The Biochemistry of Aromatic Amines

2-FORMAMIDO-1-NAPHTHYL HYDROGEN SULPHATE, A METABOLITE OF 2-NAPHTHYLAMINE

By E. BOYLAND AND D. MANSON

Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital), Fulham Road, London, S.W. 3

(Received 15 November 1965)

1. 2-Formamido-1-naphthyl hydrogen sulphate is excreted by dogs and rats dosed with 2-naphthylamine or with 2-amino-1-naphthyl hydrogen sulphate and was isolated from dog urine. 2. 2-Formamido-1-naphthol, naphtho[2,1-*d*]oxazole and *N*-formyl-2-naphthylhydroxylamine are excreted as (2-formamido-1-naphthyl glucosid)uronic acid. 2-Formamidonaphthalene is converted into conjugates of 2-amino-6-naphthol and 2-amino-8-naphthol, but 2-methylaminonaphthalene is excreted as 2-amino-1-naphthyl hydrogen sulphate and its *N*-methyl and *N*-formyl derivatives and (2-amino-1-naphthyl glucosid)uronic acid. 3. 2-Methylamino-1-naphthyl hydrogen sulphate is converted into 2-formamido-1-naphthyl hydrogen sulphate and 2-amino-1-naphthyl hydrogen sulphate on charcoal. 2-Amino-1-naphthyl hydrogen sulphate and formaldehyde react on charcoal to yield 2-methylamino- and 2-formamido-1-naphthyl hydrogen sulphate. 4. 2-Formamido-1-naphthol, 2-formamido-1-naphthyl hydrogen sulphate, *N*-formyl-2-naphthylhydroxylamine and *N*-formyl-2-naphthylhydroxylamine-*O*-sulphonic acid were synthesized.

2-Formamido-1-naphthyl hydrogen sulphate is to be added to the list of metabolites of the bladder carcinogen, 2-naphthylamine (cf. Boyland, 1963). Formamido compounds do not appear to have been described as metabolites of aromatic amines, but they occur in Nature, e.g. formylkynurenine, hadacidin (Kaczka, Gitterman, Dulaney & Folkers, 1962), gramicidin (Sarges & Witkop, 1964) and *N*-formyl-methionyl-transfer RNA (Marcker & Sanger, 1964). A preliminary report of this work has appeared (Boyland & Manson, 1965).

EXPERIMENTAL

Animals. Female Chester Beatty Albino rats (approx. wt. 200g.) were kept in cages designed for the separate collection of urine and faeces, and were injected intraperitoneally. Beagles and mongrel bitches (average wt. 10kg.) were given 2-naphthylamine in gelatin capsules by mouth.

Chromatography. Whatman no. 1 chromatography paper was used for descending development with the following solvent systems: (a) butan-1-ol-propan-1-ol-aq. 0.1*N*-NH₃ (2:1:1, by vol.); (b) butan-1-ol-acetic acid-water (2:1:1, by vol.); (c) ethanol-water-aq. NH₃ (sp.gr. 0.88) (35:13:2, by vol.) with thymol blue (0.03%) (Hartley & Lawson, 1962); (d) butan-1-ol saturated with 0.1*N*-NH₃; (e) butan-1-ol-acetic acid-water (12:3:5, by vol.); (f) butan-1-ol-benzene (4:1, v/v) saturated with 0.1*N*-NH₃ (Brill &

Radomski, 1965). Thin-layer chromatography was carried out on glass plates coated with silica gel G (E. Merck A.-G., Darmstadt, W. Germany) of 0.25mm. thickness with the following solvent mixtures: (a) above; (g) light petroleum (b.p. 40–60°)-acetone (7:3, v/v); (h) light petroleum (b.p. 40–60°)-acetone (4:1, v/v); (i) chloroform-ethyl acetate-acetic acid (6:3:1, by vol.); (j) benzene-ethanol (19:1, v/v); (k) chloroform-methanol (49:1, v/v); (l) chloroform-methanol (19:1, v/v); (m) butan-1-ol-acetic acid-water (10:1:1, by vol.). Solvents (a) and (m) were used for the thin-layer chromatography of sulphuric esters but better separations were obtained by paper chromatography. *N*-Formyl-2-naphthylhydroxylamine-*O*-sulphonic acid changed into 2-formamido-1-naphthyl hydrogen sulphate on paper chromatograms so that thin-layer chromatography was used in the detection of this compound. The *R_F* values are given in Tables 1 and 2. Compounds were detected on chromatograms with the reagents described by Boyland, Manson & Nery (1963). Colour reactions are given in Table 3. The colours given by aromatic amino compounds with *p*-dimethylaminocinnamaldehyde were much intensified by spraying with FeCl₃ solution. Additional reagents were aq. Na₂CO₃ (10%, w/v) followed by diazotized *p*-nitroaniline (0.2%, w/v, in *N*-HCl); aq. sodium aminopruesside (5%, w/v) (Boyland & Nery, 1964); aq. FeCl₃ (5%, w/v); ammoniacal AgNO₃. A solution of chromotropic acid (0.2%) in H₂SO₄ (72%, v/v) was used for the detection of formaldehyde. Hydrolysis of acetamido groups was carried out by spraying the paper chromatograms with 2*N*-HCl and heating them between glass sheets in an oven at 70° for 30min.

Table 1. R_F values of some 2-naphthylamine derivatives on paper chromatograms

Compositions of solvents and other details are given in the text. *N*-Formyl-2-naphthylhydroxylamine-*O*-sulphonic acid is converted into 2-formamido-1-naphthyl hydrogen sulphate and 2-formamido-1-naphthol during paper chromatography (see the Materials section).

Compound	Solvent ...	R_F				
		(a)	(b)	(d)	(e)	(f)
2-Naphthylamine		0.84	0.86	0.80	0.93	0.92
2-Acetamido-1-naphthol		0.87	0.88	0.77	0.95	0.95
2-Formamido-1-naphthol		0.86	0.84	0.86	0.93	0.92
2-Amino-1-naphthyl hydrogen sulphate		0.44	0.71	0.24	0.65	0.24
2-Formamido-1-naphthyl hydrogen sulphate		0.53	0.68	0.32	0.65	0.34
2-Acetamido-1-naphthyl hydrogen sulphate		0.60	0.71	0.52	0.73	0.49
2-Methylamino-1-naphthyl hydrogen sulphate		0.60	0.80	0.39	0.79	0.46
2-Dimethylamino-1-naphthyl hydrogen sulphate		0.60	0.83	0.39	0.79	0.62
2-Amino-1-naphthylmercapturic acid		0.43	0.92	0.18	0.91	0.16
(2-Amino-1-naphthyl glucosid)uronic acid		0.19	0.65	0.02	0.56	0.02
(2-Acetamido-1-naphthyl glucosid)uronic acid		0.28	0.81	0.07	0.74	0.06
(2-Formamido-1-naphthyl glucosid)uronic acid		0.25	0.70	0.05	0.63	0.06
(<i>N</i> -Formyl-2-naphthylhydroxylamine <i>O</i> -glucosid)uronic acid*		0.30	0.70	—	—	—

* Identity based on evidence given in the text.

Table 2. R_F values of some 2-naphthylamine derivatives on thin-layer chromatograms

Compositions of solvents and other details are given in the text.

Compound	Solvent ...	R_F						
		(a)	(g)	(h)	(i)	(j)	(k)	(l)
2-Amino-1-naphthyl hydrogen sulphate		0.66	—	—	—	—	—	0.60
2-Formamido-1-naphthyl hydrogen sulphate		0.73	—	—	—	—	—	0.62
2-Acetamido-1-naphthyl hydrogen sulphate		0.73	—	—	—	—	—	0.62
2-Methylamino-1-naphthyl hydrogen sulphate		0.73	—	—	—	—	—	0.62
<i>N</i> -Formyl-2-naphthylhydroxylamine- <i>O</i> -sulphonic acid		0.77	—	—	—	—	—	0.60
Ether-soluble pigment from <i>N</i> -formyl-2-naphthylhydroxylamine- <i>O</i> -sulphonic acid and NaHCO ₃		—	0.70	—	—	—	—	—
2-Naphthylamine		0.95	0.87	0.45	0.50	0.63	0.70	0.78
2-Formamidonaphthalene		—	0.65	0.30	0.75	0.30	0.42	0.61
2-Methylaminonaphthalene		—	0.93	0.60	0.90	0.87	0.96	0.96
2-Dimethylaminonaphthalene		—	0.97	0.80	0.87	0.93	0.97	0.97
<i>N</i> -Formyl-2-naphthylhydroxylamine		—	0.50	0.14	0.75	0.27	0.22	0.56
2-Formamido-1-naphthol		0.95	0.63	0.20	0.70	0.26	0.25	0.43
Oxidation product of 2-formamido-1-naphthol		0.95	0.25	0.00	0.40	0.15	0.03	0.20
2-Acetamido-1-naphthol		0.95	0.67	0.20	0.75	0.26	0.30	0.50
Oxidation product of 2-acetamido-1-naphthol		0.95	0.30	0.00	0.50	0.16	0.07	0.25
Naphtho[2,1- <i>d</i>]oxazole		—	0.77	0.60	0.82	0.77	0.83	0.96
2-Amino-3-naphthol		—	0.60	0.60	0.32	0.33	0.29	0.52
2-Amino-5-naphthol		—	0.32	0.17	0.22	0.30	0.29	0.51
2-Amino-6-naphthol		—	0.26	0.12	0.07	0.23	0.25	0.48
2-Amino-7-naphthol		—	0.27	0.00	0.00	0.23	0.21	0.43
2-Amino-8-naphthol		—	0.32	0.18	0.12	0.25	0.21	0.52

2-Naphthylhydroxylamine, *N*-acetyl-2-naphthylhydroxylamine-*O*-sulphonic acid, (*N*-acetyl-2-naphthylhydroxylamine *O*-glucosid)uronic acid (E. Boyland & D. Manson, unpublished work) and *N*-formyl-2-naphthylhydroxylamine-*O*-sulphonic acid (this paper) gave a bluish-green colour with hexylresorcinol (0.5%, w/v, in 2*N*-NaOH) alone, i.e. not preceded by aq. NaNO₂ and HCl as used in the diazotization and coupling procedure.

This colour reaction was specific for 2-naphthylhydroxylamine and these derivatives and was not given by any constituent of normal rat or dog urine.

Enzymic hydrolyses. Taka-diaxase (Parke, Davis and Co. Ltd., Hounslow, Middlesex) and Ketodase (Warner-Chilcott Laboratories, Morris Plains, N.J., U.S.A.) were used.

Infrared spectra. These were obtained by using Nujol

mulls of the samples between rock-salt plates in a Perkin-Elmer Infracord spectrophotometer, model 137.

Materials. Decolorizing charcoal was obtained from May and Baker Ltd., Dagenham, Essex (labelled as containing not more than 0.08% of Fe and having not more than 10% of ash) and sugar charcoal from Hopkin and Williams Ltd., Chadwell Heath, Essex. 2-Naphthylamine was supplied by the British Drug Houses Ltd., Poole, Dorset. The following compounds were synthesized: salts of 2-amino-1-naphthyl hydrogen sulphate by the persulphate oxidation of 2-naphthylamine (Boyland, Manson & Sims, 1953); 2-amino-1-naphthol, 2-acetamido-1-naphthol and 2-acetamido-1-naphthyl sodium sulphate (Booth, Boyland & Manson, 1955); 2-naphthylhydroxylamine and 2-amino-1-naphthylmercapturic acid (Boyland, Manson & Nery, 1962); 2-dimethylaminonaphthalene (Billman, Radike & Mundy, 1942); 2-dimethylaminonaphthalene *N*-oxide phosphate (Boyland & Manson, 1957a); 2-dimethylamino-1-naphthyl sodium sulphate (Sims, 1958). (2-Amino-1-naphthyl glucosid)uronic acid and (2-acetamido-1-naphthyl glucosid)uronic acid were obtained as described by Boyland & Manson (1957b).

2-Formamido-1-naphthol. 2-Amino-1-naphthol hydrochloride (3g.) in water (25ml.) was treated with NaOH (1 equiv.) and $\text{Na}_2\text{S}_2\text{O}_4$ (0.5g.) to precipitate 2-amino-1-naphthol, which was quickly washed with water and dried over P_2O_5 . Dicyclohexylcarbodi-imide (2g.) and formic acid (98–100%) (5ml.) were added to a solution of 2-amino-1-naphthol (1.7g.) in benzene-ether (1:1, v/v) (50ml.). After 4hr. dicyclohexylurea was filtered off and washed with benzene. The filtrate was evaporated to dryness *in vacuo* to yield a solid (1.2g.), which, after recrystallization from benzene-light petroleum (b.p. 40–60°), yielded 2-formamido-1-naphthol as needles (0.6g.), m.p. 136–138° (Found: C, 70.7; H, 4.55; N, 7.1. $\text{C}_{11}\text{H}_9\text{NO}_2$ requires C, 70.6; H, 4.85; N, 7.5%). The compound was soluble in cold 2N-NaOH but rapidly hydrolysed to 2-amino-1-naphthol, as shown by the development of a greenish-brown colour and the separation of a purple pigment; in 2N- NH_3 hydrolysis was slower. Attempted formylation of the hydrochloride of 2-amino-1-naphthol by the above method was unsuccessful.

2-Formamido-1-naphthol and 2-acetamido-1-naphthol decomposed during thin-layer chromatography to yield compounds that gave a blue colour with ammoniacal AgNO_3 immediately. The difference between the R_F values of these second spots in most solvent systems was greater than the difference between the R_F values of the parent compounds. The formamido- and acetamido-naphthol and their oxidation products all gave blue colours on chromatograms with nitrous acid and with sodium aminoprusside solution.

Naphtho[2,1-d]oxazole. 2-Amino-1-naphthol hydrochloride (4g.), sodium formate (4g.) and formic acid (15ml.) were heated under reflux for 3hr. Fischer & Römer (1906) stated that the oxazole was obtained by evaporation of the solution, but thin-layer chromatography showed that, although the oxazole was present, the major product was 2-formamido-1-naphthol. The reaction mixture was evaporated and the residue was extracted several times with hot benzene. The residue from the benzene extracts was sublimed at 180° and 15mm. The sublimate crystallized from light petroleum (b.p. 40–60°) to yield the oxazole (0.5g.), m.p. 77–79° [Fischer & Römer (1906) gave

m.p. 79°] (Found: C, 78.1; H, 4.2; N, 8.0. Calc. for $\text{C}_{11}\text{H}_7\text{NO}$: C, 78.1; H, 4.2; N, 8.3%). When heated at 100° with 2N-HCl (but not with water alone) the compound was converted into 2-amino-1-naphthol.

2-Formamido-1-naphthyl hydrogen sulphate. Formic acid (98–100%) (3ml.) was added with cooling to 2-amino-1-naphthyl ammonium sulphate (1.15g.) and dicyclohexylcarbodi-imide (3g.) in pyridine (15ml.). After 16hr. dicyclohexylurea was filtered off and the filtrate evaporated to 5ml. and diluted with water (150ml.). The pH was adjusted to 4.0 and the solution stirred with charcoal (5g.) for 30min. The charcoal was filtered off and washed with water and the sulphuric ester was eluted with methanol containing 5% (v/v) of aq. NH_3 (sp.gr. 0.88). The eluate was evaporated and the residue crystallized from aq. ethanol to yield 2-formamido-1-naphthyl ammonium sulphate (0.5g.), m.p. 169–171° (decomp.) (Found: C, 46.1; H, 4.7; N, 10.0; S, 11.5. $\text{C}_{11}\text{H}_{12}\text{N}_2\text{O}_5\text{S}$ requires C, 46.6; H, 4.3; N, 9.9; S, 11.3%). Formylation of 2-amino-1-naphthyl sodium sulphate (1g.) in the same way gave 2-formamido-1-naphthyl sodium sulphate (0.25g.), which crystallized from aq. ethanol, m.p. 175–178° (Found: C, 45.5; H, 2.9; N, 5.0; S, 11.5. $\text{C}_{11}\text{H}_9\text{NNaO}_5\text{S}$ requires C, 45.7; H, 2.8; N, 4.8; S, 11.1%). The salts were stable at 5°, but decomposed over several months at room temperature to 2-amino-1-naphthol, 2-amino-1-naphthyl hydrogen sulphate and 2-formamido-1-naphthol. When an aqueous solution of 2-formamido-1-naphthyl hydrogen sulphate was acidified with 2N-HCl the formyl group was removed and 2-amino-1-naphthyl hydrogen sulphate separated. Formic acid was identified in solvent (c) as a product of hydrolysis with 2N- H_2SO_4 ; chloride prevented the detection of formate in this solvent system. Hot mineral acids removed both the sulphate and the formyl groups, but heating in aq. acetic acid (50%, v/v) or aq. formic acid (50%, v/v) for 20min. gave mainly 2-formamido-1-naphthol (detected as described above). The formyl group was rapidly removed in 2N-NaOH at room temperature and slowly in saturated aq. NaHCO_3 or 2N- NH_3 . 2-Acetamido-1-naphthyl hydrogen sulphate behaved differently from 2-formamido-1-naphthyl hydrogen sulphate. At room temperature 2-acetamido-1-naphthyl hydrogen sulphate gave 2-acetamido-1-naphthol with 2N-HCl and was unchanged by 2N-NaOH. 2-Formamido-1-naphthyl hydrogen sulphate appeared as a u.v.-absorbing spot on paper chromatograms. Incubation with Taka-diastase or limpet arylsulphatase at pH 6.0 or with human-urine arylsulphatase at pH 5.0 or pH 7.0 at 37° did not remove the sulphate group.

2-Formamidonaphthalene. This compound was prepared by heating 2-naphthylamine (30g.) in formic acid (98–100%) (100ml.) under reflux for 4hr. (Liebermann & Jacobson, 1882). The compound was precipitated by the addition of water and was crystallized from benzene (yield 24g.), m.p. 128–129° [Liebermann & Jacobson (1882) gave m.p. 129°]. The compound reacted slowly with *p*-dimethylaminocinnamaldehyde. Aq.-acetone solutions of the compound treated with sodium persulphate in the presence of NaHCO_3 or NaOH were not oxidized to 2-formamido-1-naphthyl hydrogen sulphate, but some 2-amino-1-naphthyl hydrogen sulphate was formed in the presence of NaOH.

***N*-Formyl-2-naphthylhydroxylamine.** Formic acid (2ml.) was added with cooling to 2-naphthylhydroxylamine (1g.) and dicyclohexylcarbodi-imide (2g.) in benzene (50ml.) and

Table 3. *Colour reactions of 2-naphthylamine and some derivatives on chromatograms*

Details of the tests are given in the text.

Compound	HCl, NaNO ₂ and hexylresorcinol	<i>p</i> -Dimethyl- aminocinnam- aldehyde*	Diazotized <i>p</i> -nitroaniline and Na ₂ CO ₃	Diazotized sulphanilic acid and Na ₂ CO ₃	Reduction of ammoniacal AgNO ₃	Other tests
2-Naphthylamine	Yellow	Immediate	Red	Orange	1-2 min.	A colour is given by diazotized amine reagents without Na ₂ CO ₃ A colour is only given by diazotized amine reagents after Na ₂ CO ₃ Blue against brown background by the test for <i>N</i> -oxides of Ziegler & Pettit (1964)
2-Formamidonaphthalene	None	Slow	None	None	None	
2-Methylaminonaphthalene	Greenish yellow†	Immediate	Mauve	Orange-red	1-2 min.	
2-Dimethylaminonaphthalene	Greenish yellow†	None	Mauve	Red	1-2 min.	Blue with FeCl ₃ , nitrous acid or sodium aminopruesside
2-Dimethylaminonaphthalene <i>N</i> -oxide	None	Immediate	None	None	None	
2-Formamido-1-naphthol	Blue†	Slow	Orange-red	Red, becoming mauve	Immediate (black changing to blue)	
Oxidation product of 2-formamido-1-naphthol	Blue†	Slow	None	None	Immediate	Mauve with FeCl ₃
2-Acetamido-1-naphthol	Blue†	Slow	Red	Red, becoming yellow	Immediate (black changing to blue)	
Oxidation product of 2-acetamido-1-naphthol	Blue†	Slow	None	None	Immediate	
<i>N</i> -Formyl-2-naphthylhydroxylamine	None	Slow	None	None	Slow	Gives the reactions of 2-amino- 1-naphthyl hydrogen sulphate after treatment with 2 <i>N</i> -HCl Yields mainly 2-acetamido-1- naphthol on treatment with 2 <i>N</i> -HCl at room temperature Green with nitrous acid; bluish grey with FeCl ₃ Spot becomes brownish-red in sunlight
Naphtho[2,1- <i>d</i>]oxazole	None	Immediate	None	None	None	
2-Amino-3-naphthol	Red	Immediate	Mauve	Mauve	Immediate	
2-Amino-5-naphthol	Brownish red	Immediate	Brownish red	Orange	Immediate	
2-Amino-6-naphthol	Red	Immediate	Mauve	Pink, becoming mauve	Immediate	
2-Amino-7-naphthol	Orange	Immediate	Red	Orange	Immediate	
2-Amino-8-naphthol	Red	Immediate	Mauve	Red	Immediate	
2-Amino-1-naphthyl hydrogen sulphate	Mauve	Immediate	—	—	Slow	
2-Formamido-1-naphthyl hydrogen sulphate	None	1-2 min.	—	—	None	
2-Acetamido-1-naphthyl hydrogen sulphate	None	None	—	—	None	
2-Methylamino-1-naphthyl hydrogen sulphate	Transient mauve, becoming grey-green	Immediate	Pale yellow	None	5-10 min.	

2-Dimethylamino-1-naphthyl hydrogen sulphate	None	None	None	5-10 min.	Blue-green with alkaline hexylresorcinol
N-Formyl-2-naphthylhydroxylamine-O-sulphonic acid	Blue-green†	None	—	Immediate	Positive reaction to $K_2Cr_2O_7$ - $AgNO_3$ and platinum chloride reagents
2-Amino-1-naphthylmercapturic acid	Yellow	Immediate	—	Slow	—
(2-Amino-1-naphthyl glucosid)uronic acid	Brownish red (mauve if diazotized with $NaNO_2$ and acetic acid)	Immediate	—	Slow	—
(2-Formamido-1-naphthyl glucosid)uronic acid	None	1-2 min.	—	None	Gives the reactions of the amino compound after treatment with 2N-HCl at room temperature
(2-Acetamido-1-naphthyl glucosid)uronic acid	None	None	—	None	Gives the reactions of the amino compound after treatment with hot 2N-HCl
(N-Formyl-2-naphthylhydroxylamine O-glucosid)uronic acid	Blue-green†	—	—	—	Blue-green with alkaline hexylresorcinol

* All compounds that react give a red colour.

† These colours are produced by the nitrous acid and no further colour is given with hexylresorcinol.

‡ This colour is due to breakdown to 2-naphthylhydroxylamine, which reacts with hexylresorcinol in alkaline solution (see the Experimental section).

ether (2 ml.). After standing at room temperature for 2 hr., the dicyclohexylurea was collected and the filtrate evaporated *in vacuo* at 30°. The residue was placed over NaOH pellets *in vacuo* overnight to remove formic acid and crystallized from aq. ethanol to yield N-formyl-2-naphthylhydroxylamine as plates (0.25 g.), m.p. 146-148° (decomp.) (Found: C, 70.2; H, 4.3; N, 7.2. $C_{11}H_9NO_2$ requires C, 70.6; H, 4.85; N, 7.5%). The compound yielded 2-amino-1-naphthol on heating with 2N-HCl.

N-Formyl-2-naphthylhydroxylamine-O-sulphonic acid. N-Formyl-2-naphthylhydroxylamine (1 g.) and pyridine- SO_3 (2 g.) in dry benzene (150 ml.) were stirred for 2 hr. The precipitate that formed was collected and dissolved in methanol (100 ml.). The procedure for the isolation of the N-acetylphenylhydroxylamine-O-sulphonic acid (Boyland & Nery, 1962) could not be followed as the use of dry NH_3 gas to precipitate SO_4^{2-} caused the breakdown of the N-formyl compound. This step was omitted and potassium acetate (2 g.) in methanol (25 ml.) was added; a solid separated on cooling which was a mixture of the sulphuric ester and K_2SO_4 . It was triturated with ice-cold water to remove the inorganic matter and the undissolved material was collected and washed successively with cold water, ethanol and ether to yield potassium N-formyl-2-naphthylhydroxylamine-O-sulphonate (0.6 g.), m.p. 148-150° (decomp.) (Found: C, 43.2; H, 2.7; N, 4.4; S, 9.9. $C_{11}H_9KNO_5S$ requires C, 43.3; H, 2.6; N, 4.6; S, 10.5%). In water at room temperature potassium N-formyl-2-naphthylhydroxylamine-O-sulphonate yielded mainly 2-formamido-1-naphthol and 2-formamido-1-naphthyl hydrogen sulphate, but 2-naphthylhydroxylamine and 2-amino-1-naphthyl hydrogen sulphate were also detected. The solution contained SO_4^{2-} and became acid in reaction. The compound broke down rapidly in acid or alkaline solution. In N-acetic acid the products were the same as in water. In $NaHCO_3$ solution a blue ether-soluble material was also formed which differed on thin-layer chromatograms from that produced by oxidation of 2-formamido-1-naphthol. In 2N-HCl, 2-formamido-1-naphthol, 2-naphthylamine and 2-amino-1-naphthyl hydrogen sulphate were formed. In m- H_3PO_4 , 2-naphthylhydroxylamine and 2-amino-1-naphthyl dihydrogen phosphate were also formed. In 2N- NH_3 and 2N-NaOH solutions 2-amino-1-naphthol, 2-amino-1-naphthyl hydrogen sulphate and 2-naphthylamine were formed immediately. Like N-acetyl-2-naphthylhydroxylamine-O-sulphonic acid and 2-naphthylhydroxylamine the compound gave a bluish-green colour with alkaline hexylresorcinol alone. The compound decomposed during paper chromatography to give 2-formamido-1-naphthyl hydrogen sulphate and 2-formamido-1-naphthol.

2-Methylaminonaphthalene. This compound was prepared by the reduction of 2-formamidonaphthalene (20 g.) with $AlLiH_4$ (10 g.) in ether (70 ml.) (Wessely & Swoboda, 1951); the yield was 15.5 g. (b.p. 114-115°/0.8 mm.).

2-Methylamino-1-naphthyl hydrogen sulphate. Sodium persulphate (8 g.) in water (200 ml.) was added to 2-methylaminonaphthalene (5 g.) in acetone (500 ml.) and 2N-NaOH (30 ml.) with stirring. After standing overnight the solution was evaporated almost to dryness. Water was added, and the solution was extracted with ether and adjusted to pH 4.0 and treated with charcoal. The charcoal was filtered off and washed with methanolic NH_3 and the eluate evaporated. The residue was dissolved in ethanol (10 ml.) and 10N-NaOH (1 ml.) added. The solution was cooled

to yield crystals contaminated with some 2-amino-1-naphthyl hydrogen sulphate, but after recrystallization from aq. ethanol twice yielded 2-methylamino-1-naphthyl sodium sulphate (0.46 g.), m.p. 258–264° (decomp.) (Found: C, 48.1; H, 3.6; N, 5.3; S, 12.0. $C_{11}H_{10}NNaO_4S$ requires C, 48.0; H, 3.7; N, 5.1; S, 11.6%). Demethylation *in vivo* or on charcoal gave 2-amino-1-naphthyl hydrogen sulphate (see below). The preparation of 2-methylaminophenyl potassium sulphate from *N*-methylaniline by persulphate oxidation has been described (Boyland & Sims, 1958).

RESULTS

Isolation of 2-formamido-1-naphthyl hydrogen sulphate from dog urine

Urine from eight female beagles receiving daily oral doses of 2-naphthylamine (400 mg.) was collected until 25.6 g. of the amine had been given. The daily specimens of urine were adjusted to pH 5.0 and treated with 20 g. portions of charcoal until they were colourless. The charcoal was washed with water and stirred with five portions of methanol (200 ml.) containing 5% (v/v) of aq. ammonia (sp.gr. 0.88). The combined extracts were evaporated to dryness at 40° under reduced pressure. The residues from this procedure were dissolved in solvent (a) and made into a paste with cellulose powder, which was added to the top of a cellulose powder column (6 cm. x 55 cm.) prepared in the same solvent mixture. Elution of the column was continued with 2l. of solvent until 2-formamido-1-naphthyl hydrogen sulphate appeared. Separation from the more abundant 2-amino-1-naphthyl hydrogen sulphate was not possible and both metabolites were collected in one large fraction. The solvent (1l.) was removed under reduced pressure after the addition of aq. ammonia (sp.gr. 0.88) (10 ml.). 2-Amino-1-naphthyl ammonium sulphate (8.5 g.) was collected as evaporation proceeded. When the solvent had been removed the residue was dissolved in the minimum of water, acidified with acetic acid and allowed to stand at 5° for 2 hr. and more 2-amino-1-naphthyl hydrogen sulphate (0.5 g.) collected. The filtrate was neutralized with aq. ammonia and the solution was applied to 50 sheets of Whatman no. 3MM chromatography paper and the chromatograms were developed in solvent (a). 2-Formamido-1-naphthyl hydrogen sulphate formed a u.v.-absorbing band that was eluted with aq. methanol (90%, v/v). Aq. ammonia was added and the extracts were evaporated under reduced pressure. The residue was triturated with hot ethanol, cooled, and the solid (30 mg.) collected and washed with ethanol and ether. The material was dissolved in the minimum of ethanol and cooled. 2-Formamido-1-naphthyl ammonium sulphate crystallized as small spheres (15 mg.), m.p. 168–170° (decomp.) (Found: C, 46.6; H, 4.4.

$C_{11}H_{12}N_2O_5S$ requires C, 46.6; H, 4.3%). In another experiment the filtrate from the acetic acid precipitation was neutralized with 2N-sodium hydroxide and the sodium salt of the metabolite was isolated from the chromatograms. It crystallized from aqueous ethanol as rosettes of needles, m.p. 172–174° (decomp.) (Found: S, 11.15. $C_{11}H_8NNaO_5S$ requires S, 11.1%). The i.r. spectra and other properties of the sodium and ammonium salts of the metabolite were identical with those of the corresponding salts of synthetic 2-formamido-1-naphthyl hydrogen sulphate. The metabolite was also identified in the urine of mongrel bitches given single doses of 2-naphthylamine. Treatment of the urine with charcoal was necessary for the separation of this metabolite from 2-amino-1-naphthyl hydrogen sulphate. As 2-formamido-1-naphthyl hydrogen sulphate is formed from 2-methylamino-1-naphthyl hydrogen sulphate or from 2-amino-1-naphthyl hydrogen sulphate and formaldehyde by charcoal (see below), the possibility that the metabolite is an artifact arising from the use of charcoal was investigated. Urine from a dog dosed with 2-naphthylamine (400 mg. daily) was collected for 4 days, adjusted to pH 4.0 and extracted four times with butan-1-ol. The butanol extracts were evaporated to dryness and the residue applied to sheets of Whatman no. 3MM chromatography paper. After development with solvent (a) 2-formamido-1-naphthyl hydrogen sulphate was detected on the chromatograms. It was eluted from the paper and identified by colour tests and by the formation of 2-formamido-1-naphthol when it was heated with dilute acetic acid. 2-Methylamino-1-naphthyl hydrogen sulphate was not detected. In another experiment a series of catheter specimens was obtained over several hours. Samples of urine were run in solvent (m) on thin-layer chromatograms. The chromatograms were sprayed with alkaline hexylresorcinol, but *N*-formyl-2-naphthyl-hydroxylamine-*O*-sulphonic acid was not detected and there was no blue-green colour reaction given by other spots.

Detection of 2-formamido-1-naphthyl hydrogen sulphate in rat urine

Four female rats were dosed with 2-naphthylamine (50 mg. in 1 ml. of arachis oil) daily for 4 days. The urine was treated with charcoal which was then washed with methanolic ammonia. The metabolites in the methanol eluate were separated by chromatography on Whatman no. 3MM paper in solvent (a). 2-Formamido-1-naphthyl hydrogen sulphate was eluted with methanol and identified as described above. The region of the chromatograms that might contain (2-formamido-1-naphthyl glucosid)uronic acid (see below) was also eluted with

methanol. The methanol extracts were evaporated to dryness and the residue was dissolved in acetate buffer, pH 5.0 (5ml.). Ketodase (2ml.) was added and the solution was incubated for 16hr. and extracted with ether. Only 2-acetamido-1-naphthol was detected in the ether extract (cf. Table 4).

2-Formamido-1-naphthyl hydrogen sulphate could not be detected in the urine of hamsters or guinea pigs after the administration of 2-naphthylamine.

Metabolism of 2-amino-1-naphthyl hydrogen sulphate in rats and dogs

After the injection of 2-amino-1-naphthyl sodium sulphate (50mg. in 1ml. of water) into two female rats daily for 4 days, 2-formamido-1-naphthyl hydrogen sulphate and (2-amino-1-naphthyl glucosid)uronic acid were detected in the urine.

The urine of a dog dosed orally with 2-amino-1-naphthyl sodium sulphate (200mg. in a gelatin capsule) daily for 3 days contained the formamido derivative and (2-amino-1-naphthyl glucosid)uronic acid.

Metabolism of 2-formamido-1-naphthyl hydrogen sulphate in the rat

2-Formamido-1-naphthyl sodium sulphate (50mg. in 1ml. of water) was injected into two female rats. Urine collected after 1hr. contained a trace of the administered compound but the major

compound present was 2-amino-1-naphthyl hydrogen sulphate. In urine obtained 3 and 16hr. after injection, the free amino compound was the only metabolite detected.

Metabolism of N-formyl and N-methyl compounds

The above experiments indicate that 2-formamido-1-naphthyl hydrogen sulphate was probably formed by the formylation of 2-amino-1-naphthyl hydrogen sulphate, but the metabolism in rats of some other possible precursors was studied.

2-Formamidonaphthalene. Two female rats were each given four daily injections of 2-formamidonaphthalene (50mg. in 1ml. of arachis oil). The urine was treated with charcoal and the metabolites were eluted with methanolic ammonia. Traces of 2-amino-1-naphthyl hydrogen sulphate and (2-amino-1-naphthyl glucosid)uronic acid, but not the corresponding formamido compounds, were detected. A portion of the residue from the methanol eluate was heated with 5N-hydrochloric acid for 20min. at 100°. The solution was cooled, adjusted to pH 5.0 and extracted with ether. Thin-layer chromatography showed the presence of two major spots that had the R_f values and colour reactions of 2-amino-6-naphthol and 2-amino-8-naphthol. 2-Amino-3-naphthol, -5-naphthol and -7-naphthol were not detected. Paper chromatography of the

Table 4. 2-Amino-1-naphthol conjugates excreted by rats after being dosed with 2-naphthylamine and some derivatives

2-Acetamido-1-naphthyl hydrogen sulphate was not detected as a metabolite of any of the above compounds, nor does it appear to be a metabolite of 2-naphthylamine in the dog, rabbit, guinea pig or hamster (E. Boyland & D. Manson, unpublished work). + denotes the principal 2-amino-1-naphthol metabolites; tr. denotes 2-amino-1-naphthol metabolites that gave only faint colour reactions.

Conjugates of 2-amino-1-naphthol

	2-Amino-1-naphthyl hydrogen sulphate	2-Formamido-1-naphthyl hydrogen sulphate	(2-Amino-1-naphthyl glucosid)-uronic acid	(2-Acetamido-1-naphthyl glucosid)-uronic acid	(2-Formamido-1-naphthyl glucosid)-uronic acid
2-Naphthylamine*	+	tr.	+	+	—
2-Formamidonaphthalene	tr.	—	tr.	—	—
N-Formyl-2-naphthylhydroxylamine	+	+	tr.	—	+
2-Formamido-1-naphthol†	tr.	tr.	tr.	—	+
Naphtho[2,1-d]oxazole	tr.	tr.	tr.	—	+
2-Amino-1-naphthyl hydrogen sulphate*	+	tr.	tr.	—	—
2-Formamido-1-naphthyl hydrogen sulphate	+	tr.	—	—	—
2-Methylaminonaphthalene‡	+	tr.	tr.	—	—
2-Methylamino-1-naphthyl hydrogen sulphate†	+	—	—	—	—

* Dogs dosed with these compounds excreted the same 2-amino-1-naphthol conjugates, except for (2-acetamido-1-naphthyl glucosid)uronic acid.

† Rabbits dosed with this compound excreted the same metabolites.

‡ 2-Methylamino-1-naphthyl hydrogen sulphate was also detected in the urine.

residue from the methanol eluate showed spots that gave red colours with *p*-dimethylaminocinnamaldehyde after a few minutes, which indicated that these metabolites still carried the formamido group. (*N*-Formyl-2-naphthylhydroxylamine *O*-glucosid)uronic acid was not detected.

N-Formyl-2-naphthylhydroxylamine. *N*-Formyl-2-naphthylhydroxylamine (25 mg. in 1 ml. of arachis oil) was given to two female rats daily for 2 days. 2-Naphthylamine but neither 2-naphthylhydroxylamine nor its *N*-formyl derivative were detected in ether extracts of the urine. Paper chromatography of the urine showed the presence of 2-amino-1-naphthyl hydrogen sulphate and its formamido derivative and a trace of (2-amino-1-naphthyl glucosid)uronic acid. After two-dimensional paper chromatography in solvent (a) followed by solvent (b), 2-amino-1-naphthylmercaptopuric acid was detected. After treatment of the chromatograms with 2*N*-hydrochloric acid at 70° for 30 min. to hydrolyse *N*-acetyl or *N*-formyl derivatives there was only a slight reaction to amino group reagents. Paper chromatography also showed the presence of a compound [R_F 0.3 in solvent (a) and R_F 0.7 in solvent (b)] that gave a faint bluish-green colour with hexylresorcinol. In this colour reaction and the R_F value it resembled (*N*-acetyl-2-naphthylhydroxylamine *O*-glucosid)uronic acid (Boyland & Manson, 1963), but the colour of the spot with *p*-dimethylaminocinnamaldehyde was more intense than was expected from the hexylresorcinol reaction. When the spot was treated with 2*N*-hydrochloric acid at room temperature for a few minutes it gave a brick-red colour with hexylresorcinol after diazotization. The metabolite was further concentrated by charcoal adsorption. The residue obtained by methanol elution was then chromatographed on Whatman no. 3MM paper and the metabolite eluted with aqueous methanol. Treatment of the residue from this with Ketodase yielded only 2-formamido-1-naphthol, indicating that the major constituent of the original spot was (2-formamido-1-naphthyl glucosid)uronic acid, but the faint colour reaction with alkaline resorcinol was probably due to the presence of (*N*-formyl-2-naphthylhydroxylamine *O*-glucosid)uronic acid. Boyland & Manson (1963) showed that, although (*N*-acetyl-2-naphthylhydroxylamine *O*-glucosid)-uronic acid gave 2-naphthylamine under acid or alkaline conditions, it did not appear to rearrange to (2-acetamido-1-naphthyl glucosid)uronic acid. *N*-Formyl-2-naphthylhydroxylamine *O*-sulphonic acid was not detected on examination of untreated urine by thin-layer chromatography in solvent (m).

2-Formamido-1-naphthol. The compound was administered to two female rats (30 mg. in 1 ml. of arachis oil) daily for 5 days. The principal metabolite was (2-formamido-1-naphthyl glucosid)uronic

acid, identified by the release of 2-formamido-1-naphthol with Ketodase after separation of the metabolite by paper chromatography. Only traces of 2-amino-1-naphthyl hydrogen sulphate, 2-formamido-1-naphthyl hydrogen sulphate and (2-amino-1-naphthyl glucosid)uronic acid were found.

2-Formamido-1-naphthol was also administered to rabbits to obtain (2-formamido-1-naphthyl glucosid)uronic acid for use as a reference compound. Two male rabbits were each injected intraperitoneally with 2-formamido-1-naphthol (300 mg.) in arachis oil (5 ml.) for 8 days (total dose 4.8 g.); the doses were daily except that 3 days elapsed between the fourth and fifth doses. The urine was collected daily, adjusted to pH 5.0 and stirred with charcoal (three 4 g. portions). The charcoal was washed with water and then stirred with methanol containing 5% (v/v) of aq. ammonia (sp.gr. 0.88). The elution with methanol was repeated several times and the combined methanol extracts were evaporated to dryness under reduced pressure. The residue was dissolved in aq. methanol (50%, v/v) and applied to sheets of Whatman no. 3MM chromatography paper. The chromatograms were developed in solvent (a). The areas containing the glucosiduronic acid were cut out and the metabolite was extracted from the paper strips with aq. methanol (90%, v/v). The extracts were evaporated to dryness under reduced pressure to yield a gum (2.5 g.). Half of this was triturated with ethanol to give a white solid (700 mg.), which was filtered off and washed with ethanol and ether. The material (100 mg.) was dissolved in water (1.5 ml.) and ammonium sulphate added until the solution was saturated (cf. Williams, 1940). The solution was filtered and set aside for 24 hr. at room temperature. The crystals (plates) were collected and washed with ice-cold water, giving ammonium (2-formamido-1-naphthyl glucosid)uronate (25 mg.), m.p. 222–224° (decomp.) (Found: C, 52.9; H, 5.5; N, 7.2. $C_{17}H_{20}N_2O_8$ requires C, 53.7; H, 5.3; N, 7.4%), $[\alpha]_D -31.1^\circ$ (c 0.45 in water).

The other half of the gum obtained above was treated with diazomethane followed by acetic anhydride in pyridine, but attempts to obtain a crystalline triacetyl methyl ester were not successful.

The *N*-formyl group of the metabolite was readily removed at room temperature by 2*N*-hydrochloric acid or 2*N*-sodium hydroxide to yield (2-amino-1-naphthyl glucosid)uronic acid. Hydrolysis in 5*N*-ammonia was slow. When treated with Ketodase at 37° the metabolite gave 2-formamido-1-naphthol and glucuronic acid.

Attempts to isolate the glucosiduronic acid from urine by the lead acetate procedure were not successful, as hydrolysis of the *N*-formyl group

occurred in the acid conditions that developed during the passage of hydrogen sulphide.

Paper chromatography of the urine and of the residue from the methanol elution of the charcoal showed that (2-formamido-1-naphthyl glucosid)-uronic acid was the principal metabolite and that, as with rat urine, only traces of other 2-amino-1-naphthol conjugates were present.

Naphtho[2,1-d]oxazole. Two female rats were injected with the compound (30mg.) in arachis oil (1ml.) daily for 2 days. The metabolites were the same as those present after dosing with 2-formamido-1-naphthol, with the addition of an unidentified compound with R_f 0.18 on paper chromatography in solvent (a). This gave a red colour with *p*-dimethylaminocinnamaldehyde and a red colour with hexylresorcinol after nitrous acid.

2-Methylamino-1-naphthyl hydrogen sulphate. Two female rats were dosed with 2-methylamino-1-naphthyl sodium sulphate (40mg. in 1ml. of water) daily for 5 days. The urine was extracted with butan-1-ol at pH 4.0. 2-Methylamino-1-naphthyl hydrogen sulphate was the predominant compound detected by paper chromatography. 2-Amino-1-naphthyl hydrogen sulphate was found, but the formamido derivative was not detected.

2-Methylaminonaphthalene. Two female rats were dosed with 2-methylaminonaphthalene (30mg. in 1ml. of arachis oil) daily for 4 days. Ether extracts of the urine contained the administered compound and 2-naphthylamine. 2-Amino-, 2-formamido- and 2-methylamino-1-naphthyl hydrogen sulphate and (2-amino-1-naphthyl glucosid)-uronic acid were detected on paper chromatograms of the urine. No other amino compounds were detected but other spots on the chromatogram gave colours with ferric chloride or diazotized *p*-nitroaniline similar to those given by 2-methylaminonaphthalene and 2-methylamino-1-naphthyl sulphate, and were probably metabolites that were *N*-methyl derivatives.

Formation of 2-formamido-1-naphthyl hydrogen sulphate in the presence of charcoal

(a) *From 2-methylamino-1-naphthyl hydrogen sulphate.* During the synthesis of 2-methylamino-1-naphthyl hydrogen sulphate, it appeared to be demethylated by charcoal. Demethylation was accompanied by the formation of 2-formamido-1-naphthyl hydrogen sulphate. On bubbling air from the reaction mixture into a solution of chromotropic acid at 50°, formaldehyde was detected by the appearance of the characteristic mauve colour. 2-Methylamino-1-naphthyl sodium sulphate (300mg.) in water (50ml.) was adjusted to pH 5.0 and charcoal (2g.) added. The mixture was aerated for 4hr. at room temperature and the charcoal was

collected by filtration and washed several times with methanolic ammonia. The methanol extracts were combined with the aqueous filtrate from the charcoal and evaporated under reduced pressure at 40°. The residue was applied to Whatman no. 3MM chromatography paper and the chromatograms were developed in solvent (a). The regions containing the reaction products and unchanged starting material were cut out and extracted with aq. methanol. Each extract was evaporated to dryness under reduced pressure. 2-Methylamino-1-naphthyl sodium sulphate (72mg.) was crystallized from aq. ethanol. 2-Amino-1-naphthyl hydrogen sulphate (25mg.) was obtained by acidification of the residue from the methanol extract and was identical (i.r. spectrum and mixed m.p.) with an authentic specimen of the compound. The third compound (11mg.) had m.p. 175–178° after crystallization from ethanol, yielded 2-formamido-1-naphthol on heating with aq. acetic acid (50%) and the i.r. spectrum was identical with that of 2-formamido-1-naphthyl sodium sulphate. The mother liquors contained a further 8mg. When treated in the same way 2-dimethylamino-1-naphthyl sodium sulphate gave 2-methylamino-1-naphthyl hydrogen sulphate, 2-formamido-1-naphthyl hydrogen sulphate, 2-amino-1-naphthyl hydrogen sulphate and formaldehyde. 2-Dimethylaminonaphthalene (100mg. in 20ml. of 60% ethanol and 200mg. of charcoal) was converted into 2-methylaminonaphthalene and a trace of 2-naphthylamine. 2-Formamidonaphthalene and 2-dimethylaminonaphthalene *N*-oxide were not detected. 2-Dimethylaminonaphthalene *N*-oxide phosphate (50mg. in 10ml. of water; 150mg. of charcoal) yielded 2-dimethylaminonaphthalene, 2-methylaminonaphthalene and 2-naphthylamine. All these reactions also took place on charcoal deactivated with stearic acid; the products were eluted from this with aq. phenol (5%, w/v).

(b) *From 2-amino-1-naphthyl hydrogen sulphate and formaldehyde.* Air was passed through a solution of 2-amino-1-naphthyl sodium sulphate (2g.) and formaldehyde (36%) (15ml.) in water (250ml.) at pH 4.0 with charcoal (5g.) for 16hr. The charcoal was collected by filtration and washed with methanolic ammonia. The washings were combined with the aqueous filtrate and evaporated under reduced pressure. Examination by paper chromatography showed the presence of starting material, 2-formamido-1-naphthyl hydrogen sulphate and 2-methylamino-1-naphthyl hydrogen sulphate. These compounds were separated on Whatman no. 3MM chromatography paper as in (a); 2-methylamino-1-naphthyl sodium sulphate was crystallized from aq. ethanol and ether. The fraction also contained material with a white fluorescence that was formed from 2-amino-1-

naphthyl hydrogen sulphate and formaldehyde without the presence of charcoal. An odour of formaldehyde was present during evaporation of this fraction. From the other fractions 2-formamido-1-naphthyl sodium sulphate (160mg.) and 2-amino-1-naphthyl hydrogen sulphate (340mg.) were obtained. All the compounds isolated were identical with the authentic compounds in i.r. spectra and in other properties. Several unknown compounds, both fluorescent and u.v.-absorbing, were present on the chromatograms. When 2-naphthylamine was treated with formaldehyde in a similar manner only trace amounts of 2-formamido-naphthalene were formed.

When 2-amino-1-naphthyl hydrogen sulphate was treated with formaldehyde in the presence of sugar charcoal neither 2-formamido- nor 2-methylamino-1-naphthyl hydrogen sulphate was detected. Little adsorption of the sulphuric ester occurred.

DISCUSSION

2-Formamido-1-naphthyl hydrogen sulphate is a hitherto unknown metabolite of 2-naphthylamine. The removal of the formyl group when the compound is administered to rats indicates that the amount detected in rat or dog urine represents a small part of the metabolite actually formed. It was not detected in the urine of species (guinea pig and hamster) that excreted only small amounts of 2-amino-1-naphthyl hydrogen sulphate. It is probable that formylation of the amino group occurs after the formation of 2-amino-1-naphthyl hydrogen sulphate rather than by formylation of 2-naphthylamine itself, as 2-formamidonaphthalene was converted by rats mainly into conjugates of 2-formamido-6-naphthol and 2-formamido-8-naphthol. 2-Formamido-1-naphthol and *N*-formyl-2-naphthylhydroxylamine were metabolized mainly to (2-formamido-1-naphthyl glucosid)uronic acid. This conjugate was not detected as a metabolite of 2-naphthylamine from rats or dogs. Formylation may be carried out by the intestinal flora acting on 2-naphthylamine metabolites passing into the gut, but no 2-amino-1-naphthyl hydrogen sulphate was detected in the bile of either rats or dogs dosed with 2-naphthylamine (Boyland, Manson & Nery, 1960).

It is possible that 2-amino-1-naphthyl hydrogen sulphate is *N*-methylated and the formamido group is formed by oxidation of the methylamino group. The oxidation and demethylation of 2-methylamino-1-naphthyl hydrogen sulphate and formation of the formamido compound by oxidation of the intermediate *N*-hydroxymethyl compound occur under mild conditions in the presence of charcoal. Formaldehyde reacts with 2-amino-1-naphthyl hydrogen sulphate on charcoal more

readily than with 2-naphthylamine. 2-Methylamino-1-naphthyl hydrogen sulphate was not a metabolite of 2-naphthylamine or 2-amino-1-naphthyl hydrogen sulphate formed by rats or dogs, but was a metabolite of 2-methylaminonaphthalene in rats. Its formation was not accompanied by large amounts of 2-formamido-1-naphthyl hydrogen sulphate.

The activity or otherwise of 2-formamido-1-naphthyl hydrogen sulphate as a carcinogen is not known, but deformylation would yield 2-amino-1-naphthyl hydrogen sulphate, which is not carcinogenic when implanted into the bladders of mice (Clayson, Jull & Bonser, 1958).

No evidence for the excretion of the unstable *N*-formyl-2-naphthylhydroxylamine-*O*-sulphonic acid was found, and it appears unlikely to be a precursor of the new metabolite. This compound readily breaks down to 2-naphthylhydroxylamine, 2-formamido-1-naphthol and 2-amino-1-naphthol. Similar breakdown of the unknown 2-naphthylhydroxylamine-*O*-sulphonic acid would yield the two carcinogens 2-naphthylhydroxylamine and 2-amino-1-naphthol. Both the latter compounds have been reported free in the urine of dogs dosed with 2-naphthylamine (Troll, Belman, Nelson, Levitz & Twombly, 1959; Troll & Nelson, 1961; Boyland & Manson, 1963).

We thank Dr K. Williams of this Institute for examining the effect of human urine arylsulphatase on the metabolite, and Mr C. H. Kinder, F.R.C.S., and Mr F. Watson of the Buckston Browne Farm, Downe, Kent, for the provision of dog urine. This investigation has been supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital) from the Medical Research Council and the British Empire Cancer Campaign for Research, and by the Public Health Service Research Grant no. CA-03188-08 from the National Cancer Institute, U.S. Public Health Service.

REFERENCES

- Billman, J. H., Radike, A. & Mundy, B. W. (1942). *J. Amer. chem. Soc.* **64**, 2977.
Booth, J., Boyland, E. & Manson, D. (1955). *Biochem. J.* **60**, 62.
Boyland, E. (1963). *The Biochemistry of Bladder Cancer*, pp. 34-40. Springfield, Ill.: Charles C. Thomas.
Boyland, E. & Manson, D. (1957a). *J. chem. Soc.* p. 4689.
Boyland, E. & Manson, D. (1957b). *Biochem. J.* **67**, 275.
Boyland, E. & Manson, D. (1963). *Rep. Brit. Emp. Cancer Campgn.* **41**, 39.
Boyland, E. & Manson, D. (1965). *Biochem. J.* **95**, 7F.
Boyland, E., Manson, D. & Nery, R. (1960). *Rep. Brit. Emp. Cancer Campgn.* **38**, 52.
Boyland, E., Manson, D. & Nery, R. (1962). *J. chem. Soc.* p. 606.
Boyland, E., Manson, D. & Nery, R. (1963). *Biochem. J.* **86**, 263.

- Boyland, E., Manson, D. & Sims, P. (1953). *J. chem. Soc.* p. 3623.
- Boyland, E. & Nery, R. (1962). *J. chem. Soc.* p. 5217.
- Boyland, E. & Nery, R. (1964). *Analyst*, **89**, 95.
- Boyland, E. & Sims, P. (1958). *J. chem. Soc.* p. 4198.
- Brill, E. & Radomski, J. L. (1965). *Biochem. Pharmacol.* **14**, 743.
- Clayson, D. B., Jull, J. W. & Bonser, G. M. (1958). *Brit. J. Cancer*, **12**, 222.
- Fischer, O. & Römer, F. (1906). *J. prakt. Chem.* **73**, 440.
- Hartley, R. D. & Lawson, G. J. (1962). *J. Chromat.* **7**, 69.
- Kaczka, E. A., Gitterman, C. D., Dulaney, E. L. & Folkers, K. (1962). *Biochemistry*, **1**, 340.
- Liebermann, C. & Jacobson, P. (1882). *Liebigs Ann.* **211**, 36.
- Marcker, K. & Sanger, F. (1964). *J. molec. Biol.* **8**, 835.
- Sarges, R. & Witkop, B. (1964). *J. Amer. chem. Soc.* **86**, 1861.
- Sims, P. (1958). *J. chem. Soc.* p. 44.
- Troll, W., Belman, S., Nelson, N., Levitz, M. & Twombly, G. H. (1959). *Proc. Soc. exp. Biol., N.Y.*, **100**, 75.
- Troll, W. & Nelson, N. (1961). *Fed Proc.* **20**, 41.
- Wessely, F. & Swoboda, W. (1951). *Mh. Chem.* **82**, 621.
- Williams, R. T. (1940). *Biochem. J.* **34**, 48.
- Ziegler, D. M. & Pettit, F. H. (1964). *Biochem. biophys. Res. Commun.* **15**, 188.