can be used to calculate a deuterium isotope effect of 1.1 ± 0.1 on the protonation of the quinoid intermediate.

Glutamate decarboxylase catalyzes a very slow decarboxylation of α-methylglutamic acid.[14] Decarboxylation of 6.2 ml of 0.02 M D,L-α-methylglutamic acid in the buffer described above but containing 4×10^{-4} M pyridoxal 5′-phosphate for 10 days with 1000 units of enzyme resulted in approximately 20% decarboxylation. The product γ-aminovaleric acid was isolated, washed repeatedly with water, and pyrolyzed to the corresponding lactam. Mass spectra of the lactam are shown in Figure 1. In D_2O the product contained 0.9 deuterium atom,[15] whereas the product from the mixed solvent contained only 0.14 ± 0.01 deuterium. The hydrogen isotope effect calculated from these isotopic composition measurements is $k^H/k^D = 6.2 \pm 0.4$.

The lack of appreciable hydrogen isotope discrimination in the decarboxylation of glutamic acid indicates that the proton source for protonation of the quinoid intermediate is a monoprotic catalytic group of the enzyme which is sufficiently shielded from the solvent that hydrogen exchange between this group and the solvent does not occur during the lifetime of the quinoid intermediate. Transfer of the proton from the solvent to the catalytic group probably occurs prior to the decarboxylation step. Enzymatic decarboxylations of amino acids occur with retention of configuration at the α-carbon atom,[16] and it is possible that the catalytic group involved in protonation is the group that binds the α-carboxyl group of the substrate prior to decarboxylation. It is possible that the decarboxylation of glutamic acid is "ordered", with protonation of this catalytic group necessarily occurring prior to substrate binding.

The large hydrogen isotope effect observed in the decarboxylation of α-methylglutamic acid is in striking contrast to the lack of an effect in the decarboxylation of glutamic acid. There are two possible reasons for the presence of a large isotope discrimination in this case: Protonation of the quinoid intermediate might occur from a different proton source—either directly from the solvent or from an exposed catalytic group. Alternatively, protonation might occur from the same catalytic group as before, but the lifetime of the quinoid intermediate might be significantly longer and the conformation of the enzyme might be such as to allow hydrogen exchange between the catalytic group and the solvent.

Hydrogen isotope discrimination experiments of the type discussed here may be useful for studying a variety of enzymatic reactions involving proton transfer to carbon. Only if hydrogen discrimination is absent is it possible to make any statement about the route of the proton from solvent to substrate. Even in the absence of hydrogen discrimination, several factors must be considered: It must be shown that the lack of discrimination is not the result of readily reversible proton transfer; if such transfer takes place and the catalytic group of the enzyme is in contact with the solvent, then the enzyme will catalyze facile hydrogen exchange between solvent and product. It is possible at least in principle that the transition state for the proton transfer might be very asymmetric and that the absence of a hydrogen isotope discrimination might be the result of this asymmetry. However, such asymmetric transition states generally give rise to small, though measurable, isotope effects, and it is unlikely that an isotope effect of 1.0 would result from such a circumstance. This technique is not capable of detecting the presence of catalytic sulfhydryl groups, because such groups can give rise to appreciable isotope fractionation even if the catalytic group is shielded from the solvent.

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(2) It is possible at least in theory that the transition state for a proton transfer such as being discussed here might be very asymmetric and thus give rise to a very small isotope effect. Such situations in proton transfers to and from carbon are seldom, if ever, observed. Alternatively, small hydrogen isotope effects are observed in diffusion-controlled proton transfers. However, diffusion-controlled proton transfer to carbon occurs only when little or no electronic rearrangement of the carbon substrate takes place on protonation (see J. E. Crooks, ref 1b, p 153). Most proton transfers to carbon of interest in enzymology are accompanied by extensive electronic rearrangement, so are not expected to be diffusion controlled.
(3) If this same situation obtains except that the enzyme functional group is a lysine ammonium group, a large hydrogen isotope discrimination will be observed as a result of intramolecular competition among the various hydrogens and deuteriums of the ammonium group.
(4) Reference 1b, p 216.
(5) The fractionation factor for the sulfhydryl group is appreciably different from unity,[4] so hydrogen isotope discrimination may be observed in this case even if a shielded catalytic group is present.
(6) A similar method has occasionally been used for studying hydrogen isotope effects in organic reactions. See, for example, M. M. Kreevoy and R. A. Kretchmer, *J. Am. Chem. Soc.*, **86**, 2435 (1964); V. Gold and M. A. Kessick, *Pure Appl. Chem.*, **8**, 273 (1964); V. Gold and M. A. Kessick, *Proc. Chem. Soc., London*, 295 (1964).
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(11) This is consistent with previous studies of S. Mandeles, R. Koppelman, and M. E. Hanke, *J. Biol. Chem.*, **209**, 327 (1954).
(12) Specifically deuterated γ-aminobutyric acid was prepared by decarboxylation of L-glutamic acid in D_2O with glutamate decarboxylase. Three milliliters of 0.2 M pyridinium chloride buffer, pH 4.5, containing 1.7×10^{-6} M dithiothreitol, 1.7×10^{-6} M pyridoxal 5′-phosphate, and 0.03 M γ-aminobutyric acid-γ-D was incubated with 820 units of glutamate decarboxylase at 22 °C for 120 h. Proton-decoupled ^{13}C NMR spectroscopy was used to look for enzyme-catalyzed hydrogen exchange (such exchange would cause the γ-carbon resonance of γ-aminobutyric acid-γ-D to change from a triplet to a singlet). No exchange was observed.
(13) A previous report[11] that glutamate decarboxylase catalyzes stereospecific hydrogen exchange of γ-aminobutyric acid is apparently in error. The enzyme used in those studies was actually unfractionated bacterial acetone powder, and we assume that significant amounts of γ-aminobutyric acid transaminase were present.
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(15) Because of the need to use large amounts of enzyme in these experiments, the D_2O solvent contained more H_2O than was present in the glutamic acid experiments. Because of the large isotope effect on the protonation this led to incorporation of less than a full equivalent of deuterium into the product.
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cis-Dimethyldiazene

Sir:

Although the properties of the trans isomers of the simple diazenes, HN=NH,[1] CH_3N=NH,[1c,2] and CH_3N=NCH_3,[3] are rather well known, the only cis isomer that has been reported is that of dimethyldiazene (C). Hutton and Steel obtained small amounts of C by direct photoisomerization of the solid trans at liquid nitrogen temperature but did not secure enough material for a full characterization.[4] Nelsen prepared a mixture of cis and trans isomers by the pyrolysis of 1,2,3,6-tetrahydropyridazine.[5]

We report here the isolation of millimole quantities of pure

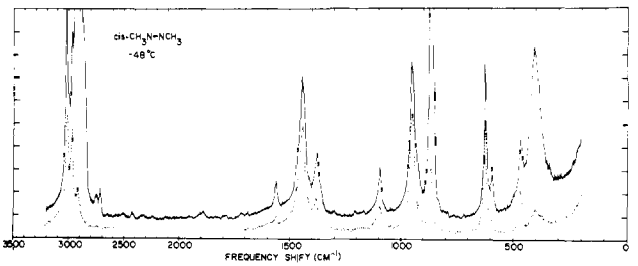

Figure 1. Raman spectrum of liquid cis-dimethyldiazene at −48 °C, 270-mW of 514.5-nm laser light: solid line, analyzer parallel to polarization of laser; dotted line, analyzer perpendicular. The peak marked t is due to some trans isomer.

C, the determination of some of its physical and chemical properties, and a provisional vibrational assignment.

We have prepared pure C by two methods. One method employed the Nelsen pyrolysis followed by bulb-to-bulb distillation at temperatures up to −50 °C on a vacuum system. The other was a naphthalene-photosensitized isomerization of the trans isomer in pentane at −20 °C with 256.0-nm radiation from low pressure mercury lamps.[6] Conversion reached 35-40%, and no impurities were produced other than small amounts of material, presumably nitrogen and ethane, that was noncondensable at liquid nitrogen temperature. Careful bulb-to-bulb distillation over the temperature range −90 to −70 °C yielded pure samples of C as shown by proton NMR spectra. Samples prepared by this photolysis method were easier to purify than those prepared by the pyrolysis method.

In contrast to the trans isomer, C is quite reactive. At room temperature it readily isomerizes to formaldehyde methylhydrazone, CH_2=$NNHCH_3$, which, in turn, dimerizes.[4,7] This isomerization reaction interferes with vibrational spectroscopy at room temperature. A gaseous sample at a pressure of a few Torr in a 1-m (metal body), folded-path cell isomerized in minutes to the hydrazone. Liquid samples sealed in capillary tubes for Raman spectroscopy isomerized more slowly and were kept unchanged for long times in spectroscopic experiments at −50 °C. The facile isomerization reaction and the low volatility (vp 10 Torr at 0 °C) of C made fractionation by gas chromatography impossible.

Table I provides a comparison of the properties of *cis*- and *trans*-dimethyldiazene. The very large difference in boiling point between the two isomers, which is exceptional even in comparison with other alkyldiazenes,[8] is understandable in view of the very large dipole moment of the cis isomer and relatively small size of dimethyldiazene. The cis isomer is rather soluble (and stable!) in water at room temperature[4] and rather insoluble in hydrocarbons. During the preparation by photolysis, the cis isomer was partly thrown out of solution. The longer wavelength of λ_{max} for the cis isomer follows the pattern of the other alkyldiazenes, but the smaller chemical shift of the cis isomer does not.[4,8]

Figure 1 shows the Raman spectrum of liquid C at −48 °C. A provisional vibrational assignment is given in Table II. This assignment was guided by frequencies predicted by normal coordinate calculations. We have computed the frequencies of the 24 fundamentals of the cis isomer from the 17 parameter potential energy function that Pearce, Levin, and Harris fitted to the trans isomer and its perdeutero modification.[9] The CH bonds in the methyl groups were assumed to eclipse the double bond,[10] despite probable steric crowding. Table II also includes characterizations of each fundamental based on the principal contribution to the potential energy distribution. The frequency assignments in Table II also incorporate observations from infrared spectra of the glassy phase at −196 °C. As expected, the NN stretching frequency for C, which probably has C_{2v} symmetry, appears in the infrared. The corresponding mode of the trans isomer, which has C_{2h} symmetry, is inactive in the infrared. The agreement of the experimental frequencies with the predicted ones is quite good for this type of calculation. Undoubtedly, most of the missing frequencies give bands which are obscured by near neighbors of high intensity.

Further spectroscopic studies are underway on the d_3 and d_6 modifications of C, from which we expect to develop a complete assignment of the vibrational fundamentals and a refined set of potential constants. We also hope that the refined knowledge of the physical and chemical properties of C will guide the search for *cis*-methyldiazene and *cis*-diazene.

Table I. Properties of the Dimethyldiazenes

	Trans	Cis
Mp, °C	−78[a]	−66
Bp, °C	1.5[a]	95[b]
Dipole moment, D	0	3.2[c]
UV, λ_{max} in hexane (nm)	352	368
NMR chem shift[d] (ppm)	3.76	3.62

[a] Handbook of Chemistry and Physics", 52nd ed, The Chemical Rubber Co., Cleveland, Ohio, 1971-72, p C-131. [b] Extrapolation of vapor pressures measured in −22 to 0 °C range using Clausius-Clapeyron equation. [c] Private communication: J. F. Stevens and R. F. Curl, Jr. [d] In $CDCl_3$, downfield relative to internal Me_4Si. In D_2O upfield relative to protons: trans 0.95, cis 1.05 ppm.

Table II. Vibrational Fundamentals of *cis*-Dimethyldiazene

		PED,[a] %	Calcd freq, cm^{-1}	Obsd freq, cm^{-1}
a_1	1	97 CH str	2975	3006
	2	93 CH str	2912	2902
	3	77 NN str	1590	1556
	4	85 CH_2 bd	1441	1438
	5	57 CH_2 bd, 48 CH rk	1364	1373
	6	79 CH rk	1052	1088
	7	81 CN str	853	862
	8	83 CNN bd	318	398
a_2	9	97 CH str	2975	
	10	81 CH_2 bd	1466	
	11	56 CH_2 bd, 47 CH rk	1179	
	12	74 NN tors	563	464
	13	61 CH_3 tors, 19 NN tors	220	
b_1	14	97 CH str	2974	
	15	93 CH str	2912	
	16	84 CH_2 bd	1442	1465[b]
	17	56 CH_2 bd, 47 CH rk	1369	~1350[b]
	18	58 CH rk, 42 CNN bd	1152	1161[b]
	19	78 CN str	997	946
	20	61 CNN bd, 19 CN str	711	623
b_2	21	98 CH str	2971	2956
	22	87 CH_2 bd	1462	1465[b]
	23	86 CH rk	997	
	24	100 CH_3 tors	165	

[a] Potential energy distribution—percentage contributed by potential constants associated with designated internal coordinates. Due to contributions from off-diagonal potential constants the partial PED can exceed 100%. [b] From glassy-phase infrared.

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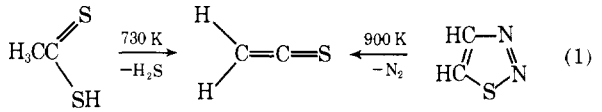
Unstable Intermediates. 5.[1] Thioketene

Sir:

The capability of photoelectron spectrometers to record "molecular finger-prints" provides an efficient analytical tool to screen thermal decompositions in the gas phase for specific low-temperature reaction channels.[1,2]

Thus the PE spectra[3] of Figure 1 prove that thioketene[4,6-8] is the only thermolysis product of both the H_2S abstraction from dithioacetic acid at 730 K and the N_2 elimination from 1,2,3-thiadiazole above 900 K:[3]



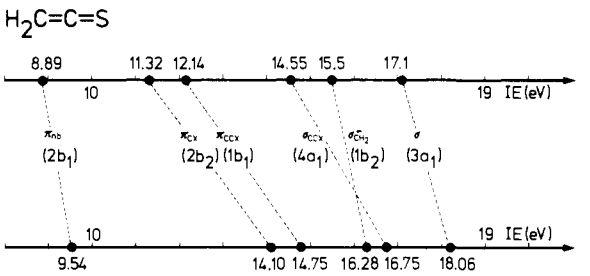
(1)

The identity of $H_2C{=}C{=}S$—and at the same time the exclusion of other possible valence isomers like ethynyl mercaptan,[8] HC≡CSH, or thiirene[8] under the reaction conditions[3]—can be established beyond doubt in many ways.[9] The rather reliable results of PNO-CEPA calculations[9,11] for individual radical cation states do reproduce the spectroscopic ionization patterns with an accuracy of better than 0.3 eV (Figure 1 and Table I). Orientating SCF calculations carried out in addition suggest that thioketene is the preferred species among its possible tautomers: According to the total energies resulting with identical basis sets for idealized geometries, thioketene is more stable by ~74 kJ/mol than ethynyl mercaptan and by ~552 kJ/mol than thiirene. The assignment of the first two PE bands to π-type ionizations is supported by radical cation stretching frequencies $\tilde{\nu}^+$ (Table I) which correspond to the reduced thioketene stretching vibration $\tilde{\nu}_{CS}$ 1760 cm^{-1} in the molecular ground state. Furthermore, comparison with the PE spectra of iso(valence)electronic molecules like ketene $H_2C{=}C{=}O$[10,13] shows the expected close resemblance. Obviously, all vertical ionization energies are reduced due to the smaller effective nuclear charge of sulfur. The electron distribution in the molecular ground state as well as the changes in the individual radical cation states are summarized in Table II.

Table II. Charge Distribution in the Ground State of $H_2C{=}C{=}S$ and Changes in RHF Gross Atomic Populations upon Ionization to the Individual Radical Cation States $H_2C{=}C{=}S^{\cdot+}$

Atom	H	C_1	C_2	S
$^1A_1$ [a]	0.868	6.233	6.054	15.974
$\tilde{X}(^2B_1)$	−0.09	−0.27	−0.04	−0.51
$\tilde{A}(^2B_2)$	−0.10	−0.11	−0.23	−0.47
$\tilde{B}(^2B_1)$	−0.10	−0.19	−0.19	−0.43
$\tilde{C}(^2A_1)$	−0.09	−0.19	−0.20	−0.42
$\tilde{D}(^2B_2)$	−0.19	−0.13	−0.13	−0.36
$\tilde{E}(^2A_1)$	−0.16	−0.13	−0.19	−0.36
$\tilde{F}(^2A_1)$	−0.12	−0.21	−0.12	−0.42
$\tilde{G}(^2A_1)$	−0.11	−0.18	−0.17	−0.43

[a] Ground state.



According to the restricted-Hartree-Fock calculations (Table II), in the ground state the H_2C carbon bears a considerable negative charge. The largest change in the sulfur atom population occurs upon ionization to the radical cation ground state $\tilde{X}(^2B_1)$; the hydrogens are most strongly influenced in the $\tilde{D}(^2B_2)$ and $\tilde{E}(^2A_1)$ states.

Both decompositions (eq 1) yield thioketene exclusively; neither starting materials nor traces of other by-products are visible in the PE spectra (cf. Figure 1). Nevertheless, as con-

Table I. Vertical Valence Ionization Energies of CH_2CS IE_n (eV) and Radical Cation Vibrational Frequencies

State	$-\epsilon^{SCF}$	RHF	CEPA	IE_n [a]	ν^+, [b] cm^{-1}
$\tilde{X}(^2B_1)$	8.98 $3b_1$ (π)	8.19	8.85	8.89	1450; 700
$\tilde{A}(^2B_2)$	11.44 $3b_2$	10.31	11.28	11.32	1660; 680
$\tilde{B}(^2B_1)$	13.65 $2b_1$ (π)	12.60	12.44	12.14	710
$\tilde{C}(^2A_1)$	15.92 $9a_1$	14.39	14.75	14.55	950
$\tilde{D}(^2B_2)$	17.03 $2b_2$	15.51	15.31	(15.5)	
$\tilde{E}(^2A_1)$	19.49 $8a_1$	18.46	17.65	(17.2)	
$\tilde{F}(^2A_1)$	27.00 $7a_1$	25.77			
$\tilde{G}(^2A_1)$	30.32 $6a_1$	28.79			
Total energies $\tilde{X}(^1A_1)$ state (eV)[c]:					
SCF	−12 907.944 84				
PNO-CI (upper bound)	−12 914.810 53				
CEPA	−12 915.711 02				

[a] Values of most intense subbands without vibrational corrections. [b] Error bounds are about ±80 cm^{-1}. [c] 1 au = 27.21167 eV.