and the more acidic 2-chloroethanol, 4-nitro-*N*-sulfinylaniline reacted with all of the alcohols studied. These results suggested that the rate of reaction of *N*-sulfinylaniline with alcohols is apparently increased by the substitution of an electron withdrawing group on the aromatic nucleus. In view of a recent report[3] indicating that 4-methoxy-*N*-sulfinylaniline does not react with ethyl alcohol, we wish to report the results of our continuing study which show that a variety of *N*-sulfinylanilines react rapidly and completely with methyl alcohol.

The ultraviolet spectra of both aromatic and aliphatic *N*-sulfinylamines have been determined in anhydrous methyl alcohol to determine the reactivity of these compounds with this solvent. The spectra of aromatic *N*-sulfinylamines indicated rapid and complete reaction. The *N*-sulfinylanilines studied were: 2- and 3-nitro-; 4-bromo-; 4-iodo-; 4-methyl-; 2,4-dimethyl-; 2,6-dimethyl-; and 4-*sec*-amyl-. This study shows that even highly hindered *N*-sulfinylanilines containing electron donating groups (*e.g.*, 2,6-dimethyl-*N*-sulfinylaniline) can undergo rapid reaction. The rapid rate of reaction of all of the above compounds precluded a study of the effect of substituents on the rate of reaction.

The aliphatic *N*-sulfinyl amines showed little or no reaction with methyl alcohol. The aliphatic *N*-sulfinylamines studied were: *n*-heptyl-; *tert*-butyl-; cyclohexyl-; 2-chloroethyl-; and 3-chloropropyl-.

To account for this difference in reactivity of the aromatic and aliphatic *N*-sulfinylamines it is proposed that an increase in the base strength of the *N*-sulfinylamino group of the aliphatic *N*-sulfinylamines causes a decrease in the reaction rate. *N*-Sulfinyl-*tert*-butylamine showed no reaction with methyl alcohol. This can be attributed to both the increased basicity of the amine and to steric hindrance of the bulky *tert*-butyl group.

EXPERIMENTAL

The ultraviolet spectra were determined using either a Beckman DU spectrophotometer or a Beckman DK recording spectrophotometer. Solutions of the *N*-sulfinylamines containing about 5×10^{-3} g./l. were measured in 1- or 2-cm. cells.

When each of the *N*-sulfinylanilines discussed above was dissolved in anhydrous methyl alcohol the spectrum obtained was essentially identical with that of the parent aniline dissolved in methyl alcohol.

SPECTRA OF RNSO

R	In CH_3OH		In cyclohexane	
	log ϵ		log ϵ	
tert-Butyl	233	3.10	233	3.03
n-Heptyl	232–234	3.24	235	3.67[a]
Cyclohexyl	237–238	2.99	235	3.83[a]
$ClCH_2CH_2$—	230	3.27	233	3.55
$Cl(CH_2)_3$—	231	3.49	231	3.82

[a] In ether solution.

The spectra of the aliphatic *N*-sulfinylamines discussed are given below.

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Reaction of Styrylquinolines with Sulfhydryl Compounds[1]

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The observation that 4-(4-dimethylaminostyryl)-quinoline (I)[2-4] and its methiodide[5] caused regression of Lymphoma 8 tumors in rats led to a study of the fate of these compounds in the animals. It was discovered that the deep purple quaternary salt was converted into a nearly colorless compound in the small intestine.[6] An aqueous solution of the latter compound produced a spot on filter paper which became deeply colored again after exposure to air and heat, especially in the presence of a trace of base. It was suggested that the original dye was reduced to a leuco form, or that amino acids in the contents of the small intestine might be reacting with it. Glycine did not decolorize a solution of the hydrochloride of I, but cysteine and mercaptoacetic acid each did. Glutathione, cysteineamine, and *p*-thiocresol also decolorized acid solutions of I.

Gilman, Towle, and Ingham[7] were able to reduce the ethylene double bond in 4-styrylquinoline and in 2-styrylquinoline, but not in 2-(4-dimethylaminostyryl)-8-methylquinoline, by a boiling solution of *p*-thiocresol in xylene. When their method was applied to I, a white crystalline reduction product melting at approximately 110° was obtained. The same compound was obtained by reduction with hydrogen and Raney nickel at room temperature and 45 p.s.i., or by reduction with a boiling solution

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of potassium benzylate in benzyl alcohol.[8] The analysis indicated that two hydrogen atoms had been added to the parent compound, and the ultraviolet absorption lacked the strong peak at 410 mμ attributed to the ethylene double bond. This compound did not revert to I on exposure to air and was not readily soluble in water.

Reaction of I with cysteine in 6% (by volume) acetic acid produced a compound which was soluble in ammonium hydroxide or in acetic acid, but which gradually deposited crystals of the original free base. The ammonium hydroxide solution produced a colorless spot which became red on standing in air.

It was possible to follow the decolorization reaction in a colorimeter, and to learn that it was more rapid in 10% (by volume) acetic acid than in glacial acetic acid, but did not seem to take place at all in neutral methanol, although hydrogen sulfide did produce a reversible decrease in the color of a neutral aqueous solution of 4-(4-dimethylaminostyryl)quinoline methochloride.

In glacial acetic acid at 29°, with initial concentration of styrylquinoline 0.005 mg./ml. and cysteine 6 mg./ml., the reactions seemed to be pseudo-first order, but to approach equilibrium when 1/2 to 3/4 of the original dye had been converted to a colorless form. The half reaction times found graphically by extrapolation ranged from fifty hours to one and a half hours in the order I > 2-(4 - dimethylaminostyryl)quinoline > 1-(4 - dimethylaminostyryl)isoquinoline > 1-(4 - morpholinostyryl)isoquinoline. Experiments carried out in 10% acetic acid indicated, as was expected, that increasing the concentration of cysteine produced a roughly proportional increase in reaction rate, and also an increase in the proportion of the decolorized dye. In one series of experiments the effect of differences in *p*H was studied by addition of different quantities of sodium acetate to reaction mixtures containing the same concentrations of acetic acid and of cysteineamine hydrochloride and measuring the rate of decolorization of I. At *p*H 4.1 the reaction was three times as rapid as at *p*H 3.2 and fifteen times as rapid as at *p*H 2.2. (The lower *p*H levels reduced the concentration of styrylquinoline mono-cations by forming di-cations and also decreased the concentration of RS^- anions.) These observations suggested that the key reaction was the addition of an RS^- ion to the ethylene double bond in an aminostyrylquinoline cation. It appeared that a bulky substituent, even a methyl group, at the 5- or 3- position on the quinoline ring should interfere with the addition. 4-(4-Dimethylaminostyryl)-5,6-benzoquinoline did react with cysteine much more slowly than I did, and so did 4-(4-dimethylaminostyryl)-3-methylquinoline (II). It was interesting to note that the reverse reaction, the decomposition of the addition compound, was retarded to a similar extent.

The significance of these observations in relation to the problem of the biological action of the styrylquinolines is not certain, but it is interesting that Myleran, used clinically in treatment of leukemia, also reacts with the —SH group in cysteine, as Roberts and Warwick[9] have announced recently. It is possible that surface phenomena may increase the stability of the addition compounds at protein surfaces, so that aminostyrylquinolines might interfere with some sulfhydryl enzyme. There is also a possibility that they may function as enzyme-like catalysts, catalyzing an unnatural reaction in the cell.

EXPERIMENTAL

4-[2-(4-Dimethylaminophenyl)ethyl]quinoline. A mixture of 4.00 g. of I and 6.00 *g.* of *p*-thiocresol was heated 16 hr. at 190–205°, under nitrogen. The mixture was dissolved in 100 ml. benzene, washed repeatedly with aqueous sodium hydroxide, then with water, and dried over sodium sulfate. (Acidification of the sodium hydroxide washings precipitated 2.5 g. of recovered *p*-thiocresol.) The hydrochloride of the reduction product was precipitated by passing hydrogen chloride gas into the benzene solution and was dried by a stream of nitrogen, wt. 3.4 g. The free base was liberated by addition of alcoholic ammonium hydroxide and was recrystallized from isohexane and from diluted ethanol, white crystals, m.p. 105.3–108.0°, yield 30%.

Anal. Calcd. for $C_{19}H_{20}N_2$: C, 82.57; H, 7.29. Found: C, 82.48, 82.35; H, 7.34, 7.28.[10]

The same compound was obtained by reduction of I with hydrogen. A solution of 12.0 g. of I in 150 ml. of 95% ethanol, with 11.5 g. of Raney nickel catalyst took up the theoretical amount of hydrogen at 45 p.s.i. and 25–30° within 2.5 hr. The catalyst was removed by filtration and the product was precipitated by addition of water. The yellow solid was dissolved in benzene and passed through an alumina column. Evaporation of the proper fraction of solution and crystallization from octane, followed by treatment with carbon in isopropyl ether, produced white crystals, m.p. 110.5–111.6°, yield 14%.

A solution of 11.2 g. of potassium hydroxide in 110 ml. of benzyl alcohol was distilled until 10 ml. of benzyl alcohol had been collected, then 0.1 mole of I was added to the solution and refluxed 3 hr. The product was separated from the potassium compounds by adding 100 ml. of water and 600 ml. of isopropyl ether, then shaking the water layer with a second portion of the ether. The residue obtained by evaporation of the ether and vacuum distillation of the benzyl alcohol was dissolved in isopropyl ether, treated with carbon, and recrystallized repeatedly from isopropyl ether to form white crystals, m.p. 110.5–111.6°, yield 24%.

Anal. C, 82.36, 82.42; H, 7.30, 7.19.[10]

Reaction of 4-(4-dimethylaminostyryl)-3-methylquinoline with cysteine. To 0.504 g. of II in 10 ml. of glacial acetic acid was added 3.08 g. of L-cysteine in enough boiled water to bring the final volume to 100 ml. and 8.0 ml. more of acetic acid was added. The resulting solution was kept in the dark in a closed flask for a period of 6 months. During this time the color faded to a light pink. A small amount of white precipitate was recovered by filtering the solution, 100 mg., m.p. 225–227°. The filtrate was brought to *p*H 8 by addition of sodium hydroxide, then to *p*H 7 by cautious addition of

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(9) J. J. Roberts and G. P. Warwick, *Nature*, **183**, 1509 (1959).

(10) Analyses by Galbraith Microanalytical Laboratory.

acetic acid. A gummy precipitate appeared which was completely soluble in sodium hydroxide, indicating absence of the starting compound and of its dihydro derivative. The sodium hydroxide solution was brought back to *p*H 7 by acetic acid and the pale pink precipitate was washed with a little water and dried at 0.1 mm. at room temperature. The dried solid decomposed gradually at about 160–170°, giving off a gas and leaving a dark red liquid. A methanol solution of the compound produced a nearly colorless spot on filter paper. After exposure to air for several weeks the spot exhibited a salmon color similar to that produced by a solution of a known sample of II.

Anal. Calcd. for $C_{23}H_{27}N_3SO_2 \cdot 1^1/_2H_2O$: C, 63.56; H, 6.96; S, 7.38. Found: C, 63.44; H, 6.70; S, 7.25, 7.32.[10]

Reaction rates were compared by use of a Bausch and Lomb Spectronic 20 colorimeter. A measured volume of glacial acetic acid containing a known concentration of the styrylquinoline was mixed with a measured volume of boiled or distilled water or of glacial acetic acid containing a known weight of cysteine or cysteineamine hydrochloride and diluted to a predetermined final volume in a volumetric flask. After mixing, the clear sample was kept at approximately 20° in a cabinet between readings. The air was swept out of the flasks and tubes with butane gas to prevent oxidation of cysteine to cystine by oxygen, but sometimes a white deposit believed to be cystine did form in the test tubes. Also, the original cysteine contained a trace of cystine and it was usually necessary to filter or centrifuge the water or acetic acid solution before mixing it with the styrylquinoline solution. Optical densities were read at 480, 510, and 540 mμ. The wave length corresponding to the greatest absorption was not the same for all the compounds, but the rate of change of absorption with time differed little from one wave length to another. The logarithms of the optical densities were plotted against time. The quantity of cysteine used was so much larger than the quantity of the styrylquinoline that a psuedo first order reaction curve was obtained and the straight line drawn through the points near the beginning of the curve plotted on semilogarithmic paper was used to estimate by extrapolation the 50% reaction time. In fact, however, the curves eventually turned to parallel the time axis, indicating that an equilibrium was approached.

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Simplified Synthesis of the C-Terminal Tripeptide Sequence of Oxytocin

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A variety of preparative methods have been utilized in the synthesis of ethyl carbobenzoxy-L-prolyl-L-leucylglycinate and carbobenzoxy-L-prolyl-L-leucylglycinamide, intermediates in the synthesis of oxytocin.[1] However, in all of these methods the use of protected L-leucine is required and at some stage in the synthesis the protecting group has to be removed. The present report describes a more direct synthesis in which the blocking of the amino group of L-leucine is not necessary.

Carbobenzoxy-L-prolyl-L-leucine was prepared by the mixed anhydride method[2] from carbobenzoxy-L-proline and L-leucine. The recrystallized product agreed well in melting point and optical rotation with the product obtained on hydrolysis of methyl carbobenzoxy - L - prolyl - L - leucinate prepared by the nitrophenyl ester method.[1f] The protected dipeptide was coupled by the mixed anhydride method with ethyl glycinate hydrochloride to yield ethyl carbobenzoxy - L - prolyl - L - leucyl glycinate or with glycinamide hydrochloride to yield carbobenzoxy - L - prolyl - L - leucylglycinamide. Melting points and optical rotations of the protected tripeptides were in close agreement with literature values.

EXPERIMENTAL

All melting points were determined in capillary tubes and are corrected.

Methyl carbobenzoxy-L-prolyl-L-leucinate. Methyl L-leucinate hydrochloride[3] (10.9 g.) was dissolved in water (100 ml.). Potassium bicarbonate (8.5 g.) was added and the resulting mixture was extracted four times with 30-ml. portions of ethyl acetate. The combined ethyl acetate extracts were dried over anhydrous magnesium sulfate and filtered. *p*-Nitrophenyl carbobenzoxy-L-prolinate[1f] (18.5 g.) was added to the filtrate. The resulting solution was concentrated *in vacuo* to about 30 ml. and was left standing at room temperature for 3 days. Ethyl acetate (100 ml.) was added and the resulting solution was extracted with 30 ml. portions of *N* ammonium hydroxide until colorless extracts were obtained. The ethyl acetate solution was washed twice with water, was dried over anhydrous magnesium sulfate, and was filtered. The filtrate was concentrated *in vacuo* and hexane was added to the residue. The crystalline product was filtered off; wt. 14.9 g., m.p. 75.5–78°. This material was recrystallized from ethyl acetate (50 ml.) and hexane (150 ml.); wt. 10.7 g., m.p. 76.5–78°, $[\alpha]_D^{21}$ −69.0° (*c* 1, ethanol).

Anal. Calcd. for $C_{20}H_{28}O_5N_2$: C, 63.8; H, 7.50; N, 7.44. Found: C, 64.0; H, 7.62; N, 7.44.

Carbobenzoxy-L-prolyl-L-leucine. Method A. Carbobenzoxy-L-proline (12.5 g.) was dissolved in tetrahydrofuran (70 ml.). Triethylamine (7.5 ml.) was added and then the solution was cooled to −10°. Isobutyl chloroformate (6.8 g.) in tetrahydrofuran (30 ml.) was added. The solution was stirred at −10° for 20 min. Then a solution of L-leucine (7.9 g.) and triethylamine (12.6 ml.) in water (65 ml.) was added. Stirring was continued without further cooling for 90 min. The reaction mixture was acidified by the slow addition of concentrated hydrochloric acid. Tetrahydrofuran was removed *in vacuo* and the resulting solid was filtered off. It was dissolved in glacial acetic acid (20 ml.) and was added to water (200 ml.). The crystalline solid was filtered off; wt. 17.0 g. It was

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