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SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF ARSONIUM AND STIBONIUM YLIDS

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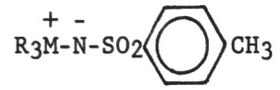
ABSTRACT

This paper reports new arsinimines and stibinimines that have been prepared. These compounds were tested against *Sarcina lutea* and *Staphylococcus epidermis* in the presence of an antibiotic medium I and IV. These compounds were shown to possess toxic effects against *Plasmodium berghei* in birds. Toxicity in mice was also studied.

INTRODUCTION

Iminoarsananes (Arsinimine $R_3As{=}N\text{-}SO_2\text{-}R_1$) are isolectronic with arsonium ylids. Mann and Chaplin (1937) found that tertiary aromatic phosphine and arsines R_3M (M=P,As) with anhydrous chloramine-T in anhydrous alcohol gave the phosphinimine or arsinimine.

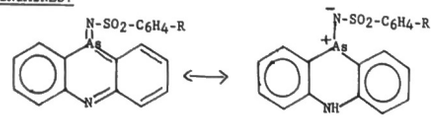


Wittig and Laib (1953) were the first to undertake a study of stibonium ylids. Wittig and Hellwinkel (1964) reported the preparation of triphenyl stibonium-N-tosylimine by reaction of triphenyl stibine with chloramine-T.

The author has found the phenarsazine chloride and triphenyl stibine to react with chloramine-B, chloramine-T and a new N-chloro-N-sodio-p-ethyl benzene sulfonamide (Shah, 1971, 1972 & 1974) to obtain the corresponding arsinimines and stibinimines. The infrared and ultraviolet spectra of these compounds have been examined, and the acute oral toxicity of some derivatives have been determined in male albino mice. These compounds were tested against *Plasmodium berghei* in birds and exhibited toxicity.[1]

Arsinimines and stibinimines were prepared from the corresponding phenarsazine chloride or stibine with chloramines. The structure of these compounds are as follows:

ARSINIMINES:



STIBINIMINES:

$(C_6H_5)_3Sb{=}N\text{-}SO_2\text{-}C_6H_4\text{-}R$

Compound No. 1 and 4	R = -H
Compound No. 2 and 5	R = $-CH_3$
Compound No. 3 and 6	R = $-C_2H_5$

These compounds were tested against *Sarcina lutea* and *Staphylococcus epidermis* in the presence of Bacto-Antibiotic Medium I (Code 0263) (Bacto Penassay seed agar) and Bacto Antibiotic Medium IV (Code 0244) (Bacto yeast beef agar) at a pH 6 phosphate buffer and with 16-20 hr. incubation at 30-31°C. This method was first described by Abraham (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 compound IV and V are less than 5.0 gm/kg of body weight.[2]

METHODS

Phenarsazine chloride or triphenyl stibine (0.01 mole) was dissolved in 30 ml of absolute ethanol. The corresponding chloramine (0.01 mole) was dissolved in 30 ml of absolute ethanol. The reaction mixture then warmed to 60-70°C for 15 minutes in a steam bath and allowed to stand overnight. The product formed on standing and after filtration was washed thoroughly with water, dried and recrystallized from ethanol or methanol.

MICROBIOLOGICAL TEST

Accurately weigh approximately 10 mg of each compound in separate 100 ml volumetric flask and dissolve in 10 ml HCl and enough pH 6 phosphate buffer to give an exact concentration of 100 μg/ml (solution a).

Dilute appropriate aliquots of stock solution, (a), with enough pH 6 buffer to obtain concentrations of 5, 4, 3, 2 & 1 μg/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 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.

[1]Walter-Reed Army Institute, Washington, D.C.

[2]Hill Top Research, Inc., Miamiville, Ohio

Place three cylinders on each plate at approximately 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. 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 mo (logarithm of the concentration in the reservoir) gives a straight line intercepting the concentration axis at ln m′ (critical concentration).

TOXICITY TESTS

Each test sample was administered orally by stomach tube to a group of 10 male albino mice. (Laboratory supply company, weight range 21.6 to 26.4 grams). Each sample was administered as a 50% weight per volume suspension in Mazola corn oil at a dosage level of 5.0 grams per kilogram of body weight.

Food was withheld from the mice for approximately 18 hours prior to dosage. All animals were observed closely for gross signs of systemic toxicity and mortality at frequent intervals during the day of dosage, and at least once daily thereafter for a total of 14 days. At the end of the 14 day observation period the surviving mice were weighed, sacrificed by cervical dislocation and gross necropsies were performed.

Compound No. 4:

The mortality results during the 14-day observation period are presented below. Values are number of animals dead/number of animals tested.

TIME OF DEATH

HOURS				DAYS			
2	3	4	24	2-5	6	7	8-14
0/10	2/10	4/10	8/10	8/10	9/10	9/10	9/10

The acute oral LD_{50}(lethal dose for 50% of the animals) is less than 5.0 gm/kg of body weight for male albino mice. All mice exhibited depression and rapid, shallow respiration approximately one hour following dosage. The two surviving mice appeared normal on the morning of the first post-dosage day. The surviving mouse exhibited depression and rapid, shallow respiration on the seventh through the twelfth day, after which this animal appeared normal. The single surviving mouse exhibited an average body weight gain of 1.5 grams which is below the normal limits for mice of the age, sex and strain used in this study. Gross necropsies performed at termination showed no gross pathology.

Compound No. 5:

The mortality results during the 14-day observation period are presented below. Values are number of animals dead/number of animals tested, cumulative.

TIME OF DEATH

HOURS				DAYS			
2	3	4	24	2-5	6	7	8-14
0/10	0/10	0/10	5/10	5/10	8/10	9/10	9/10

The acute oral LD_{50} for male albino mice is less than 5.0 gm/kg of body weight. All mice exhibited depression and rapid, shallow respiration within one hour following dosage and throughout the remainder of the day. On the first post-dosage day toxic signs among the surviving mice were confined to slight depression in one mouse. All mice appeared normal on the second day and no toxic signs were noted in the animals which died on the sixth and seventh days. The single surviving mouse exhibited an average body weight gain of 2.5 grams, which is slightly below the normal limits for mice of the age, sex and strain used in this study. Gross necropsies performed at termination showed no gross pathology.

Compound No. 4 and No. 5 can probably be classified as toxic by ingestion when tested by the method described above.

Biological Activity

The compounds were tested for their antimalarial activity against *Plasmodium berghei* in birds according to a procedure already published (Osdene, Russel and Rane, 1967). The test results are given in Table 3.

RESULTS AND DISCUSSION

Analytical and spectral data (infrared, ultraviolet) support the structure of these compounds. Figures 1 and 2 show the inhibition of *Sarcina lutea* and *Staphylococcus epidermis* by arsinimines and stibinimines. From these 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 (0.025 μg/ml) but at a high concentration (0.1 μg/ml) inhibits *Staphylococcus epidermis*. Arsinimines and stibinimines also inhibits *Sarcina lutea* at a low concentration (1 μg/ml), and at a high concentration (3 μg/ml) inhibits *Staphylococcus epidermis*.

These compounds are 40 times less active than the Penicillin G Potassium against *Sarcina lutea*, and 30 times less active than Penicillin G Potassium against *Staphylococcus epidermis*. Tables 2 and 3 show the antibacterial properties and antimalarial data of arsinimines and stibinimines.

ACKNOWLEDGEMENT

The author wishes to thank the Woodson-Tenent Laboratories for their cooperation and research facilities.

TABLE 1: *Infrared and Ultraviolet Spectra of Arsinimines and Stibinimines.*

$R\text{-}M\text{=}N\text{-}SO_2\text{-}R_1$

NUMBER	R	R1	M	MP°C	FORMULA	ANALYSES	INFRARED CM^{-1}	ULTRAVIOLET λMAX. CH_3OH
1	$C_{12}H_9ASClN$	-H	As	115	$C_{18}H_{14}N_2SO_2As$	C,H,N,S[a]	922(As=N) 1135,1155(sSO_2) 1035,1195($AsSO_2$)	272 296 305
2	$-(C_6H_5)_3-$	-H	Sb	198	$C_{24}H_{20}NSO_2Sb$	C,H,N,S[b]	920(Sb=N) 1135,1158(sSO_2) 1042,1190($AsSO_2$)	256 261 266.5
3	$C_{12}H_9ASClN$	$-CH_3$	As	122	$C_{19}H_{16}N_2SO_2As$	C,H,N,S[c]	925(As=N) 1135,1160(sSO_2) 1042,1198($AsSO_2$)	272 305
4	$-(C_6H_5)_3-$	$-CH_3$	Sb	180	$C_{25}H_{22}NSO_2Sb$	C,H,N,S	925(Sb=N) 1138,1160(sSO_2) 1045,1200($AsSO_2$)	256 261.5 266
5	$C_{12}H_9ASClN$	$-C_2H_5$	As	90	$C_{20}H_{18}N_2SO_2As$	C,H,N,S[d]	920(As=N) 1133,1157(sSO_2) 1022,1038($AsSO_2$) 1195	272.5 302
6	$-(C_6H_5)_3-$	$-C_2H_5$	Sb	190	$C_{26}H_{24}NSO_2Sb$	C,H,N,S	921(As=N) 1132 (sSO_2) 1037;1195($AsSO_2$)	251 267

S[a] = Calcd 8.55, Found 9.10, S[b] = calcd 6.30 Found 6.00
S[c] = Calcd 8.27, Found 8.60 S[d] = Calcd 7.96, Found 7.40
S = Symmetric As = Asymmetric

TABLE 2: *Microbiological Data of Arsinimines & Stibinimines.*

Compound No.	Concentration m_o (µg/ml)	Sarcina lutea Diameter of Zones x(mm)	Critical Concentration m' (µg/ml)	Concentration m_o (µg/ml)	Staphy. epidermis Diameter of Zones x(mm)	Critical Concentration m' (µg/ml)
1	3 2 1	24.4 18.2 11.8	0.30	5 4 3	29.0 26.4 23.60	1.6
2	3 2 1	25.0 17.10 9.63	0.80	5 4 3	29.35 27.0 22.10	2.2
3	3 2 1	25.70 17.50 9.0	0.82	5 4 3	31.3 26.80 21.50	2.5
4	3 2 1	24.60 17.00 10.20	0.74	5 4 3	28.8 25.40 21.8	2.2
5	3 2 1	24.20 18.30 10.56	0.70	5 4 3	28.30 25.50 22.10	2.0
6	3 2 1	25.50 18.30 11.0	0.60	5 4 3	29.20 26.20 23.30	1.6

TABLE 3: *Antimalarial Activity of Arsinimines and Stibinimines.*

COMPOUND	ANIMAL	DOSE MG/KG	CURES	MSTT*	MSTC*	T-C*	TOX.	MSTX*
1	Bird	30	-	-	8.5	-	05	1 Toxic
		120	-	-	8.5	-	05	1 Toxic
		480	-	-	8.5	-	05	1 Toxic
2	Bird	30	-	-	8.5	-	05	2 Toxic
		120	-	-	8.5	-	05	1 Toxic
		480	-	-	8.5	-	05	1 Toxic
4	Bird	30	-	8.2	8.5	0.3	00	0
		120	-	-	8.5	-	05	1 Toxic
		480	-	-	8.5	-	05	1 Toxic
5	Bird	30	-	8.4	8.5	0.1	00	0
		120	-	-	8.5	-	05	1 Toxic
		480	-	-	8.5	-	05	1 Toxic

MSTT - Means Survival Time of Treated Animals

MSTC - Means Survival Time of Controls

T-C - Changes in Survival Time (MSTT-MSTC)

Tox - Toxic Deaths. Those deaths occuring on days 2,3,4 and 5 after infection. Control animals do not die before day 6.

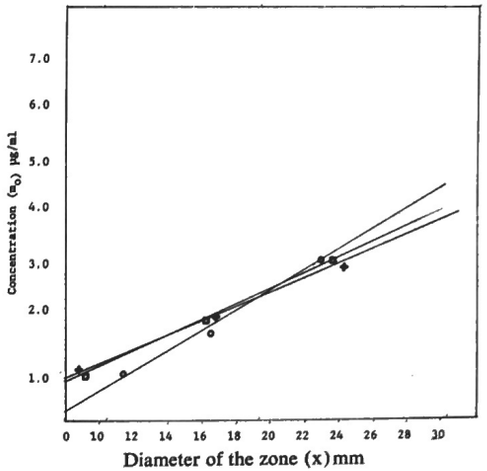


Fig. 1: *Inhibition of* Sarcina lutea *on Arsinimines.**
0 — 0 Compound #1
□ — □ Compound #2
+ — + Compound #3
0 — 0 Phenarsazine-benzene-sulfonylimine
□ — □ Phenarsazine-p-toluene-sulfonylimine
+ — + Phenarsazine-p-ethyl benzene-sulfonylimine

*Draw the graph diameter of the zone against concentration(m_o), instead of square of diameter of the zone against ln m_o(Due to the small concentration of the sample).

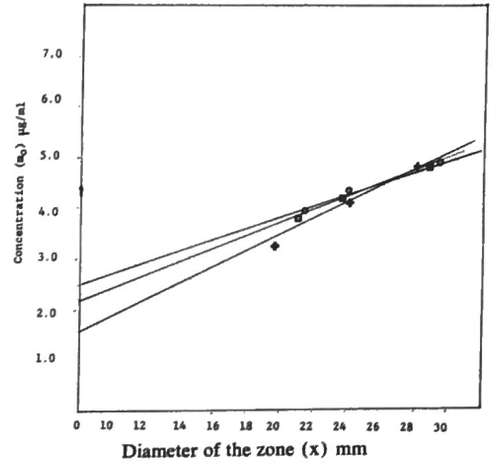


Fig. 2: *Inhibition of* Staphylococcus epidermis *on Arsinimines.**
+ — + Compound #1
□ — □ Compound #2
0 — 0 Compound #3

(N.B. * same as figure 1)

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"30-YR.-OLDS LOOK BACK AND SEE HIGH SCHOOL IRRELEVANT"

The quality of life for 30-year-olds is "quite good", but it is not because of their education. That is the conclusion drawn from a sample to follow up the massive "Project Talent" testing of 400,000 15-year-olds in 1960. Centering on the all-important criterion of "quality of life," the American Institute for Research (AIR) in Palo Alto, Cal. let the participants answer what they considered the constituents of "quality of life".

Looking at a representative national sample of 1,000 participants, the 30-year-olds looked back at their high school experience and found it largely irrelevant to their later lives. One of the ten experts who analyzed the interviews, Robert Gagne, professor of education at Florida State Univ., bluntly concluded, "The evidence of these interviews suggests that a high school education as a whole serves *no* very useful purpose."

What role did education play in these young adults that got much more education than their parents? Almost 85% graduated from high school or got an equivalent diploma, while only half of their parents did so. The results point to the glaring failures in their education that gave no help to develop goals and plans. Only 13% of the men in the study are now in the occupational category they chose for themselves as 15-year-olds. Why? It seems that the young people did not know their own interests and abilities, and they had almost no knowledge of the requirements and availability of various jobs. The results, the study shows, are "much wasted time, lack of motivation, and personal frustration."

In conclusion, the needs are in 1) more vocational guidance from well-informed, concerned counselors, 2) higher quality of teaching, 3) improved individualized instruction, 4) new courses for the non-college bound and 5) training for teachers and counselors to deal with personal and emotional problems.

Copies of the 87 page report "An Empirical Study to Aid in Formulating Educational Goals", may be obtained at $3.00 per copy from American Institute for Research, P.O. Box 1113, Palo Alto, Cal. 94302.

From Assn. for Supervision and Curric. Development, *News Exchange* Vol. 18, Number 2. May, 1976.