is obtained from the initial rate and the rate at 7% conversion.

It is of interest to note that if the equations employed above are applicable to the data obtained from the system employing 15% nitrobenzene, 85%carbon tetrachloride, the values obtained for the ratio of k_{t_1}/k_{t_2} from the degree of polymerization and from the rate expression are in the same order of magnitude as that obtained from the system employing 30% nitrobenzene, 70% carbon tetra-chloride. From the data for the 15% nitrobenzene case, the values obtained for k_{t_1}/k_{t_2} from the degree of polymerization and from the rate measurements are 2.1×10^{-2} mole $1.^{-1}$ and 2.5×10^{-2} mole $1.^{-1}$, respectively. The ratio of $k_{\rm tr}/k_{\rm p}$ obtained from the degree of polymerization data for the 30%nitrobenzene system has been employed in deriving k_{t_1}/k_{t_2} from the degree of polymerization data for the 15% nitrobenzene system. Since this ratio was the same value obtained by Endres and Overberger¹¹ for the system employing a nitrobenzene mixture (40% by volume) at a temperature of 0°, it is unlikely that the value would change in going from a 30 to a 15% mixture of nitrobenzene at 25°. The assumption of a constant value may therefore be made.

The corrected experimental curve for the effect of water on the maximum rate of polymerization for the system employing 15% nitrobenzene, 85% carbon tetrachloride is given in Fig. 6, curve a.

The various ratios of rate constants derived above may also be calculated from the data obtained without employing the corrected water concentration mentioned above. If this is done, it is found that there is essentially no difference between the constants derived from the degree of polymerization data whether the corrected or uncorrected water concentrations are employed, since it can be seen from the results obtained that the degree of polymerization is little affected by small changes of the water concentration. However, a difference between the corrected and uncorrected values obtained for the constants derived from the rate data is found and this is understandable since it has been observed that the rate of polymerization varies considerably with small changes of the water concentration.

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BROOKLYN 1, N. Y.

Synthesis of *m*-Methoxynaphthylamines as Precursors for Chromogenic Substrates

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Because of the expected greater rate of coupling as compared to naphthylamine, 3-methoxy-1-naphthylamine and 4methoxy-2-naphthylamine have been synthesized for use in the preparation of thromogenic substrates for the histochemical demonstration of amidases and peptidases. The chloroacetamides, trifluoroacetamides and L-leucyl-4-methoxy-2-naphthyl amide also were prepared. The last derivative is a superior reagent for the histochemical demonstration of leucine amino peptidase.

One of the principles most widely used for demonstrating the presence of hydrolytic enzymes in tissue sections and homogenates involves the two-step process

substrate enzyme

coupling component + diazonium salt \longrightarrow azo dye (2)

The coupling components² are aromatic hydroxy compounds^{3a} or amines,^{3b} usually of the naph-

(1) This investigation was supported by a research grant from the National Cancer Institute, National Institutes of Health, Department of Health, Education and Welfare.

(2) (a) The term "coupling component" follows the usage of H. Zollinger, Chem. Revs., **51**, 347 (1952). (b) V. Defendi and A. G. E. Pearse, J. Histochem. & Cytochem., **3**, 203 (1955), use the term "primary reaction product."

(3) (a) G. Wolf and A. M. Seligman, THIS JOURNAL, 73, 2080
(1951); A. M. Seligman, H. H. Chauncey, M. M. Nachlas, L. H. Manheimer and H. A. Ravin, J. Biol. Chem., 190, 7 (1951); M. M. Nachlas, A. Young and A. M. Seligman, J. Histochem. & Cytochem., 5, 565 (1957). (b) G. Gomori, Proc. Soc. Exptl. Biol. Med., 87, 559
(1954); M. N. Green, K. C. Tsou, R. Bressler and A. M. Seligman, Arch. Biochem. & Biophys., 57, 458 (1955); M. M. Nachlas, D. T.

thalene series. Whereas the reaction rate of the second step may be permitted to vary widely in the estimation of enzymes in homogenates, its value assumes critical importance in histochemistry. It has been observed^{2b,3b,4} that the definition of areas of enzymatic activity becomes increasingly sharp as the rate for the second step increases. Thus, for histochemical applications, it may be advantageous to accelerate reaction 2, even at the cost of retarding, somewhat, reaction 1. This is especially important when the coupling component produced in reaction 1 is soluble and diffusible.

The Hammett's equation relationship between the structures of m and p-substituted benzenediazonium salts and their second-order reaction rates with a given coupling component has been demonstrated,⁵ but the influence of structural variation in the coupling component has not been studied systematically. It is quite likely, moreover, that Crawford and A. M. Seligman, J. Histochem. & Cytochem., 5, 264 (1957).

(4) S. J. Holt, *ibid.*, 4, 541 (1956); V. Defendi, *ibid.*, 5, 1 (1957).
(5) H. Zollinger, *Helv. Chim. Acta*, 36, 1730 (1953).

[[]Contribution from the Department of Chemistry of The Johns Hopkins University and the Departments of Surgery of The Sinai Hospital of Baltimore and The Johns Hopkins University School of Medicine]

coupling component + other products (1)

such relationships would be complicated by steric effects or other factors.⁶ On the basis of modern theory,⁷ as well as of evidence in the benzene series,⁸ there was reason to believe that reaction 2 might be accelerated by introduction of an electron-releasing group ortho or para to the site of coupling. The methoxy group appeared suitable for our purposes, since hydroxy, amino and alkylamino groups would be unsuitable, because they would allow coupling with unhydrolyzed substrate. Thus, in place of the naphthylamine derivatives currently employed as histochemical substrates for amidases and peptidases,^{3b} it was proposed that derivatives of the hitherto unknown 3-methoxy-1naphthylamine and 4-methoxy-2-naphthylamine be tried. Similarly, in place of the simple naphthols, it was hoped that 3-methoxy-1-naphthol and 4-methoxy-2-naphthol could be made available. In this latter endeavor we have not yet been successful.

The *meta* di-substituted naphthalenes are accessible only with difficulty. After most of the present study had been completed, recent work by Vertalier and Sannié closely bearing on the problem came to our attention.⁹ These authors appear to have obtained superior over-all yields of 3-nitro-1-naphthol, one of the required intermediates.

Although the *m*-methoxynaphthylamines evidence far greater coupling reactivity than the simple naphthylamines, their chloroacetyl and trifluoroacetyl derivatives were hydrolyzed too slowly for use as amidase substrates,¹⁰ whereas the corresponding derivatives of 1- and 2-naphthylamine react satisfactorily with the enzyme.^{10,11} Since the L-leucyl derivative of 1-naphthylamine is not hydrolyzed by leucine aminopeptidase,^{3b} the corresponding 3-methoxy derivative was not prepared, but only the leucyl amide of 4-methoxy-2-naphthylamine. This compound proved to be a superior substrate for demonstrating the enzyme histochemically, because of the better localization and the stability of the azo dye copper chelate.¹⁰

Experimental

1,3-Dinitronaphthalene.—N-1-Naphthyl-*p*-toluenesulfonamide¹² was nitrated¹³ to give N-2,4-dinitro-1-naphthyl*p*-toluenesulfonamide. Deamination of 0.4 mole of this intermediate was carried out according to the procedure of Hodgson and Birtwell,¹⁴ and the crude product, containing copper oxide, was extracted with several portions of boiling acetone (totaling 1.3 liters), rather than ethylene dichloride,¹⁴ decolorized with charcoal and precipitated with water.

decolorized with charcoal and precipitated with water. **3-Nitro-1-naphthylamine**.—Other members of this research group,¹⁵ despite repeated efforts, had been able to

(7) C. K. Ingold, "Structure and Mechanism in Organic Chemistry," Cornell Univ. Press, Ithaca, N. Y., 1953, p. 247; L. A. Wiles, *Chem. Revs.*, **56**, 329 (1956).

(8) K. H. Saunders, "The Aromatic Diazo-Compounds and their Technical Applications," 2nd ed., Edward Arnold & Co., London, 1949, pp. 200-201.

- (9) S. Vertalier and C. Sannié, Bull. soc. chim. France, 234 (1954).
 (10) M. M. Nachlas, D. H. Rosenblatt, B. Monis and A. M. Seligman, unpublished data.
- (11) G. Gomori, Proc. Soc. Expil. Biol. Med., 85, 570 (1954).
 (12) G. T. Morgan and E. D. Evens, J. Chem. Soc., 115, 1126 (1919).
- (13) H. H. Hodgson and E. W. Smith, ibid., 1854 (1935).
- (14) H. H. Hodgson and S. Birtwell, *ibid.*, 433 (1943).
- (15) B. M. Anderson and D. Dennis, unpublished data.

isolate only traces of 3-nitro-1-naphthylamine by mono-reduction of 1,3-dinitronaphthalene according to the directions of Hodgson and Birtwell.¹⁶ A more reliable procedure was sought. Attempted use of the catalytic method of was sought. Attempted use of the catalytic method of Veselý and Rein¹⁷ gave none of the desired product. Monoreduction with sodium hydrogen sulfide reagent prepared according to Hodgson and Ward18 succeeded the first time, but on subsequent trials gave very poor yields of products requiring extensive purification. In the latter cases the pHof the reagent was found to be quite high (11.6), even considering that it contained a large proportion of methanol. Bubbling hydrogen sulfide through the reagent brought the pH down to about 8.6, with a slight yellow color showing; this treatment led to the desired results. The following procedure was therefore used: Forty grams of sodium sulfide nonahydrate was dissolved in 60 ml. of water and diluted to 100 ml.; 14 g. of powdered sodium bicarbonate was added to the stirred solution, while the temperature was kept below 20°. To the well-cooled solution 100 ml. of methanol was now added, and stirring was continued 30 minutes. The solution was filtered and the copious precipitate washed with 55 ml. of methanol. The volume of the combined precipitate and washings was adjusted to 240 ml. with methanol. Just before the reagent was to be used, hydrogen sulfide was bubbled in, with stirring, until the solution had a slight yel-low tinge. Approximately 210 ml. of this reagent was added to a stirred suspension of 20 g. of 1,3-dinitronaphthalene in 160 ml. of refluxing methanol over a period of 15 minutes. After another 15 minutes of refluxing, the mix-ture was chilled well and filtered. The precipitate was washed with a little methanol-water (1:1) and with water, and sucked dry. The nearly pure orange 3-nitro-1-naphthylamine on the filter was recrystallized from ethanol-water (yield about 55% of theory, m.p. 135.5-136.5°). From the filtrate of the reaction mixture, on dilution with water, a mixture of monoreduction products was isolated, which could be worked up according to known procedures.16,19 The employment of deficient amounts of the sodium hydrogen sulfide reagent may cause contamination of the product with starting material. In such case, the amine can be ex-tracted with boiling 9% hydrochloric acid and recovered by neutralization. Excessive quantities of the reagent diminish the yield, but apparently not the purity of the 3nitro-1-naphthylamine.

3-Nitro-1-naphthol.—As remarked by Sannié and Vertallier,⁹ addition of diazotized 3-nitro-1-naphthylamine to boiling water²⁰ gives very little of the desired 3-nitro-1naphthol. We tried boiling 30% sulfuric acid, with a resulting yield of about 10%, but did not increase the sulfuric acid concentration to the point that the aforementioned authors had.⁹ Instead, the conversion was effected by heating 1 g. of 3-nitro-1-naphthylamine with 56 ml. of 6% hydrochloric acid in a sealed tube at $160-165^{\circ}$ for 5 hours. The dark resinous product was extracted with a large volume of boiling water, from which the yellow 3-nitro-1-naphthol crystallized on cooling in a yield of about 82% (m.p. 169- 170° , literature⁹ m.p. 169°), with an additional 5% recoverable by extraction of the mother liquors with ether. This compound tends to take on an electrostatic charge.

1-Methoxy-3-nitronaphthalene.—The preceding compound (12.3 g.) was methylated²¹ by heating at reflux with 200 ml. of acetone, 30 g. of anhydrous potassium carbonate and 13 ml. of dimethyl sulfate for three hours, during which time the initially deep red-brown solution became perceptibly lighter. Water was added, acetone removed on the steam-bath, and the residue taken up with ethyl ether and washed with water until the washings were neutral. Evaporation of the solvent left an almost pure product, which was recrystallized from alcohol-water to give clusters of small yellow needles, m.p. 104–104.5°, in 93% yield. The compound was also prepared, in a lower yield, by treatment of 3-nitro-1-naphthol with diazomethane.

(17) V. Veselý and E. Rein, Collection Czechoslov. Chem. Communs., 1, 360 (1929).

- (18) H. H. Hodgson and E. R. Ward, J. Chem. Soc., 242 (1948); 1187 (1949).
- (19) H. H. Hodgson and D. E. Hathway, ibid., 385 (1944).
- (20) V. Veselý and K. Dvorák, Bull. soc. chim. France, [4] 33, 329 (1923).
- (21) Method of L. R. Row and T. R. Seshadri, Proc. Indian Acad. Sci., 21A, 155 (1945); 22A, 215 (1945).

⁽⁶⁾ R. Pütter, Angew. Chem., 63, 188 (1951); H. Zollinger, Helv. Chim. Acta, 38, 1597 (1955).
(7) C. K. Ingold, "Structure and Mechanism in Organic Chemis-

⁽¹⁶⁾ H. H. Hodgson and S. Birtwell, J. Chem. Soc., 75 (1944).

Anal. Caled. for $C_{11}H_9NO_3$: C, 65.0; H, 4.46; N, 6.9. Found: C, 65.1; H, 4.46; N, 6.7.

4-Methoxy-2-naphthylamine.—A suspension of 1-methoxy-3-nitronaphthalene (5 g.) in methanol (150 ml.) was hydrogenated over 10% palladium-on-charcoal (0.1 g.) at an initial hydrogen pressure of 2 atm. After filtration of the solution from catalyst, the methanol was distilled and the residue extracted with ligroin or petroleum ether (the latter giving a purer product) to give long large colorless prisms, m.p. 57.5-58.5°, which showed a strong blue fluorescence in methanol solution.

Anal. Caled. for $C_{11}H_{11}\rm{NO}$: C, 76.3; H, 6.40; N, 8.1. Found: C, 76.2; H, 6.28; N, 8.0.

N-4-Methoxy-2-naphthylchloroacetamide.—To a solution of 0.36 g. of 4-methoxy-2-naphthylamine in 10 ml. of acetone there was added 0.4 ml. of chloroacetyl chloride. A precipitate formed immediately, with evolution of heat. After the initial reaction had subsided, 40 ml. of 2.5% aqueous sodium bicarbonate was added gradually, with swirling, whereby the initial precipitate dissolved and a new solid precipitate fell out. This crude product was isolated and recrystallized from aqueous ethanol, giving fine colorless needles, m.p. $153-154^{\circ}$.

Anal. Caled. for C₁₃H₁₂O₂NCl: C, 62.5; H, 4.85; N, 5.6. Found: C, 62.5; H, 4.81; N, 5.6.

N-4-Methoxy-2-naphthyltrifluoroacetamide.—To a solution of 0.36 g. of 4-methoxy-2-naphthylamine in 10 ml. of acetone there was added 1.0 ml. of trifluoroacetic anhydride, with evolution of some heat. After 5 min., 40 ml. of 2.5% aqueous sodium bicarbonate was added gradually, with swirling, to precipitate an oil. The oily layer was taken up in ether, washed with water and evaporated to a viscous residue. Recrystallization from ligroin gave a solid precipitate (m.p. $131-134.5^\circ$), which was recrystallized from aqueous ethanol (charcoal) to give colorless microscopic needles, m.p. $133.5-135^\circ$.

Anal. Caled. for $C_{13}H_{10}O_2NF_3$: C, 58.0; H, 3.74; N, 5.2. Found: C, 58.0; H, 4.03; N, 5.3.

Carbobenzoxy-L-leucyl-4-methoxy-2-naphthylamide.— The synthesis was carried out with equimolar quantities of reagents according to the method of Sheehan, Goodman and Hess.²² A solution of 2.51 g. of N,N'-dicyclohexylcarbodiimide in 15 ml. of dichloromethane was added to 3.22 g. of carbobenzoxy-L-leucine and 2.10 g. of 4-methoxy-2-naphthylamine dissolved in 10 ml. of the same solvent. Reaction was immediate and rapid. After one hour, the mixture was filtered and the precipitate of dicyclohexylurea was washed with ether. The combined filtrate and washings were boiled down to give a pinkish solid residue. Trituration of the product with ether removed most of the unreacted start-

(22) J. C. Sheehan, M. Goodman and G. P. Hess, THIS JOURNAL, 78, 1367 (1956).

ing materials without dissolving much of the desired product. The latter was taken up in ethyl acetate (35 ml.), washed with 1 N hydrochloric acid, 1 N potassium bicarbonate and water, filtered and evaporated nearly to dryness. Addition of petroleum ether gave a white precipitate of carbobenzoxy-t-leucyl-4-methoxy-2-naphthylamide. This substance began to shrink at 169° and melted at 171–171.6°.

Anal. Caled. for C₂₅H₂₈N₂O₄: C, 71.4; H, 6.71; N, 6.7. Found: C, 71.5; H, 6.66; N, 6.9.

L-Leucyl-4-methoxy-2-naphthylamide.—The carbobenzoxy group was removed²³ from the preceding compound (1.52 g.) by hydrogenolysis for 3 hours in methanol solution (150 ml.) at 2 atm. pressure of hydrogen with a catalyst of 10% palladium-on-charcoal (0.1 g.). The mixture was filtered from catalyst and boiled down to a mobile oily residue. Extraction of this oil with boiling petroleum ether gave, on chilling, an oily precipitate which soon changed to a highly crystalline solid, m.p. 96.5–97.5°, $[\alpha]^{28}D + 15.1°$ (c 2.02, methanol).

Anal. Calcd. for $C_{17}H_{22}N_2O_2$: C, 71.3; H, 7.75; N, 9.8. Found: C, 71.3; H, 7.62; N, 9.9.

3-Methoxy-1-nitronaphthalene.—Methylation of 4-nitro-2-naphthol¹² by the method used for preparing 1-methoxy-3nitronaphthalene gave small yellow plates, m.p. 101-102° (literature¹² m.p. 100-103°).

3-Methoxy-1-naphthylamine.—The method used above for hydrogenating 1-methoxy-3-nitronaphthalene was applied to 3-methoxy-1-nitronaphthalene. The compound, after crystallization from ligroin, was purified for analysis by recrystallization (charcoal) from petroleum ether. The needle-like prisms begin to sinter at 78° and melt at 79– 79.5°. The compound shows a strong blue fluorescence in methanol.

Anal. Calcd. for $C_{11}H_{11}NO;\ C,\,76.3;\ H,\,6.40;\ N,\,8.1.$ Found: C,76.0; H,6.36; N,7.9.

N-3-Methoxy-1-naphthylchloroacetamide.—Prepared analogously to the isomeric derivative above, this compound occurred as very small needles melting at 144–145°.

Anal. Calcd. for $C_{13}H_{12}O_2NCl$: C, 62.5; H, 4.85; N, 5.6. Found: C, 62.2; H, 4.75; N, 5.7.

N-3-Methoxy-1-naphthyltrifluoroacetamide.—Prepared similarly to the isomeric derivative above, this compound was isolated as fine needles melting at 119–120°.

Anal. Calcd. for $C_{13}H_{10}O_2NF_3$: C, 58.0; H, 3.74; N, 5.2. Found: C, 57.8; H, 3.84; N, 5.3.

Acknowledgment.—The authors wish to acknowledge the assistance rendered by Mr. Joseph Walter, who performed the analyses.

(23) M. Bergmann and L. Zervas, Ber., 65, 1192 (1932).

BALTIMORE, MARYLAND

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The Oxidation of Unsaturated Compounds. V. The Effect of Oxygen Pressure on the Oxidation of Styrene^{1,2}

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The reaction of styrene at 50° in the presence of 0.01 M 2,2'-azobis-(2-methylpropionitrile) (ABN) has been studied at oxygen pressures ranging from 0-3200 mm. In the absence of oxygen, styrene is converted to polystyrene at the rate of 0.095 mole/l./hr. Above about 10 mm. pressure, styrene reacts at a nearly constant rate of 0.06 mole/l./hr., and the product is mostly styrene polyperoxide, $(C_3H_SO_2)_n$. The rate of reaction of styrene passes through a minimum of 0.03 mole/l./hr. at about 0.5 mm. pressure of oxygen. The $O_2: C_3H_8$ ratio in the polymer increases from zero to nearly unity as the oxygen pressure increases from 0-100 mm. Other products of oxidation also depend on pressure. A maximum of about 27% of the reacting styrene is converted to styrene oxide at 1 mm. pressure, and up to 46% to benzaldehyde and formaldehyde at 12 mm. pressure. Mechanisms for these reactions are discussed. The rate of reaction with oxygen or a peroxide radical of a radical ending in a styrene unit depends on the penultimate unit. The decomposition of styrene polyperoxide and the oxygen inhibition of bulk and emulsion polymerization are also discussed.

(1) Portions of these papers were presented at Meetings of the American Chemical Society at Minneapolis on September 13, 1955, at Atlantic City on September 18 and 20, 1956, and at New York on September 9 and 11, 1957.

(2) Because of repeated cross references in this and the four succeeding papers, sections, tables, figures, equations and footnotes are each numbered serially throughout papers V to IX.

(3) Stanford Research Institute, Menlo Park, California.