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.

Acknowledgment.—The authors are greatly indebted to Mr. George L. Perry, Sr., for excellent technical assistance. The kindness of Dr. Corwin Hansch in fitting the data by digital-computer techniques and permitting these fits and his commentaries to be included herein is highly appreciated.

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

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Received May 27, 1965

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]

(1) (a) Portions of this work were reported at the Northwest Regional Meeting of the American Chemical Society, Corvallis, Ore., June 1965. Scientific Paper No. 2589, Washington Agricultural Experiment Station, Pullman, Wash., Project 1525. (b) Supported in part by Medical and Biological Research Funds, Initiative 171, State of Washington.

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TABLE II

THE EFFECT OF SOLVENTS ON APPROXIMATE MINIMUM INHIBITORY CONCENTRATION (MIC)[a] WITH SEVERAL COMPOUNDS AND THREE TEST ORGANISMS EXPOSED FOR 15 MIN

Organism	Compd[b]	Solvent[c] Water	DMSO	Ethanol	γ-Valero-lactone	γ-Butyro-lactone
Ps. aeruginosa	Phenol	9,000	...	...	...	...
	I	>1,900	250	150	75	100
	Ia	1,000	...	...	...	50
	II	>5,000	...	...	...	40
	IIa	>467	...	...	...	75
	IIb	2,000	200	150	25	40
	III	>3,200	>1000	300	...	100
	IV	>5,000	...	...	...	150
	EMC	300	200	...	...	300
	TBTO	...	...	>6000	...	700
	LPC	...	...	50	...	50
	MDMA	...	...	...	...	50
	IAA	1,000	...	...	...	...
	Ic[d]	...	...	...	...	50
S. aureus	Phenol	13,000	...	...	...	...
	I	1,200	600	150	100	100
	Ia	2,000	...	...	...	120
	II	1,200	...	...	...	25
	IIa	500	...	...	...	100
	IIb	3,000	800	>225	100	100
	III	600	250	175	...	50
	IV	>4,500	...	...	...	400
	EMC	300	200	...	...	200
	TBTO	...	...	200	...	100
	LPC	...	...	500	...	400
	MDMA	...	...	...	...	>2000
	IAA	5,000	...	...	...	...
	Ic[d]	...	...	...	...	150
E. coli	Phenol	9,000	...	...	...	...
	I	>2,000	300	100	70	100
	Ia	1,450	...	...	...	100
	II	>5,000	...	...	...	25
	IIa	>500	...	...	...	70
	IIb	>3,000	200	50	40	35
	III	>3,300	>900	300	...	125
	IV	>3,000	...	...	...	300
	EMC	200	300	...	...	200
	TBTO	...	...	>9000	...	500
	LPC	...	...	250	...	250
	MDMA	...	...	...	...	500
	IAA	3,000	...	...	...	...
	Ic[d]	...	...	...	...	100

[a] Values are parts per million of compound. [b] The numbered compounds are listed in Table I. See text for abbreviations. [c] Indicates MIC was not determined. [d] Equivalent amounts of the reagents used in synthesis of I were dissolved in BUL and diluted.

assay tubes were prepared by dilution of these solutions with sterile water.

An assay procedure for phenol coefficient was adapted for determination of MIC.[6] The MIC for each test organism was determined for each material in one or more solvents; and the parts per million of solvent not interfering with growth of the test organisms was determined. The exposure periods were 5, 10, and 15 min. Three levels of phenol were included in each assay as a positive control. The assay tubes were scored either growth or no growth after 48 hr of incubation at 37°, and 3 days standing at room temperature. The 15-min exposure data is reported for each test organism in Table II. Data for 5- and 10-min exposures are not reported but were used to verify the more meaningful 15-min exposure data. The mode of action of these compounds, such as the study made by Stedman, *et al.*,[7] or as discussed by Albert,[8] was beyond the scope of this investigation.

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Results and Discussion

The concentration of solvent in the assay tubes was well below the highest level that permitted apparently normal growth of the test organisms. *S. aureus* grew after a 15-min exposure to 200,000 ppm of all solvents used. *E. coli* grew after a 15-min exposure to 200,000 ppm of BUL, DMSO, or ethanol, or 100,000 ppm of VAL. *Ps. aeruginosa* grew after a 15-min exposure to 200,000 ppm of ethanol or DMSO, 150,000 ppm of BUL, or 50,000 ppm of VAL. β-Propiolactone polymerized after shaking with compound I; it was not further investigated as a solvent.

The results of the MIC assays are presented in Table II. The aqueous solutions of most of the mercury–tin compounds, usually near-saturated, were relatively inactive against the test organism, particularly *E. coli* and *Ps. aeruginosa*, but, nevertheless, were as much as 25 times as active as phenol against *S. aureus*. With

diluted DMSO as solvent, the mercury–tin compounds I, IIb, and III were more active against the test organisms than the corresponding aqueous solutions. The data suggest enhanced activity of 2–10 times as a result of prior solution of the mercury–tin compounds in DMSO. With dilute ethanol as solvent, the activity of the mercury–tin compounds was further enhanced. The diluted VAL or BUL solutions of the mercury–tin compounds showed further increased activity. With BUL as a solvent the greatest increase in activity was noted with II, which was 48, 125, and 200 times as active as the corresponding aqueous solution with *S. aureus*, *Ps. aeruginosa*, and *E. coli*, respectively. In the case of the intermediate TBTO, however, the diluted BUL solution was at least 9 and 18 times more active against *Ps. aeruginosa* and *E. coli*, respectively, than the dilute ethanol solution. By comparison, when EMC was dissolved in water, dilute ethanol, or BUL, no increased antibacterial activity was apparent since the MIC was 200 or 300 ppm with all of the test organisms.

The compound IAA, used in the synthesis of all the mercury–tin compounds except IIa, prevented growth of all test organisms in the range of 1000–5000 ppm. The compound MDMA, used in preparation of I, Ia, and IV, in dilute BUL solution was highly effective against *Ps. aeruginosa*, less effective against *E. coli*, and much less effective in the case of *S. aureus*. When the reagents used to synthesize I were combined in theoretical proportions in BUL and diluted, the solution has approximately the same activity as I.

The greater antibacterial activity of the mercury–tin compounds in dilute BUL was especially evident when compared with EMC in terms of mercury content. The MIC for EMC in parts per million of mercury for *Ps. aeruginosa* was 227 compared to 22 for I and 10 for II or IIb in BUL. With *S. aureus*, the comparable values were 151 for EMC compared to 22 for I, 6 for II, or 25 for IIb. The values with *E. coli* were 151 for EMC, 22 for I, 6 for II, and 9 for IIb. Thus, one-tenth or less mercury was required to prevent growth of the test organisms compared to the amount needed from EMC. Comparison of the activity of the mercury–tin compounds with TBTO on a parts per million of tin basis would show an even greater difference.

The effectiveness of the mercury–tin compounds in diluted BUL may also be compared on the basis of mercury-containing moiety. Compound I is a derivative of MDMA, II of mercuribis(3-pyridine), and III of phenylmercuri-3-pyridine. These three are each derived from tributyltin iodoacetate. With each test organism the dipyridine derivative II in diluted BUL appears to be slightly more effective; with I and III about equal. A similar comparison in diluted BUL with compounds derived from tri-*n*-propyltin iodoacetate indicates a slightly greater activity of the dipyridine derivative IIb compared to Ia. When the effect of the alkyl-substituted tin is determined, by comparison of the activity of I and Ia, and II and IIb, the tri-*n*-butyltin compound appears to be equally as effective as the tri-*n*-propyltin compound. The change from acetate of II to propionate of IIa resulted in a slightly less active compound.

The trimethyltin compound IV is different from the others in that both nitrogens of the tertiary amine MDMA have combined with a trimethyltin iodoacetate molecule. This compound was less active than the other mercury–tin compounds, although on a parts per million of mercury basis it was more active than EMC.

Table III records the effect of the addition of sodium thioglycollate to the medium on the MIC of several compounds. The expected reduction of activity of mercury compounds by thioglycollate was confirmed with EMC, and proportionately with the mercury–tin compounds tested. The response of the test organism to TBTO was variable with thioglycollate in the media.

TABLE III

A COMPARISON OF THE APPROXIMATE MINIMUM INHIBITORY CONCENTRATION (MIC)[a] OF COMPOUNDS IN DILUTED γ-BUTYROLACTONE USING THREE TEST ORGANISMS EXPOSED 15 MIN

	Test organism		
Material	*Ps. aeruginosa*	*S. aureus*	*E. coli*
EMC	800	>1000	1100
TBTO	1100	>800	800
I	300	500	500
IIb	100	280	160

[a] Values are parts per million of compound. Sodium thioglycollate was added: 0.5 g/l. of medium.

These data suggest that the mode of action of the mercury–tin compounds is similar to other mercurials.[9] The mercury–tin compounds appeared to be effective in preventing growth of the test organisms by combination with thiol groups in the cell wall. However, in the presence of dilute BUL they may possibly act also as quaternary ammonium compounds to erode the cell wall,[10] permitting penetration of the active mercury–tin compound to prevent growth of the test organism. This may account for the increased activity per unit of mercury. The greater increased activity of TBTO and the mercury–tin compounds dissolved in BUL against *Ps. aeruginosa* and *E. coli* suggest action on the weaker cell wall of these gram-negative organisms. Surface active agents enhance the activity of mercurials,[11,12] but BUL has not been previously reported to have this effect.

Acknowledgment.—The writers thank John K. Chao for aid in synthesis of the compounds tested and Dan Watson and Robert Johnson for aid in the microbiological work.

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