114. *The Reaction of Phenylhydroxylamine and 2-Naphthylhydroxylamine with Thiols.*

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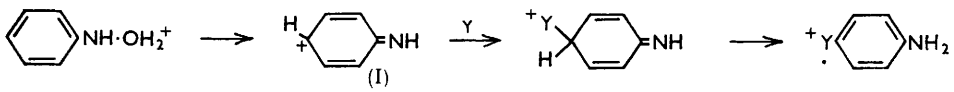
Phenylhydroxylamine with L-cysteine and *N*-acetyl-L-cysteine yields mixtures of *S*-*o*- and *S*-*p*-aminophenyl-L-cysteine and *o*- and *p*-aminophenylmercapturic acid, respectively. *N*-Acetylphenylhydroxylamine, similarly, yields the corresponding acetamidophenyl derivatives. The reactions with 2-naphthylhydroxylamine yield *S*-(2-amino-1-naphthyl)-L-cysteine and 2-amino-1-naphthylmercapturic acid. With glutathione, 2-naphthylhydroxylamine gives *S*-(2-amino-1-naphthyl)glutathione.

Recent investigations on the metabolism of some carcinogenic aromatic amines have shown that 2-acetamidofluorene,[1] 2-naphthylamine,[2] and 4-acetamidobiphenyl[3] are oxidised by *N*-hydroxylation. Carcinogenic aromatic amines, and therefore the reactions of arylhydroxyamines with biological materials, are of interest.

Bamberger and others[4] have shown that phenylhydroxylamine rearranges in acidic media to yield mainly *o*- and *p*-aminophenol, with varying amounts of azobenzene, azoxybenzene, aniline, and nitrosobenzene. When the reaction was carried out in the presence of nucleophiles other than water, *e.g.*, chloride ions, methanol, ethanol, or aniline, the corresponding *ortho*- and *para*-substituted chloroanilines, anisidines, phenetidines, and semidines, respectively, were also formed. Dewar[5] regarded the rearrangement as being intramolecular and electrophilic, but Heller, Hughes, and Ingold[6] showed that it was a nucleophilic intermolecular rearrangement, mainly of the S_Ni' type, in which the rate-



(I)

controlling step is the formation of the imide ion (I); Y is any suitable reagent. In the light of this mechanism, it is likely that the oxidation of aromatic amines[7] by perphosphoric acid to yield aminoaryl phosphates involves the initial formation of the corresponding arylhydroxylamine or its phosphate.

Although this mechanism adequately explains the purely acid-catalysed rearrangements of aromatic hydroxylamines it appears that other mechanisms, probably involving free radicals, also play a part. The presence of aniline, nitrosobenzene, azobenzene, and azoxybenzene among the products of reaction of phenylhydroxylamine in acidic or neutral media suggests an oxidation–reduction system. Similar products are also formed on thermal decomposition of phenylhydroxylamine.[8] Hydroxylamine also has a catalytic effect on the autoxidation of acraldehyde, styrolene, and sodium sulphite.[9]

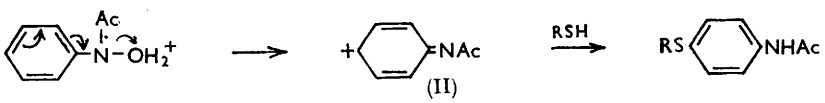
Phenylhydroxylamine reacts with L-cysteine or *N*-acetyl-L-cysteine, to yield *S*-aminophenyl-L-cysteine and aminophenylmercapturic acid, respectively. These compounds were also synthesised in the following ways: Diazotised *o*- and *p*-nitroaniline were treated with L-cysteine and *N*-acetyl-L-cysteine to yield *S*-*o*- and *S*-*p*-nitrophenyl-L-cysteine and *o*- and *p*-nitrophenylmercapturic acids, respectively. Reduction with zinc and acetic

[1] Cramer, Miller, and Miller, *J. Biol. Chem.*, 1960, **235**, 885; Miller and Miller, *Biochim. Biophys. Acta*, 1960, **40**, 380.
[2] Boyland and Manson, unpublished work.
[3] Wyatt, Miller, and Miller, *Proc. Amer. Assoc. Cancer Res.*, 1961, **3**, 279.
[4] Bamberger, *Annalen*, 1921, **424**, 233, 297; 1925, **441**, 207; Yukawa, *J. Chem. Soc. Japan*, 1950, **71**, 547.
[5] Dewar, "Electronic Theory of Organic Reactions," Oxford Univ. Press, 1949, p. 225.
[6] Heller, Hughes, and Ingold, *Nature*, 1951, **168**, 909.
[7] Boyland and Manson, *J.*, 1957, 4689.
[8] Muller and Lindemann, *Angew. Chem.*, 1933, **46**, 681.
[9] Moreu, Dufraisse, and Badoche, *Compt. rend.*, 1926, 823.

acid yielded the aminophenyl derivatives which, on acetylation, yielded the acetamidophenylmercapturic acids. By the addition of *o*- and *p*-aminothiophenol to acetamidoacrylic acid, *o*- and *p*-aminophenylmercapturic acids were obtained in high yield. L-Cysteine and *N*-acetyl-L-cysteine were also treated with *o*- and *p*-chloronitrobenzene in alkaline media, giving the nitrophenyl-L-cysteines and nitrophenylmercapturic acids. These substituted phenylmercapturic acids are crystalline, slightly soluble in water and the common organic solvents, and dissolve readily in dilute alkali. They are converted by hot N-sodium hydroxide into the corresponding thiophenols. In N-hydrochloric acid at 100°, acetamidophenylmercapturic acids are first converted almost quantitatively into the aminophenylmercapturic acids, the *para*-isomer being somewhat more resistant to hydrolysis than the *ortho*-isomer, then into *S*-aminophenylcysteines and degradation products. 2-Nitro-1-naphthylmercapturic acid was synthesised by the reaction of *N*-acetyl-L-cysteine with diazotised 2-nitro-1-naphthylamine, reduction with zinc in acetic acid yielding 2-amino-1-naphthylmercapturic acid.

An equimolar solution of phenylhydroxylamine and L-cysteine hydrochloride in 30% acetic acid, after 4 hours at 60° or several days at room temperature, gave *S*-*o*- and *S*-*p*-aminophenylcysteine, in addition to *o*- and *p*-aminophenol. The aminophenols were the main products; the *para*-isomer predominated in each case. Under similar conditions, and equimolar solution of phenylhydroxylamine and *N*-acetyl-L-cysteine in 30% acetic acid yielded *o*- and *p*-aminophenylmercapturic acid. *N*-Acetylphenylhydroxylamine with L-cysteine hydrochloride or *N*-acetyl-L-cysteine gave the corresponding *o*- and *p*-acetamidophenyl derivatives. In the presence of *N*-acetyl-L-cysteine in aqueous acetone or aqueous acetic acid 2-naphthylhydroxylamine yielded a compound which, by paper chromatography, appeared to be 2-amino-1-naphthylmercapturic acid. Similar compounds were produced by the reaction of 2-naphthylhydroxylamine with L-cysteine and glutathione. When 2-naphthylhydroxylamine was treated with hydrochloric acid only 2-amino-1-naphthol was detected. Similarly only one aminonaphthyl derivative of each of the thiols employed was detected.

These results are in agreement with the S_N1' mechanism postulated by Heller, Hughes, and Ingold.[6] It is interesting that the effect of the acetyl group on the intermediate ion (II) will be to confer resonance stabilisation by increased conjugation, compared with the ion (I).



(II)

When *N*-acetyl-L-cysteine or L-cysteine was added to an acetone solution of 2-naphthylamine treated with peracetic acid, compounds which appeared to be 2-amino-1-naphthylmercapturic acid and *S*-(2-amino-1-naphthyl)-L-cysteine, respectively, were formed. It is probable that peracetic acid oxidises 2-naphthylamine to 2-naphthylhydroxylamine which then reacts with the thiol in the manner described. It is also likely that the formation of 2-amino-1-naphthyl dihydrogen phosphate by the oxidation of 2-naphthylamine with peracetic acid in the presence of phosphoric acid, described by Boyland and Manson,[7] proceeds in the same way.

In view of the ease with which they give highly reactive intermediates and the variety of reactions which they undergo, aromatic hydroxylamines may be expected to react with suitable sites in biological systems as oxidising, reducing (cf. the ability of phenylhydroxylamine to oxidise or reduce hæmoglobin or methæmoglobin, respectively [10]), arylating, condensing, or metal-chelating agents. In view of the ability [11] of many nitrogen and

[10] Crick and Jackson, *Brit. J. Pharm. Chemotherapy*, 1953, **8**, 87; Kiese and Waller, *Arch. exp. Path. Pharmak.*, 1950, **211**, 345; Kiese and Reinwein, *ibid.*, 392, 402.
[11] Haddow, *Brit. Med. Bull.*, 1958, **14**, 79.

sulphur "mustards," epoxides, aromatic azides, polycyclic hydrocarbons, and diazo-compounds to provide electron-deficient intermediates capable of reacting with nucleophilic centres (*e.g.*, amino-, phosphate, or thiol groups) in biological systems during carcinogenesis, the known toxicity of aromatic hydroxylamines (and of the aromatic amines [1-3] which may produce them by metabolism) may be in some part due to reactions of such types *in vivo*.

The ability to form metal complexes shown by arylhydroxylamines, hydroxamic acids (which may be formed *in vivo* by the reaction of hydroxylamines with carboxylic acids [12] or phosphate esters), and other *N*-substituted arylhydroxylamines [13] (*e.g.*, cupferron) may change the normal functioning of certain enzyme systems. Their ability to condense with carbonyl groups [14] under mild conditions, to give either *N*-substituted hydroxylamines or nitrones, may alter the normal metabolic pathways of sugars and carbohydrates in the body.

Experimental

Paper Chromatography.—Chromatograms were run on Whatman No. 1 filter paper in the following solvents: (A) butan-1-ol–acetic acid–water (2 : 1 : 1); (B) butan-1-ol–propan-1-ol–ammonia (2N) (2 : 1 : 1); (C) di-isopropyl ether saturated with water.[15] Mercapturic acids and *S*-phenylcysteines were detected with (*a*) dichromate–silver nitrate spray [16] (yellow to brown colours); those containing free aromatic amino-groups were detected by (*b*) diazotisation followed by coupling with *N*-1-naphthylethylenediamine [17] (blue to purple colours) or with (*c*) 0·5% ethanolic *p*-dimethylaminobenzaldehyde containing 1% of concentrated hydrochloric acid (yellow); those containing aromatic acetamido-groups were first sprayed with 0·5N-hydrochloric acid, heated in an oven at 100° for 10—20 min., then sprayed by method (*b*) or (*c*). *S*-Substituted cysteines were also detected by (*d*) dipping the chromatogram in 1% ninhydrin in acetone, followed by heating at 100° for 5—15 min. (blue to purple). Aminophenols were detected with (*b*) (purplish-brown), (*c*) (yellowish-brown), or (*e*) a 2% solution of 2,6-dichloroquinone chloroimide in ethanol followed by 2N-sodium carbonate (blue). Other detecting reagents used were *p*-dimethylaminocinnamaldehyde [18] (2 g. in 100 ml. of 6N-hydrochloric acid and 100 ml. of ethanol), a Chromatolite lamp (Hanovia Ltd.), titanous chloride (15% w/v), and N-hydrochloric acid and sodium nitrite (0·5%) followed by hexylresorcinol (0·5% in 2N-sodium hydroxide).

S-o-*Nitrophenyl*-L-*cysteine.*—Zinc powder (4 g.) was heated with L-cystine (6·5 g.) in N-sulphuric acid (200 ml.) at 60° for 20 min., cooled, and filtered, and the filtrate was treated with freshly prepared cuprous oxide until no more dissolved. Diazotised *o*-nitroaniline (from 8 g.) in N-sulphuric acid (300 ml.) was added in 30 min. to the cuprous solution with stirring at 0°, and the mixture was kept at 0° for 2 hr., then left at room temperature overnight, and filtered. The filtrate was stirred with charcoal (10 g.), and the charcoal was filtered off and washed with water until free from sulphate. The adsorbed material was eluted with a 5% solution of ammonia (*d* 0·88) in methanol (300 ml.), and the solvent removed by distillation, to yield S-o-*nitrophenyl*-L-*cysteine*, yellow needles (5·5 g.) (from water), m. p. 164° (decomp.) (Found: C, 44·8; H, 4·0; N, 11·5; S, 12·5. $C_9H_{10}N_2O_4S$ requires C, 44·6; H, 4·2; N, 11·6; S, 13·2%).

o-*Nitrophenylmercapturic Acid.*—Acetic anhydride (3 ml.) was added dropwise in 10 min. with shaking to *S*-*o*-nitrophenyl-L-cysteine (1·2 g.) in 2N-sodium hydroxide (35 ml.) at 0°, the mixture was filtered after 1 hr., and the filtrate acidified with 3N-hydrochloric acid to pH 2 and poured into ice-water (50 ml.), to give the mercapturic acid (0·9 g.) which crystallised as yellow needles (from 10% aqueous ethanol), m. p. 157° (decomp.) (lit.,[19] m. p. 156—158°) (Found: C, 46·6; H, 4·3; N, 9·7; S, 11·7. Calc. for $C_{11}H_{12}N_2O_5S$: C, 46·5; H, 4·3; N, 9·8; S, 11·3%).

[12] Grossowicz, Wainfan, Borek, and Waelsch, *J. Biol. Chem.*, 1950, **187**, 111; Virtanen and Berg, *Acta Chem. Scand.*, 1951, **5**, 509.
[13] Schome, *Current. Sci.*, 1944, **13**, 257.
[14] Hellman and Teichmann, *Chem. Ber.*, 1956, **89**, 1134; Banfield and Kenyon, *J.*, 1926, 1612; Angeli and Bigiavi, *Atti Accad. naz. Lincei, Rend. Classe Sci. fis. mat. nat.*, 1927, **5**, 819; McQueen, U.S.P. 2,426,894/1947.
[15] Parke, *Biochem. J.*, 1960, **77**, 493.
[16] Knight and Young, *Biochem. J.*, 1958, **70**, 111.
[17] Bratton and Marshall, *J. Biol. Chem.*, 1939, **128**, 537.
[18] Harley-Mason and Archer, *Biochem. J.*, 1958, **69**, 60P.
[19] Bray, James, and Thorpe, *Biochem. J.*, 1956, **64**, 38.

S-o-*Aminophenyl*-L-*cysteine*.—A solution of *S*-*o*-nitrophenyl-L-cysteine (0·6 g.) in 2N-ammonia (10 ml.) was added to a 40% ferrous sulphate solution (15 ml.) at 80°, kept thereat for 5 min., treated with ammonia (*d* 0·88; 1 ml.), shaken for 5 min., cooled, filtered, acidified with acetic acid to pH 4, stirred with charcoal (2 g.), and filtered again. The charcoal was washed with water (500 ml.) and stirred with a 5% solution of ammonia (*d* 0·88) in methanol (100 ml.), and the methanolic ammonia solution obtained on filtration was evaporated to dryness, yielding colourless needles (0·2 g.; from water) of *S*-*o*-aminophenyl-L-cysteine, m. p. 238° (decomp.) (lit.,[20] m. p. 240°) (Found: C, 50·8; H, 5·7; N, 13·0; S, 14·8. Calc. for $C_9H_{12}N_2O_2S$: C, 50·9; H, 5·7; N, 13·2; S, 15·1%).

o-*Aminophenylmercapturic Acid*.—Zinc powder (1 g.) was shaken with a solution of *o*-nitrophenylmercapturic acid (1 g.) in 50% acetic acid (20 ml.) until the supernatant layer was colourless. The mixture was filtered and stirred with charcoal (2 g.), the charcoal was washed and eluted with methanolic ammonia (as above) (100 ml.), and the methanolic ammonia solution distilled *in vacuo*, to yield colourless needles (0·6 g.) of *ammonium* o-*aminophenylmercapturate*, m. p. 146° (decomp.) (from methanol–ether) (Goodman, Ross, and Baker [20] report m. p. 145° for the free acid) (Found: C, 48·9; H, 6·3; N, 15·9; S, 12·0. $C_{11}H_{17}N_3O_3S$ requires C, 48·7; H, 6·3; N, 15·5; S, 11·8%). When a 20% aqueous solution of the ammonium salt was adjusted to pH 4 with 4N-hydrochloric acid, the free acid, m. p. 145°, crystallised as needles. This substance was also obtained in the following manner: α-Acetamidoacrylic acid (1 g.) in dry dioxan (20 ml.) containing piperidine (1 ml.) was heated with *o*-aminothiophenol (1 g.) in dioxan (20 ml.) under reflux for 2 hr. The solvent was removed *in vacuo* and the gummy residue extracted with ether (2 × 10 ml.). The residual gum was dissolved in water (5 ml.) containing a few drops of aqueous ammonia, and the solution was filtered, adjusted to pH 4 with 4N-hydrochloric acid, and stored overnight at 0°. *o*-Aminophenylmercapturic acid (1·5 g.), m. p. and mixed m. p. 145°, crystallised.

o-*Acetamidophenylmercapturic Acid*.—*o*-Aminophenylmercapturic acid (0·5 g.), suspended in acetic anhydride (2 ml.) at 0°, was treated dropwise with dry pyridine (0·3 ml.) and after 16 hr. at 0° was diluted with water (5 ml.) and extracted with ether (2 × 5 ml.), and the aqueous layer was acidified to pH 3 with 4N-hydrochloric acid, yielding o-*acetamidophenylmercapturic acid* (0·5 g.) which crystallised as needles (from ethanol), m. p. 182° (decomp.) (Found: C, 52·5; H, 5·9; N, 9·4; S, 11·1. $C_{13}H_{16}N_2O_4S$ requires C, 52·7; H, 5·5; N, 9·5; S, 10·8%).

S-p-*Nitrophenyl*-L-*cysteine*.—This was obtained as yellow needles (3·5 g.), m. p. 173—175° (from water) (lit.,[20] m. p. 175°), by a reaction analogous to that described for preparation of the *ortho*-isomer, from L-cysteine (6·5 g.) and *p*-nitroaniline (8·3 g.) (Found: C, 44·7; H, 4·1; N, 11·7; S, 12·8. Calc. for $C_9H_{10}N_2O_4S$: C, 44·6; H, 4·2; N, 11·6; S, 13·2%).

p-*Nitrophenylmercapturic Acid*.—*S*-*p*-Nitrophenyl-L-cysteine (1·5 g.) in 2·5N-sodium hydroxide (50 ml.) and acetic anhydride (4 ml.) gave, as described for the *ortho*-isomer, *p*-nitrophenylmercapturic acid, as yellow needles (1·3 g.), m. p. 167° (decomp.) (lit., m. p. 156—158°,[19] 168—170° [20]) (Found: C, 46·5; H, 4·3; N, 9·8; S, 11·3%).

S-p-*Aminophenyl*-L-*cysteine*.—As in the preparation of the *ortho*-isomer, *S*-*p*-nitrophenyl-L-cysteine (0·6 g.) in 40% ferrous sulphate–ammonia solution gave colourless needles (0·25 g.) of S-p-*aminophenylcysteine*, m. p. 248—251° (decomp.) (Found: C, 50·4; H, 5·7; N, 12·8; S, 14·4. $C_9H_{12}N_2O_2S$ requires C, 50·9; H, 5·7; N, 13·2; S, 15·1%).

p-*Aminophenylmercapturic Acid*.—As in the preparation of the *ortho*-isomer, *p*-nitrophenylmercapturic acid (1·2 g.) gave *ammonium* p-*aminophenylmercapturate* (0·8 g.) as needles (from methanol–ether), m. p. 165° (decomp.) (Found: C, 48·9; H, 6·3; N, 15·4; S, 11·3%). The *acid*, obtained from the ammonium salt and from *p*-aminothiophenol (1·5 g.) and α-acetamidoacrylic acid (1 g.), as described above, had m. p. and mixed m. p. 155—156° (decomp.) (Found: C, 51·8; H, 5·5; N, 11·5; S, 12·7%).

p-*Acetamidophenylmercapturic Acid*.—*p*-Aminophenylmercapturic acid (0·5 g.) with acetic anhydride (2 ml.) and pyridine (0·3 ml.) (cf. above) p-*acetamidophenylmercapturic acid* (0·2 g.), needles (from ethanol), m. p. 188—190° (decomp.) (Found: C, 52·6; H, 5·4; N, 9·2; S, 9·9%). When *p*-aminophenylmercapturic acid (0·2 g.) in methanol (10 ml.) was treated with acetic anhydride (1 ml.) and a few drops of 6N-hydrochloric acid, *methyl* p-*acetamidophenylmercapturate* was obtained as plates (0·12 g.), m. p. 176° (decomp.) (Found: C, 54·7; H, 5·4; N, 8·6. $C_{14}H_{18}N_2O_4S$ requires C, 54·1; H, 5·8; N, 9·0%).

2-*Nitro*-1-*naphthylmercapturic Acid*.—Cuprous oxide (3·5 g.; commercial) was added with

[20] Goodman, Ross, and Baker, *J. Org. Chem.*, 1958, **23**, 1251.

shaking to *N*-acetyl-L-cysteine (5·0 g.) in N-sulphuric acid (150 ml.) at about 70°. The mercaptide tended to separate but was redissolved by addition of concentrated hydrochloric acid (5 ml.). The solution was cooled to 0° and added during 30 min. to a cooled solution of diazotised 2-nitro-1-naphthylamine, prepared as follows.[21] Sodium nitrite (2·1 g.) was added at 0—10° with stirring to cooled concentrated sulphuric acid (15 ml.). The mixture was heated to 70° and cooled again. A solution of 2-nitro-1-naphthylamine (5·5 g., Fundamental Research Company, Berkeley, California, U.S.A.; also prepared by the method of Saunders and Hamilton [22]) in glacial acetic acid (80 ml.) was added at 10° to the nitrite solution in 20 min. with stirring. When the diazotised amine had been added to the mercaptide solution the whole was stirred for 2 hr. and left overnight at room temperature. A yellow precipitate and tar were filtered off. This mixture, which contained a little of the cysteine derivative, was dissolved in 2N-sodium hydroxide and extracted several times with ether. The aqueous phase was added to the filtrate obtained as above, the pH adjusted to 4·0, and activated charcoal (5 g.) added with stirring. After filtration the charcoal treatment was repeated and the combined charcoals were washed with water and then with methanolic ammonia (see above) (3 × 100 ml.). The methanol washings were evaporated to dryness *in vacuo*. The residue was dissolved in hot water in which, after cooling, yellow needles of *ammonium 2-nitro-1-naphthylmercapturate* formed. The compound recrystallised from 98% aqueous ethanol as needles (1·0 g.), m. p. 184—186° (decomp.) (Found: C, 50·9; H, 4·8; N, 11·8. $C_{15}H_{17}N_3O_5S$ requires C, 51·3; H, 4·9; N, 12·0%). Acidification of the aqueous mother-liquors with concentrated hydrochloric acid yielded more (0·7 g.) of the nitronaphthyl derivative as the free acid. The compound had R_F 0·9 in solvent A and R_F 0·43 in B. The spot gave a red colour with *p*-dimethylaminocinnamaldehyde after treatment with titanous chloride.

2-Amino-1-naphthylmercapturic Acid.—Zinc dust (0·5 g.) was added to a solution of 2-nitro-1-naphthylmercapturic acid (0·5 g.) in 50% aqueous acetic acid (20 ml.). The solution was stirred until it was colourless and then filtered, and stirred with charcoal (5 g.). The charcoal was washed with water, followed by methanolic ammonia (3 × 100 ml.). The methanol washings were evaporated to dryness and the residue dissolved in hot water (5 ml.), except for a yellow substance which was filtered off. The filtrate was acidified with glacial acetic acid. Rosettes of buff-coloured needles of *2-amino-1-naphthylmercapturic acid* (30 mg.), m. p. 173—175° (decomp.), then separated (Found: C, 58·6; H, 5·0; N, 9·0. $C_{15}H_{18}N_2O_3S$ requires C, 59·2; H, 5·3; N, 9·2%). The compound had R_F 0·88 in system A and R_F 0·43 in system B. The spot was fluorescent under ultraviolet light and became brown in sunlight. It gave a red colour with *p*-dimethylaminocinnamaldehyde and, after diazotisation, a yellow colour with hexylresorcinol. The compound was soluble in methanol, ethanol, and cold dilute alkali.

2-Naphthylhydroxylamine.—2-Nitronaphthalene (10 g.; Fundamental Research Company; also prepared by the method of Hodgson and Marsden [23]) was dissolved in ether (250 ml.) saturated with water, and aluminium amalgam (3 g.) was added during 20 min. Additional water was usually required to complete the reaction. When the reaction had finished the mixture was filtered and evaporated to dryness, without overheating. The residue was dissolved in hot chloroform and cooled. 2-Naphthylhydroxylamine (2 g.) crystallised as plates which were washed successively with cold chloroform and light petroleum (b. p. 40—60°). It had m. p. 135—137° (decomp.) (preheated to 120°); Baudisch and Fürst [24] gave m. p. 126°. On prolonged heating during crystallisation or exposure to light the compound became orange. By concentration of the mother-liquors and addition of light petroleum (b. p. 40—60°) further (generally less pure) crops were obtained. After 2-naphthylhydroxylamine had been heated with 2N-hydrochloric acid for 1—2 min., 2-amino-1-naphthol was detected by the green colour produced on the addition of concentrated ammonia solution; when this solution was shaken with benzene the organic layer became mauve.[25] No other aminonaphthols were detected by paper chromatography.

Reaction of Chloronitrobenzenes with L-*Cysteine and with* N-*Acetyl*-L-*cysteine.*—A solution of *p*-chloronitrobenzene (0·5 g.) in ethanol (10 ml.) containing pyridine (5 ml.) was added to a suspension of L-cysteine hydrochloride (0·5 g.) in pyridine (5 ml.), and the whole heated at 80°

[21] Cf. Hodgson and Walker, *J.*, 1933, 1620.
[22] Saunders and Hamilton, *J. Amer. Chem. Soc.*, 1932, **54**, 636.
[23] Hodgson and Marsden, *J.*, 1944, 22.
[24] Baudisch and Fürst, *Ber.*, 1917, **50**, 324.
[25] Liebermann and Jacobson, *Annalen*, 1882, **211**, 36.

for 2 hr. Paper chromatography revealed a compound having R_F 0·52 (solvent A) and 0·33 (solvent B) and giving colour reactions similar to those given by *S*-*p*-nitrophenyl-L-cysteine. No attempt was made to isolate the product. Similarly, *o*-chloronitrobenzene gave *S*-*o*-nitrophenyl-L-cysteine (R_F 0·51 in solvent A, and 0·30 in B). With *N*-acetyl-L-cysteine under similar conditions, *p*- (R_F 0·82 in A, and 0·40 in B) and *o*-nitrophenylmercapturic acid (R_F 0·79 in A, and 0·36 in B) were formed from the corresponding chloronitrobenzenes.

Reaction of Phenylhydroxylamine with L-*Cysteine Hydrochloride and* N-*Acetyl*-L-*cysteine.*—Phenylhydroxylamine (0·50 g.) was heated with L-cysteine hydrochloride (0·20 g.) in 30% acetic acid (15 ml.) at 60° for 4 hr. A similar mixture was left at room temperature for 5 days. Paper chromatography revealed the presence of *o*- and *p*-aminophenol (R_F in C, *o*- 0·78, *p*- 0·36), *S*-*p*-aminophenylcysteine (R_F 0·33 in A, 0·71 in B), and *S*-*o*-aminophenylcysteine (trace) (R_F 0·53 in A, 0·75 in B) in both mixtures. Under similar conditions, solutions of phenylhydroxylamine (0·50 g.) and *N*-acetylcysteine (0·80 g.) formed, in addition to *o*- and *p*-aminophenol, *p*- (R_F 0·67 in A, 0·20 in B) and *o*-aminophenylmercapturic acid (trace) (R_F 0·76 in A, 0·32 in B).

Reaction of N-*Acetylphenylhydroxylamine with* N-*Acetyl*-L-*cysteine.*—Under conditions similar to those described for phenylhydroxylamine, solutions of *N*-acetylphenylhydroxylamine (0·37 g.) and *N*-acetyl-L-cysteine (0·40 g.) formed *o*- and *p*-acetamidophenols (R_F in A, *o*- 0·86, *p*-, 0·80; in B, *o*- 0·81, *p*- 0·87), *p*-acetamidophenylmercapturic acid (R_F 0·80 in A, 0·29 in B), and *o*-acetamidophenylmercapturic acid (trace) (R_F 0·82 in A, 0·35 in B). The mixture which was heated at 60° also contained traces of *o*- and *p*-aminophenol and *o*- and *p*-aminophenylmercapturic acid which were produced by acid hydrolysis of the acetamido-derivatives.

Conversion of Acetamidophenylmercapturic Acids into the Aminophenyl Derivatives.—*o*- and *p*-Acetamidophenylmercapturic acids (0·1 g.) in N-hydrochloric acid (5 ml.) were heated at 100°. Samples were removed at 5-min. intervals, neutralised with N-sodium hydrogen carbonate, and chromatographed in solvents A and B. *o*-Acetamidophenylmercapturic acid was completely converted into *o*-aminophenylmercapturic acid in about 15 min.; the *p*-compound required about 25 min. On further heating, both compounds gave the corresponding *S*-aminophenyl-L-cysteines, and unidentified decomposition products in increasing amounts.

Reaction of 2-Naphthylhydroxylamine with Thiols.—2-Naphthylhydroxylamine (100 mg.) and *N*-acetyl-L-cysteine (100 mg.) in 50% aqueous acetone (3 ml.) or 30% aqueous acetic acid (3 ml.) were left at room temperature for 2 hr. Examination of the mixtures by paper chromatography in solvents A and B showed the presence of a compound with R_F values and colour reactions identical with those of 2-amino-1-naphthylmercapturic acid. With L-cysteine hydrochloride a compound was formed with R_F 0·76 in system A and 0·40 in B. This compound gave a positive test with the dichromate–silver nitrate reagent and a yellow colour with hexylresorcinol after diazotisation, but in addition gave a blue colour with ninhydrin. It was therefore probably *S*-(2-amino-1-naphthyl)-L-cysteine. Similarly with glutathione under the same conditions paper chromatography showed the presence of a ninhydrin-positive compound, also giving a yellow colour with hexylresorcinol after diazotisation and a positive reaction to the dichromate–silver nitrate reagent. This derivative had R_F 0·67 in system A and 0·15 in B. It was probably *S*-(2-amino-1-naphthyl)glutathione.

Reaction of N-*Acetyl*-L-*cysteine and* L-*Cysteine with 2-Naphthylamine after Treatment with Peracetic Acid.*—40% Peracetic acid (0·1 ml.) was added with shaking to 2-naphthylamine (100 mg.) in acetone (3 ml.). The mixture was left at room temperature for 10 min. and *N*-acetyl-L-cysteine (100 mg.) in water (0·5 ml.) then added. After 2 hr. the mixture was examined by paper chromatography. It contained a compound identical in R_F values and colour reactions with 2-amino-1-naphthylmercapturic acid. Similarly, with L-cysteine hydrochloride under the same conditions, the mixture gave a compound with the same R_F values and colour reactions as that produced by reaction of 2-naphthylhydroxylamine with L-cysteine.

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