

828. *Curvularin. Part I. Isolation and Partial Characterisation of a Metabolic Product from a New Species of Curvularia.*

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The isolation of a new mould metabolic product, $C_{16}H_{20}O_5$, from a species of *Curvularia* is described; a second compound, $C_{16}H_{18}O_5$, is also obtained in minor amount. The compound $C_{16}H_{20}O_5$, for which the name "curvularin" is proposed, is shown by chemical and spectroscopic studies to contain two *C*-methyl groups, a dihydroxybenzoyl system, and an aliphatic carbonyl group.

DURING an examination in 1952 of the metabolic products of a number of moulds, C. T. Calam¹ isolated a crystalline compound, m. p. 201—203°, from the culture filtrate of a species of *Curvularia*. As there is no record of any previous investigation of the chemistry of the metabolic products of this genus a detailed examination of the compound was considered desirable. Through the courtesy of Dr. Calam, to whom I am glad to express my thanks, a culture of the mould and a specimen of the metabolic product were made available for further work. The mould was identified as a species of *Curvularia* at the Commonwealth Mycological Institute at Kew; it does not appear to have been described previously. Although when first isolated the mould produced spores, it later lost this capacity.

The *Curvularia* was grown on a medium containing glucose, peptone, and mineral salts. Ether-extraction of the culture filtrate afforded a solid (0.40—0.48 g./l.) which on crystallisation gave in high yield a compound $C_{16}H_{20}O_5$, m. p. 206—206.5°, $[\alpha]^{18} -36.3^\circ$, identical with that isolated by Calam. The name "curvularin" is proposed for this substance which has not been described previously. Curvularin contains two *C*-methyl groups and two active hydrogen atoms which are present in two hydroxyl groups. It exhibits strong infrared absorption at 3356 cm^{-1} (hydroxyl) and forms a crystalline dibenzoate, $C_{30}H_{28}O_7$, a crystalline di-*p*-chlorobenzoate, $C_{30}H_{26}O_7Cl_2$, and an amorphous diacetate, $C_{20}H_{24}O_7$, the spectra of which do not show this band. Curvularin possesses the properties of a phenol, giving a purple ferric reaction in aqueous ethanol, coupling with diazotised aniline in alkaline solution to give a red dye, and dissolving readily in aqueous alkali, from which it is precipitated unchanged by carbon dioxide. Treatment with ethereal diazomethane or dimethyl sulphate and potassium carbonate readily gives a dimethyl ether, $C_{18}H_{24}O_5$, showing that both hydroxyl groups are phenolic. The molecular formula of curvularin permits of the presence of only one benzene nucleus and hence both hydroxyl groups must be attached to this. The formulation of the compound as a dihydric phenol explains *inter alia* the sensitivity of its alkaline solutions to aerial oxidation. Curvularin fails to decolorise a solution of 2:6-dichlorophenolindophenol, showing that it does not contain a quinol grouping; this is confirmed by the blue colour developed in alkaline solution with 2:6-dichloroquinone chloroimide² which indicates the presence of an unsubstituted position on the aromatic ring *para* to an hydroxyl group. The hydroxyl groups must therefore be *ortho* or *meta* to one another. Curvularin reacts with diazoaminobenzene, giving bisphenylazocurvularin, $C_{28}H_{28}O_5N_4$, which implies that at least two hydrogen atoms are attached directly to the benzene nucleus.

Chemical tests have failed to indicate the functions of the three remaining oxygen atoms in the curvularin molecule. Thus both curvularin and *OO*-dimethylcurvularin give a negative reaction in the hydroxamic acid test for the carboxylic ester group and do not give ketonic derivatives under the usual conditions. However, the infrared absorption spectra of these compounds show clearly the presence of two different types of carbonyl group. Curvularin exhibits three strong absorption bands in the 6 μ region, at 1692, 1653, and 1606 cm^{-1} , which in *OO*-dimethylcurvularin are found at 1736, 1695, and 1618 cm^{-1} . The lower frequencies for curvularin are due presumably to hydrogen-bonding between

¹ Calam, unpublished work in Imperial Chemical Industries Limited, Pharmaceuticals Division.

² Gibbs, *J. Biol. Chem.*, 1927, **72**, 649.

the carbonyl and hydroxyl groups which is known³ to result in the lowering of carbonyl frequencies by *ca.* 50 cm^{-1} . Because of this complication the absorption spectrum of curvularin is unsuitable for diagnostic purposes. In *OO*-dimethylcurvularin, however, hydrogen-bonding cannot be present and the absorption bands can be assigned to specific groups with some confidence. The band at 1736 cm^{-1} is attributed to an aliphatic carbonyl group, several types of which show similar absorption (*e.g.*, unconjugated five-membered ring ketone, 1745 cm^{-1} ; unconjugated ester, 1740 cm^{-1} ; unconjugated six-membered ring lactone, 1740 cm^{-1}).⁴ Because of the lack of further information concerning this carbonyl group, no conclusion can be drawn as to its exact nature. The band at 1692 cm^{-1} appears to be due to a ketonic group conjugated with the benzene ring, as alkyl aryl ketones are known⁴ to absorb at *ca.* 1690 cm^{-1} . The band at 1618 cm^{-1} is attributed to the absorption of the conjugated aromatic system and is similar to the intense bands in the 1580—1620 cm^{-1} region shown by many alkoxyphenyl ketones. The absorption of curvularin diacetate in the 6μ region resembles that of the dimethyl ether. Strong absorption bands are found at 1770 (phenolic acetate), 1727 (aliphatic carbonyl), 1695 (aromatic ketone), and 1621 cm^{-1} (conjugated aromatic system). From the above chemical and spectral evidence it appears that the dihydroxybenzoyl system $\cdot\text{C}_6\text{H}_2(\text{OH})_2\text{CO}\cdot$ is present in curvularin.

The ultraviolet absorption spectrum is of the type expected from such a chromophore and reduction experiments provide chemical evidence in favour of it. Deoxydihydrocurvularin, $\text{C}_{16}\text{H}_{22}\text{O}_4$, can be isolated on the hydrogenation of curvularin in acetic acid with Adams catalyst, if the reaction is interrupted after the uptake of two mols. of hydrogen. The infrared spectrum of this compound in the 6μ region shows one strong band at 1704 cm^{-1} (hydrogen-bonded aliphatic carbonyl group) and only weak absorption in the 1600 cm^{-1} region, indicating that the carbonyl group conjugated with the aromatic ring has been lost, presumably by reduction to a methylene group. The ultraviolet spectrum is quite unlike that of curvularin and bears a marked resemblance to that of resorcinol (see Table).

Ultraviolet absorption spectra (in ethanol).

Resorcinol	λ_{max} 2200 (ϵ 6100), 2760 Å (ϵ 2150)
Deoxydihydrocurvularin	λ_{inf} 2260 Å (ϵ 7850); λ_{max} 2860 Å (ϵ 2850)
Sodium borohydride product	λ_{inf} 2240 Å ($E_{\text{cm}}^1\%$ 280); λ_{max} 2850 Å ($E_{\text{cm}}^1\%$ 90)

The reduction of curvularin by sodium borohydride in methanol (a specific reagent for the reduction of aldehydes and ketones⁵) gives an amorphous product, the ultraviolet spectrum of which resembles that of deoxydihydrocurvularin. In this case the aromatic carbonyl group has presumably been converted into a secondary alcohol.

After the separation of curvularin from the crude metabolic product a second compound, $\text{C}_{16}\text{H}_{18}\text{O}_5$, *m. p.* 224.5—225°, $[\alpha]_{\text{D}}^{18}$ —83°, can be isolated in small quantity. It contains two hydroxyl groups and, as it is soluble in aqueous sodium hydroxide but insoluble in aqueous sodium hydrogen carbonate and gives an orange dye on treatment with diazotised aniline in alkaline solution, it is phenolic. The presence of an unsubstituted *para*-position on the aromatic ring is shown by the positive reaction with 2 : 6-dichloroquinone chloroimide. Like curvularin the compound fails to react with carbonyl reagents and gives a negative reaction in the hydroxamic acid test for the carboxylic ester group, but the infrared spectrum reveals two types of carbonyl group. Strong absorption bands are found at 1718 (probably aliphatic carbonyl), 1623 (hydrogen-bonded aromatic carbonyl), and 1592 cm^{-1} (conjugated aromatic system). The compound therefore contains a hydroxybenzoyl group. Its solution in alkali is deep yellow in colour and, unlike such a solution of curvularin, appears to be unaffected by oxygen.

EXPERIMENTAL

Analyses are by the microanalytical laboratory of this Department; molecular weights were determined ebullioscopically in acetone. Ultraviolet absorption spectra were determined with a Hilger "Uvispek" spectrophotometer. Infrared absorption spectra were determined,

³ Gordy, *J. Chem. Phys.*, 1940, **8**, 516.

⁴ Grove and Willis, *J.*, 1951, 877.

⁵ Chaikin and Brown, *J. Amer. Chem. Soc.*, 1949, **71**, 122.

for Nujol mulls, with a double-beam spectrophotometer with rock-salt optics; the concentrations and thickness of the mulls were not measured.

Cultural Conditions.—The *Curvularia* (No. F334 in the Nobel Division culture collection; Commonwealth Mycological Institute Cat. No. I.M.I. 52,980) was grown on beer wort-agar slopes at 25° for 14 days, a thick growth of dark-grey, non-sporing mycelium being obtained. A suspension of mycelial fragments in sterile water was used for inoculation of aqueous media. The *Curvularia* showed but poor growth on media containing only glucose and mineral salts; vigorous growth resulted when an amino-acid source was added. A suitable medium contained glucose (400 g.), "Bactopeptone" (100 g.), magnesium sulphate (5 g.), dipotassium hydrogen phosphate (5 g.), and traces of ferrous, zinc, copper, and manganese sulphates and ammonium molybdate in tap water (20 l.). The cultures were grown at 24° in "Glaxo" bottles each containing 400 ml. of medium. The glucose concentration of the solution fell rapidly after 4 days and after 14 days was 1–3 g./l. The upper surface of the mycelium was then light grey with some white secondary growths, and the reverse was black.

Isolation of Curvularin.—The culture filtrate was decanted from the mycelium (dry wt. ca. 7.5–9.0 g./l.). Manual extraction of the culture filtrate (10 vol.) with ether (3 × 1 vol.) gave, after removal of the solvent, crude curvularin as colourless or pale yellow-brown crystals. Initially the yield of crude solid was found to vary widely from batch to batch. The culture of *Curvularia* then employed was observed to give rise to two types of mycelium when grown on aqueous media: a light grey form which produced curvularin, and a dark grey, faster-growing form which produced only traces of the substance; morphologically the strains appeared to be identical. Cultures prepared from the light grey strain were used in subsequent experiments and consistently high yields of crude curvularin (0.40–0.48 g./l. of the culture filtrate) were obtained.

Crude curvularin (7.03 g.) was dissolved in hot benzene containing a small quantity of methanol, and the solution was boiled until crystallisation began and then set aside. *Curvularin* (5.09 g.) separated as plates, m. p. 206–206.5° (Pyrex or evacuated soda-glass capillary), $[\alpha]_D^{18} -36.3^\circ$ (*c* 3.8 in EtOH) [Found: C, 65.9, 65.8, 65.9; H, 6.9, 6.9, 6.8; C-Me (Kuhn-Roth), 7.5; active H (Zerewitinoff), 0.57; OH (by acetylation), 11.95%; *M*, 307. C₁₈H₂₆O₅ requires C, 65.75; H, 6.9; 2C-Me, 10.3; 2 active H, 0.68; 2OH, 11.65%; *M*, 292.3], ultraviolet absorption max. in (a) EtOH at 2230, 2720, and 3045 Å (ϵ 11,300, 6350, and 5100) and inflection at 2290 Å (ϵ 10,150), unaffected by the addition of hydrochloric acid, and (b) 0.005*N*-aqueous sodium hydroxide at 2330 and 3510 Å (ϵ 10,200 and 9300) and inflections at 2580 and 3280 Å (ϵ 7250 and 8100). Concentration of the mother-liquor from the above crystallisation gave more crystalline material (0.93 g.), m. p. 202–203°, which on recrystallisation afforded curvularin (0.62 g.), the total yield being 5.71 g. (81%).

Curvularin is soluble in ethanol, methanol, dioxan, acetone, pyridine, and concentrated sulphuric acid, moderately soluble in acetic acid and ether, and sparingly soluble in benzene, light petroleum, chloroform, and water. It gives yellow solutions with aqueous ammonia, sodium carbonate, and sodium hydroxide, from which it may be recovered unchanged by acidification. Alkaline solutions of curvularin darken rapidly in air, eventually becoming purple. This sensitivity to oxygen in the presence of alkali is also demonstrated by the rapid decomposition of curvularin at its m. p. when heated in open soda-glass capillaries. Curvularin does not contain nitrogen, sulphur, or halogen (Lassaigne test) or methoxyl groups (Zeisel). It is recovered unchanged after 3 hours' refluxing with 2*N*-sulphuric acid in aqueous ethanol. Heating with 58% aqueous hydriodic acid results in decomposition. Curvularin decolorises bromine in dioxan and potassium permanganate in acetone. With tetranitromethane in ethanol it gives a deep yellow colour, as do many other phenolic compounds. It readily reduces warm Fehling's solution but not ammoniacal silver nitrate solution. It reduces Tollens's reagent only if warmed; a silver mirror is formed immediately in the cold, however, if curvularin is first warmed with alkali. There is no uptake of hydrogen when a solution in acetic acid is shaken with a palladium catalyst, indicating the absence of simple olefinic unsaturation. The 3:5-dinitrobenzoic acid-aqueous-ethanolic alkali test for the ketomethylene group is negative. Neither curvularin nor the culture filtrates from *Curvularia* show anti-bacterial activity (against *B. subtilis*, *B. coli*, and *Staph. aureus*) or antifungal activity (spore germination test with *P. digitatum*).

Esters.—A mixture of curvularin (0.30 g.), dry pyridine (5 ml.), and acetic anhydride (5 ml.) was kept for 7 days at room temperature. Working up in the usual manner gave a gum (0.35 g.) which did not crystallise from solvents and on distillation in a short-path still at 160°/0.08 mm. gave curvularin diacetate as a pale yellow glass which softened at ca. 50° (Found: C, 65.3;

H, 6.7; Ac, 21.1%; *M*, 395. Calc. for $C_{30}H_{24}O_7$: C, 63.8; H, 6.45; 2Ac, 22.9%; *M*, 376.4), ultraviolet absorption inflection in EtOH at 2350 Å (ϵ 5600). Consistent C and H analyses could not be obtained for this compound. The infrared absorption spectrum was determined on a thin film of the compound.

A mixture of curvularin (0.50 g.), dry pyridine (10 ml.), and benzoic anhydride (2 g.) was kept at room temperature for 8 days. Working up in the usual manner gave an oil which was dissolved in benzene to which was added light petroleum (b. p. 60–80°) to turbidity. *Curvularin dibenzoate* (0.30 g.) separated as needles, m. p. 133–134°, $[\alpha]_D^{18} -10.8^\circ$ (*c* 1.9 in $CHCl_3$) (Found: C, 72.05; H, 5.9. $C_{30}H_{28}O_7$ requires C, 72.0; H, 5.65%), ultraviolet absorption max. in EtOH at 2360 Å (ϵ 36,700), inflection at 2740 Å (ϵ 4400).

A solution of curvularin (0.20 g.) in aqueous sodium hydroxide (10%, 2 ml.) was shaken under nitrogen with benzoyl chloride (0.5 ml.). Cooling and scratching afforded a solid which on crystallisation from ethanol gave curvularin dibenzoate as needles, m. p. and mixed m. p. 132–133°.

Curvularin (0.50 g.), dry pyridine (10 ml.), and *p*-chlorobenzoic anhydride (1.5 g.) were warmed to effect dissolution and kept at room temperature for 5 days. Working up in the usual manner afforded the *di-p-chlorobenzoate* (0.73 g.) which crystallised from benzene–light petroleum (b. p. 60–80°) or from ethanol as felted needles, m. p. 152–153°, $[\alpha]_D^{18} -8.4^\circ$ (*c* 2.1 in $CHCl_3$) (Found: C, 63.6; H, 4.8; Cl, 12.9%; *M*, 590. $C_{30}H_{26}O_7Cl_2$ requires C, 63.3; H, 4.6; Cl, 12.45%; *M*, 569.4).

OO-Dimethylcurvularin.—(a) Curvularin (20 g.) in dry acetone (300 ml.) was heated under reflux with potassium carbonate (60 g.) and dimethyl sulphate (60 ml.) during 1.5 hr. After evaporation under reduced pressure, the mixture was shaken with water and extracted with ether, evaporation of which gave a yellow oil (22 g.). Distillation of a portion of this material in a short-path still at 170–180°/0.08 mm. gave a pale yellow glass which crystallised. The remainder of the oil crystallised from aqueous ethanol when seeded, giving *OO-dimethylcurvularin* (19.06 g.) as short rods, m. p. 72°, $[\alpha]_D^{18} -2.9^\circ$ (*c* 2.7 in $CHCl_3$) (Found: C, 67.5; H, 7.9; OMe, 19.6%; *M*, 333. $C_{18}H_{24}O_5$ requires C, 67.5; H, 7.55; OMe, 19.4%; *M*, 320.4), ultraviolet absorption max. in EtOH at 2230 and 2675 Å (ϵ 10,500 and 5100), inflection at 2910 Å (ϵ 3600). The infrared absorption spectrum was determined on a thin film of the compound.

(b) To a suspension of curvularin (2 g.) in dry ether (50 ml.) was added ethereal diazomethane [from *N*-nitrosomethylurea (10 g.), 40% aqueous potassium hydroxide (30 ml.), and ether (50 ml.)] and methanol⁶ (0.1 ml.). After 2 days in the dark, the solution was filtered and evaporated, giving a yellow oil (2.17 g.). Crystallisation from aqueous ethanol (with seeding) gave *OO-dimethylcurvularin*, m. p. 70–71°, mixed m. p. 71.5–72°.

Bisphenylazocurvularin.—To a hot solution of curvularin (0.30 g.) in ethanol (10 ml.) was added a solution of diazoaminobenzene (0.60 g.) in the same solvent (5 ml.). After 2 days at room temperature, the crystalline precipitate was collected. Crystallisation from ethanol gave *bisphenylazocurvularin* (0.26 g.) as red blades, m. p. 232.5–233° (Found: C, 67.4; H, 5.7; N, 11.5%; *M*, 515. $C_{28}H_{28}O_5N_4$ requires C, 67.2; H, 5.65; N, 11.2%; *M*, 500.5).

Deoxydihydrocurvularin.—A solution of curvularin (1.0 g.) in acetic acid (100 ml.) was shaken with hydrogen at atmospheric pressure in the presence of pre-reduced Adams catalyst⁷ (0.15 g.) until rather more than 2 mols. of hydrogen (153 ml. at N.T.P.) had been taken up. Evaporation of the filtered solution gave a semi-solid residue which on crystallisation from benzene–methanol gave *deoxydihydrocurvularin* (0.23 g.) as plates, m. p. 265.5–266° (evacuated Pyrex capillary) (Found: C, 69.4; H, 7.85%; *M*, 275. $C_{16}H_{22}O_4$ requires C, 69.05; H, 8.0%; *M*, 278.35).

Reduction of Curvularin with Sodium Borohydride.—A solution of curvularin (1.0 g.) in methanol (30 ml.) was neutralised by the addition of *N*-sodium hydroxide (6.9 ml.) and added during 10 min. to a solution of sodium borohydride (0.21 g.) in the same solvent (10 ml.), under nitrogen. After 3 hr. the solution was acidified and diluted with water. Ether-extraction gave a brown gum (0.95 g.).

Isolation of the Compound $C_{16}H_{18}O_5$.—The mother-liquors from the crystallisation of a number of batches of crude curvularin (49.5 g.), on further concentration, yielded a solid, m. p. 218° (1.84 g.). Crystallisation of this from ethyl acetate and then from benzene–methanol gave a compound as small octahedral prisms, m. p. 224.5–225° (decomp.), $[\alpha]_D^{18} -83^\circ$ (*c* 3.0 in $COMe_2$) [Found: C, 66.7, 66.5; H, 6.4, 6.1; OH (by acetylation), 10.9%; *M*, 306. $C_{16}H_{18}O_5$

⁶ Cf. Schönberg and Mustafa, *J.*, 1946, 746.

⁷ Frampton, Edwards, and Henze, *J. Amer. Chem. Soc.*, 1951, 73, 4432.

requires C, 66.2; H, 6.25; 2OH, 11.7%; *M*, 290.3], ultraviolet absorption max. in EtOH at 2250, 3015, and 3400 Å (ϵ 14,000, 5900, and 5950). The compound can also be purified slowly by sublimation at 200°/0.05 mm. It is moderately soluble in acetic acid, ethanol, methanol, ethyl acetate, and ether, and is sparingly soluble in chloroform, water, and benzene. It gives a brown ferric reaction in aqueous ethanol and decolorises a solution of potassium permanganate in acetone.

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