m.p. 118–121°. Yield, 6.6 g. A sample, after sublimation at 105°/0.2 mm., had m.p. 120–121°. (Found: C, 72.1; H, 8.8%. $C_{10}H_{14}O_2$ requires C, 72.3; H, 8.4%.)

The above substance (12 g.), dissolved in 5N-NaOH (120 ml.), was shaken with benzoyl chloride (25 ml. at once, and 10 ml. after 20 hr.). After 24 hr. the precipitated solid was collected, well washed with water, and dissolved in ether. The ether was dried (Na_2SO_4), filtered and evaporated. The residue, with l.p. formed crystals (17 g.) of m.p. 77–81°. A sample, after sublimation at 145°/0.05–0.1 mm., separated from l.p.-benzene as large colourless prisms, m.p. 83–84°, of 4-benzoyloxyphenyl-methyl-ethyl carbinol. (Found: C, 75.7; H, 6.3%. $C_{17}H_{18}O_3$ requires C, 75.5; H, 6.7%.)

(Probably) 4-Benzoyloxy- α -ethyl-styrene. An intimate mixture of the above benzoate (3 g.) with freshly dried finely powdered anhydrous $MgSO_4$ (12 g.) was heated at 160–170° for 1 hr. Dilution of the cooled product gave a water-insoluble solid, which was collected, washed, and crystallized from methanol, giving almost colourless needles (1.75 g.);

m.p. 111–112°. (Found: C, 80.7; H, 6.7%. $C_{17}H_{18}O_2$ requires C, 80.9; H, 6.4%.)

4-sec-Butyl-phenol. The above compound (2 g.) in benzene (50 ml.) was shaken with H_2 and Pd-black (0.5 g.) until no more H_2 was absorbed. The product, obtained by evaporation of the filtered solution, was an oil at 20°. It was boiled under reflux with N-NaOH (40 ml. in approx. 30% v/v ethanol-water) for 3 hr. Ethanol was then removed in steam, and the alkaline residue was acidified with HCl. The oily phenol was separated by steam distillation and formed an aqueous suspension, which crystallized at 0°. Yield, 1.3 g., m.p. 51–53°; Reilly & Hickinbottom (1920) record m.p. 59°.

SUMMARY

1. A series of substituted 2-aminophenols has been tested *in vitro* for antibacterial activity.

2. It is concluded that such activity is not increased but may be decreased by simple nuclear substitution of 2-aminophenol.

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A Metabolite of 2-Acetamidofluorene*

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Investigation of the fate of carcinogenic and related non-carcinogenic hydrocarbons in animals was begun by Boyland & Levi (1935) and by Chalmers & Peacock (1936), a few years after the discovery of the first carcinogenic compound of known structure (Kennaway, 1930). The presence of phenolic derivatives of carcinogenic hydrocarbons in the excreta of rabbits, rats and mice was recognized at an early stage of these studies, and some of the metabolites were later isolated and identified. Fieser (1941), reviewing the results of this line of cancer research, came to the conclusion that: 'biological hydroxylation represents an avenue for the elimination of carcinogens in a detoxified form', and that 'the reaction of detoxication, if indeed it may be so regarded, involves an attack of the molecule at some site other than the centre of special susceptibility to substitutions'. Relevant data obtained since

1941 conform in the main with Fieser's views. Berenblum & Schoental (1943) called attention to the fact that 'with benzantracene and dibenzanthracene, the positions in the molecule metabolically attacked are also those where sulphonation occurs *in vitro*, provided the most reactive positions are blocked'.

It seemed of interest to find whether these rules applied to 2-acetamidofluorene,* one of the most potent of the carcinogens which produce distant tumours (Wilson, DeEd & Cox, 1941; Bielschowsky, 1944; Armstrong & Bonser, 1944). Wilson *et al.* (1941) observed that the fur of the experimental rats, and the white pine shavings on which they

* Previous authors have described this compound as 2-acetylaminofluorene. It is here described as 2-acetamidofluorene, in conformity with the rules of organic nomenclature adopted.

were kept, were stained when higher dosages of acetamidofluorene were fed. They suspected that 2-aminofluorene, excreted with the urine, was responsible for this bright orange colour, but were unable to isolate aminofluorene from the urine of rats and rabbits receiving acetamidofluorene by mouth; nor could they obtain a derivative of quinonoid structure.

RESULTS

Our first efforts were directed to obtaining metabolites from the urine of rats receiving 2-acetamidofluorene, since the observations of Wilson *et al.* (1941) had made it highly probable that derivatives of the carcinogen are excreted by the kidneys. One metabolite could be isolated without great difficulty. The compound, a substance of phenolic character, crystallized in colourless needles, m.p. 232°. The analysis was in agreement with the formula $C_{15}H_{13}O_2N$. The presence of an acetyl group was established, thus indicating that the substance is a monohydroxy derivative of acetamidofluorene. The melting-point and absorption spectrum of the isolated compound were found to be identical with the melting-point and absorption spectrum of synthetic 2-acetamido-7-hydroxyfluorene. The amount of the metabolite which could be isolated corresponded to about 5–8 % of the administered carcinogen.

2-Acetamido-7-hydroxyfluorene gives a very sensitive colour reaction with nitrite, which can be applied to ether extracts of urine. By this method it was found that the excretion of 2-acetamido-7-hydroxyfluorene ceases 2–3 days after withdrawal of the drug from the diet, independently of the duration of administration.

2-Acetamido-7-hydroxyfluorene is not the only metabolite of 2-acetamidofluorene excreted with the urine, but it seems to be the derivative which is excreted in largest quantities, and others have not yet been identified.

EXPERIMENTAL

Albino rats received a daily dosage of 4–6 mg. of 2-acetamidofluorene with their diet (skimmed milk-bread). The animals were kept in metabolism cages. The collected urine was stored in a refrigerator, preserved with chloroform, until 1 l. was available.

All melting-points are uncorrected. Analyses were carried out by Dr G. Weiler (Oxford).

Isolation of 2-acetamido-7-hydroxyfluorene. The urine (1000 ml.), which was alkaline to litmus, was extracted five times with 250 ml. of ether. Ethanol (10 ml.) was added to break the emulsions, but sometimes it was necessary to centrifuge in order to obtain a satisfactory separation. The combined ether extracts were dried over sodium sulphate and concentrated to about 250 ml. The ether was washed with dilute HCl and water, further concentrated to

a volume of about 10–15 ml. and poured into a flask containing 50 ml. of water. After 2 days' standing in a refrigerator, crystallization started at the interface of the ether-water layers and increased slowly during the next few days. The crystals were collected and washed with ice-cold ether. The substance so obtained was still very impure and melted between 190 and 200°.

For purification 100 mg. of these crystals were boiled under reflux with 10 ml. of anhydrous ether (peroxide-free). The ether extracted most of the coloured impurities. The undissolved portion (80 mg.) was recrystallized first from 50 % (v/v) acetic acid with the addition of charcoal and then from aqueous ethanol, m.p. 232°. (Found: C, 75.03; H, 5.69; N, 5.95 %. $C_{15}H_{13}O_2N$ requires C, 75.28; H, 5.48; N, 5.86 %.)

Synthesis of 2-acetamido-7-hydroxyfluorene. 2-Amino-7-nitrofluorene (7-amino-2-nitrofluorene) was prepared according to the method of Diels, Schill & Tolson (1902) and diazotized as follows: 2 g. of this substance were dissolved in 500 ml. of boiling 2N- H_2SO_4 , the solution was cooled quickly to about 40° and 1 g. of $NaNO_2$ added with stirring, the temperature being maintained at 40–45°. The reaction mixture was filtered, urea added and the solution boiled under reflux for 90 min. 7-Hydroxy-2-nitrofluorene crystallized. The product was collected, washed with water and dried. After recrystallization from toluene it had m.p. 250°. (Found: N, 6.07 %. $C_{15}H_9O_3N$ requires N, 6.17 %.)

2-Amino-7-hydroxyfluorene was obtained from the crude nitro-compound. 1 g. nitro-compound was mixed with 5 g. zinc dust and 1 g. anhydrous $CaCl_2$, 100 ml. of 80 % (v/v) ethanol were added and the mixture was boiled under reflux for 60 min. on a water-bath. The dark yellow solution turned colourless. The zinc dust was filtered off and on addition of water to the hot filtrate a slightly coloured, crystalline precipitate was obtained, which was collected after standing in a refrigerator for several hours. The precipitate was dried *in vacuo*. After recrystallization from aqueous ethanol it melted with decomposition at 271°. (Found: N, 7.18 %. $C_{15}H_{11}ON$ requires N, 7.11 %.)

2-Acetamido-7-hydroxyfluorene. The crude amino-compound was dissolved in 200 ml. of water by the addition of 2 ml. of conc. HCl. The solution was filtered from a small amount of a coloured impurity and 1 g. crystalline sodium acetate and excess acetic anhydride were added. The solution was stirred for 2 hr. in an ice-bath. After a few minutes the acetyl compound began to crystallize. The product was collected and recrystallized first from aqueous acetic acid and then from aqueous ethanol, m.p. 232°. (Found: C, 75.18; H, 5.49; N, 5.85 %. $C_{15}H_{13}O_2N$ requires C, 75.28; H, 5.48; N, 5.86 %.)

Comparison of the isolated and synthetic compounds.

Both substances had the same melting-point and no depression was observed in a mixture of the two. They showed no differences in solubility and both gave the same colour reaction with nitrite. Dr F. B. Strauss (Oxford) compared the absorption spectra of the isolated and synthesized compounds and gave the following opinion: 'both spectra are approximately identical; moreover the wave-lengths of maximum absorption as well as the molar extinction coefficients of maximum absorption are identical within the limits of experimental error.'

The colour reaction. 2-Acetamido-7-hydroxyfluorene (1 mg.) was dissolved in 5 ml. glacial acetic acid. 0.5 ml. of this solution was diluted with an equal volume of water, and a drop of 2N-HCl and four drops of a freshly prepared 0.1% solution of sodium nitrite were added. A deep purple colour developed. The dye could be extracted with amyl alcohol. Amounts as small as 20 μ g. are still detectable with this reaction.

The ether extract of the urine of rats receiving a diet containing 2-acetamidofluorene showed a strong nitrite reaction: 250 ml. of urine were extracted as described above, the ether was dried over sodium sulphate, evaporated and the residue dissolved in

5 ml. of glacial acetic acid. Of this extract 0.1 ml. still showed a positive nitrite reaction. When collection of the urine of rats was commenced 48 hr. after the withdrawal of the drug from the diet and the urine was treated in the same way, the extracts did not show a positive test, even when amounts corresponding to 50 ml. of urine were taken.

DISCUSSION

The isolation of 2-acetamido-7-hydroxyfluorene from the urine of rats receiving 2-acetamidofluorene suggests that the observation of Berenblum & Schoental (1943) is not confined to benzanthracene and dibenzanthracene. When 2-aminofluorene is treated with sulphuric acid 2-amino-7-fluorene-sulphonic acid is formed (Courtot, 1930). The biological hydroxylation has thus taken place in the same position as that in which sulphonation occurs *in vitro*.

SUMMARY

One metabolite of 2-acetamidofluorene has been isolated from the urine of rats, and has been identified as 2-acetamido-7-hydroxyfluorene.

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Purification and Properties of Cytochrome c

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It was previously shown that cytochrome *c* can be extracted in a soluble form from baker's yeast (Keilin, 1930) or from heart muscle of horse or ox (Theorell, 1935; Keilin & Hartree, 1937), and that fractionation of the cytochrome *c* from heart muscle gives rise to a preparation containing 0.34% Fe. A very simple method of preparation of cytochrome *c* of this purity was described (Keilin & Hartree, 1937), and this method has been generally adopted by other workers in their studies of this substance.

Attempts to increase the iron content above 0.34% were unsuccessful, and the protein appeared to be homogeneous in cataphoretic experiments.

While determining the isoelectric point of cytochrome *c* in the Tiselius apparatus, Theorell & Åkesson (1939) noticed that at pH slightly above the isoelectric point cytochrome *c* containing 0.34% Fe no longer migrated as a homogeneous protein. The isoelectric point of cytochrome *c* was given as 10.65 at 0°, and at pH 10.68 the red cytochrome *c* moved slowly towards the anode while a colourless boundary moved more rapidly (Theorell & Åkesson, 1941). By the use of a large Tiselius (1938) apparatus and continuous electrophoresis of 9–10 days' duration, Theorell & Åkesson were able to separate the colourless fraction from a considerable quantity of