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THE TRYPANOCIDAL ACTIVITY OF SOME PYRIMIDYL-AMINOPHENYLARSONIC COMPOUNDS

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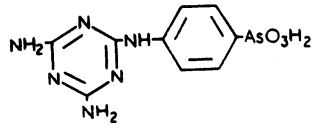
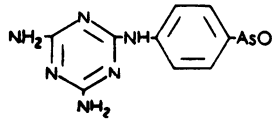
A series of substituted pyrimidylaminophenylarsonic acids have been prepared, reduced to the corresponding oxophenylarsines and finally, in certain cases, to the arsenobenzenes. The tervalent arsenicals were highly active against *Trypanosoma rhodesiense* in mice. 4-(2:6-Diaminopyrimid-4-ylamino)phenyloxoarsine either as the insoluble carbonate or the soluble isethionate was the most promising of these compounds.

The announcement by Friedheim in 1939 of the new arsenical drug, melarsen, for the treatment of human trypanosomiasis followed by the introduction in 1944 of the more highly active derivative, melarsen oxide, stimulated work in the field of arsenical compounds containing heterocyclic nuclei. Although most of the early work had been carried out against *Trypanosoma equiperdum* in mice, Friedheim stated in 1941 that he had obtained favourable results in the treatment of man infected with *T. gambiense*.



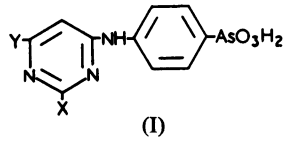
Melarsen



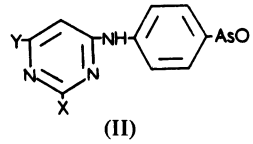
Melarsen oxide

Following the publication of the account of melarsen oxide, Banks and Controulis (1946) described analogous pyrimidylaminophenylarsonic acids and some derivatives of these compounds containing tervalent arsenic. However, the trypanocidal activity of these compounds against *T. equiperdum* in rats was inferior to that of the previously prepared arsenicals of the anilinotriazine series.

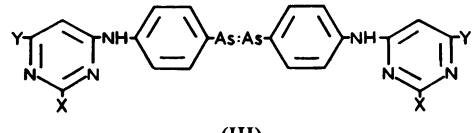
Our interest lay in determining the activity of arsenical compounds containing a pyrimidine nucleus against *T. rhodesiense* and so assessing their possible value for the treatment of human trypanosomiasis. Accordingly a series of pyrimidylaminophenylarsonic acids were prepared having various substituents in the 2- and 4-positions of the pyrimidine nucleus (I) as well as compounds containing a pyrimidinium radical. Some of these compounds were reduced (*a*) by treatment with sulphur dioxide and hydriodic acid to the corresponding oxophenylarsines (II) and (*b*) by hypophosphorous acid to the *pp'*-di(pyrimidylamino)arsenobenzenes (III). All the compounds were then tested biologically.



(I)



(II)



(III)

METHODS AND MATERIALS

Chemical

The compounds described in Tables I to V were prepared by the general procedures given for these types of substances. Analytical details are given in Table VI. In the cases where the preparative method differed appreciably from the general method the preparations are described in detail.

Pyrimidylaminophenylarsonic Acids (I)

Preparation.—The substituted pyrimidine (0.1 mole), arsanilic acid (0.1 mole), water (120 ml.) and concentrated hydrochloric acid (10 ml.) were boiled under reflux for 1.5 hr. After cooling the solid was collected and crystallized from water containing some hydrochloric acid (acid strength approximately 0.1N) to give the hydrochloride of the required pyrimidylaminophenylarsonic acid.

2-Amino-4-(p-arsonophenylamino)-1:6-dimethylpyrimidinium Chloride. — 2-Amino-4-chloro-1:6-dimethylpyrimidinium iodide (5.72 g.) was dissolved in hot water (50 ml.) and shaken vigorously for 3 min. with freshly prepared silver chloride (14.3 g.). The hot suspension was filtered, the filtrate added to the hot solution of arsanilic acid (4.34 g.) in a mixture of water (28 ml.) and concentrated hydrochloric acid (2 ml.) and this mixture boiled under reflux for 15 min. Excess sodium chloride was added and the whole cooled in ice when colourless prisms separated. These were collected and crystallized from 0.2N aqueous hydrochloric acid when colourless needles of 2-amino-4-(*p*-arsonophenylamino)-1:6-dimethylpyrimidinium chloride monohydrate were obtained, m.p. 336 to 337° with decomposition. (Found: C, 36.5; H, 4.9; N, 13.9; As, 19.1. $C_{12}H_{16}O_3N_4AsCl,H_2O$ requires: C, 36.7; H, 4.6; N, 14.3; As, 19.1%.) This substance lost hydrogen chloride on crystallization from water, particularly if the *p*H of the solution was adjusted to 5 by the addition of dilute sodium carbonate solution, to give the anhydro-base in the form of heavy, small prisms which were insoluble in water and had m.p. 344° (decomp.). The quaternary chloride was readily reformed on treatment with dilute hydrochloric acid.

2:6-Diamino-4-(p-arsonophenylamino)-1-methylpyrimidinium Chloride. — 2:6-Diamino-4-chloro-1-methylpyrimidinium iodide (5.7 g.) was dissolved in boiling water (120 ml.) and the hot solution shaken with excess freshly precipitated silver chloride. The mixture was filtered and to the filtrate arsanilic acid (8.7 g.) was added and the whole boiled under reflux for 5 hr. Fine colourless needles separated on cooling; these were collected and repeatedly crystallized from 1% aqueous hydrochloric acid when 2:6-diamino-4-(*p*-arsonophenylamino)-1-methylpyrimidinium chloride monohydrate was obtained, m.p. 265° (decomp.). (Found: C, 33.6; H, 4.8; N, 17.9; As, 19.1. $C_{11}H_{15}O_3N_5AsCl,H_2O$ requires: C, 33.6; H, 4.2; N, 17.8; As, 19.1%.)

2:4-Diamino-6-(p-arsonophenylamino)-1-methylpyrimidinium Chloride. — 2:4-Diamino-6-chloro-1-methylpyrimidinium iodide (5.7 g.) was converted to the corresponding chloride as above, the aqueous solution added to the solution of arsanilic acid (8.7 g.) in 4% sodium hydroxide solution (40 ml.) and the whole boiled under reflux for 5 hr. The solution was then treated with decolorizing carbon, filtered, acidified to congo red paper with hydrochloric acid, excess sodium chloride added and finally cooled in ice. The crystalline material which separated on cooling was collected and the filtrate allowed to stand for 2 days. The solid which slowly separated was filtered off, extracted with 10% aqueous sodium carbonate solution, filtered from a trace of insoluble matter, acidified with acetic acid and the solution saturated with sodium chloride. The solid which slowly crystallized was filtered and repeatedly crystallized from 1% aqueous hydrochloric acid to yield 2:4-diamino-6-(*p*-arsonophenylamino)-1-methylpyrimidinium chloride monohydrate, m.p. 289° (decomp.). (Found: C, 33.6; H, 4.3; N, 17.2; As, 18.8. $C_{11}H_{15}O_3N_5AsCl,H_2O$ requires: C, 33.6; H, 4.3; N, 17.8; As, 19.1%.)

2-(p-Arsonophenylamino)-4:6-dimethylpyrimidine. — 2-Chloro-4:6-dimethylpyrimidine (2.85 g.) was added to a solution of arsanilic acid (4.34 g.) in water (100 ml.) containing 8% aqueous hydrochloric acid (10 ml.) and the mixture boiled under reflux for 10 min. Sufficient sodium hydroxide solution was added to dissolve the crystalline material which separated on cooling, the solution was filtered and the filtrate acidified with acetic acid. The white solid was collected and crystallized from 8% aqueous hydrochloric acid when 2-(*p*-arsonophenylamino)-4:6-dimethylpyrimidine hydrochloride was obtained as colourless needles, m.p. 246° (decomp.). (Found: C, 40.1; H, 4.1; N, 11.7; As, 20.9. $C_{12}H_{14}O_3N_3As,HCl$ requires: C, 40.1; H, 4.2; N, 11.7; As, 20.9%.)

2-Amino-5-(4-arsono-2-nitrophenylamino)-4:6-dimethylpyrimidine. — 4-Chloro-3-nitrophenylarsonic acid (35.2 g.) was suspended in water (250 ml.), sufficient sodium hydroxide added until the solution was neutral to litmus, 2:5-diamino-4:6-dimethylpyrimidine (17.5 g.) added and the whole boiled under reflux for 3 hr. The solution was made just acid to litmus with hydrochloric acid and cooled. The crude product was collected (50 g.) and crystallized from water when 2-amino-5-(4-arsono-2-nitrophenylamino)-4:6-dimethylpyrimidine dihydrate was obtained as buff-orange needles, m.p. 180° (decomp.). (Found: C, 34.3; H, 3.6; N, 16.6; As, 18.2. $C_{12}H_{14}O_5N_5As,2H_2O$ requires: C, 34.4; H, 4.2; N, 16.7; As, 17.8%.)

Oxo(pyrimidylaminophenyl)arsines (II)

General Preparation.—The pyrimidylaminophenylarsonic acid was dissolved in 12 volumes of 20% hydrochloric acid, potassium iodide (0.5 g.) added, the mixture warmed to 60° and sulphur dioxide passed in until no iodine was liberated on standing for 30 min. with the stream of sulphur dioxide stopped. The solid which separated was collected, dissolved in a slight excess of sodium hydroxide solution, filtered and the filtrate neutralized by the addition of dilute hydrochloric acid.

In the cases of the 2:4-diamino- or methylaminopyrimidine derivatives the above conditions were unsatisfactory, the modified conditions described below for the preparation of (*p*-2:4-diaminopyrimid-6-ylaminophenyl)oxoarsine being typical of the method for those derivatives.

(p-2 : 4-Diaminopyrimid-6-ylaminophenyl)oxoarsine.—*p* - (2 : 4 - Diaminopyrimid - 6 - ylamino)phenylarsonic acid (14.4 g.) was dissolved in concentrated hydrochloric acid (500 ml.) and potassium iodide (0.5 g.) dissolved in water (5 ml.) added. The stirred solution was warmed to 40° and sulphur dioxide passed through the mixture at this temperature for 2 hr. The mixture was then allowed to stand, the supernatant liquor decanted and the residue suspended in water (200 ml.). The mixture was made alkaline to brilliant yellow by the addition of 10% aqueous sodium carbonate solution (50 ml.), filtered and the solid dissolved in 2% aqueous sodium hydroxide solution (250 ml.). Carbon dioxide was then passed through this solution until it was no longer alkaline to Clayton yellow indicator, the precipitated solid collected, well washed with water, and dried.

(p-2 : 4-Diaminopyrimid-6-ylaminophenyl)oxoarsine Isethionate.—(*p* - 2 : 4 - Diaminopyrimid - 6 - ylaminophenyl)oxoarsine carbonate (5 g.) was suspended in water (50 ml.) and isethionic acid added until the resulting solution was neutral to litmus. After filtration from a trace of insoluble material the filtrate was evaporated to dryness *in vacuo*, the residue dissolved in ethyl alcohol, and the solution diluted slowly with ether when (*p*-2 : 4-diaminopyrimid-6-ylaminophenyl)oxoarsine isethionate separated as insoluble prism aggregates, m.p. 108° (eff.) after shrinking at 74 to 75°.

2 - Amino-4-(p-arsenosophenylamino)-1 : 6-dimethylpyrimidinium Iodide.—(*p*-2-Amino-6-methylpyrimid-4-ylaminophenyl)oxoarsine (8.7 g.) was suspended in β-ethoxyethanol (100 ml.), methyl iodide (4.0 ml.) added and the whole heated under reflux in the steam bath for 5 hr. After cooling the mixture was ground with acetone, filtered, and crystallized from water when 2 - amino - 4 - (*p*-arsenosophenylamino)-1 : 6-dimethylpyrimidinium iodide hemihydrate was obtained as colourless prisms, m.p. 248 to 250° (decomp.). (Found: N, 12.9; As, 16.9. $C_{12}H_{14}ON_4AsI,\frac{1}{2}H_2O$ requires: N, 12.7; As, 17.0%.)

pp'-Di(pyrimidylamino)arsenobenzene Derivatives (III)

General Preparation.—A solution of hypophosphorous acid (prepared from sodium hypophosphite (15 g.), concentrated hydrochloric acid (30.0 ml.), methyl alcohol (200 ml.) and hydriodic acid (0.5 ml.)) was added slowly during 25 min. to a suspension of the requisite pyrimidylaminophenylarsonic acid (0.03 mole) in 3.5% aqueous hydrochloric acid (250 ml.) previously heated to 70 to 75°, the whole operation being carried out in an atmosphere of nitrogen. The mixture rapidly turned yellow and an orange precipitate was formed. After heating for 1 hr., the mixture was cooled, filtered, and the residual solid well washed with absolute ethyl alcohol and finally with ether. The product so obtained is the required dihydrochloride.

The base was prepared by suspending the hydrochloride in water, adding sodium hydroxide solution until the mixture was just alkaline to Clayton yellow, filtering and washing thoroughly with water.

pp' - Di(2 - amine - 1 : 6 - dimethyl - 4 - pyrimidinium - amino)arsenobenzene Diphosphate.—This compound was prepared similarly from 2-amino-4-(*p*-arsenosophenylamino)-1 : 6-dimethylpyrimidinium chloride and was obtained as a reddish-orange powder, m.p. 249 to 250° (decomp.). (Found: C, 36.1; H, 4.6; N, 13.8; As, 18.7. $C_{24}H_{32}O_8N_8P_2As_2,1\frac{1}{2}H_2O$ requires: C, 36.0; H, 4.4; N, 14.0; As, 18.8%.)

TABLE I

ACTIVITY AGAINST *T. RHODESIENSE* OF PYRIMIDYLAMINOPHENYLARSONIC ACIDS OF THE GENERAL FORMULA (I)

X and Y, see formula I. P = poisoned. C = cure (no trypanosomes observed in the blood during an observation period of 35 days). R = relapse (blood temporarily cleared of trypanosomes). VSL = very slight retarding effect on the course of the infection. NA = no action.

Comp. No.	X	Y	Salt	Results after a Single Subcutaneous Dose (Doses in mg./20 g.)			
				10	5	2·5	1
10,216	NH_2	CH_3	$HCl,\frac{1}{2}H_2O$	NA			
10,217	NH_2	H	HCl,H_2O	12C 2R	1C 3R	4R	
10,263	NH_2	Cl		P	NA		
10,362	NH_2	NH_2	HCl,H_2O	4C	7C 1R	2C 2R	
14,373	$NHCH_3$	$NHCH_3$	HCl			P	VSL
14,375	NH_2	$NHCH_3$	HCl		4C		1C 3R
14,376	H	$NHCH_3$	HCl,H_2O	6R			

TABLE II

ACTIVITY AGAINST *T. RHODESIENSE* OF OXO(PYRIMIDYLAMINOPHENYL)ARSINES OF THE GENERAL FORMULA (II)

X and Y, see formula II. * Doses of base. C = cure. R = relapse. VSL = very slight retarding effect on the infection.

Comp. No.	X	Y	Salt, Derivative	Results after a Single Subcutaneous Dose (Doses in mg./20 g.)						
				0·5	0·25	0·1	0·05	0·025	0·01	0·005
10,185	NH_2	H	$2HCl,\frac{1}{2}H_2O$	6C	4C	9C 1R	12C 3R	4C 6R	8R	
10,218	NH_2	CH_3				9C 2R	6C 5R	2C 8R	6R	
11,478	NH_2	CH_3	Dithioglycollate, H_2O			15C 1R	5C 5R	9R	10R	
11,523	NH_2	CH_3	1:2-Dimercaptopropanol, HCl		Toxic	3C 3R	4C 1R			
12,065/1	NH_2	NH_2	Carbonate, $2H_2O$				12C	4C 1R		
12,065/2*	NH_2	NH_2	Isethionate, $2H_2O$				35C	45C 2R	24C 5R	9C 6R
11,524	NH_2	Cl	1:2-Dimercaptopropanol	3P 1R	6VSL					
14,374	CH_3NH	CH_3NH	$\frac{1}{2}H_2O$		8C 4R	1C 8R				
14,377	NH_2	CH_3NH	H_2O			4C	9C	7C 1R	4R	
14,378	H	CH_3NH	$2H_2O$		5C 1R	4C 4R	4R			
16,523	CH_3NH	NH_2	H_2O		6C	12C	15C 1R	3C 2R		

TABLE III

ACTIVITY AGAINST *T. RHODESIENSE* OF DI(PYRIMIDYLAMINO)ARSENOBENZENES OF THE GENERAL FORMULA (III)

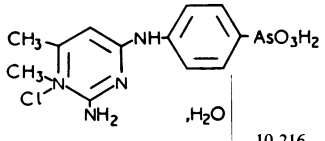
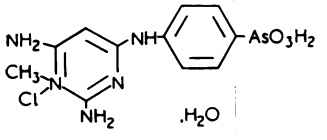
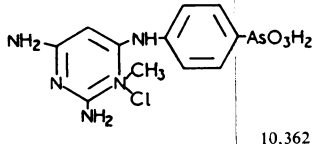
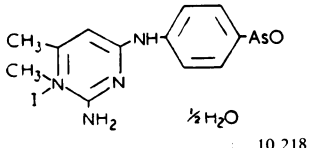
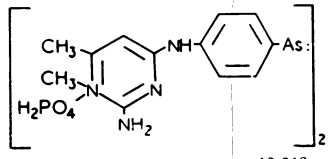
X and Y, see formula III. C = cure. R = relapse. VSL = slight retarding effect on the course of the infection. NA = no action.

Compound No.	X	Y	Salt	Results after a Single Subcutaneous Dose (Doses in mg. 20 g.)				
				0·25	0·1	0·05	0 025	0·01
10,670	NH_2	CH_3	$2HCl,3\frac{1}{2}H_2O$	4C	14C 13R	5C 23R	6R	
11,444	NH_2	H	$2HCl,3\frac{1}{2}H_2O$	4C	15C 1R	18C 6R	5C 15R	1C 7R
11,476	NH_2	Cl	$1\frac{1}{4}H_2O$	VSL	NA			
11,477	NH_2	NH_2	$1\frac{1}{2}H_2O$		20C	16C 1R	13C 4R	5C 7R

TABLE IV

ACTIVITY AGAINST *T. RHODESIENSE* OF QUATERNIZED ARSENICALS

See Table I for explanation of letters in results columns.

No. and Formula	Related to	Results after a Single Subcutaneous Dose (Doses in mg. 20 g.)				
		10	5	0·5	0·25	0·1
10,260 	10,216 (Table I)		NA			
10,669 	10,362 (Table I)	NA				
11,525 	10,362 (Table I)	VSL				
10,363 	10,218 (Table II)			3P 1C	10C 6R	7R
11,005 	10,218 (Table II)				3P 5C 2R	3C 6R

Biological

Tests for trypanocidal action were made against the Tinde (Tanganyika) strain of *T. rhodesiense*, the Busimbi (Uganda) strain of *T. congolense*, and a South American strain of *T. cruzi*. Mice were infected intraperitoneally (*T. rhodesiense* and *T. congolense*) or subcutaneously (*T. cruzi*) and, when trypanosomes were observed in the blood to the extent of 1 in 2 to 2 in 1 trypanosomes/high power field (1/6 in. objective and a ×6 ocular) of a wet thick blood film, the mice were treated subcutaneously by the injection of 0.5 ml. of a suspension or solution of the compound under test. Infections with *T. rhodesiense* and *T. congolense* were treated with one dose only; *T. cruzi* was given three to five treatments on successive days.

RESULTS

None of the compounds prepared exhibited any action against *T. cruzi* and only slight action at doses approaching the maximum tolerated was obtained against *T. congolense*. Against *T. rhodesiense*, however, several of them showed outstanding activity. The results obtained with this species are listed in Tables I to V. No figures for toxicity are given because this was not done in detail, but in general it can be stated that the arsonic acids (Table I) are less toxic than the oxoarsines (Table II). With the former, doses of 10 mg./20 g. could be given subcutaneously except in the case of 14,373 which killed some mice at 2.5 mg., whereas most of the latter did not permit doses greater than 0.5 mg. The arsenobenzenes shown in Table III appeared to be a little less toxic than the corresponding oxoarsines, and the quaternized compounds in Table IV were possibly more toxic than the unquaternized.

TABLE V

ACTIVITY AGAINST *T. RHODESIENSE* OF PYRIMIDYL-AMINOPHENYLARSONIC ACIDS LINKED IN THE 2- AND 5-POSITIONS

VSL, see Table I for explanation.

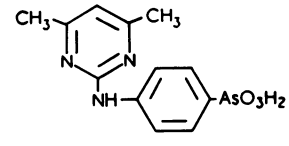
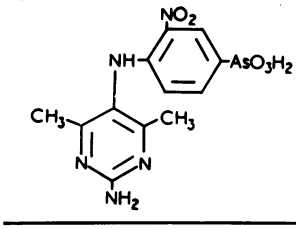
No. and Formula	Results after a Single Subcutaneous Dose (Doses in mg./20 g.)	
	10	2·5
10,219 		VSL
10,364 	VSL	

TABLE VI

ANALYTICAL DETAILS OF THE COMPOUNDS DESCRIBED IN TABLES I TO III

Ref. No.	Melting Point °C.	Analysis Calculated				Analysis Found			
		C	H	N	As	C	H	N	As
Table I									
10,216	301 (dec.)	35·7	4·1	15·1	20·3	35·7	4·6	14·8	20·3
10,217	331 ,,	32·9	3·8	15·4	20·6	32·9	4·0	15·1	20·7
10,263	293 ,,	—	—	16·3	21·8	—	—	16·4	22·0
10,362	278 ,,	—	—	18·4	19·8	—	—	18·0	19·9
14,373	283–284 (dec.)	37·0	4·4	18·0	19·3	37·1	4·8	17·9	19·3
14,375	280 (dec.)	—	—	18·7	20·0	—	—	18·9	20·2
14,376	268 ,,	34·9	4·2	14·8	19·8	34·7	4·0	14·7	20·0
Table II									
10,185	185–186	33·5	3·4	15·6	20·9	33·7	3·7	14·9	20·7
10,218	200–201 (dec.)	42 9	4·2	18·2	24·3	43·0	3·7	18·3	24·3
11,478	248 ,,	38·0	4·0	11·8	15·8	38·5	3·8	11·5	15·3
11,523	129–130 ,,	37·3	4·4		16·7	36·9	4·5		17·1
12,065 carbonate	273–275 ,,	35·2	4·2	19·5	20·9	35·3	4·0	19·3	20·7
12,065 isethionate	108 ,,	31·8	4·4		16·6	31·4	4·2		16·6
11,524	193–194	37·5	3·4		18·0	37·9	3·6		17·8
14,374	130 (dec.)	43·9	4·6	21·3	22·9	43·6	4·4	21·1	23·0
14,377	100 ,,	40·9	4·3	21·7	23·2	41·3	4·6	21·3	24·0
14,378	110 ,,	40·5	4·6	17·2	23·0	41·6	4·6	17·2	23·0
16,523	195–200 (dec.)	40·8	4·3		23·3	40·3	4·4		23·6
Table III									
10,670	270–275 ,,	38·6	4·5	16·4	21·9	38·7	4·4	16·7	21·8
11,444	260–262 ,,	36·6	4·1		22·9	36·4	4·2		22·9
11,476	224–227 ,,	39·0	3·1	18·2	24·4	38·6	2·8	18·2	24·5
11,477	286–287 ,,	41·6	4·0		26·0	41·2	3·6		26·0

The main conclusions to be drawn from the results listed in the tables are as follows:

(1) The oxoarsines (Table II) are much more active than the corresponding arsonic acids (Table I). The best results were achieved with 12,065 which has an amino group in both the 2- and 4-positions of the pyrimidine ring. Such interference as was made with either of these groups, and particularly the one in the 2-position, lessened activity. The compound 12,065 was selected for further work and it has been shown to be highly effective against infections of *T. rhodesiense* established in the nervous system of monkeys. This work will be reported separately.

(2) The di(pyrimidylamino)arsenobenzenes (Table III) have the same degree of activity as the corresponding oxoarsines, and it is probable that they break down readily to the oxoarsines.

(3) Such quaternizing as was done (Table IV) lessened activity. This is shown particularly when the activity of 10,363 is compared with that of 10,218.

(4) Linking the pyrimidine moiety in the 2- or 5-positions also lessened activity (Table V).

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