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]

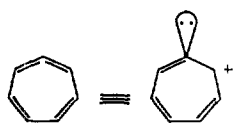
Although we do not at this time have definitive evidence for the monomer structure, circumstantial evidence, including the fact that flash pyrolysis of the sodium salt of tropone tosylhydrazone (**5**) under the conditions described above also gives heptafulvalene,[13] certainly points to cycloheptatrienylidene.[11,14]

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]

(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



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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.

(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

(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°.

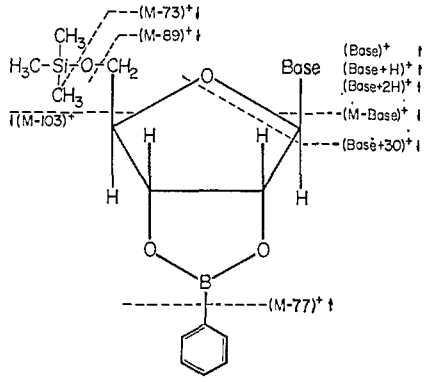

Figure 1. Fragmentation of nucleoside phenylboronate trimethylsilyl ethers.

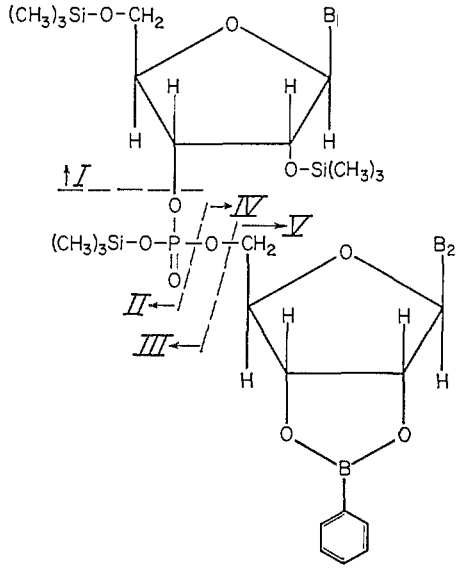

Figure 2. Fragmentation of silylated phenylboronates of dinucleoside phosphates.

fragment ions is sufficient to characterize each of the dinucleoside phosphates and, in particular, this information unequivocally differentiates between sequence isomers such as ApU and UpA. Figure 3 represents a possible fragmentation pattern to account for the ions observed. Ion I results from cleavage of the PO–C3′ bond, and ion V from cleavage of the PO–C5′ bond with charge retention on the carbon atom in each case.

Table I. Characteristic Fragment Ions of Silylated Phenylboronates of Dinucleoside Phosphates

Compd	Fragment *m/e* M+	I	II	III[a]	IV	V	Relative intensity[b]
ApA	1042	466	618	636	424	408	85:59:9:75:100
UpU	996	443	595	613	401	385	53:78:25:72:100
ApU	1019	466	618	636	401	385	66:75:7:38:100
UpA	1019	443	595	613	424	408	10:34:4:52:100
UpC	995	443	595	613	400	384	55:53:21:93:100
CpU	995	442	594	612	401	385	60:54:12:84:100

[a] Fragmentation of trialkyl phosphates with charge retention on the oxygen is accompanied by transfer of two hydrogens to form a protonated phosphate ion.[2] [b] The most abundant of the five ions = 100.

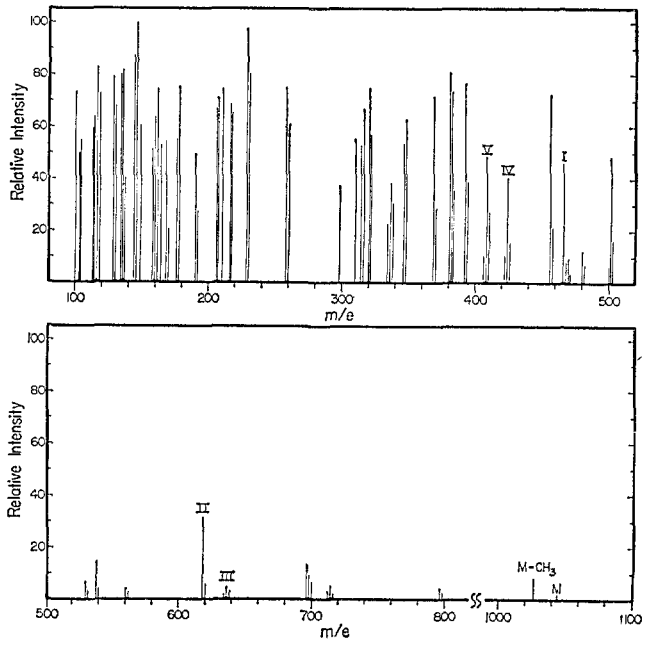

Figure 3. Mass spectrum of trimethylsilylated adenylyl-(3′–5′)-adenosine phenylboronate.

Ion III includes the structure of ion I with the addition of the phosphate–trimethylsilyl moiety. The same addition to ion V did not produce a prominent ion. However, cleavage of the P–O bond at the 5′ position of the nucleoside containing base B_2 yields ion IV. This same type of cleavage also results in ion II which includes base B_1. The key fragment ions for some dinucleoside phosphates are presented in Table I.

It may be noted that the elemental composition (indicating the presence of boron) of ion V would always yield unambiguous information as to which base is at the 3′ terminus of a particular oligonucleotide, and, furthermore, would also establish that the compound is not phosphorylated at this position. The significance of this type of information will become increasingly apparent when attempts are made to order the bases in the more complex tri- and tetranucleotides.

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A New Rearrangement Process in Methylcyclopentyl and *t*-Amyl Cations

Sir:

A reaction which interchanges the three methyl groups of *t*-amyl cation has recently been studied using nmr.[1–3] We now report an additional, slower process

(1) M. Saunders and E. Hagen, *J. Am. Chem. Soc.*, 90, 2436 (1968).
(2) D. M. Brouwer and E. L. Mackor, *Proc. Chem. Soc.*, 147 (1964).
(3) D. M. Brouwer, *Recueil*, 87, 210 (1968).