to cyanoacetaldehyde and that in turn condenses to compound VII in aqueous solution (*1, 10*), this photochemical reaction suggests an efficient pathway for the prebiotic synthesis of a potential nicotinamide-like hydrogen transfer system from cyanoacetylene (*11*).

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12. Sponsored by NIH grant GM 15915. J.P.F. is a PHS career development awardee (GM 6380) of the National Institute of General Medical Sciences.

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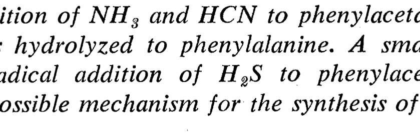
Phenylalanine and Tyrosine Synthesis under Primitive Earth Conditions

Abstract. *Phenylacetylene can be synthesized in substantial yields from various hydrocarbons by high temperatures, electric discharges, and ultraviolet light. Phenylacetylene is hydrated to phenylacetaldehyde by way of both nucleophilic and radical additions of H_2S followed by hydrolysis of the thioaldehyde. The addition of NH_3 and HCN to phenylacetaldehyde yields phenylalanine nitrile which is hydrolyzed to phenylalanine. A small yield of tyrosine is obtained from the radical addition of H_2S to phenylacetylene. This sequence of reactions is a possible mechanism for the synthesis of these amino acids on the primitive earth.*

A wide variety of processes have been used for synthesizing amino acids under primitive earth conditions (*1*), but the synthesis of the aromatic amino acids phenylalanine and tyrosine has not been demonstrated (*2*). The lack of phenylalanine and tyrosine in such experiments combined with the apparent difficulty of synthesizing them results in their absence from some lists of "primitive" amino acids (*3*).

We propose that phenylalanine was synthesized on the primitive earth from phenylacetylene, followed by hydration to phenylacetaldehyde and a Strecker synthesis with the HCN and NH_3, which are generally assumed to have been present in the primitive oceans.



Phenylacetylene can be synthesized from a variety of mixtures of simple hydrocarbons by high temperatures, electric discharges, and ultraviolet light (*4*) (Table 1). Phenylacetylene was identified by its gas chromatography retention time (*5*) and by ultraviolet and infrared spectra. Benzene, styrene, and naphthalene are also synthesized in most of these experiments. Success in these experiments is dependent on removing the products from the region of the hot wire or electric discharge by cooling the walls of the flask to approximately −20°C (*6*). This is analogous to reactions in the atmosphere where the phenylacetylene would have time to diffuse to the ocean before being broken up by various energy sources in the atmosphere. The yields of phenylacetylene in these experiments are 0.001 to 5 percent. The rate of production of phenylacetylene in the primitive atmosphere is difficult to estimate, but the phenylacetylene yields in these experiments suggest that the rate could be substantial.

The hydration of phenylacetylene is difficult to achieve under primitive earth conditions. The hydration can take place in two ways, electrophilic (acid catalyzed) to give acetophenone (*7*) and nucleophilic (base catalyzed) to give phenylacetaldehyde (*8*). A Strecker synthesis with acetophenone gives α-amino-α-phenylpropionic acid, a compound which is presumed to have played no role in the origin of life. Heating phenylacetylene in aqueous solutions buffered between *p*H 7 and 10 results in very small yields of phenylacetaldehyde and acetophenone (*9*). The hydration products were detected by converting them to the amino acids with NH_3 and HCN, followed by hydrolysis. The mixture was desalted, and the amino acids were analyzed on the Beckman-Spinco amino acid analyzer (*10*) and by paper chromatography. Heating aqueous ammonia solutions buffered between *p*H 7 and 10 also gives only small yields of phenylacetaldehyde (half life, approximately 500 years at 100°C and *p*H 8). Increasing the *p*H to 14 does not increase the yield because of decomposition of the phenylacetaldehyde produced. At lower temperatures, the rate of addition of OH^- or NH_3 to phenylacetylene would probably be too slow to have been effective in the primitive ocean.

The nucleophilic addition of HCN to phenylacetylene gives cinnamonitrile, which upon addition of NH_3 and hydrolysis, yields β-aminohydrocinnamic acid rather than phenylalanine. The addition of NH_3 to cinnamic acid gives exclusively β-aminohydrocinnamic acid at *p*H values between

Table 1. Formation of phenylacetylene from simple hydrocarbons. The hot wire source was a tungsten wire at approximately 1300°C in a 1-liter flask. The spark source was a Corona-type discharge from a Tesla coil in a 3-liter flask. The ultraviolet source was a Hanovia medium-pressure Hg ultraviolet lamp at 22°C.

Gas	Energy source	Time (hr)	Yield of C_6H_5-$C\equiv CH$ (%)
CH_4 (450 mm)	Hot wire	3	0.05
	Spark	12	.001
C_2H_6 (450 mm)	Hot wire	1.5	4.7*
	Spark	12	0.003
C_2H_4 (50 mm) + CH_4 (400 mm)	Hot wire	2	1.5†
	Spark	12	0.04†
C_2H_2 (5 mm) + CH_4 (445 mm)	Hot wire	3	.40†
	Spark	12	.02†
C_2H_2 (10 mm) + CH_4 (360 mm)	Ultraviolet	0.7	.12†

* The yield is not affected substantially on the addition of 200 mm of nitrogen or 50 mm of ammonia to the reaction flask. † Yield based on either C_2H_4 or C_2H_2.

5 and 14 (*11*). Furthermore the equilibrium constant for phenylalanine synthesis from cinnamic acid, a reaction catalyzed by phenylalanine-ammonia lyase, is unfavorable (*12*).

The nucleophilic addition of H_2S to phenylacetylene at *p*H's between 7 and 12 results in good yields of phenylacetaldehyde. The conditions and yields are given in Table 2. The second-order rate constant for HS^- as the nucleophile is about 10^4 times as great as that for the addition of OH^- (*13*). The phenylalanine was identified by its R_F value in three paper chromatography solvents, and its position on the amino acid analyzer. This identification was confirmed by preparing the phenylhydantoin derivative. Its melting point was 171° to 172°C, the same as that of authentic 3-phenyl-5-benzylhydantoin, and the mixed melting point was not depressed.

Phenylacetylene can also be hydrated to phenylacetaldehyde by way of radical addition of H_2S (*14*). An aqueous mixture of H_2S and phenylacetylene, buffered at *p*H 7.5 in the absence of air, was irradiated for 5 minutes with a Hanovia medium-pressure Hg ultraviolet lamp. Ammonia and HCN were added, and the mixture was hydrolyzed and desalted. The yields of phenylalanine are comparable to the nucleophilic addition of H_2S (Table 2). The HS radicals generated from $H_2O_2 + H_2S$ and from the action of visible light on yellow solutions of ammonium polysulfide are also effective in synthesizing phenylacetaldehyde.

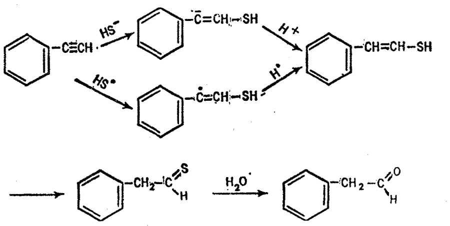


Table 2. Formation of phenylalanine from phenylacetylene.

H_2S (*M*)	*p*H	Temp (°C)	Time (hr)	Yield* (%)	Yield† (%)
3×10^{-4}	7.8	60	84	0.03	
6×10^{-3}	7.8	60	67	0.5	3.8
6×10^{-3}	8.3	80	44	6.8	20.4
6×10^{-3}	7.8	100	67	5.1	17.0
1.5×10^{-2}	7.5	22	0.08‡	1.7	10.5
1.3×10^{-3}	7.5	22	0.16‡	0.1	

* Based on initial amount of phenylacetylene added. The phenylacetylene concentration was $3 \times 10^{-3} M$ in these experiments. † Based on the amount of phenylacetylene reacted which was followed spectrophotometrically. ‡ Irradiation with a Hanovia medium-pressure Hg ultraviolet lamp.

In addition to the phenylalanine from radical addition, a small yield of tyrosine was found. No tyrosine was observed in the nucleophilic addition of H_2S to phenylacetylene. The tyrosine was isolated by elution from Dowex 50 (H+) with increasing concentrations of HCl (*15*). The tyrosine peak was evaporated to dryness. Rechromatography of this fraction on the buffered columns of the amino acid analyzer showed a peak corresponding to tyrosine. The yield of tyrosine was 2 percent of the phenylalanine yield. Both *o*- and *m*-tyrosine, which may also have been synthesized along with the tyrosine, were not isolated, but peaks corresponding to these amino acids were observed on chromatograms from the amino acid analyzer.

Tyrosine would also have been synthesized on the primitive earth by hydroxylation of phenylalanine. Solutions of phenylalanine are hydroxylated by ultraviolet light and γ-rays to tyrosine and dihydroxyphenylalanine (*16*). Various hydrogen peroxide reagents also will hydroxylate benzene rings (*17*). Molecular oxygen combined with reducing agents, such as ascorbic acid (*18*), also hydroxylate phenylalanine, but molecular oxygen was probably absent from the primitive earth except for traces.

These results suggest that the presence of H_2S in the primitive ocean is necessary for the synthesis of phenylalanine. Although H_2S is the stable form of sulfur under reducing conditions, an excess of Fe^{+2} would precipitate all the sulfide. These results imply that the sulfide may have been more abundant than iron in the primitive ocean.

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