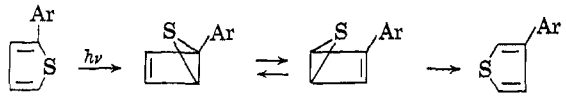

VI VIa VIb VII

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Determination of Terminal Sequences in Viral and Ribosomal Ribonucleic Acids

Sir:

The use of alkaline degradation for the determination of the terminal nucleotide in the ribonucleic acid of tobacco mosaic virus has been reported by Sugiyama and Fraenkel-Conrat[1] and by Whitfeld.[2] The terminal base was obtained as the nucleoside adenosine by the isotope dilution method although it was observed that considerable quantities of the other nucleosides were also formed. The authors suggested that this apparent heterogeneity in the terminal base resulted from phosphate exchange between the nucleoside phosphates and the nucleosides during the degradation. It is now confirmed that at least part of this effect is caused by phosphate exchange between the nucleoside and the nucleoside cyclic phosphates which are intermediates in the alkaline hydrolysis. Furthermore, experiments have been designed to minimize this effect, and the terminal sequences of some viral and ribosomal nucleic acids have been determined. Initially, the following general procedure was developed for the estimation of small amounts of nucleosides in the presence of large quantities of nucleotides in alkaline or enzymic hydrolysates.

The alkaline hydrolysate is neutralized with perchloric acid and cooled to 0°. The supernatant solution is removed from the precipitated potassium perchlorate by centrifugation and concentrated *in vacuo* to 1–2 ml. The solution is separated from further precipitated salt and applied to a DEAE–cellulose column (HCO_3^- form, 1.7 × 60 cm.) which has been prewashed with water. The salt precipitates are washed with cold water (3 × 2 ml.) and the washings are concentrated to about 2 ml. and also applied to the column. The nucleotides are retained on the column and, on elution with water at 5–10 ml./hr., the salt ($KHCO_3$) appears first in the eluate, followed by the nucleosides. Under these conditions the nucleosides are obtained free of both nucleotides and salt and can be directly fractionated by paper electrophoresis in ammonium formate (0.05 *M*, pH 3.0) and estimated spectrophotometrically. In the case of enzymic hydrolyses the solution is concentrated and added directly to the column. A control experiment, in which a mixture of one absorbancy unit of each of the four nucleosides was run through this procedure, gave better than 90% recovery.

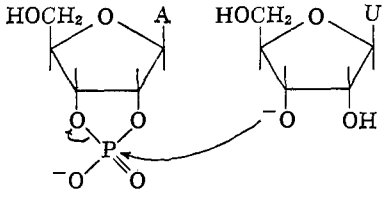
Table I lists the results of alkaline hydrolyses designed to evaluate phosphate exchange reactions between adenosine-2′,3′ cyclic phosphate and uridine

Table I. Phosphate Exchange between Adenosine-2′,3′ Cyclic Phosphate (50 mg.) and Uridine

	Uridine, mg.	Time, hr.	Temp., °C.	KOH, *N*	Volume, ml.	Adenosine formed, % of total nucleoside
I	1	20	37	0.25	10	4.4
II	0	20	37	0.25	10	0
III	1[a]	20	37	0.25	10	0
IV	1	20	37	0.25	100	2.3
V	1	20	37	0.25	200	1.3
VI	1	15	25	2	10	1.3

[a] The uridine was added after the cyclic phosphate had been opened under the conditions given, and the mixture was then kept at 37° for a further 20 hr.

under conditions which would normally result in the complete hydrolysis of ribonucleic acid. The control experiment II shows that no dephosphorylation of the nucleotide takes place in the absence of nucleoside, while experiment III shows that no phosphate exchange occurs after the cyclic phosphate has been opened. Thus it appears that phosphate exchange results from a competition between the 2′ or 3′ anions of the uridine and the hydroxyl ions in the nucleophilic attack on the cyclic phosphate, yielding uridylyl-(2′(3′)→2′(3′))-



adenosine. Attack on the phosphorus by the remaining 2′ or 3′ anion of the uridine moiety in this dinucleoside phosphate would result in the formation of adenosine and uridine-2′,3′ cyclic phosphate, the latter being subsequently hydrolyzed to the nucleotide. In the alkaline hydrolysis of ribonucleic acid this method of phosphate exchange is probably supplemented by a mechanistically analogous one involving the attack of the nucleoside anion on some of the unhydrolyzed phosphodiester linkages. With these considerations it is predicted that phosphate exchange should be reduced by lowering the nucleoside concentration relative to the hydroxyl ion concentration. A decrease in the exchange reaction is thus observed under the conditions used in experiments IV, V, and VI.

Table II lists the bases at the 3′-hydroxyl terminus of some ribonucleic acids determined by the methods described above. On alkaline hydrolysis, the RNA of the bacteriophage f2 produced essentially 1 mole equiv. of adenosine together with some cytidine. Adenosine was also obtained by hydrolysis with pancreatic ribonuclease, thus indicating that the penultimate base of f2 RNA is a pyrimidine. A similar result was obtained with the alkaline hydrolysis of the RNA from the bacteriophage MS2, confirming the terminal nucleoside

(1) T. Sugiyama and H. Fraenkel-Conrat, *Proc. Natl. Acad. Sci. U.S.*, 47, 1393 (1961).
(2) P. R. Whitfeld, *J. Biol. Chem.*, 237, 2865 (1962).

Table II. Terminal Nucleosides

RNA, 50 mg.	Method of hydrolysis	Moles of nucleosides/mole of nucleic acid[a] A	G	C	U
f2	KOH, VI	0.91	<0.05	0.18	<0.05
..	Pancreatic ribonuclease	0.78	<0.05	<0.05	<0.05
MS2	KOH, VI	0.94	<0.05	0.15	<0.05
..	.. ..	0.93	<0.05	0.16	<0.05
WG	.. ..	0.21	0.25	0.33	0.27
..	Pancreatic ribonuclease	<0.05	<0.05	<0.05	<0.05
..	Ribonuclease T_1	<0.05	0.27	<0.05	0.25

[a]Calculations were based on a chain length of 3000 nucleotides for the f2- and MS2-RNA and 1500 nucleotides for the WG-RNA.

as adenosine (recently determined by Sugiyama[3] using the isotope dilution method). The ribosomal RNA from wheat germ (WG) produced all four nucleosides on alkaline hydrolysis. On digestion with pancreatic ribonuclease, however, none of the nucleosides was liberated, showing that none of the nucleic acid molecules contains pyrimidines as their penultimate bases. Ribonuclease T_1 which splits RNA specifically at the guanosine-3′ phosphoryl positions gave quantities of guanosine and uridine equal to those obtained in the alkaline hydrolysis. Thus the majority of the chains in this ribosomal nucleic acid must possess the terminal sequences: -GpU, -GpG, -ApA, and -ApC.

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(3) T. Sugiyama, *J. Mol. Biol.*, **11**, 856 (1965).

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A Novel Oxidation Reaction of Organodisilanes with Perbenzoic Acid

Sir:

During the course of an investigation of the properties of silicon–silicon bonding, we have found a novel oxidation reaction of organodisilanes with perbenzoic acid involving direct insertion of an oxygen atom into the silicon–silicon bonding.

$$\underset{\text{I}}{R(CH_3)_2Si\text{–}Si(CH_3)_3} + PhCO_3H \longrightarrow R(CH_3)_2Si\text{–}O\text{–}Si(CH_3)_3 + PhCO_2H$$

In a typical experiment 9.9 g. (0.048 mole) of phenylpentamethyldisilane (I, R = C_6H_5) was treated with 0.096 mole of perbenzoic acid in dichloromethane at room temperature. After standing overnight to ensure complete reaction, the reaction mixture was washed with water, sodium carbonate solution, and water, dried over anhydrous magnesium sulfate, and distilled, to yield, after removal of the solvent, 9.2 g. (86%) of phenylpentamethyldisiloxane. Careful examination of the reaction mixture by v.p.c. revealed that there was no other product such as hexamethyldisiloxane or diphenyltetramethyldisiloxane. In a similar manner, hexamethyldisilane gave hexamethyldisiloxane

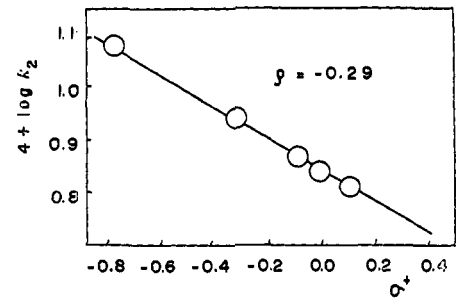

Figure 1. Correlation of 4 + log k_2 and σ^+ for the reaction of phenylpentamethyldisilanes with perbenzoic acid in benzene at 45.0°.

almost quantitatively. In the case of vinyldisilane, both the vinyl and disilanyl groups were oxidized; thus, vinylpentamethyldisilane gave pentamethyldisiloxanylepoxyethane (II) in quantitative yield, b.p. 49.0° (23 mm.), $n^{20}D$ 1.4125, d^{20}_4 0.8887, MR (calcd. 53.52) 53.38. *Anal.* Calcd. for $C_7H_{18}O_2Si_2$: C, 44.16; H, 9.53. Found: C, 44.38; H, 9.38.

$$CH_2{=}CHSi(CH_3)_2Si(CH_3)_3 \xrightarrow{2PhCO_3H} CH_2\text{—}CH\text{—}SiMe_2\text{—}O\text{—}SiMe_3 \quad \text{II}$$

The n.m.r. spectrum[1] of II in carbon tetrachloride (cyclohexane internal standard) showed a singlet at τ 9.92 for protons of five methyl groups and a typical epoxide pattern[2] consisting of a quartet centered at τ 8.00 and double quartet centered at 7.53 and 7.18 ($J_{\alpha\beta}$ = 5 c.p.s., $J_{\alpha\beta'}$ = 4 c.p.s., $J_{\beta\beta'}$ = 7.5 c.p.s.). Again there was no other siloxane.

It is advantageous in this oxidation reaction that the reaction could be carried out without cleavage of the phenyl–silicon bond, unlike other electrophilic reactions with silicon compounds such as halogenation of disilanes.[3]

The kinetics of the reaction have been studied in benzene. The extent of reaction was followed by the standard iodometric titration of perbenzoic acid.[4] The second-order law was found to be obeyed accurately in this reaction, and the results are listed in Table I. The qualitative effects of substituents are in accord with the characters of disilanes and perbenzoic acid as nucleophilic and electrophilic reagents, respectively. Figure 1 shows an excellent linear plot of log k_2 *vs.* σ^+ of Brown and Okamoto[5] for substituted phenylpentamethyldisilanes (shown in Table I). These results may indicate that certain substituents on the phenyl ring of the disilanes are capable of resonance interaction with the silicon atom, possibly through p_π–d_π conjugation,[6] to make the silicon–silicon bond more nucleophilic. Substitution of a phenyl group for

(1) We are grateful to Dr. K. Tori, Shionogi Research Laboratory, for the determination of the spectrum.

(2) R. Silverstein and G. C. Bassler, "Spectrometric Identification of Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 1963, p. 81.

(3) C. Eaborn, "Organosilicon Compounds," Butterworths Scientific Publications, London, 1960, p. 354.

(4) Y. Nagai, *J. Soc. Org. Syn. Chem. Japan*, **19**, 527, 533, 537 (1961).

(5) H. C. Brown and Y. Okamoto, *J. Am. Chem. Soc.*, **80**, 4979 (1958).

(6) For spectral evidence, see H. Sakurai and M. Kumada, *Bull. Chem. Soc. Japan*, **37**, 1894 (1964); H. Gilman, W. H. Schwebke *Chem. Ind.* (London), 1063 (1964); *J. Organometal. Chem.*, **2**, 369 (1964).