METABOLISM OF THIOSTERS OF CARCINOGENIC HYDROCARBONS

Part I. Metabolism of Dibenzothiophene

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EVER since the realisation that a chemical entity, 1 : 2 : 5 : 6-dibenzanthracene, produces cancer,[1] the compound and its structural analogues are being exhaustively studied for their chemical and physical properties, carcinogenic activity and metabolism with a view to elucidating the mechanism of carcinogenesis by hydrocarbons. Accumulated evidence of such studies led to the suggestion[2, 3] that presence of an "activated phenanthrene bridge" (9 : 10-positions in phenanthrene which are also referred to as the 'K-region') in polycylic hydrocarbons was the preponderent cause, if not the only one of their carcinogenic activity. In order to examine the validity of the above suggestion, Robinson[4] put forward an idea of synthesising certain thiophene isosters (thiosters) of carcinogenic hydrocarbons in which the phenanthrene bridge or bridges have been replaced by an isosteric thiophene nucleus as illustrated by compound (I), the thioster of the highly carcinogenic hydrocarbon, 9 : 10-dimethyl-1 : 2-benzanthracene.

Since then, thiosters of carcinogenic hydrocarbons, compound (I) and several others such as (II), (III) and (IV) have been synthesised by one of us.[5] Compounds (I) and (II) have been found to be non-carcinogenic[6, 7] by subcutaneous injection in mice, but when painted on the skin the former compound has been found to possess a weak carcinogenic activity. Compound (III) is a strong carcinogen[6] even more potent than its hydrocarbon analogue. Compound (IV) is also carcinogenic.[6] The behaviour of the compounds (III) and (IV) indicated that they were apparent exceptions to the activated phenanthrene bridge hypothesis.

A careful analysis of the available data on the carcinogenicity of hydrocarbons and their thiosters suggested that as regards carcinogenicity there

may not exist direct interrelationship between hydrocarbons and their corresponding thiosters. It seemed likely that the sulphur atom in the condensed thiophene has its own characteristic reactivity towards a biological substrate. Although a di-univalent sulphur in a heterocyclic aromatic system is electronically equivalent to a conjugated double bond in aromatics, nevertheless the sulphur atom might impart a distinctive chemical reactivity to the molecule. Thus, the sulphur atom may undergo oxidation to a sulphoxide or sulphone

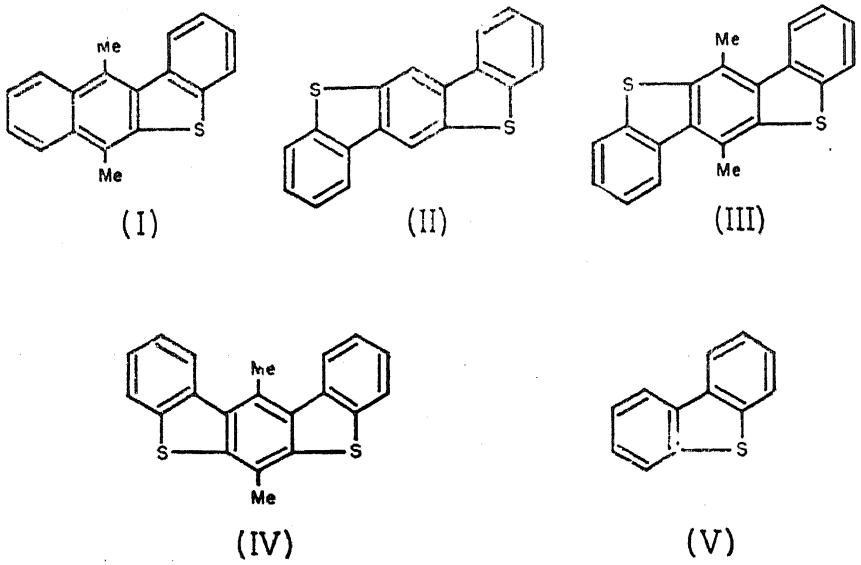

CHART I

grouping and/or it may induce facile substitution in rest of the molecule. In order to find out whether the sulphur atom in condensed thiophenes is involved in metabolism or not, metabolic studies of certain condensed thiophenes were deemed essential. Among the condensed thiophenes, thionaphthene alone has been studied for its metabolism[8] in rabbits and a thionaphthene-alpha-glucuronic acid of undetermined constitution has been reported to have been isolated from their urine. The present paper deals with the metabolism of dibenzothiophene (V) (referred to hereafter as DBT) in rats. DBT was selected for the preliminary study of the metabolism of thiosters since most of the thiosters that have been tested for carcinogenicity contain DBT as a structural unit.

DBT was incorporated in diet and fed to male rats of Wistar strain. The animals were kept in metabolic cages equipped with an arrangement for collection of urine. Pooled samples of urine were acidified to congo-red (pH 3–5), hydrolysed and extracted with ether. The ethereal extract was shaken with sodium hydroxide solution and the alkaline solution, on acidifi-

cation yielded a solid, which on crystallization gave a compound (VI), m.p. 258–59°. It formed acetyl (m.p. 210°), benzoyl (m.p. 206-07°) and O-methyl (m.p. 208–11°) derivatives.

Elementary analysis of the metabolite (VI), its derivatives and its infra-red spectrum (two, high intensity bands at 1150 cm.$^{-1}$ and 1295 cm.$^{-1}$ characteristic of the sulphone group and a strong band at 1200 cm.$^{-1}$ characteristic of the phenolic hydroxyl group, *see* Fig. 1), showed that it is a hydroxydibenzothiophene-5 : 5-dioxide. Since it is known[9] that sulphoxides exhibit a strong band in the range 1060-1040- cm.$^{-1}$ in solution with a corresponding reduction of 10-20 cm.$^{-1}$ in the solid state, while sulphones exhibit two bands: the first in the narrow range 1160–1140 cm.$^{-1}$ and the second in the range 1350–1300 cm.$^{-1}$ in solution, with only slight shifts in the solid state.

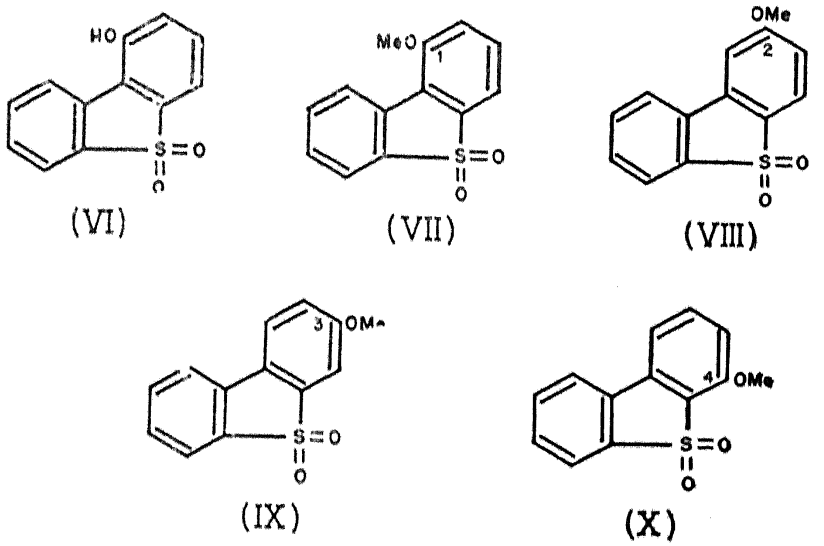

CHART II

The O-methoxy derivative (m.p. 208–11°) of the metabolite (VI) was different from 2-methoxydibenzothiophene-5: 5-dioxide (m.p. 244°, VIII) 3-methoxydibenzothiophene-5: 5-dioxide (m.p. 218–20°, IX), and 4-methoxydibenzothiophene-5: 5-dioxide (m.p. 191–92°, X). The latter compounds have been prepared by us unambiguously and will be reported in a separate communication. The ultra-violet spectra of the O-methyl derivative of the metabolite (VI) and of the compounds (VIII, IX and X) are shown in Fig. 2. The metabolite (VI) is, therefore, 1-hydroxydibenzothiophene-5 : 5-dioxide (VI).

The aqueous layer of the hydrolysed urine remaining after extraction with ether was made alkaline (pH 10·0) with sodium hydroxide solution. The alkaline urine showed the presence of dibenzothiophene-sulphonic

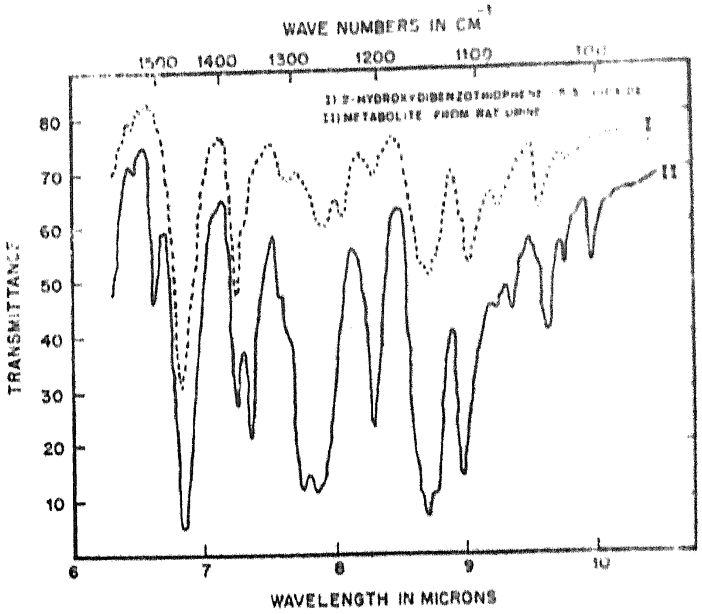

FIG. 1. Infra-Red Spectra of compounds (I) and (II) in Nujol-Mull.

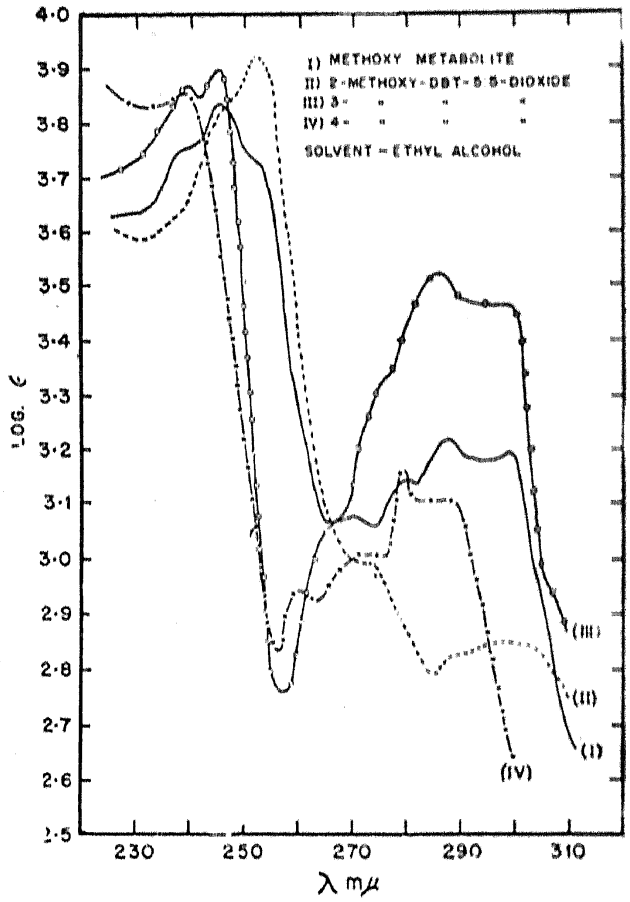

FIG. 2. Ultra-violet Spectra.

acid, as evidenced by the formation of crystalline salts with aniline hydrochloride and *p*-chloraniline hydrochloride. It seems likely that DBT is also metabolised as a water-soluble sulphonic acid derivative.

The normal metabolism of hydrocarbons in rats and mice leads to α-hydroxy derivatives[10] and an introduction of the hydroxyl group is assumed to occur through the mechanism of perhydroxylation. The metabolism of DBT in rats also resulted in hydroxylation of the molecule in the 1-position and in addition, oxidation of the di-univalent sulphur atom in (V) to the sulphone group has taken place leading to the metabolic elution of DBT (V) as 1-hydroxydibenzothiophene-5 : 5-dioxide and a water-soluble sulphonic acid derivative.

In view of Heidelberger's observations[11] that 1 : 2 : 5 : 6-dibenzanthracene is conjugated with proteins *in vivo* at the K-region and metabolized as 2-phenylphenanthrene-3 : 2′-dicarboxylic acid, and our present finding that, the sulphur atom in DBT which isosterically substitutes the K-region is also oxidised *in vivo*; it may be concluded that the K-region is not immobilized as regards carcinogenicity in thiosters since the sulphur atom, like the K-region is also implicated in an oxidative process. The present study, therefore, explains the apparently anomalous behaviour of the key thiosters such as (I), (III) and (IV) as regards their carcinogenic activity.

In conclusion, DBT is metabolized through hydroxylation in the 1-position and is oxidised at the chemically reactive sulphur atom in the molecule. These changes are such as might be expected to interfere with the oxidative mechanism of the cell.

Experimental

Preparation of Dibenzothiophene.—Dibenzothiophene required for the metabolic studies was prepared according to the method of Gilman and Jacoby.[12] The product was purified by distillation under reduced pressure and by crystallisation from ethyl alcohol, when it yielded colourless needles m.p. 99°. Yield 35%.

Metabolic Study of DBT.—A batch of 12–24 young male rats (1½–2, months) of Wistar Strain was used. The animals were kept in four to eight metabolic cages which were provided with an arrangement for collecting urine. The animals were maintained on a colony diet,* incorporating 0·25%

* Composition: Wheat 66, Gram-flour 15, Fish meal 7, Yeast 5, Groundnut 5, Shark-liver oil 1, and Sweet oil 1%. When DBT was incorporated in the diet, corresponding amount of wheat was reduced.

DBT and water *ad. libitum*. Weekly weights of the animals were recorded and these were found to be of the same order as those of animals on normal diet.

The daily output of urine of one rat, dosed with DBT, was about 1·50 to 3·0 c.c. The urine was pooled and preserved over copper sulphate crystals in a refrigerator. The collection of 3–4 months urine was worked up at a time.

Isolation of 1-*hydroxy-dibenzothiophene*-5: 5-*dioxide* (*VI*) *from rat urine*.—Urine (500 c.c.) was filtered, acidified with concentrated hydrochloric acid to congo-red and boiled for 30 minutes. The hydrolysed urine was cooled and extracted with peroxide-free ether (5×100 c.c.). The concentrated ethereal extract (100 c.c.) was exhaustively shaken with 1 N. sodium hydroxide solution (5×20 c.c.). The alkali-soluble fraction (*a*) separated, and it was worked up as described in the succeeding paragraph. The urine layer was further worked up.

The alkali-soluble fraction (*a*) was acidified with concentrated hydrochloric acid (congo-red) and the solution extracted with peroxide-free ether (5×100 c.c.). The ethereal extract yielded 1-hydroxydibenzothiophene-5: 5-dioxide (VI), which, on several crystallisations from water, gave clusters of colourless, shining needles, m.p. 258–59° (Found: C, 62·0; H, 3·7. $C_{12}H_8O_3S$ requires C, 62·0; H, 3·5%). Yield of the crude product was about 50 mg. from 2 litres of urine.

Properties of the compound (*VI*).—It was soluble in ethyl alcohol. It was insoluble in 5% sodium bicarbonate solution. An aqueous solution of (VI) did not give any colouration with ferric chloride solution.

Acetate of compound (*VI*).—The compound (0·02 g.) was refluxed with acetic anhydride (2·0 c.c.) and pyridine (2–3 drops) for one hour. The mixture was cooled, diluted with water and kept in the refrigerator. The acetate was filtered and washed with (i) 20% hydrochloric acid, (ii) 5% sodium hydroxide solution and finally with distilled water. The acetate crystallised from ethyl alcohol in plates (0·012 g. Yield 60%), m.p. 210°. (Found: C, 61·2; H, 3·88, $C_{12}H_7O_3S + COCH_3$ required C, 61·32; H, 3·68%).

Benzoate of compound (*VI*).—Compound (VI) (0·014 g.), benzoyl chloride (0·1 c.c.) and pyridine (2·0 c.c.) were refluxed on a water-bath for two hours. The reaction mixture was cooled and hydrochloric acid (10 c.c. 5 N) added to it. The solution was then extracted with peroxide-free ether and the ethereal extract was successively washed with 2% NaOH, 2% HCl and 2% $NaHCO_3$ solutions. The product obtained from ether extract crystallized

from dilute ethyl alcohol in needles (0·007 g. Yield 50%) m.p. 206-07°. (Found: C, 67·9; H, 3·4. $C_{19}H_{12}O_4S$ requires C, 67·85; H, 3·6%).

Methyl ether of compound (VI).—Compound (VI) (0·02 g.) was dissolved in anhydrous acetone (5 c.c.). To the solution, freshly ignited potassium carbonate (0·14 g.) and dimethyl sulphate (0·05 c.c.) were added and the mixture refluxed for 8 hours. After cooling, the reaction mixture was filtered and the residue washed with acetone. The product from acetone extract was then extracted with peroxide-free ether. The ether extract was washed with 5% NaOH (2×20 c.c.). Removal of ether gave the methoxy derivative, which crystallized from aqueous ethanol, in colourless long rectangular plates, (0·016 g. Yield, 66%) m.p. 208–11°. (Found C, 63·5; H, 4·7. $C_{13}H_{10}O_3S$ requires C, 63·41; H, 4·1%).

Formation of arylamine salts with the urinary sulphonic acid.—The urine layer remaining after the separation of compound (VI) was made alkaline to pH 10·0 with 10% sodium hydroxide solution. The alkaline urine was evaporated to dryness on a steam-bath. The product was dissolved in water to give a 50% solution. A portion (2 c.c.) of the solution was boiled separately with two different arylamine hydrochlorides. The resulting solutions, on cooling, gave colourless crystalline salts: (*a*) the salt with anilinehydrochloride melting at 158–61° and (*b*) the salt with *p*-chloraniline hydrochloride which melted at 150–51° (decomp.).

Summary

Dibenzothiophene (DBT) when given to rats is eliminated through urine as 1-hydroxydibenzothiophene-5: 5-dioxide and an unidentified sulphonic acid. DBT is thus metabolised in a manner similar to 1: 2: 5: 6-dibenzanthracene. Since, the sulphur atom in the thiosters, like the 'K-region' in 1: 2: 5: 6-dibenzanthracene, is implicated in an oxidative process, the 'K-region' is not immobilized as regards carcinogenicity in the thiosters. The present work explains the carcinogenic activity of the key thiosters observed by earlier workers.

Acknowledgements

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