acetate [11β,21-dihydroxypregna-1,4-dien-3,20-dione 21-acetate] in 10 mL of dry tetrahydrofuran was added via the addition funnel, and the funnel was washed with an additional 10 mL of solvent. TLC indicated the reaction was complete after 2 h and the solution was poured into water and adjusted to a pH of ca. 4 with acetic acid. From this aqueous suspension, 821 mg (2.01 mmol, 78%) of **13** slowly crystallized: mp 187–194 °C (ethyl acetate–cyclohexane); IR 3400, 2220, 1750, 1665, 1615, 1605 cm^{-1}; UV 218 nm (ϵ 25 000); NMR δ 7.35 (d, J = 10 Hz, 1 H), 6.28 (dd, J = 10, 2 Hz, 1 H), 6.04 (s, 1 H), 5.43 (s, 1 H), 4.95 (d, J = 14.5 Hz, 1 H), 4.67 (d, J = 14.5 Hz, 1 H), 4.45 (m, 1 H), 2.13 (s, 3 H), 1.48 (s, 3 H), 0.95 (s, 3 H). Anal. Calcd for $C_{25}H_{31}NO_4$: C, 73.32; H, 7.63; N, 3.42. Found: C, 73.07; H, 7.89; N, 3.37.

The Formation of the Cardenolide 14 from the 1,4-Dien-3-one 13. To a solution of **13** (350 mg, 0.86 mmol) in 50 mL of ethanol was added 2.5 mL of distilled water and 0.5 g of *p*-toluenesulfonic acid monohydrate. The reaction mixture was refluxed for 16 h, cooled, and diluted with 150 mL of distilled water. The precipitate was filtered, dried, and crystallized from methylene chloridine–ethyl acetate–petroleum ether to yield 205 mg (0.57 mmol, 65%) of cardenolide **14** as the monohydrate: mp 256–269 °C; IR 3450, 1780, 1750, 1660, 1625, 1660 cm^{-1}; UV 220 nm (ϵ 21 000), 244 (sh, 15 500); NMR (Me_2SO-d_6) δ 7.37 (d, J = 10 Hz, 1 H), 6.17 (dd, J = 10, 2 Hz, 1 H), 5.95 (m, 2 H), 4.83 (d, 2 H), 4.57 (d, exchanges with D_2O), 4.23 (m, 1 H), 1.40 (s, 3 H), 0.86 (s, 3 H). Anal. Calcd for $C_{23}H_{28}O_4 \cdot H_2O$: C, 71.48; H, 7.82. Found: C, 71.63; H, 7.64.

Registry No.—**2,** 14030-39-8; **2** enol ether, 53287-13-1; **3,** 1693-63-6; **4,** 66007-63-4; **7,** 23330-61-2; **8,** 19637-05-9; **9,** 2739-50-6; **10,** 65970-05-0; **11,** 65970-06-1; **12,** 58652-04-3; **13,** 65970-17-4; **14,** 65970-18-5; **15,** 65970-08-3; **16,** 65970-09-4; **17,** 65970-07-2; **18,** 6747-92-8; **19,** 66007-62-3; **20,** 53287-14-2; **21,** 24366-43-6; **22,** 65970-10-7; **23,** 65970-11-8; **24,** 65970-12-9; **25,** 65970-13-0; **26,** 65970-14-1; **27,** 65970-15-2; **28,** 65970-16-3; **29,** 65969-98-4; **30,** 65969-99-5; **31,** 65970-00-5; **31** 3-enol ether, 65970-01-6; **31** 11-oxo derivative, 65970-04-9; **33,** 66007-61-2; **34,** 65970-02-7; **35,** 65970-03-8; diethyl cyanomethylphosphonate, 2537-48-6; deoxycorticosterone acetate, 56-47-3.

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New Zearalenone Related Macrolides and Isocoumarins from an Unidentified Fungus

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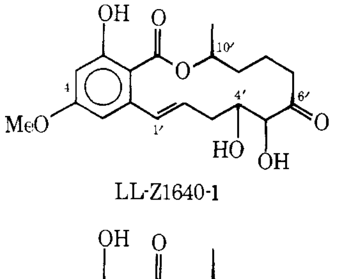
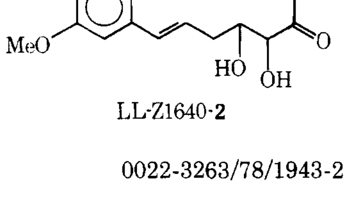
The isolation and characterization of four new zearalenone-like macrolides and three isocoumarins from an unidentified fungus, Lederle Culture Z1640, are reported. By altering the fermentation conditions this organism could be forced to produce curvularin macrolides. X-ray studies on a di-*p*-chlorobenzoyl derivative of LL-Z1640-1 showed this metabolite to be (4′*S*,5′*S*)-4′,5′-dihydroxyzearalenone 4-methyl ether.

Lederle culture Z1640 was selected for study on the basis that crude extracts of shaker-flask fermentations inhibited the growth and motility of the ciliated protozoan *Tetrahymena pyriformis*. Stirred fermentations of this unidentified fungus yielded three zearalenone[1] related metabolites LL-Z1640-1, -2, and -4. Surface fermentation yielded LL-Z1640-4 and -3 as the predominant products. In an attempt to obtain the corresponding diphenolic compounds still cultures were incubated in the presence of D,L-ethionine.[2] Under these conditions the mycelium was blanched from the normal very dark color and workup yielded curvularin and dihydrocurvularin[3] and none of the larger macrolides.



LL-Z1640-1



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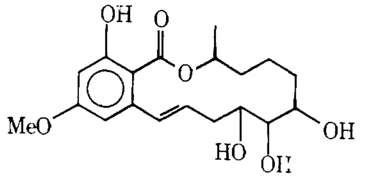
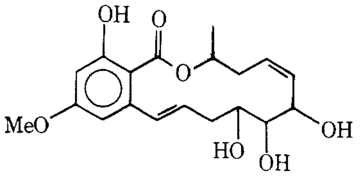


LL-Z1640-3



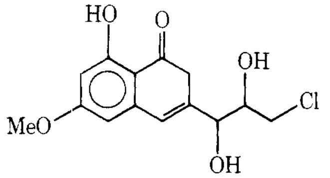
LL-Z1640-4

0022-3263/78/1943-2339$01.00/0

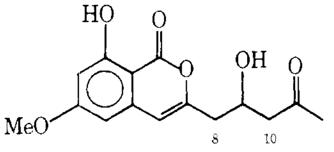
The culture failed to produce spores under a number of conditions and consequently its identity has not been established. Based on the dark-colored mycelium it exhibits, it likely belongs in the family *Dematiaciae* of the class *Deuteromyces*.

The uterotropic and anabolic properties of zearalenone are well known.[1a] Macrolides 1 through 4 revealed no particularly interesting activities except perhaps some antiviral effects associated with **4**. The presence of the phenolic esters may be responsible for this lack of activity. In our hands all attempts to remove this ether by classical methods resulted in degradation.

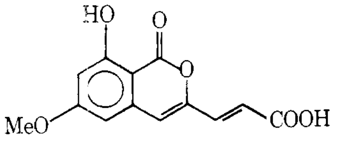
In one fermentation where very little of the macrolides was observed, three new isocoumarins LL-Z1640-**5**, -**6**, and -**7** were produced. Components **6** and **7** were obtained in some quantity whereas only a very minor yield of **5** was recovered (see Experimental Section).



LL-Z1640-**5**



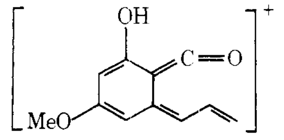
LL-Z1640-**6**



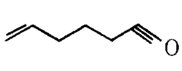
LL-Z1640-**7**

Characterization of Macrolides. Macrolide **1**, $C_{19}H_{24}O_7$, was shown to be a β resorcylate by characteristic UV maxima at 232, 270, and 311 nm (ϵ 30 900, 12 000, and 6 100). The NMR spectrum included signals indicative of a secondary methyl (δ 1.40, J = 6 Hz), a methoxy (δ 3.82) two aromatic protons (δ 6.40), and a trans double bond (eight-line pattern at δ 5.95 and a broadened doublet at δ 7.05, J = 15 Hz). An exchangeable 1 H singlet at δ 12.0 is assigned to the chelated phenolic hydrogen. The IR spectrum shows carbonyl bands at 1710 and 1655 cm^{-1} attributed to the ketone and hydrogen-bonded lactone groupings. A positive reaction with periodate and the ready formation of an acetonide indicated the vicinal glycol moiety.

The mass spectrum of the acetonide, $C_{22}H_{28}O_7$, provided decisive evidence for the structure of the macrocyclic portion. The base peak at *m/e* 190 and the second most abundant ion at *m/e* 97 are consistent only with the glycol–ketone system as depicted in **1**.



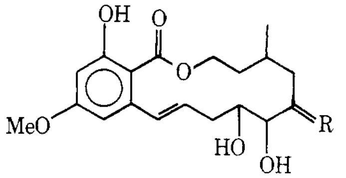
m/e 190



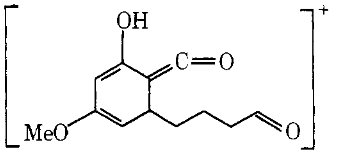
m/e 97

Lactone **2**, $C_{19}H_{22}O_7$, exhibited an UV spectrum almost identical to that of **1** except the 233 nm maxima had an ϵ of 38 200 cm^{-1} requiring the presence of an additional chromophore. The IR spectrum is also closely related to that of **1** except the 6′ ketone band is now at 1680 cm^{-1} consistent with its being part of an α,β-unsaturated system. This was supported by the NMR spectrum which showed the additional trans vinyl proton signals. The mass spectrum of the easily formed acetonide, $C_{22}H_{26}O_7$, showed in this case an *m/e* 95 ion as the base peak while the *m/e* 190 ion is only 16% of the former. The *m/e* 95 ion is consistent with the position of an additional site of unsaturation in the $C_{6'}$–$C_{11'}$ unit.

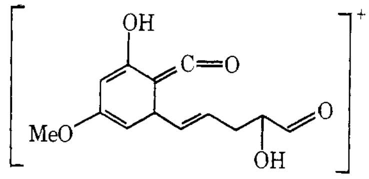
Hydrogenation of both **1** and **2** gave **8** as shown by mp, mmp, and IR spectrum. Examination of the mass spectrum of **8** revealed significant differences from that of **1**, **2**, and their acetonides in that strong ions at *m/e* 221 and 251 are observed rather than the characteristic *m/e* 190 ion seen in the spectra of the parent compounds.



8, R = O
9, R = H,OH

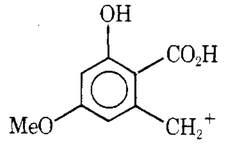


m/e 221

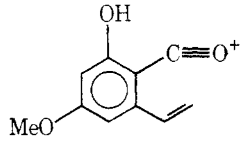


m/e 251

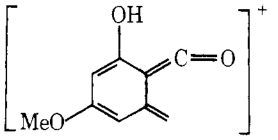
Other significant fragmentation ions are observed at *m/e* 182, 177, and 164 with structures as shown. The *m/e* 97 ion expected from the $C_{6'}$–$C_{11'}$ chain is surprisingly only 4% that of the base peak at *m/e* 128.



m/e 182



m/e 177



m/e 164

Lactones **3** and **4** showed UV spectra similar to those of the corresponding ketones although their IR spectra are devoid of the $C_{6'}$ ketone absorption. Reduction of **3** and **4** gave **9** indicating the only difference between them to be the double bond between $C_{7'}$ and $C_{8'}$ in **4**. Sodium borohydride reduction of **1** gave two triols, one of which was identical to **3** thus interrelating all four macrolides.

Macrolide **4** was further characterized by the formation of its acetonide **10**. Although the acetonides of **1** and **2** were crystalline, that of **4** was an oil despite considerable effort to crystallize it. Oxidation of **10** with Jones reagent yielded the crystalline ketone **11**. The IR spectrum of **11** has a strong band at 1735 cm^{-1} indicating the structure of **11** to be as indicated. Further verification was obtained from the NMR spectrum

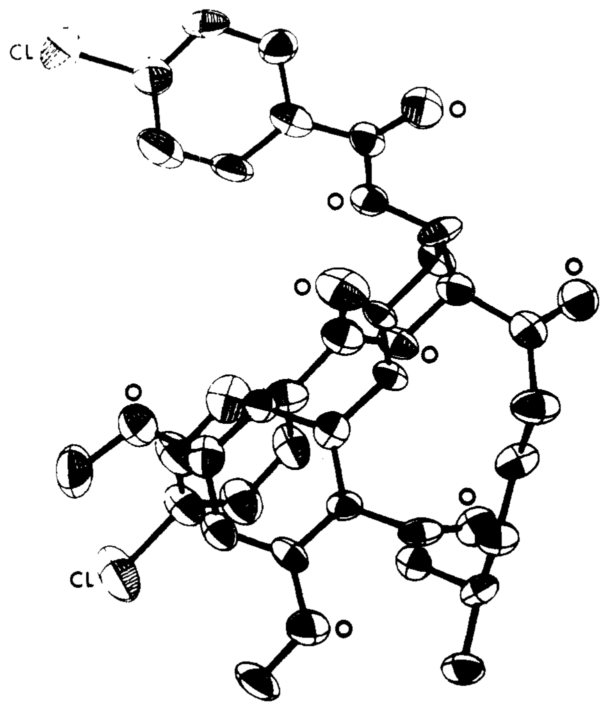
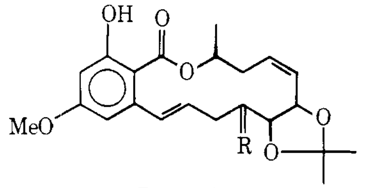


Figure 1.

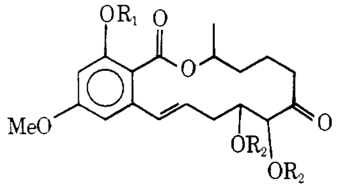
of 12 which showed a 1 H doublet at δ 4.65 (J = 8 Hz) assigned to $H_{5'}$ which is split only by $H_{6'}$. Compound 12 which is the



10, R = H,OH
11, R = O

2-methyl ether of 1 upon treatment with p-chlorobenzoyl chloride in pyridine gave the nicely crystalline 13.

X-Ray Crystallography. Prismatic crystals of 13, suitable for X-ray analysis, yielded the following data: from diffractometer measurements on 25 reflections in the range 20 < θ



12, $R_1 = CH_3$; R_2 = H
13, $R_1 = CH_3$, R_2 = C(O)-p-ClPh

< 30 °C (Cu Kα λ 1.5418 Å) a = 10.257 (3), b = 11.147 (4), c = 28.287 (11) Å; orthorhombic crystals, space group $P2_12_12_1$, C = 4; d_{obsd} = 1.336 g cm^{-3}, d_{calcd} = 1.343 g cm^{-3}; linear absorption coefficient μ = 22.6 cm^{-1}; crystal size 460 × 230 × 320 m elongated along the a axis.

Intensity data were collected on a computer controlled Enraf-Nonius CAD-3 diffractometer using the θ–2θ scan method with Ni-filtered Cu Kα radiation. In the range 3 < θ < 60 °C, 2753 independent reflections were measured of which 1736 were classified as observed by the criterion $I > 2\sigma(I)$ where $\sigma(I)$ was determined from counting statistics. No absorption corrections were applied.

A trial structure consisting of two chlorine atoms and 40 nonhydrogen atoms was obtained using the MULTAN[4] program for direct-phase determination. Isotropic refinement treating all atoms except the chlorines as carbon gave $R = [\Sigma||F_o| - |F_c||/\Sigma|F_o|]$ = 0.22. The remaining atoms of the molecule were then found in a difference electron density map. After assigning oxygen atoms on the basis of chemistry and refined isotropic temperature parameters further refinement (isotropic and anisotropic) led to R = 0.10 for observed reflections. Coordinates for all hydrogens (except on the methyl groups) were calculated and peaks corresponding to them were found in a difference map. Hydrogens were included, but not refined, in final refinement calculations; the last difference map contained no significant peaks. The anomalous dispersion effects of chlorine and oxygen were included in a series of anisotropic refinement calculations for the two enantiomers of the structure. Reliability indices of R = 0.076 and R = 0.080 were obtained which by the Hamilton[5] test are significantly different. In the structure corresponding to R = 0.076 the configurations at atoms $C_{4'}$, $C_{5'}$, and $C_{10'}$ are all S. The same configuration at $C_{10'}$ was found in the structure of 8′-hydroxyzearalenone.[6]

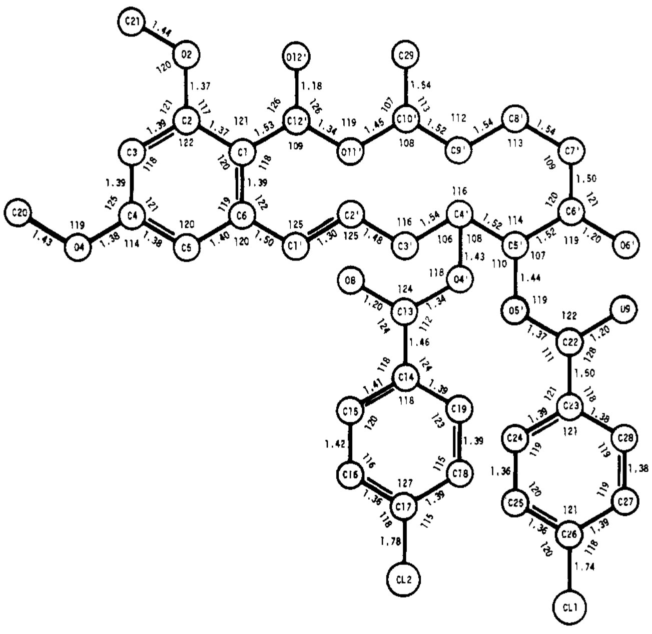


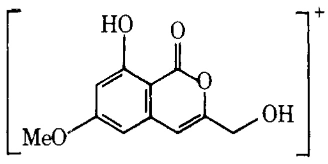
Figure 2.

Lists of coordinates and anisotropic temperature parameters for the nonhydrogen atoms and torsion angles in the structure are available as supplementary data and appear in the microfilm edition. Bond distances and angles are shown in Figure 1 (a list of structure factors has been bound into *J. Org. Chem.* 43 (1978), held in the library at Lederle Laboratories; copies may be obtained from the Librarian).

All calculations were made using the X-RAY SYSTEM (1972)[7] set of crystallographic programs; atomic scattering factors and dispersion corrections were taken from the International Tables for X-ray Crystallography.[8]

A survey of contact distances <4.0 Å in the unit cell revealed no unusually close interactions. The conformation of the molecule is shown in Figure 2. The torsion angle $O_{4'}$–$C_{4'}$–$C_{5'}$–$O_{5'}$ is −57° corresponding to a left-handed rotation looking along $C_{4'}$–$C_{5'}$; this agrees with the result obtained from circular dichroism studies on this compound.

Characterization of Isocoumarins. The isocoumarin 5, $C_{13}H_{13}O_6Cl$, has the structure shown on the basis of NMR, UV, IR, and mass spectral data. A 2,4-dihydroxyisocoumarin moiety is indicated by comparison of the UV spectrum with that of the known metabolite diaporthin.[9] The NMR of 5 shows signals attributable to the phenolic hydroxy proton at δ 11.04, the meta aromatic hydrogens as 1 H doublets at δ 6.4 and 6.5 (J = 2 Hz), the methoxy signal at δ 3.88, and the vinyl



m/e 221

proton as a sharp 1 H singlet at δ 6.58. The chloromethyl protons resonate as a 2 H doublet at δ 3.80 (J = 4 Hz) and the two remaining hydrogens at δ 4.15 and 4.45 as broad multiplets. A positive reaction with periodate confirmed the presence of the vicinol glycol moiety. The base peak in the mass spectrum occurs at *m/e* 221 which is represented as shown.

Likewise the low-field NMR signals in the spectrum of **6** are essentially identical to the corresponding ones of diaporthin. The terminal methyl signal resonates at δ 1.20 and two overlapping doublets at δ 2.65 and 2.69 (J = 5.5 Hz) are assigned to the C_8 and C_{10} methylene protons. The C_9 carbinyl proton signal is seen as a five-line pattern at δ 2.47 while the C_9 hydroxyl proton signal resonates as an exchangeable signal at δ 3.60. Prominent fragmentation ions in the mass spectrum appear at *m/e* 274, 232, and 206 consistent with the assigned structure.

The most polar isocoumarin **7** exhibited a UV spectrum similar to that of **5** and **6** but each maximum was shifted bathochromically with absorption at 259, 304, 313, 342, and 355 sh nm (ϵ 43 300, 11 800, 12 300, 15 500, and 11 800). The NMR spectrum showed signals indicative of the isocoumarin moiety but in addition signals representing an AB pair at δ 6.14 and 6.32 (J = 13 Hz) were present which were assigned to the trans olefinic protons of the double bond of the side chain. The acidic nature was indicated by its solubility in sodium bicarbonate.

Biosynthesis. A polyketide pathway from acetate is the most likely biogenetic origin of zearalenone metabolites.[1e] A similar route has been proposed for the curvularins.[3a] The close relationship of zearalenone, curvularin, and isocoumarins such as diaporthin each produced by different fungi is well known. It is noteworthy that culture Z1640 is capable of producing metabolites of each type depending on the fermentation conditions.

Experimental Section

TLC was carried out on Brinkmann silica gel plates. NMR spectra were obtained with a Varian A-100 instrument. Mass spectra were obtained with an AEI MS9 mass spectrometer. IR and UV spectra were run on an Infracord and Cary 11 spectrophotometers, respectively. CD curves run on a Jasco spectropolarimeter were supplied by Professor K. Nakanishi of Columbia University. Solvents and solutions were dried over anhydrous $MgSO_4$. Melting points were taken on a Thomas hot-plate apparatus and are uncorrected.

Isolation of Macrolides. Culture Z1640 was grown from a slant in a medium consisting of 3% corn steep liquor, 3% glucose, 1% proflo, and 0.5% $CaCO_3$ with pH adjusted to 6.5 before sterilization. Second stage inoculum was added to a 300-L fermenter containing the medium NH_4 tartrate 0.2%, $MgSO_4{\cdot}7H_2O$ 0.05%, KH_2PO_4 0.1%, KCl 0.05%, $FeSO_4{\cdot}7H_2O$ 0.001%, glucose 5%, and corn steep liquor 1% with pH adjusted to 6.0. Fermentation occurred at 28 °C with air supply at 0.5 v/v/min and impeller speed at 215 rpm. Harvesting was carried out after 11 days and the pH was usually below 5.5.

The ethyl acetate extract (400 L) of the culture filtrate (pH 4.2) from two 300-L 11-day fermentations was concentrated to a dark viscous residue. This was taken up in methanol and washed with heptane and reconcentrated to dryness. Trituration with ether provided a semicrystalline mass which was slurried in a methanol–methylene chloride mixture and the soluble portion was absorbed into the top of an acid-washed silica gel column (5.5 × 75 cm) packed in methylene chloride. The column was developed with 5% methanol–methylene chloride and monitored at 275 nm by an UV spectrum. Combination of the appropriate fractions and concentration to dryness gave on tituration with ether 842 mg of crude **2**. Recrystallization from ethyl acetate–hexane gave the analytical sample: mp 172–176 °C; $[\alpha]_D$ −75.9° (*c* 0.41, MeOH); IR (KBr) 3500, 1690, 1640, 1610, 1575 and 970 cm^{-1}; UV (MeOH) 233, 271, and 314 nm (ϵ 38 200, 11 400, and 5 960); mass spectrum *m/e* (%, composition) 362 (M, 42, $C_{19}H_{22}O_7$), 344 (2, $C_{19}H_{20}O_6$), 249 (7, $C_{13}H_{13}O_5$), 219 (41, $C_{12}H_{11}O_4$), 190 (100, $C_{11}H_{10}O_3$), and 95 (61, C_6H_7O). Anal. Calcd for $C_{19}H_{22}O_7$: mol wt 362.1365. Found: mol wt (mass spectrum) 362.1377.

That portion of the above-mentioned semicrystalline mass which was insoluble in the methanol–methylene chloride system was filtered off to give 40 g of semicrystalline material which by silica gel TLC (chloroform–methanol, 85:15) was composed of two materials with R_f's of 0.60 and 0.25. Ten grams of this mixture was dissolved in methanol–acetone and adsorbed onto silica gel. The solvent was then removed on a rotary evaporator and the silica gel was poured onto the top of a silica gel column (5.5 × 75 cm) packed in methylene chloride. The column was developed with 5% methanol–methylene chloride and monitored at 275 nm. This gave a single broad band resulting in 9 g of a crystalline mixture of **1**, **2**, and **4** as shown by TLC. Rechromatography over the same size column resolved the charge into a crystalline mixture of **1** and **2** and 2.9 g of essentially pure **4**. Fractional crystallization of **1** from benzene/hexane gave the analytical sample: mp 151–153 °C; $[\alpha]_D$ −80° (*c* 0.48, MeOH); IR (KBr) 3550, 1710, 1645, 1610, 1570, and 970 cm^{-1}; UV (MeOH) 232, 270, and 311 nm (ϵ 30 900, 12 000, 6 100); *m/e* (%, composition) 364 (M, 25, $C_{19}H_{24}O_7$), 346 (1, $C_{19}H_{22}O_6$), 249 (13, $C_{13}H_{13}O_5$), 219 (32, $C_{12}H_{11}O_4$), 190 (100, $C_{11}H_{10}O_3$), and 97 (13, C_6H_9O). Anal. Calcd for $C_{19}H_{24}O_7$: mol wt 364.1522. Found: mol wt (mass spectrum) 364.1504.

Recrystallization of **4** from ethyl acetate–hexane gave crystals: mp 193–195 °C; $[\alpha]_D$ −102° (*c* 0.49, MeOH): IR (KBr) 3500, 1645, 1620, and 1570 cm^{-1}; UV (MeOH) 236, 272, and 316 (ϵ 21 840, 9 100, and 4 000); *m/e* (%, composition) 364 (M, 16, $C_{19}H_{24}O_7$), 346 (12, $C_{19}H_{22}O_6$), 328 (7, $C_{19}H_{20}O_5$), 267 (16, $C_{13}H_{15}O_6$), 249 (36, $C_{13}H_{13}O_5$), 219 (19, $C_{12}H_{11}O_4$), 190 (100, $C_{11}H_{20}O_3$), and 97 (42, C_6H_9O). Anal. Calcd for $C_{19}H_{24}O_7$: mol wt 364.1522; Found: mol wt (mass spectrum) 364.1536.

A second crop from the above mother liquors provided an additional 283 mg of **4**.

Acetonide of 2. A mixture of 200 mg of **2** in 5 mL of 2,2-dimethoxypropane was treated with a few milligrams of *p*-toluenesulfonic acid and warmed gently on the steam bath until solution was complete. After standing overnight at room temperature, the solution was diluted with chloroform, washed with 5% sodium bicarbonate and brine, and dried (magnesium sulfate) and the solvent was removed to give a crystalline residue. Recrystallization from ethyl acetate/hexane gave 100 mg of crystals: mp 164–165 °C; $[\alpha]_D$ −71.3° (*c* 0.59, MeOH); IR 3500, 1704, 1645, 1570, 990, and 970 cm^{-1}; *m/e* (% composition) 402 (M, 2, $C_{22}H_{26}O_7$), 190 (15, $C_{11}H_{10}O_3$), and 95 (100, C_6H_7O). Anal. Calcd for $C_{22}H_{26}O_7$: mol wt 402.1678. Found: mol wt (mass spectrum) 402.1670.

Acetonide of 1. Treatment of **1** with 2,2-dimethoxypropane in the same manner as for **2** gave crystals: mp 187–188 °C; IR (KBr) 3500, 1720, 1650, 1620, and 1570 cm^{-1}; *m/e* (%, composition) 404 (M, 55 $C_{22}H_{28}O_7$), 190 (100, $C_{11}H_{10}O_3$), and 97 (92, C_6H_9O). Anal. Calcd for $C_{22}H_{28}O_7$: mol wt 404.1835. Found: mol wt (mass spectrum) 404.1830.

Acetonide of 4. A crude mixture of 2.5 g of LL-Z1640 crystalline solids containing primarily **1** and **4** by TLC was heated with 60 mL of 2,2-dimethoxypropane in the presence of a few crystals of *p*-toluenesulfonic acid. Upon solution the solvent was evaporated off and the residue was chromatographed over 100 g of acid-washed silica gel using a 50:50 hexane/ethyl acetate solvent system. About 1.9 g of the acetonide of **1** was obtained, mp 181–183 °C, as indicated by IR and NMR. The second product (1 g) eluted was an oil which by TLC was a single compound **10**: *m/e* (%, composition) 404 (M, 30, $C_{22}H_{28}O_7$), 249 (10, $C_{13}H_{13}O_5$), 219 (9, $C_{12}H_{11}O_4$), 193 (17, $C_{10}H_9O_4$), and 190 (23, $C_{11}H_{10}O_3$). Anal. Calcd for $C_{22}H_{28}O_7$: mol wt 404.1833. Found: mol wt (mass spectrum) 404.1833.

About 600 mg of this oil was dissolved in 75 mL of ether and stirred overnight with a solution of 2 g of potassium dichromate in 0.2 mL of concentrated sulfuric acid and 6 mL of water. The ether phase was evaporated to give 450 mg of residue which was chromatographed over 50 g of acid-washed silica gel using 10% ethyl acetate in hexane. This gave in addition to unreacted starting material **10** 95 mg of crystalline **11**: mp 169–170 °C; $[\alpha]_D$ −77° (*c* 0.67, ethyl acetate–methanol 30:70); *m/e* (%, composition) 402 (M, 10, $C_{22}H_{26}O_7$), 190 (68, $C_{11}H_{10}O_3$), 189 (100, $C_{11}H_9O_3$), and 167 (13, $C_{10}H_{15}O_2$). Anal. Calcd for $C_{22}H_{26}O_7$: C, 65,66; H, 6.51. Found: C, 65.73; H, 6.64.

Hydrogenation of 1 and 2. A solution of 32 mg of **1** in methanol was stirred in a hydrogen atmosphere in the presence of 10 mg of 10% palladium on charcoal until the hydrogen uptake ceased (~1 mmol). The mixture was filtered through diatomaceous earth and the filtrate was evaporated to dryness in vacuo giving a crystalline residue of **8**.

Recrystallization from benzene/hexane gave the analytical sample: mp 160–161 °C; $[\alpha]_D$ +30.5° (*c* 0.47, MeOH); IR (KBr) 1705, 1645, 1630, and 1580 cm^{-1}; UV (MeOH) 218, 265, and 305 nm (ϵ 21 400, 10 800, and 4 390); *m/e* (%, composition) 366 (M, 47, $C_{19}H_{26}O_7$), 251 (44, $C_{13}H_{15}O_5$), 221 (45, $C_{12}H_{13}O_4$), 193 (40, $C_{11}H_{13}O_3$), 192 (53, $C_{11}H_{12}O_3$), 182 (39, $C_9H_{10}O_4$), 177 (39, $C_{11}H_{13}O_2$ and $C_{10}H_9H_3$), 164 (48, $C_9H_8O_3$), 129 (75, $C_7H_{13}O_2$), 128 (100, $C_7H_{12}O_2$). Anal. Calcd for $C_{19}H_{26}O_7$: mol wt 366.1678. Found: mol wt (mass spectrum) 366.1689.

A 50-mg sample of **2** was hydrogenated in the same manner as above to give **8** identical to that obtained from **1** by IR, mp, and mmp.

Sodium Borohydride Reduction of 1. Macrolide **1** (25 mg) in methanol was treated with a few milligrams of sodium borohydride and the reaction was allowed to stand at room temperature overnight. Acidification followed by extraction with ethyl acetate gave by silica gel TLC (methanol/chloroform 15:85) two compounds. The minor component by this TLC system was identical to **3.** The IR of the crystalline mixture was very similar to that of **3.**

Hydrogenation of 4. A solution of 50 mg of **4** in ethanol was hydrogenated over 10 mg of 10% palladium on charcoal. After the uptake was complete (slight excess of 2 mol), the reaction mixture was filtered through diatomaceous earth, concentrated to dryness, and crystallized from benzene/hexane to give crude **9.** Recrystallization from benzene/hexane gave the analytical sample: mp 146–148 °C; IR (KBr) 1640, 1615, and 1575 cm^{-1}; *m/e* (%, composition) 368 (M, 42, $C_{19}H_{10}O_7$), 251 (26, $C_{13}H_{15}O_5$), 221 (98, $C_{12}H_{13}O_4$), 206 (25, $C_{12}H_{14}O_3$), 192 (52, $C_{11}H_{12}O_3$), 182 (100, $C_9H_{15}O_4$), 177 (72, $C_7H_{13}O_5$), 164 (69, $C_9H_8O_3$), and 99 (81, $C_6H_{11}O$). Anal. Calcd for $C_{19}H_{28}O_7$: mol wt 368.1833. Found: mol wt (mass spectrum) 368.1824.

Preparation of 12 and 13. About 1 g of **1** was refluxed in acetone overnight in the presence of 2 g of K_2CO_3 and 5 mL of CH_3I. About 250 mg of **12,** mp 162–164 °C, was obtained following silica gel chromatography: CD (MeOH) $\Delta\epsilon$ (325) 0, (310) −0.02, (300) 0, (285) +0.05, (275) +0.02, (245) +0.22, (232) 0, (220) −0.17, and (215) −0.10. About 210 mg of **12** in 2 mL of pyridine was treated with 0.5 mL of *p*-chlorobenzoyl chloride at room temperature overnight. Water was added and the mixture was extracted with ether. The ether extract was treated with 6 M HCl, 5% Na_2CO_3, and brine, dried, and concentrated to a solid. Preparative TLC using 20% EtOAc in benzene gave **13:** mp 159–161 °C; yield 117 mg; NMR ($CDCl_3$) δ 5.26 (1 H, m, $H_{10'}$), 5.92 (1 H, m, $H_{5'}$), and 6.06 [1 H, d ($J_{4'5'}$ = 4 Hz), $H_{4'}$] CD (MeOH) $\Delta\epsilon$ (325) 0, (295) −1.8, (285) 0, (260) +16.8, (250) 0, (245) −8.0, and (230) 0; mass spectra, molecular ions at 656 and 654.

Surface Fermentation of Culture Z1640. Slants of LL-Z1640 were used to inoculate Erlenmeyer flasks each containing 50 mL of a medium consisting of corn steep liquor 2%, cerelose 4%, $(NH_4)_2SO_4$ 1%, KH_2PO_4 0.6%, and $CaCO_3$ 0.05% with the pH adjusted to 6.2 with a potassium hydroxide solution. The Erlenmeyers were incubated at 28 °C on a rotary shaker for 6 days. Each Erlenmeyer flask was then used to inoculate two Fernbach flasks, each containing 1 L of medium consisting of NH_4 tartrate 0.2%, $MgSO_4 \cdot 7H_2O$ 0.05%, KH_2PO_4 0.1%, glucose 5.0%, corn steep liquor 1.0% in aqueous solution with pH adjusted to 6.5 with sodium hydroxide. Fermentation at ambient temperature was allowed to continue for 8 to 9 weeks. At this time a thick black matte covered the surface of the medium in each Fernbach flask. The liquid layer was drained off and the matte was reflooded with fresh medium and these reflooded flasks were harvested at the end of 4 to 5 weeks.

Workup included extraction with half-volume of ethyl acetate, concentration of the solvent extract, followed by silica gel chromatography.

From 5 L of surface fermentation 800 mg of crude solids were obtained by extraction with ethyl acetate. Silica gel chromatography yielded 400 mg of **4.** Further chromatography of the mother liquors from this and other similar preparations yielded a small amount (100 mg) of **3:** mp 175 °C; $[\alpha]_D$ −112° (*c* 0.28, MeOH); IR (KBr) 3500, 1640, 1605, and 1575 cm^{-1}; UV (MeOH) 235, 272, and 315 nm (ϵ 26 580, 11 350, and 5 490); *m/e* (%, composition) 366 (M, 14, $C_{19}H_{26}O_7$), 348 (2, $C_{19}H_{24}O_6$), 330 (2, $C_{19}H_{22}O_5$), 219 (8, $C_{12}H_{13}O_4$), 190 (100, $C_{11}H_{26}O_3$), and 99 (30, $C_6H_{11}O$). Anal. Calcd for $C_{19}H_{26}O_7$: mol wt 366.1678. Found: mol wt (mass spectrum) 366.1661.

Still fermentations were set in Fernbachs using the same medium except that D,L-ethinoine at a concentration of 200 μg/mL of medium was added at the time of inoculation. Incubation was again allowed to proceed for about 8 weeks. The mattes in these flasks were almost white in color and workup in the described manner yielded only curvularin and dehydrocurvularin as shown by mp, NMR, IR, and mass spectral data.

Isolation of Isocoumarins. A 1000-L fermentation (11 days) was extracted with ethyl acetate (400 L) at pH 5. The concentrate was defatted by partitioning between methanol.heptane. The methanol layer was concentrated to dryness to give 125 g of a dark oily residue. This was chromatographed over an acid-washed silica gel (1000 g) column (in methylene chloride) and developed with a gradient between methylene chloride/25% ether–methylene chloride. The eluate was monitored at 275 nm by UV. Compound **6** was obtained from the first band and was obtained crystalline after concentration to dryness of the appropriate fractions and crystallization from ethyl acetate/hexane. This gave 1.34 g of **6:** mp 95–98 °C; $[\alpha]_D$ +4.4° (*c* 0.23, $CHCl_3$); UV 237 (sh), 243, 256 (sh), 277, 286 (sh), and 325 nm (ϵ 49 850, 56 450, 13 700, 7 400, 4 930, and 7 120); *m/e* (% composition) 292 (M, 4, $C_{15}H_{16}O_6$), 274 (35, $C_{15}H_{14}O_5$), 232 (69, $C_{13}H_{12}O_4$), and 206 (100, $C_{11}H_{10}O_4$). Anal. Calcd for $C_{15}H_{16}O_6$: mol wt 292.0942; Found: mol wt (mass spectrum) 292.0947.

The next zone to be eluted off the column gave 2.9 g of **7** after concentration to dryness and crystallization from ethyl acetate/hexane: mp 192–194 °C; IR 3500, 1680, 1640, 1615, and 1570 cm^{-1}; UV 260, 305 (sh), 313, 342, and 354 nm (sh) (ϵ 47 160, 11 790, 12 315, 15 460, and 11 290); *m/e* (%, composition) 262 (M, 100, $C_{13}H_{10}O_6$), 244 (18, $C_{13}H_8O_5$), and 216 (12, $C_{12}H_8O_4$). Anal. Calcd for $C_{13}H_{10}O_6$: mol wt 262.0476. Found: mol wt (mass spectrum) 262.0461.

Finally a mixture of mainly **1** and a little **5** (4.1 g) was obtained from the last 275-nm band. Column chromatography over silica gel with a gradient elution [methylene chloride/methylene chloride (10% methanol)] gave after fractional crystallization from methylene chloride/ether 1.09 g of crude **1** and a little **5.** Preparative TLC (silica gel; ethyl acetate/chloroform 1:1) of 150 mg of the above material gave 5 mg of **5** after elution of the plate with acetone, concentration to dryness, and crystallization from ethyl acetate/hexane: mp 158–160 °C; IR (KBr) 3500, 1680, 1625, and 1575 cm^{-1}; UV 236 (sh), 244, 257 (sh), 276, 286 (sh), and 325 nm (ϵ 48 000, 54 000, 11 700, 6 600, 4 500, and 11 700); *m/e* (%, composition) 300 (M, 9, $C_{13}H_{13}O_6Cl$), and 221 (100, $C_{11}H_9O_5$). Anal. Calcd for $C_{13}H_{13}O_6Cl$: mol wt 300.0420; Found: mol wt (mass spectrum) 300.0413.

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Registry No.—**1,** 66018-37-9; **1** acetonide, 66036-83-7; **2,** 66018-38-0; **2** acetonide, 66018-39-1; **3,** 66018-40-4; **4,** 66018-41-5; **5,** 66018-42-6; **6,** 66018-43-7; **7,** 66018-44-8; **8,** 66018-45-9; **9,** 66018-46-0; **10,** 66018-47-1; **11,** 66018-48-2; **12,** 66018-49-3; **13,** 66018-50-6.

Supplementary Material Available: Atomic coordinates and torsion angles from the X-ray analysis of **13** (2 pages). Ordering information is given on any current masthead page.

References and Notes

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