However, only the first two have demonstrable trypanocidal activity. Substitution in the amino groups on the triazine ring does not cause any marked change in toxicity but does destroy the activity (6, 7, 8). Of the four pentavalent compounds showing trypanocidal activity (3, 4, 9, 11), the two having substituents in the benzene rings show only moderate activity (9, 11), the better of the two (11) having a curative index twice that of tryparsamide. The two *p*-arsanilic acid derivatives (3, 4) have unusually large curative indices, much larger than any experienced previously by us. Of these two compounds, the 4,6-diamino (3) compound is to be preferred because of ease of preparation and the lower effective dose level.

The trivalent compounds derived from (3) all have unusual activity (12–16) while the trivalent compound (17), having a benzene ring substituent, shows little activity. There is little to choose between these compounds. Of the compounds examined, several are being investigated more extensively to determine if their chronic toxicity will warrant clinical evaluation. The study of effectiveness in rabbit syphilis is also in progress. More detailed pharmacological reports will be made elsewhere.

Summary

1. The condensation of halotriazines with substituted anilines in acid solution has been applied to the synthesis of triazinylarsanilic acids.

2. A number of arsenicals of anilinotriazines have been prepared and studied in experimental trypanosomiasis. Several compounds have such unusual activities as to justify further study and clinical evaluation.

DETROIT, MICHIGAN RECEIVED APRIL 13, 1944

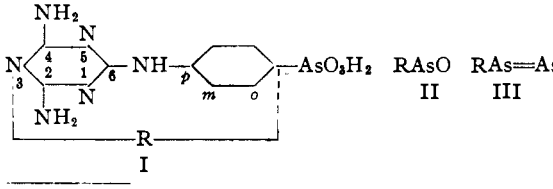
[CONTRIBUTION FROM THE LABORATORIES OF THE UNIVERSITY OF GENEVA, SWITZERLAND]

Trypanocidal and Spirochetocidal Arsenicals Derived from s-Triazine

BY E. A. H. FRIEDHEIM

This study is part of a search for therapeutic agents effective against the causative microorganisms of African sleeping sickness and syphilis. The work was guided by the consideration that it is especially important that such agents be (a) active in stages of these diseases in which the central nervous system is involved, and that (b) they should lack the toxic side effects on the central nervous system, particularly on the optic nerve, which are common to all pentavalent arsenicals used at the present time.

p-(2,4-Dichloro-s-triazinyl-6)-aminophenylarsonic acid, obtained readily from sodium arsanilate and cyanuric chloride, is a very toxic substance of no therapeutic value; however, it acquires considerable trypanocidal activity when at least one of its halogens is replaced by an amino group. In a series of fifteen arsonic acids of this type, the maximum trypanocidal effect combined with minimum toxicity, was found when both halogens were replaced by unsubstituted amino groups, *i. e.*, in *p*-(2,4-diamino-s-triazinyl-6)-aminophenylarsonic acid.[1] The biological effect of the replacement of the chlorine by other substituents is shown in Table II.



I

RAsO II RAs=AsR III

(1) Synonyms: 4-melaminylphenylarsonic acid, melarsen, triazinearsonic acid, 2,4-diamino-6(*p*-arsonoanilino)-s-triazine.

The compounds in this series have been prepared by taking advantage of the rule that in cyanuric chloride the halogens may be readily replaced by amino groups, the first chlorine at 0°, the second at room temperature and the third at 100–120°.[2]

The arsonic acids of this series are white, amorphous powders which remain unchanged when heated to 300°; they discolor and char without melting at higher temperatures ranging from 300–350°.

Reduction with sulfur dioxide, in the presence of hydriodic acid, leads to the corresponding arsine oxide (II) and reduction with hypophosphoric acid–hydriodic acid mixture to the corresponding arseno derivative (III).

I, II and III cure experimental trypanosomiasis and II cures, furthermore, experimental relapsing fever.

The therapeutic index for I, by parenteral administration, was found to be 30–50 for mice and about 10 for rabbits (*T. equiperdum, brucei, gambiense*).[3] This compound does not show the toxic effects of the classical pentavalent aromatic arsenicals on the central nervous system and does not cause in mice the symptom of "waltzing."[4] The therapeutic indices for compounds II and III, by oral administration in mice infected with *T. equiperdum*, were shown to be 20 and 25, respectively. The index for mapharsen in control experiments was found to be 1.3 (see Table III).

(2) H. E. Fierz-David and M. Matter, *J. Soc. Dyers Colourists*, **53**, 424 (1937).

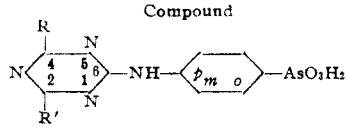
(3) E. A. H. Friedheim, *Ann. Inst. Pasteur, Paris*, **65**, 108 (1940).

(4) E. A. H. Friedheim, *Schweiz. medizin. Wochenschrift*, **71**, 116 (1941).

TABLE I

COMPOUNDS DERIVED FROM *p*-(s-TRIAZINYL-6)-AMINOPHENYLARSONIC ACID

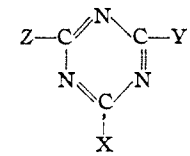
Compound



R	R'	Yield, %	Arsenic, % Calcd.	Arsenic, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
Chloro	Chloro	85	20.52	20.45	15.35	15.32
Chloro	Hydroxy	76	21.61	21.71	16.17	16.15
Hydroxy	Hydroxy	60	22.83	22.90	17.08	17.03
Amino	Chloro	69	21.68	21.57	22.96	22.92
Amino	Hydroxy	62	22.90	23.20	21.41	21.38
Amino	Amino	57	22.97	22.88	25.77	25.65
Amino	Methylamino	64	22.02	22.00	24.71	24.80
Methylamino	Methylamino	52	21.14	21.20	23.73	23.91
Diethylamino	Diethylamino	58	17.09	17.21	19.17	19.05
p-Arsanilino	Chloro	61	27.46	27.37	12.89	12.94
p-Arsanilino	Amino	46	28.47	28.43	15.98	15.86
p-Sulfanilino	Chloro	34	14.93	15.12	13.96	13.92
8'-Hydroxy-3',6'-disulfo-1'-naphthylamino	Chloro	29	11.56	11.46	10.81	10.77
m-(2,4-Diamino-s-triazinyl-6)-amino-*p*-hydroxyphenylarsonic acid		53	21.89	21.75	24.57	24.48
p-(2,4-Diamino-s-triazinyl-6)-amino-*o*-hydroxyphenylarsonic acid		49	21.89	21.84	24.57	24.49
p-(2,4-Diamino-s-triazinyl-6)-amino-phenylarsine oxide		48	25.64	25.72	28.77	28.74
p,p'-Bis-(2,4-diamino-s-triazinyl-6)-aminoarsenobenzene		32	27.13	27.09	30.44	30.37

TABLE II

EFFECT OF SUBSTITUENTS ON TOXICITY AND THERAPEUTIC ACTIVITY OF s-TRIAZINYLAMINOPHENYLARSONIC ACIDS ON *T. equiperdum* INFECTION OF MICE[a]



No.	X	Y	Z	Max. tol. dose, g./kg.	Min. cur. dose, g./kg.	Therapeutic index
1	—NH—C_6H_4—AsO_3H_2	—Cl	—Cl	0.2	..	..
2	—NH—C_6H_4—AsO_3H_2	—Cl	—OH	.2	..	..
3	—NH—C_6H_4—AsO_3H_2	—OH	—OH	.3	..	..
4	—NH—C_6H_4—AsO_3H_2	—NH_2	—Cl	1.5	0.1	15
5	—NH—C_6H_4—AsO_3H_2	—NH_2	—OH	0.2	.2	1
6	—NH—C_6H_4—AsO_3H_2	—NH_2	—NH_2	1.5	.03	50
7	—NH—C_6H_4—AsO_3H_2	—NH_2	—NH—CH_3	0.5	.05	10
8	—NH—C_6H_4—AsO_3H_2	—NH—CH_3	—NH—CH_3	.2	..	..
9	—NH—C_6H_4—AsO_3H_2	—$N(C_2H_5)_2$	—$N(C_2H_5)_2$	.05	..	..
10	—NH—C_6H_4—AsO_3H_2	—NH—$C_6H_4AsO_3H_2$	—Cl	.3	..	..
11	—NH—C_6H_4—AsO_3H_2	—NH—$C_6H_4AsO_3H_2$	—NH_2	.5	..	..
12	—NH—C_6H_4—AsO_3H_2	—NH—$C_6H_4SO_3H$	—Cl	.2	..	..
13	—NH—C_6H_4—AsO_3H_2	—H acid	—Cl	.3	..	..
14	OH(4), —NH(3) C_6H_3—AsO_3H_2	—NH_2	—NH_2	.5	.1	5
15	OH(2), —NH(4) C_6H_3—AsO_3H_2	—NH_2	—NH_2	.3[b]	.03	10
	Standards for comparison					
	Orsanine			1.0[c]	0.05	20
	Tryparsamide			2.2	.3	7

[a] Single intraperitoneal treatment with the corresponding sodium salt. [b] Crystallizes in kidney and bladder. [c] In non-lethal doses, toxic effect on central nervous system: produces "waltzing" mice.

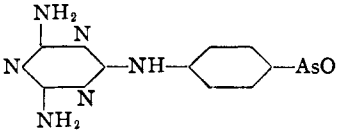
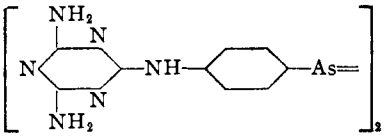
An index of 4 was established for compound II in the case of a lethal relapsing fever infection (*Sp. duttoni*) in mice; in control experiments, the index for mapharsen proved to be 1.7.

Experimental

1. ***p*-(2,4-Dichloro-s-triazinyl-6)-aminophenylarsonic Acid.**—A solution of 21.7 g. of arsanilic acid in 200 cc. of 0.5 *N* sodium hydroxide is added dropwise within one hour,

TABLE III

TOXICITY AND THERAPEUTIC EFFECT OF TRIVALENT MELAMINYL PHENYL ARSENIC COMPOUNDS ON *T. equiperdum* INFECTION OF MICE. ORAL ADMINISTRATION

Compound	50% lethal dose, g./kg.	Maximum tolerated dose, g./kg.	Minimum curative dose, g./kg.	Index
	7.5	6.0	0.3	20
	3.0	2.5	0.1	25
Standard for comparison				
Mapharsen	0.15	0.1	0.075	1.33

with efficient stirring, to a fine suspension of 20 g. of cyanuryl chloride[5] in 400 cc. of water to which 100 g. of chipped ice and 2 cc. of octyl alcohol had been added. The temperature is kept at 0–2° and the *p*H around 7.2, if necessary by addition of small portions of sodium bicarbonate. After one hour tests for primary aromatic amine become negative and the reaction mixture appears as a slightly opalescent solution. It is bone-blacked, filtered and yields on acidification with dilute hydrochloric acid a white precipitate which is filtered off and washed with water and acetone. For purification the precipitate is redissolved in 10 times its weight of aqueous 5% sodium bicarbonate and reprecipitated from the bone-blacked and filtered solution by acidification with acetic acid.

Anal. Calcd. for $C_9H_7O_3N_4Cl_2As$: As, 20.52; N, 15.35. Found: As, 20.45; N, 15.32.

2. ***p*-(2-Chloro-4-hydroxy-s-triazinyl-6)-aminophenylarsonic Acid.**—A solution of one part of the dichloro compound, described above, in ten parts of 10% sodium hydroxide is kept for eight hours at 40°. On acidification with dilute hydrochloric acid a white precipitate is formed which is filtered off, washed with water and purified as in (1).

Anal. Calcd. for $C_9H_8O_4N_4ClAs$: As, 21.61; N, 16.17. Found: As, 21.71; N, 16.15.

3. ***p*-(2,4-Dihydroxy-s-triazinyl-6)-aminophenlyarsonic Acid.**—A solution of one part of dichloro compound in ten parts of 10% sodium hydroxide is heated under pressure for five hours at 120°. On cooling and acidification of the filtered solution with dilute hydrochloric acid a white precipitate is formed which is isolated and purified as in (1).

Anal. Calcd. for $C_9H_9O_5N_4As$: As, 22.83; N, 17.08. Found: As, 22.90; N, 17.03.

4. ***p*-(2-Amino-4-chloro-s-triazinyl-6)-aminophenylarsonic Acid.**—A solution of one part of the dichloro compound in 20 parts of 10% aqueous ammonia is kept for ten hours at 30°. The excess of ammonia is driven off *in vacuo* and the cooled, bone-blacked and filtered solution yields on acidification with dilute hydrochloric acid a white precipitate which is isolated and purified as in (1). The compound is slightly soluble in an excess of dilute hydrochloric acid.

Anal. Calcd. for $C_9H_9O_3N_5ClAs$: As, 21.68; N, 22.96. Found: As, 21.57; N, 22.92.

5. ***p*-(2-Amino-4-hydroxy-s-triazinyl-6)-aminophenylarsonic Acid.**—One part of the chlorohydroxy compound (2) is heated with 20 parts of 28% aqueous ammonia in an autoclave at 120° for three hours. The product is isolated and purified as in (4).

Anal. Calcd. for $C_9H_{10}O_4N_5As$: As, 22.90; N, 21.41. Found: As, 23.20; N, 21.38.

(5) Diels, *Ber.*, **32**, 691 (1899).

6. ***p*-(2,4-Diamino-s-triazinyl-6)-aminophenylarsonic Acid.**—One part of the dichloro compound (1) is heated with 20 parts of 28% aqueous ammonia in an autoclave at 120° for three hours. The product is isolated and purified as in (4).

Anal. Calcd. for $C_9H_{11}O_3N_6As$: As, 22.97; N, 25.77. Found: As, 22.88; N, 25.65.

7. ***p*-(2-Amino-4-methylamino-s-triazinyl-6)-aminophenylarsonic Acid.**—One part of the amino-chloro compound (4) is heated with ten parts of 10% aqueous methylamine in an autoclave at 120° for two hours. The product is isolated and purified as in (4).

Anal. Calcd. for $C_{10}H_{13}O_3N_6As$: As, 22.02; N, 24.71. Found: As, 22.00; N, 24.80.

8. ***p*-[2,4-Di-(methylamino)-s-triazinyl-6]-aminophenylarsonic Acid.**—One part of the dichloro compound (1) is heated with ten parts of a 17% aqueous solution of methylamine in the autoclave at 110° for two hours. The product is isolated and purified as in (4).

Anal. Calcd. for $C_{11}H_{15}O_3N_6As$: As, 21.14; N, 23.73. Found: As, 21.20; N, 23.91.

9. ***p*-[2,4-Di-(diethylamino)-s-triazinyl-6]-aminophenylarsonic Acid.**—The preparation is analogous to (8), but diethylamine is used in place of methylamine.

Anal. Calcd. for $C_{15}H_{19}O_3N_6As$: As, 17.09; N, 19.17. Found: As, 17.21; N, 19.05.

10. **2-Chloro-s-triazinyl-4,6-di-(*p*-aminophenylarsonic Acid).**—A solution of 23.9 g. of arsanilic acid in 220 cc. of 2% sodium hydroxide is added dropwise with stirring to a solution of 36.5 g. of the dichloro compound (1) in 200 cc. of 2% sodium hydroxide. After standing for twenty-four hours at 28° the clear reaction mixture is acidified with dilute hydrochloric acid, whereupon a white precipitate is formed which is filtered off, washed with dilute hydrochloric acid and purified by precipitation with acetic acid from its bone-blacked, filtered, aqueous bicarbonate solution.

Anal. Calcd. for $C_{15}H_{14}O_6N_5ClAs$: As, 27.46; N, 12.89. Found: As, 27.37; N, 12.94.

11. **2-Amino-s-triazinyl-4,6-di-(*p*-aminophenylarsonic Acid).**—One part of the compound (10) is heated with ten parts of 20% aqueous ammonia in an autoclave at 110° for two hours. The product is isolated and purified as in (4).

Anal. Calcd. for $C_{15}H_{16}O_6N_6As$: As, 28.47; N, 15.98. Found: As, 28.43; N, 15.86.

12. ***p*-[2-Chloro-4-(*p*'-sulfanilyl)-s-triazinyl-6]-aminophenylarsonic Acid.**—This substance is prepared according to (11) by the use of 21 g. of sulfanilic acid in place of arsanilic aicd. Since it is readily soluble in water it is isolated from an ice cold solution.

Anal. Calcd. for $C_{15}H_{13}O_6N_5ClSAs$: As, 14.93; N, 13.96. Found: As, 15.12; N, 13.92.

13. ***p*-[2-Chloro-4-(3',6'-disulfo-8'-hydroxy-naphthylamine-1')-s-triazinyl-6]-aminophenylarsonic Acid.**—Thirty-eight and three-tenths grams of the disodium salt of H acid is added gradually with stirring at 35° to a solution of 36.5 g. of the dichloro compound (1) in 200 cc. of 2% sodium hydroxide. After standing for ten hours, the reaction mixture is acidified with dilute hydrochloric acid. A white precipitate is formed which is filtered off, washed with ice water and purified by precipitation with dilute hydrochloric acid from a concentrated solution in aqueous bicarbonate. The compound is soluble in cold water.

Anal. Calcd. for $C_{19}H_{15}O_{10}N_6ClS_2As$: As, 11.56; N, 10.81. Found: As, 11.46; N, 10.77.

14. ***m*-(2,4-Diamino-s-triazinyl-6)-amino-*p*-hydroxyphenylarsonic Acid.**—A solution of 22.3 g. of 4-hydroxy-3-aminophenylarsonic acid in 200 cc. of 2% sodium hydroxide is run dropwise, with stirring, within one hour, into a fine suspension of 20 g. of cyanuryl chloride in 400 cc. of water to which 100 g. of chipped ice and 1 cc. of octyl alcohol had been added. The *p*H is adjusted to 7.4. Stirring is continued at 0 to 2° until a filtered sample does not give any color reaction with diazobenzenesulfonic acid. The reaction mixture, kept at 10°, is saturated with gaseous ammonia. The temperature is then raised slowly to the boiling point of the mixture. After most of the excess ammonia is driven off, the hot solution is boneblacked and filtered. The cleared filtrate yields on acidification with dilute hydrochloric acid a white precipitate which is purified as in the described manner.

Anal. Calcd. for $C_9H_{12}O_4N_6As$: As, 21.89; N, 24.57. Found: As, 21.75; N, 24.48.

15. ***p*-(2,4-Diamino-s-triazinyl-6)-amino-*o*-hydroxyphenylarsonic Acid.**—Preparation in all ways as in (14) using 4-amino-2-hydroxyphenylarsonic acid.

Anal. Calcd. for $C_9H_{12}O_4N_6As$: As, 21.89; N, 24.57. Found: As, 21.84; N, 24.49.

16. ***p*-(2,4-Diamino-s-triazinyl-6)-aminophenylarsine Oxide.**—Ten grams of *p*-(2,4-diamino-s-triazinyl-6)-aminophenylarsonic acid is dissolved in 500 cc. of 10% hydrochloric acid. One gram of potassium iodide, dissolved in a small amount of water is added and the solution stirred and saturated with sulfur dioxide while the temperature is maintained at 35°. The arsine oxide precipitates slowly in the form of a white powder. After standing for twenty-four hours it is filtered off, washed with ice water and dried *in vacuo*.

The compound is soluble in dilute sodium hydroxide solution, and in the dissolved state it reduces Fehling solution. Hydrochloric or acetic acid precipitates the product from an alkaline solution.

Anal. Calcd. for $C_9H_9ON_6As$: As, 25.64; N, 28.77. Found: As, 25.72; N, 28.74.

17. ***p,p'*-Bis-(2,4-diamino-s-triazinyl-6)-aminoarsenobenzene.**—A mixture prepared from 30 g. of sodium hypophosphite, which had been dissolved in 60 cc. of 38% hydrochloric acid, 200 cc. of methanol and 0.5 cc. of 48% hydriodic acid is added, in a current of nitrogen, to 20 g. of *p*-(2,4-diamino-s-triazinyl-6)-aminophenylarsonic acid which had been dissolved in 300 cc. of 4% hydrochloric acid. During the addition, the solution of the arsonic acid is stirred and maintained at 70°. A yellow precipitate is formed which is filtered off and washed with water, methanol and ether and dried *in vacuo*. It is insoluble in water, methanol and ether. It remains unchanged when heated to 230°. It starts to discolor at higher temperatures and chars at around 250°.

Anal. Calcd. for $C_{18}H_{18}N_{12}As_2$: As, 27.13; N, 30.44. Found: As, 27.09; N, 30.37.

Summary

The introduction of the s-triazine ring into the amino group of arsanilic acid enhances the trypanocidal and spirochetocidal properties of this compound, provided that at least one unsubstituted amino group is attached to a carbon atom of the triazine ring.

The optimum effect is obtained in *p*-(2,4-diamino - s - triazinyl - 6) - aminophenylarsonic acid. The corresponding arsinoxide and arseno compound are described.

160 WEST 59TH STREET
NEW YORK 19, N. Y.

RECEIVED JULY 3, 1944

[CONTRIBUTION FROM THE NATIONAL MEDICAL COLLEGE OF SHANGHAI]

The Constituents of Fritillaria roylei[1]

BY YUN-HSI WU

T. Q. Chao[2] isolated two alkaloids from the roots of the *Fritillaria roylei*, the source of one variety of the common Chinese drug Pei-Mu growing in Chekiang, China. Chao named the alkaloids peimine and peiminine, and K. K. Chen studied their pharmacology.[3] An extensive analytical characterization of peimine was conducted by Chi, Kao and Chang.[4] Chao,[5] on investigating Pei-Mu of the Szechuan variety, encountered an entirely different active principle, fritimine.

In the present investigation of *Fritillaria roylei*, the author has further characterized the alkaloids peimine and peiminine and has succeeded in isolating a hitherto unknown neutral, nitrogen-free product which appears to be related to the alkaloids and for which the name propeimin is suggested.

Peimine.—Chao[2] suggested for this alkaloid the formula $C_{19}H_{30}O_2N$, but the value which he found for nitrogen was over one per cent. higher than subsequently established.[4] Chi, Kao and Chang[4] reported a total of twenty-six analyses of peimine and its salts and on the basis of the results assigned the formula $C_{26}H_{43}O_3N$.

In the present investigation the yield, 1.5 g. of peimine from 50 kg. of drug, was the same as that given by Chi, Kao and Chang. Although the previous workers state that the alkaloid is inactive, the preparation showed a rotation of $[\alpha]^{18}D$ −19.2°. Peimine is a saturated alkaloid. It contains neither methylimide nor methoxyl groups. A Zerewitinoff determination indicated the presence of two active hydrogen atoms per mole. The analyses are in better accord with the formula $C_{27}H_{45}O_3N$ than with that given by Chi, Kao and Chang, as shown in the following summary

(1) The author is greatly indebted to Professor A. Butenandt, director of the Kaiser-Wilhelm Institut für Biochemie, for his valuable suggestions in carrying out the work, to Professor G. Oddo of the University of Palermo for sending a sample of solanidine-s for comparison, and to Professor L. F. Fieser of Harvard University for arranging this paper for publication.

(2) Chao, *Chinese J. Physiol.*, **6**, 265 (1932).

(3) Ref. 2, note by K. K. Chen.

(4) Chi, Kao and Chang, THIS JOURNAL, **58**, 1306 (1936).

(5) Chao, *Chinese J. Physiol.*, **7**, 41 (1933).