CGCXVII. METABOLISM OF POLYCYCLIC COMPOUNDS.

I. PRODUCTION OF DIHYDROXYDIHYDRO-ANTHRACENE FROM ANTHRACENE.

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POLYCYCLIC aromatic hydrocarbons are known to produce two distinct pathological changes in animals. Thus under certain conditions naphthalene produces cataract in rabbits, whilst under other conditions derivatives of 1:2-benzanthracene produce cancer in some species. It is possible that animals might deal with toxic hydrocarbons in either of two ways; the compounds might either be converted into more active pathogenic substances or be detoxicated by conversion into some harmless compound. Our present knowledge of the metabolism of aromatic hydrocarbons with the exception of benzene and naphthalene is very scanty.

It has been known for some time that the urine of naphthalene-fed animals liberates naphthalene on boiling with acid [Baumann and Heuter, 1878]. Bourne and Young [1934] have isolated naphthalenemercapturic acid from the urine of naphthalene-fed rabbits. Bergel and Pschorr [1903] isolated phenanthrolyglycuronic acid from the urine of rabbits which had been given phenanthrene in their food, but they were unable to determine the constitution of this glycuronic acid compound.

EXPERIMENTAL.

The rats and rabbits received the following diet:

	Parts		Parts
Wholemeal flour	30	Wheat germ	18
Dried milk powder	10	Bran	18
Lard	16	Dried yeast	2
NaCl	2	Anthracene	4

Both rats and rabbits were given milk and a small amount of fresh green food and of cod-liver oil mixed in the diet each day. The animals were kept in metabolism cages of the Paine type and the pooled urine of each species was collected.

Isolation of products from urine. The urine was filtered through kieselguhr before any process of separation was carried out. The filtered urine from rats and rabbits gave crystalline anthracene on boiling with dilute acid. The precursor of this anthracene has not yet been isolated.

Precipitation of the urine with lead acetate and barium acetate did not yield any pure material although the urine of both species gave a positive naphthoresorcinol test for glycuronic acid, and conjugated glycuronic acids have often been isolated by means of their lead salts.

The filtered urine was extracted with ether in continuous extractors for 1, 2 or 3 days. The ether extract of this untreated urine was washed with NaOH solution and water, dried and evaporated. The brown crystalline residue was recrystallised from toluene. Products with similar chemical behaviour were

isolated from both rat and rabbit urine in yields up to 100 mg. per litre of urine. This corresponds to a yield of about 0.5% of the anthracene consumed.

Product isolated from the neutral ether extract of urine of rats. The substance from rat urine crystallised in white prisms, m.p. 160–161°, with previous decomposition; $[\alpha]_D^{21}$ in acetone –154° (c, 1), in dioxan –100° (c, 2). It was soluble in ether, alcohol and acetone, and moderately soluble in hot water and hot benzene. The solutions had a faint blue fluorescence. (Analysis (Schoeller). Found: C, 78.9; H, 5.5%; mol. wt. 227, 222. $C_{14}H_{12}O_2$ requires C, 79.2; H, 5.6%; mol. wt. 212.)

The substance was not soluble in ammonia or NaOH, hence it was not phenolic. It was acetylated with boiling acetic anhydride containing pyridine for 15 min. (without pyridine the substance was recovered unchanged after a short boiling in acetic anhydride). The diacetate (III) separated on addition of water and was recrystallised from light petroleum, m.p. 149°; $[\alpha]_D^{20}$ (c, 0.05) in benzene –375.5°; in dioxan –362° (c, 1). Insoluble in water, soluble in organic solvents with violet fluorescence. (Found (Schoeller): C, 72.9; H, 5.4%. $C_{18}H_{16}O_4$ requires C, 73.0; H, 5.4%.)

The analyses thus agree with the formula for a dihydroxydihydroanthracene (I) or (II). The optical activity indicates that the carbon atoms bearing the OH groups are the reduced ones.

Boiling the substance with dilute acid produced α -anthrol (IV), m.p. 149–151° (m.p. of α -anthrol 150–153° [Dienel, 1905]), which was soluble in alkali with a yellow colour and oxidised in air to give a blue colour and precipitate. The benzene solution had a strong blue-violet fluorescence. (Found (Schoeller): C, 86.2; H, 5.3%. $C_{14}H_{10}O$ requires C, 86.5; H, 5.2%.)

Acetylation of this with acetic anhydride in the presence of pyridine gave α -acetoxy-anthracene, m.p. 128–130° (m.p. of α -acetoxyanthracene 128–130° [Dienel, 1905]).

The dihydroxydihydroanthracene reacted with benzoyl chloride in pyridine. Recrystallisation of the dibenzoate from alcohol gave a product m.p. 138°; $[\alpha]_D -452^\circ$ (c, 2). (Found (Weiler): C, 80.1; H, 5.0%. $C_{28}H_{20}O_4$ requires C, 80.0; H, 4.7%.)

The dihydroxydihydroanthracene rapidly reduced KMnO_4 in acetone with production of heat. After dilution with water and treatment with SO_2 the product was extracted with ethyl acetate, and the acidic product was removed with NaHCO_3 and recrystallised from hot water. It had m.p. $242\text{--}243^\circ$ with a neutralising equivalent of 106 in agreement with that of naphthalene-2:3-dicarboxylic acid (V). (Theoretical equivalent = 108.) (Found (Schoeller): C, 66.8; H, 4.0%. $\text{C}_{12}\text{H}_8\text{O}_4$ requires C, 66.6; H, 3.7%.)

The acid was sublimed and gave an anhydride, m.p. 246° , mixed m.p. with an authentic specimen 246° .

The dihydroxydihydroanthracene was hydrogenated by shaking the acetone solution with palladium in the presence of H_2 . With a suitable catalyst (Pd-BaSO_4) the substance took up approximately 1 mol. of H_2 . The reduced product did not give α -anthrol on boiling with acid and was more soluble in water and benzene than the original substance. The solutions of the reduction product showed no fluorescence. It crystallised in plates, m.p. 149° , from benzene and appeared to be dihydroxytetrahydroanthracene (VI); $[\alpha]_D$ in dioxan $+99^\circ$ (c, 2). (Found (Schoeller): C, 78.5; H, 6.6%. $\text{C}_{14}\text{H}_{14}\text{O}_2$ requires C, 78.5; H, 6.5%.)

This dihydroxytetrahydroanthracene was acetylated by boiling with acetic anhydride in the presence of a little pyridine. The acetate crystallised from aqueous acetic acid in plates, m.p. 91° ; $[\alpha]_D^{20}$ in dioxan -74° (c, 1). (Found (Weiler): C, 72.7; H, 6.2%. $\text{C}_{18}\text{H}_{18}\text{O}_4$ requires C, 72.5; H, 6.4%.)

The dihydroxytetrahydroanthracene was warmed with KMnO_4 in acetone for a few minutes. An acid was isolated which crystallised from hot water in laminae, m.p. 210° , with slight decomposition. The neutralisation equivalent (120) agrees with that of naphthalene-2-carboxy-3-propionic acid (VII). Theoretical equivalent = 123. (Found (Schoeller): C, 68.8; H, 4.9%. $\text{C}_{14}\text{H}_{14}\text{O}_4$ requires C, 68.8; H, 4.9%.)

The dihydroxytetrahydroanthracene and the diacetate of this compound on boiling with acid yielded a pink crystalline substance, insoluble in alkali, optically inactive and of m.p. 149° . (Found (Weiler): C, 85.3; H, 6.3%. $\text{C}_{14}\text{H}_{12}\text{O}$ requires C, 85.5; H, 6.1%.)

This substance reacted with phenylhydrazine and appeared to be either 1- or 2-ketotetrahydroanthracene. Braun and Bayer [1929] have described 1-ketotetrahydroanthracene, melting at 95° , and 2-ketotetrahydroanthracene melting at $148\text{--}150^\circ$. The substance obtained by acid hydrolysis is therefore probably 2-ketotetrahydroanthracene (VIII).

The analytical figures and behaviour on acetylation show that the ether-soluble substance contains two hydroxyl groups and, as it yields α -anthrol on acid hydrolysis, one of the OH groups is in the α -position of the anthracene nucleus. It might therefore be either 1:4-dihydroxy-1:4-dihydroanthracene (I) or 1:2-dihydroxy-1:2-dihydroanthracene (II). It cannot be one of the meso-substituted compounds because the substance can be oxidised to naphthalenedicarboxylic acid (V) and takes up hydrogen in the presence of palladium.

Since acid hydrolysis of the dihydroxydihydroanthracene yields α -anthrol and hydrolysis of the dihydroxytetrahydroanthracene gives 1-ketotetrahydroanthracene it is very probable that the dihydro-compound is 1:2-dihydroxy-dihydroanthracene (II).

The KMnO_4 oxidation of 1:4-dihydroxy-1:2:3:4-tetrahydroanthracene would give 1:4-diketotetrahydroanthracene or naphthalene-2:3-dicarboxylic acid, whereas 1:2-dihydroxy-1:2:3:4-tetrahydroanthracene would give naphthalene-2-carboxy-3-propionic acid. The isolation of what is apparently naphthalene-2-

carboxylic-3-propionic acid (VII), an acid containing two carbon atoms more than naphthalene-2:3-dicarboxylic acid (V), by oxidation of the hydrogenated ether extract shows the neutral ether-soluble substance to be 1:2-dihydroxy-1:2-dihydroanthracene (II); the acid would accordingly be naphthalene-2-carboxy-3-propionic acid (VII).

Substance obtained from neutral ether extract of urine of rabbits.

This crystallised from benzene in plates, m.p. 184°, with previous decomposition; $[\alpha]_D$ in dioxan +16° (c, 1).

It dissolved in ether, acetone, alcohol, hot water and hot benzene to give slightly fluorescent solutions. (Found (Weiler): C, 79.1; H, 5.6%. $C_{14}H_{12}O_2$ requires C, 79.2; H, 5.7%.)

In its chemical properties it resembled the product isolated from rat urine. Acetylation with acetic anhydride and pyridine gave a diacetate, laminae, m.p. 184° from light petroleum; $[\alpha]_D$ in dioxan +309° (c, 2). (Found (Schoeller): C, 73.0; H, 5.4%. $C_{18}H_{16}O_4$ requires C, 73.0; H, 5.4%.) Acid hydrolysis gave an impure anthrol which gave an acetate, m.p. 129°, after recrystallisation (α -acetoxyanthracene, m.p. 128–130° [Dienel, 1905].)

Oxidation with $KMnO_4$ yielded naphthalene-2:3-dicarboxylic acid, m.p. 242–243°, identical with that obtained under similar conditions from the ether-soluble product of the rat urine. (Found: C, 66.0; H, 4.0%. $C_{12}H_{10}O_4$ requires C, 66.6; H, 3.7%.) Hydrogenation with hydrogen and palladium gave a dihydroxy-tetrahydroanthracene, m.p. 162°; $[\alpha]_D^{20} -1^\circ$ (c, 2). (Found (Weiler): C, 78.6; H, 6.76%. $C_{14}H_{14}O_2$ requires C, 78.6; H, 6.6%.) The dihydroxytetrahydroanthracene on acetylation with boiling acetic anhydride and pyridine gave a diacetate, m.p. 84°; $[\alpha]_D^{20} -3^\circ$ (c, 1). (Found (Weiler): C, 72.2; H, 6.4%. $C_{18}H_{18}O_4$ requires C, 72.5; H, 6.4%.)

The rotations of the dihydroxytetrahydroanthracene and the diacetoxy-tetrahydroanthracene were too small for accurate determinations with the amounts of material available. They both appeared to be slightly laevorotatory.

Oxidation of this dihydroxytetrahydroanthracene with $KMnO_4$ in acetone gave naphthalene-2-carboxy-3-propionic acid, m.p. 205°, identical with that obtained from the rat urine under similar conditions. (Found (Weiler): C, 68.5; H, 5.2%. $C_{14}H_{12}O_4$ requires C, 68.8; H, 4.9%.) The tetrahydro-compound on boiling with dilute HCl gave 2-keto-1:2:3:4-tetrahydroanthracene, m.p. 145–149°.

The chemical properties and composition of the derivatives of the compound from the rabbit are identical with those of the compound from the rat and the substance must therefore be 1:2-dihydroxy-1:2-dihydroanthracene. The differences in m.p. and optical rotation (Table I) show that the two species produce

Table I. *Properties of the 1:2-dihydroxy-1:2-dihydroanthracenes isolated from rat and rabbit urine.*

	Rat	Rabbit
M.P.	160–161°	184°
$[\alpha]_D^{20}$ in dioxan	–100°	+ 16.2°
M.P. of diacetate	149°	184°
$[\alpha]_D^{20}$ of diacetate in dioxan	–362°	+ 309°
M.P. of hydrogenated product	149°	162°
$[\alpha]_D^{20}$ of hydrogenated product	+ 98°	– 1°
M.P. of diacetate of hydrogenated product	91°	84°
$[\alpha]_D^{20}$ of diacetate of hydrogenated product	– 74°	– 3°

different stereoisomerides of the compound. As the molecule is not symmetrical about the two asymmetric carbon atoms, internal compensation is impossible, thus four stereoisomerides of substance (II) are possible. It is not definitely proved that the substances isolated were optically pure.

As the 1:2-dihydro-1:2-dihydroxyanthracene yields α -anthrol readily on acid hydrolysis and the original urine from rats gives anthracene under similar conditions it seems probable that rats excrete 1- (or 2)-hydroxy-1:2-dihydroanthracene in addition to the dihydroxy-compound. It is uncertain whether this is excreted as such or conjugated with glycuronic acid.

It is remarkable that anthracene should be attacked in the side rings in the animal body. Most chemical reactions such as halogenation, nitration or oxidation carried out *in vitro* lead to products with substituents in the 9:10 positions. It is possible that the substances isolated are formed in the gut by the action of intestinal bacteria, but if this were the case it would be very remarkable for the flora of the rat and rabbit to produce different stereoisomerides.

In addition to the two isomeric dihydroxydihydroanthracenes, two distinct glycuronic acid derivatives of anthracene have been isolated from the two species but the constitution of these has not yet been determined. Indications have also been obtained that some anthracene is excreted in the form of mercapturic acid but so far this has not been obtained in a pure condition.

Experiment in which the carcinogenic hydrocarbon 1:2:5:6-dibenzanthracene was fed to rats have led to the isolation of a substance analogous to those described in this paper. Work on its properties is now in hand.

SUMMARY.

Rats and rabbits fed on the same diet containing anthracene excrete different stereoisomerides of 1:2-dihydroxy-1:2-dihydroanthracene. The form excreted by rats is laevorotatory while that excreted by rabbits is dextrorotatory.

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