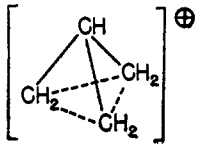
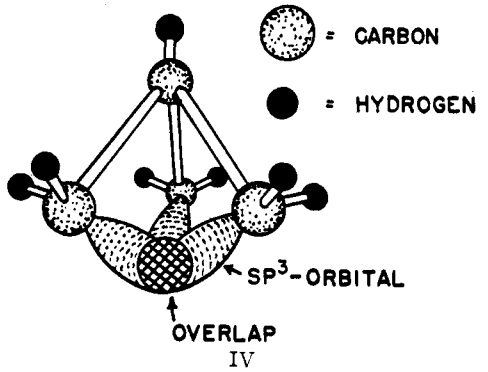
number of suggestions[2,4,5] regarding the nature of the cationic intermediate (or intermediates) which might be involved. Investigation of the course of the reactions using C^{14}-labeled cyclopropylcarbinyl derivatives reveals that the three methylene groups of the starting material achieve a degree of equivalence at some point between reactants and products which is well (although not uniquely) accounted for by a "non-classical" cationic intermediate of structure I.[4,5]



I

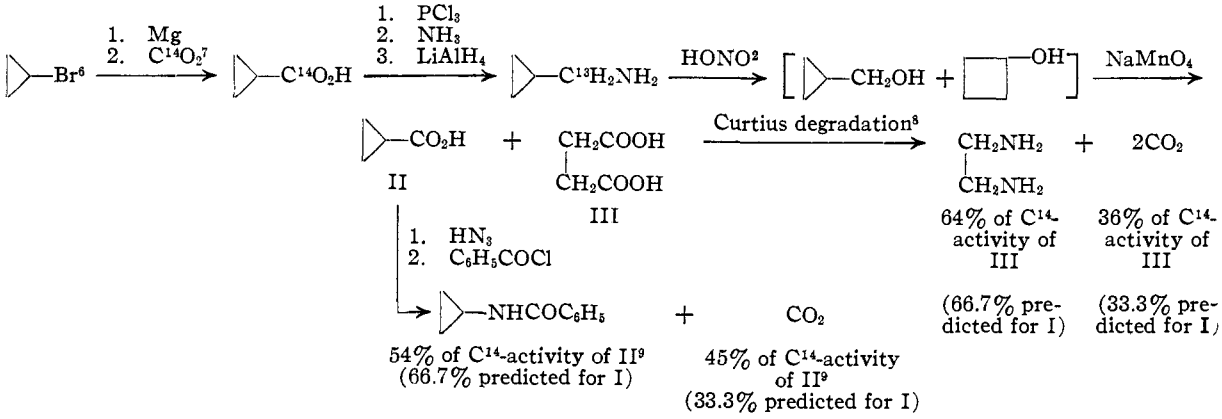
The main features of the experimental evidence for attainment of substantial equivalence between the methylene groups in the reaction of cyclopropylcarbinylamine with nitrous acid follow.

the extra electrons would have to go into less stable orbitals. It is significant that the experimental data, so far available,[2] indicate that cyclopropylcarbinyl and cyclobutyl derivatives are not interconverted in anion or free-radical reactions.



IV

A full report of this and related work will be presented in later papers.



Dr. M. J. S. Dewar (private communication) suggests that I can be very reasonably formulated by the molecular orbital theory if it is considered that all of the carbon atoms use the customary sp^3 orbitals and that the methinyl (CH) group is attached to the three methylene groups by the customary σ-bonds. The three extra sp^3 orbitals of each of the methylene groups are then positioned to overlap as shown in IV and can form one stable molecular orbital holding two electrons[10] and two vacant, considerably-less stable orbitals. This formulation is especially attractive since it permits prediction that structures analogous to II for the corresponding anion or free radical would be unfavorable because

(5) (a) V. C. Chambers, Ph.D. Thesis, M.I.T., October, 1950; (b) R. H. Mazur, Ph.D. Thesis, M.I.T., January, 1951; (c) R. B. Woodward (Harvard University), private communication.

(6) J. D. Roberts and V. C. Chambers, THIS JOURNAL, **73**, 3176 (1951).

(7) The carbon dioxide was prepared from barium carbonate-C^{14} supplied by the Oak Ridge National Laboratory on allocation from the U. S. Atomic Energy Commission. The radioactivity analyses were made by Miss Winifred Bennett and Miss Clare M. McGinnis.

(8) A. A. Benson and J. A. Bassham, THIS JOURNAL, **70**, 3939 (1948).

(9) The difference between predicted and found is likely to be due to some direct (non-carbonium ion) replacement of $-NH_2$ by $-OH$.

(10) A. D. Walsh, *Trans. Faraday Soc.*, **45**, 179 (1949); T. M. Sugden, *Nature*, **160**, 367 (1947); R. S. Mullikin, *J. Chem. Phys.*, **1**, 492 (1933). It is to be noted that the argument here is not rigorous since the three orbitals do not have D_{3h} symmetry.

DEPARTMENT OF CHEMISTRY AND LABORATORY FOR NUCLEAR SCIENCE AND ENGINEERING JOHN D. ROBERTS
MASSACHUSETTS INSTITUTE OF TECHNOLOGY
CAMBRIDGE 39, MASS. ROBERT H. MAZUR

RECEIVED MAY 25, 1951

THE VALENCE OF PRECIPITATING RABBIT ANTIBODY

Sir:

In the course of a quantitative ultracentrifugal and electrophoretic investigation of the soluble complexes formed between antigen and antibody in the region of antigen excess, we have obtained evidence that precipitating antibody to bovine serum albumin (BSA) is largely bivalent. Crystalline BSA was iodinated to an average degree of 5.1 I atoms per molecule (BSA-5I), and rabbit antisera were prepared against the un-iodinated BSA. The γ-globulin fraction of the pooled antisera was first purified by precipitation with $^1/_3$ saturated $(NH_4)_2SO_4$, and then a specific antigen-antibody precipitate was formed in the equivalence zone using BSA-5I as antigen. The precipitate was washed with cold saline and then was redissolved in antigen excess. Treatment of this solution with $^1/_2$ saturated $(NH_4)_2SO_4$ resulted in a precipitate that completely redissolved in buffered saline.

This final solution was analyzed for I and N, and thus the total antigen and total antibody content determined. Solutions in greater antigen excess were prepared by adding known amounts of BSA-5I to this solution.

Ultracentrifuge experiments were carried out in phosphate buffer, *p*H 7.6, $\mu = 0.1$, at 21 ± 1°. Several species of antigen-antibody complexes, as well as free antigen, appeared in the sedimentation diagrams, as was originally observed by Heidelberger and Pedersen[1] in similar systems. The faster-sedimenting complexes, present to a large extent in solutions in low antigen excess, were considerably diminished in solutions in high excess, and in the latter a single peak (*a* complex) became most prominent. The *a* complex appears to be the richest antigen-containing complex capable of being formed by the antibody in this system. Extrapolation of the sedimentation constants of the *a* complex to zero effective concentration yields the value $s^{w}_{20} = 8.7\ S$.

Electrophoresis experiments were performed in veronal buffer, *p*H 8.5, $\mu = 0.1$. Resolution of the free antigen, with its appropriate mobility, was readily achieved in both ascending and descending limbs, and the relative areas under the free antigen peak were accurately determined from the ascending pattern. The values so obtained agreed with the per cent. of free antigen evaluated from the ultracentrifuge diagrams. Resolution among the complexes was poor, but in solutions in which sufficiently large proportions of the *a* complex were present, a partial but definite separation of a faster-moving peak from the other complexes was observed. The relative area under this peak agreed with the per cent. of the *a* complex in the solution determined ultracentrifugally.

For several reasons which are too lengthy to be detailed here, it is unlikely that the *a* complex is the 3:1 antigen:antibody species. One reason is that such a complex, with $s^{w}_{20} = 8.7\ S$ and a molecular weight of 370,000, would be required to have a frictional ratio, f/f_0, of 2.1. This value suggests a molecular asymmetry that is larger than would be expected for a complex in which 3 antigen molecules were attached to a single antibody molecule. The *a* complex must therefore be either largely the 1:1 or 2:1 antigen:antibody species, or a mixture of about equal proportions of the two. However, 1:1 and 2:1 complexes should have observably different electrophoretic mobilities, and the fact that all of the *a* complex observed ultracentrifugally can be accounted for under a single peak electrophoretically makes it unlikely that the *a* complex is a mixture of the two species, and likely that it is rather largely one or the other.

The following considerations indicate that the *a* complex cannot be the 1:1 species. If the amount of free antigen in a given solution, determined electrophoretically, is subtracted from the total antigen present, the result is the total amount of antigen bound in all of the complexes in that solution. This, divided by the total antibody, and multiplied by the appropriate molecular weight factor, 2.3, gives $\overline{(AG/AB)}_{N,B}$, the *average* number of antigen molecules bound per antibody in *all* of the complexes in the solution. The results are presented in Table I. This average number rises well above unity as the antigen excess is increased. Solution II-2 contains about 33% of the complexes as the *a* complex, the other species in the solution being characterized by smaller antigen-antibody ratios. The *a* complex therefore cannot be the 1:1 species, since it must obviously be richer in antigen.

TABLE I

COMPOSITION OF ANTIGEN-ANTIBODY COMPLEXES

Solution	% Total antigen	% Free antigen	$\overline{(AG/AB)}_{N,B}$
I	34.7	8.6	0.92
II	35.8	12.4	0.82
I-1	50.0	28.1	1.01
I-2	62.2	42.6	1.19
II-2	68.1	50.9	1.24

We conclude that the major part of the *a* complex contains 2 antigen molecules and 1 antibody molecule, and that the antibody is therefore largely bivalent. Further studies with this and similar systems are in progress or are contemplated, and a detailed account of this investigation will be published shortly.

This work was supported by a grant from the U. S. Public Health Service.

CONTRIBUTION NO. 1579
GATES AND CRELLIN LABORATORIES OF CHEMISTRY
CALIFORNIA INSTITUTE OF TECHNOLOGY S. J. SINGER
PASADENA, CALIFORNIA DAN H. CAMPBELL

RECEIVED MAY 28, 1951

A NEW ROUTE TO HYDROPHENANTHRENE KETONES. THE SYNTHESIS OF THE C_{18} KETONE DERIVED FROM DEHYDROABIETIC ACID

Sir:

The presently available methods for the preparation of hydrophenanthrene ketones of type I are useful only in the special case where R′ = H,[1] while the synthesis of substances related to the resin acids from such intermediates would require that the R′ group be methyl. We now wish to report a simple general synthesis of these hydrophenanthrene ketones.

Alkylation of the sodium derivative of Hagemann's ester[2] in a 3:1 mixture of benzene and dimethylformamide with the required β-phenethyl bromide gave, in 70% yield, the following 2-substituted-3-methyl-4-carbethoxy-Δ^2-cyclohexenones: 2-[β-phenethyl], b.p. 178–182° (0.4 mm.), dinitrophenylhydrazone (orange needles) m.p. 132–133° ($C_{24}H_{26}N_4O_6$: C, 61.79; H, 5.62; found: C, 62.19; H, 5.68), semicarbazone m.p. 167–168° ($C_{19}H_{24}N_3O_3$: C, 66.66; H, 7.07; found: C, 66.96; H, 7.46); 2-[β-*m*-isopropylphenethyl], b.p. 190–194° (0.4 mm.), dinitrophenylhydrazone (orange-red needles) m.p. 103° ($C_{27}H_{32}N_4O_6$: C, 63.76; H, 6.34; found: C, 64.09; H, 6.34). These were decarbethoxylated

(1) M. Heidelberger and K. O. Pedersen, *J. Exp. Med.* **65**, 393 (1937).

(1) *Inter alia*, R. Robinson and J. Walker, *J. Chem. Soc.*, 747 (1936), 183 (1938); W. E. Bachmann, S. Kushner and A. C. Stevenson, THIS JOURNAL, **64**, 974 (1942).

(2) C. T. Hagemann, *Ber.*, **26**, 876 (1893); *cf.* L. I. Smith and G. F. Rouault, THIS JOURNAL, **65**, 631 (1943).