Antibacterial N-[ω,ω'-Bis(alicyclic and aryl)-*sec*-alkyl]-1,3-diamino-2-propanol Dihydrochloride Salts

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Abstract □ A series of 14 antibacterial N-[ω,ω'-bis(cycloalkyl, bicyclo[2.2.1]heptyl, and substituted phenyl)-*sec*-alkyl]-1,3-diamino-2-propanol dihydrochloride salts were synthesized as potential topical antiseptics and disinfectants. Four derivatives which were particularly effective against *Pseudomonas aeruginosa* encompassed the three diverse ring-type substituents and an 8-n-pentadecyl moiety. The calculated Hansch hydrophobic parameter (π) for the N-substituents of the more efficient compounds were in the range 7.0–9.0 and correlated with minimal inhibitory activity as a parabola for all of the products under the assay conditions. The potencies against Gram-positive and other Gram-negative bacteria were comparable to benzalkonium chloride and chlorhexidine.

Keyphrases □ Antibacterials—potential, N-[ω,ω'-Bis(alicyclic and aryl)-*sec*-alkyl]-1,3-diamino-2-propanol dihydrochloride salts □ *Pseudomonas aeruginosa*—effect of potential antiseptics and disinfectants □ Hansch hydrophobic parameter—correlation with efficiencies of potential antibacterials

Antimicrobial agents have been sought from among N-mono-(1), N,N'-bis(mono)- (2, 3), and disubstituted 1,3-diamino-2-propanol salt derivatives (4). The present report describes the synthesis of N-substituted *sec*-alkyl compounds of the first type, the *in vitro* antibacterial assay, and a potential structure–activity relationship. This study is part of an effort to develop antiseptics and disinfectants especially effective against *Pseudomonas aeruginosa* (5). The cell envelope of Gram-negative bacteria presents a permeability barrier which includes high lipid content, anionic lipopolysaccharides, and proteins (porins) apparently able to form molecular size-limiting channels trans-outer membrane (6). Variation of the *sec*-alkyl group by symmetrical substitution of the ω,ω' carbon atoms with alicyclic or aryl moieties provided products with differences in lipophilicity, geometry, and surfaces with proton or electron enrichment. Interestingly, a recent report noted that single methyl branching introduced on a long-chain alkyl group in several tertiary amines, caused a loss of inhibitory action for Gram-negative bacteria but not toward Gram-positive bacteria (7).

RESULTS AND DISCUSSION

Syntheses—The dihydrochloride salts I*a*–I*n* were prepared by condensation of the requisite symmetrical alkanone and 1,3-diamino-2-propanol to form the Schiff base, followed by reduction of the carbon–nitrogen double bond by catalytic hydrogenation or with sodium borohydride, and neutralization with hydrogen chloride in diethyl ether, 2-propanol, or binary mixtures (2, 8, 9). Yields were 40–90%. Compounds I*d* (5) and I*l* (9) were reported previously. The ketone-starting materials for I*a*, I*b*, and I*f* were purchased[1] or were obtained from earlier N-substituted triamine and tetramine syntheses (5), for I*c–e*, I*h–l*, and were known for I*m* (10). The preparation of ketones required for I*g* and I*n* is described under *Experimental.*

Microbiology—The minimal inhibitory concentrations of the compounds were determined by an agar dilution method. Stock solutions of the 14 compounds containing 1 or 10 mg/ml in dimethyl sulfoxide were added to melted sterile nutrient agar[2] to give the desired final concentrations, and 10 ml was pipetted into Petri plates. The concentrations tested were 200, 100, 80, 60, 50, 40, 30, 20, 10, 8, 6, 4, 2, and 1 μg/ml (5, 11). The dimethyl sulfoxide had no inhibitory effect on the test bacteria. The test bacteria were shaken at 220 rpm for 20 hr at 37° in 10 ml of brain–heart broth[2], diluted to 10^{-3} in fresh brain–heart broth, and spot inoculated onto the surfaces of the agar plates containing the test compounds. The number of colony-forming units in each inoculated spot was $\sim 10^5$. The plates were scored after a 20-hr incubation at 37°. The minimum inhibitory concentration is the lowest concentration of compound that completely inhibited growth by macroscopic examination. All assays were run in duplicate. The results are shown in Tables I and II.

The more potent pseudomonad inhibitors of the series (Table I) represented practically all substituent variations; namely, linear alkyl (I*b*), alicyclic (I*d* and I*e*), and electron-donor substituted aryl (I*i*). The efficiency, comparable to chlorhexidine, could be linked to the lipid–water partition property and apparently was not sensitive to steric, electronic, and bulk factors. However, no attempt was made to analyze structure–activity relationships by molecular connectivity (12) or polarizability parameters (13).

Structure–Activity—The calculated hydrophobic parameter, π, (14) for the $[R(CH_2)_n]^2$ groups of the best inhibitors was in the range 7–9. Regression analysis using the least-squares method indicated, within 95% confidence limits, a parabolic relationship with minimal inhibitory concentration for both pseudomonad strains and provided the predicted curves: minimum inhibitory concentration (μg/ml) = 1340.68 (±279.13) − 343.39 (±78.11) π + 22.40 (±5.25) π^2 for strain MB418 (n = 14, r = 0.8048, and s = 55.65) (Fig. 1); minimum inhibitory concentration (μg/ml) = 1343.55 (±285.83) − 346.38 (±79.99) π + 22.69 (±5.38) π^2 for strain MB2245 (n = 14, r = 0.7985, and s = 56.90); where n is the number of assayed compounds used, r is the correlation coefficient, and s the standard deviation from the regression line.

Use of a lipid group-calculated π for correlation purposes (the polar base moiety remaining constant in the series) avoided problems associated with whether the active molecular species was neutral, ionic (15), or metal chelated (16).

The broad spectrum assay (Table II) showed a similar ordering in which compounds with high inhibitory potency for Gram-negative bacteria (*e.g., Escherichia coli*) were comprised of diverse substituent groups.

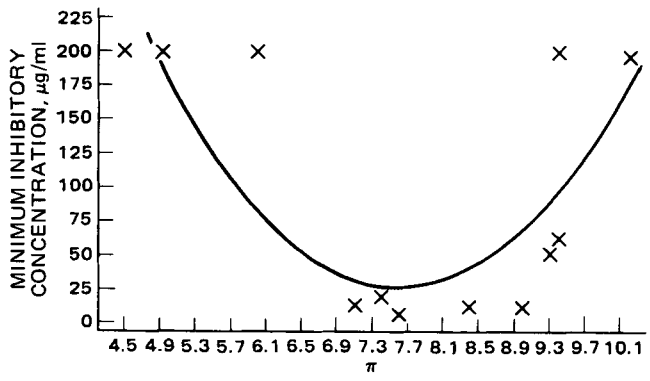

Figure 1—*The calculated Hansch hydrophobic parameter (x) for the lipoidal substituent, $[R(CH_2)_n]_2$ (Table I), of* N-*substituted 1,3-diamino-2-propanol dihydrochlorides and* in vitro *minimal inhibitory concentration; (—) regression analysis predicted curve:* P. aeruginosa *MB418.*

[1] Aldrich Chemical Co.

[2] Difco, brain–heart infusion agar.

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Table I—*N*-(Substituted *sec*-alkyl)-1,3-diamino-2-propanol Dihydrochloride Salts: Physicochemical and Antipseudomonad Screen Data {$[R(CH_2)_n]_2CHNHCH_2CHOHCH_2NH_2 \cdot 2HCl$}

Compound	R	*n*	Melting Point[a]	Minimum Inhibitory Concentration μg/ml *Pseudomonas aeruginosa* MB 418	MB 2245	$[R(CH_2)_n]_2$ π[b]	Empirical Formula	Analysis Calc.	Found
I*a*	Methyl	3	203–205[c]	>200	>200	4.5	$C_{12}H_{30}Cl_2N_2O$	C 49.80	49.67
								H 10.45	10.22
								Cl 24.50	24.52
								N 9.68	9.50
I*b*	Methyl	6	273–276	6	6	7.6	$C_{18}H_{42}Cl_2N_2O$	C 57.88	58.02
								H 11.33	11.65
								N 7.50	7.18
I*c*	Cyclohexyl	1	262–264[c]	80	40	6.1	$C_{18}H_{38}Cl_2N_2O$	C 58.53	58.63
								H 10.37	10.63
								N 7.58	7.18
I*d*	Cyclohexyl	2	254–255[c]	10	8	7.1	$C_{20}H_{42}Cl_2N_2O$	C 60.45	60.04
								H 10.65	10.82
								N 7.05	7.06
I*e*	3,3-Dimethyl-bicyclo[2.2.1]-heptan-2-yl	2	236–237[c]	10	10	9.0	$C_{26}H_{50}Cl_2N_2O$	C 65.40	65.55
								H 10.55	10.95
								N 5.86	5.68
I*f*	Phenyl	1	185–186	>200	>200	4.9	$C_{18}H_{26}Cl_2N_2O$	C 60.50	60.70
								H 7.33	7.48
								Cl 19.84	19.47
								N 7.84	8.17
I*g*	4-Phenylphenyl	2	241–243[d]	>200	>200	9.4	$C_{32}H_{38}Cl_2N_2O$	C 71.50	71.48
								H 7.13	7.27
								N 5.21	5.03
I*h*	4-Methylphenyl	2	265–267	40	50	6.9	$C_{20}H_{34}Cl_2N_2O$	C 64.09	63.94
								H 8.25	8.16
								N 6.74	6.39
I*i*	4-(1-Methylethyl)phenyl	2	260–262	10	10	8.4	$C_{26}H_{42}Cl_2N_2O$	C 66.60	66.65
								H 9.02	9.23
								Cl 15.10	14.89
								N 5.97	6.00
I*j*	4-(1-Methylethyl)phenyl	3	244–246[c]	60	60	9.4	$C_{28}H_{46}Cl_2N_2O$	C 67.59	67.58
								H 9.32	9.69
								N 5.63	5.70
I*k*	4-(1,1-Dimethylethyl)phenyl	2	270–272[e]	50	50	9.3	$C_{28}H_{46}Cl_2N_2O$	C 67.59	67.34
								H 9.32	9.60
								N 5.63	5.55
I*l*	4-(1,1-Dimethylethyl)phenyl	3	267–269[c]	>200	>200	10.3	$C_{30}H_{50}Cl_2N_2O$	C 68.53	67.99
								H 9.59	9.46
								Cl 13.48	13.19
								N 5.33	5.12
I*m*	4-Methoxyphenyl	2	240–242	200	200	6.0	$C_{22}H_{34}Cl_2N_2O$	C 59.32	59.43
								H 7.69	7.91
								N 6.29	6.40
I*n*	4-Chlorophenyl	2	263–265[e]	20	20	7.4	$C_{20}H_{28}Cl_4N_2O$	C 52.88	52.86
								H 6.21	6.46
								Cl 31.22	31.20
								N 6.17	6.00
	Benzalkonium chloride			>200	>200				
	Chlorhexidine			6	8				

[a] Salts were crystallized or recrystallized from isopropanol except as otherwise noted. [b] Hansch hydrophobic parameter (14). [c] Purified by column chromatography of the base on silica gel (1 g:75 g) with methylene chloride–methanol–ammonium hydroxide (35:4:1), conversion to the dihydrochloride in ether with dry HCl in 2-propanol (29% w/w), and isolation upon precipitation or by solvent removal. [d] Recrystallized from ether–2-propanol. [e] Ethanol.

Several of the most lipophilic salts were highly effective against the nine genera; I*j* at ≤6 μg/ml and I*k* exhibited similar patterns, but they were only moderately active for the two *Pseudomonas* strains, suggesting a more critical partitioning requirement.

Many examples of antibacterial activity–lipophilicity parabolic findings have been reported previously for homologs of inhibitors (17). Several explanations have been proposed. Highly hydrophilic compounds in a series cannot penetrate a cell envelope lipid barrier, whereas the more hydrophobic members remain entrapped in the lipid phase and fail to reach target sites. These postulates may account for each low activity side of the parabolic curve, with molecule size and colloidal associations as other possible contributors. More recent studies using quaternary ammonium salts of increasing alkyl chain length, in a simple salts test medium with and without varying sensitivity mutants of *Ps. aeruginosa*, indicate a positive linear correlation between lipophilicity and antimicrobial activity (18). However, the probable test medium imposed artifactual results of the present work may better anticipate activities under conditions of potential use. Preliminary *in vitro* and *in vivo* antibacterial studies on skin (excised and intact cow udder) with Table I derivatives appear to confirm the findings[3].

[3] To be published elsewhere.

EXPERIMENTAL

Melting points were taken in open capillary tubes[4] and are uncorrected. IR spectra for solids were run as mulls in mineral oil[5]. NMR spectra[6] (60 MHz) were obtained in dimethyl sulfoxide-d_6 with tetramethylsilane as an internal standard. Spectral data for all reported compounds appeared consistent with assigned structures. TLC was performed on precoated gel GF glass plates (250 μm)[7], using dioxane–methanol–ammonium hydroxide (15:4:1) as the developing solvent for the diamine hydrochloride salts and ether–petroleum ether (1:1) or hexane–ethyl acetate (1:1) mixtures for ketones (iodine staining). The amine salts were colorless solids, easily hydrated, and were dried for 4 hr at 60–80° (0.05 mm) before analysis. All hydrogenations were run at 20–25° and 2.8–3.5 kg/cm^2 until theoretical hydrogen uptake, requiring from 1 to 4 hr. After catalyst removal by filtration, the solvent was distilled *in vacuo*.

Ketones—1,5-Bis(aryl)-1,4-pentadien-3-ones were prepared by literature methods.

Bis(4-chlorophenyl) Derivative—A mixture of 4-chlorobenzaldehyde

[4] Thomas-Hoover melting point apparatus.
[5] Perkin-Elmer 137 spectrometer.
[6] Varian A-60 spectrometer.
[7] Analtech.

Table II—*In Vitro* Antibacterial Assay

Compound	Minimal Inhibitory Concentration, μg/ml								
	S. aureus[a]	*S. pyogenes*[b]	*B. bronchiseptica*[c]	*K. aerogenes*[d]	*B. subtilis*[e]	*C. pseudodiphtherium*[f]	*P. multocida*[g]	*E. coli*[h]	*S. schottmuelleri*[i]
I*a*	—[j]	60	60	—	—	200	200	—	—
I*b*	6	2	2	6	6	2	2	6	6
I*c*	40	1	6	80	40	6	20	20	50
I*d*	4	2	2	6	4	2	2	8	8
I*e*	6	1	6	2	6	1	4	6	6
I*f*	—	200	50	200	—	50	60	—	—
I*g*	6	4	8	200	6	60	50	60	200
I*h*	30	4	4	30	30	30	6	40	40
I*i*	40	4	6	2	4	2	6	40	40
I*j*	6	2	2	6	6	4	4	6	6
I*k*	8	2	4	20	4	2	2	8	8
I*l*	6	4	40	200	6	6	6	40	8
I*m*	60	40	40	200	40	60	40	80	200
I*n*	2	1	1	20	6	1	1	2	20
Benzalkonium chloride[k]	1	1	8	6	6	1	1	—	200
Chlorhexidine	1	1	1	2	1	1	1	2	6

[a] Merck Bacteria (MB); genus and strain, Staphylococcus MB 2865. [b] Streptococcus MB 3738. [c] Bordetella MB 3551. [d] Klebsiella MB 1503. [e] Bacillus MB 964. [f] Corynebacterium MB 261. [g] Pasturella MB 7. [h] Escherichia MB 2884. [i] Salmonella MB 2837. [j] Greater than 200 μg/ml. [k] Hyamine 3500, Rohm & Haas, which contains alkyl (50% C-14, 40% C-12, 10% C-16) dimethylbenzylammonium chloride as an 80% solution in ethanol.

(15.2 g, 0.11 mole) and acetone (3.1 g, 0.05 mole) in 60 ml of ethanol was added dropwise (15 min) to a solution of sodium hydroxide (12 g, 0.3 mole) in 120 ml of water diluted with 100 ml of ethanol. The temperature during addition was maintained at 10–13° and then at 20–25° for 4 hr. The precipitated solids were filtered, washed with water, and air dried. Recrystallization of 0.2 g of the yellow product (13.5 g, 83%) from benzene gave pure bis(4-chlorobenzal)acetone (0.14 g); mp 191–193° [lit. (19) mp 193–194°].

1,5-Bis(4-chlorophenyl)-3-pentanone—A mixture of the di-unsaturated ketone (6 g, 0.02 mole) and 2.5 g of Raney nickel in 100 ml of ethanol was shaken at 20° under 3.5 kg/cm^2 hydrogen for 2 hr until near theoretical uptake was observed. The catalyst was filtered and the solvent was removed by distillation. The residual oil was dissolved in 400 ml of methylene chloride and chromatographed on a column containing 450 g of silica gel. The product was obtained in fractions four and five (400 ml each) as a solid (4 g, 75%) after solvent removal. One gram was recrystallized from 5 ml of 2-propanol to yield colorless plates (0.7 g); mp 65–67°, IR: 1690 (C=O) cm^{-2}.

Anal.—Calc. for $C_{17}H_{16}Cl_2O$: C, 66.46; H, 5.25; Cl, 23.08. Found: C, 66.58; H, 5.20; Cl, 23.02.

***N*-(ω,ω'-Disubstituted *sec*-alkyl)-1,3-diamino-2-propanol Dihydrochlorides (I*a*–I*n*)**—Schiff bases were obtained by condensation of ω,ω'-disubstituted alkanones with 1,3-diamino-2-propanol in refluxing toluene and azeotropic water removal (5), or by reaction in ethanol without water separation. The carbon–nitrogen double bond was reduced catalytically with hydrogen or with sodium borohydride. Both procedures are exemplified with the preparations, I*i* and I*m*. Table I lists the physical and analytical data.

1-Amino -3- {[3-[4-(1-methylethyl)phenyl] -1- [2-[4-(1-methylethyl)phenyl]ethyl]-propyl}amino}-2-propanol Dihydrochloride (I*i*)—A solution of 1,5-bis[4-(1-methylethyl)-phenyl]-3-pentanone (6.4 g, 0.02 mole) in 20 ml of ethanol was added dropwise (45 min) to a stirred solution of 1,3-diamino-2-propanol (10.8 g, 0.12 mole) in 80 ml of ethanol maintained at 90°. The resulting solution was heated at 90° for 12 hr, diluted with 50 ml of ethanol and cooled to 5°. Sodium borohydride (1.2 g, 0.03 mole) was added portionwise (10 min), and the reaction mixture, after 2 hr at 5°, was stirred for 2 hr at 20°. The solvent was removed *in vacuo* and the residual oil partitioned between 75 ml of ether and 75 ml of water. The ether solution was washed with 75 ml of brine, dried over sodium sulfate and concentrated *in vacuo*. The residual base was dissolved in 15 ml of 2-propanol, the solution cooled to 5° and mixed with 25 ml of 2-propanol saturated with hydrogen chloride. The solids, after mixing at 5° for 6 hr, were filtered and recrystallized from 35 ml of 95% 2-propanol to yield 6.9 g of Ic; NMR (dimethyl sulfoxide-d_6): δ 3.47 ppm [quintet, 1H of *sec*-alkyl(—CH_2)$_2$C*H*—NH—].

1-Amino -3- [3-(4-methoxyphenyl) -1- [2-(4-methoxyphenyl)ethyl]propylamino]-2-propanol Dihydrochloride (I*m*)—Powdered 1,5-bis(4-methoxyphenyl)-3-pentanone (6.0 g, 0.02 mole) was added slowly (30 min) to a stirred solution of 1,3-diamino-2-propanol (9.0 g, 0.1 mole) in 100 ml of ethanol maintained at 90°. After 12 hr the solution was cooled to 20°, diluted with 50 ml of ethanol, and mixed with 0.75 g of platinum oxide. Hydrogenation was run at 20–25° and 3.5 kg/cm^2 hydrogen. Within 2.5 hr, theoretical uptake was observed, and the reaction mixture was shaken an additional 0.5 hr. The catalyst was removed by filtration and the solvent distilled *in vacuo*. The residual oil was dissolved in 100 ml of ether. The product precipitated when mixed with 5 ml of 2-propanol which was previously saturated with hydrogen chloride. After 3 hr of mixing, the salt was filtered to yield 7.9 g (89%) of I*n* which was recrystallized from 2-propanol; NMR (dimethyl sulfoxide-d_6): δ 3.24 ppm [quintet, 1 H of *sec*-alkyl(—CH_2)$_2$C*H*—NH—].

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