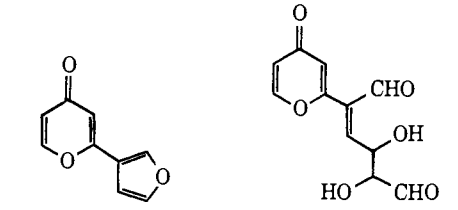
proton (δ 7.18 (doublet of doublets, J = 4.0, 1.2 Hz)), and four protons which unfortunately overlapped each other, in the region δ 4.0–3.4.

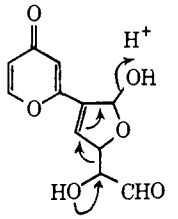
When a small amount of the shift reagent Eu(fod)$_3$ [tris(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedione)europium(III)][12] was added, sufficient chemical shifts were induced to produce first-order multiplets which were in accord with the epoxide ring protons (the four protons were separated by 0.45, 0.59, and 0.65 ppm) in **1**. Using the indicated numbering system, the following (absolute) values for the coupling constants were measured: J_{23} = 4.0, J_{26} = 1.2, J_{34} = 2.8, J_{45} = 2.8, J_{56} = 3.4 Hz. The small J_{45} coupling constant suggests a trans configuration of epoxide groups since the dihedral angle of the corresponding protons would be ~50° for the trans and 0° for the cis arrangements.

In an effort to obtain further evidence to confirm **1**, the antibiotic was treated with acid to open the epoxide rings and then was oxidized with periodate. The resulting product had: mp 123–127°; λ_{max}^{MeOH} 277 nm (ϵ 12,300); *m/e* 162.0309 (calcd for $C_9H_6O_3$, 162.0316). The ir (1658 cm^{-1}) and the nmr (characteristic three-proton pattern) spectra indicated that this product still had the γ-pyrone ring. The remaining C_4H_3O part of the molecule had an nmr spectrum (δ 8.26, 7.72, and 6.93, each signal as an apparent one-proton doublet of doublets with J values < 2.5 Hz) expected for a β-substituted furan.[13] The periodate oxidation product was therefore assigned structure **3**. This product could be rationally derived from **1** through intermediates **4** and **5**. The furan was best obtained by dissolving the antibiotic in dilute aqueous acid and treating the resulting solutions with sodium periodate. It was not formed by acid treatment alone nor by attempted sublimation of the product from the acid treatment.

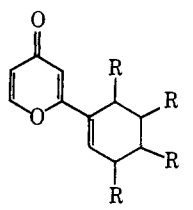


3

4



5



6, R = OH, OH, Cl, OCH_3

Additional evidence for structure **1** was obtained from treating the antibiotic with hydrogen chloride in methanol to obtain **6**, an expected product from the reaction of epoxide groups with this reagent.[14] The molecular formula of **6** was based on the mass spectrum which had a parent ion at *m/e* 272.04487 (calcd for $C_{12}H_{13}O_5Cl$, 272.04515) and expected chlorine isotope peaks for the parent ion and an M − 18 fragment ion. The ir and nmr spectra had adsorptions characteristic for the pyrone ring. In addition, the nmr spectrum had a doublet (δ 6.43, J = 2.3 Hz) of the olefinic proton, a system of four protons (δ 3.9–4.9) attributed to the protons on carbons bearing oxygen and chlorine, and a three-proton singlet (δ 3.55) of an OMe group. The uv spectrum, λ_{max}^{MeOH} 264 nm (ϵ 16,000), was similar to that of the starting antibiotic. The substitution pattern of the groups on the cyclohexane ring is unknown.

LL-Z1220 appears to be the first reported compound containing a cyclohexene diepoxide ring system. Recently a plant product, crotepoxide, has been reported to contain a closely related ring system, a cyclohexane diepoxide.[15] Levopimaric acid dioxide[16] and pseudoascaridole[17] are chemically modified natural products which contain cyclohexane diepoxide ring systems.

Other unique chemical features of the cyclohexene diepoxide ring system of LL-Z1220 will be reported in the near future.

Acknowledgments. We wish to thank Mr. W. Fulmor and staff for spectral data and Mr. L. Brancone and staff for elemental analyses.

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Effect of Pressure on the Visible Absorption Spectrum of Metmyoglobin Fluoride

Sir:

We have investigated the effects of pressures up to 6500 kg/cm^2 on the visible absorption spectrum (450–700 mμ) of aqueous metmyoglobin fluoride. Some typical results are presented in Figure 1.

The spectrum of aqueous metmyoglobin fluoride at 1 atm (1.03 kg/cm^2) is characteristic of a high-spin hemoprotein with absorption maxima at 490 and 605 mμ.[1] Upon pressurization to 2250 kg/cm^2 there is little change in the spectrum other than an increase in absorbance due to compression of the solvent. As the pressure is further increased, however, the spectrum begins to change significantly with time. A definite equilibrium spectrum is reached eventually at each pressure. On release of the pressure to 1 atm virtually 100% return to the initial spectrum is observed. It appears that the protein undergoes a reversible change. Above 5500 kg/cm^2 the change is rapid and virtually complete and the spectrum obtained is substantially different from that observed at atmospheric pressure.

The visible spectrum of metmyoglobin fluoride at 6375 kg/cm^2 shows an absorption maximum near 540

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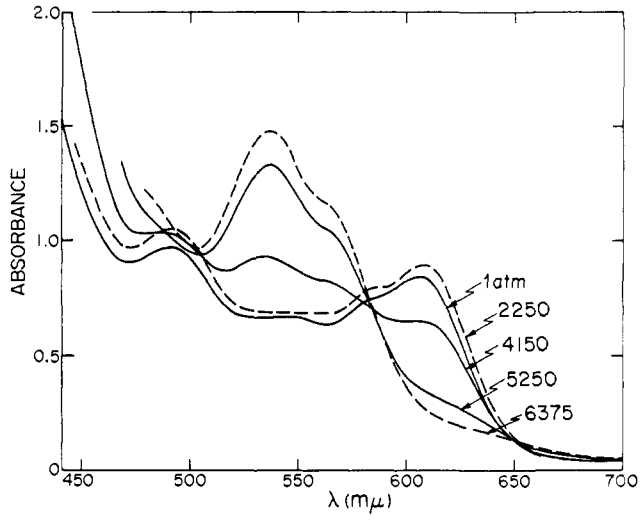

Figure 1. Pressure dependence of the visible spectrum (20°) of aqueous metmyoglobin fluoride (*ca.* 3×10^{-5} *M*; prepared from Sigma type II sperm whale myoglobin without further purification). All curves were taken after the system had reached equilibrium except for that at 5250 kg/cm², which was taken after 2 min.

mμ similar to that of a hemoprotein with the Fe^{III} atom of the porphyrin nucleus in a low-spin configuration.[1] It is characteristic of heme in which the Fe^{III} atom is coordinated to six nitrogen-containing ligands (hemochromogen),[2] and is virtually identical with the spectrum of metmyoglobin imidazole obtained by adding an excess of imidazole to metmyoglobin at atmospheric pressure. The spectrum of the metmyoglobin imidazole complex is essentially unaffected by pressurization to 6500 kg/cm². This observation suggests that the spectral change is due to a conformational change in the protein, in which the fluoride ligand is replaced by a nitrogen of an imidazole side chain in the high-pressure conformation. The possibility of a pressure-induced process such as this was suggested by Fabry and Hunt[3] in connection with the pressurization of hemoglobin and its derivatives to 2000 kg/cm². Fabry studied only shifts in the position of the Soret band. Therefore, he could not determine whether the high-spin ⇄ low-spin transition was caused by the replacement of the ligand in the sixth coordination position by imidazole (possibly that at E7) or simply by a compression of the iron–ligand bond.

We have also studied the effects of pressure on the visible absorption spectrum of methemoglobin and several of its derivatives and observe spectral changes similar to those obtained for metmyoglobin fluoride. The changes occur at considerably lower pressure (500–1500 kg/cm² for methemoglobin fluoride) and are only partially reversible.

The results shown in Figure 1 were obtained using 0.05 *M* cacodylate buffer at pH 6.25. It is well established that the pH of a buffer solution will change upon pressurization. From dilatometric measurements made by Dr. Frank Gasparro in this laboratory and from compressibilities estimated by the method of Lown, *et al.*,[4] we estimate that the pH of this cacodylate buffer will be lowered by no more than 1.3 units at 6500 kg/cm². Although this pH change will cause slight shifts in the absorption maxima, a pH 5 buffer at 1 atm does not lead to the spectral changes described above.

Since metmyoglobin fluoride was stable toward aggregation or precipitation for periods of at least 4–5 hr under the conditions employed, it was possible to obtain both rate and equilibrium constants for the process

$$\underset{\text{(high spin)}}{\text{metmyoglobin fluoride}} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \underset{\text{(low spin)}}{\text{high-pressure conformation}}$$

From the effect of pressure on the equilibrium constant the overall ΔV of this reaction was calculated to be *ca.* -90 cm³/mol. Apparent first-order rate constants for the forward reaction (k_1) ranged from *ca.* 4×10^{-5} to *ca.* 3×10^{-4} sec^{-1} over the pressure range 3850–4400 kg/cm², and gave a value of $\Delta V^{\ddagger}$ essentially the same as that for ΔV.[5]

Similar studies on ribonuclease[6] and chymotrypsinogen[7] have been reported. These results were interpreted in terms of pressure-induced conformational changes, and it would appear that similar changes are taking place here. We are examining other heme proteins and extending our measurements over wider temperature, pH, and spectral ranges.

Acknowledgment. We thank the National Science Foundation for support of these studies.

(5) At a given pressure, spectra used to obtain rate data showed isosbestic points at 485, 507, and 580 mμ supporting the above two-state interpretation of this equilibrium. NOTE ADDED IN PROOF. Although these data have been interpreted in terms of a two-state equilibrium, subsequent kinetic studies of the reversal reaction yield biphasic plots indicating that more than two states must be involved in the kinetics.

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Computer-Aided Mass Spectrometric Identification of Stereoisomeric Monosaccharides[1]

Sir:

The identification of microgram amounts of monosaccharides is an intricate problem in the structure determination of oligo- and polysaccharides because of the occurrence of a large number of stereoisomers. Although mass spectrometry has been widely applied in the field of carbohydrate research,[2–4] the influence of the configuration on the fragmentation is still obscure. Some authors reported a correlation between the mass spectra and the configuration,[5,6] whereas others indi-

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