Comparison of the Diuretic Activities of Benzimidazolyl-toluenesulfonamide Compounds and Acetazolamide

By THOMAS R. CASTLES and HAROLD E. WILLIAMSON

The diuretic activities of α-(2-benzimidazolyl)*p*-toluenesulfonamide (BTS) and α-(5-methyl-2-benzimidazolyl)-*p*-toluenesulfonamide (MBTS) were compared with the diuretic activity of acetazolamide using a rat diuretic assay. Acetazolamide was about 83 times more potent than BTS and about 56 times more potent than MBTS on a weight basis. Both BTS and MBTS exhibited a greater kaluretic action than acetazolamide when equivalent natruretic doses were employed, suggesting that BTS and MBTS affect renal distal tubular mechanisms to a greater degree than acetazolamide.

SOUTHWORTH (1) first reported that sulfanilamide produced an alkaline urine. Subsequently, sulfanilamide was found to inhibit carbonic anhydrase (2); this led to the development of acetazolamide as a diuretic agent (3). Miller *et al.* (4) found that *p*-toluenesulfonamide was a more potent inhibitor than sufanilamide. Hosein *et al.* (5) reported that benzimidazole was also an inhibitor of carbonic anhydrase. It was of interest to learn whether the incorporation of two structurally different inhibitory groups into the same compound would result in an addition of activities. Therefore, the benzimidazole derivatives of *p*-toluenesulfonamide, α-(2-benzimidazolyl)-*p*-toluenesulfonamide (BTS) and α-(5-methyl-2-benzimidazolyl)-*p*-toluenesulfonamide (MBTS), were examined *in vivo* using a rat diuretic assay. 2-Acetylamino-1,3,4-thiadiazole-5-sulfonamide (acetazolamide), a potent carbonic anhydrase inhibitor both *in vitro* and *in vivo* (3), was used as the reference compound.

METHODS

Drugs were evaluated by the assay procedure described by Lipschitz *et al.* (6). Male albino (Holtzman) rats, 140–200 Gm., were fasted for 16 hours prior to the experiment and given an oral load of 0.9% NaCl or water, 50 ml./Kg., at the beginning of the assay. Drugs were administered orally in the load as a solution (acetazolamide) or as a suspension (BTS, MBTS). For urine collection, three rats were placed in each metabolism cage, and urine was collected over a 5-hour period. At the end of the collection period, the bladders of the rats were emptied manually. Urine samples were analyzed for sodium and potassium with a Coleman flame photometer (model 21), and chloride was determined using a Buchler-Cotlove chloridometer.

Data in Tables I and II were analyzed using Duncan's new multiple-range test following analysis of variance (7). Figures 1 and 2 were analyzed using a four-point parallel-line bioassay (8). The 0.05 level of probability was used as the criterion of significance.

RESULTS

The results of Finney's four-point parallel-line assay comparing the natruretic actions of BTS, MBTS, and acetazolamide in rats given a saline load are shown in Figs. 1 and 2. No significant deviation from parallelism was found. Acetazolamide was calculated to be 83 times more potent than BTS (95% confidence limits, 46–310) and 56 times more potent than MBTS (95% confidence limits, 28–97) on a weight basis.

Table I summarizes the effects of BTS, MBTS, and acetazolamide on the excretion of water, chloride, and potassium in these same rats. All three compounds produced a significant increase in the excretion of water but did not alter the excretion of chloride significantly. MBTS at both doses and BTS at the higher of the two doses employed, produced a significant increase in the excretion of potassium. The small increases in the

TABLE I.—EFFECT OF BTS, MBTS, AND ACETAZOLAMIDE ON THE EXCRETION OF WATER, CHLORIDE, AND POTASSIUM IN RATS GIVEN AN ORAL LOAD OF SALINE

	Treatment, mg./Kg							Coefficient of Variability, %
Excretion of	None	BTS 75	MBTS 1.25	Acetazolamide 1.25	BTS 150	MBTS 150	Acetazolamide 2.5	
Water, % of load	47[a]	57	62	65	65	79	89	14
Chloride, % of load	67	63	61	62	60	66	62	28
	None	Acetazolamide 1.25	Acetazolamide 2.5	BTS 75	BTS 150	MBTS 75	MBTS 150	
Potassium, meq./Kg.	1.5	1.8	1.9	2.0	2.2	2.7	2.8	20

[a] Data were analyzed by Duncan's multiple-range test following analysis of variance using a randomized complete block design. Any two values in each row which are underscored by the same line are not significantly different. Any two values in each row which are not underscored by the same line are significantly different ($p < 0.05$). Five groups of rats were employed for each treatment.

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TABLE II.—EFFECT OF EQUIVALENT NATRURETIC DOSES OF BTS OR MBTS AND ACETAZOLAMIDE ON THE URINARY EXCRETION OF WATER, CHLORIDE, AND POTASSIUM IN RATS GIVEN AN ORAL LOAD OF WATER

Excretion of	Treatment: Control	BTS, 140 mg./Kg.	Acetazolamide, 1.75 mg./Kg.	Coefficient of Variability, %
Water, % of load	79[a]	91	93	8
Sodium, meq./Kg.	0.5	3.2	2.8	17
Chloride, meq./Kg.	0.5	2.4	1.5	23
Potassium, meq./Kg.	0.7	4.0	2.3	32
	Control	MBTS, 150 mg./Kg.	Acetazolamide, 2.5 mg./Kg.	
Water, % of load	93	114	119	6
Sodium, meq./Kg.	0.6	2.7	2.4	19
Chloride, meq./Kg.	0.5	0.9	1.1	23
Potassium, meq./Kg.	0.8	2.3	1.6	13

[a] Data were analyzed by Duncan's new multiple-range test following analysis of variance using a completely randomized block design. Any two values in each row which are underscored by the same line are not significantly different. Any two values in the same row which are not underscored by the same line are significantly different ($p < 0.05$). Four groups of rats were used for each treatment.

excretion of potassium seen with acetazolamide were not significantly different from the control rate of excretion of this ion.

Table II summarizes the effect of equivalent natruretic doses of BTS or MBTS and acetazolamide on the excretion of sodium, water, chloride, and potassium in rats given an oral load of water. Equivalent natruretic doses (as determined from Figs. 1 and 2) were used to compare the effects of these drugs on the excretion of potassium at equivalent rates of excretion of sodium. Both BTS and MBTS produced a greater kaluresis than acetazolamide at equivalent rates of natruresis. All three agents increased the excretion of chloride. Only the increase seen with BTS was significantly greater than that seen with acetazolamide.

DISCUSSION

Diuretic agents which act by inhibiting renal carbonic anhydrase produce a characteristic change in the urinary excretory pattern of water and electrolytes. They increase the excretion of sodium and potassium ions, bicarbonate, and water; decrease the excretion of hydrogen and ammonium ions; and do not markedly alter the excretion of chloride. The two benzimidazolyl-toluenesulfonamide compounds, when administered to saline-loaded animals, increased the excretion of sodium, potassium, and water, but did not alter the excretion of chloride. Thus, these compounds appear to induce changes consistent with inhibition of renal carbonic anhydrase.

Miller *et al.* (4) found that acetazolamide on a molar basis was about 39 times more potent an inhibitor of carbonic anhydrase than *p*-toluenesulfonamide *in vitro*. Converting our data to a molar basis shows that acetazolamide is about 64 times more potent than BTS and about 41 times more potent than MBTS. Thus, it would appear that the addition of a benzimidazole moiety does not enhance the carbonic anhydrase inhibitory activity of *p*-toluenesulfonamide.

The kaluresis seen with BTS and MBTS in the saline-loaded animals appeared to be greater than that seen with acetazolamide. This was examined further using equinatruretic doses of these agents. Both BTS and MBTS produced a greater kaluresis than acetazolamide when the natruretic responses were equal. This could be due to a difference in inhibition in various portions of the tubule. Carbonic anhydrase inhibitors have been reported to act on both the proximal and distal tubules (9–11). By blocking carbonic anhydrase in the proximal tubule, and consequently the supply of hydrogen ions for exchange with sodium, proximal reabsorption of sodium ion is depressed. By blocking carbonic anhydrase in the distal tubule, the decreased supply of hydrogen ions to the hydrogen ion-potassium ion exchange mechanism for sodium ion favors a potassium-for-sodium exchange. From this, an agent acting predominantly on the distal tubules should cause a greater kaluresis than nat-

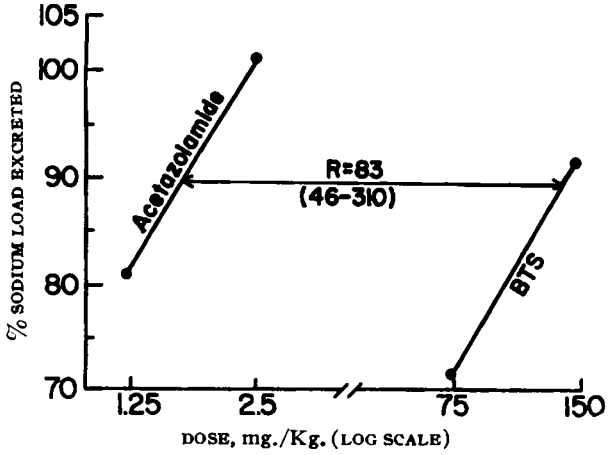

Fig. 1.—The natruretic action of BTS compared to that of acetazolamide using a four-point parallel-line bioassay. *R* equals potency ratio with 95% confidence limits in parentheses.

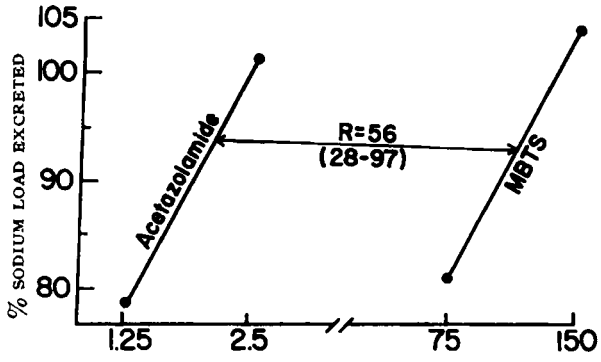

Fig. 2.—The natruretic action of MBTS compared to that of acetazolamide using a four-point parallel-line bioassay. *R* equals potency ratio with 95% confidence limits in parentheses.

ruresis. The greater kaluresis produced by BTS and MBTS when equivalent natruretic doses of these agents and acetazolamide were employed therefore suggests that these benzimidazolyl-toluenesulfonamide compounds exert a greater effect on the distally located exchange mechanism than does acetazolamide.

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Use of 3-Azabicyclo[3.2.2]nonane in the Mannich Reaction IV. Additional Derived Products

By C. DEWITT BLANTON, JR., and W. LEWIS NOBLES

Additional β-amino ketones, phenol Mannich bases, allylamines, propylamines, and γ-aminoalkyl esters involving the use of 3-azabicyclo[3.2.2]nonane in the Mannich reaction are reported. Results of biological tests are cited.

ADDITIONAL MANNICH bases derived from 3-azabicyclo[3.2.2.]nonane are reported in Tables I–V. Results of biological tests are cited.

EXPERIMENTAL

The Mannich reaction was carried out as previously described (1). Preparation of the phenolic Mannich bases was achieved by the procedure of Burckhalter *et al.* (2) or by that of Bruson and MacMullen (3). The γ-amino secondary and γ-amino tertiary alcohols used as intermediates for the preparation of the compounds reported herein were prepared as described earlier (4, 5). The procedure of Pohland and Sullivan (6), Method D, was adopted as a general one for the esterification of γ-amino secondary alcohols. The method employed for the dehydration of the tertiary alcohols was patterned after that of Adamson (7). The procedure adopted as a general one for the reduction of allylamines to propylamines was patterned after that of Adamson and Billinghurst (8). For the preparation of the allylamines 7 and 10 (Table III) the tertiary alcohols, 3 - [3 - (3 - azabicyclo[3.2.2.]-nonyl)] - 1,1 - di - (2 - thienyl)propan - 1 - ol hydrochloride and 3 - [3 - (3 - azabicyclo[3.2.2]nonyl)]-1 - (4′ - propoxyphenyl) - 1 - phenylpropan - 1 - ol hydrochloride, underwent dehydration during their isolation from the Grignard reaction medium.

BIOLOGICAL TEST RESULTS[1]

During the preliminary screening program, compounds reported in this series of papers exhibited a broad spectrum of antimicrobial activity against such organisms as *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Candida albicans, Trichophyton mentagrophytes,* and *Trichomonas foetus.* The activity is particularly pronounced against *Trichophyton* and *Trichomonas;* in this regard, the compound β-3-(3-azabicyclo [3.2.2.] nonyl) - 2,5 - dimethyl-

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