493. *The Chemistry of Fungi. Part XX.* Metabolites of* Chaetomium indicum *Corda.*

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The isolation of a tribasic acid $C_{14}H_{12}O_7$ and two nitrogenous acids from the metabolic liquors of *Chaetomium indicum* Corda grown on Czapek–Dox medium is recorded. The acid $C_{14}H_{12}O_7$ is shown to be 4-carboxy-2-oxo-3-phenylhept-3-enedioic acid (I), or, less probably, the δ-lactone of type (III) derived from it.

IN an examination of the metabolic products of the genus *Chaetomium* of the Ascomycetes Fungi the growth of *C. indicum* Corda on a Czapek–Dox medium gave a clear orange metabolic liquor from which an acid $C_{14}H_{12}O_7$ was isolated along with small amounts of two nitrogenous products (A) and (B). This acidic metabolite, which had an intense green ferric reaction, was optically inactive and on potentiometric titration behaved as a tribasic acid with, curiously, only one point of inflexion on the pH titration curve, indicating that the three acidic groups are of similar strength; on esterification with ethereal diazo-

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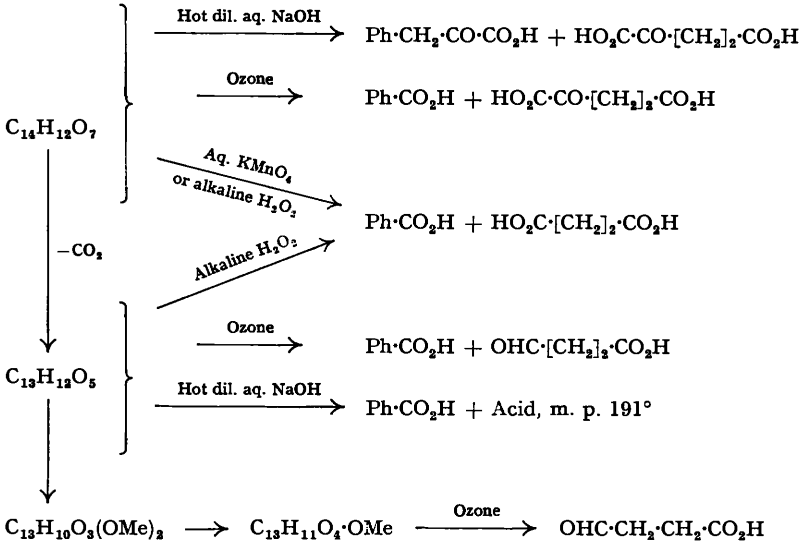
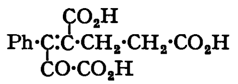
* Part XIX, preceding paper.

methane it gave a trimethyl derivative. The metabolite did not react with the usual carbonyl reagents and behaved as a saturated compound towards perbenzoic acid, bromine in acetic acid or chloroform, and hydrogen in the presence of palladium–charcoal or Raney nickel. On being heated in a vacuum at 180° or boiled with dilute sulphuric acid the compound lost a molecule of carbon dioxide and yielded a dibasic acid $C_{13}H_{12}O_5$, which had the same green ferric reaction and was characterised by the formation of a dimethyl derivative. Like the parent compound this acid failed to react with carbonyl reagents, bromine, perbenzoic acid, or hydrogen in the presence of a catalyst. The annexed scheme summarises the reactions and degradations of the metabolite $C_{14}H_{12}O_7$ and its decarboxylation product $C_{13}H_{12}O_5$.

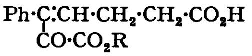


The production of the decomposition products indicated strongly supports the view that the acid $C_{14}H_{12}O_7$ is 4-carboxy-2-oxo-3-phenylhept-3-enedioic acid (I), and similarly its decarboxylation product is 2-oxo-3-phenylhept-3-enedioic acid (II; R = H). These formulations, however, do not account for the characteristic intense ferric reactions of the acids which, curiously, are not given by their methyl derivatives.

(I)



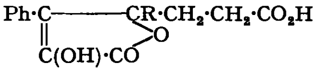
$\mathrm{Ph{\cdot}\underset{CO{\cdot}CO_2H}{C}{:}\overset{CO_2H}{C}{\cdot}CH_2{\cdot}CH_2{\cdot}CO_2H}$

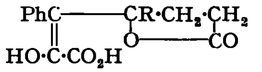


$\mathrm{Ph{\cdot}\underset{CO{\cdot}CO_2R}{C}{:}CH{\cdot}CH_2{\cdot}CH_2{\cdot}CO_2H}$

(II)

(III)





(IV)

Clearly the acids formulated as (I) and (II; R = H) could give rise to lactones of two types (III and IV; R = CO_2H), and (III and IV; R = H) which would account for the ferric reactions exhibited by the metabolite and its decarboxylation product. Nevertheless, on the basis of the degradation experiments recorded, apart from the ferric reactions, we regard these acids as being represented by formula (I) and (II; R = H) respectively. In support of this it may be noted, *inter alia*, that on ozonolysis the metabolite gives α-oxoglutaric acid whilst the monomethyl ester of its decarboxylation product yields β-formylpropionic acid, which also serves to show that this ester has formula (II; R = Me). In an attempt to differentiate further between the structures of types (I) and (III) the infra-red absorption spectra of the metabolite and its decarboxylation product were kindly examined for us by Dr. A. J. Ham of the Shell Petroleum Company Ltd. Although there appears to be some evidence against the lactone formula, in the absence of data for analogous compounds the results were inconclusive. With regard to the ferric reactions exhibited by these compounds it may well be that they are due to the presence of small amounts of the lactone forms.

Owing to lack of material the nature of the acid, m. p. 191°, formed in small amounts along with benzoic acid by treatment of the acid $C_{13}H_{12}O_5$ with alkali has not been defined. It does not react with carbonyl reagents and has a negative ferric reaction. By analogy with the formation of α-oxoglutaric acid from (I) the expected product would have been β-formylpropionic acid. This compound (Wislicenus, Boklen, and Reuthe, *Annalen*, 1908, **363**, 353; Perkin and Sprankling, *J.*, 1899, **75**, 16; Carrière, *Ann. Chim.*, 1922, **17**, 38) is described as a liquid rapidly forming a solid trimer, m. p. 167°. By boiling this with aqueous alkali Carrière (*loc. cit.*) obtained an acidic product as its semicarbazone but was unable to isolate the parent acid. We have found that on being kept or on treatment with warm alkali as described by Carrière β-formylpropionic acid forms a polymeric substance, m. p. 184°, which depresses the melting point of the acidic compound, m. p. 191°. The product, m. p. 167°, was not observed.

The two nitrogenous metabolites (A), m. p. 159°, and (B), m. p. 146°, have the properties of saturated dibasic acids containing a hydroxamic acid residue and will be the subject of further investigation when sufficient material has been accumulated.

EXPERIMENTAL

Isolation of the Metabolic Products.—Cultures of the mould *Chaetomium indicum* Corda were maintained on slopes of glucose–peptone–agar. For production of the metabolites the organism was grown on liquid Czapek–Dox medium (sterile, with initial pH 4·2) at 30° for 22—24 days.

The filtered orange metabolic liquors (100 l.) were acidified (pH 1·0) with 10% sulphuric acid and 24 hr. later the resulting flocculent brown precipitate, which had settled, was collected and the aqueous filtrate concentrated in a "climbing-film" evaporator. After the addition of ammonium sulphate (1·5 kg.) the resulting concentrate (20—25 l.) was exhaustively extracted with ether in a counter-current extractor, and the gummy residue left on the evaporation of the dried extract was crystallised from hot chloroform (*ca.* 700 ml.). Repeated recrystallisation of the product from chloroform–light petroleum (b. p. 60—80°) then gave *4-carboxy-2-oxo-3-phenylhept-3-enedioic acid* (25—50 g.) in colourless, rectangular prisms containing chloroform of crystallisation which was removed on the steam-bath during 1 hr. The compound then had m. p. 170° (decomp.), λ_{max} 288 ($\log_{10} \varepsilon$ 4·25) and $\lambda_{min.}$ 237 ($\log_{10} \varepsilon$ 3·32) in EtOH [Found: C, 57·6; H, 4·2%; *M* (Rast), 291; equiv., by potentiometric titration, 97·4. $C_{14}H_{12}O_7$ requires C, 57·5; H, 4·1%; *M*, 292; equiv., for a tribasic acid, 97·3). This acid is moderately soluble in cold water and readily soluble in hot water and in the usual organic solvents except chloroform or light petroleum. It slowly dissolves in aqueous sodium hydrogen carbonate with the evolution of carbon dioxide.

Treatment of the acid (1 g.) dissolved in ether at 0° with an excess of ethereal diazomethane for 15 min. gave the *trimethyl ester* which was purified by being washed in ethereal solution with aqueous sodium hydrogen carbonate followed by distillation and obtained as a pale yellow viscous oil (1 g.), b. p. 187—189°/0·4 mm., insoluble in aqueous sodium hydroxide [Found: C, 61·5; H, 5·2; OMe, 26·0. $C_{14}H_9O_4(OMe)_3$ requires C, 61·6; H, 5·4; OMe, 27·8%].

The brown precipitate, obtained on acidification of the metabolic liquor, was extracted with light petroleum (b. p. 60—80°) and on concentration this extract deposited *substance* (A) in small, pale yellow needles which, after repeated purification from chloroform–light petroleum, had m. p. 159°, $[\alpha]_D^{20}$ +11·4° (*c*, 1·022 in $CHCl_3$), $\lambda_{max.}$ 286 ($E_{1\,cm.}^{1\%}$ 346) (yield, 1·5—2·0 g. from 100 l. of liquor) [Found, in specimen dried in a high vacuum at 70°: C, 67·7; H, 8·0; N, 3·1%; equiv. (potentiometrically), 231; *M* (Rast), 445. $C_{26}H_{37}O_6N$ requires C, 68·0; H, 8·1; N, 3·1%; equiv., 229·8; *M*, 459·6]. This compound, which readily dissolved in aqueous sodium hydrogen carbonate, gave a wine-red ferric reaction in alcohol and a highly insoluble green-blue copper derivative.

When a culture aged 18 months was employed for the production of 4-carboxy-2-oxo-3-phenylhept-3-enedioic acid, the brown precipitate contained in place of (A) smaller amounts of a *substance* (B) which was isolated and purified by the method employed for (A). Compound (B) separated from light petroleum (b. p. 60—80°) in small rosettes of colourless rectangular prisms, m. p. 146°, $[\alpha]_D^{20}$ +120° (*c*, 1·01 in $CHCl_3$), $\lambda_{max.}$ 287 ($E_{1\,cm.}^{1\%}$ 305) [Found, in specimen dried in a high vacuum at 70° : C, 68·1; H, 8·2; N, 2·7; *C*-Me, 12·0%; equiv., 245·3; *M* (Rast), 482·8]. This compound had the same wine-red ferric reaction as (A) and formed an insoluble green copper derivative.

2-*Oxo*-3-*phenylhept*-3-*enedioic Acid*.—When a solution of (I) (2 g.) in 2N-sulphuric acid (50 ml.) was boiled in nitrogen for 2 hr. and then cooled, crystalline 2-*oxo*-3-*phenylhept*-3-*enedioic acid* (1·4 g.) separated and, on purification from acetone–light petroleum (b. p. 60—80°), formed small colourless, rectangular prisms, m. p. 178°, $\lambda_{max.}$ 285 ($\log_{10} \varepsilon$ 4·35) and $\lambda_{min.}$ 233 ($\log_{10} \varepsilon$ 2·62) in EtOH, having an intense green ferric reaction in alcohol [Found: C, 62·5; H, 5·0%; *M* (Menzies–Wright), 245 in EtOH. $C_{13}H_{12}O_5$ requires C, 62·9; H, 4·9%; *M*, 248]. The same product (160 mg.) was formed by heating the parent acid (200 mg.) at 180° in a high vacuum and had m. p. and mixed m. p. 178° after purification from ether and then acetone–light petroleum. It is readily soluble in the usual solvents except water and light petroleum and dissolves slowly in aqueous sodium hydrogen carbonate.

When the effluent gases from the boiling sulphuric acid solution were led into aqueous barium hydroxide a precipitate of barium carbonate separated, whereas with aqueous 2 : 4-dinitrophenylhydrazine sulphate a precipitate was not formed.

Treatment of 2-oxo-3-phenylheptadienoic acid (1 g.) in a mixture of methanol (20 ml.) and ether (25 ml.) at 0° with an excess of ethereal diazomethane for 15 min. gave an oil. A solution of this in ether (25 ml.) was washed with aqueous sodium hydrogen carbonate, dried, and evaporated, leaving the *dimethyl* ester which slowly solidified and then separated from light petroleum (b. p. 40—60°) in rosettes of long, slender needles, m. p. 52—53°, insoluble in aqueous sodium hydroxide [Found: C, 65·5; H, 5·8; OMe, 21·2. $C_{13}H_{10}O_3(OMe)_2$ requires C, 65·2; H, 5·8; OMe, 22·5%]. This ester behaved as a saturated compound and failed to react with carbonyl reagents. The same ester (1 g.), m. p. and mixed m. p. 52—53°, was formed by esterification of the acid (1 g.) with methyl iodide (5 ml.) and potassium carbonate (7·5 g.) in boiling acetone (60 ml.) for 15 hr.

When a solution of this ester (1 g.) in methanol (15 ml.) was treated with 2N-aqueous sodium hydroxide (15 ml.) the initial precipitate rapidly dissolved and 5 min. later the mixture was acidified with concentrated hydrochloric acid, diluted with water (125 ml.), and extracted with ether (25 ml. × 3). The ethereal extracts were washed with 2N-sodium hydrogen carbonate (30 ml. × 3), and the washings acidified and extracted with ether. Evaporation of the combined, dried extracts left *methyl hydrogen* 2-*oxo*-3-*phenylhept*-3-*enedioate* which solidified on trituration with light petroleum (b. p. 60—80°), and then crystallised from the same solvent in rosettes of colourless needles (0·75 g.), m. p. 127—128°, having a negative ferric reaction (Found: C, 64·0; H, 5·2; OMe, 13·0. $C_{13}H_{11}O_4{\cdot}OMe$ requires C, 64·1; H, 5·4; OMe, 11·8%). Treatment of this compound with ethereal diazomethane regenerated the parent dimethyl ester, m. p. and mixed m. p. 52—53°.

Degradation of 4-*carboxy*-2-*oxo*-3-*phenylhept*-3-*enedioic Acid with Aqueous Sodium Hydroxide*.—A solution of the acid (1 g.) in 2N-sodium hydroxide was boiled in nitrogen for ½ hr., cooled, acidified with 2N-sulphuric acid, and extracted with ether in a continuous extractor for 20 hr. The semi-solid orange-red product left on evaporation of the ethereal extract was repeatedly extracted with boiling benzene, leaving an intractable residue (*ca.* 400—500 mg.). Concentration of the combined benzene extracts gave a crystalline product, part of which readily redissolved in a little hot benzene. On cooling, this solution deposited phenylpyruvic acid in colourless plates (250 mg.), m. p. 158°, identical with an authentic specimen and having an intense green ferric reaction (Found: C, 66·2; H, 5·0. Calc. for $C_9H_8O_3$: C, 65·9; H, 4·9%). The 2 : 4-dinitrophenylhydrazone separated from dilute alcohol in small yellow needles, m. p. 190—191°, identical with an authentic specimen (Found: C, 53·3; H, 3·6; N, 16·2. Calc. for $C_{15}H_{12}O_6N_4$: C, 52·3; H, 3·5; N, 16·3%).

The crystalline residue, sparingly soluble in hot benzene, was purified by repeated sublimation in a high vacuum and gave α-oxoglutaric acid in colourless needles, m. p. 115—116°, undepressed on admixture with an authentic specimen (Found: C, 41·1; H, 4·2. Calc. for $C_5H_6O_5$: C, 41·1; H, 4·1%). The 2 : 4-dinitrophenylhydrazone formed rosettes of yellow needles, m. p. 227° (decomp.), from dilute alcohol, identical with an authentic specimen (Found: C, 40·1; H, 3·4; N, 17·6. Calc. for $C_{11}H_{10}O_3N_4$: C, 40·5; H, 3·1; N, 17·2%).

When the alkaline hydrolysate (25 ml.) from the acid (500 mg.) was acidified, diluted with water, and treated with 2 : 4-dinitrophenylhydrazine (1 g.), and the precipitate was dissolved in concentrated sulphuric acid (25 ml.) and water (200 ml.), and heated on the steam-bath for 10 min., a yellow precipitate of the mixed 2 : 4-dinitrophenylhydrazones of phenylpyruvic acid and α-oxoglutaric acid was obtained. This was resolved by means of hot benzene, and the components were identified by comparison with authentic specimens. When the acidified hydrolysate was diluted with water and distilled the distillate did not contain a volatile acid.

Oxidation of 4-*Carboxy*-2-*oxo*-3-*phenylhept*-3-*enedioic Acid.*—(*a*) 5% Aqueous potassium permanganate (50 ml.) was added dropwise to a solution of the acid (1 g.) in acetone (50 ml.) during ½ hr. and, after the mixture had been cleared with sulphur dioxide and the greater part of the acetone evaporated, the aqueous liquor was extracted continuously with ether for 12 hr. From the semi-crystalline product (0·6 g.) left on evaporation of the ethereal extract boiling light petroleum (b. p. 60—80°) extracted benzoic acid (0·4 g.), m. p. and mixed m. p. 122°, after crystallisation from water and sublimation at 100—110°/2·4 mm.

The residue, insoluble in light petroleum, was triturated with a little chloroform and then crystallised from acetone–light petroleum (b. p. 60—80°), giving succinic acid in colourless needles (0·1 g.), m. p. and mixed m. p. 185° (Found : C, 41·3; H, 5·1. Calc. for $C_4H_6O_4$: C, 40·7; H, 5·1%). The *p*-toluidide had m. p. and mixed m. p. 262—263° (Found : N, 9·7. Calc. for $C_{18}H_{20}O_2N_2$: N, 9·5%).

The same products were formed when the acid was oxidised in 4% aqueous sodium hydrogen carbonate.

(*b*) A solution of the acid (1 g.) in 2N-sodium hydroxide (20 ml.), containing hydrogen peroxide (3 ml. of 100-vol.), was kept for 72 hr., acidified, and continuously extracted with ether for 72 hr. The product left on evaporation of the ethereal extract was resolved into benzoic (0·2 g.) and succinic acid (150 mg.) by the method employed in (*a*).

(*c*) A moderate stream of ozone and oxygen was led into a solution of the acid (1 g.) in ethyl acetate (75 ml.) at 0° for 1 hr., the solvent evaporated in a vacuum, and the residue dissolved in water containing manganese dioxide (200 mg.). After 12 hr. the mixture was heated on the steam-bath for 40 min., cooled, and extracted with ether. Evaporation of the extracts left a mixture of benzoic (30 mg.) and succinic acid (100 mg.). The presence of volatile acids or carbonyl compounds could not be detected.

When the ozonide was decomposed with a solution of 2 : 4-dinitrophenylhydrazine (1 g.) in concentrated sulphuric acid (25 ml.) and water (200 ml.) a red-orange gummy solid (0·5 g.) separated which was collected after 18 hr., dried, and extracted with hot benzene. The 2 : 4-dinitrophenylhydrazone of α-oxoglutaric acid was obtained from the extract and on purification from aqueous alcohol and then ethyl acetate–light petroleum (b. p. 60—80°) had m. p. 227° (decomp.), identical with an authentic specimen (Found : N, 17·6. Calc. for $C_{11}H_{10}O_8N_4$: N, 17·2%).

Degradation of 2-*Oxo*-3-*phenylhept*-3-*enedioic Acid.*—(*a*) This acid (1 g.) was boiled with 2N-aqueous sodium hydroxide (50 ml.) in nitrogen for ½ hr. and the cooled, acidified liquor extracted with ether in a continuous extractor. A small amount of a colourless substance (100 mg.) separated from the ethereal extract and on recrystallisation from acetone–light petroleum (b. p. 60—80°) formed small colourless needles, m. p. 191°, having a negative ferric reaction (Found : C, 55·4; H, 5·5%). After the removal of this compound evaporation of the ether gave phenylpyruvic acid, having m. p. 158° on purification from benzene (Found : C, 65·8; H, 5·0. Calc. for $C_9H_8O_3$: C, 65·9; H, 4·9%). The 2 : 4-dinitrophenylhydrazone had m. p. and mixed m. p. 191—192°.

(*b*) 5% Aqueous potassium permanganate (50 ml.) was added dropwise to a solution of the acid (1 g.) in acetone during ½ hr., the mixture cleared with sulphur dioxide, the acetone evaporated, and the residue extracted with ether. The product from the ethereal extracts was separated into benzoic acid (0·4 g.) (soluble) and oxalic acid (50 mg.) (insoluble) [by means of hot light petroleum (b. p. 60—80°)] which were purified and identified respectively by comparison with authentic specimens.

(*c*) Oxidation of the acid (1 g.) in 2N-sodium hydroxide (20 ml.) with hydrogen peroxide (3 ml. of 100-vol.) at room temperature for 72 hr. followed by extraction of the acidified mixture with ether gave benzoic acid (400 mg.) and succinic acid (150 mg.).

(*d*) Ozonolysis of this acid by the procedure employed in the case of (I) gave a mixture of succinic and benzoic acid. Volatile acidic or carbonyl compounds could not be detected.

When the ozonide from the acid (1 g.) was decomposed with a solution of 2 : 4-dinitrophenylhydrazine (1 g.) in concentrated sulphuric acid (25 ml.) and water (200 ml.), purification of the resulting yellow precipitate from aqueous alcohol and then ethyl acetate–light petroleum (b. p. 60—80°) gave the 2 : 4-*dinitrophenylhydrazone* of β-formylpropionic acid in orange-yellow needles (0·3 g.), m. p. 202—203° (Found : C, 42·9; H, 3·7; N, 19·8. $C_{10}H_{10}O_6N_4$ requires C, 42·6; H, 3·6; N, 19·9%). On being kept the aqueous liquors deposited a further quantity of this derivative (0·15 g.).

(*e*) When the monomethyl ester of 2-oxo-3-phenylhept-3-enedioic acid (1 g.) was ozonised in methyl acetate at 0° and the oily ozonide decomposed with a cold solution of 2 : 4-dinitrophenylhydrazine sulphate (1 g.) in concentrated sulphuric acid (25 ml.) and water (200 ml.), the 2 : 4-dinitrophenylhydrazone (0·3 g.) of β-formylpropionic acid was obtained. This had m. p. 203° and was identical with an authentic specimen.

Prepared from authentic β-formylpropionic acid (Carrière, *Ann. Chim.*, 1922, **17**, 38), the 2 : 4-dinitrophenylhydrazone formed orange-yellow needles, m. p. 202—203°, from ethyl acetate–light petroleum (Found : C, 42·4; H, 3·8; N, 19·6%).

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