167. *Leuco-anthocyanins and Leuco-anthocyanidins. Part I. The Isolation of Peltogynol and its Molecular Structure.*

By (Mrs.) G. M. Robinson and Robert Robinson.

The discussions on the problem of the origin of anthocyanins in the plant have been much obscured in the past by the arbitrary assumption that the chromogens (Palladin's respiratory prochromogens; *Biochem. Z.*, 1909, **18**, 151) are the flavones and flavonols or their glycosides. This was a natural suggestion in 1911 (Wheldale, *J. Genetics*, **1**, 133), but it is not supported by any physiological evidence or by a statistical comparison of the constitutions of congeneric pyrones and pyrylium salts. Indeed, when the chemical relations of these groups became clear as the result of the work of Willstätter on the anthocyanins, the flavone-chromogen obsession was so potent that the acceptable hypothesis of anthocyanin formation by oxidation was discarded by many authors and in some quarters it was maintained that the pigments owe their formation to the reduction of γ-pyrones such as apigenin and quercetin or their glycosides.

Doubtless the details of the oxidase theory of Keeble and Armstrong (*Proc. Roy. Soc.*, 1912, *B*, **85**, 214; *J. Genetics*, 1912, **2**, 277; cf. Keeble, Armstrong, and Jones, *Proc. Roy. Soc.*, 1913, *B*, **86**, 308) require adjustment in the light of present knowledge, but in general terms it is surely in better harmony with the whole of the circumstances of anthocyanin formation than the view attributing a predominant rôle to reduction. Even the experiments *in vitro* cannot be logically interpreted in favour of the reduction hypothesis. The flavones can only be reduced to flavylium salts by metals in strongly acidic media and the yields are very poor; thus no close analogy with the assumed reduction *in vivo* has been provided (Everest, Willstätter, Combes, Shibata *et alii*).

The occurrence in flowers and fruits of colourless substances affording anthocyanin-like pigments on treatment with acids has been frequently noticed, but the significance of these observations has not been fully recognised and the pigments have often been dismissed as "phlobaphenes," a term which in respect of its precision may be classed with "humic acid."

Laborde (*Compt. rend. Acad. Sci.*, 1908, **146**, 1411; **147**, 753, 993) found that unripe green or red grapes contain colourless chromogens which are converted into pigment on heating with dilute hydrochloric acid; Malvezin (*ibid.*, 1908, **147**, 384) showed that the same chromogen developed colour on heating with water at 85°, but only in the presence of air. Dezani (*Staz. sper. agr. Ital., Modena*, 1910, **43**, 428), Tswett (*Biochem. Z.*, **1913**, **58**, 225; formaldehyde or acetaldehyde used in addition to hydrochloric acid), Rosenheim (*Biochem. J.*, 1920, **14**, 178), Jonesco (*Compt. rend. Acad. Sci.*, 1921, **173**, 1006; 1922, **175**, 592), and Kozlowski (*ibid.*, 1921, **173**, 855) have also observed the formation of pigments of anthocyanidin-like nature from colourless or pale yellow chromogens without the use of a reducing agent.

Rosenheim (*loc. cit.*) prepared a white amorphous powder of glycosidic nature from the unripe berries of purple grapes or from ripe white grapes and stated that anthocyanidin could be obtained from it by the action of hydrochloric acid in the absence of oxygen. He proposed the expression "leuco-anthocyanin" for the new group of natural products. No indication of the nature of the anthocyanidin obtained from the leuco-anthocyanins was forthcoming in any of the work cited above.

The present authors (*Biochem. J.*, 1933, **27**, 206) have investigated the distribution of leuco-anthocyanins in various parts of plants and have applied a series of qualitative but characteristic tests to the identification of the anthocyanidins produced from the chromogenic material.

The leuco-anthocyanins were thus found to be of widespread occurrence in the vegetable kingdom; the majority of them yielded cyanidin on treatment with hot hydrochloric acid. Curiously enough, the leuco-anthocyanin of white grapes yielded cyanidin whereas the pigment of purple grapes, namely, oenin, is a malvidin glucoside; we had, however, already found that the skins of a red South African grape contained a cyanidin monoglycoside (*Biochem. J.*, 1932, **26**, 1663).

The whole subject appeared so nebulous and yet of such fundamental interest that a careful investigation of particular cases was urgently required.

The leuco-anthocyanins are divided roughly into three classes: (a) those that are insoluble in water and the usual organic solvents, or give only colloidal solutions; (b) those readily soluble in water and not extracted from the solution by means of ethyl acetate; (c) those capable of extraction from aqueous solution by means of ethyl acetate. Probably class (b) consists of relatively simple glycosides or diglycosides, whereas members of class (c) are sugar-free and should be regarded as leuco-anthocyanidins. We directed our attention in the first place to an example in class (c), although the anthocyanidin obtained in this case was clearly not identical with any known aglucone of a plant pigment.

The heartwoods of *Peltogyne porphyrocardia*, *P. pubescens*, and *Copaifera pubiflora* are light brown when freshly cut, but acquire a characteristic purplish-red colour on exposure to light and air (hence the expression "purpleheart"). Aqueous extracts of the saw-dust contain the substance responsible for this property, and on heating such solutions after the addition of hydrochloric acid, a relatively large amount of an anthocyanidin is readily produced. This peltogynidin rather closely resembles a 5-*O*-substituted cyanidin in its

properties, but the acid solutions are a little brighter and more blue-toned red. A full description of this and related flavylium salts is reserved for a future communication. The leuco-anthocyanidin can be isolated by extraction of aqueous solutions with ethyl acetate following a procedure described in the Experimental section. The colourless crystalline substance is named *peltogynol*; it has the composition $C_{16}H_{14}O_6$ and furnishes crystalline *tetra-acetyl*, *tetrabenzoyl*, and *tetra-anisoyl* derivatives; the substance is dextrorotatory.

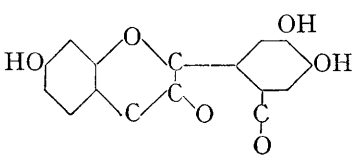
On methylation by means of aqueous sodium hydroxide and methyl sulphate, a characteristic *tetramethyl* ether was obtained, but on some occasions this was mixed with a *trimethyl* ether, the two substances being readily separable. It was noticed that the trimethyl ether was produced when the methyl sulphate was in excess and the product had been kept for some hours in contact with the acid solution. Actually the trimethyl ether could be prepared from the pure tetramethyl ether by hydrolysis with hydrochloric acid under mild conditions. The trimethyl ether is insoluble in aqueous alkalis and gives no ferric reaction in alcoholic solution; it is therefore non-phenolic and obviously the fourth methyl group must be associated with a semi-acetal structure of methylglucoside type. This was confirmed by the behaviour of the tetramethyl ether, which was recovered unchanged after treatment with an excess of ethylmagnesium bromide in benzene–ether solution. On the other hand peltogynol itself furnishes a 2 : 4-*dinitrophenylhydrazone*.

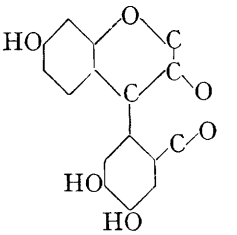
Important confirmation of our view of the composition of peltogynol and *O*-tetra-acetylpeltogynol was provided by the optical and *X*-ray crystallographic examination of the substances. This was very kindly undertaken by Miss D. Crowfoot, and her report shows clearly the great value of the new methods for the determination of the molecular weights. Peltogynol and its derivatives showed abnormal cryoscopic behaviour in camphor.

When peltogynol is heated with nitric acid, the combined oxidation and nitration that ensues results in the formation of 2 : 4 : 6-trinitroresorcinol (styphnic acid); on the other hand, the *O*-trimethylpeltogynol afforded 4 : 5-dinitroveratrole. Only traces of the latter were obtained, but on oxidation of the trimethyl ether with potassium permanganate considerable quantities of *m*-hemipinic acid could be isolated. A second product of this degradation was *trimethylpeltogynic acid*, $C_{15}H_8O_2(OMe)_3{\cdot}CO_2H$ [or $C_{15}H_{10}O_2(OMe)_3{\cdot}CO_2H$], which retains the capacity to furnish a red flavylium salt on oxidation in the presence of acid.

These facts show that peltogynol contains a resorcinol and a catechol nucleus and that the catechol nucleus is 4 : 5-disubstituted, one of the substituting groups being $CH_2{\cdot}OH$ or CHO. The very close similarity of the peltogynidin salts to cyanidin chloride being taken into consideration, it is evident that the skeletons (I) and (II) must be considered. The hydroxyl in the resorcinol nucleus cannot be in the alternative position 5 of the benzopyran ring on account of the fluorescence properties exhibited by methylated peltogynidin salts; the 5-methoxy-flavylium salts are non-fluorescent. Similarly the alkali-colour reaction of peltogynidin speaks for a close analogy with fisetinidin.

(I.)



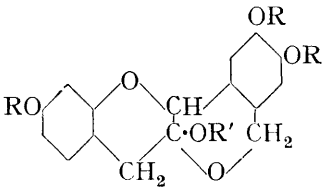


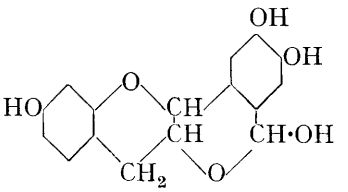
(II.)

Here the *X*-ray evidence favoured (I), as Miss Crowfoot found it difficult to arrange models based on (II) according to the usual stereochemical assumptions in such a way as to include four molecules per unit cell. Furthermore a pyrylium salt with highly characteristic properties is obtainable from trimethyl or tetramethyl peltogynol, and what appears to be the same substance can be synthesised from fisetinidin chloride tetramethyl ether by condensation with formaldehyde in the presence of concentrated sulphuric acid. The formal

identification of the two specimens is not yet quite complete owing to the difficulties caused by traces of impurities. On the basis of (I), the complete formula for peltogynol should be (III; R, R′ = H) or (IV, in semiacetal-type configuration).

(III.)





(IV.)

However, (IV) should be no more readily convertible into a flavylium salt than is catechin, whereas (III) represents a true potential leuco-anthocyanidin, the state of oxidation being that of a dihydroflavylium salt. There is, of course, a marked contrast between the behaviour of catechin and peltogynol and their derivatives in respect of the relative ease of conversion into flavylium salts; in the former case the transformation can only be accomplished under special conditions (Appel and Robinson, this vol., p. 426), whereas in the latter case the leuco-anthocyanidin nature of the substance is its most obvious characteristic.

The formula (III) fits all the facts hitherto ascertained and, provisionally accepting it, we may note that, as in the case of cyanomaclurin (see following paper), the dihydroanthocyanidin state of oxidation is stabilised by the ketose group. We adopt as a working hypothesis the view that a corresponding structure will be found in other leuco-anthocyanins and that the formation of the anthocyanidins from these precursors is actually the result of oxidation.

The experiments designed to exclude the latter possibility were hardly conclusive; a mere trace of oxygen is required and in any case disproportionation is a possibility that must be reckoned with. No information is available in regard to the quantitative relations of leuco-anthocyanin to pigment produced under different conditions.

If we have correctly interpreted the evidence, the formation of peltogynidin from peltogynol must involve oxidation, but we have not been able to inhibit the appearance of pigment on boiling with hydrochloric acid by taking ordinary measures for the exclusion of oxygen.

In the case of the leuco-anthocyanins a plausible suggestion is that a hydroxyl group of a sugar-chain is involved in the semi-acetal structure with the carbonyl group in position 3 of the pyran nucleus.

Experimental.

Peltogynol.—The first small specimens of woods of *Peltogyne* species were kindly supplied by Miss M. M. Chattaway and Dr. L. Chalk of the Department of Forestry, and the material used in this investigation was a log of the wood of *P. porphyrocardia* obtained from Trinidad through the Department of the Conservator of Forests. It was found that the reddening of the wood on exposure does not occur in the dark even during 4 hours at 40°. In the earlier experiments extraction with 0·5% hydrochloric acid was practised, but the procedure is not advantageous (the wood contains free acetic and butyric acids). Direct fractional extraction with organic solvents gave a large yield of material, but this was very difficult to purify. We are much indebted to Mr. W. Campbell of the Forests Products Research Laboratory, Princes Risborough, for kindly undertaking the comminution of the wood. This proved worth while, as the yields on the weight of wood used were: fine shavings, 1·3%; powdered wood (60—80 or 100 mesh/sq. in.), 2·3%. The finely divided wood (50 g.) was heated on the steam-bath with water (500 c.c.) for 20 minutes and after filtration the residue was treated in the same way. The combined extracts were saturated with sodium chloride, sodium hydrogen carbonate (10 g.) added, and the cold solution shaken with three successive volumes of ethyl acetate (250 c.c. each). The extracts were kept over anhydrous sodium sulphate (10 g.) and sodium hydrogen carbonate (10 g.) (the latter absorbing a yellow substance, soluble in ether and exhibiting strong co-pigmenting properties in solutions of pelargonidin or cyanidin chlorides) for *ca.* 12 hours, filtered, and concentrated under diminished pressure to about 50 c.c. The solution was filtered through dry sodium sulphate (5 g.) and sodium hydrogen carbonate (5 g.) into light petroleum (300 c.c. of

b. p. 40—60°) with constant stirring. The light mauve solid was triturated with light petroleum (200 c.c. of b. p. 60—70°) and then ground with the convenient minimum of pure ethyl acetate; the isolated solid was then pure enough for many purposes.

Alternatively the solid washed with light petroleum was triturated with water (10 c.c.) for the removal of a soluble yellow impurity, then taken up in ethyl acetate, the solution dried by means of sodium sulphate (2 g.) and sodium hydrogen carbonate (2 g.), and a little benzene added to precipitate a further impurity. The filtered solution was added to light petroleum, and the recovered solid treated twice again in the same manner. It was then almost colourless and was dissolved in warm water and crystallised by concentration of the solution in a desiccator. The colourless, elongated, flat prisms became pink at 200° and darker red at about 240° with gradual softening and decomposition (Found in material dried at 80° in a vacuum : C, 63·7, 63·7, 63·7; H, 4·9, 4·9, 4·9; N, 0·0; MeO, 0·0. $C_{16}H_{14}O_6$ requires C, 63·6; H, 4·6%). $[\alpha]_D^{21°}$ + 273° (c = 0·6 in ethyl acetate). The high value for hydrogen is due to the hygroscopic character of the substance.

Another method of purification consisted in washing the crude substance successively with light petroleum (b. p. 60—70°), benzene, chloroform, and a little ethyl acetate. It was then dissolved in ethyl acetate, and the solution concentrated in a vacuum desiccator; the pale bluish crystals were collected, partly dissolved in ethyl acetate (leaving behind most of the coloured impurity), and the solution filtered through a little activated alumina. This removed the whole of the colour, and colourless crystals were obtained on concentration of the solution. If too much alumina is employed, the peltogynol is itself adsorbed and cannot be removed by continuous extraction with acetone or ethyl acetate. As the purification of peltogynol proceeds, it becomes much more sparingly soluble in ethyl acetate, but this is not reflected in changed analytical results and all specimens afforded the same tetra-acetate. Peltogynol is sparingly soluble in cold water, moderately readily in hot water; it is very sparingly soluble in ether, benzene or chloroform, readily soluble in acetone, and freely soluble in the simple alcohols. The plates separating from ethyl acetate become syrupy in contact with water, but the substance quickly crystallises again in a new form. An aqueous or alcoholic solution develops a bright green coloration on the addition of ferric chloride. Pure peltogynol is not discoloured on exposure to light and air.

A full description of peltogynidin and its derivatives will be submitted later, but it may be stated here that the salt obtained by the action of hot hydrochloric acid or cold alcoholic hydrogen chloride on peltogynol is undoubtedly a mixture containing some bimolecular condensation products. The action of bromine on the substance in peroxide-containing dioxan solution (cf. Appel and Robinson, *loc. cit.*) gives a purer product. All specimens of peltogynidin give somewhat bluer-red solutions than cyanidin, but the colour reactions with sodium acetate and sodium carbonate closely resemble those of fisetinidin. The ferric reaction is pure blue in alcoholic solutions and is identical with that displayed by cyanidin. Characteristic of peltogynidin is the bright red coloration produced (through blue) on the addition of sodium hydroxide; no other anthocyanidin exhibits this behaviour.

O-*Tetra-acetylpeltogynol*.—This derivative was prepared on many occasions, substantially as described below, but the reaction was often completed by heating with consequent diminution of the yield. The substance is sparingly soluble in cold methyl and ethyl alcohols, ether, water, and light petroleum; it is readily soluble in benzene, chloroform, and ethyl acetate and moderately readily soluble in hot ether or alcohol. It crystallises from aqueous acetic acid in colourless, flat, rectangular prismatic needles and best from benzene–alcohol in a similar form or in hair-fine needles on rapid separation, m. p. 173° (Found in material dried at 80° in a vacuum : C, 61·3, 61·3, 61·3; H, 4·8, 4·8, 4·8. $C_{24}H_{22}O_{10}$ requires C, 61·3; H, 4·7%). The determination of acetyl groups invariably gave a high value; for example, Dr. G. Weiler used the method of alkaline hydrolysis, and estimation of the acetic acid isolated by distillation (Found : MeCO, 39·4, 39·3. $C_{24}H_{22}O_{10}$ requires 4MeCO, 36·6%). But peltogynol itself gave MeCO 1·5—2%, and it is evident that a small proportion of a volatile acid is produced by its degradation. Miss Crowfoot's results confirmed the composition attributed to the derivative.

The following is the best method of preparation. A mixture of peltogynol (1 g.), pyridine (1 c.c.), and pure acetic anhydride (12 g.) was made with cooling, kept for 48 hours, and then decomposed with water. The solid was washed with aqueous sodium carbonate and water, triturated with light petroleum, and dried (0·95 g.); after further washing with ether (40 c.c.) and one crystallisation, an almost pure product was obtained (0·64 g. of m. p. 169°) m. p. 173°, after crystallisation from benzene–alcohol ($[\alpha]_D^{20°}$ + 125°; c = 0·4 in chloroform). Tetra-acetylpeltogynol is not convertible into an acetylpeltogynidin salt, even by means of bromine

and technical dioxan; on heating with alcoholic hydrogen chloride it is gradually hydrolysed and the solution slowly reddens, becoming ultimately intense bluish-red.

The solution of the substance in concentrated sulphuric acid is momentarily colourless, but a pink colour and a bright green fluorescence very quickly make their appearance; the colour deepens to brownish-red and on the addition of water it becomes magenta and the fluorescence disappears. The preparation of a methoxyacetyl derivative of peltogynol was attempted but the product could not be purified.

Peltogynol from Peltogyne pubescens.—A small specimen of the wood was reduced to sawdust, and the peltogynol isolated in the usual way. The substance had the superficial character of the leuco-anthocyanidin previously examined and it was identified by conversion into the tetra-acetyl derivative. This had m. p. 173°, alone or mixed with a specimen from *P. porphyrocardia* (Found: C, 61·3; H, 4·8%). The woods of *Copaifera pubiflora* and *Trachylobium Hornemanianum* contain a leuco-anthocyanidin showing the reactions of peltogynol.

O-*Tetrabenzoylpeltogynol*.—Benzoyl chloride (6 g.) was added to a cooled solution of peltogynol (2 g.) in the minimum quantity of dry pyridine and after 2 days the mixture was decomposed with water, and benzoyl chloride eliminated by means of sulphanilic acid and aqueous sodium carbonate. The solid product was washed with water, and light petroleum, precipitated from benzene solution by means of light petroleum, washed with cold ether, and crystallised from acetic acid, forming colourless plates, m. p. 244° (Found in material dried at 80° in a vacuum: C, 73·6; H, 4·2. $C_{44}H_{30}O_{10}$ requires C, 73·5; H, 4·2%). The derivative is readily soluble in cold chloroform and moderately readily soluble in ethyl acetate. Direct conversion into peltogynidin salts is very slow, but this may readily be demonstrated after hydrolysis by means of alcoholic potash.

O-*Tetra-anisoylpeltogynol*.—An anhydrous mixture of peltogynol (1·5 g.), anisoyl chloride (9 g.), and pyridine (6 drops) was kept for 2 days and then decomposed with water. The product was washed with aqueous sodium hydrogen carbonate, water, and light petroleum; it crystallised from acetic acid in colourless plates, m. p. 218° with decomposition to a red solid (Found: C, 68·6; H, 4·5; MeO, 14·5. $C_{48}H_{38}O_{14}$ requires C, 68·7; H, 4·5; 4MeO, 14·8%). The substance is sparingly soluble in methyl alcohol and ether, moderately readily so in acetone, and freely soluble in benzene; it resembles the benzoate in many respects.

O-*Tetramethylpeltogynol* (III; R, R′ = Me).—Methyl sulphate (25 g.) and the requisite amount of aqueous potassium hydroxide (10%) to maintain alkalinity were alternately added in three portions to a mechanically stirred solution of peltogynol (5 g.) in methyl alcohol (25 g.; acetone was used with identical results) in an atmosphere of nitrogen. After 2 hours, water was added, the product taken up in benzene, and the extract washed with dilute aqueous potassium hydroxide and water. It was concentrated to a small volume and on the addition of light petroleum the crude product (4·5 g.) was precipitated. The derivative was crystallised from aqueous alcohol (50%) and then from methyl or ethyl alcohol, forming colourless, glistening, long, rectangular plates, m. p. 175° (Found: C, 66·8; H, 5·9; MeO, 33·5. $C_{20}H_{22}O_6$ requires C, 67·0; H, 6·1; 4MeO, 34·6%). The low value for methoxyl has been observed on several occasions with both the tetramethyl and the trimethyl ether; it is doubtless due to the observed formation of insoluble, partly methylated flavylium iodides. The derivative is moderately readily soluble in most organic solvents ($[\alpha]_D^{20°}$ + 264°; c = 0·73 in chloroform) and some of its properties have been already mentioned. Especially characteristic is the brilliant green fluorescence of the trimethylpeltogynidin salts obtained by the usual methods (hot hydrochloric acid, cold alcoholic hydrogen chloride or bromine–dioxan); there is little doubt that these salts contain the tetranuclear heterocyclic system of the formula (III).

O-*Trimethylpeltogynol* (III; R = Me, R′ = H).—(A) The formation of this substance in methylation experiments when the solution was allowed to become acid and then kept for some hours was fortuitous and can easily be avoided as described in the last section. Most of the substance examined was, however, obtained in this way and separated from the tetramethyl ether by taking advantage of its more sparing solubility in organic solvents; it crystallised from ethyl acetate in long flat needles or plates, m. p. 198° (Found: C, 66·2; H, 5·8; MeO, 26·9. $C_{19}H_{20}O_6$ requires C, 66·3; H, 5·8; 3MeO, 27·0%). The micro-Zeisel treatment was specially prolonged and lower results were usually obtained (25—26%). The pure substance is sparingly soluble in cold benzene and crystallises from the hot solvent in needles (complete separation from tetramethyl ether); it is readily soluble in cold chloroform ($[\alpha]_D^{20°}$ + 254°; c = 0·236 in chloroform).

(B) A cold solution of *O*-tetramethylpeltogynol (1 g.) in acetic acid (20 c.c.) and hydrochloric acid (10 c.c. of 15%) was kept under nitrogen for 12 hours; water and benzene were

then added and the product was precipitated from the organic layer by means of light petroleum. The solid was triturated with 50% aqueous acetone, a small quantity of a colourless substance, m. p. above 230°, remaining undissolved; the solution was added to water, and the precipitate washed with methyl alcohol and a little cold benzene and crystallised from benzene and from alcohol. The long, flat, colourless needles had m. p. 198°, alone or mixed with the specimen previously obtained. Direct comparison of the properties also showed that the samples were identical. The significance of this experiment is explained in the introductory section.

The trimethyl ether affords the same trimethylpeltogynidin salt as the tetramethyl ether.

2 : 4-*Dinitrophenylhydrazone of Peltogynol.*—The derivative separated as an orange-red amorphous precipitate when aqueous solutions of peltogynol and of 2 : 4-dinitrophenylhydrazine in 1% hydrochloric acid were mixed and kept for a few hours. On trituration with a little acetic acid a crystalline powder was obtained, but this could not be recrystallised. A warm mixture of peltogynol and dinitrophenylhydrazine in acetic acid was seeded with the powder and, on scratching, the substance crystallised to a small extent on the sides of the vessel; the tube was washed out without removing the adhering crystals and a solution of peltogynol (0·5 g.) and dinitrophenylhydrazine (0·35 g.) in acetic acid (10 c.c.) was filtered into it. The mixture was then heated on the steam-bath for 15 minutes and allowed to cool. Almost the whole of the product separated from the hot solution; it was collected and washed with acetic acid and ether and formed an orange microcrystalline powder consisting of dense aggregates of needles. On heating, darkening commenced at about 190°, the substance was chocolate-brown at 220°, and it exploded at 224° (Found in material dried at 110° in a vacuum over phosphoric oxide : C, 54·5; H, 3·8; N, 11·5. $C_{22}H_{18}O_9N_4$ requires C, 54·8; H, 3·7; N, 11·6%). The substance is very sparingly soluble in most organic solvents, for example, in boiling acetic acid or ethyl acetate, but when impure it dissolves readily in alcohol; it is deposited very slowly indeed when its hot saturated solutions are cooled. The characteristic crimson peltogynidin chloride is obtained on boiling with concentrated hydrochloric acid. The solution in aqueous sodium hydroxide quickly develops an intense violet-brown colour. The ferric reaction in dioxan is an intense yellowish-ivy green coloration.

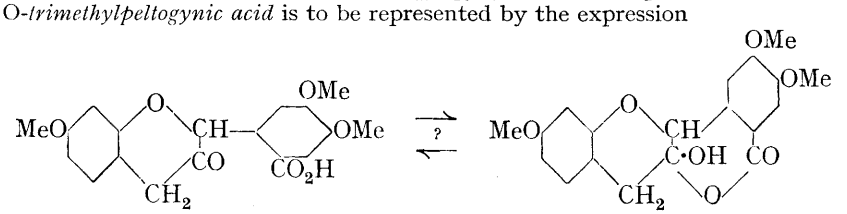
Oxidation of Peltogynol with Nitric Acid. Formation of Styphnic Acid.—A mixture of peltogynol (1 g.) and nitric acid (10 c.c., *d* 1·42) was heated over a gauze for 1 minute; dense fumes accompanied the vigorous oxidation. The reaction was allowed to subside and the process twice repeated. After the addition of water (50 c.c.) the solution was extracted with benzene (20 c.c.) and then with toluene (20 c.c.) and the combined extracts were concentrated to 10 c.c. The crude styphnic acid was precipitated by the addition of light petroleum and crystallised twice from ethyl acetate and again from benzene–toluene, forming almost colourless, flat needles and rectangular plates, m. p. 175°, alone or mixed with an authentic specimen. The substance tallied with styphnic acid in its properties and for further confirmation the highly characteristic compound with naphthalene and acetone was prepared. This crystallised from acetone in long yellow needles, which were washed with ether and then had m. p. 163—164°, alone or mixed with an authentic specimen. With the aid of this derivative more styphnic acid could be obtained from the aqueous solution already extracted with benzene and toluene by further extraction with ethyl acetate. This procedure gave a product too impure for direct crystallisation.

Attempts to demonstrate the resorcinol nucleus by potash fusion of peltogynol were only partly successful; the products gave the fluorescein reaction.

Oxidation of Trimethylpeltogynol with Nitric Acid.—*O*-Trimethylpeltogynol (2·2 g.) was heated on the steam-bath with nitric acid (50 c.c., *d* 1·42) and water (25 c.c.) until the vigorous evolution of fumes ceased; the liquid was then concentrated to one half its bulk and refluxed for 15 minutes. Finally most of the nitric acid was removed by distillation, and the residue mixed with water and extracted with ether. The ethereal layer was washed with aqueous sodium carbonate (probably removing styphuric acid) until the washings were colourless, then dried, and evaporated; the residue crystallised and was thrice recrystallised from ethyl alcohol, eventually forming pale yellow needles, m. p. 131° alone or mixed with authentic 4 : 5-dinitroveratrole. 2 : 3-Dimethoxyphenanthraphenazine was also prepared from the dinitroveratrole obtained as above and this derivative exhibited the characteristic violet fluorescence in benzene solution and magenta coloration in sulphuric acid solution.

Oxidation of Trimethylpeltogynol with Potassium Permanganate.—Although dioxan is not really adequately stable to permanganate, the oxidation was conducted in this solvent. A solution of potassium permanganate (10 g.) in water (300 c.c.) was gradually added to one of *O*-trimethylpeltogynol (3·5 g.) in pure dioxan (75 c.c.), mixed with saturated aqueous sodium

carbonate (10 c.c.), and heated on the steam-bath. The oxidation was slow at first, and when the permanganate was reduced, the straw-yellow filtered solution was concentrated to 100 c.c. and acidified with hydrochloric acid. The precipitated ochreous acid (filtrate A) was washed with ether and dried (0·56 g.). The substance was dissolved in acetone, the solution decolorised with the minimum of activated alumina and added to a large volume of pure ether, and the filtered ethereal solution thoroughly washed with water. It was then dried over sodium sulphate and concentrated to a small volume. The acid separated as a colourless microcrystalline crust and it was further purified by a repetition of the process (Found in material dried at 110° in a high vacuum over phosphoric oxide : C, 63·5; H, 5·3; MeO, 25·2. $C_{19}H_{18}O_7$ requires C, 63·7; H, 5·0; 3MeO, 26·0%. $C_{19}H_{20}O_7$ requires C, 63·3; H, 5·5%). The substance, which gradually decomposes and chars above 200° without melting, is readily soluble in alcohol and acetone, sparingly so in benzene and ether; it yields a red flavylium salt on treatment with bromine in hot technical dioxan solution. The formula $C_{19}H_{18}O_7$ is the more probable and we consider that this O-*trimethylpeltogynic acid* is to be represented by the expression



The Congo-red reaction of the filtrate (A) was removed by the addition of sodium acetate, and calcium oxalate then precipitated by means of calcium chloride; the clear centrifuged liquid was saturated with sodium chloride, strongly acidified with hydrochloric acid, and repeatedly extracted with ether. The ethereal solution was dried and evaporated, leaving a considerable crystalline residue of *m*-hemipinic acid. This was recognised by the formation of the characteristic silver hydrogen *m*-hemipinate and by that of the anhydride, which sublimed in leaflets, m. p. 175°. The acid was dissolved in an excess of aqueous ethylamine, and the solution distilled, finally by strong heating in a glass tube. The ethylimide obtained as a distillate and sublimate crystallised from alcohol in almost colourless needles, m. p. 230° alone or mixed with an authentic specimen (Found : C, 61·2; H, 5·6; N, 5·9. Calc. for $C_{12}H_{13}O_4N$: C, 61·3; H, 5·5; N, 5·9%).

The manganese precipitate was suspended in water, and the manganese dioxide dissolved by passing sulphur dioxide. The pale pink flocculent residual precipitate consisted of unchanged trimethylpeltogynol mixed with a large proportion of a much more sparingly soluble substance; it was not further examined, as similar material has been obtained in other ways and it is surmised that condensation of two molecules is responsible for such deterioration.

O-*Tetramethylfisetinidin Chloride.*—This salt was obtained in quantitative yield by the passage of hydrogen chloride into a cold solution of *p*-methoxysalicylaldehyde (0·5 g.) and **ω : 3 : 4**-trimethoxyacetophenone (0·7 g.) in ethyl acetate (10 c.c.). It crystallised, in deep brown needles with a green glance, almost completely from the reaction mixture; it crystallised well from 6% hydrochloric acid in chocolate needles (Found in air-dried material : C, 53·3, 53·4; H, 6·1, 6·2; MeO, 28·7. $C_{19}H_{19}O_5Cl,3{\cdot}5H_2O$ requires C, 53·6; H, 6·1; 4MeO, 29·2%). The salt exhibits the usual properties of its type; it gives readily a colourless pseudo-base and its solutions have the usual bluish-red (alcoholic) to orange-red (aqueous) colour, less blue-toned than cyanidin derivatives and much less blue-toned than peltogynidin salts under similar conditions. The aqueous and alcoholic solutions are non-fluorescent. If a solution of formaldehyde, trioxymethylene or methylene sulphate in sulphuric acid is added to a cold solution of the salt in the same solvent, a rapid change towards a bluish-red tone is observed; at the same time, intense green fluorescence is developed and on dilution a fluorescent solution is obtained which tallies closely with one of trimethylpeltogynidin salts. The further stages of this investigation will be reported subsequently, but the significance of even the qualitative observation cannot be overlooked.

The following report has been submitted by Miss D. Crowfoot, to whom we are greatly indebted.

Peltogynol.—Monoclinic needles elongated along *b*, flattened on (001). Birefringence positive and very high. Large optic axial angle.

X-*Ray data.* $a = 7{\cdot}91$ (limits 7·89, 7·99), $b = 7{\cdot}32$ (limits 7·30, 7·35), $c = 23{\cdot}25$; $\beta = 72°$. $c \sin\beta = 22{\cdot}34$ (limits 22·22, 22·46), $a \sin\beta = 7{\cdot}52$. $P = 1{\cdot}535$ (limits 1·531, 1·538). $M = 301$ *

* $C_{16}H_{14}O_6$ requires *M*, 302; $C_{24}H_{22}O_{10}$ requires *M*, 470.

(limits 296, 307) with $n = 4$. Space group $P2$, from $(0k0)$ halved when k odd. There are therefore two molecules in the asymmetric unit. There is also a halving of $(00l)$ with l odd not required by symmetry, which suggests that these two molecules are related approximately by a screw axis along $\perp$ (001). The dimensions of the unit cell and the kind of optics suggest lath-shaped molecules (*i.e.*, that one dimension is decidedly longer than the other two), arranged flat in b—the α optic direction.

The requirements fit very closely with formula (I) (p. 746), from which a plausible structure can be devised. Formula (II) is very difficult to fit at all. Its great breadth can just be fitted by allowing considerable slanting in b, but even so the length is shorter than would be expected.

Acetyl Derivative of Peltogynol.—Long needles. Orthorhombic elongated along b with m faces developed. {101}. Birefringence high, positive. Small optic axial angle. $a = \beta$, $b = \alpha$, $c = \gamma$.

X-*Ray data*. $a = 13{\cdot}26$, $b = 5{\cdot}70$, $c = 30{\cdot}10$. $(h00)$, $(0k0)$, $(00l)$ halved for h, k, l odd respectively. Space group $P2$, 2, 2. $n = 4$. $M = 473 \pm 6$.*

The dimensions found and the optics associated with them immediately suggest a parallel with certain types of crystallographic arrangement found in the sterols. Such an analogy would be reasonable if the structure of this compound were based on (I), not on (II).

The breadth of (II) again is difficult to fit into the $\alpha\beta$ section as would be required if the straightforward interpretation is given to the optics. This is not required, however, by orthorhombic symmetry as it is by monoclinic. But any structure based on (II) seems to be much less satisfactory than those derived from (I).

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