371. *Trypanocidal Action and Chemical Constitution. Part XIII. Arylthioarsinites from Cysteine and Glutathione.*

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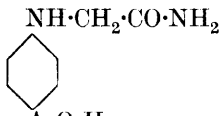
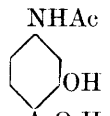
In Part X (J., 1931, 3043) it was shown that acetanilide- and benzamide-*p*-arsenoxides could be converted into thioarsinites of greater therapeutic efficiency by condensation with thiol compounds. A study of the full protocols of these substances shows that the best is di(β-amino-β-carboxyethyl)benzamide-*p*-thioarsinite, $NH_2 \cdot CO \cdot C_6H_4 \cdot As[S \cdot CH_2 \cdot CH(NH_2) \cdot CO_2H]_2$.

The favourable therapeutic properties of this thioarsinite rendered necessary an investigation of the effect of condensing cysteine with arsonic acids of recognised therapeutic value such as *N*-phenylglycineamide-*p*-arsonic acid (I; "tryparsamide"), 4-acetamido-2-hydroxyphenylarsonic acid (II; "orsanine"), and 3-amino-4-hydroxyphenylarsonic acid (III) from which salvarsan is derived.



(I.)



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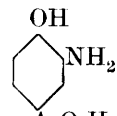

(III.)

As the tripeptide, glutathione, contains cysteine as one of its component amino-acids, and as moreover it plays an important rôle in one theory of the mode of action of arsenicals, it was also decided to condense the arsenoxides of these acids with glutathione.

N-Phenylglycineamide-*p*-arsenoxide or the dichloroarsine cannot be prepared by reduction of (I) in acid solution, since arsenic is split off from the nucleus. *N*-Phenylglycine-*p*-arsonic acid can, however, be reduced to phenylglycine-*p*-dichloroarsine (D.R.-P. 251104), but when this substance is esterified with methyl-alcoholic hydrogen chloride, only methyl phenylglycine is obtained. Fortunately the ester, methyl phenylglycine-*p*-arsonic acid, can be reduced to methyl phenylglycine-*p*-dichloroarsine, isolated as its *arsenoxide*, and this ester on treatment with concentrated aqueous ammonia gives the required N-*phenylglycineamide*-p-*arsenoxide*. This arsenoxide is a relatively labile substance, for when warmed in suspension in 3% acetic acid it is converted into *tri*(*phenylglycineamide*)*arsine* (IV),

which on oxidation with iodine gives a well-crystallised *tri(phenylglycineamide)arsine oxide dihydrate* (V).

$(NH_2{\cdot}CO{\cdot}CH_2{\cdot}NH{\cdot}C_6H_4)_3As$ (IV.) $(NH_2{\cdot}CO{\cdot}CH_2{\cdot}NH{\cdot}C_6H_4)_3AsO,2H_2O$ (V.)

This behaviour finds a parallel in the case of *p*-aminophenylarsine oxide, which under more severe conditions gives triaminotriphenylarsine (Ehrlich and Bertheim, *Ber.*, 1910, **43**, 923).

Phenylglycineamide-*p*-arsenoxide condenses smoothly with cysteine to give crystalline *di*(β-*amino*-β-*carboxyethyl*) N-*phenylglycineamide*-p-*thioarsinite* and with glutathione to give the amorphous *diglutathionyl* derivative.

The alternative method for the preparation of thioarsinites as employed by Barber (J., 1929, 1020), namely, treatment of an arsonic acid with 4 molecular proportions of a thiol compound, failed in the case of phenylglycineamide-*p*-arsonic acid (I) and cysteine, since the two normal products, di(β-amino-β-carboxyethyl) phenylglycineamide-*p*-thioarsinite and cystine, are non-separable by any simple means and more severe treatment leads to the formation and isolation of tricysteinylarsine $[CO_2H{\cdot}CH(NH_2){\cdot}CH_2{\cdot}S]_3As$, and cystine.

4-Acetamido-2-hydroxyphenylarsenoxide can be obtained in 50% yield by cautious reduction of the corresponding arsonic acid (II) at 0°, and it condenses readily in aqueous solution with glutathione and with cysteine to give *di*(*glutathionyl*) 4-*acetamido*-2-*hydroxyphenylthioarsinite* and *di*(β-*amino*-β-*carboxyethyl*) 4-*acetamido*-2-*hydroxyphenylthioarsinite* respectively. The non-acetylated parent substance, 4-amino-2-hydroxyphenylarsonic acid, cannot be reduced to the arsenoxide in acid solution without loss of arsenic from the nucleus, and this instability of the arsenoxide is so great that when the arsonic acid, as the monosodium salt, is treated, at room temperature throughout, with 4 molecular proportions of cysteine hydrochloride, tricysteinylarsine is formed.

The remaining arsonic acid, 3-amino-4-hydroxyphenylarsonic acid (III), can be readily reduced to 3-amino-4-hydroxyphenyldichloroarsine, from which the free oxide can be obtained. The latter condenses with cysteine and with glutathione to yield *di*(β-*amino*-β-*carboxyethyl*) and *di*(*glutathionyl*) 3-*amino*-4-*hydroxyphenylthioarsinites* respectively.

A summary of the results obtained when these thioarsinites are tested on an experimental infection of *Trypansoma equiperdum* in mice is given in the following table, *T* signifying the maximum dose tolerated, expressed in milligrams per gram of mouse, *C* the minimum curative dose, and *r* the number of days during which the bloodstream remains free from trypanosomes.

	T.	*C.*	*r.*
Di(β-amino-β-carboxyethyl) phenylglycineamide-*p*-thioarsinite	0·09	0·03	>30
Diglutathionyl phenylglycineamide-*p*-thioarsinite	0·35	0·075	>30
Di(β-amino-β-carboxyethyl) 4-acetamido-2-hydroxyphenylthioarsinite	0·05	0·04	23
Diglutathionyl 4-acetamido-2-hydroxyphenylthioarsinite	0·15	0·01	>30
4-Acetamido-2-hydroxyphenylarsonic acid	1·0	0·15	>30
Di(β-amino-β-carboxyethyl) 3-amino-4-hydroxyphenylthioarsinite	0·07	0·02	>30
Diglutathionyl 3-amino-4-hydroxyphenylthioarsinite	0·2	0·025	>30
3-Amino-4-hydroxyphenylarsenoxide	0·04	0·008	>30
Di(carboxymethyl) phenylglycineamide-*p*-thioarsinite	0·05	0·02	>30
Di(β-amino-β-carboxyethyl) benzamide-*p*-thioarsinite	0·1	0·01	>30
Benzamide-*p*-arsonic acid	1·0	0·3	>30
Tricysteinylarsine	0·025	[inactive]	

Of the new substances now described, only diglutathionyl 4-acetamido-2-hydroxyphenylthioarsinite equals or surpasses di(β-amino-β-carboxyethyl) benzamide-*p*-thioarsinite in therapeutic efficiency. Out of 58 infected mice treated with the former substance in doses between 0·01 and 0·15 mg. per g., 55 were "free" from trypanosomes at the end of 31 days, including 8 mice out of 9 receiving 0·01 mg. per g., the lowest dose so far tried. Out of 22 mice treated with di(β-amino-β-carboxyethyl) benzamide-*p*-thioarsinite on doses between 0·01 and 0·1, 21 were free from trypanosomes at the end of 30 days; on a dose of 0·005, 2 out of 4 mice had not relapsed at the end of 30 days.

It is proposed to test these two substances on different species of trypanosomes and to extend their trial to more generalised trypanosome infections such as occur in rabbits.

Experimental.

N-*Phenylglycine*-p-*arsenoxide Methyl Ester*.—*p*-Arsonophenylglycine methyl ester (36 g.) (MeO, 10·8. Calc., 10·7%) was added to 16% HCl aq. (360 c.c.), containing a trace of KI, at −5° and the suspension was saturated with SO_2 below 0° and kept for 24 hrs. The needles produced were washed with ice-cold 16% HCl aq. and added to sat. $NaHCO_3$ aq. (250 c.c.) below 0°, further solid being added if necessary to keep the reaction alkaline. The *arsenoxide*, washed with H_2O and dried in vac., was a pale primrose-yellow solid (24·7 g.) (Found in different preps.: As, 27·3, 27·3; MeO, 11·1, 11·0, 10·9. $C_9H_{10}O_3NAs$, H_2O requires As, 27·4; MeO, 11·4%). Titration with iodine in $NaHCO_3$ suspension is unsatisfactory owing to the very sparing solubility of the oxide.

N-*Phenylglycineamide*-p-*arsenoxide*.—The preceding methyl ester (24 g.) was added all at once to NH_3 aq. (200 c.c., *d* 0·88) at −10° in a stoppered bottle, and the temp. allowed to rise to that of the room; the viscous product became white and cryst. after a few days (yield, 16·5 g.) (Found: N, 11·1; As, 30·5. $C_8H_9O_2N_2As$ requires N, 11·7; As, 31·2. $C_8H_9O_2N_2As,\frac{1}{4}H_2O$ requires N, 11·5; As, 30·7%).

The product is difficult to free from adhering NH_3 and in one case digestion with warm 3% AcOH converted it into needles with considerable loss in wt. Fission of As had taken place, since the N : As ratio had now risen to 5·7 : 1. The cryst. solid was extracted (Soxhlet) with MeOH, and the residual *tri(phenylglycineamide)arsine* (9·0 g.) crystallised from formamide, separating in small needles, m. p. about 225° (decomp.) (Found: N, 15·8; As, 14·3. $C_{24}H_{27}O_3N_6As$ requires N, 16·1; As, 14·3%). On oxidation in suspension in $NaHCO_3$ aq. it consumed 97% of the calc. amount of $N/10$-I. The oxidation product, *tri(phenylglycineamide)arsine oxide*, separated in square anisotropic plates which required 55 vols. of boiling H_2O for its solution. The recryst. product separated in prisms which effervesced at about 160° [Found: C (micro), 50·1; H, 5·3; N (macro), 14·4; As, 13·0; H_2O, 6·3. $C_{24}H_{27}O_4N_6As$, $2H_2O$ requires C, 50·1; H, 5·4; N, 14·6; As, 13·0; H_2O, 6·3%].

Di(β-amino-β-carboxyethyl) Phenylglycineamide-p-thioarsinite.—Phenylglycineamide-*p*-arsenoxide (2·8 g.; As, 26·7%) was added to cysteine hydrochloride (3·15 g.) in water (50 c.c.). By gentle warming, a clear solution was obtained, which on addition of sat. Na_2CO_3 aq. to remove the acidity to Congo paper began to deposit a gelatinous solid. Abs. EtOH (50 c.c.) was immediately added and after being kept for a few hrs. at 0° the product (4·2 g.) was collected and washed with cold 50% EtOH. The amorphous product had $[\alpha]_{5461}$ + 9·1° (c = 0·4 in N-HCl) and As, 16·3 (calc. for anhydr. substance, 16·2%). It was obtained cryst. by solution in 250 c.c. of dil. HCl and removal of the acidity to Congo-paper by Na_2CO_3 aq. A gelatinous solid separated, but when kept for a week this crystallised completely in microscopic woolly needles (Found: N, 11·5, 11·6; As, 15·2. $C_{14}H_{21}O_5N_4S_2As$,$1\frac{1}{2}H_2O$ requires N, 11·4; As, 15·3%). $[\alpha]_{5461}$ + 9·3° (c = 0·46 in N-HCl). This thioarsinite gives a weak coloration with nitroprusside in $NaHCO_3$ aq.

p-*Arsonophenylglycineamide and Cysteine. Isolation of Tricysteinylarsine and Cystine Hydrochloride.*—"Tryparsamide" (12·2 g. of commercial Na salt) and cysteine hydrochloride (25·2 g.) were dissolved in warm H_2O (1 l.), and the reaction of the solution adjusted to acidity to litmus paper but non-acidity to Congo-paper. A cryst. crop soon began to separate and was collected after 24 hrs.; yield 22·5 g., $[\alpha]_{5461}$ − 75·6 (c = 0·4 in N-HCl). Many attempts to isolate the condensation product of cysteine and reduced "tryparsamide" from such a product failed. The course of fractionation can be followed polarimetrically, but cystine and di(β-amino-β-carboxyethyl) phenylglycineamide-*p*-thioarsinite appear to form a non-separable series of mixed crystals. When such a product was dissolved in cold 16% HCl aq., crude cystine hydrochloride, $[\alpha]_{5461}$ − 246°, separated, and from the mother-liquor by addition of Na_2CO_3 a product of rotation $[\alpha]_{5461}$ + 6·1°. When the latter product was recrystallised from boiling H_2O, it gave tricysteinylarsine, needles, m. p. 253—255° (decomp.). It gave a weak nitroprusside reaction in $NaHCO_3$ aq. (Found: C, 25·2; H, 4·0; N, 9·6; As, 17·5. Calc.: C, 24·8; H, 4·2; N, 9·6; As, 17·2%).

Attempted Esterification of N-*Phenylglycine*-p-*dichloroarsine. Isolation of* N-*Phenylglycine Methyl Ester.*—The hydrochloride of this dichloroarsine was dissolved in dry synthetic MeOH (5·8 g. in 40 c.c.) and saturated with HCl at 0° and then at the b. p. On removal of the solvent and addition of excess of sat. $NaHCO_3$ aq. a cryst. solid (2·0 g.) separated, m. p. 47°. It was free from As, sol. in Et_2O, and proved to be phenylglycine methyl ester (Found: OMe, 18·3. Calc., 18·8%).

Di(glutathionyl) Phenylglycineamide-p-thioarsinite.—To glutathione (3·18 g.; 97% purity by iodine titration) dissolved in H_2O (20 c.c.), phenylglycineamide-*p*-arsenoxide (1·25 g.) was added. On slight further dilution a clear solution was obtained, which was evaporated to a syrup over H_2SO_4 and then stirred with excess of EtOH. The pptd. amorphous *dithioarsinite* was dried in vac. (yield, 4·6 g.). It gave no reaction with nitroprusside in $NaHCO_3$ aq. (Found: As, 8·5, 8·6. $C_{28}H_{41}O_{13}N_8S_2As$ requires As, 8·9%).

4-Acetamido-2-hydroxyphenylarsenoxide.—The Na salt of the parent arsonic acid (8·35 g. of " Orsanine sodique ") was added to 16% HCl aq., containing a trace of KI, below 0°, and the solution saturated with SO_2 below 0°. The flask was sealed and the contents were kept below 0° for 3 days: the cryst. ppt. had then become replaced by an amorphous product. This was collected, washed with cold 16% HCl aq., and then added to sat. $NaHCO_3$ aq. (75 c.c.) at 0°. The finely divided solid was washed with H_2O (yield, 2·55 g.) (Found: N, 5·7; As, 29·8. Calc. for $C_8H_8O_3NAs,\frac{1}{2}H_2O$: N, 5·6; As, 30·0%). This oxide has also been described by Baranger (Thesis, Paris, 1931), but no yield is recorded.

Di(β-amino-β-carboxyethyl) 4-Acetamido-2-hydroxyphenylthioarsinite. — The preceding arsenoxide (0·6 g.) was added to cysteine hydrochloride (0·77 g.; 2 mols.) in H_2O (20 c.c.). The insol. arsenoxide soon changed to a weakly anisotropic gelatinous product. On removal of acidity to Congo-paper a further deposition of solid occurred. The total solid was washed with H_2O (yield, 1·0 g.) [Found: C (micro), 33·4; H, 5·4; H_2O, 9·0. $C_{14}H_{20}O_6N_3S_2As$, $2\frac{1}{4}H_2O$ requires C, 33·2; H, 4·9; H_2O, 8·0%]. The product gave a weak nitroprusside reaction in $NaHCO_3$ aq.

Di(glutathionyl) 4-Acetamido-2-hydroxyphenylthioarsinite—This was prepared as described above for the analogous product from phenylglycineamide-*p*-arsenoxide. The product was a fine white amorphous powder which retained H_2O tenaciously (Found: N, 10·3; As, 8·0, whence N : As = 6·9 : 1. $C_{28}H_{40}O_{14}N_7S_2As$ requires N, 11·7; As, 8·9%). The nitroprusside reaction was very weak in $NaHCO_3$ aq. but intense in NaOH aq.

Action of Cysteine Hydrochloride on 4-Amino-2-hydroxyphenylarsonic Acid. Isolation of Tricysteinylarsine.—The arsonic acid (0·96 g.), dissolved in *N*-NaOH (3·3 c.c.; 1 mol.), was added to cysteine hydrochloride (2·1 g.; 4 mols.) in H_2O (80 c.c.). On removal of the acidity to Congo-paper a solid (1·55 g.) gradually separated, $[\alpha]_{5461}$ — 67·4° (*c* = 0·42 in *N*-HCl). This crop was dissolved in the rotation liquors and made up to 40 c.c. with H_2O. When the acidity to Congo-paper was again removed, the product (1·25 g.) had $[\alpha]_{5461}$ — 60·4°. Repetition of the process, but adjustment of the reaction to faint acidity to Congo-paper, gave a product (0·7 g.), $[\alpha]_{5461}$ + 7·0°, crystallising in tufts of needles. Two further repetitions of the same process gave 0·4 g. and 0·3 g. with $[\alpha]_{5461}$ + 37·3° and 35·3° respectively. The product now crystallised very readily in fan-shaped tufts of needles which proved to be identical with tricysteinylarsine [Found: N (micro), 9·7. Calc.: N, 9·7%].

3-Amino-4-hydroxyphenyldichloroarsine Hydrochloride.—The parent arsonic acid (10 g.) was suspended in H_2O (90 c.c.), and a very small crystal of KI added, followed by conc. HCl (35 c.c.). The solution was saturated at 0° with SO_2, kept for some hrs. at 0°, and then resaturated. On addition of conc. HCl (100 c.c.) the hydrochloride of the required dichloroarsine separated in lustrous leaflets. The product (8·5 g.) when dried in vac. was anhydr. (Found: Cl, 35·9; N, 4·9; As, 25·6. $C_6H_6ONCl_2As,HCl$ requires Cl, 36·6;

N, 4·8; As, 25·8%). This *substance* has been previously described (D.R.-P. 272289), but no analyses are given.

Di(β-amino-β-carboxyethyl) 3-Amino-4-hydroxyphenylthioarsinite.—The preceding dichloroarsine (2·9 g.) was dissolved with 2 equivs. (3·1 g.) of cysteine hydrochloride in H_2O (100 c.c.), and the acidity to Congo-paper just removed. Within 10 mins. the solution had set to a gelatinous mass. When kept at 0° for 12 hrs., partial crystn. had set in on the surface, and when stirred and kept for a further 24 hrs., complete transformation to the cryst. form had taken place. The product (2·75 g.) was recrystallised from 200 c.c. of boiling H_2O and separated readily in asbestos-like needles (2·35 g.), m. p. 237—238° (decomp.). The nitroprusside reaction was very weak in $NaHCO_3$ aq., but intense in NaOH aq. [Found for solid dried in vac.: C (micro), 33·3; H, 4·7; N, 9·7; H_2O, 3·0. Calc. for $C_{12}H_{18}O_5N_3S_2As,\frac{1}{2}H_2O$: C, 33·3; H, 4·7; N, 9·7; H_2O, 2·1%]. $[\alpha]_{5461} + 10·2$ ($c = 0·43$ in *N*-HCl). The above method of preparation is superior to that of Johnson and Voegtlin (*J. Biol. Chem.*, 1930, **89**, 27), who give m. p. 225—227° (uncorr.). As stated by these authors, this substance can be accurately titrated in dil. acid solution with *N*/10-I.

Di(glutathionyl) 3-Amino-4-hydroxyphenylthioarsinite.—3-Amino-4-hydroxyphenylarsenoxide was obtained from the corresponding dichloroarsine hydrochloride by dissolving the latter (2·9 g.) in H_2O (10 c.c.) at 0° and slowly adding $NaHCO_3$ (2·6 g.). The pptd. arsenoxide was washed with a little ice-water and quickly transferred to a vacuum desiccator. The product (1·5 g.) was free from chloride and contained 35% of arsenic as shown by titration with iodine, corresponding to a 93% content of arsenoxide. Of this oxide, 0·6 g. was added to glutathione (1·85 g.) in H_2O (20 c.c.). When solution was complete, the clear liquid was evaporated over H_2SO_4 to a syrup, which was ground under abs. EtOH (yield, 2·2 g.) (Found: N, 11·4; As, 8·7, whence N : As = 7·05 : 1. $C_{26}H_{38}O_{13}N_7S_2As$ requires N, 12·3; As, 9·4%).

Di(carboxymethyl) Phenylglycineamide-p-thioarsinite.—Dithioacetic acid (3·64 g.) was reduced at 35—40° with Zn dust (3·0 g.) in 3*N*-HCl (40 c.c.) during 1 hr. The clarified liquid was treated with sodium phenylglycineamide-*p*-arsonate ("tryparsamide") (2·76 g.), and the acidity of the solution gradually reduced by addition of 2*N*-NaOH. The required thioarsinite slowly separated in prismatic needles, m. p. 90° in agreement with Barber's description (J., 1929, 1023). This method of preparation avoids the isolation of free thiolacetic acid, which is very sensitive to atmospheric oxygen.

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