RESEARCH PAPERS

THE METABOLISM OF 5-*p*-AMINOBENZENESULPHONAMIDO-3-METHYLISOTHIAZOLE (SULPHASOMIZOLE)

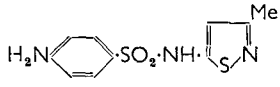
BY J. W. BRIDGES AND R. T. WILLIAMS

From the Department of Biochemistry, St. Mary's Hospital Medical School, University of London, London, W.2.

Received March 15, 1963

The fate of the sulphonamide drug, sulphasomizole, in man, dog, rabbit and rat has been studied. In man, rat and rabbit, the major urinary metabolite, is N^4-acetylsulphasomizole together with the unchanged drug. In man about 60 per cent of an oral dose of 30 mg./kg. is excreted in the urine in 24 hr., just over a third being acetylated. In the rabbit about 80 per cent of an oral dose (150 mg./kg. is excreted in 24 hr. and about two-thirds is acetylated. In the rat about 70 per cent of the dose (150 mg./kg.) is excreted in 24 hr. and just under one third is acetylated. In the dog, the main excretory product is the unchanged drug, there being no acetylation. All four species excrete small amounts (1 per cent) of the N^4-glucuronide of sulphasomizole. Other minor metabolites detected were the N^4-sulphate of sulphasomizole which was found in rat and dog urine, and an unidentified oxidation product present as a glucuronide which was detected in rabbit and dog urine.

5-*p*-AMINOBENZENESULPHONAMIDO-3-METHYLISOTHIAZOLE (I) is an antibacterial drug containing a new heterocyclic system, namely the isothiazole or 1,2-thiazole ring system. This compound and some of its derivatives were first described by Adams and Slack (1959) and its antibacterial properties were reported by Adams and others (1960).



I

The compound is marketed as a sulphonamide drug of moderate duration of action under the name of "Bidizole". The substance has been given the approved common name, sulphasomizole.

EXPERIMENTAL

Materials

Sulphasomizole, m.p. 189°, 5-*p*-aminobenzenesulphonacetamido-3-methylisothiazole (N^1-acetylsulphasomizole), m.p. 168–169°, 5-amino-3-methylisothiazole, m.p. 41–42°, 4-amino-3-hydroxybenzenesulphonic acid, m.p. 266° (decomp.) and 4-amino-2-hydroxybenzenesulphonic acid were the gifts of May & Baker, Ltd., Dagenham, Essex. 5-*p*-Acetamidobenzenesulphonamido-3-methylisothiazole (N^4-acetylsulphasomizole), m.p. 272–273° and 5-acetamido-3-methylisothiazole, m.p. 179–180°, were prepared by acetylating the corresponding amines in sodium carbonate solution with acetic anhydride (cf. Adams and Slack, 1959).

Sulphasomizole N^4*-glucuronide.* A solution of sulphasomizole (9·42 g.) in dimethylformamide (20 ml.) was mixed with a solution of sodium glucuronate (6·9 g.) in ethylene glycol (30 ml.). The resulting solution was adjusted to pH 3–4 with glacial acetic acid (0·5 ml.) and then heated for 10 min. at 70°. The solution was kept in the dark for 24 hr. at room temperature. The crystals (4·3 g.), which had separated, were filtered, washed with dimethylformamide and dried with acetone followed by ether. They were purified by dissolving in 0·02N ammonium hydroxide (10 ml.), adding acetone (25 ml.) and keeping at 0° overnight (yield, 2·9 g.). The compound decomposed on heating to 150–200° and showed $[\alpha]_D^{20}$ — 65·9° (*c*, 1 in 0·02N NH_4OH) which remained constant over 24 hr. It analysed as the *sodium salt* of 5-p-*aminobenzenesulphonamido*-3-*methylisothiazole* N^4*-glucosiduronic acid monohydrate.* (Found: Na, 4·9; H_2O, 3·7 (drying *in vacuo* at 78°); $C_6H_{10}O_7$ (glucuronic acid), 37·0; $C_{10}H_{11}O_2N_3S_2$ (sulphasomizole), 55·1 per cent. $C_{16}H_{18}O_8N_3S_2Na$, H_2O requires Na, 4·7; H_2O, 3·7; $C_6H_{10}O_7$, 40·0; $C_{10}H_{11}O_2N_3S_2$, 55·5 per cent). The compound reduced Fehling's solution on warming, gave an intense naphthoresorcinol test for uronic acids, and a test for aromatic amines on diazotising in acid solution and coupling with *N*-1-naphthylethylenediamine. It was unstable in dilute acid, i.e. below pH 7, but stable in dilute alkali, i.e. above pH 7 up to 2N sodium hydroxide.

Sulphasomizole N^4*-sulphate.* Chlorosulphonic acid (1·3 ml.) was added slowly to pyridine (20 ml.) cooled in ice. To the solution, sulphasomizole (5·38 g.) was added with stirring until the drug had dissolved. After keeping the mixture at room temperature for 48 hr., it was poured into aqueous potassium hydroxide solution (5·6 g. KOH in 100 ml. water). The alkaline solution was now extracted with ether (5 × 100 ml.) to remove pyridine and concentrated under reduced pressure at 45° to 3 ml. The concentrate was then banded on Whatman seed test paper (1·6 mm. thick) and irrigated for 60 hr. by ascending chromatography with the solvent system n-butanol: ammonia solution (s.g. 0·88): water (4:1:5, by vol.). The portion of the paper which gave a negative *p*-dimethylaminocinnamaldehyde test, a positive diazo test (see p. 567) and a positive test for sulphate only after hydrolysis, was cut out of the chromatogram and eluted with 0·05N potassium hydroxide. The eluate was decolourised by heating with charcoal and filtered. To the filtrate, there was added slowly 1 litre of acetone, and on standing white crystals (0·86 g.) separated. The crystals were collected and purified by dissolving in water (3 ml.) and precipitating with acetone (500 ml.) (yield, 0·68 g.). The *dipotassium salt* of 5-(*p-sulphoaminobenzenesulphonamido*)-3-*methylisothiazole* was very soluble in water and crystallised as a monohydrate. (Found: K, 17·85; sulphamate-S, 6·9; sulphasomizole, $C_{10}H_{11}O_2N_3S_2$, 61·15 per cent. $C_{10}H_9O_5N_3S_3K_2$, H_2O requires K, 17·6; sulphamate-S, 7·2; sulphasomizole, 60·7 per cent).

Methods

Free and total aromatic amines in urine. These were determined by the method of Bratton and Marshall (1939). Urine containing 20–150 mg.

of sulphasomizole/100 ml. was diluted with water so as to contain between 20–40 μg./ml. Free aromatic amine (which included any N^4-glucuronide or N^4-sulphamate) in this diluted urine was determined directly by the Bratton and Marshall method. The recovery of sulphasomizole added to urine (20–100 mg./ml.) was 99–100 per cent. For total aromatic amine, the diluted urine (2 ml.) was mixed with 2N hydrochloric acid (0·5 ml.) and heated on a boiling water-bath for 1 hr. and the total aromatic amine determined by the Bratton and Marshall method. The recovery of N^4-acetylsulphasomizole added to urine (40–100 mg./100 ml.) was 99–100 per cent.

Determination of metabolites on strip chromatograms. Paper chromatography of urines of animals receiving sulphasomizole revealed the presence of up to five metabolites. Two of these were major metabolites and were isolated and identified as sulphasomizole and N^4-acetylsulphasomizole; the other three were minor products, one of which was identified as sulphasomizole N^4-glucuronide and the other two suspected to be the N^4-sulphamate and an oxidation product containing a diazotisable amino-group.

The urine (0·2–0·4 ml.) was banded across a piece of Whatman No. 31 paper (12 inches wide). The paper was chromatographed for 5 hr. with a mixture of n-propanol and ammonia solution (s.g. 0·88), in the proportion of 7:3. The appropriate strips (position determined with reference spots of the known compounds) of the paper were cut out and eluted with water (1–2 ml.) in "eluting tubes" (made by Howard Rawson Ltd.). Sulphasomizole, its N^4-glucuronide, N^4-sulphamate and suspected oxidation product were determined directly on the eluates by the Bratton and Marshall method. The eluate of the N^4-acetylsulphasomizole was hydrolysed with acid as before and the liberated amine determined. Tests on various other parts of the paper showed that no other metabolites related to sulphasomizole were present.

Determination in faeces. Faeces from rats and rabbits dosed with sulphasomizole were also analysed. The faeces were homogenized with water and the homogenate centrifuged. Free and total aromatic amines were determined on the supernatant. The recovery of sulphasomizole added to faeces (10–40 mg. to 20 g. wet faeces) was 72–86 per cent. Chromatography of faecal extracts showed that small amounts of sulphasomizole and its N^4-acetyl derivative only were present.

Chromatography

The R_F values of sulphasomizole and its possible metabolites are shown in Table I.

The details of the colour tests were as follows.

Diazo test. The paper was sprayed with 1 per cent sodium nitrite solution which had been freshly mixed with 0·5 vol. of 2N hydrochloric acid. After 3 min. the paper was sprayed with 0·1 per cent solution of *N*-1-naphthylethylenediamine dihydrochloride in water. With this reagent aromatic amines and the N^4-glucuronide and N^4-sulphamate show up as red or red-purple spots.

TABLE I

CHROMATOGRAPHY AND COLOUR REACTIONS OF SULPHASOMIZOLE AND ITS POSSIBLE METABOLITES

Descending chromatography on Whatman No. 4 paper was used. The solvent systems were, A, n-propanol:ammonia solution (s.g. 0·88) (7:3); B, ethanol: n-butanol:ammonia solution (s.g. 0·88):water (12:4:1:1); C, n-butanol: acetic acid:water (4:1:5); D, n-butanol:water (1:1); E, ethyl methyl ketone:water:acetic acid (200:100:1); F, ethyl methyl ketone:ammonia solution (s.g. 0·88) (200:1). The proportions of solvents are by volume. Chromatograms in solvents A, B, C, and D were run for 7 hr. and in E and F for 2 hr.

Compound	R_F values in solvent						Colour reaction‡		
	A	B	C	D	E	F	Dimethylamino-cinnamaldehyde	Diazo test	Fluorescence u.v.
4-Amino-3-hydroxybenzene-sulphonic acid	0·19	0·45	0·06	—	0·01	0·93	red	red	violet
4-Amino-2-hydroxybenzene-sulphonic acid	0·36	0·50	—	—	0·04	0·91	red	red	violet
Sulphanilic acid	0·39	0·52	0·09	0·87	0·89	0·83	red	red- purple	violet
Sulphasomizole	0·69	0·72	0·84	0·84	0·94	0·89	red	red-purple	weak violet
N^4-Acetylsulphasomizole	0·79	0·83	0·82	0·86	0·93	0·90	none	none	quench
N^1-Acetylsulphasomizole	0·86	0·74	0·80	—	0·91	0·90	red	orange-red	quench
Sulphasomizole N^4-glucuronide*	0·08	0·10	—†	0·08	—†	0·09	red slowly	red slowly	weak violet
N^4-Sulphosulphasomizole	0·36	0·32	0·22	—	0·43	0·01	red very slowly	red very slowly	none
5-Amino-3-methyl-isothiazole	0·85	0·84	0·85	0·69	0·04	0·91	red	red	weak violet
5-Acetamido-3-methyl-isothiazole	0·70	0·86	0·83	—	0·94	0·93	none	none	quench

* Gives naphthoresorcinol test.
† Hydrolysed to free amine in these solvents.
‡ See text.

p-*Dimethylaminocinnamaldehyde test.* A 1 per cent solution of *p*-dimethylaminocinnamaldehyde in ethanol was used. This solution was acidified with 0·1 vol. of 2N hydrochloric acid just before spraying the paper. Aromatic amines and urea show up immediately as red spots. The weak N^4-conjugates (glucuronide and sulphamate) show up as red spots more slowly (5–10 min. for glucuronide, 20–60 min. for sulphamate), whilst the red spot due to urea turns yellow on keeping (0·5–1 hr.). If 2N acetic acid is used instead of hydrochloric acid, the weak conjugates do not show up with the reagent.

Glucuronide test. The paper was sprayed with a freshly prepared 1 per cent aqueous solution of naphthoresorcinol in 20 per cent aqueous trichloroacetic acid and then heated in an oven at 140° for 10 min. Easily hydrolysable glucuronides show up as blue spots.

Ultra-violet light. The paper was examined with ultra-violet light of wavelength 254 mμ from a Hanovia "Chromatolite" lamp. Some compounds showed a weak violet fluorescence, others, particularly the N^4-acetylsulphasomizole, quenched the background fluorescence of the paper and showed up as dark spots.

Ultra-violet and infra-red absorption and fluorescence spectra were determined with the Unicam spectrophotometer S.P. 500, the Perkin-Elmer infracord Spectrometer and the Aminco-Bowman Spectrophoto-fluorometer, respectively.

Detection and Isolation of Metabolites

Isolation of sulphasomizole and its N^4*-acetyl derivative.* A chinchilla rabbit (4·3 kg.) was fed with 2 g. of sulphasomizole. The 24-hr. urine was brought to pH 10 with 2N ammonium hydroxide, filtered by suction, and evaporated to 5 ml. at 40–45° under pressure. The concentrated urine was banded on heavy Whatman No. 3 MM paper and chromatographed with solvent A (propanol: ammonia solution, see Table I), with reference spots of the drug and its N^4-acetyl derivative suitably placed on the paper. When the chromatogram had developed, strips corresponding to these two compounds were cut out and eluted with acetone. The sulphasomizole fraction on evaporation yielded 0·57 g. of the drug, m.p. 189–190°, mixed m.p. 190–191°, after recrystallisation from methanol and water (1:1). It was further identified by ultra-violet absorption spectra (see Table II).

TABLE II

ULTRA-VIOLET ABSORPTION SPECTRA OF SULPHASOMIZOLE AND SOME OF ITS DERIVATIVES

Compound	Absorption maxima in			
	0·1N HCl		Water or 0·1N NaOH*	
	λ max	ε max $^{10-3}$	λ max	ε max $^{10-3}$
Sulphasomizole†	245	9·3	257	14·8
	301	10·7	280	14·7
N^4-Acetylsulphasomizole†	254	16·7	255	16·8
	305	9·1	275–276	13·5
5-Amino-3-*methylisothiazole*† ..	230	7·0	235	5·4
	281	12·7	262	7·7

* The pK_a of the sulphonamide group of sulphasomizole is 5·03 and of N^4-acetylsulphasomizole is 4·9 (Dr. P. O. Kane of May & Baker, Ltd.). Therefore, these compounds are ionised in water.

† These compounds did not fluoresce in aqueous solution when examined in the Aminco-Bowman spectrofluorimeter. Sulphanilamide in aqueous solution is fluorescent (fluorescence maximum, 350 mμ; excitation, 275 mμ) (Bridges and Williams, 1962b).

The N^4-acetyl fraction was evaporated and the crystals were purified by dissolution in dilute sodium hydroxide and precipitation by acidification with 2N hydrochloric acid. The N^4-acetylsulphasomizole had m.p. 271–272° and mixed m.p. 271–272° and had ultra-violet and infra-red spectra identical with a synthetic sample (see Table II).

Both these compounds were isolated similarly from the urines of man and rat, after administration of the drug, but only the free drug was isolated from dog urine.

Detection of sulphasomizole N^4*-glucuronide.* The N^4-glucuronide of sulphasomizole was detected chromatographically using for solvents (A, B, D and F, Table I) in human, dog, rabbit and rat urine, as a minor metabolite. The spot corresponding to this compound gave the naphthoresorcinol reaction. The diazo and cinnamaldehyde tests were given slowly indicating a weak *N*-conjugate. On eluting the spot with water and chromatographing the eluate with the acid solvents C and E (Table I), spots with R_F corresponding to sulphasomizole were found. In solvent E a spot (R_F 0·02) corresponding to glucuronic acid was also found. Aqueous

eluates of the glucuronide spot were not hydrolysed by snail β-glucuronidase at pH 7, although the same enzyme solution was shown to be effective in hydrolysing the *O*-glucuronide, *o*-aminophenylglucuronide, at the same pH (it is to be noted that the optimum pH of β-glucuronidase is 5·2, but at this pH the N^4-glucuronide is unstable). It is believed that β-glucuronidase does not hydrolyse N^4-glucuronides (Bridges and Williams, 1962a; see also Axelrod, Inscoe and Tompkins, 1957).

N^4-Sulphosulphasomizole. A spot with similar R_F values and reactions to the synthetic N^4-sulphosulphasomizole was found in the urine from all rats (3) and dogs (2) tested, from 3 human urines out of 5, and one rabbit urine out of 4. These spots were eluted in appropriate experiments and estimated.

The possible oxidation product. A minor diazotisable spot with the R_F values of 0·4 in solvent A and 0·27 in solvent B was found in all the rabbit and dog urines examined and in one human urine out of five, but in none of the rat urines. The spot gave immediate tests for aromatic amine and a positive naphthoresorcinol test for glucuronic acid. This material was prepared by the elution with water of the appropriate area from a large number of preparative chromatograms of the urine of rabbits receiving sulphasomizole (total, about 8 g.) and concentration of the eluate. Attempts to isolate it were unsuccessful. However, the compound was stable to boiling for 0·5 hr. with 2N sodium hydroxide. It was hydrolysed by boiling with 0·1N hydrochloric acid for 0·5 hr. to yield a diazotisable compound of R_F 0·47 in solvent B.

Quantitative Results

Rabbits. The results for rabbits are shown in Table III. The drug is excreted in the urine mainly as sulphasomizole and its N^4-acetyl derivative. The acetyl derivative accounts for about ⅔ of the drug excreted in the first 24-hr. after dosing. The two ways of estimating the acetylated amine,

TABLE III

THE FATE OF SULPHASOMIZOLE IN THE RABBIT

Sulphasomizole administered orally; the figures quoted are per dose excreted in 24 hr. after dosing.

	Rabbit No.	1*	2*	3*	4†
	Dose, mg./kg.	150	150	150	750
	"Free" amine by direct determination	25·2	19·1	31·5	21·5
	Total amine by direct determination (24 hr. excretion)	93·8	57·0	87·8	63·7
	Acetylated amine (by diff.)	68·6	37·9	56·3	42·2
By analysis of chromatograms of 24 hr. urine	N^4-Glucuronide	1·0	1·0	0·7	0·7
	N^4-Sulphamate	0·0	1·0	0·0	0·0
	"Oxidation product"	1·0	2·9	1·6	1·0
	Free sulphasomizole	21·4	13·6	29·2	21·6
	"Free amine (sum of above 4 items)	23·4	18·5	31·5	23·3
	Acetylated amine	64·2	34·1	51·5	39·5
	Total urinary amine after 48 hr.	99·1	78·0	88·2	65·2
	Total urinary amine after 5 days	99·9	79·0	89·2	69·4
	Total amine in faeces after 5 days	0·0	0·0	0·0	12·4‡
	Total drug accounted for	99·9	79·0	89·2	81·8

* Female chinchilla rabbits.
† Female of mixed breed of unknown origin.
‡ This figure may be the result of contamination of faeces by urine.

i.e. by the difference between "free" and total aromatic amine and by determining the acetylated amine separated by chromatography, agreed with one another. The amounts of "free" amine obtained by two methods also agreed. It will be noted from the Table that the "free" amine is largely sulphasomizole, the rest being made up of small smounts of sulphasomizole N^4-glucuronide (about 1 per cent) and a diazotisable "oxidation product" (1–2 per cent). The presence of the N^4-sulphamate was doubtful. In the chinchilla rabbits about 80 per cent of the dose of 150 mg./kg. was excreted in the first day and most of the drug was excreted by the second day. It seems likely that there is no faecal excretion of the drug in the rabbit.

Rats. The results for female Wistar rats are shown in Table IV. The major metabolites of sulphasomizole in rats are again the free drug and the N^4-acetyl derivative. In 48-hr. about 60–90 per cent of the drug fed was excreted, but the acetylated drug accounted for only about 30 per cent of the material excreted and the main excretory product was unchanged sulphasomizole. No "oxidation product" was detected in rat urine, but the N^4-glucuronide and the "N^4-sulphamate" (each about 1 per cent) was detected in all the urines. Faecal excretion of the drug is uncertain, since the values in Table IV could be due to urinary contamination.

TABLE IV

THE FATE OF SULPHASOMIZOLE IN RATS

Sulphasomizole administered orally at a dose of 150 mg./kg. Results expressed as per cent of dose excreted in 24 hr.

	Rat No.	1	2	3
By analysis of chromatograms of 24 hr. urine	"Free" amine (direct)	—*	49·0	9·1
	Total amine in 24 hr. (direct)	—*	67·8	17·7
	Acetylated amine (by difference)	—*	18·8	8·6
	N^4-Glucuronide	1·2	1·3	0·7
	"N^4-Sulphamate"	1·0	1·6	0·4
	"Oxidation product"	0·0	0·0	0·0
	Free sulphasomizole	19·5	42·0	7·3
	"Free" amine (sum of previous 4 items)	21·7	44·9	8·4
	Acetylated amine	9·3	19·8	4·8
	Total amine excreted in 48 hr.	60·6	88·3	65·7
	(acetylated	22·3	29·0	10·6)
	Total amine excreted in 5 days	60·6	97·3	65·7
	Total amine in faeces in 5 days	19·8†	0·0	29·0†
	Total drug accounted for	80·4	97·3	94·7

* Not determined.
† Possibly due to contamination with urine.

Man. Five healthy male students (19–24 years old) were given tablets of sulphasomizole (dose, 30 mg./kg.) and the 24 hr. urine was concentrated and analysed on paper chromatograms. The results are shown in Table V. The results are fairly consistent, an average of 58 per cent (range 57–62) of the dose was excreted in 24 hr. Just over a third (36 per cent) of the excreted material occurred as the N^4-acetyl derivative and the rest was mainly the unchanged drug. Small amounts of the N^4-glucuronide (0·1–0·9 per cent) were also excreted; traces of the "N^4-sulphamate" were found in three cases, but the oxidation product was not found in most cases.

TABLE V

THE FATE OF SULPHASOMIZOLE IN MAN

Five healthy male subjects took 27–32 mg./kg. of sulphasomizole. The urine was collected for 24 hr., concentrated and chromatographed. The results are expressed as per cent of dose.

	Metabolite	Subjects				
		J.W.B.	J.B.	W.J.	R.W.	P.S.
Fractions obtained chromatographically	N^4-Glucuronide	0·7	0·1	0·1	0·9	0·2
	"N^4-Sulphamate"	0·1	0·0	0·2	0·0	0·1
	"Oxidation product"	0·0	0·0	0·0	0·0	0·1
	Sulphasomizole	40·9	43·2	35·1	30·1	35·1
	N^4-Acetylsulphasomizole	16·6	18·2	24·1	31·8	21·1
	Total in 24 hr.	58·3	61·5	59·5	62·8	56·6
	Total in 48 hr.	76·4	—	—	—	—
		19·5*				

* Acetylated

Dogs. Two male Corgi dogs each received sulphasomizole, 5 g. daily, during chronic toxicity studies. A 24 hr. sample of urine collected on the 1st and 5th day was sent to us by May & Baker, Ltd.

These urines were concentrated as before and chromatographed. The results are shown in Table VI. No acetylation product was found and most of the drug was excreted unchanged, about 36 per cent being eliminated in 24 hr. All the minor metabolites were also found and they amounted to 2–4 per cent of the dose or nearly 10 per cent of the material excreted.

TABLE VI

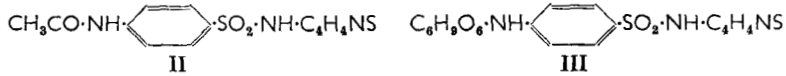
THE FATE OF SULPHASOMIZOLE IN THE DOG (CORGI)

	Metabolite	Dog 1*	Dog 2†
Fractions obtained chromatographically	N^4-Glucuronide	0·3	0·7
	"N^4-Sulphamate"	1·1	1·7
	"Oxidation product"	1·1	2·0
	Sulphasomizole	33·8	31·0
	N^4-Acetylsulphasomizole	0·0	0·0
	Total for 24 hr.	36·3	35·4

* This dog (10 kg.) received 5 g. of the drug, 24 hr. previously.
† This dog (10 kg.) had received 5 g. of the drug daily for 5 days, the urine analysed was for the fifth day.

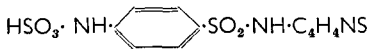
DISCUSSION

In Table VII the data on each species of animal has been averaged and the amount of each metabolite found in the urine has been calculated as a percentage of the total drug excreted. The major transformation product of sulphasomizole is N^4-acetylsulphasomizole (II) and it is clear that the extent of acetylation varies with species. About two-thirds of the excreted drug is acetylated in the rabbit, just over one-third in man, just under one-third in the rat and none at all in the dog. The N^4-glucuronide of sulphasomizole (III) is a minor constituent of the urine and we are of the opinion that it is an artifact and not a true metabolite. Other work carried out in this department (Bridges and Williams, 1962a) on the formation of N^4-glucuronides of aromatic amines strongly suggests that these compounds

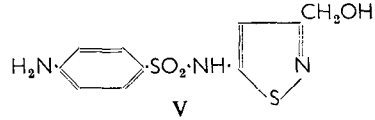


II III

are formed in the urine non-enzymically. The occurrence of an aryl sulphamate (N^4-sulphosulphasomizole, IV) was suspected in rat and dog urine and these compounds (aryl sulphamates) are known metabolites of some aromatic amines *in vivo* (Boyland, Manson and Orr, 1957; Parke, 1960) and *in vitro* (Roy, 1960; 1961). There are also reports in the Japanese literature of the occurrence in urine of the sulphamates of sulphathiazole (Uno and Veda, 1960), sulphisoxazole (Uno and Kono, 1960), and sulfamethylthiadiazole (Uno and Okazaki, 1960). A minor metabolite, which we suspect is the glucuronide of an oxidation product



IV



V

of sulphasomizole, was found by paper chromatography in rabbit and dog urine. This compound did not appear to be a phenolic glucuronide and we suggest that it may be derived from sulphasomizole oxidized at the 3-methyl group (V).

TABLE VII

URINARY METABOLITES OF SULPHASOMIZOLE IN VARIOUS SPECIES

Average values have been calculated from the other Tables

	Chinchilla rabbits	Wistar* rats	Corgi dogs	Man
Number of Animals	3 ♀	3 ♀	2 ♂	5 ♂
Oral dose of drug, mg./kg.	150	150	500	30
Total drug excreted in 24 hr. in per cent of dose	79·5	71·5	35·9	59·7
Metabolites found in urine	Per cent of total drug excreted			
Free drug	26·9	68·1	90·2	61·8
N^4-Acetylated drug	68·3	29·0	0·0	36·8
N^4-Glucuronide	1·1	1·5	1·4	0·7
"N^4-Sulphamate"	0·0	1·4	3·9	0·1
"Oxidation product"	2·2	0·0	4·2	0·0

* The figures for rats are for 48 hr. after dosing.

Acknowledgement. The work was supported by a grant from May & Baker, Ltd. We are grateful to Dr. H. J. Barber, Dr. J. P. Johnson and Dr. R. Slack of May & Baker Ltd., for materials and assistance.

REFERENCES

Adams, A., Freeman, W. A., Holland, A., Hossack, D., Inglis, J., Parkinson, J., Reading, H. W., Rivett, K., Slack, R., Sutherland, R. and Wien, R. (1960). *Nature, Lond.*, **186**, 221–222.
Adams, A. and Slack, R. (1959). *J. chem. Soc.*, 3061–3072.
Axelrod, J., Inscoe, J. K. and Tompkins, M. (1957). *J. biol. Chem.*, **232**, 835–841.
Boyland, E., Manson, D. and Orr, S. F. D. (1957). *Biochem. J.*, **65**, 417–423.
Bratton, A. C. and Marshall, F. K. (1939). *J. biol. Chem.*, **128**, 537–550.
Bridges, J. W. and Williams, R. T. (1962*a*). *Biochem. J.*, **83**, 27P.
Bridges, J. W. and Williams, R. T. (1962*b*). *Nature, Lond.*, **196**, 59–61.
Parke, D. V. (1960). *Biochem. J.*, **77**, 493–503.
Roy, A. B. (1960). *Ibid.*, **74**, 49–56.
Roy, A. B. (1961). *Ibid.*, **79**, 253–261.
Uno, T. and Kono, M. (1960). *J. Pharm. Soc. Japan*, **80**, 201–204.
Uno, T. and Okazaki, Y. (1960). *Ibid.*, **80**, 1682–1686.
Uno, T. and Veda, M. (1960). *Ibid.*, **80**, 1785–1788.