The Reaction of Aralkyl Sulphate Esters with Glutathione Catalysed by Rat Liver Preparations

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1. Rat liver supernatant preparations catalyse the reactions of some aralkyl sulphate esters with GSH to yield *S*-aralkylglutathione derivatives. 2. A glutathione *S*-transferase that catalyses these reactions has been purified 16-fold. 3. The purified enzyme preparation catalyses the release of sulphate ions from benzyl sulphate, 1-menaphthyl (naphth-1-ylmethyl) sulphate and phenanthr-9-ylmethyl sulphate only in the presence of GSH. It does not cause the release of sulphate ions from prop-1-yl sulphate, L-serine *O*-sulphate, phenyl sulphate or oestrone 3-sulphate even when GSH is added. 4. The stability and specificity of the enzyme and its response to inhibitors and to changes of pH were studied. 5. The activity of the preparation was compared with the activities of glutathione *S*-transferases described previously.

Hyde & Young (1968) and Clapp & Young (1970) demonstrated that aralkyl esters of the type $R'-CO-O-CH_2-R$ (where *R* is aromatic and *R'* is either aliphatic or aromatic) when administered to the rat give rise to mercapturic acids. In addition Clapp & Young (1970) obtained evidence for the presence of *S*-(1-menaphthyl)glutathione (a derivative of 1-methylnaphthalene) in the bile of rats to which several carboxylic acid esters of 1-menaphthyl alcohol had been given. It therefore seemed likely that mercapturic acid formation from aralkyl esters is initiated by the aralkylation of GSH with the liberation of the acid and that a glutathione *S*-transferase might be involved (see Boyland & Chasseaud, 1969*a*). Work by Boyland & Chasseaud (1969*b*) indicated that although rat liver preparations catalyse the reactions of certain aralkyl esters (e.g. 1-menaphthyl acetate) with GSH the rate of reaction was very low.

Gillham, Clapp, Morrison & Young (1970) have since shown that the acid sulphate esters of benzyl alcohol and 1-menaphthyl alcohol are converted by the rat, in relatively high yield, into the corresponding aralkylmercapturic acids. These workers also demonstrated that rat liver contains an enzyme that catalyses the interaction of 1-menaphthyl sulphate and GSH. The purification and properties of this enzyme are the subject of the present paper.

MATERIALS

Melting points are uncorrected.

Sodium benzyl sulphate and sodium 1-menaphthyl sulphate were prepared as described by Clapp & Young (1970). Sodium phenanthr-9-ylmethyl sulphate was

synthesized from phenanthr-9-ylmethanol in a similar manner.

S-Benzylglutathione, m.p. 199–200°C (decomp.), and *S*-(1-menaphthyl)glutathione, m.p. 210°C (decomp.), were prepared by the method described for the latter compound by Hyde & Young (1968). An attempt was made to form *S*-(phenanthr-9-ylmethyl)glutathione by this method, but the product could not be crystallized. The discoloured powder that was obtained showed a major chromatographic spot with the properties expected of an *S*-aralkylglutathione derivative.

S-Benzyl-L-cysteine, m.p. 208–209°C (decomp.), was prepared by the method used for *S*-benzylglutathione except that L-cysteine was used in place of GSH.

The method of Cramer & Weimann (1961) was used to prepare benzyl orthophosphate as the bis-cyclohexylammonium salt, m.p. 234–236°C (decomp.).

Samples of *S*-(1-menaphthyl)-L-cysteine and 1-menaphthylmercapturic acid were gifts from Dr J. J. Clapp. Specimens of potassium phenyl sulphate and sodium phenylmethanesulphonate were provided by Mr A. R. Morrison. Sodium prop-1-yl sulphate was provided by Mr J. Woodman. Dr J. H. Thomas furnished a sample of potassium L-serine *O*-sulphate. All other compounds used were commercially available.

METHODS

Animals. Male barrier-maintained C.F.E. albino rats, weight 230–250 g, were used throughout.

Chromatography. Chromatograms were prepared by the descending method on Whatman no. 3 paper by the use of the solvent mixtures: *A*, butan-1-ol–acetic acid–water (12:5:3, by vol.); *B*, propan-1-ol–water–aq. NH_3 (sp.gr. 0.88) (80:20:1, by vol.); *C*, butan-1-ol–pyridine–3*M*-aq. NH_3 (4:3:3, by vol.). Two detecting reagents were used: the platinum reagent of Toennies & Kolb (1951) and ninhydrin, both as used by Barnsley, Thomson & Young (1964). A list of typical R_f values is given in Table 1.

Table 1. *Paper chromatography of S-aralkylglutathione derivatives and related compounds*

For experimental details see the Methods section.

Compound	Solvent ...	R_F		
		A	B	C
<i>S</i> -(Benzyl)glutathione		0.58	0.25	0.30
<i>S</i> -(1-Menaphthyl)glutathione		0.62	0.19	0.40
<i>S</i> -(Phenanthr-9-ylmethyl)glutathione		0.66	0.28	0.39
<i>S</i> -(Benzyl)-L-cysteine		0.71	0.66	0.60
<i>S</i> -(1-Menaphthyl)-L-cysteine		0.69	0.63	0.72
Acid hydrolysis product, believed to be <i>S</i> -(phenanthr-9-ylmethyl)-L-cysteine		0.75	0.75	0.68
GSH		0.30	0.00	0.00
GSSG		0.05	0.00	0.00
Glutamic acid		0.33	0.09	0.10
Glycine		0.29	0.19	0.20

Ultraviolet spectroscopy. The Hilger Uvispek spectrophotometer and the Unicam SP.800 spectrophotometer (fitted with Honeywell Controls Ltd. external recorder, range -1.0 to $+10.0$ mV) were used. In the experiments with enzymes a cuvette carrier was used through which water from a constant-temperature bath was circulated.

Determination of sulphate. The BaSO₄-gelatin method described by Dodgson (1961) was used for the determination of inorganic sulphate.

Determination of protein. A modification of the biuret method due to Cleland & Slater (1953) was used for protein measurements on fractions obtained during the purification of the enzyme. The biuret method gave an extinction of 1.0/18.6 mg of protein in a final volume of 5 ml (calibrated with bovine serum albumin).

Demonstration of the formation of S-aralkylglutathione derivatives from aralkyl sulphate esters in the presence of a rat liver supernatant preparation. A non-particulate fraction was obtained from rat livers in the following manner. The livers of two freshly killed animals were placed in ice-cold 0.25 M-sucrose, pH 7.3 (40 ml). All subsequent operations were performed at 4°C. The livers were shredded with scissors and homogenized in a glass-Teflon homogenizer (A. H. Thomas Co., Philadelphia, Pa., U.S.A.). Centrifuging was performed in an MSE Super-speed 40 refrigerated ultracentrifuge with an 8 × 25 ml rotor. The following fractions were removed: nuclear and cell debris (5000g for 10 min), mitochondria and lysosomes (22500g for 15 min) and microsomes (100000g for 1 h). The postmicrosomal supernatant was made up to 50 ml with 0.25 M-sucrose, pH 7.3, and used in the incubations described below. Separate experiments showed that such preparations contained about 80% of the total glutathione *S*-transferase activity towards 1-menaphthyl sulphate found in a whole homogenate.

The supernatant fraction (5 ml) was mixed in a total volume of 10 ml containing final concentrations of 3.75 mM-GSH, 2.5 mM-1-menaphthyl sulphate, 0.25 mM-EDTA and 50 mM-triethanolammonium chloride-NaOH buffer, pH 8.0. The mixture was incubated for 1 h at 37°C in open flasks and any reaction was terminated by the addition of 15% (w/v) trichloroacetic acid (1 ml). Reaction products were extracted from the deproteinized solution by

the addition of activated charcoal (100 mg). The charcoal extract was washed twice with water and menaphthyl compounds were eluted with methanol-benzene-aq. NH₃ (sp.gr. 0.88) (87:10:3, by vol.). The eluate was concentrated and reaction products present were identified by paper chromatography in solvent mixtures A, B and C. The compound that appeared to correspond chromatographically to *S*-(1-menaphthyl)glutathione in this experiment was further characterized in the following way. The compound was purified by preparative paper chromatography in solvent mixture C, and hydrolysed in 5 M-HCl by heating in a boiling-water bath for 3 h. The constituents of the tripeptide were identified by means of paper chromatography. Similar experiments were performed with benzyl sulphate and phenanthr-9-ylmethyl sulphate as substrates.

Assay of the enzyme-catalysed reaction of 1-menaphthyl sulphate with GSH. Unless specifically noted assays were performed in 50 mM-triethanolammonium chloride-NaOH buffer, pH 8.3, at 37°C. Stock GSH solutions normally contained EDTA (2 mM) and were purged with N₂ before use. The assays were usually performed in the presence of 5 mM-GSH and 0.5 mM-1-menaphthyl sulphate. The pH value used was optimum and the substrate concentrations were saturating. Rates of reaction were measured during the initial linear period of the reaction, which lasted for at least 10 min.

The ϵ values of 1-menaphthyl sulphate and *S*-(1-menaphthyl)glutathione at 298 nm are 14001 $\cdot$ mol⁻¹ $\cdot$ cm⁻¹ and 39001 $\cdot$ mol⁻¹ $\cdot$ cm⁻¹ respectively and these values are independent of pH value in the range 6.0-10.0. This difference was made the basis of a spectrophotometric assay for the interconversion of the compounds. It was found that solutions containing various proportions of the substrate and product at a final fixed total concentration have extinctions that increase in a linear fashion as the fraction of *S*-(1-menaphthyl)glutathione in the mixture increases. This was found to be the case at final total concentrations of 0.15, 0.30 and 0.60 mM, and at 1.20 mM as far as it was possible to determine extinctions accurately.

A unit of enzyme activity is defined as that amount of enzyme that forms 1 μ mol of *S*-(1-menaphthyl)glutathione/10 min from 1-menaphthyl sulphate and GSH under the

Table 2. *Purification of 1-menaphthyl sulphate-dependent glutathione S-transferase from rat liver*

Stage	Vol. (ml)	Total protein (mg)	Total activity (units)	Recovery (%)	Specific activity (unit/mg)	Purification
(1) 60000g Supernatant ('extract')	320	7230	56.9	100	0.008	1.0
(2) 15% (v/v) Ethanol supernatant	360	3380	45.6	80	0.014	1.7
(3) 2.2 mM-ZnSO ₄ supernatant	340	1090	38.9	68	0.036	4.5
(4) 15 mM-ZnSO ₄ precipitate	178	710	31.6	56	0.045	5.6
(5) (NH ₄) ₂ SO ₄ precipitate (45-55% saturation)	10	90	11.7	21	0.130	16.3

above assay conditions. The specific activity of the enzyme is expressed as the number of enzyme units/mg of protein.

Assay of other types of glutathione S-transferase. Glutathione *S*-aryltransferase activity was assayed with 1,2-dichloro-4-nitrobenzene (1 mM) and GSH (5 mM) as substrates in 50 mM-triethanolammonium chloride-NaOH buffer, pH 8.3, at 37°C. The course of the reaction was followed by the spectrophotometric method of Booth, Boyland & Sims (1961), initial rates being measured.

The conjugations of GSH with other substrates, catalysed by other glutathione *S*-transferases, were determined titrimetrically as described by Boyland & Chasseaud (1967). All these assays were performed in 50 mM-KH₂PO₄-NaOH buffer, pH 6.5, at 25°C in the presence of 3 mM-GSH. The substrates used for each transferase were: glutathione *S*-alkyltransferase, iodomethane (3 mM); glutathione *S*-alkenyltransferase, diethylmaleate (3 mM); glutathione *S*-aralkyltransferase, 1-menaphthyl chloride (1 mM); glutathione *S*-epoxidetransferase, 2,3-epoxypropyl phenyl ether (3 mM). The amounts of enzyme added were such that not more than 20% of the substrates was converted during the reactions.

Enzyme purification. The stages in the purification of the enzyme (usually from the livers of eight rats) were essentially those described by Johnson (1966) for glutathione *S*-alkyltransferase. The stages were briefly as follows. Stage (1): preparation of a rat liver 60000g supernatant fraction (the 'extract'). Stage (2): denaturation of inactive protein at pH 6.0 by the rapid addition of ethanol-water (9:1, v/v) to give a final ethanol concentration of 15% (v/v). Stage (3): precipitation of inactive protein by 2.2 mM-ZnSO₄ at pH 6.0. Stage (4): precipitation of active protein by increasing the concentration of ZnSO₄ to 15 mM at pH 6.2. Stage (5): (NH₄)₂SO₄ fractionation at pH 7.0, when active protein separated between 45% and 55% saturation. The fraction obtained at stage (5) was used for the investigation of the properties of the enzyme (referred to as 'the purified enzyme').

It was found that enzyme fractions obtained after stage (2) increased in activity by two- to four-fold if they were dialysed overnight at 4°C against 2% (w/v) NaCl, pH 7.0, containing 2 mM-EDTA. Dialysis was therefore performed at stages (3)-(5). The increase in activity was probably due to the removal of sulphate ions, for subsequent experiments indicated that they inhibit the enzyme.

The course of a typical enzyme purification is outlined in Table 2.

RESULTS

Formation of S-aralkylglutathione derivatives. Benzyl sulphate, 1-menaphthyl sulphate and phenanthr-9-ylmethyl sulphate were all found to react with GSH at pH 8.0 to give compounds that were chromatographically indistinguishable from the corresponding *S*-aralkylglutathiones. The reaction was stimulated markedly by the presence of the rat liver supernatant preparation, but traces of the GSH derivatives were found when the enzyme was denatured by boiling. The metabolites, which corresponded chromatographically to the *S*-aralkylglutathiones, on complete acid hydrolysis yielded only glutamic acid, glycine and the appropriate *S*-aralkyl-L-cysteine.

Stoichiometry of the aralkylation of GSH. When the purified enzyme was incubated at pH 8.0 and 37°C in the presence of 1-menaphthyl sulphate (1 mM) and GSH (5 mM) the production of *S*-(1-menaphthyl)glutathione and the release of sulphate ions were equivalent. In two experiments with separate batches of purified enzyme (enzyme protein concentration 1.2 and 2.8 mg/ml) the results were: *S*-(1-menaphthyl)glutathione, 0.24 and 0.15 μmol/mg of protein formed in 15 min: sulphate ions, 0.22 and 0.13 μmol/mg of protein released in 15 min.

Substrate specificity. Three general types of compounds were tested as possible substrates for the enzyme: thiols other than GSH, sulphate esters other than aralkyl sulphates, and other esters.

The ϵ values for 1-menaphthylmercapturic acid and *S*-(1-menaphthyl)-L-cysteine are 50001·mol⁻¹·cm⁻¹ and 48001·mol⁻¹·cm⁻¹ respectively at 298 nm and pH 8.0, i.e. about three times the value for 1-menaphthyl sulphate. Thus any interaction of either L-cysteine or *N*-acetyl-L-cysteine with 1-menaphthyl sulphate ought to be revealed by an increase in the absorption at 298 nm.

Table 3. *Substrate specificity of the 1-menaphthyl sulphate-dependent glutathione S-transferase of rat liver*

The final concentrations in the reaction mixtures (total vol. 1.0 ml) were 5.0 mM-GSH, 1.0 mM-sulphate ester, 50 mM-triethanolammonium chloride-NaOH buffer, pH 8.3, and 3.3 mg of enzyme protein/ml. Incubations were for 20 min at 37°C. The reactions were stopped by the addition of 200 μ l of 25% (w/v) trichloroacetic acid, and 500 μ l portions of the deproteinized supernatant were taken for assay of sulphate ions released.

Substrate	SO ₄ ²⁻ ions liberated (μ mol)
Benzyl sulphate	0.79
1-Menaphthyl sulphate	0.60
Phenanthr-9-ylmethyl sulphate	0.36
L-Serine O-sulphate	0.07
Phenyl sulphate	0.00
Oestrone 3-sulphate	0.00
Prop-1-yl sulphate	0.00

No increase in the absorption of the reaction mixture at 298 nm was observed when L-cysteine (5 mM) or N-acetyl-L-cysteine (5 mM) was incubated at pH 8.0 and 37°C in the presence of 1-menaphthyl sulphate (1 mM) and the purified enzyme. With the same experimental technique it was found the 1-menaphthyl alcohol (ϵ 8401 $\cdot$ mol⁻¹ $\cdot$ cm⁻¹ at 298 nm) was not able to replace its sulphate ester as a substrate for the enzyme.

Table 3 shows the amount of sulphate ions released from a series of sulphate esters when incubated at pH 8.3 and 37°C in the presence of the purified enzyme and GSH. If GSH or the enzyme was omitted from the complete reaction mixture containing an aralkyl sulphate no significant release of sulphate ions was observed. The results indicate that of the sulphate esters tested only the aralkyl derivatives were substrates for the enzyme. Although some sulphate ions were released from L-serine O-sulphate, this occurred in the absence of GSH and was probably due to the desulphating enzyme found by Thomas & Tudball (1967) in the supernatant fraction from rat liver.

Two non-sulphate esters were incubated at pH 8.3 and 37°C in the presence of the purified enzyme and GSH (5 mM): benzyl orthophosphate (2 mM) and 1-menaphthyl acetate (2 mM). [The second compound was added to the reaction mixture in ethanol because of its low solubility in water, but the final concentration of ethanol, 3% (v/v), had no effect on the activity of the 1-menaphthyl sulphate-dependent glutathione S-transferase.] When the reaction mixtures were examined chromatographically for S-substituted glutathione derivatives as described for S-(1-menaphthyl)glutathione no

Table 4. *Inhibition of the 1-menaphthyl sulphate-dependent glutathione S-transferase of rat liver*

The final concentrations in the reaction mixtures (total vol. 3.0 ml) were 1.0 mM-GSH, 0.1 mM-1-menaphthyl sulphate, 50 mM-triethanolammonium chloride-NaOH buffer, pH 8.3, 0.5 mg of enzyme protein/ml and inhibitor to the final concentration indicated. Initial rates were determined at 37°C.

Compound added	Concn. (mM)	Inhibition (%)
S-(1-Menaphthyl)glutathione	0.1	60
Sodium sulphate	10.0	58
Sulphanilic acid	1.0	41
p-Aminobenzoic acid	1.0	33
Benzyl sulphate	1.0	30
S-(Benzyl)glutathione	1.0	24
L-Lysine	1.0	17
3,5-Diaminobenzoic acid	1.0	11
Phenylmethanesulphonic acid	1.0	11
δ -Amino-n-valeric acid	1.0	10
Sebacic acid	1.0	6
Toluene-p-sulphonic acid	1.0	6
Sulphanilamide	1.0	0
1-Menaphthyl alcohol	1.0	0
GSSG	2.0	0

evidence was found for the formation of GSH derivatives.

Effect of substrate concentration. The method of Florini & Vestling (1957) was used to determine the K_s values at pH 8.3 for the purified enzyme. Lineweaver & Burk (1934) plots were constructed for four GSH concentrations between 0.53 and 5.0 mM at each of six 1-menaphthyl sulphate concentrations between 0.07 and 0.55 mM. This gave pencils of straight lines intersecting at a point in the fourth quadrant giving a K_s value for 1-menaphthyl sulphate of 0.06 mM. Similar graphs were prepared at four 1-menaphthyl sulphate concentrations between 0.06 and 0.28 mM for each of six GSH concentrations between 0.65 and 5.0 mM. This also gave pencils of straight lines intersecting in the third quadrant giving a K_s value for GSH of 1.0 mM.

Effect of inhibitors. The effects of several classes of possible inhibitors on the purified enzyme were tested. Compounds that are similar to the substrates, e.g. GSSG and phenylmethanesulphonic acid, were examined. The inhibitory effect of reaction products was tested. In addition the effects of some amino compounds were investigated. The results with a range of inhibitors are given in Table 4.

Comparison of the 1-menaphthyl sulphate-dependent enzyme with other glutathione S-transferases. To compare the 1-menaphthyl sulphate-dependent enzyme with other glutathione S-transferases the specific activities of all of these enzymes were

determined at stages (1) and (5) of the enzyme purifications described in the Methods section. The ratios of the specific activity of the 1-menaphthyl sulphate-dependent enzyme to those of the other transferases were calculated for each purification stage. This activity ratio was set at 1.0 for stage (1) and any significant deviation of the ratio from unity indicated the separation of two distinct enzymes. The technique clearly distinguishes three enzymes, glutathione *S*-aralkyltransferase, *S*-aryltransferase and *S*-alkenyltransferase, from the 1-menaphthyl sulphate-dependent enzyme, for the activity ratios at stage (5) of the purification were found to be in the range 5–7. The other two enzymes, glutathione *S*-alkyltransferase and *S*-epoxide-transferase, were not distinguished as clearly from the 1-menaphthyl sulphate-dependent enzyme, but an activity ratio of 7.4 was obtained for glutathione *S*-alkyltransferase if the preparation at stage (5) was dialysed overnight at 4°C against water. There are therefore good grounds for believing that the present enzyme differs from four of the five main classes of glutathione *S*-transferase described by Boyland & Chasseaud (1969a).

DISCUSSION

Role of the enzyme in mercapturic acid synthesis. The work of Hyde & Young (1968) and Clapp & Young (1970) led to the conclusion that the aralkylation of GSH is an early step in the formation of aralkylmercapturic acids from aralkyl esters. Clapp & Young (1970) suggested that the aralkyl ester or a substance derived from it probably reacts with GSH to give an *S*-substituted glutathione; the subsequent conversion of this compound into an *S*-substituted cysteine that undergoes *N*-acetylation to give the mercapturic acid would then follow the general pathway outlined by Barnes, James & Wood (1959) and Bray, Franklin & James (1959a,b).

The nature of the proximal aralkylating agent was not clear from the experiments of Hyde & Young (1968) and Clapp & Young (1970) because both the ester and the parent aralkyl alcohol were converted into mercapturic acid when administered to the rat. The finding by Gillham *et al.* (1970) that rat liver contains an enzyme that catalyses the interaction of 1-menaphthyl sulphate with GSH helped to throw light on this subject. It has been shown in the present work that this enzyme, when purified, catalyses the conversion of 1-menaphthyl sulphate, but not that of the alcohol, into a GSH derivative. Also, if the sulphate ester is incubated with the purified enzyme in the absence of GSH there is no significant release of sulphate ions. Finally, there is a stoichiometric relationship between the amount of sulphate ions released and the quantity of *S*-(1-menaphthyl)glutathione formed when GSH

and 1-menaphthyl sulphate are incubated in the presence of the purified enzyme. Additional though indirect evidence that this enzyme is the one responsible for an early step in mercapturic acid formation *in vivo* stems from the fact that the purified enzyme is more active towards benzyl sulphate than towards 1-menaphthyl sulphate, and Clapp & Young (1970) were able to show a higher conversion, in terms of metabolite isolated, of benzyl sulphate than of 1-menaphthyl sulphate into the corresponding mercapturic acid.

Identity of the purified enzyme. The results obtained during the present work indicate that the glutathione *S*-transferase purified is distinct from the glutathione *S*-transferases described previously (see Boyland & Chasseaud, 1969a). Apart from the distinct separation of four of the known glutathione *S*-transferases obtained during the enzyme purification, some of the transferases can be ruled out on other grounds. Glutathione *S*-aralkyltransferase and *S*-epoxidetransferase have pH optima at 6.8 and 6.5 respectively (Suga, Ohata, Kumaoka, & Akagi, 1967; Boyland & Williams, 1965), whereas the present enzyme shows optimum activity at pH 8.3. Glutathione *S*-alkyltransferase appears to be ruled out because of its instability to dialysis, in contrast with the present enzyme. On dialysis overnight an 'extract' lost 80% of its glutathione *S*-transferase activity towards iodomethane, but only 10% of its activity towards 1-menaphthyl sulphate. Glutathione *S*-aryltransferase is not found in the rat kidney (Booth *et al.* 1961), whereas the specific activity of the present enzyme is similar in the rat liver and kidney (B. Gillham, unpublished work).

Possible mechanism of the 1-menaphthyl sulphate-dependent glutathione S-transferase. Boyland & Chasseaud (1969b) suggested that the interaction of benzyl chloride with GSH catalysed by glutathione *S*-aralkyltransferase proceeds by an S_N1 type mechanism. These workers envisaged a two-step reaction in which a carbonium ion, generated by the release of a chloride ion, reacts with GSH. Such a mechanism at the enzymic level would be equivalent kinetically to a Ping Pong reaction (Cleland, 1963). This type of reaction does not appear to be mediated by the enzyme that catalyses the interaction of 1-menaphthyl sulphate and GSH, because the families of double-reciprocal plots obtained with both of these substrates were not parallel lines as required for a Ping Pong reaction, but rather converged to single points.

Clark, Darby & Smith (1967) showed that the glutathione *S*-aryltransferase obtained from grass grubs could be inhibited by dicarboxylic acids (e.g. sebacic acid and traumatic acid) in which the carboxyl groups are superimposable (in models) on the carboxyl groups in GSH. For the present

enzyme sebacic acid was found to be a poor inhibitor whereas sulphanilic acid and *p*-aminobenzoic acid were strongly inhibitory. The carboxyl (sulphonate) and amino groups in these compounds are superimposable on the α -carboxyl group of glutamic acid and the α -amino group of glycine. It is noteworthy that two compounds closely related to sulphanilic acid (sulphanilamide and toluene-*p*-sulphonic acid) produced little or no inhibition of the present enzyme. Both of these compounds possess only one ionizable group. This suggests that the enzyme binds GSH and inhibitors such as sulphanilic acid and *p*-aminobenzoic acid by the positively and the negatively charged groups on these compounds, both groups being necessary for optimum binding.

Role of glutathione S-transferases. The view has been expressed by Boyland & Chasseaud (1968) that a function of the glutathione *S*-transferases is to protect cellular nucleophiles by conjugating electrophiles with GSH. These electrophiles may be of endogenous or exogenous origin. If these electrophiles are exogenous it seems unlikely that they are present in the liver in high concentration. Therefore it might be expected that for their efficient enzymic conjugation with GSH the K_s value for the electrophile would be low (in the μ M range). The K_s value (0.06 mM) found for 1-menaphthyl sulphate with the present enzyme suggests that this enzyme may indeed be effective in the metabolism of trace amounts of compounds of the aralkyl sulphate type.

It seems possible that a route for the metabolism of ingested aralkyl alcohols might involve sulphation, conjugation with glutathione and subsequent conversion of the conjugate into a mercapturic acid. An example of this pathway might be the metabolism of 3,5-di-*tert*.-butyl-4-hydroxytoluene, since two of the metabolites of this compound in the rat have been shown by Daniel, Gage & Jones (1968) to be the corresponding benzyl alcohol derivative and 3,5-di-*tert*.-butyl-4-hydroxybenzylmercapturic acid.

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