amine features of sulfonamide binding directly related to the presence of the metal ion in this enzyme. Only the Zn(II) and Co(II) enzymes induce the hypochromic-bathochromic shift and anomalous dispersion in the visible absorption bands of the azosulfonamide. Although the metal ions Mn(II), Ni(II), Cu(II), Cd(II), and Hg(II) have been shown to bind at the active site,[7,13,14] they do not induce the specific binding of the azosulfonamide which results in the visible Cotton effects. The ORD of Hg(II) carbonic anhydrase plus azosulfonamide given in Figure 1 is a typical example of this group. There is a small perturbation of the ORD in the near-ultraviolet region which may reflect weak binding of the aromatic portion of the molecule. Slight binding of [^{3}H] acetazolamide to the inactive metallocarbonic anhydrases as well as the apoenzyme has been observed to begin at inhibitor concentrations of *ca.* 2 × 10^{-5} *M*.[5] The Hg(II) enzyme, however, induces no changes in the visible absorption bands above 350 mμ (Figure 1). These observations support the findings with [^{3}H]acetazolamide.

While Zn(II) and Co(II) carbonic anhydrase induce identical shifts in the azosulfonamide absorption bands, large differences in the magnitude of the 525-mμ Cotton effect indicate subtle differences in the binding of the sulfonamide to the two metallocarbonic anhydrases (Figure 1). Both the amplitude and the position of the Cotton effects of the carbonic anhydrase–azosulfonamide complexes prove extremely sensitive to small changes in protein structure. Carbonic anhydrase isozymes B and C from three different species each show different visible ORD patterns for their complexes with the azosulfonamide. One of these, the azosulfonamide complex of the monkey enzyme B, is illustrated in Figure 2 by the CD spectrum of the complex compared to that of the native enzyme.[15] As is not immediately apparent from the ORD, most if not all of the bands of the bound azosulfonamide show induced optical activity. In contrast to the human isozyme B, the complex of this isozyme has several ultraviolet bands which have rotatory strengths comparable to or greater than those of the protein itself. Hence, the small chromophore with optical activity induced only by the dissymmetric molecular environment dominates the entire rotatory dispersion curve of the large protein complex. The phenomenon is particularly striking in carbonic anhydrase, since the rotatory powers of all the dichroic bands of the protein above 215 mμ are relatively weak.

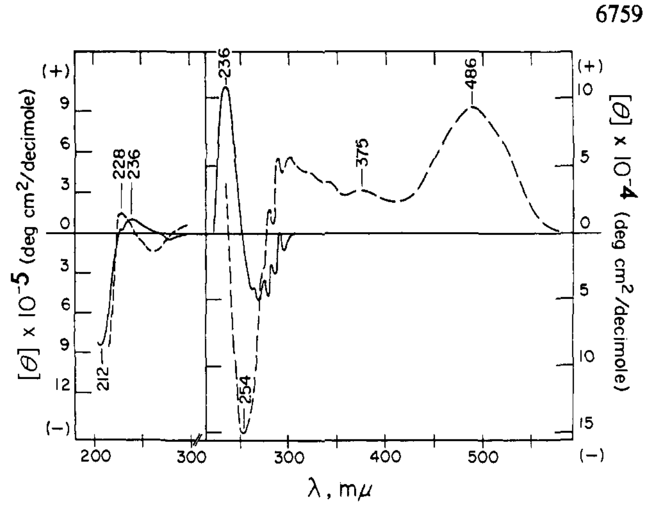

Figure 2. CD spectra of *Macaca mulata* carbonic anhydrase B (———) and its 1:1 azosulfonamide complex (— — —). Protein concentration was 4.5 × 10^{-5} *M* in 0.025 *M* Tris, pH 7.5, 25°. Measurements were made with a Durrum–Jasco ORD/UV-5 spectropolarimeter equipped with the CD attachment. Maximum deflection was ±0.002° with a maximum deviation between runs of ±0.00005° above 230 mμ and ±0.0001° at 215 mμ. The slit width above 300 mμ was 0.3 mm or less. Path lengths varied from 0.2 to 1.0 cm. Ellipticity is expressed as degrees square centimeter per decimole. Concentrations were expressed as decimoles of protein per cubic centimeter and values of ellipticity are uncorrected for the index of refraction of the medium. Spectral positions of the main dichroic bands in millimicrons are indicated by the numbers on the figure.

Sulfonamides have complex ultraviolet spectra associated with the variety of ring structures that have been synthesized. Some of the bands are associated with very large Cotton effects induced on binding of the sulfonamides to carbonic anhydrase. These may be positive, negative, or absent depending on the structure of the inhibitor. With the number of structures and incorporated chromophores available in these molecules, spectral and ORD studies of their carbonic anhydrase complexes provide a sensitive probe for the topography surrounding the active site of this enzyme.[16] The determination of the structure of some of these carbonic anhydrase–sulfonamide complexes seems feasible in light of progressing work on the structure of the crystalline enzyme by X-ray diffraction[13] and may provide information on the sort of protein interactions that induce the dissymmetry in the sulfonamide molecules.

Acknowledgment. I thank Dr. Thomas H. Maren of the University of Florida for many helpful discussions and suggestions concerning the properties of the sulfonamide inhibitors. The circular dichroism instrument was kindly made available by Dr. Yash P. Myer of the State University of New York, Albany, N. Y. This work was supported by Grant No. AM-09070-03 from the U. S. Public Health Service.

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(14) S. Lindskog and B. G. Malmström, *J. Biol. Chem.*, **237**, 1129 (1962).

(15) The CD spectrum of the monkey isozyme B is similar to that for the human isozyme B, as previously reported by S. Beychok, J. M. Armstrong, C. Lindblow, and J. T. Edsall, *ibid.*, **241**, 5150 (1966). The origins of the ellipticity bands of the native protein have been discussed in detail by these authors. The molecular ellipticity, [θ], has been expressed per mole of protein rather than per mole of amino acid residue in view of the fact that the ellipticity bands of the complex arise largely from the incorporation of 1 mole of azosulfonamide. The values in Figure 2 can be compared to those calculated on a mean residue basis by dividing by 256.

(16) J. E. Coleman, in preparation.

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A Novel Oxidative Rearrangement with Manganese Dioxide

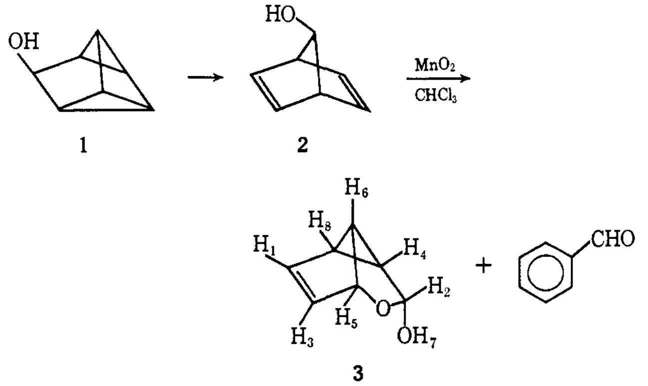
Sir:

During an investigation of the possible use of manganese dioxide as an oxidizing agent for quadricyclanol (**1**), a very interesting reaction and rearrangement were discovered which are also potentially significant mechanistically both in the study of manganese dioxide oxidations and in carbonium ion chemistry.

Treatment of **1** with manganese dioxide in chloroform caused a rapid, quantitative rearrangement to norbornadienol (**2**). Treatment of **2** with manganese dioxide in chloroform at 45° resulted in the formation of only two products, a tricyclic alcohol (**3**), bp 55° (3 mm), in yields as high as 75%, and benzaldehyde.[1] **3** proved to be thermally labile, having a half-life of less than 30 hr at 140°, and smoothly rearranged to form one product, a diether (**4**). Analysis of both **3** and **4** showed that an oxygen atom had been incorporated during the oxidation, giving each the molecular formula $C_7H_8O_2$.[2]

The infrared spectrum of **3** gave $\nu_{\max}^{CCl_4}$ at 3555, 3470, 3065, 2975, 1610 (very strong), 1395, 1096, 1035, and 716 cm^{-1}, showing the presence of unsymmetrical olefin, hydroxy, and ether groups and suggesting the presence of a cyclopropyl ring.

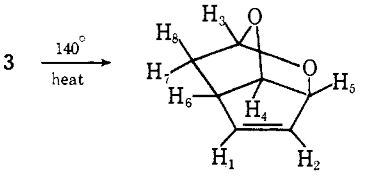
Even though each proton in **3** gave rise to discrete multiplets, the nmr spectrum was not readily interpretable except in light of a complete double resonance study. A detailed analysis of the results of this study was consistent only with structure **3** as drawn. The chemical shifts (τ) for H_1 through H_8, respectively, are: 3.41 (q), 3.98 (d), 4.60 (q), 5.19 (t), 5.82 (s, broad), 6.03 (s, broad), 7.13 (s, broad), and 7.75 (m). The experimental coupling constants extracted are: $J_{1-3} = 5$ cps, $J_{1-8} = 3$ cps, $J_{2-4} = 6$ cps, $J_{3-5} = 3$ cps, $J_{4-8} = 6$ cps, and $J_{6-8} = 1$–2 cps. H_5 and H_6 were too close in chemical shift to be decoupled. H_7 is the OH proton.



The infrared spectrum of the diether, **4**, showed $\nu_{\max}^{CCl_4}$ at 3060, 3010, 2970, 2940, 1345, 1062, 1030, 960, 930, 910, 860, and 730 cm^{-1}, thus demonstrating the lack of hydroxy and carbonyl groups and the presence of olefinic and ether linkages in the rearranged product.

The nmr spectrum of **4** was also extremely complicated, but again a detailed analysis based on a complete double-resonance study permitted unambiguous assignment of structure **4** as written. The chemical shifts (τ) for H_1–H_8 are, respectively: 3.40 (q), 3.87 (q), 4.33 (s, broad), 4.79 (t), 5.99 (m), 7.64 (m, broad), and two hydrogens at 8.35 (m, broad). The experimental coupling constants are: $J_{1-2} = 6$ cps, $J_{1-6} = 3$ cps, $J_{1-5} = 1$ cps, $J_{2-5} = 2.5$ cps, $J_{3-6} = 1$ cps, $J_{3-8} = 2$ cps, $J_{4-6} = 4$ cps, $J_{4-5} = 4$ cps, $J_{6-7} = 3$ cps, $J_{6-8} = 9$ cps, and $J_{7-8} = 13$ cps.

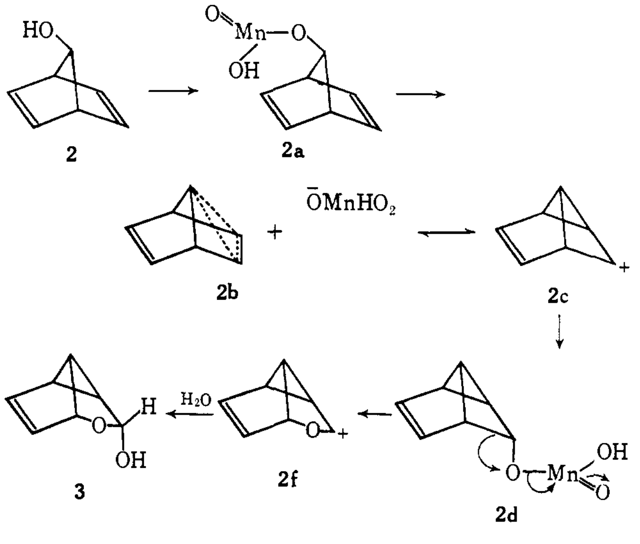
It is worth noting that the coupling constants involving hydrogens 3, 6, 7, and 8 are typical for the bicyclo[2.2.1] structure. J_{6-8} is large (9 cps), consistent with a dihedral angle of about 0°. J_{6-7} is quite small, however, in keeping with a dihedral angle of about 110°. Also, the bridgehead hydrogen is characteristically coupled to the *exo* hydrogen ($J_{3-8} = 2$ cps), but not to the *endo* hydrogen, H_7. Both the coupling scheme and the chemical shift of H_3 are most consistent with an acetal formulation for structure **4**. The olefinic hydrogens, H_1 and H_2, form an additionally coupled AB quartet, as do the geminally located H_7 and H_8. The geminal relationship of H_7 and H_8 is indicated by the large value of the coupling constant ($J_{7-8} = 13$ cps).



4

Hydrogenation of **3** on 5% Pd–$CaCO_3$ gave a saturated ether alcohol (**5**) as the sole product, resulting from quantitative consumption of 1 mole of hydrogen. The infrared spectrum of **5** gave $\nu_{\max}^{CCl_4}$ at 3560, 3450, 2950, 2865, 1160, 1085, and 1045 cm^{-1}. The lack of a band attributable to cyclopropyl or olefinic groups beyond 3000 cm^{-1} suggests that the molecule has rearranged during hydrogenation, and since the nmr spectrum has not yet been resolved, no structural assignment has been made for **5**. **5** was stable to pyrolysis at 140° for 6 hr.

The solvolysis of **3** was attempted in methanol, ethanol, 50% aqueous acetone, and pyridine. In each case unchanged **3** was recovered after 3 hr. However, even trace amounts of mineral acid resulted in immediate decomposition of **3**, giving a multitude of products, with none predominating to any significant extent. The rather remarkable transformation of **2** to **3** can be readily rationalized in terms of the following mechanism.



This sequence requires formation of a manganese ester of 7-norbornadienol (**2**) or, perhaps, even ioniza-

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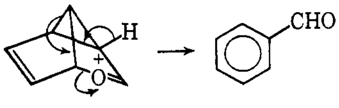
(1) Using manganese dioxide prepared by a modification of the procedure of G. Rosenkranz, F. Sondheimer, and O. Mancera, *J. Chem. Soc.*, 2189 (1953), variable yields of **3** and benzaldehyde were obtained; in some cases the conversion of **2** to products was as low as 5%. However, without exception, **3** and benzaldehyde were always formed, and **3** was the major product. Manganese dioxide prepared by other methods was ineffective.

(2) Satisfactory elemental analyses were obtained for both **3** and **4**; the mass spectra were consistent with the proposed structures and will be discussed in detail in the full paper.

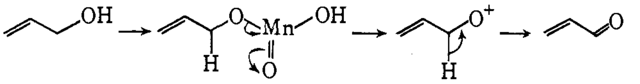
tion of a Lewis acid complex of the alcohol. In view of the proposed structure of the 7-norbornadienyl carbonium ion[3] and the tendency of 7-norbornadienyl derivatives to isomerize to the tricyclic structure (as in **2d**),[4] it is proposed that **2a** isomerizes to **2d** *via* the 7-norbornadienyl carbonium ion (**2b**, **2c**). Transformation of **2d** to **2f** is most readily understood in terms of heterolytic cleavage of the oxygen–manganese bond to donate electrons to manganese and thus effect its reduction. This leaves oxygen, at least formally, with a positive charge, and the resulting 1,2 shift gives **2f** in a manner analogous to the Baeyer–Villiger rearrangement.[5] The ion **2f** is particularly favorable, being both an oxonium ion and a cyclopropylcarbinylcarbonium ion. Addition of water to **2f** should lead exclusively to *endo*-hydroxy in **3**.[4] The ion, **2f**, can also give rise to benzaldehyde through a rearrangement involving loss of a proton.



2f

It should be noted that the type of rearrangement leading to **3** is not without precedent. Greene has reported the conversion of a cyclopropyl alcohol ring system[6] to a β-lactone. This type of rearrangement, again, suggests an ionic mechanism similar to the one we have proposed herewith, in the case of the cyclopropyl alcohol, initial rearrangement to a lactol followed by straightforward oxidation to the observed lactone product.

If, indeed, this mechanism is operative in our system and in Greene's, the implication is clear that manganese dioxide oxidations of ordinary allylic and benzylic alcohols may proceed in a similar fashion. However, in those cases where removal of an α proton is not difficult, as it would be in **2a** or **2d**, elimination takes place to form the conjugated carbonyl compound. We would also suggest that product stability plays an important role in the reaction and accounts, at least in part, for the lack of reactivity of saturated alcohols. It is unlikely, of course, that a discrete electron-deficient oxygen is formed; it is likely, however, that cleavage of the oxygen–manganese bond leads in the transition state.



The interpretation offered here is perfectly consistent with the data of Pratt and Van de Castle,[7] who suggested a free-radical mechanism to explain the relative insensitivity of benzylic alcohols to changes in substituent when undergoing manganese dioxide oxidation. According to our mechanistic scheme, this insensitivity can be interpreted as arising from the fact that the positive charge in the transition state is on oxygen and not at the benzylic carbon. Other systems which may show the type of rearrangement encountered in this work are currently under investigation to test the general applicability of the mechanistic hypothesis outlined here.

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[3] P. R. Story, L. C. Snyder, D. C. Douglas, E. W. Anderson, and R. L. Kornegay, *J. Am. Chem. Soc.*, **85**, 3630 (1963).

[4] P. R. Story, *ibid.*, **83**, 3347 (1961); H. C. Brown and H. M. Bell, *ibid.*, **85**, 2324 (1963); H. Tanida, T. Tsuji, and T. Irie, *ibid.*, **88**, 864 (1966).

[5] P. A. S. Smith in "Molecular Rearrangements," P. de Mayo, Ed., John Wiley and Sons, New York, N. Y., 1964, Chapter 8.

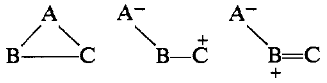
[6] Obtained from the intramolecular photodimerization of dianthracylcarbinol: F. Greene, *Bull. Soc. Chim. France*, 1356 (1960).

[7] E. F. Pratt and J. F. Van de Castle, *J. Org. Chem.*, **26**, 2973 (1961).

Di-*t*-butyloxadiaziridine, the Cyclic Form of an Azoxy Group. Ring–Chain Isomerism in Three-Membered Rings

Sir:

Observations that tertiary alkyl groups stabilize small rings[1] have directed our attention to the possibility of preparation of new ring systems and to the problem of ring–chain (I–II,III) isomerism in small rings.



I II III

Known examples embrace three-atom systems isolable only in the closed form (I,[2] A = B = C = carbon), only in the open form (III,[2] A = C = oxygen, B = nitrogen), or, much more rarely, in both forms (A = oxygen, B = nitrogen, C = carbon).[3] We wish to report the preparation of di-*t*-butyloxadiaziridine (V = I, A = oxygen, B = C = nitrogen), the first well-characterized[4] member of a new ring system and possibly unique in its lack of carbon as one of the three ring atoms.

Irradiation of azoxy-*t*-butane[5,6] (IV) in pentane at 10° with a Hanovia Type L lamp resulted in the disappearance of the ultraviolet absorption. Removal of solvent, trap-to-trap distillation of the residue, and chromatography on Woelm alumina at 10° with pentane eluent afforded an oil of composition $C_8H_{18}N_2O$ (*Anal.* Calcd: C, 60.72; H, 11.47; N, 17.70; mol wt, 158. Found: C, 60.62; H, 11.33; N, 17.70; mol wt, 166 [cryoscopic in benzene]); infrared (CCl_4)[7] 2980, 2930, 2860, 1475, 1450, 1385, 1365, 1235, and 1210 cm^{-1}; nmr (CCl_4) singlet at 1.0 ppm; major species in the mass

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[1] F. D. Greene and J. C. Stowell, *J. Am. Chem. Soc.*, **86**, 3569 (1964); J. C. Sheehan and J. H. Beeson, *ibid.*, **89**, 362, 366 (1967); J. F. Pazos and F. D. Greene, *ibid.*, **89**, 1030 (1967).

[2] For I, II, and III, the appropriate number of substituents are assumed to be attached to A, B, and C, corresponding to the usual valence states of these atoms.

[3] W. D. Emmons, *J. Am. Chem. Soc.*, **79**, 5739 (1957). See also E. Schmitz, *Advan. Heterocyclic Chem.*, **2**, 83 (1963).

[4] The nitrogen–nitrogen–oxygen three-membered ring frequently has been postulated in the older literature. It has also been proposed as an intermediate in the Wallach rearrangement by M. M. Shemyakin, V. I. Maimind, and Ts. E. Agadzhanyan, *Chem. Ind.* (London), 1223 (1961). However, see also C. S. Hahn, K. W. Lee, and H. H. Jaffé, *J. Am. Chem. Soc.*, **89**, 4975 (1967), and references cited therein.

[5] J. P. Freeman, *J. Org. Chem.*, **28**, 2508 (1963).

[6] Ultraviolet maximum at 221 $m\mu$.

[7] Azoxy-*t*-butane has strong bands at 1285 and 1490 cm^{-1}.