ΔpK_a of 0.01 and $\Delta\sigma^*$ of 0.10, Et and Me derivatives should have similar optimum partition coefficients. The data in this report substantiate this observation.

This study demonstrates the usefulness of the extrathermodynamic approach in understanding structure–activity relationships. Thus, the antibacterial activities of leucomycins, as well as lincomycin and clindamycin analogs depend on their relative hydrophobic properties. Through use of dummy variables, steric effects could be examined in the lincomycin series. Finally, a comparison of the optimum π value for one subseries with that of another subseries results in the indication of an electronic effect. In the case of the leucomycin series the electronic and steric effects could not be separated because of lack of variety in the derivatives tested.

Antimalarial Agents. 8. Ring-Substituted Bis(4-aminophenyl) Sulfones and Their Precursors[1]

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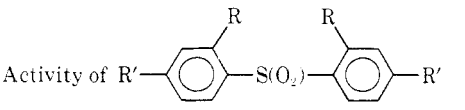
Received June 5, 1971

Forty-four compounds related to bis(4-aminophenyl) sulfone (DDS) were tested against *Plasmodium berghei* in mice. Seven of them were better antimalarial agents than DDS or equal to bis(4-acetamidophenyl) sulfone (DADDS). Ortho substitution to the NH_2 group of DDS resulted in total loss of activity. Meta substitution or N-acetylation did not lead to any general trend.

The acetylation of bis(4-aminophenyl) sulfone (I, 4,4′-diaminodiphenyl sulfone, DDS) to bis(4-acetamidophenyl) sulfone (II, *N*,*N*′-diacetyl-DDS, DADDS) resulted in a considerable improvement of activity against *Plasmodium berghei* in mice.[2,3]

Attempts for additional significant improvements of the antimalarial activity of DDS-related structures by replacing one or both NH_2 functions of DDS with various other groups have not been successful.[1a,3-6] The effect of ring substitution of I is described in this paper. Monosubstitution in the ortho or meta position to one of the NH_2 groups was expected to alter the nucleophilicity primarily of that group, to distort the apparent symmetry of the DDS-molecule, and to change its polarity. Meta mono- and meta,meta′ disubstitution (again relative to NH_2) should alter the shape of the molecule by H bonding or by steric or electromeric interactions of the substituent(s) with the adjacent SO_2 group much more than ortho substitution. Ortho substituents, however, could have a steric effect on the adjacent NH_2 group. Finally, symmetrical substitution in both Ph rings should render both amino moieties equally different from the NH_2 groups of DDS. The activity data obtained by a previously published method[7] and listed in Tables I–IV were evaluated in view of the expected changes of the DDS molecule brought about by the ring substituents CH_3, CF_3, CO_2Me, NH_2, NHAc, NO_2, OMe, and Cl.

TABLE I



Compound: Structure R	R′	CIST[f] or (% cures) at mg/kg 40	160	640
I[a] H	NH_2	8.0	(40)	(20)[g]
II[a] H	NHAc	(20)	(100)	(100)
III[b] NH_2	NH_2	8.8	(40)	(100)
IV NHC(O)H	NHC(O)H	0.9	4.9	12.3
V[c] Cl	NO_2	0.7	3.5	13.9
VI[c] Cl	NH_2	4.7	(40)	(60)
VII[d] Me	NH_2	1.5	9.9	(60)[h]
VIII CO_2Me	NO_2	0.3	0.3	0.5
IX CO_2Me	NH_2	0.2	0.6	0.8
X CO_2Me	NHAc	0.9	0.9	1.1
XI[e] CF_3	NO_2	0	0	0.2
XII[e] CF_3	NH_2	0.2	0.2	0.4

[a] Test data supplied by Dr. B. Poon of Walter Reed Army Institute for Research. [b] H. Bradburry and F. J. Smith, *J. Chem. Soc.*, 793 (1956). [c] S. S. Berg, *ibid.*, 1991 (1949). [d] M. Balasubramanian and V. Baliah, *ibid.*, 1251 (1955). [e] G. W. Stacy, C. R. Bresson, R. E. Harmon, and R. C. Thamm, *J. Org. Chem.*, **22**, 298 (1957). [f] Change in survival time, *i.e.*, mean survival time of treated mice minus the mean survival time of the control. [g] 80% toxic deaths at 640 mg/kg. [h] 40% toxic deaths at 640 mg/kg.

Ortho mono- and ortho,ortho′ disubstitutions of I resulted in a general trend, *i.e.*, they rendered the DDS structure inactive (XXXII–XXXV, XL, XLIII, and XLVI). Since both electron-withdrawing and -donating substituents had the same effect, it appears that internal H bonding or distortion of a favorable spacial NH_2 arrangement of DDS caused the deactivation. The significance of the position of a substituent is well illustrated by the test data for CF_3-substituted structures XXIII and XLIII. Meta mono- and meta,meta′ disubstitutions, however, did not lead to any general conclusion on the antiplasmodial activity effect of substituents and did not indicate any structure–activity

† In memory of my teacher, Professor Clemens Schöpf, deceased December 17, 1970.

(1) (a) Part 7: *J. Med. Chem.*, **14**, 550 (1971); (b) this study was supported by U. S. Army Medical Research and Development Command; This is Contribution No. 948, from the Army Research Program on Malaria. (c) the compounds were tested by Dr. L. Rane of the University of Miami, Florida; (d) analyses are indicated by symbols of the elements, since analytical results obtained for these elements were within ±0.4% of the theoretical values.

(2) Test data supplied by Dr. Bing Poon of Walter Reed Army Institute for Research.

(3) E. F. Elslager, Z. B. Gavrilis, A. A. Phillips, and A. F. Worth, *J. Med. Chem.*, **12**, 357 (1969).

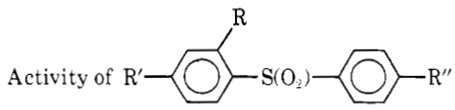
(4) I. C. Popoff and G. H. Singhal, *ibid.*, **11**, 631, 886 (1968).

(5) B. Serafin, T. Urbanski, and D. C. Warhurst, *ibid.*, **12**, 336 (1969).

(6) H. Bader, J. F. Hoops, J. H. Biel, H. H. Koelling, R. G. Stein, and T. Singh, *ibid.*, **12**, 709 (1969).

(7) T. S. Osdene, P. B. Russell, and L. Rane, *ibid.*, **10**, 431 (1967).

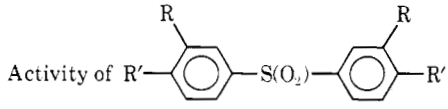
TABLE II



Compound				CIST[d] or (% cures) at mg/kg		
	Structure					
No.	R	R′	R″	40	160	640
XIII[a]	NO_2	NO_2	NHAc	1.1	6.9	(40)[e]
XIV	NO_2	NHAc	NHAc	0.4	4.8	9.0
XV[a]	NO_2	NHAc	NH_2	5.2	(40)	(40)[f]
XVI	NO_2	NH_2	NH_2	9.4	(40)	(100)
XVII	Cl	NO_2	NHAc	0.3	5.1	(80)[g]
XVIII	Cl	NH_2	NHAc	(60)	(80)	(100)
XIX	Cl	NO_2	NH_2	0	0.2	0.6
XX[b]	Cl	NH_2	NH_2	4.2	(60)	(*h*)
XXI	CF_3	NO_2	NHAc	1.0	1.4	1.8
XXII	CF_3	NH_2	NHAc	4.9	(20)	(60)[i]
XXIII[c]	CF_3	NH_2	NH_2	7.1	(100)	(100)[j]
XXIV	NH_2	NHAc	NHAc	0.7	5.7	(60)
XXV	NH_2	NH_2	NHAc	2.0	8.2	(60)
XXVI	NHAc	NHAc	NHAc	1.6	(20)	(60)
XXVII	NH_2	NH_2	NH_2	0.2	1.8	2.6
XXVIII	CO_2Me	NO_2	NHAc	3.4	5.0	(60)
XXIX	CO_2Me	NH_2	NHAc	0.3	0.5	0.5
XXX	CO_2Me	NH_2	NH_2	12.4	(80)	(60)[k]

[a] U. P. Basu and K. R. Chandran, *J. Indian Chem. Soc.*, **27**, 123 (1950); *Chem. Abstr.*, **45**, 8469*e* (1951). [b] See footnote *c* of Table I. [c] See footnote *e* of Table I. [d] See footnote *f* of Table I. [e] 40% cures at 320 mg/kg and 40% toxic deaths at 320 and 640 mg/kg. [f] 40% and 20% toxic deaths at 640 and 160 mg/kg, respectively. [g] 20% toxic deaths at 320 and 640 mg/kg. [h] 100% toxic deaths at 640 mg/kg, 100% cures at 320 mg/kg. [i] 20% and 40% toxic deaths at 320 and 640 mg/kg, respectively; 80% cures at 320 mg/kg. [j] 100% and 20% cures at 320 and 80 mg/kg, respectively. [k] 40% and 20% toxic deaths at 640 and 160 mg/kg, respectively; 80% cures at 80 mg/kg.

TABLE III

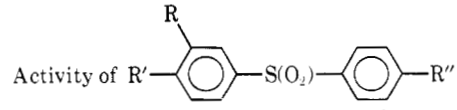


Compound			CIST[c] or (% toxic deaths) at mg/kg		
	Structure				
No.	R	R′	40	160	640
XXXII	CF_3	NO_2	4.8	(100)	(100)
XXXII	CF_3	NH_2	0.1	0.1	0.5
XXXII[a]	NO_2	NH_2	0.2	0.4	0.6
XXXIV[a]	NH_2	NH_2	0.3	0.7	4.1
XXXV[b]	Cl	NH_2	0	0	0
XXXVI	Cl	NHAc	0.2	0.2	0.4

[a] F. Ullmann and J. Korselt, *Ber.*, **40**, 641 (1907). [b] I. G. Farbenind. A. G., French Patent 829,926 (1938); *Chem. Abstr.*, **33**, 1960[9] (1939). [c] See footnote *f* of Table I.

relationship; this is confirmed by comparing the test data within the group of structures XV(NO_2), XXIII (CF_3, most active), XX (Cl, least active), and XXX (CO_2Me), or the group of XXII (CF_3), XVIII (Cl, most active), and XXIX (CO_2Me, least active), or of XII (CF_3, inactive), VI (Cl, most active), IX (CO_2Me, inactive), and VII (Me); these structures are listed within each group in descending order of electron-withdrawing power of their ring substituents given in parentheses. Furthermore, it should be noted that, contrary to the expectations based on the high activity and low toxicity of DADDS (II) or on the improved activity of monoacetylated DDS,[1a] N- and N,N′-acetylation of the ring-substituted DDS structures studied by us did not necessarily result in activity improvement. Thus,

TABLE IV



Compound				CIST[b] or (% cures) at mg/kg		
	Structure					
No.	R	R′	R″	40	160	640
XXXVII[a]	OMe	NO_2	NHAc	3.8	(40)	(40)
XXXVIII	OMe	NO_2	NH_2	0.3	4.3	(*c*)
XXXIX[a]	OMe	NH_2	NHAc	1.5	5.9	(40)[d]
XL[a]	OMe	NH_2	NH_2	0.5	0.7	0.7
XLI	CF_3	NO_2	NHAc	1.4	4.0	7.4
XLII	CF_3	NO_2	NH_2	1.0	4.2	9.0
XLIII	CF_3	NH_2	NH_2	3.4	7.2	(20)
XLIV	Cl	Cl	NHAc	0.3	0.5	0.5
XLV	Cl	Cl	NH_2	0.3	0.5	0.9
XLVI	Cl	NH_2	NH_2	0.9	0.9	1.3

[a] H. Bauer, *J. Amer. Chem. Soc.*, **73**, 2113 (1951). [b] See footnote *f* of Table I. [c] 100% toxic deaths at 640 mg/kg; 20% cures at 320 mg/kg. [d] 60% and 20% toxic deaths at 640 and 320 mg/kg, respectively.

monoacetylation of XXIII to XXII, or of XXX to XXIX, and mono- or diacetylation of XVI to XV and XIV, respectively, was accompanied by considerable or total loss of activity. Monoacetylation of XX to XVIII, however, caused a relative gain of activity equal to that achieved by diacetylation of DDS to DADDS. Considering both the antiplasmodial and the toxicity data, several of the compounds (III, VI, XVI, XVII, XXIII, and XXX) were better than DDS (I) but none was superior to DADDS (II) in the mice tests.

Experimental Section

4-Acetamido-4-aminophenyl 4-acetamidophenyl sulfone (XXIV), mp 265–267° (from EtOH), was obtained in 65% yield by the hydrogenation of XIV in DMF as described for XXII. *Anal.* ($C_{16}H_{17}N_3O_4S$): C, H, N. 4-Aminophenyl 2,4-diaminophenyl sulfone (XXVII), mp 138–140° [from MeOH–H_2O, *Anal.* ($C_{12}H_{13}N_3O_2S$): C, H, N, S], was prepd in 42% yield by the hydrolysis of XXVI as described for XVI; XXVII has been reported[8] to melt at 118°, resolidify and remelt at 150°. The hydrogenation procedure used for the prepn of XXIV converted XXVIII to 4-acetamidophenyl 4-amino-2-carbomethoxyphenyl sulfone (XXIX), mp 111–113° (from 50% MeOH), in 75% yield. *Anal.* ($C_{16}H_{16}N_2O_5S$): C, H, N. A 15-min refluxing of XXIX in 12% HCl gave 61% yield of 4-aminophenyl 4-amino-2-carbomethoxyphenyl sulfone (XXX), mp 160.5–162.5° (from 50% MeOH). *Anal.* ($C_{14}H_{14}N_2O_4S$): C, H, N. Following are the syntheses of the remaining new compounds.

Bis(2,4-diformamidophenyl) Sulfone (IV).—A soln of 4.0 g (0.014 mole) of bis(2,4-diaminophenyl) sulfone (III) in 100 ml of HCOOH was refluxed for 6 hr, treated with Darco, filtered hot, and poured into 1.5 l. of ice H_2O. The pptd solid was recrystd from DMF–H_2O (5:1) to obtain 2.0 g (36%) of IV, mp 285–288° dec. *Anal.* ($C_{16}H_{14}N_4O_6S$): C, H, N.

Bis(2-carbomethoxy-4-nitrophenyl) Sulfone (VIII).—A mixt of 22.7 g (0.1 mole) of methyl 2-chloro-5-nitrobenzoate and 17.0 g g (0.1 mole) of KS(S)COEt in 300 ml of 95% MeOH was refluxed for 24 hr and then chilled. The resulting ppt (17.0 g, 83%, mp 168–171°) was recrystd from Me_2CO to yield bis(2-carbomethoxy-4-nitrophenyl) sulfide, mp 170–172°. *Anal.* ($C_{16}H_{12}N_2O_8S$): C, H, S.

A soln of 7.1 g (0.045 mole) of $KMnO_4$ in 300 ml of H_2O was slowly added at 40–45° to a mixt of 11.8 g (0.03 mole) of the above sulfide in 500 ml of AcOH and stirred for 8 hr at 50–55°. The reaction mixt was decolorized with $NaHSO_4$ at room temp and the solid (10.0 g, 79%, mp 174–176°) was recrystd from 80% Me_2CO (Darco) to furnish the sulfone VIII, mp 174.5–176°. *Anal.* ($C_{16}H_{12}N_2O_{10}S$): C, H, N.

(8) B. R. Baker, M. V. Querry, and A. F. Kadish, *J. Org. Chem.*, **15**, 402 (1950).

Bis(4-amino-2-carbomethoxyphenyl) Sulfone (IX) and Bis(4-acetamido-2-carbomethoxyphenyl) Sulfone (X).—A soln of 7.0 g of VIII in 150 ml of THF and 25 ml of EtOH was hydrogenated over Raney Ni at 4.2 kg/cm². The solid (mp 208–212°), recovered by evapn of the Darco-treated hydrogenation soln, was recrystd from MeOH to render 4.8 g (80%) of IX, mp 214–215°. *Anal.* ($C_{16}H_{16}N_2O_6S$): C, H, N.

Acetylation of IX by refluxing in AcOH–AcOAc resulted in 90% yield of X, mp 218–219° (from DMF–H_2O). *Anal.* ($C_{20}H_{20}N_2O_8S$): N.

4-Acetamidophenyl 4-Acetamido-2-nitrophenyl Sulfone (XIV) and 4-Aminophenyl 4-Amino-2-nitrophenyl Sulfone (XVI).—A soln of 36.5 g (0.165 mole) of sodium 4-acetamidobenzenesulfinate and 32.2 g (0.150 mole) of 4-chloro-3-nitroacetanilide in 100 ml of DMF was stirred and refluxed for 5 hr. The reaction mixt was poured in 1.5 l. of ice H_2O. The orange crude sulfone XIV was dissolved in 750 ml of hot dioxane–DMF (2:1), then Darco-treated, and dild with 750 ml of H_2O to yield 39.5 g (70%) of XIV, mp > 300°. *Anal.* ($C_{16}H_{15}N_3O_6S$): N.

Hydrolysis of XIV in refluxing 18% HCl followed by diln of the reaction mixt with H_2O gave a 69% yield of recrystd 4-aminophenyl 4-amino-2-nitrophenyl sulfone (XVI), mp 151–153° (from 50% EtOH). *Anal.* ($C_{12}H_{11}N_3O_4S$): C, H, N.

4-Acetamidophenyl 2-Chloro-4-nitrophenyl Sulfone (XVII), 4-Acetamidophenyl 4-Amino-2-chlorophenyl Sulfone (XVIII), and 4-Aminophenyl 2-Chloro-4-nitrophenyl Sulfone (XIX).—A mixt of 50.0 g (0.25 mole) of 4-acetamidobenzenesulfinic acid, 10.0 g (0.25 mole) of NaOH, 48.0 g (0.25 mole) of 3,4-dichloronitrobenzene, 0.5 g of Cu powder, and 0.5 g of I_2 in 300 ml of 95% EtOH was refluxed for 24 hr and filtered. The filter cake was washed with Me_2CO. The residue obtained by evapn of the combined filtrate was triturated with hot Me_2CO. The soln was evapd, and the residue was dissolved in a hot mixt of 500 ml of dioxane and 200 ml of Me_2CO. The Darco-treated hot soln was dild with 950 ml of H_2O to obtain 22.5 g (26%) of XVII, mp 188–190°; recrystn from AcOH did not change the mp. This product is reported[8] to melt partially at 115–120° then resolidify and melt again at 178–180°. *Anal.* ($C_{14}H_{11}ClN_2O_5S$): C, H, N, S.

A soln of 17.7 g (0.05 mole) of XVII in 100 ml of dioxane and 20 ml of EtOH was hydrogenated over Raney Ni at 4.2 kg/cm²; 16.1 g (100%) of crude product XVIII, mp 270–280° dec, was obtained by diluting the filtered hydrogenation mixt with 1.5 l. of H_2O. It was dissolved in 1600 ml of Me_2CO–MeCN (2:1), treated with Darco, and concd to 700–800 ml to recover 6.5 g of pure XVIII, mp 284–287° dec. *Anal.* ($C_{14}H_{13}ClN_2O_3S$): N.

The hydrolysis of XVII in refluxing 48% H_2SO_4 followed by diln of the reaction mixt with 2 l. of ice H_2O and washing of the solid with 5% Na_2CO_3 gave 96% of XIX, mp 215–217°; recrystn from Me_2CO–H_2O (2:1) did not change the mp. *Anal.* ($C_{12}H_9ClN_2O_4S$): C, H, Cl, N.

4-Acetamidophenyl 4-Nitro-2-trifluoromethylphenyl Sulfone (XXI), 4-Acetamidophenyl 4-Amino-2-trifluoromethylphenyl Sulfone (XXII).—A soln of 36.5 g (0.165 mole) of sodium 4-acetamidobenzenesulfinte and 33.9 g (0.153 mole) of 2-chloro-5-nitrobenzotrifluoride in 100 ml of DMF was stirred and refluxed for 6 hr. The reaction mixt was poured in 1.5 l. of ice H_2O and the oily product was washed with H_2O until it solidified. The solid was recrystd (3x) from 95% EtOH to give 15.3 g (26%) of XXI, mp 219–221°. *Anal.* ($C_{15}H_{11}F_3N_2O_5S$): F, N.

A soln of 13.0 g (0.033 mole) of XXI in 100 ml of DMF–dioxane (1:1) was hydrogenated over Raney Ni at 4.2 kg/cm² and 40°. The filtered soln was poured in 1.5 l. of H_2O, and the solid was recrystd from 70% EtOH to render 8.2 g (68%) of XXII, mp 218–220°. *Anal.* ($C_{15}H_{13}F_3N_2O_3S$): C, H, F, N.

4-Acetamidophenyl 2,4-Diaminophenyl Sulfone (XXV) and 4-Acetamidophenyl 2,4-Diacetamidophenyl Sulfone (XXVI).—To a refluxing mixt of 18.3 g (0.05 mole) of 4-acetamidophenyl 2,4-dinitrophenyl sulfone (XIII) and 300 ml of 90% AcOH was added in small portions 33.6 g (0.60 g-atom) of Fe powder. After an addl 30-min refluxing, the reaction mixt was filtered and divided in 2 equal portions (A and B).

Portion A was poured slowly in 2 l. of ice H_2O to give 3.0 g (40%) of XXV, mp 108–110° dec. *Anal.* ($C_{14}H_{16}N_3O_3S$): C, H, N.

Portion B was evapd to dryness, the residue was triturated with 500 ml of hot Me_2CO, and the filtrate was evapd to dryness. The residue was refluxed for 2 hr in a mixt of 100 ml of AcOH and 10 ml of AcOAc and poured in 2 l. of ice H_2O to furnish 7.0 g (72%) of crude XXVI. It was recrystd from 33% AcOH to recover 4.0 g (41%) of pure XXVI, mp 261–262° dec. *Anal.* ($C_{18}H_{19}N_3O_5S$): C, H, N.

4-Acetamidophenyl 2-Carbomethoxy-4-nitrophenyl Sulfone (XXVIII).—A soln of 43.0 g (0.20 mole) of methyl 2-chloro-5-nitrobenzoate and 48.5 g (0.22 mole) of sodium 4-acetamidobenzenesulfinate in 100 ml of DMF was refluxed and stirred for 3 hr and then poured slowly into 2 l. of ice H_2O. The resulting solid was recrystd from 85% AcOH (Darco) to obtain 47.8 g 64%) of XXVIII, mp 243–245°. *Anal.* ($C_{16}H_{14}N_2O_7S$): C, H, N.

Bis(4-nitro-3-trifluoromethylphenyl) Sulfone (XXXI) and Bis(4-amino-3-trifluoromethylphenyl) Sulfone (XXXII).—A mixt of 112.8 g (0.5 mole) of 5-chloro-2-nitrobenzotrifluoride and 80.0 g (0.5 mole) of KS(S)COEt in 500 ml of 95% EtOH was refluxed for 24 hr, and then dild with 100 ml of H_2O to yield 23.0 g (22%) of bis(4-nitro-3-trifluoromethylphenyl) sulfide, mp 137–138.5° (from AcOH). *Anal.* ($C_{14}H_6F_6N_2O_4S$): C, H, N, S.

To a soln of 25.0 g (0.085 mole) of $K_2Cr_2O_7$ in 300 ml of H_2SO_4 and 60 ml of H_2O was slowly added 26.0 g (0.063 mole) of bis(4-nitro-3-trifluoromethylphenyl) sulfide at 5°. After the addn of 100 ml of H_2SO_4, the mixt was stirred for 1.5 hr at 20–25° and poured into 3 l. of ice H_2O. The ppt was dissolved in Me_2CO and the clear soln was dild with H_2O to obtain 29.0 g of crude XXXI, mp 230–240°. The crude product was triturated with $CHCl_3$ to recover 23.5 g (84%) of pure XXXI, mp 246–248°. *Anal.* ($C_{14}H_6F_6N_2O_6S$): C, H, S.

A soln of 11.1 g (0.025 mole) of XXXI in a mixt of 100 ml of dioxane, 20 ml of DMF, and 20 ml of EtOH was hydrogenated over Raney Ni at 4.2 kg/cm². The filtered reaction mixt was dild with 1 l. of 5% NaCl soln to obtain 8.5 g of product, mp 185–193°. It was dissolved in 200 ml of concd HCl and dild with 200 ml of H_2O to recover 5.0 g (52%) of XXXII, mp 196–200°. *Anal.* ($C_{14}H_{10}F_6N_2O_2S$): C, H, N, S.

Bis(4-acetamido-3-chlorophenyl) Sulfone (XXXVI).—A mixt of 4.3 g (0.014 mole) of bis(4-amino-3-chlorophenyl) sulfone (XXXV), 15 ml of AcOAc, and 25 ml of AcOH was stirred and refluxed for 1.5 hr. The solid was filtered off, washed with EtOEt, and recrystd from DMF–H_2O to produce 3.5 g (65%) of XXXVI, mp 247–249°. *Anal.* ($C_{16}H_{14}Cl_2N_2O_2S$): C, H, N.

4-Aminophenyl 3-Methoxy-4-nitrophenyl Sulfone (XXXVIII).—A soln of 5.4 g (0.015 mole) of 4-acetamidophenyl 3-methoxy-4-nitrophenyl sulfone (XXXVII) in 50 ml of glacial AcOH and 25 ml of concd HCl was refluxed for 4 hr and poured into 1.5 l. of ice H_2O. The solid was washed with H_2O and recrystd from 70% EtOH to give 3.3 g (69%) of XXXVIII, mp 181–183°. *Anal.* ($C_{13}H_{12}N_2O_5S$): C, H, N, S.

4-Acetamidophenyl 4-Nitro-3-trifluoromethylphenyl Sulfone (XLI), 4-Aminophenyl 4-Nitro-3-trifluoromethylphenyl Sulfone (XLII), and 4-Aminophenyl 4-Amino-3-trifluoromethylphenyl Sulfone (XLIII).—A mixt of 44.2 g (0.2 mole) of sodium 4-acetamidobenzenesulfinate, 45.1 g (0.2 mole) of 5-chloro-2-nitrobenzotrifluoride, a trace of Cu powder, and 100 ml of DMF was refluxed for 3 hr and filtered hot, and the filtrate was poured into 1.5 l. of ice H_2O. The gummy solid was triturated with boiling EtOH and recrystd from *i*-PrOH and then from Me_2CO to obtain 8.3 g (11%) of XLI, mp 221–222°. *Anal.* ($C_{15}H_{11}F_3N_2O_5S$): C, H, N.

Hydrolysis of XLI as described for the prepn of XXXVIII resulted in 56% of XLII, mp 188–190° (from 70% EtOH). *Anal.* ($C_{13}H_9F_3N_2O_4S$): F, N.

A mixt of 15.0 g of $SnCl_2 \cdot 2H_2O$, 15 ml of concd HCl, and 5.0 g (0.013 mole) of XLI was refluxed for 45 min and dild with 75 ml of 50% NaOH under ice cooling. The solid (3.9 g, 99%, mp 183–186°) was washed with H_2O and recrystd from THF and from EtOH to recover 1.9 g (48%) of XLIII, mp 192–193.5°. *Anal.* ($C_{13}H_{11}F_3N_2O_2S$): C, H, N.

4-Acetamidophenyl 3,4-Dichlorophenyl Sulfone (XLIV), 4-Aminophenyl 3,4-Dichlorophenyl Sulfone (XLV), and 4-Aminophenyl 4-Amino-3-chlorophenyl Sulfone (XLVI).—To a melt of 15 g of urea, 100 g of $AlCl_3$, and 13.5 g (0.1 mole) of acetanilide was added slowly and with stirring 24.5 g (1.1 moles) of 3,4-dichlorobenzenesulfonyl chloride. The melt was stirred to complete the HCl evoln and then was poured on 1 kg of ice. The solid was dissolved in THF, the sol was dried ($MgSO_4$) and evapd. The residue was recrystd (2x) from Me_2CO–petr ether (bp 60–110°) to recover 12.2 g (34%) of XLIV, mp 215–216° *Anal.* ($C_{14}H_{11}Cl_2O_3S$): C, H, N.

A mixt of 5.0 g (0.015 mole) of XLIV and 150 ml of concd NH_4OH was shaken for 96 hr in a 300-ml autoclave at 200°

under autogeneous pressure. The solid (3.6 g, 88%, mp 202–205°) was recrystd from Me_2CO–petr ether (bp 60–110°) to yield 2.0 g (49%) of XLVI, mp 204–206°. *Anal.* ($C_{12}H_{11}ClN_2O_2S$): C, H, N.

An attempt to shorten the reaction time for the prepn of XLVI at 270–300° (19 hr) resulted in 2.6 g (56%) of XLV, mp 216–219° (from Me_2CO). *Anal.* ($C_{12}H_9Cl_2NO_2S$): N. Acetylation of XLV gave XLIV, mp 214–215° (no mp depression with authentic XLIV).

Acknowledgment.—Miss P. M. Thomas assisted us in the preparation of a few of the compounds. The analyses were carried out by Pennwalt's Analytical Department.

Inhibition of Electron Transport by Substituted Salicyl-*N*-(*n*-octadecyl)amides†

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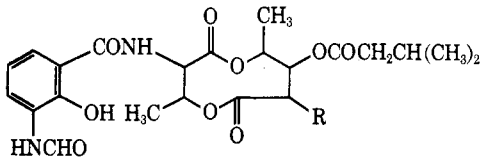
Received March 11, 1971

Several new analogs of antimycin A have been synthesized which were inhibitory toward submitochondrial succinate oxidase, NADH-oxidase, and succinate–cytochrome *c* reductase. The degree of inhibition was generally greatest when the substituted salicyl-*N*-(*n*-octadecyl)amides were preincubated with the electron transport particles. Inhibitions greater than 90% were observed with 5-nitro-, 5-formamido-, and 3,5-dinitrosalicyl-*N*-(*n*-octadecyl)amides. Salicyl-*N*-(*n*-octadecyl)amide was not inhibitory. The analog inhibitory site was similar to the antimycin A site as determined by an increased reduced cytochrome *b* (562 nm) peak and a decreased reduced cytochrome *c* (550 nm) peak in the inhibited difference spectra. The results demonstrated that a formamido substituent ortho to the phenolic OH of antimycin A is not required for inhibitory activity provided there is either a formamido or nitro group para to OH.

The characteristic inhibitory activity of antimycin A(I) on the electron transport system of higher animals is associated with the substituted aromatic moiety[1,2] while the remaining dilactone portion seeminly provides a required lipophilicity. Dickie, *et al.*,[2] reported that 3-formamidosalicyl-*N*-(*n*-octadecyl)amide inhibited the reduction of cytochrome *c* in a manner analogous to antimycin.



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antimycin A

The observation that an analog having an NO_2 group substituted for the formamido function retained a diminished inhibitory activity,[2] suggested that an acylated amino group[3] was functionally replaceable. The phenolic OH of antimycin is strongly acidic[4] and required for inhibitory activity.[5] If the function of the NHCHO and NO_2 groups ortho to the phenolic OH is merely to enhance acidity, then analogs containing combinations of ortho and para substituents should also show inhibitory properties. The synthesis of several previously unreported substituted salicyl-*N*-(*n*-octadecyl)amides and their inhibitory capacity toward submitochondrial electron transport are reported in this paper.

Experimental Section‡

Substituted Salicyl-*N*-(*n*-octadecyl)amides.—These compds were prepd from the appropriately substituted salicylic acid by a modification of the method of Dickie, *et al.*[2] As an example, the prepn of 3-formamido-5-nitrosalicyl-*N*-(*n*-octadecyl)amide is described in detail.

To 30 ml of 88% HCO_2H contg 2.0 g of sodium formate was added 2.5 g (12 mmoles) of 3-amino-5-nitrosalicylic acid monohydrate, mp 227–228°, prepd by the method of Meldola, *et al.*[6] After heating in a water bath for 1 hr, 30 ml of H_2O was added and the heating contd for 5 min. Upon cooling to room temp the formylated product pptd and was collected by filtration to yield 1.2 g (5.0 mmoles). This product was immediately dissolved in 25 ml of THF and 2.86 g (10.5 mmoles) of *n*-octadecylamine was dissolved with stirring. A THF soln (75 ml) contg 1.2 g (5.8 mmoles) of DCI was slowly added, and the soln was stirred at room temp for 20 hr. The reaction mixt was acidified with 25 ml of glacial AcOH followed by addn of 12 ml of H_2O to ppt dicyclohexylurea which was filtered and discarded. The filtrate was evapd in a rotary evaporator, and the residue was crystd from AcOH–H_2O. Recrystn from EtOH–H_2O yielded 1.55 g (3.2 mmoles, 27%) of a bright yellow product, mp 115.5–116.5°. *Anal.* ($C_{26}H_{43}N_3O_5 \cdot H_2O$) C, H, N.

Prepn of Mitochondria and Submitochondrial Particles.—Beef heart mitochondria were prepd by the methods described by Crane, *et al.*[7] These prepns remained stable for several weeks when stored at −20°. Submitochondrial particles were prepd by a modification of the method of Urban, *et al.*,[8] Thawed mitochondrial prepns were adjusted to pH 8.0 with 1 *N* NaOH followed by sonication with a Bronwill Biosonik Model 11 at a probe intensity of 70 for 2 min at 4°. The sonicate was centrifuged at 40,000*g* for 10 min, and the supernatant was decanted and saved. The pellet was suspended in a pH 7.5, 0.01 *M* Tris–succinate buffer soln which contd 0.25 *M* mannitol and 0.2 m*M* EDTA. The suspension was adjusted to pH 8.0, resonicated, and centrifuged at 40,000*g*. The 2 supernatants were combined and centrifuged at 70,000*g* for 30 min. The pellet was washed 3 times with 0.05 *M* phosphate buffer (pH 7.5) and suspended in the Tris–mannitol soln at a protein concn of 10 mg/ml. Protein concns were detd by the method of Folin and Ciocalteau.[9]

Assay for Inhibition of NADH and Succinate Oxidase.—

† Supported in part by Grant No. AI 08621 from the National Institutes of Health.

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