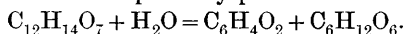


LXXVI.—*Apiin and Apigenin.*

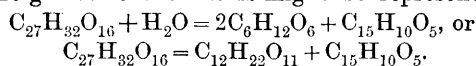
By ARTHUR GEORGE PERKIN, F.R.S.E.

APIIN, a substance which is found in the leaves, stem, and seeds of parsley (*Apium petroselinum*), was so named by Braconnot (*Ann. Chim. Phys.*, 1843, [iii], 9, 250), although its existence was first indicated by Rump (Bechner's *Repert. f. Pharm.*, 1836, 6, 6). Both authors obtained it as a gelatinous mass by extracting parsley seeds with boiling water, and, considering the readiness with which it gelatinised, Braconnot included it among the members of the pectin class. Subsequently, Planta and Wallace (*Annalen*, 1850, 74, 262) obtained apiin as a colourless mass melting at 180° , and to it they assigned the formula $C_{24}H_{28}O_{13}$; by the action of boiling dilute acids, it was converted into a brown substance, $C_{24}H_{20}O_9$, apparently without formation of sugar, although Braconnot had previously considered that this was formed. Lindenhorn (*Inaugural Dissertation*, Würzburg, 1867) was the first who succeeded in obtaining apiin in a crystalline and pure condition, the product yielding on analysis $C=53.57$, $H=5.35$ per cent., numbers which agree closely with those found by later workers; by the action of dilute acids, he found that it was decomposed into glucose, and apigenin $C=66.13$, $H=3.9$ per cent., and considered that the reaction probably proceeded as follows:



In my preliminary notice on this subject in the "Proceedings" (1897), I erroneously assigned the discovery of the true nature of apiin and the isolation of pure apigenin to von Gerichten instead of to Lindenhorn, who has very kindly forwarded me a copy of his dissertation; and I wish to express my regret at this mistake.

The latest publication on this subject is by von Gerichten (*Ber.*, 1876, 9, 1124), who found for apiin and apigenin numbers almost identical with those already obtained by Lindenhorn. By fusion with alkali, apiin yielded phloroglucinol and an acid not closely examined, but which, by the further action of the alkali, gave protocatechuic acid, besides some oxalic, formic, and parahydroxybenzoic acids. Oxidised with chromic acid at the ordinary temperature, formic and carbonic acids were produced, and with nitric acid apiin yielded picric and oxalic acids. From these results, von Gerichten considered the most probable formula of apigenin to be $C_{15}H_{10}O_5$, and that the decomposition of the glucoside with acids might be represented as follows:



It appeared to me possible, judging by the above reactions, that

apigenin was a yellow mordant dye-stuff, although no information on this point has, so far as I am aware, been given by previous workers. Experiment proved this to be the case, and the following investigation was therefore instituted.

EXPERIMENTAL.

The material employed throughout this investigation was a preparation of apiin sold by Merck, of Darmstadt, and was practically pure. In case, however, further purification should be necessary, the following method was devised for this purpose. To water kept continually boiling, the apiin was added so as to form a strong solution, and the yellow liquid very rapidly concentrated until a semi-solid, crystalline mass was obtained; this was collected while hot with the aid of the pump, washed with a little hot water, and finally pressed, these operations being performed with rapidity. From its hot aqueous solution, apiin always separates on cooling as a light yellow, transparent jelly, and its preparation in the crystalline form as above is probably due to a difference of the hydration of the product when thus caused to separate at the boiling heat. Obtained in this way, it forms a colourless mass of fine, silky needles, giving on analysis numbers which agree closely with those previously obtained by Lindenhorn and von Gerichten (*loc. cit.*).

0.1268, dried 100°, gave 0.2470 CO₂ and 0.0595 H₂O. C = 53.13; H = 5.21.

C₂₇H₃₂O₁₆ requires C = 52.94; H = 5.22 per cent.

The object of this portion of the investigation being the study of apigenin rather than that of its glucoside apiin, it was subsequently found that no advantage was gained by this purification of the commercial substance, which was consequently employed in its original condition.

Decomposition of the Glucoside.—The results of previous investigators have shown that apiin, in comparison with most glucosides, is but slowly decomposed by dilute acids, the reaction being completed only after prolonged digestion. To obviate this difficulty, if possible, numerous experiments were carried out with acids of varying concentration, but without advantage, and, ultimately, with slight modifications, von Gerichten's method (*loc. cit.*) was employed. To 3 litres of boiling dilute hydrochloric acid (sp. gr. 1.04), 30 grams of finely powdered apiin were added, and the clear yellow solution was boiled, using a reflux condenser to prevent concentration of the acid. By using this large volume of liquid, the risk of bumping, and probable fracture of the flask, was reduced to a minimum. In a few minutes, a yellow, flocculent product began to separate, which gradually became darker in appearance, and finally of a dirty-brown colour; analyses of this pro-

duct, after purification, were made at various stages of the operation, with the result that 20 hours' digestion was found preferable to the shorter term of 10 hours recommended by von Gerichten. The crude apigenin was then collected, washed with water, dried, extracted with a large volume of boiling alcohol, alcoholic lead acetate being added drop by drop to the light brown extract until the supernatant liquid was almost colourless. After removal of the small quantity of lead precipitate, the filtrate was treated with a few drops of acetic acid, evaporated to a small bulk, and boiling water added, until a faint turbidity was perceptible. On evaporation, fine, yellow needles began to separate, and when no further deposition could be observed, the mixture was cooled, and the product collected, washed, and dried. The yield of pure apigenin thus obtained was only equal to about 40 per cent. of the apiin employed, an amount considerably less than that indicated by theory. This loss is evidently to be accounted for by the production of the dark-brown, amorphous substance above indicated, which appears to be formed principally, if not entirely, from the glucoside, rather than from apigenin itself. The following are analyses of apigenin made at various stages of the operation :

0.1256	gave	0.2962 CO ₂	and	0.0441 H ₂ O.	C = 64.31 ; H = 3.90.
0.1258	„	0.3033 CO ₂	and	0.0455 H ₂ O.	C = 65.75 ; H = 4.01.
0.1241	„	0.3018 CO ₂	and	0.0426 H ₂ O.	C = 66.32 ; H = 3.81.
0.1160	„	0.2822 CO ₂	and	0.0404 H ₂ O.	C = 66.34 ; H = 3.87.
C ₁₅ H ₁₀ O ₅ requires C = 66.66 ; H = 3.70 per cent.					

The above numbers are in close agreement with those of Lindenhorn and von Gerichten (*loc. cit.*), who obtained respectively C = 66.13 ; H = 3.9, and C = 66.21 ; H = 3.75 per cent.

The apigenin thus obtained consists of minute, straw-coloured needles, sparingly soluble in ether or boiling water, somewhat readily in alcohol. Dilute alkalis dissolve it with a pale yellow coloration, and its alcoholic solution yields a yellow precipitate with lead acetate. The greater portion of the apigenin is, however, not so deposited, and this is evidently due to the presence of the acetic acid liberated in the formation of the lead compound first produced. An alcoholic solution of apigenin gives with ferric chloride a brownish-black, and with ferrous sulphate a brown-red, coloration. Treated with mineral acids in the presence of acetic acid, no acid compounds were produced. As stated above, apigenin is a mordant yellow dye-stuff, and its properties in this respect are described at the conclusion of this paper. It was found to contain no methoxyl group. The solubilities of apigenin not being serviceable for the determination of its molecular weight by the usual methods, endeavours were made to obtain derivatives which would yield some insight into this question.

Dibromapigenin.—To finely powdered apigenin, suspended in acetic acid, an amount of bromine slightly in excess of that required for the formation of a dibromo-compound was added drop by drop, rise of temperature being avoided. Although hydrobromic acid was evolved, the product did not appear to pass into solution, and the reaction proceeded as in the bromination of quercetin, luteolin, and allied substances. After standing for 48 hours, the mixture was drained upon a porous tile, washed with a little acetic acid, and crystallised from nitrobenzene.

0.1144 gave 0.1766 CO_2 and 0.0230 H_2O . $\text{C} = 42.09$; $\text{H} = 2.23$.

0.1218 „ 0.1877 CO_2 and 0.0235 H_2O . $\text{C} = 42.02$; $\text{H} = 2.14$.

$\text{C}_{15}\text{H}_8\text{O}_5\text{Br}_2$ requires $\text{C} = 42.05$; $\text{H} = 1.87$ per cent.

Dibromapigenin forms pale yellow needles, which melt above 290° . It is sparingly soluble in boiling nitrobenzene, dissolves in dilute alkalis with a pale yellow colour and possesses dyeing properties, but of a feebler nature than those of apigenin itself.

Dis-azobenzeneapigenin.—The addition of diazobenzene sulphate solution to apigenin dissolved in dilute sodium carbonate solution caused the separation of a red precipitate, which after some hours was collected, washed, and dried. To purify it, it was first extracted with boiling alcohol to remove a resinous impurity, and subsequently with nitrobenzene diluted with a little acetic acid, until the residue was of an orange colour. Finally, it was crystallised from nitrobenzene.

0.1581 gave 16.2 c.c. nitrogen at 13° and 726 mm. $\text{N} = 11.56$.

0.1300 „ 13.1 c.c. „ „ 17° and 751 mm. $\text{N} = 11.54$.

0.1262 „ 12.7 c.c. „ „ 14° and 758 mm. $\text{N} = 11.75$.

0.1218 „ 0.3029 CO_2 and 0.0457 H_2O . $\text{C} = 67.82$; $\text{H} = 4.16$.

$\text{C}_{15}\text{H}_3\text{O}_5(\text{C}_6\text{H}_5\text{N}_2)_2$ requires $\text{C} = 67.78$; $\text{H} = 3.76$; $\text{N} = 11.71$ per cent.

Thus obtained, it formed an orange-coloured mass of fine needles, melting at 290 — 292° , almost insoluble in the usual solvents, and but sparingly in nitrobenzene. If dissolved in boiling acetic acid with the aid of a few drops of sulphuric acid and the hot solution cautiously treated with boiling water, it is obtained as a glistening mass of red needles having a slightly metallic lustre, thus resembling maclurin-azobenzene, which can be crystallised in a similar manner (this vol., p. 186).

The further study of this substance is being carried on in conjunction with a diazobenzene derivative of chrysin, the colouring matter of poplar buds which I have lately obtained.

These bromine and diazobenzene derivatives of apigenin indicate that the formula $\text{C}_{15}\text{H}_{10}\text{O}_5$ correctly represents the molecular weight of apigenin. Certainly it cannot be $\text{C}_{17}\text{H}_{12}\text{O}_6$, the alternative formula

suggested by von Gerichten, and on which, in fact, he himself placed but little value.

Tribenzoylapigenin.—As experiments on the acetylation of apigenin in the ordinary way yielded, as previously noticed by von Gerichten, a viscous product difficult to purify, attempts were made to prepare a benzoyl compound. For this purpose, the Baumann and Schotten method was employed, a 10 per cent. solution of potassium hydroxide being found the most serviceable. The colourless, viscous product, after being washed with dilute alkali and spread in a thin layer upon a porous tile to harden, was extracted with ether and crystallised from benzene.

0.1125 gave 0.3070 CO_2 and 0.0423 H_2O . $\text{C} = 74.41$; $\text{H} = 4.17$.

$\text{C}_{15}\text{H}_7\text{O}_5(\text{C}_7\text{H}_5\text{O})_3$ requires $\text{C} = 74.23$; $\text{H} = 3.78$ per cent.

Tribenzoylapigenin crystallises in colourless, silky needles melting at $210\text{--}212^\circ$, readily soluble in hot benzene, sparingly so in alcohol. Apigenin thus contains three hydroxyl groups.

Decomposition Products of Apigenin.—As stated in the introduction, von Gerichten has previously studied the action of strong alkali on apiin, with the results there given; unfortunately, he gives no details in his paper as to the temperature, time, or concentration of the alkali employed.

As a preliminary experiment, air was aspirated through an alkaline solution of the colouring matter, this method having given useful results in the hands of Herzig and others, but apigenin is so stable that even after 48 hours little, if any, of it was attacked. By digestion with a boiling solution of caustic potash in its own weight of water, a more satisfactory result was obtained. The yellow liquid gradually became brown, and decomposition was complete in three-quarters of an hour, a sample giving no precipitate of apigenin on the addition of an acid. To the main bulk, a slight excess of acid was now added, and the opaque liquid, after neutralisation with sodium hydrogen carbonate, was thoroughly extracted with ether (extract A), the aqueous liquid being set aside for subsequent examination. The ethereal solution, on evaporation, left a brownish oil, which on standing for some hours deposited crystals; these, after purification, were obtained as colourless needles melting at 210° .

0.1178 gave 0.2446 CO_2 and 0.0493 H_2O . $\text{C} = 56.62$; $\text{H} = 4.64$.

$\text{C}_6\text{H}_6\text{O}_3$ requires $\text{C} = 57.14$; $\text{H} = 4.76$ per cent.

Its aqueous solution gave a violet coloration with ferric chloride. From the above results, it evidently consisted of *phloroglucinol*.

The oily matter above referred to, which had been drained from the crystals of phloroglucinol, still contained a small quantity of this substance, which was removed as far as possible by dissolving it in hot water, cooling, separating the deposited crystals from the accompanying

oil, and repeating the process with the latter. Eventually, however, these solutions, from which some oily drops had separated at once on cooling, deposited on standing overnight long, prismatic needles melting in their impure condition slightly below 100° . These were purified by recrystallisation from water, care being taken that the solution was not too concentrated, otherwise the product separated in an oily condition. The purification, moreover, was facilitated by previously digesting the hot solution with a trace of barium carbonate, from which it appeared that a small quantity of some acid impurity hindered the ready crystallisation of this substance. It was finally purified by crystallisation from benzene, and could in this way be freed from adhering traces of phloroglucinol, as the latter is but little soluble in this liquid. Thus obtained, it formed colourless, prismatic needles melting at 107° , and giving a faint violet coloration with ferric chloride in aqueous solution.

0.1203 gave 0.3097 CO_2 and 0.0655 H_2O . $\text{C} = 70.10$; $\text{H} = 6.04$.

$\text{C}_6\text{H}_4(\text{OH}) \cdot \text{CO} \cdot \text{CH}_3$ requires $\text{C} = 70.58$; $\text{H} = 5.88$ per cent.

The analytical numbers and the melting point of the substance pointed to its identity with parahydroxyacetophenone (Klingel, *Ber.*, 1885, 18, 2691), but this is said to give a brown coloration with aqueous, ferric chloride. Some parahydroxyacetophenone was therefore prepared by Klingel's method, and was found to be in every way identical with the above decomposition product of apigenin melting at 107° . This was confirmed by preparing the semicarbazide derivatives of the two ; they were found to be identical, both crystallising from water in colourless needles melting at 197 – 198° . The statement of Klingel, that this substance yields a brown coloration with ferric chloride, is evidently incorrect.

A trace of the parahydroxyacetophenone from apigenin being still available for experiment, it was fused with alkali at 190 – 210° for 15 minutes, and from the melt parahydroxybenzoic acid melting at 209 – 210° was obtained.

The benzene mother liquors obtained during the purification of the above substance contained a trace of a more readily soluble product. Owing to the difficulty in obtaining any quantity, however, it was impossible to identify it, but further experiments will be carried out in this direction.

The aqueous liquid from which the ethereal extract A had been obtained was neutralised with acid and again extracted with ether. On evaporation, a small quantity of a crystalline residue was left, which, on purification, was obtained in almost colourless needles melting at 209 – 210° ; these gave a brown coloration with ferric chloride, and consisted of *parahydroxybenzoic acid*.

In purifying the hydroxybenzoic acid just mentioned, a trace of a

dark-coloured precipitate was obtained on adding lead acetate to the aqueous solution of the crude substance ; on decomposing this with sulphuric acid, a brown, viscid product was obtained, but the quantity was too small to admit of purification. Ferric chloride solution gave with this a dark coloration having a perceptible greenish tint, which suggested the presence of protocatechuic acid.

These results indicate that by the gentle action of alkali, apigenin yields phloroglucinol and parahydroxyacetophenone, for these were the main products thus formed ; the parahydroxybenzoic acid found, most probably was produced by the decomposition of the latter.

Action of Fused Alkalis at 200°.—Von Gerichten has stated in his paper (*loc. cit.*) that, besides phloroglucinol, apiin yields, on decomposition, an acid, which, by further action of the alkali, is transformed into protocatechuic, parahydroxybenzoic, formic, and oxalic acids. No such acid appears to be formed from apigenin by the above treatment, and, as before stated, owing to absence of detail, a repetition of his experiments was impossible, it was deemed necessary to study the action of alkali at a higher temperature. This was carried out at 180° to 200° for one hour, and the resulting acid A and phenolic substances B were separated by the method previously employed. The portion B consisted almost entirely of phloroglucinol, although the presence of a trace of hydroxyacetophenone could be detected.

A hot solution of the acid product (A) when treated with lead acetate gave a light-coloured precipitate, which was collected, washed with boiling water, and decomposed with sulphuric acid. From this, in the usual way, a crystalline product was obtained which melted at 195—196°, gave with ferric chloride in aqueous solution a green coloration, and was evidently *protocatechuic acid*. The filtrate from the above lead compound contained *parahydroxybenzoic acid*.

The formation of protocatechuic acid in this manner was somewhat puzzling, for although von Gerichten's acid was not found in the previous experiments, yet this, mentioned by him as its principal decomposition product, appeared as a result of the action of the alkali at a higher temperature. Von Gerichten did not detect the formation of parahydroxyacetophenone, and it appeared just possible that by the prolonged action of alkali this would be oxidised to a dihydroxyacetophenone and subsequently converted into protocatechuic acid. Experiments in this direction gave nothing but parahydroxybenzoic acid with traces of phenol, and all attempts with the object of converting the acid itself into protocatechuic acid were also unsuccessful. This point is further discussed at the conclusion of this paper.

Methylation of Apigenin.

Apigenin dissolved in a solution of its own weight of potassium hydroxide in methylic alcohol was boiled with excess of methylic iodide for 36 hours. After removal of unattacked methylic iodide and the greater portion of the alcohol by distillation, the residue was treated with water, extracted with ether, and the ethereal solution washed with dilute alkali to remove any partially methylated substance. On evaporation, a pale yellow crystalline product was left, which was purified by crystallisation first from alcohol and then from acetone until the melting point was constant.

0.1198 gave 0.2996 CO_2 and 0.0550 H_2O . $\text{C} = 68.20$; $\text{H} = 5.09$.

$\text{C}_{15}\text{H}_8\text{O}_3(\text{OCH}_3)_2$ requires $\text{C} = 68.45$; $\text{H} = 4.70$ per cent.

It formed a glistening mass of pale yellow needles melting at $171-172^\circ$, sparingly soluble in alcohol, more readily in acetone, insoluble in aqueous alkalis. The methoxy-groups present were estimated by Zeisel's method.

0.1937 gave 0.3180 AgI . $\text{CH}_3 = 10.48$.

$\text{C}_{15}\text{H}_8\text{O}_3(\text{OCH}_3)_2$ requires $\text{CH}_3 = 10.07$ per cent.

It having been shown by means of the tribenzoyl compound that apigenin contains three hydroxyl groups, whereas only a dimethyl ether could be obtained, it appeared evident that the latter contains an hydroxyl group which resists methylation. To make sure of this, a mixture of apigenin dimethyl ether (1 part), anhydrous sodium acetate (1 part), and acetic anhydride (6 parts), after being boiled for an hour, was diluted with acetic acid, poured into water, and the whole left for 12 hours; the colourless, semicrystalline precipitate was then collected, dried, and crystallised, first from alcohol and then from acetone.

0.1202 gave 0.2952 CO_2 and 0.0536 H_2O . $\text{C} = 66.97$; $\text{H} = 4.86$.

$\text{C}_{15}\text{H}_7\text{O}_3(\text{OCH}_3)_2 \cdot \text{C}_2\text{H}_5\text{O}$ requires $\text{C} = 67.05$; $\text{H} = 4.71$ per cent.

Monacetylapiogenin dimethyl ether crystallises in colourless needles melting at $195-196^\circ$, and is somewhat readily soluble in acetone.

A determination of the acetyl group by Liebermann's method gave the following result :

0.5164 $\text{C}_{15}\text{H}_7\text{O}_3(\text{OCH}_3)_2\text{C}_2\text{H}_5\text{O}$ gave 0.4542 $\text{C}_{15}\text{H}_8\text{O}_3(\text{OCH}_3)_2$.

Found : 87.95 per cent. Theory requires 87.64 per cent.

The regenerated dimethyl ether melted at $171-172^\circ$.

As referred to in previous papers, Kostanecki and others have found that it is not possible to methylate by any known method the hydroxyl

group occupying the ortho-position relatively to the carbonyl group present in members of the xanthone, flavone, alizarin, and other groups; he has, moreover, shown that such ethers, when treated with alcoholic potash in alcoholic solution, yield yellow potassium salts which are decomposed by water into potassium hydroxide and the free ether. Apigenin dimethyl ether behaved in a similar manner; on adding alcoholic potash to the boiling alcoholic solution, yellow needles of the potassium salt were deposited, which were decomposed by water, the ether being regenerated.

It thus appears that apigenin contains an hydroxyl in the ortho-position relatively to the carbonyl group. The mother liquors obtained during the purification of the above dimethyl ether yielded on evaporation yellow needles of the same substance, but in an impure condition, being accompanied by a trace of a second product which depressed its melting point considerably. Experiments will be made to determine the nature of this impurity.

Decomposition of Apigenin Dimethyl Ether with Alcoholic Potash.

In view of the somewhat peculiar behaviour of apigenin when treated with fused alkali, it was of special interest to determine the behaviour of the dimethyl ether towards alcoholic potash, as among the products of this more gentle action one would expect to find an acid containing the nucleus originally present in the molecule; that is, an ether of protocatechuic, parahydroxybenzoic, or of von Gerichten's acid. It will be remembered that Herzig (*Ber.*, 1895, 28) determined the constitution of fisetin in this way.

Apigenin dimethyl ether was heated with dilute alcoholic potash at 160—170° for an hour, as little or no decomposition ensued at 100°. The pale brown solution thus obtained, which on cooling deposited glistening scales of a potassium salt, was evaporated to dryness, dissolved in water, and extracted with ether (A), the aqueous solution being then saturated with carbonic anhydride, and again extracted with ether (B). The residual liquid, on acidification, deposited a small quantity of a crystalline product which, after being purified first by crystallisation from water and then from benzene, was obtained in colourless needles melting at 179—180°, a point intermediate between that of protocatechuic acid dimethyl ether 179·5° and that assigned to anisic acid (184·2 corr.).

0·0748 gave 0·1744 CO₂ and 0·0360 H₂O. C = 63·58; H = 5·34.

C₆H₄(OCH₃)·COOH requires C = 63·15; H = 5·26 per cent.

C₆H₃(OCH₃)₂·COOH „ C = 59·01; H = 6·01 „

This substance could not therefore be veratric acid, but was apparently anisic acid.

To decide this point, a portion was treated with fused alkali at 200° for half an hour, the product dissolved in water, acidified, and extracted with ether. The crystalline product thus obtained, after recrystallisation from water, melted at $209-210^{\circ}$, and had all the properties of parahydroxybenzoic acid. The acid formed by the decomposition of apigenin dimethyl ether with alcoholic potash is thus without doubt *anisic acid*.

Whether the melting point assigned to anisic acid by Oppenheim and Pfaff (*Ber.*, 8, 893) is too high, or that found above was due to slight impurity, could not be decided with certainty, as no opportunity was found for its preparation from parahydroxybenzoic acid. It is however, significant that the melting points of two distinct preparations from the apigenin ether were identical, and a sample of the acid obtained from Merck gave the same result.

The extract (A), on evaporation, yielded a trace of oily matter, having the characteristic persistent odour of *anisic aldehyde*, showing that in this decomposition the formation of the acid is preceded by that of the aldehyde. In the following paper on "Rhamnazin," a quercetin dimethyl ether, which gives in a similar way vanillic acid, the formation of vanillin has also been noticed, and it is, therefore, possible that such a reaction is general with this class of substance.

From extract B, a small quantity of a viscous residue was obtained apparently not susceptible of ready purification. An aqueous extract gave the *phloroglucinol* reaction, and in general characteristics reminded one of a product obtained in the same way by the decomposition of luteolin with alcoholic potash (*Trans.*, 1896, 69, 802).

Ethylation of Apigenin.

This reaction was carried out by a similar process to the methylation above described, and the portion of the product insoluble in alkali was purified by crystallisation, first from alcohol and subsequently from acetone, until the melting point was constant. When alcohol alone was used, considerable difficulty was experienced in the purification, suggesting the presence of a substance of lower melting point. Possibly this was due to the employment of ordinary absolute alcohol for the ethylation, although such was not formerly found to be the case. The nature of this impurity will be investigated. Analysis of the ethyl ether gave the following result:

0.1208 gave 0.3085 CO_2 and 0.0625 H_2O . C = 69.65; H = 5.74.

0.1128 „ 0.2890 CO_2 and 0.0587 H_2O . C = 69.87; H = 5.78.

$\text{C}_{15}\text{H}_8\text{O}_3(\text{OC}_2\text{H}_5)_2$ requires C = 69.94; H = 5.52 per cent.

A determination of the ethoxyl groups gave the following result:

0.2188 gave 0.2900 AgI. $C_2H_5 = 16.35$ per cent.

$C_{15}H_8O_3(OC_2H_5)_2$ requires $C_2H_5 = 17.79$ per cent.

This result, although clearly indicating the presence of two groups, is somewhat low, and is due to the difficulty experienced in completely decomposing the ether, for although in this instance the operation was maintained fully an hour longer than usual, and a higher temperature employed, yet, as seen below, a trace of the ether was found to be unattacked.

As shown at the commencement of this paper, the analyses of apigenin, as carried out by myself and others, gave numbers invariably slightly lower than those required by theory ; as this is possibly due to their still containing a trace of undecomposed apiin, it was considered desirable to examine apigenin obtained by the decomposition of one of its ethers, which should readily be obtained of chemical purity. The hydriodic acid mother liquors from the above determination were therefore poured into water containing sodium hydrogen sulphite solution, and the yellow, flocculent deposit collected, washed, and dried. To remove any unaltered diethyl ether, it was extracted with boiling benzene, the ether being readily soluble in this solvent, even in the cold ; as indicated above, a trace was found to be present. The residual apigenin, after further purifying by crystallisation from dilute alcohol, was analysed.

0.1119 gave 0.2734 CO_2 and 0.0432 H_2O . $C = 66.63$; $H = 4.28$.

$C_{15}H_{10}O_5$ requires $C = 66.66$; $H = 3.70$ per cent.

Apigenindiethyl ether crystallises in yellow, glistening needles melting at $161-162^\circ$, sparingly soluble in alcohol, readily in benzene. In its properties, it closely resembles the corresponding methyl ether, for although insoluble in aqueous alkalis, it yields, with alcoholic potash, a yellow potassium salt, which is decomposed by water into potassium hydroxide and the unchanged ether.

On acetylation, a colourless product was formed, which, after purification by crystallisation from acetone, was obtained as colourless needles melting at $181-182^\circ$.

0.1128 gave 0.2836 CO_2 and 0.0597 H_2O . $C = 68.56$; $H = 5.88$.

$C_{15}H_7O_3(OC_2H_5)_2 \cdot C_2H_5O$ requires $C = 68.48$; $H = 5.43$ per cent.

It was thus evidently *monacetyl apigenin diethyl ether*.

Decomposition with Alcoholic Potash.—This was carried out in a similar manner to the decomposition of the methyl ether described above. The solution was evaporated to dryness, dissolved in water, saturated with carbonic anhydride, and extracted with ether (A). The aqueous liquid, on acidification, deposited a crystalline precipitate, which, on being purified by recrystallisation from water, was obtained

in colourless needles melting at 192° when rapidly heated. This is the ethyl ether of parahydroxybenzoic acid, as on fusion with alkali at 200° the product gave the reactions of the hydroxy-acid itself.

On evaporating the extract A, a pale brown, somewhat viscid residue was left, closely resembling that produced from the methyl ether, an aqueous extract giving the phloroglucinol reaction. As a small quantity only was available, its purification was not attempted.

Action of Nitric Acid on Apigenin.

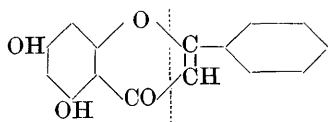
From apiin, by means of nitric acid, von Gerichten (*loc. cit.*) obtained picric and oxalic acids, but did not study the behaviour of apigenin in this respect. This substance is not violently attacked when added to nitric acid (sp. gr. 1.54), but dissolves, forming a brown solution. If such a liquid saturated with apigenin be very gently warmed, and the source of heat removed as soon as action has set in, a small quantity of a crystalline product separates. This was collected upon a porous tile, purified by digestion with nitric acid (sp. gr. 1.42), again drained upon a porous tile, and, finally, crystallised from toluene. It formed glistening, yellow needles, which, when heated, became opaque at about 230° , and melted at $244\text{--}245^{\circ}$. The yield of this substance being exceedingly small (equal to about 1 per cent. of the apigenin employed), it has not been at present possible to submit it to analysis. If it be a derivative of parahydroxybenzoic acid, as appears possible, it most closely resembles metadinitroparahydroxybenzoic acid, although, according to Salkowski (*Annalen*, 1872, 163, 36), the melting point of this acid is $235\text{--}237^{\circ}$. This product will be further investigated.

Theoretical Considerations.

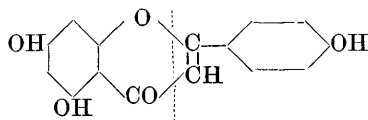
It is shown in this paper that apigenin, $C_{15}H_{10}O_5$, reacts with 2 molecules of diazobenzene, and yields dibromo- and tribenzoyl-derivatives. Although containing three hydroxyl groups, two only are capable of etherification, the dimethyl and diethyl ethers, when treated with acetic anhydride, forming monacetyl compounds. It thus appears that in apigenin one hydroxyl is in the ortho-position relatively to the carbonyl group. The products of the decomposition of apigenin with alkali at a low temperature consist principally of parahydroxyacetophenone and phloroglucinol, whereas, by a more energetic action, protocatechuic acid, parahydroxybenzoic acid, and phloroglucinol are obtained. From the dimethyl ether, by the action of alcoholic potash, anisic aldehyde, anisic acid, and a phloroglucinol derivative were obtained, the diethyl ether behaving similarly with production of the ethyl ether of parahydroxybenzoic acid.

Apigenin, therefore, in its principal reactions, bears a striking

resemblance to chrysin, $C_{15}H_{10}O_4$, the yellow colouring matter of poplar buds. This contains two hydroxyl groups, one only of which is capable of etherification, and yields, by decomposition with alkali, acetophenone, benzoic acid, and phloroglucinol. On account of the production of acetophenone from this substance, Kostanecki (*Ber.*, 1893, 26, 2901) assigned to it the constitution of a dihydroxyflavone or phenylated pheno- γ -pyrone.



Such a compound would yield acetophenone and phloroglucinol-carboxylic acid, or, under the circumstances of the decomposition, phloroglucinol and carbonic anhydride. It would thus appear that *apigenin* is very probably a *hydroxychrysin*,



its decomposition into phloroglucinol and parahydroxyacetophenone being then understood.

A point at first sight not in harmony with this constitution is the production of protocathechuic acid from apigenin by means of alkali at 200° . Several facts, however, indicate that this colouring matter does not contain a catechol nucleus, one of these being the absence of this acid from the decomposition products formed by a similar means at a low temperature. Most important, however, is the formation from the ethers of apigenin of derivatives of parahydroxybenzoic acid only, no compounds of protocathechuic acid being here obtained. Taking into consideration the fact that neither parahydroxyacetophenone nor parahydroxybenzoic acid yield catechol derivatives with fused alkali, it appears very possible that, during the action, the phenol nucleus in a portion of the apigenin itself is oxidised to catechol, and that the substance so produced being incapable of existence at the temperature of the melt, is at once decomposed with formation of protocathechuic acid and phloroglucinol. In connection with this portion of the investigation, an interesting point suggests itself; that is, the possibility of introducing a second hydroxyl into apigenin, which, if successful, should yield results of much value. Work in this direction I desire to reserve to myself, as also the further study of the decomposition products and derivatives of apigenin described in this paper.

Dyeing Properties.

In studying the dyeing properties of apigenin, woollen cloth, previously mordanted with aluminium, iron, chromium and tin, was employed. The probable constitution of this substance indicated above made it interesting to carry out, at the same time, comparative trials with both chrysin and quercetin. The results are embodied in the following table :

	Aluminium.	Chromium.	Iron.	Tin.
Apigenin.	Pure yellow.	Yellow, having faint orange tint.	Chocolate brown.	Undyed.
Chrysin.	The same, slightly weaker.	Ditto, slightly weaker.	Pale chocolate.	Undyed.
Quercetin.	Brown orange inclining to yellow.	Red-brown.	Green-black.	Bright yellow.

It was at once evident that the shades yielded by apigenin bore a striking resemblance to those given by chrysin, and thus a further connecting link between these colouring matters must be added to those I have attempted to establish above. The slight difference observable, other than that of strength, as apigenin is evidently a slightly stronger colouring matter than chrysin, is in the browner tint given by the iron mordant. That both chrysin and apigenin are somewhat feeble dyes is evidently due to the non-existence in them of two hydroxyls in the ortho-position relatively to one another, for quercetin which contains two such groups is characterised by the much greater strength and brilliancy of shade which it yields with the same mordants.

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