112. *The Structure of Clavatol, a Metabolic Product of* Aspergillus clavatus.

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Clavatol, isolated from cultures of *Aspergillus clavatus* by Bergel, Moss, Morrison, and Rinderknecht (*J*, 1944, 417), has been identified by degradation and synthesis as 2 : 4-*dihydroxy*-3 : 5-*dimethylacetophenone*.

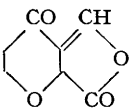
In the course of experiments on the production of clavatin (patulin) (I) by the mould *Aspergillus clavatus*, when grown on synthetic media containing glucose, Bergel, Moss, Morrison, and Rinderknecht (*J.*, 1944, 417) observed the formation of small quantities of a phenolic substance,

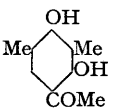
clavatol, which could be separated from clavatin by reason of its low solubility in water. As clavatol had no antibiotic properties and was produced in very small amount by the mould it was not exhaustively studied by these authors. It seemed, however, desirable that its structure should be investigated and its relationship, if any, to clavatin established, and we have therefore carried out the investigation here reported.

Clavatol, $C_{10}H_{12}O_3$, behaved on titration with alkali as a monohydric phenol, and in accordance with this behaviour it yielded a *monomethyl ether* and a *mono-acetate*. Since it resisted catalytic hydrogenation at atmospheric pressure it evidently contained no ethylenic linkage. From these facts it was evident that it must contain a second ring in addition to the phenolic nucleus, or, alternatively, one of the two oxygens, as yet unaccounted for, must be present in a carbonyl group resistant to hydrogenation. Clavatol failed to react with the usual carbonyl reagents, but the possibility that it might nevertheless be a ketone remained; it gave a strong purple ferric reaction and a positive iodoform reaction, although it gave no colouration with the nitro-chromic reagent (Fearon and Mitchell, *Analyst*, **1932**, **57**, 372) for alcoholic hydroxyl groups. An added point in favour of the presence of a concealed keto-group (as in, *e.g.*, various resacetophenone derivatives) was the stability of clavatol to heating with concentrated mineral acid or alkali fusion; such stability would hardly be expected in a compound containing oxygen either in a heterocyclic nucleus or in a simple ether linkage.

Taking all the above facts into account it seemed to us that clavatol might well be a resacetophenone derivative in which only one of the phenolic hydroxyl groups was capable of ready detection. Accordingly a small amount of the substance was treated in alkaline solution with hydrogen peroxide. The solution thus obtained yielded on acidification and treatment with 2 : 4-dinitrophenylhydrazine *3-hydroxy-2 : 6-dimethylbenzoquinone 2 : 4-dinitrophenylhydrazone*, identical with a synthetic specimen. It was therefore concluded that clavatol itself is 2 : 4-*dihydroxy*-3 : 5-*dimethylacetophenone* (II) (3 : 5-*dimethylresacetophenone*). Synthesis of (II) by applying the Hoesch reaction to 2 : 4-dimethylresorcinol gave a product identical in all respects with natural clavatol.

The production of clavatol together with clavatin by *A. clavatus* recalls the observation that clavatin is also accompanied by two simple phenolic compounds (gentisyl alcohol and gentisic acid) in cultures of *P. patulum* (Birkinshaw, Bracken, and Raistrick, *Biochem. J.*, **1943**, **37**, 726). Clavatol seems to us of particular interest since it is the first simple resacetophenone derivative identified as a product of mould metabolism. Compounds of this type might well act as intermediates in the biosynthesis of anthoxanthin pigments; such pigments have not as yet been found in fungi, although one mould pigment, citromycetin, appears on present evidence to be a related benzo-γ-pyrone derivative (Raistrick, *Phil. Trans.*, **1931**, *B*, **220**, 1).

(I.) 



(II.)

Experimental.

Clavatol.—Isolated as described by Bergel, Moss, Morrison, and Rinderknecht (*loc. cit.*), clavatol forms needles from aqueous methanol. It can be sublimed in a high vacuum and then forms lustrous colourless plates, m. p. 183° [Found : C, 66·3; H, 6·8; *M* (Rast), 188. Calc. for $C_{10}H_{12}O_3$: C, 66·7; H, 6·7%; *M*, 180]. It dissolves in sodium hydroxide (equiv. by titration, 176—182), sodium carbonate, or ammonia with a yellow colour and gives a purple ferric reaction destroyed by acids. Clavatol gives no reaction with diazotised sulphanilic acid or with 2 : 6-dichloroquinone-chloroimide. On oxidation with potassium permanganate it yields acetic and oxalic acids.

Clavatol Monomethyl Ether.—Prepared by treating clavatol with diazomethane or with methyl sulphate and sodium hydroxide, the *ether* has m. p. 35—36° (b. p. 90°/0·6 mm.) (Found : C, 68·2, 68·6; H, 7·3, 7·2; OMe, 15·0, 16·6. $C_{11}H_{14}O_3$ requires C, 68·0; H, 7·2; OMe, 16·0%).

Clavatol Acetate.—Prepared in the usual manner with acetic anhydride in pyridine solution, the *acetate* crystallises from aqueous methanol in colourless prisms, m. p. 95—96° (Found : C, 64·2; H, 6·2. $C_{12}H_{14}O_4$ requires C, 64·9; H, 6·3%).

Oxidation of Clavatol with Hydrogen Peroxide.—Clavatol (0·188 g.) was dissolved in a minimum amount of 0·1N-sodium hydroxide, and aqueous hydrogen peroxide (5 c.c. containing 0·034 g.) was added dropwise during 15 minutes. The intensely purple solution was set aside for two hours then acidified with dilute sulphuric acid. Unchanged clavatol (0·1 g.) was removed by filtration and the bright yellow filtrate extracted with ether. Evaporation of the ethereal extract gave orange needles (20 mg.) which on treatment with saturated aqueous 2 : 4-dinitrophenylhydrazine hydrochloride gave a red precipitate. Thrice recrystallised from alcohol, the 2 : 4-*dinitrophenylhydrazone* formed deep red plates or needles, m. p. 243° (decomp.) (Found : C, 51·0; H, 3·7; N, 16·6. $C_{14}H_{12}O_6N_4$ requires C, 50·6; H, 3·6; N, 16·8%). When 3-hydroxy-2 : 6-dimethylbenzoquinone (Fittig and Sieperman, *Annalen*, 1875, **180**, 27) was treated with 2 : 4-dinitrophenylhydrazine hydrochloride in the same way, it yielded a

2 : 4-dinitrophenylhydrazone identical in m. p. (243°) and mixed m. p. with the above product from clavatol (Found : C, 50·9; H, 3·9; N, 16·7%).

2 : 4-*Dihydroxy*-3 : 5-*dimethylacetophenone* (*Clavatol*).—2 : 4-Dimethylresorcinol was prepared by catalytic hydrogenation of resorcinol-2 : 4-dialdehyde (Baker, Kirby, and Montgomery, *J.*, 1932, 2876) using a palladium oxide catalyst. This catalyst was much more effective (yield 94%) than the palladised charcoal recommended by Asahina and Nonomura (*J. Pharm. Soc. Japan*, 1934, **54**, 79), the use of which was accompanied by extensive polymerisation.

2 : 4-Dimethylresorcinol (1·8 g.) was dissolved in dry ether (10 c.c.) to which had been added methyl cyanide (1·0 g.) and powdered anhydrous zinc chloride (0·8 g.). The mixture was cooled in ice, and dry hydrogen chloride was passed through it for 3 hours, by which time a red solid had separated. The flask containing the reaction mixture was sealed and set aside overnight in a refrigerator. The red solid was then collected, washed with ether, and dissolved in water (50 c.c.), and the solution was boiled for 30 minutes. The solid (m. p. 175°) which had separated (1·48 g.; 68%) was collected, dried, and purified by sublimation in a high vacuum; it then formed lustrous plates, m. p. 183°, undepressed in admixture with natural clavatol (Found : C, 66·8; H, 6·5. $C_{10}H_{12}O_3$ requires C, 66·7; H, 6·7%). The acetate of the synthetic 2 : 4-*dihydroxy*-3 : 5-*dimethylacetophenone* had m. p. 95—96°, undepressed by clavatol acetate (m. p. 95—96°).

The clavatol used in this investigation was obtained through the courtesy of the Research Department, Roche Products Ltd.; we are also indebted to the members of that Department for making available such analytical and other data as were obtained by them in their earlier work on *A. clavatus*.

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