Biochemical Pharmacology, Vol. 30, No. 17, pp. 2499-2502, 1981.
Printed in Great Britain.

0006-2952/81/172499-04$02.00/0.

PRELIMINARY COMMUNICATIONS

THE METABOLIC FORMATION OF N-ACETYL-S-2-HYDROXYETHYL-L-CYSTEINE FROM TETRADEUTERO-1,2-DIBROMOETHANE. RELATIVE IMPORTANCE OF OXIDATION AND GLUTATHIONE CONJUGATION *IN VIVO*

P.J. van Bladeren, D.D. Breimer, J.A.T.C.M. van Huijgevoort,
N.P.E. Vermeulen and A. van der Gen.
Departments of Pharmacology and Organic Chemistry, University of Leiden,
P.O. Box 9502, 2300 RA Leiden, The Netherlands

The metabolism of 1,2-dibromoethane has received considerable attention in recent years. Its principal urinary metabolites are derived from glutathione adducts. The most important one is the mercapturic acid derivative, N-acetyl-S-2-hydroxyethyl-L-cysteine[1,2], but a number of related metabolites have been described as well[2,3,4]. Two possible routes have been described for the formation of the mercapturic acid derivative, both involving a reactive, and therefore potentially harmful intermediate. Microsomal oxidation, followed by loss of hydrogen bromide, leads to bromoacetaldehyde[5]. Direct conjugation of 1,2-dibromoethane with glutathione, catalyzed by the glutathione transferases, gives rise to S-2-bromoethyl-glutathione[1]. Conjugation of bromoacetaldehyde with glutathione, followed by reduction leads to a glutathione conjugate identical to the one formed by reaction of water with S-2-bromoethyl-glutathione. (Figure 1). Ultimately identical mercapturic acids are excreted in urine.

With the aid of tetradeutero-1,2-dibromoethane it should in principle be possible to distinguish between the two biotransformation pathways described above by determining the number of deuterium atoms preserved in the mercapturic acid derivative, formed from it *in vivo*. Direct conjugation of 1,2-dibromoethane with glutathione would result in retention of all four deuterium atoms, since no C-D bonds are broken in this process. During the formation of bromoacetaldehyde however, at least one deuterium atom is lost, while

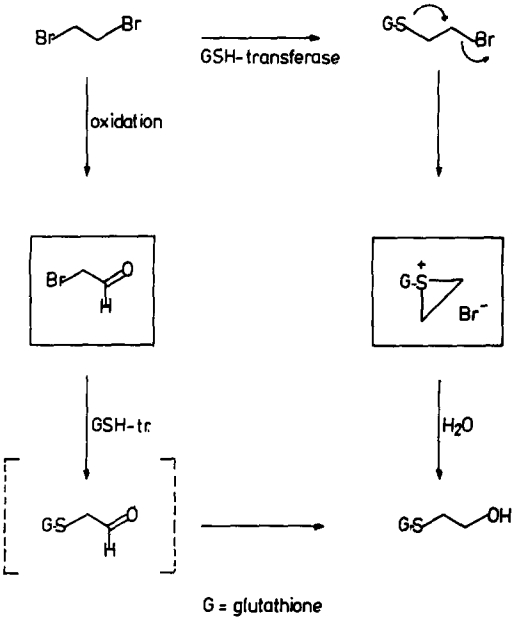


Fig. 1
Metabolic pathways of 1,2-dibromoethane.

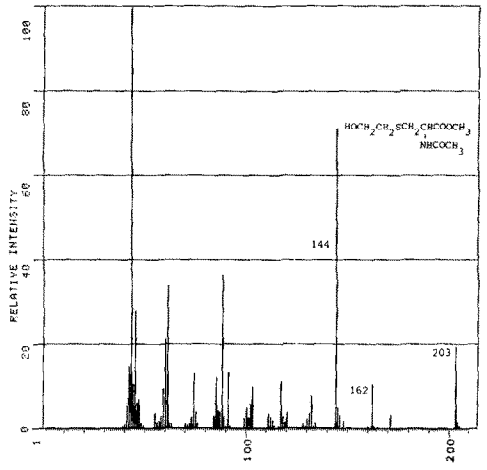
enolization of the aldehyde may result in the loss of two additional deuterium atoms. Tetradeutero-1,2-dibromoethane has recently been used successfully by Livesey and Anders[6] to show that ethene is formed from it by glutathione conjugation. In the present paper experiments are described in which the extent of occurrence of the two possible pathways could be estimated from the deuterium content of the mercapturic acid derivatives.

RESULTS AND DISCUSSION

The mass spectrum of the main metabolite *in vivo* of 1,2-dibromoethane, N-acetyl-S-2-hydroxyethyl-L-cysteine methyl ester[1] is depicted in Fig. 2 (A). The fragment ion with the highest mass, at m/z 203 is formed by loss of a water molecule from the molecular ion (m/z 221). The second important fragment ion, at m/z 162, arises through loss of 59 from the molecular ion. High resolution mass measurements of this ion indicate that it is formed for 16% by loss of a $C_2H_3O_2$-radical and for 84% by loss of a C_2H_5NO fragment. The fragment ion at m/z 162 can in turn lose a water molecule, while the ion at m/z 203 can lose 59, both resulting in formation of a fragment ion at m/z 144. The middle spectrum of Fig. 2 was obtained from synthetic N-acetyl-S-1,1,2,2,--tetradeutero-2-hydroxyethyl-L-cysteine methyl ester. The same fragmentations occur:loss of HDO from the molecular ion at m/z 225 gives rise to the fragment ion with the highest mass, at m/z 206; loss of 59 results in formation of the ion at m/z 166; from both these ions m/z 147 is formed.

Since no molecular ion could be detected at 70 eV, or at lower electron energies, a problem not unknown with this type of molecule[7], the deuterium content of the mercapturic acid formed from 1,2-dibromoethane-D4 by rats had to be estimated from a fragment ion. A reliable fragment in this case is the one originating from loss of 59 from the molecular ion. The fact that from the tetradeutero-mercapturic acid also 59 is lost indicates that this process does not involve the 2-hydroxyethyl part of the molecule. Loss of a water molecule takes place from this portion of the mercapturic acid, so that differences between loss of H_2O and HDO could occur. When looking at the fragment ions resulting from loss of 59 from the molecular ion, it is assumed that no isotope effect influences the ratio of loss of water and loss of 59. Proof that this assumption is justified can only be obtained by a technique which would show the molecular ion, such as chemical ionization mass spectrometry[7].

Part (C) of Fig. 2 depicts a mass spectrum obtained from the mercapturic acid isolated from urine of rats treated with 1,2-dibromoethane-D4. It is clear that isomers with a different deuterium content are present. No isomer without deuterium was detectable. Compounds containing four deuterium atoms (m/z 206, 166 and 147) as well as those containing one deuterium atom (m/z 204, 163 and 145) are well represented. For a series of rats treated with 1,2-dibromoethane-D4, the mercapturic acids were isolated and the relative intensities of the fragment ions 163, 164, 165 and 166 (containing one to four deuterium atoms) determined accurately by GCMS by scanning from m/z 150 to m/z 215 and corrected for natural abundance isotope peaks.



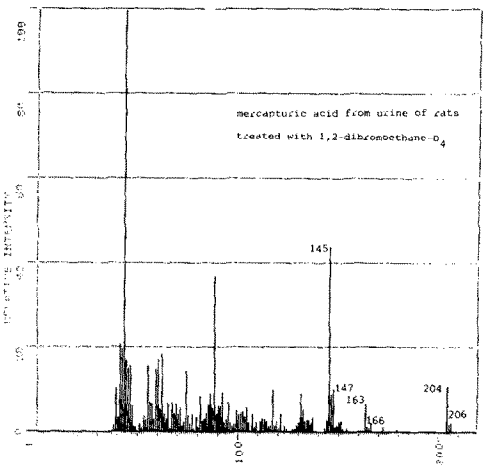


Fig. 2 70 eV EI mass spectra of synthetic N-acetyl-S-2-hydroxyethyl-L-cysteine methyl ester (A), synthetic N-acetyl-S-1,1,2,2-tetradeutero--2-hydroxyethyl-L-cysteine methyl ester (B) and the mercapturic acid from urine of rats given 0.10 mmol 1,2-dibromoethane-D4 (C). 1,2-Dibromoethane-D4 was obtained from MSD-isotopes (Montreal) (99+% pure, containing 99 atom % deuterium). N-acetyl-S-2--hydroxyethyl-L-cysteine methyl ester was synthesized from 1,2-dibromoethane as described previously[10]. Similarly, the 1,1,2,2-tetradeutero-analogue was obtained from 1,2-dibromoethane-D4. The isolation of N-acetyl-S-2-hydroxyethyl-L-cysteine methyl ester from urine and its mass spectral analysis, using a GCMS Computer Combination, were carried out as described before[7].

The results are presented in Table 1. With both male and female rats the most abundant peak is m/z 163, corresponding to a mercapturic acid-containing one deuterium atom (ca 60%). Fragment ion m/z 166 corresponding to the fully deuterated mercapturic acid, is present for ca 22%. Only minor amounts of isomers containing two and three deuterium atoms are present in the mixture.

Table 1. Relative intensities of fragment ions 163, 164 and 166 (loss of 59 from the molecular ion) in the mass spectra of N-acetyl-S-2-hydroxyethyl-L-cysteine methyl ester, isolated from urine of rats given 0.10 mmol 1,2-dibromoethane-D4.

	1D (163)[A)]	2D (164)	3D (165)	1+2+3D	4D (166)
Male rats, oral (n = 4)[B)]	63.0 ± 6.4%	6.7 ± 2.6%	8.5 ± 3.0%	78.2 ± 2.6%	21.8 ± 2.7%
Male rats, i.p. (n = 3)	54.4 ± 8.7%	4.2 ± 2.3%	10.0 ± 1.0%	68.6 ± 6.4%	31.4 ± 6.4%
Female rats, oral (n = 4)	51.5 ± 3.3%	10.5 ± 1.0%	13.7 ± 0.9%	75.7 ± 1.5%	24.3 ± 1.5%

A) Values are corrected for natural abundance isotope peaks. Total intensity (1+2+3+4D) was taken as 100%.
Values given are means ± SEM

B) Numbers in brackets give the number of rats.

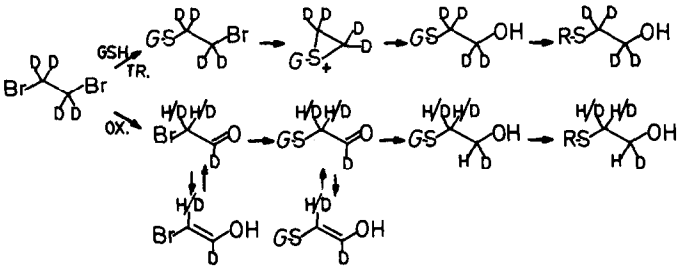


Fig. 3 The metabolic pathways of 1,2-dibromoethane-D4, showing the fate of the deuterium atoms. They are retained when 1,2-dibromoethane is conjugated directly with glutathione, but if oxidation occurs first, one deuterium is certainly lost, and two more are lost partly by enolization of the intermediate aldehydes.

The implications of these findings can be seen in Fig. 3. Retention of four deuterium atoms in the mercapturic acid is only compatible with direct conjugation of 1,2-dibromoethane with glutathione, a route in which no C-D bonds are broken. This implies that the reactive conjugate, S-2-bromoethyl-glutathione plays an important role in the in vivo biotransformation of 1,2-dibromoethane, and may well be responsible for some of the adverse effects of this compound[8,9]. On the other hand, the fact that the majority of mercapturic acids has retained only one deuterium atom and that only small amounts have two or three is in agreement with the intermediacy of aldehydes during the oxidative metabolism[5]. One deuterium atom is lost during the oxidation process, which presumably takes place via oxygen insertion in a C-D bond, followed by loss of D-Br. Two more deuterium atoms can easily be exchanged for hydrogen via subsequent enolization.

In conclusion, these investigations have shown that during the metabolism of tetradeutero--1,2-dibromoethane in the rat, the oxidative and conjugative routes occur in a ratio of about 4:1. If a difference exists in the involvement of the two intermediates in mutagenesis and carcinogenesis this finding might have important implications.

ACKNOWLEDGEMENT The authors thank dr. W.C.M.M. Luijten for the high resolution measurements.

REFERENCES

1. P.J. van Bladeren, D.D. Breimer, G.M.T. Rotteveel-Smijs, R.A.W. de Jong, W. Buijs, A. van der Gen and G.R. Mohn, *Biochem. Pharmacol. 29*, 2975 (1980).
2. E. Nachtomi, E. Alumot and A. Bondi, *Israel J. Chem. 4*, 239 (1966).
3. A.R. Jones and K. Edwards, *Experientia 24*, 1100 (1968).
4. S. Yllner, *Acta Pharmac. Tox. 30*, 257 (1971).
5. D.L. Hill, T.W. Shih, T.P. Johnson and R.F. Stuck, *Cancer Res. 38*, 2438 (1978).
6. J.C. Livesey and M.W. Anders, *Drug Metab. Disp. 7*, 199 (1979).
7. N.P.E. Vermeulen, J. Cauvet, W.C.M.M. Luijten and P.J. van Bladeren, *Biomed. Mass Spectrom. 7*, 413 (1980).
8. W.A. Olson, R.T. Habermann, E.K. Weisburger, J.M. Ward and J.H. Weisburger, *J. Natn. Cancer Inst. 51*, 1993 (1973).
9. H.B. Plotnick, *J. Am. Med. Assn. 239*, 1609 (1978).
10. P.J. van Bladeren, W. Buijs, D.D. Breimer and A. van der Gen, *Eur. J. Med. Chem. 15*, 495 (1980).