PAJANEELIN, A BITTER COMPONENT OF *PAJANEELIA RHEEDII*

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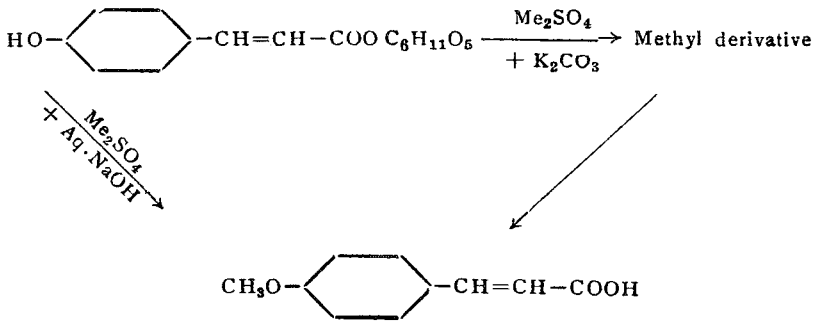
Pajaneelia Rheedii (Bignoniaceæ) is a tree of medium size found in the Malabar coast, Eastern Bengal and Burmah. It is sometimes used for dug-out canoes in Travancore, but its chief use is as a support for the pepper-vine. We had occasion to test small quantities of the root and stem barks received from Travancore and we found them to be highly bitter. The bitter principle has now been isolated and studied; it is given the name 'pajaneelin'. The yield is frequently as high as 5% of the weight of the dry stem bark though it varies considerably.

Pajaneelin is readily isolated by extracting the bark in the cold with alcohol, concentrating the extract and adding water; it can be crystallised from hot water or alcohol and is highly bitter. It melts at 237–39°, has the molecular formula $C_{15}H_{18}O_8$, and is strongly lævorotatory. It is insoluble in aqueous sodium carbonate but dissolves readily in dilute sodium hydroxide. It gives a yellowish brown colour with ferric chloride. It undergoes hydrolysis in dilute acid, but considerable resinification takes place. Cold aqueous sodium hydroxide is far more convenient for this reaction and it yields besides a hexose, an aglucone melting at 210–12° (decomp.) and having the composition $C_9H_8O_3$. The aglucone occurs also free to some extent in the bark (0·1%). It is an acid dissolving readily in sodium bicarbonate solution and giving a brownish yellow colour with ferric chloride. Methylation with methyl iodide or dimethyl sulphate and potassium carbonate yields a dimethyl derivative (ether-ester) insoluble in alkali and melting at 90–91°. This undergoes saponification to form a monomethyl ether which is an acid melting at 174° but clarifying at 188–89°. From its properties and reactions the aglucone has been identified as *p*-hydroxy-cinnamic acid (para-coumaric acid) and the methyl ether as *p*-methoxy-cinnamic acid and this has been confirmed by a comparison with synthetic samples obtained by the method of Robinson.[1] The sugar is identified as *d*-fructose since it is lævorotatory, yields an osazone identical with glucosa-zone and gives the special tests,

Pajaneelin yields on hydrolysis one molecule of para-coumaric acid and one molecule of *d*-fructose. It is neutral in reaction and as already pointed out, its hydrolysis is smoothly effected with cold dilute alkali. Methylation with excess of dimethyl sulphate and potassium carbonate yields a derivative which undergoes hydrolysis with alkali forming *p*-methoxy-cinnamic acid. On the other hand methylation with aqueous sodium hydroxide and dimethyl sulphate yields directly *p*-methoxy-cinnamic acid due obviously to the removal of the sugar group during the process. It is therefore clear that pajaneelin is a fructose ester of para-coumaric acid and not a glycoside.



Some sugar esters of this type involving aromatic acids are already known. Probably the most widely occurring are the gallic acid derivatives which are present as components of tannins. Vaccinin,[2] which is a constituent of whortle and other berries, is a benzoic acid ester yielding glucose and benzoic acid on hydrolysis. Another benzoyl ester of the sugars is the dibenzoyl-glucoxylose obtained by Power and Salway[3] from *Daiviesia latifolia.* A more complex case is populin[4] which is a monobenzoyl ester of salicin. In this the sugar part of the glucoside is esterified with benzoic acid. To a similar category belong the complex anthocyanins like salvianin (monardaein), delphinin, gentianin and violanin. The first is a derivative of pelargonidin and the others are derived from delphinidin. Willstatter and Mieg[5] originally expressed the opinion that *p*-hydroxy-benzoic acid in delphinin is attached to the glucose residue and not to the phenolic hydroxyl groups of the pigment. The methylation experiments of Kondo[6] seem to have been inconclusive though he was inclined to a different view. As the result of the complete methylation and hydrolysis of salvianin Karrer and Widmer[7] obtained a dimethyl ether of pelargonidin. Though on the earlier view of Karrer that a disaccharose unit was present in the 3-position of the anthocyanin molecule this seemed to indicate the linking of the para-hydroxy cinnamic acid unit to the phenolic hydroxyl in the 5 or 7 position, on the later and correct 3 : 5-dimonoside structure of Robinson[8] the acid

group should be combined to a sugar hydroxyl. In accordance with this Karrer and Meuron[9] later showed conclusively that in violanin the *p*-hydroxycinnamic acid unit is attached to the sugar part. There is no doubt that the combination of the acids with the sugar hydroxyls represents a stabler condition than esterification of a phenolic hydroxyl.

Another very interesting example of a naturally occurring sugar ester is the pigment of saffron, crocin. In it the unsaturated long-chain dicarboxylic acid crocetin is esterified with 2 molecules of gentiobiose.

p-Coumaric acid has been previously found in the leaves and stem bark of *Catalappa ovata*[10] in which it occurs along with ferulic acid. It has also been isolated from green tea leaves[11] and from the urine of special diseases.[12] It should therefore be considered to be an important compound of the 9-carbon (C_6-C_3) system occurring in nature. The bark of *Pajaneelia Rheedii* seems to be one of the richest sources.

The remarkable feature about pajaneelin is that it is the first instance where fructose has been found in combination with an aromatic acid in ester form. Though this sugar is present in nature in considerable quantities both free and in combination as sucrose and inulin, simple fructosides and fructose esters have not been known. Pajaneelin is highly bitter and in this respect it has resemblance to vaccinin.

Experimental

Isolation of Pajaneelin

The stem and root barks of *Pajaneelia Rheedii* were obtained from the forests of Travancore. There was considerable variation in quality; a sample of the stem bark collected in July 1945 gave the best results and the experiments carried out with this sample are described below:

The dry bark powder (2 kg.) was extracted thrice in the cold with alcohol keeping the material immersed in the solvent for 24 hours each time. The combined extract was distilled to recover the solvent and the concentrate thoroughly extracted with ether. The ether solution was marked (A). The ether extracted concentrate (B) was allowed to stand for a week. A heavy crystalline solid was deposited; it was filtered and washed with cold water. Some more of it was obtained on diluting the filtrate with water. When recrystallised from water or alcohol it melted at 237–39° and the melting point was not improved by further crystallisation. The total yield of the pure product (pajaneelin) was 100 g. (5%). The aqueous mother-liquor and washings were put together and marked (C).

The ether extract (A) on evaporation yielded an amorphous solid which was extracted with aqueous sodium carbonate and filtered. On acidifying the filtrate, extracting it with ether and evaporating the ether extract a small amount of crystalline solid was obtained. When re-crystallised from hot water it melted at 210–12° (decomp.) and was found to be identical with the compound (aglucone) obtained by the hydrolysis of pajaneelin.

In one experiment a portion of the aqueous mother-liquor (C) was treated with excess of basic lead acetate and the pale yellow precipitate of the lead salt filtered and washed with water. It was then ground up with excess of water, the mixture warmed and repeatedly saturated with hydrogen sulphide. The precipitate of lead sulphide was filtered and washed with boiling water. The filtrate was concentrated to small bulk, the syrupy residue dissolved in a small amount of alcohol and treated with excess of ether. The ether layer was separated, evaporated and the resulting residue extracted with small quantities of aqueous sodium carbonate. This extract was filtered and acidified when a colourless crystalline precipitate was obtained. It melted at 210–12° with decomposition and was identical with the aglucone obtained from pajaneelin. The total yield of the free aglucone from (A) and (C) was approximately 0·1%.

Mother-liquor (C) seemed to contain some more pajaneelin besides the aglucone since in another experiment on subjecting it to treatment with aqueous potash it gave the aglucone readily and in much better yield. Enough potash was added to (C) in order to make the strength 5%. The solution was allowed to stand for 24 hours, acidified with hydrochloric acid and extracted thoroughly with ether. When the ether solution was shaken with aqueous sodium carbonate (5%) and the alkaline solution acidified the aglucone was obtained in a crystalline condition.

Properties and Reactions of Pajaneelin

Pajaneelin was soluble in alcohol and acetone and insoluble in ether. It was soluble in boiling water and separated out on cooling in the form of rectangular prisms melting at 237–39°. It was highly bitter to the taste and gave a yellowish brown colour with alcoholic ferric chloride. It formed a pale yellow bulky precipitate with basic lead acetate. It was insoluble in cold aqueous sodium bicarbonate and carbonate and its solution in hot water was neutral to litmus. But it dissolved in cold aqueous sodium hydroxide with a pale yellow colour. It underwent charring in concentrated sulphuric acid and formed a yellow solution in strong nitric acid. Its solution in alcohol was strongly lævorotatory (Found: C, 55·7; H, 5·8; $C_{15}H_{18}O_8$ requires C, 55·2; H, 5·5%.) $[\alpha]_D^{29}$, −173°.

Pajaneelin Acetate

This was prepared by boiling pajaneelin with acetic anhydride and a few drops of pyridine for 2 hours. It crystallised from ethyl acetate-petroleum ether mixture as colourless needles melting at 108–9° (Found: C, 56·4; H, 5·0; $C_{25}H_{28}O_{13}$ requires C, 56·0; H, 5·2%).

Hydrolysis of Pajaneelin

(i) *Acid hydrolysis.*—Pajaneelin was boiled with 7% sulphuric acid for 2 hours; a dark brown solid separated out during the course of this hydrolysis. The mixture was extracted repeatedly with ether. When the ether solution was evaporated a pale brown solid was left; it crystallised from hot water as colourless needles and melted at 210–12° and was identical with the aglucone obtained by alkaline hydrolysis (see below). The yield was poor. The aqueous acid solution left after ether extraction was treated with excess of barium carbonate and the precipitated barium sulphate filtered off. After concentration to small bulk the filtrate yielded an osazone identical with fructosazone (glucosazone).

(ii) *Alkaline hydrolysis.*—Pajaneelin (2·0 g.) was dissolved in 10% aqueous potash (100 c.c.) and the solution allowed to stand for 24 hours. It was then acidified with acetic acid and repeatedly extracted with ether. The ether extract was marked (A) and the aqueous solution (B).

The aglucone.—(A) was evaporated and the residue taken up with aqueous sodium carbonate. The solution was filtered and acidified with hydrochloric acid. The crystalline precipitate was then recrystallised from boiling water when it came out as colourless needles melting with decomposition at 210–12°. It was readily soluble in organic solvents and its solution in water was acidic to litmus. The solid dissolved in aqueous sodium bicarbonate with effervescence and its solution in aqueous potash was yellow. In the presence of mineral acids (hot) it was unstable and underwent discolouration and resinification. Its solution in concentrated sulphuric acid was pale brown. It gave a yellowish brown colour with alcoholic ferric chloride and a pale yellow precipitate with basic lead acetate (Found: C, 66·0; H, 4·8; $C_9H_8O_3$ requires C, 65·9 and H, 4·8%).

The percentage of aglucone in pajaneelin was estimated by effecting the saponification quantitatively as follows: A weighed quantity of pajaneelin was treated with excess of N/10 aqueous potash and allowed to stand in the cold for 24 hours. The excess of alkali remaining was then titrated with standard acid. The alkali used up in the saponification corresponded to the

aglucone (Found: aglucone 50·2%. Calculated for $C_9H_8O_3$ in $C_{15}H_{18}O_8$, 50·3%).

The acetyl derivative was prepared by boiling the aglucone with acetic anhydride and a few drops of pyridine for 2 hours. It crystallised from ethyl acetate-petroleum ether mixture in the form of colourless rhombohedral plates and prisms melting at 206–7°.

The aglucone (2 g.) was dissolved in dry acetone (30 c.c.) and dimethyl sulphate (10 c.c.) and anhydrous potassium carbonate (15 g.) added. The mixture was kept gently boiling for 20 hours. The inorganic salts were then filtered off and washed with warm acetone. The acetone filtrate was concentrated to small bulk and excess of water added. The precipitated solid was collected and recrystallised from alcohol. It came out in the form of colourless, thin and broad rectangular plates with a tendency to taper at the ends and melted at 90–91°. The substance was insoluble in aqueous sodium carbonate and cold aqueous sodium hydroxide and was the ether-ester, [Found: OCH_3, 31·5; $C_{11}H_{12}O_3$ requires OCH_3 (2), 32·3%].

The ether-ester was hydrolysed by boiling with alcoholic potash (8%) for 30 minutes. As much of the alcohol as possible was distilled off, the residue dissolved in water and acidified with sulphuric acid. The colourless precipitate was recrystallised from alcohol when it was obtained as colourless elongated rhombohedral prisms melting at 174–75°, but the melt became clear only at 188–89°. The substance was acidic in reaction, dissolved in sodium bicarbonate with effervescence and could be recovered by acidification [Found: OCH_3, 18·0%; equivalent weight by titration 178·9 and by the silver salt method 184·2. $C_{10}H_{10}O_3$ requires OCH_3 (1), 17·4% and molecular weight 178·0].

Comparison was effected between the aglucone, its acetate, methyl ether and ether-ester on the one hand and synthetic samples of *p*-hydroxycinnamic acid, its acetate, methyl ether and ether-ester on the other. The identity was established by the determination of mixed melting points; there was no depression.

The sugar —The aqueous solution (B) was evaporated almost to dryness on a water-bath. The residue dissolved completely in cold water indicating the absence of pajaneelin. The solution was treated with a little activated charcoal to effect clarification. It was strongly lævorotatory and strongly reducing, Fehling's and Tollen's reagents being reduced even in the cold. It yielded readily an osazone crystallising as sheaves of golden yellow needles and melting at 204–5°. It also gave the special tests for *d*-fructose

(Pinoff's and Seliwanoff's tests). Comparison with a solution of an authentic sample of *d*-fructose established the identity. In an experiment done quantitatively, from the value of the optical rotation of the final sugar solution the yield of the sugar was calculated to be 57·0%; yield required for $C_6H_{12}O_6$ in $C_{13}H_{18}O_8$, 55·2%.

The estimation of the sugar was also made directly as follows: Saponification of a weighed quantity of pajaneelin was effected with 5% aqueous sodium hydroxide in the cold during 24 hours. The solution was light brown in colour. On rendering it slightly acidic with hydrochloric acid the colour became very pale yellow. The solution was then made up to a definite volume and the rotation taken after filtering off the aglucone that separated out. The yield of *d*-fructose thus found was 56·5%.

Methylation of Pajaneelin and Hydrolysis

Pajaneelin (1 g.) was dissolved in dry acetone (30 c.c.), dimethyl sulphate (5 c.c.) and anhydrous potassium carbonate (10 g.) added and the mixture boiled under reflux for 20 hours. At the end of this period the potassium salts were filtered and washed with a small quantity of warm acetone. The filtrate was concentrated to small bulk, treated with excess of cold water and allowed to stand. An oily liquid separated out and it did not solidify. It was therefore ether extracted and the ether distilled off. The residual oily product did not solidify on allowing to stand for a long time. It could not be crystallised from any solvent. It was therefore directly saponified by treatment with cold alcoholic potash for 24 hours. As much alcohol as possible was evaporated, the solution diluted with water and acidified. The precipitate was purified by again dissolving it in sodium carbonate solution and reprecipitating it. After a final crystallisation from dilute alcohol it came out as colourless elongated prisms melting at 174° and clarifying at 188–89°. It was found to be identical with a synthetic sample of *p*-methoxy-cinnamic acid.

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Summary

Pajaneelin, the crystalline bitter principle of the bark of *Pajaneelia Rheedii* is strongly lævorotatory and yields on hydrolysis with dilute alkali molecular proportions of *p*-hydroxy-cinnamic acid and *d*-fructose. Methylation and subsequent hydrolysis produces *p*-methoxy-cinnamic acid. It is a neutral compound. It is therefore an ester of *p*-hydroxy-cinnamic acid with *d*-fructose. The occurrence of similar sugar esters is briefly reviewed.

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