

XV.—*A Chemical Investigation of the Constituents of Indian and American Podophyllum (Podophyllum emodi and Podophyllum peltatum).*

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Podophyllum emodi is a small, herbaceous plant which grows abundantly in Northern India; it is figured and briefly described in Royle's *Illustrated Botany of the Himalayas*. The root, or, more strictly, the rhizome, has long been used in Indian medicinal practice for the same purpose as that for which the allied American plant, *Podophyllum peltatum*, is employed in Europe and America. It acts as a powerful purgative, and, in particular, beneficially affects the liver. It has been frequently suggested that the constituents of both rhizomes are very similar, but they have never been completely examined (Watt's *Dictionary of the Economic Products of India*, 6, Part I.; Dymock, *Imperial Institute Handbooks of Commercial Products*, No. 3; Umney, *Pharm. Journal*, [iii], 12, 217; Thompson, *Amer. Journ. Pharm.*, 1891).

The chemical constituents of the rhizome of the American *Podophyllum peltatum* have, however, been made the subject of several investigations. First, in 1832, Hodgson prepared the mixture of resins known and largely used in medicine as "podophyllin," by precipitating a concentrated alcoholic extract of the rhizome with water; this is now manufactured on a large scale, and is the form in which 'podophyllum' is usually administered as a drug. The first important contribution to the chemistry of podophyllum was that made by Podwyssotski (*Pharm. Journ.*, [iii], 12, 217, 1011); this chemist showed that the rhizome did not, as was previously supposed, contain the alkaloid berberine; he isolated from it three substances which he named *podophyllotoxin*, *podophyllic acid*, and *podophylloquercetin* respectively. To the first of these he attributed the characteristic purgative action of the drug, but the others he believed to be physiologically inert; the first two he did not succeed in obtaining in a crystalline condition, the third was a crystalline, yellow colouring matter.

He further showed that when podophyllotoxin is acted on by alkaline solutions, it is decomposed, furnishing two new substances, one crystallising in long needles with a silky lustre, which he named *picropodophyllin*, and the other a gelatinous substance having the characters of an acid, which he called *picropodophyllic acid*.

Kursten (*Arch. Pharm.*, 1891, 229, 220—248), who considerably

amplified the work of Podwyssotski, succeeded in crystallising podophyllotoxin, and ascribed to it the composition represented by the formula $C_{23}H_{24}O_9, 2H_2O$. He showed also that by the action of alkalis it is converted into an isomeride picropodophyllin, and concluded that the picropodophyllin acid of Podwyssotski simultaneously produced is an oxidation product which can be prepared by the action of permanganate in alkaline solution. He expressed its composition by the formula $C_{20}H_{24}O_9$ and called it podophyllin acid. He obtained this acid crystalline, and ascertained its molecular weight through the analysis of its copper salt; he also determined that both picropodophyllin and podophyllotoxin have the same composition, and contain three methoxyl groups. The composition of Podwyssotski's podophylloquercetin he represented by the formula $C_{23}H_{16}O_{10}$, and prepared crystalline hexacetyl and hexabenzoyl derivatives from it; from an examination of these derivatives, and of the methyl ether of the colouring matter, he came to the conclusion that podophylloquercetin and quercetin (from quercitron bark) are not identical.

Very little has so far been done in the chemistry of *Podophyllum emodi*. The amount of resin in the rhizome has been estimated by Dymock and Hooper (*Pharmacographia Indica*), and also by J. C. Umney (*Pharm. Journ.*, [iii], 23, 207), who showed that it contains Podwyssotski's amorphous podophyllotoxin, but in smaller quantity than the American rhizome.

The objects of the present investigation were to decide whether or not the constituents of the American and Indian plants are identical, to determine their constitutions as far as possible, and to ascertain whether the rhizome of the latter plant could be used as a source of the medicinal resin 'podophyllin.' In addition to the chemical examination, physiological and therapeutical investigations of the action of the several constituents have been instituted, and the results of these will be separately published.

Briefly stated, the principal results we have obtained are as follows. We have proved that the constituents of *P. emodi* are identical with those of *P. peltatum*. Crystalline podophyllotoxin is shown to have the composition represented by the formula $C_{15}H_{14}O_6, 2H_2O$; when acted on by aqueous alkalis it is converted into the isomeric picropodophyllin; the gelatinous acid first produced in this reaction is the acid corresponding to the lactone picropodophyllin, and not an oxidation product, as Kursten supposed. It is also shown that both podophyllotoxin and picropodophyllin contain two methoxyl groups, that both furnish monobromo-derivatives, and, when fused with potash, yield orcinol and acetic acid, also that both furnish dimethylnaphthalene when distilled with zinc dust. The formula $C_{15}H_{14}O_6$ is assigned to podophyllotoxin and picropodophyllin, and $C_{15}H_{16}O_7$ to

podophyllic acid, and structural formulæ are suggested for these substances, exhibiting them as derivatives of a substituted phenylated hydro- γ -pyrone. The yellow colouring matter has been completely purified, analysed, and shown to have the composition represented by the formula $C_{15}H_{10}O_7$; it yields a pentacetyl derivative melting at 192° , a tetramethyl ether (m. p. 156°), and a compound with sulphuric acid corresponding exactly with the quercetin compound. When fused with potash, it yields phloroglucinol and protocatechuic acid. From this experimental evidence it is concluded that the colouring matter is identical with the quercetin of quercitron bark, and that, therefore, the use of the name podophylloquercetin is unnecessary.

The removal of podophyllotoxin and podophylloquercetin from 'podophyllin' left a dark coloured, resinous powder, which was still physiologically active; attempts were therefore made to isolate this active substance from the residue. By treating it with absolute alcohol, followed by fractional precipitation of the solution with water, a viscid, brown resin was obtained, which proved to be active as a purgative; all attempts to crystallise this viscid resin were unsuccessful. In the purest form in which we have obtained it, it is a transparent, reddish-brown substance, softening and becoming semi-liquid a few degrees above the atmospheric temperature. It yields a crystalline acetyl derivative, and from this we have ascertained indirectly that the podophyllo-resin probably has the formula $C_{12}H_{12}O_4$.

We are indebted to Dr. H. W. G. Mackenzie, assistant physician to St. Thomas's Hospital, and Mr. W. Dixon, M.B., Salters' Research Fellow in Pharmacology at St. Thomas's Hospital, for having examined the therapeutic effects and the physiological action of the constituents we have obtained from *Podophyllum emodi*. An account of their work will be published in a separate form, and only the general conclusions will be indicated here. Therapeutic trial has proved that the podophyllin prepared from *Podophyllum emodi* is as valuable a purgative as the podophyllin obtained from *P. peltatum*. The action of this resinous mixture is due partly to the podophyllotoxin it contains, and partly to the active 'podophyllo-resin' mentioned above. Owing to its intensely irritating action internally, even when given in small doses, podophyllotoxin is unsuitable as a medicinal substitute for podophyllin, whilst podophyllo-resin would seem to present no therapeutic advantage as compared with the podophyllin now employed. Picro-podophyllin, picropodophyllic acid, and the quercetin are very slightly, if at all, active as purgatives.

Since *P. emodi* furnishes more podophyllin than *P. peltatum*, the Indian plant is of greater value as the source of this resin. Appended to this paper are results of determinations of the amount of resin contained in the rhizomes of plants collected in different districts of the

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Punjab under the supervision of Dr. George Watt, C.I.E., Reporter on Economic Products to the Government of India.

Podophyllotoxin.

Podwyssotski had shown that podophyllotoxin is precipitated when a chloroform extract of the rhizome of *P. peltatum* is added to a large excess of light petroleum, and Kursten had found that by fractionally precipitating a chloroform solution of this crude podophyllotoxin a crystalline substance (podophyllotoxin) could be obtained. The method we finally adopted, after many trials, consisted in preparing the mixture of resins (podophyllin) from the rhizome of *P. emodi* and then percolating this, in a Soxhlet extractor, with chloroform; the chloroform was distilled from the percolate, the dark brown extract boiled with benzene, and the hot benzene solution filtered into a cold flask and allowed to cool somewhat; by this means, a good deal of resin was easily removed. The solution was then poured from the deposited resin, boiled for some time with animal charcoal, filtered, and set aside for several days, when almost colourless crystals of podophyllotoxin were obtained. These were purified by recrystallisation either from a mixture of chloroform and petroleum, or from alcohol and water.

The pure substance, which forms colourless needles melting at 117° , is easily soluble in alcohol, acetone, chloroform, and hot benzene, but only slightly in water. Its taste is very bitter. Analysis of a carefully purified specimen, which had been dried at 110° until no further loss of water occurred, gave the following results.

0.1121 gave 0.2534 CO_2 and 0.514 H_2O . C = 61.65; H = 5.09.

0.142 ,, 0.3246 CO_2 ,, 0.611 H_2O . C = 62.24; H = 4.78.

0.1328 ,, 0.3025 CO_2 ,, 0.0667 H_2O . C = 62.13; H = 5.57.

Mean C = 62.00; H = 5.11. $\text{C}_{15}\text{H}_{14}\text{O}_6$ requires C = 62.06; H = 4.82 p. c.

We therefore adopt this formula as simpler than that suggested by Kursten, $\text{C}_{23}\text{H}_{24}\text{O}_9 \cdot 2\text{H}_2\text{O}$.

Specific Rotation of Podophyllotoxin.—For the determination of this constant, an alcoholic solution containing 2.417 grams in 100 c.c. of absolute alcohol was used in a 2-decimetre tube with Laurent's half-shade polarimeter. The compound is strongly laevorotatory.

The mean of ten readings was $-4^{\circ} 35'$.

Hence the specific rotation $[\alpha]_D$ is $\frac{100 \times 4^{\circ} 35'}{2 \times 2.417} = -94^{\circ} 48'$.

Water of Crystallisation.—When podophyllotoxin is crystallised from a mixture of alcohol and water, it separates as a hydrate. A determination of the water gave the following result.

0.2988 dried at 100° for 2 hours lost 0.0302; further dried at 110° until the weight was constant, it lost 0.0013; no further loss occurred at 120° . 0.2988 therefore contains $0.0315 = 10.5$ per cent. $C_{15}H_{14}O_6 \cdot 2H_2O$ requires 11.2 per cent. of water of crystallisation.

Anhydrous Podophyllotoxin.—The dried podophyllotoxin obtained in the previous experiment was dissolved in absolute alcohol, and the solvent then removed by exposure in a vacuous desiccator. As the solution became concentrated, crystals of anhydrous podophyllotoxin melting at 157° were obtained; these pass into the ordinary hydrated substance when recrystallised from alcohol by adding water. It was afterwards found that a very convenient method of obtaining anhydrous podophyllotoxin consists in heating the hydrated substance at its melting point (117°) for a few minutes, dissolving the product in dry chloroform, and adding dry light petroleum until the mixture is slightly turbid; after standing a few hours, the anhydrous substance crystallises out. On exposure to air and light for several weeks, anhydrous podophyllotoxin acquires a purple colour; the hydrated substance, on the contrary, appears to be quite stable under these conditions. The specific rotation of an alcoholic solution of anhydrous podophyllotoxin was found to be $[\alpha]_D = -78^{\circ} 4'$.

Picropodophyllin.

If podophyllotoxin is boiled with aqueous alkalis until completely dissolved, and the liquid is then acidified with dilute mineral acids, a white precipitate is thrown down; this is soluble in hot alcohol, and on cooling is deposited in the form of silky masses of long needles, melting at 227° . It has all the properties of Podwyssotski's picropodophyllin. It was found to be soluble in chloroform, acetone, and hot alcohol, and nearly insoluble in cold alcohol and water. Like podophyllotoxin, it has an extremely bitter taste, but has no rotatory action on polarised light. Analysis of a carefully purified specimen gave the following results.

0.0895 gave 0.203 CO_2 and 0.0436 H_2O . $C = 61.86$; $H = 5.42$.

0.1253 „ 0.2841 CO_2 „ 0.0604 H_2O . $C = 61.74$; $H = 5.35$.

Mean $C = 61.8$; $H = 5.38$. $C_{15}H_{14}O_6$ requires $C = 62.06$; $H = 4.82$ percent.

Picropodophyllin, therefore, appears to be isomeric with podophyllotoxin. As further proof of the isomerism of podophyllotoxin and picropodophyllin, it should be added that when podophyllotoxin is heated in sealed tubes with water or dilute hydrochloric acid, some picropodophyllin is formed, and although decomposition products, such as methylic alcohol, &c., have been carefully searched for, none have been found.

The difference of even a methyl group makes a considerable difference

in the percentage composition of this molecule ; thus, supposing picro-podophyllin to be methylpodophyllotoxin, $C_{15}H_{13}O_6 \cdot CH_3$, this formula would require C = 63·15 ; H = 5·2 per cent.

The results of the combustion of picropodophyllin recorded above cannot be reconciled with this, and the presence of an extra methoxyl or acetyl group would make the difference greater still.

On the other hand, the combustions of podophyllotoxin are uniformly slightly lower in carbon than those of picropodophyllin.

Podophyllotoxin ...	61·64	61·53	61·13	61·51	61·40	mean 61·4
Picropodophyllin...	61·86	61·74	61·93			„ 61·8

But we believe that this is explained by the difficulty of obtaining podophyllotoxin completely anhydrous. The facts recorded subsequently greatly strengthen the supposition that the two compounds are isomeric.

From the experiments we have made, it does not appear that picro-podophyllin is an actual constituent of the plant.

Podophyllic Acid.

If the solution obtained by boiling podophyllotoxin with alkali is carefully cooled and then acidified by adding dilute acetic acid, no precipitation occurs, but on standing for several hours (unless the solution is very concentrated) the whole solidifies to a transparent jelly. If this jelly is dissolved in alcohol and the solution allowed to stand, almost the whole of the podophyllotoxin originally used is obtained in the form of picropodophyllin. It is therefore evident that a very close connection exists between picropodophyllin and the gelatinous acid, which is probably the principal constituent of Podwyssotski's picro-podophyllic acid, and Kursten's podophyllic acid. We propose to retain for it the name of *podophyllic acid*.

If picropodophyllin itself is boiled with alkalis it dissolves, and if this solution is cooled and acidified with acetic acid, it also gelatinises.

All attempts to isolate this gelatinous substance failed ; when dissolved, it invariably gives only picropodophyllin on removing the solvent, and when exposed in the vacuous desiccator, it leaves only a mass of crystals of picropodophyllin. It is apparently an acid, since it redissolves on the addition of alkaline solutions, but as it does not react with any of the ordinary indicators, it is difficult to prepare salts by direct neutralisation. It was found possible, however, to prepare crystalline salts by the following method. Picropodophyllin or podophyllotoxin is dissolved in hot alcohol, and a sufficient quantity of alcoholic soda added to this solution, the mixture is then boiled for some time, and when crystals begin to form the mixture is allowed to cool, and the crystalline precipitate filtered off, washed once or twice

with alcohol and ether, and dried. It can be recrystallised from water or alcohol.

An aqueous solution of this substance gelatinises when acidified with acetic acid, and from this gelatinous material picropodophyllin is obtained by dissolving it in hot alcohol, and the substance when heated in a dry test tube yields a residue of sodium carbonate; the substance must therefore be the sodium salt of podophyllic acid.

From this crystalline sodium salt, a *silver* salt was prepared by double decomposition. Silver podophyllate is somewhat soluble in water, and is very unstable, becoming dark grey in a few minutes when exposed to light.

Analysis of two specimens gave the following results.

$$\begin{array}{l} 0.0745 \text{ gave } 0.0195 \text{ Ag. Ag.} = 26.17 \\ 0.0706 \text{ ,, } 0.0178 \text{ Ag. Ag.} = 25.21 \end{array} \left. \vphantom{\begin{array}{l} 0.0745 \\ 0.0706 \end{array}} \right\} \text{Mean Ag} = 25.69 \text{ per cent.}$$

$$\text{C}_{14}\text{H}_{15}\text{O}_5 \cdot \text{COOAg} \text{ requires Ag} = 25.82 \text{ per cent.}$$

The copper salt was prepared by adding solution of copper acetate to a solution of sodium podophyllate and slowly removing the water by evaporation in a desiccator; it is thus easily obtained in light green prisms. When boiled with alcohol or water, the copper salt decomposes, giving copper oxide, picropodophyllin crystallising from the alcoholic solution; this decomposition was made use of for the determination of the amount of copper contained in the salt.

$$0.1030 \text{ gave } 0.0128 \text{ CuO} = 12.42 \text{ per cent.}$$

$$(\text{C}_{15}\text{H}_{15}\text{O}_7)_2\text{Cu} \text{ requires Cu} = 11.8 \text{ per cent.}$$

The aqueous solution of sodium podophyllate is laevorotatory; a determination of the specific rotation, using a solution containing 2.7365 grams in 100 c.c. of solution in a 2-decimetre tube with Laurent's half-shade polarimeter, gave as the mean of ten readings $4^\circ 33'$.

Hence the specific rotation

$$[\alpha]_D = \frac{100 \times 4^\circ 33'}{2.7365 \times 2} = -83^\circ 8'.$$

From the experimental facts mentioned, it is clear that picropodophyllin must be the anhydride of podophyllic acid. These facts may be summarised thus.

- (1) Picropodophyllin, when boiled with alkali, gives podophyllic acid.
- (2) Podophyllic acid loses water and becomes picropodophyllin.
- (3) The copper salt of podophyllic acid is decomposed on boiling with alcohol into cupric oxide and picropodophyllin, water being eliminated; picropodophyllin must therefore contain the group $-\text{CO}\cdot\text{O}-$.

It was thought that possibly the gelatinous acid produced from podophyllotoxin might be an optical isomeride of that obtained from

picropodophyllin, but the determination of the specific rotation of the former shows that the two are identical; this determination was carried out under the conditions mentioned above, using a solution containing 1.267 grams in 100 c.c.

The mean of ten readings was $-2^{\circ} 7'$.

Specific rotation $[\alpha]_D = -83^{\circ} 31'$.

From these observations, we conclude that Podwyssotski's picropodophyllin acid, is not, as Kursten supposes, an oxidation product of podophyllotoxin having the formula $C_{20}H_{24}O_9$, but when pure is a monobasic acid of the formula $C_{15}H_{16}O_7$, of which picropodophyllin ($C_{15}H_{14}O_6$) is the anhydride or lactone.

Determination of Methoxyl in Podophyllotoxin and Picropodophyllin.

This estimation was carried out by Zeisel's method; two experiments gave the following results.

0.203 podophyllotoxin gave 0.3159 AgI. $CH_3O = 20.61$.

0.1226 " " 0.1918 AgI. $CH_3O = 20.71$.

$C_{13}H_8O_4(OCH_3)_2$ requires $CH_3O = 21.2$ per cent.

The determination of methoxyl groups in picropodophyllin gave the following results.

0.2068 picropodophyllin gave 0.3346 AgI. $CH_3O = 21.43$.

0.134 " " 0.2204 AgI. $CH_3O = 21.7$.

$C_{13}H_8O_4(OCH_3)_2$ requires $CH_3O = 21.2$ per cent.

Podophyllotoxin and picropodophyllin, therefore, both contain two methoxyl groups.

Action of Fused Potash on Podophyllotoxin.

Guareschi (*Ber.*, 1879, 12, 683) has examined the action of fused potash on "podophyllin," which he recognises to be a mixture, and found that paroxybenzoic acid, pyrocatechol and protocatechuic acids were the principal products.

When podophyllotoxin is added to melted potash and the mixture kept just fused for about half an hour, a dark brown "melt" is formed. This was dissolved in water, dilute sulphuric acid added in excess, the precipitate dissolved in ether, and after the ethereal solution had been decolorised with animal charcoal, the solvent was removed by distillation, and the residue dissolved in boiling water, and precipitated with a solution of basic lead acetate. This precipitate was decomposed with dilute sulphuric acid, the mixture shaken with ether, the ethereal solution dried over fused calcium chloride, and light petroleum added until

the mixture was just turbid. At first a resinous substance was deposited, but the later fractions consisted of a substance crystallising in colourless needles, and melting, after having been dried in the desiccator for some days, at 107° . It gave a violet coloration with ferric chloride solution. It had, therefore, the properties of *orcinol*, but the quantity obtained was insufficient for analysis.

The solution of the melt, which had been acidified with dilute sulphuric acid, was distilled, and the distillate, after being exactly neutralised with soda, was concentrated. Silver nitrate was then added and the white, crystalline precipitate collected, dried, and analysed.

0.0892 silver salt gave 0.0573 Ag. $\text{Ag} = 64.2$.

Silver acetate requires $\text{Ag} = 64.61$ per cent. The volatile acid is therefore *acetic acid*.

No derivatives were obtained when podophyllotoxin or picropodophyllin was allowed to react with acetic anhydride, benzoic chloride, or hydroxylamine; in each case the substance was recovered unchanged.

When solutions of podophyllotoxin and phenylhydrazine interact, an oily precipitate forms on standing, but this could not be obtained in a crystalline condition, and has not been further examined.

Action of Bromine on Podophyllotoxin.

Finely powdered podophyllotoxin was made into a paste with glacial acetic acid, and bromine added until a slight excess was present; the acetic acid was then removed by exposure in a vacuum desiccator, the residue dissolved in ether, the solution decolorised with charcoal, and sufficient light petroleum added to cause slight turbidity on standing. In this way, colourless crystals of a bromo-derivative melting above 250° were obtained; this, after recrystallisation from ether and petroleum, was analysed by Carius' method.

0.116 gave 0.0568 AgBr. $\text{Br} = 20.9$.

$\text{C}_{15}\text{H}_{14}\text{O}_6\text{Br}$ requires $\text{Br} = 21.6$ per cent.

Action of Bromine on Picropodophyllin.

A bromo-derivative of picropodophyllin was formed on treating it with bromine in the same manner as podophyllotoxin, but it could not be obtained in a crystalline condition except from alcohol, and solution in this liquid was always accompanied by liberation of free bromine. A specimen which had been crystallised from alcohol was dissolved in ether, and the solution fractionally precipitated with light petroleum; the second fraction was colourless and melted at 138° , and no change in the melting point was observed after fractional precipitation from

ether. A portion of this specimen was analysed, with the following result.

0.0347 gave 0.0143 AgBr. Br = 18.85

$C_{15}H_{14}O_6Br$ requires Br = 21.6 per cent.

This monobromopodophyllin, therefore, appears to be an isomeric of monobromopodophyllotoxin.

Action of Nitric Acid on Podophyllotoxin and on Picropodophyllin.

On adding strong nitric acid to podophyllotoxin dissolved in glacial acetic acid, a deep red coloration was produced, which, after a few hours, changed to deep yellow; after the nitric and acetic acids had been partly removed by heating on a water bath, a colourless, crystalline substance separated, which was shown to be *oxalic acid* by an analysis of the silver salt.

Found Ag = 70.5. $Ag_2C_2O_4$ requires Ag = 70.8 per cent.

It should be added here that the substance melted at 100° before drying, and at 187° after drying, which is characteristic of oxalic acid.

The residue left after complete removal of the nitric acid and acetic acid was an amorphous, yellow resin; from this, no crystalline substance could be isolated.

Picropodophyllin was found to yield exactly the same products as podophyllotoxin when attacked by nitric acid.

Distillation of Podophyllotoxin with Zinc Dust.

When podophyllotoxin is mixed with a large excess of zinc dust and the mixture heated to redness in a tube, a small quantity of a yellow oil is obtained; by using considerable quantities of podophyllotoxin, enough of this oil was finally accumulated for analysis. It was purified by dissolving in ether, and after shaking with dilute alkali, which removed some phenolic substance, the ether was distilled off, the last traces being removed by exposure in a vacuum, and the oily residue distilled. The portion boiling between 256° and 258° (about 80 per cent. of the product) was pale yellow, and had a slight phenolic odour; when exposed to the air for several days, it darkened and somewhat resinified; on adding picric acid to its ethereal solution, it became deep red and deposited orange-red crystals melting at 134° (dimethylnaphthalene picrate melts at 136°).

The oil gave the following numbers on analysis.

0.1547 gave 0.5181 CO_2 and 0.1166 H_2O . C = 91.3; H = 8.3.

Dimethylnaphthalene requires C = 92.2; H = 7.69 per cent.

Picropodophyllin also furnishes dimethylnaphthalene when distilled with zinc dust.

The Colouring Matter.

The residue left after removal of the ether from the ethereal solution obtained from the resin previously extracted by chloroform was treated with a small quantity of ether to remove resinous substances. The residue was then dissolved in hot glacial acetic acid, from which, on cooling, it crystallised in needles; these were then recrystallised from ether and chloroform. Prepared in this way, the colouring matter has the appearance of ordinary quercetin, and agrees with it in properties and composition.

Analysis gave the following numbers.

0.1239 gave 0.2730 CO_2 and 0.042 H_2O . $\text{C} = 60.09$; $\text{H} = 3.71$.

0.1034 „ 0.2293 CO_2 „ 0.034 H_2O . $\text{C} = 60.44$; $\text{H} = 3.46$.

$\text{C}_{15}\text{H}_{10}\text{O}_7$ requires $\text{C} = 59.6$; $\text{H} = 3.31$ per cent.

A compound of the colouring matter with sulphuric acid was prepared according to the method described by A. G. Perkin (Trans., 1895, 67, 647), the addition of sulphuric acid to a saturated solution of the colouring matter in hot acetic acid; on cooling, this solution deposited rosettes of a brilliantly orange coloured compound. These crystals were dried on a porous tile, and after being exposed for some time in a vacuum desiccator to remove acetic acid, were decomposed with water, and the amount of sulphuric acid liberated determined by titration.

0.112 gave sulphuric acid requiring 2.9 c.c. N/5 soda for neutralisation. $\text{S} = 8.196$ per cent.

0.075 gave sulphuric acid requiring 1.9 c.c. N/5 soda for neutralisation. $\text{S} = 8.11$ per cent.

$\text{C}_{15}\text{H}_{10}\text{O}_7 \cdot \text{H}_2\text{SO}_4$ requires $\text{S} = 8.0$ per cent.

The colouring matter in the first case was collected on a tared filter, washed, dried at 110° , and weighed.

0.112 gave 0.0849 colouring matter = 75.803 per cent. $\text{C}_{15}\text{H}_{10}\text{O}_7 \cdot \text{H}_2\text{SO}_4$ requires 75.5 per cent.

Action of Acetic Anhydride. — About 1 gram of the colouring matter was heated on the water bath for about 2 hours with 4 c.c. of acetic anhydride and some anhydrous sodium acetate; the mixture was then poured into excess of water, the precipitate collected, and after repeatedly washing with water, was dissolved in hot alcohol, the solution decolorised with charcoal, and set aside. On cooling, the substance crystallised out in silky masses of long needles, which, after recrystallisation from alcohol, melted at 192° ; pentacetylquercetin melts at 192° .

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It was analysed by dissolving it in a mixture of sulphuric and acetic acids, precipitating with water, and weighing the colouring matter which separated on cooling.

0.1586 acetyl derivative gave 0.093 colouring matter = 58.65 per cent. Acetylquercetin, $C_{15}H_5O_7(C_2H_3O)_5$, requires 58.98 per cent.

Action of Fused Potash.—About 0.5 gram of the colouring matter was heated with 10 grams of potassium hydroxide dissolved in 5 c.c. of water for about 20 minutes. The dark chocolate-brown "melt" was dissolved in water, neutralised with dilute sulphuric acid, and extracted with ether; the ethereal solution was distilled, the residue dissolved in water, and after the solution had been decolorised with animal charcoal, lead acetate was added, and the white precipitate thus formed was collected, decomposed with dilute sulphuric acid, and the mixture extracted with ether. The ethereal solution deposited colourless crystals melting at 192° , and an aqueous solution gave a green coloration with solution of ferric chloride. It was therefore *protocatechuic acid* (m. p. 193°).

The filtrate from the lead acetate precipitate was mixed with dilute sulphuric acid, the mixture extracted with ether, the latter distilled off, and the residue dissolved in water; this solution contained *phloroglucinol*, as, on the addition of hydrochloric acid, it stained a pine shaving a deep magenta colour.

Methylation of the Colouring Matter.—The methylic ether of the colouring matter was obtained by boiling a solution in methylic alcohol with potash and methylic iodide for about 15 hours on the water bath; the excess of methylic iodide was then removed by distillation, the residue boiled with benzene, the solution filtered, and the benzene distilled off. The residue, on crystallisation from hot methylic alcohol, gave a mass of glistening, yellow needles melting at 156° ; quercetin methylic ether melts at 157° .

	Formula from combustions.	Acetyl derivative.	Potash fusion products.	Methyl ether.	Composition of H_2SO_4 compound.
Colouring matter of <i>Podophyllum</i> <i>emodi</i>	$C_{15}H_{10}O_7$	m. p. 195° col. matt. = 58.65 per cent.	protocatechuic acid and phlo- roglucinol	m. p. 156°	S = 8.196 per cent. col. matter = 75.803 per cent. orange rosettes
Quercetin (from quercitron bark)	$C_{15}H_{10}O_7$	m. p. 195° col. matt. = 58.98 per cent.	protocatechuic acid and phlo- roglucinol	m. p. 158°	S = 8 per cent. col. matter = 75.5 per cent. orange rosettes

This experimental evidence leaves no doubt that the yellow colouring matter of the rhizome of *Podophyllum emodi* is *quercetin*.

These facts tabulated as page 220.

Professor Hummel kindly undertook to ascertain the value of *Podophyllum emodi* as a dye-stuff. He has compared it with quercitron bark and states that the results are most satisfactory. It is therefore probable that this plant will prove to be of commercial value as a dye-stuff and as a source of the dye quercetin, in addition to podophyllin.

Podophyllo-resin.

The physiological action of the resin left after exhaustion of 'podophyllin' with chloroform and with ether showed that it still contained some substance which was very active as a purgative, although all the podophyllotoxin had been removed. The only solvent for this resinous residue was alcohol, and by fractional precipitation of such a solution with water, it was found possible to separate the residue into two fractions, one a soft, transparent, brownish-red resin, and the other a black powder almost insoluble in alcohol and containing calcium and magnesium. The former of these two was found to be active as a purgative, and has been named provisionally *podophyllo-resin*, whereas the latter is quite inert. All attempts to isolate a crystalline substance from podophyllo-resin failed, and as it was impossible to ensure its homogeneity, attention was turned to its derivatives in the hope of obtaining some crystalline substance which could be purified, and the analysis of which could be utilised for the determination of the composition of the active resin.

Action of Acetic Anhydride on Podophyllo-resin.—About 0.5 gram of resin was boiled for about an hour with acetic anhydride and sodium acetate, the mixture poured into excess of water, allowed to stand for some hours, and the precipitate after being collected and washed with water, was dissolved in boiling alcohol, the solution decolorised with animal charcoal, and set aside. On cooling, a white, somewhat gelatinous, precipitate formed which under the microscope was seen to consist of rosettes of crystals; this was recrystallised from alcohol, in which it is sparingly soluble even on boiling. The recrystallised substance melted at 198° and the melting point was not altered by further recrystallisation.

0.1328 gave 0.3023 CO_2 and 0.0667 H_2O . C = 62.12; H = 5.57.

0.1217 „ 0.2787 CO_2 „ 0.0560 H_2O . C = 62.44; H = 5.09.

Mean C = 62.28; H = 5.33. $\text{C}_{16}\text{H}_{18}\text{O}_6$ requires C = 62.7; H = 5.8 per cent.

The amount of acetic acid produced on hydrolysis was determined. For this purpose, after a weighed quantity had been boiled with N/10

soda solution for several hours, it was acidified with dilute sulphuric acid, and the acetic acid distilled off.

0.1251 acetyl derivative gave 0.0474 acetic acid = 38.6 per cent.

$C_{12}H_{10}O_4(C_2H_3O)_2$ requires 39.4 per cent.

The resin therefore probably has the formula $C_{12}H_{12}O_4$ or $C_{12}H_{10}O_2(OH)_2$.

Action of Fused Potash on Podophyllo-resin.—About 0.3 gram of the resin dissolved in 5 grams of potash and 2 c.c. of distilled water was heated for half an hour; the melt was then dissolved in water, neutralised with dilute sulphuric acid, and the mixture shaken with ether. The ethereal solution was distilled, the residue dissolved in boiling water, and lead acetate solution added; the white precipitate thus formed was decomposed by dilute sulphuric acid, the mixture shaken with ether, and the ether removed by distillation, when a residue was obtained which became crystalline after a time. This residue, when dissolved in water, gave a green coloration with ferric chloride, and therefore contained *protocatechuic acid*, but the recrystallised residue melted at 200° (protocatechuic acid melts at 193°). It was therefore probable that some other substance was present, and in order to determine what this was, a larger quantity (about 2 grams) of the resin, which, however, was not quite so pure as the specimen first experimented with, was fused with potash; the crystalline residue in this case was separated into two fractions, one melting at 210° and the other at 192° (protocatechuic acid). Enough of the former could not be obtained for analysis, but the melting point, and the fact that it gives no coloration with ferric chloride solution and is precipitated by bromine water, makes it probable that it is *parahydroxybenzoic acid* (m. p. 210°). The presence of this substance has already been noted among the products of the decomposition of podophyllin by fused potash (Guareschi, *Ber.*, 1879, 12, 683).

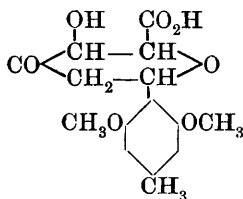
Constituents of Podophyllum peltatum.

The whole of the work described above in connection with *Podophyllum emodi* has been repeated with the rhizome of *Podophyllum peltatum*. The constituents of the two plants are identical, but the proportions in which they occur differ considerably. Thus, estimation of the amount of the crude substance 'podophyllin' contained in the two rhizomes shows that the American rhizome may contain from 4 to 6 per cent. and the Indian rhizome from 10 to 12 per cent. These results are referred to in the last section of the paper.

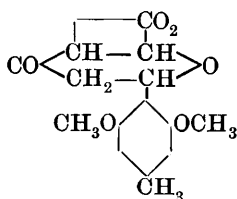
Constitution of Podophyllotoxin, Picropodophyllin and Picropodophyllic Acid.

The facts which have been recorded make it possible to discuss the constitution of podophyllotoxin, picropodophyllin and podophyllic acid. There is little doubt that podophyllotoxin and picropodophyllin are isomerides, and that the latter is the lactone or anhydride of podophyllic acid, into which it has been converted and from which it has been obtained. It has been shown that podophyllotoxin, when dissolved in alkalis, passes into a salt of this acid, and that on heating this acid picropodophyllin is produced, which reverts to the acid again when dissolved in alkalis. There is no evidence to show that podophyllotoxin changes into picropodophyllin before it passes into the acid, although this may be suggested as highly probable, since picropodophyllin, and not podophyllotoxin, is formed when the acid is dehydrated. The nature of the isomerism of podophyllotoxin and picropodophyllin is at present obscure; the chief difference between the two compounds is that podophyllotoxin is levorotatory, whilst picropodophyllin is optically inactive, podophyllic acid is, however, like podophyllotoxin, levorotatory.

The principal facts which must be represented by any structural formula for podophyllotoxin are (1) the composition expressed by the empirical formula $C_{15}H_{14}O_6$, (2) the optical activity, (3) the existence of two methoxyl groups, (4) the hydration by alkalis producing an unstable acid, (5) the formation of a monobromo-derivative, (6) the production of oreinol and acetic acid on fusion with potash, (7) the production of oxalic acid on oxidation with nitric acid. We believe that these facts are satisfactorily represented if podophyllotoxin and its congeners are regarded as the derivatives of a hydrogenated pyronecarboxylic acid. It appears highly probable that podophyllic acid is the carboxylic acid of a *dimethoxymethyl-phenylhydro-γ-pyrone*, of which picropodophyllin is the lactone, whilst podophyllotoxin must have a very similar constitution; possibly it is an optically active form of the racemic lactone. We therefore propose the following formulæ for these substances.



Podophyllic acid.



Picropodophyllin.

Since quercetin accompanies podophyllotoxin in the plant, it is interesting to recall the fact that, according to Herzig and others, quercetin is also a derivative of a phenylated pyrone.

*Estimation of "Podophyllin" and "Podophyllotoxin" in
P. emodi and P. peltatum.*

Podophyllin.—As the rhizome of podophyllum is chiefly used for the preparation of *Resina podophylli* (podophyllin), the estimation of the amount of resin in the rhizome is of some commercial importance.

The process of estimation used consisted in taking a weighed portion of the powdered rhizome, extracting this in a Soxhlet percolator with boiling absolute alcohol, evaporating the percolate to a thin syrup, adding excess of water, and allowing the precipitated resin to settle. The water was then poured off, the process being repeated until the water ceased to extract any sugar. The resin was then dried at 100° and weighed.

The following is a tabular statement of the amounts of resin contained in samples derived from different districts of the Punjab.

District.	Percentage of resin.
Kulu	9.55
Bashahr	9.003
Chamba { Mature roots	11.12
{ Young roots.....	12.03
Hazara	9.06

Estimations of resin in four specimens of the rhizome of *Podophyllum peltatum* gave respectively 4.17 per cent., 5.2 per cent., 5.4 per cent., 5.2 per cent. Dymock and Hooper (*loc. cit.*) found 10 per cent. of resin in the Indian rhizome, and Umney (*loc. cit.*) 12 per cent.

Podophyllotoxin.—An attempt was first made to estimate directly the amount of podophyllotoxin in podophyllum rhizome by percolation with suitable solvents, but this had to be abandoned owing to the difficulty of purifying the material without loss.

The conversion of podophyllotoxin into its very insoluble and easily purified isomeride picropodophyllin next suggested itself as an indirect method of estimation, and preliminary experiments showed that by taking a weighed quantity of podophyllotoxin and converting it into picropodophyllin by treatment with lime, 98 per cent. of the amount taken could be recovered in the form of picropodophyllin.

The process finally adopted is carried out as follows. A weighed quantity of the ground rhizome dried at 100° is mixed with lime, and the mixture percolated with chloroform in a Soxhlet extraction apparatus, the residue left on evaporating the chloroform

solution to dryness is dissolved in absolute alcohol, the solution made into a thin paste with slaked lime, and the whole evaporated to complete dryness; more alcohol is then added, and the process repeated. The dried residue is then mixed with absolute alcohol, the mixture boiled and filtered through a jacketed funnel, and the residue and the filter paper containing it, after being allowed to dry somewhat, is packed into an extraction apparatus, percolated with alcohol, and the percolate mixed with the original alcoholic filtrate; the mixed liquids are then evaporated to dryness in a tared dish and the residue of picropodophyllin weighed. The residue thus obtained is almost colourless and is usually well crystallised, but contains a small quantity of calcium picropodophyllate due to incomplete dissociation of the salt during the drying of the chloroform extract with lime. On incineration, the residue gives on an average about 1 per cent. of calcium oxide, but as this is responsible for only a very small error in an estimation, it may be neglected.

The following table includes the results of a series of estimations of podophyllotoxin in Indian and American podophyllum, and for convenience of comparison the percentages of resin (podophyllin) are also given.

District yielding the specimens.	Quantity of rhizome used.	Percentage of podophyllotoxin found.	Percentage of resin found.	
Kulu	11·92	grams 2·8	9·55	} <i>Podophyllum emodi.</i>
	15·17	„ 2·9		
Bashahr	32·46	„ 3·5	9·00	
	27·12	„ 3·6		
Chamba. {	10·97	„ 5·17 }	3	
Young roots... {	13·46	„ 5·3 }		
Old roots {	9·81	„ 4·7 }	11·12	} <i>Podophyllum peltatum.</i>
	10·59	„ 4·51 }		
Hazara	11·6	„ 2·9	—	
	14·5	„ 3·08		
United States of America {	11·35	„ 0·77	5·2	
	12·02	„ 0·82		
	23·55	„ 0·995	4·17	
	22·6	„		

Taking the average yield of resin and of crystallised podophyllotoxin from the rhizome of *Podophyllum emodi* to be 10 per cent. and 3·5 per cent. respectively, the amount of podophyllotoxin in podophyllin from the Indian drug must be about 38 per cent., whilst that contained in American podophyllin is only about 20 per cent., although in extracting podophyllotoxin itself from the plant nothing like this quantity is obtained, owing to the difficulty experienced in purifying it from the

viscous resinous substances also removed by the solvents. Although there is a difference of nearly 20 per cent. in the amount of podophyllotoxin contained in the podophyllin from these two sources, it is remarkable that they differ comparatively little in physiological activity, a fact which supports the view that podophyllotoxin cannot be regarded as the only active constituent of the resin.

Since the Indian resin contains more podophyllotoxin than the American, it behaves somewhat differently when warmed with alkalis, owing to the larger amount of the insoluble picropodophyllin which is formed and crystallises out. The test of the British Pharmacopœia, which requires podophyllin to be soluble in aqueous ammonia, therefore needs modification. In preparing the ammoniacal tincture from the Indian resin, the mixture should not be heated. Nor, indeed, should heat be used in any case in preparing the tincture, since even with the American resin some of the active podophyllotoxin will be changed into the inert picropodophyllin.

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