It has been suggested that these complexes are closely related structurally to those of the complexes formed by silver ion and aromatic substances. At the present time a structure is favored for the product of interaction of bromine and the aromatic ring in which the centers of the ring and the two halogen atoms lie on a straight line.

DAVIS, CALIFORNIA   RECEIVED MARCH 6, 1950

Copolymerization. XV.[1] Copolymerization of Acetylene Derivatives with Olefins. Retardation by Radicals from Acetylenes

BY KENNETH W. DOAK

Much work has been done to determine the relative reactivities, in free radical copolymerization, of various olefinic monomers with different radicals. Mayo, Lewis and Walling[2] have established a general reactivity series based on a consideration of monomer reactivity ratios. A polarity series, in which monomers are placed according to their ability to donate or accept electrons, has been established by a consideration of the products of the reactivity ratios. No quantitative data have been determined for the radical copolymerization of acetylene derivatives which would enable this class of monomers to be placed in the reactivity and polarity series for olefins. It was the objective of this work to determine the reactivity ratios for the copolymerization of the representative acetylenes, hexyne-1, phenylacetylene, and diphenylacetylene, with the olefins acrylonitrile and methyl acrylate. The copolymerization of styrene and phenylacetylene was also studied. In order to compare the reactivity of the double and triple bond, hexene-1 was copolymerized with acrylonitrile and methyl acrylate.

Since the acetylene derivatives caused marked retardation in most copolymerizations, some experiments were carried out in order to gain some information upon the mechanism of chain termination. The rate of polymerization of three systems was determined as a function of the catalyst concentration, in order to test for the formation of radicals which do not propagate the kinetic chain. Thus, it was shown by Bartlett and Altschul[3] that the rate of polymerization of allyl acetate is proportional to the catalyst concentration, instead of being a square root function, as occurs for most polymerizations. This presumably is due to the formation of allyl radicals which do not propagate the chain. The molecular weight of the polymers is independent of the catalyst concentration.

Experimental

Monomers.—The acetylene derivatives were obtained commercially. The diphenylacetylene (m. p. 59–61°) was used without further purification. Hexyne-1 and phenylacetylene were carefully fractionated before being used. The physical constants were: hexyne-1, b. p. 71.8–72.0 (760 mm.), $n^{20}D$ 1.3993; phenylacetylene, b. p. 75.2 (90 mm.), $n^{20}D$ 1.5485. Styrene, acrylonitrile and methyl acrylate were commercial samples, redistilled before use. Physical constants closely checked literature values.

Copolymerization for Reactivity Ratios.—Copolymerizations were carried out in evacuated tubes, with benzoyl peroxide as catalyst, as previously discussed by Mayo and Lewis.[4] The amount of peroxide used was 0.1 mole %,

TABLE I
COPOLYMERIZATION OF ACETYLENES

Expt.	$[M_1]_0{}^a$	$[M_1]^a$	$[M_2]_0{}^a$	$[M_2]^a$	Time, hr.	Polymer analysis
		Methyl Acrylate (M_1)–Phenylacetylene (M_2)				
1	85.22	71.53	14.20	11.25	70	63.66, 63.49% C
2	85.23	71.73	14.32	11.38	70	63.61, 63.71
3[b]	40.42	37.68	57.27	54.72	145	75.97, 75.89
4[b]	40.62	37.95	55.35	52.72	145	76.41, 76.44
		Acrylonitrile (M_1)–Phenylacetylene (M_2)				
5	83.46	76.42	14.09	11.40	97	15.40, 15.19% N
6	84.63	77.54	14.09	11.23	97	14.96, 14.73
7[b]	39.13	36.87	54.72	51.96	145	7.90
8[b]	39.58	37.32	54.99	52.22	145	7.88
		Acrylonitrile (M_1)–Diphenylacetylene (M_2)				
9	80.44	72.78	20.17	20.03	15	25.0, 24.7% N
10	50.62	46.48	50.04	49.76	160	21.56
11	51.02	47.96	49.85	49.63	160	21.31
		Methyl Acrylate (M_1)–Diphenylacetylene (M_2)				
12	79.67	65.58	19.980	19.913	15	56.11, 56.26% C
13	50.95	42.48	49.92	49.74	48	57.57, 57.33
14	50.91	42.07	50.01	49.84	48	57.26, 57.25
		Acrylonitrile (M_1)–Hexyne-1 (M_2)				
15	121.13	114.68	73.74	73.05	54	22.65% N
16	120.14	114.27	73.91	73.26	54	22.51
17	49.94	48.42	45.82	45.61	32	21.80
		Acrylonitrile (M_1)–Hexene-1 (M_2)				
18	59.29	44.11	38.02	37.19	3.5	24.30% N
19	59.75	47.12	37.97	37.20	3.5	24.08
		Methyl Acrylate (M_1)–Hexyne-1 (M_2)				
20	50.54	33.39	46.72	45.35	75	58.10% C
21	35.59	16.51	31.64	29.49	115	58.90
22	35.23	14.83	34.72	32.31	115	59.03
		Methyl Acrylate (M_1)–Hexene-1 (M_2)				
23	50.02	18.56	43.41	38.72	4.5	59.59% C
24	49.66	17.71	43.27	39.22	4.5	59.08
25	49.49	18.39	43.32	39.36	4.5	59.09
		Phenylacetylene (Homopolymerization)				
26[b]			45.31	44.28	160	

^{a} Millimoles. ^{b} 0.2 mole % benzoyl peroxide.

(1) This paper was presented at the Atlantic City Meeting of the American Chemical Society, September, 1949. For paper XIV in this series, see Walling, Cummings, Briggs and Mayo, THIS JOURNAL, **72**, 48 (1950).

(2) Mayo, Lewis and Walling, *ibid.*, **70**, 1529 (1948).

(3) Bartlett and Altschul, *ibid.*, **67**, 816 (1945).

(4) Mayo and Lewis, *ibid.*, **66**, 1594 (1944).

except for a few experiments, indicated in Table I, in which 0.2 mole % was used.

The copolymers of methyl acrylate with hexyne-1, hexene-1, diphenylacetylene, and phenylacetylene (low proportion), and of styrene with phenylacetylene, were purified by the frozen benzene technique.[5] Those from methyl acrylate and a higher proportion of phenylacetylene had a lower molecular weight, and the monomer mixture was removed under vacuum, at room temperature, from the polymer which formed thin layers in flasks. Drying was finished at 140° for a few minutes. A homopolymer of phenylacetylene was dried in the same manner. The copolymers of acrylonitrile with diphenylacetylene, hexyne-1, and hexene-1 precipitated from the reaction mixture as insoluble powders, which were extracted with alcohol and hexane and dried at 60° and 1 mm. The copolymers of acrylonitrile and phenylacetylene (low proportion) were dissolved in acetone and precipitated (three times) from hexane as finely divided powders. Those from acrylonitrile and phenylacetylene (higher proportion) were freed from most of the monomer mixture by vacuum distillation, then were precipitated as finely divided powders and dried. The data for these experiments appear in Table I. The graphical determination of reactivity ratios for phenylacetylene with methyl acrylate and acrylonitrile is shown in Fig. 1.
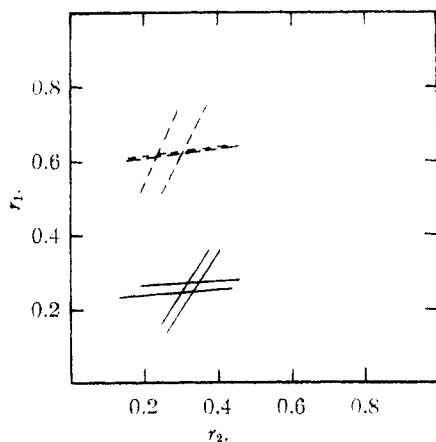

Fig. 1.—Reactivity ratios for copolymerization of phenylacetylene with methyl acrylate (– – –) and acrylonitrile (—); r_2 is phenylacetylene radical.

Rates of Copolymerization.—Some experiments were carried out to determine the effect of small amounts of phenylacetylene upon the rate of polymerization of styrene. These experiments are summarized in Table II.

TABLE II

EFFECT OF PHENYLACETYLENE UPON POLYMERIZATION OF STYRENE[a]

Styrene, g.	Phenyl-acetylene, g.	Yield, g.	Molecular weight[b]
9.37	1.007	1.38	63,000
9.78	0.585	1.68	96,000
9.99	.408	1.90	112,000
10.17	.199	2.26	141,000
10.37	.000	2.60	177,000

[a] Seventeen hours at 60°, with 0.025 g. of benzoyl peroxide. [b] Calculated from intrinsic viscosity in benzene, by the method of Gregg and Mayo, THIS JOURNAL, 70, 2373 (1948).

More carefully controlled rate measurements were made to determine the rate of polymerization of a mixture of styrene and phenylacetylene as a function of catalyst con-

(5) Lewis and Mayo, Ind. Eng. Chem., Anal. Ed., 17, 134 (1945).

centration. The catalyst chosen was 1-azo-bis-1-cyclo-hexanecarbonitrile, which has a half-life at 60° of about 1300 hours.[6] The experiments were carried out by pipetting aliquot parts of a mixture of the two monomers into tubes containing different amounts of the catalyst. The monomers were degassed three times, sealed at 10^{-4} mm., and polymerized at 60°. The styrene copolymers were precipitated from hexane (three times for the phenylacetylene system, four times for the diphenylacetylene system) and dried by the frozen benzene technique. The methyl acrylate copolymers were freed from the monomers by vacuum distillation from very thin layers of polymer, the drying being completed at 65°. This drying procedure appears to be adequate, since the methyl acrylate-phenylacetylene system, and phenylacetylene alone, have an extremely low rate of polymerization (cf. Tables I and III). The rate data are recorded in Table III.

TABLE III

RATES OF COPOLYMERIZATION

Moles/l. × 100	Time, hr.	Yield, g.	%/hr.[a] × 100	Mol. wt. × 10⁻³
Phenylacetylene–Styrene[b]				
0.000	100	0.043		
0.193	100	.223	2.04	53
.373	100	.296	2.89	...
.721	100	.513	5.46	46
1.205	64	.510	8.74	41
1.553	64	.661	11.58	36
2.38	64	.821	14.69	37
3.52	29.6	.561	21.46	...
4.88	29.6	.702	27.7	34
6.09	29.6	.837	33.0	33
0.00[e]	123.3	.108		63
.410	123.3	.568	4.32	51
.811	123.3	.718	5.82	...
1.66	48.3	.510	11.21	42
2.86	48.3	.729	16.70	39
4.08	48.3	.948	22.29	37
Phenylacetylene–Methyl Acrylate[c]				
0.000	108	0.0187	0.00	
0.385	108	.1731	1.53	
0.855	108	.312	2.94	
1.668	85.5	.441	5.43	
3.29	85.5	.745	9.45	
4.88	85.5	.961	12.37	
Diphenylacetylene–Styrene[d]				
0.434	24.5	0.458	19.6	...
0.794	24.5	.591	25.9	...
1.604	24.5	.927	42.4	167
3.19	18.3	1.043	65.3	142
4.77	18.3	1.283	82.2	126
6.35	18.3	1.491	97.6	113
0.0	67.5	0.075		

[a] Corrected for blank, and for small decrease in amount of monomers. [b] 10.0-cc. samples (9.00 g.) of mixture of 100 g. of styrene and 24.0 g. of phenylacetylene. [c] 10.0-cc. samples (9.40 g.) of mixture of 51.6 g. of methyl acrylate and 5.3 g. of phenylacetylene. [d] 10.0-cc. samples (9.31 g.) of mixture of 50.0 g. of styrene and 21.0 g. of diphenylacetylene. [e] Two sets of experiments were carried out at different times to check reproducibility.

Discussion

Relative Reactivities of Acetylenes and Olefins.—The monomer reactivity ratios for the

(6) Lewis and Matheson, THIS JOURNAL, 71, 747 (1949).

<div align="center">

TABLE IV

MONOMER REACTIVITY RATIOS AND RELATIVE REACTIVITIES

</div>

M$_1$	r$_1$	M$_2$	r$_2$	Relative radical reactivities (M$_2$) Acrylo-nitrile	Methyl acrylate
Acrylonitrile	0.26 ± 0.03	Phenylacetylene	0.33 ± 0.05	1.00	
Methyl acrylate	0.62 ± 0.02	Phenylacetylene	.27 ± 0.04		1.00
Acrylonitrile	13.6 ± 1.0	Diphenylacetylene	(.0)^a	0.019	
Methyl acrylate	55 ± 5	Diphenylacetylene	(.0)^a		0.011
Acrylonitrile	5.4 ± 0.3	Hexyne-1	(.0)^a	0.048	
Methyl acrylate	11.2 ± 2	Hexyne-1	(.0)^a		0.055
Acrylonitrile	12.2 ± 2.4	Hexene-1	(.0)^a	0.021	
Methyl acrylate	8.5 ± 2	Hexene-1	(.0)^a		0.073
Acrylonitrile^b	0.04 ± 0.04	Styrene	.40 ± 0.05	3.2^d	
Methyl acrylate^c	0.18 ± 0.02	Styrene	.75 ± 0.03		3.5

^{a} These r$_2$ values were assumed to be zero, and r$_1$ was calculated from the intersection with the r$_1$ axis accordingly. ^{b} Ref. 8b. ^{c} Ref. 8a. ^{d} Calculated assuming maximum r$_1$ of 0.08, ref. 8b.

systems studied are recorded in Table IV. Relative reactivities of the acetylenes with the acrylonitrile and methyl acrylate radicals were determined from the reciprocals of r$_1$, phenylacetylene being taken as unity. Data for hexyne-1 and styrene (data for the latter taken from the literature) make it possible to compare the reactivities of the acetylenic and olefinic bonds toward attack by free radicals.

The acetylenes have the following order of reactivity toward both the acrylonitrile and methyl acrylate radicals: phenylacetylene > hexyne-1 > diphenylacetylene. Thus, substituting a phenyl group for an alkyl group greatly increases the reactivity of the acetylenic bond, possibly because of the contribution of structures such as HC=C=⟨◯⟩· to the transition state. The second phenyl group causes a large decrease in the reactivity. This decrease presumably is due to steric hindrance. Nozaki[7] postulated that symmetrical substitution always caused deactivation due to steric hindrance, although Mayo, Lewis, and Walling[2] found that diethyl fumarate is more reactive than methyl acrylate toward the styrene radical, which is a good donor, but less reactive toward a poorer donor radical such as vinylidene chloride.

Hexyne-1 and hexene-1 have comparable reactivities (within experimental error) toward the methyl acrylate radical, but hexyne-1 is about twice as reactive as hexene-1 toward the acrylonitrile radical, which is a stronger acceptor than methyl acrylate. It thus appears that hexyne-1 is a better donor than hexene-1, presumably because the aliphatic triple bond is more easily polarized than the double bond. Styrene is over three times as reactive as phenylacetylene toward both the acrylonitrile and methyl acrylate radical, showing that in a conjugated system the olefinic bond is more reactive. Thus, it appears that resonance structures such as R—CH$_2$—CH=⟨◯⟩. in styrene make a greater contribution to the ac-

(7) Nozaki, *J. Polymer Sci.*, 1, 455 (1946).

tivated complex than do structures such as R—CH=C=⟨◯⟩· in phenylacetylene.

A comparison of the products of r$_1$ and r$_2$ indicates that styrene and phenylacetylene have about the same alternating tendency with methyl acrylate. With acrylonitrile, a better acceptor, styrene appears to alternate better than phenylacetylene. A conjugated double bond thus appears to be more reactive and a better donor than a conjugated triple bond.

Mechanism of Chain Termination.—The data in Table I show qualitatively that the rates of copolymerization of phenylacetylene with methyl acrylate and acrylonitrile are much slower than the corresponding systems containing styrene.[8] The data in Table II show that adding small amounts of phenylacetylene (up to about 10%) reduce the over-all rate of polymerization of styrene by a factor of about two, accompanied by a decrease in the molecular weight of the polymer. This relation suggested that phenylacetylene formed radicals which do not readily propagate the chain, but have a strong tendency to terminate chain growth. Experiments (Table III) were carried out to determine the rate of polymerization of a mixture of styrene and phenylacetylene as a function of the catalyst concentration. 1-Azo-bis-1-cyclohexanecarbonitrile was used as a catalyst; because of the long half-life, about 1300 hours,[6] a constant rate of radical formation will result over fairly long periods of time.

In a monomer mixture containing 19.7 mole % phenylacetylene, the rate of copolymerization, when plotted against the catalyst concentration (Fig. 2), gives a curve which obeys the empirical relationship

$$\text{Rate} = K_1[\text{C}]^{1/2} + K_2[\text{C}]$$
$$K_1 = 0.334, \quad K_2 = 4.06$$

in which rate is expressed in %/hour by weight, and [C], the concentration of the catalyst, in moles/liter. This expression can be derived by

(8) (a) Lewis, Walling, Cummings, Briggs and Mayo, THIS JOURNAL, **70**, 1519 (1948); (b) Lewis, Mayo and Hulse, *ibid.* **67**, 1701 (1945).
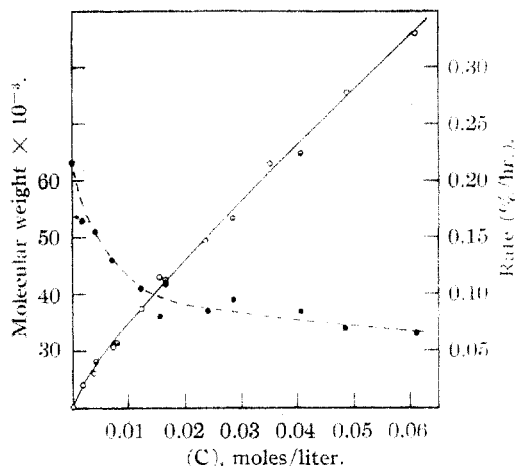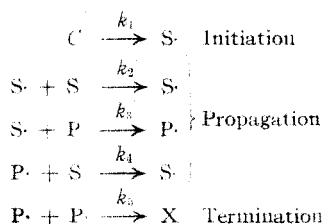
Fig. 2.—Rate of polymerization of styrene and phenylacetylene: —, calcd. for rate = 0.334 $\sqrt{C}$ + 4.06C; ○, one set of simultaneous experiments; ◐, second set of experiments, Table III; ─ ─ ─, ●, molecular weights.

making the following assumptions: (a) constant rate of radical formation; (b) chain propagation occurs by attack of radicals upon both styrene and phenylacetylene; (c) the radicals formed from a phenylacetylene unit are relatively unreactive, some propagating the chain by attack principally upon styrene, others coupling to terminate the chain. (d) In the steady state, the concentration of any species of radicals is constant. The reactions are

$$C \xrightarrow{k_i} S\cdot \quad \text{Initiation}$$

$$S\cdot + S \xrightarrow{k_2} S\cdot$$

$$S\cdot + P \xrightarrow{k_3} P\cdot \quad \bigg\} \text{Propagation}$$

$$P\cdot + S \xrightarrow{k_4} S\cdot$$

$$P\cdot + P \xrightarrow{k_5} X \quad \text{Termination}$$

C is catalyst concentration, S and S· are styrene and styrene radical concentrations, P and P· are phenylacetylene and phenylacetylene radicals. The reactions of catalyst with P, and P· with P are neglected, since they are probably small in a system containing a four-fold excess of styrene.

If the rate of formation of radicals equals the rate of destruction, then

$$k_1 C = k_5 P\cdot^2 \tag{1}$$

Since the rate of formation of P· is equal to the rate of destruction

$$k_3 S\cdot P = k_4 P\cdot S + k_5 P\cdot^2 \tag{2}$$

The rate of polymer formation, neglecting the addition of phenylacetylene to the phenylacetylene radical, is represented by

$$d\,Pol/dt = k_2 S\cdot S + k_3 S\cdot P + k_4 P\cdot S \tag{3}$$

The rate of formation of radicals is

$$dC/dt = k_1 C \tag{4}$$

Dividing (3) by (4) and eliminating S· and P·, one obtains

$$\frac{d\text{Pol}}{dC} = \frac{1}{\sqrt{k_1 k_5 C}}\left[\frac{k_2 k_4 S^2}{k_3 P} + 2k_4 S\right] + \left[1 + \frac{k_2 S}{k_3 P}\right] \tag{5}$$

Since the rate constants, and S and P are constant

$$\frac{d\text{Pol}}{dC} = \frac{K'}{\sqrt{C}} + K'' \tag{6}$$

Equation (6) is the differential form of the empirical relationship observed experimentally.

If k_4 is assumed to be zero (in the case that the phenylacetylene radical does not add to styrene), (6) becomes d Pol/dC = K'', equivalent to the polymerization of allyl acetate.

If one assumes that P· is a radical formed from phenylacetylene by chain transfer instead of by copolymerization, the kinetics are unchanged. They merely indicate that phenylacetylene forms radicals which do not readily propagate the kinetic chain.[9]

The molecular weights of the polymers decrease with increasing catalyst concentration, then approach a constant value. Thus, at high catalyst concentration, the chain termination appears to be analogous to that in the polymerization of allyl acetate.[3]

The extent of copolymerization in this system is unknown, due to lack of a satisfactory analytical method. However, if phenylacetylene is nearly one-third as reactive as styrene toward the styrene radical (Table IV), the polymers should contain nearly one-twelfth phenylacetylene (assuming no alternating tendency). This much cannot be incorporated as end-groups, since the molecular weights are over 30,000. Price and Greene[10] have found the reactivity ratios for the system 2-vinylpyridine and phenylacetylene to be 4.0 and 0.2, respectively, for the two radicals, showing copolymerization as well as retardation. Walling, et al.,[10a] have shown 2-vinylpyridine and styrene to have the reactivity ratios 0.55 and 1.14, respectively, for the two radicals.

The system styrene–diphenylacetylene shows a rate of polymerization which is proportional to the square root of the catalyst concentration (Fig. 3), indicating normal radical termination.[11] The rates of polymerization are higher than for the system styrene–phenylacetylene by a factor of 7–13. Since the reactivity data with methyl acrylate and acrylonitrile indicate that diphenylacetylene is much less reactive than phenylacetylene, it is likely that diphenyl-

(9) A calculation of k_2/k_3 gives an unreasonable value, if k_3 is assumed to be the rate constant for addition of the styrene type radical to phenylacetylene. If one assumes that phenylacetylene forms radicals both by copolymerization and transfer, and that the latter type radicals do not readily propagate the kinetic chain, a relationship of the form shown by (6) can be derived, in which the constants have a more complex form.
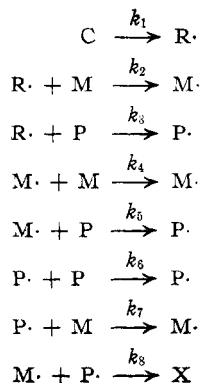
(10) Price and Greene, J. Polymer Sci., in press.

(10a) Walling, Briggs and Wolfstirn, THIS JOURNAL, 70, 1543 (1948).

(11) The curve does not extrapolate to the origin. The reason for this is unkown at the present time.

acetylene copolymerizes with styrene to a negligible extent. The number of diphenylacetylene radicals formed, even if they are relatively unreactive, is thus probably too small to affect the normal square root termination.

The system phenylacetylene–methyl acrylate, with 20 mole % of phenylacetylene, also shows a rate of copolymerization which is proportional to the square root of the catalyst concentration (Fig. 3).[11] These two monomers were shown by reactivity ratios to copolymerize readily with a considerable tendency to alternate. Thus, chains ending in phenylacetylene radicals are definitely formed, but the square root relationship indicates that there is no specific coupling of phenylacetylene radicals. However, if chain termination is by cross-termination, the square root relationship will result, as shown in the following derivation

$$C \xrightarrow{k_1} R\cdot$$

$$R\cdot + M \xrightarrow{k_2} M\cdot$$

$$R\cdot + P \xrightarrow{k_3} P\cdot$$

$$M\cdot + M \xrightarrow{k_4} M\cdot$$

$$M\cdot + P \xrightarrow{k_5} P\cdot$$

$$P\cdot + P \xrightarrow{k_6} P\cdot$$

$$P\cdot + M \xrightarrow{k_7} M\cdot$$

$$M\cdot + P\cdot \xrightarrow{k_8} X$$

C, M, M·, P, and P· represent concentrations of catalyst, methyl acrylate monomer and radical and phenylacetylene monomer and radical. The steady state relationships are:

$$k_5M\cdot P = k_7P\cdot M \qquad (7)$$
$$k_1C = k_8P\cdot M\cdot \qquad (8)$$

The rate of polymer formation is

$$dPol/dt = (k_4M + k_5P)M\cdot + (k_6P + k_7M)P\cdot \qquad (9)$$

Eliminating M· and P· from (7), (8) and (9), and dividing by (4), the following relationship is obtained

$$\frac{dPol}{dC} = \left[\frac{k_4k_7M^2}{k_5P} + 2k_7M + k_6P \right] \left(\frac{k_5P}{k_1k_7k_8M} \right)^{1/2} \frac{1}{\sqrt{C}} \qquad (10)$$

$$\frac{dPol}{dC} = \frac{Const.}{\sqrt{C}} \qquad (11)$$

Perhaps the best test for such cross-termination would be the method of Walling,[12] who showed that methyl acrylate and styrene have an abnormally high rate of cross-termination. If this occurs in the system phenylacetylene–methyl acrylate, it conceivably could cause retardation.

Hexyne-1 causes marked retardation, as seen by a comparison of the rates of copolymerization of acrylonitrile and methyl acrylate with hexyne-1
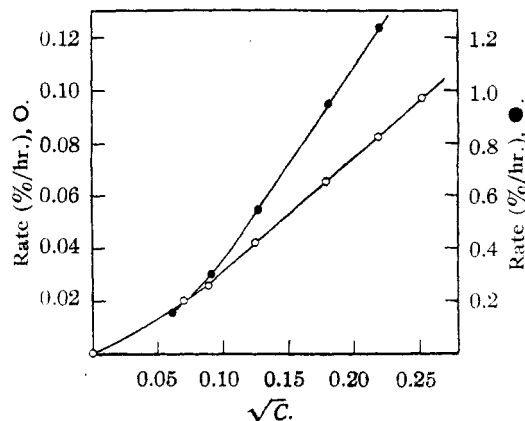
(12) Walling, THIS JOURNAL, 71, 1930 (1949).

Fig. 3.—Rate of polymerization of styrene and diphenylacetylene, methyl acrylate and phenylacetylene (lower curve).

and hexene-1 (Table I). Here again, it appears that the radicals formed from an acetylene derivative do not readily propagate the kinetic chain.

In the kinetic treatments of chain termination in systems containing phenylacetylene, the models used may be oversimplified, since certain factors have been neglected, such as chain transfer with the catalyst or second monomer, and termination by interaction of two radicals ending with the second monomer. However, the method appears to be useful in explaining the abnormal copolymerization behavior of acetylene derivatives, or other monomers which can copolymerize or transfer to form radicals which may not readily propagate the kinetic chain.

Acknowledgment.—The author wishes to express his appreciation to Dr. F. R. Mayo and Dr. F. M. Lewis for helpful discussions, and to Mrs. L. L. Olivo for carrying out much of the experimental work.

Summary

1. Monomer reactivity ratios have been determined for phenylacetylene, diphenylacetylene, hexyne-1 and hexene-1 with methyl acrylate and acrylonitrile.

2. Phenylacetylene is less than one-third as reactive as styrene toward the acrylonitrile and methyl acrylate radicals, but hexyne-1 and hexene-1 are more nearly equally reactive. Diphenylacetylene is 0.011 to 0.019 as reactive as phenylacetylene, while hexyne-1 is 0.05 as reactive.

3. The polymerization of styrene in the presence of phenylacetylene, with 1-azo-bis-cyclohexanecarbonitrile as catalyst, obeys the relationship Rate $= K_1\sqrt{C} + K_2C$. A kinetic treatment suggests that phenylacetylene radicals are formed, either by copolymerization or transfer, which do not readily propagate the kinetic chain, but tend to specifically react with each other, causing retardation.

4. The systems phenylacetylene–methyl acrylate and styrene–diphenylacetylene copolymerize with a rate proportional to the square root of catalyst concentration. In the former case, abnormally high cross-termination is suggested.

PASSAIC, NEW JERSEY   RECEIVED MARCH 17, 1950

Studies on the Structure of Nucleic Acids. I. Interaction of Rosaniline with Desoxypentose Nucleic Acid[1]

BY LIEBE F. CAVALIERI AND ALICE ANGELOS

Introduction

The question of the structure of nucleic acids as they exist in solution, and in particular as they exist in living cells, has received a considerable amount of attention from various quarters. Organic and biochemical evidence has aided greatly in the elucidation of the nature of the covalent linkages, while physical chemical data, such as electrophoretic patterns, sedimentation rates, titration curves and X-ray studies have been more useful in the understanding of the secondary forces which are of importance in any detailed analysis of structure. Notwithstanding the abundance of data which confronts us, many questions regarding the fine structure remain unanswered. The more important problems deal with the sequence of the nitrogenous bases, the point of attachment in the sugar molecule of the various nucleotides and the extent and nature of the branching. The question as to whether nucleic acids are mixtures of polynucleotides rather than single entities is unanswered and has rendered the problem of structure still more nebulous.

The ability of nucleic acids to bind cationic dyes has been known for some time, but not until recently has any systematic and quantitative attempt been made to study this phenomenon. Thus, Michaelis[2] has observed and correlated spectrophotometric changes of basic dyes in the presence of nucleic acids. More recently the interaction of certain antimalarial drugs with pentose nucleic acid has been discussed.[3] Since the application of binding techniques to the study of protein structure[4,5] has resulted in a measure of success, we felt that a quantitative approach to the problem on hand would yield useful information which could be correlated with existing physico-chemical data.

Experimental

Materials.—The desoxypentose nucleic acid was a sample generously supplied by Dr. Aaron Bendich, prepared from calf thymus by a slight modification of the method of Hammarsten. The material was characterized as to purines, pyrimidines, and phosphorus content by Chargaff and co-workers.[6]

N, %	13.4	Guanine, %	7.4
P, %	8.0	Cytosine, %	4.7
Adenine, %	10.0	Thymine, %	8.4

The molecular weight of thymus DNA has been variously reported, but a value of 35,000 was used for calculations, based on the measurements of Jungner, Jungner and Allgen[7] and Hammarsten.[8] The acid- and alkali-treated samples of DNA were prepared according to Gulland, Jordan and Taylor.[9] Analysis for alkali-treated sample: N, 14.1; P, 9.0; for acid-treated sample N, 13.3; P, 8.0.

The rosaniline was a commercial sample obtained from the Allied Chemical and Dye Corporation. After recrystallization from water, the nitrogen and chlorine analyses indicated 98 to 100% purity. In 0.05 M potassium phosphate buffer the extinction coefficient was 79,600 at pH 5.6 and 66,300 at pH 6.7 (5400 Å.). It was shown to obey Beer's law under the conditions of the study.

Anal. Calcd. $C_{20}H_{20}N_3Cl$: N, 12.48; Cl, 10.51. Found: N, 12.63; Cl, 10.31.

Method.—The binding of dye by DNA was determined by the method of equilibrium dialysis. Experiments were carried out at pH 5.6 and 6.7 in 0.05 M potassium phosphate buffer. DNA solutions varied from 0.05 to 0.2%. Five milliliters of DNA solution in 0.05 M buffer contained in a Visking cellophane bag were immersed in 5 ml. of dye in 0.05 M buffer. A group (ca. 24) of test-tubes was placed in a shaking device overnight which was sufficient time for equilibrium to be attained. The optical density of the solution of free dye (outside the bag) was determined in a Beckman spectrophotometer, Model DU, at a wave length of 540 mμ and the concentration calculated. Results were reproducible to within about 3%. Experiments were carried out at 3 ± 0.5°, 27 ± 1° and 32 ± 1°. Concentrations of DNA and dye were chosen such that a large proportion of dye was bound with respect to free dye concentration. The amount of dye adsorbed by the cellophane casing at each equilibrium concentration was determined from separate runs and found to be about 20% of the free dye concentration at pH 6.7. At pH 5.6 the cellophane adsorption ranged from about 30% at low dye concentrations to 15% at high dye concentrations.

Results

The data on the binding of rosaniline hydrochloride by desoxypentose nucleic acid (DNA) are presented in Figs. 1 and 2 and Tables I and II. The figures in the fifth column of Table I represent free dye concentrations to which have been added the values for the casing adsorption. This facilitates the calculations of the figures in

(1) The authors wish to acknowledge the support of the National Cancer Institute of the United States Public Health Service, the James Foundation of New York, Inc., and the joint support of the Office of Naval Research and the Atomic Energy Commission, contract N6-ori-99.

(2) Michaelis, *Cold Spring Harbor Symposia on Quantitative Biology*, **XII**, 131 (1947).

(3) Irvin, Irvin and Parker, *Science*, **110**, 426 (1949).

(4) Karush and Sonenberg, THIS JOURNAL, **71**, 1369 (1949); Karush, *ibid.*, **72**, 2705 (1950).

(5) Klotz and Urquhart, *ibid.*, **71**, 847 (1949).

(6) Chargaff, Vischer, Doniger, Green and Misani, *J. Biol. Chem.*, **177**, 405 (1949).

(7) Jungner, Jungner and Allgen, *Nature*, **163**, 849 (1949).

(8) Hammarsten, *Acta Med. Scand. Supp.*, **196** (1947).

(9) Gulland, Jordan and Taylor, *J. Chem. Soc.*, 1131 (1947).