Hartman, P. E. & Liu, C. (1954). *J. Bact.* **67**, 77.
Herbert, E., Potter, V. R. & Takagi, Y. (1955). *J. biol. Chem.* **213**, 923.
Hunter, F. E. jun. & Ford, L. (1955). *J. biol. Chem.* **216**, 357.
Kornberg, A. (1950). *J. biol. Chem.* **182**, 805.
Lehninger, A. L. (1953). *Harvey Lect.* **49**, 176.
Lindegren, C. C. (1949). *The Yeast Cell, its Genetics and Cytology*. St Louis: Educational Publishers.
Linnane, A. W. & Still, J. L. (1955). *Arch. Biochem. Biophys.* **59**, 383.
Lohmann, K. & Jendrassik, L. (1926). *Biochem. Z.* **178**, 419.
Maley, G. F. & Plaut, G. W. E. (1953). *J. biol. Chem.* **205**, 297.
Massey, V. (1955). In *Methods in Enzymology*, vol. 1, p. 729. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
Mudd, S., Brodie, A. F., Winterscheid, L. C., Hartman, P. E., Beutner, E. H. & McLean, R. A. (1951). *J. Bact.* **62**, 729.
Nossal, P. M. (1953). *Aust. J. exp. Biol. med. Sci.* **31**, 583.
Nossal, P. M. (1954). *Biochem. J.* **57**, 62.
Nossal, P. M., Keech, D. B. & Morton, D. J. (1956*a*). *Biochim. biophys. Acta*, **22**, 412.
Nossal, P. M., Keech, B. & Utter, M. F. (1956*b*). *Fed. Proc.* **15**, 321.
Pinchot, G. B. (1953). *J. biol. Chem.* **205**, 65.
Pinchot, G. B. & Racker, E. (1951). In *Phosphorus Metabolism*, vol. 1, p. 366. Ed. by McElroy, W. D. & Glass, B. Baltimore: Johns Hopkins Press.
Racker, E. (1950). *Biochim. biophys. Acta*, **4**, 20.
Robinson, R. W. & Hogden, C. G. (1940). *J. biol. Chem.* **135**, 707.
Slater, E. C. (1953). *Nature, Lond.*, **172**, 975.
Slater, E. C. & Cleland, K. W. (1952). *Nature, Lond.*, **170**, 118.
Slonimski, P. P. & Ephrussi, B. (1949). *Ann. Inst. Pasteur*, **77**, 47.
Tissières, A. & Slater, E. C. (1955). *Nature, Lond.*, **176**, 736.
Utter, M. F. & Kurahashi, K. (1954). *J. biol. Chem.* **207**, 787.
Woodward, G. E. & Hudson, M. T. (1955). *J. Franklin Inst.* **259**, 543.

Metabolism of Polycyclic Compounds

12. AN ACID-LABILE PRECURSOR OF 1-NAPHTHYLMERCAPTURIC ACID AND NAPHTHOL: AN *N*-ACETYL-*S*-(1:2-DIHYDROHYDROXYNAPHTHYL)-L-CYSTEINE*

BY E. BOYLAND AND P. SIMS
Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, Fulham Road, London, S.W. 3

(*Received* 8 *August* 1957)

The presence of acid-labile precursors of mercapturic acids in the urines of animals dosed with bromobenzene and chlorobenzene was first noticed by Baumann & Preusse (1879) and Jaffe (1879). In studying the metabolism of naphthalene in rats and rabbits, Boyland & Wiltshire (1953) observed a metabolite in the urine which gave the colour reactions of 1-naphthol, but which was not extracted from neutral solution by ether. This so-called 'ether-insoluble 1-naphthol' was not present in the urine of animals dosed with 1-naphthol. In these experiments the excreted 1-naphthol was estimated colorimetrically, either by coupling with diazotized *p*-nitroaniline or by interaction with 2:6-dichloroquinonechloroimide. In both methods of estimation the urine was acidified before the addition of the reagents.

In a further investigation Boyland & Solomon (1956) estimated a number of the metabolites of naphthalene by measurement of the areas of the spots produced by the various metabolites on paper chromatograms. In the course of this work a method was developed for the determination on chromatograms of 1-naphthylmercapturic acid (I), first isolated by Bourne & Young (1934), depending on the pink fluorescence seen in ultraviolet light in the presence of ammonia. It was noticed that the urines of rats and rabbits treated with naphthalene contained little or no 1-naphthylmercapturic acid, but that after acidification of the urines the acid was liberated from a precursor (Solomon, 1954).

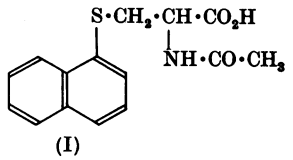
Knight & Young (1957) have recently detected the presence of acid-labile mercapturic acid precursors (for which they propose the name pre-mercapturic acids) in the urine of animals after the administration of benzene, anthracene, fluorobenzene, chlorobenzene, bromobenzene and iodobenzene, as well as with naphthalene. They also noticed that benzyl chloride was excreted directly as the corresponding mercapturic acid after administration to animals.

It has now been shown (Boyland, Sims & Solomon, 1957) that the 'ether-insoluble 1-naphthol' and the acid-labile precursor of 1-naphthylmercapturic acid are the same substance, treatment of the urine with mineral acid giving

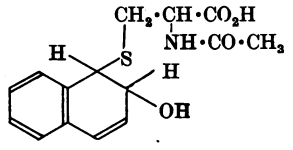
* Part 11: Boyland & Sims (1957).

rise to the mercapturic acid and to 1- and 2-naphthol. The present paper describes the isolation and properties of the precursor.

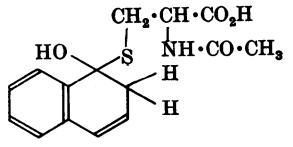
From a consideration of the evidence presented below it seems likely that the precursor is *N*-acetyl-*S*-(1:2-dihydro-2-hydroxy-1-naphthyl)-L-cysteine (II).



(I)



(II)



(III)

EXPERIMENTAL

Melting points are uncorrected.

Materials. *S*-1-Naphthyl-L-cysteine and 1-naphthylmercapturic acid were prepared from 1-naphthylamine by the method of du Vigneaud, Wood & Binkley (1941) as needles from aqueous ethanol, m.p. 182–183° and 169–170° respectively. 2-Naphthylmercapturic acid was the gift of Mr R. H. Knight. *N*-Acetylcysteine formed prisms from water, m.p. 110°, when prepared by the method of Pirie & Hele (1933). *NN'*-Diacetylcystine was prepared as described by Hollander & du Vigneaud (1931), as a colourless powder which became gummy on keeping. *trans*-1:2-Dihydro-2-hydroxy-1-naphthyl glucosiduronic acid was isolated from the urine of rabbits dosed with naphthalene (E. Boyland & P. Sims, unpublished work). 1-Naphthyl glucosiduronic acid was isolated from the urine of rabbits dosed with 1-naphthol by the method of Berenbom & Young (1951).

Paper chromatography. Whatman no. 1 chromatography paper was used throughout. Descending chromatograms were developed with either solvent system 1 or 2 for 15 hr. and ascending chromatograms with solvent system 3 for 5 hr. (see Table 1). After development the chromatograms were dried at room temperature and examined under u.v. light after exposure to NH_3. The chromatograms were then usually sprayed with a solution of freshly diazotized *p*-nitroaniline (0·2% in 0·1N-HCl), allowed to dry at room temperature and sprayed with aq. 10% Na_2CO_3. Occasionally the chromatograms were sprayed with diazotized sulphanilic acid (0·05% in 0·1N-HCl) or with a saturated solution of 2-chloro-4-nitrobenzenediazonium naphthalene-2-sulphonate [N.N.C.D. reagent (Boyland & Sims, 1953)] in 0·1N-HCl. In each case the air-dried chromatograms were then treated with aq. 10% Na_2CO_3. The properties on paper chromatograms of the compounds mentioned in this paper are summarized in Table 1.

Table 1. *Paper-chromatographic properties of compounds related to the 1-naphthylmercapturic acid precursor*

Solvent systems: 1, butanol saturated with aq. 2N-NH_3 (downward development); 2, the organic phase of a mixture of butanol–ethanol–water (17:3:20, by vol.) (downward development); 3, aq. 0·1N-NH_3 (upward development). The R_F values refer to the pure compounds. R_F values of the compounds detected in urine are usually slightly higher.

Metabolite	R_F in solvent 1	2	3	Fluorescence	Diazotized *p*-nitroaniline	Diazotized sulphanilic acid	N.N.C.D. reagent
1-Naphthylmercapturic acid precursor*	0·22	0·38	0·81	Dark-absorption	Blue	Red	Blue
1-Naphthylmercapturic acid*	0·37	0·35	0·69	Orange or pink†	—	—	—
2-Naphthylmercapturic acid*	0·36	0·36	0·72	Orange or pink†	—	—	—
1-Naphthol	0·96	0·96	0·62	Blue†	Blue	Red	Blue
2-Naphthol	0·96	0·96	0·55	Violet†	Orange	Orange	Orange
N-Acetylcystine*‡	0·01	—	0·86	Dark-absorption	—	—	—
NN'-Diacetylcystine*	0·01	—	0·86	Dark-absorption	—	—	—
1-Naphthyl glucosiduronic acid	—	—	0·80	Dark violet†	—	—	—

* Also detected by the platinic iodide reagent of Toennies & Kolb (1951) and by spraying with 1% $K_2Cr_2O_7$ in 5% aqueous acetic acid followed by saturated aqueous silver acetate (Knight & Young, 1957).

† After exposure to NH_3 vapour.

‡ After chromatography the compound failed to react immediately with the sodium nitroprusside reagent of Toennies & Kolb (1951) and is presumed to have become oxidized to *NN'*-diacetylcystine.

Dosing of animals and collection of urine. Six rabbits (body wt. approx. 2 kg.), maintained on oats, rat cake, cabbage and water, were each injected intraperitoneally on five consecutive days with a solution of naphthalene in arachis oil (5 ml. of 20%, w/v). The rabbits and other animals were housed in metabolism cages designed for the collection of urine separately from faeces. The urines were collected up to the sixth day and were stored at 0°.

Paper-chromatographic examination of the rabbit urines. Urines were examined by means of paper chromatography with the solvent systems described in Table 1, both before and after the addition of 10 N-HCl. Inspection of the papers showed that fresh urine contained the 1-naphthylmercapturic acid precursor, but no detectable amount of 1-naphthylmercapturic acid, whereas the acidified urine contained 1-naphthylmercapturic acid but no 1-naphthylmercapturic acid precursor. If the urines were kept either at room temperature or at 0°, detectable amounts of 1-naphthylmercapturic acid were present after a few days. Examination of the various solutions obtained during the working-up procedures described below showed that decomposition of the precursor was continually occurring, with the formation of 1-naphthylmercapturic acid and 1- and 2-naphthol.

Isolation of the 1-naphthylmercapturic acid precursor. The pooled urines were centrifuged to removed solid matter and acidified to pH 4 with acetic acid. Activated charcoal (500 g.) was added and the mixture was stirred for about 10 min. The charcoal was filtered off and washed with water (about 10 l.) to remove urea and inorganic salts, then with aq. 2 N-NH_3 (300 ml.), and finally with hot methanol containing benzene (5%, v/v) until most of the naphthalene metabolites had been eluted (about 10 l. of the solvent mixture was usually required). The methanol–benzene eluates were evaporated to dryness under reduced pressure to a brown gum. The material eluted from the charcoal by aq. 2 N-NH_3 contained some naphthalene metabolites together with large amounts of naturally occurring compounds, including allantoin, and was discarded.

A column was prepared by dry-packing cellulose powder (600 g., Whatman standard grade) into a glass chromatography column (100 cm. × 7 cm.) by the method of Hough, Jones & Wadman (1949). A mixture of butanol–*cyclo*hexane–aq. 2 N-NH_3 (2 l. of 9:2:1, by vol.) was passed through the column. The gum containing the naphthalene metabolites was dissolved in a small volume of methanol and the solution was adsorbed on cellulose powder (30 g.) which was packed in a layer on top of the cellulose in the column. The column was developed with the above solvent mixture and the eluate was collected in fractions of about 500 ml. volume.

A few drops of aq. NH_3 (sp.gr. 0·88) were added to the fractions, which were then separately evaporated to volumes of about 20 ml. under reduced pressure. The solutions thus obtained were examined by means of paper chromatography with the solvent systems described in Table 1. It was not possible to separate completely the naphthalene metabolites from one another on a column as described above; nor was it possible to reproduce absolutely the chromatographic conditions. All the known metabolites of naphthalene were, however, detected in one or more of the fractions, by methods already published (e.g. Boyland & Solomon, 1956). The results obtained from a typical separation are shown in Table 2.

The fractions containing the 1-naphthylmercapturic acid precursor were combined and rechromatographed on a smaller cellulose-powder column (prepared in the manner already described from about 150 g. of powder), fractions of about 200 ml. being collected. The fractions in which the 1-naphthylmercapturic acid precursor was detected also contained at least two unknown phenolic substances and gave positive Tollens tests for glucuronide; naphthalene, as well as 1-naphthylmercapturic acid, was liberated after acidification of the fractions with HCl. These fractions were combined and, after the addition of a little aq. NH_3 (sp.gr. 0·88), evaporated to about 10 ml. under reduced pressure. When the solution was kept at 0°, the ammonium salt of the 1-naphthylmercapturic acid precursor separated, and was filtered off and washed with a few drops of ice-cold ethanol. The mother liquors were kept at 0° in an open

Table 2. *Naphthalene metabolites eluted from a typical cellulose powder column with butanol–cyclohexane–*2 N-NH_3 (9:2:1, *by vol.*)

Fractions of 500 ml. were collected and examined by paper chromatography (see Table 1). 1-Naphthol was detected in all the fractions up to no. 12. A number of substances giving red or purple colours with diazotized *p*-nitroaniline were present in all the fractions; these are presumed to be naturally occurring phenols.

Fraction no.	Metabolite
1, 2	1- and 2-Naphthol
3	*trans*-1:2-Dihydro-1:2-dihydroxynaphthalene
4, 5, 6	1-Naphthyl sulphate, 2-hydroxy-1-naphthyl sulphate and an unknown*
7, 8	The compounds in fractions 4, 5 and 6 and 1-naphthylmercapturic acid
9	1-Naphthylmercapturic acid precursor and an unknown†
10, 11, 12	1-Naphthylmercapturic acid precursor
13	—
14, and later fractions	1-Naphthyl glucosiduronic acid, 1:2-dihydro-2-hydroxy-1-naphthyl glucosiduronic acid and two unidentified substances‡

* This substance (R_F 0·53 in solvent 1) gave an immediate blue colour with diazotized *p*-nitroaniline and an immediate purple colour with this reagent when the chromatogram was sprayed with 2 N-HCl and heated at 70° for 20 min. It is believed to be 1-hydroxy-2-naphthyl sulphate.

† This substance (R_F 0·24 in solvent 1) gave a blue colour with diazotized *p*-nitroaniline, and on two-dimensional chromatograms which were sprayed with 2 N-HCl between the first and second runs gave rise to 1-naphthol and a compound which was either 1- or 2-naphthylmercapturic acid. The substance was possibly *N*-acetyl-*S*-(1:2-dihydro-1-hydroxy-2-naphthyl)-L-cysteine.

‡ These two substances (R_F 0·16 and 0·05 in solvent 1) gave blue and yellow colours repectively with diazotized *p*-nitroaniline. After the chromatograms were treated with 2 N-HCl as described above, both substances gave purple colours with diazotized *p*-nitroaniline. They are thought to be 1-hydroxy-2-naphthyl and 2-hydroxy-1-naphthyl glucosiduronic acid respectively.

vessel for several days, when more of the precursor crystallized. When no more crystals separated from the mother liquors (paper chromatography showed that the precursor was slowly decomposing into 1-naphthylmercapturic acid and 1- and 2-naphthol), the liquors were evaporated under reduced pressure and rechromatographed on a fresh cellulose-powder column. By this means the ammonium salt of the precursor was obtained in yields of between 300 and 900 mg. from each batch of urine collected from rabbits dosed with 30 g. of naphthalene.

Properties of the 1-naphthylmercapturic acid precursor. The ammonium salt obtained above was recrystallized from propanol in needles, m.p. 142° (decomp.), which were dried at 100°/0·5 mm. for 15 min., to yield the ammonium salt of N-*acetyl*-S-(1:2-*dihydro*-2-*hydroxy*-1-*naphthyl*)-L-*cysteine* (II) (Found: C, 55·8; H, 6·5; N, 8·1; S, 9·9. $C_{15}H_{20}O_4N_2S$ requires C, 55·55; H, 6·2; N, 8·6; S, 9·9%). Crystals dried at 25°/0·5 mm. for 2 hr. apparently contained propanol of crystallization (Found: C, 56·3; H, 7·2. Calc. for $C_{15}H_{20}O_4N_2S,C_3H_8O$: C, 56·2; H, 7·3%). If the compound was heated at 100°/0·5 mm. for periods of more than 30 min. decomposition occurred, and it was shown by paper chromatography that the gum thus formed contained 1-naphthylmercapturic acid and 1- and 2-naphthol. When the ammonium salt was separated from methanol by means of ether, the product formed needles, m.p. 142° (decomp.), and appeared to contain ether of crystallization (Found, after drying at 25°/0·5 mm.: C, 56·8, 56·7; H, 7·0, 7·2; N, 7·6; S, 8·4. Calc. for $C_{15}H_{20}O_4N_2S,\frac{1}{2}C_4H_{10}O$: C, 56·5; H, 7·0; N, 7·75; S, 8·9%). Similarly, from methanol–benzene the product formed needles, m.p. 142° (decomp.), which appeared to contain benzene of crystallization (Found, after drying at 25°/0·5 mm. for 3 hr.: C, 59·2; H, 6·3; N, 7·5; S, 8·8. Calc. for $C_{15}H_{20}O_4N_2S,\frac{1}{2}C_6H_6$: C, 59·5; H, 6·4; N, 7·7; S, 8·8%). When the samples crystallized from the last two solvent mixtures were heated to above 70° at 0·5 mm. for more than 15 min. decomposition occurred.

The product obtained by crystallization from methanol–benzene had (after drying at 25°/0·5 mm.) $[\alpha]_D^{22} - 338 \pm 2°$ in water (*c*, 0·5) and the u.v. absorption, measured in water, showed $\lambda_{max.}$ 258 mμ and $\epsilon_{max.}$ 6310. The infrared spectrum, measured as a mull in liquid paraffin, showed a band at 800 cm.$^{-1}$, whereas the infrared spectrum of 1-naphthylmercapturic acid itself has two bands, at 771 and 798 cm.$^{-1}$. It is probable that the simplification of these bands in the precursor is due to the reduction of one of the rings of the naphthalene nucleus in this compound.

Decomposition of the 1-naphthylmercapturic acid precursor by acid. When the ammonium salt of the precursor (50 mg.), in water (0·5 ml.), was treated with a few drops of 10 N-HCl, 1-naphthylmercapturic acid separated within a few seconds. After 5 min. the crystals were filtered off and recrystallized from aqueous ethanol in needles, m.p. 169°, undepressed in admixture with samples prepared (*a*) from the urine of rabbits injected with naphthalene by the method of Bourne & Young (1934), and (*b*) synthetically (Found: N, 4·8; S, 11·0. Calc. for $C_{15}H_{15}O_5NS$: N, 4·8; S, 11·1%). The presence of 1- and 2-naphthol in the mother liquors was demonstrated by means of paper chromatography with solvent 3.

In a second experiment a weighed amount of the ammonium salt (which had been recrystallized from methanol–benzene) was treated as before, and the precipitated 1-naphthylmercapturic acid was filtered off, washed with a little ice-cold water and weighed. The combined filtrate and washings were extracted with ether (3 × 2 ml.) and the ethereal solution was applied along the base line of chromatography paper and developed upward with solvent 3 (Table 1) for 6 hr. The paper was dried and the positions of the 1- and 2-naphthol bands determined under u.v. light. These bands were cut out and eluted separately with ethyl acetate (3 × 3 ml.). The solutions were each made up to 10 ml. with ethyl acetate and 1- and 2-naphthol determined on a Unicam SP. 500 spectrophotometer with 2:6-dichloroquinonechloroimide reagent (Gibbs, 1927), the colours being measured at 605 mμ. Standard curves were prepared by chromatographing and eluting known amounts of the naphthols. The results of duplicate determinations are shown in Table 3.

The aqueous layers from the ether extractions were spotted on paper chromatograms, which were developed with solvent 3 (Table 1). A compound was detected with the $K_2Cr_2O_7$–silver acetate reagent which had R_F 0·86 and did not give an immediate colour with the sodium nitroprusside reagent of Toennies & Kolb (1951). When chromatographed under these conditions, *N*-acetylcysteine also failed to give a colour with sodium nitroprusside (see Table 1).

The precursor (10 mg.), in water (0·2 ml.), was also treated with 10 N-H_2SO_4, with 10 N-H_3PO_4 and with dry methanol saturated with HCl (0·2 ml. of each reagent): in each case immediate decomposition occurred, 1-naphthylmercapturic acid and 1- and 2-naphthol being detected in the products by paper chromatography. When the precursor was similarly treated with acetic acid no decomposition was detected within 24 hr.

Table 3. *Acid decomposition of the 1-naphthylmercapturic acid precursor*

Precursor (assumed to be $C_{15}H_{20}O_4N_2S,\frac{1}{2}C_6H_6$) (mg.)	1-Naphthylmercapturic acid (mg.)	1-Naphthol (mg.)	2-Naphthol (mg.)
25·0	17·2 (86·5%)	0·09	0·18
24·6	16·8 (85·5%)	0·06	0·13

Table 4. *Acid decomposition of* trans-1:2-*dihydro*-2-*hydroxy*-1-*naphthyl glucosiduronic acid*

Treatment	1-Naphthol	2-Naphthol	1-Naphthyl glucosiduronic acid
Kept at 25° for 24 hr.	–	Trace	+
Heated at 60° for 10 min.	–	+	+ +
Heated at 100° for 30 min.	+ +	+	–

Decomposition of trans-1:2-*dihydro*-2-*hydroxy*-1-*naphthyl glucosiduronic acid by acid.* The glucosiduronic acid (15 mg.) in 2 N-HCl (0·2 ml.) was treated as shown in Table 4 and the products were examined by means of paper chromatography with solvent 3 (Table 1).

Decomposition of the 1-*naphthylmercapturic acid precursor with alkali.* The ammonium salt of the precursor (50 mg.) was heated to 100° with aqueous N-NaOH (1 ml.) for 5 min. Ammonia was evolved and examination of the products on paper chromatograms showed the presence of 1-naphthylmercapturic acid, 2-naphthol and a substance [which could only be detected when solvent 3 (Table 1) was used] which was presumed to be 1-thionaphthol. This substance was also present in the products from the alkaline hydrolysis (under similar conditions) of 1-naphthylmercapturic acid itself. 1-Naphthol was not detected in the products of the alkaline hydrolysis of the precursor.

When the ammonium salt (50 g.) was heated at 100° with N-NaOH (1 ml.) for 45 min. and the solution diluted and aerated as described by Bourne & Young (1934), then 1:1-dinaphthyl disulphide (15 mg.), m.p. 88–89°, was obtained. Bourne & Young obtained a similar product from the alkaline hydrolysis and oxidation of 1-naphthylmercapturic acid.

Paper chromatography showed that, when the ammonium salt of the precursor was kept overnight at room temperature with aq. N-NaOH, some decomposition with the formation of 1-naphthylmercapturic acid and 2-naphthol occurred. On the other hand, a solution of the ammonium salt in pyridine at room temperature was stable for at least 48 hr. Attempts to prepare the sodium and potassium salts of the precursor from the ammonium salts by the addition of one equivalent of alkali resulted in the formation of gums, in which the presence of the breakdown products of the precursor was demonstrated.

Action of silver salts on the 1-*naphthylmercapturic acid precursor.* The work of Peters & Wakelin (1947) suggested that it might be possible to split the thio ether linkage of the precursor to yield a 1:2-dihydro-1:2-dihydroxynaphthalene. The precursor (10 mg.) in water (0·2 ml.) and 10% aq. $AgNO_3$ (0·05 ml.) was treated with 0·1 N-NaOH (0·5 ml.). The solution was filtered and the filtrate extracted with ether (2 × 1 ml.). The aqueous and ether layers were examined on paper chromatograms, revealing 1- and 2-naphthol and 1-naphthylmercapturic acid. No diol was detected. The precursor (in 10 mg. portions) in water (0·2 ml.) was also treated with 10% aq. $AgNO_3$ (0·05 ml.) alone, saturated aqueous silver acetate (0·1 ml.) and freshly prepared Ag_2O (0·1 g.). In each experiment the products, identified on paper chromatograms, were 1- and 2-naphthol and 1-naphthylmercapturic acid.

Attempted preparation of derivatives of the 1-*naphthylmercapturic precursor.* (*a*) The methyl ester. The ammonium salt of the 1-naphthylmercapturic acid precursor (100 mg.) was suspended in ether and treated with an excess of diazomethane in ether. The compound slowly dissolved with evolution of gas. When the reaction had finished the ether was distilled off, leaving a colourless gum which could not be crystallized. Examination of the gum on paper chromatograms showed the presence of a little 2-naphthol, together with a substance which had R_F 0·85 in solvent 1 (Table 1), and which showed as a dark absorbent region in ultraviolet light and gave a blue colour with diazotized *p*-nitroaniline and Na_2CO_3. These are the properties expected of the methyl ester of the precursor.

(*b*) The *O*-acetyl derivative. The ammonium salt of the precursor (100 mg.), in pyridine (1 ml.), was treated with acetic anhydride (0·5 ml.) at 0° for 5 hr. The yellow solution was poured into water and the mixture extracted with ether (3 × 50 ml.). The ether was evaporated, leaving a clear gum which could not be crystallized. The gum was dissolved in a little saturated aq. $NaHCO_3$ and the solution was washed with ether, acidified with conc. HCl and extracted with ether. The ether was evaporated and the residual gum examined by paper chromatography both before and after heating with 5 N-HCl for 15 min. 1-Naphthylmercapturic acid and a trace of 2-naphthol were detected in the acid-treated material.

Detection of the 1-*naphthylmercapturic acid precursor in species other than rabbit.* The animals listed in Table 5 were dosed with a solution of naphthalene in arachis oil (20%, w/v) as shown in the table. The urines produced in the 24 hr. after treatment were examined on paper chromatograms before and after acidification with HCl. For the examination of the human urine, the metabolites were first concentrated by absorption on charcoal and elution as described above. Estimates of the amounts of the mercapturic acid and its precursor were obtained by a comparison of the sizes of the spots produced with those of the spots produced by the other known naphthalene metabolites.

Table 5. *Formation of* 1-*naphthylmercapturic acid and its precursor in different species*

The rats were dosed orally, the other animals by intraperitoneal injection. The human subjects were dosed orally.

Species	Dose (naphthalene in arachis oil; 20%, w/v) (g.)	1-Naphthylmercapturic acid precursor	1-Naphthylmercapturic acid	
			Before acidification of the urine	After acidification of the urine
Rabbit	1·0	+ +	–	+ +
Rat (male)	0·1	+ +	–	+ +
Rat (female)	0·1	+ +	–	+ +
Mouse	0·02	+ +	Trace	+ +
Hamster	0·1	+ +	–	+ +
Guinea pig	0·4	+	–	+
Man 1	0·5	Trace	–	Trace
Man 2	0·5	Trace	–	Trace
Man 3	0·5	+	–	+

Examination of possible intermediates in the formation of the 1-naphthylmercapturic acid precursor in the rabbit. *S*-1-Naphthyl-L-cysteine (0·5 g. in arachis oil), (±)-*trans*-1:2-dihydro-1:2-dihydroxynaphthalene (1 g. in arachis oil), 1-naphthol (1 g. in arachis oil), 2-naphthol (0·5 g. in arachis oil) and 1:2-dihydronaphthalene (0·5 ml.) were each injected intraperitoneally into rabbits and the urines collected for 24 hr. after injection. The urines were examined by means of paper chromatography both before and after acidification with 10 N-HCl. With *S*-1-naphthyl-L-cysteine, 1-naphthylmercapturic acid was detected in the urine both before and after acidification, whereas none was present in the urines (either before or after acidification) from the animals treated with (±)-*trans*-1:2-dihydro-1:2-dihydroxynaphthalene or with 1- or 2-naphthol. None of these urines contained the 1-naphthylmercapturic acid precursor. A trace of the 1-naphthylmercapturic acid precursor was found in the unacidified urine and of 1-naphthylmercapturic acid in the acidified urine of the animal treated with 1:2-dihydronaphthalene.

DISCUSSION

The 1-naphthylmercapturic acid precursor represents a new type of metabolite of aromatic compounds, although the earlier work of Baumann & Preusse (1879) and Jaffe (1879) suggested that acid-labile precursors of the mercapturic acids of the halogenated benzenes were formed when the halogenobenzenes were fed to animals. The work of Knight & Young (1957) and experiments with bromobenzene and anthracene (E. Boyland & P. Sims, unpublished observations) suggest that the formation of these precursors is a general metabolic pathway of aromatic compounds in animals. The apparent direct combination of benzyl chloride (Knight & Young, 1957) and some chloronitrobenzenes (Bray, James & Thorpe, 1955*a*, *b*, 1956; Bray & James, 1957) with cysteine (with elimination of chlorine) in the body might well be due to the presence of reactive chlorine atoms in these compounds.

The structure of the 1-naphthylmercapturic acid precursor has not yet been unequivocally established. The extreme ease with which the compound breaks down has made the preparation of derivatives difficult. The fact that the compound is decomposed immediately by mineral acids suggests a 1:2-dihydrohydroxynaphthalene type of structure, similar to that of 1:2-dihydro-1-naphthyl glucosiduronic acid (Boyland & Solomon, 1955), which is immediately decomposed by acid to yield naphthalene. The 1:2-dihydronaphthalene structure is supported by the light-absorption data (see Fig. 1). The spectrum of the precursor resembles that of 1:2-dihydronaphthalene rather than that of 1:4-dihydronaphthalene. The two most likely structures for the precursor are (II) or (III) above. Both of these compounds would be expected to lose the elements of water readily, to give 1-naphthylmercapturic acid. The simultaneous formation of naphthols suggests that elimination of *N*-acetylcysteine can also take place. We have not detected this compound in the decomposition products, but there is evidence for the presence of *NN'*-diacetylcysteine, which may have been produced from *N*-acetylcysteine during the paper chromatography. Knight & Young (1957), using animals treated with ^{35}S-labelled cysteine as well as with naphthalene, found that a ^{35}S-labelled compound other than 1-naphthyl[^{35}S]mercapturic acid was produced when the precursor was decomposed by acid. The formation of both 1- and 2-naphthol from the precursor on acidification is difficult to explain by assuming the existence of either structure (II) or (III). There is possibly an acid-induced migration of a hydroxyl radical, but in the analogous case of 1:2-dihydro-2-hydroxy-1-naphthyl glucosiduronic acid decomposition with cold mineral acid occurs, at a very much slower rate, with the formation of 1-naphthyl glucosiduronic acid and 2-naphthol; no 1-naphthol has been detected. The formation of 1-naphthylmercapturic acid and 2-naphthol by the action of alkali on the precursor can be readily explained if the precursor has structure (II). The breakdown of the precursor by either acid or alkali gives rise mainly to 1-naphthylmercapturic acid; the formation of the naphthols and *N*-acetylcysteine is

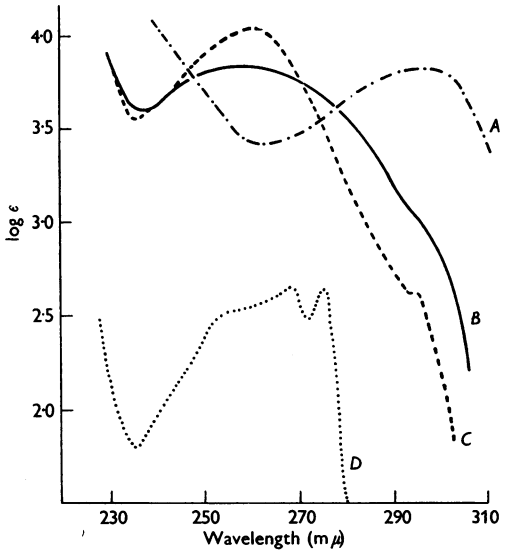


Fig. 1. Ultraviolet-absorption spectra of 1-naphthylmercapturic acid (*A*), the 1-naphthylmercapturic acid precursor (*B*), 1:2-dihydronaphthalene (*C*) and 1:4-dihydronaphthalene (*D*). (*A*) was measured in aq. 0·1 N-NH_3, (*B*) in water, and (*C*) and (*D*) in ethanol.

clearly a secondary reaction. The evidence so far obtained favours structure (II) for the precursor, but attempts to confirm this structure by the formation either of 2-naphthyl acetate on acetylation and acid decomposition or of a 1:2-dihydronaphthalene-1:2-diol by the action of silver salts have not been successful, the precursor being too readily decomposed by these reagents. A compound of structure (II) could exist in *cis* and *trans* forms, but there is no evidence as to which form the precursor possesses.

Baumann & Preusse (1879) and Jaffe (1879) found that the lead salts of the precursors of *p*-bromo- and *p*-chloro-benzenemercapturic acid possessed high optical rotations, whereas those of the mercapturic acids themselves are low. For the naphthalene derivatives the value of $[\alpha]_D^{22} - 338°$ of the precursor may be compared with that of $[\alpha]_D^{22} - 25°$ obtained by Bourne & Young (1934) for 1-naphthylmercapturic acid.

The mechanism by which the precursor arises in the body is not known. Of the possible intermediates tested, only 1:2-dihydronaphthalene gave rise to any detectable amount of precursor, and it is possible that the precursor arose from naphthalene produced by the dehydrogenation of the dihydro compound *in vivo*. It seems likely from previous work (e.g. du Vigneaud *et al.* 1941; West & Mathura, 1954) that the acetylation of the amino group occurs after the addition of cysteine (or a compound containing a cysteine residue) to the naphthalene nucleus. The formation of a compound such as (II) above would require the addition of cysteine together with a hydroxyl group to naphthalene, or the direct addition of the unknown cysteine sulphenic acid

$$(HO_2C \cdot CH(NH_2) \cdot CH_2 \cdot S \cdot OH).$$

The work of Stekol (1937), Gutmann & Wood (1950) and Mills & Wood (1956) suggests that with the halogenobenzenes reaction occurs between the aromatic compound and tissue-bound amino acid sulphur, whereas Barnes & James (1957) and Bray & Franklin (1957) favour reaction of the aromatic compound with the SH group of glutathione followed by hydrolysis with glutathionase. If either of these reactions occur with naphthalene, the reaction between the hydrocarbon and an S–OH group, rather than with an SH group, would seem to be necessary.

An alternative mechanism would be the reaction between the SH group of cysteine (or a tissue-bound or glutathione SH group) and the unknown naphthalene-1:2-epoxide. Such an intermediate has already been postulated (Boyland, 1950) to account for the formation of 1:2-dihydro-1:2-dihydroxynaphthalene in naphthalene-treated animals. From such a reaction the formation of the isomeric *N*-acetyl-*S*-(1-hydroxy-1:2-dihydro-2-naphthyl)-L-cysteine might be expected. We have detected a small amount of a substance in rabbit urine which might well be this compound (see Table 2). This substance and the postulated epoxide could be reduced in the body to 1:2-dihydro-1-hydroxynaphthalene, the glucosiduronic acid derivative of which has been described (Boyland & Solomon, 1956).

The 1-naphthylmercapturic acid precursor has been detected in the urine of all the species which we have examined, and it is probable that it also formed in the other species in which the formation of 1-naphthylmercapturic acid is known to occur.

SUMMARY

1. A substance has been detected in and isolated from the urine of rabbits dosed with naphthalene which on acidification with mineral acid gives rise to 1-naphthylmercapturic acid and 1- and 2-naphthol.

2. From a consideration of the acid decomposition products and the fact that it gives 1-naphthylmercapturic acid and 2-naphthol on treatment with alkali it is suggested that the compound is *N*-acetyl-*S*-(1:2-dihydro-2-hydroxynaphthyl)-L-cysteine.

3. A number of physical properties of the compound are recorded.

4. The compound was not produced when rabbits were injected with 1- and 2-naphthol, (±)-*trans*-1:2-dihydro-1:2-dihydroxynaphthalene or *S*-1-naphthyl-L-cysteine. Traces were produced with 1:2-dihydronaphthalene.

5. Rats, mice, hamsters, guinea pigs and man all excreted the precursor after treatment with naphthalene.

6. Possible modes of formation of the precursor in the body are discussed.

We wish to thank Miss M. Biggs for injecting the animals, Mr R. E. S. Prout for skilled technical assistance, Dr S. F. D. Orr for the infrared spectra and Mr P. Baker of Wellcome Research Laboratories for some of the microanalyses. This investigation has been supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research, Royal Cancer Hospital) from the British Empire Cancer Campaign, the Jane Coffin Childs Memorial Fund for Medical Research, the Anna Fuller Fund and the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service.

REFERENCES

Barnes, M. M. & James, S. P. (1957). *Biochem. J.* **66**, 3P.
Baumann, E. & Preusse, C. (1879). *Ber. dtsch. chem. Ges.* **12**, 806.
Berenbom, M. & Young, L. (1951). *Biochem. J.* **49**, 165.
Bourne, M. C. & Young, L. (1934). *Biochem. J.* **28**, 803.

Boyland, E. (1950). *Biochem. Soc. Symp.* no. 5, p. 40.
Boyland, E. & Sims, P. (1953). *J. chem. Soc.* p. 2966.
Boyland, E. & Sims, P. (1957). *Biochem. J.* **66**, 38.
Boyland, E., Sims, P. & Solomon, J. B. (1957). *Biochem. J.* **66**, 41P.
Boyland, E. & Solomon, J. B. (1955). *Biochem. J.* **59**, 518.
Boyland, E. & Solomon, J. B. (1956). *Biochem. J.* **63**, 679.
Boyland, E. & Wiltshire, G. H. (1953). *Biochem. J.* **53**, 636.
Bray, H. G. & Franklin, T. J. (1957). *Biochem. J.* **66**, 3P.
Bray, H. G. & James, S. P. (1957). *Biochem. J.* **66**, 45P.
Bray, H. G., James, S. P. & Thorpe, W. V. (1955*a*). *Biochem. J.* **60**, xxiii.
Bray, H. G., James, S. P. & Thorpe, W. V. (1955*b*). *Biochem. J.* **61**, v.
Bray, H. G., James, S. P. & Thorpe, W. V. (1956). *Biochem. J.* **64**, 38.
du Vigneaud, V., Wood, J. L. & Binkley, F. (1941). *J. biol. Chem.* **138**, 369.
Gibbs, H. O. (1927). *J. biol. Chem.* **72**, 649.
Gutmann, H. R. & Wood, J. L. (1950). *Cancer Res.* **10**, 8.
Hollander, L. & du Vigneaud, V. (1931). *J. biol. Chem.* **94**, 243.
Hough, L., Jones, J. K. N. & Wadman, W. H. (1949). *J. chem. Soc.* p. 2511.
Jaffe, M. (1879). *Ber. dtsch. chem. Ges.* **12**, 1092.
Knight, R. H. & Young, L. (1957). *Biochem. J.* **66**, 55P.
Mills, G. C. & Wood, J. L. (1956). *J. biol. Chem.* **219**, 1.
Peters, R. A. & Wakelin, R. W. (1947). *Biochem. J.* **41**, 555.
Pirie, N. W. & Hele, T. S. (1933). *Biochem. J.* **27**, 1716.
Solomon, J. B. (1954). Ph.D. Thesis: University of London.
Stekol, J. A. (1937). *J. biol. Chem.* **121**, 87.
Toennies, G. & Kolb, J. J. (1951). *Analyt. Chem.* **23**, 823.
West, H. D. & Mathura, G. R. (1954). *J. biol. Chem.* **208**, 315.

The Preparation of Barium Monophosphotaurocyamine

By J. F. MORRISON, A. H. ENNOR and D. E. GRIFFITHS*
Department of Biochemistry, John Curtin School of Medical Research, The Australian National University, Canberra, A.C.T., Australia

(*Received* 10 *July* 1957)

The synthesis and isolation of monophosphotaurocyamine (*N*-phosphoguanidoethanesulphonic acid) has not been described, although the compound is of interest as the naturally occurring phosphagen in certain annelids (van Thoai, Roche, Robin & van Thiem, 1953). These authors reported its isolation as a calcium salt after the phosphorylation of taurocyamine by phosphoryl chloride in an alkaline medium. A later communication (van Thoai & van Thiem, 1957*a*) reported the isolation of phosphotaurocyamine as a crystalline ammonium salt, but no details were given. In view of the difficulties which have been encountered by the present workers, because at least two products are formed in the phosphorylation reaction, it was considered worth while to report details of the synthesis and isolation.

This communication deals with the synthesis of taurocyamine, the products of the reaction between phosphoryl chloride and taurocyamine, the separation in pure form of barium monophosphotaurocyamine and some of its properties.

MATERIALS AND METHODS

Taurine (British Drug Houses Ltd.) was used without further purification.

Taurocyamine was estimated by the method described by Rosenberg, Ennor & Morrison (1956) for the determination of arginine. Bound taurocyamine was estimated by the same method after hydrolysis of the N–P bond in N-HCl at 100° for 10 min.

Inorganic phosphate was estimated by the method described by Ennor & Stocken (1950).

All hydrolyses and enzyme experiments were carried out with the sodium salts of monophosphotaurocyamine and fraction II (see later); these were prepared by passage of solutions of the barium salts through columns of Zeo-Karb 225 (Na^+ form).

Myosin was prepared by Dr H. Rosenberg of this Department from rabbit skeletal muscle as described by Bailey (1942). It was stored in 50% (v/v) glycerol at −10° and contained 2·5 mg. of protein/ml.

Taurocyamine phosphokinase (TPK) was purified from aqueous extracts of *Arenicola assimilis* by a method which will be described later and which involved fractionation with acetone, ammonium sulphate and ethanol. The enzyme was stable and was stored in 50% (v/v) glycerol at −10°; the solution contained 1·86 mg. of protein/ml.

The purified intestinal alkaline phosphatase was a gift from Professor R. K. Morton, University of Adelaide.

Taurocyamine was prepared by allowing *S*-methyl*iso*thiourea to react with taurine in an alkaline medium in the technique described by Schutte (1943) for the preparation of guanidines. Thus 72 g. of taurine was dissolved in 250 ml. of aq. 17N-NH_3 soln. and 120 g. of *S*-methyl*iso*thiourea sulphate added. The mixture was heated to 60° and stirred vigorously until the reactants dissolved. There was a vigorous evolution of methanethiol and the mixture was allowed to cool to room temperature. Crystallization of taurocyamine commenced after a few minutes and the reaction was allowed to proceed for 20 hr. The crystalline material was filtered off, washed with ice-cold water and

* Australian National University Research Scholar.