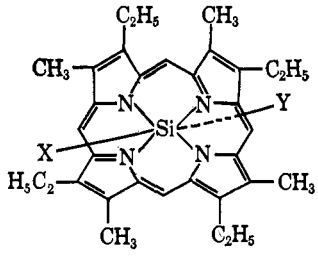
Table I. Gas Chromatographic Retention Times[a] and Predominant 70-ev Mass Spectral Ions[b] of X,Y-Si^{IV}-Etioporphyrins



Compd	X	Y	m/e M$^+$	m/e (M − (X or Y))$^+$
A	OH	Y = X	538	521
B	$OSi(CH_3)_3$	Y = X	682	593
C	OCH_2CH_3	Y = X	594	549
D	$OSi(OCH_2CH_3)_3$	OH	700	683,521
E	$OSi(OCH_2CH_3)_3$	$OSi(CH_3)_3$	772	683,593
F	$OSi(OCH_2CH_3)_3$	Y = X	862	683
G	$OSiOC(CH_3)_3(OCH_2CH_3)_2$	Y = X_H[c]	946	739,711
H	$OSi(OC(CH_3)_3)_2OCH_2CH_3$	Y = X	974	739
I	$OSi(OC(CH_3)_3)_2OH$	Y = X	918	711
J	$OSi(OC(CH_3)_3)_2OSi(CH_3)_3$	Y = X	1062	783
K	$OSi(OC(CH_3)_3)_3$	Y = X	1030	767

[a] The following gas chromatographic retention times were observed: compound B (11 min), E (14 min), F (17 min), G (21 min), H (25 min), J (>27 min), K (>27 min). [b] All mass spectra were obtained using an AEI MS-12 mass spectrometer equipped with a solid sample probe. [c] X_H = ligand X on compound H.

Compounds C, D, F, G, H, I, K (Table I) were isolated from a complex mixture of products produced when the contents of the sealed tube reaction were treated with ethyl or *t*-butyl alcohol. Trimethylsilylation of D and I gave compounds E and J, respectively. Mass spectral analysis indicated that the predominant high mass fragmentation was due to loss of one ligand. In silicon complexes where two different ligands were present, such as in compounds D, E, and G, predominant high mass fragmentation ions occurred at both mass (M − X) and (M − Y).

Preliminary gas chromatographic analysis of the silicon complex porphyrins was accomplished on an 11 ft × 0.25 in. i.d. glass column packed with 1% (OV-1) (methylsilicone phase) coated 100–120 mesh smooth glass beads. The temperature was kept isothermal at 250° and a helium carrier flow of 27 cc/min was used. Each chromatographic peak was collected and identified by mass spectrometry. Etioporphyrin I, Ni^{II}-etioporphyrin I, and diethoxy-Si^{IV}-etioporphyrin I (C) would not chromatograph under the described conditions. However, bis(trimethylsiloxy)-Si^{IV}-etioporphyrin I (B) showed a marked increase in volatility and could be chromatographed despite an increase in molecular weight of 88 mass units over the diethoxysilicon complex. The increased volatility can be attributed, at least in part, to the shielding of the aromatic porphyrin ring by the bulky trimethylsiloxy groups above and below the porphyrin plane, thus preventing the close approach and reducing the natural attraction between the planes. Consideration of the gas chromatographic retention times (Table I) indicates that additional ligand bulkiness (E to H, J, K) does not render the porphyrin nucleus more volatile than B but the volatility seems to vary directly with the molecular weight.

In the past, standard gas chromatography has had no practical use in porphyrin separations.[6] The results of our preliminary study indicate that conversion of porphyrins into volatile tetravalent silicon derivatives should provide a means by which microquantities of complex mixtures of homologous series, such as those found in ancient biogenetic deposits, can be separated and structurally studied using gas chromatography–mass spectrometry combinations. This work will also be expanded to include development of a rapid quantitative means for positive identification of porphyrins and chlorins extracted from recent biological sources.

Acknowledgment. We are grateful to Dr. G. Eglinton for useful discussions.

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Structural Analysis of Polynucleotides by Sequential Base Elimination. The Sequence of the Terminal Decanucleotide Fragment of the Ribonucleic Acid from Bacteriophage f2

Sir:

Recently we reported a method by which the terminal fragments produced by specific cleavage of ribonucleic acids could be oxidized by periodate, selectively absorbed on aminoethylcellulose, and subsequently recovered in a pure condition.[1] The application of this method to a ribonuclease T_1 digest of the ribonucleic acid from bacteriophage f2 produced a terminal fragment which was assigned the structure (3Cp,2Up,-2Ap)Cp by virtue of a preliminary base ratio analysis. In this report we show by analysis of the products formed by hydrolysis of the fragment with pancreatic ribonuclease that the composition of the oligonucleotide is, in fact, (2Cp,2Up,2ApCp)Cp. In addition, we have developed an improved method for sequence analysis of polynucleotides based on the classical periodate oxidation–β-elimination approach.[2] The application of this method to the analysis of the terminal fragment demonstrates that the terminal undecanucleotide sequence of f2 RNA is -GpUpUpApCpCpAp-CpCpCpA.

For the ribonuclease hydrolysis, the fragment (2.1 $ODU_{260m\mu}$) and the enzyme (0.05 mg) in 0.1 *M* ammonium bicarbonate buffer, pH 9 (0.2 ml), were kept at 25° for 12 hr. The products were identified by chromatography on a column (0.4 × 42 cm) of DEAE-Sephadex A25. On such a column, using ribonuclease digests of model compounds, mononucleotides were shown to be eluted separately with 0.2 *M* ammonium bicarbonate pH 9, while dinucleotides required the same buffer at 0.4 *M* concentration for separation and elution. The products obtained from the fragment were cytidine 3′-phosphate, 83 mμmoles; uridine 3′-phosphate,

(1) J. C. Lee and P. T. Gilham, *J. Am. Chem. Soc.*, **88**, 5685 (1966).
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55 mμmoles; and adenylyl-(3′–5′)-cytidine 3′-phosphate, 55 mμmoles; Cp:Up:ApCp, 3.02:2:2.

The improved analytical technique involving periodate oxidation and base elimination is based on modifications of the methods described by Neu and Heppel.[3] Cyclohexylamine is used for the elimination reaction in preference to lysine since the latter reacts with periodate. In addition, it is considered advantageous to buffer the elimination reaction mixture at pH 8 with di-*n*-propylmalonic acid. Another modification involves the removal of the phosphatase enzyme. The whole procedure requires that, between the removal of one base and the removal of the next, this enzyme should be added in order to cleave off the terminal phosphate group. It also requires that, prior to the next oxidation step, the enzyme should be completely removed. In this regard it has been found that, in solution, this enzyme can be inactivated *in situ* by treatment with EDTA in the free acid form, and that it does not recover activity when the pH of the solution is brought back to neutrality. This procedure was based on the observation that phosphatase is denatured at low pH and requires the presence of zinc ions for the recovery of activity at neutral pH.[4] The third modification involves the method of analysis of the products formed by the base elimination reaction. In a study of the periodate oxidation of model compounds it was found that, if the reaction mixtures were applied to small DEAE-Sephadex columns and washed through with water, the liberated bases were eluted in the order cytosine, uracil, adenine, and guanine. The bases could then be easily recognized by their position of elution and by their spectra. The column serves a second purpose in that, subsequent to the elution of the base, the polynucleotide fragment can be eluted by a solution containing a salt gradient, and thus the fragment is obtained in a pure condition ready for the next elimination cycle.

In a typical oxidation–elimination cycle the oligonucleotide (10–100 mμmoles) in water (0.1 ml) was treated with EDTA (free acid, 4.5 mg) to inactivate residual phosphatase from the previous cycle. The mixture was allowed to stand for 1 hr with occasional shaking. A buffered cyclohexylamine solution (0.05 ml) and 0.1 *M* sodium periodate (0.05 ml) were added and the mixture was kept at 45° for 90 min. The amine solution was prepared by dissolving di-*n*-propylmalonic acid (5 mmoles) in water and adjusting the pH to 8.0 with sodium hydroxide. To this solution was added cyclohexylamine (10 mmoles) and water such that the final volume was 10 ml. Subsequent to the oxidation reaction the solution (pH 8–8.2) was treated with 0.2 *M* ethylene glycol (0.05 ml). After 20 min water (0.65 ml) and phosphatase solution[5] (0.1 ml, 0.6 unit) were added and the mixture was kept at 37° for 2 hr and then applied to a column (0.6 × 25 cm) of DEAE-Sephadex (HCO_3^- form). The column was eluted with water to recover the liberated base and was then eluted with a linear gradient (600 ml) of 0.2 to 1.0 *M* triethylamine bicarbonate to recover the oligonucleotide fragment. The bases released from six cycles carried out on the fragment from f2 RNA are given in Table I. These results, taken together with the analysis of the ribonuclease digest and together with earlier considerations[1,6] regarding the last two bases of the RNA molecule, are sufficient to define the terminal eleven bases of the RNA as shown above.

Table I. Sequential Periodate Oxidation and Elimination of Bases from the Terminal Fragment of f2 RNA

Cycle	Bases released, mμmoles C	U	A	G
1	109	<2	<5	<3
2	62	<2	<3	<3
3	43	<2	6	<3
4	<4	<2	25	<3
5	17	<2	<3	<3
6	15	<2	<1	<3

Acknowledgment. This work was supported by the National Institutes of Health.

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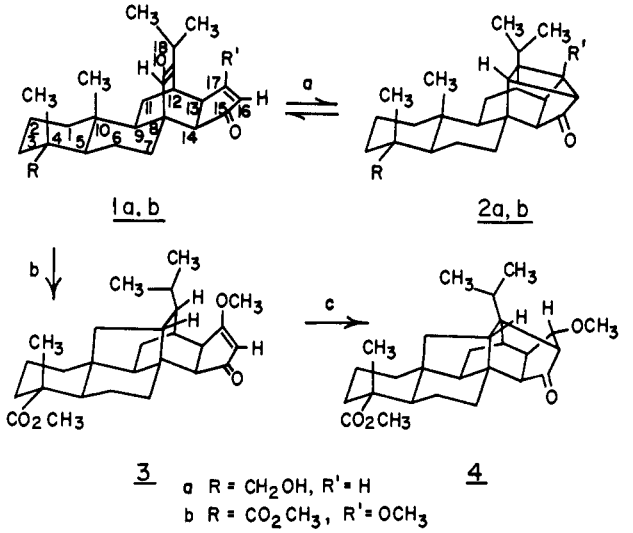
(4) M. J. Schlesinger, A. Torriani, and C. Levinthal, *Cold Spring Harbor Symp. Quant. Biol.*, **28**, 539 (1963).
(5) Bacterial alkaline phosphatase was obtained from Worthington Biochemical Corp., Freehold, N. J. One unit is that amount of enzyme which liberates 1 μmole of *p*-nitrophenol from *p*-nitrophenyl phosphate per minute under the assay conditions given by A. Garen and C. Levinthal, *Biochim. Biophys. Acta*, **38**, 470 (1960).

A Remarkable Case of Intramolecular Energy Transfer

Sir:

Photolysis of the levopimaric acid–*p*-benzoquinone adduct yields[1] the expected cage product.[2] Similarly, photoisomerization[3] (Pyrex filter) of **1a**[4] [infrared bands at 3475, 1670, and 1600 cm^{-1}, λ_{max} (cyclohexane) 235 and 310 mμ (ϵ_{max} 7340 and 100), significant nmr signals



(1) W. Herz, R. C. Blackstone, and M. G. Nair, *J. Org. Chem.*, **32**, 2992 (1967).
(2) R. C. Cookson, E. Crundwell, and J. Hudec, *Chem. Ind.* (London), 1003 (1958); R. C. Cookson, E. Crundwell, R. R. Hill, and J. Hudec, *J. Chem. Soc.*, 3062 (1964).
(3) Irradiations were carried out in methanol using a Hanovia 679A-36 lamp in a quartz immersion well with or without a Pyrex filter, but proceeded equally well in cyclohexane.
(4) Synthesis and structure proof of **1a**, **1b**, and related compounds will be presented in our detailed paper. All substances analyzed within acceptable limits.