Biochemical Pharmacology, 1962, Vol. 11, pp. 881–886. Pergamon Press Ltd., Printed in Great Britain.

STUDIES OF N-DEALKYLATION OF SOME AROMATIC SULFONAMIDES*

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(*Received* 30 *March*; *accepted* 3 *June* 1962)

Abstract—The stability *in vivo* of N-methyl substituents on sulfonamide groups in a series of related benzothiadiazine-1,1-dioxides and disulfamylbenzenes has been studied. N-methyl substituents in the 2-position in benzothiadiazines and in the 3-position in disulfamylanilines were shown to be metabolically stable; a 7-methylsulfamyl substituent was smoothly and quantitatively demethylated in the benzothiadiazine studied, while the corresponding methyl group in the analogous disulfamylaniline was removed more slowly. The metabolically removed methyl groups were expired as carbon dioxide. Replacement of the amino function of the disulfamylaniline with a methyl group caused the methyl groups on both the 1- and 3-sulfamyl substituents to be liable to removal *in vivo*.

THE OBSERVATION that compounds possessing a free aromatic sulfonamide group were specific inhibitors of the enzyme, carbonic anhydrase, was first made by Mann and Keilin.[1] These and other workers showed[2, 3] that activity was retained if the aromatic nucleus was replaced by a heterocyclic ring, but that substitution on the sulfonamide nitrogen destroyed the ability to inhibit carbonic anhydrase.

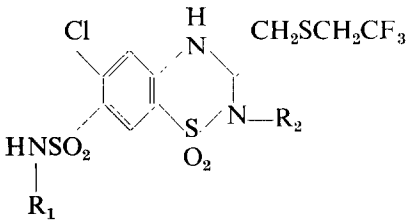
After the introduction of the potent carbonic anhydrase inhibitor, acetazolamide,[4] several derivatives bearing various alkyl groups on the sulfonamide nitrogen were synthesized. These were devoid of any activity *in vitro* against carbonic anhydrase[5] but, after administration to rats and dogs, the urine of these animals contained an inhibitor of this enzyme. The active component was not identified but was considered to be acetazolamide itself, produced by N-dealkylation *in vivo* of the administered derivative.

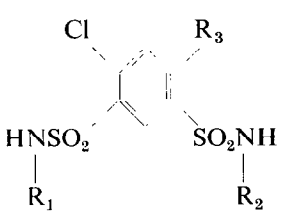
More recently, in an extensive study of aromatic sulfamyl derivatives with diuretic action,[6, 7] it was found that several compounds bearing alkyl substituents on the sulfonamide nitrogen, and hence devoid of carbonic anhydrase activity *in vitro*, regained activity *in vivo*. Again an *in vivo* N-dealkylation was postulated.

Such activation by dealkylation of a latent center of activity against carbonic anhydrase brings into question the role N-demethylation might have in the action of a 2-methyl thiazide diuretic such as polythiazide (I)†[8]. This paper deals with the metabolic fate of methyl groups attached to the nitrogen of sulfamyl groups in polythiazide (I) and some of its congeners[9–11] (II–VI) and in a related benzenesulfamyl compound (VII).

* A preliminary account of these studies was presented at the Fall Meeting of the American Society for Pharmacology and Experimental Therapeutics, Rochester, N.Y. (1961).

† Renese®, Chas. Pfizer & Co., Inc.





I $R_1 = H$; $R_2 = CH_3$
II $R_1 = R_2 = CH_3$
III $R_1 = R_2 = H$

IV $R_1 = H$; $R_2 = CH_3$; $R_3 = NH_2$
V $R_1 = R_2 = CH_3$; $R_3 = NH_2$
VI $R_1 = R_2 = H$; $R_3 = NH_2$
VII $R_1 = R_2 = R_3 = CH_3$

EXPERIMENTAL

High level dosage experiments were carried out in dogs.* Mongrels or beagles of either sex were maintained in metabolism cages with free access to water and were fed the standard laboratory diet. The drugs were administered by injection into the femoral vein. Urine was collected from the cages at 24-hr intervals.

Radiochemical experiments were carried out on male albino rats. These were housed in a Roth metabolism cage, the atmosphere of which was maintained at a slight positive pressure by means of a slow stream of air. The effluent air from the cage was passed through two traps containing ethanolamine, to absorb expired carbon dioxide. Drugs were administered orally by stomach tube. The rats were fed the standard laboratory diet with water *ad libitum*. Urine was collected from the cages.

Compounds I through VI were prepared by Dr. J. M. McManus of these laboratories. The synthesis of isotopically-labeled polythiazide (I) has been described[12]; the di-^{14}C-methyl compound II was isolated as a secondary product in this reaction. The ^{14}C-labeled sulfonamides IV and V were obtained by alkaline hydrolysis of I and II respectively. The identity of the labeled materials was established by comparison with authentic nonisotopically-labeled materials.

1,3-*bis*(*methylsulfamyl*)-4-*methyl*-6-*chlorobenzene* (VII) was prepared by treating 1,3-*bis*(chlorosulfamyl)-4-methyl-6-chlorobenzene[13] (2·3 g) with excess methylamine at room temperature for 1 hr. Evaporation and recrystallization from toluene gave VII (2·0 g), m.p. 181–183°. Analysis for $C_9H_{13}ClN_2O_4S_2$: Calculated, C,34·5; H, 4·1; N, 8·9. Found, C, 34·8; H, 4·2; N, 9·3.

Analytical methods

Radiochemical. All samples were assayed in duplicate by liquid scintillation counting and were corrected for counting efficiency by internal standards. Extraction recoveries were verified by using biological materials to which known amounts of the compound has been added.

Urine. Samples were assayed directly by dissolving 0·2 ml of specimen in 15 ml of a scintillator solution composed of ethanol–toluene (30 : 70) which contained 0·3 per cent diphenyloxazole and 0·01 per cent *p-bis*-2-(5-phenyloxazolyl)benzene.

* The authors wish to thank Dr. C. S. Delahunt for treating and maintaining the dogs; also to acknowledge gratefully the technical assistance of Miss J. Chiaini, Miss D. Baumgartner and Mr. R. Seidell.

Exhaled air. Exhaled air was bubbled through ethanolamine, and this solution was assayed for radioactivity by the method of Jeffay and Alvarez.[14]

Chemical. The basic method is that of Baer *et al.*,[15] incorporating the modifications described previously for related compounds.[12]

Paper chromatography

System 1: A descending system on Whatman no. 4 paper treated with 40%-formamide in methanol. The mobile phase is chloroform-ethyl acetate (3 : 1) saturated with formamide.

System 2: A descending system on dry Whatman no. 4 paper. The mobile phase is chloroform-formic acid-ethanol (2 : 2 : 1). The components are mixed, allowed to stand 24 hr, and the lower phase used.

All compounds were detectable on the UV fluorescent screen scanner. Compounds having free aromatic amino groups (IV, V, and VI) could also be detected by spraying with freshly prepared nitrous acid solution followed by 0·1%-N-(1-naphthyl)ethylenediamine in 95%-1-butanol.[16] Compounds I, II, and III react with this spray only after hydrolysis with a 1%-sodium hydroxide spray followed by neutralization (1 N hydrochloric acid spray) and drying.

RESULTS AND DISCUSSION

The metabolic stability of an N-methyl substituent on a sulfonamide group is clearly influenced by the nature of the other substituents in the molecule. The methyl group of polythiazide (I) was metabolically stable, the sole metabolic reaction being degradation of the thiadiazine ring to yield the disulfamylaniline IV (80 per cent). The balance of the drug was excreted unchanged, and no detectable $^{14}CO_2$ was expired in the breath when ^{14}C-methyl polythiazide (I) was administered. In the compound bearing an additional N-methyl substituent on the 7-sulfamyl group (II), however, demethylation did occur. The methyl group on the 7-sulfamyl function was quantitatively removed and appeared in the breath as carbon dioxide. The 2-methyl group was unaffected, the sole urinary metabolites being the monodemethylation product I and the corresponding disulfamylchloroaniline (IV) resulting from thiadiazine ring degradation. Opening the thiadiazine ring did not affect the metabolic stability of the methyl group in the position corresponding to the 2-position of the parent compound. Such methyl groups remained unaltered whether contained in compounds formed metabolically by thiadiazine ring cleavage or administered directly. A methylsulfamyl group corresponding to that in the 7-position of the parent benzothiadiazine was still demethylated in the monocyclic derivative V, although apparently less readily, since only 40 per cent of carbon dioxide theoretically required for monodemethylation was found in the breath. Only the unchanged compound and the monodemethylation product IV were detected in the urine. These results are summarized in Table 1.

The fate of the dimethylbenzothiadiazine II was also studied in the dog. The dose in this species (60 mg/kg) was far in excess of that used in the radiochemical experiments in rats (3·5 mg/kg), but the urinary metabolites were again solely the mono- and bicyclic products from demethylation of the extracyclic methylsulfamyl group (I and IV). At the higher dose levels, the ratio of I to IV in the urine from animals receiving II was higher than in the low dose level (radiochemical) experiments. In the high level

experiments, approximately 60 per cent of the excreted material was the monomethylbenzothiadiazine I, whereas in the radiochemical experiments excretion of I accounted for only 20 per cent of the administered compound. This may be due simply to an overloading of the metabolic mechanism that degrades I to IV. Similar effects have been noted with high doses of I.[12]

TABLE 1. DEMETHYLATION OF N-^{14}C-METHYLSULFONAMIDES BY THE RAT

Compound administered	Dose (mg/kg)	Administered activity in breath (%)	Compounds detected in urine* I	II	IV	V
I	5·3	<0·1	+	−	+	−
II	3·5	51	+	−	+	−
V	6·0	20	−	−	+	+

* Present, +; Absent, −.

The possibility that the disulfamylchloroaniline IV might be subject to further metabolism was tested in the dog. Paper chromatographic examination of urines revealed only unchanged IV, with no evidence for products arising either from acetylation of the 4-amino group or from demethylation of the 3-methylsulfamyl group. Modification of the 4-amino group was considered possible, since N-acetylation is a known metabolic reaction of a number of *p*-aminobenzenesulfonamides. However, N-acetylation was shown not to occur, since alkaline hydrolysis prior to assay failed to increase the concentration of IV.[12] The results of experiments in the dog are shown in Table 2.

TABLE 2. URINARY METABOLITES AFTER ADMINISTRATION OF N-METHYLSULFONAMIDES TO THE DOG

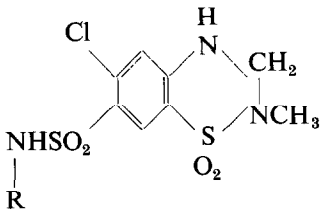
Compound administered	Dose (mg/kg)	Compounds detected in urine* I	II	IV	V	Recovery (%)
II	60	+	−	+	−	30–50
IV	100	+	−	+	−	50–80

* Present, +; absent, −.

The metabolic stability of an N-methylsulfonamide is therefore dependent on the other substituents in the molecule. In the dihydrobenzothiadiazines studied, a methyl substituent in the 2-position was unaffected, while a methylsulfamyl group in the 7-position was quantitatively demethylated. This stability at the 2-position was retained upon opening the thiadiazine ring (IV and V), but the 7-sulfamyl group was now less prone to demethylation. Upon replacing the *o*-amino group by a methyl group (VII), both the 2- and 7-positions were subject to demethylation.

Logemann and Giraldi[17] noted the abscence of VI in the urines of rats receiving V, paper chromatography showing only a more polar material, designated "hydroxy-derivatives." Since our work has shown that V is subject to monodemethylation (on the sulfamyl group *para* to the amino function), and the product of monodemethylation

(VI) has the same chromatographic behavior* as has the supposed "hydroxy-derivative" we suggest that it is, in fact, the monodemethylation product IV. These authors further concluded, since after hydrolysis to open the thiadiazine ring, urine of animals treated with VIII did not contain the disulfamyl chloroaniline VI, that *no* demethylation had occurred.

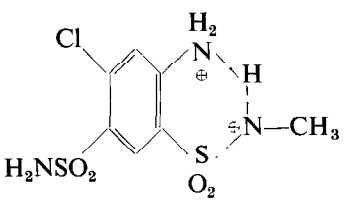


VIII R = CH_3
IX R = H

Our results suggest that *monodemethylation* had taken place to form IX which would yield IV on hydrolysis. Assignment of the structure IV to an unidentified substance that appears on chromatograms presented by Logemann and Giraldi is in accord with the chromatographic evidence previously cited.

Thus, assertions by other workers[6, 18] that demethylation *in vivo* of N-methyl-sulfonamides imparts diuretic action to compounds that lack activity against carbonic anhydrase *in vitro* is partially true. However, the carbonic anhydrase inhibitory activity of its suggested metabolite IX is probably of minor importance to the diuretic action of VIII, since the 2-methyl thiazides are potent diuretics[9, 19] but, compared with acetazolamide, are relatively weak inhibitors of carbonic anhydrase.

The observation that neither a 2-methylbenzothiadiazine nor an *o*-N-methyl-sulfamylaniline is demethylated *in vivo* suggests that a *free* hydrogen atom on the sulfonamide nitrogen is a prerequisite for metabolic dealkylation. Although, formally, the *o*-methylsulfamylaniline V has a hydrogen atom on the sulfonamide nitrogen, it is scarcely fully available owing to the contribution of the hydrogen bonded form:[20]



This view is strengthened by the fact that replacement of the *o*-amino function by a methyl group which is of comparable bulk but cannot participate in such a system results in a compound, VII, that is demethylated *in vivo*.

* Estimated R_f values from the chromatograms shown by Logemann and Giraldi are: V, 0·87; "hydroxy-derivatives," 0·69; VI, 0·30. Values determined in this laboratory in the same system were V, 0·87; IV, 0·61; and VI, 0·25.

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