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SAPONARIN

A New Glucoside, Coloured Blue with Iodine

BY

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CXXIII.—*Saponarin, a New Glucoside, Coloured Blue with Iodine.*

By George Barger.

The epidermal cells of the leaves of certain flowering plants, belonging to various natural orders, have long been known to contain, dissolved in their cell sap, a substance which is coloured blue by iodine. The colour disappears on warming and returns on cooling, as is the case with starch; it is, however, not confined to well-marked grains, but extends uniformly throughout the cell as a fine blue precipitate. On this account the substance was regarded as an amorphous variety of starch by Sanio, its discoverer (*Botanische Zeitung*, 1857, **15**, 420). Sanio found the substance in the leaf-epidermis of *Gagea lutea*, and showed by plasmolysis that it is confined to the cell sap. The publication of this note led Schenk to record similar observations on the closely allied genus *Ornithogalum* (*Botanische Zeitung*, 1857, **15**, 497, 555). Schenk doubted whether the substance was identical with starch, because fragments of the epidermis of *Ornithogalum* leaves,

when coloured blue by iodine, lost their colour when placed in water.

The substance was next observed in a species of *Ornithogalum* by Trecul (*Bull. Soc. bot. de France*, 1858, **5**, 711), and later it was studied in the same genus by Nägeli, who definitely declared against it being starch (*Beiträge zur wissensch. Botanik*, 1860, **2**, 187). The next reference to the substance is by Kraus, who found it in Arum (*Botanische Mittheilungen*, Halle, 1885). A detailed account of "soluble starch" was published by the Swiss botanist Dufour (*Bull. Soc. vaud. Sci. nat.*, 1885, **21**, 227). He found it in about twenty species of *Phanerogams*, but, like his predecessors, he did not isolate it.

The present work was started in 1901 in the botanical laboratory of Brussels University, at the suggestion of the late Professor L. Errera, to whose stimulating interest the author owes much, and some preliminary results were communicated at the meeting of the British Association in 1904. *Saponaria officinalis* was chosen as the source of material; this plant is relatively rich in the substance, and is grown on the continent for pharmaceutical purposes, so that large quantities were easily obtainable. The substance proved to be a glucoside, and the name *Saponarin* was suggested for it, leaving open the question of its probable identity with the "soluble starch" of other plants.

Experimental.

The method of isolation was as follows. Dried shoots of *Saponaria* were boiled several times with 10—20 times their weight of water for half an hour. Since the saponarin is confined to the epidermis, there is no advantage in powdering the leaves. The various decoctions were strained through linen and concentrated on the water-bath to one-quarter of their original volume; they were next acidified with acetic acid and left standing for several weeks, during which time a grey deposit was slowly formed, the saponins present hindering precipitation. The precipitate was separated, made into a thin cream with water, and poured into a hot one per cent. sodium carbonate solution (1 litre per kilogram of dry leaves), in which nearly the whole of the precipitate dissolved with a yellow colour. The alkaline solution was so dilute, that it could be acidified with acetic acid without causing any immediate precipitation (because of the presence of the saponins). Neutral lead acetate solution was then added, which produced a bulky precipitate of gums and other impurities. These were separated and the acid filtrate was left standing for several weeks in order to allow the crude saponarin to precipitate. This substance has a tendency to adhere to the sides of glass vessels. It still contained more than 30 per

cent. of impurities; the complete elimination of these was at first very difficult, for the glucoside is almost or quite insoluble in nearly all organic solvents. It could to some extent be purified by repeated solution in alkalis and precipitation by acids, when it separated in minute sphaero-crystals. The only organic solvent available was pyridine, in which it is extremely soluble on warming. On cooling, the substance does not separate out, even when a large quantity of water or alcohol is added. With ether and light petroleum, an amorphous precipitate is formed, but the following method proved eminently successful. The crude saponarin was dissolved in boiling pyridine; the dark brown solution was filtered and the filtrate was evaporated in a vacuum on the water-bath. A dark syrup remained behind, which retained traces of pyridine and was readily soluble in hot water (although the glucoside itself is insoluble in water containing pyridine). The aqueous solution was diluted, and on standing deposited isolated microscopic needles (not sphaero-crystals, as when an alkaline solution is acidified).

The needles were collected on a hardened filter paper by means of the pump and washed by suspension in hot water. When dried in the air, saponarin is a white powder; after being dried in a vacuum it becomes pale yellow. It is quite insoluble in cold water, but readily dissolves, with an intense yellow coloration, in dilute solutions of caustic alkalis and alkali carbonates, especially on warming. Hence, if boiled for some time in a glass vessel, a little is dissolved by the alkali of the glass. The glucoside is also soluble with a bright yellow colour in concentrated mineral acids, the solution in sulphuric acid showing a blue fluorescence. On acidifying an alkaline solution and on diluting a solution in concentrated acids with water, the yellow colour disappears, but the glucoside is not immediately precipitated if the solution is dilute. This power of remaining in a state of pseudo-solution is a characteristic property of saponarin, and in this condition it gives with iodine in potassium iodide the blue or violet coloration which led to its discovery. This coloration disappears on warming, but returns again on cooling, and in other respects closely resembles that produced by iodine and starch; the differences are merely quantitative, in the sense that saponarin has by far the smaller power of absorbing iodine. Accordingly the blue colour disappears completely on dilution with a little alcohol or much water, and can then be regenerated by adding starch solution. It is possible to obtain crystalline saponarin, coloured blue with iodine, by allowing a solution of the glucoside in dilute acetic acid containing a little iodine to evaporate slowly on a watch-glass. Blue needles separate out. A solution of saponarin can further be distinguished from a starch solution by the fact that it is coloured reddish-brown by ferric

chloride and bright yellow by alkalis. Normal lead acetate does not precipitate it, but basic lead acetate gives a bulky, yellow precipitate. Saponarin is almost or quite insoluble in nearly all organic solvents. It dissolves to a small extent in hot glacial acetic acid and in aniline, more readily in the chloroacetic acids, in piperidine, quinoline, and phenylhydrazine; it is very soluble in pyridine. When heated slowly, it melts and decomposes at 231—232°, but if the bath is previously heated to 230° the melting point is 236°.

The glucoside is lævorotatory. A solution containing 0·924 gram in 100 c.c. of pyridine gave α_D −0·73° in a 1-dcm tube, whence $[\alpha]_D$ −7·90°.

The air-dried substance contains water of crystallisation which cannot be removed by heat alone, but which is completely expelled on standing for a week, at the ordinary temperature, in a vacuum over sulphuric acid, so that no further loss occurs when the substance is heated in a vacuum at 100°. The anhydrous substance is extremely hygroscopic; when left in the balance case for half an hour, it absorbs the whole of the water of crystallisation which it had lost. On this account some difficulty was at first experienced in analysing the anhydrous substance, and the boat had always to be weighed enclosed in a stoppered weighing bottle. The various samples were recrystallised two or three times by the pyridine-water method and dried in a vacuum.

0·1883 gave 0·3706 CO_2 and 0·0862 H_2O. C = 53·68; H = 5·09.
0·1446 „ 0·2846 CO_2 „ 0·0675 H_2O. C = 53·68; H = 5·19.
0·1192 „ 0·2358 CO_2 „ 0·0559 H_2O. C = 53·95; H = 5·22.
0·1714 „ 0·3380 CO_2 „ 0·0784 H_2O. C = 53·78; H = 5·08.
0·1465 „ 0·2898 CO_2 „ 0·0653 H_2O. C = 53·95; H = 4·95.
$C_{21}H_{24}O_{12}$ requires C = 53·85; H = 5·13 per cent.

The water of crystallisation in the air-dried substance was determined by drying in a vacuum until constant.

At the ordinary temperature 0·1854 lost 0·0131 H_2O. H_2O = 7·07.
„ „ „ „ 0·1580 „ 0·0115 H_2O. H_2O = 7·28.
„ „ „ „ 0·3529 „ 0·0248 H_2O. H_2O = 7·03.
At 100° 0·2553 „ 0·0181 H_2O. H_2O = 7·09.

The air-dried substance was also analysed.

1. Obtained by exposing the moist substance to the atmosphere: 0·2166 gave 0·3942 CO_2 and 0·1105 H_2O. C = 49·64; H = 5·67.

2. Obtained by exposing the anhydrous substance to the atmosphere: 0·1735 gave 0·3216 CO_2 and 0·0846 H_2O. C = 50·55; H = 5·42.

$C_{21}H_{24}O_{12},2H_2O$ requires H_2O = 7·14. C = 50·00; H = 5·56 per cent.

The molecular weight was determined* in pyridine solution (*a*) with the substance dried at 100° : 0·299 gram in 2·96 grams of pyridine was between 0·223 and 0·238 mol., benzil as standard, ordinary temperature, hence M = 424—453, mean 438 ; (*b*) with the substance dried in a vacuum, the pyridine dried over caustic potash : 0·209 gram in 1·98 grams pyridine was between 0·22 and 0·23 mol., benzil as standard, temperature 90°, hence M = 459—480, mean 469.

$C_{21}H_{24}O_{12}$ requires 468.

The lead salt was also analysed. It was prepared by precipitating a solution of the glucoside in dilute ammonia with neutral lead acetate, filtering, and washing the yellow precipitate by suspension in hot water. The lead salt was dried in a vacuum until constant in weight and completely decomposed by heating with fuming nitric acid in a sealed tube, the lead being then estimated as sulphate.

0·3489 gave 0·2444 $PbSO_4$. Pb = 47·8.

$C_{21}H_{30}O_{12}Pb_2$ requires Pb = 47·2 per cent.

Ennea-acetylsaponarin.

The glucoside is readily acetylated by boiling for a few seconds with a large excess of acetic anhydride and a few drops of concentrated sulphuric acid. The acetyl derivative is easily soluble in hot alcohol and crystallises on cooling in microscopic, curved needles, melting at 183—185°. It is extremely soluble in ethyl acetate, benzene, and chloroform, and was purified by dissolving in ethyl acetate, which leaves a small quantity of an impurity behind, and then crystallising from a dilute solution in alcohol ; 0·915 gram in 10 c.c. of ethyl acetate gave, in a 1-dcm. tube, α_D −4·90°, whence $[\alpha]_D$ −5·33°.

0·1436 gave 0·2911 CO_2 and 0·0618 H_2O. C = 55·29 ; H = 4·78.

0·2078 gram in 0·8586 gram of ethyl acetate was intermediate between 0·2875 and 0·300 mol. benzil.

Hence M = 807—842, mean = 825.

$C_{21}H_{15}O_{12}(C_2H_3O)_9$ requires C = 55·32 ; H = 4·96 per cent. M = 846.

The number of acetyl groups was determined by A. G. Perkin's ethyl acetate method (Trans., 1905, **87**, 107).

1. 0·4246 gram gave 0·2748 gram acetic acid = 64·8.
2. 0·7223 „ „ 0·4632 „ „ „ = 64·1.

Theory for nine acetyl groups requires 63·8 per cent.

* All molecular weight determinations in this paper were made by the author's microscopic method (Trans., 1904, **85**, 286 ; 1905, **87**, 1756).

Acetyl-saponarin does not give a blue coloration with iodine, neither is it coloured by alkalis or ferric chloride. When boiled with acids in alcoholic solution, the acetyl groups are not only eliminated, but the glucoside itself is hydrolysed. Saponarin was, however, easily recovered by pouring the alcoholic solution of the acetyl compound into hot dilute caustic soda solution, which was kept stirred. The acetyl compound is thus precipitated in a finely-divided condition and at once undergoes saponification, the glucoside dissolving with a yellow colour. On acidifying, the saponarin is precipitated, and on recrystallisation from pyridine and water is found to be identical with the purest saponarin previously obtained, melting at exactly the same temperature.

Hydrolysis of Saponarin; Formation of Vitexin and Saponaretin.

When saponarin is boiled with dilute mineral acids it is slowly hydrolysed according to the equation:

$$C_{21}H_{24}O_{12} + H_2O = C_{15}H_{14}O_7 + C_6H_{12}O_6.$$

A sugar is formed which reduces Fehling's solution and gives an osazone melting at 205°. This osazone, after recrystallisation from alcohol, was mixed with an equal quantity of pure glucosazone, and the melting points of the mixture and of both constituents were determined simultaneously and found to be identical. The sugar is therefore glucose. In accordance with its glucosidic nature, saponarin gives Molisch's furfuraldehyde reaction with sulphuric acid and α-naphthol or thymol.

An estimation (by Fehling's solution) of the amount of sugar produced did not yield a very satisfactory result. As usual in such cases, it fell considerably short of the theoretical quantity.

1·2692 gram saponarin, dried in a vacuum, gave on hydrolysis 0·4253 gram glucose = 33·5.

$C_{21}H_{24}O_{12}$ requires glucose = 38·5 per cent.

The isolation of the phenolic product of the hydrolysis was attended with much difficulty, as it appeared for the most part as an uncrystallisable resin, however much the conditions were varied. The best results were obtained by boiling 10 grams of the glucoside for sixteen hours with 200 c.c. of 2 per cent. sulphuric acid. The saponarin was slowly dissolved, forming a dark yellow solution, which at first still gave the blue coloration with iodine. When this reaction was no longer obtained, a small quantity of a black, resinous decomposition product was separated and the solution was cooled. Oily drops appeared and, on standing for some hours, collected at the bottom of

the flask as a viscid, brown mass. The aqueous solution was decanted and the brown syrup dissolved in a little absolute alcohol. On standing, the alcoholic solution deposited a small quantity of a crystalline substance, which was ultimately found to be identical with vitexin, a colouring matter obtained by A. G. Perkin from the dye-wood of the New Zealand tree *Vitex littoralis* (Trans., 1898, **73**, 1030; and 1900, **77**, 416).

After decantation, the sulphuric acid in the aqueous solution was precipitated with baryta. On separating the barium sulphate, the solution was found to be dark yellow, owing to a very slight excess of baryta having been employed, which formed a salt of the colouring matter, but left the solution neutral to litmus. On boiling, this solution also deposited a quantity of vitexin, which at first separated in isolated characteristic leaflets, as from the alcoholic solution already referred to. Later, groups of such leaflets and sphaero-crystals were formed, of a much darker (brown) colour. The highest yield of vitexin obtained in several experiments only amounted to 13 per cent. of the theoretical. It was often much lower, and this poor yield suggested that vitexin was present as, or derived from, an impurity in the saponarin employed. Accordingly 1 gram of a sample of the glucoside, which had been purified for analysis by two crystallisations from pyridine water and consisted entirely of isolated needles, was hydrolysed with 2 per cent. sulphuric acid. This sample also yielded more than 10 per cent. of vitexin, so that the latter substance is undoubtedly a product of the hydrolysis of saponarin.

The rest of the glucoside had been converted into another amorphous substance, which remained dissolved in the alcohol, and may be called *saponaretin* (in analogy to saliretin).

Often, especially if the alcohol is dilute, some of the saponaretin was eventually deposited along with the vitexin as a spongy, yellow mass. A separation can, however, be readily effected by means of boiling alcohol, which dissolves the amorphous saponaretin and leaves the crystals of vitexin behind.

Vitexin (from Saponarin).

This substance crystallises in microscopic, rhomb-shaped, glistening plates of a pale yellow colour, melting at 260° with characteristic frothing. The bath was heated to 240° before the introduction of the substance. Vitexin is soluble in water, and slightly so in alcohol. Like the parent substance, saponarin, it readily dissolves in pyridine, and in dilute alkali with a golden-yellow colour. The solubility in boiling alcohol is so small that this is not a satisfactory solvent for recrystallisation. This difficulty can be overcome by acidifying an

alkaline solution in hot alcohol with acetic acid, but better results are obtained by dissolving in pyridine, adding a little water, and extracting repeatedly with light petroleum until crystallisation takes place in the aqueous layer. With ferric chloride, its alcoholic solution gives the same reddish-brown coloration as saponarin itself, but it does not give the blue coloration with iodine. The substance contains no water of crystallisation. For analysis it was dried in a steam oven, and then did not undergo a further loss of weight when dried in a vacuum at 100°.

0·1395 gave 0·3022 CO_2 and 0·0606 H_2O. C = 59·08; H = 4·83.
0·0922 „ 0·1993 CO_2 „ 0·0395 H_2O. C = 58·95; H = 4·75.
$C_{15}H_{14}O_7$ requires C = 58·82; H = 4·57 per cent.

The molecular weight was determined at the ordinary temperature in pyridine solution.

A solution of 0·0634 gram in 1·849 grams pyridine was intermediate between 0·103 and 0·1065 mole., azobenzene as standard.

Hence M = 327; $C_{15}H_{14}O_7$ requires 306.

Acetylvitexin.

As Perkin observed, the acetylation of vitexin proceeds best in the absence of sodium acetate; he therefore boiled the substance with acetic anhydride for six hours. If, however, a few drops of sulphuric acid are added, the change is complete in a few seconds.

The acetyl derivative is white, difficultly soluble in hot alcohol, readily soluble in glacial acetic acid, and crystallises from the latter solvent in stout, microscopic crystals melting at 257—258°.

0·1533 gave 0·3256 CO_2 and 0·0648 H_2O. C = 57·93; H = 4·70.
$C_{15}H_9O_7(C_2H_3O)_5$ requires C = 58·14; H = 4·65 per cent.

The molecular weight was determined in chloroform solution, using azobenzene as standard. 0·3076 gram dissolved in 3·135 grams of chloroform was intermediate between 0·18 and 0·19 mole. Hence M = 516—545, mean 530.

$C_{15}H_9O_7(C_2H_3O)_5$ requires M = 516.

The number of acetyl groups was determined by Perkin's ethyl acetate method:

0·4006 gave 0·2292 acetic acid = 57·2;

and also by weighing the regenerated vitexin (in a Gooch crucible)

0·2040 gave 0·1194 vitexin = 58·5.

Theory for five acetyl groups requires acetic acid = 58·1 and vitexin = 59·3 per cent.

The same acetyl derivative is obtained by the use of acetyl chloride in pyridine solution.

Saponaretin.

This substance is the chief product when saponarin is hydrolysed with dilute acids. Most of it separates from the acid solution on cooling in the form of a dark yellow syrup, and can be freed from accompanying vitexin by alcohol as already described. If the alcoholic solution after separation of the vitexin is evaporated on the water-bath in a vacuum, a viscous syrup is left behind, but if this is redissolved in absolute alcohol and again evaporated, so as to remove all the water, the saponaretin can be obtained as a light yellow powder.

After most of the saponaretin has been deposited as a thick syrup from the acid solution on cooling, further quantities separate out in the course of a few days as a pale yellow solid, which consists of minute, sphaero-crystals embedded in a gelatinous matrix. After filtering it can be purified by redissolving in boiling water and allowing to cool, when it separates again in the same form, without ever assuming a definitely crystalline form. When the moist substance is heated, it melts below 100° and forms a resin. If, however, it is first dried at the ordinary temperature in a vacuum, the jelly shrinks very much and darkens. It can then be heated without melting to above 200°, and it is gradually decomposed at a higher temperature without showing a true melting point. Saponaretin has not yet been obtained pure. From its hot aqueous solution it separates as a syrup, from a cold solution as a jelly. In alcohol it is extremely soluble, and, although crystals are formed when a concentrated solution is allowed to evaporate slowly, these crystals could not be freed from the mother liquor. Saponaretin is insoluble in most other organic solvents, and from mixtures of such solvents with alcohol it always separates as a jelly.

On acetylation, a very soluble acetyl compound is obtained which melts at a low temperature and could not be crystallised, nor could other crystalline derivatives be isolated.

In other respects the substance closely resembles vitexin, for instance, in its behaviour with alkalis and with ferric chloride.

At first it was supposed that the two substances differed by a molecule of water, but saponaretin also seems to have the formula $C_{15}H_{14}O_7$, although, as the substance was amorphous, conclusive proof is wanting. For analysis, the substance was twice allowed to separate in the gelatinous condition from water, and was then dried in air and finally in a vacuum over sulphuric acid, when it underwent a further

considerable loss of weight. As in the case of saponarin, saponaretin is very hygroscopic, so that the boat had to be weighed while enclosed in a stoppered tube.

0·2062 gave 0·4412 CO_2 and 0·0853 H_2O. C = 58·36; H = 4·60.
0·1221 ,, 0·2595 CO_2 ,, 0·0527 H_2O. C = 57·97; H = 4·78.
$C_{15}H_{14}O_7$ requires C = 58·82; H = 4·57 per cent.

When dried at 130—160°, the substance loses a little more water and corresponds with the formula $C_{15}H_{12}O_6,\frac{1}{2}H_2O$, but prolonged heating at this temperature decomposes saponaretin, and in no case did it approximate to the formula $C_{15}H_{12}O_6$.

It may be that saponaretin is identical with Perkin's homovitexin, to which he assigns the formula $C_{16}H_{16}O_7$ or $C_{18}H_{18}O_8$. Both substances differ from vitexin in being readily soluble in boiling alcohol; the composition of saponaretin dried at 160° approximates to that of homovitexin. Both substances yield, on fusion with caustic alkali, phloroglucinol and *p*-hydroxybenzoic acid. Both separate from the vitexin mother liquor on exposure to air as an amorphous product, but homovitexin was finally obtained by Perkin in a crystalline form.

Decomposition by Caustic Alkali.

Caustic potash (7 grams), to which a little water had been added, was melted in a nickel crucible and finely-powdered saponarin (1·6 grams) was gradually dropped in. The temperature was kept between 190° and 200°. After each addition of the glucoside, the fused mass was stirred with a platinum wire. The fused mass, which was dull red, was dissolved in water, acidified with hydrochloric acid, and extracted with ether; the ethereal solution was shaken with sodium carbonate and then evaporated. It left a minute quantity of a substance readily soluble in water, which reddened a pine wood splinter, previously dipped in hydrochloric acid; the presence of phloroglucinol was thus indicated. The sodium carbonate solution on acidification and extraction with ether gave a crystalline acid, which was recrystallised from water containing a little animal charcoal and then melted at 210°. With ferric chloride, no coloration, but a yellow precipitate, was produced. When rapidly heated, phenol was formed, and was detected by its odour and by the tribromophenol test. The substance was evidently *p*-hydroxybenzoic acid.

The quantity available was used for the determination of the water of crystallisation.

0·0574 of the air-dried crystals lost 0·0069 gram when heated to 110°. Hence $H_2O = 12·0$.

$C_7H_6O_3,H_2O$ requires $H_2O = 11·5$ per cent.

A larger quantity of the acid was then prepared in the same way and analysed :

0·1398 gave 0·3102 CO_2 and 0·0539 H_2O. C = 60·51 ; H = 4·28.
$C_7H_6O_3$ requires C = 60·87 ; H = 4·34 per cent.

Although sufficient of the acid was thus obtained, the amount of the other substance (phloroglucinol) was too small for identification, so that experiments were next made with aqueous caustic alkali.

Several grams of saponaretin were boiled for one and a half hours with a solution containing equal parts of caustic potash and water. At the end of that time, the solution no longer became bright yellow on diluting with water. It was slightly acidified, neutralised with sodium bicarbonate, and extracted with ether. The bicarbonate solution was found to contain a little *p*-hydroxybenzoic acid. The ethereal extract left a residue, which gave a very intense crimson coloration with pine wood. This residue was recrystallised from water containing animal charcoal, and, on standing in a vacuum, needles which did not give the pine wood reaction appeared, and finally a few large, stout prisms were obtained, which effloresced, gave an intense crimson colour with pine wood and with vanillin, a bluish-violet colour with ferric chloride, and melted at 214°. Doubtless this substance was phloroglucinol. In an attempt to recrystallise the needles which accompanied the phloroglucinol, they melted in boiling water. On leaving the aqueous solution in a vacuum, stout, glassy prisms melting at 106° were obtained.

The substance was prepared in the pure state by recrystallisation from benzene ; it then melted at 108°. It was *p*-hydroxyacetophenone.

0·1108 gave 0·2860 CO_2 and 0·0575 H_2O. C = 70·40 ; H = 5·77.
$C_8H_8O_2$ requires C = 70·59 ; H = 5·88 per cent.

The substance gave with ferric chloride a brownish-violet coloration. The semicarbazone was prepared and melted at 199° ; the benzoyl derivative melted at 134°.

The *p*-hydroxybenzoic acid produced by the fusion with potash is formed by secondary decomposition of the *p*-hydroxyacetophenone.

Identity of the Vitexin from Saponaria with that from Vitex.

Mr. A. G. Perkin, who was good enough to read this paper in manuscript before the identity of the two substances was suspected, pointed out their close resemblance, and sent the author a small specimen of vitexin, the absolute purity of which he could not guarantee. The probability of the two substances being identical was at once evident from the following comparison of their properties.

Vitexin.	*Substance from Saponarin.*
$C_{21}H_{20}O_{10}$ or $C_{15}H_{14}O_7$, melts at 264—265°.	$C_{15}H_{14}O_7$, melts at 260°.
Prismatic or hair-like needles of a canary-yellow colour.	Pale yellow, glistening plates.
Hepta- or penta-acetyl derivative melts at 251—256°.	Penta-acetyl derivative melts at 252°, after a second crystallisation at 257—258°.

Both substances give in alcoholic solution with ferric chloride the same reddish-brown coloration, dissolve in alkali with a light yellow colour, yield on hydrolysis with caustic potash phloroglucinol and *p*-hydroxyacetophenone, are reduced by sodium amalgam to a brown solution, which turns scarlet on neutralisation, and both dissolve in sulphuric acid in the cold to a pale yellow solution, which becomes dull olive-green on heating.

The chief differences were in the formulæ assigned to the two substances, and in their crystalline form. Perkin had adopted the formula $C_{21}H_{20}O_{10}$, because he obtained from his substance tetranitroapigenin, and regarded vitexin as a very stable glucoside of apigenin ($C_{15}H_{10}O_5$). He did not, however, determine the molecular weight of the substance. This determination was therefore attempted with the minute specimen received from Mr. Perkin, using pyridine, the only solvent in which vitexin is readily soluble. 0·0466 gram in 0·6726 gram pyridine was intermediate between 0·24 and 0·25 mole., using benzil as standard. Hence

$$M = \frac{0{\cdot}0466 \times 1000}{0{\cdot}6726 \times 0{\cdot}245} = 283.$$

$C_{15}H_{14}O_7$ requires 306.
$C_{21}H_{20}O_{10}$,, 432.

The specimen was dark yellow and crystallised in sphaerites, but it was not perfectly pure. In order to recrystallise it, a few drops of water were added to the pyridine solution used in the molecular weight determination.

As in the case of saponarin, the addition of water did not produce a precipitate, but when nearly all the pyridine had been removed by shaking with light petroleum, crystallisation began in the lower (aqueous) layer. The substance now separated in isolated, glistening leaflets which had been found to be so characteristic of the substance obtained from saponarin.

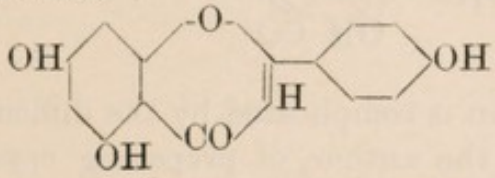
The substance was light yellow and melted at 260° when placed in the bath at 240° and rapidly heated. Vitexin, obtained from

saponarin and purified in the same way, also melted at this temperature, as did a mixture of the two substances in equal proportions. The difference from the melting point, or rather from the temperature of decomposition, given by Perkin (264—265°), is probably not due to an impurity, but to differences in manipulation, and the identity of the two substances is therefore well established.

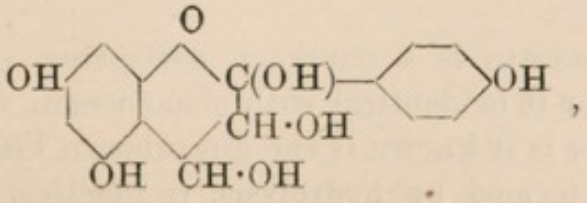
Mr. Perkin very kindly examined the tinctorial properties of the vitexin from both sources and of saponaretin. The three specimens gave similar greenish-yellow shades on woollen cloth, with chromium as mordant, and pale brown shades with iron; all three possessing only slight tinctorial properties.

Constitution of Vitexin.

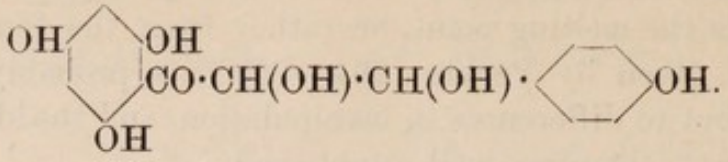
The production of phloroglucinol and *p*-hydroxyacetophenone by the action of caustic alkali, and especially the production of tetranitroapigenin by the action of nitric acid on vitexin, proved that this substance is closely related to apigenin, as Perkin pointed out (Trans., 1898, **73**, 1030), suggesting that it might be apigenin with a side chain attached; later (Trans., 1900, **77**, 422), he regarded this side chain as a sugar, and vitexin as a very stable glucoside of apigenin. The molecular weight determination of vitexin (from both sources) and of acetylvitexin, together with the production of vitexin by hydrolysis from saponarin, prove that vitexin has only the formula $C_{15}H_{14}O_7$, so that there can be no side chain. Vitexin therefore only differs from apigenin by the elements of two molecules of water. That apigenin is 1 : 3 : 4′-trihydroxyflavone,



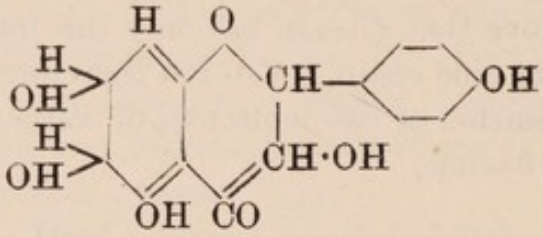
has been established by the researches of Perkin and by the synthesis due to Czajkowski, von Kostanecki, and Tambor (*Ber.*, 1900, **33**, 1988). The two additional hydroxyl groups which vitexin contains are very likely not in either of the two benzene nuclei; for otherwise it is difficult to understand the fission into phloroglucinol and *p*-hydroxybenzoic acid. Hence, these two hydroxyls would appear to be in the pyrone ring or in a chain which can give rise to it (in the formation of tetranitroapigenin). This would lead to a reduced flavanone formula:



or to the corresponding reduced chalkone formula :



Both these formulæ contain six hydroxyl groups, whereas both Perkin's determinations and those of the author show that there are but five acetyl groups in acetylvitexin. As the number of hydroxyl groups is unusually large, it may be that one escapes acetylation. Acetylsaponarin contains nine acetyl groups. Assigning to the glucose part of the molecule the structure of an $\alpha\gamma$-anhydride, this would have four hydroxyl groups; the vitexin part would therefore have five, and, after hydrolysis, six, hydroxyl groups. The formation of *p*-hydroxyacetophenone from an $\alpha\beta$-hydroxypyrone compound of the type figured would probably take place through the elimination of water, yielding the complex $CH_2{:}C(OH)\cdot C_6H_4\cdot OH$, the process being analogous to the formation of lævulic acid from dextrose. Mr. Perkin has, by letter, raised objections to the above constitution for vitexin, and has suggested the presence of a reduced phloroglucinol nucleus, which would lead to a formula of the following type, with five hydroxyl groups :



The study of vitexin is complicated by the difficulty experienced, by Perkin as well as by the author, of preparing crystalline derivatives other than the acetyl compound. Numerous attempts were made to prepare a crystalline azobenzene derivative and an alkyl ether; in the former case, a resinous product was formed, and in the latter the reaction was very slow and incomplete, whether methyl iodide or dimethyl sulphate were used.

Vitexin seems to belong to a new class of colouring matters which are closely allied to the flavone group, and differ from the corresponding flavone derivatives by two molecules of water. Scoparin, which, according to Perkin, is probably methoxyvitexin, would also belong to this group.

Whether saponaretin is a chalkone derivative corresponding to vitexin, or whether it is identical with homovitexin, cannot at present be determined, nor is it known if the glucoside in *Vitex littoralis*, from which vitexin is formed by hydrolysis, is identical with saponarin.

In conclusion, the author gratefully acknowledges his indebtedness to Mr. A. G. Perkin, F.R.S., for a specimen of vitexin, for having carried out some dyeing experiments, and especially for his valuable criticism after reading this paper in manuscript.

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