The Metabolism of Acetamidothiazoles in the Rat

2-ACETAMIDO-4-CHLOROMETHYLTHIAZOLE

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2-Acetamido-4-chloromethylthiazole is metabolized in the rat to (2-acetamido-4-thiazolylmethyl)mercapturic acid and 2-acetamidothiazole-4-carboxylic acid. 2-Acetamido-4-methylthiomethylthiazole and the corresponding sulfoxide and sulphone are also produced as minor metabolites. The identification of the metabolites is described and their formation investigated. Quantitative results on the excretion of the metabolites labelled with ^{14}C are reported.

In the preceding paper we described studies on the metabolism and excretion, by the rat, of some 4-substituted 2-acetamidothiazoles (Chatfield & Hunter, 1973). These compounds were part of a series of anti-inflammatory compounds (Evans, 1972) of which the most active member was the 4-chloromethyl compound (I). The metabolic fate of this compound was therefore of interest and particularly the extent to which the reactive chloromethyl group might act as the centre for metabolic attack.

All the acetamidothiazoles that we had previously investigated were metabolized by extensive fission and degradation of the thiazole ring but none of them contained a chemically reactive group.

Materials and Methods

The reagents used, the extraction methods, the radioisotope methods and spectroscopic methods were as described by Chatfield & Hunter (1973).

T.l.c. was conducted on 20cm $\times$ 20cm plates, 0.25 or 2.00mm thick, coated with silica gel G, fluorescent (E. Merck, Darmstadt, Germany). Plates were

developed for 15cm in benzene–methanol (9:1, v/v) R_F values are shown in Table 1.

Sulphur-containing compounds were detected in t.l.c. by the method described by Knight & Young (1958). Thiols were detected by spraying the plates with *N*-ethylmaleimide (Block *et al.*, 1958). Sulphoxides were detected with hydriodic acid–starch reagent (Thompson *et al.*, 1963) and the amino acids and aminothiazoles with ninhydrin reagent (0.5%, w/v in acetone).

Preparation of compounds

2-Acetamido-4-chloromethyl[2- ^{14}C]thiazole (I). This was prepared (Bhargava & Singh, 1960) from thio[^{14}C]urea (570mg, 0.05mCi). The product, yield 970mg, had a specific radioactivity of 15.2 $\mu\text{Ci/g}$ and was 99.2% radiochemically pure on t.l.c.

S-(2-Acetamido-4[2- ^{14}C]thiazolylmethyl)-*N*-acetyl-L-cysteine (II). This was prepared by treating sodium (33mg), ethanol (15ml) and *N*-acetyl-L-cysteine (123mg) with 2-acetamido-4-chloromethyl[2- ^{14}C]thiazole (I) (144mg). The mixture was kept at 20°C

(IV) R = HS—CH₂—

(VI) R = HO—CH₂—

(VIII) R = Me—SO₂—CH₂—

(III) R = Me—S—CH₂—

(V) R = HO₂C—

(VII) R = Me—SO—CH₂—

for 16h, then the solvent was evaporated and the residue dissolved in 0.1 M-NaOH (5ml). The solution was acidified and the product was extracted with ethyl acetate (4 × 10ml). Evaporation of the solvent gave a crystalline material that was recrystallized from acetone–water (3:1, v/v), yield 150mg, m.p. 230–232°C. The i.r. spectrum and the n.m.r. spectrum (Table 2) were consistent with the structure (II) proposed for this compound.

The product was 92% radiochemically pure by t.l.c. and contained 6% of 2-acetamido-4-hydroxymethyl-[2-¹⁴C]thiazole produced by hydrolysis of the chloromethyl compound.

2-Acetamido-4-methylthiomethylthiazole (III). A solution of sodium (1.15g, 50mmol) in ethanol (100ml) was saturated with methanethiol. 2-Acetamido-4-chloromethylthiazole (9.5g, 50mmol) was added, then the solution was heated under reflux for 3h. The solvent and excess of MeSH were removed by distillation and the solid residue was crystallized from ethanol, m.p. 120–122°C, yield 4.1g (Found: C, 41.7; H, 5.1; N, 13.9; C₇H₁₀N₂OS₂ requires C, 41.6; H, 5.0; N, 13.9%).

2-Acetamido-4-methylsulphinylmethylthiazole (VII). This was prepared by oxidation of compound (III) (3.4g, 20mmol) in acetic acid (50ml) with H₂O₂

Table 1. *R_F* values, spectral data and % excretion of 2-acetamido-4-chloromethylthiazole and its metabolites

R_F values refer to t.l.c. on silica gel G, fluorescent plates (Merck) 0.25 or 2.0mm thick in benzene–methanol (9:1, v/v).

Compound	pH of extraction	<i>R_F</i>	$\lambda_{\max.}$ in water (nm)		% of dose
			Neutral	Alkaline	
2-Acetamido-4-chloromethylthiazole (I)	—	0.89	267	288	0
2-Acetamido-4-hydroxymethylthiazole (VI)	—	0.27	269	287	0
2-Acetamido-4-methylthiomethylthiazole (III)*	7.0	0.81	271	290	2
2-Acetamido-4-methylsulphonylmethylthiazole (VIII)*	7.0	0.40	268	289	2
2-Acetamido-4-methylsulphinylmethylthiazole (VII)*	7.0	0.21	269	289	11
<i>S</i> -(2-Acetamido-4-thiazolylmethyl)- <i>N</i> -acetyl-L-cysteine (II)	2.0	0.05	272	290	42
2-Acetamidothiazole-4-carboxylic acid (V)*	2.0	0	270	268	18

* Confirmed by identification with authentic specimen.

Table 2. *N.m.r.* spectra in C²HCl₃ of 2-acetamido-4-chloromethylthiazole and its metabolites

Compound	Chemical shift (τ)	Multiplicity	Exchange with ² H ₂ O	Number of protons	Assignment
2-Acetamido-4-chloromethylthiazole (I)	7.75	Singlet	None	3	—CO—Me
	5.49	Singlet	None	2	—CH ₂ Cl
	3.11	Singlet	None	1	H—C(=S)—
2-Acetamido-4-methylthiomethylthiazole (III)	7.93	Singlet	None	3	Me—S—
	7.76	Singlet	None	3	—CO—Me
	6.32	Singlet	None	2	—X—CH ₂ —
	3.25	Singlet	None	1	H—C(=S)—
	3.07	Singlet	None	1	H—C(=S)—
<i>S</i> -(2-Acetamido-4-thiazolylmethyl)- <i>N</i> -acetyl-L-cysteine (II)	8.13	Singlet	None	3	—CO—Me
	7.87	Singlet	None	3	—CO—Me
	7.1–7.4	Doublet	None	2	—CH ₂ —S—
	6.25	Singlet	None	2	—CH ₂ —S—
	5.59	Multiplet	Triplet	1	>CH—
	3.07	Singlet	None	1	H—C(=S)—
	1.85	Doublet	Rapid	1	—NH—CO—
	5.70+1.66	Broad	Rapid	2	>NH and —CO ₂ H

(2.35 ml of 30% w/v, 20 mmol) for 48 h at 20°C. The unchanged H_2O_2 was removed by SO_2 and the residue was crystallized from ethanol–light petroleum (b.p. 60–80°C) (9:1, v/v) m.p. 159–161°C (Found: C, 38.9; H, 4.9; N, 12.8; $\text{C}_7\text{H}_{10}\text{N}_2\text{O}_2\text{S}_2$ requires C, 38.5; H, 4.6; N, 12.8%).

2 - Acetamido - 4 - methylsulphonylmethylthiazole (VIII). This was prepared similarly, from compound (III) (3.4 g, 20 mmol), acetic acid (50 ml) and H_2O_2 (12 ml of 30% w/v, 100 mmol). The product, a mixture of the sulphone (VIII) and the corresponding *N*-oxide, was crystallized three times from methanol to give pure compound (VIII), m.p. 205–206°C, yield 1.0 g (Found: C, 36.1; H, 4.5; N, 12.1; $\text{C}_7\text{H}_{10}\text{N}_2\text{O}_3\text{S}_2$ requires C, 35.9; H, 4.3; N, 12.0%). 2-Acetamidothiazole-4-carboxylic acid was prepared as described by Traumann (1960) and 2-acetamido-4-hydroxymethylthiazole as described by Chatfield & Hunter (1973).

Reverse isotope-dilution analysis

Urine (2 ml) containing 2-acetamido[2- ^{14}C]thiazole-4-carboxylic acid formed metabolically from 2-acetamido-4-chloromethyl[2- ^{14}C]thiazole was diluted 1:5 with water and authentic 2-acetamidothiazole-4-carboxylic acid (500 mg) was added. The suspension was warmed to 60°C and the acid was dissolved by addition of 5 M-KOH. The solution was cooled and the carboxylic acid precipitated by addition of 5 M-HCl. The precipitate was filtered off, washed with 0.1 M-HCl (5 × 2 ml) and the dissolution, precipitation and filtration steps were repeated twice more. The sample of acid was dried *in vacuo* and its specific radioactivity was determined as 28.0 nCi/g. It was then crystallized from ethanol–dimethylformamide (3:1, v/v) and had a specific radioactivity of 29.8 nCi/g.

Glutathione conjugation studies

These were conducted by incubating the appropriate substrates (3 mm in 0.2 ml of ethanol) with GSH (5 mm) and soluble liver fraction (2.0 ml) made up to 6.0 ml with sodium–potassium phosphate buffer (pH 8.0). Incubation was conducted for 1 h at 37°C and then terminated by addition of acetic acid (1.0 ml).

Control incubations were run from which GSH, substrate or liver fraction were omitted. In other experiments GSSG, L-cysteine or *N*-acetyl-L-cysteine replaced GSH.

The organic materials were adsorbed from the solutions with charcoal (75 mg) and eluted with 10 ml of methanol–benzene– NH_3 (sp.gr. 0.880) (90:7:3, by vol.). The eluate was evaporated and the residue chromatographed by t.l.c. on silica gel in butan-1-ol–acetic acid–water (12:1:2, by vol.).

Isolation of metabolites

2-Acetamido-4-chloromethylthiazole was administered intraperitoneally to four male Lodgement Wistar rats (200 g) for 3 days at a daily dose of 100 mg/kg. Urine was collected over 72 h and extracted at pH 7 and at pH 2, with ethyl acetate. The neutral extract contained three metabolites and two further metabolites were in the acidic extract. T.l.c. and u.v. data for these are in Table 1.

The neutral metabolites were isolated by chromatography on a silica gel column eluted with benzene–methanol (3:1, v/v) which was gradually changed to 100% methanol. Preparative t.l.c. as in Table 1 gave pure samples of the metabolites.

The metabolites from the acid extraction were separated by ion-exchange chromatography on Permutit Zeo-Carb 225 cation-exchange resin (Chatfield & Hunter, 1973) to give white needles that were crystallized from methanol–water (3:1, v/v), yield 27 mg, m.p. 230°C decomp. The other metabolite was purified by preparative t.l.c. on silica gel eluted with butan-1-ol–acetic acid–water (12:1:2, by vol.), R_f 0.32.

The relative proportions of the metabolites were established by administration of 2-acetamido-4-chloromethyl[2- ^{14}C]thiazole to rats as single doses of 100 mg/kg orally, intraperitoneally or subcutaneously.

Results

2-Acetamido-4-chloromethylthiazole was rapidly absorbed by rats, 75% of the dose appearing in the urine and 10% in the faeces in the first 24 h. A further 7% of the dose appeared over the next 72 h. There was no evidence of $^{14}\text{CO}_2$ in the exhaled air.

A similar pattern of excretion was observed after administration by the intraperitoneal and subcutaneous routes.

T.l.c. separation showed that none of the original compound was excreted unchanged and that five metabolites were formed.

The i.r. spectrum of the major metabolite, R_f 0.05 (Table 1), suggested that it contained a carboxyl group (1850–2600 cm^{-1} , 1705 cm^{-1}) and a secondary amide group (1640 cm^{-1} and 1690 cm^{-1}). The presence of both was verified by the n.m.r. spectrum (Table 2) which indicated that the metabolite was the mercapturic acid conjugate of compound (I). The i.r. spectrum was identical with that of authentic compound (II).

The second metabolite extracted at pH 2 was also a carboxylic acid and was shown by t.l.c., u.v. and i.r. spectra, to be 2-acetamidothiazole-4-carboxylic acid (V). The presence of this acid in the urine was confirmed by reverse isotope-dilution analysis. This showed that compound (V) was indeed a metabolite and over a 24 h period accounted for 18.7% of an oral

The minor metabolite of R_F 0.81 gave an i.r. spec-

Table 4. *Metabolites of S-(2-acetamido-4[2-¹⁴C]thiazolymethyl)-N-acetyl-L-cysteine (II) on administration to rats at 100mg/kg orally*

R_F values refer to t.l.c. on silica gel G, fluorescent plates (Merck), 0.25 mm thick in butan-1-ol-acetic acid-water (12:1:2, by vol.).

Metabolites of compound (II) identified	R_F	% of dose
Unchanged compound (II)	0.47	47
2-Acetamido-4-methylthiomethylthiazole (III)	0.89	1
2-Acetamido-4-methylsulphonylmethylthiazole (VIII)	0.72	4
2-Acetamido-4-methylsulphinylmethylthiazole (VII)	0.54	11
2-Acetamidothiazole-4-carboxylic acid (V)	0.32	7
Total recovery in 24h urine sample		70

Discussion

Formation of mercapturic acids from reactive alkyl and aralkyl halides is well known (Bray *et al.*, 1958) so the large proportion of compound (II) formed was expected.

The conversion of the chloromethyl group into a methylthiomethyl group is, we believe, a novel route of metabolism and may proceed from the breakdown of the mercapturic acid. The mercapturic acid (II) is presumably formed by breakdown of the glutathione conjugate (Bray *et al.*, 1959). We were able to demonstrate the formation of the corresponding glutathione conjugate *in vitro*, by using the soluble fraction of liver homogenate to catalyse the reaction between compound (I) and GSH. No compound (II) was formed in this reaction.

Trace amounts of the glutathione conjugate were formed without the presence of the liver soluble fraction, so it is possible for chemical reaction alone to account for some of the glutathione conjugate formed.

The mercapturic acid (II), however, was not formed either by enzymic or by direct chemical reaction when compound (I) was incubated with *N*-acetyl-L-cysteine, with or without the soluble liver fraction.

The further metabolism of the major metabolite, compound (II), gave the three minor metabolites compounds (III), (VII) and (VIII) identified by their u.v. spectra and R_F values (Table 4). The relative proportions of these metabolites in the urine from rats dosed with the mercapturic acid conjugate (II) were very similar to those of the metabolites of the original chloro compound (I) if a correction was applied for the direct hydrolysis of the latter compound.

The occurrence of the methylthio compound (III) as a metabolite of the mercapturic acid was confirmed also by its isolation from urine of rats that had received unlabelled compound (II). The i.r. spectrum of this metabolite was identical with that of an authentic specimen of compound (III).

We could not detect any *S*-glucuronide formation in these experiments, as might be expected if the thiol compound (IV) were an intermediate in the conversion of compound (II) into compound (III).

The formation of thiol compounds during the metabolism of thiazoles may account for some of the actions of thiazole drugs. Thus the generation of the thiourylene grouping $>N-CS-N<$ *in vivo* as a common feature of the metabolism of acetamidothiazoles (Chatfield & Hunter, 1973) could well explain the anti-thyroid action of such compounds (Bovet *et al.*, 1946; Liberti & Stanbury, 1971).

Similarly, the formation of thiol-containing metabolites such as compound (IV), even if only transient, may be significant in the anti-inflammatory action of thiazoles such as compound (I). The thiol-disulphide equilibrium during the maintenance and suppression of the inflammatory response has been studied by Butler *et al.* (1969) and Gerber *et al.* (1967) have discussed the effect of protein thiol concentrations on the development of arthritic lesions.

The metabolism of other thiazoles that are devoid of anti-inflammatory action may well provide some basis for assessing this hypothesis.

The metabolism of 2-acetamido-4-chloromethylthiazole, which contains the chemically reactive chloromethyl group, shows that the oxidative metabolism of thiazole compounds that we have reported previously can be modified considerably if reactive substituents are present.

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