Substituted Salicylanilides III

New Salicylanilides and Related Compounds with Antimicrobial Activity

By ROBERT G. TABORSKY† and ROLAND J. STARKEY

Eighteen substituted salicylanilides and related compounds have been synthesized and screened for antimicrobial activity. Most of these compounds have been prepared for the first time. The relationship of structure and activity for the series is discussed.

INTEREST IN SUBSTITUTED salicylanilides was a result of the observation of a high degree of antimicrobial activity of halonitrosalicylanilides (1) and other substituted salicylanilides (2). The first objective of the present investigation was to attempt to define further the structural requirements necessary for antimicrobial activity for this series. Some of the previously prepared salicylanilides possessed limitations to their usefulness because of toxicity, solubility, color, and inactivation by blood serum. Thus the second objective was to see whether new and more favorable members of the series could be developed.

All of the compounds under discussion are summarized and described in Table I. A considerable group (I–VIII) of those salicylanilides were synthesized by the reaction of a suitably substituted salicoyl chloride with an amine in an appropriate organic solvent, usually benzene.

Salicylanilides substituted in the "three" or "five" positions by an amino or acetylamino group were prepared by reduction of the respective nitro analogs. The reducing agent used for the former was sodium dithionate (3), and for the latter it was zinc and glacial acetic acid. The amino compound was converted to the diazo derivative by a normal diazotization (3).

The phthalyl-mono-chloroanilides were prepared by the reaction of a substituted or unsubstituted phthalic anhydride with *para*-chloroaniline (4). The imine, N-(5-nitrosalicylidene) *meta*-chloroaniline was prepared by the condensation of 5-nitrosalicylaldehyde and *para*-chloroaniline in ethanol.

4′ - Chloro - 5 - (N - *para* - chlorophenyl - sulfamido)salicylanilide was prepared by refluxing together 5-sulfosalicylic acid and *para*-chloroaniline with phosphorus trichloride in chloroform. 4′-Chloro-5-sulfamidosalicylanilide was prepared in a similar manner from 5-sulfamidosalicylic acid and *para*-chloroaniline.

All of the compounds have been synthesized for the first time with the exception of III, XI, XII, and XIII. The compounds were screened against two significant organisms by the cup-plate method. The results of that primary screening are summarized in Table II.

In the ensuing discussion, it must be remembered that all of the antimicrobial screening data are qualitative. The philosophy of the investigation has been to prepare compounds where certain parts of the previously prepared active halonitrosalicylanilides have been replaced by other groups, holding the remaining portions of the molecule unchanged. Therefore, in the following discussion, the halonitrosalicylanilides are viewed somewhat as parent compounds.

Several generalizations can be made from the results in Table II. In halonitrosalicylanilides where the *para*-chloroaniline moiety has been replaced by morpholine, picoline, or *meta*-carboxyaniline (*meta*-aminobenzoic acid), all activity was lost. Since 3′-chloro-5-nitrosalicylanilide has previously been shown to be active (1), it is apparent that a carboxyl group cannot replace a chlorine atom. On the other hand, the activity of III does show that a trifluoromethyl group can replace a chlorine atom with retention of activity. Furthermore, activity was retained when an aminothiazole entity replaced the *para*-chloroaniline portion of the molecule.

A degree of lipid solubility has at times been postulated as requisite for antimicrobial or drug activity (5, 6) in order to enable the molecule to cross or else combine with the lipid portion of the cell membrane. The halogen or nitro groups needed as substituents on salicylanilide to make it generally microbicidal probably increases its lipid solubility.

Since 4′-bromo-5-bromosalicylanilide is a known antimicrobial (7), 4′-dimethylamino-5-bromosalicylanilide (VII) was prepared with the idea that the dimethylamino group would confer

Received August 23, 1962, from the Ben Venue Laboratories, Inc., Bedford, Ohio.
Accepted for publication October 30, 1962.
† Present address: Cleveland Clinic Foundation, Cleveland 6, Ohio.

TABLE I.—SUBSTITUTED SALICYLANILIDES AND RELATED COMPOUNDS

No.	Compound	Formula	Anal. Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N	M.p., °C. (uncor.)	Yield, %
I	5-Nitrosalicylmorpholide	$C_{11}H_{12}N_2O_5$	52.3	4.8	11.1	52.7	4.8	10.9	241–244	98
II	5-Nitrosalicylpiperide	$C_{12}H_{14}N_2O_4$	57.5	5.6	11.2	57.7	5.5	11.3	245–246	99
III	3′-Trifluoromethyl-5-nitrosalicylanilide	$C_{14}H_9F_3N_2O_4$	51.5	2.8	8.6	51.6	2.9	8.7	180–182.5 (Reported, 182 (11))	86
IV	3′-Carboxy-5-nitrosalicylanilide	$C_{14}H_{10}N_2O_6$	55.6	3.3	9.3	55.3	3.6	9.2	316	67
V	N-Methyl-5-nitrosalicylanilide	$C_{14}H_{12}N_2O_4$	61.9	4.4	10.3	61.4	4.4	10.0	135–137	..
VI	N-2′-Thiazolyl-5-nitrosalicylamide	$C_{10}H_7N_3O_4S$	45.4	2.3	15.9	45.4	2.6	15.5	289[a]	..
VII	4′-Dimethylamino-5-bromosalicylanilide	$C_{15}H_{15}BrN_2O_2$	53.7	4.5	8.4	53.9	4.7	7.4	226	100
VIII	4′-Dimethylamino-5-bromosalicylanilide hydrochloride	$C_{15}H_{16}ClBrN_2O_2$	Chlorine, 9.4	..	...	...	Chlorine, 8.8	...	223[a]	100
IX	4′-Chloro-5-acetylaminosalicylanilide	$C_{15}H_{13}ClN_2O_3$	59.5	4.2	9.0	59.7	4.4	8.8	280	81
X	4′-Chloro-3-acetylaminosalicylanilide	$C_{15}H_{13}ClN_2O_3$	59.5	4.2	9.0	58.5	4.3	8.9	216–218	81
XI	4′-Chloro-5-aminosalicylanilide	$C_{13}H_{11}ClN_2O$	...	..	...	...	..	...	200–203 (Reported, 208–210 (3))	92
XII	4′-Chloro-5-diazosalicylanilide	$C_{13}H_9ClN_2O$	...	..	...	...	..	...	151[a] (Reported, 143[a] (3))	78
XIII	4′-Chloro-5-mono-phthalylanilide	$C_{14}H_{10}ClNO_3$	...	..	...	...	..	...	182–183 (Reported, 183–184 (4))	100
XIV	4′-Chloro-mono-3-nitrophthalylanilide	$C_{14}H_9ClN_2O_5$	52.5	2.8	8.8	52.0	3.0	8.1	183–185	100
XV	N-(5-Nitrosalicylidene)3′-chloroaniline	$C_{13}H_9ClN_2O_3$	56.5	3.3	10.2	56.0	3.1	10.0	205–206	79
XVI	4′-Chloro-5-(N-*para*-chlorophenylsulfamido) salicylanilide (monohydrate)	$C_{19}H_{14}Cl_2N_2O_5S$	50.3	3.1	6.2	50.1	3.7	6.3	250–251	73
XVII	4′-Chloro-5-sulfamidosalicylanilide	$C_{13}H_{11}ClN_2O_4S$	50.0	3.5	4.5	50.2	3.5	4.9	234–237	72
XVIII	5-(N-*para*-Chlorophenylsulfamido) salicylic acid	$C_{13}H_{10}NO_5S$	48.0	3.7	4.3	47.9	3.1	4.0	228	92

[a] Melted with decomposition.

equivalent or greater lipid solubility as the bromine atom to the molecule. However, the results were disappointing because the compound and its hydrochloride were both completely inactive. The hydrochloride was water insoluble which indicated that probably the dimethylamino group did increase lipid solubility. However, solubility versatility in both lipid and water may possibly be more important than either extreme as was shown to be the case for 8-hydroxyquinoline (oxine) (5).

Attempts to replace the nitro group of the active halonitrosalicylanilides by a sulfamido (XVII) or N-*para*-chlorophenylsulfamido group (XVI) produced compounds lacking antimicrobial activity. When an imine linkage replaced the amide linkage of 3′-chloro-5-nitrosalicylanilide, the resultant anil retained activity.

Recently, there has appeared in the literature an attempted correlation of pKa and activity for several salicylanilides with the idea that greater pKa represents greater chelating potential, which is possibly important to activity (8). For this reason and because of their ready synthesis, two phthalyl-mono-*para*-chloroanilides were prepared (XIII and XIV). In essence, compound XIV is equivalent to 4′-chloro-5-nitrosalicylanilide where, however, a carboxyl group has replaced the *ortho*-hydroxyl radical. However, such compounds were completely without antimicrobial activity. Although the acidity of the *ortho* group has been increased, the hydroxyl portion is farther in space and its connections to the ring lack the rigidity of the hydroxyl. Further, it would have to form a seven-membered ring with a chelating metal (hydroxyl-metal-carbonyl oxygen). Therefore, the hydroxyl group on the carboxyl would have a higher spatial entropy content (less ordered) and would require greater energies to enter into a chelate bond which probably would not be sufficiently offset by the stronger chelate bond of the ionic portion of the chelate ring due to a higher pKa. The inactivity of these latter compounds, therefore, does not necessarily rule out chelation as a factor for the active members.

A nitrosalicylanilide (V) was prepared having the amide nitrogen methylated with the resulting compound being inactive. A similar observation was made by

TABLE II.—ANTIMICROBIAL ACTIVITY OF SOME SUBSTITUTED SALICYLANILIDES AND RELATED COMPOUNDS

Compound No.	Antimicrobial Activity[a] *Staphylococcus aureus*[b]	*Epidermophyton floccosum*[c]
I	0	0
II	0	0
III	16	4
IV	0	0
V	0	0
VI	12	1
VII	0	0
VIII	0	0
IX	9	3
X	8	1
XI	2	0
XII	7	2 soln.
	4	24 solid
XIII	0	1
XIV	0	0
XV	8	5
XVI	0	0
XVII	0	0
XVIII	0	0

[a] Activity was determined by the agar cup-plate technique. The width of the zones of inhibition are expressed in mm. with no activity being represented by 0. [b] ATCC No. 6438. [c] ATCC No. 10227.

other investigators with the halogenated salicylanilides (2). In that article some of the requirements for activity among these compounds were discussed.

As a result of the present study, it has been possible to broaden some of the past known requisites for activity in the series. Of the compounds prepared that were active but did not possess a nitro group were those that yet retained a nitrogen on the "five" or "three" positions, in an amino (XI), acetylamino (IX, X), or diazo (XII) group. These latter compounds seem worthy of further investigation since they may possess physical and biological characteristics superior to other salicylanilides previously prepared.

EXPERIMENTAL[1]

Reactions of 5-Nitrosalicoyl Chloride and Amines.—Compounds I through VI were prepared by adding a benzene solution of 5-nitrosalicoyl chloride (1) to organic solutions of 2.5 molar excesses of morpholine, piperidine, *meta*-trifluoromethylaniline, *meta*-aminobenzoic acid, N-methylaniline, and 2-aminothiazole, respectively. VII was prepared from 5-bromosalicoyl chloride and N,N-dimethylphenylene diamine.

The amines were dissolved into benzene with the exception of *meta*-aminobenzoic acid, where diglyme and dimethylformamide were used as solvents. In each case the reaction mixture was vigorously stirred during the admixture and then allowed to stand at room temperature overnight. In each case a precipitate formed (usually very thick) consisting of the amine hydrochloride or a mixture of it with the respective product. The solids were filtered and varying amounts of further product could then be recovered by evaporating the filtrate to dryness. A preliminary purification was carried out by washing the product with 10% aqueous hydrochloric acid which removed the amine hydrochloride and amine. The treated solid was then dried and crystallized from boiling ethanol, sometimes adding either water or dimethylformamide to modify its solvency as necessary. The yields ranged from 67 to 100%.

Preparation of 4'-Chloro-3-acetylamino- and 5-Acetylaminosalicylanilide.—Both of these compounds were prepared in identical fashion which can be exemplified by the method for 4'-chloro-3-acetylaminosalicylanilide.

A solution of 5 Gm. (0.02 mole) of 4'-chloro-3-nitrosalicylanilide in 50 ml. of glacial acetic acid was heated to 85° and 20 Gm. of zinc dust was slowly added; vigorous action resulted. The mixture was then refluxed for 16 hours and vacuum filtered while hot to remove the zinc. The zinc was washed on the Büchner with small portions of boiling glacial acetic acid, the washings were added to the filtrate. Upon cooling the filtrate 4 hours at 5°, 5.3 Gm. (86.3% yield) of 4'-chloro-3-acetylaminosalicylanilide was obtained from the cooled solution upon filtration, m.p. 216–220°. Crystallization from chloroform gave a white product, m.p. 220–220.5°.

In a similar fashion, 5.0 Gm. of 4'-chloro-5-nitrosalicylanilide yielded 3.1 Gm. of 4'-chloro-5-acetylaminosalicylanilide which crystallized from alcohol as a white solid, m.p. 280–284°.

Preparation of 4'-Chloro-5-aminosalicylanilide and the Respective 5-Diazo Derivative.—This compound was prepared according to a recently reported procedure of reducing 4'-chloro-5-nitrosalicylanilide with sodium dithionate (3). It was then treated with nitrous acid according to the same reference to yield 4'-chloro-5-diazosalicylanilide.

Preparation of N-(5-Nitrosalicylidene) *meta*-Chloroaniline.—Two grams (0.012 mole) of 5-nitrosalicylaldehyde in 35.0 ml. of ethanol was added to 2.0 ml. (2.4 Gm.) (0.02 mole) of *meta*-chloroaniline in 10 ml. of ethanol. An immediate precipitate formed that filled the flask. The mixture was filtered after 2 hours to obtain a quantitative yield (3.3 Gm.) of the product. Crystallization from ethanol-dimethylformamide yielded yellow crystals, m.p. 205–206°.

Preparation of Monoanilides of Phthalic Acid.—Phthalyl-mono-*para*-chloroanilide was prepared from phthalic anhydride and *para*-chloroaniline according to a method described in the literature (4). In a similar manner the 3-nitrophthalic acid analog was prepared.

Twenty-one grams (0.1 mole) of 3-nitrophthalic anhydride was suspended in 100 ml. of chloroform and 12.7 Gm. (0.1 mole) of *para*-chloroaniline was added. An immediate warming and deepening of color and very heavy precipitation occurred which solidified the mixture. More chloroform was added to bring the total volume to about 300 ml. The mixture was allowed to stand overnight at room temperature, vacuum filtered, washed with chloroform, and dried to give a quantitative yield (32.0 Gm.) of 3-nitrophthalyl-mono-*para*-chloroanilide, m.p. 183.5°.

[1] Antimicrobial screening was carried out by the Wisconsin Alumni Association.

Preparation of 5-Sulfamidosalicylic Acid and N-Substituted Derivatives.—5-Chlorosulfonylsalicylic acid was prepared by adding dried salicylic acid to chlorosulfonic acid (9). A purified product was obtained upon crystallization from benzene, m.p. 171–174° (reported m.p. 171–172° (9)).

5-Sulfamidosalicylic acid was prepared by reacting the above chloride with aqueous ammonium hydroxide. Isolation and crystallization yielded a product, m.p. 256–259°, (reported m.p. 253–255° (10)).

Preparation of 4′-Chloro-5-sulfamidosalicylanilide.—Six-hundred milligrams (0.003 mole) of 5-sulfamidosalicylic acid was ground in a pestle to an intimate mixture with 300 mg. (0.004 mole) of *para*-chloroaniline. The mixture was placed into 8.0 ml. of chloroform along with 0.4 ml. of phosphorus trichloride and refluxed 72 hours. Upon cooling and filtering, 880 mg. of mixed products were obtained. These were washed with a small amount of water and crystallized from 1:2 water-ethanol to obtain the product, m.p. 234–237°.

Preparation of 5-(N-*para*-Chlorophenylsulfamido)-salicylic Acid.—Anhydrous 5-sulfosalicylic acid was prepared by taking the monohydrate and drying at 100° under a vacuum of 3 mm. for 18 hours. Ten grams (0.08 mole) of *para*-chloroaniline, 10.0 Gm. (0.05 mole) of the above anhydrous 5-sulfosalicylic acid, 75.0 ml. of chloroform, and 5.0 ml. of phosphorus trichloride were refluxed for 3 days. The mixture was then cooled in ice for several hours and filtered to obtain 14.6 Gm. of dried product. A portion was suspended in water, filtered, and crystallized from ethanol. A bright yellow insoluble material was removed by filtration during this operation. The filtrate produced white crystals of the title compound (as a monohydrate) m.p. 250–251°.

Preparation of 4′-N,N-Dimethylamino-5-bromosalicylanilide.—Twenty grams (0.09 mole) of 5-bromosalicylic acid were refluxed overnight with 15 ml. of thionyl chloride, 0.1 Gm. of anhydrous aluminum chloride, and 80 ml. of benzene. The mixture was filtered and all of the solvents removed under vacuum. The residual 5-bromosalicoyl chloride was brought into solution with pure benzene. One-third of this benzene solution (0.03 mole) was added to 13.0 Gm. (0.1 mole) of *para*-N,N-dimethylphenylenediamine in 60 ml. of benzene. The mixture was shaken and allowed to stand overnight to yield 18.7 Gm. of a dark green product obtained by filtering and drying the reaction mixture.

A 5-Gm. portion of material was crystallized from 80 ml. of ethanol to yield 4.3 Gm. of a dark green product. A considerable amount of residue had been removed by filtration. The material was crystallized a second time from 45.0 ml. of 3:1 ethanol-dimethylformamide to yield a dark green crystalline 4′-dimethylamino-5-bromosalicylanilide, m.p. 226°.

A second 5-Gm. quantity of the original product was shaken with 50 ml. of water and 15.0 ml. of concentrated hydrochloric acid. This treatment considerably lightened the product from a dark green to white with a greenish cast. The 4.3 Gm. of product from this treatment was crystallized from 36 ml. of 1:1 ethanol-water to yield 3.8 Gm. of the hydrochloride of 4′-N,N-dimethylamino-5-bromosalicylanilide as light green granules, m.p. 223° (eff.).

REFERENCES

(1) Taborsky, R. G., Darker, G. D., and Kaye, S., THIS JOURNAL, **48**, 503(1959).
(2) Lemaire, H., Schramm, C. H., and Cahn, A., *ibid.*, **50**, 831(1961).
(3) Ioffe, I. S., and Zal'manovich, M. Z., *Zh. Obshch. Khim.*, **31**, 199(1961).
(4) Sherill, M. J., Schaeffer, F. J., and Shoyer, E. P., *J. Am. Chem. Soc.*, **50**, 474(1928).
(5) Block, S. S., *J. Agr. Food Chem.*, **3**, 229(1954); Byrde, R. J. W., Clifford, D. R., and Woodcock, D., *Ann. Appl. Biol.*, **46**, 167(1958).
(6) Barlow, R. B., "Introduction to Chemical Pharmacology," John Wiley and Sons, New York, N. Y., 1955, Chapter II.
(7) Kraushaar, A., *Arzneimittel-Forsch.*, **4**, 548(1954).
(8) Baichwal, R. S., Baxter, R. M., Kandel, S. I., and Walker, G. C., *Can. J. Biochem. Physiol.*, **38**, 245(1940).
(9) "Beilsteins Handbuch der Organischen Chemie," Springer-Verlag, Berlin, Germany, 1950, Vol. 11, I, p. 106.
(10) *Op. cit.*, p. 107.
(11) Bilger, X., and Pechmeze, J., French pat. 1, 120,111 (1956).