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1. *Puberulic and Puberulonic Acids. Part I. The Molecular Formula of Puberulonic Acid and Consideration of Possible Benzenoid Structures for the Acids.*

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The molecular formula of puberulonic acid, recorded by earlier workers as $C_8H_4O_6$, has been revised to $C_9H_4O_7$. A possible trihydroxyformylbenzoic acid structure for puberulic acid has been shown to be untenable by the non-identity of 2 : 3 : 5-*trihydroxybenzaldehyde* with the decarboxylation product of puberulic acid. Benzenoid formulæ for these acids are therefore excluded.

PUBERULIC and puberulonic acids were first isolated by Birkinshaw and Raistrick (*Biochem. J.*, 1932, **26**, 441) from cultures of the moulds *Penicillium puberulum* and *P. aurantio-virens* and the formulæ $C_8H_6O_6$ and $C_8H_4O_6$ respectively were assigned to them. Both compounds titrated as dibasic acids, and the almost colourless puberulic acid, m. p. 316—318°, could be separated from mixtures of the two as its diacetyl derivative, m. p. 212°, whereas the yellow puberulonic acid, m. p. 298°, was unaffected by acetylating agents. Puberulic acid formed a tetramethyl derivative with diazomethane, and diacetylpuberulic acid formed a dimethyl derivative under the same conditions, so that the molecule of puberulic acid contained four acidic groups of which at least two were enolic or phenolic hydroxyl groups. No evidence was obtained for the presence of a carbonyl group, and the authors were unable to suggest a satisfactory structure either for puberulic or for puberulonic acid. A little later Barger and Dorrer (*ibid.*, 1934, **28**, 11) carried out a further investigation of the two acids but likewise were unable to advance structural formulæ to explain all of the experimental results. They showed that puberulic acid almost certainly contained a carboxyl group since it readily lost carbon dioxide when heated, yielding a crystalline product, $C_7H_6O_4$. Puberulonic acid changed from yellow through pink to colourless in the course of neutralisation with sodium hydroxide and, on the assumption of a formula $C_8H_4O_6$, irregular values were given for the basicity : a value of 2·3 was obtained when the acid was titrated directly, whereas back titration of alkaline solutions gave values varying from 2·9 to 3·5. This seemed to suggest the presence of a lactone or pseudo-acid grouping. In a footnote to their paper Barger and Dorrer (*loc. cit.*) stated that Dr. Hoyer had obtained evidence that puberulonic acid is structurally related to puberulic acid but the nature of this evidence has never been divulged.

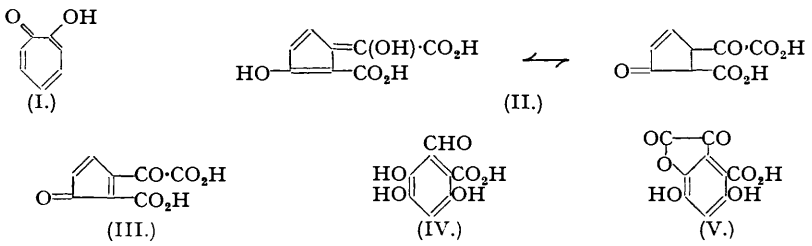
No further work on the chemistry of these compounds has been published. Oxford, Raistrick, and Smith (*Chem. and Ind.*, 1942, **61**, 485) have described the isolation of the same acids from *P. Johannioli* Zaleski and *P. cyclopium–viridicatum* series and have reported that these mould metabolic products have a significant antibiotic activity against various Gram-positive organisms. Dewar (*Nature*, 1945, **155**, 479) suggested that puberulic acid might be a hydroxystipitatic acid but adduced no evidence to support this formulation; stipitatic acid itself was claimed by the same author (*ibid.*, p. 50) to be a derivative of tropolone (I). More recently McGowan (*Chem. and Ind.*, 1947, **66**, 205) advanced structures (II) and (III) for puberulic and puberulonic acid respectively, but again without experimental support.

In the present work, the two acids were isolated from the culture medium of *P. aurantio-virens* essentially as described by Birkinshaw and Raistrick (*loc. cit.*), although the yield was increased by using a relatively high temperature (30°) for the growth of the mould. The formula for puberulonic acid, $C_8H_4O_6$, put forward by earlier workers has been amended to $C_9H_4O_7$, the results of analyses being supported by the preparation of a *complex*, $C_9H_4O_7,C_5H_5N$, with pyridine, of a condensation *product*, $C_{15}H_8O_5N_2$, with *o*-phenylenediamine, and by experiments on the hydrolysis of the acid. When puberulonic acid is heated in dilute sulphuric acid solution, one

molecular equivalent of carbon dioxide is evolved and puberulic acid is obtained in quantitative yield :

$$C_9H_4O_7 + H_2O \longrightarrow C_8H_6O_6 + CO_2$$

Thus the relationship between the acids has been finally established.

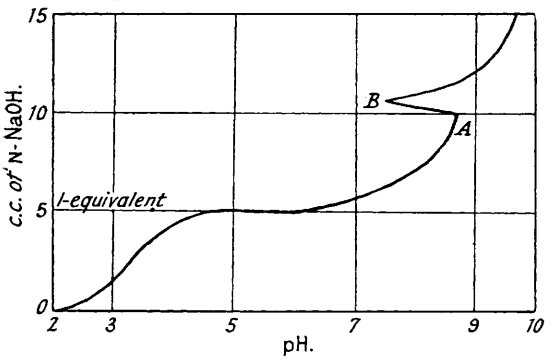


The potentiometric titration (Fig. 1) of puberulonic acid gives some support to the suggestion that a lactone grouping is present in the molecule, for during the neutralisation of the free acid a decrease in the pH was apparent on leaving the solution to stand or on further slow addition of small amounts of alkali at any point between about pH 7 and pH 10. An illustration of this effect is shown in Fig. 1.

Since in many respects puberulic acid has properties not unlike those of an aromatic compound, it seemed advisable to consider at the outset whether the evidence available supported a benzenoid formula. Birkinshaw and Raistrick (*loc. cit.*) and Barger and Dorrer (*loc. cit.*) had rejected the possibility that puberulic acid might be a dihydroxybenzenedicarboxylic acid because the decarboxylation product they obtained was not identical with any of the dihydroxybenzoic acids. Several pieces of evidence suggest that puberulic acid contains three phenolic hydroxyl groups; the dimethyl derivative formed on alkaline hydrolysis of " tetramethylpuberulic acid " gives a strong ferric reaction, and the *diacetyl* derivative of decarboxylated puberulic acid likewise gives an immediate coloration with ferric chloride. Furthermore, the isolation of a compound containing only one carbomethoxy-group from the hydrogenation product of the tetramethyl derivative suggests that puberulic acid itself contains only one carboxyl group. For these reasons structure (IV) for puberulic acid appeared worthy of consideration; on this basis the formulation of puberulonic acid as (V) might account for its condensation with *o*-phenylenediamine in the manner of an α-diketone, its yellow colour, and, if somewhat less readily, its conversion into puberulic acid on acid hydrolysis.

FIG. 1.

Potentiometric titration of puberulonic acid. The portion A—B of the curve shows the decrease in pH during slow addition of alkali.



The most serious objections to formulæ (IV) and (V) were that no carbonyl derivatives of either puberulic or decarboxylated puberulic acid could be prepared and that puberulonic acid could not be acetylated, but nevertheless it was felt that the hypothesis should be tested by comparison of decarboxylated puberulic acid with 2 : 3 : 5-*trihydroxybenzaldehyde*. This aldehyde had not been previously described and it has now been obtained in 20% overall yield by the Rosenmund reduction of 2 : 3 : 5-triacetoxybenzoyl chloride and subsequent hydrolysis of the 2 : 3 : 5-*triacetoxybenzaldehyde* produced. A comparison of the properties of 2 : 3 : 5-trihydroxybenzaldehyde and the decarboxylation product of puberulic acid shows that there is little resemblance between them (Table I). The ultra-violet absorption spectra of the two compounds are shown in Fig. 2, and those of puberulic and puberulonic acids in Fig. 3.

The other trihydroxybenzaldehydes have been described in the literature and all are different from the decarboxylation product of puberulic acid. The only possible benzenoid structures, $C_8H_6O_6$, which accommodate two phenolic groups and a carboxyl group are those of the

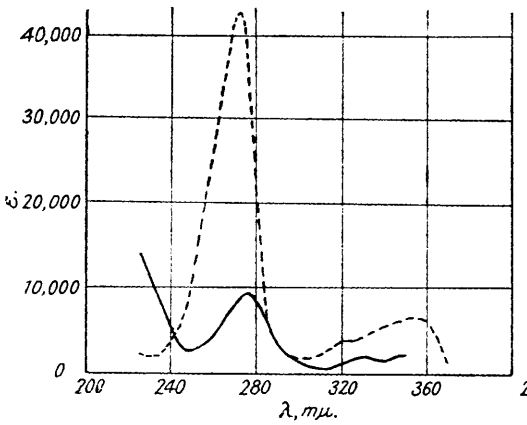
dihydroxybenzenedicarboxylic acids and the trihydroxyformylbenzoic acids (*e.g.*, IV); these structures have now been shown to be untenable for puberulic acid. Other ring structures must be considered and we have, therefore, taken up afresh degradative studies on both puberulic and puberulonic acids; the results will be presented in a separate communication.

TABLE I.

	2 : 3 : 5-Trihydroxybenzaldehyde.	Decarboxylated puberulic acid.
Colour	Canary yellow	Colourless
M. p.	187° (decomp.)	237—238°
2 : 4-Dinitrophenylhydrazone	Orange-red derivative	No reaction
Ferric reaction	Olive green	Deep blood-red
Condensation with ω-hydroxyacetophenone	Wine-red colour	No reaction
Coloration with aqueous sodium hydroxide	Golden-yellow	Light brown; rapidly darkens

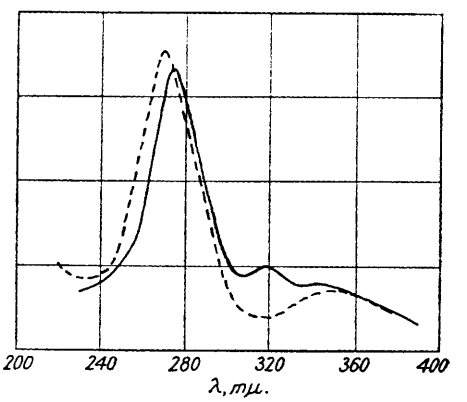
2 : 3 : 5-Trihydroxybenzaldehyde was also prepared, albeit in poor yield, by oxidation of α-resorcylaldehyde with potassium persulphate. There are several recorded examples of the

FIG. 2.



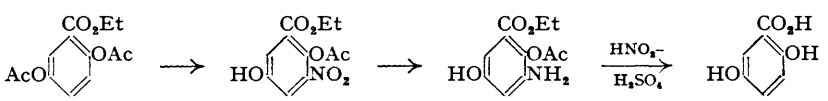
——— 2 : 3 : 5-*Trihydroxybenzaldehyde in ethanol.*
— — *Decarboxylated puberulic acid in ethanol.*

FIG. 3.



——— *Puberulonic acid in water.*
— — *Puberulic acid in water.*

preparation of aromatic hydroxyaldehydes by this reaction (*e.g.*, Baker and Brown, *J.*, 1948, 2303) but this is the first instance in which it has been used to hydroxylate a dihydroxybenzaldehyde. The oxidation was carried out in an atmosphere of nitrogen and the product isolated by the method of Baker, Brown, and Scott (*J.*, 1939, 1922). In connection with the synthesis of 2 : 3 : 5-triacetoxybenzoyl chloride required for the preparation of the trihydroxybenzaldehyde, a number of routes to 2 : 3 : 5-*trihydroxybenzoic acid* were investigated and it was eventually obtained in 15% yield by persulphate oxidation of α-resorcylic acid. The conditions for this oxidation were worked out in preliminary experiments on the oxidation of salicylic acid to gentisic acid (cf. Schering, D.R.-P. 81,297; Graebe and Martz, *Annalen*, 1905, **340**, 213; Nandor and Mauthner, *J. pr. Chem.*, 1940, **156**, 150) which was itself required for another possible route to 2 : 3 : 5-trihydroxybenzoic acid *via* 3-amino-2-acetylgentisic acid. This latter compound, in



the form of its *ethyl* ester, was diazotised by the method of Hodgson and Walker (*J.*, 1933, 1620), but heating the diazonium salt with 60% sulphuric acid under reflux gave gentisic acid (30%). This behaviour recalls that of aminogallic acid which under similar conditions is deaminated giving gallic acid (Power and Shedden, *J.*, 1902, **81**, 73). On the other hand 5-aminosalicylic acid was successfully converted into gentisic acid by this method.

Experimental.

Puberulic and Puberulonic Acid.—The acids were isolated from the culture medium of *Penicillium aurantio-virens* by a modification of the method of Birkinshaw and Raistrick (*loc. cit.*). The mould was grown at 30° rather than at 24—25° as previously recommended, thereby increasing the yield of the acid and decreasing the production of mould mycelium. After separation of the medium from the associated mycelial mat, and clarification of the solution by passage through a Sharples centrifuge, the pH was adjusted to 5·5 by the addition of sodium hydroxide solution. N-Nickel sulphate solution (900 c.c. for each 15 l. of mould solution) was then added, the pH again brought to 5·5, and the red nickel complex which separated was collected on the Sharples centrifuge. Decomposition of the nickel complex and separation of the mixed acids were carried out by the method of Barger and Dorrer (*loc. cit.*). From 30 l. of culture fluid 12 g. of mixed puberulic and puberulonic acid were obtained.

Puberulic acid (2 g.) was crystallised from methanol (50 c.c.), dissolution being effected in a hot extraction apparatus. The acid formed almost colourless plates, m. p. 318—320° (Found: C, 48·6; H, 3·0. Calc. for $C_8H_6O_6$: C, 48·5; H, 3·05%). The ultra-violet absorption of puberulic acid showed maxima at 2700 A. (ε, 35,200) and 3500 A. (ε, 7,160).

Puberulonic acid crystallised from ethyl malonate as fine yellow needles, m. p. 298° (decomp.) (Found: C, 48·0; H, 2·0. $C_9H_4O_7$ requires C, 48·2; H, 1·8. $C_8H_4O_6$ requires C, 49·0; H, 2·1%). Light absorption: Maxima at 2745 A. and 3170 A. (ε, 33,400 and 9,900 respectively). For the potentiometric titration of puberulonic acid, a well stirred suspension of the acid (1·12 g.) in water (20 c.c.) was neutralised with N-sodium hydroxide. The titration was followed potentiometrically by means of a calomel electrode–glass electrode system. The results are recorded in Fig. 1. The solution became pink at pH 7—8. This colour change was most marked after the addition of two equivalents of sodium hydroxide, and after the addition of three equivalents of alkali (pH 9·6) the solution remained orange in colour. No colour change was observed during the back-titration, and puberulonic acid was recovered unchanged from the acid solution. Barger and Dorrer (*loc. cit.*) claimed that the solution became colourless at pH 8—9.

Condensation of Puberulonic Acid with o-Phenylenediamine.—The acid (112 mg.) and *o*-phenylenediamine (60 mg.) were dissolved in just sufficient ethanol and heated under reflux for 4 hours, during which time a vermilion-red crystalline solid (125 mg.) separated. This *product* charred without melting on being heated to 350° and it was soluble with difficulty in water and organic solvents (Found: C, 60·6; H, 3·0; N, 9·3. $C_{15}H_8O_5N_2$ requires C, 60·8; H, 2·7; N, 9·4%).

Puberulonic Acid–Pyridine Complex.—Puberulonic acid (90 mg.) was heated with pyridine (2 c.c.) on a water-bath for 6 hours. Excess of pyridine was removed under reduced pressure, and the residual yellow solid was extracted with acetone to give relatively soluble unchanged puberulonic acid (10 mg.), leaving an insoluble *product* (70 mg.) which formed yellow needles, m. p. 257—258° (decomp.), from glacial acetic acid (Found: C, 55·5; H, 3·2; N, 4·6. $C_9H_4O_7,C_5H_5N$ requires C, 55·4; H, 3·0; N, 4·6%).

Conversion of Puberulonic Acid into Puberulic Acid.—A continuous stream of nitrogen, freed from carbon dioxide and oxygen, was passed through a boiling suspension of puberulonic acid (295 mg.) in 2N-sulphuric acid (50 c.c.). The carbon dioxide evolved in the reaction was collected quantitatively in barium hydroxide solution (90 c.c. of N/10) and after 8 hours the excess of barium hydroxide was determined by titration. Continuation of the hydrolysis for a further 2 hours did not change the titre (volume of barium hydroxide neutralised by carbon dioxide, 28 c.c.; calc. for 1 molar equivalent of carbon dioxide, 30 c.c.). The brown crystalline solid (243 mg.) formed in the course of reaction was separated and mixed with a further small quantity of solid (25 mg.) obtained by continuous ether extraction of the filtrate for 8 hours. The crude mixture, m. p. 305°, was repeatedly crystallised from acetone to give a faintly yellow micro-crystalline powder, m. p. 315° alone or mixed with an authentic specimen of puberulic acid (Found: C, 48·8; H, 3·2%).

Hydrogenation of Tetramethylpuberulic Acid (cf. Barger and Dorrer, *loc. cit.*).—Tetramethylpuberulic acid (534 mg.), m. p. 112°, was hydrogenated in pure glacial acetic acid at atmospheric temperature and pressure with a platinum catalyst. Six moles of hydrogen were absorbed and the solution was decolorised after the addition of 5 moles. The residue (500 mg.) obtained on removal of the acetic acid under reduced pressure was treated with water (5 c.c.), and the solution extracted with ether (2 × 10 c.c.). When the residual aqueous solution was treated with 2 : 4-dinitrophenylhydrazine a voluminous red precipitate separated. This precipitate was amorphous and oxidised in air; all attempts to purify it were unsuccessful. The ethereal extract was dried and evaporated, yielding a light-coloured oil (260 mg.) which on distillation gave a volatile fraction (160 mg.) and a residue which decomposed when further heated. The volatile fraction was redistilled; it had b. p. 120—125°/0·1 mm. and left only a trace of residue [Found: C, 59·7; H, 8·9; sap. val., 202, 204; active H (Zerewitinoff), 0·30%. $C_8H_{15}O_2(CO_2CH_3)$ requires C, 59·5; H, 8·9%; sap. val., 202; 1 active H, 0·49%].

Acetylation of Decarboxylated Puberulic Acid.—Decarboxylated puberulic acid (150 mg.) (Barger and Dorrer, *loc. cit.*), acetic anhydride (1·0 g.), and sodium acetate (0·6 g.) were heated under reflux at 140—150° for 30 minutes. The mixture was then diluted with water (20 c.c.) and acidified with hydrochloric acid. The clear solution was evaporated under reduced pressure at 25° and the residue extracted with ether. Removal of the solvent from the ethereal extract gave a brown resin which was distilled (110°/0·4 mm.) to give a light-yellow resin (70 mg.). The latter crystallised from ether–light petroleum (b. p. 40—60°) as colourless prisms, m. p. 124·5—125° [Found: C, 55·1; H, 4·4. $C_7H_4O_2(O{\cdot}CO{\cdot}CH_3)_2$ requires C, 55·4; H, 4·2%]. The *diacetyl* derivative gave an immediate purple coloration with ferric chloride.

Gentisic Acid.—(a) *From salicylic acid.* Salicylic acid (18 g., 1 mol.) was dissolved in water (375 c.c.) containing sodium hydroxide (25 g., 5 mols.). The solution was brought to 35—40° and maintained at this temperature during the course of the experiment. Potassium persulphate (35 g., 1·1 mols.) was added portionwise during 3 hours. The reaction mixture, which rapidly developed a deep chocolate-brown colour, was left for 36 hours at room temperature and then acidified with concentrated hydrochloric acid until just acid to Congo-red. This solution was extracted with ether (3 × 300 c.c.), and the combined

ethereal extracts dried and evaporated to yield unchanged salicylic acid (9·5 g.). A further quantity of hydrochloric acid (100 c.c.) was added to the mother-liquors which were then kept at room temperature for 48 hours and extracted with ether (5 × 300 c.c.). The combined ethereal extracts were dried, clarified with charcoal, and evaporated, to yield crude gentisic acid (9·5 g.), m. p. 195—198°, usually of sufficient purity for conversion into the ester. Further purification was effected by recrystallisation of the crude acid from water (charcoal).

(b) *From 5-aminosalicylic acid.* 5-Aminosalicylic acid (Puxeddu, *Gazzetta*, 1929, **59**, 10) (4·3 g.) was dissolved in concentrated sulphuric acid (30 c.c.) and the solution cooled to below 10°. A solution of sodium nitrite (2·5 g.) in concentrated sulphuric acid (15 c.c.) was prepared by stirring the nitrite into the acid at 0° and completing dissolution by raising the temperature to 70°. After cooling to 5°, this solution was stirred into the sulphuric acid solution of 5-aminosalicylic acid, and the mixture added portionwise to glacial acetic acid (55 c.c.) during an hour, the temperature being kept below 20°. Anhydrous ether (50 c.c.) was then added, whereupon a fine white precipitate of sodium sulphate was obtained and after 3 hours at 0° this was removed (4·0 g.). A further quantity of ether (300 c.c.) was stirred into the mixture, and after 24 hours at 0° a large quantity of feathery crystals had separated which were shown to be the diazonium salt from 5-aminosalicylic acid. The crystalline solid was separated, and washed with 50% glacial acetic acid–ether (20 c.c.) and finally with ether (20 c.c.). The still moist crystals were dissolved in sulphuric acid (15 c.c. of 20%), and the solution added dropwise to boiling sulphuric acid (70 c.c. of 60%). After the mixture had been heated under gentle reflux for 45 minutes, evolution of nitrogen ceased and the solution was cooled and extracted with ether (5 × 100 c.c.). The dried ethereal extract was clarified with charcoal and evaporated to yield gentisic acid (2·1 g.), m. p. 196—198°.

2 : 3 : 5-*Trihydroxybenzoic Acid.*—The experimental procedure was similar to that described for gentisic acid. α-Resorcylic acid (32 g.) yielded unchanged starting material (12 g.) and crude 2 : 3 : 5-*trihydroxybenzoic acid* (10 g.). The crude acid was washed with ice-water and recrystallised repeatedly from water (charcoal) to give the pure acid (2·5 g.) as lemon-yellow needles, m. p. 223° (decomp.) (Found : C, 49·8; H, 3·6. $C_7H_6O_5$ requires C, 49·4; H, 3·5%). The acid gave a reddish-brown colour with ferric chloride.

2 : 3 : 5-*Triacetoxybenzoic Acid.*—2 : 3 : 5-Trihydroxybenzoic acid (7·2 g.), sodium acetate (15 g.), and acetic anhydride (30 c.c.) were heated initially at 85—90° until the acid dissolved, and then under reflux for 3 hours. The cooled solution was poured into water (150 c.c.), acidified with sulphuric acid (21 c.c. of 10N.), and extracted with ether (6 × 200 c.c.). After removal of the ether, the *triacetoxy-acid* remained as a white crystalline solid (11·4 g.) and after recrystallisation from benzene had m. p. 139—140° (Found : C, 53·1; H, 4·1. $C_{13}H_{12}O_8$ requires C, 52·7; H, 4·1%).

2 : 3 : 5-*Triacetoxybenzoyl Chloride.*—2 : 3 : 5-Triacetoxybenzoic acid (6·1 g.) and thionyl chloride (20 c.c.) were heated on a water-bath under reflux for 40 minutes, by which time evolution of sulphur dioxide had ceased. Removal of the excess of thionyl chloride *in vacuo* gave a gum which crystallised slowly in the ice-chest. The crystals were washed with light petroleum (b. p. 60—80°), and dried on porous tile and then in a vacuum-desiccator over phosphoric oxide. The product (6·3 g.) had m. p. 70—75°. Further purification could not be effected; attempted micro-distillation caused decomposition, and crystallisation could not be achieved from light petroleum, benzene, or xylene. With *p*-toluidine, the acid chloride gave 2 : 3 : 5-*trihydroxybenzo*-p-*toluidide*, which had m. p. 166—167° after recrystallisation from ethanol (Found : C, 62·7; H, 5·2; N, 3·7. $C_{20}H_{19}O_7N$ requires C, 62·3; H, 5·0; N, 3·6%).

2 : 3 : 5-*Triacetoxybenzaldehyde.*—2 : 3 : 5-Triacetoxybenzoyl chloride (5·5 g.), xylene (30 c.c.), and palladium–barium sulphate (2 g.; 2% of Pd) were placed in a 150-c.c. round-bottomed flask equipped with a reflux condenser, a sealed Hershberg stirrer, and a gas inlet tube reaching to the bottom of the flask. A brisk stream of hydrogen was passed through the rapidly stirred mixture, and the temperature raised to reflux. The exit gases were bubbled into water, and the evolved hydrogen chloride titrated from time to time. After 5 hours no more hydrogen chloride was formed, 85% of the theoretical amount having been collected. After cooling, catalyst was removed and washed thoroughly with dry ether (150 c.c.). The ether and xylene extracts were combined, washed with sodium hydrogen carbonate solution (50 c.c.) and then water (2 × 50 c.c.), and dried (Na_2SO_4). After removal of the solvent *in vacuo*, the resinous residue crystallised partly on prolonged cooling. The crystals were washed with dry ether (10 c.c.) and recrystallised first from benzene and then from ether–light petroleum (b. p. 40—60°) or from benzene. The *product* (3·2 g.) had m. p. 90—91° (Found : C, 55·6; H, 4·6. $C_{13}H_{12}O_7$ requires C, 55·7; H, 4·3%). The 2 : 4-*dinitrophenylhydrazone* crystallised from acetic acid (90%) in canary-yellow feathery needles, m. p. 240° (decomp.) (Found : C, 49·3; H, 3·6; N, 12·5. $C_{19}H_{16}O_{10}N_4$ requires C, 49·7; H, 3·3; N, 12·2%).

2 : 3 : 5-*Trihydroxybenzaldehyde.*—(a) *From* 2 : 3 : 5-*triacetoxybenzaldehyde.* The triacetoxy-aldehyde (1·2 g.) and anhydrous potassium acetate (4·8 g.) were dissolved in dry methanol (50 c.c.) and heated under reflux in a current of hydrogen for 2 hours. The cooled solution was acidified to Congo-red with sulphuric acid (30 c.c. of 2N.) and kept at 0° for 3 hours. Sodium sulphate was separated by filtration, and the filtrate extracted with ether (5 × 50 c.c.). The extract was dried and the solvent removed *in vacuo*, leaving a black tar. This was dried by distillation with benzene and again extracted with dry ether (100 c.c.). Removal of the ether gave a lemon-yellow solid (200 mg.) which was further purified by sublimation at 130°/10^{-2} mm., giving a yellow micro-crystalline solid, m. p. 187° (decomp.), alone or mixed with the product prepared by persulphate oxidation (see below).

(b) *From α-resorcylaldehyde.* α-Resorcylaldehyde (22·5 g., 1 mol.) was dissolved in aqueous sodium hydroxide [32·6 g. (5 mols.) in 450 c.c. of water] which had been freed from air by passing nitrogen through it for some time. The solution was warmed to 35—40° and maintained at this temperature during the course of the experiment. Potassium persulphate (48 g., 1·1 mols.) was added in portions during 3 hours, the reaction mixture rapidly becoming deep chocolate-brown. The mixture was set aside at room temperature for 36 hours, and then acidified with concentrated hydrochloric acid until just acid to Congo-red. The copious chocolate-coloured precipitate which separated was collected (18 g.); it was mainly unchanged starting material. The mother-liquors were extracted with ether

(3 × 300 c.c.), and the extract gave a further small amount of unchanged starting material. Concentrated hydrochloric acid (150 c.c.) and ether (300 c.c.) were added to the aqueous layer, and the mixture was heated under reflux on a water-bath at 70° for 1 hour. The ethereal layer was separated, dried, and clarified with charcoal. After removal of the solvent the dark-coloured residue was extracted with boiling benzene, and the benzene extract on cooling deposited a small amount of yellow crystalline material (20 mg.). Further extraction of the mother-liquors with chloroform (4 × 300 c.c.) and removal of the chloroform under reduced pressure gave a second crop of crude material (20 mg.). Purification by sublimation at 130°/10^{-2} mm. gave a yellow micro-crystalline solid which charred at 160° and decomposed at 187° (Found: C, 54·9; H, 4·0. $C_7H_6O_4$ requires C, 54·5; H, 3·9%).

The *aldehyde* gave an olive-green colour with ferric chloride and a wine-red colour (pyrylium salt) with ω-hydroxyacetophenone under the conditions used in anthocyanin synthesis (*e.g.*, Robinson and Todd, *J.*, 1932, 2293). The 2 : 4-*dinitrophenylhydrazone*, m. p. >255° (decomp.), was very sparingly soluble in the common organic solvents (Found: N, 16·8. $C_{13}H_{10}O_7N_4$ requires N, 16·8%).

2-*Hydroxy*-3 : 5-*dimethoxybenzoic Acid*.—2 : 3 : 5-Trihydroxybenzoic acid (500 mg.) and methyl sulphate (6 c.c.) were stirred vigorously during dropwise addition of 5% potassium hydroxide solution. The addition was carried out at such a rate that the solution remained acid to litmus. A slight excess of potassium hydroxide solution was then added and the mixture heated at 70° for 1½ hours. The cooled mixture was acidified with 3N-hydrochloric acid and continuously extracted with ether for 6 hours. The ethereal extract was dried and then decolorised with charcoal, and the solvent removed *in vacuo*, yielding a brown solid. After two recrystallisations from water this gave colourless needles, m. p. 180·5° (Found: C, 54·7; H, 5·6. $C_9H_{10}O_5$ requires C, 54·5; H, 5·1%). The acid gave a deep greenish-blue ferric chloride reaction. This *acid* appeared to be identical with the hydroxydimethoxybenzoic acid obtained by Calam, Clutterbuck, Oxford, and Raistrick (*Biochem. J.*, 1947, **41**, 458) in the course of their degradation of methylated geodin.

Ethyl Diacetylgentisate.—Ethyl gentisate (21·5 g.; Juch, *Monatsh.*, 1905, **26**, 841) was dissolved in acetic anhydride (115 c.c.), concentrated sulphuric acid (2 c.c.) added, and the mixture set aside at room temperature for 36 hours. The solution was poured into ice–water (400 c.c.) and set aside in the ice-chest; the colourless oil which separated deposited crystals of the *diacetyl* ester (27·0 g.), which had m. p. 69·5° after recrystallisation from light petroleum (b. p. 60—80°) (Found: C, 58·6; H, 5·2; *O*-Ac, 34·1. $C_{13}H_{14}O_6$ requires C, 58·7; H, 5·3; *O*-Ac, 32·3%).

Ethyl 3-*Nitro*-2-*acetylgentisate*.—Ethyl diacetylgentisate (14·8 g.) was added in small portions during 90 minutes to well-stirred fuming nitric acid (14·3 c.c.; *d*, 1·5) maintained at 10—15° by ice-cooling. After addition was complete, the mixture was set aside for 40 minutes and the temperature allowed to rise to 20°. The mixture was then poured on crushed ice, whereupon the *nitro*-ester separated as an amorphous solid. Crystallisation from 50% ethanol (charcoal) yielded fine lemon-yellow needles (7·5 g.), m. p. 104·5° (Found: C, 49·3; H, 4·0; N, 5·3. $C_{11}H_{11}O_7N$ requires C, 49·0; H, 4·1; N, 5·2%).

Ethyl 3-*Amino*-2-*acetylgentisate*.—The foregoing nitro-ester (5 g.) was dissolved in methanol (300 c.c.) and hydrogenated using a Raney nickel catalyst (3 g.) at atmospheric pressure. The hydrogen uptake, which was slightly in excess of the calculated value for the hydrogenation of the nitro-group to an amino-group, was complete in 2 hours. After the wine-red solution had been decolorised with charcoal, methanol was removed *in vacuo* to yield the *amino*-ester (3·7 g.) as colourless plates, m. p. 139·5—140° after crystallisation from 80% methanol (Found: C, 55·4; H, 5·2; N, 6·0; *O*-Ac, 21·0. $C_{11}H_{13}O_5N$ requires C, 55·2; H, 5·4; N, 5·9; *O*-Ac, 18·0%).

Diazotisation and Hydrolysis of Ethyl 3-*Amino*-2-*acetylgentisate*.—The above amino-ester (1·25 g.), dissolved in glacial acetic acid (12·5 c.c.), was stirred into a mixture of sodium nitrite (0·6 g.) and concentrated sulphuric acid (6·5 c.c.), the temperature being kept below 15° by external cooling. The mixture was allowed to warm to room temperature, and after 30 minutes diazotisation was complete as shown by coupling tests with α-resorcylic acid. The solution was diluted with an equal volume of water and added dropwise to boiling sulphuric acid (50 c.c. of 60%) during 10 minutes. After cooling, the solution was continuously extracted with ether for 6 hours, and the ethereal extract dried and clarified with charcoal. Evaporation yielded crude gentisic acid (0·22 g.). Purified by sublimation it had m. p. 200° alone or mixed with an authentic specimen.

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