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SOME METABOLITES OF BROMOCYCLOPENTANE, BROMOCYCLOHEXANE AND BROMOCYCLOHEPTANE IN THE RABBIT

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Abstract—Rabbits have been dosed with bromocyclopentane, bromocyclohexane and bromocycloheptane and the excretion of bromine (estimated as bromide), glucosiduronic acid and ethereal sulphate measured. With all three compounds there is a relatively rapid excretion of bromide accounted for by the formation of a bromine-containing glucosiduronic acid. The glucosiduronic acids isolated as their tri-acetyl methyl esters are conjugates of bromocycloalkanols. Evidence is presented that hydroxylation of the bromocycloalkane occurs at position 2. The extent of hydroxylation increases with increasing ring size.

POLYHALOGENATED derivatives of alicyclic compounds are used widely as insecticides and herbicides but few detailed studies of their metabolic fate in mammals have been reported. Grover and Sims[1] found that hexachlorocyclohexane (Gammexane) was dehydrochlorinated and hydroxylated by the rat with the formation of trichlorophenols. Few accounts of the metabolism of partly halogenated derivatives of alicyclic compounds have been given although the monohalogenoparaffins have been extensively studied. This paper describes an investigation of those hydroxylated derivatives which are formed from bromocyclopentane, bromocyclohexane and bromocycloheptane and excreted conjugated with glucuronic and sulphuric acids. A preliminary account[2] of the excretion of a bromine-containing glucosiduronic acid by rabbits dosed with bromocyclohexane has been given. The compounds studied also give rise to sulphur-containing metabolites which will be reported separately.

MATERIALS AND METHODS

Bromocyclohexane was purchased from B.D.H.; bromocyclopentane, bromocycloheptane, cyclopentene, cycloheptene, the cyclohexane diols and β-glucuronidase from Koch-Light Laboratories Ltd.

Cyclopentene epoxide was prepared either by the method of Durbetaki[3] or that of Goodman *et al.*[4] The product had b.p. 99–101°, n_D^{20} 1·4380 in close agreement with the properties recorded in the literature[3] (b.p. 99–102°, n_D^{16} 1·4370). Cycloheptene epoxide was prepared by the same methods and had b.p. 80–81° at 50 mm, n_D^{20} 1·4617 (lit.[5] gives b.p. 77–78° at 50 mm, n_D^{25} 1·4620).

cis-Cyclopentane-1,2-diol prepared by the method of Owen and Smith[6] had b.p. 105–106° at 16 mm (lit.[6] b.p. 104° at 15 mm).

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trans-Cyclopentane-1,2-diol prepared according to the method of Owen and Smith[6] had b.p. 116–117° at 16 mm and solidified to give material m.p. 51–52° (lit.[6] b.p. 118° at 16 mm m.p. 52°).

cis- and *trans*-Cycloheptane-1,2-diols were prepared as for the cyclopentane analogues except that chloroform was used for the final extraction.

cis-Cycloheptane-1,2-diol had m.p. 46–48° after recrystallisation from ethyl acetate (lit.[7] m.p. 49–51°). *trans*-Cycloheptane-1,2-diol had m.p. 61–62° (lit.[8] m.p. 62–63°).

Animals. Doe rabbits (2·5 kg) were maintained as described by Bray *et al.*[9] They were housed in metabolism cages constructed to separate faeces from urine. For experiments where the rate of excretion of bromine was observed, the cages were placed over a fraction collector as described by Bray *et al.*;[10] urine being collected at given time intervals and analysed. All doses were administered by stomach tube as suspensions in water.

Determination of metabolites in urine

Sulphate. This was determined by the method of Folin.[11]

Glucosiduronic acid. The modified naphtharesorcinol method described by Bray *et al.*[12] was used except that the colours were read on an EEL colorimeter with filter 601.

Bromine. This was determined as bromide by the method of Hunter.[13] In general, 24-hr samples of urine were collected, diluted to 400 ml and aliquots (1 ml) analysed, this procedure continuing until bromide was no longer excreted. For experiments where the rates of excretion of "ether-soluble glucuronide" and "ether-soluble bromine" were determined, each sample was diluted to 100 ml and a portion (20 ml) was acidified with 2N H_2SO_4 (4 ml) and extracted continuously with ether for 16 hr. Water was then added to each extract, the ether removed by evaporation and the aqueous solution diluted to 10 ml. Duplicate samples were analysed for bromide and glucuronide. Control experiments showed that not more than 10% of bromide added to normal urine as sodium bromide appeared in the extract so that the value found for "ether-soluble bromine" may include not more than 10% of the inorganic bromide excreted.

Gas chromatography of synthetic alicyclic diols

A column of 10% XF-1150 (Cyanosilicone fluid: Wilkens Instruments and Research

TABLE 1. RETENTION TIMES* OF CYCLOALKANE DIOLS AT 150°

Compound	Retention time (min)
cis-1,2-cyclopentane-diol	4·0
trans 1,2- „ „	4·8
cis 1,2-cyclohexane-diol	5·4
trans 1,2- „ „	6·5
cis 1,3-cyclohexane-diol	11·3
trans 1,3- „ „	12·1
1,4- „ „	12·2
cis-1,2-cycloheptane-diol	7·1
trans 1,2 „ „	8·9

* See Methods section for details of the column used.

Inc.) on acid-washed silanized Chromosorb W (80–100 mesh), similar to that of Casselman and Bannard,[14] was used in a PYE series '104' gas chromatograph.

Samples were dissolved in methanol and injected on to the column maintained at 150°, the argon gas flow rate being 45 ml/min. The retention times are given in Table 1.

The retention times for the cyclohexane diols are lower than those reported by Casselman and Bannard[14] who used a 30% XF-1150 column. The ratios of the retention times for the *cis* and *trans* 1,2 and 1,3-diols are in close agreement for the two sets of data.

RESULTS

Isolation and characterisation of glucosiduronic acids

(a) *From urine of rabbits dosed with bromocyclopentane.* This metabolite was isolated as methyl bromocyclopentyl-tri-*O*-acetyl-D-glucosiduronate following essentially the method of Kamil *et al.*[15] The urine of six rabbits which had each been dosed with bromocyclopentane (685 mg) was adjusted to pH 4·2 with glacial acetic acid and a saturated aqueous solution of normal lead acetate added until precipitation was complete. The precipitate was filtered at the pump and discarded. The filtrate was adjusted to pH 8 with ammonia (sp. g. 0·880) and saturated basic lead acetate added to excess. The precipitate was collected and suspended in water, then saturated with H_2S and the precipitated lead sulphide removed by filtration. The resulting filtrate was evaporated to small volume under reduced pressure maintaining the water bath temperature below 40°. The colourless gummy residue was dissolved in water (5 ml) and inorganic material precipitated by addition of absolute ethanol. The precipitate was discarded and the filtrate evaporated to give a gum; this was dissolved in methanol (4 ml) and diazomethane in ether added until nitrogen ceased to be evolved. Excess ether and diazomethane were evaporated off and dry pyridine/glacial acetic acid (1:1 v/v) added. The solution was left to stand overnight and then poured over ice giving a fine precipitate. The precipitate was extracted with chloroform and the extract washed three times with ice-cold N-HCl to remove traces of pyridine and three times with water (20 ml). Small needle-like crystals gradually separated from the concentrated extract and were recrystallized from acetone–light petroleum (b.p. 60–80°) to give crystals (0·12 g) m.p. 108–111°. Found C, 45·30; H, 5·43; Br, 16·42%. $C_{18}H_{25}O_{10}Br$ requires C, 44·92; H, 5·20; Br, 16·65%.

(b) *From urine of rabbits dosed with bromocyclohexane.* By a similar procedure the glucuronide present in the urine of three rabbits which had each been dosed with bromocyclohexane (665 mg) was isolated as the triacetyl methyl ester (73 mg) m.p. 121–122° (after recrystallization from aqueous ethanol). Found: C, 45·93; H, 5·19; Br, 16·3. $C_{19}H_{27}O_{10}Br$ requires C, 46·05; H, 5·46; Br, 16·18%.

The mass spectrum of this product showed pairs of peaks corresponding to ionic species containing the isotopes of bromine. Thus peaks occurred at M/e 479,481 and at 435,437 corresponding to the loss of CH_3 and $COOCH_3$ respectively from the molecular ions. Peaks at M/e 161,163 corresponded to $C_6H_{10}Br$ and a large peak at M/e 317 corresponded to the loss of $C_6H_{10}BrO$ from the molecular ions. Peaks at M/e 121,123 may correspond to C_2H_2BrO and at 56 to C_4H_8, species which could arise by fission of the aglycone.

(c) *From urine of rabbits dosed with bromocycloheptane.* The analogous glucuronide derivative isolated from the urine of rabbits dosed with bromocycloheptane had m.p.

114–116°. Found: C, 47·48; H, 5·86; Br, 15·25%. $C_{20}H_{29}O_{10}Br$ requires C, 47·15; H, 5·70; Br, 15·75%.

Degradation of glucosiduronic acids

A sample (20 mg) of the crystalline glucosiduronic acid derivative was warmed with 0·5 N NaOH (0·5 ml) for 10 min to remove the *O*-acetyl and methyl ester groups. The solution was neutralized with 2N HCl and mixed with 0·2 M acetate buffer pH 4 (10 ml), β-glucuronidase (4500 Fishman Units) was added and the solution was incubated at 37° for 24 hr. The resulting solution was neutralized with 2N NaOH, silver oxide (200 mg) added and heated on a water bath overnight. The solution was evaporated to dryness and the residue extracted with absolute ethanol (0·5 ml). Samples of this ethanolic solution were examined by gas chromatography on the XF-1150 column previously described and the retention times observed compared with those of the appropriate synthetic diols. The diol formed had the same retention time as that of the expected *trans*-1,2-diol in all three cases.

Samples of the urines from rabbits dosed with the monobromocycloalkanes were treated with β-glucuronidase followed by silver oxide as described for the glucosiduronic acids obtained by hydrolysis of their crystalline derivatives. The cycloalkane diols obtained were examined by G.L.C. Only the *trans*-1,2-isomer was detected in the preparations from the urine of rabbits dosed with bromocyclohexane or bromocycloheptane while the preparation from the urine of rabbits dosed with bromocyclopentane contained *trans*-1,2-cyclopentane-diol and a small amount (about 5 per cent of the total diol) of *cis*-1,2-cyclopentane diol.

QUANTITATIVE RESULTS

The percentages of the doses of the bromocycloalkanes excreted as glucurosiduronic acids and ethereal sulphates are given in Table 2 with the percentages of the total

TABLE 2. AMOUNT OF METABOLITES EXCRETED IN URINE BY RABBITS

Compound administered	Dose (m-mole/kg)	Total* bromide	Glucosiduronic acid	Ethereal sulphate
Bromocyclopentane	1·8	62 $(54–73)_6$	27 $(16–38)_6$	13 $(12–16)_4$
Bromocyclohexane	1·6	64 $(58–76)_6$	61 $(35–84)_3$	9 $(8–10)_2$
Bromocycloheptane	1·5	64 $(56–71)_3$	78 $(69–85)_6$	19 $(17–22)_3$

Results are means expressed as percentage of dose, with ranges in parentheses; the numbers of determinations are indicated by inferior figures.

* This includes the bromide excreted plus organically bound bromine which is also estimated as bromide.

bromide excreted. In Fig. 1 the excretion of total bromine by rabbits dosed with the bromocycloalkanes is contrasted with the excretion of total bromine by animals dosed with 1-bromopentane, 1-bromohexane and sodium bromide. In Fig. 2 detailed excretion curves for the 24 hr after dosing show the total bromine excretion by a rabbit dosed with bromocyclohexane and the partition of this material into organically bound (ether-soluble) bromine and bromide. In Fig. 3 the close similarity of the

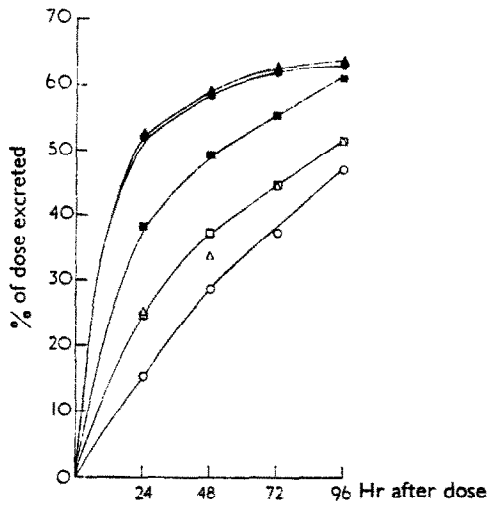

FIG. 1. Excretion of total bromine by dosed rabbits. All doses 1·6 ± 0·2 m-mole/kg. ○ Sodium bromide; △ 1-bromohexane; □ 1-bromopentane; ● bromocyclohexane; ▲ bromocycloheptane; ■ bromocyclopentane.

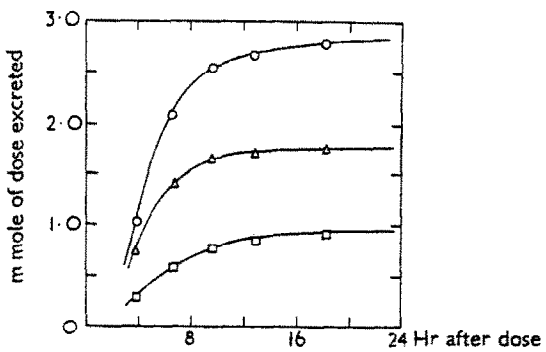

FIG. 2. Excretion of bromine by rabbit dosed with bromocyclohexane. ○ Total bromine; △ 'ether-soluble' bromine; □ bromide.

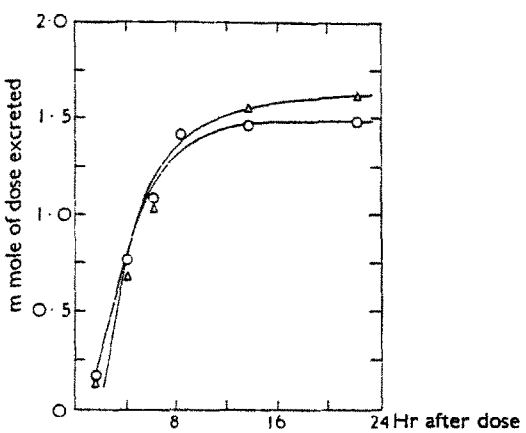

FIG. 3. Excretion of ether-soluble bromine and ether-soluble glucuronide by rabbit dosed with bromocyclohexane. ○ Ether-soluble glucuronide; △ ether-soluble bromine.

excretion curves for ether-soluble bromine and ether-soluble glucosiduronic acid expressed as milli-moles of bromocyclohexane administered is shown. It was found that equivalent amounts of bromine and ether-soluble glucosiduronic acids are present in the ethereal extracts of the acidified urine of rabbits dosed with bromocyclopentane or bromocycloheptane.

DISCUSSION

The results show that the bromine present in the bromocycloalkanes examined is excreted more rapidly than that in the corresponding 1-bromoalkanes. In rabbits dosed with these compounds the pattern of bromine excretion resembles that found after dosing with sodium bromide.[16] In both cases the excretion is slow and still incomplete after 4 days, animals dosed with 1-bromoalkanes giving a slightly greater amount of bromide than those with an equivalent amount of sodium bromide. This indicates that the 1-bromoalkanes probably undergo complete debromination. The demonstration of the equivalence of the bromine and glucuronic acid in the ethereal extracts of the urine of dosed rabbits suggests that bromine-containing glucosiduronic acids are metabolites of the bromocycloalkanes. This was confirmed by the isolation of these compounds as their crystalline triacetyl methyl esters. The formation of these metabolites accounts for the relatively rapid rate of bromine excretion observed in rabbits dosed with bromocyclopentane, bromocyclohexane or bromocycloheptane, the glucosiduronic acids being excreted more quickly than bromide ions (see Fig. 1). This observation suggests that hydroxylation of the alicyclic ring occurs while the bromine atom is still present.

The crystalline tri-acetyl methyl ester derivatives of the glucosiduronic acids were converted to the free acids and the aglycones liberated by hydrolysis with β-glucuronidase. On treatment with silver oxide the aglycones were converted to diols shown by gas chromatography to be identical with the corresponding *trans*-1,2-diols. When samples of the urines were treated with β-glucuronidase and the aglycones similarly converted to diols the *trans*-1,2-diols were again found to be present in all three cases and in the product from the urine of rabbits dosed with bromocyclopentane a small amount of *cis*-1,2-pentane-diol was also detected. The results suggest that the three monobromocycloalkanes examined undergo hydroxylation at carbon atom 2 and that the trans isomer is predominantly formed. This may be contrasted with the hydroxylation of methyl-cyclohexane[17] where hydroxylation occurs in positions 2,3 and 4, that in position 4 predominating. The results suggest that the extent of the hydroxylation increases with increasing ring size.

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