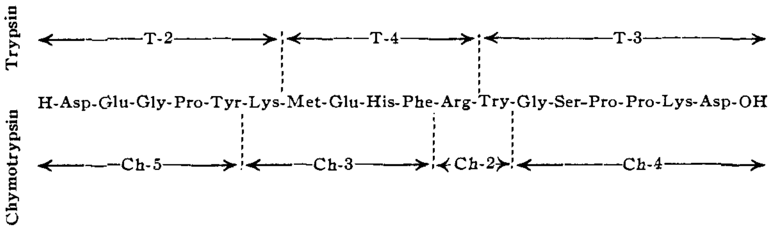

spectrophotometric method.[7] The amide content as determined by two different procedures[8,9] was 0.0. Thus, the minimum molecular weight calculated from these data is 2,177. Moreover, the isoionic point is computed[8,10] to be at *p*H 5.9. This value is in good agreement with the experimentally determined isoelectric point, which was found by zone electrophoresis on starch to be at *p*H 5.8, after correction for electroösmosis.[11]

For structural determinations, 20-mg. aliquots were digested with either crystalline trypsin (Armour) or crystalline chymotrypsin (Armour), and in each case the entire hydrolysate was submitted to electrophoresis on paper in a collidine-acetic acid buffer.[12] All peptides were located by means of guide strips sprayed with ninhydrin, and were subsequently eluted from the paper with dilute ammonia. The homogeneity of each peptide area was demonstrated by paper chromatography of aliquots in two different solvent systems. All peptides were analyzed for the N-terminal amino acid[13] and for quantitative amino acid composition.[6] Aliquots of the peptides arising from chymotryptic digestion were also submitted to digestion with carboxypeptidase to determine the C-terminal sequence. Carboxypeptidase was also employed for C-terminal analysis[14] of the MSH molecule itself, as was stepwise degradation from the N-terminus by the paper-strip modification[15] of the phenyl isothiocyanate[16] reaction. The latter procedure was successfully employed for eight successive steps. From these data, a structure for the MSH molecule is proposed (see above).

The most striking aspect of this formulation is that the sequence . . . Met . Glu . His . Phe . Arg . Try . Gly . . . also occurs, identically, in all the corticotropins[17,18,19] that have been studied hitherto. This, in all probability, explains the intrinsic melanocyte-stimulating activity of pure corticotropin preparations. In the corticotropin molecule, this central sequence apparently acquires, by virtue of a different specific order of amino acids on each side of it, an adrenal-stimulating activity as well. The significance of these results will be the subject of subsequent communications.

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RECEIVED JULY 24, 1956

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(7) T. W. Goodwin and R. A. Morton, *Biochem. J.*, **40**, 628 (1946).

(8) A. L. Levy, I. I. Geschwind and C. H. Li, *J. Biol. Chem.*, **213**, 187 (1955).

(9) E. O. P. Thompson, *ibid.*, **207**, 563 (1954).

(10) A. D. McLaren and C. Lewis, *J. Polymer Sci.*, **5**, 379 (1950).

(11) I. D. Raacke and C. H. Li, *J. Biol. Chem.*, **215**, 277 (1955).

(12) I. M. Lockhart and E. P. Abraham, *Biochem. J.*, **58**, 633 (1954).

(13) F. Sanger, *ibid.*, **39**, 507 (1945).

(14) Aspartic acid was the only C-terminal amino acid that could be demonstrated in intact MSH when a modification (C. I. Niu and H. Fraenkel-Conrat, THIS JOURNAL, **77**, 5882 (1955)) of the hydrazinolysis procedure (S. Akabori, K. Ohno and K. Narita, *Bull. Chem. Soc., Japan*, **25**, 214 (1952)) was employed.

(15) H. Fraenkel-Conrat, THIS JOURNAL, **76**, 3606 (1954).

(16) P. Edman, *Acta Chem. Scand.*, **4**, 283 (1950).

(17) P. H. Bell, THIS JOURNAL, **76**, 5565 (1954).

(18) W. F. White and W. A. Landmann, *ibid.*, **77**, 1711 (1955).

(19) C. H. Li, I. I. Geschwind, R. D. Cole, I. D. Raacke, J. I. Harris and J. S. Dixon, *Nature*, **176**, 687 (1955).

TEMPERATURE EFFECTS ON NUCLEAR MAGNETIC RESONANCE ABSORPTION OF HYDROGENS ATTACHED TO NITROGEN

Sir:

The nuclear magnetic resonance absorption of hydrogens attached to nitrogen (^{14}N) may show sharp, broad or triplet lines. Single sharp lines are obtained with amines and ammonia where exchange is rapid.[1] Broad lines (10–75 c.p.s. at 40 mc.) are observed for many amides and pyrrole,[2] while sharp triplet absorption has been observed for gaseous and completely anhydrous ammonia[2] and ammonium ions in acid solution,[3] where N–H exchanges are slow.[4] Triplet absorption is expected from spin–spin interactions between the protons and ^{14}N ($I = 1$).

The broad lines associated with N–H absorption of amides and pyrrole could be due to intermediate rates of exchange[4] or relaxation of the ^{14}N nuclei by interaction between their quadrupoles and surrounding asymmetrical electrical fields.[5] Indeed, significant sharpening of N–H lines by exchange was achieved through adding small amounts of sodium to pyrrole and concd. ammonia to formamide. However, the broad lines in the pure liquids are *not* the result of intermediate exchange rates because the line widths *decrease* with increasing temperature and, with several substances, triplet N–H absorption has been observed at higher temperatures. This behavior is opposite to that expected for any exchange process[3] having a positive temperature coefficient. That exchange was not involved was demonstrated by the spectrum of an acidified pyrrolidine hydrochloride solution at 0°. This had a broad singlet N–H absorption and showed sharp spin–spin splitting ($\sim$7 c.p.s.) of the α-hydrogens by the N–H hydrogens. At 50°, the N–H absorption was a broadened triplet without change in the splitting of the α-hydrogens.

In Table I, approximate temperatures where the N–H absorption was intermediate between singlet and triplet as well as line appearances at 30° are listed for several substances. With several amides,

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(1) *Cf.*, R. A. Ogg, *J. Chem. Phys.*, **22**, 560 (1954).

(2) Unpublished observations of Dr. J. N. Shoolery, Varian Associates.

(3) R. A. Ogg, *Faraday Society Discussion*, **17**, 215 (1954).

(4) H. S. Gutowsky and A. Saika, *J. Chem. Phys.*, **21**, 1688 (1953).

(5) For refs. see J. E. Wertz, *Chem. Revs.*, **55**, 829 (1955).

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 smear 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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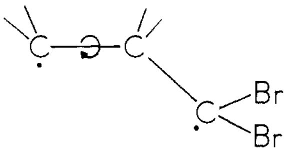
RECEIVED JUNE 25, 1956

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 resonance-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]



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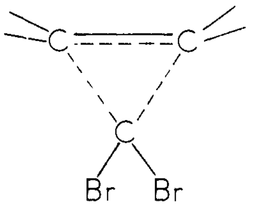
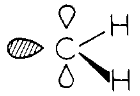


II

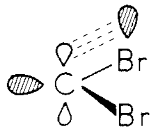
From the evidence presented[1,2] we were inclined to assign to CBr_2 a structure with paired electrons rather than two unpaired electrons. However, the presence of two Br atoms in the vicinity of the nonbonding electrons introduced an uncertainty in this conclusion which we have removed through study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



III



IV

The criterion of stereospecific addition to the 2-

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(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).