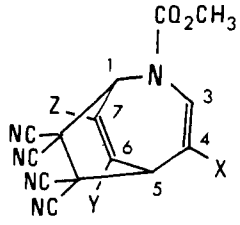
conformations. Nitrene **3** from **1** should be formed in the sp conformation **3a** by the least motion principle, whereas **9** is in the *ap* conformation as shown by NMR[11] and conformation **3b** should be generated at the cryogenic temperature of the glass.



6a, X = Y = Z = H
6b, X = Z = CH_3; Y = H
6c, X = Z = H, Y = CH_3

By analogy with the photochemistry of triptycenes,[2] most of the reactions at ambient temperatures are considered to be those of singlet nitrene **3** from the excited singlet state of **1**. Support for this interpretation was obtained by the finding that, when the ground-state triplet of the nitrene was populated at low temperature (runs 8 and 9 in Table I), the products were rich in the aniline derivative **10**[8] characteristic of the triplet species.

We were not able to detect any photoproducts due to a carbene species. Thus the formation of nitrene **3** in preference to carbene **11** is to be noted. There are several possible explanations for the observed high bridging regioselectivity, and they can occur at several points along the reaction coordinate of the di-π-methane rearrangement. The first point would be the geometrical nonequivalence of the two ends of the *o*-benzeno moieties in this rather rigid molecule. The C–N bond distance is expected to be somewhere between 1.42 (observed for triphenylamine) and 1.45 Å (for trimethylamine) and should be decidedly shorter than the 1.53 Å observed for the bridgehead-to-benzene C–C bond in many triptycenes.[12] Therefore, the distance between the two benzene rings for π–π overlap can be more effective at the end near the nitrogen bridgehead. Higher electronegativity of nitrogen vs. carbon may be employed to rationalize the higher stability of the initially formed aziridine-2,3-dicarbinyl diradical as compared to cyclopropyldicarbinyl diradical.[13] Rationalization for this argument is based on the inductive effect of a heteroatom on strengthening the opposite C–C bond of the three-membered ring and is supported by the findings on the effect of heteroatom on the equilibrium of the tropylidine–norcaradiene valence tautomerization. The norcaradiene structure gets more stabilized as we go from CH_2 through NH to O at position 7.[14] If the next step of rearrangement of the initially formed diradicals can be product controlling or the second step takes place concertedly with the first bridging, the weaker C–N bond energy relative to the C–C would be invoked. Conclusions on these points must be postponed until a more systematic study on the effect of substituents at the "methane" position on the bridging regioselectivity is completed.

(11) The ^1H NMR spectrum characteristic of the *ap* conformation was observed for **9**.[2] Since the spectrum did not change in the temperature range −50–70 °C, **9** was considered to be present in the *ap* form only. Amine **10** was found to be in two forms in a ratio of *ap*:*sp* = 4:1. From the coalescence point (−11 °C) of the two singlet lines due to the 9-H of the fluorene ring, a rough estimate of the rate and the activation free energy was given as 25 s^{-1} and 13.6 kcal mol^{-1}, respectively. These values may approximate those for **3**.

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(13) We thank a referee for pointing out this possibility. This explanation might, however, get into trouble if we note the finding of Zimmerman (Zimmerman, H. E.; Welter, T. R. *J. Am. Chem. Soc.* **1978**, *100*, 4131) that the methane carbon becomes electron deficient as bridging occurs to form the S_1 cyclopropyldicarbinyl diradical. An electron-deficient nitrogen will be less favored than a similar carbon.

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2-(Hydroxymethyl)aspartic Acid: Synthesis, Crystal Structure, and Reaction with a Transaminase

Sir:

The interaction of transaminases with analogues of the natural substrates has been the subject of numerous investigations. Thus, dicarboxylic acids are effective inhibitors of the well-known transaminase, aspartate aminotransferase.[1-4] Of special interest is 2-methylaspartate which inhibits but appears to undergo "transimination", the initial stage in the normal enzymatic reaction.[3,5] In this paper we describe the synthesis and X-ray crystal structure of 2-(hydroxymethyl)aspartic acid and the preliminary evaluation of its interaction with cytosolic aspartate aminotransferase of pig heart. This compound is of interest both as an inhibitor and because of the possibility of attaching it through the hydroxyl group to a suitable polymeric matrix to form a material suitable for affinity chromatography of proteins that bind aspartate. Although several variations of the Strecker synthesis were attempted, as was synthesis via the hydantoin, we were able to prepare the compound only in a 3% yield by the following procedure.

To a solution of 7.50 g (73 mmol) of sodium bisulfite in 16 mL of water was added 10 g (53 mmol) of ethyl 4-acetoxyacetoacetate prepared as described by DeGraw.[6] The resulting solution was treated with 2.60 g (73 mmol) of sodium cyanide in 7.0 mL of water. After 10 min in an ice bath, the upper organic phase was separated, dried over sodium sulfite, and filtered. The colorless liquid was placed in a glass-lined pressure vessel and 100 mL of liquid ammonia was added. The mixture was allowed to stand for 24 h in the sealed reaction vessel at room temperature. After the pressure was released, the contents of the vessel were transferred to a flask with a small amount of water and were concentrated to dryness. The residue was treated with 50 mL of cold, concentrated hydrochloric acid and was allowed to warm to room temperature overnight. The solution was refluxed for 2.5 h, filtered through a small bed of Norit A charcoal, and concentrated to dryness. The residue was triturated with 20 mL of absolute ethanol and the precipitated inorganic salts were filtered off and washed with two 7-mL portions of absolute ethanol. Ethyl ether (15 mL) was added to the filtrate and the supernatant was concentrated to dryness. The residue was dissolved in 50 μL of water. The solution was neutralized with silver carbonate, filtered through Norit A, and treated with gaseous H_2S. The precipitated silver sulfide was removed by filtration through Norit A and the filtrate was concentrated to 10 mL. The pH was adjusted to 3.0 by addition of Dowex 50W-8X in the hydrogen form, and the final product was crystallized by addition of ethanol. The amino acid was recrystallized three times from methanol–water mixtures. The colorless needles (0.25 g, 3% yield) of racemic 2-(hydroxymethyl)aspartic acid decomposed at 188 °C; NMR (H_2O; NaOD) δ 2.28 (d, J = 16 Hz, 1 H), 2.65 (d, J = 16 Hz, 1 H), 3.44 (d, J = 12 Hz, 1 H), 3.66 (d, J = 12 Hz, 1 H).

The α subform of the cytosolic isoenzyme of aspartate aminotransferase was isolated from pig hearts by previously described methods.[7] Other chemicals were of reagent grade.

A rectangularly shaped, colorless crystal of approximate dimensions 0.2 × 0.2 × 0.4 mm was chosen for data collection. The positions of ten reflections which were obtained from four preliminary ω-oscillation photographs taken at various ϕ settings on a four-circle diffractometer were put into an automatic indexing program.[8] The resulting reduced cell and reduced-cell scalars indicated an orthorhombic system which was subsequently confirmed by axial photographs. Lattice constants (a = 8.921 (2), b = 9.608 (1), and c = 7.937 (1) Å) were determined by a least-squares fit to $\pm 2\theta$ measurements of 11 high-angle reflections

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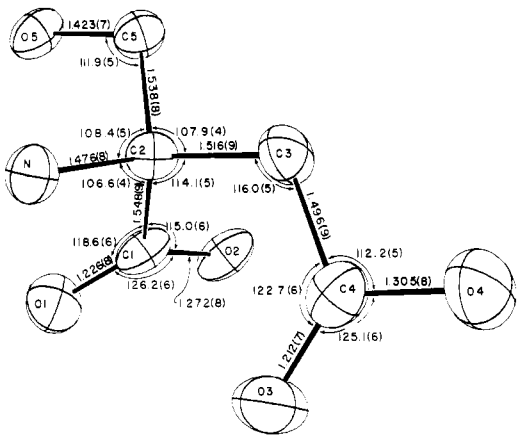

Figure 1. Structure of 2-(hydroxymethyl)aspartic acid as determined by X-ray crystallography.

with graphite-monochromated Mo Kα radiation, $\lambda = 0.70954$ Å. Intensity data were collected at room temperature on an automated, four-circle diffractometer designed and built in this laboratory.[9] Two octants of data were collected within a 2θ sphere of 50° by using graphite-monochromated Mo Kα radiation and an ω-scan data collection technique. The intensity data were corrected for Lorentz and polarization effects. Equivalent data were averaged with a total of 526 observed ($F_o > 3\sigma_F$) reflections retained for structural refinement.

A major portion of the structure was determined by using the program MULTAN.[10] The positions of the remaining nonhydrogen atoms were found by structure factor and electron-density map calculations. The positions of all but one of the hydrogen atoms were determined from an electron-density difference map. The positional parameters of all atoms and the anisotropic thermal parameters of the nonhydrogen atoms were refined by the full-matrix least-squares technique minimizing the function $\sum\omega(|F_o| - |F_c|)^2$, with $\omega = 1/\sigma_F^2$. An analysis of the weights was performed with the requirement[11] that $\omega\Delta^2$ should be a constant function of $(\sin\theta)/\lambda$. Reflections at low and high $(\sin\theta)/\lambda$ were shown to be overweighted, and the weights were subsequently adjusted. Successive iterations of refinement using the adjusted weights reduced the conventional residual to $R = 0.065$.

Bond distances and angles are shown in Figure 1. The rather short C(3)–C(4) distance can be attributed to sp^2 hybridization on C(4). Both O(5) and C(5) are less than 0.11 Å from the least-squares plane of atoms C(2), C(3), C(4), O(3), and O(4). In the crystalline state, hydrogen bonds contribute greatly to the overall bonding scheme. Both oxygens O(1) and O(5) are within intramolecular hydrogen bonding range of nitrogen [N–O(1) 2.594 (8) Å and N–O(5) 2.848 (7) Å]. The molecules crystallize in hydrogen-bonded sheets, with alternate pairs of sheets held by hydrogen bonds.

2-(Hydroxymethyl)aspartate binds to aspartate aminotransferase as is indicated by the changes in absorption spectrum in Figure 2 and the inhibition of the normal enzymatic activity in a standard assay. As is shown in Figure 2, the free enzyme at pH 8.3 has a single absorption band attributable to a Schiff base of the coenzyme pyridoxal phosphate with the amino group of lysine-258 at the active site. The peak position is at 362 nm. Upon addition of 2-(hydroxymethyl)aspartate, this peak decreases in intensity while a peak arises at 440 nm. When the reciprocals of the absorbance increases at 440 nm were plotted against the reciprocals of the (hydroxymethyl)aspartate concentrations, a straight line was obtained. From its slope and intercept, a dissociation constant of 1.3 mM was estimated for the enzyme–inhibitor complex. The 440-nm band has a strong circular dichroism (CD) comparable to that of the free enzyme at low pH.[3] There is also a weaker positive band in the CD spectrum at 366 nm. The absorption spectrum appears to contain the 366-nm band also as well as an overlapping band at 330 nm devoid of CD. There are irregularities in the absorption spectrum at the highest inhibitor concentrations. The enzyme is a dimer, and it is possible that there is some interaction between the two binding sites.

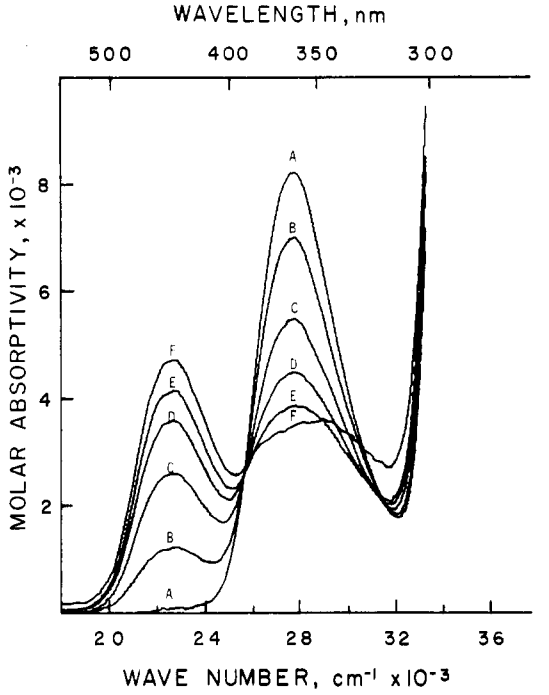

Figure 2. Titration of aspartate aminotransferase with DL-2-(hydroxymethyl)aspartate at pH 8.3. The millimolar concentrations of the L isomer of the amino acid were (A) 0, (B) 0.407, (C) 1.41, (D) 3.37, (E) 7.26, (F) 36.6. The initial enzyme concentration was 5.58×10^{-5} M with respect to the monomeric protein and decreased somewhat during the titration.

The dissociation constant of the enzyme–(hydroxymethyl)aspartate complex was also estimated from the inhibition of the normal transamination reaction. The rate of this reaction was measured by using 1×10^{-9} M enzyme, 3.2 mM 2-ketoglutarate, and 2.0–10.0 mM L-aspartate in 0.06 M triethanolamine hydrochloride buffer, pH 8.3. Double-reciprocal plots of velocity vs. aspartate concentrations were prepared for enzyme in the absence of inhibitor and in the presence of 3.0, 4.5, and 6.0 mM (hydroxymehyl)aspartate. From these plots a dissociation constant for the enzyme–inhibitor complex of 1.4 mM was obtained, in good agreement with that estimated from Figure 2.

At first glance, the reaction of 2-(hydroxymethyl)aspartate would appear to be entirely similar to that of 2-methylaspartate which is thought to first form an enzyme–inhibitor complex (I) analogous to the Michaelis complex with a true substrate. A proton transfer within complex I then yields II. The latter forms an adduct (III) which undergoes elimination of the lysine side chain attached to the protein to form Schiff base IV between the inhibitor and the coenzyme. Recent studies by X-ray diffraction[12,13] indicate that 2-methylaspartate binds in front of the pyridoxal phosphate ring as indicated and there may be a rotation of the ring accompanying the formation of Schiff base IV.[13,14] The phosphate, which is tightly liganded to a number of protein side chains, would remain in a fixed position and relatively small movement of the inhibitor would also occur. The latter is probably bound to positively charged arginine side chains at both ends. The spectrum of the 2-methylaspartate complex resembles that in

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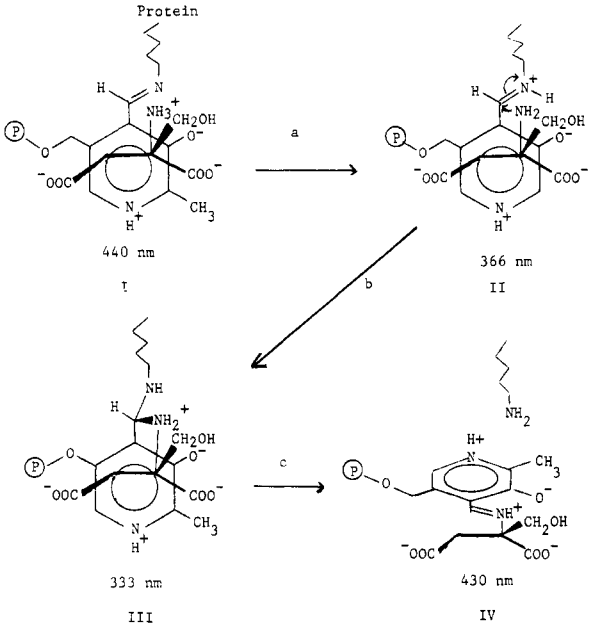

Figure 2, but the 430-nm band is devoid of CD. The absence of CD seem characteristic of substrate Schiff bases such as IV. Therefore, we suggest that the 440-nm band with a positive CD observed with 2-(hydroxymethyl)aspartate is the first ES complex I rather than IV. A smaller amount of II may be present and absorbing at 366 nm while the 330-nm band may be the adduct III. It is also possible that 2-(hydroxymethyl)aspartate binds in a different conformation than do the normal, less sterically hindered substrates. Thus the 440-nm complex may not be strictly analogous to a Michaelis complex with a substrate and may therefore not go on to give IV.

The dissociation constant 1.4 mM for the L-2-(hydroxymethyl)aspartate complex with the enzyme is substantially lower than the 5–10 mM reported for L-2-methylaspartate.[5,15] Thus, the presence of the hydroxyl group in some way enhances the binding of this inhibitor.

(Hydroxymethyl)aspartate has been cocrystallized with aspartate aminotransferase, as has been described for 2-methylaspartate.[14] The crystals have the same morphology as those of the 2-methylaspartate crystals, indicating that they are probable isomorphous with the native enzyme whose structure is being determined by Arnone et al.[16]

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Supplementary Material Available: Tables I, II, and III listing structural parameters and observed and calculated structure factors (5 pages). Ordering information is given on any current masthead page.

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Quenching of Triplet Ketones by Alcohol OH Bonds

Sir:

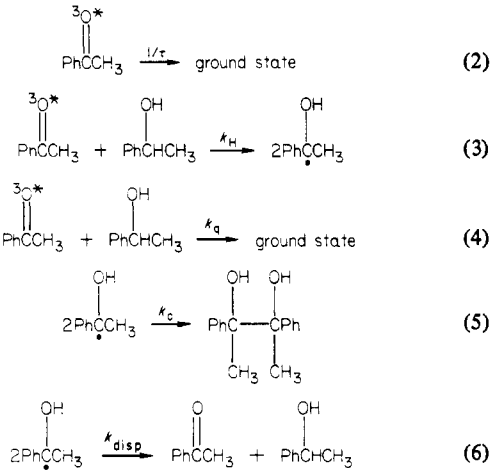
The photoreduction of ketones by alcohols is one of the oldest[1] and best known[2] photoreactions. It has long been known to involve hydrogen atom abstraction by triplet ketones.[3] We have now found that in the photoreduction of acetophenone (AP) by 1-phenylethanol (PE),[4] half of the ketone triplets are quenched by the OH bond rather than react with the α-C–H bond. This conclusion results from several independent experimental findings.

We have reconfirmed[4b] that the maximum quantum yield for photoreduction in degassed solvents is only 0.59 in benzene and 0.50 in acetonitrile. The double reciprocal plot in Figure 1 is described by eq 1 with the rate constants appropriate to the reactions in Scheme I.

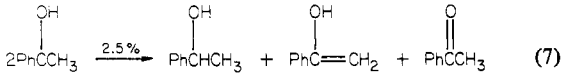
$$\Phi^{-1}(\text{pinacol}) = \left(\frac{k_c + k_{disp}}{k_c}\right)\left(1 + \frac{1}{(k_H + k_q)\tau[PE]}\right)\left(\frac{k_H + k_q}{k_H}\right) \quad (1)$$

Since the photoreduction of benzophenone by benzhydrol proceeds in close to 100% quantum efficiency,[5] and hemibenzpinacol radicals are known not to disproportionate at room temperatures,[6] inefficiency in similar photoreductions is usually assumed to involve disproportionation of radical intermediates back to ground-state reactants rather than quenching of triplet ketone.

Scheme I



In this case, a 50:50 disproportionation/coupling ratio of the 1-phenyl-1-hydroxyethyl radicals would explain the entire inefficiency. Such a large fraction of disproportionation is unusual for a benzylic radical, but might be favored by formation of acetophenone enol.[7] However, we have shown that the actual percentage of disproportionation is only 2.5% (eq 7).



The experimental basis for the above conclusion comes from irradiation of 0.15 M AP in benzene containing 0.2 M 1-phenyl-1-propanol, which yields PE and acetophenone pinacol as the only significant products in a ratio of 0.025/1, as measured by GC analysis at low conversions. At the relatively high AP

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