new compound as well as the other compounds were within ±0.4% of the calculated.

Due to the inherent complex nature of the antileukemia test, the biological variation, and the relatively low potency of most of the compds examined, no meaningful quantitative structure–activity correlation could be obtained from the limited amount of data available. From Table II it is clear that the only compd which showed promising results is 1,2,6-trimethylquinolinium iodide (*N*-methiodide of 2,6-dimethylquinoline) which gives 6/6 survival rate and about 13% increase in survival days at 37.5 mg/kg level, and 6/6 survival rate and about 30% increase in survival days at 150 mg/kg level. It is interesting to note that this compd has π_x and log K_{app} values higher than all the other quinoline derivatives but lower than these of the acridine derivative (see Table I). Unfortunately, no quantitative correlation could be obtained at the present. With the exception of *N*-methiodide of acridine, the surface of the substituent (A_w) parallels π_x and log K_{app}. All of the compds examined, with the exception of *N*-methiodide of 2-iodoquinoline, possessed toxicity at elevated dosages (80–400 mg/kg).

The electronic effect of the substituent on the activity is difficult to assess, since only 2 compds with slightly positive σ values (see Table I) were studied and these 2 compds did not show significant antileukemia activity.

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Aminobenzoic Acid Diuretics. 2.[1] 4-Substituted-3-amino-5-sulfamylbenzoic Acid Derivatives

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The synthesis of 42 N-alkylated 4-substituted-3-amino-5-sulfamylbenzoic acids is detailed. Reasons for the interest in these new aminobenzoic acid derivatives are discussed. Diuretic screening results in the dog assay for the compounds are summarized and compared with 3-benzylamino-4-chloro-5-sulfamylbenzoic acid (**76**) and the corresponding *N*-Bu compd (**77**) recently described as diuretics. For the most active 4-R_1-3-R_2NH-5-sulfamylbenzoic acids (**85**, R_1 = NHC_6H_5; R_2 = $CH_2C_6H_5$; **86**, R_1 = NHC_6H_5; R_2 = *n*-Bu; **97**, R_1 = SC_6H_5; R_2 = $CH_2C_6H_5$; **98**, R_1 = SC_6H_5; R_2 = *n*-Bu; **103**, R_1 = OC_6H_5; R_2 = $CH_2C_6H_5$; **110**, R_1 = OC_6H_5; R_2 = $CH_2CBr{=}CH_2$; **111**, bumetamide (pINN), R_1 = OC_6H_5; R_2 = *n*-Bu; **114**, R_1 = OC_6H_5; R_2 = 2-furylmethyl; **118**, R_1 = OC_6H_4-4-OH; R_2 = $CH_2C_6H_5$) the results at different doses after both iv and oral administration are given. Comparison with 4-chloro-*N*-(2-furylmethyl)-5-sulfamylanthranilic acid (furosemide) has revealed that these new highly efficacious compounds possess a level of activity hitherto unknown for "high-ceiling" diuretics.

Except for some carbonic anhydrase inhibitors, all benzenesulfonamide diuretics with saluretic action (including the thiazides and related bicyclic compounds), which have found clinical application, have as a common structural feature Cl or CF_3 in the ortho position to a sulfonamide group.[2–4] It is generally held that outstanding sulfonamide saluretics should possess halogen or pseudohalogen in this position besides an electronegative group meta to the sulfonamide group or as part of a condensed ring. Even 4-chloro-*N*-(2-furylmethyl)-5-sulfamylanthranilic acid[5] (furosemide), a well-established, nonthiazide type, high-ceiling diuretic,[4] was found to fit this broad generalization.[3] On the other hand, this empirical rule, given with some reservations,[3] had been based mainly on varied structures of thiazide type diuretics and consequently the question still remained, whether it was of general validity and applicable to sulfonamide diuretics with a different type of action.

In the preceding paper of this series some 3-amino-4-halogeno-5-sulfamylbenzoic acid derivatives have been described as powerful high-ceiling diuretics.[1] At this stage in pursuing the structure–activity relationships of highly efficacious diuretics and for reasons given above efforts have been directed towards an alteration of the 4 substituent of these aminobenzoic acid derivatives.

Chemistry.—The principal synthetic route for the preparation of the 4-R_1-3-R_2NH-5-sulfamylbenzoic acids[6] (Table IV) is outlined in Scheme I and detailed in the Experimental Section. The starting material, 4-chloro-3-nitro-5-sulfamylbenzoic acid[1] (**1**), was easily available. In the first step the relatively high reactivity of the chloro substituent of **1** was utilized for the alkylation of various amines, phenols, thiophenols, mercaptans, and alcohols to yield most of the described 4-R_1-3-nitro-5-sulfamylbenzoic acids (Table I). Special reaction conditions were required in those cases where the introduced substituent was reactive itself. For example, the phenoxy compd **22** is easily hydrolyzable in 1 *N* NaOH to yield the phenol **26**. The *n*-butylsulfinyl compd **18** was prepared by oxidation of the

(1) Part 1: P. W. Feit, H. Bruun, and C. Kaergaard-Nielsen, *J. Med. Chem.*, **13**, 1071 (1970); in this ref the term metanilic acid has been used erroneously for 3-aminobenzoic acid throughout.

(2) See G. de Stevens, "Medicinal Chemistry," Vol. I, Academic Press, New York, N. Y., 1963, Chapters V and VI.

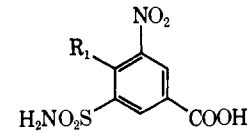
(3) See J. M. Sprauge, "Topics in Medicinal Chemistry," Vol. II, J. L. Rabinowitz and R. M. Myerson, Ed., Wiley, New York, N. Y., 1968, pp 22–24.

(4) See R. Muschaweck and K. Sturm, "Arzneimittel," G. Ehrhart and H. Ruschig, Ed., Vol. I, Verlag Chemie, Weinheim, West Germany, 1968, Chapter 16, pp 694–703.

(5) K. Sturm, W. Siedel, R. Weyer, and H. Ruschig, *Chem. Ber.*, **99**, 328 (1966).

(6) Løvens Kemiske Fabrik Produktionsaktieselskab, Belgian Patent 743,744 (1970).

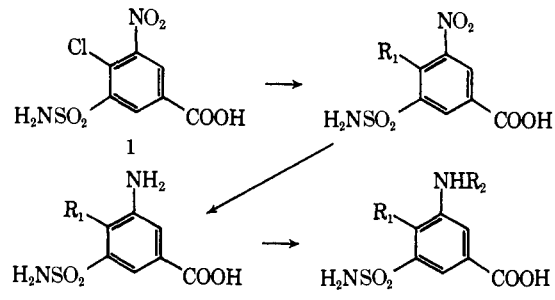
TABLE I
PHYSICAL PROPERTIES OF



No.	R_1	Method[a]	Mp, °C	Recryst solvent[b]	Yield,[c] %	Formula	Analysis[d]
2	NH-*i*-Pr	1A	206 dec	Aq EtOH–H_2O[e]	63	$C_{10}H_{13}N_3O_6S$	C, H, N
3	NH-*n*-Bu	1B	192.5	Aq MeOH	47	$C_{11}H_{15}N_3O_6S$	C, H, N
4	$NCH_2CH_2CH_2CH_2CH_2$	1B	237–238 dec	Aq MeOH	68	$C_{12}H_{15}N_3O_6S \cdot H_2O$	C, H, N
5	$NCH_2CH_2OCH_2CH_2$	1B	273 dec	Aq MeOH	64	$C_{11}H_{13}N_3O_7S \cdot H_2O$	C, H, N
6	NHC_6H_{11}	1B	186–187	Aq EtOH	38[f]	$C_{13}H_{17}N_3O_6S$	C, H, N
7	$NHCH_2C_6H_5$	1C	188 dec	Aq MeOH	37	$C_{14}H_{13}N_3O_6S$	C, H, N
8	$NHCH_2CH_2C_6H_5$	1D	208–208.5	Aq EtOH	72	$C_{15}H_{15}N_3O_6S$	C, H, N
9	NHC_6H_5	1E	261–262	Aq EtOH	60	$C_{13}H_{11}N_3O_6S$	C, H, N
10	NHC_6H_4, 4-Cl	1E	241–241.5	EtOH	51[g]	$C_{13}H_{10}ClN_3O_6S$	C, H, N
11	NHC_6H_4, 4-Me	1E	259–260	EtOH	61[h]	$C_{14}H_{13}N_3O_6S$	C, H, N
12	NHC_6H_4, 3-Me	1E	256–259	MeOH[i]	47[h]	$C_{14}H_{13}N_3O_6S$	C, H, N
13	NHC_6H_4, 4-OMe	1E	246 dec	Aq EtOH	40	$C_{14}H_{13}N_3O_7S \cdot H_2O$	C, H, N
14	NHC_6H_3, 2,4-Me_2	1E	224–225.5	EtOH	45	$C_{15}H_{15}N_3O_6S \cdot C_2H_5OH$	C, H, N
15	NHC_6H_4, 4-COOH	1F	297 dec	EtOH–H_2O[e]	47	$C_{14}H_{11}N_3O_8S \cdot H_2O$	C, H, N*
16	NH-β-naphthyl	1G	262 dec	EtOH–H_2O	78	$C_{17}H_{13}N_3O_6S \cdot H_2O$	C, H, N
17	S-*n*-Bu	1H	173–173.5	Aq EtOH	67[f]	$C_{11}H_{14}N_2O_6S_2$	C, H, N
18	SO-*sec*-Bu	1I	165 dec	Aq EtOH	81	$C_{11}H_{14}N_2O_7S_2$	H,* N, C_5
19	SC_6H_5	1J	247–248	Aq EtOH	64	$C_{13}H_{10}N_2O_6S_2$	C, H, N
20	SC_6H_4, 2-Me	1K	165–166	Aq EtOH	59	$C_{14}H_{12}N_2O_6S_2$	C, H, N
21	OCH_2CF_3	1L	195–197	Aq EtOH	39[h]	$C_9H_7F_3N_2O_7S$	C, H, N
22	OC_6H_5	1M	255–256	Aq EtOH	53	$C_{13}H_{10}N_2O_7S$	C, H, N
23	OC_6H_4, 4-OMe	1N	229–230	Aq EtOH	50[h]	$C_{14}H_{12}N_2O_8S$	C, H, N
24	OC_6H_4, 3-CF_3	1N	205–206	MeOH–H_2O	29[h]	$C_{14}H_9F_3N_2O_7S$	C, H, N
25	OC_6H_4, 4-$OCH_2C_6H_5$	1O	247	Aq EtOH[i]	14[k,l]	$C_{19}H_{16}N_2O_8S$	C, H, N
26	OH	1P	268–270 dec	H_2O[m]	40[f]	$C_7H_6N_2O_7S$	C, H, N

[a] The letters relate to the general procedure given in the Experimental Section. [b] Several recrystallizations were performed, if necessary while treating with decolorizing carbon. [c] The yield of the analytically pure compd is given. Except where otherwise stated the compounds were dried in air. Although most runs were repeated using larger quantities, no attempts were made to optimize the yield. [d] Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.25% of the theoretical values or when marked with an asterisk within ±0.4% of the theoretical values. [e] EtOH at 60° was used to dissolve the compd. [f] Dried *in vacuo* (10–14 mm) at 78° for 2 hr. [g] Dried *in vacuo* (10–14 mm) at 78° for 8 hr. [h] Dried *in vacuo* (10–14 mm) at 115° for 2–4 hr. [i] The compd was rather unstable; C: calcd 37.70; found: 37.00. [j] After *i*-PrOH was used for first purification. [k] Over two steps. [l] After warm Me_2CO–petroleum ether was used for first purification. [m] Dried in air for several days.

SCHEME I



thioether **17**. The reduction of NO_2 acids to the corresponding amino acids (Table II) was performed according to generally known methods.

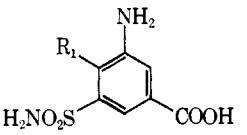
The last step, resulting in most of the desired 4-R_1-3-R_2NH-5-sulfamylbenzoic acids, (Table IV), was mainly achieved by appropriate modification of procedures previously used for alkylation of the 3-amino-4-chloro-5-sulfamylbenzoic acid.[1] Where the conditions involved simultaneous esterification, the intermediate esters (Table III) were isolated and purified if convenient.

It is apparent that besides the presence of the sulfonamide group both the nature of the substituent R_1 and the alkylating group influenced the choice of method. In the easily alkylated aminobenzoic acid derivatives bialkylation of the aromatic amino group could be suppressed to such a degree as to make the purification of the monalkylated acid possible.

The *N*-allyl side chain of the ester **68** was hydrogenated to the parent *n*-propyl compd as intermediate for the acid **107**. Br_2 addition to **68** resulted in the dibromo compd **70** which on saponification under simultaneous dehydrobromination yielded the acid **110**. Oxidation of the thioethers **97** and **98** provided the phenylsulfinyl benzoic acids **99** and **100**, respectively. The 4-(4-benzyloxyphenoxy)-3-benzylamino-5-sulfamylbenzoic acid (**117**) could be partially O-debenzylated to the corresponding phenol **118**. The 4-(4-sulfamylphenoxy) compd **119** was obtained after sulfochlorination of the 4-phenoxybenzoic acid **111** and amidation of the resultant 4′-sulfochloride.

Although the structure of the described N-substituted aminobenzoic acid derivatives seems to be established by the synthetic route, attempts were made to confirm the position of the *n*-Bu side chain in **111**. The iso-

TABLE II
PHYSICAL PROPERTIES OF



No.	R_1	Method[a]	Mp, °C	Recryst solvent[b]	Yield,[c] %	Formula	Analysis[d]
27	NH-*i*-Pr	2A	226 dec	Aq MeOH	75	$C_{10}H_{15}N_3O_4S$	C, H, N
28	NH-*n*-Bu	2A	211–211.5	Aq MeOH	64	$C_{11}H_{17}N_3O_4S$	C, H, N
29	$NCH_2CH_2CH_2CH_2CH_2$	2A	279 dec	Aq MeOH	78	$C_{12}H_{17}N_3O_4S$	C, H, N
30	$NCH_2CH_2OCH_2CH_2$	2A	297 dec	Aq MeOH	46	$C_{11}H_{15}N_3O_5S$	C, H, N
31	NHC_6H_{11}	2A	238 dec	Aq EtOH	72	$C_{13}H_{19}N_3O_4S$	C, H, N
32	$NHCH_2C_6H_5$	2A	217 dec	Aq MeOH	53	$C_{14}H_{15}N_3O_4S$	C, H, N
33	$NHCH_2CH_2C_6H_5$	2A	194.5–195	EtOH–H_2O	55	$C_{15}H_{17}N_3O_4S$	C, H, N
34	NHC_6H_5	2A	251	EtOH–H_2O	82	$C_{13}H_{13}N_3O_4S$	C, H, N
35	NHC_6H_4, 4-Cl	2B	273–273.5	Aq EtOH	30[e]	$C_{13}H_{12}ClN_3O_4S$	C, H, N
36	NHC_6H_4, 4-Me	2A	249–252	EtOH–H_2O	68	$C_{14}H_{15}N_3O_4S$	C, H, N
37	NHC_6H_4, 3-Me	2A	280–282.5	EtOH–H_2O	67[f]	$C_{14}H_{15}N_3O_4S$	C, H,* N*
38	NHC_6H_4, 4-OMe	2A	214	Aq EtOH	74[f]	$C_{14}H_{15}N_3O_5S$	C,* H, N
39	NHC_6H_3, 2,4-Me_2	2A	241–241.5	EtOH–H_2O[g]	47	$C_{15}H_{17}N_3O_4S$	C, H, N
40	NHC_6H_4, 4-COOH	2A	282 dec	Aq EtOH	52	$C_{14}H_{13}N_3O_6S$	C, H, N*
41	NH-β-naphthyl	2A	248–248.5	EtOH–H_2O	40	$C_{17}H_{15}N_3O_4S$	C, H, N
42	S-*n*-Bu	2C	223–224	Aq EtOH[h]	29	$C_{11}H_{16}N_2O_4S_2$	C, H, N
43	SO-*n*-Bu	2D	237 dec	Aq EtOH	59	$C_{11}H_{16}N_2O_5S_2$	C, H, N*
44	SC_6H_5	2E	284–285	MeOH–H_2O	52[e]	$C_{13}H_{12}N_2O_4S_2$	C, H, N
45	SC_6H_4, 2-Me	2F	277–277.5	Aq EtOH	44	$C_{14}H_{14}N_2O_4S_2$	C, H, N
46	OCH_2CF_3	2A	253–255	Aq EtOH	72	$C_9H_9F_3N_2O_5S$	C, H, N
47	OC_6H_5	2G[i]	256–257	Aq EtOH	78	$C_{13}H_{12}N_2O_5S$	C, H, N
48	OC_6H_4, 4-OMe	2G	260–261	EtOH–H_2O	79[f]	$C_{14}H_{14}N_2O_6S$	C, H, N
49	OC_6H_4, 3-CF_3	2G	270	EtOH–H_2O	61[f]	$C_{14}H_{11}F_3N_2O_5S$	C, H, N
50	OC_6H_4, 4-$OCH_2C_6H_5$	2H	264–265	Aq EtOH	36	$C_{20}H_{18}N_2O_6S$	C, H, N, S

[a–d] See corresponding footnotes in Table I. [e] Dried *in vacuo* (10–14 mm) at 78° for 2–5 hr. [f] Dried *in vacuo* (10–14 mm) at 115° for 2–5 hr. [g] Warm EtOH was used to dissolve the compd. [h] *i*-PrOH was used for first purification. [i] For large scale preparations the general method of W. A. Jacobs and M. Heidelberger, *J. Amer. Chem. Soc.*, **33**, 1435 (1917), was adapted.

mer, 3-amino-5-*n*-butylsulfamyl-4-phenoxybenzoic acid (**122**), was prepared from 4-chloro-5-chlorosulfonyl-3-nitrobenzoic acid[1] and proved to be not identical with **111** by ir and nmr spectroscopy. Furthermore, in contrast to **122**, **111** could not be diazotized and coupled with β-naphthol.

Diuretic Effect and Structure–Activity Relationships.—The N-substituted 3-aminobenzoic acids prepared in this study were screened for their diuretic properties in dogs. The urinary volume and electrolyte excretion following iv administration (soln in NaOH) from a 3-hr test period are summarized in Table IV. The onset of diuresis was observed within the first hour after injection and became, except for the most potent compounds, almost negligible after 3 hr.

A comparison of the different N-benzylated compounds (R_2 = $CH_2C_6H_5$) shows the influence of the substituent in the 4 position (R_1). When one considers the variety of these substituents it is remarkable that almost all of the relevant derivatives are much more active than the corresponding 4-Cl compound[1] (**76**).

The effect of N substitution was investigated in the alkylated 3-amino-4-phenoxy-5-sulfamylbenzoic acids. As far as the *N*-*n*-alkyl side chain is concerned the maximum activity was found for **111** (R_2 = *n*-Bu), thus showing a striking similarity to the 4-Cl 3-aminobenzoic acid series where **77** was found to be the most active compd.[1] In the present series, however, **103** (R_2 = $CH_2C_6H_5$) and **114** (R_2 = 2′-furylmethyl) show the same order of activity as **111**.

Nine of the most active compounds (**85**, **86**, **97**, **98**, **103**, **110**, **111**, **114**, and **118**) were more thoroughly tested in the dog assay. The results after iv and oral administration of different doses are given in Table V for a 6-hr test period and compared with those of 4-chloro-*N*-(2-furylmethyl)-5-sulfamylanthranilic acid (furosemide). The data reveal that these new, highly efficacious compounds possess a level of activity hitherto unknown for "high-ceiling" diuretics.

Compd **111** (bumetanide, pINN) was selected for dose–response studies and pharmacological investigation.[7] In healthy adults **111** was proved to be 40 times more active than furosemide.[8] Clinical trials with **111** are in progress.

Experimental Section†

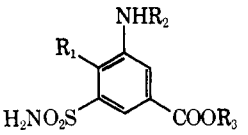
4-R_1-3-nitro-5-sulfamylbenzoic Acids (2–26, Table I). Method 1A.—To *i*-$PrNH_2$ (120 ml) 1 (22.8 g) was added in portions followed by H_2O (8 ml) with stirring and cooling. After the stirring had been contd at room temp for 4 days the excess of *i*-$PrNH_2$ was distd off *in vacuo*. The residue was triturated with 4 *N* HCl (45 ml), and the sepd crude **2** was collected.

† Anal. were performed by G. Cornali and W. Egger of these labs. Mp were cor and taken in open glass capillaries using a Hershberg apparatus. The nmr spectra were obtained with a Varian Associates spectrometer, Model A-60-A. Anal. data are given as defined in footnote *d*, Table I; anal. results were within 0.25% or when marked with an asterisk, within 0.4% of calcd values. Tech assistance was given by T. Parbst, W. Schlichtkrull, H. Dannacher, N. Hornum, and F. Frederiksen.

(7) E. H. Østergaard, M. P. Magnussen, C. Kaergaard Nielsen, E. Eilertsen, and H.-H. Frey, to be published.

(8) K. Roholt, unpublished work.

TABLE III
PHYSICAL PROPERTIES OF



No.	R_1	R_2	R_3	Method[a]	Mp, °C	Recryst solvent[b]	Yield,[c] %	Formula	Analysis[d]
51	$NCH_2CH_2OCH_2CH_2$	$CH_2C_6H_5$	Et	3A	185–186	Abs EtOH	29	$C_{20}H_{25}N_3O_5S$	H, N, C[e]
52	NHC_6H_{11}	$CH_2C_6H_5$	Et	3B	176–177	Abs EtOH	19	$C_{22}H_{29}N_3O_4S$	C, H, N
53	$NHCH_2CH_2C_6H_5$	$CH_2C_6H_5$	Et	3A	80–82	Abs EtOH	(20)	$C_{24}H_{27}N_3O_4S$	*f*
54	NHC_6H_5	$CH_2C_6H_5$	Et	3B	161–161.5	Abs EtOH	56[g]	$C_{22}H_{23}N_3O_4S$	C, H, N
55	NHC_6H_4, 4-Cl	$CH_2C_6H_5$	Et	3B	187–187.5	EtOH	48[g]	$C_{22}H_{22}ClN_3O_4S$	C, H, N
56	NHC_6H_4, 4-Me	$CH_2C_6H_5$	Et	3B	159–160	Abs EtOH	47[g]	$C_{23}H_{25}N_3O_4S$	C, H, N
57	NHC_6H_4, 3-Me	$CH_2C_6H_5$	Et	3B	169–170.5	Abs EtOH	31[h]	$C_{23}H_{25}N_3O_4S$	C, H, N
58	NHC_6H_4, 4-OMe	$CH_2C_6H_5$	Et	3B	145	Abs EtOH	53	$C_{23}H_{25}N_3O_5S$	C, H, N
59	NHC_6H_4, 4-OMe	$CH_2CH{=}CH_2$	Et	3C	176–177	Abs EtOH	30	$C_{19}H_{23}N_3O_5S$	C*, H, N
60	NHC_6H_3, 2,4-Me	$CH_2C_6H_5$	Et	3B	167.5–168	EtOH	49	$C_{24}H_{27}N_3O_4S$	C, H, N
61	NHC_6H_4, 4-COOEt	$CH_2C_6H_5$	Et	3A	161–161.5	Abs EtOH	68	$C_{25}H_{27}N_3O_6S$	C, H, N
62	NH-β-naphthyl	$CH_2C_6H_5$	Et	3B	199.5–201.5	Abs EtOH	45	$C_{26}H_{25}N_3O_4S$	C, H, N
63	S-*n*-Bu	$CH_2C_6H_5$	Et	3A	151–152	Abs EtOH	38	$C_{20}H_{26}N_2O_4S_2$	C, H, N
64	SC_6H_4, 2-Me	$CH_2C_6H_5$	Et	3A	166–167	Abs EtOH	37	$C_{23}H_{24}N_2O_4S_2$	C, H, N
65	OCH_2CF_3	$CH_2C_6H_5$	Et	3A	163–165	Abs EtOH	47[g]	$C_{18}H_{19}F_3N_2O_5S$	C, H, N
66	OC_6H_5	$CH_2C_6H_5$	Et	3B	178	Abs EtOH	59[g]	$C_{22}H_{22}N_2O_5S$	C, H, N
67	OC_6H_5	*n*-Pr	Et	3D	150–151	EtOH	56[g]	$C_{18}H_{22}N_2O_5S$	C, H, N
68	OC_6H_5	$CH_2CH{=}CH_2$	Et	3E	153–154	Abs EtOH	44	$C_{18}H_{20}N_2O_5S$	C, H, N
69	OC_6H_5	$CH_2C{\equiv}CH$	Et	3C	190–191	Abs EtOH	34	$C_{18}H_{18}N_2O_5S$	C, H, N
70	OC_6H_5	$CH_2CHBrCH_2Br$	Et	3F	163–164	Abs EtOH	36[g]	$C_{18}H_{20}Br_2O_5S$	C, H, N
71	OC_6H_5	*n*-Bu	*n*-Bu	3G	146–147	*n*-BuOH	35[h]	$C_{21}H_{28}N_2O_5S$	C, H, N
72	OC_6H_5	*n*-Am	*n*-Am	3G	138–139	*n*-AmOH	47[h]	$C_{23}H_{32}N_2O_5S$	C, H, N
73	OC_6H_5	n-C_6H_{13}	n-C_6H_{13}	3H	137–138	n-$C_6H_{13}OH$	35[g]	$C_{25}H_{36}N_2O_5S$	C, H, N
74	OC_6H_4, 4-OMe	$CH_2C_6H_5$	Et	3B	189–190	Me_2CO	7[i]	$C_{23}H_{24}N_2O_6S$	C, H, N
75	OC_6H_4, 4-$OCH_2C_6H_5$	$CH_2C_6H_5$	Et	3B	166	Abs EtOH	30[g]	$C_{29}H_{28}N_2O_6S$	C, H, N

[a–d] See corresponding footnotes in Table I. [e] C: calcd 57.26; found: 56.67. [f] Analytically pure **53** could not be obtained. [g] Dried *in vacuo* (10–14 mm) at 115° for 2–6 hr. [h] Dried *in vacuo* (10–14 mm) at 78° for 2–6 hr. [i] Crude **74** (mp 186–187°) was obtained in 72% yield.

Method 1B.—To a mixt of the appropriate R_1H (0.04 mole) and H_2O (8 ml for **3**, 13 ml for **4**, **5**, and 60 ml for **6**, resp) **1** (2.8 g) was added with stirring. The mixt was refluxed for 2 hr, and after cooling, the crude reaction product was pptd by acidification with 4 *N* HCl.

Method 1C.—The reaction mixt was prepd following method 1B using 13 ml of H_2O. Instead of refluxing, it was stirred for 2 hr and then left for 16 hr in a refrigerator. The pptd amine salt was collected by filtration and recrystd several times from H_2O. The salt was then dissolved in hot H_2O, and crude **7** was liberated by acidification with 1 *N* HCl.

Method 1D.—A mixt of **1** (2.8 g), the appropriate R_1H (0.03 mole), and H_2O (25 ml) was refluxed for 2.5 hr. After cooling the mixt was made strongly alk by addn of 2 *N* NaOH and extd with Et_2O. The aq layer was acidified to ppt crude **8**.

Method 1E.—A mixt of **1** (2.8 g), the appropriate R_1H (0.03 mole), and H_2O (25–100 ml) was refluxed for several hours. After cooling the crude material was pptd by acidification with 4 *N* HCl.

Method 1F.—A suspension of **1** (22.4 g, 0.08 mole) and the appropriate R_1H (0.08 mole) in H_2O (200 ml) was adjusted to pH 7 by addn of solid $NaHCO_3$. The resulting soln was refluxed for 10 hr. After cooling, 4 *N* HCl (25 ml) was added with stirring to ppt crude **15**.

Method 1G.—A mixt of **1** (2.8 g), the appropriate R_1H (0.03 mole), abs EtOH (25 ml), and anhyd AcONa (0.82 g) was refluxed for 5 hr. After cooling, the solvent was distd off, and the residue was triturated with hot 1 *N* HCl (35 ml). Pptd crude **16** was collected by filtration while the temp was maintained at 80°.

Method 1H.—To a soln of **1** (2.8 g) in 1 *N* $NaHCO_3$ (30 ml) the appropriate R_1H (1.1 ml) was added, and the mixt was heated to 90° for 22 hr. The cooled soln was acidified with 4 *N* HCl to ppt crude **17**.

Method 1I.—To a soln of **17** (10.02 g) in AcOH (150 ml) H_2O_2 (30 ml, 30% in H_2O) was added, and the mixt was left for 24 hr. H_2O (150 ml) was added causing pptn of crude **18**.

Method 1J.—A mixt of **1** (14 g), the appropriate R_1H (5.5 g), and 1 *N* NaOH (100 ml) was stirred at room temp for 2 days. After treatment with decolorizing carbon and filtration, crude **19** was pptd by acidification of the filtrate with 4 *N* HCl.

Method 1K.—To a soln of **1** (28 g) in 1 *N* $NaHCO_3$ (300 ml) R_1H (12.4 g) was added, and the mixt was refluxed for 3 hr. After cooling and standing for 16 hr the pptd Na salt of **20** was collected. The Na salt was recrystd from 1 *N* $NaHCO_3$ (150 ml) using satd NaCl (80 ml) for completing the crystn. The Na salt (28 g) was dissolved in hot H_2O (250 ml), and crude **20** was liberated by acidification with 4 *N* HCl.

Method 1L.—**1** (4.2 g) was added to a soln of CF_3CH_2ONa in CF_3CH_2OH (45 ml contg 2.1 g of Na), and the mixt was refluxed for 4 days. After evapn *in vacuo* H_2O (50 ml) was added, and the pH was adjusted to 1.5 by addn of 4 *N* HCl. After standing at 5° for several days the ppt was collected, washed with H_2O, and dried. The material was redissolved in Me_2CO (35 ml), and crude **21** was pptd by slow addn of petroleum ether to the filtered soln.

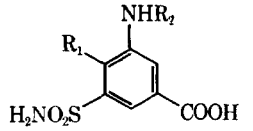
Method 1M.—To a suspension of **1** (14 g) in H_2O (100 ml) $NaHCO_3$ (17 g) was added cautiously followed by C_6H_5OH (10 g). The resulting soln was kept at 85° for 16 hr. After cooling, the pptd Na salt of **22** (7.9 g) was collected. The sodium salt was dissolved in hot H_2O (110 ml), and crude **22** was liberated by acidification with 4 *N* HCl.

Method 1N.—To a soln of **1** (14 g) in 1 *N* $NaHCO_3$ (200 ml) the appropriate R_1H was added, and the mixt was heated to 85–95° for 5 hr. After cooling and acidification with 4 *N* HCl the crude material crystd with stirring. For **23** 9.3 g of R_1H and for **24** 40 g of R_1H were used. For **24** the excess of R_1H was removed by steam distn before crystn.

Method 1O.—The *n*-Bu ester of **1** (33.6 g) [prepd from **1** in boiling *n*-BuOH with H_2SO_4 as catalyst and recrystd from *n*-BuOH, mp 140–141°. *Anal.* ($C_{11}H_{13}ClN_2O_6S$) C, H, N] and 4-benzyloxyphenol (20 g) were added to a soln of *n*-BuONa (from 2.1 g of Na) in abs *n*-BuOH (300 ml), and the mixt was refluxed

Table IV

Physical Properties and Screening Results of



No.	R_1	R_2	Method[a]	Mp, °C	Recryst solvent[b]	Yield,[c] %	Formula	Analysis[d]	Diuretic screening in dogs[e] Urinary excretion after 0.25 mg/kg iv in NaOH soln: ml/kg per 3 hr H_2O	mequiv/kg per 3 hr Na^+	mequiv/kg per 3 hr K^+	mequiv/kg per 3 hr Cl^-
Control									2[f]	0.2[f]	0.1[f]	0.1[f]
76	Cl	$CH_2C_6H_5$	*g*						5[h]	0.5[h]	0.2[h]	0.6[h]
77	Cl	*n*-Bu	*g*						3[i]	0.5[i]	0.2[i]	0.5[i]
78	NH-*i*-Pr	$CH_2C_6H_5$	4A	233–234	EtOH–H_2O	24	$C_{17}H_{21}N_3O_4S$	C, H, N	7	0.7	0.2	1.0
79	NH-*n*-Bu	$CH_2C_6H_5$	4B	198.5–199	EtOH	17[j]	$C_{18}H_{23}N_3O_4S$	C, H, N*	7	1.1	0.2	0.9
80	$NCH_2CH_2CH_2CH_2CH_2$	$CH_2C_6H_5$	4A	196	Aq EtOH	8[j]	$C_{19}H_{23}N_3O_4S \cdot 0.5H_2O$	C, H, N			*k*	
81	$NCH_2CH_2OCH_2CH_2$	$CH_2C_6H_5$	4C	237	Aq EtOH	62[j]	$C_{18}H_{21}N_3O_5S$	C, H, N			*l*	
82	NHC_6H_{11}	$CH_2C_6H_5$	4C	249–250	EtOH	56[j]	$C_{20}H_{25}N_3O_4S$	C, H, N	8[m]	0.9[m]	0.2[m]	1.2[m]
83	$NHCH_2C_6H_5$	$CH_2C_6H_5$	4D	205	Aq EtOH	63	$C_{21}H_{21}N_3O_4S$	C, H,* N			*n*	
84	$NHCH_2CH_2C_6H_5$	$CH_2C_6H_5$	4C	203	Aq EtOH	89	$C_{22}H_{23}N_3O_4S$	C, H, N			*o*	
85	NHC_6H_5	$CH_2C_6H_5$	4C	248–249	Aq EtOH	78[j]	$C_{20}H_{19}N_3O_4S$	C, H, N	36[p]	3.5[p]	0.8[p]	5.3[p]
86	NHC_6H_5	*n*-Bu	4E	231–231.5	Aq EtOH	14	$C_{17}H_{21}N_3O_4S$	C, H, N	32[p]	3.0[p]	0.5[p]	4.3[p]
87	NHC_6H_4, 4-Cl	$CH_2C_6H_5$	4C	244–245.5	Aq EtOH	74[j]	$C_{20}H_{18}ClN_3O_4S$	C, H, N	26	2.9	0.6	4.0
88	NHC_6H_4, 4-Me	$CH_2C_6H_5$	4C	217–218	Aq EtOH	60	$C_{21}H_{21}N_3O_4S$	C, H, N	28	3.0	0.3	4.2
89	NHC_6H_4, 3-Me	$CH_2C_6H_5$	4C	226–227.5	EtOH	72	$C_{21}H_{21}N_3O_4S$	C, H, N	24	3.3	0.3	3.5
90	NHC_6H_4, 4-OMe	$CH_2C_6H_5$	4C	207–208	Aq EtOH	78[q]	$C_{21}H_{21}N_3O_5S$	C, H, N	18	1.8	0.5	2.6
91	NHC_6H_4, 4-OMe	$CH_2CH{=}CH_2$	4C	216–217	Aq EtOH	74[q]	$C_{17}H_{19}N_3O_5S$	C, H, N*	12	1.1	0.2	1.3
92	NHC_6H_3, 2,4-Me_2	$CH_2C_6H_5$	4C	245–246	Aq EtOH	61[q]	$C_{22}H_{23}N_3O_4S$	C, H, N	2	0.4	0.4	0.4
93	NHC_6H_4, 4-COOH	$CH_2C_6H_5$	4C	300	Aq EtOH	42[q]	$C_{21}H_{19}N_3O_6S \cdot 0.5H_2O$	C, H, N	8	1.0	0.3	1.3
94	NH-β-naphthyl	$CH_2C_6H_5$	4C	261–262	Aq Me_2CO	28	$C_{24}H_{21}N_3O_4S$	C, H, N	15	1.7	0.4	2.5
95	S-*n*-Bu	$CH_2C_6H_5$	4C	210–211	Aq EtOH	85[q]	$C_{18}H_{22}N_2O_4S_2$	C, H, N	5	0.4	0.1	0.6
96	SO-*n*-Bu	$CH_2C_6H_5$	4F	182 dec	Aq EtOH	8[j]	$C_{18}H_{22}N_2O_5S_2 \cdot H_2O$	C, H, N	11	1.0	0.3	1.4
97	SC_6H_5	$CH_2C_6H_5$	4B	226–227	EtOH	35[j]	$C_{20}H_{18}N_2O_4S_2$	C, H, N	36[p]	3.6[p]	0.9[p]	5.0[p]
98	SC_6H_5	*n*-Bu	4E	203–204	Aq EtOH	56[q]	$C_{17}H_{20}N_2O_4S_2$	C, H, N	31[p,r]	3.3[p,r]	0.6[p,r]	4.3[p,r]
99	SOC_6H_5	$CH_2C_6H_5$	4G	243	Aq MeOH	45[j]	$C_{20}H_{18}N_2O_5S_2$	C, H, N	24	2.7	0.6	3.8
100	SOC_6H_5	*n*-Bu	4C	203–204 dec	Aq EtOH	19[q]	$C_{17}H_{20}N_2O_5S_2$	C, H, N	25	2.6	0.5	3.4
101	SC_6H_5, 2-Me	$CH_2C_6H_5$	4C	228–229	Aq EtOH	54[q]	$C_{21}H_{20}N_2O_4S_2$	C, H, N	24	3.1	0.4	4.0
102	OCH_2CF_3	$CH_2C_6H_5$	4C	230–232	Aq EtOH	90[q]	$C_{16}H_{15}F_3N_2O_5S$	C, H, N	10	1.0	0.5	1.5
103	OC_6H_5	$CH_2C_6H_5$	4C	264–265	Aq EtOH	61[q]	$C_{20}H_{18}N_2O_5S$	C, H, N	40[p]	4.1[p]	1.0[p]	5.8[p]
104	OC_6H_5	$CH_2C_6H_4$, 4-OMe	4H	248–250	MeOH	38[q]	$C_{21}H_{20}N_2O_6S$	C, H, N	1	0.1	0.2	0.2
105	OC_6H_5	$CH_2C_6H_3$, 3,4-OCH_2O	4H	229–230	MeOH	35[q]	$C_{21}H_{18}N_2O_7S$	C, H, N	2	0.1	0.3	0.3
106	OC_6H_5	Et	4I	236–237	EtOH	14	$C_{15}H_{16}N_2O_5S$	C, H, N	11	1.1	0.3	1.8
107	OC_6H_5	*n*-Pr	4C	223–225	Aq EtOH	85[q]	$C_{16}H_{18}N_2O_5S$	C, H, N	15	1.3	0.2	1.9
108	OC_6H_5	$CH_2CH{=}CH_2$	4J	223–225	Aq EtOH	79	$C_{16}H_{16}N_2O_5S$	C, H, N	11	1.0	0.3	1.5
109	OC_6H_5	$CH_2C{\equiv}CH$	4J	222–223	Aq EtOH	78[q]	$C_{16}H_{14}N_2O_5S$	C, H, N	7	0.7	0.2	1.0

110	OC_6H_5	$CH_2CBr{=}CH_2$[s]	4J	193–194	EtOH–H_2O	49	$C_{16}H_{15}BrN_2O_5S$	C, H, N	30[p]	3.6[p]	0.6[p]	4.7[p]
111	OC_6H_5	*n*-Bu	4C, 4E	230–231	Aq EtOH	71[q,t]	$C_{17}H_{20}N_2O_5S$	C, H, N, nmr[u]	39[p,v]	4.0[p,v]	0.8[p,v]	5.7[p,v]
112	OC_6H_5	*n*-Am	4C	223–224	Aq EtOH	73	$C_{18}H_{22}N_2O_5S$	C, H, N	29	2.9	0.6	4.5
113	OC_6H_5	*n*-C_6H_{13}	4C	221–223	Aq EtOH	50	$C_{19}H_{24}N_2O_5S$	C, H, N	4	0.5	0.1	0.5
114	OC_6H_5	$CH_2CCHCHCHO$	4K	215–216	Aq EtOH	14[q]	$C_{18}H_{16}N_2O_6S$	C, H, N	40[p]	4.2[p]	0.7[p]	6.0[p]
115	OC_6H_4, 4-OMe	$CH_2C_6H_5$	4C	230–232	Aq EtOH	75	$C_{21}H_{20}N_2O_6S$	C, H, N	15	1.6	0.2	2.2
116	OC_6H_4, 3-CF_3	$CH_2C_6H_5$	4L	220–222	Aq EtOH	23[q]	$C_{21}H_{17}F_3N_2O_5S$	C, H, N	16	2.0	0.4	2.9
117	OC_6H_4, 4-$OCH_2C_6H_5$	$CH_2C_6H_5$	4J	250.5–251.5	Aq EtOH	70[q]	$C_{27}H_{24}N_2O_6S$	C, H, N	3	0.3	0.1	0.4
118	OC_6H_4, 4-OH	$CH_2C_6H_5$	4M	275–277	Aq EtOH	51[q]	$C_{20}H_{18}N_2O_6S$	C, H, N	43[p]	4.7[p]	0.7[p]	6.1[p]
119	OC_6H_4, 4-SO_2NH_2	*n*-Bu	4N	265	EtOH–H_2O[w]	31[q]	$C_{17}H_{21}N_3O_7S_2$	C, H, N, S	13	1.3	0.2	1.6

[a–d] See corresponding footnotes in Table I. [e] The screening proredure is described by P. W. Feit, H. Bruun, and C. Kaergaard-Nielsen, *J. Med. Chem.*, **13**, 1071 (1970); when not otherwise stated single test only. [f] Average of 3 tests is given. [g] See footnote *e*. [h] Results after 10 mg/kg, see ref cited in footnote *e*. [i] The dose–response curve is given in the ref cited in footnote *e*. [j] Dried *in vacuo* (10–14 mm) at 78° for 2–4 hr. [k] Results after 10 mg/kg: 14, 1.5, 0.4, 2.0, resp. [l] Results after 10 mg/kg: 12, 1.1, 0.5, 1.7, resp. [m] Results in a 6-hr test period. [n] Results after 10 mg/kg: 13, 1.3, 0.4, 2.0, resp. [o] Results after 1 mg/kg: 11, 1.5, 0.6, 1.7, resp. [p] For further results see Table V. [q] Dried *in vacuo* (10–14 mm) at 115° for 2–6 hr. [r] Average of 2 tests is given. [s] The position of Br was proved by nmr spectroscopy. [t] The yield obtained following method 4C is given. [u] Nmr [$(CD_3)_2SO$, TMS] 0.78 (t, J = 6.5 Hz, 3 H, CH_3), 0.9–1.7 (m, 4 H, CH_2), 3.10 (m, 2 H, CH_2N), 4.90 (bt, J = 7 Hz, 1 H, $NHCH_2$), 6.7–7.6 (m, 5 H, C_6H_5O), 7.48 (d, J = 2.0 Hz, 1 H, arom H), 7.78 (d, J = 2.0 Hz, 1 H, arom H), 7.28 (bs, 3 H, H_2NSO_2, COOH). [v] Average of 4 tests is given. [w] Warm EtOH was used to dissolve the compd.

for 3 hr. From the hot filtered soln the *n*-Bu ester of **25** (11.5 g) pptd on cooling. This crude ester of **25** was dissolved in 1 *N* NaOH (165 ml) and sapond by standing for 18 hr at room temp. Acidification with 4 *N* HCl pptd crude **25**.

Method 1P.—A soln of **22** (5 g) in 1 *N* NaOH (60 ml) was kept at 90–95° for 6 hr. After cooling 1 *N* HCl (60 ml) was added to ppt crude **26**.

4-R_1-3-NH_2-5-sulfamylbenzoic Acids (27–50, Table II). Method 2A.—An approx 10% aq alk soln (NaOH or LiOH) (adjusted to about pH 9.5) of the corresponding 4-R_1-3-nitro-5-sulfamylbenzoic acid (Table I) was hydrogenated at room temp after addn of (10%) Pd/C, adapting the amt of catalyst to the speed of hydrogenation. When the H_2 uptake became negligible the catalyst was removed by filtration, and the crude amino acid deriv was pptd by addn of 4 *N* HCl until pH 2.5–3; for **39**, pH 1.5.

Method 2B.—To a stirred mixt of the corresponding 4-R_1-3-nitro-5-sulfamylbenzoic acid (0.02 mole), concd aq NH_3 (15 ml), and H_2O (50 ml) a soln of $Na_2S_2O_4$ (13.5 g) in H_2O was added dropwise at 20–25°. After addnl stirring for 1 hr 4 *N* HCl was added until pH 2.5 to ppt the crude material.

Method 2C.—To a stirred soln of $Na_2S_2O_4$ (16 g) in H_2O (100 ml) concd aq NH_3 (50 ml) was added followed by **17** (8.35 g) in small portions. After the addn had been completed the mixt was heated on a steam bath for 30 min. After the pH had been adjusted to 8 the soln was cooled to ppt the Na salt of **42**. The salt was redissolved in warm H_2O (100 ml), and crude **42** was pptd by addn of 4 *N* HCl until pH 2.5.

Method 2D.—Method 2B was followed except that, after addn of 4-R_1-3-nitro-5-sulfamylbenzoic acid, the mixt was acidified with 4 *N* HCl and heated under SO_2 evolution for 20 min. The pH 2.5 was adjusted by addn of 2 *N* NaOH.

Method 2E.—To a stirred mixt of Fe powder (8 g), NH_4Cl (0.84 g), H_2O (20 ml), and 1 *N* HCl (0.4 ml), **19** (3.5 g) was added in portions during 1 hr. After stirring for an addnl 1 hr, the mixt was filtered hot, and the filter cake was extd 3 times with hot 1 *N* NaOH. From the combined filtrates **44** was pptd by addn of 4 *N* HCl until pH 2.5.

Method 2F.—Method 2C was followed using **20** (7.5 g) except that no salt pptn occurred. Cooling and acidification with 4 *N* HCl to pH 2.5 pptd crude **45.**

Method 2G.—Method 2A was followed except that an aq soln of pH 7.5–8 was prepared using LiOH.

Method 2H.—Method 2A was followed except that an approx 5% alk soln and PtO_2 catalyst were used.

4-R_1-3-R_2NH-5-sulfamylbenzoates (51–75, Table III). Method 3A.—To a suspension of the corresponding 4-R_1-3-NH_2-5-sulfamylbenzoic acid in abs EtOH (10–30 ml/g of benzoic acid deriv) $C_6H_5CH_2Br$ (2.5 equiv) was added. The stirred mixt was refluxed and refluxing continued for several hours after a soln had been achieved. Addnl $C_6H_5CH_2Br$ was added in order to replace $C_6H_5CH_2Br$ converted to $C_6H_5CH_2OEt$, and the mixt was refluxed for a further 6 hr. The esterification and benzylation processes were controlled by tlc.‡ If necessary this operation was repeated several times. After cooling the mixt was left to ppt the crude material.

Method 3B.—Method 3A was followed except that no addnl $C_6H_5CH_2Br$ was added.

Method 3C.—Method 3B was adapted using $CH_2{=}CHCH_2Br$ (1.2 equiv) for **59** and $CH{\equiv}CCH_2Br$ (4 equiv) for **69**, resp. For **69** the refluxing was extended to 2 days.

Method 3D.—68 (1.65 g) was hydrogenated in EtOH (125 ml) using PtO_2 (0.05 g) as catalyst. After removal of the catalyst the soln was evapd *in vacuo* to yield crude **67**.

Method 3E.—Method 3A was followed except that $CH_2{=}CHCH_2Br$ was used instead of $C_6H_5CH_2Br$.

Method 3F.—To a soln of **68** (1 g) in $CHCl_3$ (5 ml), a soln of Br_2 (0.5 g) in $CHCl_3$ (10 ml) was added, and the mixt was stirred for 2.5 hr at room temp. Then the pptn of crude **70** was completed by addn of Et_2O (20 ml).

Method 3G.—47 was alkylated using *n*-BuOH for **71** and *n*-AmOH for **72**, resp, as described[1] for the corresponding 4-Cl compd except that the reaction mixt was cooled without sapon to ppt the crude material.

Method 3H.—A mixt of **47** (4.62 g), *n*-$C_6H_{13}Br$ (5 g), *n*-$C_6H_{13}OH$ (40 ml), and $MeSO_3H$ (0.05 ml) was refluxed for 2.5 days. Cooling pptd crude **73**.

‡ Silica gel (HF 254, Merck): $CHCl_3$ (80), AcOH (10), C_6H_{12} (10), MeOH (2.5).

TABLE V

DIURETIC AND SALURETIC ACTIVITY OF SOME 4-R_1-3-R_2NH-5-SULFAMYLBENZOIC ACIDS IN DOGS

		Urinary excretion[a]							
		iv[c]				po[d]			
No.[b]	Treatment mg/kg	ml/kg per 6 hr H_2O	mequiv/kg per 6 hr Na^+	K^+	Cl^-	ml/kg per 6 hr H_2O	mequiv/kg per 6 hr Na^+	K^+	Cl^-
Control[e]		3	0.3	0.2	0.2				
85	0.5	64	4.3	0.9	7.1	48	4.8	1.3	5.9
	0.25	43	3.9	1.2	5.9	38	4.6	0.8	6.2
	0.1	44	4.5	1.0	8.9	39	3.7	0.6	4.8
	0.05	29	2.5	1.1	4.0				
	0.01					7.7	1.2	0.3	1.3
86	0.5					59	6.7	1.6	8.7
	0.25	40	3.6	0.7	5.3	35	3.7	0.6	5.4
	0.1					31	3.2	0.9	4.5
	0.01					13	1.2	0.2	2.0
97	0.5					47	5.0	1.0	6.9
	0.25	46	4.3	1.7	6.0	44	4.8	1.0	6.7
	0.1					38	3.1	0.9	5.0
	0.01					11	1.0	0.4	1.4
98	0.25[f]	39	3.7	0.9	5.1	40	4.0	1.1	5.7
	0.05	30	3.5	0.6	4.4	20	2.5	0.4	3.2
103	0.25[e]	52	5.0	1.5	6.9	41	4.2	0.9	7.5
	0.1	28	3.0	0.8	3.9	36	4.0	2.1	5.9
	0.05	23	2.4	0.5	3.2	35	4.1	0.9	5.5
	0.025	22	2.2	0.5	3.0	23	3.7	0.6	3.2
	0.01					21	1.7	0.4	2.1
110	0.25	32	3.7	0.7	4.9				
	0.05	15	1.5	0.4	1.6	5	0.7	0.2	0.8
111	0.25[g]	45	4.3	1.1	6.2	51	4.8	1.0	7.0
	0.01[g]	6	1.0	0.3	1.4	13	1.2	0.3	1.4
114	0.25	43	4.3	0.8	6.0				
	0.05	19	2.0	0.4	2.5	27	3.2	0.6	3.9
	0.01					10	1.2	0.3	1.8
118	0.25	46	4.8	0.8	6.3	33	3.8	0.8	4.9
	0.1	41	3.5	1.2	4.8				
	0.01					2	0.2	0.1	0.1
4-Chloro-*N*-(2-furylmethyl)-anthranilic acid[h] (furosemide)	10.0[i]	33	3.7	0.8	4.8				
	4.0[i]	26	2.4	0.7	3.1				
	0.5	13	1.1	0.3	1.4	8	1.5	0.2	1.8
	0.1	6	0.5	0.3	0.7				

[a] See footnote *e*, Table IV. [b] For structure see Table IV. [c] As soln in NaOH. [d] In gelatin capsules. [e] Average of 3 tests. [f] Average of 2 tests. [g] Average of 4 tests. For the dose response see E. H. Østergaard, M. P. Magnussen, C. Kaergaard Nielsen, E. Eilertsen, and H.-H. Frey, to be published. [h] See K. Sturm, W. Siedel, R. Weyer, and H. Ruschig, *Chem. Ber.*, **99**, 328 (1966). [i] Results of a 3-hr test period.

4-R_1-3-R_2NH-5-sulfamylbenzoic Acids (78–119, Table IV). Method 4A.—Method 3A was followed except that the Et ester failed to ppt. The reaction mixt was therefore evapd *in vacuo* and the residue was sapond by heating with excess of 1 *N* NaOH on a steam bath for 45 min. After cooling and extn with Et_2O the aq soln was adjusted to pH 2.5–3 by addn of 1 *N* HCl to ppt the crude material.

Method 4B.—The appropriate 4-R_1-3-NH_2-5-sulfamylbenzoic acid was treated with the theor amt of $C_6H_5CH_2Br$ in aq soln at pH 7.5 using an automatic end-point titrator as described[1] for the parent 4-Cl compd, except that after the end of the reaction the pH was adjusted to 2–3 to ppt the crude material.

Method 4C.—The appropriate ester of 4-R_1-3-R_2NH-5-sulfamylbenzoic acid (Table III) was sapond in excess of 1 *N* NaOH (15 ml/g of ester) by heating on a steam bath for 45 min. After cooling, the crude material was pptd by addn of the corresponding amt of 4 *N* HCl. The first purification of **84** was accomplished through the Na salt prepd in H_2O using 1 *N* $NaHCO_3$. **113** was first isolated as Na salt at pH 7.5.

Method 4D.—A suspension of **32** (1 g) in H_2O (20 ml) was adjusted to pH 7.5 with 1 *N* NaOH using an automatic endpoint titrator. After addn of $C_6H_5CH_2Br$ (0.54 g) the pH was kept constant with stirring at room temp. When the NaOH consumption had ceased the pptd Na salt (0.85 g) was collected. The Na salt was heated in aq EtOH (60 ml), and AcOH (2 ml) was added to liberate the acid. Cooling pptd crude **71**.

Method 4E.—The method described[1] for the parent 4-Cl compd was adapted with an increased amt of *n*-BuOH.§ For **86** the reaction mixt obtained after sapon was evapd *in vacuo*. The residue was redissolved in H_2O and filtered, and the filtrate was adjusted to pH 7.5. After standing in a refrigerator for 3 days crude Na salt of **86** pptd. The salts might be purified by recrystn from H_2O. *Anal.* Na salt of **86** ($C_{17}H_{20}NaN_3O_4S \cdot 3H_2O$): C, H, N, H_2O. *Anal.* Na salt of 111 ($C_{17}H_{19}NaN_2O_5 \cdot 3H_2O$), C, H, N, H_2O*.

Method 4F.—Method 3B was followed except that the Et ester failed to ppt. The reaction mixt was therefore evapd *in vacuo*, and the residue was sapond with excess of 1 *N* NaOH by

§ The amt of H_2SO_4 was not increased in order to avoid higher concn of formed $(n\text{-Bu})_2SO_4$ which causes butylation of the sulfonamide N during the sapon process.

standing in the dark at room temp for 18 hr. After extn with Et_2O the aq soln was adjusted to pH 7.5 by addn of 4 *N* HCl followed by NaCl to ppt crude Na salt of **96**. After several recrystn from H_2O the Na salt was dissolved in warm aq EtOH and crude **96** pptd by addn of 4 *N* HCl until pH 3.

Method 4G.—A mixt of the appropriate 4-R_1-3-R_2NH-5-sulfamylbenzoic acid (0.8 g of **97** and 0.5 g of **98**, resp), AcOH (20 20 ml for **99** and 5 ml for **100**, resp), and H_2O_2 (30% aq soln; 1.5 ml for **99** and 2.5 ml for **100**, resp) was stirred at room temp for several days, after which the crude reaction product was isolated by filtration.

Method 4H.—**47** was reductive alkylated using the appropriate aldehyde as described[1] for the parent 4-Cl compd.

Method 4I.—A mixt of **47** (3 g), EtI (20 ml), and abs EtOH (20 ml) was refluxed for 5 days. After evapn *in vacuo* the residue was triturated with EtOH, and the solid contg the Et ester of **106** was collected by filtration. This crude ester was sapond and worked up following method 4C to yield crude **106**.

Method 4J.—Method 4C was followed except that the sapon was performed for 18 hr at room temp in the dark. For **110**, **70** was used as dehydrobromination occurred simultaneously.

Method 4K.—A mixt of the Na salt of **47** (4.95 g dried *in vacuo* at 115°; prepd by heating **47** in the calcd amt of 2.5 *N* NaOH followed by cooling and isolating of the pptd salt), abs MeOH (75 ml), and furfural (2.16 g) was refluxed for 5 hr. After cooling, $NaBH_4$ (2.5 g) was added in portions at 0–5° with stirring. After standing for 18 hr the mixt was evapd *in vacuo*, and the residue was redissolved in H_2O (45 ml). Adjusting the pH to 7.5 pptd crude Na salt of **114** (1.4 g), which was recrystd from H_2O. From a 0.5% soln of this Na salt in warm H_2O crude **114** pptd after addn of AcOH.

Method 4L.—Method 3B was followed, and the crude ester was sapond according to method 4C.

Method 4M.—An aq soln of **117** (0.5 g) was hydrogenated at pH 11 using PtO_2 catalyst (0.025 g). After the H_2 uptake had ceased, the catalyst was removed, and crude **118** was pptd by addn of 4 *N* HCl until pH 1.5.

Method 4N.—**111** (1 g) was added to HSO_3Cl (5 ml) with stirring. The mixt was allowed to warm to 50°. After stirring for 10 min it was poured on ice to ppt crude **111** 4′-sulfochloride. The crude sulfochloride was added to concd aq NH_3 with stirring. Then the reaction mixt was heated on a steam bath to remove most of the excess of NH_3. After cooling the pH was adjusted to 2.5 to ppt crude **119**.

5-*n*-Butylsulfamyl-4-chloro-3-nitrobenzoic Acid (120).—To a cooled mixt of *n*-$BuNH_2$ (2.2 g) and 1 *N* NaOH (60 ml) 4-chloro-5-chlorosulfonyl-3-nitrobenzoic acid[1] was added in portions at −4 to −2° with stirring. The stirring was continued while the mixt was allowed to reach room temp. Filtration and addn of 1 *N* HCl (50 ml) to the filtrate provided sep of oily **120** crystg on standing for 16 hr. It was twice recrystd from aq EtOH to yield **120** (4.1 g), mp 196–198°. *Anal.* ($C_{11}H_{13}ClN_2O_6S$) C, H, N.

5-*n*-Butylsulfamyl-3-nitro-4-phenoxybenzoic Acid (121).—Method 1N was followed using **120** as starting material except that the Na salt failed to sep. Therefore crude **121** was pptd by acidification of the cooled reaction mixt. It was recrystd from aq EtOH and EtOH to yield **121** (49%), mp 191–192°. *Anal.* ($C_{17}H_{18}N_2O_7S$) C, H, N.

3-Amino-5-*n*-butylsulfamyl-4-phenoxybenzoic Acid (122).—Method 2G was followed using **121** as starting material. It was twice recrystd from aq EtOH to yield **122** (61%): mp 188–189°; nmr [$(CD_3)_2SO$, TMS], δ 0.8 (t, J = 6.3 H, 3 H, CH_3), 0.9–1.6 (m, 4 H, CH_2), 2.85 (m, 2 H, CH_2N), 5.20 (bs, 2 H, NH_2C), 6.8–7.5 (m, 5 H, C_6H_5O), 7.67 (s, 2 H, arom H), ~7.0 (b line, 2 H, COOH, $HNSO_2$). *Anal.* ($C_{17}H_{20}N_2O_5S \cdot 0.5H_2O$)C*, H, N, H_2O.

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Effect of 2-Anilinopyridines on Protein Synthesis

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A series of 2-(R-substituted anilino)pyridines has been synthesized by the interaction of 2-halopyridines with R-substituted anilines at elevated temperatures. These compounds have been assayed for their effect on protein synthesis. The 2-(4-bromo, 4-chloro, and 3,4-dichloroanilino)-6-methylpyridines were found to be as effective as chloramphenicol and *p*-fluorophenylalanine in their ability to block utilization of phenylalanine in a cell-free system. A structure–activity relationship was studied but no correlation between protein synthesis inhibition and *in vitro* microbial activity could be established.

During the course of studies with a cell-free system prepared from *Escherichia coli*, 2-(*m*-chloroanilino)-6-methylpyridine was found to inhibit the incorporation of phenylalanine into protein. Since protein synthesis inhibition is recognized as one of the major biochemical modes of action of a number of prominent antibiotics[1] (the tetracyclines, the erythromycins, chloramphenicol, lincomycin, novobiocin, etc.), a number of 2-anilinopyridines and related substances were prepared and assayed for their inhibitory effects on bacterial protein synthesis under cell-free conditions. The compounds were further evaluated for their ability to inhibit the growth of cultures of *E. coli* and *Staphylococcus aureus*. The results of these screens are recorded in Table I.

Selected members of the series (**2**, **3**, **5**, **8**, **10**, **11**, **18**, **26**, **27**) were screened *in vivo* for activity against *S. aureus*. These were found to be uniformly inactive.

Chemistry.—The 2-anilinopyridines were synthesized by cautiously heating to 150–170° a mixture of R-substituted-2-chloro- or -2-bromopyridine and 2 molar equiv of Y-substituted aniline. Alternatively, the second equivalent of Y-substituted aniline could be replaced with dimethylaniline. The reaction failed with *o*- and *p*-nitroaniline, and the derivatives **15** and **17**

(1) The following references are among many dealing with this topic: (a) E. F. Gale, *Pharmacol. Rev.*, **15**, 481 (1963); (b) D. S. Feingold, *N. Engl. J. Med.*, **206**, 900, 957 (1963); (c) I. H. Goldberg, *Amer. J. Med.*, **39**, 722 (1965); (d) T. J. Franklin, in "Biochemical Studies of Antimicrobial Drugs," Cambridge University Press, New York, N. Y., 1966, p 192 f; (e) F. E. Hahn, *Int. Congr. Microbiol., 9th, 1966*, 41 (1966); (f) R. E. Monro and D. Vazquez, *J. Mol. Biol.*, **28**, 161 (1967); (g) B. Weisblum and J. Davies, *Bacteriol. Rev.*, **32**, 493 (1968).