mercapto-4-thiazoleacetic acid in 300 ml. of water was added dropwise a solution containing 63 g. (0.275 mole) of ammonium persulfate in 147 ml. of water over a 30-minute period at 25–30°. The reaction mixture was stirred for two additional hours.

For the ester, the upper aqueous layer was decanted, and the lower layer was dissolved in 400 ml. of ethyl ether. The ether solution was washed with 200 ml. of 2% aqueous sodium hydroxide, then with water until the washings were neutral to litmus, dried over sodium sulfate, and ether removed *in vacuo*. The product, a tan colored solid, m.p. 32–33°, was obtained in 65.4% yield.

For the acid, the precipitate was filtered and dried at 50°. The product, a tan colored solid, m.p. 150–151° dec., was obtained in 95.6% yield.

Anal. Calcd. for the ester $C_{14}H_{16}N_2O_4S_4$: N, 6.93; S, 31.70. Found: N, 7.03; S, 31.77. Calcd. for the acid $C_{10}H_8N_2O_4S_4$: N, 8.04; S, 36.81. Found: N, 7.88; S, 36.53.

Ethyl 2-(N,N-Diethylthiocarbamoylthio)-4-thiazoleacetate.—To a stirred solution containing 50.8 g. (0.25 mole) of ethyl 2-mercapto-4-thiazoleacetate, 10 g. (0.25 mole) of sodium hydroxide and 500 ml. of acetone was added dropwise 38 g. (0.25 mole) of N,N-diethylthiocarbamoyl chloride[6] dissolved in 200 ml. of acetone at 28–32° over a 10-minute period. The reaction mixture was stirred for four hours. The sodium chloride was collected by filtration, and the acetone removed *in vacuo*. The residue was dissolved in 500 ml. of ethyl ether, the ether solution was washed with 200 ml. of 2% aqueous sodium hydroxide, then with water until the washings were neutral to litmus, dried over sodium sulfate and the ether removed *in vacuo*. The product, a dark amber colored oil, was obtained in 84.3% yield.

Anal. Calcd. for $C_{18}H_{18}N_2O_2S_3$: N, 8.80; S, 30.20. Found: N, 8.69; S, 29.84.

2,4,6-Tris-(4-carbethoxymethyl-2-thiazolylthio)-*s*-triazine.—To a stirred solution containing 50.9 g. (0.25 mole) of ethyl 2-mercapto-4-thiazoleacetate, 14 g. (0.25 mole) of potassium hydroxide and 400 ml. of acetone was added 15.4 g. (0.083 mole) of cyanuric chloride[7] dissolved in 100 ml. of acetone. An exothermic reaction set in, causing the temperature to rise from 25 to 42°. The stirred reaction mixture was heated at 55–56° for five hours and after cooling to room temperature the potassium chloride was removed by filtration. Upon removal of the acetone *in vacuo*, the desired product was obtained as a resinous solid in a yield of 88%

Anal. Calcd. for $C_{24}H_{24}N_6O_6S_6$: N, 12.27. Found: N, 12.10.

Ethyl 2-(Chloroalkenyl or alkynylthio)-4-thiazoleacetates.—A 0.25-mole solution of the potassium salt of ethyl 2-mercapto-4-thiazoleacetate was prepared by dissolving the thiazoleacetate and potassium hydroxide in 300 ml. of acetone and 10 g. of water. To this stirred solution, 0.25 mole of either 1,3-dichloro-2-butene,[8] 2,3-dichloro-1-propene,[9] 1,3-dichloropropene[9] or 3-bromo-1-propyne[10] was added. Immediately, an exothermic reaction set in, the temperature rising from 25 to about 50°. The stirred reaction mixture was heated at 55–56° for six hours, cooled to room temperature, and filtered to remove the potassium chloride. The acetone was removed *in vacuo*. The residues were dissolved in 500 ml. of ethyl ether and washed with water until the washings were neutral to litmus, dried over sodium sulfate and the ether removed *in vacuo*. The data are summarized in Table I.

Acknowledgment.—The writers wish to acknowledge their indebtedness to Messrs. R. O. Zerbe and J. M. Hildebrand for assistance during the course of this investigation. Grateful acknowledgment also is made for the analyses by Mr. E. E. Null.

(6) Kindly supplied by Sharples Chemicals, Inc., Philadelphia, Penna.

(7) Kindly supplied by American Cyanamid Company, New York, N. Y.

(8) Kindly furnished by E. I. du Pont de Nemours and Company, Wilmington, Del.

(9) Kindly supplied by Shell Chemical Corporation, Emeryville, Calif.

(10) Kindly furnished by General Aniline and Film Corporation, New York, N. Y.

NITRO, WEST VIRGINIA

NOTES

Nucleophilic Displacement in the Biphenyl Series

BY ERNST BERLINER, BERNICE NEWMAN[1] AND TATIANA M. RIABOFF[1]

RECEIVED JUNE 7, 1954

4-Bromo-4′-nitro-, 4-bromo-2′-nitro- and 2-bromo-4′-nitrobiphenyl are unreactive toward piperidine in a typical nucleophilic displacement reaction, while under the same experimental conditions displacement of bromine in 4-bromo-3-nitrobiphenyl proceeds practically to completion.[2] From the unreactivity of the bromine in the heteronuclear biphenyl derivatives, Campbell, *et al.*, concluded that the activating effect of the nitro group was not transmitted from one ring into the other, and that the two rings in biphenyl act independently of each other, a view prevalent at that time.

Since then it has been shown that substituent effects can be transmitted through the biphenyl system, and that substituents affect the dissociation constants of biphenylcarboxylic acids and the rates of hydrolysis of their esters across the biphenyl system qualitatively in the same way as they affect the corresponding reactions in the benzene series, but that transmission in biphenyl is quantitatively less than in the benzene series.[3] In the light of these results, it seemed that the conclusion drawn from the unreactivity of the bromine in the aforementioned biphenyl derivatives was not justified, because under the same conditions 4-bromobiphenyl was also completely unreactive. The only permissible generalization is that the transmission of the effect of the 4′-nitro group must be less than in the benzene series.

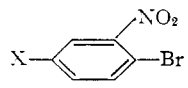
In order to bring the biphenyl derivatives up to the level of reactivity, a nitro group was introduced *ortho* to the bromine, as has been done before in

(1) Taken in part from M.A. theses submitted to the Chemistry Department of Bryn Mawr College, June, 1950 (T.M.R.) and June, 1953 (B.N.).

(2) N. Campbell, W. Anderson and J. Gilmore, *J. Chem. Soc.*, 446 (1940).

(3) E. Berliner and E. A. Blommers, THIS JOURNAL, **73**, 2479 (1951); E. Berliner and L. H. Liu, *ibid.* **75**, 2417 (1953).

the benzene series,[4,5] and the compounds thus compared were *o*-nitrobromobenzene (I), 4-bromo-3-nitrobiphenyl (II) and 4-bromo-3,4′-dinitrobiphenyl (III). In these systems the bromine is



I, X = H–
II, X = C_6H_5–
III, X = *p*-NO_2–C_6H_4–

sufficiently reactive to allow a direct comparison of the reactivity of all three compounds at 25°. Because of the low solubility of the last mentioned compound, reactions could not be conducted in piperidine as solvent,[4] but a mixture of piperidine and dioxane was used. The results are listed in Table I.

TABLE I

THE REACTION BETWEEN 4-X-2-NITROBROMOBENZENE AND PIPERIDINE AT 25°

Substituent X	$k_1 \times 10^5$, sec.$^{-1}$	Rel. rates
H–	1.30 ± 0.02	1
C_6H_5–	2.87 ± 0.02	2.21
p-NO_2–C_6H_4–	35.2 ± 1.9	27.1

The introduction of a nitro group in the *para* position of the second nucleus of biphenyl produces a not insignificant increase in reactivity. 4-Bromo-3,4′-dinitrobiphenyl reacts 27 times faster than *o*-nitrobromobenzene and 12.3 times faster than 4-bromo-3-nitrobiphenyl. The effect of the unsubstituted phenyl group, acting here as an electron attracting group, is to increase the reactivity by a factor of 2.2. The twelve-fold increase in reactivity in the 4′-nitro over the 4′-H compound is an indication of some measure of transmission of substituent effects across biphenyl, but the transmission is very much greater in benzene, where a *para* nitro group introduced into *o*-nitrobromobenzene has been estimated to raise the reactivity by a factor of about 10^5.[4,6] A similar estimate has been made for the chloro[5,6] and the fluoro compound.[7,8] But the expediency of introducing the *ortho* nitro group shows that some transmission through biphenyl does occur, and a similar method can also probably be used to show that the various positions in naphthalene are not as isolated chemically as they seem to be on the basis of similar displacement reactions.[9]

As in the case of the biphenylcarboxylic acids and esters,[3] the fact that transmission does occur, little as it may be, does not in itself distinguish between an electrostatic-inductive mechanism, caused by the electronegativity of the phenyl and *p*-nitrophenyl groups, or a conjugation mechanism, or a combination of both. Plausible as the latter may seem, the data do not favor one possibility over the other, although in this particular reaction, as in electrophilic substitution in substituted biphenyls,[3] the development of conjugation in the transition state would favor the reaction. If conjugation is responsible for the transmission, at the demand of the reaction, it must be of a very small order of magnitude.

The effect of the phenyl substituents in the *para* position of *o*-nitrobromobenzene is somewhat larger in the nucleophilic displacement reaction than in the reactions of the biphenylcarboxylic acids and esters, as would be expected from a reaction involving a direct attack on the benzene ring. A ρ-value[10] is not available for the reaction of substituted *o*-nitrobromobenzenes under the chosen conditions, but for the similar nucleophilic displacement in piperidine alone, rho is +4.95. With this value, the sigma values for 4-phenyl and 4-*p*-nitrophenyl are calculated as +0.070 and +0.29, respectively, both somewhat larger than sigmas obtained from the corresponding side-chain reactions.[3]

Experimental

The biphenyl derivatives were prepared from the corresponding amines by the Sandmeyer reaction according to the procedure of Hodgson and Walker.[11] The following modification of the mononitration of 4-acetylaminobiphenyl[2] was found to afford satisfactory results. Dry, pulverized 4-acetaminobiphenyl (20 g.) was suspended in 280 ml. of acetic anhydride in a one-l., three-necked flask, equipped with thermometer, dropping funnel and stirrer. The nitrating mixture, carefully prepared by adding in the cold 15 ml. of acetic anhydride to 15 ml. of concd. nitric acid, was added slowly through the dropping funnel, keeping the temperature at 0–5°. The material gradually dissolved and the temperature was not allowed to rise above 20°. A yellow solid soon precipitated, and the contents of the flask were poured into a 2-l. beaker half filled with ice. The filtered and thoroughly washed nitro compound weighed 24.1 g. (98%). Four crystallizations from ethanol afforded 18.1 g. (74%) of long yellow needles, melting at 130–132° (uncor.). Hydrolysis[2] of the above compound afforded the amine (98%) in the form of red, small needles of m.p. 167–169° (uncor.). Vacuum-distilled 4-bromo-3-nitrobiphenyl was crystallized from methanol to give small, peach-colored needles of m.p. 43.2–43.7° (cor.) in 52.1% yield (lit.[2] 41–42°). 4-Bromo-3,4′-dinitrobiphenyl, prepared from 3,4′-dinitro-4-aminobiphenyl[12] in an 88% yield of crude product, was crystallized twice from benzene and three times from acetone, and the yellow needles (42%) melted at 202.5–205.3° (cor.) (lit.[13] 207–208°, 210–211°). *o*-Nitrobromobenzene was a commercial sample and was crystallized three times from 70% ethanol; m.p., 40.9–41.3° (cor.) (lit.[4] 40.9–41.3°). Piperidine[14] and dioxane[15] were purified according to published procedures.

The kinetic runs were conducted as described before,[4] except that the amine was dissolved in previously thermostated dioxane in a 5-ml. volumetric flask, 2 ml. of piperidine, also thermostated, was added from a pipet, and the solution was filled to the mark with dioxane. The flask was thoroughly shaken and returned to the thermostat (25 ± 0.05°); the initial time of the reaction was taken from the mid-point of delivery of the piperidine, and the whole

(4) E. Berliner and L. C. Monack, THIS JOURNAL, **74**, 1574 (1952).

(5) J. F. Bunnett, F. Draper, P. R. Ryason, P. Noble, R. G. Tonkyn and R. E. Zahler, *ibid.*, **75**, 642 (1953).

(6) J. Miller, *J. Chem. Soc.*, 3550 (1952); B. A. Bolto and J. Miller, *Chem. & Ind.*, 640 (1953).

(7) C. W. L. Bevan, *J. Chem. Soc.*, 655 (1953).

(8) A better comparison between benzene and biphenyl in this reaction could be made if a ρ value for the nucleophilic displacement in the biphenyl series were available. Not enough substituents were studied to establish rho for this reaction, but taking only σ for *p*-nitro (the phenolic value) and *p*-H, ρ for the biphenyl reaction is +0.86, or about 5.8 times less than rho in the benzene series[4] (assuming that the change in solvent from piperidine to a mixture of piperidine and dioxane does not significantly alter rho). With the non-phenolic sigma value for *p*-nitro, rho becomes +1.4 and is 3.5 times less than rho in the benzene series.

(9) N. McLeish and N. Campbell, *J. Chem. Soc.*, 1103 (1937). For instance, see H. W. Talen, *Rec. trav. chim.*, **47**, 329 (1928).

(10) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940, Chapter VII.

(11) H. H. Hodgson and J. Walker, *J. Chem. Soc.*, 1620 (1933).

(12) R. J. W. Le Fevre and E. E. Turner, *ibid.*, 244 (1928).

(13) F. Case, THIS JOURNAL, **64**, 1850 (1942).

(14) T. E. Young and E. D. Amstutz, *ibid.*, **73**, 4773 (1951).

(15) L. F. Fieser, "Experiments in Organic Chemistry," 2nd ed., D. C. Heath and Co., Boston, Mass., 1941, p. 368.

contents of one flask were used in one kinetic determination. The concentration of I was about 0.00049 mole, that of II about 0.0004 mole and of III 0.00024 mole, the largest amount that could be dissolved. The concentrations did not affect the rate constants within that range, because the use of lower concentrations for the first two compounds (0.00038 for I and 0.00027 for II) did not alter them. Rate constants were calculated from the integrated form of the first-order rate equation; the errors reported in Table I are average deviations. Data for one determination are listed in Table II. With an initial concentration of 0.0002736 of bromo compound the rate constant was 2.81×10^{-5} sec.$^{-1}$.

TABLE II

THE REACTION BETWEEN 3-NITRO-4-BROMOBIPHENYL WITH PIPERIDINE IN DIOXANE AT 25°

Time, sec.	Moles × 10^4 of bromide	$(a - x) \times 10^4$	$k \times 10^5$, sec.$^{-1}$
3600	4.042	3.629	(2.99)
7200	4.020	3.267	2.88
9000	4.017	3.099	2.88
12600	4.017	2.799	2.87
15720	4.009	2.538	2.91
18060	4.042	2.412	2.86
21660	4.060	2.182	2.87
25020	4.070	2.022	2.80
31440	4.048	1.644	2.87
31980	4.035	1.631	2.83
50100	4.042	0.946	2.90

Results qualitatively similar to those reported in Table I were obtained in preliminary experiments conducted in boiling benzene (40 ml.) to which 4 ml. of piperidine had been added.

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Absence of Wall Effects in a Typical α-Chymotrypsin Catalyzed Hydrolysis[1]

BY RICHARD A. BERNHARD AND CARL NIEMANN[2]

RECEIVED AUGUST 20, 1954

In the past[3,4] it has been tacitly assumed that α-chymotrypsin catalyzed hydrolyses, which are conducted under the conditions ordinarily used in *in vitro* studies of the mode of action of this enzyme, proceed entirely in solution and that wall effects, arising from the interaction of the reactants with the walls of the container, are unimportant. However, it appears that no one has ever determined whether or not the above assumption is a valid one. Therefore, we have, in this investigation, examined a representative α-chymotrypsin catalyzed hydrolysis with respect to possible wall effects arising from the nature and surface area of the container.

The initial velocities were determined by the method of Jennings and Niemann from both zero- and first-order plots,[5] for the α-chymotrypsin catalyzed hydrolysis of acetyl-L-tyrosinhydroxamide,[4,6,7] in aqueous solution at 25° and *p*H 7.6 and 0.3 *M* in the THAM[8] component of a THAM–HCl buffer, under conditions where the enzyme concentration was maintained at 0.0266 mg. protein-nitrogen/ml., *i.e.*, *ca.* 0.76×10^{-5} *M*,[9] the initial specific substrate concentration at 10×10^{-3} *M*, and where only the nature or the surface area of the container was varied. It will be seen from the data presented in Table I, for experiments 1 to 3, inclusive, that there is no significant difference in the initial velocities when either Pyrex or Kimble glass or polyethylene containers of equivalent surface area were employed. Furthermore, the addition of powdered Kimble glass to either the Kimble or Pyrex glass containers, or powdered Pyrex glass to the Pyrex container (*cf.* Table I, experiments 4 to 8, inclusive) was without effect even though the increase in surface area was of the order of twenty-fold in the extreme cases.

TABLE I

α-CHYMOTRYPSIN CATALYZED HYDROLYSES OF ACETYL-L-TYROSINHYDROXAMIDE[a]

Expt.	Nature of vessel	Powd. glass added Nature	Weight[b]	Total area[c]	v_0[d]
1	Pyrex glass	.....	..	22	0.160
2	Kimble glass	.....	..	22	.159
3	Polyethylene	.....	..	22	.156
4	Kimble glass	Kimble[e]	0.30	83	.153
5	Kimble glass	Kimble	1.50	412	.157
6	Kimble glass	Pyrex[f]	0.20	61	.160
7	Pyrex glass	Pyrex	0.27	81	.157
8	Pyrex glass	Pyrex	1.33	406	.157
				Average of all values	0.157

[a] In aqueous solutions at 25° and *p*H 7.62 and 0.3 *M* in the THAM component of a THAM–HCl buffer, [E] = 0.0266 mg. protein–nitrogen/ml., $[S]_0 = 10 \times 10^{-3}$ *M*. [b] In g. [c] In units of cm.^{2} [d] In units of 10^{-3} *M*/min. [e] 150–200 mesh, density 2.5 g./cm.^{3}. [f] 150–200 mesh, density, 2.25 g./cm.^{3}.

Therefore, it may be concluded from the results of this study that, for the case at hand, wall effects are experimentally unimportant under the conditions which are employed generally in *in vitro* studies with α-chymotrypsin and that it is reasonable to assume that in all α-chymotrypsin catalyzed reactions which are studied under these conditions the reaction can be postulated as proceeding in solution in so far as can be determined within the limits of experimental error.

The average of the eight values of v_0 which are given in Table I, *i.e.*, 0.158×10^{-3} mole/min., may be compared with the value of $0.166 \pm 0.028 \times 10^{-3}$ mole/min. calculated on the basis of [E] = 0.0266 mg. protein-nitrogen/ml., $[S]_0 = 10 \times 10^{-3}$ *M*, $K_S = 43 \pm 4 \times 10^{-3}$ *M* and $k_3 = 33 \pm 3 \times 10^{-3}$ *M*/min./mg. protein-nitrogen/ml.[4]. The fact that these two values agree, within the limits of experimental error, can be taken as evidence of the consistency of the value of v_0 reported in this communication with those determined earlier.[4,6,7]

Experimental

Containers.—The Pyrex and Kimble glass containers used in this study were standard 10-ml. glass-stoppered volumetric flasks which had been cleaned with hot water containing a detergent and then thoroughly washed with distilled water. The polyethylene container was a 60-ml. screw cap bottle which had been treated similarly.

(1) Supported in part by a grant from the National Institutes of Health, Public Health Service.

(2) To whom inquiries regarding this article should be sent.

(3) H. Neurath and G. W. Schwert, *Chem. Revs.*, **46**, 69 (1950).

(4) R. J. Foster and C. Niemann, THIS JOURNAL, **77**, in press.

(5) R. R. Jennings and C. Niemann, *ibid.*, **75**, 4687 (1953).

(6) D. S. Hogness and C. Niemann, *ibid.*, **75**, 884 (1953).

(7) R. J. Foster and C. Niemann, *Proc. Natl. Acad. Sci.*, **39**, 999 (1953).

(8) Tris-(hydroxymethyl)-aminomethane

(9) Based upon a molecular weight of 22,000 and a nitrogen content of 16.0% for monomeric α-chymotrypsin.[4]