

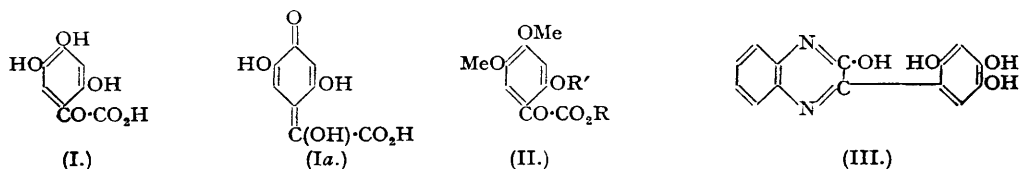
660. *The Chemistry of Fungi. Part XIV. 2 : 4 : 5-Trihydroxyphenylglyoxylic Acid from Polyporus tumulosus Cooke.*

By B. J. RALPH and ALEXANDER ROBERTSON.

The metabolic liquor of *Polyporus tumulosus* Cooke, grown on an artificial medium containing "Marmite," has been shown to contain oxalic acid, homoprotocatechuic acid, and 2 : 4 : 5-trihydroxyphenylglyoxylic acid. Syntheses of the last and of its 4 : 5-dimethyl ether are described.

IN the course of an extensive investigation of the metabolic products of Basidiomycetes fungi (including the wood-rotting fungi) at present in progress in these laboratories it has been found that, when grown on a synthetic medium containing "marmite," *Polyporus tumulosus* Cooke, the cause of "brown rot" in fallen jarrah (*Eucalyptus marginata*) logs, gives a clear bright-

yellow metabolic liquor from which ether extracted a complex mixture. By fractionation of this product there has been isolated oxalic acid, homoprotocatechuic acid, and a new acid $C_8H_6O_6$ shown to be 2 : 4 : 5-trihydroxyphenylglyoxylic acid (I) by the following reactions.



On being warmed with concentrated sulphuric acid the glyoxylic acid (I) gives carbon monoxide and 2 : 4 : 5-trihydroxybenzoic acid which was identified by its conversion into methyl asarylate. By the methyl sulphate-potassium carbonate method (I) gives the methyl ester (II; $R = R' = Me$) in which the carbonyl group is established by the formation of an oxime and a 2 : 4-dinitrophenylhydrazone. Hydrolysis of (II; $R = R' = Me$) gave the acid (II; $R = H$, $R' = Me$) which formed a 2 : 4-dinitrophenylhydrazone and on oxidation with alkaline hydrogen peroxide gave asarylic acid. With hot aqueous sodium hydroxide the acid (I) gave oxalic acid and 2 : 4 : 5 : 2' : 4' : 5'-hexahydroxydiphenyl which was isolated as the hexamethyl ether and which clearly arose by the oxidation of hydroxyquinol initially formed. In agreement with the behaviour of *o*-hydroxyphenylglyoxylic acid (Marchlewski and Sosnowski, *Ber.*, 1901, 34, 2291) the acid (I) gave the hydroxyquinoxaline (III). The same compound (III) was obtained from the ester of a monomethyl ether of (I) formed by means of diazomethane with which (I) curiously enough appears to react abnormally. In this respect and in its failure to form diagnostic carbonyl derivatives the acid (I) closely resembles the 2 : 4-dihydroxyphenylglyoxylic acids (Hunsberger and Amstutz, *J. Amer. Chem. Soc.*, 1948, 70, 671). Noteworthy in this connection is the possibility of the acid (I) existing in tautomeric forms of which (Ia) is an example (cf. Hunsberger and Amstutz, *loc. cit.*).

The structure of the acid (I) was confirmed by its synthesis from hydroxyquinol and ethyl cyanoformate and also by the synthesis of (II; $R = R' = H$) which on methylation gave (II; $R = R' = Me$).

The production, by the mould, of the two related catechol derivatives, *viz.*, homoprotocatechuic acid and (I), is noteworthy and, although the hydroxylation of the catechol nucleus can be effected by biological agents, it is not possible at present to say whether their occurrence in the present conditions implies a direct phytochemical relationship. This question is being investigated and it may be significant that in old cultures (100 days) only traces of homoprotocatechuic acid are present.

EXPERIMENTAL.

Isolation of the Crude Metabolic Products.—The mould *Polyporus tumulosus* Cooke was grown on the liquid medium (sterile, with initial pH 4.44) described by Williams and Saunders (*Biochem. J.*, 1934, 28, 1887), modified by the substitution of glucose (50 or 100 g. per l.) and glycine (2 g. per l.) respectively for sucrose and asparagine and by the addition of 0.1% of "marmite" (yeast extract); the growing conditions were a temperature of 30° for 40–60 days. An extensive study of the effects of variations in the medium and conditions of growth on the metabolic products produced by this mould will be reported elsewhere.

To the clear yellow acidified metabolic liquor (batches of 15–30 l.) ammonium sulphate (100 g. per l.) was added and the filtered mixture exhausted in a counter-current extractor with ether. The extract (1.5–2 l.) was filtered through a bed of sodium sulphate and evaporated; the yield was about 3.3 g. per l. of liquor. When a solution of a sample of this semi-solid in toluene was distilled the distillate deposited oxalic acid which was identified by analysis and comparison with an authentic sample.

Depending on the use of benzene-acetone, various fractionation procedures were employed for the separation of homoprotocatechuic acid and 2 : 4 : 5-trihydroxyphenylglyoxylic acid from the ethereal extract of the metabolic liquor. The following procedure, however, was found to be more convenient. A solution of the crude extract (7.85 g.) in saturated aqueous sodium hydrogen carbonate (100 ml.; pH 7.95) was exhausted with ether by the continuous method and the extract evaporated, leaving an oily residue (100 mg.). The acidity of this aqueous liquor was then adjusted to pH 4.0 with hydrochloric acid and extraction with ether continued; evaporation of this extract gave a protocatechuic acid fraction (1.7 g.). The aqueous phase was then acidified to pH 1.5 and again exhausted with ether during several days, giving a sticky red crystalline product (3.83 g.) consisting mainly of 2 : 4 : 5-trihydroxyphenylglyoxylic acid.

Homoprotocatechuic Acid.—A solution of the crude acid (1 g.) in hot ethyl acetate (25 ml.) was mixed with hot ligroin (50 ml.), filtered to remove a small dark red precipitate, and cooled, giving homoprotocatechuic acid in glistening colourless plates (0.8 g.), m. p. 125–126°, which on recrystallisation had

m. p. 128.5°, identical with an authentic specimen (Pictet and Gams, *Ber.*, 1909, **42**, 2949) [Found : C, 57.2; H, 4.8%; *M* (by titration), 167.3. Calc. for $C_8H_8O_4$: C, 57.1; H, 4.8%; *M*, 168]. Prepared with methyl sulphate and aqueous sodium hydroxide, the dimethyl ether formed lustrous plates, m. p. 98°, identical with authentic 3 : 4-dimethoxyphenylacetic acid [Found : C, 61.1; H, 6.0; OMe, 30.8. Calc. for $C_8H_8O_2(OMe)_2$: C, 61.2; H, 6.1; OMe, 31.6%].

2 : 4 : 5-Trihydroxyphenylglyoxylic Acid.—Repeated crystallisation of the crude compound gave the acid, m. p. 193°, giving a slightly turbid solution in water. A solution of this product (1 g.) in water (50 ml.) was clarified by repeated filtration and the acid isolated from the liquor with ether in a continuous-extraction apparatus. Recrystallised from hot benzene-acetone, 2 : 4 : 5-trihydroxyphenylglyoxylic acid formed bright red prisms, m. p. 193° (decomp.), readily soluble in water, alcohol, or acetone, sparingly soluble in benzene or chloroform, and almost insoluble in light petroleum (b. p. 60–80°), giving a deep green ferric reaction in water or alcohol, decomposing Tollens's reagent, and reducing boiling Fehling's solution (Found, in specimen dried in a vacuum at room temperature: C, 48.3, 48.4; H, 2.6, 3.4. $C_8H_6O_6$ requires C, 48.5; H, 3.0%). From moist acetone-benzene this acid separated as a hydrate in long yellow needles, initial m. p. 197° (decomp.) changing to 193° (decomp.) (Found : C, 44.8; H, 3.7. $C_8H_6O_6 \cdot H_2O$ requires C, 44.4; H, 3.7%). Kept at 80° or in a vacuum over phosphoric oxide, this hydrate lost water with the regeneration of the anhydrous red form. On potentiometric titration the compound behaved as a dibasic acid, giving a curve with two points of inflection; equiv., 99.3 and 98.9 (theory for dibasic acid, 99.0). Warmed with ethyl acetate (20 ml.) and pyridine (5 ml.), the acid gave an almost quantitative yield of the pyridine salt which crystallised from water, containing a little pyridine, as a hydrate in massive, greenish yellow prisms, m. p. 188° (decomp.) (Found : C, 53.2; H, 4.7; N, 4.6. $C_8H_6O_6 \cdot C_5H_5N \cdot H_2O$ requires C, 52.9; H, 4.4; N, 4.7%).

On being heated in a vacuum with zinc dust (1.0 g.) at 210°/0.1 mm. the acid (0.5 g.) gave a dark red crystalline sublimate of 5 : 6-dihydroxycoumaran-1 : 2-dione, m. p. 232°, soluble in water, alcohol, and ethyl acetate and in aqueous sodium hydrogen carbonate and giving with alcoholic ferric chloride an intense amethyst colour which changed to a dirty-brown (Found : C, 53.3; H, 2.2. $C_8H_4O_6$ requires C, 53.3; H, 2.2%).

A cooled solution of 2 : 4 : 5-trihydroxyphenylglyoxylic acid (5 g.) in water (500 ml.) was treated with bromine (25 ml., dropwise) and then diluted with water (250 ml.). Next day the pale yellow turbid solution was mixed with more water (750 ml.) and decolorised with sodium hydrogen sulphite. On isolation the crystalline precipitate (5.65 g.) was washed, dried, and crystallised from carbon disulphide (by spontaneous evaporation) and then from aqueous acetic acid, giving hexabromoacetone in characteristic silky needles, m. p. 109°, identical with an authentic specimen (Levy and Jedlicka, *Annalen*, 1888, **249**, 66) (Found : C, 7.5; H, 0.14; Br, 90.4. Calc. for C_6OBr_6 : C, 6.8; Br, 90.2%). From the aqueous liquor left after the separation of hexabromoacetone oxalic acid was isolated with ether and identified by the preparation of the anilide.

Attempts to acetylate 2 : 4 : 5-trihydroxyphenylglyoxylic acid by acetic anhydride-sulphuric acid or -pyridine were unsuccessful. When the compound (1 g.) was heated with sodium acetate (0.5 g.) and acetic anhydride (5 ml.) on the steam-bath for 2 hours and poured on ice crystallisation of the product (100 mg.; isolated with ether) from nitrobenzene gave a substance in pale orange rhombohedra, m. p. 202° (slight decomp.) (Found : C, 55.3; H, 3.4%).

A solution of 2 : 4 : 5-trihydroxyphenylglyoxylic acid (1 g.) and *o*-phenylenediamine (1 g.) in 2*N*-hydrochloric acid (50 ml.) was heated on the steam-bath for $\frac{1}{2}$ hour. Purified from hot aqueous pyridine the dark red crystalline product (1.35 g.) gave 2-hydroxy-3-(2 : 4 : 5-trihydroxyphenyl)quinoxaline in long dark red needles, m. p. above 350°, soluble in aqueous sodium hydroxide and insoluble in aqueous sodium carbonate, alcohol, ethyl acetate, or benzene (Found : C, 62.7; H, 4.0; N, 10.1. $C_{14}H_{10}O_4N_2$ requires C, 62.2; H, 3.7; N, 10.4%).

On being warmed on the steam-bath the red solution of 2 : 4 : 5-trihydroxyphenylglyoxylic acid (1 g.) in concentrated sulphuric acid (10 ml.) evolved carbon monoxide and, when the evolution of gas had ceased (about 10 minutes), the mixture was poured on ice (150 g.). On isolation with ether the resulting 2 : 4 : 5-trihydroxybenzoic acid (0.51 g.) separated from nitrobenzene in silky needles, m. p. 207°, which by the methyl sulphate-potassium carbonate methylation process furnished methyl asarylolate. Purified from hot ligroin and then by distillation in a high vacuum followed by recrystallisation from ligroin, this ester formed stout colourless prisms, m. p. 92.5°, undepressed on admixture with an authentic specimen (Found : C, 58.8; H, 6.3. Calc. for $C_{11}H_{14}O_5$: C, 58.4; H, 6.2%).

2 : 4 : 5-Trimethoxyphenylglyoxylic Acid.—(a) A mixture of the 2 : 4 : 5-trihydroxyphenylglyoxylic acid (20 g.), potassium carbonate (100 g.), methyl sulphate (50 ml.), and acetone (500 ml.) was heated under reflux for 3½ hours; the reddish-brown mixture finally became pale yellow. On isolation the resulting methyl 2 : 4 : 5-trimethoxyphenylglyoxylate (19.9 g.) was purified by distillation in a vacuum at 145°/0.25 mm. and then by crystallisation from 50% alcohol, forming colourless needles, m. p. 135–135.5°, insoluble in alkali [Found : C, 56.6; H, 5.3; OMe, 47.5. $C_8H_2O_2(OMe)_4$ requires C, 56.7; H, 5.5; OMe, 48.8%]. Prepared by the pyridine method at room temperature, the oxime separated from 20% alcohol in colourless thick needles, m. p. 154° [Found : N, 5.3; OMe, 46.6. $C_8H_3O_2N(OMe)_4$ requires N, 5.2; OMe, 46.1%]. The 2 : 4-dinitrophenylhydrazone formed bright red needles, m. p. 232°, from dioxan [Found : C, 50.2; H, 4.2; N, 12.6; OMe, 26.4. $C_{14}H_4O_5N_4(OMe)_4$ requires C, 49.8; H, 4.2; N, 12.9; OMe, 28.6%].

Hydrolysis of the foregoing ester (1 g.) with boiling 2*N*-sodium hydroxide (50 ml.) gave 2 : 4 : 5-trimethoxyphenylglyoxylic acid (0.92 g.), m. p. 144–145°, in pale greenish-yellow needles which had m. p. 186° (decomp.) with sintering at 180°, after purification by means of sodium hydrogen carbonate and then aqueous sodium hydroxide [Found : C, 55.0; H, 5.2; OMe, 35.4. Calc. for $C_8H_3O_3(OMe)_3$: C, 55.0;

H, 5.0; OMe, 38.8%] (cf. Smith and Haller, *J. Amer. Chem. Soc.*, 1934, **56**, 238). This compound, which had a negative ferric reaction and did not react with *o*-phenylenediamine, gave a 2 : 4-dinitrophenylhydrazone, separating from alcohol and then ethyl acetate–light petroleum (b. p. 60–80°) (1 : 1) in slender light red needles which, on being heated slowly, decomposed at 234–235° after shrinking at 208°. Rapidly heated, this derivative had m. p. 208° [Found: C, 48.9; H, 4.2; OMe, 21.3. $C_{14}H_7O_6N_4(OMe)_3$ requires C, 48.6; H, 3.8; OMe, 22.1%].

Oxidation of 2 : 4 : 5-trimethoxyglyoxylic acid (from 2 g. of ester) in 2*N*-aqueous sodium hydroxide (60 ml.) with hydrogen peroxide (10 ml.) at room temperature for 1 hour and then on the steam-bath for 10 minutes gave rise to asaric acid. Crystallised from water (charcoal), this acid formed colourless needles (1.28 g.), m. p. 144–145°, identical with an authentic specimen [Found: C, 57.0; H, 5.7; OMe, 42.3. Calc. for $C_7H_5O_6(OMe)_3$: C, 56.6; H, 5.7; OMe, 43.9%].

(b) Methylation of 2 : 4 : 5-trihydroxyphenylglyoxylic acid (6 g.), dissolved in ether (60 ml.) and methanol (6 ml.), with an excess of ethereal diazomethane for 1 hour gave a tarry product from which warm xylene extracted the methyl ester (3.3 g.), m. p. 122–123°, of a monomethyl ether of the glyoxylic acid. On repeated recrystallisation this compound formed bright yellow needles, m. p. 124–124.5°, from xylene [Found: C, 52.7; H, 4.3; OMe, 26.2. $C_8H_5O_4(OMe)_2$ requires C, 53.1; H, 4.4; OMe, 27.4%], soluble in aqueous sodium hydroxide, insoluble in aqueous sodium carbonate and giving only a faint green ferric reaction in alcohol. On methylation by the methyl sulphate–potassium carbonate method it gave methyl 2 : 4 : 5-trimethoxyphenylglyoxylate, m. p. and mixed m. p. 135°. Boiled with a solution of *o*-phenylenediamine (0.5 g.) in alcohol (10 ml.) for 10 minutes the compound (0.5 g.) gave rise to 3-hydroxy-2-(2 : 4 : 5-trihydroxyphenyl)quinoxaline, m. p. above 350°, after purification from aqueous pyridine [Found: C, 62.5; H, 3.9; N, 9.9%]. Hydrolysis of this ester (0.5 g.) with boiling 20% alcoholic potassium hydroxide (20 ml.) for $\frac{1}{2}$ hour and isolation of the product with ether from the diluted and acidified hydrolysate gave rise to a monomethyl ether (0.45 g.) of 2 : 4 : 5-trihydroxyphenylglyoxylic acid which formed yellowish-green needles, m. p. 174–174.5°, from warm xylene [Found: C, 51.2; H, 3.8; OMe, 14.1; *M* (by potentiometric titration), 212.5. $C_8H_5O_5OMe$ requires C, 50.9; H, 3.8; OMe, 14.6%; *M*, 212]. With alcoholic or aqueous ferric chloride this compound gave a deep green colour which rapidly changed to greenish-brown.

(c) Obtained by the interaction of ethyl cyanoformate (Gluud *et al.*, *Chem. Zentr.*, 1934, **105**, II, 3437) (1.5 ml.) and 3 : 4-dimethoxyphenol (2.3 g.) in ether (100 ml.), saturated with hydrogen chloride, during 2½ hours, followed by evaporation of the reaction mixture in a vacuum, the product was a reddish oil, containing yellow crystals, which was heated under reflux with 10% aqueous potassium hydroxide (100 ml.) for $\frac{1}{2}$ hour. The cooled mixture was acidified and the resulting 2-hydroxy-4 : 5-dimethoxyphenylglyoxylic acid isolated with ether, and purified with the aid of aqueous sodium hydrogen carbonate and then by crystallisation from benzene–ligroin, forming orange prisms (1.6 g.), m. p. 142–144°, which had a transient green ferric reaction in alcohol or water. Methylation of this acid by the methyl sulphate–potassium carbonate method gave rise to methyl 2 : 4 : 5-trimethoxyphenylglyoxylate, m. p. 135–135.5°, identical with a specimen obtained by method (a) [Found: C, 56.6; H, 5.5; OMe, 50.0%].

2 : 4 : 5 : 2' : 4' : 5'-Hexamethoxydiphenyl.—2 : 4 : 5-Trihydroxyphenylglyoxylic acid (1 g.) was boiled with 20% aqueous sodium hydroxide (25 ml.) for $\frac{1}{2}$ hour and the cooled mixture acidified with 8*N*-sulphuric acid and extracted with ether. Obtained by evaporation of the dried extracts, the dark green product was methylated in boiling acetone (40 ml.) with methyl sulphate (5 ml.) and potassium carbonate for 4 hours, and the dark crystalline mixture washed with a little alcohol and then extracted with boiling water. Crystallisation of the light brown residue from 50% alcohol and then from light petroleum (b. p. 60–80°)–benzene gave a compound in colourless needles, m. p. 178°, the properties of which were identical with those of 2 : 4 : 5 : 2' : 4' : 5'-hexamethoxydiphenyl (Schüler, *Arch. Pharm.*, 1907, **245**, 263) [Found: C, 64.7; H, 6.5; OMe, 49.9; *M* (Rast), 358. Calc. for $C_{12}H_4(OMe)_6$: C, 64.7; H, 6.6; OMe, 55.7%; *M*, 334] (Schüler, *loc. cit.*, and Fabini and Szeki, *Ber.*, 1910, **43**, 2682, respectively give m. p. 177° and m. p. 180°).

(With K. R. HARGREAVES) *Synthesis of 2 : 4 : 5-Trihydroxyphenylglyoxylic Acid*.—A stirred mixture of hydroxyquinol (Healy and Robinson, *J.*, 1934, 1625), ethyl cyanoformate (*loc. cit.*) (4 ml.), and ether (100 ml.) was saturated at 0° with dry hydrogen chloride during 2½ hours. Next day more ether was added to the reaction mixture, the solvent was decanted, and a solution of the viscous red product in water (30 ml.) was heated on the steam-bath for 20 minutes, cooled, and extracted with ether (20 ml. \times 3). The combined ethereal solutions were extracted with aqueous sodium hydrogen carbonate (20 ml. \times 4), and the extracts acidified with hydrochloric acid, giving a yellow precipitate of 2 : 4 : 5-trihydroxyphenylglyoxylic acid (1.2 g.); more acid (2.0 g.) was isolated from the acidic filtrate with ether. Crystallised from acetone–benzene, the anhydrous compound formed bright red prisms, m. p. 193° (decomp.), identical with the natural product [Found: C, 48.2; H, 3.1%]. Methylation of this acid by the methyl sulphate–potassium carbonate method gave methyl 2 : 4 : 5-trimethoxyphenylglyoxylate, m. p. 135°, undepressed on admixture with a specimen obtained from the natural acid.