sequent chromatographic separations of the individual constituents. The partitioning of brain lipids between aqueous methanolic and chloroform-rich phases, according to the method of Folch *et al.* (1957), resulted in the complete transfer of the strandin into the upper phase. In unpublished work, we have shown that negligible amounts of other lipids are lost in this way. However, the water-soluble non-lipid impurities do pass into the upper phase (cf. also Folch *et al.* 1957). The carbohydrate content is about 30% greater than would be expected from the composition of strandin (Bogoch, 1958), and this excess probably represents water-soluble sugars and their derivatives.

The presence of this extra carbohydrate in the upper phase almost certainly accounts for the extra peak at 440 m μ (Fig. 2), after the orcinol reaction. Although small in absolute amount, this carbohydrate would also contribute significantly to the absorption at 570 m μ , so giving an erroneously high value for the neuraminic acid content based on direct readings of E_{570} . The use of 'difference' extinctions, depending on the selective destruction of neuraminic acid by hot-acid treatment, satisfactorily removes this interference.

SUMMARY

1. Synthetic *N*-acetylneuraminic acid and partially purified ox-brain strandin have been subjected

to Bial's orcinol reaction and the characteristics of the absorption spectra have been studied.

2. Crude ox-brain lipids under similar conditions showed anomalous spectra, which were largely traced to the presence of cerebroside.

3. Partitioning of ox-brain lipids between aqueous methanolic and chloroform-rich phases caused the strandin to pass completely into the upper aqueous methanolic phase. The neuraminic acid content of this phase was determined by measuring the 'difference' extinction at 570 m μ of the orcinol-reaction products (untreated minus acid-treated).

4. Neuraminic acid has been determined in the lipids of several rat tissues. It was found only in the brain.

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Metabolism of Polycyclic Compounds

14. THE CONVERSION OF NAPHTHALENE INTO COMPOUNDS RELATED TO *TRANS*-1:2-DIHYDRO-1:2-DIHYDROXYNAPHTHALENE BY RABBITS*

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The conversion of aromatic hydrocarbons into dihydrodihydroxy compounds by 'perhydroxylation' was first recognized when Boyland & Levi (1935, 1936) isolated (+)- and (-)-1:2-dihydro-1:2-dihydroxyanthracene, and the corresponding glucosiduronic acids, from the urine of rabbits and rats respectively which had been dosed with anthracene. Boyland & Wolf (1950) showed that phenanthrene is converted by rats into *trans*-9:10-dihydro-9:10-dihydroxyphenanthrene and by rabbits into this

diol and into (-)-*trans*-1:2-dihydro-1:2-dihydroxyphenanthrene. Young (1947) found that naphthalene is converted by rats into (-)-*trans*-1:2-dihydro-1:2-dihydroxynaphthalene and Booth & Boyland (1949) obtained both the (+) and the (-)-forms of this diol from rabbits treated with naphthalene. It has been shown that naphthalene is converted into 1:2-dihydro-2-hydroxy-1-naphthylglucosiduronic acid in rabbits (Corner, Billett & Young, 1954) and in rats (Corner & Young, 1954). The product from rabbits yielded (+)-*trans*-1:2-dihydro-1:2-dihydroxynaphthalene on hydrolysis

* Part 13: Booth & Boyland (1958).

with β -glucuronidase, whereas that from rats yielded a substance which was a mixture of the ($\pm$)- and the (-)-forms of the diol.

The present work has shown that naphthalene is converted by rabbits into the 1-glucosiduronic acids of both (+)- and (-)-*trans*-1:2-dihydro-1:2-dihydroxynaphthalene and into the 2-glucosiduronic acid of at least one of these forms. Evidence for the formation of 1-hydroxy-2-naphthyl sulphate by rabbits treated with either naphthalene or with ($\pm$)-*trans*-1:2-dihydro-1:2-dihydroxynaphthalene is also presented.

EXPERIMENTAL

Melting points are uncorrected.

Paper chromatography. This was carried out on Whatman no. 1 chromatography paper. Descending chromatograms were developed with solvent 1 or 2 (Table 1) for 15 hr. and ascending chromatograms with solvent 3 (Table 1) for 5 hr. The chromatograms were dried at room temperature, examined under u.v. light after exposure to NH_3 and then sprayed with a solution of freshly diazotized *p*-nitroaniline (0.02% in 0.1N-HCl), followed by aq. 10% Na_2CO_3 soln. either immediately, or after being sprayed with aq. 2N-HCl soln. and heated in an oven to 70°. On chromatograms heated with acid, compounds which had given rise to 1-naphthol and 1:2-dihydroxynaphthalene appeared as purple and blue spots respectively without further treatment.

($\pm$)-1:2-Dihydro-1:2-dihydroxynaphthalene. This compound was prepared by the method of Booth, Boyland & Turner (1950) and formed silky needles, m.p. 101°.

(-)-1:2-Dihydro-1:2-dihydroxynaphthalene. This compound was isolated from the urine of rats dosed with naphthalene as described below for the (+)-isomer and formed plates from benzene, m.p. 128°, $[\alpha]_D^{25} - 157^\circ$ (c, 0.5 in

ethanol). It formed a diacetate, m.p. 77°, $[\alpha]_D^{25} - 435^\circ$ (c, 0.5 in ethanol).

Potassium 1-hydroxy-2-naphthyl sulphate. 1-Naphthol (10 g.) was oxidized with $\text{K}_2\text{S}_2\text{O}_8$ (20 g.) as described for 2-naphthol (Boyland & Sims, 1957). The oxidation products eluted from charcoal with methanol were shown on paper chromatograms to contain two substances with R_F values of 0.40 and 0.45 respectively in solvent 1 (see Table 1), and which gave purple and blue colours respectively with diazotized *p*-nitroaniline and aq. Na_2CO_3 soln. The products were chromatographed on a cellulose-powder column [prepared from cellulose powder (250 g.) as described previously (Boyland & Sims, 1958)] with butanol-cyclohexane-aq. 2N- NH_3 soln. (9:2:1, by vol.) as the developing solvent, and the eluate, collected in fractions of 250 ml., was examined on paper chromatograms. The fractions in which the substance of R_F 0.45 was predominant were combined and evaporated and the residue was rechromatographed on a smaller cellulose-powder column. The fractions rich in this substance were combined, a small excess of aq. 2N-KOH was added and the solution evaporated. The residue was recrystallized several times from aq. ethanol to yield potassium 1-hydroxy-2-naphthyl sulphate (56 mg.) in pale-pink plates (Found: S, 11.3. $\text{C}_{10}\text{H}_7\text{O}_6\text{SK}$ requires S, 11.5%). A little of the ester was heated to 100° with 5N-HCl (0.1 ml.) for 15 min. and the product which separated on cooling was acetylated with acetic anhydride in pyridine to yield the diacetate of 1:2-dihydroxynaphthalene, m.p. and mixed m.p. 108–109°.

The second product from the oxidation, which was not isolated, is presumed to be 4-hydroxy-1-naphthyl sulphate. Desai & Sethna (1951) isolated 1:4-dihydroxynaphthalene from the persulphate oxidation of 1-naphthol after the reaction mixture had been heated with mineral acid.

Dosing of animals. The methods used in the dosing of animals, in the collection of urine and in the treatment of the urine with charcoal have already been described (Boyland & Sims, 1958).

Table 1. Paper chromatography of *trans*-1:2-dihydroxynaphthalene and related compounds

Solvent systems: 1, butanol saturated with aq. 2N- NH_3 soln. (downward development); 2, butanol-propan-1-ol-aq. 2N- NH_3 soln. (2:1:1, by vol.; downward development); 3, aq. 0.1N- NH_3 soln. (upward development). The colours in parentheses refer to those obtained after the chromatograms had been treated with 2N-HCl (see text).

Metabolite	R_F in solvent			Fluorescence	Colour with diazotized <i>p</i> -nitroaniline
	1	2	3		
1-Naphthol	0.96	0.97	0.62	Blue	Blue
2-Naphthol	0.96	0.97	0.55	Violet	Orange
<i>trans</i> -1:2-Dihydro-1:2-dihydroxynaphthalene	0.86	0.89	—	Dark absorption	— (blue)
1-Naphthyl sulphate	0.51	0.62	—	Dark blue	Violet (blue)
2-Hydroxy-1-naphthyl sulphate	0.49	0.58	—	Light blue	Brown* (purple)
1-Hydroxy-2-naphthyl sulphate	0.46	0.55	—	Light blue	Blue (purple)
2-Naphthyl sulphate	0.46	0.53	—	Dark blue	— (orange)
1-Naphthylglucosiduronic acid	0.11	0.28	—	Dark blue	— (blue)
1:2-Dihydro-2-hydroxy-1-naphthylglucosiduronic acid†	0.08	0.18	—	Dark absorption	Pale orange‡ (blue)
1:2-Dihydro-1-hydroxy-2-naphthylglucosiduronic acid	0.08	0.18	—	Dark absorption	Pale blue

* After 24 hr.

† The optically active forms could not be distinguished on paper chromatograms.

‡ These colours are presumably those of the naphthols produced by the decomposition of the glucosiduronic acids. 1:2-Dihydro-1-hydroxy-2-naphthylglucosiduronic acid is more readily detected on two-dimensional chromatograms (see text).

Treatment of urine extracts. This procedure, with cellulose-powder columns, was described by Boyland & Sims (1958) and an indication of when the various metabolites of naphthalene would be eluted from such columns was also given. Not all the naphthalene metabolites were eluted from charcoal by methanol-NH₃. Paper chromatography showed that some 1-naphthylmercapturic acid precursor and some 1:2-dihydro-2-hydroxy-1-naphthylglucosiduronic acids were eluted when the charcoal was subsequently washed with aq. phenol (5%, w/v). The separations described below are confined to the material eluted from the charcoal with methanol-NH₃.

Separation of the various metabolites on cellulose-powder Columns. The separations were carried out on the products obtained as indicated above when six rabbits were given a total of 30 g. of naphthalene during 5 days. The first two fractions from the column were evaporated under reduced pressure to a brown gum, which was extracted several times with boiling light petroleum (b.p. 80–100°). The solvent was removed under reduced pressure and the extracted solid recrystallized from water to yield 2-naphthol in plates, m.p. and mixed m.p. 120–121°. Yields of 110, 150 and 120 mg. of 2-naphthol were obtained from three separate experiments. 1-Naphthol could not be isolated, although it was detected by paper chromatography.

Fractions containing *trans*-1:2-dihydro-1:2-dihydroxy-naphthalene were combined and evaporated, and the residual gum was extracted several times with boiling cyclohexane. The product which separated on cooling was usually a mixture of the optically active forms of the compound with the (+)-form predominating: after one recrystallization from benzene, a typical specimen had m.p. 110–112°, $[\alpha]_D^{25} + 68^\circ$ (c, 1 in ethanol). After this had been recrystallized three times from benzene, the (+)-form was obtained, m.p. 127°, $[\alpha]_D^{25} + 158^\circ$ (c, 1 in ethanol), which formed a diacetate in plates (from aqueous methanol), m.p. 78°, $[\alpha]_D^{25} + 430^\circ$ (c, 0.5 in ethanol). Evaporation of the benzene mother liquors gave a solid which was recrystallized from benzene to yield (±)-*trans*-1:2-dihydro-1:2-dihydroxy-naphthalene in silky needles, m.p. 101°. Yields of the dihydrodihydroxy compound were variable: 520 and 110 mg. were obtained from two separate experiments. On a third occasion none was isolated but paper chromatography showed the presence of traces in the untreated urine. On one occasion only the (±)-form was obtained.

Sulphuric acid esters of 1:2-dihydroxy-naphthalene. Fractions containing these esters also contained 1-naphthyl sulphate: they were combined, evaporated and treated with aq. NaOH as described by Boyland & Sims (1957) to yield the sodium salt of 2-hydroxy-1-naphthyl sulphate. The mother liquors, when examined on paper chromatograms, were seen to contain this sulphate, together with 1-naphthyl sulphate and a substance which had the properties of 1-hydroxy-2-naphthyl sulphate. Attempts to isolate this last substance were unsuccessful.

Glucosiduronic acid derivatives of *trans*-1:2-dihydro-1:2-dihydroxy-naphthalene. After fractions containing the 1-naphthylmercapturic acid precursor (Boyland & Sims, 1958) had been collected, elution of the column was continued and a number of fractions were obtained which were shown on paper chromatograms to contain compounds which gave naphthol reactions after the paper was heated with acid. During the evaporation of some of the earlier of these fractions a crystalline compound was obtained which

gave rise on paper chromatograms to two spots, one of which gave 1-naphthol reactions after acid hydrolysis. The second spot was identical with that of creatinine. Consideration of the evidence presented below shows the compound to be the *creatinine salt* (or complex) of (–)-*trans*-1:2-dihydro-2-hydroxy-1-naphthyl-D-glucosiduronic acid: from aq. ethanol it formed elongated plates, m.p. 238–239° (decomp.), $[\alpha]_D^{20} - 233^\circ$ (c, 0.5 in water) (Found: C, 53.5; H, 5.9, N, 9.3. C₂₀H₂₅O₉N₃ requires C, 53.2; H, 5.6; N, 9.3%). On one occasion it was found that the naturally occurring creatinine was eluted from the column before the glucosiduronic acids. The fractions containing these acids were therefore combined and evaporated to a small volume and creatinine (2 g.) was added. The mixture was heated to 100° for a few minutes and cooled, when the creatinine salt described above separated.

The later fractions from the cellulose-powder column were evaporated to yield gums which gave strongly positive naphtharesorcinol tests for glucosiduronic acids. These fractions and the evaporated mother liquors from the earlier fractions were combined and re-chromatographed on a smaller cellulose column with butanol-cyclohexane-aq. 2N-NH₃ soln. (22:2:3). Fractions shown by paper chromatography to contain the glucosiduronic acids of *trans*-1:2-dihydro-1:2-dihydroxy-naphthalene were combined and evaporated to dryness under reduced pressure, and the gum thus obtained was dissolved in water (100 ml.). The aqueous solution was passed through a layer of the ion-exchange resin Zeo-Karb 225 (in the acid form) and evaporated to dryness under reduced pressure. The residue was recrystallized from ethanol-light petroleum (b.p. 80–100°) to yield a colourless powder, m.p. 146–158° (decomp.) which was raised to 152–153° by several recrystallizations from the above-mentioned solvents. It appears from evidence presented below that the compound is mainly (+)-*trans*-1:2-dihydro-2-hydroxy-1-naphthyl-D-glucosiduronic acid, $[\alpha]_D^{20} + 50^\circ$ (c, 0.5 in ethanol) (Found: C, 56.4; H, 5.8. Calc. for C₁₆H₁₈O₈: C, 56.8; H, 5.4%).

Also detected in the glucosiduronic acid fractions by means of paper chromatography were 1-naphthylglucosiduronic acid and two substances believed to be the glucosiduronic acid derivatives of 1:2-dihydroxy-naphthalene. The structures of the two last-named compounds, which were present in traces in rabbit urine but in much greater amount in the urine of animals of other species, will be discussed in a later paper.

Examination of the creatinine salt of the glucosiduronic acid. A solution of the salt (600 mg.) in water (50 ml.) was passed through a layer of Zeo-Karb 225 (in the acid form). The solution was evaporated to dryness under reduced pressure to yield (–)-*trans*-1:2-dihydro-2-hydroxy-1-naphthyl-D-glucosiduronic acid (425 mg.), which was recrystallized from ethanol-light petroleum (b.p. 80–100°) in needles, m.p. 172–173° (decomp.), $[\alpha]_D^{25} - 171 \pm 5^\circ$ (c, 0.5 in ethanol) (Found: C, 56.5; H, 5.25. C₁₆H₁₈O₈ requires C, 56.8; H, 5.4%). The ion-exchange resin was then eluted with aq. 2N-NH₃ soln. and the eluate was evaporated to dryness and the residue recrystallized from aq. ethanol to yield creatinine (102 mg.), m.p. 260° (decomp.) (Found: N, 37.3. Calc. for C₄H₇O₃N₃: N, 37.15%). The picrate formed yellow needles from water, m.p. and mixed m.p. 217–218° (decomp.) (Found: N, 24.6. Calc. for C₁₀H₁₀O₈N₄: N, 24.6%).

The glucosiduronic acid (100 mg.) was heated to 60° with 2N-HCl (2 ml.) for 15 min. and the solution was cooled and

extracted with ether. The ethereal layer was shown by paper chromatography to contain 2-naphthol. The aqueous layer was treated with charcoal (1 g.) and the charcoal was filtered off, washed with water and eluted with methanol (50 ml.) containing 5% (v/v) of aq. NH_3 soln. (sp.gr. 0.88). Removal of the methanol under reduced pressure afforded a gum which was shown by paper chromatography to contain 1-naphthylglucosiduronic acid. The gum was esterified with diazomethane in ether and acetylated with acetic anhydride in pyridine to yield methyl (1-naphthyltri-*O*-acetyl-D-glucosid)uronate (110 mg.), separating from aq. ethanol in needles, m.p. and mixed m.p. 158–159°. When the glucosiduronic acid was heated to 100° with 10N-HCl for 30 min. and the products were examined on paper chromatograms only 1- and 2-naphthol were detected.

The glucosiduronic acid (100 mg.) was incubated with β -glucuronidase (Ketodase, supplied by Warner-Chilcott Laboratories; 10 ml.) at 37° for 24 hr. The solution was extracted twice with ether, the ether was evaporated and the product was recrystallized from benzene to yield (–)-*trans*-1:2-dihydro-1:2-dihydroxynaphthalene (32 mg.) in plates, m.p. and mixed m.p. 127°, $[\alpha]_D^{25} - 157^\circ$ (c, 0.5 in ethanol). The diacetate formed plates from light petroleum (b.p. 60–80°), m.p. and mixed m.p. 77°.

The glucosiduronic acid was esterified and acetylated as before to yield (–)-methyl (*trans*-2-acetoxy-1:2-dihydro-1-naphthyltri-*O*-acetyl-D-glucosid)uronate, separating from ethanol in needles, m.p. 194°, $[\alpha]_D^{25} - 293 \pm 5^\circ$ (c, 0.5 in CHCl_3) (Found: C, 58.0; H, 5.6. $\text{C}_{25}\text{H}_{28}\text{O}_{12}$ requires C, 57.7, H, 5.4%).

Separation of the tetra-acetyl methyl ester derivatives of the glucosiduronic acids of 1:2-dihydro-1:2-dihydroxynaphthalene. In one experiment the fractions containing the glucosiduronic acids were combined and evaporated and the gum, in methanol, was treated with an excess of diazomethane. The solution was evaporated to dryness and the residue dissolved in pyridine and an excess of acetic anhydride was added. After some hours the reaction mixture was poured into a large volume of water and the product which separated was dissolved in a minimum amount of boiling ethanol and the solution allowed to crystallize. The crystals (850 mg.) were recrystallized from ethanol to yield (+)-methyl (*trans*-2-acetoxy-1:2-dihydro-1-naphthyltri-*O*-acetyl-D-glucosid)uronate in long needles, m.p. 209–210°, $[\alpha]_D^{25} + 100 \pm 5^\circ$ (c, 0.5 in CHCl_3) (Found: C, 57.4; H, 5.7. $\text{C}_{25}\text{H}_{28}\text{O}_{12}$ requires C, 57.7; H, 5.4%).

The mother liquors were evaporated to a small volume and the solution was allowed to crystallize. The product which separated was recrystallized three times from ethanol to yield (–)-methyl (*trans*-2-acetoxy-1:2-dihydro-1-naphthyltri-*O*-acetyl-D-glucosid)uronate (280 mg.) in needles, m.p. 194°, undepressed in a mixture with the product obtained earlier (Found: C, 57.9; H, 5.5. Calc. for $\text{C}_{25}\text{H}_{28}\text{O}_{12}$: C, 57.7; H, 5.4%). The combined mother liquors were evaporated to dryness and the residue was recrystallized twice from aq. ethanol and then from ethanol to yield a product which is believed to be (–)-methyl (1-acetoxy-1:2-dihydro-2-naphthyltri-*O*-acetyl-D-glucosid)uronate (115 mg.) in needles, m.p. 168°, $[\alpha]_D^{25} - 228 \pm 5^\circ$ (c, 0.5 in CHCl_3) (Found: C, 57.5; H, 5.7; H, 5.3, 5.7. $\text{C}_{25}\text{H}_{28}\text{O}_{12}$ requires C, 57.7; H, 5.4%).

*Hydrolysis of (+)-methyl(2-acetoxy-1:2-dihydro-1-naphthyltri-*O*-acetyl-D-glucosid)uronate.* The derivative (650 mg.), in a solution of barium methoxide in methanol (52 ml.;

0.125N) (Bollenback, Long, Benjamin & Lindquist, 1955), was kept overnight at room temperature and the precipitate was collected, dissolved in water (250 ml.) and the solution passed through a layer of Zeo-Karb 225 (in the acid form). The solution was evaporated under reduced pressure and the product was recrystallized from ethanol–light petroleum (b.p. 80–100°) to give (+)-*trans*-1:2-dihydro-2-hydroxy-1-naphthyl-D-glucosiduronic acid (380 mg.) in needles, m.p. 151–152°, $[\alpha]_D^{25} + 106 \pm 2^\circ$ (c, 0.5 in ethanol) (Found: C, 56.7; H, 5.8. $\text{C}_{16}\text{H}_{18}\text{O}_8$ requires C, 56.8; H, 5.4%). Esterification and acetylation as before yielded the original derivative, m.p. and mixed m.p. 209–210°.

The glucosiduronic acid (100 mg.) was heated with 2N-HCl (2 ml.) at 60° for 15 min. and the products were treated with charcoal as described above. 2-Naphthol was detected in the methanol eluate by paper chromatography and when the gum obtained by evaporation of the methanol was treated with diazomethane followed by acetic anhydride in pyridine, methyl (1-naphthyltri-*O*-acetyl-D-glucosid)uronate (98 mg.), m.p. and mixed m.p. 158–159°, was obtained.

Hydrolysis of the glucosiduronic acid with 10N-HCl at 100° afforded 1- and 2-naphthol (detected on paper chromatograms). When the glucosiduronic acid (100 mg.) was hydrolysed with β -glucuronidase as previously described, (+)-*trans*-1:2-dihydro-1:2-dihydroxynaphthalene (28 mg.) was obtained, separating from benzene in plates, m.p. and mixed m.p. 127°, $[\alpha]_D^{25} + 156^\circ$ (c, 0.5 in ethanol). The diacetate formed plates from light petroleum (b.p. 60–80°), m.p. and mixed m.p. 77°.

Hydrolysis of the derivative of m.p. 168°. The derivative (70 mg.) was hydrolysed with barium methoxide in methanol (5.6 ml.; 0.125N) as before to yield finally a colourless powder which appeared to consist mainly of *trans*-1:2-dihydro-1-hydroxy-2-naphthylglucosiduronic acid. A little of the acid was heated to 60° with 2N-HCl for 15 min. and the products were examined on paper chromatograms. 1-Naphthol was detected but no 2-naphthylglucosiduronic acid appeared to have been formed. Hydrolysis of the acid with 10N-HCl at 100° for 15 min. also afforded only 1-naphthol, and examination on paper chromatograms of the products of the hydrolysis with β -glucuronidase showed the presence of *trans*-1:2-dihydro-1:2-dihydroxynaphthalene. When the compound was examined on two-dimensional chromatograms developed with either solvent 1 or 2, which was sprayed with 2N-HCl between the first and second runs, some decomposition occurred to yield 1-naphthol: the formation of 1-naphthol in this way was used to detect the presence of the glucosiduronic acid in urine.

Metabolism of compounds other than naphthalene. Two rats were each injected intraperitoneally with 5 ml. of an aqueous solution of sodium 2-hydroxy-1-naphthyl sulphate (10%, w/v) and the urine was collected for 24 hr. after the injection. Examination of the urine on paper chromatograms showed the presence of unchanged 2-hydroxy-1-naphthyl sulphate, together with a little of the isomeric 1-hydroxy-2-naphthyl sulphate.

A rabbit, a rat and a guinea pig each was injected intraperitoneally with 5, 0.5 and 2 ml. respectively of a solution of *trans*-1:2-dihydro-1:2-dihydroxynaphthalene in arachis oil (10%, w/v) and the urines were collected for 24 hr. after the injections. When the urines were examined on one- and two-dimensional paper chromatograms it was seen that in

each case 1-hydroxy-2- and 2-hydroxy-1-naphthyl sulphate and 1-naphthyl sulphate were present, together with 2-naphthol, 1-naphthylglucosiduronic acid, 1:2-dihydro-2-hydroxy-1-naphthyl- and 1:2-dihydro-1-hydroxy-2-naphthyl-glucosiduronic acid and unchanged *trans*-1:2-dihydro-1:2-dihydroxynaphthalene.

A rat was given a solution of (–)-*trans*-1:2-dihydro-2-hydroxy-1-naphthylglucosiduronic acid (500 mg.) in water (2 ml.) by mouth. The urine was collected for the 24 hr. after treatment and examined on paper chromatograms. The urine contained 2-naphthol, the two isomeric sulphuric acid esters of 1:2-dihydroxynaphthalene, 1:2-dihydro-2-hydroxy-1-naphthylglucosiduronic acid and a little 1-naphthyl sulphate.

Attempted preparation of the sulphuric acid esters of (±)-trans-1:2-dihydro-1:2-dihydroxynaphthalene. Chlorosulphonic acid (0.3 g.) was added carefully to pyridine (5 ml.), followed by (±)-*trans*-1:2-dihydro-1:2-dihydroxynaphthalene (0.5 g.). The solution was kept at about 40° for 3 hr., when two layers of liquid formed. The mixture was poured into an ice-cold solution of KOH (1 g.) in water (20 ml.) and the solution was evaporated under reduced pressure until crystals separated. These were collected and extracted with boiling methanol. The methanol was removed under reduced pressure and the solid (120 mg.) recrystallized from ethanol-ether in flat needles, m.p. 164°, which appeared to consist of the potassium salt of (±)-*trans*-1:2-dihydro-2-hydroxy-1-naphthyl sulphate, together with some of the 2-isomer. Because of their lability, attempts to separate the isomers were unsuccessful. On paper chromatograms developed with either solvent 1 or solvent 2 (Table 1) (R_F 0.23 in solvent 1), the crystals formed dark absorbent spots when viewed under ultra-violet light, from which streaked a bright-blue fluorescent compound which, from its colour reactions, appeared to be 1-naphthyl sulphate. 1-Naphthol was also detected. The appearance of the chromatograms suggested that the sulphuric acid esters were decomposing on the chromatograms during development. Further evidence that decomposition was occurring was obtained from the light-absorption curves. A freshly prepared solution of the crystals in water (at pH 6.5) had a maximum at 264 m μ (ϵ 3900), the expected maximum for a 1:2-dihydro-naphthalene type of compound. By the end of 6 hr., this had been replaced by maxima at 272 and 282 m μ and the whole curve was identical with that of potassium 1-naphthyl sulphate. When solutions of the crystals in water were acidified with HCl and examined on paper chromatograms, 1-naphthyl sulphate and a little 1- and 2-naphthol were

detected. No 2-naphthyl sulphate was found, but it is not easy to detect small amounts of this ester in the presence of large amounts of 1-naphthyl sulphate. When the ester was heated at 100° with 5*N*-HCl for 15 min., only 1- and 2-naphthol were detected by paper chromatography and the mother liquors contained inorganic sulphate.

RESULTS

In agreement with Corner & Young (1954), 2- but not 1-naphthol was isolated from the urine of rabbits dosed with naphthalene, although the presence of 1-naphthol was detected on paper chromatograms. The amounts of free *trans*-1:2-dihydro-1:2-dihydroxynaphthalene and the relative proportions of the optically active forms of this compound in the urine of rabbits treated with naphthalene are extremely variable: presumably they depend in part on the methods of collection and storage of urine.

Although 1-hydroxy-2-naphthyl sulphate has not been isolated from the urine of rabbits treated with naphthalene, paper chromatography shows the presence of a compound with the same properties as the synthetic material in the urine of these animals. The compound is also formed when rats are dosed with the isomeric 2-hydroxy-1-naphthyl sulphate, when rabbits, rats and guinea pigs are dosed with (±)-*trans*-1:2-dihydro-1:2-dihydroxynaphthalene and when rats are dosed with (–)-*trans*-1:2-dihydro-2-hydroxy-1-naphthylglucosiduronic acid. 1-Hydroxy-2-naphthyl sulphate couples much more readily with diazo reagents than its isomer and it is presumably this compound which Sato, Yamada, Suzuki, Fukuyama & Yoshikawa (1956) detected by the diazo reaction, in their experiments on the action of rat-liver slices and homogenates on naphthalene and *trans*-1:2-dihydro-1:2-dihydroxynaphthalene and in the urine of rats treated with naphthalene.

The structure of the two forms of *trans*-1:2-dihydro-2-hydroxy-1-naphthylglucosiduronic acid are confirmed by the nature of the decomposition products obtained: mild acid treatment yielded 1-naphthylglucosiduronic acid and a little 2-naphthol,

Table 2. *Ultraviolet absorption of compounds related to trans-1:2-dihydro-1:2-dihydroxynaphthalene*

Measurements were made in ethanol unless otherwise stated.

Compound	$\lambda_{\max.}$ (m μ)	$\epsilon_{\max.}$
1:2-Dihydronaphthalene*	262	10 230
(±)- <i>trans</i> -1:2-Dihydro-1:2-dihydroxynaphthalene (synthetic)†	263	6 830
(–)- <i>trans</i> -1:2-Dihydro-2-hydroxy-1-naphthylglucosiduronic acid	264	6 600
(–)- <i>trans</i> -1:2-Dihydro-2-hydroxy-1-naphthylglucosiduronic acid (creatinine salt)	262‡	25 400‡
(+)- <i>trans</i> -1:2-Dihydro-2-hydroxy-1-naphthylglucosiduronic acid	265	6 900
(–)-Methyl (2-acetoxy-1:2-dihydro-1-naphthyltri- <i>O</i> -acetylglucosid)uronate	262	8 200
(+)-Methyl (2-acetoxy-1:2-dihydro-1-naphthyltri- <i>O</i> -acetylglucosid)uronate	264	7 800
(–)-Methyl (1-acetoxy-1:2-dihydro-2-naphthyltri- <i>O</i> -acetylglucosid)uronate	262	7 900

* Morton & de Gouveia (1934).

† Beale & Roe (1951).

‡ In water.

strong acid treatment yielded 1- and a little 2-naphthol and hydrolysis with β -glucuronidase yielded either (+)- or (-)-*trans*-1:2-dihydro-1:2-dihydroxynaphthalene. 1:2-Dihydro-1-hydroxy-2-naphthylglucosiduronic acid, on the other hand, yielded 1-naphthol (but no detectable 2-naphthylglucosiduronic acid) after both mild and vigorous acid treatment. It is generally found that, with derivatives of 1:2-dihydronaphthalene of this type, treatment with acid yields mainly 1-substituted naphthalene derivatives. The light-absorption curves of the glucosiduronic acids and their derivatives are similar to one another and to that of 1:2-dihydronaphthalene itself (cf. Beale & Roe, 1951) (see Table 2). Although the (-)-form of *trans*-1:2-dihydro-2-hydroxy-1-naphthylglucosiduronic acid readily formed a highly crystalline creatinine salt, the (+)-form failed to do so under all the conditions tried.

The product obtained by the action of chlorosulphonic acid on ($\pm$)-*trans*-1:2-dihydro-1:2-dihydroxynaphthalene decomposed spontaneously in aqueous solutions at room temperature in the course of a few hours, immediately with dilute mineral acid and even in the solid state. The breakdown products in each case were 1-naphthyl sulphate, together with a little 1- and 2-naphthol. By analogy with the mild acid decomposition of the corresponding glucosiduronic acids, the original product appears to be a mixture of the two isomeric sulphuric acid esters, the 1-substituted ester breaking down to 1-naphthyl sulphate and 2-naphthol and the 2-substituted ester to 1-naphthol. The sulphuric acid ester has not been detected in the urine of animals treated with naphthalene.

DISCUSSION

In experiments *in vitro* in which naphthalene is incubated with rabbit- or rat-liver microsomal fractions (Mitoma, Posner, Reitz & Udenfriend, 1956; Booth & Boyland, 1958) and with rat-liver slices (Booth, Boyland & Sims, unpublished observations), 1-naphthol or 1-naphthyl sulphate is detected but not the 2-isomers. In animals treated with naphthalene or with *trans*-1:2-dihydro-1:2-dihydroxynaphthalene, free 2-naphthol is present (usually in greater amounts than free 1-naphthol), but 2-naphthyl sulphate or glucosiduronate has not been detected. As Berenbom & Young (1951) have shown that injected 2-naphthol is readily converted into these conjugates in the body, it seems likely that 2-naphthol is not formed by the direct hydroxylation of naphthalene in the way that some at least of the 1-naphthol is formed. Corner & Young (1955) suggested that 2-naphthol, together with 1-naphthyl sulphate and glucosiduronate, arose from the decomposition of the 1-sulphuric acid and gluc-

uronic acid conjugates of *trans*-1:2-dihydro-1:2-dihydroxynaphthalene.

Evidence presented in this paper supports this view. Moreover, the 2-glucuronic acid conjugate of *trans*-1:2-dihydro-1:2-dihydroxynaphthalene does not break down to give 2-naphthylglucosiduronate and probably the corresponding sulphuric acid ester does not yield 2-naphthyl sulphate. Although alcohols do not apparently conjugate with sulphuric acid in the body, the formation of such conjugates of *trans*-1:2-dihydro-1:2-dihydroxynaphthalene, which are subsequently decomposed in the manner indicated above or dehydrogenated to the sulphuric acid esters of 1:2-dihydroxynaphthalene, would account for the observed pattern of excreted sulphuric acid esters. A similar breakdown of some of the glucuronic acid conjugates of *trans*-1:2-dihydro-1:2-dihydroxynaphthalene would account for the pattern of glucosiduronic acids excreted. In both cases, some at least of the 1-naphthyl conjugates probably arise directly from 1-naphthol. Boyland & Wiltshire (1953) suggested that *trans*-1:2-dihydro-1:2-dihydroxynaphthalene itself [which gives rise to 1- and 2-naphthol with mineral acid (Boyland & Sims, 1953)] might be a precursor of the naphthols, and the 1-naphthylmercapturic acid precursor (Boyland & Sims, 1958) is known to give rise to the naphthols on acidification. In the first case, however, rather drastic conditions are required to effect the breakdown and in the second the formation of the naphthols is accompanied by the formation of 1-naphthylmercapturic acid, which is not usually present in freshly voided urine.

The present work supports the findings of Corner & Young (1955) that the conjugates of 1:2-dihydroxynaphthalene, which are found, together with 2-naphthol and 1-naphthyl sulphate and glucosiduronic acid, in the urine of animals treated with naphthalene are also formed when *trans*-1:2-dihydro-1:2-dihydroxynaphthalene is administered. Sato *et al.* (1956) have found that *trans*-1:2-dihydro-1:2-dihydroxynaphthalene is converted into conjugates of 1:2-dihydroxynaphthalene by the supernatant of rat-liver homogenates and

Fernley & Evans (1958) have shown that naphthalene is converted into 1:2-dihydroxynaphthalene itself by soil pseudomonads. The conjugates of 1:2-dihydroxynaphthalene are not found in the urine of animals treated with 1- or 2-naphthol. It is probable therefore that the conjugates of 1:2-dihydroxynaphthalene found in the urine after the administration of naphthalene arise via *trans*-1:2-dihydro-1:2-dihydroxynaphthalene. This aromatization of non-aromatic compounds is not uncommon. Beer, Dickens & Pearson (1951) have shown for example that hexahydrobenzoic acid is converted into benzoic acid by rabbits. There is no evidence as yet whether it is the free or conjugated dihydro-dihydroxy compounds or both which are dehydrogenated.

The metabolism of naphthalene in the rabbit appears to proceed by three main routes: (1) A direct hydroxylation to 1-naphthol followed by conjugation with sulphuric acid or glucuronic acid. (2) A 'perhydroxylation' followed by conjugation and breakdown as outlined above, or dehydrogenation of either the free or conjugated 'perhydroxylated' product to give sulphuric acid and probably glucuronic acid conjugates of 1:2-dihydroxynaphthalene. (3) The reaction of some reactive intermediate with the SH groups of cysteine, glutathione or a protein to give, after hydrolysis and acetylation, the mercapturic acid precursor. The nature of this reactive intermediate is still uncertain, but if it is the epoxide, as suggested by Boyland (1950), then it might be that both routes 2 and 3 start from this same intermediate.

SUMMARY

1. The isolation of the (+)- and (-)-forms of *trans*-1:2-dihydro-2-hydroxy-1-naphthylglucosiduronic acid from the urine of rabbits dosed with naphthalene is described. The (-)-form forms a well-defined creatinine salt.

2. Both the glucosiduronic acids were also isolated in the form of their tetra-acetyl methyl ester derivatives and a third derivative was shown to be that of *trans*-1:2-dihydro-1-hydroxy-2-naphthylglucosiduronic acid.

3. Rabbits convert naphthalene into 1-hydroxy-2-naphthyl sulphate, which is also formed when rabbits, rats and guinea pigs are treated with *trans*-

1:2-dihydro-1:2-dihydroxynaphthalene. The ester was synthesized by the persulphate oxidation of 1-naphthol.

4. Possible mechanisms of formation of the above-mentioned compounds in the animal body are discussed.

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