atmosphere for 20 hr. The solvent was removed and the residue was stirred with diethyl ether. The ether phase was separated from the crystalline material (sodium fluoroborate, 0.44 g). By crystallization and chromatographic techniques the ether fraction was found to contain methyl phenyl sulfone (0.22 g), methyl benzenesulfinate[13] (0.21 g), and *N*,*N*-dimethylbenzenesulfinamide[12] (0.11 g).

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Chemical Evolution. IV. An Evaluation of Cyanovinyl Phosphate as a Prebiotic Phosphorylating Agent[1]

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Abstract: The structure of the phosphate adduct of HC≡CCN is *cis*-β-cyanovinyl phosphate (CVP) (I). The cis configuration was assigned on the basis of an especially characteristic nmr spectrum with signals at δ 4.73 (q, $J = \sim 5$, 1.3 Hz) and 7.33 (q, $J = 7.8$, 6.2 Hz) for H-2 and H-3, respectively. Pyrophosphate is obtained when aqueous solutions of CVP and orthophosphate are heated. Pseudo-first-order kinetics were observed for the addition of orthophosphate and hydroxide to $10^{-4}\,M$ HC≡CCN. The bimolecular rate constants (l. mol^{-1} min^{-1}), $\Delta H^{\ddagger}$ (kcal), and $\Delta S^{\ddagger}$ (eu) were found to be, respectively: HPO_4^{2-}, 2.5×10^{-1}, +22.0, −4.0; $H_2PO_4^-$, 7.5×10^{-2}, +24.6, +1.8; OH^-, 8.8×10, +19.0, −0.6. The first-order rate constant for the hydrolysis of CVP at 60° is 10^{-4} min^{-1} ($\Delta H^{\ddagger} = 28$ kcal, $\Delta S^{\ddagger} = -0.6$ eu). The rate of hydrolysis is pH independent between pH 7 and 11 and increases only fourfold going from pH 7 to 1.3. There is a small positive salt effect and a small solvent isotope effect ($k_{H_2O}/k_{D_2O} = 1.3$) on the solvolysis rate. These data and other results established that the rate-determining step in the solvolysis is P–O cleavage with the formation of the anions of metaphosphate and cyanoacetaldehyde. It was calculated that cyanovinyl phosphate may have accumulated in the oceans of the primitive earth with orthophosphate concentrations as low as $10^{-6}\,M$. However, the concentration of orthophosphate on the primitive earth would have to exceed $10^{-3}\,M$ before appreciable quantities of pyrophosphate would be formed from CVP.

The origin of life is believed to have been the end result of the interaction of light, electrical discharges, and other forms of energy with the earth's primitive atmosphere. It is postulated that reactive organic molecules were produced which condensed in the primitive ocean to yield the biological molecules from which the first living system originated. One such reactive organic molecule may have been cyanoacetylene. It is produced when an electrical discharge is passed through a mixture of methane and nitrogen.[3] In aqueous solution, cyanoacetylene reacts with inorganic compounds to yield cytosine, uracil, aspartic acid, asparagine, and 6-aminonicotinamide.[3-5] The genesis of such a wide array of biological molecules from one source prompted our further investigation of the chemistry of cyanoacetylene.

The Structure of β-Cyanovinyl Phosphate

A phosphate adduct of cyanoacetylene, β-cyanovinyl phosphate (CVP) (I), is produced when cyanoacetylene is dissolved in orthophosphate buffers.[6] This adduct was characterized by an absorption maximum at 225 nm. Initially we were reluctant to assign structure I to the adduct since enol phosphates are known to be unstable in aqueous solution.[7] However, this structure is the only one consistent with the analytical data, the ultraviolet (uv) maximum at 225 nm,[8] and the infrared (ir) absorption bands at 2240 (C≡N) and 1660 cm^{-1} (C=C).

$$HC{\equiv}CCN + HPO_4^{2-} \longrightarrow {}^{2-}O_3POCH{=}CHCN$$

The nuclear magnetic resonance (nmr) spectrum of CVP in D_2O exhibits a quartet at δ 4.73 ($J = \sim 5$,[9] 1.3 Hz) and 7.33 ($J = 7.8$, 6.2 Hz). The signal at δ 7.33 collapses to a doublet ($J = 7.7$ Hz) when the center of the quartet at 4.73 is irradiated. From these data and the nmr spectra of related compounds[10] we assigned the δ 4.73 resonance to H-2 with a 1.3-Hz coupling to phosphorus. The H-3 proton is at δ 7.33 with a 6.2-Hz coupling to H-2 and a 7.7-Hz coupling to phosphorus.

This cis geometry was assigned to the double bond on the basis of the magnitude of the H-2,H-3 coupling constant. The cis-coupling constants of vinyl phosphates are 4.1–5.8 Hz and the trans-coupling constants are 11.1–13.2 Hz.[10] The observation of the *cis*-olefin is in agreement with the general rule that nucleophilic

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(1) For the previous paper in this series see J. P. Ferris and J. E. Kuder, *J. Amer. Chem. Soc.*, **92**, 2527 (1970). Supported by Grant No. GP 8254 from the National Science Foundation.

(2) (a) USPHS Research Career Development Awardee (GM 6380) of the National Institute of General Medical Sciences; to whom correspondence should be addressed; (b) NSF Summer Undergraduate Research Participant, 1969; (c) NSF Summer Undergraduate Research Participant, 1968.

(3) R. A. Sanchez, J. P. Ferris, and L. E. Orgel, *Science*, **154**, 784 (1966).

(4) J. P. Ferris, R. A. Sanchez, and L. E. Orgel, *J. Mol. Biol.*, **33**, 693 (1968).

(3) J. P. Ferris, Abstracts, 156th National Meeting of the American Chemical Society, Atlantic City, N. J., Sept 1968, p ORG-73.

(6) J. P. Ferris, *Science*, **161**, 53 (1968).

(7) F. Cramer and T. Hata, *Justus Liebigs Ann. Chem.*, **692**, 22 (1966).

(8) E. Y. Spencer, Sir A. Todd, and R. F. Webb, *J. Chem. Soc.*, 2968 (1958).

(9) It is difficult to evalute this coupling because of the overlap of this quartet with the signal from HOD.

(10) W. Brügel, T. Ankel, and F. Krückeberg, *Z. Electrochem.*, **64**, 1121 (1960); A. R. Stiles, C. A. Reilly, G. R. Pollard, C. H. Tieman, L. F. Ward, Jr., D. D. Phillips, S. B. Soloway, and R. R. Whetstone, *J. Org. Chem.*, **26**, 3960 (1961).

additions to acetylenes usually go trans to form cis products.[11] Previously we observed trans addition of the anion of cyanoacetaldehyde to cyanoacetylene to give *cis,cis*-dicyanovinyl ether (II).[3] The nmr spectrum of this compound in dimethyl sulfoxide-d_6 is a quartet (δ 5.25 and 7.57) with the characteristic cis-coupling constant of 6.3 Hz.[12]

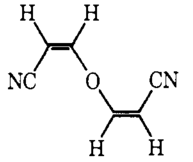

II

Table I. Kinetics of the Addition of Orthophosphate to $HC\equiv CCN$

	Phos-phate, *M*	pH (25 ± 3°)	Reaction temp, °C	Corrected pH at reaction temp	$k \times 10^3$, min^{-1}
1	0.50	6.40	59.0 ± 0.1	6.37	47.5
2	0.50	6.40	59.0 ± 0.1	6.37	46.5
3	0.50	6.70	59.0 ± 0.1	6.68	57.4
4	0.50	6.70	59.0 ± 0.1	6.68	57.4
5	0.50	7.70	59.0 ± 0.1	7.67	98.0
6	0.50	7.70	59.0 ± 0.1	7.67	97.0
7	0.10	6.71	60	6.68	11
8	0.05	6.80	59.0 ± 0.1	6.76	6.74
9	0.05	6.80	59.0 ± 0.1	6.76	6.98
10	0.05	7.80	59.0 ± 0.1	7.77	11.5
11	0.05	7.80	59.0 ± 0.1	7.77	11.4
12	0.50	6.74	40.9 ± 0.1	6.70	8.1
13	0.50	6.74	40.9 ± 0.1	6.70	8.6
14	0.50	6.74	40.9 ± 0.1	6.70	8.3
15	0.50	7.73	40.9 ± 0.1	7.69	13.6
16	0.50	7.73	40.9 ± 0.1	7.69	13.1
17	0.10	6.71	40	6.67	1.4

We observed that in aqueous solution uridine is converted to uridine monophosphate (UMP) and orthophosphate is converted to pyrophosphate by CVP.[6] However, uridine is phosphorylated at the same rate as water suggesting that little or no UMP formed on the primitive earth by the route. The observation that the phosphorylation of orthophosphate was 4.2–9.4 times as fast as the phosphorylation of water[6] encouraged us to investigate further the synthesis and reactions of CVP.

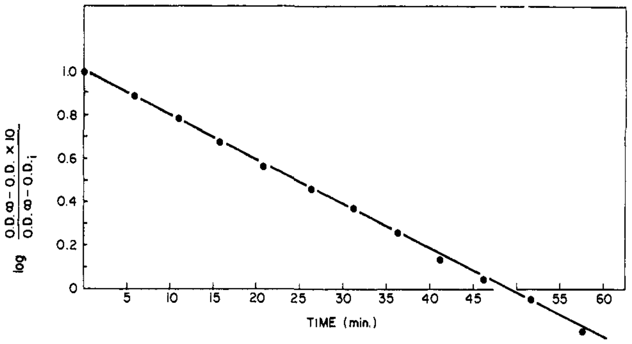


Figure 1. The addition of 0.5 *M* orthophosphate to HC═CCN at pH 6.40 and 59.0 ± 0.1°

Kinetics of Phosphate and Hydroxide Addition to Cyanoacetylene

The rate of addition of 0.10–0.5 *M* phosphate to 10^{-4} *M* cyanoacetylene was measured in water between pH 6.4 and 7.8 by the rate of formation of the 225-nm maximum of CVP. At higher pH or lower phosphate concentrations the hydrolysis of cyanoacetylene to cyanoacetaldehyde is a competing reaction. Pseudo-first-order kinetics were observed (Table I, Figure 1) and from these data the second-order rate constants and the activation parameters for HPO_4^{2-} and $H_2PO_4^-$ addition were calculated (Table II).

It had been established previously that the rate of hydrolysis of cyanoacetylene to cyanoacetaldehyde is first order in both hydroxide and cyanoacetylene.[4] These studies were extended to higher temperatures (Table III) and from these data the activation parameters and the second-order rate constants were calculated (Table II).

The activation parameters for the addition of mono- and dihydrogen phosphate and hydroxide to cyanoacetylene are very similar (Table II), a result which suggests that reaction mechanism is the same in each case. One distinguishing characteristic of these reactions is that the entropy of activation is essentially zero. This would suggest that bond formation is

Table II. Rate Constants and Activation Parameters for the Nucleophilic Addition to Cyanoacetylene[a]

	k_2, l. mol^{-1} min^{-1}				
Addend	59.0°	40.9°	0°[b]	Δ*H*, kcal	Δ*S*, eu
HPO_4^{2-}	$(2.6 \pm 0.6) \times 10^{-1}$	$(3.5 \pm 0.5) \times 10^{-2}$	1.6×10^{-4}	22.0	−4.0
$H_2PO_4^-$	$(7.4 \pm 0.5) \times 10^{-2}$	$(8.2 \pm 1.2) \times 10^{-3}$	1.5×10^{-4}	24.6	+1.8
OH^-	$(8.88 \pm 0.26) \times 10^{1}$[c]	$(1.82 \pm 0.30) \times 10^{1}$[d]	1.4×10^{-1}	19.0	−0.6

[a] Error limits at the 95% confidence level. [b] Value obtained by extrapolation from an Arrhenius plot. [c] At 59.4°. [d] At 40.4°.

Table III. Kinetics of the Addition of 10^{-2} *M* Hydroxide to 10^{-4} *M* $HC\equiv CCN$

Run no.	Temp, °C	$k \times 10^2$, min^{-1}
1	59.4 ± 0.1	87.6
2	59.4 ± 0.1	91.3
3	59.4 ± 0.1	88.2
4	59.4 ± 0.1	88.2
5	40.4 ± 0.1	18.9
6	40.4 ± 0.1	18.7
7	40.4 ± 0.1	16.9
8[a]	30	5

[a] Reference 4.

(11) W. E. Truce, H. G. Klein, and R. B. Kruse, *J. Amer. Chem. Soc.*, **83**, 4636 (1961).

(12) The same conclusions concerning the structure of dicyanovinyl ether were reached independently by T. Sasaki and K. Shoji, *Tetrahedron Lett.*, 1811 (1969). The nmr spectrum of cyanoacetaldehyde in dimethyl sulfoxide-d_6 shows both cis (δ 7.91, 3.37, J = 4.7 Hz) and trans (δ 8.21, 3.54, J = 11.7 Hz) coupling. The cis isomer must predominate in aqueous solution to account for the formation of only the *cis,cis*-dicyanovinyl ether. A similar solvent effect has already been noted for β-aminocrotononitrile.[1]

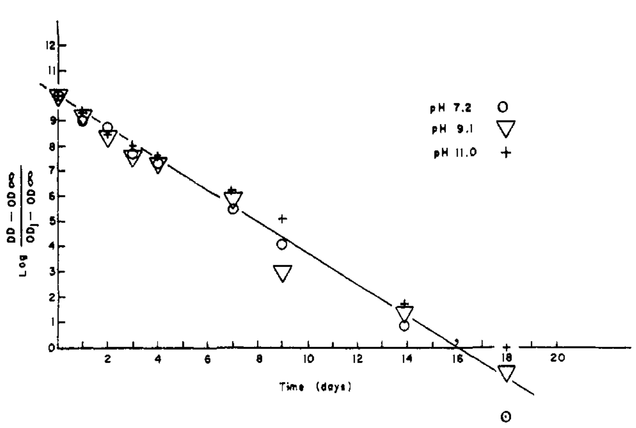
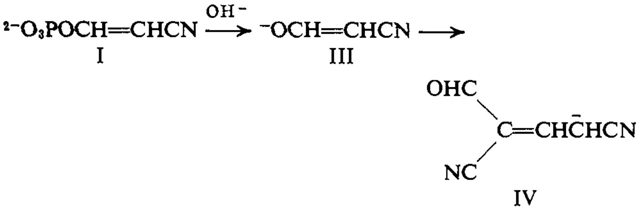


Figure 2. Hydrolysis of cyanovinyl phosphate at 60°.

essentially complete in the transition state of the addition reaction.[13] An alternative explanation is that the entropy change due to the orientation of the inorganic anion and cyanoacetylene for reaction is balanced by the entropy increase resulting from desolvation of the transition state before reaction can take place.

Solvolysis of β-Cyanovinyl Phosphate

Our preliminary observation that CVP is much more resistant to hydrolysis than phosdrin[8] and other enol phosphates[7] prompted a detailed investigation of the mechanism of the hydrolysis of this compound.[6] The product of the hydrolysis is cyanoacetaldehyde (III) which was identified by its uv maximum at 248 nm. The subsequent aldol condensation of cyanoacetaldehyde yields compound IV (λ_{max} 310 nm).[4]



$$\underset{\text{I}}{{}^{2-}O_3POCH{=}CHCN} \xrightarrow{OH^-} \underset{\text{III}}{{}^-OCH{=}CHCN} \longrightarrow$$

IV

The rate of hydrolysis of CVP, as measured by the disappearance of the uv maximum at 225 nm, is first order and pH independent from pH 6 to 11 (Figure 2). The rate of hydrolysis increases gradually as the pH is lowered from 6 to 1.3 (Table IV). This small pH effect parallels the titration of the phosphate grouping of CVP which has a pK_2 = 4.6.

Phosphate hydrolysis reactions either involve a phosphorus–oxygen cleavage with the formation of metaphosphate or carbon–oxygen fission to yield orthophosphate. The solvolysis of CVP was performed in ethanol to distinguish between these two possibilities. Phosphorus–oxygen fission of CVP would give cyanoacetaldehyde while carbon–oxygen fission would yield β-ethoxyacrylonitrile (V). That the solvolysis of CVP proceeded by phosphorus–oxygen fission was established by the quantitative solvolysis of CVP to cyanoacetaldehyde (Table V). The only other product observed was compound IV which undoubtedly formed by the subsequent aldolization of cyanoacetaldehyde. The possibility that β-ethoxyacrylonitrile formed first and then hydrolyzed to cyanoacetaldehyde was eliminated by the observation that β-ethoxyacrylonitrile is stable under the solvolysis conditions.

Table IV. Kinetics of the Hydrolysis of Barium Cyanovinyl Phosphate[a]

Run no.	pH	Buffer	Ionic strength	Temp, °C	$k \times 10^3$, min^{-1}
1	7.0	None		80	1.20
2	6.8	None		80	1.28
3	10.7	pHydrion		80	1.29
4	10.7	pHydrion		80	1.28
5	9.0	pHydrion		80	1.32
6	9.0	pHydrion		80	1.25
7	8.9	pHydrion		80	1.22[b]
8	7.98	0.02 *M* phosphate	0.06	80.6	1.01
9	7.98	0.02 *M* phosphate	0.06	80.6	1.01
10	7.0	pHydrion		80	1.35
11	7.0	pHydrion		80	1.29
12	6.9	pHydrion		80	1.28[b]
13	7.0	pHydrion		80.6	1.10
14	7.0	pHydrion		80.6	1.15
15	7.5	pHydrion		80	0.98[c]
16	7.5	pHydrion		80	0.93[c]
17	5.93	0.02 *M* phosphate	0.06	80.6	1.56
18	5.93	0.02 *M* phosphate	0.06	80.6	1.56
19	5.3	pHydrion		80.6	2.07
20	5.3	pHydrion		80.6	2.11
21	5.2	pHydrion		80	2.12
22	3.89	KOH–HCOOH	0.013	80.6	2.76
23	3.89	KOH–HCOOH	0.013	80.6	2.84
24	1.41	0.04 *M* HCl	0.04	80.6	4.04
25	1.41	0.04 *M* HCl	0.04	80.6	4.76
26	1.30	0.05 *M* HCl	0.05	80	4.02
27	1.30	0.05 *M* HCl	0.05	80	4.84
28	1.30	0.05 *M* HCl	0.05	80	4.35
29	6.87	0.02 *M* $NaClO_4$	0.02	80.6	1.20
30	6.78	0.2 *M* $NaClO_4$	0.2	80.6	1.20
31	6.76	0.2 *M* $NaClO_4$	0.2	80.6	1.24
32	6.48	2 *M* $NaClO_4$	2	80.6	2.10
33	6.52	2 *M* $NaClO_4$	2	80.6	2.20
34	1.50	$NaClO_4$	~1.5	80.6	13[d]
35	6.90	0.01 *M* phosphate	0.02	80	1.32
36	6.80	0.1 *M* phosphate	0.2	80	1.28
37	6.57	1.0 *M* phosphate	2.0	80	2.20
38	6.72	0.08 *M* $Mg(ClO_4)_2$	0.2	80	1.42
39	6.40	0.08 *M* $Mg(ClO_4)_3$	0.2	80.6	1.56
40	6.89	0.8 *M* $Mg(ClO_4)_2$	2	80.6	1.84
41	7.56	0.8 *M* $Mg(ClO_4)_2$	2	80.6	1.95
42	1.52	0.13 *M* $Mn(ClO_4)_2$	0.32	80.6	2.48
43	1.52	0.13 *M* $Mn(ClO_4)_2$	0.32	80.6	2.52
44	1.42	0.37 *M* $Mn(ClO_4)_2$	0.93	80.6	3.22
45	1.44	0.37 *M* $Mn(ClO_4)_2$	0.93	80.6	3.58
46	11.0	pHydrion		100	10.7
47	9.0	pHydrion		100	10.0
48	7.2	pHydrion		100	9.5
49	11.0	pHydrion		60	0.10
50	9.1	pHydrion		60	0.099
51	7.2	pHydrion		60	0.093

[a] For reaction conditions see Experimental Section. [b] Sodium cyanovinyl phosphate. [c] D_2O solvent. [d] pH adjusted by addition of $HClO_4$.

Table V. Ethanolysis of Triethylammonium Cyanovinyl Phosphate

	Run 1	Run 2
Reaction time, hr	46	78
Cyanovinyl phosphate, %	34.5	22
Cyanoacetaldehyde, %	70.5	73
IV, %	Trace	10
Total products, %	105	105
k, min^{-1}	0.35×10^{-3}	0.35×10^{-3}

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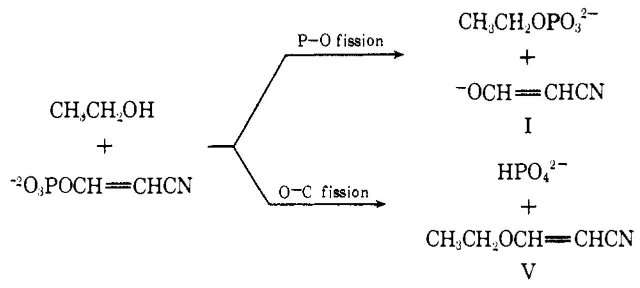
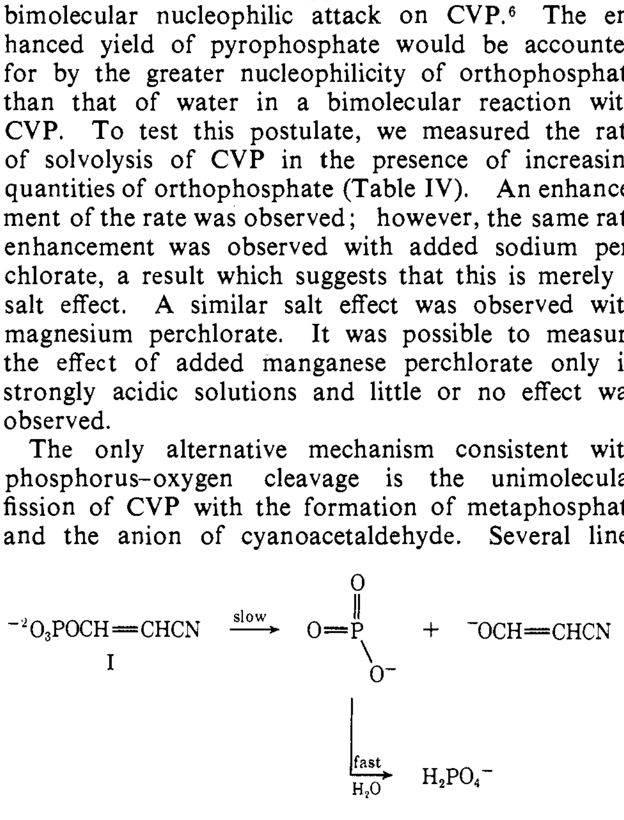
(13) A low entropy of activation was observed in the bimolecular solvolysis of ethylene oxide. L. L. Schaleger and F. A. Long, *Advan. Phys. Org. Chem.*, 1, 1 (1963).



Having established that the solvolysis proceeds by phosphorus–oxygen fission, we then set out to determine the mechanism of the cleavage. The observation that phosphate is more effectively phosphorylated than water with CVP suggested the possibility of a bimolecular nucleophilic attack on CVP.[6] The enhanced yield of pyrophosphate would be accounted for by the greater nucleophilicity of orthophosphate than that of water in a bimolecular reaction with CVP. To test this postulate, we measured the rate of solvolysis of CVP in the presence of increasing quantities of orthophosphate (Table IV). An enhancement of the rate was observed; however, the same rate enhancement was observed with added sodium perchlorate, a result which suggests that this is merely a salt effect. A similar salt effect was observed with magnesium perchlorate. It was possible to measure the effect of added manganese perchlorate only in strongly acidic solutions and little or no effect was observed.

The only alternative mechanism consistent with phosphorus–oxygen cleavage is the unimolecular fission of CVP with the formation of metaphosphate and the anion of cyanoacetaldehyde. Several lines of evidence are consistent with this mechanism. First the pH independence of the rate of hydrolysis between pH 6 and 11 suggests a unimolecular reaction. Second, we observed a small solvent isotope effect ($k_{H_2O}/k_{D_2O} = 1.3$) when the solvolysis was performed in D_2O. Similar isotope effects have been observed in the unimolecular solvolyses of acyl phosphates,[14a] acid chlorides, and sulfonyl chlorides.[14b] Third, the entropy of activation for the solvation of the dianion is essentially zero (−0.6 eu) suggesting that there is no organization of solvent molecules in the transition state. Furthermore, this value is in agreement with $\Delta S^{\ddagger}$ values observed for the unimolecular solvolyses of acyl phosphates and phenyl phosphates.[14a,15] By comparison, the bimolecular solvolysis of acetyl phenyl phosphate has a $\Delta S^{\ddagger} = -28.8$ eu.[15a]



A linear relationship between the log k and pK_a of the leaving group has been observed for the unimolecular solvolysis of the dianions of acyl and phenyl phosphates. From the slope of the line it has been concluded that the rate of solvolysis is strongly dependent on the nature of the leaving group, a conclusion which suggests that bond breaking is well advanced in the transition state.[16] Our results are in qualitative agreement with this interpretation. Cyanoacetaldehyde is a relatively weak acid, pK_a = 8.05,[17] so that the rate of solvolysis of CVP is correspondingly slow. However, from the pK_a of cyanoacetaldehyde, one would predict a rate constant of 5×10^{-8} min^{-1} for the solvolysis of CVP at 39°.[16] An extrapolated rate constant of 6×10^{-6} min^{-1} was obtained from the Arrhenius plot of our data for CVP. An explanation of this discrepancy is not immediately apparent.

Prebiological Phosphorylation

A number of pathways have been proposed for the conversion of phosphate to pyrophosphate on the primitive earth.[18] Cyanoformamide and cyanogen are effective in aqueous solution; however, the rate of phosphorylation is the same as the rate of hydrolysis of the reagent.[19,20] Cyanoguanidine has been proposed as a condensing agent;[21] however, the yields are only 0.1% using 10^{-2}–10^{-3} M phosphate and cyanoguanidine.[22] Cyanate is effective on an apatite surface;[23] however, it has been pointed out that apatite catalyzes the hydrolysis of nucleoside phosphates.[24] Another approach to prebiotic phosphorylation is the synthesis of higher phosphates by heating phosphoric acid or monobasic phosphates under anhydrous conditions.[25–28] The polymeric phosphates so produced serve as phosphorylating agents after dissolution in water.[26] However, little or no phosphorylation was observed when dibasic and tribasic phosphates were heated.[27,28] It is difficult to account for the formation of monobasic phosphates if the pH of the primitive ocean was in the 8–9 range. One possible alternative is the synthesis of ammonium hydrogen phosphate salts which on heating lose ammonia to form dihydrogen phosphates.[26]

It should be possible to evaluate the possible significance of CVP as a prebiotic phosphorylating agent by determining the rates of potential phosphorylation reactions, the rate of synthesis of CVP, and the rates of competing reactions. The absolute rate of phosphorylation by CVP was not measured; however, the

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[14] (a) D. R. Phillips and T. H. Fife, *J. Org. Chem.*, **34**, 2710 (1969); (b) C. A. Bunton and V. J. Shiner, Jr., *J. Amer. Chem. Soc.*, **83**, 3207 (1961).

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[17] The pK_a of cyanoacetaldehyde has been found to be 8.05 and not 8.4 as previously reported[4] (unpublished results of Mr. O. Zamek).

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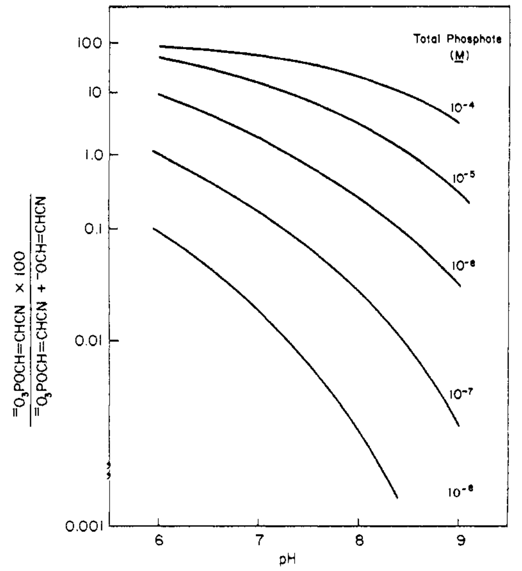

Figure 3. Yield (%) of cyanovinyl phosphate as a function of pH and orthophosphate concentration.

relative rates of phosphorylation of phosphate *vs.* water were measured and found to be in the range 4.5–9.2:1.[6]

The proportion of CVP produced on the primitive earth would depend on the relative rates of reaction of cyanoacetylene with orthophosphate as compared with the rates of competing reactions. As a first approximation, we assumed that addition of hydroxide ion to cyanoacetylene would be the only other competing reaction. The family of curves in Figure 3 was calculated from the rate constants in Table III and the pK_a of dihydrogen orthophosphate. Since the hydrolysis of CVP is 10^3–10^4 times slower than the rate of addition of hydroxide and phosphate to cyanoacetylene the rate of hydrolysis was not included in these calculations.

The present concentration of phosphate in the ocean is regulated at approximately 10^{-6} *M* by equilibration with insoluble minerals. Some variation occurs due to its consumption by plankton and other microorganisms.[29] If the phosphate concentration in the primitive ocean was determined by the equilibrium with the mineral apatite then the concentration did not exceed 3×10^{-6} *M*.[23] If we assume a concentration of phosphate of 10^{-5}–10^{-6} *M*, then, as shown in Figure 3, 1–14% of the cyanoacetylene is converted to CVP. This is an appreciable amount and one could envisage that considerable CVP would build up in the primitive ocean over a period of 10^6–10^9 years. However, at low phosphate concentrations very little pyrophosphate would be produced from this CVP because of the relatively indiscriminate nature of CVP as a phosphorylating agent. For example, assuming a selectivity of phosphate over water of 5.5, then only 10^{-4}% yield of pyrophosphate would be obtained from 10^{-5} *M* orthophosphate. It is obvious from these data that significant yields of pyrophosphate would have been obtained only if the concentration of phosphate was greater than 10^{-3} *M*.

It is conceivable that the selectivity of the phosphorylation reaction was improved by metal or surface catalysis. We found that the yield of pyrophosphate was not improved if the reaction was carried out in the presence of apatite.[6,23] Furthermore, we observed that the rate of hydrolysis of CVP was not enhanced in the presence of added manganese or magnesium ion. Finally, we observed that the sodium and barium salts of CVP hydrolyzed at the same rates (Table IV, runs 7 and 12). Therefore, barium ion has no effect on the reaction. Consequently, in the absence of any logical reason for postulating oceans with a phosphate concentration greater than 10^{-3} *M* or any mechanism for the selective reaction of orthophosphate with CVP, we are forced to conclude that very little pyrophosphate was formed on the primitive earth by the reaction of orthophosphate with CVP.

Experimental Section[30]

Cyanovinyl Phosphate. A mixture of cyanoacetylene[31] (0.51 g, 0.01 mol) and 100 ml of 1 *M* Na_2HPO_4 was heated at 60° and the course of the reaction was followed by absorption maxima of the product at 225 nm. At the end of 1 hr a maximal absorbance of 1.35 was reached (1000-fold dilution). The reaction mixture was then diluted to 400 ml and to this was added 100 ml of 1.5 *M* barium acetate. The precipitated barium phosphate was filtered and 350 ml of ethanol was added. The product (1.1 g, 35%) precipitated and was removed by filtration. An analytical sample was prepared by dissolution in water and precipitation with ethanol: uv λ_{max} 225 nm (14,000); ir (cm^{-1}) 3600, 3100, 3000 (broad), 2235, 1650, 1265, 1175, 1125, 1000, 970, 960, 850, 815, 740, 670.

Anal. Calcd for $C_3H_2NO_4PBa \cdot H_2O$: C, 11.91; H, 1.33; N, 4.63; P, 10.21. Found: C, 11.67; H, 1.55; N, 4.97; P, 10.69.

The cyclohexylamine salt was prepared by slurrying the barium salt with 1 g of Dowex 50 (H^+) in 2 ml of H_2O. The resulting solution was decolorized with Norit, filtered, and then passed through a column of 1.5 g of Dowex 50 (H^+). The column was washed with water until no further cyanovinyl phosphate was eluted as shown by the absence of uv absorption in the 225-nm region. The elutants were titrated to pH 7.8 with cyclohexylamine and then freeze dried to a solid which was purified by precipitation from methanol by addition of ethyl acetate: mp 186°; ir (cm^{-1}) broad absorption with fine structure 3400–2550, 2220, 1625, 1550, 1480, 1460, 1400, 1385, 1265, 1200, 1125 (broad), 1050, 985, 965, 825, 730, 635.

Anal. Calcd for $C_{15}H_{30}N_3O_4P$: C, 51.86; H, 8.71; N, 12.10; P, 8.92. Found: C, 51.88; H, 8.81; N, 12.15; P, 8.58.

The pyridine and triethylamine salts were prepared in the same way as the cyclohexylamine salt. These derivatives did not crystallize; however, it was possible to characterize these compounds as CVP salts by a uv absorption maximum at 225 nm.

Sodium cyanovinyl phosphate was prepared by adding Na_2SO_4 (142 mg, 1 mmol) to a suspension of barium cyanovinyl phosphate (350 mg, 0.89 mmol). After thorough mixing, the precipitated barium sulfate was precipitated by centrifugation and the aqueous solution containing the sodium salt was decanted from the precipitate. The precipitate of barium sulfate was washed with 1 ml of water and centrifuged, and the combined aqueous layers were freeze dried to yield sodium cyanovinyl phosphate; λ_{max} 225 nm. The ammonium salt was prepared by a similar procedure using $(NH_4)_2SO_4$.

The acid dissociation constants of CVP were measured by titration of the acid form with base and by titration of the disodium salt with acid using a Radiometer Model 26 pH meter that was standardized at pH 6.86 and 4.01. A 10^{-2} *M* solution of the acid form of CVP was eluted from a Dowex 50 (H^+) ion exchange resin and

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[29] H. U. Sverdrup, "Oceans, Their Physics, Chemistry and General Biology," Prentice-Hall, Englewood Cliffs, N. J., 1942, pp 257–260.

[30] Nmr spectra were measured on a Varian HR-60 or A-60 spectrometer, uv spectra were measured in water on a Unicam SP 800 spectrophotometer, and ir spectra were measured in KBr on a Perkin-Elmer 237B spectrophotometer. Tetramethylsilane was used as internal standard for nmr spectra determined in dimethyl sulfoxide-d_6 and sodium 2,2-dimethyl-2-silapentanesulfonate was used for those spectra determined in D_2O.

[31] S. Murahashi, T. Takizawa, S. Kurioka, and S. Maekawa, *Nippon Kagaku Zasshi*, 77, 1689 (1956); *Chem. Abstr.*, 53, 5163 (1959).

titrated immediately with 0.0110 N NaOH. Values of pK_1 of 2.4, 2.4 and pK_2 of 4.60, 4.55 were obtained from duplicate runs. As a check on these values the 0.025 M solutions of disodium salt of CVP were titrated with 0.144 N HCl. Values of pK_1 of 2.0, 2.0 and pK_2 of 4.66, 4.43 were obtained from duplicate determinations. From these data it is possible to assign the value of pK_2 as 4.6 ± 0.1 with certainty.[32]

The nmr spectrum was measured on a sample of the sodium salt which had been prepared in D_2O solution. The sample was twice dissolved in D_2O and freeze dried to remove as much of the H_2O and HDO as possible before determining the nmr spectrum. The relative intensity of the vinyl protons did not change after standing in D_2O solution for 7 months, a result which indicated the absence of hydrogen–deuterium exchange. The ultraviolet spectrum after 7 months indicated a 12% conversion to IV. In a double resonance experiment irradiation at 155-Hz upfield from the quartet at δ 7.33 resulted in its collapse to a doublet. Little effect was observed at δ 7.33 when the irradiating frequency was 154- or 156-Hz upfield.

Kinetic Measurements. Preliminary kinetic measurements were carried out in a covered Thelco Model 83 Sero-Utility bath with temperature control to ±1°. More precise measurements were carried out in a thermostated uv cell or the thermostated cell holder of the Unicam SP-800 spectrophotometer. Temperature control to ±0.1° was maintained with a Haake Model FS constant temperature circulator. The temperature in the cuvette was measured with an iron–constantan thermocouple.

The rate constants at 0° were obtained by extrapolation of the Arrhenius plot (log K *vs.* $1/T$). The activation parameters were obtained from the expression[35]

$$\ln k/T = -\Delta H^{\ddagger}/RT + \ln K/h + \Delta S^{\ddagger}/R$$

$-\Delta H^{\ddagger}/R$ was obtained from the slope of the plot of $\ln k/T$ *vs.* $1/T$. Substitution in the original expression gave $\Delta S^{\ddagger}$.

Kinetics of the Synthesis of Cyanovinyl Phosphate. Phosphate solutions were prepared from Fisher Reagent grade sodium phosphate salts in distilled water. The phosphate solutions (1.5–2.5 ml) were brought to constant temperature in a thermostated uv cell and 15–25 μl of 10^{-2} M cyanoacetylene was added to give a final concentration of 10^{-4} M. The spectrum was monitored periodically at 225 nm. Pseudo-first-order rate constants were obtained from the slope of a plot of $\log (OD_\infty - OD)/(OD_\infty - OD_i)$ *vs.* time and are given in Table I and Figure 1. Average values of the pseudo-first-order rate constants were used in subsequent calculations.

The concentrations of HPO_4^{2-} and $H_2PO_4^-$ were calculated from the pH of the solutions and the relationship $pH = pK_a + \log [HPO_4^{2-}]/[H_2PO_4^-]$. The p$K_a$ of orthophosphate was taken as 7.20 and the pH of the phosphate buffers measured at room temperature was corrected for the reaction temperature using the data of Bates and Acree.[36]

The pseudo-first-order rate constant k is equal to the second-order rate constants for HPO_4^{2-} (k_1) and $H_2PO_4^-$ (k_2) by the relationship $k = k_1[HPO_4^{2-}] + k_2[H_2PO_4^-]$. The slope of the linear plot of $k/[HPO_4^{2-}]$ *vs.* $[H_2PO_4^-]/[HPO_4^{2-}]$ gave k_2. Substitution of k_2 in the original expression gave k_1. Values of k_1 and k_2 were obtained by the method of least squares and the activation parameters given in Table II.

Kinetics of the Addition of 10^{-2} M Hydroxide to $HC{\equiv}CCN$. The kinetics for the addition of 10^{-2} M hydroxide ion to cyanoacetylene were measured spectrophotometrically by the increase in absorption due to the cyanoacetaldehyde anion at 248 nm. The results are given in Table III.

The second-order rate constants and activation parameters are given in Table II.

Hydrolysis of Cyanovinyl Phosphate. The hydrolysis of CVP was investigated in the pH range 1.31–11. The hydrolysis products are cyanoacetaldehyde (III) and the aldol condensation product of cyanoacetaldehyde (IV). For example, at pH 7 and 100° a 57% yield of IV was obtained as measured by its characteristic ultraviolet absorption at 310 nm.[4] The presence of cyanoacetaldehyde anion was observed, when the pH of the reaction mixture exceeded 8.05 (the pK_a of cyanoacetaldehyde[16]), by its ultraviolet absorption at 248 nm.[4] This absorption was quenched by adjusting to pH 7.[6] The yield of cyanoacetaldehyde was not measured because it was slowly converted to IV and because its uv absorption overlapped with that of CVP.

The kinetics of the hydrolysis of CVP were measured by following the disappearance of its absorption maximum at 225 nm and the rate constants are given in Table IV. Representative kinetic data are given in Figure 2. If the kinetics were measured at a pH above 8 the pH was adjusted to 6–7 with 2 M NaH_2PO_4 before measuring the ultraviolet absorption at 225 nm. This was necessary to quench the uv absorption of the cyanoacetaldehyde produced by hydrolysis. Buffer solutions were prepared as described in Table IV or else commercial buffer capsules (pHydrion, Micro Essential Laboratory, Brooklyn, N. Y.) at one-tenth the prescribed dilution [1 capsule (which is approximately 1 g of solid buffer) was diluted to 1 l. with H_2O].

An Arhennius plot of the rate constants in the pH 7–10 range extrapolated to a value of 4.4×10^{-8} min^{-1} at 0°. The half-life of CVP at 0° and pH 7–10 is 30 years; $\Delta H^{\ddagger} = 28.0$ kcal and $\Delta S^{\ddagger} = -0.6$ eu.

Ethanolysis of Triethylammonium Cyanovinyl Phosphate. Triethylammonium cyanovinyl phosphate (10^{-4} M in ethanol) was heated at the reflux temperature of ethanol and the rate of solvolysis was measured by the decrease in absorption at 225 nm. The yield of IV was determined from the intensity of the absorption at 310 nm and the yield of III was determined from the intensity of the absorption at 248 nm, after the solution was made basic. The results of duplicate runs are given in Table V. First-order kinetics were observed; however, the values for the first-order rate constant are approximate because temperature control was maintained only by the reflux temperature of ethanol. The solvolysis rate in ethanol is one-third that in water at 80°. This differential appears to be significant and cannot be due to only the 2° temperature difference between the temperature of refluxing ethanol (78°) and 80°. This difference is not due to some anomalous properties of the triethylammonium salt since the latter exhibited the usual first-order kinetics at 80° in aqueous solution ($k = 1.1 \times 10^{-3}$ min^{-1}). It is not possible to draw any conclusions as to the source of this rate difference until more precise kinetic data in nonaqueous solvents are available.[37]

One other possible reaction course is the conversion of cyanovinyl phosphate to β-ethoxyacrylonitrile which in turn undergoes hydrolysis to cyanoacetaldehyde. However, the intensity of the uv absorption (λ_{max} 227 nm, ϵ 16,000) of β-ethoxyacrylonitrile did not decrease after refluxing for 72 hr in ethanol.[38]

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[32] Nitrophenyl phosphates[33] and acylphosphates[34] have pK_1 values of 1–1.5 and pK_2 values of 4.5–5.2. The pK_2 of CVP is in good agreement with the pK_2 of these compounds; however, the pK_1 value is somewhat high. Further investigation is needed before the pK_1 of CVP can be assigned with certainty.

[33] K. A. Holbrock and L. Ovellet, *Can. J. Chem.*, **36**, 686 (1958); A. Desjobert, *Bull. Soc. Chim. Fr.*, 683 (1963); C. A. Bunton, E. J. Fendler, and J. H. Fendler, *J. Amer. Chem. Soc.*, **89**, 1221 (1967).

[34] F. Lipmann and L. C. Tuttle, *Arch. Biochem. Biophys.*, **13**, 373 (1947); D. P. Phillips and T. H. Fife, *J. Org. Chem.*, **34**, 2710 (1969).

[35] F. W. Cagle, Jr., and H. Eyring, *J. Amer. Chem. Soc.*, **73**, 5628 (1951).

[36] R. G. Bates and S. F. Acree, *J. Res. Nat. Bur. Stand.*, **30**, 129 (1943).

[37] The effects of alcohols on the rate of solvolysis of enol phosphates has been investigated in some detail.[15c]

[38] An authentic sample of β-ethoxyacrylonitrile was provided by Dr. G. Kesslin, Kay-Fries Chemicals,