im-benzylhistidine was detectable in the hydrolysate by electrophoresis at pH 3.5.

This product (100 mg) was purified in the same way as described for the benzyl derivative. The flow rate was 28 ml/hr. The gradient was started after 18.5 hr (520 ml) and the main peak was eluted between 980 and 1040 ml. The combined fractions were lyophilized to give 65 mg. After purification *via* the picrate salt there was 45 mg of white powder. A sample hydrolyzed in 5.5 *N* HCl at 110° for 72 hr had the following amino acid composition: Gly 2.03, Val 1.03, Tyr 0.95, Ile 0.97, His 0.93, Pro 1.05, Phe 1.00. A sample hydrolyzed for 40 hr with aminopeptidase-M[21] had Gly 2.00, Val 1.10, Tyr 1.00, Ile 0.85, His 0.33, Pro 0.41, Phe 0.94. Electrophoresis at pH 1.85 showed one spot, E_H 0.57; tlc showed one spot, $R_{f\,III}$ 0.48, $R_{f\,III}$ 0.72; positive reaction with ninhydrin, Pauly reagent, chlorination,[23] and 1-nitroso-2-naphthol.[24]

Ac-Gly-Gly-Val-Tyr-Ile-His-Pro-Phe.—To 15 mg of purified Gly-Gly-Val-Tyr-Ile-His-Pro-Phe in 2 ml of DMF was added 0.05 ml of Ac_2O and 0.05 ml of Et_3N. The solution was stirred for 2 hr at room temperature. A small amount of insoluble material was removed by centrifugation then 20 ml of Et_2O was added to the supernatant giving a voluminous white precipitate. The precipitated peptide was washed with Et_2O (two 20-ml portions) then dried *in vacuo* over KOH pellets at room temperature yielding 10 mg of an off white powder. Electrophoresis at pH 1.85 showed one spot, E_H 0.30, ninhydrin −, Pauly +. There was no detectable free peptide at E_H 0.57 under conditions where 1% could have been detected. A 5-mg portion of the peptide was dissolved in 0.2 ml of 0.1 *M* NaOH and kept at room temperature for 30 min to saponify any acetyl groups on tyrosine. The solution was neutralized with 0.2 ml of 0.1 *M* HCl giving a gelatinous precipitate. Normal saline containing 0.1% polyvinylpyrrolidone (20 ml) was added to prevent adsorption on glass. This solution was used directly for biological assay.

Synthesis and Microbiological Properties of Dipeptides Containing Cyclopentaneglycine and β-2-Thienylalanine[1a]

JIM T. HILL[1b] AND FLOYD W. DUNN[1c]

Department of Biochemistry, University of Tennessee Medical Units, Memphis, Tennessee

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Dipeptides synthesized included L-cyclopentaneglycyl-β-2-thienyl-L-alanine, β-2-thienyl-L-alanyl-L-cyclopentaneglycine, L-phenylalanyl-L-isoleucine, and glycyl-L-cyclopentaneglycine. Cyclopentaneglycine and β-2-thienylalanine may be viewed as structural analogs of isoleucine and phenylalanine, respectively. The effect of the dipeptides on the growth of three organisms, *Escherichia coli*, *Leuconostoc mesenteroides*, and *Lactobacillus arabinosus*, was studied. Under the test conditions, the peptides had greater growth-stimulating or growth-inhibiting effects than equivalent amounts of the corresponding free amino acids.

Peptides often display unique growth-stimulating effects in microorganisms.[2] Other peptides, containing amino acid analogs such as β-2-thienylalanine, may be more effective growth inhibitors than the free amino acid analog.[3] Where an organism was inhibited by a mixture of β-2-thienylalanine and one of its peptides, the inhibition was more effectively nullified by phenylalanine peptides than by free phenylalanine.[4]

Kihara and Snell[5] described the "double inhibition" of *Leuconostoc mesenteroides* by high levels of L-alanine and L-leucine. This "double inhibition" was reversed by the addition of a mixture of glycine and L-isoleucine and, more effectively, by a single dipeptide, glycyl-L-isoleucine. This concept of "double inhibition" suggested to us the preparation of a dipeptide containing two amino acid analogs in order to test whether such peptides would be more effective growth inhibitors than the constituent free amino acid analogs. The amino acid analogs chosen were cyclopentaneglycine[6] and β-2-thienylalanine,[7] structural analogs of isoleucine and phenylalanine, respectively.

This report describes the synthesis and microbiological properties of L-cyclopentaneglycyl-β-2-thienyl-L-alanine, β-2-thienyl-L-alanyl-L-cyclopentaneglycine, glycyl-L-cyclopentaneglycine, and L-phenylalanyl-L-isoleucine.

Experimental Section[8]

Peptide synthesis employed standard coupling and deprotection procedures.[9] The following compounds used in the present work were prepared earlier in this laboratory: L-cyclopentaneglycine, carbobenzoxy-β-2-thienyl-L-alanine, carbobenzoxy-L-phenylalanine, carbobenzoxy-L-isoleucine, β-2-thienyl-L-alanine methyl ester hydrochloride, L-phenylalanine methyl ester hydrochloride, L-isoleucine methyl ester hydrochloride, carbobenzoxyglycine, glycyl-L-isoleucine, glycyl-L-phenylalanine, glycyl-β-2-thienyl-DL-alanine, and L-isoleucyl-L-phenylalanine.

Carbobenzoxy-L-cyclopentaneglycine (I).—To a solution of 4.45 g of L-cyclopentaneglycine (31 mmoles) in an equivalent amount of 2 *N* NaOH were slowly added, with stirring at 0°, 6.39 g (36 mmoles) of carbobenzoxy chloride and 18.5 ml (37 mmoles) of 2 *N* NaOH, maintaining a pH of approximately 8. The product obtained upon acidifying the reaction mixture was purified by dissolving in ethyl acetate, extracting into 0.5 *M* $KHCO_3$, and reprecipitating with HCl; yield 6.8 g (85%), mp

(1) (a) Presented in part at the 48th annual meeting of the Federation of American Societies of Experimental Biology, Chicago, Ill., April 1964. This investigation was supported by U. S. Public Health Service Grant No. AI03710 from the National Institute of Allergy and Infectious Diseases. (b) Part of this work was taken from the M.S. thesis of Jim T. Hill, University of Tennessee, June 1964. (c) To whom requests for reprints should be sent: Department of Chemistry, Abilene Christian College, Abilene, Texas 79601.

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82–84°, $[\alpha]^{22}D$ −4.62° (*c* 2, EtOH). *Anal.* ($C_{15}H_{19}NO_4$) C, H, N.

L-Cyclopentaneglycine Methyl Ester Hydrochloride (II).—A suspension of 6.8 g of L-cyclopentaneglycine in 80 ml of MeOH was saturated with dry HCl at 0°. The cold solution was allowed to stand for 3 hr before the MeOH was removed from the ester under reduced pressure at 50°. The residue was repeatedly dissolved in absolute MeOH and concentrated to dryness until the odor of HCl was gone. After recrystallization from MeOH–Et_2O the yield was 6.8 g (73%), mp 161–163°. *Anal.* ($C_8H_{16}ClNO_2$) C, H, N.

Carbobenzoxy-L-cyclopentaneglycyl-β-2-thienyl-L-alanine Methyl Ester (III).—A solution of 2.77 g of **I** (0.01 mole) in 50 ml of PhMe was treated with 0.01 mole of Et_3N, chilled to −5°, and treated with 1.36 g (0.01 mole) of isobutyl chloroformate. After about 30 min, a cold solution of 2.36 g (0.01 mole) of β-2-thienyl-L-alanine methyl ester hydrochloride and 0.01 mole of Et_3N in 50 ml of $CHCl_3$ was added and the reaction mixture was allowed to stand overnight at room temperature. The resulting protected peptide was washed (5% $KHCO_3$, 5% HCl, H_2O) and isolated from the organic solvent. After recrystallization from warm EtOH–H_2O the yield was 3.7 g (84%), mp 151–153°, $[\alpha]^{22}D$ −10.50° (*c* 2, EtOH). *Anal.* ($C_{23}H_{28}N_2O_5S$) C, H, N.

Carbobenzoxy-β-2-thienyl-L-alanyl-L-cyclopentaneglycine Methyl Ester (IV).—In the usual way, 3.03 g (0.01 mole) of carbobenzoxy-β-2-thienyl-L-alanine and 1.96 g (0.01 mole) of II were coupled to yield 3.56 g (80%) of the desired product, mp 118–121°, $[\alpha]^{22}D$ −24.75° (*c* 2, EtOH). *Anal.* ($C_{23}H_{28}N_2O_5S$) C, H, N.

Carbobenzoxy-L-phenylalanyl-L-isoleucine Methyl Ester (V).—Coupling of 2.58 g (0.01 mole) of carbobenzoxy-L-phenylalanine with 1.93 g (0.01 mole) of L-isoleucine methyl ester hydrochloride resulted in a yield of 3.5 g (80%), mp 98–100°, $[\alpha]^{22}D$ −10.10° (*c* 2, EtOH). *Anal.* ($C_{24}H_{30}N_2O_5$) C, H.

Carbobenzoxyglycyl-L-cyclopentaneglycine (VI).—By the usual procedure, 2.09 g (0.01 mole) of carbobenzoxyglycine and 1.93 g of II were coupled to yield 2.5 g of ester in the form of an oil which resisted crystallization. The resulting ester was saponified with NaOH in H_2O–AcMe to yield 1.3 g of VI (52%), mp 74–78°. *Anal.* ($C_{15}H_{22}N_2O_5$) N.

Glycyl-L-cyclopentaneglycine (VII).—A 1.2-g sample of VI was hydrogenated in the presence of Pd black to yield 0.6 g (82%) of the dipeptide, mp 256–258° dec. *Anal.* ($C_9H_{16}N_2O_3$) C, H, N.

Carbobenzoxy-L-cyclopentaneglycyl-β-2-thienyl-L-alanine (VIII).—In warm AcMe, 3.30 g of **III** (7.0 mmoles) was saponified by intermittent addition of 1 *N* NaOH to maintain a pH of 7.5–9.0, yield 1.67 g (54%), mp 166–167°, $[\alpha]^{22}D$ +0.56° (*c* 2, EtOH). *Anal.* ($C_{22}H_{26}N_2O_5S$) C, H.

L-Cyclopentaneglycyl-β-2-thienyl-L-alanine (IX).—A 1.51-g sample of VIII was treated with 10 ml of a solution of glacial HOAc saturated with anhydrous HBr. After about 1 hr the solution was poured into an excess of anhydrous Et_2O, and the resulting solid was thoroughly washed with Et_2O to remove excess HBr. The free peptide precipitated when an ethanolic solution of the peptide hydrobromide was neutralized with NH_4OH; yield 0.95 g (85%), mp 274–276° dec, $[\alpha]^{22}D$ +17.76° (*c* 2, 2 *N* HCl). *Anal.* ($C_{14}H_{20}N_2O_3S$) C, H, N.

Carbobenzoxy-β-2-thienyl-L-alanyl-L-cyclopentaneglycine (X).—Saponification of 3.0 g (6.5 mmoles) of IV resulted in a yield of 1.5 g (54%), mp 183–184°, $[\alpha]^{22}D$ −11.90° (*c* 2, EtOH). *Anal.* ($C_{22}H_{26}N_2O_5S$) C, H.

β-2-Thienyl-L-alanyl-L-cyclopentaneglycine (XI).—Deprotection of 1.3 g of X with anhydrous HBr in glacial HOAc produced 0.5 g (54%) of the peptide, mp 261–264° dec, $[\alpha]^{22}D$ +17.65 (*c* 2, 2 *N* HCl). *Anal.* ($C_{14}H_{20}N_2O_3S$) C, H, N.

Carbobenzoxy-L-phenylalanyl-L-isoleucine (XII).—Saponification of 3.0 g (7.0 mmoles) of V yielded 1.5 g (52%), mp 141–143°, $[\alpha]^{22}D$ −1.31° (*c* 2, EtOH). *Anal.* ($C_{28}H_{28}N_2O_5$) C, H.

L-Phenylalanyl-L-isoleucine (XIII).—Hydrogenation of 1.05 g of XII in absolute EtOH in the presence of 150 mg of Pd black resulted in 0.45 g of the peptide (68%), mp 248–250° dec, $[\alpha]^{22}D$ +14.67° (*c* 2, 2 *N* HCl). *Anal.* ($C_{15}H_{22}N_2O_3$) C, H, N.

Biological Methods.—Growth studies were performed on *Escherichia coli* (ATCC 9723), *Leuconostoc mesenteroides* P-60 (ATCC *Streptococcus* spp. 8072), and *Lactobacillus arabinosus* 17-5 (ATCC strain 8014).

The usual growth medium for *E. coli* consisted of a salt–glucose mixture previously used in peptide studies.[4] The previously described medium[10] used in studies with *L. arabinosus* 17-5 was modified to contain L-isoleucine and L-phenylalanine at concentrations of 40 and 4 mμmoles/ml, respectively. The *L. mesenteroides* P-60 assay medium[11] was modified to contain L-isoleucine and L-phenylalanine at a concentration of 40 mμmoles/ml, except as noted in Tables I–III and Figure 1.

TABLE I

INHIBITION OF GROWTH OF VARIOUS MICROORGANISMS DUE TO L-CYCLOPENTANEGLYCINE AND β-2-THIENYL-L-ALANINE AND DIPEPTIDES OF THESE AMINO ACID ANALOGS

	ID_{50},[b] mμmoles/ml		
Inhibitor[a]	*E. coli*	*L. arabinosus*	*L. mesenteroides*
Cyc	2.0	1500	300
β-2-Thi	0.4	300	1500
Cyc-β-2-thi	0.5	30	12
β-2-Thi-cyc	0.3	6	4
Cyc + β-2-Thi	0.4	300	300
Gly-cyc			30
Gly-β-2-thi			40[c]

[a] Abbreviations: Cyc, L-cyclopentaneglycine; β-2-Thi, β-2-thienyl-L-alanine; Cyc-β-2-thi, L-cyclopentaneglycyl-β-2-thienyl-L-alanine; β-2-Thi-cyc, β-2-thienyl-L-alanyl-L-cyclopentaneglycine; Gly-cyc, glycyl-L-cyclopentaneglycine; Gly-β-2-thi, glycyl-β-2-thienyl-DL-alanine. [b] Amount of inhibitor required to produce half-maximal growth when added to the basal medium of the respective organism. [c] Added as DL form; calculated for L form.

TABLE II

COMPARISON OF GROWTH INHIBITION BY CYCLOPENTANEGLYCINE AND β-2-THIENYLALANINE AND THEIR DIPEPTIDES[a] ON *E. coli* 9723, INCUBATED FOR 16 HR AT 37°

Amt of inhib., mμmoles/5 ml	Galvanometer readings[d]					
	Inhibitor[b]			Inhibitor[c]		
	Cyc + β-2-Thi	Cyc-β-2-thi	β-2-Thi-cyc	Cyc + β-2-Thi	Cyc-β-2-thi	β-2-Thi-cyc
0	60	60	59	47	50	47
100		60	57		50	47
300	61	57	29	42	30	45
1000	57	47	5	34	0	26
2000		5	5		0	10
3000	48			36		

[a] For abbreviations, see Table I. [b] Added to the basal medium supplemented with 5000 mμmoles of L-cyclopentaneglycine, 5000 mμmoles of β-2-thienyl-L-alanine, and 300 mμmoles of isoleucyl-L-phenylalanine/5 ml. [c] Added to the basal medium supplemented with 5000 mμmoles of L-cyclopentaneglycine, 5000 mμmoles of β-2-thienyl-L-alanine, and 300 mμmoles of L-phenylalanyl-L-isoleucine/5 ml. [d] Distilled water reads 0, and an opaque object reads 100.

Bacterial growth was measured as turbidity in an instrument previously described.[12] All results were confirmed by at least three independent growth studies.

Results and Discussion

Two of the organisms used, *L. arabinosus* and *L. mesenteroides*, require an exogenous supply of amino acids, while the third, *E. coli*, does not. Table I shows that the isomeric dipeptides, L-cyclopentaneglycyl-β-2-thienyl-L-alanine and β-2-thienyl-L-alanyl-L-cyclopentaneglycine, were about equal to a mixture of the two free analogs or to free thienylalanine in producing 50% inhibition of *E. coli* growth. However, for 50% inhibi-

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TABLE III

UTILIZATION OF ISOLEUCINE, PHENYLALANINE, AND THEIR DIPEPTIDES FOR REVERSAL OF INHIBITION BY DIPEPTIDES OF CYCLOPENTANEGLYCINE AND β-2-THIENYLALANINE[a] ON *L. mesenteroides* P-60, INCUBATED FOR 16 HR AT 30°

mμmole of reversing agent/5 ml	Galvanometer readings[d]									
	Reversing agent[b]					Reversing agent[c]				
	Ileu	Phe	Ileu + Phe	Phe-ileu	Ileu-phe	Ileu	Phe	Ileu + Phe	Phe-ileu	Ileu-phe
0	9	11	9	9	10	7	5	8	9	5
10				22	24				23	10
30			10	49	56			9	50	39
100	12	10	12	66	66	9	7	14	64	64
300	27	9	52			32	7	45		
500	47	10	69			38	8	68		
1000	48	10				41	7			

[a] Abbreviations: Ileu, L-isoleucine; Phe, L-phenylalanine; Phe-ileu, L-phenylalanyl-L-isoleucine; Ileu-phe, L-isoleucyl-L-phenylalanine. [b] Added to the basal medium supplemented with 500 mμmoles of L-cyclopentaneglycyl-β-2-thienyl-L-alanine/5 ml. [c] Added to the basal medium supplemented with 500 mμmoles of β-2-thienyl-L-alanyl-L-cyclopentaneglycine/5 ml. [d] Distilled water reads 0, and an opaque object reads 100.

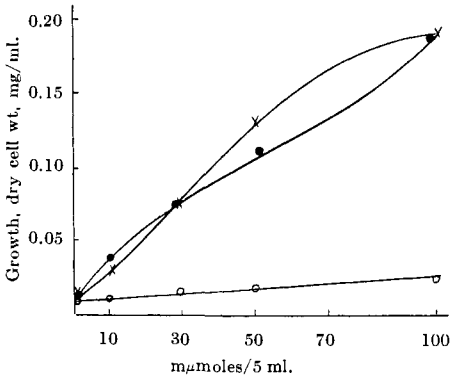

Figure 1.—Growth stimulation of *L. mesenteroides* P-60 by a mixture of L-phenylalanine and L-isoleucine (○), L-isoleucyl-L-phenylalanine (●), and a mixture of glycyl-L-isoleucine and glycyl-L-phenylalanine (X) in the basal medium supplemented with 2000 mμmoles of β-2-thienyl-L-alanine, 2000 mμmoles of L-cyclopentaneglycine, and 300 mμmoles of L-cyclopentaneglycyl-β-2-thienyl-L-alanine/5 ml. The tubes were incubated for 16 hr at 30°.

tion of *L. arabinosus* and *L. mesenteroides* growth the dipeptides were 10–70 times more potent than either of the constituent amino acid analogs or an equimolar mixture of both amino acid analogs. Table I also shows that the dipeptide β-2-thienyl-L-alanyl-L-cyclopentaneglycine was more toxic than its isomer in all three organisms. The glycyl peptides of β-2-thienyl-L-alanine and L-cyclopentaneglycine, tested in *L. mesenteroides*, were found to be about 10–40 times more toxic than either of the constituent amino acid analogs alone and nearly ten times more toxic than an equivalent amount of a mixture of both analogs.

When the basal medium for *E. coli* was supplemented with inhibitory levels of both L-cyclopentaneglycine and β-2-thienyl-L-alanine growth could be made dependent on the addition of both L-phenylalanine and L-isoleucine or their dipeptides. In the experiments described in Table II, these two natural amino acids were provided in the form of their dipeptides at such levels as needed to support growth. In such a system, the inhibitory effect of either L-cyclopentaneglycyl-β-2-thienyl-L-alanine or β-2-thienyl-L-alanyl-L-cyclopentaneglycine was much more pronounced than a mixture of the corresponding free amino acid analogs. Since in these experiments growth of the organism is dependent on isoleucylphenylalanine or phenylalanylisoleucine, it may be inferred that peptide utilization was blocked in a specific way by the peptide analogs at a site yet to be determined.

The complete reversal of the growth inhibition due to the peptides containing both amino acid analogs might be expected to require the presence in the medium of both of the corresponding natural amino acids, as is shown in Table III. *L. mesenteroides* growth inhibition due to either L-cyclopentaneglycyl-β-2-thienyl-L-alanine or β-2-thienyl-L-alanyl-L-cyclopentaneglycine was completely nullified only with the addition of both isoleucine and phenylalanine either in the form of a mixture or in the dipeptide form. It can be seen in Table III that either of the dipeptides containing the required natural amino acids was more effective in reversing the inhibition than was an equimolar mixture of the two natural amino acids. Isoleucine alone reversed the inhibitory effects of the dipeptide analogs better than phenylalanine alone, but only a combination of both amino acids allowed full growth.

It seemed of interest to determine whether or not the "double inhibition" by the dipeptide analogs containing both cyclopentaneglycine and β-2-thienylalanine and the pronounced growth stimulation by the dipeptides containing isoleucine and phenylalanine specifically required that the amino acids be in the same peptide. When growth of *L. mesenteroides* was inhibited by the dipeptide analog L-cyclopentaneglycyl-β-2-thienyl-L-alanine and a mixture of L-cyclopentaneglycine and β-2-thienyl-L-alanine, an equimolar mixture of L-phenylalanine and L-isoleucine showed little growth-promoting effect (Figure 1). The dipeptide L-isoleucyl-L-phenylalanine completely nullified the growth inhibition, as did an equimolar mixture of two dipeptides, glycyl-L-isoleucine and glycyl-L-phenylalanine. Growth was promoted equally well by supplying phenylalanine and isoleucine in a single dipeptide or in a mixture of two different dipeptides. However, the two free amino acids did not promote growth at similar levels of concentration. In promoting growth of *L. mesenteroides*, under the testing conditions, the required amino acids are more effective when presented in peptide form, but it is not obligatory that they be in the same peptide.

Resting cell studies demonstrated that all dipeptides herein reported were hydrolyzed by all three microorganisms. Therefore, the bacteria possess the capability for membrane transport and hydrolysis of these

peptides. The unusual growth-stimulating and growth-inhibiting effects observed for these peptides could be accounted for in terms of membrane transport and competition for transport by two peptides, *e.g.*, L-isoleucyl-L-phenylalanine and L-cyclopentaneglycyl-β-2-thienyl-L-alanine.

Kinetics and Mechanisms of Action of Drugs on Microorganisms. VIII. Quantification and Prediction of the Biological Activities of *meta*- and *para*-Substituted N_1-Phenylsulfanilamides by Microbial Kinetics

EDWARD R. GARRETT, JOBST B. MIELCK, JOACHIM K. SEYDEL, AND HANS J. KESSLER

College of Pharmacy, University of Florida, Gainesville, Florida 32601

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The apparent first-order generation rate constants, k_{app}, were determined in the steady-state growth of *Escherichia coli* in presence of graded concentrations, S, of a series of systematically substituted N_1-phenylsulfanilamides. From the expression $k_{app} = k_0 - k_0k_bS/(1 + k_bS)$, where k_0 is the determined generation rate constant in absence of drug, the bacteriostatic activity parameters, k_b in l. μmole^{-1}, were calculated. A good linear relationship was obtained between log k_b values and modified Hammett substituent parameters, $\rho = 1.12 \pm 0.11$, with the exception of the parent compound and the N_1-3,5-dinitrophenylsulfanilamide. A systematic dependency of k_b values on pH in the range of 6.0–7.4, where k_0 was constant, was not observed. The k_{app} values obtained in the presence of sulfonamide when extrapolated to zero drug concentration yielded calculated values for k_0 that showed dependence on pH. The activity parameters were independent of the determined chloroform-water distribution coefficients and applications of these data to the Hansch equation did not improve the correlation of k_b with substituent constants.

A recent review article[1] has reported on the many investigations that have been made to evaluate relationships between the structure and antibacterial activity of substituted sulfonamides (SA). A linear correlation was observed between the logarithm of the minimum inhibitory concentration (MIC) for *Escherichia coli* and various physicochemical properties within the closely chemically related series of the substituted N_1-phenylsulfanilamides. The pertinent physicochemical parameters were Hammett substituent constants (σ), dissociation constants of the free amino (pK_{a_2}') and the sulfonamido groups (pK_{a_1}'),[2] and measures of the electron density of the nitrogen atom in the substituted anilines that were the SA precursors, determined from ir[2,3] and nmr[4] measurements. A recent analysis[5] of the structure activity relationships of a series of SA has indicated that these σ, pK_{a_2}' values, and partition coefficients estimated from a series of compounds with the same substituents[6] are statistically correlated with MIC data. This indicated a statistically significant effect not only of σ but of the fraction of the drug in the undissociated form in the nutrient media and its oil/water partition coefficient on these MIC values.

The action of SA on drug-equilibrated microbial growth in an individual cell has been described by a model that involves the partitioning of SA between the medium and the cell interior to establish a concentration of SA inside the cell (S') which can react with receptor sites (R).[7,8] The apparent first-order generation rate

$$S \overset{K_1}{\rightleftharpoons} S' + R \overset{K_2}{\rightleftharpoons} RS' \qquad (1)$$

constant, k_{app}, in sec^{-1}, of a bacterial culture in steady-state growth and affected by SA concentration, S, adheres to the equation

$$k_{app} = k_0 - k_0k_bS/(1 + k_bS) \qquad (2)$$

where k_0 is the generation rate constant in absence of drug and k_b is defined as the product of the two equilibrium constants of eq 1. The parameter k_b may be calculated from positive k_{app} values observed at different concentrations of SA in the media and is independent of the SA concentration. The determination of MIC (μmole l.$^{-1}$), however, involves the observation of the absence of some degree of turbidity considered as a manifestation of an increase to a constant number of organisms after an arbitrarily fixed time interval. The activity parameters k_b, of two drugs can be related to their MIC values when the ideal condition of $k_{app} = 0$ is considered and where S_i might be equal to MIC_i. From simultaneous equations for each drug according to eq 2, the ratio of the activity parameters can be calculated as

$$k_{b_1}/k_{b_2} = MIC_2/MIC_1 \qquad (4)$$

$$k_b = K_1K_2 \text{ (l. } \mu\text{mole}^{-1}) \qquad (3)$$

Thus under the conditions specified the ratio of the k_b values is equal to the reciprocal of the ratio of their corresponding MIC values. However, the validity of the MIC value *per se* is limited by the sensitivity of the turbidimetric method and the normal error of the serial dilution techniques used. It is essentially a one-point method. In addition, at the dosage levels of SA which completely inhibit bacterial growth, as was shown for the specific case of sulfisoxazole-affected growth rates,[7] an additional phenomenon of kill or death of microorganisms occurs. Thus the MIC value might be based

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