Structure and Valence Isomerization of LL-Z1220. An Antibiotic Containing a Benzene Dioxide Moiety[1]

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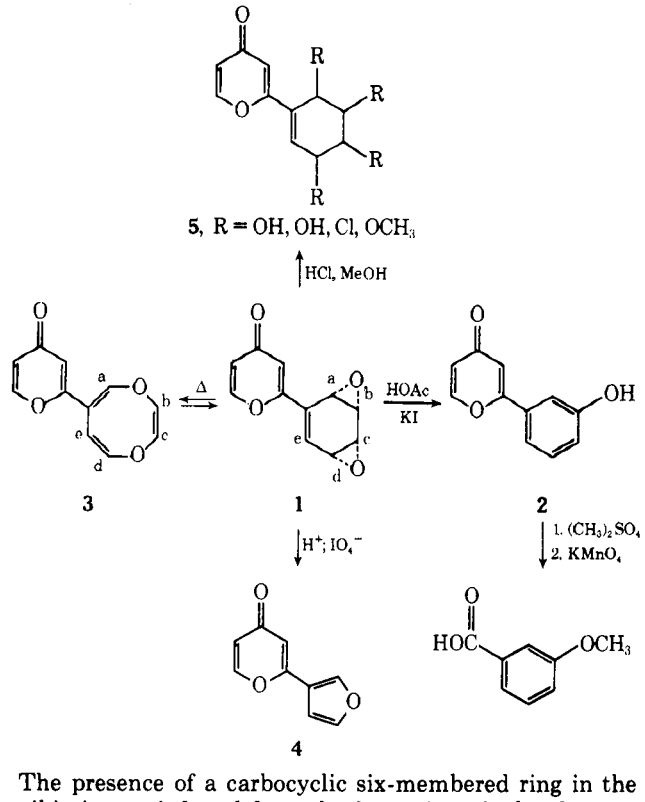
The structure 2-(3,8-dioxatricyclo[5.1.0.0^{2,4}]oct-5-en-5-yl)-4*H*-pyran-4-one is proposed for the novel antibiotic LL-Z1220. It appears to be the first reported natural product containing a benzene dioxide moiety. The antibiotic readily undergoes valence isomerization to give a 1,4-dioxocin. Chemical and spectral properties of the antibiotic indicate that the benzene dioxide has the syn configuration.

The isolation and preliminary characterization of LL-Z1220, an antibiotic produced by an undetermined fungal species, was reported previously.[2] It has *in vitro* activity against gram-negative and gram-positive bacteria and fungi. The antibiotic was obtained as a colorless, crystalline solid, $C_{11}H_8O_4$, mp 148° dec, $[\alpha]^{25}D$ − 123° (*c* 0.591, $CHCl_3$), which gave a positive test for an epoxide group. Evidence is now presented which allows structure proposal 1 for the antibiotic and indicates that it undergoes valence isomerization to a 1,4-dioxocin. This involves a revision from the *anti*-diepoxide structure favored earlier.

Discussion

Nmr spectroscopy, mass spectrum, and elemental analyses established the molecular formula of LL-Z1220 as $C_{11}H_8O_4$.[2] When the antibiotic was heated in acetic acid–potassium iodide, it formed a deoxy compound **2**, $C_{11}H_8O_3$, which was methylated and then oxidized with permanganate to yield *m*-methoxybenzoic acid. These results established the presence of a meta-substituted phenolic moiety in **2**. On the basis of spectral data, the remaining $C_5H_3O_2$ portion of **2** was deduced to be an α-substituted γ-pyrone and it was concluded that deoxy LL-Z1220 had gross structure **2**.[1]



The presence of a carbocyclic six-membered ring in the antibiotic was inferred from the formation of **2** by the acetic acid–potassium iodide reaction and of **5** by the reaction of hydrogen chloride in methanol.[1] The acetic acid–potassium iodide reagent is known to convert epoxides to carbon–carbon double bonds; however, in this case one of the epoxides yielded a phenolic hydroxyl group.

The oxygens of the carbocyclic ring were assumed to be in the form of epoxides by a consideration of ^{13}C and proton nmr spectra and chemical reactions; therefore, gross structure **1** was proposed for LL-Z1220.[1] This assignment is consistent with nmr data (100 MHz, $CDCl_3$) recently reported by Vogel and coworkers on synthetic benzene dioxide, which shows olefinic protons at δ 6.47 (2 H) and epoxide protons as multiplets at δ 3.71 (2 H) and 3.39 (2 H).[3] The benzene dioxide moiety of LL-Z1220 (100 MHz, $CDCl_3$) has corresponding signals at δ 7.18 (1 H, dd, J = 4.3, 1.2 Hz), 3.85 (2 H, m), and 3.64 (2 H, m).

When the antibiotic was heated in acetic anhydride, it rearranged in ~60% yield to a compound with the same molecular formula as the starting antibiotic. The nmr spectrum of the rearrangement product **3** clearly indicated the presence of eight protons, none of which exchanged in the presence of CD_3OD. The lowest field proton had a chemical shift (δ 7.68) and apparent splitting (J = 7.0 Hz) similar to those of the pyrone moiety proton of the starting antibiotic; however, the other pyrone protons of **3** appeared as a complex two-proton multiplet at δ 6.30. Spin-decoupling studies established that the low-field proton was coupled to the proton responsible for the multiplet at δ 6.30; a shift reagent resolved the nmr spectrum of this multiplet into the typical two-proton pattern anticipated for the β protons of the α-substituted γ-pyrone. The rearrangement product had ir absorption near 1652 cm^{-1} as expected for a γ-pyrone.[4] This evidence suggested that the rearrangement had not affected the α-substituted γ-pyrone moiety of the antibiotic.

The nmr spectrum of the remaining $C_6H_5O_2$ portion of **3** had absorptions of a one-proton singlet (δ 7.23) and two sets of one-proton doublets, δ 5.92 and 6.03 with J_{bc} = 4.1 Hz and δ 5.46 and 6.55 with J_{de} = 6.8 Hz. These coupling patterns were confirmed by a decoupling study. Investigation of the ^{13}CH satellite nmr spectrum of **3** indicated that the high-field protons, H_b, H_c, and H_e, were bonded to sp^2 carbons as deduced from the magnitude of the ^{13}CH splitting constants (192, 196, and 161 Hz, respectively). This is consistent with reported data for olefinic compounds, benzene, and furans.[5] The chemical shifts of the other two protons in this portion of the molecule were to lower field and could be designated as aromatic or olefinic. Unfortunately, the ^{13}CH couplings of H_a and H_d were obscured by other signals.

A structure which accommodates five aromatic or olefinic protons with the observed coupling pattern is a 6-substituted 1,4-dioxocin. Consequently, the proposed structure for the rearrangement product is **3** and the rear-

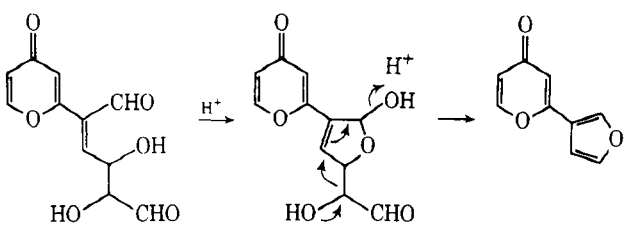
rangement is a benzene dioxide–1,4-dioxocin valence isomerization.

Refluxing 3 in ethyl acetate caused reversion to a product that was identical with the original antibiotic in all respects except for its lack of optical activity. Again, the conversion was not complete and the ratio of isolated antibiotic to rearrangement product under these conditions was approximately 1:9.

Recently Vogel, Altenbach, and Cremer reported the synthesis of 1,4-dioxocin and benzene dioxide.[3] Their observation that the coupling constant for the adjacent protons of the two epoxide rings of *syn*-benzene dioxide was 2.83 Hz led them to the conclusion that our previous assignment of anti stereochemistry to the benzene dioxide moiety of LL-Z1220 (J = 2.8 Hz) might be in error.[3] The suspected syn configuration is confirmed by the reaction described above in which the valence isomer of LL-Z1220 (3) isomerized on heating to a racemic mixture of LL-Z1220 which was not diastereomeric with the original antibiotic. This would be expected if the antibiotic had the syn configuration, since Vogel and coworkers showed that the 1,4-dioxocin isomerized only to *syn*-benzene dioxide.[3]

It is interesting to note that both LL-Z1220 (1) and its valence isomer 3 have *in vitro* antimicrobial activity. Both compounds are especially active against the fungi *Microsporum canis* and *Trichopyhyton mentrophytes*.

When the antibiotic was exposed to acid and then oxidized with periodate, a product having the molecular formula $C_9H_6O_3$ was obtained. Spectral data on this material indicated that the γ-pyrone was still intact and that the remaining C_4H_3O moiety was a β-substituted furan. The periodate oxidation product was therefore assigned structure 4 and could be rationally derived from 1 through intermediates 6 and 7.[1]



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LL-Z1220 appears to be the first reported natural product containing a benzene dioxide moiety. Although a number of natural products are known to have a multiple epoxide functionality, even the related cyclohexane diepoxide seems to be rare in nature. However, it has been observed in crotepoxide[6] and in the fused ring system of triptolide and tripdiolide,[7] substances obtained from plants.

Experimental Section

Melting points were determined on a Fisher-Johns hot stage and are uncorrected. Proton magnetic resonance spectra were determined on Varian A-60A, A-60D, and HA-100 spectrometers, and the ^{13}C nuclear magnetic resonance spectrum was obtained at 22.63 MHz on a Bruker HFX-90 spectrometer. Spin decoupling was accomplished by the frequency-sweep method with the Varian HA-100. Chemical shifts are reported as δ units with tetramethylsilane as an internal reference. Infrared spectra were obtained on a Perkin-Elmer infrared spectrophotometer, Model 21, and ultraviolet spectra on a Cary Model 11M. Mass spectra were determined with an AEI MS-9 mass spectrometer. Low-resolution spectra were at 1000 $m/\Delta m$ and high-resolution spectra were at 10,000 $m/\Delta m$. Perfluorokerosine was used as a standard for high-resolution spectra. Unless otherwise stated, thin layer chromatography was performed on precoated plates of silica gel (E. Merck F_{254}) supplied by Brinkmann Instruments, Inc. Zones were detected by uv quenching.

LL-Z1220. The nmr spectrum of LL-Z1220, along with other analytical data, were reported previously.[2] When a small amount of shift reagent, tris(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedione)europium(III),[8] was added to the nmr sample, sufficient chemical shifts were induced to produce first-order multiplets for the high-field proton. The four protons were separated by 0.45, 0.59, and 0.65 ppm and had the following coupling constants: J_{de} = 4.0, J_{ae} = 1.2, J_{cd} = 2.8, J_{bc} = 2.8, J_{ab} = 3.4 Hz.

Deoxy LL-Z1220 (2). LL-Z1220 (300 mg) was mixed with 600 mg of potassium iodide and 30 ml of glacial acetic acid[9] at room temperature for 1 hr. By the end of this time, the potassium iodide had dissolved and the solution had turned dark brown. The reaction mixture was passed through a 1.2 × 24 cm column containing a mixed bed resin of sulfonic (H^+) and quatarnary (OH^-) resins. The column was rinsed with 10 ml of acetic acid and 10 ml of water and the combined effluents were evaporated *in vacuo* to a semicrystalline residue. When this material was mixed with approximately 10 ml of water, an almost colorless precipitate was obtained which weighed 68 mg. The aqueous solution was freeze dried to obtain 271 mg of resinous material which by thin layer chromatography appeared to be the same as the precipitated material but less pure. LL-Z1220 had R_f 0.47 and the reaction product (deoxy LL-Z1220) had R_f 0.60 when the layer was developed with acetone. The precipitated deoxy LL-Z1220 could be further purified by sublimation to obtain an almost white powder: mp 241–243°; uv max (CH_3OH) 278 nm (ϵ 13,900); mass spectrum (70 eV) m/e (rel intensity) 188 (100), 160 (74), 147 (4), 131 (24), 121 (26), 118 (89). The nmr spectrum (DMSO-d_6) showed pyrone protons at δ 8.24 (1 H, d, J = 6.0 Hz), 6.83 (1 H, d, J = 2.4 Hz), and 6.34 (1 H, dd, J = 6.0, 2.4 Hz), an exchangeable proton at δ 9.71 (1 H, s), and aromatic protons at δ 7.30 (3 H, m) and 6.98 (1 H, m). The ir spectrum (KBr) contained characteristic peaks at 3380–2700 (OH), 1645 (C=O), and 784 cm^{-1} (meta-disubstituted phenyl).

Anal. Calcd for $C_{11}H_8O_3$: mol wt, 188.0473. Found: mol wt, 188.0468 (mass spectrum).

***m*-Methoxybenzoic Acid from the Oxidation of 2.** The deoxy compound 2 (100 mg) was heated at 60° for 8 hr with 100 ml of acetone, 3.0 g of anhydrous K_2CO_3, and 1.1 ml of dimethyl sulfate according to a methylation procedure described by Rao and Axelrod.[10] The resulting brown solution was filtered to remove inorganic salts and evaporated to a brown, semicrystalline residue weighing 101 mg, assumed to be the methylated deoxy LL-Z1220.

The residue was then mixed with 5 ml of water, 1 drop of 50% aqueous NaOH, and 300 mg of $KMnO_4$. The mixture was refluxed for 1 hr, cooled to room temperature, adjusted to pH 1 with 4 N H_2SO_4, and then heated to boiling. $NaHSO_3$ was added to dissolve the remaining MnO_2. The resulting solution was extracted three times with 3-ml portions of $CHCl_3$. The combined extracts were washed with water and evaporated to 11 mg of crystalline residue, crude *m*-methoxybenzoic acid.

This material was purified by preparative tlc (R_f 0.52) on a layer developed with methanol–benzene–acetic acid (8:45:4) and crystallized from water to obtain the colorless, crystalline acid: mp 101–104° (reported[11] mp 109–110°); ir (KBr) 3200–2500 (OH), 1698 (C=O), and 747 cm^{-1} (CH); mass spectrum (70 eV) m/e 152.0488 (calcd for $C_8H_8O_3$, m/e 152.0473); uv max (CH_3OH) 228 nm (ϵ 4200), 292 (1800). This acid was shown to be identical with authentic *m*-methoxybenzoic acid by tlc and ir and uv spectra.

Valence Isomerization of LL-Z1220. A solution of 5 g of LL-Z1220 in 100 ml of acetic anhydride was heated on a steam bath for 50 min, cooled to room temperature, and poured into 1200 ml of ice water. After the mixture had stood for 1 hr at room temperature, it was evaporated to a semicrystalline residue. The residue was extracted with ethyl acetate to obtain 2.0 g of insoluble residual antibiotic and 3.0 g of soluble material, the crude rearrangement product 3.

Crude rearrangement product, 4.8 g from two runs, dissolved in a small volume of ethyl acetate was charged onto a 2.4 × 27 cm column of silica gel, 60–200 mesh, Davison Chemical Co. The column was eluted with ethyl acetate at a flow rate of 90 ml/hr and the rearrangement product emerged in 126–207 ml of column effluent. Concentration of this fraction *in vacuo* yielded 3.2 g of light-tan crystals which could be recrystallized from ethyl acetate to yield the colorless, optically inactive rearrangement product 3: mp 84–87°; uv max 268 nm (ϵ 13,100); mass spectrum (70 eV) m/e (rel intensity) 204 (47), 175 (100), 147 (32), 134 (15), 121 (15), 118 (14), 105 (36); ir (KBr) 1652 cm^{-1} (C=O). The nmr spectrum ($CDCl_3$) had pyrone protons at δ 7.68 (1 H, d, $J \cong 7$ Hz, virtual coupling) and 6.30 (2 H, m) and protons from the 1,4-dioxocin ring at δ 7.23 (1 H, s), 5.92 (1 H, d, J = 4.1 Hz), 6.03 (1 H, d, J = 4.1 Hz), 5.46 (1 H, d, J = 6.8 Hz), and 6.55 (1 H, d, J = 6.8 Hz).

Addition of tris(dipivalomethanate)europium(III) resulted in chemical shifts which revealed the characteristic coupling pattern for the γ-pyrone protons: α-H, d, J = 5.8 Hz; β-H, d, J = 2.4 Hz; and β-H, dd, J = 5.8 and 2.4 Hz.

Anal. Calcd for $C_{11}H_8O_4$: C, 64.71; H, 3.95; O, 31.34; mol wt, 204.0422. Found: C, 64.69; H, 3.98; O, 31.44; mol wt, 204.0411 (mass spectrum).

Formation of LL-Z1220 from 3 by Valence Isomerization. Rearrangement product 3 (100 mg) was dissolved in 5 ml of ethyl acetate and heated at reflux for 60 min. The reformed antibiotic 1 (11 mg) crystallized from the solution on cooling. These crystals had nmr, ir, and uv spectra identical with those of the original antibiotic but were optically inactive. The material that remained in solution was shown to be 3 by tlc. Layers developed with ethyl acetate gave the antibiotic, R_f 0.08, and the rearrangement product, R_f 0.27.

Periodate Oxidation Reaction of LL-Z1220. LL-Z1220 (75 mg) was added to 25 ml of H_2O containing 0.75 ml of 18 N H_2SO_4. The mixture was stirred for ~20 min to effect solution and 50 ml of 0.02 M sodium metaperiodate was added. The resulting solution started turning yellow almost immediately. Aliquots (5 ml) were removed from the reaction at intervals, mixed with 40 ml of 1% potassium iodide, and immediately titrated with 0.100 N sodium thiosulfate. A rapid uptake of 0.82 mol of periodate/mol of LL-Z1220 was observed. Tlc studies showed that the major product from the periodate reaction remained the same from several minutes to 3⅓ hr reaction time.

The remaining reaction mixture (45 ml), after 3⅓ hr of reaction time, was passed through a 1.2 × 24 cm column of Amberlite XAD-2. The column was washed with 50 ml of water and eluted with 20 ml of methanol. The eluate was evaporated to 15 mg of semicrystalline residue 4, which was sublimed at 90–120° (0.05 mm) to obtain 2.5 mg of colorless sublimate: mp 133–137°; uv max 210 nm (ε 16,200), 277 (12,300); mass spectrum (70 eV) m/e (rel intensity) 162 (100), 134 (33), 105 (9), 95 (29), 92 (60); ir (KBr) 1658 cm^{-1} (C=O). The nmr spectrum (CD_3OD) showed protons characteristic of the pyrone ring at δ 8.11 (1 H, d, J = 5.8 Hz), 6.67 (1 H, d, J = 2.5 Hz), 6.42 (1 H, dd, J = 5.8, 2.5 Hz), and three 1-H multiplets for the furan at 8.23, 7.69, and 6.91.

Anal. Calcd for $C_9H_6O_3$: mol wt, 162.0316. Found: mol wt, 162.0309 (mass spectrum).

Reaction of LL-Z1220 with Methanolic HCl. An anhydrous methanolic HCl solution was prepared by adding 1.5 ml of acetyl chloride to 25 ml of methanol cooled in a Dry Ice bath. The solution was allowed to warm to room temperature, 100 mg of LL-Z1220 was added, and the resulting solution was heated at reflux for 5 min. The reaction mixture was evaporated to a residue, which was purified by preparative tlc on Analtech silica gel CG, 2 mm, layers. After the layers were developed with acetone, the major zone was detected at R_f 0.5 by uv quenching. Sections of the layers containing the zones were removed and eluted with acetone. The extracted product was then rechromatographed on the same type of layer developed with 95% ethanol. From the major zone (R_f 0.7) was obtained 42 mg of clear, slightly yellow oil 5: uv max 264 nm (ε 16,000); mass spectrum (70 eV) m/e (rel intensity) 272 (6), 254 (6), 242 (5), 237 (8), 225 (8), 213 (37), 207 (41), 177 (100), 163 (28); ir (KBr) 1658 cm^{-1} (C=O). The nmr spectrum (acetone-d_6) had peaks for a γ-pyrone at δ 8.07 (1 H, d, J = 5.8 Hz), 6.66 (1 H, d, J = 2.5 Hz), and 6.29 (1 H, dd, J = 2.5, 5.8 Hz), an olefinic proton at δ 6.43 (d, J = 2.3 Hz), protons on sp^3 carbons bearing O or Cl at δ 4.80 (1 H, dd, J = 8.0 and 3.1 Hz) and 4.5–3.9 (3H, m), and an OMe singlet at δ 3.55.

Anal. Calcd for $C_{12}H_{13}O_5Cl$: mol wt, 272.04515. Found: mol wt, 272.04487 (mass spectrum).

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Registry No. 1, 36431-52-4; 2, 36162-59-1; 3, 49664-64-4; 4, 36162-60-4; 5, 36153-69-2.

References and Notes

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Total Synthesis of β-Lactam Antibiotics. IV. Epimerization of 6(7)-Aminopenicillins and -cephalosporins from α to β[1]

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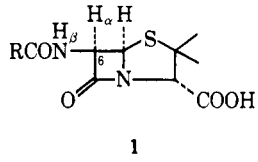
6α-Aminopenicillins and 7α-aminocephalosporins can be epimerized largely to their β epimers by treatment of their *p*-nitrobenzaldehyde Schiff bases with phenyllithium followed by protonation under kinetically controlled conditions. This reaction enables the completion of new total syntheses of both penicillins and cephalosporins.

The most direct total syntheses of penicillins 1 and cephalosporins 2 utilize the cycloaddition of a masked glycine moiety to a thiazoline[2] or thiazine[1] for the construction of the azetidinone ring. A major drawback, however, is that the newly created stereochemistry of the lactam hydrogen atoms is trans instead of cis, as it is in the natural substances. The trans epimers are biologically inactive.[3-5] Since the thermodynamically more stable forms are trans, probably because 6(7) substituents are more crowded in the β configuration than in the α, they cannot be epimerized from trans to cis by simple equilibration, *e.g.*, through the enolate anion, beyond the equilibrium amount, which ranges from 0 to 47% cis for various derivatives.[6]



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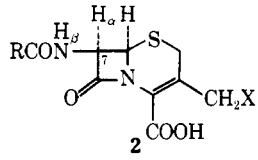

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