CLXXIV. METABOLISM OF POLYCYCLIC COMPOUNDS.

III. ANTHRYLMECAPTURIC ACID.

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ALTHOUGH phenylmercapturic acid has not been isolated there are indications that benzene is excreted partly in that form in dogs [Callow & Hele, 1926]. Bourne & Young [1934] have isolated α -naphthylmercapturic acid from the urine of rabbits and Stekol [1935] from the urine of dogs fed on naphthalene. It has been shown that anthracene is excreted as different stereoisomerides of dihydroxydihydroanthracene [Boyland & Levi, 1935] and it was stated at that time that there were indications of anthrylmercapturic acid in the urine of rats and rabbits fed on anthracene. We have now isolated this mercapturic acid and determined its properties and structure.

EXPERIMENTAL.

Rats and rabbits were fed on the diet described in the previous paper [Boyland & Levi, 1935] which contained anthracene. Sulphur compounds in the urine were determined according to Folin [1905] and some results are shown in Table I. The feeding of anthracene to rats appears to cause an increase in the

Table I. *Sulphur content of urines.*

	Number of animals yielding pooled urine	Free SO ₄	Ethereal SO ₄	Organic S	Total
Normal rats mg. BaSO ₄ per 100 ml. urine	10	436	76	232	744
As % total S		58	11	31	—
Rats fed with anthracene mg. BaSO ₄ per 100 ml. urine	20	381	114	301	796
As % total S		48	14	38	—

proportion of organic sulphur excreted. If the increased organic sulphur were due entirely to anthrylmercapturic acid there should be about 1 g. of that substance per litre urine. Actually only 100 mg. have been isolated in a pure condition from each litre of urine. We have isolated a maximum amount of 20 mg. per litre from rabbit urine.

Isolation of anthrylmercapturic acid.

The method used for the isolation of the anthrylmercapturic acid is in part similar to that used by Bourne & Young [1934] for α -naphthylmercapturic acid. The urine after the removal of the dihydroxydihydroanthracene by prolonged

ether extraction as previously described was acidified with HCl and extracted four or five times by shaking with chloroform. Unfortunately a continuous extractor could not be used because the chloroform tended to form stable emulsions with the acidified urine. The united chloroform extracts were filtered and extracted with aqueous NaHCO_3 . The alkaline extract was acidified with HCl and the precipitated crude mercapturic acid was redissolved in chloroform. The chloroform solution was dried, evaporated to small volume and treated with 3-4 vol. of hot benzene. On standing, brown crystals of mercapturic acid separated. This product was purified by means of the sodium salt as described by Bourne & Young for α -naphthylmercapturic acid, using 4*N* NaOH instead of 2*N* NaOH. The product was pure enough for most purposes and for analysis was twice recrystallized from a large volume of toluene or from a mixture of benzene and alcohol.

Properties.

The substances isolated from both rat and rabbit urines were similar in appearance and properties, crystallizing in pale yellow needles, soluble in sodium bicarbonate solution, insoluble in cold water, slightly soluble in hot water and soluble in hot concentrated HCl and in 30% toluenesulphonic acid solution, sparingly soluble in benzene, moderately soluble in chloroform and ether and easily soluble in alcohol, acetone and acetic acid. Other properties of the pure products are given in Table II.

Table II.

	M.P. ° C.	$[\alpha]_D^{20}$ in $\text{C}_2\text{H}_5\text{OH}$	$[\alpha]_D^{20}$ in dioxan	Equiv. wt.		
Anthrylmercapturic acid from rat urine	195	- 6° ($c=0.8\%$)	+ 45° ($c=0.8\%$)	358		
Anthrylmercapturic acid from rabbit urine	193	- 10° ($c=1.5\%$)	+ 46° ($c=1\%$)	351		
Theory	—	—	—	339		
<i>Analysis of acid from rat urine.</i>						
	C	H	N	S	Acetyl (Roth)	
Found	Weiler	67.7	5.3	3.9	9.0	—
	Schoeller	67.4	5.1	3.9	9.2	—
	Roth	—	—	—	—	10.5, 10.3
$\text{C}_{19}\text{H}_{15}\text{O}_3\text{NS}$ requires	67.8	5.0	4.1	9.4	—	12.7

The figures for the acetyl content are too low but the compound was extremely difficult to hydrolyse and the acetyl content difficult to determine. Attempts to isolate the deacetylated compound have so far been unsuccessful, as the mercapturic acid resisted boiling 10% HCl or 30% toluenesulphonic acid for 24 hours. On heating in a sealed tube with 10% HCl at 130° decomposition with formation of H_2S , anthracene and other products occurred. The acetyl determinations were made by hydrolysis with alcoholic KOH which would cause breakdown to thioanthrol; they do, however, indicate that the molecule contains an acetyl group.

Alkaline hydrolysis.

Hydrolysis with boiling 2*N* NaOH caused liberation of ammonia which continued for several hours. The solution was then allowed to oxidize in air and a little of a previously undescribed dianthryldisulphide was precipitated.

When 0.5 g. mercapturic acid was boiled with 4*N* NaOH for 10 min. a yellow precipitate was formed. The mercaptan and disulphide were extracted from the cooled hydrolysis mixture with ether after acidifying. The ether was evaporated and the residue oxidized in cold acetic acid with H_2O_2 . On addition of water the

dianthryldisulphide was precipitated, yield 0.25 g. After crystallization from glacial acetic acid it formed pale yellow prisms, m.p. 191–193°. It was soluble in benzene, ether and acetic acid but only slightly soluble in C_2H_5OH .

Analysis. (Found (Schoeller): C, 80.0; H, 4.48; S, 15.2%. $C_{28}H_{18}S_2$ requires C, 80.4; H, 4.3; S, 15.3%.)

The structure of the dianthryldisulphide.

The dianthryldisulphide could not be the 9:9-dianthryldisulphide, which is an orange-yellow compound melting at 223° [Cooke *et al.* 1925] which on oxidation yields anthraquinone.

The position of the sulphur atom in the molecule was determined by oxidation of the disulphide with boiling HNO_3 to anthraquinonesulphonic acid. After driving off the excess HNO_3 the anthraquinonesulphonic acid was treated with boiling HCl and $KClO_3$ when a chloroanthraquinone separated. The chloroanthraquinone was recrystallized from C_2H_5OH and melted at 158°.

(Found: (Schoeller) Cl, 14.4%. $C_{14}H_7O_2Cl$ requires Cl, 14.7%.)

The substance was identified (m.p. and mixed m.p.) with an authentic specimen of α -chloroanthraquinone, whence it follows that the disulphide must be $\alpha\alpha$ -dianthryldisulphide and the product isolated from urine is probably 1-anthrylmercapturic acid.

In addition to the metabolic products of anthracene which we have described, the urine of rats and rabbits given food containing anthracene contains a labile compound which readily yields crystalline anthracene on boiling with acid. This "anthracenogen" is probably a derivative of hydroxydihydroanthracene.

From the $CHCl_3$ extract of urine from rats and rabbits fed with anthracene a small amount of 9:10-anthraquinone has been isolated, which is possibly a contaminant of anthracene present in the animals' food and not a true metabolic product.

The probable metabolic products of anthracene are therefore (1) 1:2-dihydroxy-1:2-dihydroanthracene, (2) the glycuronic acid of (1), (3) a hydroxydihydroanthracene, (4) 1-anthrylmercapturic acid, (5) possibly 9:10-anthraquinone.

SUMMARY.

The same 1-anthrylmercapturic acid has been isolated from the urines of rats and rabbits fed on a diet containing anthracene. Rats produce more of the mercapturic acid than do rabbits under the same conditions.

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