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SHORT COMMUNICATIONS

Observations on the metabolism of *cis*- and *trans*-indane-1, 2-diols

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THE METABOLISM of aromatic hydrocarbons in the animal body is of interest since many of these compounds are carcinogenic and others are in widespread use in industry as solvents.

Aromatic hydrocarbons are metabolised to dihydrodiols in animals. Most of the dihydrodiols identified as metabolites of aromatic hydrocarbons have the *trans*- configuration. Indene[1] and acenaphthylene[2] are metabolised to a mixture of *cis*- and *trans*- dihydrodiols. After the separate administration of carefully purified *cis*- or *trans*- acenaphthene-1, 2-diol or *cis*- or *trans*- indane-1, 2-diol to animals a mixture of the *cis*- and *trans*- forms of the dihydrodiol administered was found in the urine.[3] With indane-1, 2-diols the interconversion of the dihydrodiol forms may occur either through the intermediate ketol,[4] namely 2-hydroxyindan-1-one or by the hydrolysis of a hydroxyindanyl sulphuric acid.[4] Synthetic potassium *cis*- and *trans*- hydroxyindanyl sulphates are hydrolysed by boiling water to a mixture of *cis*- and *trans*- indane-1, 2-diols.[4] Two sulphuric acid conjugates are found in the urine of animals that have been administered separately with either *cis*- or *trans*- indane-1,2-diol. One metabolite is the conjugated ketol, 1-oxoindan-2-yl sulphuric acid.[4] The second metabolite was not identified although preliminary experiments have suggested that it may be a hydroxyindanyl sulphuric acid.[4]

This investigation was designed to identify the unknown sulphuric acid conjugate of the indane-1,2-diols and to investigate its role as a possible intermediate in the interconversion of *cis*- and *trans*-indane-1,2-diols.

cis- Dihydrodiols may be of some metabolic importance since *cis*- acenaphthene-1,2-diol is metabolised to 1,8-naphthalic acid.[5]

Experimental and results

The identification of the sulphuric acid conjugate found in the urine of rats that have been administered with either cis *or* trans- *indane*-1, 2-*diol* two rats were each injected subcutaneously with a suspension of 75 mg of *cis*- indane-1,2- diol in 1 ml of arachis oil in the left lumber region. In a second pair each was injected with a 1 ml oil suspension containing 75 mg of *trans*-indane-1,2-diol. In a third pair each was injected with 1 ml of arachis oil. All rats were injected in the right lumber region with an aqueous solution of carrier free $Na_2{}^{35}SO_4$ immediately after the first injection. The 24-hr urines from each pair were collected and diluted to 15 ml. Two ml of each urine was applied to Whatman 3 MM paper strips and the chromatograms developed in butan-1-ol saturated with 2N-NH_3. The radioactive metabolites were detected by preparing radioautographs on Kodak X-ray paper. No metabolites were detected on the control chromatograms but two metabolites, R_f 0·40 and R_f 0·42, were detected on each of the two experimental chromatograms. Duplicate chromatograms were sprayed with the Burma reagent[4] and a red spot appeared on each of the two experimental chromatograms in the R_f 0·40–0·42 region. This suggested that one of the metabolites present was 1-oxoindan-2-yl sulphuric acid, identified in a previous investigation.[4]

Paper discs (R_f 0·40–0·42) were removed from the unsprayed experimental and control chromatograms. The discs were extracted exhaustively with methanol–water–ethyl acetate–acetic acid (150:10:40:1 by vol.).[6] The three extracts were evaporated to dryness *in vacuo*. The residues were each dissolved in 2 ml of water and incubated for 12 hr at 37° with a rat liver aryl sulphatase preparation.[7] The hydrolysates were extracted exhaustively with ether and the extracts examined by paper chromatography for *cis*- and *trans*- indane-1,2-diols. *trans*-Indane-1,2-diol, but not *cis*- indane-1,2-diol was detected in the two experimental ether extracts. No metabolites were detected in the control extract.

The experiment was repeated using a bacteria aryl sulphatase preparation [Boehringer Corp. (London) Ltd.]. The results of this experiment were similar to the first one. The second sulphuric acid metabolite originally present in the experimental urines would appear to be *trans*- hydroxyindanyl sulphuric acid. As the other metabolite was 1-oxoindan-2-yl sulphuric acid[4] the new metabolite is probably *trans*- 1-hydroxyindan-2-yl sulphuric acid.

An attempt to form cis- *and* trans-*hydroxyindanyl sulphuric acids by using rat liver preparations.* A particle free supernatant was prepared from rat liver homogenates,[8] and portions (0·5 ml) were added to 1 ml of a buffered ATP solution (0·3 M, KH_2PO_4; 30 mM K_2SO_4; 5 mM $MgCl_2$; 40 mM ATP, 3:3:3:1, by vol.) (pH 6·8) in stoppered test tubes. With the exception of the controls the tubes also contained 300 μg of either *cis*- indane-1,2-diol or *trans*- indane-1,2-diol. After incubation at 37° for 2 hr in a waterbath the reaction was stopped by the addition of 5 ml of methanol to each tube. The amount of sulphate ester present was determined by the methylene blue method, described by Roy.[9] Synthetic potassium *cis*- and *trans*- hydroxyindanyl sulphates were used as reference substances. The results (Table 1) show that *trans*- indane-1,2-diol stimulated ester sulphate formation, whereas *cis*-

TABLE 1. FORMATION OF HYDROXYINDANYL SULPHATES BY RAT LIVER PARTICLE FREE SUPERNATANTS

Substrate	Sex of liver preparation	Conjugates formed (expressed as μg of hydroxyindanyl sulphate ± S.E.M.)
cis-indane-1,2-diol	Male	11 ± 1 (4)*
trans-indane-1,2-diol	Male	19 ± 2 (4)
None (control)	Male	11 ± 1 (4)
cis-indane-1,2-diol	Female	13 ± 0 (4)
trans-indane-1,2-diol	Female	24 ± 1 (4)
None (control)	Female	13 ± 0 (4)

* The number of observations are given in parentheses.

indane-1,2-diol did not. A further experiment was carried out to determine the identity of the ester sulphate formed. The experiment previously described was repeated except that carrier free $Na_2{}^{35}SO_4$ was added to the incubation medium. Portions of the final aqueous methanol extracts were examined for radioactive metabolites by paper chromatography as described above. The metabolites were detected by "scanning" the chromatograms on a Packard Model 7200 Radiochromatogram Scanner. One metabolite, R_f 0·42, was detected on the chromatogram of the extract of the medium that had included *trans*- indane-1,2-diol. No metabolites were detected on the chromatograms of the control and *cis*- indane-1,2-diol extracts.

Paper discs of the R_f 0·42 region were removed and the metabolites eluted and hydrolysed by arylsulphatase preparations as described above. The hydrolysates were examined for dihydrodiols by paper chromatography and gas–liquid chromatography.[10] *trans*- Indane-1,2-diol was detected on the chromatogram of the hydrolyates from chromatogram of the buffered enzyme solution to which *trans*- indane-1,2-diol was added. The results were confirmed by gas–liquid chromatography. The dihydrodiol present in the hydrolysate was found to have a retention time of 35·9 min at 150° identical to that of synthetic *trans*- indane-1,2-diol. *cis*- Indane-1,2-diol has a retention time of 21·6 min under identical conditions. No dihydrodiols were detected in the hydrolysates from the chromatograms of the control and *cis*- indane-1,2-diol samples.

Clearly the arylsulphatransferase present in the rat liver supernatant was active in conjugating *trans*- indane-1,2-diol with sulphuric acid but was inactive towards *cis*- indane-1,2-diol. In addition the enzymic hydrolysis of *trans*- hydroxyindanyl sulphate did not result in an inversion of configuration.

Discussion

Since *cis*- hydroxyindanyl sulphuric acid was not detected as a metabolite of either *cis*- or *trans*- indane-1,2-diol the conversion of *cis*- indane-1,2-diol to *trans*- indane-1,2-diol cannot have occurred

through the hydrolysis of an hydroxyindanyl sulphuric acid intermediate. *trans*- 1-Hydroxyindanyl-2-sulphuric acid was identified as a metabolite of *cis*- and *trans*- indane-1,2-diol. The mechanism for the chemical hydrolysis of *trans*- 1-hydroxyindan-2-yl sulphuric acid appears to differ from the mechanism for the enzymic hydrolysis. Boiling aqueous solvents hydrolyses the individual hydroxyindanyl sulphates, to a mixture of the *cis*- and *trans*- dihydrodiols,[4] but the enzymic hydrolysis of *trans*-1-hydroxyindan-2-yl sulphuric acid produced only *trans*- indane-1,2-diol. It is possible that the chemical hydrolysis occurred through an epoxide intermediate since some 1,2-dihydroxy groups in sugars are converted to epoxides through treatment with aqueous reagents.[11] Indene epoxide is hydrolysed *in vivo* and *in vitro* to a mixture of *cis*- and *trans*- diols.[4] The enzymic hydrolysis of *trans*-1-hydroxyindan-2-yl sulphate was carried out over 12 hr at 37° and yielded only *trans*- indane-1,2-diol. As this reaction was at physiological temperature it is unlikely that *trans*- 1-hydroxyindan-2-yl sulphate was the intermediate in the conversion of *trans*- indane-1,2-diol to *cis*- indane-1,2-diol. The direct chemical interconversion of one distereoisomer to the other is unlikely since the chemical interconversion of the dihydrodiol forms requires elevated temperature and low pH.[1] The most likely route for the interconversion of *cis*- and *trans*- indane-1,2-diol is through 2-hydroxyindan-1-one as previously suggested.[4]

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Inhibition of rat liver homogenate arginase activity *in vitro* by the hepatotoxic amino acid indospicine

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IT HAS previously been reported that acute administration of the hepatotoxic amino acid indospicine (L-2-amino-6-amidino hexanoic acid) to rats depressed the incorporation of ^{14}C-leucine into liver and serum protein *in vivo*.[1] Subsequently it was shown, using cell-free reaction systems, that a competitive antagonism existed between indospicine and arginine such as to limit the incorporation of the latter into protein.[2] Further, because esterification of arginine to transfer RNA (*t*RNA) was inhibited in