peak at τ 3.88 (C_1' proton). This photoproduct is probably formed by interaction of the proximal C_2'–H bond of the ribose moiety with the intermediate carbene VI.[8] The photolysis of other pyrimidine methylides and their evaluation for cancerostatic and antiviral activity are now in progress.

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(8) B. M. Trost, *J. Am. Chem. Soc.*, **88**, 1587 (1966).

(9) Fellow in the Visiting Program of the U. S. Public Health Service, 1966–1970.

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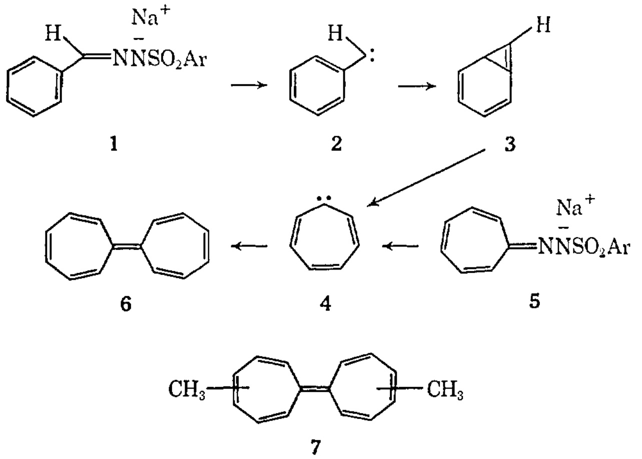
The Rearrangement of Phenylcarbenes to Cycloheptatrienylidenes[1]

Sir:

Singlet carbenes generated in inert media normally stabilize themselves by undergoing either intramolecular rearrangement to give valence-satisfied products or intersystem crossing to give lower energy triplets.[2] At this time we wish to report evidence for the rearrangement of phenylcarbenes to cycloheptatrienylidenes, reorginizations which constitute examples of carbenes stabilizing themselves by rearranging to lower energy carbenes.[3]

Rearrangements were observed upon flash pyrolysis of the sodium salts of aromatic aldehyde tosylhydrazones. In a typical experiment the sodium salt of benzaldehyde tosylhydrazone (**1**) was pyrolyzed at 250° (40 mm) in a stream of nitrogen (introduced into the column at 0.5 l./min) and the volatile products were collected in a trap cooled with liquid nitrogen. The resulting light beige colored solid (about 45% yield) turned a deep blackish brown (without gas evolution) when warmed to room temperature. Nmr analysis of the dark product showed a mixture consisting of about 33% stilbenes (identified by spectra and vpc) and 67% (corresponds to 30% yield) of a black crystalline product which upon isolation was found to be identical (nmr: structured singlet at τ 4.15; λ_{max} (EtOH) 234 (ϵ 22,000) and 362 mμ (ϵ 21,000)) with known heptafulvalene[11] (**6**). Under similar conditions (225°) the sodium salt of *p*-methylbenzaldehyde tosylhydrazone also gave a light colored material at liquid nitrogen temperature which turned black on warming. Nmr analysis of the condensed product showed 53% of the volatile product (24% yield) to be a material which, upon isolation, was a black noncrystalline solid with spectral properties (nmr: τ 8.20 (singlet with shoulder, six protons), 4.28 (structured singlet, ten protons); λ_{max} (EtOH) 233 (ϵ 23,100) and 368 mμ (ϵ 21,700)), which leaves little doubt but that it is dimethylheptafulvalene (**7**). As in the case of heptafulvalene itself the dimethyl product is too unstable (complete decomposition in air in 15 min) for elemental analysis. However, after separation by column chromatography, the substance was readily hydrogenated to a product that had all of the anticipated properties (including correct analysis) of dimethylbicycloheptyl (significant fragments *m/e* 222 (M), 111). Finally, although the heptafulvalenes have not been isolated due to low yields, nmr results point to the same reaction for *p*-chlorophenylcarbene and *p*-methoxyphenylcarbene.



In an attempt to determine the point in the reaction at which the heptafulvalenes are formed, the three following experiments were run. In the first, a mixture of the sodium salts of benzaldehyde tosylhydrazone and *p*-methylbenzaldehyde tosylhydrazone were introduced together into the pyrolysis column. Separation of the mixture of black heptafulvalenes by chromatography of the volatile residues on basic alumina followed by catalytic (Pt) reduction gave a mixture of bicycloheptyl, dimethylbicycloheptyl, and a new material whose mass spectrum (*m/e* 208 (M), 111, 97) and retention time leave no question but that it is the monomethylbicyclo-

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(1) This research was supported by the National Science Foundation to whom the authors are deeply grateful.

(2) *Cf.* W. Kirmse, "Carbene Chemistry," Academic Press, New York, N. Y., 1964; J. Hine, "Divalent Carbon," The Ronald Press Co., New York, N. Y., 1964; B. Capon, M. J. Perkins, and C. W. Rees, "Organic Reaction Mechanisms. 1965," Interscience Publishers, New York, N. Y., 1966, pp 222–236; B. Capon, M. J. Perkins, and C. W. Rees, "Organic Reaction Mechanisms. 1966," Interscience Publishers, New York, N. Y., 1967, pp 279–306; C. Capon, M. J. Perkins, and C. W. Rees, "Organic Reaction Mechanisms. 1967," Interscience Publishers, New York, N. Y., 1968, pp 278–304.

(3) Interconversion of carbenes is not without precedence. For example, reaction products led Skattebøl[4] to suggest the rearrangement of vinylcyclopropylidenes to cyclopentenylidenes, and Crow and Wentrup[5] have reported carbazole products from 2-pyridylcarbene (500° (0.02 mm)) which were rationalized by an azacycloheptatrienylidene (in equilibrium with nitrogen analogs of **3**) as either an intermediate or a transition state. Moreover, ring expansion of phenylnitrene to give substituted azepines is a reaction that has been known for many years[7] and has recently received thorough investigation.[6–9] Although it has been recognized that the products might arise from an azacycloheptatrienylidene (which would constitute a ring expansion analogous to the one reported in this note), a nitrogen analog of **3** appears to be the preferred structure for the reactive intermediate. In another case, deprotonation of ferrocenyltropylium fluoroborate and phenyltropylium fluoroborate with diisopropylethylamine has been reported to give products that were "cautiously" suggested to have arisen from ring contraction of ferrocenylcycloheptatrienylidene to ferrocenylphenylcarbene.[10] Under the same conditions ferrocenylphenylcarbene was not reported to undergo ring expansion.

(4) L. Skattebøl, *Tetrahedron*, **23**, 1107 (1967).

(5) W. D. Crow and C. Wentrup, *Tetrahedron Lett.*, 6149 (1968).

(6) W. von E. Doering and R. A. Odum, *Tetrahedron*, **22**, 81 (1966).

(7) *Cf.* R. Huisgen, D. Vossins, and M. Appl, *Chem. Ber.*, **91**, 1 (1958).

(8) R. A. Odum and M. Brenner, *J. Am. Chem. Soc.*, **88**, 2074 (1966); R. J. Sundberg, B. P. Das, and R. H. Smith, Jr., *ibid.*, **91**, 658 (1969), and references therein.

(9) J. I. G. Cadogen, *Quart. Rev.* (London), 222 (1968), and references therein.

(10) P. Ashkenasi, S. Lupan, A. Scharz, and M. Cais, *Tetrahedron Lett.*, 817 (1969).

(11) W. von E. Doering in "Theoretical Organic Chemistry. The Kekule Symposium," Academic Press, New York, N. Y., 1959, p 44; W. M. Jones and C. L. Ennis, *J. Am. Chem. Soc.*, **91**, 6391 (1969).

heptyl. In the second and third experiments, the individual tosylhydrazone salts were introduced into separate pyrolysis columns and the volatile products were allowed to mix in one case in the gas phase after exiting the columns but before condensation and in the other case in the condensed phase (by alternately introducing small amounts of the different sodium salts into separate pyrolysis columns and condensing the volatile products in a common trap). In neither of the latter two cases was any mixed dimer formed (as indicated by the absence of the monomethylbicycloheptyl after the work-up and reduction). From these experiments, three conclusions can be drawn: (1) dimerization of the carbenes occurs in the gas phase; (2) dimerization is complete before the monomer reaches the end of the pyrolysis column; and (3) the beige deposit is not a monomer.[12]

Although we do not at this time have definitive evidence for the monomer structure, circumstantial evidence, including the fact that flash pyrolysis of the sodium salt of tropone tosylhydrazone (**5**) under the conditions described above also gives heptafulvalene,[13] certainly points to cycloheptatrienylidene.[11,14]

Finally, although pyrolyses of the salts of benzaldehyde tosylhydrazones give some aromatic products, the salt of tropone tosylhydrazone gives essentially none.[16] This suggests that if the rearrangement is reversible the seven-membered-ring carbene is favored.[17,18] This is not surprising since the carbocyclic aromatic carbene would certainly be expected to be stable relative to phenylcarbene.[18]

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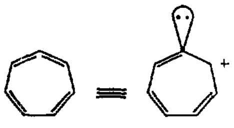
[12] The observed color change is a curiosity since the material that is presumed to be pure heptafulvalene is nearly black,[11] and the observed color change is irreversible. At the present time we do not have an explanation for this.

[13] The condensate from this reaction shows the same color changes mentioned above.

[14] Other possible heptafulvalene precursors include cycloheptatetraene (i) and the cyclopropane **3**. The allene as a possibility is probably trivial since ring strain would be expected to bend the allene moiety and twist its terminal carbons to such an extent that it would probably become tantamount to a resonance form of **4**. The strained cyclopropene 3 could also give heptafulvalene by a "2 + 2" cycloaddition of the cyclopropene double bonds followed by appropriate bond shifts. Although the credibility of this possibility is increased by the fact that both of the required reactions have been observed for substituted biscyclopropenyl systems, it is unlikely that it is the sole intermediate in these reactions for the following reason.[15] Dimerization of the cyclopropane double bonds should be able to occur either "head-to-head" (to give heptafulvalene) or by the more sterically favored "head-to-tail" route which should give benzenoid products. All evidence to date indicates that, with the exception of stilbenes, benzenoid products from any of these reactions are formed in, at most, trace amounts.



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[15] R. Breslow and P. Gall, *J. Am. Chem. Soc.*, **81**, 4747 (1959).

[16] Traces of *cis*-stilbene have been detected, but these probably arise from traces of benzaldehyde contamination in our tropone.

[17] This is particularly interesting in light of the chemistry of ferrocenyltropylium fluoroborate and ferrocenylphenylcarbene.[10]

[18] This rearrangement finds a very interesting analogy in the thoroughly documented rearrangement of the benzyl cation to the tropylium cation in the mass spectrometer.[19]

[19] *Cf.* R. W. Kiser, "Introduction to Mass Spectrometry," Prentice-Hall, Inc., Englewood Cliffs, N. J., 1965, pp 272–273.

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Mass Spectrometry of Trimethylsilyl Derivatives of Nucleoside and Dinucleotide Phenylboronates. Application to Oligonucleotide Sequence Analysis[1]

Sir:

The use of mass spectrometry in the determination of base sequence in ribonucleic acid will be dependent upon the ability (1) to volatilize the short-chain oligonucleotide components and (2) to obtain fragment ions which yield information on the order of the bases in the chain. A recent report[2] cataloged fragment ions from silylated derivatives of dinucleoside phosphates which might be used in sequence analysis. Although it was noted that the fragment ions from isomers such as adenylyl-(3′–5′)-uridine (ApU) and uridylyl-(3′–5′)-adenosine (UpA) were isomeric and thus their mass alone was not sufficient to distinguish the two sequence isomers, it was suggested that the abundance ratios of the ions might be useful in making this differentiation. We now report a method which involves the sequential use of two reagents to asymmetrically derivatize dinucleoside phosphates such that the fragment ions of the products clearly distinguish the order of the bases. In this method the dinucleoside phosphate is converted to its phenylboronic ester, a procedure which tags the *cis*-1,2-diol position, and, subsequently, trimethylsilyl groups are introduced into the remaining reactive groups of the molecule to enhance volatility.

Initial model experiments on nucleosides established the feasibility of this approach. Phenylboronic esters of uridine, adenosine, cytidine, and guanosine were synthesized by a method[3] which was modified to milligram scale. The phenylboronate was then silylated by reaction with N-trimethylsilylimidazole.[4] Mass spectra[5] from these derivatives yielded fragment ions corresponding to the fragmentation pattern in Figure 1. The phenylboronate trimethylsilyl ethers of U, A, C, and G contain the following characteristic ions: M, $M - CH_3$, $M - (CH_3)_3Si$, $M - C_6H_5$, $M - (CH_3)_3SiO$, $M - (CH_3)_3SiOCH_2$, M − base, base + 30, base, base + H, and base + 2H. The elemental composition of the ions was verified by high-resolution mass spectrometry. Major fragment ions not shown which are common to all four derivatized nucleosides are those at mass 172 ($C_{10}H_9O_2B$), 159 ($C_9H_8O_2B$), and 147 ($C_8H_8O_2B$) and these arise from cleavages of the ribose moiety.

Dinucleoside phosphates were derivatized in the same manner as for the nucleosides and mass spectrometric analyses yielded complex fragmentation profiles (Figure 2). However, the information derived from only five

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(1) This work was supported by the National Science Foundation (Grant No. GB 7864).

(2) D. F. Hunt, C. Hignite, and K. Biemann, *Biochem. Biophys. Res. Commun.*, 33, 378 (1968).

(3) A. M. Yurkevich, I. Kolodkina, L. Varshavskaya, V. Borodulina-Shvetz, I. Rudakova, and N. Preobrazhenski, *Tetrahedron*, **25**, 477 (1969). Equimolar quantities of nucleoside (2 mg) and phenylboronic acid in dry pyridine (or dimethylformamide for guanosine) were heated 2 hr at 120° in sealed tubes. The mixture was evaporated to dryness, the residue washed several times with ether, and the product recrystallized from hot acetone. Purity of the product was confirmed by characteristic values for the melting point and the ultraviolet spectrum.

(4) M. G. Horning, A. Moss, and E. Horning, *Biochim. Biophys. Acta*, **148**, 597 (1967).

(5) A capillary containing the sample (evaporated *in vacuo*) was introduced through the vacuum lock into the ion source of a CEC-110 high-resolution mass spectrometer. Low-resolution spectra were obtained by using the direct inlet probe on the Hitachi–Perkin-Elmer RMU-6D spectrometer. All derivatives were volatile at 200–240°.