The Biochemistry of Aromatic Amines

2. THE CONVERSION OF ARYLAMINES INTO ARYLSULPHAMIC ACIDS AND ARYLAMINE-N-GLUCOSIDURONIC ACIDS*

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Aromatic amines are known to be metabolized by conversion into acetamido derivatives and oxidation to aminophenols. The aminophenols may be conjugated or further metabolized. In studying the metabolism of 2-naphthylamine by paper chromatography two products were detected which gave the free amine on acidification (Boyland & Manson, 1955). One of these substances could be prepared by treating 2-naphthylamine with sodium glucuronate in aqueous solution and is presumably sodium 2-naphthylamine *N*-glucosiduronate. The other product was found to be 2-naphthylsulphamic acid; this product appears to be produced in greater amounts by rabbits than by rats and an analogous reaction occurs with aniline and 1-naphthylamine. Aliphatic sulphamate or *N*-sulphonate groups are probably present in naturally occurring polysaccharides such as heparin (Reinert & Winterstein, 1939). 2-Naphthylamine and other aromatic amines may be excreted as the *N*-glucosiduronic acids and as the *N*-sulphonic acids or sulphamates.

EXPERIMENTAL

Methods

Animals were kept in metabolism cages and the urine was collected daily. Rabbits (approx. 2.5 kg.) and guinea pigs (approx. 400 g.) were fed on cabbage, bran, rat cake and water, rats (approx. 200 g.) on rat cake, bread and water, and hamsters (approx. 85 g.) on rat cake, mash, mixed seeds and water. Amines were injected intraperitoneally in arachis oil. Rabbits were given 0.3 g. of aniline, 4-aminodiphenyl, 1-naphthylamine or 0.5 g. of 2-naphthylamine in 10 ml. of oil; rats received 0.03 g. of each of the first three amines and 0.05 g. of 2-naphthylamine in 1 ml. of oil; guinea pigs received 0.06 g. of 2-naphthylamine in 1.5 ml. of oil; hamsters received 0.02 g. of 2-naphthylamine in 0.5 ml. of oil.

Paper chromatography. Upward and downward development was employed with Whatman no. 1 chromatography paper. Solvent systems used were (a) *n*-butanol-propan-1-ol-0.1N-NH₃ soln. (2:1:1, by vol.) (Booth, Boyland & Manson, 1955), (b) benzene-methanol-amyl alcohol (B.S. 696)-2N-HCl (35:35:17.5:12.5, by vol.) (Ekman, 1948) and (c) *n*-butanol-acetic acid-water (2:1:1, by vol.).

Metabolites were detected on paper chromatograms by spraying with (1) N-HCl, then NaNO₂ (0.5%), followed by hexylresorcinol (0.5% in 2N-NaOH) and (2) Ehrlich's reagent: *p*-dimethylaminobenzaldehyde (0.5% in ethanol containing 1 ml. of conc. HCl/100 ml.). *R_F* values and colour reactions are listed in Table 1.

Materials

Melting points are uncorrected. Aniline, 4-aminodiphenyl (*p*-xenyamine), 1-naphthylamine and 2-naphthylamine were from commercial sources.

Potassium 1-amino-2-naphthylsulphate and potassium 2-amino-1-naphthyl sulphate were prepared by oxidation of 1- and 2-naphthylamine respectively with potassium persulphate (Boyland, Manson & Sims, 1953), and potassium 4-amino-3-diphenyl sulphate by oxidation of 4-aminodiphenyl with potassium persulphate (Boyland & Sims, 1954). Potassium 2-aminophenyl sulphate was prepared by reduction of potassium 2-nitrophenyl sulphate (Burkhardt & Wood, 1929).

Potassium salts of phenylsulphamic acid, 4-diphenylsulphamic acid, 1-naphthylsulphamic acid and 2-naphthylsulphamic acid were all prepared by treatment of the parent amines with chlorosulphonic acid in dry pyridine. Chlorosulphonic acid (5 g.) was added to pyridine (50 ml.) at 15°, followed by the amine (1/20 mole) and after keeping the mixture at room temperature overnight it was poured into 100 ml. of N-KOH. The solution was extracted several times with ether and the aqueous phase concentrated to low volume and cooled, when the compounds crystallized out as plates. They were recrystallized from aqueous ethanol: potassium phenylsulphamate (Found: N, 6.9. Calc. for C₆H₅O₂NSK: N, 6.6%); potassium 4-diphenylsulphamate (Found: N, 4.8; S, 11.4. C₁₅H₁₀O₂NSK requires N, 4.9; S, 11.15%); potassium 1-naphthylsulphamate (Found: N, 5.3. Calc. for C₁₀H₇O₂NSK: N, 5.4%); potassium 2-naphthylsulphamate (Found: N, 5.4; S, 12.5. Calc. for C₁₀H₇O₂NSK: N, 5.4; S, 12.3%).

Each gave a precipitate after slight warming with N-HCl and BaCl₂. All diazotized and coupled without prior hydrolysis but reacted only slowly with Ehrlich's reagent.

Dipotassium salt of the sulphuric ester of 1-hydroxy-2-naphthylsulphamic acid. This compound was prepared (1) by the action of chlorosulphonic acid on 2-amino-1-naphthol, and (2) by oxidation of potassium 2-naphthylsulphamate with potassium persulphate.

(1) 2-Amino-1-naphthol hydrochloride (3 g.) in water was treated with NaOH (1 equivalent) and Na₂S₂O₄ (0.5 g.) and the precipitated 2-amino-1-naphthol (plates) was washed with water and ether and dried over P₂O₅. The dry 2-amino-

* Part 1: Boyland, Manson, Sims & Williams (1956).

1-naphthol (2 g., m.p. 135–138° with decomposition) was added to carbon disulphide (50 ml.), dimethylaniline (10 ml.) and chlorosulphonic acid (1.4 g.). After standing overnight the solution was added to excess of 2N-KOH, extracted with ether and the aqueous phase evaporated to low volume. The dipotassium salt of the sulphuric ester of 1-hydroxy-2-naphthylsulphamic acid separated and was recrystallized from aqueous methanol (0.4 g., plates). (Found: N, 3.4; S, 16.5. $C_{16}H_7O_7NS_2K_2$ requires N, 3.5; S, 16.2 %.) One equivalent only of chlorosulphonic acid had been employed in the hope of obtaining the unknown 1-hydroxy-2-naphthylsulphamic acid but this was not achieved. 2-Amino-1-naphthol hydrochloride gave predominantly 2-amino-1-naphthylsulphuric acid with chlorosulphonic acid (Boyland *et al.* 1953; and unpublished observations). (2) $K_2S_2O_8$ (7 g.) in water (75 ml.) was added to potassium 2-naphthylsulphamate (7 g.) and KOH (2.5 g.) in 50% aqueous acetone (250 ml.) over 1 hr. with stirring. After standing overnight, 2N-KOH (60 ml.) was added and the solution evaporated almost to dryness. The residue was extracted several times with a total of 150 ml. of hot methanol. After filtering and cooling the solution a mixture of product and starting material (1.5 g.) separated. Concentration to 100 ml. gave a further 1 g. of mixture and further concentration to 50 ml. gave 0.6 g. of almost pure product which after recrystallization from aqueous methanol gave the dipotassium salt of the sulphuric ester of 1-hydroxy-2-naphthylsulphamic acid. (Found: N, 3.6; S, 16.0. Calc. for $C_{16}H_7O_7NS_2K_2$: N, 3.5; S, 16.2 %.) The concentration procedure and purification were guided by paper chromatography.

Products from methods (1) and (2) gave 2-amino-1-naphthol on boiling with dilute HCl, detected by the characteristic colours given when the solution was made alkaline with NH_3 soln. and extracted with benzene (Liebermann & Jacobson, 1882). The compound diazotized and coupled with hexylresorcinol to yield a mauve colour but reacted only slowly with Ehrlich's reagent. It could be run without decomposition in solvent system (a) but partially decomposed in solvent system (c) to yield 2-amino-1-naphthylsulphuric acid.

N-Glucosiduronic acids. The method of Smith & Williams (1949b) was adapted for the synthesis of 2-naphthylamine *N*-glucosiduronic acid as ammonium and sodium salts and for the preparation of a solution of 2-amino-1-naphthylsulphuric acid *N*-glucosiduronic acid. Analyses of the 2-naphthylamine *N*-glucosiduronic acid salts indicated that the compounds consisted of one molecule of amine and one molecule of *N*-glucosiduronic acid with the water of crystallization dependent on the derivative (cf. Smith & Williams, 1949b). *N*-Glucosides are also known to crystallize with water, alcohol or amine of crystallization (Mitts & Hixon, 1944).

Ammonium 2-naphthylamine N-glucosiduronate. D-Glucuronolactone (1 g.), dissolved in 0.3N-Ba(OH)₂ (20 ml.), was kept at room temperature for 3 hr. and then treated with $(NH_4)_2SO_4$ (saturated solution, 1 ml.). Barium sulphate was removed by centrifuging and the solution of ammonium glucuronate filtered (Ford's clarifying pad, grade F.C.B.). 2-Naphthylamine (freshly recrystallized, 1 g.) in ethanol (10 ml.) was added, and any precipitated amine removed by filtration and the filtrate allowed to stand for 1 hr. at room temperature, when the crystalline product (plates) was filtered off and washed successively with water, ethanol and

Table 1. R_f values and colour reactions of some aromatic amines and derivatives

All the compounds gave yellow colours with Ehrlich's reagent. For composition of solvent systems see text.

Compound	Fluorescence in ultraviolet light	Colour after diazotization and coupling with hexylresorcinol	R_f in solvent system			
			(a)		(b)	(c)
			Descending	Ascending	Ascending	Ascending
Aniline	None	Yellow	Volatilized	—	0.75	—
Phenylsulphamic acid	None	Yellow	0.33	—	Hydrolysis	—
2-Aminophenylsulphuric acid	None	Red	0.33	—	0.66	—
4-Aminodiphenyl	Faint blue	Yellow	0.96	—	0.93	—
4-Diphenylsulphamic acid	Faint blue	Yellow	0.40	—	Hydrolysis	—
4-Amino-3-diphenylsulphuric acid	None	Red	0.47	—	0.70	—
1-Naphthylamine	Blue-white	Orange-yellow	0.94	—	0.90	—
1-Naphthylsulphamic acid	Blue	Orange-yellow	0.39	—	Hydrolysis	—
1-Amino-2-naphthylsulphuric acid	Blue-white	Blue	0.40	—	0.70	—
2-Naphthylamine	Blue-white	Yellow	0.94	0.95	0.90	0.98
2-Naphthylsulphamic acid	Blue	Yellow	0.40	0.55	Hydrolysis	0.68
2-Amino-1-naphthylsulphuric acid	Blue-white	Mauve	0.42	0.58	0.70	0.73
2-Naphthylamine- <i>N</i> -glucosiduronic acid	Blue-white	Yellow	—	0.28 (Partial hydrolysis)	—	Complete hydrolysis
2-Amino-1-naphthylsulphuric acid	Blue-white	Mauve	0.08	0.15	—	0.30 (Partial hydrolysis)
1-Hydroxy-2-naphthylsulphamic acid sulphuric ester	Blue-white	Mauve	0.16	0.16 (Partial hydrolysis)	—	0.35 (Partial hydrolysis)

ether. Yield of ammonium 2-naphthylamine *N*-glucosiduronate, 1.2 g., m.p. 138–140° (decomp.). Recrystallization was difficult as prolonged heating with 50% ethanol caused decomposition, but a small amount was recrystallized for analysis, without altering the melting point. (Found: C, 61.9, 61.8; H, 6.45, 6.5; N, 8.4, 8.5. $C_{26}H_{29}O_6N_3 \cdot 1.5H_2O$ requires C, 61.75; H, 6.4; N, 8.3%.)

As the compound was insoluble in cold water, ethanol and pyridine the optical rotation could not be determined. It gave a rapid positive reaction for glucuronic acid with naphthoresorcinol solution. It was diazotized with HCl and $NaNO_2$ and the diazo compound coupled with hexylresorcinol. Cold 10*N*-NaOH liberated NH_3 . On boiling with water the material decomposed and crystals of 2-naphthylamine (identified by m.p. and mixed m.p.) separated on cooling. On keeping in the dry state it became brown if exposed to daylight. Paper chromatography of the substance with solvent system (a) caused some decomposition, 2-naphthylamine and the *N*-glucosiduronic acid being detected. With a two-dimensional chromatogram further decomposition occurred during the second development.

Sodium 2-naphthylamine *N*-glucosiduronate. D-Glucuronolactone (1.7 g.) in water (10 ml.) was converted into sodium glucuronate by addition of NaOH (0.39 g.) and allowing the solution to stand at room temperature for 3 hr. An equal volume of a phosphate buffer (pH 7.0, 0.2*M*) was then added followed by 2-naphthylamine (0.7 g.) in ethanol (50 ml.). The volume was made up to 100 ml. with water and the solution kept at 0° overnight. Elongated plates of sodium 2-naphthylamine *N*-glucosiduronate (0.5 g.) separated, the m.p., 191–192° (decomp.), being unchanged by recrystallization from aqueous ethanol.

$$[\alpha]_D^{23} - 115^\circ \xrightarrow{24 \text{ hr.}} -25^\circ$$

(value then constant). (c, 0.08 in water.) (Found: C, 62.0; H, 5.6; N, 6.0, 5.7; Na, 4.6, 4.8. $C_{26}H_{25}O_6N_2Na \cdot H_2O$ requires C, 62.1; H, 5.4; N, 5.6; Na, 4.6%.)

Aqueous solutions of the compound became pinkish brown in colour after exposure to light.

Reaction of 2-amino-1-naphthylsulphuric acid with D-glucuronic acid. D-Glucuronolactone (1 g.) was converted into ammonium glucuronate as above and 2-amino-1-naphthylsulphuric acid (1.3 g., in aqueous ethanol, neutralized with dilute NH_3 soln.) was added. Paper chromatography showed the presence of a substance which diazotized and coupled with hexylresorcinol to give the same mauve colour as 2-amino-1-naphthylsulphuric acid. It had an R_F of 0.15 in solvent system (a) (ascending) and 0.3 in solvent system (c). The corresponding values for 2-amino-1-naphthylsulphuric acid were 0.58 (ascending) and 0.73. By analogy with the formation of 2-naphthylamine *N*-glucosiduronic acid this probably represented the corresponding derivative of the sulphuric ester. The spot believed to be the reaction product was cut out, re-attached to fresh paper and redeveloped in solvent system (a), when both the initial substance and 2-amino-1-naphthylsulphuric acid could be detected. The compound is thus unstable like 2-naphthylamine *N*-glucosiduronic acid. If the spot was sprayed with dilute HCl and heated at 70° for 5 min. before running again, only 2-amino-1-naphthylsulphuric acid was detectable after development of the chromatogram.

Attempts to isolate the compound were unsuccessful; gradual evaporation of the solution over P_2O_5 gave only ammonium 2-amino-1-naphthyl sulphate.

Infrared spectra

Infrared spectra of the samples as mulls in liquid paraffin or a fully fluorinated oil were measured on a Perkin-Elmer model 112 spectrometer, fitted with a NaCl prism.

RESULTS

2-Naphthylamine metabolism in rat, rabbit, guinea pig and hamster

Aromatic amines are one of a group of compounds which cause excretion of large amounts of reducing material, probably mainly glucuronic acid (Bray, Hybs & Thorpe, 1951). 2-Naphthylamine produces the same effect and the glucuronic acid excreted might react with the amine and its metabolites to yield *N*-glucosiduronic acids. The reaction of *p*-toluidine with glucuronic acid has been described by Smith & Williams (1949*a, b*). Paper chromatography of urine from rats and rabbits injected with 2-naphthylamine showed that a spot of the same R_F and colour reactions as synthetic 2-naphthylamine *N*-glucosiduronic acid was present.

A two-dimensional chromatogram was carried out, being developed first in solvent system (a), and the paper dried and then sprayed with *N*-HCl along the line of the separated metabolites. The chromatogram was heated at 70° for 5 min. and dried. After treatment with ammonia vapour to neutralize residual acid (which causes distortion of the chromatogram) the sheet was developed in solvent system (c), which gives better definition of 2-naphthylamine than solvent system (a). 2-Naphthylamine was released from the spot corresponding to the *N*-glucosiduronic acid. The *N*-glucosiduronate could be detected by paper chromatography of urine immediately after it was passed. The urine was centrifuged and the deposit washed with water, ethanol and ether. This deposit gave a strong positive reaction with the Tollens test for glucuronic acid and chromatography showed the presence of the *N*-glucosiduronic acid. The presence of the compound in the deposit accords with the low solubility of the synthetic compounds. Guinea-pig and hamster urines were not examined for this compound.

The two-dimensional chromatogram described above also gave 2-naphthylamine from a spot (R_F 0.6 in the first development) which diazotized and coupled with hexylresorcinol to give a yellow colour. In addition, a spot having the same colour reactions as 2-amino-1-naphthylsulphuric acid but having an R_F value of 0.15 in solvent system (a) gave, by the above technique, 2-amino-1-naphthylsulphuric acid, R_F 0.73 in system (c).

Realization that the amino group of 2-naphthylamine could be substituted by a very acid-labile group suggested that 2-naphthylsulphamic acid

(I) might be a metabolite. This was found to behave in exactly the same manner as the compound with R_f 0.6, that is, although the amino group was substituted it could be readily diazotized and coupled and reacted slowly with Ehrlich's reagent. It was readily hydrolysed on the chromatograms, even by cold mineral acid, to yield the parent amine.

2-Naphthylsulphamic acid could be detected in rabbit urine without recourse to two-dimensional chromatography although the spot overlapped with 2-amino-1-naphthylsulphuric acid. Its presence was confirmed by its isolation from rabbit urine after administration of the amine. Only small amounts were present in rat urine, in which it could be detected only by the two-dimensional technique employed above, but none was detected in the urine of guinea pigs and hamsters.

The labile precursor of 2-amino-1-naphthylsulphuric acid was present in the urine of rats and rabbits (the urine of hamsters and guinea pigs was not examined for this metabolite) and might be a derivative in which the amino group is substituted (II) by glucuronic acid or (III) by sulphuric acid, or 1-hydroxy-2-naphthylsulphamic acid (IV) which could give 2-amino-1-naphthylsulphuric acid by migration of the sulphate group in the presence of acid.

The precursor cannot be 2-acetamido-1-naphthylsulphuric acid as this has a different R_f value [0.63 in solvent system (a), upward development] and it has not been detected as a metabolite of 2-naphthylamine in rats or rabbits (unpublished observations).

The *N*-sulphonic acid and *N*-glucosiduronic acid of 2-amino-1-naphthylsulphuric acid have similar R_f values in the solvent systems employed (ascending or descending development). Both compounds yielded 2-amino-1-naphthylsulphuric acid on acid treatment of the chromatogram in the same way as the unknown metabolite, but the R_f value of the metabolite was closer to that of the *N*-glucosiduronic acid. 1-Hydroxy-2-naphthylsulphamic acid (IV) is not known and could not be synthesized. However, after treatment of 2-hydroxyphenylsulphamic acid with *N*-HCl no 2-aminophenyl-

sulphuric acid could be detected so that migration of a sulphate ($-\text{SO}_3\text{H}$) group appears unlikely. If the spot due to the metabolite was cut out and extracted with methanol and the residue from the extract developed on a second chromatogram, or if the spot was cut out and run again after re-attachment to fresh paper, 2-amino-1-naphthylsulphuric acid could be detected. This decomposition on the chromatogram with the *n*-butanol-propan-1-ol-0.1*N*- NH_3 soln. solvent system without any further treatment was characteristic of the synthetic *N*-glucosiduronic acid. Repeated running in this solvent system of a spot derived from the synthetic *ON*-disulphate (III) did not cause any decomposition although it is hydrolysed in acetic acid-butanol systems. This metabolite is therefore 2-amino-1-naphthylsulphuric acid *N*-glucosiduronic acid. The corresponding spot in rabbit urine behaved in the same way.

Isolation of sodium 2-naphthylamine N-glucosiduronate. 2-Naphthylamine (0.5 g. in arachis oil) was injected intraperitoneally into each of four rabbits for 5 days (total dose 10 g.) and the urine collected and stored in the cold. The urine (2 l.) was centrifuged and the supernatant preserved for the isolation of 2-naphthylsulphamic acid (below). The deposit was washed three times with 10 ml. portions of water, flocculent material being decanted, and then washed twice with ethanol and twice with ether. The deposit was then extracted twice with hot (60°) 50% ethanol, and the extract filtered and concentrated to dryness in a desiccator at room temperature. The residue gave a rapid positive

Tollens reaction. Paper chromatography showed the presence of a substance identical with synthetic 2-naphthylamine *N*-glucosiduronic acid. After three crystallizations from 50% ethanol to remove amorphous and inorganic impurities, crystalline material (1.5 mg.) was obtained, m.p. 180–185°.

Infrared spectral analysis (Fig. 1) also indicated that the substance was identical with synthetic sodium 2-naphthylamine *N*-glucosiduronate, except for some slight further absorption in the isolated sample at 1045 and 1115 cm^{-1} ; this must be due to the presence of an impurity in the isolated sample.

Isolation of sodium 2-naphthylsulphamate. The supernatant from the isolation of the *N*-glucosiduronic acid was filtered and passed through a charcoal column (approx. 150 g. of activated

charcoal, British Drug Houses Ltd.) under slight suction. The eluate was almost colourless and showed no detectable naphthylamine metabolites. The charcoal was then washed with water (1.5 l.). This eluate also contained no detectable metabolites. Hot methanol was then passed through the column in six 500 ml. portions, which were collected separately and examined by paper chromatography. Some separation of 2-amino-1-naphthylsulphuric acid from 2-naphthylsulphamic acid occurred as the first and second fractions contained more of the sulphuric ester than the third and fourth, which were richer in the sulphamic acid derivative. The fifth and sixth fractions contained much less of both compounds and the sixth contained 2-acetamido-6-naphthylglucosiduronic acid (isolation of this compound will be described separately). The third, fourth and fifth fractions were combined, distilled to dryness after making alkaline with 2*N*-NaOH, and the gummy residue was extracted several times with hot methanol to separate inorganic material. The combined methanol extracts were evaporated to dryness, the residue was dissolved in the minimum of *n*-butanol-propan-1-ol-water (2:1:1, by vol.) and Whatman cellulose powder added to form a paste. The paste was added to the top of a cellulose column (23 cm. $\times$ 5 cm.) prepared in the same solvent mixture and the column developed over 18 hr., the eluate being collected in 50 ml. portions. Paper chromatography showed that the first two fractions contained 2-acetamidonaphthalene, 2-acetamido-6-naphthol and a compound believed to be 2-acetamido-5:6-dihydroxy-5:6-dihydronaphthalene. The third, fourth and fifth fractions contained 2-amino-1-naphthylsulphuric acid and 2-naphthylsulphamic acid and were evaporated to dryness and the residue was crystallized from 70 % ethanol. After cooling overnight crystals (plates) of almost pure sodium 2-naphthylsulphamate were deposited which were recrystallized from 70 % ethanol. Yield, 0.023 g. (Found: N, 5.7; S, 13.1. Calc. for $C_{10}H_8O_3NSNa$: N, 5.7; S, 13.1 %.) In another experiment 0.019 g. of sodium 2-naphthylsulphamate was isolated after injection of 6 g. of 2-naphthylamine. The R_f value was identical with that of the synthetic compound, and it could be diazotized and coupled with hexylresorcinol to give a yellow colour without prior hydrolysis, but it reacted slowly with Ehrlich's reagent. The compound yielded inorganic sulphate upon warming with dilute HCl. It gave a persistent yellow colour to a Bunsen flame. Hydrolysis of the compound with dilute HCl, and evaporation of the solution and treatment of the residue with benzoyl chloride in pyridine, gave 2-benzamidonaphthalene, m.p. 160–163° with no depression of m.p. with an authentic specimen of 2-benzamidonaphthalene.

Concentration of the mother liquors of the first experiment gave only mixtures of sodium 2-amino-1-naphthyl sulphate and 2-naphthylsulphamate, which could not be separated. Cellulose-column chromatography of the material in the first and second methanol washings of the charcoal column gave mainly sodium 2-amino-1-naphthyl sulphate. To obtain the sulphuric ester all these fractions were combined and acidified with dilute HCl. The precipitate was collected and recrystallized by solution in dilute alkali and precipitation with dilute acid to yield 2-amino-1-naphthylsulphuric acid (0.049 g.), m.p. 224–226°, which gave no depression of melting point with an authentic specimen. The second experiment gave 0.029 g. of the ester. 2-Amino-1-naphthylsulphuric acid has not previously been isolated from urine of rabbits injected with 2-naphthylamine.

Aniline metabolism in rabbits and rats

Phenylsulphamic acid could not be separated from 2-aminophenylsulphuric acid by paper chromatography in solvent system (a) or (c). The sulphuric ester was present in both species after administration of the amine. Aniline could not be detected after development with solvent system (a) or (c) owing to its volatility. Hence phenylsulphamic acid could not be identified (as aniline) after two-dimensional chromatography with an intermediate acid hydrolysis as described for 2-naphthylsulphamic acid. After application of the urine the chromatogram was developed first in solvent system (a) for 18 hr. and then with solvent system (b) for 6 hr., when phenylsulphamic acid is hydrolysed but 2-aminophenylsulphuric acid is not. After treatment of rabbit urine in this manner aniline was detectable, released from a spot with an R_f value corresponding to that of phenylsulphamic acid. Phenylsulphamic acid could not be detected in rat urine.

4-Aminodiphenyl metabolism in rabbits and rats

4-Diphenylsulphamic acid and 4-amino-3-diphenylsulphuric acid were separable by paper chromatography in solvent system (a). No sulphamic acid derivative was detected in rat or rabbit urine with this solvent system or by two-dimensional chromatography in this solvent system followed by solvent system (b).

1-Naphthylamine metabolism in rabbits and rats

1-Naphthylsulphamic acid was detected in rabbit urine by the method used for the detection of 2-naphthylsulphamic acid. None was detected in rat urine.

*Infrared-absorption spectra of 2-naphthylamine
and the carbohydrate derivatives*

The main features of the several spectra (Fig. 1) are the sharp bands between 750 and 850 cm^{-1} due to the out-of-plane deformation modes of the aromatic CH groups, the strong absorption at 950–1100 cm^{-1} in the sugar derivatives, the pair of very strong bands at about 1400 and 1600 cm^{-1} due to the carboxylic ion in the *N*-glucosiduronates and the strong absorption at about 1450 cm^{-1} due to the ammonium ion.

The band at about 1630 cm^{-1} , present in 2-naphthylamine itself, persists in each of the sugar derivatives. Although partly due to the amino group in the amine, it must also be connected with the CC vibrations of the naphthalene nucleus, and a band at this position is present in the spectrum of 2-naphthol. If the aromatic nucleus were conjugated with a $\text{N}=\text{C}$ bond, this band should be shifted to lower frequencies, and another band at about 1660 cm^{-1} would be expected (Bellamy, 1954*a*). The persistence of the band at 1630 cm^{-1} therefore suggests the presence of the $-\text{NH}-\text{C}\equiv$ structure in each of the sugar derivatives. The similarity of their absorption at 745, 810 and 836 cm^{-1} , due to the CH deformation modes, confirms this similarity in structure. No band due to the NH group itself could be assigned with certainty; the stretching vibration at 3 μ . is overlapped with absorption due to the sugar hydroxyl groups, and the deformation vibration does not show any well-defined characteristic absorption band (Bellamy, 1954*b*).

DISCUSSION

Arylsulphamic acids appear to be a new type of arylamine metabolite; these are stable in neutral solution but hydrolysed by cold acid.

Fig. 1. Infrared spectra of (a) 2-naphthylamine, (b) 2-naphthylamine *N*-glucoside, (c) sodium 2-naphthylamine *N*-glucosiduronate and (d) ammonium 2-naphthylamine *N*-glucosiduronate.

Bushby & Woiwod (1955, 1956) have described the isolation of an *N*-glucosiduronic acid of 4:4'-diaminodiphenylsulphone from rabbit urine after administration of the parent amine. The excretion of labile glucosiduronic acids was proposed by Smith & Williams (1949*a, b*) to account for the presence of free glucuronic acid in the glucosiduronic acid fractions of aniline, *p*-phenetidine, *o*-, *m*- and *p*-anisidine urines. They considered the excretion of free glucuronic acid unlikely though not impossible. Bray *et al.* (1951) have shown, however, that reducing material, probably glucuronic acid, is present in rabbit urine after administration of several aromatic compounds. Some of this must be free and not conjugated, because the amount produced is too great to be accounted for by the dose of foreign compound administered, and the excretion continues after cessation of dosing. The formation of the *N*-glucosiduronic acid may occur in the urine by combination of the free amine and glucuronic acid after excretion rather than in the body tissues, but this would seem to be unlikely, particularly as Bushby & Woiwod (1956) found evidence of an *N*-glucosiduronic acid present in the blood of rabbits.

Booth *et al.* (1955) reported the formation of *N*-glucosides as artifacts during a study of the metabolism of 2-naphthylamine by rat-liver and kidney slices. The compounds arose by reaction of the amine and its derivatives with glucose in the Ringer solution employed.

A practical point in paper chromatographic analysis arising from the identification of *N*-sulphonic and *N*-glucosiduronic acids is that substances which diazotize and couple on chromatograms are not necessarily free aromatic amines. This may have some importance in the identification of naturally occurring aromatic amines.

SUMMARY

1. The urine of rabbits dosed with 2-naphthylamine contains two acid-labile derivatives of 2-naphthylamine: 2-naphthylsulphamic acid and 2-naphthylamine *N*-glucosiduronic acid.

2. Sodium 2-naphthylsulphamate, which readily yields 2-naphthylamine in cold acid solution, has been isolated from the urine of rabbits and detected by paper chromatography in the urine of rats but not of hamsters or guinea pigs dosed with 2-naphthylamine.

3. Phenylsulphamic acid was detected in urine of rabbits but not of rats dosed with aniline. 1-Naphthylsulphamic acid was detected in the urine of rabbits but not of rats dosed with 1-naphthylamine. 4-Diphenylsulphamic acid did not appear to be formed from 4-aminodiphenyl by either rats or rabbits.

4. Sodium 2-naphthylamine *N*-glucosiduronate was isolated from urine of rabbits dosed with 2-naphthylamine and detected in urine of rats dosed with the amine.

5. The 2-naphthylamine *N*-glucosiduronate was shown by infrared absorption to have a structure similar to 2-naphthylamine *N*-glucoside.

6. Urine of rats and rabbits dosed with 2-naphthylamine contains an acid-labile derivative of 2-amino-1-naphthylsulphuric acid. This compound is not the sulphuric ester of 1-hydroxy-2-naphthylsulphamic acid, which was synthesized, but is 2-amino-1-naphthylsulphuric acid *N*-glucosiduronic acid.

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Glutathione Metabolism

2. THE OXIDATION AND REDUCTION OF GLUTATHIONE IN INTACT ERYTHROCYTES*

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The sulphhydryl-disulphide oxidation-reduction system is involved in a large number of biological processes. The metabolic activity of many of these processes is dependent, to a large extent, on the level of oxidation of the sulphur constituent. The glutathione system ($\text{GSH} \rightleftharpoons \text{GSSG}$), as the chief soluble sulphhydryl-disulphide component of the cell, is thought to play an important role in the regulation of the oxidation-reduction equilibrium of the cell by virtue chiefly of its effect on other sulphhydryl-disulphide systems (see Barron, 1951). Thus reduced glutathione has been found to activate or protect sulphhydryl enzymes or coenzymes under a variety of conditions, whereas a number of reactions dependent on an intact disulphide group are inhibited by this substance. Conversely, oxidized glutathione is inhibitory to many sulphhydryl

systems. The relative proportion of reduced to oxidized glutathione within the cell, as well as the factors which control this steady state, may therefore have an important influence on the regulation of intracellular metabolism.

The oxidation and reduction of glutathione by cell-free tissue preparations has been extensively investigated (see Vennesland & Conn, 1954). Less is known, however, of the factors which control the SS-SH equilibrium within the intact cell. As an approach to this problem, an investigation of the oxidation and reduction of glutathione in the intact erythrocyte was undertaken.

The exposure of intact erythrocytes to oxygen leads to a fall in the reduced glutathione level (Meldrum, 1932). In addition, the glyoxalase activity of the cells is decreased (Jowett & Quastel, 1933). Since reduced glutathione is a specific cofactor in the glyoxalase system (Lohmann, 1932), it was suggested (Jowett & Quastel, 1933) that the

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