c.c. *N*/10-KOH. N.V. = 64·9.

A dicarboxylic acid, $C_6H_4O_6$, requires N.V. = 65·2.

The molecular weight of the substance was determined by Barger's microscopic method, with the following result:

0·0700 in 5·6 c.c. alcohol was between 0·065 and 0·075 mol.

Mean M.W. = 179. $C_6H_4O_6$ requires M.W. = 172.

It is evident from these results that the above substance is a dibasic acid possessing the empirical formula $C_6H_4O_6$. The only compounds of this formula hitherto recorded are tetrahydroxybenzoquinone, $CO<\begin{matrix}C(OH):C(OH)\\C(OH):C(OH)\end{matrix}>CO$, and 3:5-dihydroxy-4-pyrone-2-carboxylic acid, $CO<\begin{matrix}C(OH):C(CO_2H)\\C(OH)=\!=\!=CH\end{matrix}>O$, neither of which, however, is identical with the above-described substance. Since the latter is therefore a new compound, it is proposed to designate it *sarsapic acid*, with reference to the source from which it has been obtained.

Sarsapic acid, $C_4H_2O_2(CO_2H)_2$, is sparingly soluble in cold water or ether, but very soluble in alcohol. It is moderately soluble in hot water, from which it separates, on cooling, in colourless needles. It gives no coloration with ferric chloride. Its *methyl* ester, $C_4H_2O_2(CO_2{\cdot}CH_3)_2$, prepared by passing dry hydrogen chloride into a hot methyl-alcoholic solution of the acid, crystallises from alcohol in colourless leaflets, melting at 121°. This substance is volatile in steam, and, when gently heated, possesses an odour suggestive of safrole. It was analysed with the following result:

0·0995 gave 0·1746 CO_2 and 0·0363 H_2O. C = 47·9; H = 4·1.

$C_8H_8O_6$ requires C = 48·0; H = 4·0 per cent.

From the above-mentioned properties of sarsapic acid it would appear to contain two carboxylic groups, which would account for the state of combination of four of the oxygen atoms. In order to ascertain the manner in which the two remaining oxygen atoms are combined, methyl sarsapate was heated for some time with acetic anhydride, but no change took place. The same ester was heated for several hours with sodium acetate and hydroxylamine hydrochloride in aqueous alcohol, but no reaction occurred. It would appear, therefore, that sarsapic acid contains neither a hydroxyl nor a carbonyl group. With consideration of these facts it is highly probable that the acid possesses the constitution:

$$O<\begin{matrix}CH:C(CO_2H)\\CH:C(CO_2H)\end{matrix}>O.$$

The ethereal liquid, which had been shaken with aqueous ammonium carbonate for the removal of the sarsapic acid, as described above, was subsequently extracted with aqueous solutions of sodium carbonate and sodium hydroxide. Both of these extracts, on acidi-

fication, yielded only a small amount of a brown, amorphous solid, whilst the ethereal liquid remaining after the treatment with alkalis also contained nothing definite in character.

The aqueous liquid, after being extracted with ether as above described, gave on agitation a copious and persistent froth, and evidently contained some saponaceous substance. In order to isolate the latter, if possible, the aqueous liquid was extracted repeatedly with amyl alcohol. The combined extracts were first washed with a little water, and then concentrated under diminished pressure, when a considerable quantity (20 grams) of a pale brown, amorphous solid was deposited. This substance was found to be glucosidic in character, and also to possess saponin-like properties, but no definite compound could be isolated directly from it. With the object of ascertaining whether any definite hydrolytic product could be obtained from the substance, the latter was heated for some time with dilute sulphuric acid, when a brown solid soon began to separate. After the hydrolysis was complete the mixture was extracted with ether, when some indefinite material remained undissolved. The ethereal solution was then first washed with aqueous sodium hydroxide, which removed some colouring matter, and subsequently with water, after which it was dried and the ether removed, when about 1 gram of a crystalline solid remained. This substance was purified by recrystallisation from alcohol, when it formed colourless, prismatic needles, melting at 183°. It was found to be identical with sarsasapogenin, $C_{26}H_{42}O_3$, the hydrolytic product of the glucoside (sarsasaponin) which was subsequently isolated, as described below.

The aqueous liquid obtained in the above-described hydrolysis was treated with baryta water for the removal of the sulphuric acid, and concentrated to a small volume. It then readily reduced Fehling's solution, but no osazone could be prepared from it.

It is evident from the above results that the brown, amorphous solid which had been obtained by extraction with amyl alcohol contained a small amount of sarsasaponin, together with a considerable proportion of indefinite material.

The amyl-alcoholic filtrate remaining after the removal of the above-described brown, amorphous solid also yielded a small amount of sarsasapogenin on hydrolysis with sulphuric acid.

The original aqueous liquid, after having been extracted with amyl alcohol as above described, was treated with an excess of basic lead acetate, when an abundant, dark brown precipitate was produced. This was collected and washed with water, then suspended in water, and decomposed by hydrogen sulphide. The filtered liquid, which was very darkly coloured, was concentrated

to a small volume, but nothing crystalline separated on keeping. A portion of the liquid was therefore heated for a short time with aqueous potassium hydroxide, after which treatment the mixture was acidified and extracted with ether. The ethereal liquid was then shaken with aqueous ammonium carbonate, which removed a small quantity of a crystalline acid. This substance, after purification, melted at 305°, and was identified as sarsapic acid, $C_6H_4O_6$, which had previously been isolated as described above. Since this small amount of acid was only obtained after heating with alkali, it must have been present in the aqueous liquid in some form of combination. The ethereal liquid, after the removal of the sarsapic acid, contained only indefinite colouring matter.

The filtrate from the basic lead acetate precipitate was treated with hydrogen sulphide for the removal of the excess of lead, and the filtrate concentrated to a syrup. On keeping the latter for some time about 10 grams of a crystalline solid separated in long needles. This substance consisted of potassium nitrate, which had previously been observed to occur in sarsaparilla root. The syrup also contained an abundance of sugar, since it readily yielded *d*-phenylglucosazone, melting and decomposing at 212°. In order further to confirm the identity of the sugar a portion of the syrup was heated with acetic anhydride. An acetyl derivative was thus obtained, which, when crystallised several times from alcohol, melted at 130—131°, and consisted of β-penta-acetyldextrose. The above-mentioned syrup gave no precipitate with potassium-mercuric iodide, iodine, phosphomolybdic acid, or mercuric nitrate, but, when heated with potassium hydroxide, ammoniacal vapours were evolved. Since it still possessed saponaceous properties, a portion of the syrup was heated with 5 per cent. aqueous sulphuric acid, and the mixture subsequently extracted with ether, when a small amount of sarsasapogenin, $C_{26}H_{42}O_3$, was obtained, thus indicating the presence of sarsasaponin in the original aqueous liquid.

Examination of the Resin (B).

The crude resin, which had been separated from the aqueous liquid (*A*) by means of amyl alcohol as above described, was dissolved in alcohol, mixed with purified sawdust, and the dried mixture successively extracted in a large Soxhlet apparatus with light petroleum, ether, chloroform, ethyl acetate, and alcohol.

Petroleum Extract of the Resin.

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

The petroleum extract of the resin was a dark-coloured, viscid solid, amounting to 125 grams. Since it consisted chiefly of fatty

matter, it was heated for some time with 50 grams of potassium hydroxide in the presence of alcohol. The greater portion of the alcohol was then removed, water added, and the mixture subsequently extracted many times with ether. The combined ethereal extracts yielded on evaporation 10 grams of an oily residue, which rapidly solidified. This material was dissolved in a hot mixture of alcohol and ethyl acetate, when, on cooling, a substance separated in colourless needles, melting at 135—136°, which gave the colour reaction of the phytosterols:

0·0958, heated at 110°, lost 0·0040 H_2O. $H_2O = 4·2$.

0·0918 * gave 0·2818 CO_2 and 0·0995 H_2O. $C = 83·7$; $H = 12·0$.

$C_{27}H_{46}O,H_2O$ requires $H_2O = 4·5$ per cent.

$C_{27}H_{46}O$ requires $C = 83·9$; $H = 11·9$ per cent.

A determination of the optical rotatory power of the substance gave the following result:

0·3048,* made up to 20 c.c. with chloroform, gave α_D −0°50′ in a 2-dcm. tube, whence $[\alpha]_D$ −27·3°.

The acetyl derivative, when crystallised from a mixture of alcohol and ethyl acetate, separated in stellar clusters of colourless needles, melting at 126—127°.

It is evident from these results that the above-described substance is sitosterol.

*Isolation of Sitosterol-*d*-glucoside (Phytosterolin)*, $C_{33}H_{56}O_6$.

The alkaline liquid, from which the above-described sitosterol had been removed by ether, was acidified with dilute hydrochloric acid, and the precipitated material extracted with ether. A portion of the precipitate, amounting to about 3 grams, was very sparingly soluble in ether. This was therefore separately collected, and then purified by crystallisation from amyl alcohol. It was thus obtained in microscopic needles, melting and decomposing at 280—285°:

0·1062 gave 0·2802 CO_2 and 0·0984 H_2O. $C = 72·0$; $H = 10·3$.

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

From the analysis and properties of the above-mentioned substance it appeared to consist of a phytosterolin (phytosterol glucoside). The correctness of this supposition was confirmed by hydrolysing the substance in amyl-alcoholic solution with hydrochloric acid (compare T., 1913, **103**, 403), when it was resolved into dextrose, which was identified by means of its osazone, and a substance melting at 136°. The latter gave the characteristic colour reaction of the phytosterols, possessed an optical rotation in chloroform of $[\alpha]_D$ −35·2°, and yielded an acetyl derivative melting

* Anhydrous substance.

at 124°. This hydrolytic product was thus identified as sitosterol, and the substance from which it was obtained was consequently sitosterol-*d*-glucoside.

Identification of the Fatty Acids.

The above-mentioned ethereal solution, from which the phytosterolin had been removed by filtration, yielded, on evaporation, about 70 grams of crude fatty acids. These were converted into their methyl esters, and then subjected to fractional distillation under diminished pressure, when the greater portion passed over at 210—230°/15 mm., but a small fraction was collected above 230°/15 mm. The latter fraction solidified in the receiver, and, when crystallised from ethyl acetate, separated in glistening leaflets, melting at 58—59°:

0·1174 gave 0·3354 CO_2 and 0·1381 H_2O. C = 77·9; H = 13·1.
$C_{23}H_{46}O_2$ requires C = 78·0; H = 13·0 per cent.

The above substance appeared to consist of methyl behenate, and this view of its character was confirmed on hydrolysis, when a fatty acid was obtained melting at 76—77°, and possessing a neutralisation value of 157 ($C_{22}H_{44}O_2$ requires N.V. = 165). The fatty acid was thus identified as behenic acid.

The fraction of methyl esters distilling at 210—230°/15 mm. was hydrolysed, and the resulting fatty acids separated into liquid and solid portions by conversion into the lead salts, and treating the latter with ether.

The Solid Acids.—This portion of acid, amounting to about 30 grams, was crystallised once from a mixture of alcohol and ethyl acetate, when it melted at 54—56°. The product was analysed with the following result:

0·1148 gave 0·3168 CO_2 and 0·1289 H_2O. C = 75·3; H = 12·5.
$C_{16}H_{32}O_2$ requires C = 75·0; H = 12·5 per cent.
$C_{18}H_{36}O_2$ „ C = 76·1; H = 12·7 „ „

It is thus evident that the solid acids consisted of a mixture of palmitic and stearic acids.

The Liquid Acids.—These acids, when distilled under diminished pressure, passed over between 230° and 240°/15 mm., and amounted to 20 grams. An analysis and a determination of the iodine value gave the following results:

0·1132 gave 0·3178 CO_2 and 0·1170 H_2O. C = 76·6; H = 11·5.
0·1492 absorbed 0·2333 iodine. Iodine value = 156.
$C_{18}H_{34}O_2$ requires C = 76·6; H = 12·1 per cent. Iodine value = 90·1.
$C_{18}H_{32}O_2$ „ C = 77·1; H = 11·4 „ „ Iodine „ = 181·4.

From these results it would appear that the liquid acids consisted of a mixture of oleic and linolic acids, the latter predominating.

Ethereal Extract of the Resin.

This extract was a dark-coloured solid, amounting to 32 grams. It was digested with a considerable volume of ether, when a portion was found to be very sparingly soluble. The mixture was therefore filtered, and the insoluble material purified by crystallisation from a mixture of amyl and ethyl alcohols, when about 1 gram of a microcrystalline solid, melting at 295—300°, was obtained. This substance was identified as sitosterol-*d*-glucoside, since it yielded, on hydrolysis, sitosterol and dextrose.

The ethereal liquid from which the above-mentioned glucoside had been removed was next shaken with aqueous ammonium carbonate. The alkaline liquid, on acidification, yielded 1·5 grams of an oily acid, which became partly crystalline on keeping. The crystals were freed from oily matter by pressing on a porous plate, and then recrystallised from dilute alcohol, when colourless needles, melting at 300—305°, were obtained. This acid yielded a methyl ester, melting at 121°, and was thus identified as sarsapic acid, $C_6H_4O_6$, which had previously been isolated from the aqueous liquid.

The ethereal liquid was subsequently shaken with aqueous sodium carbonate, but only a small amount of a brown, amorphous solid was thus removed. It was then treated with a 10 per cent. solution of sodium hydroxide, when an extract was obtained which, on acidification, yielded a quantity of a brown, amorphous solid. This was collected and dissolved in hot alcohol, from which it separated in an indistinctly crystalline form. Attempts were made to obtain this substance in a purer condition, but after several separations from alcohol it still retained a brown colour, and was not definitely crystalline. It began to sinter at 78°, and melted completely at 145°. It was glucosidic in character, for, on heating with hydrochloric acid in the presence of alcohol, it yielded an aqueous liquid which readily reduced Fehling's solution, and also a hydrolytic product melting at 50—55°. The latter possessed the properties of a fatty alcohol, but the amount obtained was not sufficient for its complete characterisation. It would appear probable, however, from the result of the hydrolysis, and also from the properties of the glucoside, that the latter was somewhat impure cetyl-*d*-glucoside, which is known to sinter at 78°, and become completely melted at 150° (Salway, T., 1913, **103**, 1028).

Identification of Stigmasterol, $C_{30}H_{50}O$.

The ethereal liquid, which had been shaken with alkalis, as above described, was finally washed with water, dried, and the ether removed. A crystalline solid, amounting to 2 grams, was thus obtained, which, when recrystallised from a mixture of ethyl acetate and alcohol, separated in colourless leaflets, melting at 140°. The substance possessed the properties of a phytosterol, and evidently consisted for the most part of sitosterol, which had previously been isolated from the petroleum extract of the resin. Its high melting point indicated, however, the presence of some stigmasterol, and the substance was therefore successively acetylated and brominated, according to the method described by Windaus and Hauth (*Ber.*, 1906, **39**, 4378; 1907, **40**, 3681). In this manner a sparingly soluble bromo-derivative was isolated, which crystallised from a mixture of alcohol and chloroform in colourless leaflets, decomposing at 208°:

0·0656 gave 0·1162 CO_2 and 0·0408 H_2O. C = 48·3; H = 6·9.

$C_{32}H_{52}O_2Br_4$ requires C = 48·7; H = 6·6 per cent.

From the analysis and properties of the above compound it is evidently identical with tetrabromoacetyl stigmasterol, thus proving the presence of stigmasterol in sarsaparilla root.

Chloroform and Ethyl Acetate Extracts of the Resin.

These extracts were dark-coloured, brittle solids, amounting to 55 and 20 grams respectively. Both of these extracts were glucosidic in character, but no definite glucoside could be isolated from them. The ethyl acetate extract of the resin, however, on heating with dilute sulphuric acid, yielded a small amount of sarsapic acid, $C_6H_4O_6$.

Alcohol Extract of the Resin.

This extract was a dark brown solid, amounting to 45 grams. It was dissolved in alcohol, and the solution kept for some time, when a quantity of a crystalline solid separated, which was collected. The alcoholic liquid was then concentrated to a convenient bulk, and heated for some time with aqueous hydrochloric acid. After removing the alcohol in a current of steam, the remaining aqueous liquid was separated by filtration from a dark-coloured resin, and the filtrate extracted with ether. The resin also was digested with ether, which, however, dissolved but a small proportion of it. The two ethereal liquids were united, washed first with aqueous sodium hydroxide, subsequently with water, then dried, and the solvent

removed. A crystalline residue (0·2 gram) was thus obtained, which melted at 183°, and was found to be identical with the sarsasapogenin described below.

Isolation of Sarsasaponin, $C_{44}H_{76}O_{20},7H_2O$.

The above-mentioned crystalline solid, which separated from the alcoholic solution of the resin, was purified by several crystallisations from alcohol, when colourless, elongated needles were obtained, which began to sinter at about 200°, and melted completely at 248°. The substance was glucosidic in character, and its aqueous solution, when agitated, yielded a copious and persistent froth. It thus possessed the properties of a saponin. The amount of pure substance isolated from the above extract of the resin was 1·5 grams. It was analysed, with the following results:

0·1089, on heating at 125°, lost 0·0130 H_2O. $H_2O = 11·9$.
0·0934 * gave 0·1967 CO_2 and 0·0663 H_2O. $C = 57·4$; $H = 7·9$.
0·1048 * „ 0·2213 CO_2 „ 0·0758 H_2O. $C = 57·6$; $H = 8·0$.

$C_{44}H_{76}O_{20},7H_2O$ requires $H_2O = 12·0$ per cent.
$C_{44}H_{76}O_{20}$ requires $C = 57·1$; $H = 8·2$ „
$C_{44}H_{72}O_{20}$ „ $C = 57·4$; $H = 7·8$ „

The above analytical figures will be seen to be in somewhat better agreement with the formula $C_{44}H_{72}O_{20}$ than with $C_{44}H_{76}O_{20}$, but the data subsequently obtained by the hydrolysis of the glucoside are more in accordance with the latter formula, and this has therefore been adopted as having the greater probability of correctness.

W. v. Schulz (*loc. cit.*) had previously isolated from sarsaparilla root a saponin glucoside, which possessed nearly the same percentage composition as that above described (Found, $C = 57·1$; $H = 8·1$), and was designated sarsasaponin. This compound, to which the formula $(C_{22}H_{36}O_{10})_{12},24H_2O$ was assigned, was stated to melt at 220·26°, to have $[\alpha]_D - 16·25°$, and to yield an indefinite hydrolytic product of variable composition. Its hydrolysis was, however, considered to result in the formation of sarsasapogenin, $C_{28}H_{46}O_4$, dextrose, and an undetermined acid or mixture of acids, $C_4H_6O_6$, in accordance with the following equation:

$$2(C_{22}H_{36}O_{10}) + 2H_2O = C_{28}H_{46}O_4 + 2C_6H_{12}O_6 + C_4H_6O_6.$$

The saponin glucoside, which has now been isolated from sarsaparilla root, yields a well-defined, crystalline, hydrolytic product, which will subsequently be described. Although this glucoside differs in some of its other characters, such as melting point and optical rotation, from the sarsasaponin of v. Schulz, and

* Dried at 125°.

a somewhat different formula has now been assigned to it, there can be no doubt of the fundamental identity of the compounds. It is therefore deemed desirable that the name sarsasaponin should be retained for the glucoside which is here described.

Sarsasaponin is readily soluble in water or hot alcohol, but only very sparingly soluble in ether. It cannot be crystallised from water, and is best purified by crystallisation from 95 per cent. alcohol. It shows, however, a great tendency to separate from concentrated alcoholic solutions in a gelatinous form.

Sarsasaponin can be removed for the most part from its aqueous solution by shaking the latter with finely divided substances which are insoluble in water, and it had been observed by v. Schulz (*loc. cit.*) that when its lead compound was decomposed by hydrogen sulphide, the glucoside was contained to a large extent in the precipitated lead sulphide. In the course of the present investigation sarsasaponin could only be isolated in a pure state from the resinous material, notwithstanding the fact that it is readily soluble in water, and was evidently contained, in part, in the aqueous liquid obtained by treating the alcoholic extract of the root with water. From the facility with which it is mechanically precipitated, as well as from the results of cryoscopic observations, it seems highly probable that sarsasaponin forms with water only colloidal solutions. The property of forming such solutions would also account for the persistent froth which is produced by the substances designated as saponins when shaken with water.

When sarsasaponin is dissolved in acetic anhydride, and subsequently a few drops of concentrated sulphuric acid added, a yellow colour is produced, and the liquid rapidly develops a green fluorescence. On keeping for some time, or on the addition of a larger amount of sulphuric acid, the liquid acquires a reddish-brown colour.

The specific rotatory power of sarsasaponin was determined with the following result:

0·2130,* made up to 20 c.c. with water, gave α_D −1°2′ in a 2-dcm. tube, whence $[\alpha]_D$ −48·5°.

v. Schulz (*loc. cit.*) has recorded that the substance designated by him as sarsasaponin had a rotation of $[\alpha]_D$ −16·25°.

Hydrolysis of Sarsasaponin.

Formation of Sarsasapogenin, $C_{26}H_{42}O_3$, *and Dextrose.*

One gram of sarsasaponin, in aqueous solution, was heated with 5 per cent. sulphuric acid, when, after a short time, a gelatinous,

* Anhydrous substance.

hydrolytic product separated, which gradually became crystalline. The heating was continued for several hours, and the mixture then distilled in a current of steam, but no volatile product of hydrolysis was found in the distillate. After this operation there remained in the distillation flask a crystalline solid, which was collected by filtration, the filtrate being set aside for the subsequent examination of the sugar. The solid substance was washed with water, and recrystallised from alcohol, when it separated in slender, colourless needles, melting at 183°, and containing water of crystallisation:

0·1936, heated at 120°, lost 0·0113 H_2O. $H_2O = 5·8$.

0·0961 * gave 0·2724 CO_2 and 0·0906 H_2O. $C = 77·3$; $H = 10·5$.

The molecular weight of the substance was determined by both the cryoscopic and the microscopic method, with the following results:

0·1681,* in 19·8 c.c. benzene, gave Δt −0·100°, whence M.W. = 424.

0·1820,* in 10 c.c. alcohol, was between 0·05 and 0·04 mol. Mean M.W. = 404.

$C_{26}H_{42}O_3,1\frac{1}{2}H_2O$ requires $H_2O = 6·3$ per cent.

$C_{26}H_{42}O_3$ requires $C = 77·6$; $H = 10·4$ per cent. M.W. = 402.

From the above results it is evident that the hydrolytic product of sarsasaponin, for which the name sarsasapogenin may be retained, possesses the empirical formula $C_{26}H_{42}O_3$. This is further confirmed by the analysis of its acetyl derivative, described below.

It has been stated by W. v. Schulz (*loc. cit.*) that the complete hydrolysis of sarsasaponin is only effected with great difficulty, and that the resulting hydrolytic product, sarsasapogenin, possesses the formula $C_{28}H_{46}O_4$. Inasmuch as the sarsasaponin obtained in the present investigation was easily and completely hydrolysed by the above-described treatment, it is probable that the glucoside of v. Schulz was contaminated with some phytosterolin, such as is now known to be present in sarsaparilla root, and which is only very slowly hydrolysed by aqueous acids.

Sarsasapogenin, $C_{26}H_{41}O_2{\cdot}OH$, is readily soluble in chloroform or benzene, but only moderately so in ether or cold alcohol. When the substance is dissolved in acetic anhydride, and a few drops of concentrated sulphuric acid subsequently added, a yellow coloration is produced, and the liquid soon develops a green fluorescence. On keeping for some time, or on the addition of a larger amount of sulphuric acid, the liquid acquires a reddish-brown colour. Sarsasapogenin is optically active:

0·1133, made up to 20 c.c. with methyl alcohol, gave α_D −0°41′ in a 2-dcm. tube, whence $[\alpha]_D$ −60·3°.

* Anhydrous substance.

Monoacetylsarsasapogenin, $C_{26}H_{41}O_3 \cdot CO \cdot CH_3$. — This compound was prepared by heating sarsasapogenin for an hour with acetic anhydride, the solution being then concentrated to a small bulk, and a little alcohol subsequently added. After a short time an acetyl derivative separated, which, when collected and recrystallised from alcohol, was obtained in colourless needles, melting at 137°. It was analysed and its specific rotation determined, with the following results:

0·0968 gave 0·2684 CO_2 and 0·0872 H_2O. C = 75·6; H = 10·0.
$C_{28}H_{44}O_4$ requires C = 75·7; H = 9·9 per cent.

0·1160, made up to 20 c.c. with chloroform, gave α_D −0°40′ in a 2-dcm. tube, whence $[\alpha]_D$ −57·5°.

The acid, aqueous liquid resulting from the hydrolysis of sarsasaponin was treated with just sufficient barium hydroxide to precipitate the sulphuric acid completely, and the mixture then filtered. The filtrate, on evaporation, yielded a syrup, from which *d*-phenylglucosazone (m. p. 214°) was prepared, thus proving the presence of dextrose. No evidence could be obtained of the formation of any other sugar as a product of hydrolysis of the glucoside.

The above results have shown that sarsasaponin is resolved on hydrolysis into sarsasapogenin, $C_{26}H_{42}O_3$, and dextrose. In order to ascertain the relative proportion of sarsasapogenin which is yielded by the glucoside, a definite quantity of the latter, dried at 120°, was hydrolysed, and the respective hydrolytic product collected, dried at 110°, and weighed. The amount of sarsasapogenin thus obtained was 38 per cent. of the weight of sarsasaponin employed ($C_{44}H_{76}O_{20}$ requires $C_{26}H_{42}O_3$ = 43·5 per cent.). The hydrolysis of the glucoside may thus be represented by the following equation:

$$C_{44}H_{76}O_{20} + H_2O = C_{26}H_{42}O_3 + 3C_6H_{12}O_6.$$

The assumption of v. Schulz (*loc. cit.*) that by the hydrolysis of sarsasaponin an acid or a mixture of acids is formed, together with the other products mentioned, cannot be confirmed, and is obviously incorrect. The acid observed by him was doubtless only such as is produced by the action of the hydrolysing agent on the sugar.

Examination of "Smilacin" ("Smilasaponin" of v. Schulz).

The substance described by v. Schulz (*loc. cit.*) as "smilasaponin," and recorded in the literature under that name or as "smilacin," was evidently regarded by him as a distinct glucoside of sarsaparilla root. Although an amorphous product, he assigned to it the

formula $(C_{20}H_{32}O_{10})_5,12H_2O$, and observed its optical rotation to be $[\alpha]_D$ −26·25°. Inasmuch as the "smilasaponin" examined by v. Schulz was stated to have been the preparation known in commerce as "smilacin," it was deemed desirable in connexion with the present investigation to determine its character. A small quantity of the preparation was therefore obtained from the same source of supply as had been indicated by v. Schulz.

"Smilacin," as procured from the source indicated, was a pale brownish-yellow, amorphous powder. It dissolved readily in water, yielding a yellow solution, which frothed strongly on agitation, and did not reduce Fehling's solution. The substance, when heated in a capillary tube, began to sinter at 140°, and decomposed with evolution of gas at 160°. When heated at 110°, it lost 17·7 per cent. of its weight, and on ignition it left a small amount of inorganic residue. In view of the character of the substance, it was not deemed suitable for analysis, and on account of the colour of its aqueous solution the optical rotatory power could only be determined with approximate accuracy.

0·2250 of anhydrous substance, made up to 20 c.c. with water, gave α_D about −1°0′ in a 2-dcm. tube, whence $[\alpha]_D$ −44·4°.

When a little of the substance was dissolved in acetic anhydride, and a few drops of concentrated sulphuric acid subsequently added, a reddish-brown coloration was produced, the liquid also showing a faint green fluorescence.

Hydrolysis of "Smilacin."—Three grams of the substance were dissolved in 30 c.c of amyl alcohol, and 10 c.c. of 15 per cent. aqueous hydrogen chloride added, together with sufficient alcohol (about 1 c.c.) to render the mixture homogeneous. This solution was heated for three hours, and the amyl alcohol then removed in a current of steam, when a dark-coloured solid remained in the distillation flask. The solid was extracted with ether, and the ethereal solution washed with aqueous sodium carbonate, which removed a small amount of brown, amorphous material. The ethereal liquid was then dried, and the solvent removed, when 0·3 gram of a substance was obtained, which, when crystallised from alcohol, separated in colourless, feathery needles, melting at 184—185°. When the substance was mixed with a specimen of pure sarsasapogenin (m. p. 183°) no depression of the melting point ensued. It also showed the same behaviour as the last-mentioned compound when dissolved in acetic anhydride and a drop of concentrated sulphuric acid subsequently added. The identity of the substance with sarsasapogenin was further confirmed by analysis:

0·0712 gave 0·2035 CO_2 and 0·0665 H_2O. C = 77·9; H = 10·4.
$C_{26}H_{42}O_3$ requires C = 77·6; H = 10·4 per cent.

The aqueous liquid resulting from the above-described hydrolysis was exactly neutralised with sodium carbonate, and then evaporated to dryness under diminished pressure. This residue was digested with hot alcohol, the mixture filtered, and the filtrate evaporated. A syrupy liquid was thus obtained, which readily reduced Fehling's solution, and yielded an osazone melting at 212°.

It is evident from the above results that the so-called "smilacin," which has now been examined, contained a relatively small proportion of the glucoside sarsasaponin, but that it consisted for the most part of indefinite amorphous products.

Summary and Conclusions.

The material used for the present investigation consisted of a good quality of grey Jamaica sarsaparilla root.

The root was found to contain a small amount of an enzyme, which slowly hydrolysed amygdalin.

An alcoholic extract of the root, when distilled in a current of steam, yielded an amount of essential oil equivalent to about 0·01 per cent. of the weight of root employed. This essential oil was a pale yellow liquid, which distilled between 70° and 200°/15 mm., and had a density of 0·977 at 15°/15°.

The alcoholic extract was found to contain the following definite compounds: (i) a crystalline glucoside, sarsasaponin, $C_{44}H_{76}O_{20},7H_2O$ (m. p. 248°; $[\alpha]_D$ −48·5°), which, on hydrolysis, is resolved into sarsasapogenin, $C_{26}H_{42}O_3$ (m. p. 183°; $[\alpha]_D$ −60·3°), and dextrose. Sarsasapogenin yields a *monoacetyl* derivative, $C_{26}H_{41}O_3{\cdot}CO{\cdot}CH_3$ (m. p. 137°; $[\alpha]_D$ −57·5°); (ii) sitosterol-*d*-glucoside (phytosterolin), $C_{33}H_{56}O_6$ (m. p. 280—285°); (iii) sitosterol, $C_{27}H_{46}O$ (m. p. 135—136°; $[\alpha]_D$ −27·3°); (iv) stigmasterol, $C_{30}H_{50}O$, identified by its tetrabromoacetyl derivative, $C_{30}H_{49}OBr_4{\cdot}CO{\cdot}CH_3$ (m. p. 208°); (v) a new, crystalline, dicarboxylic acid, *sarsapic acid*, $C_4H_2O_2(CO_2H)_2$, melting at 305°, and yielding a *dimethyl* ester, $C_8H_8O_6$ (m. p. 121°); (vi) dextrose, from which β-penta-acetyl dextrose (m. p. 131—132°) and *d*-phenylglucosazone (m. p. 212°) were prepared; (vii) a mixture of fatty acids, consisting of palmitic, stearic, behenic, oleic, and linolic acids. The alcoholic extract contained, furthermore, a small quantity of a substance which possessed the characters of cetyl-*d*-glucoside, and a considerable quantity of potassium nitrate was also present. The total amount of resinous material was equivalent to about 1·25 per cent. of the weight of the root.

The results of the present investigation have shown that Jamaica sarsaparilla root contains but one definite saponin glucoside, namely, sarsasaponin. This is accompanied, however, in the root by a phytosterolin (sitosterol-*d*-glucoside), which represents a class of compounds that has only quite recently been known to occur in plants (compare T., 1913, **103**, 399, 1022). It is probable that the same or similar conditions exist with respect to the constituents of other commercial varieties of sarsaparilla root, and the composition and properties of the compound designated by v. Schulz and earlier investigators as parillin would, in fact, indicate that it consisted of a mixture of sarsasaponin and a phytosterolin. The so-called "smilacin" ("smilasaponin" of v. Schulz), as examined by the present authors, has been ascertained not to be a homogeneous substance, but to contain some sarsasaponin, together with indefinite amorphous products.

It may finally be noted that v. Schulz (*loc. cit.*) had found sarsasaponin to be, physiologically, the most active of the glucosidic products described by him, and this observation is quite in accordance with the above conclusions respecting the character of "parillin" and "smilasaponin."

THE WELLCOME CHEMICAL RESEARCH LABORATORIES,
LONDON, E.C.

R. CLAY AND SONS, LTD., BRUNSWICK ST., STAMFORD ST., S.E., AND BUNGAY, SUFFOLK