containing NaI (~0.1 mole) as a hold-back carrier for I^{131}. The I^{131}-labeled amino acid was redissolved in methanol and precipitated again by adding water or acetone containing stable NaI. In this way I^{131}-labeled amino acids were obtained with constant specific activity.

Radiochemical Purity Determinations. F^{18}-Labeled Compounds. I. γ-Spectrum Scan Using a γ Spectrometer, 3 × 3 in. NaI(Tl) Crystal, R.C.L. 512 Multichannel Pulse-Height Analyzer.—The spectrum was taken with an energy spread of approximately 2.5 mev to ensure that there was no interference from high-energy activities such as Cl^{38} ($t_{1/2}$ = 37.3 min, 1.67 mev, 2.19 mev) and Na^{24} ($t_{1/2}$ = 15.0 hr, 1.37 mev). Only one peak appeared in the γ spectrum (at 0.51 mev) corresponding to the annihilation radiation of the F^{18} positrons.

II. Decay Curves.—Activity measurements of the F^{18}-labeled compounds for about 6 hr after irradiation at intervals of 1 hr yielded a straight-line decay curve on semilogarithmic paper, with a half-life value of 105–120 min. Twenty-four hours after the irradiation no activity was detected.

Br^{82}-Labeled Compounds. I.—γ-Spectrum scan (0.55 mev, 0.61 mev, 0.76 mev).

II.—Decay curves (half-life = 36 ± 0.5 hr).

Paper Chromatography of Br^{82}- and I^{131}-Labeled Amino Acids.—Radiobromo- and radioiodo-labeled amino acids were subjected to ascending paper chromatography[15] to ensure the absence of free Br^{82} or I^{131} ions. The strip papers were then scanned in a 4π radiochromatogram scanner, Picker X-ray Corp. The R_f values obtained corresponded to those of the pure amino acids.

Quantification and Prediction of the Biological Activities of Chloramphenicol Analogs by Microbial Kinetics[1]

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The rate constants, k, for the growth of *Escherichia coli* are linearly related to the concentrations of the chloramphenicol analog, [I], *i.e.*, $k = k_0 - k_I[I]$ where the inhibitory constant k_I gives a precise estimate of the substituent effect on biological activity by a total-count technique with the Coulter counter. The substituent effects for a series of ring-substituted chloramphenicol analogs of the D-*threo* configuration may be ranked p-NO_2 > p-SCH_3 > p-I > p-Br ~ m-NO_2 > p-OCH_3 > p-Cl > p-*i*-C_3H_7 > p-SO_2CH_3 > p-NH_2, where the p-SCH_3, p-SO_2CH_3, and m-NO_2 analogs are incongruent with the Hansch equation. The individual inhibitory constants, k_i, can be used to predict quantitatively the rate of *E. coli* growth in any admixture of analogs, *e.g.*, $k = k_0 - \Sigma_i k_i[I]_i$ and clearly demonstrate that, in this series, dose effects are additive with respect to rates.

The interest in correlating substituent constants and partition coefficients with biological activity as evidenced by the recent publications of Hansch and coworkers[2] has shown the vital need for the quantitative and precise evaluation of the biological activity of various substituted compounds. Only when such data are available can the models for such correlations be adequately tested and modified.

The complaint[2a] is legitimate that there is a severe limitation on the number of adequate examples in the literature where a particular compound has been modified with a wide variety of substituents and where these compounds have been tested under a standard set of conditions to yield quantitative results. In fact, an outstanding exception is considered[2a] to be a study of the action of chloramphenicol analogs on microorganisms by the serial-dilution method where the accuracy of the determinations was ±25%.[3] The need for quantification at a higher order of accuracy is readily apparent when parameters are to be correlated in the intermediate ranges of activities, *i.e.*, 25–100% of the most active analog.

It has been demonstrated that the total counts obtained with the use of the Coulter counter (Coulter Electronics, Hialeah, Fla.) of a growing *Escherichia coli* culture in the logarithmic growth phase in the presence of chloramphenicol and/or tetracycline are coincident with the viable counts by the colony-count technique[1c] and the mode of action is inhibitory. This provides a counting technique that could estimate growth rates rapidly and precisely (±2%) in the presence of various chloramphenicol analogs and permit the quantification of substituent effects on biological activity with confidence.

The apparent first-order generation rate constant can be determined from the slope of a plot of the logarithm of numbers, N, of *E. coli* inoculated in the constant growth phase since

$$N = N_0 e^{kt} \quad (1)$$

so that

$$\log N = \log N_0 + kt/2.303 \quad (2)$$

where t is in seconds. In the specific cases of chloramphenicol and tetracycline it has been shown[1] that the apparent first-order generation rate constant after the addition of antibiotic is linearly related to the antibiotic concentration

$$k = k_0 - k_I[I] \quad (3)$$

where k_0 is the rate constant in the absence of antibiotic and k_I is the specific inhibitory constant for the antibiotic I. These procedures permit the determination of various k_I values as more precise estimates of the effects of a compound's substituents on biological activity. Previous studies have shown that

(1) This is number IV in a series entitled Kinetics and Mechanisms of Action of Antibiotics on Microorganisms. The previous publications in this series were (a) E. R. Garrett and M. R. W. Brown, *J. Pharm. Pharmacol.*, **15**, 185T (1963); (b) M. R. W. Brown and E. R. Garrett, *J. Pharm. Sci.*, **53**, 179 (1964); (c) E. R. Garrett and G. H. Miller, *ibid.*, **54**, 427 (1965).

(2) (a) C. Hansch, R. M. Muir, T. Fujita, P. P. Maloney, F. Geiger, and M. Streich, *J. Am. Chem. Soc.*, **85**, 2817 (1963); (b) C. Hansch and T. Fujita, *ibid.*, **86**, 1616 (1964); (c) C. Hansch and A. R. Steward, *J. Med. Chem.*, **7**, 691 (1964); (d) T. Fujita, J. Iwasa, and C. Hansch, *J. Am. Chem. Soc.*, **86**, 5175 (1964); (e) J. Iwasa, T. Fujita, and C. Hansch, *J. Med. Chem.*, **8**, 150 (1965).

(3) M. N. Shemyakin, M. N. Kolosov, M. M. Levitov, K. I. Germanova, M. G. Karapetyan, Yu. B. Shvetsov, and E. M. Bamdas, *J. Gen. Chem. USSR*, **26**, 885 (1956).

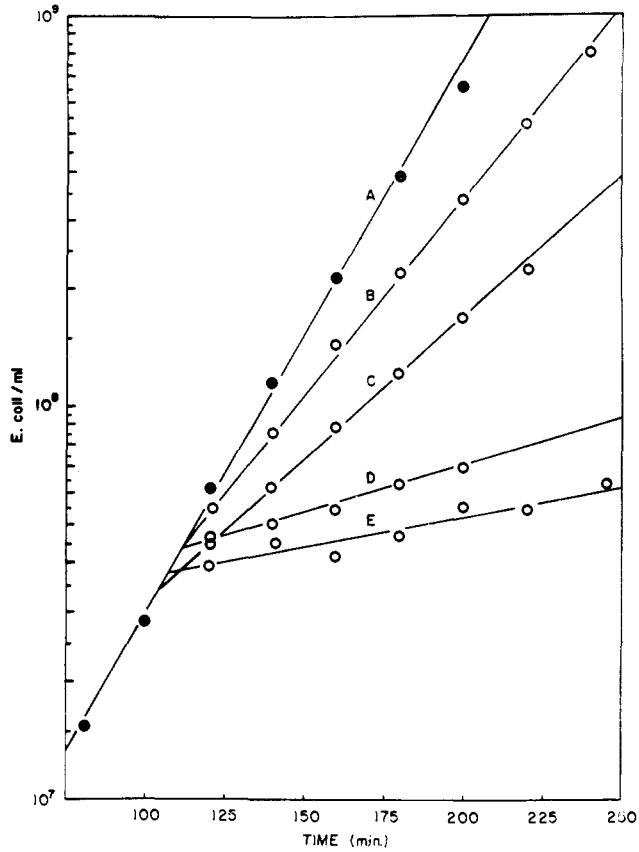

Figure 1.—Typical set of generation curves of *E. coli* in the presence of graded concentrations of a chloramphenicol analog. The curves for the various molar concentrations of the *p*-methoxy analog and their apparent rate constants are: curve, 10^6[I], 10^5k; A, 0.0, 55.6; B, 16.6, 37.9; C, 33.2, 27.5; D, 49.8, 9.2; E, 66.6, 5.9. The initial inoculum was 1.1×10^{-6} organisms/ml.

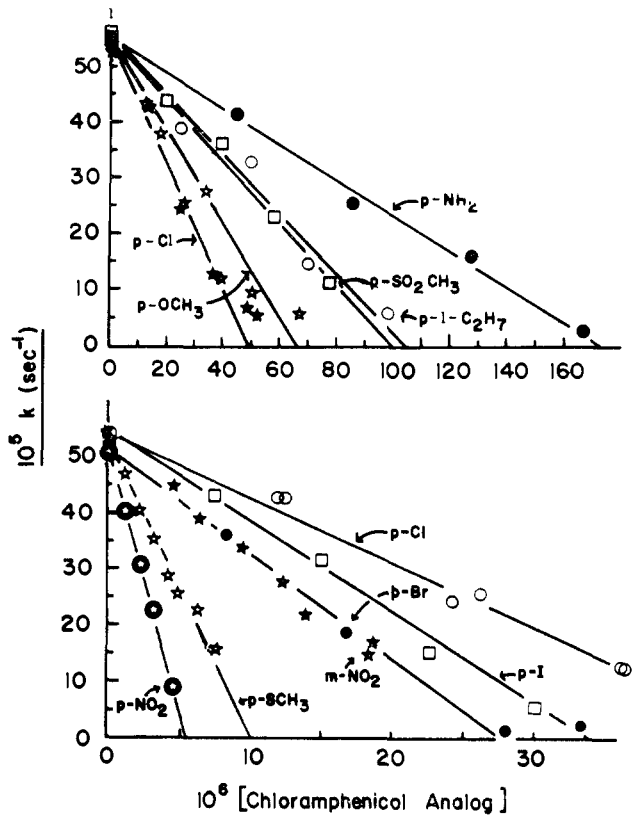

Figure 2.—Plots of apparent generation rate constants for *E. coli* as a function of the concentration of various chloramphenicol analogs.

doubling and halving the nutrients, decreasing the metal content, and reducing and increasing the phosphate concentrations had no significant effects on *E. coli* growth under the studied conditions.[1c]

Another item of importance that may be obtained from such a kinetic analysis of structure–activity relations is the quantitative prediction of the biological activity of complex mixtures in accordance with the expression

$$k = k_0 - \Sigma k_i[\mathrm{I}]_i \qquad (4)$$

where the k_i are the respective inhibitory rate constants for the ith antibiotic, I_i. The demonstration of the validity of predictions from eq 4 for various mixtures of differently potent antibiotics should serve as verification as to the reliability of k_I values as estimates of intrinsic biological activity. It has been shown that if rate effects are additive, it is highly improbable that estimates of potency to achieve stated values of biological response such as the minimum inhibitory concentration are, in themselves, additive when administered in combination.[4]

Experimental Section

The preparation of the chloramphenicol analogs used in these studies, all of *threo* geometry, has been previously reported.[3,5] The chloramphenicol analogs cited in this paper were obtained through the good offices of Dr. Harry M. Crooks, Jr., and Parke, Davis and Company Research Laboratories, Ann Arbor, Mich.

Methods.—Replicate slants were prepared from *E. coli* strain B/r that was isolated from a culture on an agar plate. These slants were then stored frozen, and a new one was used for each experiment. A broth culture was allowed to grow for 16 hr in a peptone broth, U.S.P, available as Bacto Antibiotic Medium 3, Difco Laboratories, Detroit, Mich. The sample was diluted tenfold in fresh broth and the generation was followed turbidimetrically with a Klett–Summerson colorimeter until a reading was reached that corresponded to a concentration of 10^8 organisms/ml in the logarithmic growth phase. The samples were diluted into five replicate 20-ml volumes of fresh broth in 125-ml loosely capped erlenmeyer flasks to achieve a concentration of 10^6 organisms/ml. The replicate cultures were maintained at 37.5° in a 50-gal constant-temperature water bath equipped with a shaker.

Aliquots of each culture (0.5 ml) were transferred at 20-min intervals to sterilized vials containing 1 drop of formaldehyde. The samples were appropriately diluted with properly filtered (through a double thickness of filter paper, type HA millipore) and sterilized 0.9% saline solution so that the total organism count in 50 μl would not exceed 30,000. The coincidence error was thus less than 0.5% on the Coulter counter, Model B equipped with a 30-μ orifice. The mean of five total counts was obtained on each sample. Further details of these methods have already been published.[1]

Effects of Antibiotic Concentration on Growth Rates.—Various concentrations of a specific antibiotic were added to four of the five replicate cultures at 90 min after inoculation to obtain relatively equally spaced, apparent growth rates between zero and the maximum that was obtained without added antibiotic. A typical semilogarithmic plot of such data is given in Figure 1 for various concentrations of the DL-*p*-methoxy analog of chloramphenicol. The linearity of such plots after the antibiotic was added conformed to eq 2. The rate constants, k, for the generation

(4) E. R. Garrett, *Antibot. Chemotherapy*, **8**, 8 (1958).

(5) M. L. Long and H. D. Troutman, *J. Am. Chem. Soc.*, **71**, 2473 (1949); L. L. Bambas, H. D. Troutman, and L. M. Long, *ibid.*, **72**, 4445 (1950); M. C. Rebstock and E. L. Pfeiffer, *ibid.*, **74**, 3207 (1952); R. A. Cutler, R. J. Stenger, and C. M. Suter, *ibid.*, **74**, 5475 (1952); M. C. Rebstock, C. D. Stratton, and L. L. Bambas, *ibid.*, **77**, 24 (1955); M. C. Rebstock and C. D. Stratton, *ibid.*, **77**, 3082, 4054 (1955).

TABLE I

APPARENT FIRST-ORDER GENERATION CONSTANTS, k (IN SEC^{-1}), FOR THE GROWTH OF *E. coli* IN THE PRESENCE OF CHLORAMPHENICOL ANALOGS AT 37.5°

10^5k	10^5[p-NH_2]	10^5k	10^6[p-i-C_3H_7]	10^5k	10^6[p-SO_2CH_3]	10^5k	10^6[p-OCH_3]		
54.9	0.0	56.0	0.0	55.0	0.0	55.6	0.0		
41.2	4.4	38.8	24.5	43.9	19.2	37.9	16.6		
25.4	8.6	32.8	49.0	36.0	38.3	27.5	33.2		
16.0	12.7	14.2	70.2	22.6	57.5	9.2	49.8		
2.4	16.7	5.8	98.0	10.9	76.6	5.9	66.6		
10^5k	10^6[p-Cl]	10^5k	10^6[p-Br]	10^5k	10^6[m-NO_2]	10^5k	10^7[p-SCH_7]	10^5k	10^6[p-I]
53.7	0.0	51.4	0.0	44.9	4.6	47.1	10.5	53.5	0.0
43.0	12.1	36.0	8.3	38.9	6.3	40.3	21.0	43.0	7.5
42.3	13.1	18.9	16.7	34.2	9.2	35.2	31.5	31.4	15.0
25.5	26.1	1.2	28.0	27.6	12.3	28.5	41.5	15.0	22.5
24.1	24.2	2.2	33.3	21.9	13.8	25.6	46.3	5.1	30.0
12.9	39.0			17.0	18.6	22.3	61.8		
12.6	36.4			16.0	18.3	15.3	77.2		
6.5	48.5								
5.1	52.1								

rates of *E. coli* in the presence of various conentrations of chloramphenicol analogs are given in Table I. The linear dependence of the k_f on antibiotic concentrations [I] is shown in Figure 2.

Effects of Combinations of Antibiotics.—A series of solutions A, B, C, and D of various chloramphenicol analogs of widely varying potencies were prepared so that each component in a mixture was in such concentration that it was approximately equipotent with all others in the given mixture. The composition of these mixtures after addition to the *E. coli* system in the logarithmic growth phase is given in Table II. The calculated relative potency of each chloramphenicol analog concentration in the mixture with respect to the concentration of the most potent analog in the solution is also given. Each mixture was added to the growing *E. coli* cultures and counts were obtained in the manner described previously. Several dilutions were made prior to the addition so that the concentrations of the antibiotics in each culture would be a fraction, f, of each antibiotic concentration in those cultures where the concentrations of antibiotics were as given. Typical, semilogarithmic plots for the kinetic studies on various concentrations of a mixture (*viz.*, C) are given in Figure 3. The linearity of such plots after the mixtures of antibiotics were added conformed to eq. 2. The rate constants, k, for the generation rates of *E. coli* in the presence of various concentrations of the chloramphenicol analog mixtures are also given in Table II.

Results and Discussion

The linearity of plots of the logarithms of numbers of *E. coli vs.* time for various concentrations of chloramphenicol analogs, demonstrated by the typical plots of Figure 1 for the DL-*p*-methoxy analog of chloramphenicol, conforms to the expectation of eq 2. The generation rate constants, k, in the presence of the various concentrations of the chloramphenicol analogs are given in Table I. The plots of these rate constants *vs.* the concentrations of the various chloramphenicol analogs are given in Figure 2 and are linear in accordance with the expectations of eq 3. The slopes of these plots represent the inhibitory constants, k_I, and are listed in Table III with the stereochemistry of the analogs used. Table III also includes the σ_m and σ_p Hammett substituent constants for *m*- and *p*-phenyl substituents, respectively, as obtained from the compilation of Jaffe.[6] The π values listed which represent the logarithm of the ratio of the partition coefficient of a correspondingly substituted phenoxyacetic acid in octanol–water to that of the partition coefficient of the parent unsubstituted compound were derived

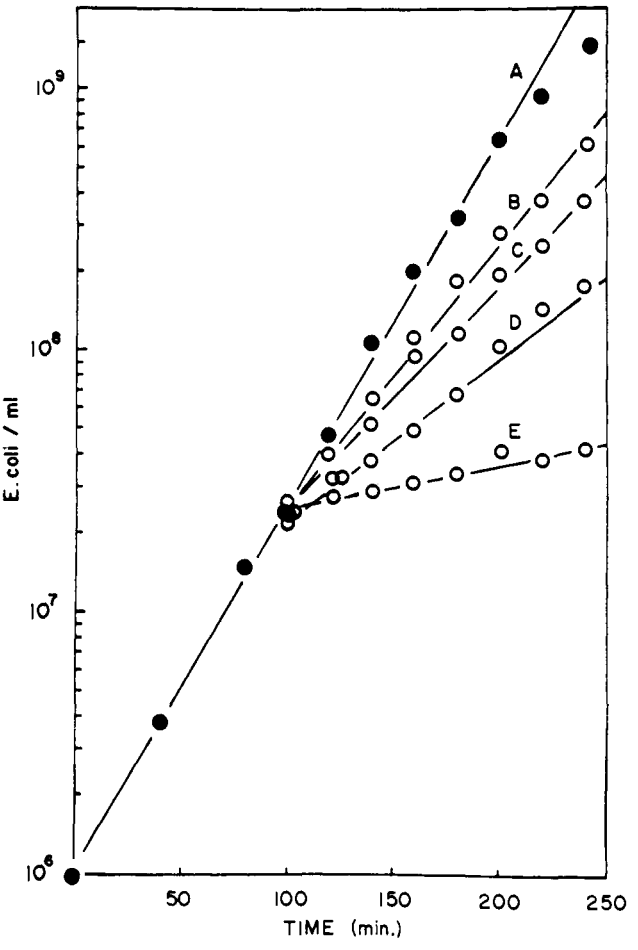

Figure 3.—Typical set of generation curves for *E. coli* in the presence of graded concentrations of a mixture of chloramphenicol analogs. The concentrations for the various curves are $f[I]_0$ where the curve, f, and 10^5k are: A, 0.0, 54.3; B, 0.34, 36.8; C, 0.45, 32.4; D, 0.68, 23.5; E, 1.00, 6.4. The $10^6[I]_0$ of the chloramphenicol analogs in the mixture C were: *p*-NH_2, 54.2; *p*-Br, 10.1; *p*-OCH_3, 24.9.

from the studies of Hansch, *et al.*[2] The logarithm of the per cent relative activity values, *i.e.*, log A, listed represent the logarithm of the per cent of the inhibitory constant for chloramphenicol, $k_{p\text{-}NO_2}$, that may be obtained by a chloramphenicol analog, k_I, so that

$$\log A = \log 10^2 k_I/k_{p\text{-}NO_2} \quad (5)$$

(6) H. H. Jaffe, *Chem. Rev.*, **53**, 191 (1953).

TABLE II

EXPERIMENTAL[a] AND CALCULATED[b] RATE CONSTANTS, k (IN SEC^{-1}), FOR GROWTH OF *E. coli* IN VARIOUS MIXTURES OF CHLORAMPHENICOL ANALOGS

	A		B		C		D	
Analog	10^6[Analog]	Rel[c] pot.	10^6[Analog]	Rel[c] pot.	10^6[Analog]	Rel[c] pot.	10^6[Analog]	Rel[c] pot.
p-NO_2	3.02	1.00	2.01	1.00			1.51	1.00
p-NH_2	80.1	0.93	54.2	0.95	54.2	0.89	40.0	0.94
p-Cl			12.6	0.78				
p-Br					10.1	1.00	7.68	1.07
p-OCH_3					24.9	1.06	20.0	1.21
f[d]	10^6k^b	10^6k^a	10^6k^b	10^6k^a	10^6k^b	10^6k^a	10^6k^b	10^6k^a
0.22	42.6	44.1	43.4	44.1				
0.34	36.8	38.4	37.8	38.3	35.3	36.8	35.2	37.6
0.45					32.8	32.4	26.8	30.9
0.68	18.9	20.7	21.4	21.6	16.2	23.5	16.0	23.6
1.00	1.9	6.9	5.2	7.4	−0.6	6.4	−3.0	4.2

[a] Determined from slopes of ln *E. coli* counts *vs.* time in seconds after addition of antibiotic mixtures at 37.5°. [b] Calculated from $k = k_0 - \Sigma_i k_i$[chloramphenicol analog] where k_0 is the growth rate constant without antibiotic and was 54.3×10^{-5} sec^{-1}, where the k_i had been determined as a function of the concentration of each chloramphenicol analog. [c] The relative potency of each concentration of chloramphenicol analog is calculated in the mixture with respect to the most potent in the mixture, *viz.*, k(most potent)/k(analog) × [analog]$_0$/[most potent] = relative potency. Except for mixture C, the most potent is chloramphenicol itself. The undiluted concentrations of the mixture are equipotent with the following amounts of 10^6[chloramphenicol]: A, 5.82; B, 5.49; C, 6.30; D, 6.35. [d] Since the antibiotic mixtures were diluted and added to the growing *E. coli* cultures, the actual concentrations for each of the antibiotics in a given mixture are this fraction, f, times the concentration given in the table.

TABLE III

ACTIVITIES OF CHLORAMPHENICOL ANALOGS

						Log A[d]		
							Calcd	
Substituent	Isomer	k_I[a]	σ_m[b]	σ_p[b]	π[c]	Exptl	(a)	(b)
p-NH_2	D	3.16	−0.17	−0.66		0.55		
p-*i*-C_3H_7	DL	5.36		−0.15	1.4[e]	0.78	0.57	
	D	10.8[f]				1.08		1.27
p-SO_2CH_3	D	5.60	0.65	0.73	−1.26	0.80	3.15	1.77
					−0.47[g]	0.80	2.21[g]	1.60[g]
p-OCH_3	DL	8.22	0.12	−0.27	−0.04	0.96	0.98	
	D	16.4[f]				1.26		1.22
p-Cl	D	11.2	0.37	0.23	0.70	1.10	1.24	1.21
p-I	DL	16.3	0.35	0.28	1.26	1.26	1.37	
	D	32.6[f]				1.56		1.40
p-Br	D	19.0	0.39	0.23	1.02	1.32	1.34	1.31
m-NO_2	D	19.0	0.71	0.78	0.10	1.32		
p-SCH_3	D	51.5	0.14	−0.05	0.62[h]	1.76	0.84	1.18
p-NO_2	D	90.0	0.71	0.78	0.06	2.00	1.96	2.06

[a] As determined from the slope of the plot of the observed generation rate constants for *E. coli*, k (in sec^{-1}), against the chloramphenicol analog concentration, [I], *i.e.*, $k = k_0 - k_I$[I], where k_0 in sec^{-1} is the rate constant in the absence of antibiotic and k_I is in liters/mole/second.[1b] [b] As taken from the compilation of Jaffe.[6] [c] As taken from the compilation of Hansch, *et al.*,[2a] for the logarithm of the ratio of the partition between octanol and water for the substituted phenoxyacetic acid (similarly substituted as the chloramphenicol analog) to the partition of the unsubstituted phenoxyacetic acid. [d] The calculated values are from an attempt to fit the data to the Hansch expression[2a] $a\pi^2 + b\pi + c\sigma_m + d = \log 10^2 k_I/k_{p\text{-}NO_2} = \log A$. The values of calculations (a) are obtained from this expression for $a = 0.4$, $b = -0.5$, $c = 1.75$, and $d = 0.75$ for experimental values of log A. The values of calculation (b) are obtained from this expression for $a = 0.7$, $b = -0.1$, $c = 1.25$, and $d = 1.11$ for the D-*threo* configurations on the premise that the L configuration of the racemates has no activity. [e] Assumed to be similar to the 3-*n*-propyl-substituted compound.[2a] [f] Since the biologically active configuration is the D-*threo*,[7] the k_I for the D configuration in these racemates would be twice the experimentally determined values that are given above. [g] Hansch, *et al.*, prefer −0.47 for best fit of their data.[2a] [h] This value is for the 3-substituted phenoxyacetic acid, but it has been argued that the π values should be similar for the 4-substituted compound.[2a]

In the cases where only the DL racemate was used, two experimental log A values are reported, the one observed for the DL racemate and another calculated for the D-*threo* isomer on the basis that the k_I value is twice that experimentally observed, since it has been well established that only the D-*threo* configuration of the chloramphenicols possesses biological activity.[3,7]

The recent work of Hansch and associates[2] has resulted in the development of an equation in the form of (6) for the correlation of chemical constitution with biological activity

$$\log A = a\pi^2 + b\pi + c\sigma + d \qquad (6)$$

where the lettered parameters have been obtained by regression analysis. In an application to correlate phenyl-substituent effects on chloramphenicol activity,[2a] the π values were derived from phenoxyacetic acid partition studies. The log A values related to minimum inhibitory concentrations determined from serial dilution studies of the action of chloramphenicol analogs on various microorganisms were taken[2a] from the work of Shemyakin, *et al.*[3] The data

(7) R. E. Maxwell and V. S. Nickel, *Antibiot. Chemotherapy*, **4**, 289 (1954); J. Controulis, M. C. Rebstock, and H. M. Crooks, Jr., *J. Am. Chem. Soc.*, **71**, 2463 (1949).

of Table III on the activities of chloramphenicol analogs provides a much higher degree of quantification and precision to test the advantages and limitations of such an equation (6).

An algebraic technique to determine the parameters of eq 6 for the data on the *para*-substituted analogs (Table III) was used.

In the specific cases of the *p*-NO_2 and *p*-OCH_3 chloramphenicol analogs, $\pi \sim 0$ and eq 6 reduces to

$$c\sigma + d = \log A \qquad (7)$$

whence the parameters c and d can be calculated from simultaneous equations. The *meta* substituent constant σ_m is used in accordance with Hansch, *et al.*[2a] A series of equations may then be calculated in the two unknown parameters a and b for each *para* substituent.

$$a = (\log A - c\sigma_m + d)/\pi^2 - b(1/\pi) \qquad (8)$$

and a plotted against b with respective slopes, $-(1/\pi)$, and intercepts, $(\log A - c\sigma_m + d)/\pi^2$. If these are consistent equations they will all intersect at one point for specific values of a and b. Valid approximations of these specific values of a and b within the range of the experimental errors of π and log A values (if eq 6 is an absolute representation of the functional dependencies) may be obtained from the centrum of the area of intersection of the plotted straight lines of the various versions of eq 8.

Iterative procedures to determine better values of c and d are also possible when the best estimates of a and b are substituted into eq 6. The calculated log A values (column a, Table III) were derived from $a = 0.4$, $b = -0.5$, $c = 1.75$, and $d = 0.75$ which were determined from the above procedures where the experimental values of k_I were used. The column b values were derived from $a = 0.7$, $b = -0.1$, $c = 1.25$, and $d = 1.11$ where the k_I values, actual and derived, for only the D-*threo* activities were used.

The inability to correlate the π, σ_m, and log A values for the *p*-SO_2CH_3 and *p*-SCH_3 chloramphenicol analogs with the obtained parameters are evident from the several comparisons of experimental and calculated log A values and also from the wide displacement of the plot of eq 8 for these analogs from the nexus of intersections for the other *para*-substituted chloramphenicols. In fact, the possibility of a technological mistake in that these two compounds were mislabeled was considered. However, the infrared spectra of the contents of the labeled materials which were ascertained as having been properly used showed conclusively that this was not the case. The characteristic sulfone bands 1205, 1140, and 1115 cm^{-1} were present in the labeled *p*-SO_2CH_3 analog and absent in the labeled *p*-SCH_3 analog.

When the data were fitted by more elegant statistical and digital computer techniques[2] similar results were obtained. Examples of such fits with detailed comments in the footnotes are given in Table IV.

The data of Table III and that of the literature[2,3] demonstrate that linear relations such as eq 6 do exist among some substituent and partition parameters for approximate correlation with biological activities. There yet remains the facts that log A values calculated from the different premises and parameters of calculation a or calculation b (Table III) (*i.e.*, activities are equal to or twice that of the racemates) give similar approximations for the more precise experimental log A values (Table III). Also, some substituents, *e.g.*, *p*-SO_2CH_3 and *p*-SCH_3, cannot be related to log A by the same set of parameters. Within a simple homologous series, *e.g.*, *p*-NO_2, *p*-OCH_3, *p*-Cl, *p*-Br, and *p*-I, there is no one set of parameters that can give precise estimates of log A within the range of good biological data. The facts that the π values for both the *m*-nitro and *p*-nitro analogs are the same and that σ_m is almost the same value as σ_p might lead to the expectation of equipotency for these two analogs. The more than fourfold difference in such potencies clearly makes it impossible to include *meta* and *para* substituents under the umbrella of the same equation (6). If it is assumed that the σ_m constants are the effective substituent parameters in the *para*-substituted analogs to activate the *meta*, *i.e.*, the 2 position,[2a] then a *meta* substituent might use the σ_p constant to activate the same 2 position. An argument may be presented on statistical grounds that a *m*-NO_2 has only one position to activate *ortho* to the other phenyl substituent, whereas a *p*-NO_2 has two such positions.

TABLE IV

PARAMETERS OF BEST COMPUTER FIT TO THE HANSCH[a] EQUATION[2]: LOG $A = a\pi^2 + b\pi + c\sigma + d$ FOR THE CORRELATION OF RELATIVE ACTIVITY VALUES OF SUBSTITUTED CHLORAMPHENICOLS (A), LOGARITHMS OF RELATIVE PARTITION COEFFICIENTS (π), AND HAMMETT SUBSTITUENT CONSTANTS (σ)

Item	Compd excluded	No. of compds	Eq unknowns: a	b	c	d	σ^b	R^c
1[d]	None	10	−0.29	0.35	0.13	1.31	0.35	0.74
2[e]	*p*-SCH_3	9	−0.13	0.28	0.46	1.07	0.34	0.76
			0.00	0.23	0.68	0.91	0.32	0.75
3[f]	*p*-SCH_3, *p*-NH_2	8	−0.45	0.69	0.57	1.00	0.34	0.69
	p-SCH_3, *p*-NH_2, *p*-CH_3SO_2	7	0.65	−0.74	1.08	0.99	0.29	0.75
			0.00	0.055	0.78	1.05	0.28	0.69
4[g]	*p*-SCH_3, *p*-NH_2, *p*-CH_3SO_2, *m*-NO_2	6	1.28	−1.63	1.71	0.98	0.005	1.00
			0.00	−0.02	0.99	1.08	0.25	0.83
			0.00	−0.20	0.00	1.54	0.37	0.33
			0.00	0.00	1.00	1.06	0.22	0.83

[a] These parameters of best fit for the specified data were obtained on the digital computer by Dr. Corwin Hansch of Pomona College, Claremont, Calif., to whom we are sincerely indebted for the basis of the commentary included in the footnotes of this table. The log A values for the D-*threo* forms were used. The σ_m value used for *p*-*i*-C_3H_7 was −0.07 and −0.17 for the *p*-NH_2. The other values are as given in Table III. [b] Standard deviation of predicted log A values for the fit cited. [c] Correlation coefficient for the fit cited. [d] This is the best fit for all ten compounds of Table III. A high discrepancy is contributed by the *p*-SCH_3 compounds. Perhaps this compound is readily oxidizable in biological systems and its anomalous activity is due to chemically derived species. [e] It is observed that whether the π^2 function is included or excluded little effect is shown on the efficiency of the data fitting. This is in contradistinction to the fitting[2a] of minimum inhibitory concentration data.[3] [f] No improvement in fit is observed on omission of the *p*-NH_2 and *p*-SCH_3 compounds or on the exclusion of *p*-NH_2, *p*-SCH_3, and *p*-CH_3SO_2, all of which may be considered anomalous in their effects.[3] It is again observed that the omission of the π^2 function has little effect on the efficiency of the data fitting. [g] When four constants are fitted to six points, it is almost inevitable that an excellent correlation will result. The positive sign for this fit indicates that large values for π would give great activity, which is, of course, impossible. The subsequent fits in this set where some of the parameters are omitted for these most consistent compounds show that the π function plays an exceedingly small role in contradistinction to the fit[2a] of other minimum inhibitory concentration data.[3]

In general, it can be concluded that although linear free energy and partition relations may serve as good first approximations for the prediction of substituent effects on biological activities, other factors must be considered in the complexities of drug distributions and receptor sites even in a highly reproducible and simple biological system such as this *E. coli*. It is hoped that more precise measurements of such activities as obtained from the methods given here in detail may help dissociate and quantify these several effects, among which may be included molecular size and binding constants peculiar to substituents.

The premise that the action of combined chloramphenicol analog concentration is additive on the rates of *E. coli* growth is well substantiated for a wide range of mixtures of such antibiotics. The predictive eq 4, where the k_i obtained from studies on the individual antibiotics, predicts k values in the inhibitory range of bacterial growth which have been experimentally verified by the data of Table II.

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Antibacterial Activity of Compounds with Both Mercury and Tin in Organic Combination[1]

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Several organic compounds containing both mercury and tin were tested for activity against three common bacterial species. The activities of these compounds and ethylmercuric chloride, laurylpyridinium chloride, and some of the intermediates used in the synthesis of the organic mercury–tin compounds were compared. Solutions of the organic mercury–tin compounds in a small amount of solvent, especially γ-butyrolactone, followed by dilution with water, were as much as 200 times as active as a solution prepared solely with water. On a mercury content basis, the mercury–tin compounds were 10–20 times as active as ethylmercuric chloride. Thioglycollate reduced the activity of these compounds.

The activity of organic mercury compounds[2] and organic tin compounds[3] against microflora is well known, but no information is available on the microbiological activity of organic compounds containing both mercury and tin.[4] This paper reports some determinations of the minimum inhibitory concentration (MIC) of several mercury–tin compounds against three species of bacteria as test organisms. Comparison is made with the MIC of a mercurial, an organic tin compound, and a quaternary ammonium compound.

Methods

The test organisms used were field strains isolated from viscera of diseased poultry: *Escherichia coli* and *Staphylococcus aureus* from chickens, *Pseudomonas aeruginosa* from a turkey. The organisms were identified at this Station and were maintained according to usual microbiological procedures. The assay medium was Difco phenol red broth with 0.75% added glucose. The well-known action of thioglycollate on mercurials was determined with ethylmercuric chloride (EMC) and compounds I and IIb (Table I), using assay broth with 0.5 g of sodium thioglycollate added/l. that had been prepared within 20 hr of use.

The mercury–tin compounds, synthesized by the method of Miller and Chan,[4] are listed in Table I. For MIC assay these compounds were dissolved in β-propiolactone and the solvents listed in Table II by shaking at least 10 hr. The solutions were filtered, analyzed for mercury,[5] and the parts per million of original compound in solution was calculated. The butyrolactone (BUL) and valerolactone (VAL) solutions used contained 1000–9000 ppm of the mercury–tin compounds except compound IV solution, which contained 15,000 ppm. The ethanol solutions contained 1500–3000 ppm and the dimethyl sulfoxide (DMSO) solutions, 4000–9000 ppm. Reagents used to prepare compound I, mercuribis(*p*-dimethylaniline) (MDMA), iodoacetic acid (IAA), and bis(tri-*n*-butyl)tin oxide (TBTO), were dissolved in BUL, water, or ethanol. Solutions of a well-known highly active mercurial, EMC, and a commercial quaternary ammonium compound, laurylpyridinium chloride (LPC), were also made. The

TABLE I

LIST OF COMPOUNDS TESTED

No.	Tin-containing moiety	Mercury-containing moiety
I	Tri-*n*-butyltin salt of	Carboxymethyl[*p*-(*p*-dimethylaminophenylmercuri)phenyl]-dimethylammonium iodide
Ia	Tri-*n*-propyltin salt of	Carboxymethyl[*p*-(*p*-dimethylaminophenylmercuri)phenyl]-dimethylammonium iodide
II	Tri-*n*-butyltin salt of	1-Carboxymethyl-3-(3-pyridylmercuri)pyridinium iodide
IIa	Tri-*n*-butyltin salt of	1-(2-Carboxyethyl)-3-(3-pyridylmercuri)pyridinium iodide
IIb	Tri-*n*-propyltin salt of	1-Carboxymethyl-3-(3-pyridylmercuri)pyridinium iodide
III	Tri-*n*-butyltin salt of	1-Carboxymethyl-3-(phenylmercuri)pyridinium iodide
IV	Bis(trimethyltin)salt of	Mercuridi-*p*-phenylenebis[(carboxymethyl)dimethylammonium iodide]

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