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STUDIES IN THE BIOCHEMISTRY OF MICRO-ORGANISMS—VI*

THE STRUCTURE AND STEREOCHEMISTRY OF THE PHTHALAN CURVULOL, AND OTHER METABOLIC PRODUCTS OF *CURVULARIA SIDDIQUI*

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Abstract—Curvulol, a metabolite of *Curvularia Siddiqui* sp.novo, is shown to be 3(S)-4,5-dihydroxy-6-methoxy-3-methylphthalan (II), the first naturally-occurring example of this ring system. 2-Acetyl-3,5-dimethoxyphenylacetic and 3,5-dimethoxybenzoic acids are produced by the same organism, in addition to the compounds described previously.[1-5]

Earlier papers have described the isolation[1,2] and characterization[3,4,5] of various metabolic products of *Curvularia Siddiqui* sp. novo[6] when grown on a fully synthetic medium or a synthetic medium enriched with carrot extract. The secondary metabolites are primarily hydroxylated 2-acetyl-phenylacetic acid derivatives, with the exception of curvulol, m.p. 204°, which was best produced[1,2] from the enriched medium and was suggested[3] to be 4-acetyl-5-(β-hydroxyethyl)-resorcinol (I). However, a recently-determined proton magnetic resonance (PMR) spectrum was incompatible with this structure. Re-examination of authentic material now leads to the structure and absolute stereochemistry (II) for curvulol, 3(S)-4,5-dihydroxy-6-methoxy-3-methylphthalan, which is thus the first example of a naturally occurring phthalan. Biogenetically, it probably arises by enzymic modification of the corresponding, fully acetate-derived, hydroxylated 2-acetylphenylacetic acid.

Contrary to the earlier report,[3] we find curvulol, $C_{10}H_{12}O_4$, to be optically active, $[\alpha]_D^{19}$ $+18{\cdot}6^\circ$ (c. 0·42%). The compound was previously[3] shown to lack carbonyl activity towards hydroxylamine or 2,4-dinitrophenylhydrazine, although it gave a

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* Part V, ref. 5.

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[1] A. Kamal, N. Ahmad, M. Ali Khan and I. H. Qureshi, *Tetrahedron* **18**, 433 (1962).

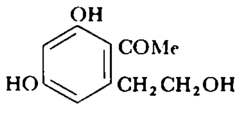
[2] A. Kamal, A. Ali Qureshi, M. Ali Khan and F. Mohd Khan, *Tetrahedron* **19**, 117 (1963).

[3] A. Kamal, M. Ali Khan and A. Ali Qureshi, *Tetrahedron* **19**, 111 (1963).

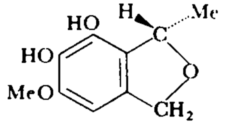
[4] A. Kamal and M. Akram Sandhu, *Tetrahedron Letters* 611 (1963).

[5] A. Kamal, A. Ali Qureshi, A. Ahmad and R. W. Rickards, *Tetrahedron* **21**, 1411 (1965).

[6] S. Iftikhar Ahmad and M. Sayeed Qureshi, *Pakistan J. Sci. Ind. Res.* **3**, (3), 177 (1960).

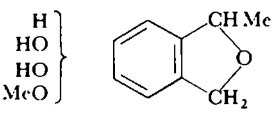


I

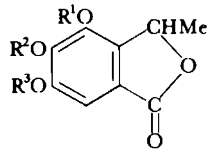


II

positive iodoform test and ferric reaction. Analysis shows the presence of one methoxyl and one C-methyl group. Its UV spectrum, λ_{max} 276 mμ (ε 1360) (lacking the reported[3] maximum at 285 mμ), resembles that of an unconjugated phenolic system,[7] whilst IR absorption indicates the presence of hydroxyl groups but no carbonyl group. Acetylation afforded a diacetate, $C_{14}H_{16}O_6$, $[\alpha]_D^{23}$ +21·4°, whose IR spectrum lacked hydroxyl absorption but had one carbonyl maximum at 1783 cm^{-1}, necessitating that both acetyl groups were phenyl esters.[8] Confirmation of these features, and evidence that the remaining oxygen was in a cyclic ether linkage, came from the PMR spectrum of curvulol (in pyridine), which showed resonances corresponding in chemical shift[9] and intensity to the groupings ArCH(C$\underline{H}_3$)O—(1 : 1 doublet, J = 6·5 c/s, τ 8·17), ArOC$\underline{H}_3$ (singlet, τ 6·19), ArC$\underline{H}_2$O— (multiplet, τ 4·79), ArC$\underline{H}$MeO— (quartet, J = 6·5 c/s, with further unresolved splitting, τ 4·19), and Ar$\underline{H}$ (singlet, τ 3·48). The hydroxylic protons, obscured by rapid exchange in pyridine, could be seen in dioxan solvent as singlets at τ 2·60 and 2·80, which were removed by exchange with deuterium oxide. These data permit us to write curvulol as the phthalan, partial structure (III). The hydrogens of the heterocyclic ring undergo mutual long-range spin coupling, possibly of the homo-allylic type[10] transmitted by the aromatic ring, thus contributing to the observed multiplicity of their resonances.



III



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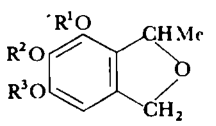
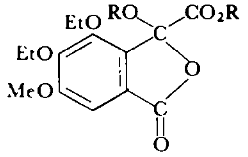


V



VI

Methylation of curvulol with methyl iodide and potassium carbonate in acetone gave 0,0-dimethylcurvulol, $C_{12}H_{16}O_4$, which as expected closely resembled curvulol in UV absorption but lacked hydroxyl absorption in the IR. Its PMR spectrum showed resonances due to three methoxyl groups at τ 6·02 (singlet, 3H) and 6·08

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[7] A. I. Scott, *Interpretation of the Ultraviolet Spectra of Natural Products* p. 91, Pergamon Press, Oxford (1964).

[8] L. J. Bellamy, *The Infrared Spectra of Complex Molecules* p. 178, Methuen, London (1958).

[9] L. M. Jackman, *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry* p. 50, Pergamon Press, Oxford (1959).

[10] cf. S. Sternhell, *Rev. pure appl. Chem., Aust.* **14**, 15 (1964).

(singlet, 6H), in addition to other resonances as in curvulol itself. Oxidation of this phthalan derivative with potassium permanganate in acetone yielded the expected phthalide, $C_{12}H_{14}O_5$, ν_{max} 1765 cm^{-1} (cf. Ref. 8), showing singlet proton resonances at τ 2·58 (Ar$\underline{H}$), 5·80, 5·85 and 5·88 (3 $ArOC\underline{H}_3$) and the 1:3:3:1 quartet, τ 4·23 and 1:1 doublet, τ 8·26, of the AX_3 pattern (J_{AX} = 7·0 c/s) due to the $ArC\underline{H}(C\underline{H}_3)OCO$— system. This product, $[\alpha]_D^{24}$ +22·9°, was identified as (+)-4,5,6-trimethoxy-3-methylphthalide (IV: R^1,R^2,R^3 = Me) by comparison of its IR spectrum with that of the synthetic (±)-phthalide,[11] thus permitting development of the partial structure (III) of curvulol to (V: R^1,R^2,R^3 = H,H,Me) in which only O-methyl group remains to be located.

Similar permanganate oxidation of diacetyl curvulol (V: R^1,R^2,R^3 = Ac,Ac,Me) afforded a diacetoxy-methoxy-3-methylphthalide (IV: R^1,R^2,R^3 = Ac,Ac,Me), $C_{14}H_{14}O_7$, $[\alpha]_D^{25}$ +24·5, ν_{max} 1773 and 1781 cm^{-1} (cf. Ref. 8), showing singlet proton resonances at τ 2·68 (Ar$\underline{H}$), 6·12 ($ArOC\underline{H}_3$), and 7·67 (2 $ArOCOC\underline{H}_3$), in addition to the 1:3:3:1 quartet at τ 4·55 and 1:1 doublet at τ 8·47 of the $ArC\underline{H}(C\underline{H}_3)OCO$— system ($J$ = 7·0 c/s). After hydrolysis of the ester and lactone groups, alkali fusion cleaved the resulting benzyl alcohol system in a retro-aldol reaction triggered by the phenolic nucleus, yielding acetaldehyde, characterized as its 2,4-dinitrophenylhydrazone, together with a dihydroxymethoxybenzoic acid, $C_8H_8O_5$. From the oxygenation pattern of the phthalide (IV: R^1,R^2,R^3 = Ac,Ac,Me) this must be an O-methyl gallic acid, and its PMR spectrum (in acetone) revealed the aromatic protons as an AB system, a pair of doublets (J = 2·0 c/s) centred at τ 2·70 and 2·78, together with the methoxyl singlet at τ 6·11. The occurrence of spin coupling between the *m*-related hydrogen nuclei necessitates that they are in different environments, and that the acid is therefore the unsymmetrically-substituted 3,4-dihydroxy-5-methoxybenzoic acid. This conclusion was confirmed by direct comparison with synthetic material.[5,12] Curvulol is accordingly the phthalan (V: R^1 = Me, R^2 = R^3 = H) or (V: R^1 = R^2 = H, R^3 = Me).

Of these two possible structures, curvulol was shown to be the latter, 4,5-dihydroxy-6-methoxy-3-methylphthalan (V: R^1 = R^2 = H, R^3 = Me) by degradation of its O,O-diethyl derivative, $C_{14}H_{20}O_4$, prepared with ethyl iodide and potassium carbonate in acetone. Successive alkaline oxidation with potassium permanganate and then peroxide gave an acid, $C_{13}H_{16}O_7$, ν_{max} 3700–2300 and 1707 cm^{-1}, which was identified as 3,4-diethoxy-5-methoxyphthalic acid by pyrolysis to the corresponding phthalic anhydride, $C_{13}H_{14}O_6$, ν_{max} 1850 and 1781 cm^{-1} (cf. Ref. 13), and comparison with an authentic sample[14] of the latter.

The intermediate in this degradative sequence, isolable after the oxidation with alkaline permanganate, is the lactol carboxylic acid (VI: R = H), $C_{14}H_{16}O_8$. The structure of this compound follows from its analytical data, in conjunction with IR maxima at 1765 and 1730 cm^{-1} corresponding to the phthalide[8,15,16] and acid carbonyls, and an UV chromophore, λ_{max} 263 and 294 mμ, closely resembling that

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[11] E. Maekawa and S. Nanya, *Bull. chem. Soc. Japan* **32**, 1311 (1959).
[12] W. Bradley, R. Robinson and G. Schwarzenbach, *J. chem. Soc.* **133**, 793 (1930).
[13] ref. 8, p. 125.
[14] M. Tomita and S. Uyeo, *J. chem. Soc. Japan* **64**, 77 (1943).
[15] J. F. Grove and H. A. Willis, *J. Chem. Soc.* 877 (1951).
[16] D. S. Erley, W. J. Potts, P. R. Jones and P. J. Desio, *Chemy Ind.* 1915 (1964).

of 4,5,6-trimethoxy-3-methylphthalide, λ_{max} 259 and 298 mμ. Treatment with methanolic hydrogen chloride gave the corresponding methyl ether methyl ester (VI: R = Me), $C_{16}H_{20}O_8$, showing similar UV absorption and phthalide[8, 15, 16] and aliphatic ester carbonyl maxima at 1775 and 1756 cm^{-1}. PMR spectroscopy now showed the presence of the expected three methoxyl singlets at τ 6·03, 6·18 and 6·62, the latter being attributable (cf. Ref. 16) to that of the pseudo-ester, in addition to the aromatic proton singlet at τ 2·80 and the A_2X_3 systems of the two ethoxyl groups as a multiplet at τ 5·81 (2 MeC$\underline{H}_2$O—) and two overlapping triplets (1:2:1, J = 7·0 c/s) centred at τ 8·60 and 8·63 (2 C$\underline{H}_2$CH$_2$O—). In alkaline solution, the lactol carboxylic acid (VI: R = H) would yield the di-anion of the more acidic acyclic form, and such phenylglyoxylic acids are known[3, 17] to be relatively stable towards alkaline permanganate but are readily cleaved by alkaline peroxide.

THE ABSOLUTE CONFIGURATION OF CURVULOL

The (+)-4,5,6-trimethoxy-3-methylphthalide derived from curvulol showed a negative plain optical rotatory dispersion curve in methanol. Comparison with the similar curves reported by Nagai and collaborators[18] for 3(S)-3-methyl and 3(S)-3-*n*-butylphthalide indicates that the absolute configuration at C_3 in curvulol should be S, as depicted in structure (II), assuming no interference from the aromatic methoxyl substituents.

This conclusion was confirmed by degradation to optically pure L-(+)-lactic acid. In order to avoid the necessity of cleaving a relatively stable ether linkage to the asymmetric centre, the (+)-4,5-diacetoxy-6-methoxy-3-methylphthalide (IV: $R^1 = R^2$ = Ac, R^3 = Me) rather than a phthalan was chosen as the starting material. The degradative sequence involved destructive ozonolysis of the aromatic ring, followed by peroxide treatment of the ozonide in acetic acid. This route would be anticipated to afford lactic acid via an intermediate equivalent to an oxalate ester of this acid, which would cleave readily either by hydrolysis or oxidation under the mild acidic conditions without interference at the asymmetric centre.

OTHER METABOLITES FROM C. SIDDIQUI

Further investigation of the previously-described[2] acidic fraction produced by growth of *C. Siddiqui* on a synthetic medium enriched with carrot extract led to the isolation of two further metabolites, 3,5-dimethoxybenzoic acid and an acid $C_{12}H_{14}O_5$.

This latter acid showed IR maxima at 3440–2400, 1710 and 1660 cm^{-1} indicative of an aliphatic carboxyl and an aromatic ketone, whilst its UV spectrum, λ_{max} 270 and 304 mμ (ε 7140 and 5180) closely resembled that reported[1] for curvulin (VII: R^1 = Et, R^2 = H)[3, 4] λ_{max} 270 and 303 mμ (ε 7470 and 5640). Its PMR spectrum (in pyridine) showed singlet resonances at τ 7·18, 6·35 and 5·97 corresponding in chemical shift and in intensity to an aromatic methyl ketone, two superimposed aromatic methoxyl groups, and a phenylacetyl methylene group (cf. Ref. 5). Diazomethylation gave the corresponding methyl ester, $C_{13}H_{16}O_5$, ν_{max} 1740 (aliphatic ester) and 1685

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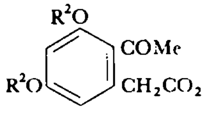
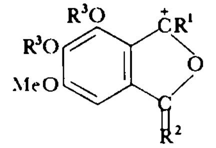
[17] cf. O. C. Musgrave, *J. Chem. Soc.* 1104 (1957).

[18] U. Nagai, T. Shishido, R. Chiba and H. Mitsuhasi, *Tetrahedron* **21**, 1701 (1965).

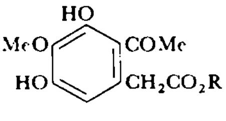
cm^{-1} (aromatic ketone), without alteration to the UV chromophore. PMR spectroscopy now showed two *m*-related aromatic hydrogens as a pair of doublets (J_{AB} = 2·5 c/s) centred at τ 3·23 and 3·30, in addition to singlets at τ 7·30 ($ArCOC\underline{H}_3$), 6·10 ($ArOC\underline{H}_3$ plus $ArC\underline{H}_2CO$—), 5·96 and 5·93 (2 $ArOCH_3$). The acid is clearly 2-acetyl-3,5-dimethoxyphenylacetic acid (VII:R^1 = H, R^2 = Me), the synthetically known[1,3,4] dimethyl ether of curvulinic acid (VII: R^1 = R^2 = H)[3,4] which is itself a metabolite[1,2] of *C. Siddiqui*. This conclusion was confirmed by direct comparison with authentic O,O-dimethylcurvulinic acid.[4]



VII



VIII



IX

MASS SPECTRA OF C. SIDDIQUI METABOLITES

The mass spectra of curvulol and its derivatives are all characterized by loss of the substituent at C_3 on the heterocyclic ring, forming the ion (VIII) which is stabilized by resonance with both the aromatic ring and the adjacent oxygen atom. Thus, in curvulol (II) and O,O-dimethylcurvulol (V: R^1,R^2,R^3 = Me) the base peak resulting from loss of a methyl radical from the strong molecular ions (at 196 and 224 respectively) dominates the spectrum; further weak loss of CO is the only other notable fragmentation. In the phthalide (IV: R^1,R^2,R^3 = Me) (M^+ 238), this decarbonylation is more effective and provides the base peak of the spectrum. In the lactol methyl ester (V: R = Me) (M^+ 340), loss of the carbomethoxyl group provides the ion (VIII: R^1 = OMe, R^2 = O, R^3 = Et), ten times more intense than any other ion, which again undergoes weak loss of CO.

The mass spectroscopic fragmentation of 2-acetyl-3,5-dimethoxyphenylacetic acid (VII: R^1 = H, R^2 = Me) (M^+ 238) parallels that of the unmethylated metabolite curvulinic acid (VII: R^1 = R^2 = H) (M^+ 210), except for a considerably enhanced loss from the molecular ion of a methyl radical, presumably from one of the methoxyl groups. The base peak, M-43, the these spectra arises by loss of the $COCH_3$ group by both single-stage (—$COCH_3$) and two-stage processes (—CH_3, —CO) as evidenced by metastable transitions. The only other major peaks, M-59 and M-60, correspond to the loss of CH_3 together with CO_2 or with CO + OH, respectively, whilst other losses of H_2O, CO_2 and H_2O + CO, are relatively weak. In curvulic acid (IX: R = H) (M^+ 240), on the other hand, this M-59 ion is now the base peak of the spectrum, ions due to loss of CO_2, H_2O + CO, CH_3 + CO + OH, and CH_3 + CO + H_2O are intense, whilst direct losses of CH_3 and H_2O are again weak.

The naturally occurring ethyl esters curvulin (VII: R^1 = Et, R^2 = H) (M^+ 238) and curvin (IX: R = Et) (M^+ 268) show similar mass spectra with strong ions at M-42, M-45 and M-46 due to the loss of CH_2CO (together with corresponding metastable ions), C_2H_5O, and C_2H_5OH from the molecular ions. The base peaks at M-74 arise by decarbonylation of this last species, as confirmed by the presence of appropriate metastable ions in both spectra. The remaining intense ions at M-88 represent loss of C_2H_5OH from the M-42 species, again verified by metastable transitions. The only essential difference between the two fragmentations is the much greater extent of cleavage of the ethyl-oxygen bond in curvulin compared to curvin, to give M-29 and M-(42 + 29) ions, the latter approaching the intensity of the molecular ion.

EXPERIMENTAL

M.p's were taken on a Kofler hot-stage, and are uncorrected. Light petroleum refers to the fraction b.p. 40–60°. UV spectra were measured in ethanol on Perkin Elmer 137 or Unicam SP800 spectrometers. IR spectra were determined with Perkin Elmer 237 or Unicam SP200 instruments, in carbon tetrachloride unless otherwise stated. PMR spectra were recorded at 60 Mc/s. for ca. 12% solutions in deuterochloroform (unless otherwise stated) containing tetramethylsilane as internal reference on Varian A60 or Perkin Elmer R10 machines. Optical rotations were measured in ethanol on a Bendix Ericsson ETL–NPL Automatic Polarimeter 143A. Microanalyses were by Dr. A. Bernhardt, Mülheim.

Curvulol. Curvulol used was authentic material.[3] ν_{max} (in Nujol) 3410, 3340 and 3150 (OH), 1623 and 1598 cm^{-1} (Ar). (Found: OMe, 15·2; CMe, 7·5. $C_{10}H_{12}O_4$ requires: 1 OMe, 15·3; 1 CMe, 7·7%.)

Diacetyl curvulol. Acetylation of curvulol (196 mg) with acetic anhydride (2 ml) and pyridine (2 ml) overnight at room temperature gave, after chromatography in ether on neutral alumina, sublimation at 140°/10^{-2} mm and crystallization from ether–light petroleum, diacetyl curvulol (159 mg), prisms m.p. 100°, $[\alpha]_D^{23}$ +21·4° (c. 0·57%), λ_{max} 278 mμ and λ_{infl} 296 mμ (ε 2680, 482), ν_{max} (in Nujol) 1764 cm^{-1} (ArOAc), ν_{max} (in CCl_4) 1783 (ArOAc) and 1624 cm^{-1} (Ar). (Found: C, 59·8; H, 5·9. Calc. for $C_{14}H_{16}O_6$: C, 60·0; H, 5·8%) The PMR spectrum showed singlets at τ 3·08 (Ar$\underline{H}$) 6·03 (ArOC$\underline{H}_3$), and 7·58 (2 ArOCOC$\underline{H}_3$), a doublet at τ 8·43 (1:1, J = 6·5 c/s, ArCH(C$\underline{H}_3$)O—), and multiplets at τ 4·54 (ArC$\underline{H}$MeO—) and 4·77 (ArC$\underline{H}_2$O—). IR and UV spectra were identical with the previously described preparation.[3]

O,O-*Dimethylcurvulol.* Curvulol (392 mg) and anhydrous potassium carbonate (100 mg) were heated in refluxing acetone (25 ml) and methyl iodide (2 ml) for 16 hr, additional methyl iodide (0·5 ml) being added every 2 hr. The product, isolated by removal of the solvent, dilution with water, extraction with ethyl acetate and washing with aqueous sodium hydroxide, on distillation at 90°/0·1 mm afforded O,O-*dimethylcurvulol* (327 mg), an oil $[\alpha]_D^{23}$ +21·1° (c. 0·45%), λ_{max} 279 mμ (ε 1570), ν_{max} (liquid film) 1614 and 1583 cm^{-1} (Ar). (Found: C, 64·0; H, 7·2. $C_{12}H_{16}O_4$ requires: C, 64·3; H, 7·2%.)

Oxidation of O,O-dimethylcurvulol. Powdered potassium permanganate (700 mg) was added in portions (100 mg) to O,O-dimethylcurvulol (224 mg) in refluxing acetone (25 ml) over 12 hr, when a permanent colour persisted. After filtration, the solvent was removed, water added, and the product extracted with ether and then washed with aqueous sodium bicarbonate. Chromatography on neutral alumina in ether–light petroleum (3:1), distillation at 130°/10^{-2} mm, and crystallization from ether–light petroleum yielded colourless needles of (+)-4,5,6-*trimethoxy-3-methylphthalide* (105 mg), m.p. 41°, $[\alpha]_D^{24}$ +22·9° (c. 0·33%), λ_{max} 259 and 298 mμ (ε 8470, 4080), ν_{max} 1765 (phthalide) and 1605 cm^{-1} (Ar). (Found: C, 60·4; H, 5·8. $C_{12}H_{14}O_5$ requires: C, 60·5; H, 5·9%.) ORD in methanol (c. 0·05%): $[\alpha]_{450}$ +0·52°, $[\alpha]_{400}$ −1·1°, $[\alpha]_{350}$ −4·0°, $[\alpha]_{330}$ −8·6°, $[\alpha]_{310}$ −20·4°. The IR spectrum of this product was identical with that of (±)-4,5,6-trimethoxy-3-methylphthalide, m.p. 51°, prepared after Maekawa and Nanya.[10]

Oxidation of diacetyl curvulol. Powdered potassium permanganate (1·20 g) was added in portions (100 mg) to diacetyl curvulol (280 mg) in refluxing acetone (25 ml) over 8 hr, when a permanent colour persisted. The excess of oxidant was destroyed with methanol, and the mixture was filtered. The filtrate was evaporated, water added, and the product was extracted with ether. After washing with aqueous sodium bicarbonate, the extract was chromatographed over Florisil in ether and sublimed at 120°/10^{-2} mm. Crystallization from ether gave the (+)-*diacetoxy-methoxy-3-methylphthalide* (122 mg), needles m.p. 150°, $[\alpha]_D^{25}$ +24·5° (c. 0·65%), λ_{max} 224, 245 and 299 mμ (ε 6150, 6320 and 4060), ν_{max} (in Nujol) 1781 and 1773 (ArOAc and phthalide), and 1620 cm^{-1} (Ar). (Found: C, 56·9; H, 4·9; OMe, 9·5. $C_{14}H_{14}O_7$ requires: C, 57·1; H, 4·8; 1 OMe 9·0%.)

Alkaline degradation of the (+)-diacetoxy-methoxy-3-methylphthalide. The phthalide (98 mg) in aqueous sodium hydroxide (20%, 10 ml) was heated under reflux for 4 hr, when the water was evaporated and the temperature raised to 160°. A stream of nitrogen carried volatile products into acidic 2,4-dinitrophenylhydrazine solution, affording acetaldehyde 2,4-dinitrophenylhydrazone (11 mg), m.p. and mixed m.p. with authentic material 165° after crystallization from methanol. The alkaline residue was acidified and extracted with ether. The extract on fractionation with aqueous sodium bicarbonate yielded 3,4-dihydroxy-

5-methoxybenzoic acid (28 mg), needles m.p. 220° from water, λ_{max} 275 mμ (ε 13250), ν_{max} (in Nujol) 3230 (OH), 3500–2350 and 1660 ($ArCO_2H$), and 1613 cm^{-1} (Ar), identified by thin-layer chromatography, mixed m.p. and comparison of UV and IR spectra with authentic material, m.p. 220°, synthesized by the method of Bradley *et al.*[5, 12]

O,O-Diethylcurvulol. Ethylation of curvulol (392 mg) for 16 hr in refluxing acetone (25 ml) containing ethyl iodide (2 ml) and anhydrous potassium carbonate (200 mg), adding additional ethyl iodide (0·5 ml) hourly, followed by work-up as for the corresponding O,O-dimethyl derivative, gave an oil (437 mg). Chromatography on Florisil in ether–light petroleum (4:1) and distillation at 80°/10^{-2} mm yielded O,O-*diethylcurvulol* (412 mg), $[\alpha]_D^{31}$ +17·2° (c. 0·41 %), λ_{max} 280 mμ (ε 1830), ν_{max} (liquid film) 1610 and 1583 cm^{-1} (Ar). (Found: C, 66·6; H, 7·9; O, 25·2. $C_{14}H_{20}O_4$ requires: C, 66·6; H, 8·0; O, 25·4 %.) The PMR spectrum showed singlets at τ 3·51 (Ar$\underline{H}$) and 6·16 (ArOC$\underline{H}_3$), a doublet at τ 8·48 (1:1, J = 6·0 c/s, ArCH(C$\underline{H}_3$)O—), triplets at τ 8·63 and 8·66 (1:2:1, J = 7·5 c/s, 2 C$\underline{H}_3$CH$_2$O—), and multiplets centred at τ 4·66 (ArC$\underline{H}$MeO—), 4·98 (ArC$\underline{H}_2$O—), and 5·96 (J = 7·5 c/s, 2 MeC$\underline{H}_2$O—).

Alkaline permanganate oxidation of O,O-diethylcurvulol. Powdered potassium permanganate (780 mg) was added in portions to O,O-diethylcurvulol (252 mg) in refluxing aqueous potassium hydroxide (5 %, 20 ml) over 12 hr, when a permanent colour persisted. After destruction of the excess of oxidant with methanol, the filtered solution was acidified, saturated with ammonium sulphate, and extracted with ethyl acetate. The fraction of this extract which was soluble in aqueous sodium bicarbonate afforded on recovery *the lactol carboxylic acid* (VI: R = H) (122 mg), needles m.p. 154° from ether–light petroleum, λ_{max} 263 and 294 mμ (ε 19,900 and 4180) shifting upon addition of alkali to λ_{max} 252 and 269 mμ, ν_{max} (in Nujol) 3370 and 3160 (OH), 1765 (phthalide), 1730 (CO_2H), and 1607 cm^{-1} (Ar). (Found: C, 53·7; H, 5·2; O, 41·0. $C_{14}H_{16}O_8$ requires: C, 53·8; H, 5·1; O, 41·6 %.) The PMR spectrum showed singlets at τ 2·86 (Ar$\underline{H}$) and 6·08 (ArOC$\underline{H}_3$), triplets at τ 8·61 and 8·68 (1:2:1, J = 7·5 c/s, 2 C$\underline{H}_3$CH$_2$O—), and quartets at τ 5·76 and 5·84 (1:3:3:1, J = 7·5 c/s, 2 MeC$\underline{H}_2$O—).

The lactol carboxylic acid (VI: R = H) (10 mg) with methanolic hydrogen chloride at room temperature overnight gave *the lactol methyl ether methyl ester* (VI: R = Me) (7 mg), m.p. 121–122° after purification by chromatography in ether on Florisil, distillation at 120°/0·1 mm, and crystallization from ether–light petroleum, λ_{max} 266 mμ and λ_{infl} 295 mμ (ε 15,600 and 7850), ν_{max} 1775 (phthalide), 1756 (CO_2Me), and 1605 cm^{-1} (Ar). (Found: C, 56·6; H, 6·1. $C_{16}H_{20}O_8$ requires: C, 56·5; H, 5·9 %.)

Alkaline peroxide oxidation of the lactol carboxylic acid (VI: R = H). The lactol carboxylic acid (VI: R = H) (104 mg) was oxidized with hydrogen peroxide (30 %, 7·5 ml) in aqueous sodium hydroxide (0·1 N, 15 ml) for 4 hr. The excess of oxidant was destroyed with palladized charcoal in the presence of a trace of ferric chloride, and the solution filtered and acidified. Ethyl acetate extracted 3,4-diethoxy-5-methoxyphthalic acid (67 mg), plates m.p. 180° from ether–benzene (lit.[14] m.p. 178–180° dec.), λ_{max} 259 mμ and λ_{infl} 287 mμ (ε 7690 and 2610), ν_{max} (in $CHCl_3$) 3700–2300 and 1707 ($ArCO_2H$), and 1590 cm^{-1} (Ar). (Found: C, 55·0; H, 5·5. Calc. for $C_{13}H_{16}O_7$: C, 54·9; H, 5·7 %.) The PMR spectrum (in pyridine) showed a singlet at τ 6·16 (ArOC$\underline{H}_3$), triplets at τ 8·55 and 8·63 (1:2:1, J = 7·5 c/s, 2 C$\underline{H}_3$CH$_2$O—), and overlapping quartets centred at τ 5·57 and 5·82 (1:3:3:1, J = 7·5 c/s, 2 MeC$\underline{H}_2$O—).

Sublimation of this phthalic acid at 140°/10^{-2} mm gave 3,4-diethoxy-5-methoxyphthalic anhydride, needles m.p. 97° from light petroleum, λ_{max} 247 and 321 mμ (ε 1800 and 412), ν_{max} 1850 and 1781 cm^{-1} (phthalic anhydride[13]). (Found: C, 58·8; H, 5·4. Calc. for $C_{13}H_{14}O_6$: C, 58·6; H, 5·3 %.) A mixed m.p. with authentic material[14] showed no depression, and IR and UV spectra of the compounds were identical.

Ozonolysis of (+)-*4,5-diacetoxy-6-methoxy-3-methylphthalide.* The (+)-phthalide (IV: R^1 = R^2 = Ac, R^3 = Me) (98 mg) was ozonized in acetic acid (5 ml) for 16 hr at room temperature, when hydrogen peroxide (30 %, 7·5 ml) was added. After 2 hr, excess of oxidant was destroyed with palladized charcoal in the presence of a trace of ferric chloride, and the solution was filtered. The solvent was removed under reduced pressure and the residue evaporated four times with benzene (10 ml) to remove acetic acid. Preparative thin-layer chromatography on Kieselgel-G (Merck) in ether–benzene (1:1) separated L-lactic acid, $[\alpha]_D^{26}$ +3·85° (c. 0·57 % in H_2O) (lit.[19] $[\alpha]_D^{15}$ +3·82° in H_2O). The acid was identified by its IR spectrum in comparison with authentic (±)-material, and by conversion with diazomethane into the methyl ester which was identical with authentic (±) methyl lactate in IR spectrum, R_f value of thin-layer chromatography, and retention time on gas–liquid chromatography on a polyethylene glycol adipate column at 125°.

Isolation of other metabolites from C. Siddiqui. Curvularia Siddiqui sp. novo was grown on a synthetic

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[19] I. Heilbron and M. Bunbury, *Dictionary of Organic Compounds* Vol. 3, p. 160. Eyre and Spottiswoode, London (1953).

medium enriched with carrot extract, harvested, and the acidic fraction soluble in aqueous sodium bicarbonate was separated, as described previously.[2] Extraction with boiling ether left a residue of fairly pure curvulic acid, whilst the ether extract on fractional crystallization from ether–light petroleum gave succinic and curvulinic acids. Extraction of the evaporated mother-liquors with hot chloroform, after filtration and cooling, afforded colourless needles of 3,5-dimethoxybenzoic acid, m.p. 180° (lit.[20] m.p. 185–186°), λ_{max} 259 and 304 mμ (ε 1330, 1320), ν_{max} (in Nujol) 3580–2550 and 1680 cm^{-1} ($ArCO_2H$). (Found: C, 59·0; H, 5·9; O, 35·1; OMe 33·7; M (Rast) 180. Calc. for $C_9H_{10}O_4$: C, 59·[illegible]; H, 5·5; O, 35·1; 2 OMe, 34·1 %; M, 182.) The acid, identified by mixed m.p. and IR spectrum in comparison with authentic material, showed PMR absorption in pyridine at τ 6·23 (singlet, 2 $\mathrm{ArOC}\underline{\mathrm{H}}_3$) and in dioxane three aromatic protons as an A_2B system at τ 2·70 (doublet, J_{AB} = 2·5 c/s) and τ 3·20 (triplet, J_{AB} = 2·5 c/s).

Chromatography of the chloroform mother-liquors on silica gel in ether gave a crystalline fraction, m.p. 99° shown to contain two components by thin-layer chromatography. Preparative thin-layer chromatography on Kieselgel G (Merck) in ether–benzene (1:1) separated the major component. Re-chromatography on silica gel in ether–benzene (1:2) afforded 2-acetyl-3,5-dimethoxyphenylacetic acid, m.p. 119°, λ_{max} 222, 270 and 304 mμ (ε 12,800, 7140 and 5180), ν_{max} (in Nujol) 3440–2400 and 1710 (CO_2H) and 1660 cm^{-1} (ArCOMe), identified by mixed m.p., thin-layer chromatography and IR spectrum in comparison with synthetic material,[4] m.p. 120°. (Found: C, 60·4; H, 6·2; O, 33·4; OMe, 26·3; CMe, 5·5; M (Rast) 225. Calc. for $C_{12}H_{14}O_5$: C, 60·5; H, 5·9; O, 33·6; 2 OMe, 26·1; 1 CMe 6·3 %; M, 238.)

Diazomethylation of this phenylacetic acid (50 mg) in ether gave *methyl 2-acetyl-3,5-dimethoxyphenylacetate* (47 mg) an oil b.p. 70–73°/0·1 mm, λ_{max} 222, 268 and 303 mμ (ε 16,300, 7730 and 5890), ν_{max} 1740 (CO_2Me) and 1685 cm^{-1} (ArCOMe). (Found: C, 62·0; H, 6·14. $C_{13}H_{16}O_5$ requires: C, 61·9; H, 6·4 %.)

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[20] Ref. 19, 2, 270.