Biochemical Pharmacology, Vol. 36, No. 11, pp. 1829–1835, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00
Pergamon Journals Ltd.

1,2-DIBROMO COMPOUNDS

THEIR MUTAGENICITY IN *SALMONELLA* STRAINS DIFFERING IN GLUTATHIONE CONTENT AND THEIR ALKYLATING POTENTIAL*

CLEMA E. M. ZOETEMELK†, GEORGES R. MOHN‡, ARNE VAN DER GEN§ and DOUWE D. BREIMER†

† Division of Pharmacology of the Center for Bio-Pharmaceutical Sciences, ‡ Departments of Radiation Genetics and Chemical Mutagenesis and § Organic Chemistry of the University of Leiden, P.O. Box 9503, 2300 RA Leiden, The Netherlands

(*Received* 28 *August* 1986; *accepted* 5 *December* 1986)

Abstract—The mutagenic activities of several structurally related dibromo compounds were compared in *Salmonella* strains sensitive to base substitution mutagenesis (TA1535 and/or TA100) and in the glutathione (GSH)-deficient derivative TA100/NG-57, using a preincubation procedure. The compounds tested were 1,2-dibromoethane (DBE), 1,2-dibromopropane (DBP), 1,2-dibromo-1-phenylethane (DBPE) and model compounds for the half-mustards resulting from their conjugation with GSH, i.e. the *N*-acetyl-*S*-2-bromoalkyl-L-cysteine methyl esters SBE, SBP, and SBPE, respectively. The alkylating potential of all compounds was assayed with the 4-(*p*-nitrobenzyl)pyridine (NBP) alkylation test. Five of the compounds showed a good correlation between relative mutagenic activity in TA100 and electrophilic reactivity in the NBP-test, the order of decreasing potency being SBE > SBP >DBE > DBPE > DBP. SBPE displayed the highest reactivity in the NBP-test, but was devoid of mutagenic activity.

The mutagenic activity of DBE was substantially decreased in the GSH-deficient strain TA100/NG-57 and could be restored by pretreating the cells with GSH. None of the other chemicals showed different mutagenic activities in TA100 and TA100/NG-57.

From the results it can be concluded that 2-bromothioethers possess higher alkylating activities than the 1,2-dibromo compounds. Methyl substitution has a deactivating effect on the mutagenic activity. The results with the phenyl-substituted analogue, DBPE, show that a higher alkylating activity does not always lead to a higher mutagenic activity.

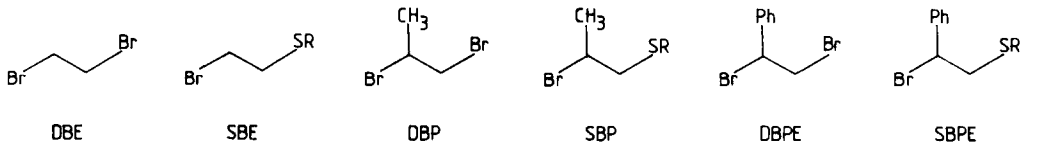
Organohalides are widely used in industry, agriculture and medicine. They are applied as lead scavengers and antiknock preparations in motor fuels, as soil and grain fumigants, anesthetics and industrial solvents or reactants. Recently, some of their toxic, mutagenic and carcinogenic properties have been elucidated [1, 2]. The mechanisms of halocarbon metabolism have been reviewed by Macdonald [3]. Two metabolic pathways are principally responsible for their mammalian metabolism: oxidation by the cytochrome P-450 dependent mixed-function oxidases and conjugation with glutathione (GSH),‖ catalyzed by the GSH *S*-transferases. The vicinal dihalogen alkanes 1,2-dichloroethane and 1,2-dibromoethane (DBE) are particularly interesting in this respect, since a clear relationship between metabolic pathways and mutagenicity was observed which led to the conclusion that conjugation with GSH is a major step in the bioactivation of vicinal dihalogen compounds in mammals [4–9].

The purpose of the present study was to investigate the influence of substituents on the bioactivation of vicinal dibromo compounds, particularly in relation to their conjugation with GSH. In addition to DBE itself, we selected the methyl- and phenyl-substituted analogues 1,2-dibromopropane (DBP) and 1,2-dibromo-1-phenylethane (DBPE) (see Scheme 1) to be included in the assays, for the following reasons. In recent studies aimed at determining the *in vivo* formation of mercapturic acids after treatment of rats with DBP [10] and DBPE,¶ we found that GSH conjugation only plays a minor role in the biotransformation of DBP. A strikingly different pattern was observed with DBPE, which is metabolized for almost 60% via GSH conjugation. Previous studies have shown that in the case of DBE this pathway contributes to approximately 20% in the *in vitro* metabolism [11]. It was assumed that the profound influence of substituents on the GSH conjugation of 1,2-dibromo alkanes would be reflected in differences in mutagenic potency of the parent compounds as well as their presumptive GSH-conjugates. We assessed the mutagenic activity of DBE, DBP, and DBPE in sensitive bacterial strains

* Part of this work was presented at the Dutch Pharmacological Meeting 1984, and was published in *Pharmac. Weekblad, Scientific Ed.* **6**, 182 (1984).

‖ Abbreviations used: DBE, 1,2-dibromoethane; DBP, 1,2-dibromopropane; DBPE, 1,2-dibromo-1-phenylethane; SBE, *N*-acetyl-*S*-(2-bromoethyl)- L-cysteine methyl ester; SBP, *N*-acetyl-*S*-(2-bromopropyl)-L-cysteine methyl ester; SBPE, *N*-acetyl-*S*-(2-bromo-2-phenylethyl)-L-cysteine methyl ester; GLC, gas liquid chromatography; GSH, glutathione; RSH, *N*-acetyl-L-cysteine methyl ester; Tween-80, Polyoxyethylene-sorbitan-tristearate; NBP, 4-(*p*-nitrobenzyl)pyridine; DMSO, dimethyslulfoxide.

¶ Submitted for publication.



R = $CH_2CH(COOCH_3)NHCOCH_3$)

Ph = C_6H_5

Scheme 1. Structures of the dibromo compounds and their corresponding mercapturic acid methyl esters

(*Salmonella typhimurium* TA1535 and TA100) and also determined the alkylating potential of these chemicals in the NBP-test [12, 13]. Information about the influence of GSH conjugation on the mutagenic potency was obtained by using the GSH-deficient strain TA100/NG-57, described by Kerklaan *et al.* [14]. To assess the properties of the primary GSH-conjugates, we synthesized the corresponding *N*-acetyl-*S*-2-bromoalkyl-L-cysteine methyl esters SBE, SBP and SBPE (see Scheme 1) as model compounds for the reactive 2-bromothioethers formed by GSH conjugation from DBE, DBP, and DBPE, respectively. Their alkylating and mutagenic activities were compared with those of the three parent dibromo compounds.

MATERIALS AND METHODS

Chemicals

DBE was obtained from J. F. Baker Chemicals (Deventer, The Netherlands) and purified by preparative GLC to more than 99.9% purity. DBP was obtained from Fluka A.G. (Buchs, Switzerland), purified by distillation and checked by GLC before use to be of more than 99.5% purity. DBPE and NBP were purchased from Aldrich Chemie S.A. (Brussels, Belgium). DBPE was further purified by recrystallization from dichloromethane/ether. A sample of SBE, synthesized according to the method of van Bladeren *et al.* [6], was kindly provided by W. Buijs (University of Leiden, The Netherlands); SBP and SBPE were synthesized following the same method from propene and styrene, respectively.

SBP: yield 47% from propene (pale yellow oil). ^{1}H-NMR ($CDCl_3$, TMS): 1.73 ppm *d* 3H J = 7 Hz $CHCH_3$, 2.08 ppm *s* 3H $COCH_3$, 2.98 ppm *m* 4H CH_2SCH_2, 3.80 ppm *s* 3H OCH_3, 4.11 ppm *m* 1H $CHCH_3$, 4.80 ppm *m* 1H $NHCHCO$, 6.33 ppm broad 1H NH.
MS: *m/z* 297/299 ($M^{+\cdot}$,−), *m/z* 238/240 ($M^{+\cdot}$ − CH_3CONH_2 and/or $\cdot COOCH_3$, 9%), *m/z* 218 ($M^{+\cdot}$ − HBr, 55%), *m/z* 196/198 (*m/z* 238/240 − CH_2CO, 7%), *m/z* 176 (RS^+, 25%), *m/z* 159 (*m/z* 218 − CH_3CONH_2 and/or $\cdot COOCH_3$, 22%), *m/z* 144 ($RS^+ - CH_3OH$, 13%), *m/z* 127 (*m/z* 159 − CH_3OH, 37%), *m/z* 117 (RS^+ − CH_3CONH_2 and/or $\cdot COOCH_3$, 25%), *m/z* 88 ($^+NH_2CHCOOCH_3$, 42%), *m/z* (CH_3CO^+, 100%).

SBPE: yield 54% from styrene (pale yellow solid). ^{1}H-NMR ($CDCl_3/CCl_4$, TMS): 2.00 and 2.12 ppm *s* and *s* 3H $COCH_3$ (two diastereoisomers), 2.85 ppm *m* 2H CH_2CHCO, 3.34 ppm *d* 2H J = 6 Hz $CHBrCH_2S$, 3.75 ppm *s* 3H OCH_3, 4.74 ppm *m* 1H CH_2CHCO, 4.99 ppm *m* CHBr, 6.75 ppm broad 1H NH, 7.37 ppm *m* 5H C_6H_5.
MS: *m/z* 359/361 ($M^{+\cdot}$, −) *m/z* 279 ($M^{+\cdot}$ − HBr, 5%), *m/z* 220 (*m/z* 279 − CH_3CONH_2 and/or $\cdot COOCH_3$, 2%), *m/z* 135 ($C_6H_5CHCHS^+$, 12%), *m/z* 104 ($C_6H_5CHCH_2^{+\cdot}$, 100%), *m/z* 103 ($C_6H_5CHCH^+$, 38%), *m/z* 84 ($HCCN^+H_2COCH_3$, 13%), *m/z* 78 ($C_6H_6^{+\cdot}$, 28%), *m/z* 77 ($C_6H_5^+$, 21%), *m/z* 43 (CH_3CO^+, 27%).

Bacterial strains

Salmonella typhimurium strains TA1535 and TA100, known to be sensitive to the mutagenic action of dihalogen compounds [6] were kindly provided by Prof. B. N. Ames (Berkeley, CA). The GSH-deficient derivative TA1535/NG-19 was obtained from P. R. M. Kerklaan (University of Leiden, The Netherlands). The GSH-deficient derivative TA100/NG-57 was isolated as described previously [14].

Growth and suspension media

The composition of complete agar medium, nutrient broth and phosphate–saline buffer (pH 7.0) has been described by Adams [15]. Top agar contained 0.6% Bacto-Difco agar and 0.5% sodium chloride per litre; it was kept at 50° before use. His^+ agar medium contained 1.5% Bacto-Difco agar, 0.5% D-glucose, trace elements, 0.5 μg/ml L-histidine, 50 μg/ml cysteine [14] and 1 μg/ml D-biotin in Vogel-Bonner medium E [16]. 0.2 M sodium phosphate buffer (pH 7.4) was prepared as described by Maron and Ames [17].

Mutagenicity tests

Preincubation test. Stock solutions of the test compounds were prepared in ethanol, except for DBPE which was dissolved in ethanol/Tween-80 (2:1). The concentration of ethanol and Tween-80 in the final incubation mixtures never exceeded 3% (v/v) and 1% (v/v), respectively. Overnight cultures of the strains were centrifuged and resuspended in phosphate–saline buffer, or in phosphate–saline buffer containing 1 mM GSH. The suspensions were then incubated for 15 min at 37° in a rotary shaker in the dark to allow penetration of GSH into the cells. Thereafter the bateria were centrifuged, washed with buffer and resuspended in phosphate–saline buffer. From these suspensions approx. 10^8 bacterial cells were incubated with varying doses of the compounds in sodium phosphate buffer (total volume 1 ml) for 1 hr at 37° in a rotary shaker. Subsequently, 2.5 ml of molten top agar were added to the incubation

mixtures and the total content was poured over His$^+$ agar plates. The plates were incubated at 37° for 48 hr in the dark, after which the revertant colonies were counted. Each experiment was done in triplicate and at least five separate experiments were performed with each compound.

Spot test. Overnight cultures of strain TA100 were centrifuged, washed and resuspended in phosphate–saline buffer. From these suspensions, about 10^8 bacterial cells were spread over the surface of His$^+$ and complete agar plates. After drying, 10 μl of the test compounds dissolved in DMSO or DMSO/phosphate-saline buffer (1:1) were spotted on the middle of the plates. After incubation for 48 hr at 37° the plates were examined for zones of growth inhibition and the number of revertant colonies outside the zones of inhibition were counted.

Determination of reactivity in the NBP-alkylation test

The method was essentially the same as that described by Eder *et al.* [13]. Solutions of the test compounds were prepared in ethyl methyl ketone. Of each solution 0.05–0.5 ml (adjusted with ethyl methyl ketone to 0.5 ml) were mixed with 0.5 ml of a 200 mM solution of NBP in ethyl methyl ketone, and water was added to a final concentration of 4% (v/v). The reaction mixtures were refluxed for 20 min in a boiling water bath and subsequently cooled in ice. Then 0.5 ml of a mixture of triethylamine and ethyl methyl ketone (1:1) were added to each sample and its extinction against water at 560 nm was determined within 2 min on a Hitachi UV-VIS spectrophotometer. The extinction values were in the range of 0.2–1.2 under the present conditions.

RESULTS

The mutagenic activities of DBE, DBP and DBPE were compared in strain TA1535 and its GSH-deficient derivative TA1535/NG-19, either with or without pretreatment of the bacterial cells with GSH. The results for DBE, shown in Fig. 1, are in agreement with those previously reported by Kerklaan *et al.* [18] and confirm the mutagenic activity of DBE in strain TA1535, while the GSH$^-$ derivative is much less sensitive to the mutagenic action of this compound. After pretreatment of the cells with GSH, which results in increased intracellular GSH levels, both strains were mutated to the same extent by DBE. Neither DBP (0–8 mM) nor DBPE (0–4 mM) induced mutant frequencies significantly higher than the level of spontaneous revertants (5–10 per plate). The only noticeable effects were the absence of residual background growth at exposure concentrations of $\geq$7 mM with DBP and of $\geq$3.2 mM with DBPE (results not shown), which are indicative of cytotoxicity. The possibility thus remained that the apparent non-mutagenicity of DBP was caused by their toxicity and/or by the relative insensitivity of strain TA1535 to these compounds. The availability of strain TA100, a derivative of TA1535 more sensitive to dihalogen alkane mutagenesis [6, 19, 20] and the recent isolation of a GSH-deficient mutant of TA100 [14] prompted us to perform further experiments with these latter two strains. DBE, DBP and DBPE were tested in both strains either with or without pretreatment with GSH. The results are presented in Fig. 2. DBE (Fig. 2A) exerts a mutagenic behaviour which is comparable to that observed in TA1535 and TA1535/NG-19 as previously reported by Kerklaan *et al.* [14]. Also in the TA100 strains, the presence of GSH appears to be crucial for the expression of mutagenic activity of DBE in *Salmonella*. DBP, on the contrary, was neither mutagenic in strain TA100 nor in TA100/NG-57, independent of pretreatment with GSH (results not shown). At exposure levels above 6 mM the cytotoxicity of DBP became apparent by reduction of the residual background growth on the mutation plates. As shown in Fig. 2B, DBPE is equally mutagenic in TA100 and TA100/NG-57, independent of the presence of GSH. Toxicity becomes noticeable at exposure concentrations of 3.2 mM or higher.

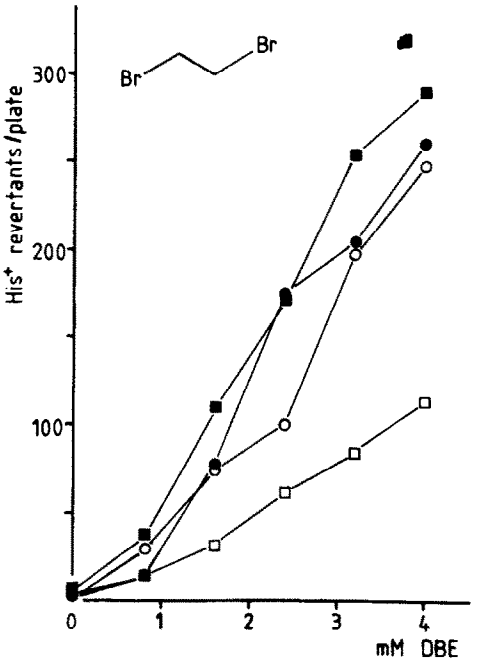


Fig. 1. Mutagenicity of DBE in *Salmonella typhimurium* strains TA1535 (○, ●) and TA1535/NG-19 (□, ■). The cells were pretreated with 1 mM GSH (●, ■) or not (○, □) as described in Materials and Methods. The experimental points represent average values obtained from at least three separate experiments.

Figure 3 shows the results of the experiments performed with the model compounds SBE and SBP. SBE (Fig. 3A) and SBP (Fig. 3B) induced high mutant frequencies in strain TA100 as well as in strain TA100/NG-57, and the mutagenic activity was not influenced by the presence of GSH in the bacterial cells, since no or marginal differences in the numbers of His$^+$ revertants were observed in the two strains at equimolar concentrations of the compounds. Contrary to our expectations, the benzylic bromide SBPE, exerted neither mutagenic nor toxic effects (results not shown). This lack of biological activity may be due to rapid deactivation through reaction of the compound with water, before reaching the critical macromolecules in the cells.

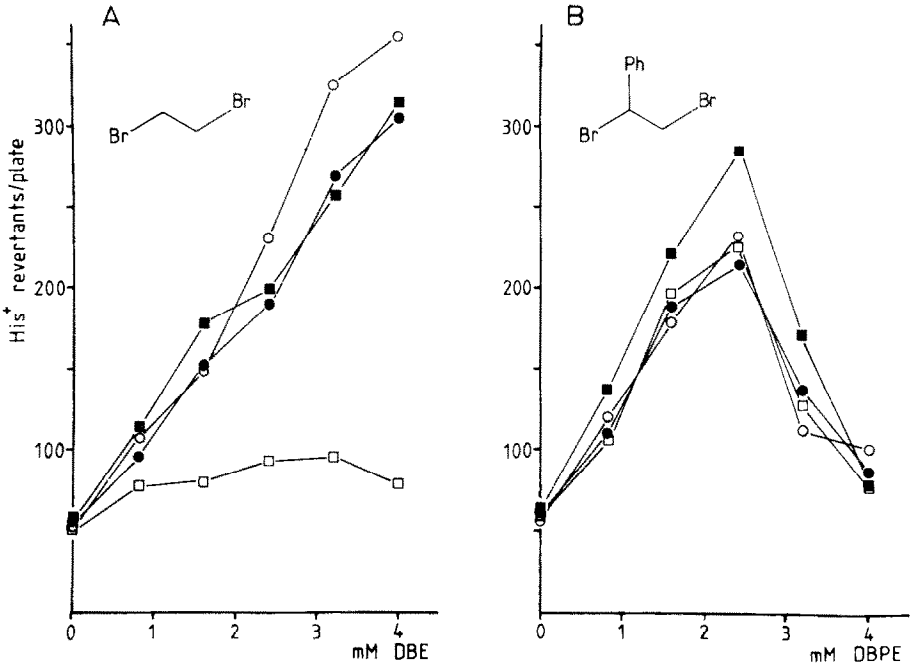


Fig. 2. Mutagenic activity of DBE (A) and DBPE (B) in *Salmonella typhimurium* strains TA100 (○, ●) and TA100/NG-57 (□, ■). As described in Materials and Methods, the cells were pretreated with 1 mM GSH (●, ■) or not (○, □). The points represent average values obtained from 3 to 6 separate experiments.

Therefore the influence of the solvent systems on the mutagenicity and toxicity of SBPE was assessed in spot tests. For comparison the other compounds were included in the assays, except DBE and DBP, which are too volatile to be applied in spot test procedures. The results are shown in Table 1. Using DMSO as a solvent, all four compounds caused growth inhibition; however when dissolved in a mixture of DMSO and phosphate–saline buffer (1:1); only SBE and SBP exhibited cytotoxic effects. As to

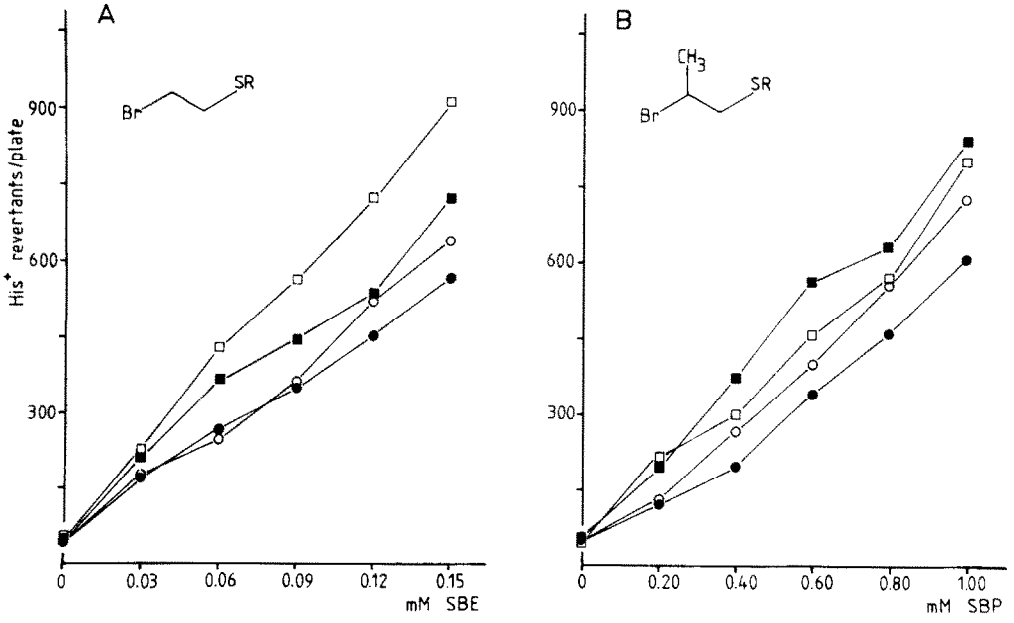


Fig. 3. Mutagenic activity of SBE (A) and SBP (B) in *Salmonella typhimurium* strains TA100 (○, ●) and TA100/NG-57 (□, ■). Pretreatment of the cells with 1 mM GSH (●, ■) or not (○, □) was as described in Materials and Methods. The points represent average values obtained from 3 to 6 separate experiments.

Table 1. The influence of solvent on the activity of bromo compounds in spot tests

Compound	μmol/plate	DMSO Ø*	DMSO Revertants†	DMSO/PO_4-saline Ø*	DMSO/PO_4-saline Revertants†
DBPE	1.9	18	412	0‡	254‡
SBE	1.8	21	249	19	265
SBP	1.7	21	265	18	203
SBPE	1.4	18	121	0	100
Control	—	0	115	0	96

* Zone of colony inhibition in mm.
† Number of revertants outside the zone of inhibition.
‡ DBPE forms a suspension under these conditions; the zone is partly overgrown.

DBPE this was not unexpected, since this chemical formed a suspension already in the solvent mixture and was obviously too insoluble to diffuse into the (aqueous) agar medium. SBPE, did not show any growth inhibiting effects in spite of its good solubility. In contrast to the other three compounds, SBPE was devoid of mutagenic activity in spot tests regardless of the solvent mixture used.

In order to investigate the relationship between mutagenic and alkylating potential, the compounds were also compared in the NBP-test. In preliminary experiments not reported here, using ethyl methyl ketone as the solvent [13], each chemical exhibited a characteristic exponential dose-dependent pattern of reactivity. This non-linearity of response may be caused by the polarity of the solvent used, since both the transition state and the intermediate product have a polar character (see Fig. 4). To increase the polarity of the medium, we repeated the tests in the presence of water (4%, v/v). This resulted in a linear dose-dependent increase of NBP-alkylation and the compounds could then be compared on the basis of the slopes of their respective linear regression curves. The results are presented in Table 2. SBPE has by far the highest NBP-alkylating potency, the ranking order of reactivity being SBPE > SBE > SBP > DBE > DBPE > DBP. This is identical to the order of mutagenic activity (pre-incubation procedure) with the notable exception of SBPE which showed no activity in the latter tests.

Table 2. The alkylating activity of bromo compounds in the NBP-test

Compound	A_{560}/mmol, l^{-1}	N†	r‡	Concentration range (mM)
DBE	0.080*	11	99.7	0–9.1
DBP	0.0049	10	99.3	0–137.0
DBPE	0.0114	11	99.3	0–75.2
SBE	1.893	11	98.9	0–0.37
SBP	0.513	10	99.5	0–1.28
SBPE	4.887	8	99.3	0–0.27

* The extinction at 560 nm was taken as a measure for the amount of product formed in 20 min at 100°. From the slopes of the curves the alkylating activity was determined per mmol/l.
† N is the number of concentration used to measure the reactivity, at each concentration 3–6 determinations were done, independently of each other.
‡ r is the correlation coefficient.

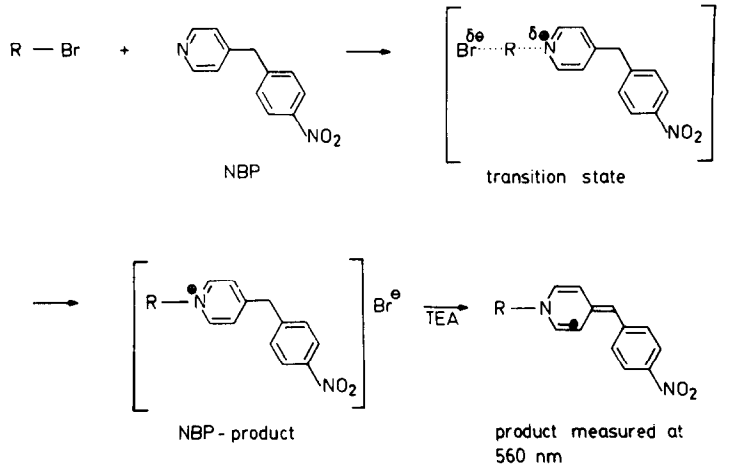


Fig. 4. The reaction of 4-(p-nitrobenzyl)pyridine (NBP) and an alkylating agent as studied in the NBP-test: TEA = triethylamine; R = alkyl.

DISCUSSION

The objective of these studies was to explore the relationship between structure and mutagenic and alkylating activities, within a series of 1,2-dibromo compounds, with special regard for the influence of GSH conjugation. Our interest in the latter had arisen from previous studies concerning the bioactivation of DBE. In these studies it was found that the two metabolic pathways which are largely responsible for the mammalian biotransformation of DBE [11, 21] can both produce reactive intermediates [7]. Oxidation of DBE, through microsomal monooxygenases, generates bromoacetaldehyde [22] and conjugation with GSH produces a reactive sulfur half-mustard, *S*-2-bromoethyl-glutathione [5]. In addition it has been demonstrated that mainly conjugation with GSH can be held responsible for the mutagenic activity of DBE [5] and for the DNA-binding of DBE *in vitro* [8, 9].

To study the influence of GSH conjugation on the mutagenic activity of DBE, DBP and DBPE, we synthesized the bromothioethers SBE, SBP and SBPE as model compounds for the primary GSH-conjugates formed from DBE, DBP and DBPE respectively. Furthermore, we assessed the mutagenic potency of the bromo compounds not only in *Salmonella* strain TA100 but also in its GSH-deficient derivative, strain TA100/NG-57. DBP showed no mutagenic activity under the present conditions, a result in line both with the low activity of GSH and GSH S-transferases towards DBP *in vitro* [6] and with the minor role of GSH conjugation in its *in vivo* metabolism. In this context it should be noted that in earlier investigations in rats we found that, during the biotransformation of DBP, primary GSH conjugation accounted for only 1% or less of the excreted metabolites [10]. In Fig. 2A it can be seen that DBE exerted a different mutagenic behaviour in the two strains, which was in agreement with the previous reports of Kerklaan *et al.* [14]. In contrast, DBPE induced equal mutant frequencies in both strains, independent of the presence or absence of GSH (Fig. 2B). This was an unexpected result, since DBPE is known to be a better substrate for GSH S-transferases than DBE [6]. Also, in biotransformation studies in rats, DBPE was found to be metabolized via primary GSH conjugation for almost 60%* while in DBE metabolism this route accounts for only 20% or less [11, 21]. The synthesized model conjugates (SBE, SBP and SBPE) tested in the same strains gave a better insight in the possible origins of this intriguing finding (Fig. 3). As expected, the 2-bromothioethers SBE and SBP induced high mutagenic activities, independent of the absence or presence of GSH. However, SBPE, the benzylic bromo-compound, showed no mutagenic effects, in spite of its chemical reactivity, as exemplified by the high alkylating activity in the NBP-test (see Table 2). We presumed that this lack of mutagenic activity of SBPE was caused by its high reactivity and that SBPE could have been inactivated by reaction with water or other weak nucleophiles before it had reached the target molecules. Further experiments with spot tests, in which an aqueous solvent was avoided, revealed that SBPE could induce growth inhibition but we observed no mutagenic effects. Additional evidence for this presumption was obtained by ^{13}C NMR measurements, which showed that, upon the addition of water to a solution of SBPE in DMSO, SBPE was completely hydrolysed within 10 min.

With five compounds a good correlation was found between the relative mutagenicity in TA100 (Figs. 2 and 3) and the electrophilic reactivity in the NBP-test (Table 2), the order of decreasing potency being SBE > SBP > DBE > DBPE > DBP. A linear dose-dependent increase of activity in the NBP-test, could be obtained by raising the polarity of the medium through the addition of water (4%, v/v). The amount of water was found to be critical, since in the presence of higher concentrations (8%. v/v) the activity of SBPE decreased strongly, again indicating a deactivation by the medium. The activities of the other compounds increased under these conditions, which is as expected when, in a reaction with a separation of charges in the transition state (see Fig. 4), a higher dielectric constant of the medium is applied [23].

Taken together it can be stated that: (1) the 2-bromothioethers, model compounds for GSH conjugation show a higher alkylating activity and with the notable exception of SBPE, a higher mutagenic activity than the parent dibromo compounds; (2) only DBE is obviously (bio-)activated by GSH conjugation as shown by different mutagenic potencies in TA100 and TA100/NG-57. DBP is a poor substrate for GSH conjugation. If significant conjugation had occurred, leading to formation of the corresponding 2-bromothiother, this would have resulted in a different mutagenic activity in the two *Salmonella* strains. DBPE is a good substrate for GSH conjugation. The lack of mutagenic activity of the highly SBPE is probably due to scavenging before critical biomacromolecules are reached. These results demonstrate that bioactivation of vicinal dibromo compounds critically depends on the ability of formation of intermediates and on the chemical reactivity of these intermediates.

Acknowledgements—We thank Mr P. R. M. Kerklaan for his helpful discussions and Mrs B. van Meeteren-Wälchli for synthesizing the model compounds. The investigations were supported by a grant from the Foundation for Medical Research (FUNGO) which is subsidized by the Netherlands Organization for the Advancement of Pure Research (ZWO).

REFERENCES

1. L. Fishbein, *J. Toxic. Environ. Hlth* **6**, 1133 (1980).
2. R. J. Laib, *Drug Metab. Disp.* **4**, 1 (1982).
3. T. L. Macdonald, *CRC Crit. Rev. Toxic.* **11**, 85 (1983).
4. U. Rannung, *Mutat. Res.* **76**, 269 (1980).
5. 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. Pharmac.* **29**, 2975 (1980).
6. P. J. van Bladeren, D. D. Breimer, G. M. T. Rotteveel-Smijs, P. de Knijff, G. R. Mohn, B. van Meeteren-Wälchli, W. Buijs and A. van der Gen, *Carcinogenesis* **2**, 499 (1981).

* Submitted for publication.

7. T.-W. Shih and D. L. Hill, *Res. Commun. Chem. Pathol. Pharmac.* **33**, 449 (1981).
8. D. W. Sundheimer, R. D. White, K. Brendel and I. G. Sipes, *Carcinogenesis* **3**, 1129 (1982).
9. N. Ozawa and F. P. Guengerich, *Proc. natn. Acad. Sci. U.S.A.* **80**, 5266 (1983).
10. C. E. M. Zoetemelk, I. H. Oei, B. van Meeteren-Wälchli, W. Onkenhout, A. van der Gen and D. D. Breimer, *Drug Metab. Dispos.* **14**, 601 (1986).
11. P. J. van Bladeren, D. D. Breimer, J. A. T. C. M. van Huijgevoort, N. P. E. Vermeulen and A. van der Gen, *Biochem. Pharmac.* **30**, 2499 (1981).
12. J. Epstein, R. W. Rosenthal and R. J. Ess, *Analyt. Chem.* **27**, 1435 (1955).
13. E. Eder, T. Neudecker, D. Lutz and D. Henschler, *Chem.-Biol. Interact.* **38**, 303 (1982).
14. P. R. M. Kerklaan, C. E. M. Zoetemelk and G. R. Mohn, *Biochem. Pharmac.* **34**, 2151 (1985).
15. M. H. Adams, in *Bacteriophages*. Wiley/Interscience, New York (1959).
16. H. J. Vogel and D. M. Bonner, *J. biol. Chem.* **218**, 97 (1956).
17. D. M. Maron and B. N. Ames, *Mutat. Res.* **113**, 173 (1983).
18. P. Kerklaan, S. Bouter and G. Mohn, *Mutat. Res.* **122**, 257 (1983).
19. U. Rannung, A. Sundvall and C. Ramel, *Chem.-Biol. Interact.* **20**, 1 (1978).
20. P. J. van Bladeren, A. van der Gen, D. D. Breimer and G. R. Mohn, *Biochem. Pharmac.* **28**, 2521 (1979).
21. E. Nachtomi, *Biochem. Pharmac.* **19**, 2853 (1970).
22. D. L. Hill, T.-W. Shih, T. P. Johnston and R. F. Struck, *Cancer Res.* **38**, 2438 (1978).
23. C. Reichardt, in *Solvent Effects in Organic Chemistry*. Verlag Chemie, Weinheim/New York (1979).