TABLE I

DATA ON COPOLYMERIZATIONS BY DIFFERENT CATALYSTS OF 0.02 MOLE OF STYRENE AND 0.02 MOLE METHYL METHACRYLATE

Catalyst	Mole % catalyst	Temp., °C.	Time, hr.	Yield, % by wt. of monomers used	C, %	H, %	Mole % styrene in polymer
Na	[a]	Room	65	11	60.17 ± 0.04	8.16 ± 0.01	0.6 ± 0.1
$(C_6H_5)_3CNa$	1.0	Room	24.5	18.4	60.27 ± .24	8.12 ± .23	0.80 ± 0.65
$(C_6H_5)_3CNa$	0.5	Room	65	11.8	60.88	6.11	2.79
$(C_6H_5COO)_2$	0.1	60	7.7	12.3	76.18	7.99	50.19
$(p\text{-}ClC_4H_4COO)_2$	0.1	60	16	21.3	75.70	8.31	48.70
$(t\text{-}C_4H_9O)_2$	0.1	100	2.5	17.5	75.81 ± .58	8.15 ± .15	49.04 ± 1.80

[a] On shaking machine.

because its solubility in ether ensured separation from excess sodium) was prepared from triphenylmethyl chloride and sodium in ether solution.[8]

Procedure.—Techniques previously reported were used for the preparation[9] and purification[10] of the polymers. In the reaction with triphenylmethylsodium, an ether solution of the catalyst was added to the monomer mixture. The degassing process was carried out with a conventional high vacuum system and a pressure of 2.5×10^{-4} mm. was obtained at least three times before the sample was allowed to polymerize for the indicated time. Repetition of polymerizations previously reported[3] was undertaken to determine the accuracy in duplication of the previous work, and good agreement was obtained. The results are given in Table I.

(8) *Org. Syntheses*, **19**, 83 (1939).

(9) F. R. Mayo and F. M. Lewis, THIS JOURNAL, **66**, 1594 (1944).

(10) F. M. Lewis and F. R. Mayo, *Ind. Eng. Chem., Anal. Ed.*, **17**, 134 (1945).

MORLEY CHEMICAL LAB.
WESTERN RESERVE UNIVERSITY
CLEVELAND, OHIO

1,3,5-Trichloro-2,4,6-tribromocyclohexane

BY H R. FRISCH

RECEIVED MAY 13, 1954

Benzene reacts with chlorine to form a mixture of isomers of benzene hexachloride and with bromine to yield a mixture of the hexabromides, but chlorine does not react with benzene hexabromide with the replacement of any bromine atoms. Under the conditions described below the reaction of benzene with a mixture of chlorine and bromine yields a product which is probably a mixture of isomers of 1,3,5-trichloro-2,4,6-tribromocyclohexane, eight of which are theoretically possible with a cyclohexane of planar configuration. On alkaline hydrolysis 3 moles of the alkali bromide and 1,3,5-trichlorobenzene were obtained. The formation of 1,3,5-trichloro-2,4,6-tribromocyclohexane is best explained if a chemical combination of chlorine and bromine is assumed; the existence of chlorine bromide has been suggested, but not conclusively demonstrated.

The insecticidal potency of the mixture against roaches and mites is about the same as that of crude benzene hexachloride.

Experimental

Under actinic irradiation and at temperatures below 10°, benzene reacted quantitatively with stoichiometric amounts of chlorine and bromine to yield a solid product which was recrystallized from hot glacial acetic acid as colorless plates of 1,3,5-trichloro-2,4,6-tribromocyclohexane, m.p. 171° with slight decomposition.[1]

Anal. Calcd. for $C_6H_6Cl_3Br_3$: C, 16.99; H, 1.43; Cl, 25.07; Br, 58.51; mol. wt., 424.24. Found: C, 16.3; H, 1.47; Cl, 22.38; Br, 56.42; mol. wt., 427.

(1) Tests were carried out by courtesy of Niagara Chemical Division of Food Machinery and Chemical Corporation, Middleport, N. Y.

The compound is soluble in most organic solvents with the exception of carbon tetrachloride. On alkaline hydrolysis it yielded 3 moles of alkali bromide and a water-insoluble oil, 1,3,5-trichlorobenzene; b.p. 208° (760 mm.), Cl 58%.

INSTITUTE OF ORGANIC CHEMISTRY
UNIVERSITY OF DELFT (NETHERLANDS)

Pyrosynthesis of Aspartic Acid and Alanine from Citric Acid Cycle Intermediates[1]

BY SIDNEY W. FOX, JOSEPH E. JOHNSON AND MAVIS MIDDLEBROOK

RECEIVED JUNE 21, 1954

Experiments directed toward synthesis of true protein, and elucidation of its primordial origin[2] and subsequent evolution[3] have been performed. In the course of these experiments, almost all chromatographic evaluations of pyropolymerization of various pairs of DL-amino acids revealed, following hydrolysis, a number of ninhydrin-reactive spots which exceeded the number of reactants. These results indicate that the conditions which produce polymers[2,4] from unsubstituted amino acids lead also to the formation of additional amino acids (and in part possibly to amines).

Attention was diverted to a possible thermal origin of aspartic acid from the citric acid cycle by the postulate that those features of current biochemistry which are biologically relatively ubiquitous were also part of a primordial biochemistry.[5] Over a century ago, aspartic acid had been prepared by heating ammonium fumarate or ammonium malate[6,7] although of course not with reference to the now recognized citric acid cycle.

In the present experiments, ammonium salts of two additional acids from the citric acid cycle were each heated for up to three hours at 200°, chromatographed, and also hydrolyzed and chromatographed. Faint ninhydrin spots were obtained from heated ammonium fumarate and ammonium malate, although there were none from ammonium citrate or ammonium succinate either before or

(1) Journal paper No. J-2546 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project 1111, supported by the Rockefeller Foundation.

(2) S. W. Fox and M. Middlebrook, *Federation Proc.*, **13**, 211 (1954).

(3) S. W. Fox, *Am. Naturalist*, **87**, 253 (1953).

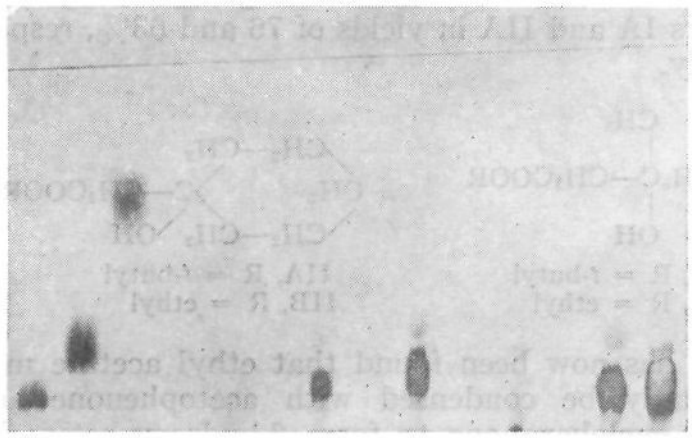
(4) H. Schiff, *Ann.*, **307**, 231 (1899).

(5) Additional reasons for this particular study were the facts that aspartic acid is an *early* or the earliest amino acid in biosynthesis and that its metabolic origin is from the citric acid cycle (see E. Baldwin, "Dynamic Aspects of Biochemistry," the University Press, Cambridge, 1952).

(6) J. Wolff, *Ann.*, **75**, 294 (1850).

(7) Dessaignes, *Compt. rend.*, **30**, 324 (1850).

after hydrolysis. The fumarate and malate products however yielded greatly increased ninhydrin reactions after hydrolysis (Fig. 1). The low intensities prior to hydrolysis are explainable on the basis that the conditions employed for synthesis of aspartic acid are identical to those which cause rapid pyrohomopolymerization of aspartic acid to an acid-hydrolyzable product.[2,4] The conversion of malate to aspartate was visibly greater than that of fumarate at 120, 160 and 200°.



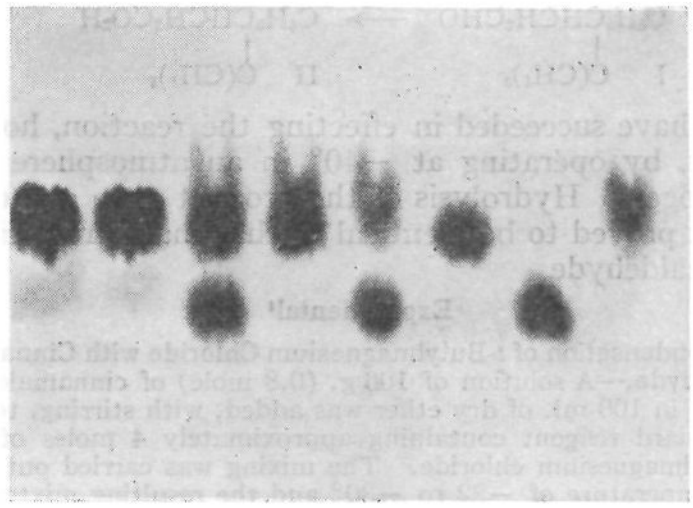
1 2 3 4 5 6 7 8 9 10 11 12 13

Fig. 1.—1, 10 λ of aspartic acid standard; 2, 10 λ of alanine standard; 3, 10 λ of leucine standard, to permit comparisons of R_F; 4, 10 λ of unheated monoammonium fumarate; 5, 10 λ of unheated monoammonium malate; 6, 10 λ of heated monoammonium fumarate; 7, 2 λ of hydrolyzed heated monoammonium fumarate (2 λ); 8, 10 λ of heated monoammonium malate; 9, 1 λ of hydrolyzed heated monoammonium malate showing faint spot with R_F of alanine; 10, 10 λ of hydrolyzed heated monoammonium succinate; 11, 10 λ of hydrolyzed heated ammonium citrate showing non-ninhydrin spot at origin; 12, 10 λ of hydrolyzed heated monoammonium fumarate (this amount shows alanine spot better than does spot 7); 13, same as 12 for monoammonium malate instead of spot 9. Heating was for 3 hours at 200° in tubes of 16 × 150 mm. size in oil-bath. Hydrolyses were performed with concd. hydrochloric acid at 15 lb. steam pressure for 12 hours. The dried products from 1.0 g. of reactant were dissolved in 15.0 ml. of water. Standards were from solutions containing 1.0 mg. of amino acid per ml. Chromatographic solvent was 4 butanol:1 acetic acid:1 water.

In all chromatograms showing additional ninhydrin spots following pyropolymerizations of amino acids and hydrolysis, spots with the R_F of alanine were observed. Inasmuch as the R_F values of α-alanine and of β-alanine, in the butanol–acetic acid solvent used, are the same,[8] new chromatograms were run in pyridine–water (7:3), in which the R_F values differ. The results from the hydrolyzate of heated ammonium malate (Fig. 2) confirm in the second solvent system that the spot is alanine and that this is partly or entirely α-alanine. In this latter system, β-alanine has the same R_F as aspartic acid.

The same conditions can thus yield aspartic acid from a citric cycle acid, can lead to the formation of one or more additional amino acids, and can result in a *proteinoid* polymer from which these amino acids are recovered following hydrolysis. Any concepts of prebiological chemistry must necessarily be highly speculative. If origins of biochemistry are to be considered,[9] however, the experiments reported here indicate that thought must be accorded to a thermal origin of biochemistry. In this connection, it is of interest that, on the basis of taxonomic studies, Copeland[10] suggested the origin of biology in thermal waters.

(8) H. K. Berry, H. E. Sutton, L. Cain and J. S. Berry, Univ. of Texas Publication No. 5109, 1951, p. 22.



1 2 3 4 5 6 7 8

Fig. 2.—1, 10 λ of aspartic acid; 2, 10 λ of α-alanine; 3, 10 λ of β-alanine; 4, 10 λ each of aspartic acid and α-alanine; 5, 10 λ each of aspartic acid and β-alanine; 6, 10 λ each of aspartic acid, α-alanine, and β-alanine; 7, 20 λ of product from ammonium malate heated at 160°; 8, 20 λ of product from ammonium malate heated at 200°. Chromatographic solvent was 7 pyridine:3 water.

(9) S. L. Miller, *Science*, **117**, 528 (1953).
(10) J. J. Copeland, *Ann. N. Y. Acad. Sci.*, **36**, 1 (1936).

DEPARTMENT OF CHEMISTRY
IOWA STATE COLLEGE
AMES, IOWA

Conjugate Addition of *t*-Butylmagnesium Chloride to Cinnamaldehyde and Ethyl Cinnamate

BY REYNOLD C. FUSON AND LEWIS I. KRIMEN

RECEIVED OCTOBER 15, 1954

The only report of the conjugate addition of a Grignard reagent to an α,β-unsaturated aldehyde was made by Stevens, who succeeded in condensing *t*-butyl- and *t*-amylmagnesium chloride with crotonaldehyde in the 1,4-manner.[1] By the use of the *t*-butyl reagent we have been able to achieve a similar result with cinnamaldehyde. The 1,4-addition product, β-*t*-butylhydrocinnamaldehyde (I), isolated by way of the sodium bisulfite addition compound, underwent oxidation when exposed to the air to give the corresponding acid.

We were able to synthesize β-*t*-butylhydrocinnamic acid (II), which had been described earlier by Koelsch,[2] from ethyl cinnamate and *t*-butylmagnesium chloride. These compounds had been shown by others not to combine in the 1,4-manner under the conditions ordinarily employed for such condensations.[3]

(1) P. G. Stevens, THIS JOURNAL, **57**, 112 (1935).
(2) C. F. Koelsch, *ibid.*, **65**, 1640 (1943).
(3) C. R. Hauser, R. S. Yost and B. I. Ringler, *J. Org. Chem.*, **14**, 26 (1949); A. D. Petrov and P. S. Bataev, *J. Gen. Chem.* (*U.S.S.R.*), **20**, 2236 (1950).