Structure and Species as Factors Affecting the Metabolism of some Methoxy-6-sulphanilamidopyrimidines

By J. W. BRIDGES,* M. R. KIBBY,† S. R. WALKER AND R. T. WILLIAMS
Department of Biochemistry, St Mary's Hospital Medical School, London, W. 2

(Received 5 August 1968)

1. A comparative study was made in man, rhesus monkey, rat and rabbit of the urinary excretion of 2-, 4- and 5-methoxy- and 2,4-, 2,5- and 4,5-dimethoxy-6-sulphanilamidopyrimidines given orally. 2. In the rabbit, 70–80% of the dose of each drug was excreted in 2 days, mainly as *N*⁴-acetyl derivatives, except 2,5-dimethoxy-6-sulphanilamidopyrimidine, which was mainly excreted unchanged. 3. In the rat, 50–70% of the dose of each drug was excreted in 2 days, except the 2-methoxy and 2,4-dimethoxy compounds, whose excretion was about 30%. The *N*⁴-acetyl derivatives accounted for 20–70% of the drugs excreted, except the 2,5-dimethoxy derivative, which was excreted unchanged. 4. In the rhesus monkey, some 40–60% of the dose of the 2-methoxy, 2,4-dimethoxy and 2,5-dimethoxy compounds was excreted in 2 days, but the 4-methoxy, 5-methoxy and 4,5-dimethoxy compounds were excreted at less than half this rate. The 4-methoxy, 5-methoxy and 4,5-dimethoxy compounds were highly acetylated (80–90%) whereas the 2-methoxy compound was poorly acetylated (17%) and the 2,5-dimethoxy compound hardly at all. The major metabolite of the 2,4-dimethoxy compound in the monkey was the *N*¹-glucuronide. 5. In man, 30% of the dose of the 4-methoxy and 2,4-dimethoxy compounds was excreted in 24 hr., whereas the 4,5-dimethoxy compound (Fanasil) was very slowly excreted (12% in 2 days). The 4-methoxy compound was well acetylated (65%), but the 2,4- and 4,5-dimethoxy compounds were not (20–30%). The main metabolite of the 2,4-dimethoxy compound in man was the *N*¹-glucuronide. 6. *N*¹-Glucuronide formation occurred extensively only with the 2,4-dimethoxy compound and only in man and the rhesus monkey. It did not occur in the rabbit and only to a minor extent in the rat. 7. The 2,5-dimethoxy compound was not significantly acetylated *in vivo* in the rabbit, rat or monkey, but acetylation occurred *in vitro* in rabbit or monkey liver homogenates. 8. These findings are discussed.

The newer sulphonamide drugs include several methoxy-6-sulphanilamidopyrimidines such as sulphadimethoxine or Madribon (2,4-dimethoxy), sulphathodimethoxine or Fanasil (4,5-dimethoxy) and sulphamonomethoxine (4-methoxy). In earlier papers (Bridges, Kibby & Williams, 1965; Adamson, Bridges & Williams, 1966; Bridges, Kibby, Walker & Williams, 1968) we found that sulphadimethoxine showed a remarkable species difference in metabolism, since it formed an *N*¹-glucuronide as a major metabolite in man and other primates but not in certain lower animals. In the present paper, the metabolism of 2-, 4- and 5-methoxy- and 2,4-, 2,5- and 4,5-dimethoxy-6-sulphanilamidopyrimi-

dines in four species, man, monkey, rat and rabbit, is described. It is shown that, not only does the metabolism of these compounds vary with species, but also, within a species, with the position of the methoxyl groups attached to the pyrimidine ring.

MATERIALS AND METHODS

Compounds. 2-Methoxy- (m.p. 186–187°), 5-methoxy- (m.p. 200–201°), 2,5-dimethoxy- (m.p. 190–193°), 2,4-dimethoxy- (m.p. 200–201°) and 4,5-dimethoxy-6-sulphanilamidopyrimidine (m.p. 195–197°) were gifts from Roche Products Ltd., Welwyn Garden City, Herts. 4-Methoxy-6-sulphanilamidopyrimidine, m.p. 201–203°, was a gift from Imperial Chemical Industries Ltd., Alderley Park, Cheshire.

The *N*⁴-acetyl derivatives of 2-methoxy- (m.p. 225–227°), 4-methoxy- (m.p. 219–220°; hemihydrate; Found: C, 47.1; H, 4.6; N, 16.9; S, 9.7. Calc. for C₁₃H₁₄N₄O₄S, 0.5H₂O: C, 47.1; H, 4.6; N, 16.7; S, 9.7%), 5-methoxy- (m.p. 215–217°;

* Present address: Department of Biochemistry, University of Surrey, 14 Falcon Road, London, S.W. 11.

† Present address: Department of Biochemistry, University of Strathclyde, Glasgow, C. 1.

hemihydrate; Found: C, 47.1; H, 4.6; N, 16.7; S, 9.5%, 2,4-dimethoxy- (m.p. 201–202°), 4,5-dimethoxy- (m.p. 214–215°; Found: C, 47.7; H, 4.7; N, 16.0; S, 9.0. Calc. for $C_{14}H_{16}N_4O_8S$: C, 47.7; H, 4.6; N, 15.9; S, 9.1%) and 2,5-dimethoxy-6-sulphanilamidopyrimidine (m.p. 158–160°) were prepared by standard methods. These compounds are known and appear in the patent literature.

Sulphamonomethoxine N^1 -glucuronide. Sodium 4-methoxy-6-sulphanilamidopyrimidine (sodium sulphamonomethoxine; 3.16g.) and methyl 2,3,4-tri-*O*-acetyl-1-bromoglucuronate (4.13g.), m.p. 106–109° (Bollenback, Long, Benjamin & Lindquist, 1955), were dissolved in aq. 75% (v/v) acetone (50 ml.) and the solution was kept at 20° in the dark for 6 days. It was then poured on a column (2.5 cm. $\times$ 25 cm.) of activated alumina (type H; 100–200 mesh) (P. Spence Ltd., Widnes, Lancs.), eluted with acetone (200 ml.) and the eluate evaporated to dryness. The product was recrystallized from propan-2-ol to give white needles of methyl 2,3,4-tri-*O*-acetyl-1-deoxy-1-[N^1 -(4-methoxy-pyrimidin-6-yl)sulphanilamidoglucosid]uronate, m.p. 183–184° and $[\alpha]_D^{20} + 68^\circ$ (c 1 in acetone) (Found: C, 47.1; H, 5.0; N, 9.1; S, 5.4; H_2O (loss at 100°), 2.7; $C_{24}H_{28}N_4O_{12}S.H_2O$ requires C, 46.9; H, 4.9; N, 9.1; S, 5.2; H_2O , 2.9%). The ester (0.95g.) in methanol (30 ml.) was treated with 0.5N-barium methoxide in methanol (5 ml.). The mixture was kept at 0° for 7 days and then treated with 2N- H_2SO_4 (1.25 ml.) and water (15 ml.). The precipitated $BaSO_4$ was filtered and the solution banded on Whatman seed-test paper and chromatographed (descending) with propan-2-ol-aq. ammonia (sp.gr. 0.88) (7:3, v/v). The band of R_F 0.4–0.5 was cut out and eluted with 0.05N-ammonia solution. The eluate was rechromatographed and the band of R_F 0.44–0.51 eluted as before and the solution evaporated to dryness *in vacuo* at 45° (0.4g.). The solid was dissolved in methanol (5 ml.) and precipitated with propan-2-ol. The precipitate was recrystallized from methanol to give white needles

(0.23g.) of ammonium 1-deoxy-1-[N^1 -(4-methoxypyrimidin-6-yl)sulphanilamidoglucosid]uronate, m.p. 145–153° and $[\alpha]_D^{20} + 2.3^\circ$ (c 4.5 in water) (Found: C, 41.6; H, 5.0; N, 14.1; S, 6.6; $C_{17}H_{28}N_5O_9S.H_2O$ requires C, 41.6; H, 5.1; N, 14.3; S, 6.5%). The compound did not reduce Fehling's or Benedict's solution.

Sulphorthodimethoxine N^1 -glucuronide. Sodium 4,5-dimethoxy-6-sulphanilamidopyrimidine (sodium sulphorthodimethoxine, 3.6g.) and methyl 2,3,4-triacetyl-1-bromoglucuronate (4.13g.) were dissolved in aqueous acetone as above and kept at 0° for 3 days in the dark. The solution was banded on Whatman seed-test paper and chromatographed (descending) with the propan-2-ol-aq. ammonia solution above and the u.v.-light-absorbing band at R_F 0.82–0.93 was cut out and eluted with acetone. The chromatography and elution were repeated on the acetone eluate, which was then evaporated to dryness under diminished pressure to yield a solid (0.9g.), which on recrystallization from propan-2-ol gave white platelets of methyl 2,3,4-tri-*O*-acetyl-1-deoxy-1-[N^1 -(4,5-dimethoxy-pyrimidin-6-yl)sulphanilamidoglucosid]uronate, m.p. 161–163° and $[\alpha]_D^{20} + 81^\circ$ (c 1 in methanol) (Found: C, 48.0; H, 5.2; N, 8.5; $C_{25}H_{30}N_4O_{13}S$ requires C, 47.9; H, 4.8; N, 8.9%). Attempts to convert this ester with barium methoxide into a crystalline free glucuronide were not successful, but the non-crystalline product was used for chromatography (see Table 1).

Sulphamates. The N^4 -sulphates of the 4-methoxy-, 5-methoxy- and 4,5-dimethoxy-6-sulphanilamidopyrimidines were prepared by the method of Bridges *et al.* (1968). Their dipotassium salts were recrystallized from ethanol. Potassium 4-(4-methoxypyrimidin-6-yl)sulphamoylphenylsulphamate hemihydrate (Found: C, 29.7; H, 2.7; K, 17.6; N, 12.6; $C_{11}H_{10}K_2N_4O_6S_2.0.5H_2O$ requires C, 29.6; H, 2.5; K, 17.5; N, 12.6%), potassium 4-(5-methoxypyrimidin-6-yl)sulphamoylphenylsulphamate (Found: C, 30.3; H, 2.3; K,

Table 1. R_F values and colour reactions of methoxy-6-sulphanilamidopyrimidines

Whatman no. 1 paper with the descending technique was used. The solvent was propan-2-ol-aq. ammonia (sp.gr. 0.88) (7:3, v/v). DMAC, *p*-Dimethylaminocinnamaldehyde reagent; NR, naphtharesorcinol reagent (see Bridges *et al.* 1965); BCSR, barium chloride-sodium rhodizonate spray (Feigl, 1947). — Means no colour reaction.

Position(s) of methoxyl group(s) ... Compound	R_F values						Colour reactions with		
	2	4	5	2,4	2,5	4,5	DMAC	NR	BCSR
Methoxy-6-sulphanilamidopyrimidine (MS)	0.64	0.63	0.68	0.72	0.60	0.56	Purple	—	—
N^4 -Acetyl-MS†	0.76	0.75	0.77	0.84	0.72	0.71	—	—	—
MS N^4 -glucuronide	0.10	0.07	0.08	0.11	0.07	0.09	Purple after 5 min.	Blue	—
MS N^1 -glucuronide	0.35	0.48	*	0.54	*	0.32	Purple	Blue-brown	—
MS N^4 -sulphate	*	0.36	0.30	0.42	0.30	0.20	Purple after 30 min.	—	White on a pink background

* These compounds were not made.

† The acetyl derivatives were detected on paper as dark spots when the paper was illuminated by ultraviolet light (254 m μ).

18.0; N, 12.9; $C_{11}H_{10}K_2N_4O_6S_2$ requires C, 30.3; H, 2.3; K, 17.9; N, 12.8%) and *potassium 4-(4,5-dimethoxy-2-pyrimidin-6-yl)sulphamoylphenylsulphamate* (Found: C, 31.0; H, 2.5; K, 16.7; N, 12.3; $C_{12}H_{12}K_2N_4O_7S_2$ requires C, 30.9; H, 2.6; K, 16.8; N, 12.0%). The N^4 -sulphate of the 2,5-dimethoxy compound was also made, but insufficient was available to purify it fully, so that the slightly impure compound, which contained a little free sulphate, was used for chromatographic purposes.

N^4 -Glucuronides. The N^4 -glucuronides of the methoxy-sulphanilamidopyrimidines were prepared as sodium salts as described by Bridges *et al.* (1965) for that of sulphadimethoxine. The crystalline products obtained were impure because of the lability of the glucuronides, but they could be used for chromatographic purposes. They readily reduced Benedict's solution and were easily distinguishable from the N^4 -glucuronides by paper chromatography (see Table 1).

Animals. The animals used were Chinchilla rabbits, Wistar albino rats (Porton strain) and rhesus monkeys (*Macaca*). The drugs, suspended in water, were administered orally by stomach tube. Human subjects took the drugs in gelatin capsules.

Chromatography. The R_F values and colour reactions of the drugs and their possible metabolites are given in Table 1.

Determination of metabolites. Free and total amines in urine were determined by the Bratton & Marshall (1939) method as described by Bridges *et al.* (1968) for sulphadimethoxine. Recoveries of the drugs and their acetyl derivatives (0.02–1.0 mg./ml.) were satisfactory (99–100%).

Separation of metabolites. Urines (0.1–0.5 ml.) were banded on Whatman no. 1 paper (20 cm. bands), dried and chromatographed with propan-2-ol-aq. ammonia (Table 1) against reference spots of the expected metabolites. The metabolites were located on strips of the paper as quenching spots under u.v. light (254 m μ) from a Hanovia Chromatolite scanner (Engelhard Industries Ltd., Slough, Bucks.). The metabolites were eluted from the appropriate position on the paper with 0.1 N-ammonia and estimated as above. The recoveries of the synthetic compounds (0.1–10 mg.) from paper were 96–100%. The rest of the paper was also ex-

amined, but no free or total diazotizable amine was found in this part of the paper with any of the drugs used.

Solubility and pK_a determinations. The drug or its acetyl derivative was added to 0.1 M-citrate–0.2 M-phosphate buffer (20 ml.) of pH 4.2, 5.2, 6.2, 7.2 or 7.4 in a conical flask which was then shaken for 2 hr. on a Griffin shaking machine (Griffin and George, Wembley, Middx.) at room temperature (20°). The solution was filtered and the sulphonamide in solution determined by the Bratton & Marshall (1939) method. The pH of the solution was checked on a Pye Master pH-meter. pK_a values were calculated from the solubilities at various pH values by the methods of Krebs & Speakman (1945). Partition coefficients were determined by shaking a saturated solution of the sulphonamide in citrate-phosphate buffer, pH 7.4, with an equal volume of $CHCl_3$ for 1 hr. and then estimating the sulphonamide remaining in the buffer.

Acetylation in vitro. Livers were removed from the animals immediately after they had been killed and placed in ice. Portions (10 g.) were homogenized in 0.25 M-sucrose solution (30 ml.) and then centrifuged in an MSE Super-Speed centrifuge at 2000g for 10 min. Incubation mixtures consisted of sulphonamide drug (0.3 μ mole in 1 ml. of 0.1 M-citrate–0.2 M-phosphate buffer, pH 7.4) and the 2000g supernatant (1 ml.) in stoppered centrifuge tubes. The tubes were incubated at 37° for 1 hr. on a shaking water bath. Trichloroacetic acid solution (1 ml. of 20%, w/v) and water (7 ml.) were added to each tube, which was then centrifuged for 5 min. at 2000g. Samples of the supernatant (4 ml.) were withdrawn and free and total amine determined by the Bratton & Marshall (1939) method.

RESULTS AND DISCUSSION

Our interest in the metabolic fate of the methoxy-6-sulphanilamidopyrimidines was stimulated by the finding that the 2,4-dimethoxy derivative (sulphadimethoxine) showed a remarkable species difference in metabolism in that in man and other primates, but not in lower species, the main metabolite

Table 2. *Urinary excretion of methoxy-6-sulphanilamidopyrimidines in various species*

The drugs, suspended in water, were administered orally at a dose of 100 mg./kg. Urine was collected daily and its total aromatic amine content determined. Average values for three animals are given with the standard errors; where no standard error is given only two animals were used.

Species	Days after dosing	Position(s) of methoxyl group(s) ...	% of dose recovered in the urine after the methoxy-6-sulphanilamidopyrimidine					
			2	4	5	2,4	2,5	4,5
Man	1		—	29 $\pm$ 4	—	25 $\pm$ 3*	—	5.5 $\pm$ 0.6†
	2		—	—	—	—	—	12
Monkey	1		57 $\pm$ 3	12 $\pm$ 10	17 $\pm$ 3	42 $\pm$ 4	42 $\pm$ 3	25 $\pm$ 4
	2		59	14†	19 $\pm$ 8	45 $\pm$ 2	44 $\pm$ 6	—
Rat	1		19 $\pm$ 7	40 $\pm$ 12	20 $\pm$ 1	13 $\pm$ 1	58 $\pm$ 12	19 $\pm$ 1
	2		30	65	56	29	68	46
Rabbit	1		67 $\pm$ 8	75 $\pm$ 5	82 $\pm$ 3	43 $\pm$ 5	66 $\pm$ 12	63 $\pm$ 4
	2		71	82	84	73	72	75

* Dose 33 mg./kg.

† Dose 17 mg./kg.

‡ These animals showed oliguria.

Table 3. *Metabolites of methoxy-6-sulphanilamidopyrimidines in urine of various species*

The values given for each metabolite are as percentages of the material excreted in 24 hr. (the values of the 24 hr. excretion in percentages of dose are given in Table 2) and are the averages for three animals. tr. = trace, just detectable in some individuals.

Species	Metabolite determined in urine	Position(s) of methoxyl group(s) ...	% of 24 hr. excretion of methoxy-6-sulphanilamidopyrimidine given					
			2	4	5	2,4	2,5	4,5
Man	Free drug		—	18	—	8	—	56
	<i>N</i> ⁴ -Acetyl		—	65	—	21	—	29
	<i>N</i> ¹ -Glucuronide		—	8	—	62	—	0
	<i>N</i> ⁴ -Sulphate		—	6	—	0	—	9
Monkey	Free drug		76	7	8	6	94	8
	<i>N</i> ⁴ -Acetyl		17	89	86	21	3	85
	<i>N</i> ¹ -Glucuronide		3	2	2	70	1	2
	<i>N</i> ⁴ -Sulphate		0	tr.	3	1	tr.	1
Rat	Free drug		76	54	31	40	99	26
	<i>N</i> ⁴ -Acetyl		20	40	63	46	0	66
	<i>N</i> ¹ -Glucuronide		2	1	1	7	0	2
	<i>N</i> ⁴ -Sulphate		0	2	0	2	0	0
Rabbit	Free drug		33	2	6	1	92	9
	<i>N</i> ⁴ -Acetyl		67	98	92	92	7	89
	<i>N</i> ¹ -Glucuronide		0	0	0	0	0	0
	<i>N</i> ⁴ -Sulphate		0	0	tr.	0	0	0

in urine was a stable glucuronide in which the glucuronic acid residue was attached to the sulphonamide nitrogen atom (Bridges *et al.* 1965, 1968; Adamson *et al.* 1966). As far as man and the rhesus monkey are concerned, the work in this paper shows that the formation of an *N*¹-glucuronide as a major metabolite occurs only with the 2,4-dimethoxy derivative of 6-sulphanilamidopyrimidine and not with the 2-, 4- or 5-methoxy- or the 2,5- or 4,5-dimethoxy derivatives. The only other related compound that forms an *N*¹-glucuronide *in vivo* is 4-methoxy-2-methyl-6-sulphanilamidopyrimidine (sulphamethomidine) (Bridges, Walker & Williams, 1967, 1969).

The urinary excretion of the six methoxysulphanilamidopyrimidines is shown in Table 2. In the rabbit all six are excreted in the urine to the extent of 70–80% of the dose in 2 days. In the rat the excretion is less, amounting to about 29–68% in 2 days, the 2-methoxy (30%) and 2,4-dimethoxy (29%) compounds being the least rapidly excreted. In the monkey, excretion is lower than in the rat, ranging from 14 to 59% in 2 days. The 4-methoxy (14%), 5-methoxy (19%) and 4,5-dimethoxy (25%) derivatives are slowly excreted by the monkey, yet the monkey excretes the 2-methoxy derivative (59%) more rapidly than the rat and at a rate comparable with that by the rabbit (71%). In man, only three of the compounds were studied because of the unknown toxicity of the others for man. 4,5-Dimethoxy-6-sulphanilamidopyrimidine (Fanasil) was very slowly excreted in man, about 5–6% of the dose being excreted in 1 day and only 12% in 2 days.

Table 2 shows that these compounds vary in their rates of excretion, not only with species, but also with the position of the methoxyl groups in the pyrimidine ring in an apparently haphazard manner. The 4,5-dimethoxy compound is excreted slowest in man, the 4- and 5-methoxy compounds in the monkey, and the 2,4-dimethoxy compound in the rat and rabbit.

The rates of excretion could be related to the nature of the metabolites, the percentages of which in a 24 hr. excretion are shown in Table 3. The important metabolites quantitatively are the free drugs, the *N*⁴-acetyl derivatives and the *N*¹-glucuronides. The *N*⁴-sulphates were found only occasionally and in small amounts. The *N*⁴-glucuronides were found in small amounts in all the urines from all the drugs, but these are probably artifacts (see Bridges & Williams, 1962; Bridges *et al.* 1968). In the rabbit all the drugs, except the 2,5-dimethoxy derivative, are extensively acetylated (about 90%, except the 2-methoxy compound, which is still extensive at 67%). The amount of acetylated 2,5-dimethoxy compound is only 7% of the 24 hr. excretion and most of the drug is excreted unchanged. The rabbit forms no *N*⁴-sulphate or *N*¹-glucuronide with any of the drugs. In the rat all the drugs, except the 2,5-dimethoxy compound, are well acetylated (generally 40–60%, but the 2-methoxy compound again is rather less at 20%). The 2,5-dimethoxy compound is excreted entirely unchanged by the rat, there being no detectable acetyl derivative in the urine. Small amounts of *N*¹-glucuronide (1–7% of the 24 hr. excretion) are

excreted after all the compounds, except the 2,5-dimethoxy compound. The *N*⁴-sulphates were detected in the urines after dosing with the 4-methoxy- and 2,4-dimethoxy compounds. In the monkey, the 4- and 5-methoxy and 4,5-dimethoxy compounds are highly acetylated (85–90%), whereas the 2-methoxy (17%) and 2,5-dimethoxy (3%) compounds are poorly acetylated. The 2,4-dimethoxy compound is excreted mainly as the *N*¹-glucuronide (see Bridges *et al.* 1968), but the amount of *N*¹-glucuronide excreted after the other five is very small (1–3%). In man, the fate of the 4-methoxy and 2,4-dimethoxy compounds is similar to that in the monkey, but the 4,5-dimethoxy compound is poorly acetylated (29%) in man compared with the other species. Table 3 suggests that (1) only the 2,4-dimethoxy compound forms an *N*¹-glucuronide extensively and this occurs only in man and the monkey and (2) all the compounds are appreciably acetylated in all the species except the 2,5-dimethoxy compound.

The fact that, of all of the six compounds studied, 2,4-dimethoxy-6-sulphanilamidopyrimidine is the only one to form an appreciable amount of an *N*¹-glucuronide does not appear to be due to any special electronic property of its sulphonamide nitrogen atom, for this nitrogen atom has the same free valence and electronic charge as the corresponding nitrogen atom in 4-methoxy-6-sulphanilamidopyrimidine, which forms virtually no *N*¹-glucuronide *in vivo* (see Table 6). Nor does this reaction appear to depend on lipid solubility (see Table 5) for, although the 2,4-dimethoxy compound is the most lipid-soluble of the six compounds studied, the 4,5-dimethoxy compound is also highly lipid-soluble but forms virtually no *N*¹-glucuronide *in vivo*. The explanation for this unique reaction must depend on very subtle steric factors involving both the enzyme and the compound.

Acetylation of the methoxy-6-sulphanilamidopyrimidines. The rate of acetylation of the six

drugs *in vitro* by liver homogenates is shown in Table 4. All are acetylated by both rabbit and monkey liver preparations. In rabbit liver the 2-, 4- and 5-methoxy and 2,4-dimethoxy compounds are acetylated three to four times as fast as the 2,5- and 4,5-dimethoxy derivatives, whereas in the monkey liver the monomethoxy compounds are acetylated at about twice the rate of the dimethoxy compounds. Although the 2,5-dimethoxy compound is acetylated *in vitro* at a lower rate than some of the others, its rate is not much different from that of the 4,5-dimethoxy compound. According to Perault & Pullman (1963) the rate of enzymic acetylation of aromatic amines *in vitro* parallels the electronic charge on the amino group. This does not appear to be true in the series of compounds studied here, for the electronic charge on the aromatic nitrogen atom is the same for all of them (Table 6), yet there is up to a fourfold variation in the rate of acetylation *in vitro* (Table 4). It appears probable that some unknown steric factors are involved. There is, however, a vague correlation between the rate of acetylation *in vitro* and the *pK_a* values of the sulphonamide groups (Table 5), which vary slightly from 6.4 for the 2-methoxy compound and 6.0 for the 4,5-dimethoxy compound. The 4,5-dimethoxy compound is highly acetylated *in vivo*, whereas the 2,5-dimethoxy compound is hardly acetylated at all. Other factors are involved *in vivo*, such as distribution, tissue binding and excretion by the kidney. Table 5 shows the *pK_a* values, the solubilities at pH 7.4 and the distribution ratios of these drugs. The *pK_a* values of these compounds are not very different. The 2,5-dimethoxy compound has a much greater water-solubility at pH 7.4 than the others and at the same time its distribution

Table 4. *Rates of acetylation of methoxy-6-sulphanilamidopyrimidines by liver homogenates*

The results are the averages for three animals. Details of the incubations are given in the text.

6-Sulphanilamidopyrimidine used	Rate of acetylation (μmole/g. of liver/hr.)	
	Rabbit	Monkey
2-Methoxy	0.26 (0.21–0.30)	0.235 (0.20–0.27)
4-Methoxy	0.32 (0.30–0.34)	0.31 (0.29–0.35)
5-Methoxy	0.28 (0.23–0.31)	0.29 (0.26–0.34)
2,4-Dimethoxy	0.235 (0.20–0.27)	0.165 (0.14–0.205)
2,5-Dimethoxy	0.07 (0.04–0.11)	0.13 (0.09–0.17)
4,5-Dimethoxy	0.085 (0.05–0.13)	0.155 (0.125–0.19)

Table 5. *Solubilities and pK_a values of the methoxy-6-sulphanilamidopyrimidines and their N⁴-acetyl derivatives*

Solubilities were measured in citrate-phosphate buffer, pH 7.4 (see the text).

6-Sulphanilamidopyrimidine	<i>pK_a</i>	Solubility in buffer (mg./l.)	Distribution ratio CHCl ₃ /buffer
2-Methoxy	6.40	1479	0.08
<i>N</i> ⁴ -Acetyl-2-methoxy	6.28	—	—
4-Methoxy	6.36	267	0.23
<i>N</i> ⁴ -Acetyl-4-methoxy	—	900	0.09
5-Methoxy	6.24	1048	0.52
<i>N</i> ⁴ -Acetyl-5-methoxy	—	768	0.19
2,4-Dimethoxy	6.26	343	5.25
<i>N</i> ⁴ -Acetyl-2,4-dimethoxy	6.16	190	0.38
2,5-Dimethoxy	6.18	4507	0.21
<i>N</i> ⁴ -Acetyl-2,5-dimethoxy	—	3429	0.08
4,5-Dimethoxy	6.00	2428	2.03
<i>N</i> ⁴ -Acetyl-4,5-dimethoxy	—	2050	0.24

Table 6. *Electronic charge and free valence of the N¹- and N⁴-positions of the methoxy-6-sulphanilamidopyrimidines*

Molecular-orbital calculations were carried out on an I.C.T. 1905 computer (International Computers Ltd.) by using the parameters and programme of Krüger-Thiemer & Hansen (1966).

6-Sulphanilamido-pyrimidine	Charge on		Free valence	
	N ⁴ (amino)	N ¹ (imido)	N ⁴ (amino)	N ¹ (imido)
2-Methoxy	1.84	1.635	1.010	0.489
4-Methoxy	1.84	1.637	1.010	0.491
5-Methoxy	1.84	1.635	1.010	0.493
2,4-Dimethoxy	1.84	1.637	1.010	0.491
2,5-Dimethoxy	1.84	1.635	1.010	0.493
4,5-Dimethoxy	1.84	1.638	1.010	0.497

ratio suggests that it is poorly lipid-soluble. This suggests that it would be more readily excreted by the kidney in the unchanged state than the others. Both the 2,4- and 4,5-dimethoxy compounds are relatively easily lipid-soluble (their distribution ratios are 25 and 10 times respectively that of the 2,5-dimethoxy compound) and would tend to be re-absorbed in the kidney tubule, whereas their less lipid-soluble acetyl derivatives would be more readily excreted. It is probable therefore that the very poor acetylation of the 2,5-dimethoxy compound *in vivo* can be partly explained in terms of its relatively high water-solubility and low lipid-solubility and its low rate of acetylation.

It has been stated that variations in the pH of the

urine can affect the rate of excretion of weak acids and bases (Milne, 1965). Variations in the pH of the urine might therefore explain some of the differences in the rate of excretion of the free and acetylated sulphonamides studied here. However, in this work the pH of the urine in a given species remained relatively constant regardless of the isomer given.

This work was supported by a grant from May and Baker Ltd., Dagenham, Essex.

REFERENCES

- Adamson, R. H., Bridges, J. W. & Williams, R. T. (1966). *Biochem. J.* **100**, 71 p.
- Bollenback, G. N., Long, J. W., Benjamin, D. G. & Lindquist, J. A. (1955). *J. Amer. chem. Soc.* **77**, 3310.
- Bratton, A. C. & Marshall, E. K. (1939). *J. biol. Chem.* **128**, 537.
- Bridges, J. W., Kibby, M. R., Walker, S. R. & Williams, R. T. (1968). *Biochem. J.* **109**, 851.
- Bridges, J. W., Kibby, M. R. & Williams, R. T. (1965). *Biochem. J.* **96**, 829.
- Bridges, J. W., Walker, S. R. & Williams, R. T. (1967). *Biochem. J.* **103**, 23 p.
- Bridges, J. W., Walker, S. R. & Williams, R. T. (1969). *Biochem. J.* **111**, 173.
- Bridges, J. W. & Williams, R. T. (1962). *Biochem. J.* **83**, 27 p.
- Feigl, F. (1947). *Qualitative Analysis by Spot Tests*, p. 237. New York and Amsterdam: Elsevier Publishing Co. Inc.
- Krebs, H. A. & Speakman, J. C. (1945). *J. chem. Soc.* p. 593.
- Krüger-Thiemer, E. & Hansen, R. (1966). *Arzneim. Forsch.* **16**, 1453.
- Milne, M. D. (1965). *Proc. R. Soc. Med.* **58**, 961.
- Perault, A. M. & Pullman, B. (1963). *Biochim. biophys. Acta*, **66**, 86.