Biochem. J. (1973) **134**, 1093–1101
Printed in Great Britain

Biosynthesis of Mercapturic Acids from Allyl Alcohol, Allyl Esters and Acrolein

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(*Received* 22 *March* 1973)

1. 3-Hydroxypropylmercapturic acid, i.e. *N*-acetyl-*S*-(3-hydroxypropyl)-L-cysteine, was isolated, as its dicyclohexylammonium salt, from the urine of rats after the subcutaneous injection of each of the following compounds: allyl alcohol, allyl formate, allyl propionate, allyl nitrate, acrolein and *S*-(3-hydroxypropyl)-L-cysteine. 2. Allylmercapturic acid, i.e. *N*-acetyl-*S*-allyl-L-cysteine, was isolated from the urine of rats after the subcutaneous injection of each of the following compounds: triallyl phosphate, sodium allyl sulphate and allyl nitrate. The sulphoxide of allylmercapturic acid was detected in the urine excreted by these rats. 3. 3-Hydroxypropylmercapturic acid was identified by g.l.c. as a metabolite of allyl acetate, allyl stearate, allyl benzoate, diallyl phthalate, allyl nitrite, triallyl phosphate and sodium allyl sulphate. 4. *S*-(3-Hydroxypropyl)-L-cysteine was detected in the bile of a rat dosed with allyl acetate.

1-Menaphthylmercapturic acid has been isolated from the urine of rats after the subcutaneous injection of various 1-menaphthyl (α-methylnaphthalene) compounds including 1-menaphthyl acetate and 1-menaphthyl benzoate (Hyde & Young, 1968), and these appear to have been the first examples of mercapturic acid formation *in vivo* arising from the administration of carboxylic acid esters to animals. More recently benzylmercapturic acid and 1-menaphthylmercapturic acid have been isolated from the urine of rats that had been injected subcutaneously with sodium benzyl sulphate and sodium 1-menaphthyl sulphate respectively (Clapp & Young, 1970). The present work was undertaken to extend these observations to the metabolism of allyl esters, since it is known that the esters of allyl alcohol are able to transfer the allyl group to a strongly nucleophilic anion such as the thiol ion (Albert, 1965). Preliminary reports of some of this work have appeared (Clapp *et al.*, 1969; Kaye & Young, 1970, 1972).

Experimental

Materials

All melting and boiling points are uncorrected. Elementary analyses were carried out by the Microanalytical Laboratory, School of Pharmacy, London W.C.1, U.K. (see Table 1). Allyl alcohol, acrolein, allyl acetate, diallyl phthalate and 1,2-epoxypropane were purchased from BDH Chemicals Ltd., Poole, Dorset, U.K. Allyl formate and allyl propionate were purchased from Kodak Ltd., Kirkby, Liverpool, U.K.; allyl stearate and allyl benzoate were purchased from K & K Laboratories Inc., Plainview, N.Y., U.S.A.; triallyl phosphate was purchased from R. N. Emanuel Ltd., Wembley, Middx., U.K. All the liquid compounds, except 1,2-epoxypropane, were redistilled before use. Diazomethane was prepared by the reaction between alcoholic KOH heated to 65°C and an ethereal solution of Diazald (*N*-methyl-*N*-nitrosotoluene-*p*-sulphonamide) which was purchased from R. N. Emanuel Ltd.

Preparation of sodium allyl sulphate. Chlorosulphonic acid (2ml) was added slowly to dry pyridine (100ml) cooled in an ice-bath, and allyl alcohol (1.9ml) was added with stirring to the mixture, which was then removed from the ice-bath. On the following day the mixture was again cooled and 60% (w/v) NaOH was added with stirring until the apparent pH of the pyridine layer had risen to 8. The pyridine layer was removed, evaporated to a small volume, 10vol. of acetone was added and the mixture was left at 5°C overnight. The crystals that had formed were filtered off and recrystallized from hot propan-1-ol. After washing with acetone and drying over P_2O_5 *in vacuo* the crystals weighed 1.5g (Found: C, 23.0; H, 3.4; ester sulphate S, 20.1; $C_3H_5NaO_4S$ requires C, 22.5; H, 3.1; ester sulphate S, 20.0%).

Preparation of allyl nitrate. The method used was based on that described by Ferris *et al.* (1953) for the general preparation of alkyl nitrates. Allyl bromide (8.5ml) was added to a cooled solution of $AgNO_3$ (18.7g) in dry acetonitrile (30ml). The mixture was shaken, left for 24h at room temperature, and the precipitate of AgBr was removed by filtration. The filtrate was poured into 1 litre of ice-cold water, and the

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Table 1. *Properties of the synthetic mercapturic acids and some related compounds*

Compound	M.p. (°C)	$[\alpha]_D^{20-25}$	Elementary analysis: Found (%) C	H	N	S	Required (%) C	H	N	S
Allylmercapturic acid	124	−33±2*	47.2	6.3	6.8	15.9	47.3	6.5	6.9	15.8
Dicyclohexylammonium allylmercapturate	165	+4±1*	62.1	9.6	7.4	8.2	62.4	9.4	7.3	8.3
Dicyclohexylammonium allylmercapturate sulphoxide	157	+10±3*	59.5	9.4	7.3	7.9	60.0	9.1	7.0	8.0
S-Allylglutathione	195–196	−22±2*	44.5	6.2	11.6	9.2	45.0	6.1	12.1	9.2
S-Allyl-L-cysteine	222–224	−8±2†	44.5	7.1	8.9	20.2	44.7	6.9	8.7	19.9
Dicyclohexylammonium 2-hydroxypropylmercapturate	167	−5±1‡	59.4	9.4	7.1	7.9	59.7	9.5	7.0	8.0
Dicyclohexylammonium 2-hydroxy-1-methylethylmercapturate	166	+6±2†	59.3	9.4	6.9	8.0	59.7	9.5	7.0	8.0
Dicyclohexylammonium 3-hydroxypropylmercapturate	176	−4±1†	59.6	9.1	6.6	7.9	59.7	9.5	7.0	8.0
S-(3-Hydroxypropyl)-L-cysteine	197–201	−32±3†	40.1	7.3	7.9	18.3	40.2	7.3	7.9	17.9

* *c* 1 in ethanol; † *c* 1 in water; ‡ *c* 1.5 in water.

mixture was extracted with methylene chloride (3×50ml). After the extract had been dried over anhydrous Na_2SO_4, the methylene chloride was removed by distillation at 40°C and the allyl nitrate, obtained by fractional distillation at 75–78°C/260mmHg (0.35×10^5 Pa), weighed 5.8g (Found: C, 35.4; H, 4.7; N, 13.1; $C_3H_5NO_3$ requires C, 35.0; H, 4.9; N, 13.6%).

Preparation of allyl nitrite. The method of preparation was based on that described by Noyes (1943) for the general preparation of alkyl nitrites. A solution of 38g of $NaNO_2$ in 150ml of water was cooled to 0°C and to it was added slowly with stirring a mixture of water (10ml), conc. H_2SO_4 (13.6ml) and allyl alcohol (34.1ml) at 0°C. The alcohol mixture was added over a 1h period so that hardly any gas was evolved and the temperature of the reaction mixture remained almost constant. The mixture was kept at 0°C until it had separated into two layers, the greenish upper layer being the allyl nitrite. This was removed and washed several times with 10ml portions of a solution of $NaHCO_3$ (2g) and NaCl (25g) dissolved in water (100ml), yielding a yellowish liquid which was dried over anhydrous Na_2SO_4 and redistilled under slightly reduced pressure. The product was extremely volatile and lachrymatory, and distilled at 41–42°C/660mmHg (0.88×10^5 Pa), which was indicative of it being an alkyl nitrite, for these compounds in general have low boiling points, e.g. propyl nitrite boils at 47°C/760mmHg (1.01×10^5 Pa). The boiling point of the allyl nitrite at 760mmHg (1.01×10^5 Pa) was not determined because of the risk of explosion, and density determinations as well as elementary analysis could not readily be performed because of its extreme volatility. The product had properties consistent with it being allyl nitrite and it gave positive reactions in tests for nitrite esters with diphenylamine and conc. H_2SO_4 (Feigl, 1954*a*), and for allyl compounds with phloroglucinol and conc. HCl (Feigl, 1954*b*).

Allylmercapturic acid, allylmercapturic acid sulphoxide and its dicyclohexylammonium salt, S-allylglutathione, S-allyl-L-cysteine and dicyclohexylammonium 3-hydroxypropylmercapturate. These compounds were prepared as described by Kaye *et al.* (1972).

Preparation of dicyclohexylammonium allylmercapturate. Allylmercapturic acid (0.23g) was dissolved in acetone (10ml) and dicyclohexylamine (0.23ml) was added slowly with stirring. After 30min at 5°C the crude dicyclohexylammonium salt which had precipitated was filtered off and dissolved in hot ethanol. Crystallization occurred after the addition of 3vol. of hot acetone and more crystals were obtained by adding a further portion of acetone to the mother liquor and standing at 5°C. After drying over P_2O_5 *in vacuo* a total yield of 0.24g was obtained.

Dicyclohexylammonium 2-hydroxypropylmercapturate, dicyclohexylammonium 2-hydroxy-1-methylethylmercapturate and S-(3-hydroxypropyl)-L-cysteine. These compounds were prepared by the method of Barnsley (1966) without separation of the (+)- and (−)-isomers of the first two compounds.

S-(2-Hydroxypropyl)-L-cysteine. This compound was a gift from Dr. E. A. Barnsley (Department of

Biochemistry, Memorial University of Newfoundland, St. John's, Newfoundland, Canada).

Methods

Animals and dosing. Male albino rats of the CFE strain (supplied by the Michael and Sidney Herbert Research Laboratories of St. Thomas's Hospital Medical School, London SE1 7EH, U.K.), body weight 200–250g, were used throughout the work. The animals were fed on rat cakes (Spiller's Autoclaved Small Animal Diet) and had access to water at all times. They were housed in metabolism cages which permitted the collection of urine separately from faeces. When the urine of rats dosed with an allyl compound was to be examined by paper chromatography the compound was administered to the animals as follows. Allyl alcohol, allyl formate, allyl acetate, allyl propionate, allyl benzoate, allyl nitrite and acrolein were each injected subcutaneously into the lumbar region as 1% (v/v) solutions in arachis oil. Diallyl phthalate was injected as a 2% (v/v) solution in arachis oil, allyl nitrate as a 5% (v/v) solution in arachis oil and triallyl phosphate as a 10% (v/v) solution in arachis oil. Allyl stearate (0.060g) was liquefied by warming before its injection into rats and sodium allyl sulphate was administered as a 6% (w/v) solution in water. *S*-(3-Hydroxypropyl)-L-cysteine was administered subcutaneously as an aq. 10% (w/v) suspension. In all cases except allyl stearate, the rats were given 1ml of one of these mixtures. Allyl propionate and triallyl phosphate were also injected intraperitoneally or given by stomach tube. While being dosed the rats were lightly anaesthetized with ether and their urine was collected for two successive 24h periods immediately after dosing.

Experiments with rats fed with ^{35}S-labelled yeast. ^{35}S-labelled yeast was prepared from a medium containing [^{35}S]sulphate (Knight & Young, 1958) and it was fed to rats as a 5% (w/w) mixture with the diet described by Maw (1953). The urine was chromatographed in the four solvent mixtures *A*, *B*, *C* and *D* (see below) and the chromatograms were examined for ^{35}S-labelled compounds with a Packard model 7200 radiochromatogram scanner.

Chromatography. Chromatograms were developed overnight on Whatman 3MM paper by the descending method. The following solvent mixtures were used: *A*, butan-1-ol–water–acetic acid (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–3M-NH_3 soln. (4:3:3, by vol.); *D*, butan-2-one–2-methylpropan-2-ol–water–diethylamine (10:10:5:1, by vol.). The R_F values of the reference compounds in these four solvent mixtures are given in Table 2. The platinum reagent of Toennies & Kolb (1951) as modified by Barnsley *et al.* (1964) was used to detect sulphur-containing compounds, and amino acids were detected on the same chromatogram with the ninhydrin reagent (Smith, 1969). A dipping procedure was used for applying both reagents. Hydroxyalkylmercapturic acids were converted into their methyl esters by the action of diazomethane in ether, and methanolic solutions of these esters were examined, as described by James *et al.* (1967), by g.l.c. with a Perkin–Elmer F-11 gas chromatograph, which was equipped with a dual flame ionization detector. The columns used were stainless-steel coils 2m long and

Table 2. *R_F values and retention of some of the synthetic compounds*

The chromatography solvents *A*, *B*, *C* and *D* and the g.l.c. columns I and II shown are those described in the section on 'Chromatography'.

Compound	R_F values in solvent mixtures				Retention time of methyl esters (min)	
	A	*B*	*C*	*D*	Column I at 185°C	Column II at 210°C
Allylmercapturic acid*	0.88	0.61	0.62	0.69		
Allylmercapturic acid sulphoxide †	0.67	0.42	0.42	0.40		
S-Allylglutathione‡	0.40	0.11	0.19	0.22		
S-Allyl-L-cysteine‡	0.59	0.46	0.52	0.56		
2-Hydroxypropylmercapturic acid*	0.78	0.48	0.49	0.52	3.2	7.5
2-Hydroxy-1-methylethylmercapturic acid*	0.80	0.51	0.51	0.55	3.6	8.0
3-Hydroxypropylmercapturic acid*	0.78	0.48	0.49	0.51	5.0	11.1
S-(2-Hydroxypropyl)-L-cysteine‡	0.37	0.26	0.34	0.34		
S-(3-Hydroxypropyl)-L-cysteine‡	0.37	0.26	0.34	0.34		

* Compound detected as a white spot with the platinum reagent.
† Compound detected as a yellow spot on heating the chromatogram dipped in the platinum reagent.
‡ Compound detected as a white spot with the platinum reagent and as a blue spot with ninhydrin.

2.2mm internal diameter, and they were packed with acid-washed silanized Chromosorb G coated with 2.5% silicone gum rubber E301 (column I) or with 5% fluorosilicone oil FS-1265 (column II) which were supplied by Perkin–Elmer Ltd., Beaconsfield, Bucks., U.K. The retention times of the methyl esters are shown in Table 2.

Determination of ester sulphate sulphur. A solution of the sulphate ester (about 0.020g) in 10ml of 1M-HCl was evaporated to dryness on a boiling-water bath and the residue was dissolved in water. The sulphate was precipitated as the benzidine salt, which was separated and titrated with 20mM-NaOH solution as described by Hawkins & Young (1954).

Deacetylation of mercapturic acids. Mercapturic acids in the urine of dosed animals were deacetylated by adjusting samples of the urine to pH7 and incubating them at 37°C for 48h with pig acylase I, which had been purchased from Sigma Chemical Co., St. Louis, Mo., U.S.A.

Cannulation of the bile duct of a rat dosed with allyl acetate. The bile duct of a rat was cannulated by Dr. D. J. Riches, Department of Anatomy, St. Thomas's Hospital Medical School, by a method similar to that described by Boyland *et al.* (1961). The upper part of the bile duct was intubated so as to avoid contamination with pancreatic juice, and the bile was collected by passing the cannula into a glass saddle-shaped vessel (Van Zyl, 1958) strapped with adhesive tape to the back of the animal. The rat was housed in a metabolism cage which allowed the separate collection of urine and faeces, and the animal had access at all times to a 2.5% (w/v) solution of glucose in 0.9% (w/v) NaCl. Urine and bile were collected for 24h immediately before the subcutaneous injection of 1ml of a 2% (v/v) solution of allyl acetate in arachis oil, and thereafter for two successive 24h periods.

Isolation procedures. The urine was made just acid to Congo Red by the addition of conc. HCl and the acidified urine was shaken for 1h periods with five separate portions of butan-2-one saturated with water, each portion being equal to three times the volume of urine. The butan-2-one extracts were concentrated to a gum, which was dissolved in a small volume of 0.1M-sodium formate buffer, pH4.0, and the solution was chromatographed on a column of Amberlite CG 400 (formate form). The column was washed with the buffer, fractions (15ml) were collected, and every tenth fraction was examined for the presence of mercapturic acids by subjecting 100μl of it to descending paper chromatography in solvent mixture *B* followed by dipping the dried chromatogram in the platinum reagent.

When fractions were shown to contain an hydroxypropylmercapturic acid, they were pooled and desalted by passage through a column of Zeo-Karb 225 (H^+ form). The eluate was collected until its pH had risen to 5, and was then evaporated to dryness on a rotary film evaporator at 40–50°C under reduced pressure. The residue was dissolved in water and the solution was left under reduced pressure over NaOH for a few days. The solution was evaporated to dryness and re-evaporated several times after addition of acetone. The residue was treated with acetone and the precipitate which formed on standing for 10min was removed by centrifuging. A 2% (v/v) solution of dicyclohexylamine in acetone was added to the acetone supernatant until the apparent pH of the mixture had risen to 7. The precipitate that formed overnight at 5°C was centrifuged off, washed with acetone and recrystallized from the minimum volume of hot ethanol by the addition of excess of acetone. Samples for g.l.c. were obtained from the acidified urine of dosed rats by the above method except that the amounts used were much smaller. After standing over NaOH under reduced pressure for several days the aqueous solution containing the hydroxypropylmercapturic acid was evaporated to dryness and the residue was dissolved in methanol to be methylated.

When fractions eluted from the Amberlite column were shown to contain allylmercapturic acid they were treated as described above for fractions containing a hydroxypropylmercapturic acid, except that in some cases crystals of allylmercapturic acid formed while the aqueous solution was left under reduced pressure over NaOH. When this occurred the crystals were removed, washed with a little ice-cold water, and if coloured they were recrystallized from hot acetone. The aqueous mother liquor was combined with any washings and the allylmercapturic acid remaining in this mixture was recovered as its dicyclohexylammonium salt.

Results

Chromatographic studies

When the urines of rats that had been dosed with the allyl compounds were examined by paper chromatography in the four solvent mixtures described above the following results were obtained. No mercapturic acids were detected in samples other than those excreted in the first 24h after dosing. Allylmercapturic acid was found in the urine of rats injected with triallyl phosphate, sodium allyl sulphate or allyl nitrate. Also detected in these urines were allylmercapturic acid sulphoxide and a hydroxypropylmercapturic acid. This last compound was also detected in the urines of rats dosed with allyl alcohol, allyl formate, allyl acetate, allyl propionate, allyl stearate, allyl benzoate, diallyl phthalate, allyl nitrite, acrolein and *S*-(3-hydroxypropyl)-L-cysteine, but allylmercapturic acid and its sulphoxide were not detected in these urines. The same results as above were obtained on paper-chromatographic examination of the urine of rats dosed with allyl propionate

or triallyl phosphate by intraperitoneal injection or by stomach tube, as well as by subcutaneous injection.

Further evidence for the presence of allylmercapturic acid and a hydroxypropylmercapturic acid in the urines of rats dosed with sodium allyl sulphate or allyl nitrate was obtained after these urines had been treated with acylase I. Under these circumstances paper chromatography followed by application of the platinum reagent revealed two compounds as white spots with R_F values, in the four solvent mixtures previously described, corresponding to those given by *S*-allyl-L-cysteine and *S*-(2- or 3-hydroxypropyl)-L-cysteine. These spots became blue when the chromatograms were dipped in the ninhydrin reagent and then heated. When this procedure was adopted for samples of urine excreted by rats dosed with allyl stearate, diallyl phthalate, allyl nitrite or acrolein only one such spot was revealed and this corresponded to *S*-(2- or 3-hydroxypropyl)-L-cysteine.

In all cases the hydroxypropylmercapturic acid metabolite was further examined by obtaining a sample of a purity such that when methylated it could satisfactorily be examined by g.l.c. In every case the methylated metabolite had the same retention time as the methyl ester of 3-hydroxypropylmercapturic acid. No evidence was obtained for the presence of the methyl ester of either of its two isomers, namely 2-hydroxypropylmercapturic acid or 2-hydroxy-1-methylethylmercapturic acid. On adding the methyl ester of synthetic 3-hydroxypropylmercapturic acid to the methylated metabolite no new peak was obtained on the gas chromatograms with either column. When the methyl ester of either of the other two hydroxymercapturic acids was added to the methylated metabolite, however, an extra peak was obtained. To show that this result was not a necessary consequence of the method used, exactly the same procedure was adopted for the urine excreted by five rats each dosed subcutaneously with 1ml of a 2% (v/v) solution of 1,2-epoxypropane in arachis oil. In this case the metabolite was shown by g.l.c. to be 2-hydroxypropylmercapturic acid, i.e. the same result as Barnsley (1966) obtained by other methods.

Experiments with rats fed with ^{35}S-labelled yeast

Paper chromatograms of the urine of rats fed with ^{35}S-labelled yeast and dosed with allyl alcohol, allyl acetate, allyl stearate or just arachis oil alone were run in the four solvent mixtures. The dried chromatograms were scanned for ^{35}S and in all cases the R_F values of the radioactive peaks, not present in the control urine, supported the results obtained by detection of sulphur-containing compounds with the platinum reagent. No further sulphur-containing metabolites of these three allyl compounds were detected on the radiochromatograms.

Isolation studies

3-Hydroxypropylmercapturic acid was shown to be excreted by rats dosed with allyl alcohol, all the esters of allyl alcohol studied, acrolein and *S*-(3-hydroxypropyl)-L-cysteine, but the isolation of this metabolite, as its dicyclohexylammonium salt, was not attempted for all these compounds. Allylmercapturic acid was shown to be excreted by rats when they were dosed with triallyl phosphate, sodium allyl sulphate or allyl nitrate, and with these compounds attempts to isolate this metabolite from the urine were

Table 3. *Amounts of 3-hydroxypropylmercapturic acid and allylmercapturic acid isolated from the urine of rats dosed with some allyl compounds or with S-(3-hydroxypropyl)-L-cysteine*

For experimental details see the text.

Compound administered	No. of rats	Total dose (g)	3-Hydroxypropylmercapturic acid isolated (g)	Allylmercapturic acid isolated (g)	Percentage conversion
Allyl alcohol	72	0.613	0.255*		6.3
Acrolein	144	0.606	0.457*		10.5
Allyl formate	80	0.758	0.242*		6.8
Allyl propionate	90	1.500	0.480*		9.1
Triallyl phosphate	50	5.377		0.579	3.9
Sodium allyl sulphate	40	8.000		0.404†	4.0
Allyl nitrate	100	5.380	0.555*	0.628†	2.6, 5.9
S-(3-Hydroxypropyl)-L-cysteine	6	1.200	0.472*		17.4

* Isolated entirely as its dicyclohexylammonium salt, and this is the weight given.

† Isolated in part as the free acid and in part as its dicyclohexylammonium salt; the value given is corrected so as to give the actual weight of the mercapturic acid present in the isolated material.

made. The amounts of 3-hydroxypropylmercapturic acid and allylmercapturic acid isolated from the urine of rats after they had been dosed with the allyl compounds are shown in Table 3 (3-hydroxypropylmercapturic acid was always isolated as its dicyclohexylammonium salt, whereas the allylmercapturic acid was isolated partly as the free acid and partly as its dicyclohexylammonium salt for two of the three compounds). In every case the identity of the isolated product was confirmed by its m.p. and by its mixed m.p. with the appropriate synthetic material and by its i.r. spectrum obtained as a 1% KBr disc. The chromatographic properties, specific optical rotation and elementary analysis of every specimen of mercapturic acid isolated were also determined and were found to be in agreement with those given by authentic samples and with theoretical values, and provided confirmatory evidence of identity. Elementary analysis does not distinguish dicyclohexylammonium 3-hydroxypropylmercapturate from dicyclohexylammonium 2-hydroxypropylmercapturate or dicyclohexylammonium 2-hydroxy-1-methylethylmercapturate, and so g.l.c. of the methyl ester of the isolated mercapturic acid was also used to identify the 3-hydroxypropylmercapturic acid. The amounts of allylmercapturic acid and 3-hydroxypropylmercapturic acid present in the urine of the dosed rats may well have been substantially more than was indicated by the amounts isolated, for the chief purpose of the isolation studies was the separation of the mercapturic acids in pure form. When these two mercapturic acids were isolated as dicyclohexylammonium salts from normal rat urine to which they had been added, 57.7% of the 3-hydroxypropylmercapturic acid and 55.8% of the allylmercapturic acid was recovered by the isolation procedure.

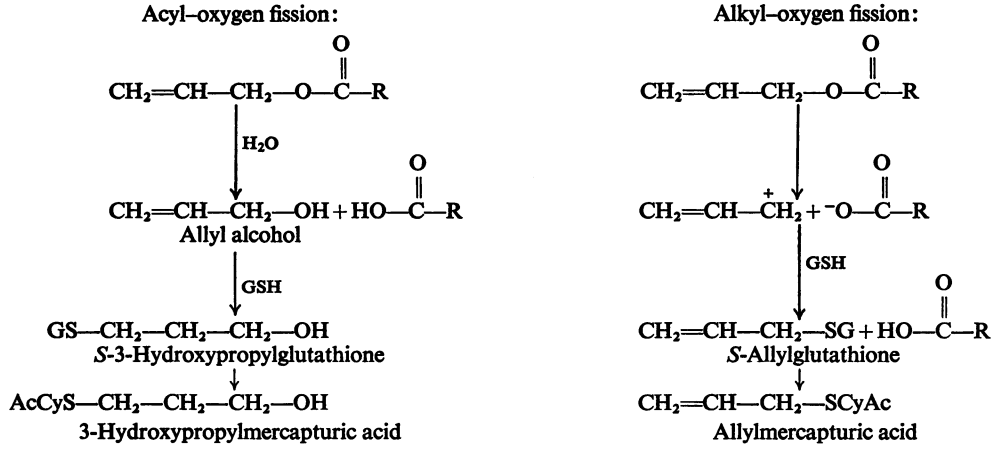
Chromatographic examination of the bile and urine of a rat dosed with allyl acetate

Samples of the bile obtained before and after dosing a rat subcutaneously with 1ml of a 2% (v/v) solution of allyl acetate in arachis oil were examined by paper chromatography in the four solvent mixtures *A*, *B*, *C* and *D*. The platinum reagent revealed the presence of three compounds in the bile collected 24h after administering allyl acetate which were not present before dosing. Two of these compounds gave positive ninhydrin reactions, one of which had R_F values corresponding closely to those given by *S*-(3-hydroxypropyl)-L-cysteine. The third compound was present in very small amount; it gave no reaction with ninhydrin and its R_F values corresponded closely to 3-hydroxypropylmercapturic acid. The urine excreted by the rat in the 24h period immediately after dosing was shown by paper chromatography to contain a compound, revealed by the platinum reagent but not by ninhydrin, which had the same R_F values as 3-hydroxypropylmercapturic acid in solvent mixtures *A*, *B*, *C* and *D*.

Discussion

The isolation of 3-hydroxypropylmercapturic acid (as its dicyclohexylammonium salt) from the urine of rats dosed with allyl alcohol represents the first example of the isolation of a mercapturic acid from the urine of animals dosed with a non-halogenated aliphatic alcohol. The failure to detect allylmercapturic acid as a metabolite of allyl alcohol suggests that allyl alcohol is not metabolized by alkylation of glutathione with the allylic double bond remaining intact. Boyland & Chasseaud (1966, 1967, 1968) have demonstrated the enzymic conjugation of glutathione with a number of $\alpha\beta$-unsaturated carbonyl compounds *in vitro*, including diethylacetal, which is related to acrolein (allyl aldehyde), and this raises the possibility that a derivative of allyl alcohol, containing a carbonyl moiety, such as acrolein, is the molecule that conjugates with glutathione. (Being a very reactive molecule acrolein would probably react with glutathione non-enzymically as well as enzymically.) Support for this possibility is provided by the isolation of 3-hydroxypropylmercapturic acid, as its dicyclohexylammonium salt, from the urine of rats dosed subcutaneously with acrolein. Also, it is known from experiments *in vitro* that allyl alcohol is oxidized by NAD^+ and an alcohol dehydrogenase present in rat liver (Serafini-Cessi, 1972) and there are grounds therefore for believing that this might occur *in vivo*. It has been suggested by Rees & Tarlow (1967) that the hepatotoxicity produced by treating rats with allyl formate is due to the conversion of this allyl ester into acrolein via allyl alcohol. The presence of the double bond in allyl alcohol appears to be required if a mercapturic acid metabolite is to be formed *in vivo*, for it was not possible to detect a mercapturic acid in the urine of rats dosed with propane-1,3-diol or 1,3-diacetoxypropane (Kaye, 1971). The presence of the carbonyl moiety in acrolein would probably polarize the $\alpha\beta$ double bond such that conjugation would occur by addition of the nucleophilic glutathione anion to the β-carbon atom of the allylic double bond of acrolein. This process is likely to be catalysed by one of the many glutathione *S*-alkenetransferases described by Boyland & Chasseaud (1968). At some stage during the conversion of the glutathione conjugate into a mercapturic acid, reduction of the carbonyl moiety to an alcoholic group would need to occur since the metabolite excreted is 3-hydroxypropylmercapturic acid. Evidence for a similar mechanism in the metabolism of crotyl alcohol and crotonaldehyde has been obtained by Gray & Barnsley (1971).

The type of fission undergone *in vivo* by the esters



Scheme 1. *Two possible metabolic pathways of organic esters of allyl alcohol*

Similar metabolic pathways can be drawn for the inorganic esters of allyl alcohol. Abbreviation: AcCyS; *N*-Acetyl-L-cysteine.

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of allyl alcohol may be deduced from the mercapturic acid excreted in the urine (see Scheme 1). If allylmercapturic acid is a metabolite of the allyl ester then alkyl–oxygen fission of the ester is likely to have occurred, whereas if 3-hydroxypropylmercapturic acid is a metabolite then acyl–oxygen fission is likely to have occurred. Of the esters of allyl alcohol studied only three were metabolized to allylmercapturic acid and these were triallyl phosphate, sodium allyl sulphate and allyl nitrate. Thus only these three compounds exhibited the property, suggested by Albert (1965), that esters of allyl alcohol are able to transfer an allyl group to a strongly nucleophilic anion, such as the thiol ion.

The formation of allylmercapturic acid from triallyl phosphate *in vivo* is not altogether unexpected, for a number of phosphotriesters have been shown by Hutson *et al.* (1968) to alkylate glutathione *in vitro*, and Jackson & Jones (1968) and Jones & Jackson (1969) have demonstrated that the rat metabolizes a number of trialkyl phosphates to dialkyl phosphates and the corresponding *S*-alkyl-L-cysteines.

It has been shown (Woodman & Young, 1971) that sodium *n*-butyl sulphate, *n*-pentyl sulphate and *n*-hexyl sulphate are metabolized by rats to their corresponding alkylmercapturic acids. These biosynthetic reactions are unlikely to proceed via the corresponding *n*-alkyl alcohols because these compounds were not metabolized to mercapturic acids. Clapp & Young (1970) isolated benzylmercapturic acid and 1-menaphthylmercapturic acid from the urine of rats dosed with sodium benzyl sulphate and sodium 1-menaphthyl sulphate. These biosynthetic reactions could proceed via the corresponding alcohols, since evidence has been obtained that benzyl alcohol is metabolized to benzylmercapturic acid (A. R. Morrison & L. Young, unpublished work) and that 1-menaphthyl alcohol is metabolized to 1-menaphthylmercapturic acid (Clapp & Young, 1970), although the results of Gillham (1971) suggested that the alcohols were not intermediates in the conversion of these aralkyl sulphate esters into their corresponding mercapturic acids. The result obtained in the present work with sodium allyl sulphate suggests that the corresponding alcohol, allyl alcohol, is not involved in the metabolism of this sulphate ester to its corresponding mercapturic acid, allylmercapturic acid, since this mercapturic acid was not detected in the urine of rats dosed with the alcohol.

Triallyl phosphate, sodium allyl sulphate and allyl nitrate were also shown in the present work to be metabolized to 3-hydroxypropylmercapturic acid. The results obtained do not distinguish between the various pathways by which this may occur. The situation is similar to that found in the metabolism of allyl halides (Kaye *et al.*, 1972) where these compounds were found to be metabolized to allylmercapturic acid and 3-hydroxypropylmercapturic acid. The formation of these two mercapturic acids from allyl halides and allyl esters derived from strong acids might be expected to occur because these molecules may form transient carbonium ions which can react with OH^- and GS^-. Since the hydrolysis of esters into their corresponding alcohols is a well recognized reaction *in vivo*, the pathway involving allyl alcohol as an intermediate in the metabolic conversion of

Table 4. *Summary of the formation of allylmercapturic acid and 3-hydroxypropylmercapturic acid from the allyl esters studied*

+, Present; −, not detected.

Allyl ester administered	Acid from which the ester is derived	pK_a* value of the acid	Mercapturic acid present in the urine of the dosed rats: Allylmercapturic acid	Mercapturic acid present in the urine of the dosed rats: 3-Hydroxypropylmercapturic acid
Allyl nitrate	Nitric acid	0	+	+
Sodium allyl sulphate	Sodium hydrogen sulphate	1.9	+	+
Triallyl phosphate	Diallylphosphoric acid	<2.1†	+	+
Diallyl phthalate	Monoallylphthalic acid	<2.9†	−	+
Allyl nitrite	Nitrous acid	3.4	−	+
Allyl formate	Formic acid	3.8	−	+
Allyl benzoate	Benzoic acid	4.2	−	+
Allyl acetate	Acetic acid	4.7	−	+
Allyl propionate	Propionic acid	4.9	−	+
Allyl stearate	Stearic acid	‡	−	+

* Values given in the *Handbook of Chemistry and Physics* (The Chemical Rubber Co.).
† See the text.
‡ No value given, although Jukes & Schmidt (1935) estimated a value of 4.95.

triallyl phosphate, sodium allyl sulphate and allyl nitrate into 3-hydroxypropylmercapturic acid appears to be the most likely. It seems probable that this process is independent of the route of administration of the ester, for the same chromatographic results were obtained when triallyl phosphate was administered to rats by subcutaneous or intraperitoneal injection, or by stomach tube.

The failure to detect allylmercapturic acid in the urine of rats dosed with allyl formate, allyl acetate, allyl propionate, allyl stearate, allyl benzoate, allyl nitrite and diallyl phthalate suggests that these esters of allyl alcohol do not undergo alkyl–oxygen fission *in vivo*. A possible pathway by which these esters are metabolically converted into 3-hydroxypropylmercapturic acid might involve allyl alcohol and *S*-(3-hydroxypropyl)-L-cysteine as intermediates. Support for this pathway comes from the findings that allyl alcohol itself and its esters are metabolized to the same mercapturic acid, 3-hydroxypropylmercapturic acid, and that paper-chromatographic evidence was obtained for the presence of *S*-(3-hydroxypropyl)-L-cysteine in the bile of a rat dosed with allyl acetate. It seems probable that the metabolic conversion of allyl esters into 3-hydroxypropylmercapturic acid is independent of the route of administration of the ester, for the urine obtained from rats dosed with allyl propionate by subcutaneous or intraperitoneal injection, or by stomach tube, when examined by paper chromatography revealed the same results after application of the platinum reagent.

A summary of the results obtained for the excretion of mercapturic acids in the urines of rats dosed with the esters of allyl alcohol studied is given in Table 4. The esters are listed in order of the increasing pK_a values of the acids from which the esters can be said to be derived. With two esters, however, namely triallyl phosphate and diallyl phthalate, assigning a pK_a value to the acid raises some difficulty. For triallyl phosphate the acid esterifying allyl alcohol would be diallylphosphoric acid, the pK_a of which is not known. Kumler & Eiler (1943), however, have determined the pK_a values for a number of dialkyl esters of phosphoric acid and have shown them to range from 1.3 for dimethylphosphoric acid to 1.7 for di-*n*-butylphosphoric acid. Thus each of the dialkylphosphoric acids (dimethyl-, diethyl-, di-*n*-propyl- and di-*n*-butylphosphoric acid) is a stronger acid than phosphoric acid itself (pK_a 2.1). Diallylphosphoric acid is therefore likely to have a pK_a value of less than 2.1. By applying a similar argument to monoallylphthalic acid, the acid from which diallyl phthalate can be said to be derived, the pK_a value of this acid is likely to be less than the pK_a value of phthalic acid itself, which is 2.9. From Table 4 it can be concluded that, with the esters of allyl alcohol studied, alkyl–oxygen fission of the ester occurs *in vivo*, as indicated by the excretion of allylmercapturic acid in the urine of dosed rats, only when the acid from which the ester can be said to be derived is a strong acid with a pK_a value of less than about 2.0.

I thank Professor L. Young for his interest and advice. I am also grateful to Dr. D. H. Riches of the Department of Anatomy, St. Thomas's Hospital Medical School, for cannulating the bile duct of a rat, and to Mr. A. R. Morrison for some technical guidance.

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