ACKNOWLEDGMENTS AND ADDRESSES

Received August 16, 1975, from the *Quality Control Department, Endo Laboratories, Inc., Garden City, NY 11530*

Accepted for publication December 1, 1975.

The authors thank Mr. J. Fernandez, Technicon Corp., for technical assistance.

[x] To whom inquiries should be directed.

Inhibition of *Streptococcus faecalis* by Long Chain Aliphatic Monoamines: Quantitative Structure–Activity Studies

G. E. BASS [x], LARRY J. POWERS, and E. O. DILLINGHAM

Abstract □ Primary, secondary, and tertiary long chain aliphatic amines were synthesized, and their activity against *Streptococcus faecalis* was determined. Quantitative structure–activity analyses were carried out based on the Hansch extrathermodynamic model, using partition coefficients, CMC's, quantum mechanical charges on the amine nitrogen, and the Taft steric parameter. The best correlations were obtained with the CMC. Steric properties of the ammonium head become important for tertiary amines. The structural feature of these compounds that dominates biological activity is the length of the alkyl tail. Ammonium head substituents are of only secondary importance.

Keyphrases □ *Streptococcus faecalis*—inhibition by long chain aliphatic monoamines, quantitative structure–activity relationships □ Amines, long chain aliphatic—inhibition of *Streptococcus faecalis*, structure–activity relationships □ Structure–activity relationships—long chain aliphatic monoamine inhibition of *Streptococcus faecalis*

The antibacterial activity of amines carrying long aliphatic chains has been recognized (1, 2). Although the quaternary compounds have been studied the most, simple primary, secondary, and tertiary long chain aliphatic amines, $R_1R_2N—C_nH_{2n+1}$, are also potent antibacterials. Relationships between structures and biological activities of these compounds have been investigated (3–7); but in most of these studies, structure variations were limited primarily to the length of the long aliphatic chain or "tail."

Hansch and coworkers (4–6) carried out quantitative structure–activity correlations on published activity data for primary amines using the extrathermodynamic model:

$$\log(\text{biological activity}) = a(\log P)^2 + b(\log P) + c \quad \text{(Eq. 1)}$$

where P is the octanol–water partition coefficient. Biological data included antibacterial (Gram-positive and Gram-negative species) (4, 5), antifungal (6), and hemolytic (5) activities. Very good correlations were always found.

Weiner *et al.* (3) suggested that activity variations for these compounds may be explained within the framework of the Ferguson principle (8), utilizing surface activity parameters such as the critical micelle concentration (CMC) and the surface concentration. These studies involved the inhibition of *Micrococcus pyogenes* var. *aureus*, *Escherichia coli*, and *Candida albicans* by three substituted dodecylamines.

To develop a more complete understanding of the structure–activity relationships for these compounds, the effect of alkyl substitution on the ammonium head ($R_1R_2N—$) was investigated. Physicochemical properties that might play a role in, or reflect, the bioactivities of these compounds include the partition coefficient, CMC, the Taft steric parameter (E_s) for the ammonium head, and the quantum mechanical charge (Q_N) calculated for the amine nitrogen.

Long chain aliphatic amines (primary, secondary, and tertiary) were synthesized, and their ability to inhibit the growth of *Streptococcus faecalis* was determined. The physicochemical parameters noted were evaluated, and quantitative structure–activity correlations were carried out using the generalized Hansch equation:

$$\log(\text{activity}) = a(\log P)^2 + b(\log P) + c(\log \text{CMC})^2 + d(\log \text{CMC}) + eE_s + fQ_N + g \quad \text{(Eq. 2)}$$

Not all of these parameters can be used together in a single correlation. For example, log P and log CMC reflect similar properties. In the opinion of these authors, little can be gained from these empirical correlations if more than three parameters are involved.

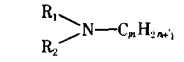
EXPERIMENTAL[1]

The amine salts were prepared by reacting the acyl chloride of the appropriate long chain carboxylic acid with amines to obtain the corresponding amides. After recrystallization, the amides were reduced with lithium aluminum hydride to the desired amines. The amines were isolated and converted to the hydrochloride salts. Melting points and IR spectra were in agreement with available literature data.

Partition coefficients were calculated from substituent contributions obtained from the literature (5, 9, 10). The reported value of log P = 1.85 was assumed for dodecylamine. Substituent contributions for $—NH_2$ and $—N(CH_3)_2$ were −1.85 and −0.95, respectively. The value for $—NHCH_3$ was interpolated as −1.40. Methylene groups were assumed to contribute 0.50, and branching corrections

[1] Melting points were determined on a Thomas-Hoover capillary melting-point apparatus. IR spectra were determined on a Perkin-Elmer model 257 spectrophotometer. Turbidity measurements were made using a Bausch & Lomb Spectronic 20. Surface tension was determined using a Fisher surface tensiometer. Regression analyses were performed on an IBM 1130 system.

Table I—*S. faecalis* Inhibitory Activities and Physicochemical Parameters for a Series of Long Chain Aliphatic Monoamines



n	R_1	R_2	$ID_{50} \times 10^6$ [a]	Log P [b]	Log (CMC × 10^7) [c]	E_s [d]	Q_N [e]
8	H	H	1800	−0.150	6.26	2.48	0.131
10	H	H	230	0.850	5.54	2.48	0.131
11	H	H	84.8	1.35	4.89	2.48	0.131
12	H	H	38.0	1.85	4.15	2.48	0.131
12	H	CH_3	30.0	2.30	4.32	1.24	0.349
12	H	C_2H_5	25.0	2.80	4.02	1.17	0.347
12	H	n-C_4H_9	58.5	3.80	3.27	0.85	0.347
12	H	*tert*-C_4H_9	18.0	3.33	4.31	−0.30	0.345
12	CH_3	CH_3	60.0	2.75	4.48	0.00	0.529
12	C_2H_5	C_2H_5	31.5	3.75	4.38	−0.14	0.525
12	iso-C_3H_7	iso-C_3H_7	34.5	4.35	4.29	−0.94	0.523
12	iso-C_4H_9	iso-C_4H_9	120.0	5.35	4.19	−1.86	0.525
12	C_2H_5	n-C_4H_9	25.0	4.75	4.35	−0.46	0.525
14	H	CH_3	4.0	3.30	3.36	1.24	0.349
14	H	n-C_3H_7	3.8	4.30	2.17	0.88	0.347
14	H	$C_2H_4OCH_3$	4.2	2.82	3.35	0.47	0.348
16	H	H	2.5	3.85	2.73	2.48	0.131
16	H	C_2H_5	3.3	4.80	1.80	1.17	0.347
17	H	H	1.9	4.35	2.55	2.48	0.131
18	H	H	10.0	4.85	2.83	2.48	0.131
18	H	CH_3	3.5	5.30	2.77	1.24	0.349
18	CH_3	CH_3	3.6	5.75	2.60	0.00	0.529

[a] Amine concentration, moles per liter × 10^6, required for 50% growth inhibition. [b] P = octanol–water partition coefficient, calculated. [c] CMC in isotonic saline. [d] E_s = Taft steric factor for onium head, R_1R_2N—. [e] Q_N = quantum mechanical charge on the amine nitrogen in proton units.

Table II—Statistics for Correlation of *S. faecalis* Inhibitory Activities with Physicochemical Parameters of Long Chain Aliphatic Monoamines Using the Extrathermodynamic Model

Physicochemical Parameters	All Compounds (n = 22)		Primary Amines (n = 7)		Secondary Amines (n = 9)		Tertiary Amines (n = 6)		Dodecylamines (n = 10)	
	R^2	F	R^2	F	R^2	F	R^2	F	R^2	F
log CMC	0.81	87	0.96	117	0.46	6.0	0.71	9.8	0.06	0.5
(log CMC)², log CMC	0.84	51	0.96	51	0.47	2.6	0.80	5.8	0.08	0.3
log P	0.44	16	0.86	30	0.28	2.7	0.19	0.9	0.13	1.2
(log P)², log P	0.57	12	0.95	39	0.28	1.2	0.25	0.5	0.34	1.8
E_s	0.00	0.0	—	—	0.03	0.2	0.38	2.5	0.11	0.9
log CMC, E_s	0.81	41	—	—	0.47	2.6	0.88	11	0.24	1.1
log P, E_s	0.60	14	—	—	0.28	1.2	0.94	25	0.13	0.5
Q_N	0.01	0.1	—	—	—	—	—	—	0.07	0.6
log CMC, Q_N	0.81	41	—	—	—	—	—	—	0.20	0.9
log P, Q_N	0.54	11	—	—	—	—	—	—	0.13	0.5

of −0.20 and −0.47 were employed for iso- and *tert*-substituents, respectively.

The CMC's of the amines in isotonic saline were determined by the du Nouy ring method (11, 12). The quantum mechanical charge on the amine nitrogens was calculated using the Del Re sigma electron method (13).

Growth inhibition by the amine salts was measured turbidimetrically at 660 nm. A minimum of 12 amine concentrations (five replicates each) in NIH thioglycolate broth was assayed along with controls. Each tube (containing 5 ml of broth) was loop inoculated from an overnight growth of *S. faecalis*[2] and incubated at 37° for 8 hr. Percent growth *versus* amine concentration plots were constructed to obtain concentrations required for 50% inhibition (ID_{50}).

Quantitative structure–activity correlations using the generalized Hansch model (Eq. 2) were carried out by regression analysis, and goodness of fit was assessed statistically (14).

RESULTS AND DISCUSSION

Inhibitory activities and physicochemical parameters for 22 long chain aliphatic amines are presented in Table I. Octylamine and heptadecylamine were the least active and most active compounds tested, respectively. For the primary amines, activity increased steadily with chain length up to heptadecylamine and then began to decrease. Solubilities of $C_{20}H_{41}$—NH_2 and $C_{22}H_{45}$—NH_2 were not sufficient to allow ID_{50} determinations ($ID_{50} > 5 \times 10^{-5}$ M). No easily recognized pattern was exhibited by the secondary and tertiary amines.

Results for the quantitative structure–activity correlations are summarized in Table II. When all 22 compounds in Table I were included in the analyses, the best correlations were found with log CMC. The log P parameter was considerably less effective. The Taft steric parameters, E_s, and the quantum mechanical charge on the amine nitrogen, Q_N, taken alone, were totally incapable of explaining activity variations. These parameters did not significantly improve correlations when used together with log CMC or log P. The essence of these results is that the rather wide range of activities displayed by these compounds is best correlated with parameters sensitive to the length of the long alkyl tail. Activity correlates better with the substituent contribution for the tail, π (tail) (R^2 = 0.75, F = 58.7), than with log $P = \pi$ (head) + π (tail).

Analyses involving only the primary amines again showed log CMC to be the best parameter while appreciable correlations with log P were also obtained. No significant correlations were obtained

[2] Oral isolate was supplied by Dr. Albert T. Brown, School of Dental Medicine, University of Connecticut Health Center, Hartford, Conn.

for the secondary amines considered alone. Best correlations were found with log CMC.

Notable results were obtained for the tertiary amines. Fair correlations were found with log CMC (linear and quadratic) but not log P or E_s. However, very significant improvements were obtained when E_s was used together with either log CMC or log P. The clear implication is that for tertiary amines the bulk of the head group must be considered along with the overall hydrophobic properties of the molecule and has a deactivating effect. Somewhat similar results were found for a series of alcohols (15). Hansch and Glave (5) also noted structure–activity similarities for long chain amines and alcohols. They proposed that both classes of compounds be classified as "membrane-perturbing" agents.

The dodecylamines, like the secondary amines, yielded no significant correlations. This finding underscores the inability of the physicochemical parameters studied to represent adequately the biologically important properties of the ammonium head. However, the range of activities for this series is rather narrow.

In summary, the activity is dominated by the length of the long aliphatic tail. Best correlations are found with physicochemical properties sensitive to this feature. Substitution of small alkyl groups on the amine nitrogen has only a secondary effect on activity. For secondary amines, no rationale for the direction or magnitude of this effect was uncovered. For tertiary amines, the bulk of the ammonium head appears to be important when considered with hydrophobic properties. CMC was superior to the partition coefficient for quantitative structure–activity correlations.

REFERENCES

(1) G. Domagk, *Deut. Med. Wochenschr.*, **61**, 829(1935); through *Chem. Abstr.*, **29**, 7018(1935).

(2) A. T. Fuller, *Biochem. J.*, **36**, 548(1942).

(3) N. D. Weiner, F. Hart, and G. Zografi, *J. Pharm. Pharmacol.*, **17**, 350(1965).

(4) E. J. Lien, C. Hansch, and S. M. Anderson, *J. Med. Chem.*, **11**, 430(1968).

(5) C. Hansch and W. R. Glave, *Mol. Pharmacol.*, **7**, 337(1971).

(6) C. Hansch and E. J. Lein, *J. Med. Chem.*, **14**, 653(1971).

(7) J. J. Kabara, A. J. Conley, and J. P. Truant, *Antimicrob. Ag. Chemother.*, **2**, 492(1972).

(8) J. Ferguson, *Proc. Roy. Soc., Ser. B*, **127**, 387(1939).

(9) J. Iwasa, T. Fujita, and C. Hansch, *J. Med. Chem.*, **8**, 150(1965).

(10) C. Hansch, J. E. Quinlan, and G. L. Lawrence, *J. Org. Chem.*, **33**, 347(1968).

(11) A. W. Adamson, "Physical Chemistry of Surfaces," 2nd ed., Interscience, New York, N.Y., 1967, pp. 24–491.

(12) L. J. Powers, E. O. Dillingham, and G. E. Bass, *J. Pharm. Sci.*, **64**, 883(1975).

(13) G. Del Re, in "Electronic Aspects of Biochemistry," B. Pullman, Ed., Academic, New York, N.Y., 1964, p. 221. G. Del Re, *J. Chem. Soc.*, **1958**, 4031.

(14) W. P. Purcell, G. E. Bass, and J. M. Clayton, "Strategy of Drug Design," Wiley, New York, N.Y., 1973, pp. 69–71.

(15) E. O. Dillingham, R. W. Mast, G. E. Bass, and J. Autian, *J. Pharm. Sci.*, **62**, 22(1973).

ACKNOWLEDGMENTS AND ADDRESSES

Received September 9, 1974, from the *Department of Molecular Biology, College of Pharmacy, University of Tennessee Center for the Health Sciences, Memphis, TN 38163*

Accepted for publication February 11, 1975.

Supported in part by U.S. Public Health Service Grants DE-03429 and HL-09495.

The authors gratefully acknowledge the technical assistance of J. N. Warren and I. Bilsky.

[x] To whom inquiries should be directed.

5-Aryloxy-6-methoxy-8-aminoquinolines as Potential Prophylactic Antimalarials

KALIDAS PAUL and C. DeWITT BLANTON, Jr. [x]

Abstract □ 5-(*p*-Anisyloxy)-6-methoxy-8-(5-isopropylaminopentylamino)quinoline was resynthesized for evaluation in the *Plasmodium berghei* and monkey prophylactic (*Plasmodium cynomolgi*) tests. A new primary amine, three secondary amines, and one structurally modified side-chain analog of the 5-aryloxy series were also prepared. None of these compounds showed significant antimalarial or prophylactic activity.

Keyphrases □ 8-Aminoquinolines, 5- and 6-substituted—series synthesized, screened for antimalarial activity □ Antimalarial agents, potential—5- and 6-substituted 8-aminoquinolines synthesized and screened □ Structure–activity relationships—5- and 6-substituted 8-aminoquinolines synthesized and screened for antimalarial activity

During studies (1, 2) on the synthesis and evaluation of various derivatives of the 8-aminoquinoline nucleus as potential prophylactic antimalarials, a known compound, 5-(*p*-anisyloxy)-6-methoxy-8-(5-isopropylaminopentylamino)quinoline (I) (3, 4), was proposed for resynthesis and evaluation in the *Plasmodium berghei* and monkey prophylactic (*Plasmodium cynomolgi*) tests. In *Plasmodium gallinaceum* studies (4), I was relatively nontoxic and highly active, displaying the highest therapeutic index (177) of any 8-aminoquinoline tested. It was suggested that I and related compounds may be more conveniently converted to the *o*-quinoid structure *in vivo*, a structural feature proposed for the active metabolite (5, 6). Primaquine and pentaquine exhibited therapeutic indexes of 30 and 57, respectively, in this test (4).

Although the 8-aminoquinolines are quite toxic, primaquine has had a prominent place in malaria prophylaxis. The possibility that 5-aryloxy-substituted 8-aminoquinolines may offer a lead in the search for more effective and less toxic agents led to a reexamination of I as well as a few selective, structurally modified analogs (II–VI). For example, the 5-aryloxy analog (II) of primaquine was considered since primaquine has been recognized as the least toxic and most effective 8-aminoquinoline tested in humans (5, 7). It was reported (7, 8) that occasional aromatic interruption in