162. *Aryl-2-halogenoalkylamines. Part VI. Reactions with Certain Organic Sulphur Compounds.*

By W. C. J. Ross.

The reaction of *NN*-di-2-chloroethyl-*p*-anisidine with 2-mercaptoethanol and thiophenol in aqueous alcohol solutions of different pH has been studied. Thioethers react with arylhalogenoalkylamines to give sulphonium compounds. 4-Aryl-substituted thiazans are conveniently prepared by the reaction of aryl-di-2-halogenoalkylamines and sodium sulphide in aqueous acetone.

THE reactivity of the carcinogenic and tumour-growth inhibitory arylhalogenoalkylamines towards various anions and amines was discussed in Parts III and IV (*J.*, 1949, 2589, 2824) of this series. In the present communciation some reactions of the halogenoalkylamines with certain organic sulphur compounds are described.

It has already been established in Part III that the carbonium ion derived from an " aromatic nitrogen mustard " could not react with an undissociated carboxyl group and it was thought that reaction with an undissociated thiol group was also improbable. To test this point it was necessary to allow the arylhalogenoalkylamines to react in solutions of different pH containing thiols of known dissociation constants. Since it had been found previously that aqueous acetone solutions could not be used for the quantitative study because of mercaptal formation it was necessary to determine the pK of typical thiols in aqueous alcoholic solution. Thiophenol and 2-mercaptoethanol were chosen as representative of aromatic and aliphatic thiols and found to have pK_a 8·05 and 10·8, respectively, in 50% alcohol.

Table I shows the results obtained when *NN*-di-2-chloroethyl-*p*-anisidine reacted with these thiols in solutions of varying pH. Under the experimental conditions all the halide will react and there is 100% excess over the amount required to react with the added thiol.

TABLE I.

Reaction of NN-*di-2-chloroethyl-*p-*anisidine in* 50% *ethanol with thiophenol and 2-mercaptoethanol. Concn. of amine* 0·01M. *Concn. of thiol* 0·01M. *Temp.* 85°. *Time* 30 *mins.*

Added salts.	pH change during reaction.	PhSH, % reacted.	$CH_2(SH)\cdot CH_2\cdot OH$, % reacted.
Nil	4·0—2·3	nil	nil
0·01N-HOAc + 0·01M-NaOAc	6·3—6·0	—	10
0·05M-NaOAc	8·6—6·6	90	33
0·20M-Na_2HPO_4	9·6—8·8	—	75
0·05M-Na_2CO_3	11·0—10·8	97	98

It is not easy to make experiments of this type very precise because in order to control the pH of the solution during the reaction various salts have to be added and the anions derived from each type will have characteristic competition factors towards the carbonium ions. The pH values, measured with a glass electrode, are only apparent values but as the pKs of the thiols were measured under identical conditions an estimate of the amount of thiol in the dissociated form can be made. Notwithstanding the imperfections of the method it is clear that the reaction with thiols is dependent on pH. In acid solutions where dissociation is suppressed there is no reaction but as the pH rises more thiol will ionise and as was expected more reaction occurs.

The results also indicate that a negatively charged sulphur atom is a powerful nucleophilic centre having a great affinity for the carbonium ion, since Table I shows considerable reaction with mercaptoethanol under conditions where only a small proportion of the thiol group will be dissociated. If this small fraction reacts rapidly with the halide then more will dissociate and eventually appreciable reaction will occur. A number of compounds which have high competition factors for carbonium ions, for example, dithiophosphates, mono- and di-thiophosphonates, thiosulphate, and dithiocarbamate (Ogston, *Trans. Faraday Soc.*, 1948, **44**, 45), are highly dissociated in solution giving ions containing, in effect, negatively charged sulphur atoms.

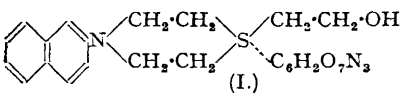
If only the dissociated form of a thiol can react then the competition factor for any thiol at a given pH should be $f \times F_{S^-}$, where f is the fraction of the thiol in the ionic form—an activity coefficient—and F_{S^-} is the competition factor of the ion. Some idea of the order of the competition factor of a negatively charged sulphur atom can be obtained from Ogston's figures for mustard gas. The competition factor of cysteine at pH 7 is given as $1{\cdot}2 \times 10^3$ and since

cysteine has $pK_a = 10{\cdot}5$ the value of f will be about $10^{-3{\cdot}5}$ at pH 7·0. The value of the competition factor F_{S^-} of the ionised sulph-hydryl group will be $1{\cdot}2 \times 10^3/10^{-3{\cdot}5}$ or about 4×10^5. It is interesting to note that the value of the competition factor for dithiophosphate at pH 7—when f will be approximately unity—is of the same order, namely $1{\cdot}3 \times 10^5$. This high value may be compared with the values for organic-acid anions which range from 20 to 100.

Quite generally, the competition factor of a compound at any pH will be given by the expression $f_n \times F_A$ where f_n is the fraction of the substance in the reactive form at pH $= n$, which can be calculated from the dissociation constant, and F_A is the competition factor of the reactive form A—this will be the anion for an acid or a thiol and the free base for an amine.

In a mixed biological system at physiological pH—usually about 7·5—the extent to which any substance will react with the carbonium ion derived from an " aromatic nitrogen mustard " will be proportional to $f_{7{\cdot}5} \times F_A \times c$ where c is the concentration of the substance being considered. When an arylhalogenoalkylamine is administered to an animal in the relatively low dosage required to inhibit tumour growth or to induce tumour formation, in the affected cells a small amount of reagent is distributed amongst a large excess of reacting groups. It is clear that the various substances present will react in proportion to the value of the expression given above. As the mustard-gas type of compound is not an efficient inhibitor of so-called SH enzymes (compare Dixon and Needham, *Nature*, 1946, **158**, 432) despite the high value of F_{S^-} one must conclude that the value of c for the ·SH group in the tissues involved is low. Another factor is that the ·SH groups in proteins are situated on the relatively short cysteine side-chains and may not be as accessible for reaction as the more exposed carboxy- and amino-groupings. Consideration of the factors mentioned above suggests that the groups most likely to react are the acid groups of proteins and the phosphoryl and aromatic type amino-groups in nucleic acids.

The isolation of *NN*-di-2-phenylthioethyl-*p*-anisidine from the reaction between *NN*-di-2-chloroethyl-*p*-anisidine and thiophenol in alkaline aqueous acetone solution at 66° was described in Part IV; the same product is obtained when the reaction is carried out at 37° and the β-*naphthyl* derivative has now been prepared in a similar manner from β-naphthyldi-2-chloroethylamine. No crystalline product has been isolated from the reaction between *NN*-di-2-chloroethyl-*p*-anisidine and mercaptoethanol but when sodium picrate is added to the product of the reaction between β-naphthyldi-2-chloroethylamine and this thiol a small quantity of the *picrate* (I) of a thiazan derivative is obtained. In Part IV it was shown that when the chlorine atom on one side-chain of an aryl-dihalogenoalkylamine was replaced by ·NHR the rate of ionisation of the second chlorine atom was increased. It was of interest to discover the effect of replacing the first chlorine atom by ·SR. Unfortunately it was not possible to ascertain this effect in as direct a way as that used in the case of the amines since the reaction has to be carried out in the presence of a buffer salt and the total halide reacting cannot be determined directly in the presence of the thiol. Table II shows the results obtained when *NN*-di-2-chloroethyl-*p*-anisidine reacted under various conditions in 50% acetone: an excess of thiol is present in this experiment so that the small loss by mercaptal formation is not significant.



(I.)

TABLE II.

Reaction of NN-*di-2-chloroethyl*-p-*anisidine in* (a) 50% *acetone*; (b) 50% *acetone containing sodium acetate* (0·20M.); (c) *and* (d) *as* (b) *but also containing thiophenol* (0·04M.). *Concn. of amine*, 0·01M. *Temp.* 66°. *Time:* (a), (b), *and* (c), 30 *mins.*, (d) 2 *hours.*

	(*a*).	(*b*).	(*c*).	(*d*).*
H^+, %	58	6·8	76	95
Cl^-, %	58	79·5	—	—

* No extra acidity was developed if this solution was heated for a longer period, hence all the halide had reacted.

Column (*d*) indicates that the carbonium ion derived from the arylhalogenoalkylamine reacts with the thiol—and possibly to a small extent with water—and the acetate anion in the ratio 95 : 5. If one makes the reasonable assumption that thiol and acetate react in the same ratio in (*c*) then the total extent of carbonium-ion formation can be shown to be 80% in 30 minutes. It was shown in Part III that the amount of chloride ion produced when a dihalide reacted in the presence of 0·2M-sodium acetate closely represented the actual extent of the formation of the carbonium ion. Column (*b*) indicates that under the conditions chosen,

78·5% of the dihalide ionises; this is practically identical with the extent of ionisation in the presence of thiol. It can therefore be concluded that the replacement of the first chlorine atom in an aryldi(halogenoalkyl)amine by ·SR does not significantly affect the rate at which the second chlorine atom ionises.

The possible formation of sulphonium compounds by the reaction of halogenoalkylamines with thioethers is of interest because of the natural occurrence of such ethers, *e.g.* methionine. When *NN*-di-2-chloroethyl-*p*-anisidine reacts at 66° in aqueous acetone containing an excess of phenyl methyl sulphide titration of the liberated hydrogen and chloride ions indicates that about 9% of the halide has formed a sulphonium salt, the reaction of the halide with the thioether giving chloride ion but no hydrogen ion :

$$NR_2{\cdot}CH_2{\cdot}CH_2Cl \longrightarrow NR_2{\cdot}CH_2{\cdot}\overset{+}{C}H_2 + Cl^- + R'SR'' \longrightarrow NR_2{\cdot}CH_2{\cdot}CH_2{\cdot}\overset{+}{S}\begin{matrix}R'\\R''\end{matrix}$$

Approximately the same proportion of β-naphthyldi-2-chloroethylamine reacts to form sulphonium salt at 37°. In the presence of dibutyl sulphide the *p*-anisidine derivative reacts to give 26% of the sulphonium salt. The readier formation of sulphonium halides from dialkyl sulphides than from aryl alkyl sulphides has already been noted (cf. Hickinbottom, "Reaction of Organic Compounds," London, 1940, p. 114). The formation of the thiazan derivative (I) involves internal sulphonium salt formation in the monosubstitution product.

Good yields of aryl-substituted thiazans are obtained when aryldi-2-halogenoalkylamines are heated with aqueous acetone solutions of sodium sulphide. The method appears to be of general application and is a very convenient one for the preparation of such thiazans as 4-phenyl-, 4-m- and 4-p-*tolyl*-, 4-p-*chlorophenyl*-, 4-p-*methxoyphenyl*-, and β-*naphthyl-thiazan.*

There is some discrepancy in the literature concerning the properties of 4-phenylthiazan. Helfrich and Reid (*J. Amer. Chem. Soc.*, 1920, **42**, 1208, quoted in Heilbron and Bunbury's "Dictionary of Organic Compounds," London, 1943) obtained a compound, m. p. 108—111°, by the action of 2-dichloroethyl sulphide on aniline in the cold whilst Okáč (*Chem. Listy*, 1934, **28**, 227) obtained an oil when the same reactants were heated under reflux with sodium acetate and sodium carbonate in ethanol solution. Korshak and Strepikheev (*J. Gen. Chem. Russia*, 1944, **14**, 312) prepared the thiazan as a solid, m. p. 32·3—32·6°, by heating *NN*-di-2-chloroethylaniline with sodium sulphide in absolute alcohol and Cerkovnikov and Stern (*Archiv Kem.*, 1946, **18**, 12) obtained the same product by a similar method using *NN*-di-2-bromoethylaniline hydrobromide. The present results are in agreement with the findings of the Russian workers.

Experimental.

Determination of the pK_a *of Thiophenol and 2-Mercaptoethanol.*—2-Mercaptoethanol (780 mg.) or thiophenol (1·10 g.) was dissolved in aqueous ethanol (200 ml.; 50% by volume) and titrated with potassium hydroxide solution (0·82N. in 50% ethanol). The pH of the solution was measured after each addition of alkali by use of a glass-electrode system with a Cambridge portable electrometer valve pH meter. The results for 2-mercaptoethanol were :

ml. of KOH.	% neutralisation.	pH.	pK_a.	ml. of KOH.	% neutralisation.	pH.	pK_a
0	0	7·1	—	7·5	53	10·8	10·8
1·5	11	9·9	10·8	9·0	63	11·0	10·8
3·0	21	10·2	10·8	10·5	74	11·4	10·9
4·5	31	10·4	10·8	12·0	84	12·0	—
6·0	42	10·6	10·7				

The average pK_a value of 2-mercaptoethanol in 50% ethanol is 10·8 and the corresponding value for thiophenol is 8·05.

Reaction of NN-*Di-2-chloroethyl*-p-*anisidine with Mercaptoethanol and Thiophenol in* 50% *Ethanol.*—*NN*-Di-2-chloroethyl-*p*-anisidine (500 mg.), contained in a small specimen tube, was added to boiling aqueous ethanol (200 ml.; equal volumes) containing either mercaptoethanol (156 mg.) or thiophenol (200 mg.), and the mixture was heated under reflux for 30 minutes. (A thermometer in the boiling liquid registered 85°.) The cooled solution was diluted with an equal volume of water and, after the addition of glacial acetic acid (1 ml.), the amount of unreacted thiol present was determined by titration with 0·1N-iodine (starch indicator). The experiment was repeated with various salts present; Table I shows the proportion of thiol reacting under different conditions. The pH of the solution was measured before and after the reaction on a parallel run using the method alread described.

Reaction of β-Naphthyldi-2-chloroethylamine with Thiophenol.—β-Naphthyldi-2-chloroethylamine (500 mg.), thiophenol (0·5 ml.), and sodium hydroxide (5 ml.; N.) were dissolved in aqueous acetone (500 ml.; 50%) and kept at 37° for 4 days. During this time the solution became turbid and oily droplets separated. After removal of the acetone under reduced pressure the product was extracted with benzene, and the dried extract was passed through a column of activated alumina. The eluates

contained ***β-naphthyldi-(2-phenylthioethyl)amine*** which crystallised from benzene–light petroleum (b. p. 60—80°) as prisms, m. p. 113—114° (Found : C, 74·9; H, 6·2. $C_{26}H_{25}NS_2$ requires C, 75·2; H, 6·1%).

Reaction of β-Naphthyldi-2-chloroethylamine with 2-Mercaptoethanol.—Naphthyldi-2-chloroethylamine (1·4 g.), mercaptoethanol (3 ml.), sodium hydroxide (25 ml.; N.), and 50% acetone (500 ml.) were heated under reflux for 5 hours. Removal of the acetone yielded an oil which was dissolved in aqueous methanol containing picric acid and sodium picrate. After several days a small quantity of *picrate* had separated from the solution; it recrystallised from acetone–methanol as rhombs, m. p. 200° (decomp.) (Found: C, 52·6; H, 4·2; N, 11·6. $C_{22}H_{22}O_8N_4S$ requires C, 52·6; H, 4·4; N, 11·2%).

Reaction of NN-*Di-2-chloroethyl*-p-*anisidine with Thiophenol in 50% Acetone Solution at* 66°.—*NN*-Di-(2-chloroethyl)-*p*-anisidine (500 mg.), dissolved in 50% acetone (200 ml.) (*a*) alone, (*b*) containing crystalline sodium acetate (5·44 g.), or (*c*) containing thiophenol (880 mg.) and sodium acetate (5·44 g.), was heated under reflux for 30 minutes. The cooled mixture was titrated first with 0·1N-sodium hydroxide (phenolphthalein indicator) and then, after the addition of glacial acetic acid (1 ml.), with 0·1N-iodine (starch indicator). The difference between these titres represents the amount of acid produced by the reaction of the arylhalogenoalkylamine. The results are shown in Table II.

Reaction of NN-*Di-2-chloroethyl*-p-*anisidine and β-Naphthyldi-2-chloroethylamine with Thioethers in 50% Acetone Solution at* 66°.—*NN*-Di-2-chloroethyl-*p*-anisidine (500 mg.), dissolved in 50% acetone (200 ml.) containing (*a*) phenyl methyl sulphide (620 mg.) or (*b*) dibutyl sulphide (730 mg.), was heated at 66° for 30 minutes. Titration of the hydrogen ion and chloride ion indicated (*a*) H, 52·5; Cl, 57·5%: thus 5/57·5 or 8·8% of the halide reacting formed a sulphonium salt, and (*b*) H, 43·0; Cl, 58·0%: *i.e.* 15/58 or 26% of the halide yielded a sulphonium compound.

When β-naphthyldi-2-chloroethylamine (536 mg.) was dissolved in 50% acetone (400 ml.) containing phenyl methyl sulphide (620 mg.) and the solution kept at 37° for 24 hours titration indicated H, 18·5; Cl, 20·5%: the amount of halide converted into sulphonium salt being 2/20·5 or 9·8%.

Preparation of 4-*Arylthiazans.*—*NN*-Di-2-chloroethyl-*p*-anisidine (1 g., 4 millimols.) and sodium sulphide (5 g.) dissolved in 50% acetone (200 ml.) were heated under reflux for 2 hours. The solid which separated from the cooled mixture after removal of the acetone under reduced pressure was crystallised from light petroleum (b. p. 40—60°). 4-p-*Methoxyphenylthiazan* was obtained as long prismatic needles, m. p. 76—77° (Found : C, 63·1; H, 7·3. $C_{11}H_{15}ONS$ requires C, 63·1; H, 7·2%). Other thiazans were obtained by a similar procedure except that longer times of reaction were required for the more slowly reacting arylhalogenoalkylamines (*e.g.*, 15 hours were allowed for the reaction with *p*-chloro-*NN*-di-2-chloroethylaniline). The following have been prepared : 4-Phenylthiazan, m. p. 31°; picrate, m. p. 141° (Korshak and Strepikheev, *loc. cit.*, give m. p. 141—142°). 4-*m*-Tolylthiazan, an oil which gives a *picrate* (prisms from acetone–methanol), m. p. 187—188° (Found : C, 48·7; H, 4·4. $C_{17}H_{18}O_7N_4S$ requires C, 48·4; H, 4·3%). 4-p-*Tolylthiazan* [prisms from light petroleum (b. p. 40—60°)], m. p. 33—35° (Found : C, 68·4; H, 8·0. $C_{11}H_{15}NS$ requires C, 68·4; H, 7·8%); *picrate* (large plates from acetone), m. p. 155° after sintering at 143° and resolidifying (Found : C, 48·7; H, 4·5%). 4-p-*Chlorophenylthiazan* [prisms from light petroleum (b. p. 40—60°)], m. p. 70—71° (Found : C, 56·2; H, 5·7. $C_{10}H_{12}NClS$ requires C, 56·2; H, 5·7%). 4-*β-Naphthylthiazan* [prisms from light petroleum (b. p. 60—80°)], m. p. 82—83° (Found : C, 73·1; H, 6·7. $C_{14}H_{15}NS$ requires C, 73·2; H, 6·6%)

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THE CHESTER BEATTY RESEARCH INSTITUTE,
FULHAM ROAD, LONDON, S.W.3.

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