TABLE I

PROTON NUCLEAR MAGNETIC RESONANCE ABSORPTION CHARACTERISTICS OF SOME N-H COMPOUNDS[a]

Compound	N–H absorption at 35°	Approx. transition temp., singlet to triplet absorption, °C.	J of triplet absorption, c.p.s.[b]
Formamide	Broad singlet	50 ± 10[c]	60 ± 4
Acetamide	Broad singlet[d]	175 ± 10[e]	56 ± 5
N-Methylformamide	Broad singlet	Not obsd. to 250	..
N-Methylacetamide	Broad singlet	225 ± 20	60 ± 5
Succinimide	....	>250	..
Pyrrole	Very broad singlet	50 ± 25	55 ± 5
Methylammonium chloride[f]	Broad triplet[g]	<0	49 ± 2
Ethylammonium chloride[f]	Broad triplet[g]	<0	50 ± 2
Dimethylammonium chloride[f]	Very broad triplet[g]	≤0	53 ± 3
Pyrrolidine hydrochloride[f]	Very broad triplet[g]	25 ± 5	52 ± 4

[a] Varian Associates V-4300 High Resolution Nuclear Magnetic Spectrometer and 12-in. electromagnet at 40 mc. with a vacuum-jacketed sample holder. [b] The limits are large because of broad peaks. [c] Triplet absorption disappeared at 150–175°. [d] At m.p. [e] Triplet absorption disappeared around 250°. [f] Approximately 50% solutions containing two drops of concentrated hydrochloric acid per 0.5 ml. [g] The triplet pattern persisted at 125° even though N–H exchange was rapid enough to sinear the N–H, C–H spin–spin interactions.

the triplet absorption disappears at high temperatures, possibly because of N–H exchange. In agreement with this idea, the formamide triplet absorption persists to 175° in dioxane but not in the pure liquid.

The temperature effects on ^{14}N–H absorption indicate that slow molecular motions are most effective for quadrupole relaxation of ^{14}N dipole. Structural influences appear to be important also.

Applications should be obvious of the foregoing to studies of molecular motions, qualitative analysis by nuclear magnetic resonance, estimation of t_1 for ^{14}N, and determination of optimum conditions for minimization of quadrupole relaxation in observations of ^{14}N absorption.

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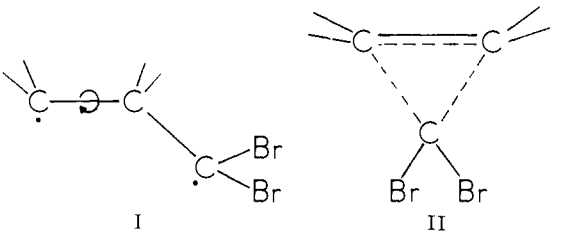
STRUCTURE OF CARBENE, CH_2

Sir:

The literature contains numerous statements regarding the paired or unpaired condition of the two electrons in carbenes (*e.g.*, methylene, CH_2). In forthcoming publications[1,2] the stereospecificity and reactivity in CBr_2 additions to olefins are considered as proof that (I) rather than (II) is the correct structure of the intermediate complex.

Although it is generally agreed that knowledge of the structure of reaction products is a poor guide to an understanding of the ground state of a reso-



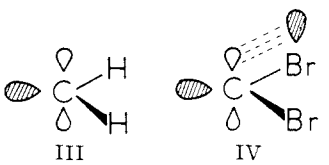
I II

nance-hybrid reactant, this particular type of system provides a rather unique contradiction. The intercombination of the singlet and triplet states is improbable if the two electrons are in the same region of space[3] and do not move in the vicinity of perturbing atoms of large atomic number[4] or paramagnetic ions.[5] Thus reactions which involve a change in multiplicity under these restrictive conditions are slow.[3]

From the evidence presented[1,2] we were inclined to assign to CBr_2 a structure with paired electrons rather than two unpaired electrons. However, the presence of two Br atoms in the vicinity of the nonbonding electrons introduced an uncertainty in this conclusion which we have removed through study of CH_2.

Photolysis of diazomethane in the presence of excess *cis*-2-butene in gas or liquid phase does not isomerise the unreacted butene and yields two products, *cis*-1,2-dimethylcyclopropane and *cis*-2-pentene, both free of isomer contamination. Similarly *trans*-2-butene yields *trans*-1,2-dimethylcyclopropane and *trans*-2-pentene.

Since the rate of ring formation is large relative to the rate of rotation about the central C–C bond (structure II), the quantum mechanical restrictions preclude a structure for CH_2 in which the non-bonded electrons have parallel spins. We propose structures (III) for carbene and are



III IV

inclined toward (IV) for dibromocarbene. These structures represent planar molecules having sp^2 hybridisation and a vacant p-orbital. This structure assignment based on chemical evidence is consistent with those proposed on the basis of spectroscopic evidence[6] and quantum mechanical calculations.[7]

The criterion of stereospecific addition to the 2-

(1) P. S. Skell and A. Y. Garner, THIS JOURNAL, **78**, 3409 (1956).

(2) P. S. Skell, A. Y. Garner and R. C. Woodworth, *ibid.*, **78**, in press (1956).

(3) For references see A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," John Wiley and Sons, New York, N. Y., 1953, pp. 61, 107, 198, 326.

(4) D. S. McClure, *J. Chem. Phys.*, **17**, 908 (1949); D. S. McClure, N. W. Blake and P. L. Hanst, *ibid.*, **22**, 255 (1954); M. Kasha, *Discussions Faraday Soc.*, **9**, 14 (1950); *J. Chem. Phys.*, **20**, 71 (1952).

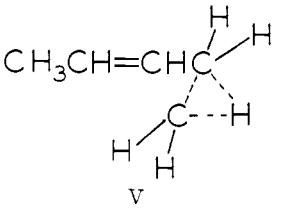
(5) E. Gelles and K. S. Pitzer, THIS JOURNAL, **77**, 1974 (1955).

(6) G. Herzberg, *Rev. Mod. Phys.*, **14**, 195 (1942); *Astrophys., J.* **96**, 314 (1942).

(7) J. Lennard-Jones, *Trans. Faraday Soc.*, **30**, 70 (1934); H. H. Voge, *J. Chem. Phys.*, **4**, 581 (1936); K. J. Laidler and E. J. Casey, *ibid.*, **17**, 213 (1949); J. Lennard-Jones and J. A. Pople, *Discussions Faraday Soc.*, **10**, 9 (1951).

butenes has been used to detect other carbene reaction intermediates. For example, photolyses of ethyl diazoacetate in the presence of these olefin produces carbethoxycarbene, $:CHCOOC_2H_5$, since the ethyl 2,3-dimethylcyclopropanecarboxylates are obtained in stereospecific reactions. Copper-bronze catalysis produces the same results as photolysis.

Conversion of C–H bonds to C–CH_3 bonds has been described.[8] The failure to observe isomerization in the products *cis*- and *trans*-2-pentene from $:CH_2$ and *cis*- and *trans*-2-butene, respectively, suggests that the intermediate in this substitution reaction has structure (V). This intermediate for



V

the substitution reactions is similar to that proposed for the $CH_2 + H_2$ reaction.[9]

(8) A. Meerwein, H. Rathjen and H. Werner, *Ber.*, **75**, 1610 (1942); W. von E. Doering, R. G. Buttery, R. G. Laughlin and N. Chandhuri, THIS JOURNAL, **78**, 3224 (1956).

(9) J. Chanmugam and M. Burton, *ibid.*, **78**, 509 (1956). See also for references to earlier discussions of this mechanism.

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GLUTAMINE REQUIREMENT FOR THE INOSINIC ACID TRANSFORMYLASE REACTION

Sir:

Flaks and Buchanan[1] described the purification of pigeon liver inosinic acid transformylase which catalyzed the following reaction: IMP-5′[2] + glycine ⇌ serine + AICR. These investigators demonstrated that leucovorin was a required co-factor. It is the purpose of this communication to present data to show that L-glutamine is also required in this reaction.

The pigeon liver extracts were prepared according to the method of Greenberg[3] except that bicarbonate was omitted from the buffer and the homogenization performed at 4° in a blender for 15 seconds. The supernate after centrifugation at 36,000 × *g* was absorbed twice with norite (20 mg./ml.) at 0°. This extract required either ATP and CF or anhydroleucovorin *per se* as co-factors for the transformylase reaction. The reaction was followed by aryl amine liberation.[4] Greenberg[5,6] has shown that CF must be activated by ATP before it functioned as a transformylation co-factor. Precipitation of the norite-treated extract with 50% acetone at 0° resolved the enzymatic activity into two fractions. Both the acetone precipitate and the acetone supernate were necessary for transformylation activity. The activity of the latter was replaced by L-glutamine. Although glutamic acid and NH_4^+ ions had a slight effect, asparagine, NH_4^+ or glutamic acid were ineffective. Vitamins, purines, pyrimidines, nucleotides, nucleosides, DPNH, TPNH, DPN and TPN were incapable of replacing glutamine. The activity of the apo-enzyme was limited by the concentration of L-glutamine in the reaction mixture. The results are shown in the table.

TABLE I

THE EFFECT OF GLUTAMINE ON THE FORMATION OF ARYL AMINE BY INOSINIC ACID TRANSFORMYLASE OF PIGEON LIVER

The reaction vessels were incubated for 30 minutes at 37° and the reaction stopped by adding 2 ml. of 0.2 *N* HCl and 0.2 ml. of 20% trichloroacetic acid. The acetone precipitate of the extract represented 1 ml. of the original cell-free extract; total volume 1.0 ml. at *p*H 7.5. The substrates were: Na inosinate, 0.6 μM.; glycine, 100 μM.; leucovorin, 0.1 μM.; $CuSO_4 \cdot 5H_2O$, 0.1 μM.; ATP, 0.2 μM.

		μmoles aryl amine (AIC) I	II[b]	III[c]
1	Acetone precipitate + CF[a] + ATP + glycine + inosinate	0.011	0.007	0.007
2	1 + 0.08 μM. L-glutamine	.027	...	...
3	1 + 0.2 μM. L-glutamine	.068	...	...
4	1 + 0.4 μM. L-glutamine	.104	...	...
5	1 + 0.8 μM. L-glutamine	.104	.054	.077
6	1 + 0.8 μM. L-asparagine	.011	...	...
7	1 + 0.8 μM. DL-glutamic acid	.011	...	...
8	1 + 2 μM. NH_4Cl	.016	...	...
9	1 + 0.8 μM. DL-glutamic acid + 2μM. NH_4Cl	.035	...	...
10	5 minus ATP	.038	.025	.045
11	5 minus CF	.026	.022	...
12	5 minus glycine	.045	.018	.028
13	5 minus inosinate	.050	.017	.028
14	5 + 15 μM. DL-serine	.053	.007	.007
15	5 minus acetone ppt.	.005	...	.001

[a] Anhydroleucovorin can replace CF. [b] Assay contained 14 μM. glycine. [c] Acetone reprecipitated enzyme; 14 μM. glycine in reaction vessel.

The following criteria were used to show that the liberation of aryl amine was due to the inosinic acid transformylase reaction: (a) serine specifically inhibited aryl amine liberation; (b) both glycine and inosinic acid were required for optimal activity (some enzyme preparations contained small amounts of these substrates); (c) the reaction required both ATP and CF[7]; (d) the liberated aryl amine could not be acetylated by acetic anhydride[8]; and (e) the acid-hydrolysis product of the liberated aryl amine was identified as AIC when chromatographed according to the method of Greenberg and Spilman.[9]

(1) J. G. Flaks and J. M. Buchanan, THIS JOURNAL, **76**, 2275 (1954).

(2) Abbreviations: ATP, adenosine triphosphate; CF, leucovorin; DPN, diphosphopyridine nucleotide; TPN, triphosphopyridine nucleotide; AIC, 4-amino-5-imidazolecarboxamide; IMP-5′, inosinic acid; AICR, 4-amino-5-imidazolecarboxamide ribotide.

(3) G. R. Greenberg, *J. Biol. Chem.*, **190**, 611 (1951).

(4) A. C. Bratton and E. K. Marshall, Jr. *ibid.*, **128**, 537 (1939).

(5) G. R. Greenberg, THIS JOURNAL, **76**, 1458 (1954).

(6) G. R. Greenberg, *Fed. Proc.*, **13**, 221 (1954).

(7) L. Warren and J. G. Flaks, *ibid.*, **15**, 379 (1956).

(8) J. M. Ravel, R. E. Eakin and W. Shive, *J. Biol. Chem.*, **172**, 67 (1948).

(9) G. R. Greenberg and E. L. Spilman *ibid.*, **219**, 411 (1956).