Acknowledgements

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SYNTHESIS AND STUDIES OF BACTERIOSTATIC ACTIVITY OF SULFILIMINES

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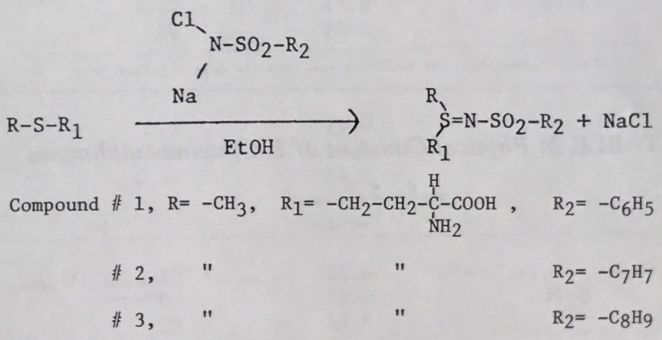
Abstract

A new series of Amino Acids-Sulfilimine have been prepared. They were tested against *Escherichia coli* and *Sarcina lutea* in presence of an antibiotic medium. Also studied was the toxicity in mice.

Introduction

The amino acids, L-Methionine and L-Cysteine recently have been found to react with Chloramine-B, Chloramine-T and N-chloro-N-sodio-*p*-ethyl benzenesulfonamide to obtain the corresponding sulfilimines. Inhibition of the growth of *Escherichia coli* and *Sarcina lutea* by these amino acid derivatives of sulfilimines have been described by others. (Dakin, Cohen, *et al*, derivatives may function as competitive inhibitors in the metabolism of the corresponding amino acids. The acute oral toxicity of some amino acid derivatives have been determined in male albino mice.

Several N-*p*-tolyl sulfonyl derivatives of sulfilimines have been described by others. (Dakin, Cohen, *et al*, 1916; Inglis, 1918; and Nicolet and Willard, 1921). Recently there has been prepared a new Chloramine (N-chloro-N-sodio-*p*-ethyl benzene sulfonamide) (Shah and Claypool, 1972) and a new series of sulfilimines called N-*p*-ethyl benzene sulfonyl sulfilimines. The sulfilimines of L-Methionine are given in Table 1, were prepared by treating the corresponding sulfide in the N-sulfonyl halomide reaction.

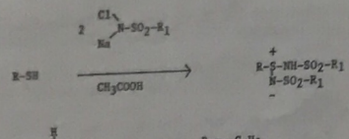


The sulfilimines of L-Cysteine are given in Table 2, were prepared by the action on N-sulfonyl halomide on solutions of mercaptans in glacial acetic acid.

These amino acid derivatives were tested against *Escherichia coli* and *Sarcina lutea* in the presence of antibiotic medium (I) and (IV), pH 6 phosphate buffer, with 16-20 hr incubates at 30-31°C. This method was first described by Abraham et. al, (1941) for the assay of Penicillin. It was later modified by Foster and Woodruff (1943) and by Schmidt and Moyer. (1944) The acute oral LD_{50} for compounds (2 & 5) are greater than 5.0 gm/kg of body weight.[1]

In summary, analytical data are not adequate proof for

[1] Hill-Top Research, Inc., Miamiville, Ohio 45147.



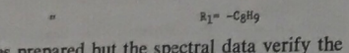
Compound # 4, R= $-CH_2-\underset{NH_2}{\overset{H}{C}}-COOH$ R_1= $-C_6H_5$

5, " R_1= $-C_7H_7$

6, " R_1= $-C_8H_9$

the sulfilimines prepared but the spectral data verify the preparation of the new sulfilimines in this work. Such verification has been successfully utilized by K. Tsujihara (1969) in his work with sulfilimines. The diameter of the zones are directly dependent upon the concentration of the antimicrobial agents. Figures 1 & 2 show that the inhibition of *Sarcina lutea* and *Escherichia coli* on L-Methionine & L-Cysteine-sulfilimines. From these

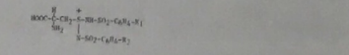
TABLE 1: *Physical Constants of L-Methionine-Sulfilimines*



Compound #	R	MP, °C	Yield	Infrared, cm^{-1}	Ultraviolet MeOH λ max	Analysis
(1)	-H	138-139	25	1210, 1027 1170 925	255, 262 267, 272	C,H,N,S[a].
(2)	$-CH_3$	134-135	28	1205, 1030 1167 928	256, 262 268, 273	C,H,N,S[d].
(3)	$-C_2H_5$	98-99	18	1203, 1032 1166 930	256, 261 267, 272	C,H,N,S[e].

[a] Calcd, 23.5, Found 22.9. [d] Calcd, 22.3, Found, 21.8 [e] Calcd, 20.8, Found, 20.3.

TABLE 2: *Physical Constant of L-Cysteine Sulfilimines*



Compound #	R_1 & R_2	MP, °C	Yield	Infrared, cm^{-1}	Ultraviolet CHCl$_3$ λ max	Analysis
(4)	-H	136-136.5	70	1220, 1040 1168 932	257, 261 269, 274	C,H,N,S[f]
(5)	$-CH_3$	138	65	1218, 1038 1167 930	256, 263 268, 274	
(6)	$-C_2H_5$	107-108	45	1211, 1030 1160 921	255, 267 268, 273	

[f] Calcd, 22.5, Found, 22.1.

figures the critical concentration of each compound can be determined. (Kavanagh, 1963) The Penicillin G Potassium readily inhibits *Sarcina lutea* at a very low concentration (0.025μg/ml), but at high concentration (0.1μg/ml) inhibits *Escherichia coli*. These amino acid derivatives inhibit Sarcina lutea at a low concentration (30μg/ml), but at a high concentration (50μg/ml) inhibits *Escherichia coli*. Amino acids themselves do not inhibit either *Sarcina lutea* or *Escherichia coli*.

These amino acid derivatives are (1200:1) more than a thousand times less active than the Penicillin G Potassium in *Sarcina lutea*, and (500:1) 500 times less active than Penicillin G Potassium in *Escherichia coli*. These results suggest that these amino acid derivatives have some antimicrobial and toxic properties.

MATERIALS AND METHODS

Sulfilimines of analytical purity as listed in Table 1 & 2 were prepared from appropriate Chloramine and Sulfides or Mercaptans either by known methods (cf Tables) or by the following general procedure.

(a) *From Sulfides:* (McCall, Tarbell and Havill, 1951):

The mixed solutions of 0.02 mol of sulfide (in 50 ml ethyl alcohol) and of 0.03 mol of chloramine (in 50 ml of 50% ethyl alcohol-water solution) were heated in a water bath for 30 minutes. It was then covered and allowed to stand overnight at room temperature. When product formed on standing the product was filtered, washed thoroughly with water, dried and recrystallized from EtOH or MeOH (once or twice).

(1) *Methionine-benzene sulfonylimine:—*

The recrystallized product was obtained by the general procedure outlined above; yield 25%, mp 138-139°, ir (CHCl$_3$): (Asymm SO_2); 1210, 1027 cm^{-1}, (Symm SO_2): 1170 cm^{-1}, (S=N) 925 cm^{-1}. *Anal.* calcd for $C_{11}H_{16}N_2S_2O_4$; C, 48.60; H, 5.88; N, 10.29; S, 23.5, Found, C, 48.60; H, 5.73: N. 9.98; S, 22.9.

(2) *Methionine-p-toluene-sulfonylimine:—*

The recrystallized product was prepared by the general procedure; yield 28%, 134-135°, ir (CHCl$_3$): (Asymm SO_2): 1203, 1030 cm^{-1}, (Symm SO_2): 1167 cm^{-1}, (S=N): 928 cm^{-1}. *Anal.* calcd for $C_{12}H_{18}N_2S_2O_4$: C, 50.35; H, 6.30; N, 9.80; S, 22.3. Found: C, 50.10; H, 5.98; N, 9.98; S, 21.8.

(3) *Methionine-p-ethyl benzene sulfonylimine:—*

The recrystallized product was prepared by the general procedure; yield 18%, mp 98-99°, ir (CHCl$_3$): (Asymm SO_2): 1230, 1032 cm^{-1}, (Symm SO_2): 1166 cm^{-1}, (S=N): 930 cm^{-1}. *Anal.* calcd for $C_{13}H_{20}N_2S_2O_4$: C, 52.00; H, 6.67; N, 9.30; S, 20.8. Found: C, 52.40; H, 6.50; N, 8.98; S, 20.3.

From Mercaptans—(Clark, Kenyon and Phillips, 1930):

The mixed solutions of 1.10 mol of mercaptan (in 200 ml glacial acetic acid) and of 1.1452 mol of chloramine (in 100 ml glacial acetic acid) were heated in water bath for 20 min., the mixture was poured into water. The solid product which separated was extracted with benzene to remove disulfide, and then digested with hot dilute sulfuric acid. A heavy viscous oil remained which crystallized on cooling and recrystallized from EtOH or MeOH (twice).

(4) *Cysteine-benzene sulfonimido sulfine-benzene-sulfonylimine:—*

The recrystallized product was prepared by the general procedure; yield 70%, mp 136-136.5°, ir (CHCl$_3$): (Asymm SO_2): 1200, 1040 cm^{-1}, (Symm SO_2): 1168 cm^{-1}, (S=N): 932 cm^{-1}. *Anal.* calcd for $C_{15}H_{17}N_3S_3O_6$; C, 44.30; H, 5.10; N, 8.60; S, 22.5. Found: 44.70; H, 4.86; N, 8.07; S, 21.4.

(5) *Cysteine-p-toluene sulfonimido sulfine-p-toluene sulfonylimines:—*

The recrystallized product was prepared by the general procedure; yield 65%, mp 138°, ir (CHCl$_3$): (Asymm SO_2): 1218, 1038 cm^{-1}, (Symm SO_2): 1167 cm^{-1}, (S=N): 930 cm^{-1}. Mol, Formula: $C_{17}H_{21}N_3S_3O_6$.

(6) *Cysteine-p-ethyl benzene sulfonimido sulfine-p-ethyl benzene sulfonylimine:—*

The recrystallized product was prepared by the general procedure; yield 45%, mp 107-108°, ir (CHCl$_3$): (Asymm SO_2): 1211, 1030 cm^{-1}, (Symm SO_2): 1160 cm^{-1}, (S=N): 921 cm^{-1}. Mol. Formula: $C_{19}H_{25}N_3S_3O_6$.

MICROBIOLOGICAL TEST[2]

Accurately weigh ca. 10 mg of each amino acid derivative in separate 100 ml volumetric flask and dissolve in 10 ml HCl and enough pH 6 phosphate buffer to give an exact concentration of 100μg/ml(solution a). Dilute approximate aliquots of solution (a), with enough pH 6 buffer to obtain concentrations of 70-, 60-, 50-, 40-, 30μg/ml. Add 10 ml melted Bacto-antibiotic-medium I to sterile petri dishes, distribute evenly and let harden on perfectly level surface. For actual assay approximate amounts of organism-suspensions are added to (1 ml) Bacto-antibiotic-medium-IV previously melted and cooled to 48°C. Mix thoroughly and add 4.0 ml to each plate containing base layer of Bacto antibiotic-medium-I. Distribute media evenly by tilting plates from side to side with circular motion and let harden.

Place three cylinders on each plate at ca 60° intervals on a 2.8 cm radius. Fill all 3 cylinders with the test solution. Incubate plates overnight at 30-31°C and measure the diameters of zones of inhibition by means of mm ruler, calipers or calibrated projection device. Three plates were used for each assay solution and three plates for the standard solution. Determine the corrected value of the sample and standard. Plotting values of x^2 (square of the zone size) against ln m_0 (logarithm of the concentration in the reservoir) gives a straight line intercepting the concentration axis at ln m_1 (critical concentration).

TOXICITY TESTS

Each sample was administered to male albino mice (Laboratory Supply-Company), weight range 18 to 27 gms, at a dosage level of 5.0gm/kg of body weight. The samples were administered as 50% weight/volume suspensions in corn oil (Mazola). Food was withheld from the mice for approximately 18 hrs. prior to dosage. Following dosage, food consisting of commercial pellets and water were available *ad libitum*. The mice were housed in conventional box-type mouse cages in groups of ten. All animals were observed closely for gross signs of systemic toxicity and mortality on the day of dosage, and at least once daily thereafter for a total of 14 days. Gross necropsies were performed on the animals that died. At the end of the 14-day observation period the surviving mice were weighed, sacrificed by cervical dislocation and gross necropsies were performed.

With compound # 2, no mortalities occurred. Therefore the acute oral LD_{50} for male albino mice is greater than 5.0 gm/kg of body weight. On the day of dosage the mice appeared depressed and showed depressed righting and placement reflexes, ataxia and a rapid shallow respiration. On the following day and for the remainder of the 14-day observation period the mice exhibited normal behavior and appearance. The mice showed an average weight gain of seven gms which is within normal limits for mice of the age, sex and strain used in this study. Gross necropsies performed at termination showed no gross pathology.

In compound #5, three mice died during the study. Two deaths occurred within two hrs. of dosage. The third death occurred on the third-post dosage day. Therefore, the acute oral LD_{50} for male albino mice is greater than 5.0gm/kg of body weight. On the day of dosage the mice appeared depressed and exhibited depressed righting and placement reflexes, ataxia and rapid shallow respiration. These signs persisted without substantial change through the second post-dosage day. On the third-post-dosage day and for the remainder of the 14-day observation period the surviving mice appeared grossly normal. The mice showed an average body weight gain of eight gms

TABLE 3: *Microbiological Data of Sulfilimines*

Compound #	Sarcina lutea Concentration m_0 (μg/ml)	Sarcina lutea Diameter of zones x (mm)	Sarcina lutea Critical-Concentration m' (μg/ml)	Escherichia coli Concentration m_0 (μg/ml)	Escherichia coli Diameter of Zones x (mm)	Escherichia coli Critical-Concentration m' (μg/ml)
(1)	50	17.1		70	21.0	
	40	14.6	21.5	60	17.6	39.4
	30	12.1		50	13.8	
(2)	50	18.1		70	19.0	
	40	15.2	22.5	60	16.6	32.0
	30	12.2		50	14.2	
(3)	50	17.4		70	21.6	
	40	14.9	20.9	60	18.0	31.8
	30	12.3		50	14.8	
(4)	50	17.8		70	21.0	
	40	14.75	24.0	60	18.0	34.8
	30	11.8		50	14.8	
(5)	50	17.8		70	20.1	
	40	15.2	18.5	60	17.0	38.0
	30	12.8		50	13.8	
(6)	50	17.8		70	21.4	
	40	14.6	18.0	60	17.8	38.0
	30	12.5		50	14.3	

[2] (Horwitz, 1970)

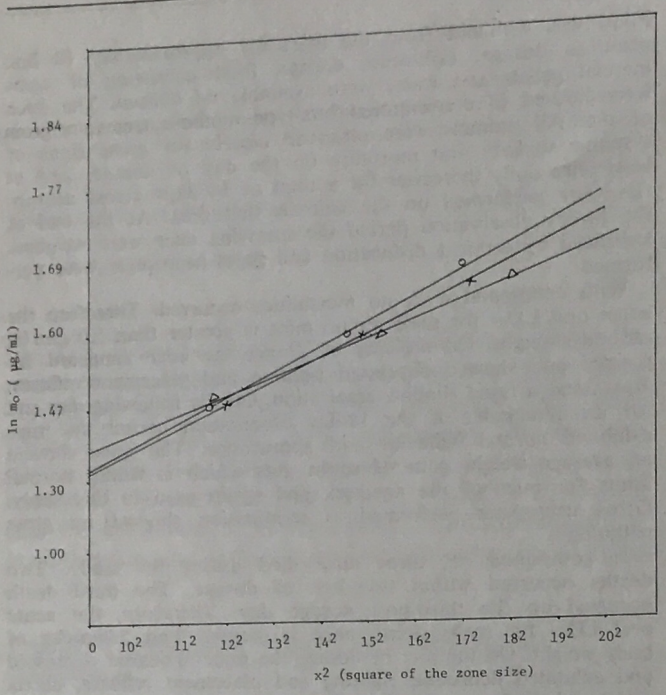


Fig. 1: *Inhibition of* Sarcina lutea *on L-Methionine-sulfilimines.*

o — o Methionine-benzene-sulfonylimine
Δ — Δ Methionine-p-toluene-sulfonylimine
x — x Methionine-p-ethyl benzene sulfonylimine

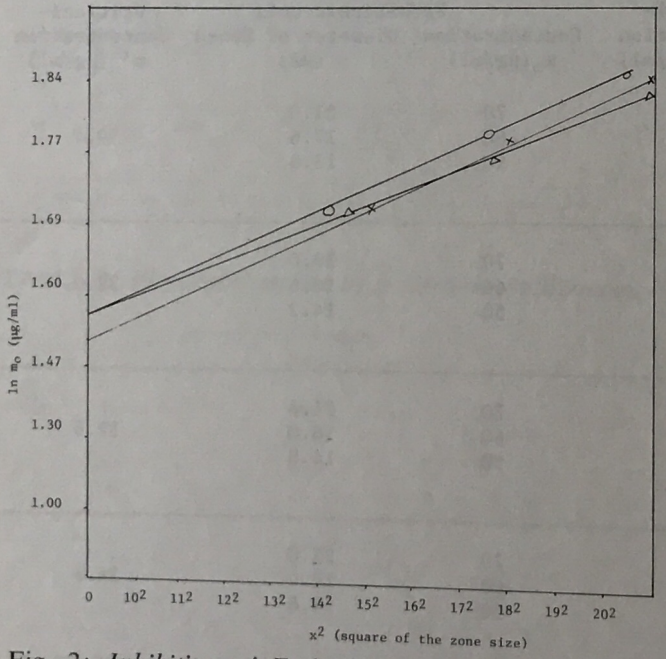


Fig. 2: *Inhibition of* Escherichia coli *on L-Cysteine-sulfilimines.*

x — x Cysteine-benzene sulfonimido sulfine-benzene-sulfonylimine
o — o Cysteine-p-toluene sulfonimido sulfine-p-toluene sulfonylimine
Δ — Δ Cysteine-p-ethyl benzene sulfonimido sulfine-p-ethyl benzene sulfonylimine

which is within normal limits for mice of the age, sex and strain used in this study. Gross necropsies performed on the surviving mice at termination showed no gross pathology.

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