CCCXIII.—*Trypanocidal Activity and Chemical Constitution. Part II. New Sulphur Derivatives of Aromatic Organic Arsenicals (cont.). Derivatives of 2-Thiolbenziminazole-5-arsinic Acid.*

By JOHN GARWOOD EVERETT.

THE outstanding trypanocidal value of 2-thiolbenziminazole-5-arsinic acid (I) and its arseno-compound (Part I, J., 1929, 674) made it seem possible that the free thiol (SH) group had some significance in the cure of trypanosomiasis. Accordingly, various derivatives of this acid have now been prepared, in which the hydrogen of the thiol group has been replaced by other groups.

(I.) HS·C<N—NH>C$_6$H$_3$·AsO$_3$H$_2$ [·S·C<N—NH>C$_6$H$_3$·AsO$_3$H$_2$]$_2$ (II.)

Benziminazole-5-arsinic acid 2-disulphide (II) was obtained in the form of its *monohydriodide* by oxidation of a dilute aqueous solution of (I) with iodine. The monohydriodide is crystalline and orange in colour, the free acid being white and amorphous. The non-arsenated analogue, *benziminazole 2-disulphide* (III), was prepared from 2-thiolbenziminazole in a similar manner, the disulphide itself being precipitated in this case.

2-*Carboxymethylthiolbenziminazole-5-arsinic acid* (IV) and the corresponding *carbamyl* compound (VI) were prepared by the inter-

(III.) [·S·C<N—NH>C$_6$H$_4$]$_2$ CO$_2$H·CH$_2$·S·C<N—NH>C$_6$H$_3$·AsO$_3$H$_2$ (IV.)

action of (I) with chloroacetic acid and chloroacetamide respectively. They did not decolorise iodine solution in presence of sodium bicarbonate, showing that the carboxymethyl (or carbamylmethyl) group is attached to sulphur, and not to nitrogen. On reduction with sodium hyposulphite they gave respectively 5 : 5′-*arseno*-(2-*carboxymethylthiolbenziminazole*) (V), soluble in sodium bicarbonate

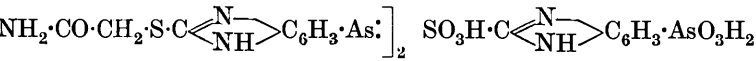
(V.) [CO$_2$H·CH$_2$·S·C<N—NH>C$_6$H$_3$·As:]$_2$

solution, and 5 : 5′-*arseno*-(2-*carbamylmethylthiolbenziminazole*) (VII), soluble in dilute sodium hydroxide solution. The acids (IV) and (VI) differed from (I) in being readily soluble in dilute mineral acids.

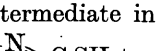
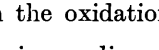
(VI.) NH$_2$·CO·CH$_2$·S·C<N—NH>C$_6$H$_3$·AsO$_3$H$_2$

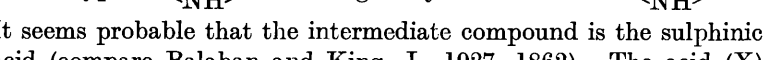
2-*Sulphobenziminazole-5-arsinic acid* (VIII) was obtained by oxidation of (I) with alkaline potassium permanganate solution.

On reduction with sodium hyposulphite it gave 5 : 5′-*arseno*-(*benziminazole*-2-*sulphonic acid*) (IX), soluble in sodium bicarbonate solution. The non-arsenated analogue, *benziminazole*-2-*sulphonic acid* (X), was obtained by oxidising similarly 2-thiolbenziminazole. The acids (VIII) and (X) are both very stable and extremely resistant to hydrolysis. This supports the view that the sulphonic



(VII.) (VIII.)

acid is not the intermediate in the oxidation of thiol compounds of the type  to give cyclic amidines  . It seems probable that the intermediate compound is the sulphinic acid (compare Balaban and King, J., 1927, 1862). The acid (X) is not hydrolysed at all by boiling with 25% sodium hydroxide

(IX.)  (X.)

solution for 2 hours, and only to the extent of 7·3% when heated with hydrochloric acid at 170° for 3 hours, the product being benziminazole (compare Lamb and Pyman, J., 1924, **125**, 707; Barnes and Pyman, J., 1927, 2712). The acid (VIII) shows 6·2% hydrolysis after boiling for 2 hours with 25% sodium hydroxide solution, and 40·8% hydrolysis when heated with hydrochloric acid at 170° for 3 hours. The action of 3% nitric acid on these two acids is being studied.

Therapeutic Results.—The above-mentioned compounds have been tested against an experimental infection of *T. equiperdum* in mice with the following results : T = maximum tolerated dose in mg./g. of mouse, c = minimum curative dose in mg./g. of mouse, r = average number of days elapsing between disappearance and reappearance of trypanosomes in the peripheral blood-stream, i = intravenous, o = oral.

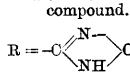
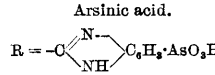
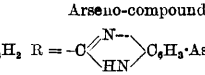
[*Note on the test for curative action.* Eight mice are used for the test. The minimum curative dose (c) is the smallest single dose which will cause the disappearance of all trypanosomes from the peripheral blood-stream of all eight mice for r days. The cure is usually temporary. Experience in this laboratory, however, has shown that when r is greater than 30 days the cure is permanent.

If c is greater than T, this indicates that disappearance of all trypanosomes has occurred in fewer than 8 mice, the number of mice in which it has occurred being given by the figure in parentheses.

2-Thiolbenziminazole, for example, does not cause the disappearance of all the trypanosomes in a single case, shown by the figure 0 in parentheses.]

		T.	c (8 mice).	T/c.	r.
2-Thiolbenziminazole-5-arsinic acid (I)	*i*	0·5	0·5	1·0	>30
	o	10·0	0·1	100·0	15
Benziminazole-5-arsinic acid 2-disulphide (II)	*i*	0·3	0·3	1·0	17
	o	10·0	0·1	100·0	20
2-Carbamylmethylthiolbenziminazole-5-arsinic acid (VI)	*i*	2·0	2·0	1·0	13
	o	5·0	4·0	1·25	9
2-Carboxymethylthiolbenziminazole-5-arsinic acid (IV)	*i*	2·0	>2·0 (0)	<1·0	—
	o	10·0	>10·0 (0)	<1·0	—
2-Sulphobenziminazole-5-arsinic acid (VIII)	*o*	1·0	> 1·0 (0)	<1·0	—
	o	10·0	>10·0 (0)	<1·0	—
5 : 5′-Arseno-(2-thiolbenziminazole)	*i*	0·1	0·005	20·0	>30
	o	>10·0	0·05	>200·0	12
5 : 5′-Arseno-(2-carbamylmethylthiolbenziminazole) (VII)	*i*	0·1	0·025	4·0	6
	o	5·0	2·5	2·0	9
5 : 5′-Arseno-(2-carboxymethylthiolbenziminazole) (V)	*i*	0·1	0·1	1·0	3
	o	5·0	5·0	1·0	20
5 : 5′-Arseno-(benziminazole-2-sulphonic acid) (IX)	*i*	0·1	0·1	1·0	7
	o	5·0	1·0	5·0	7
Non-arsenated compounds.					
2-Thiolbenziminazole	*i*	0·1	>0·1 (0)	<1·0	—
	o	1·0	>1·0 (0)	<1·0	—
Benziminazole-2-sulphonic acid (X)	*i*	1·0	>1·0 (0)	<1·0	—
	o	10·0	>10·0 (0)	<1·0	—

These results may be summarised as follows :

	Non-arsenated compound. 	Arsinic acid. 	Arseno-compound. 
R·SH	Inactive	Very active	Very active
R·S–S·R		Very active	
$R·S·CH_2·CO·NH_2$		Slightly active	Moderately active
$R·S·CH_2·CO_2H$		Inactive	Slightly active
$R·SO_3H$	Inactive	Inactive	Slightly active

Conclusions. A comparison of the arsinic acids with the parent acid (I) shows that only benziminazole-5-arsinic acid 2-disulphide (II) approaches it in therapeutic activity. This acid is very susceptible to hydrolysis, particularly in alkaline solution, giving the thiol acid (I), and on reduction it gives 5 : 5′-arseno-(2-thiolbenziminazole). It is therefore probably present in the blood-stream and tissues as the free thiol type R·SH. In the other cases replacement of the hydrogen of the thiol group causes adverse modification of therapeutic activity in both arsinic acid and arseno-compound.

B. Tuberculosis.—My thanks are due to Dr. Hesse, of the Pharmacological Institute, Göttingen, for kindly testing all the compounds mentioned in Part I of this series against *B. tuberculosis*. They were all inactive.

EXPERIMENTAL.

Benziminazole-5-arsinic Acid 2-Disulphide (II).—2-Thiolbenziminazole-5-arsinic acid (5·48 g.) was dissolved in water (300 c.c.) by boiling, cooled to 50°, *N*-iodine solution (20 c.c.) added all at once, and the flask rapidly cooled. The precipitate of rosettes of small, deep orange, prismatic needles proved to be *benziminazole-5-arsinic acid 2-disulphide monohydriodide* (yield, 3 g.; 45%) (Found: As, 22·3; N, 8·2; S, 9·6; I, 19·0. $C_{14}H_{12}O_6N_4S_2As_2,HI$ requires As, 22·2; N, 8·3; S, 9·5; I, 18·8%). The orange mother-liquor, on standing, rapidly became colourless, depositing 2-thiolbenziminazole-5-arsinic acid (2 g.).

The sodium salt of (II) was obtained in fine white needles by dissolving the monohydriodide in just sufficient sodium bicarbonate to make the solution neutral to litmus paper, and precipitating the salt with alcohol, in which sodium iodide is soluble. When a solution of the salt in a small amount of water was acidified with dilute sulphuric acid till faintly blue (Congo-red), it yielded *benziminazole-5-arsinic acid 2-disulphide* (II) as an orange amorphous powder (Found: As, 27·4; N, 10·3; S, 11·6. $C_{14}H_{12}O_6N_4S_2As_2$ requires As, 27·5; N, 10·3; S, 11·7%).

Benziminazole-5-arsinic acid 2-disulphide so obtained was insoluble in water, and soluble in sodium bicarbonate solution with effervescence. On reduction with sodium hyposulphite it gave 5 : 5′-arseno-(2-thiolbenziminazole). It was rapidly hydrolysed by cold dilute acids or alkalis, or even boiling water, with formation of 2-thiolbenziminazole-5-arsinic acid (I) (compare benziminazole 2-disulphide, III). This ready conversion into (I) is shown by boiling with thiolacetamide, the product being the thiolacetamide derivative of 2-thiolbenziminazole-5-arsinic acid (m. p. 245°, decomp.).

Thiolacetamide Derivative of 2-Thiolbenziminazole-5-arsinic Acid.—2-Thiolbenziminazole-5-arsinic acid (2·75 g.) was boiled with thiolacetamide (3·64 g.), the solution filtered hot, and cooled (compare Barber, J., 1929, 2335). The *thiolacetamide* derivative crystallised in tufts of fine needles. It was recrystallised from hot water; m. p. 245° (decomp.) (Found: As, 18·5; N, 13·8; S, 23·9. $C_{11}H_{13}O_2N_4S_3As$ requires As, 18·6; N, 13·9; S, 23·8%).

2-Carboxymethylthiolbenziminazole-5-arsinic Acid (IV).—Benziminazole-5-arsinic acid (27·4 g.) in water (100 c.c.) and chloroacetic acid (9·4 g.) in water (100 c.c.) were separately neutralised with sodium hydroxide solution, and the two solutions mixed and boiled. Alkalinity was maintained by the addition of sodium hydroxide solution (20%) as required. The resulting solution was

treated with charcoal, filtered, and hydrochloric acid added till faintly blue (Congo-red). The acid obtained was dissolved in sodium bicarbonate solution, and reprecipitated with hydrochloric acid (yield, 25 g.) (Found in acid dried at 80° : As, 22·6; N, 8·4; S, 9·8. $C_9H_9O_5N_2SAs$ requires As, 22·6; N, 8·5; S, 9·7%).

2-*Carboxymethylthiolbenziminazole-5-arsinic acid* so obtained occurred in rosettes of white needles, and gave no colour with an alkaline solution of sodium nitroprusside. It was insoluble in cold water, moderately easily soluble in hot water, and readily soluble in cold dilute mineral acids. The calcium salt formed microcrystalline rosettes and the barium salt was amorphous.

2-*Carbamylmethylthiolbenziminazole-5-arsinic Acid* (VI).—Prepared as described under (IV) above, except that solid chloroacetamide was used in place of a solution of chloroacetic acid, the temperature of reaction being 80°, the *acid* (VI) was obtained in bunches of colourless needles (yield, 90%) (Found in acid dried at 80° : As, 22·6; N, 12·5; S, 9·7. $C_9H_{10}O_4N_3SAs$ requires As, 22·7; N, 12·7; S, 9·7%). It resembled (IV) in solubility and in its behaviour with sodium nitroprusside. The barium salt formed rosettes of needles and the magnesium salt was amorphous.

2-*Sulphobenziminazole-5-arsinic Acid* (VIII).—A solution of 2-thiolbenziminazole-5-arsinic acid (5·6 g.) in water (25 c.c.) and sodium hydroxide solution (50%; 12 c.c.) was boiled, and potassium permanganate (6·4 g.) added gradually; a vigorous reaction ensued after each addition. Boiling was continued for 5 minutes. The filtered solution was made just acid with hydrochloric acid (Congo-red) and cooled in ice. The precipitated acid was removed, the filtrate concentrated further, and the combined precipitates recrystallised from glacial acetic acid (yield, 4 g.) (Found in acid dried at 100° : As, 23·2; N, 8·7; S, 9·9. $C_7H_7O_6N_2SAs$ requires As, 23·3; N, 8·7; S, 9·9%).

2-*Sulphobenziminazole-5-arsinic acid* so obtained occurred in bunches of fine white needles, very soluble in cold water. It did not absorb iodine in presence of excess of sodium bicarbonate solution, showing the absence of unchanged 2-thiol compound, and an acidified solution did not decolorise a dilute solution of potassium permanganate, showing that the sulphur was fully oxidised. The acid did not react with phosphorus pentachloride and was very resistant to hydrolysis both by acids and by alkalis (compare Lamb and Pyman, *loc. cit.*; and benziminazole-2-sulphonic acid, X). The barium salt formed bunches of fine needles.

Acid hydrolysis. The acid (1 g. quantities) was heated with hydrochloric acid (10 c.c.) at 170° for 3 hours and at 110° for 6 hours. Estimation of sulphate in the filtered solutions gave barium sulphate

0·296 g. and 0·2796 g., representing 40·8% and 38·6% hydrolysis, respectively.

Alkaline hydrolysis. 1 G. was boiled for 2 hours with 25% sodium hydroxide solution (20 c.c.), no de-arsenication occurring. The cooled diluted acidified solution gave 0·045 g. of barium sulphate, representing 6·2% hydrolysis.

5 : 5′-*Arseno*-(2-*carboxymethylthiolbenziminazole*) (V).—2-Carboxymethylthiolbenziminazole-5-arsinic acid was reduced with sodium hyposulphite as described in Part I (*loc. cit.*). The relatively insoluble sodium salt precipitated from the reduction mixture was washed with alcohol and dissolved in water. The solution on acidification with hydrochloric acid (Congo-red) yielded 5 : 5′-*arseno*-(2-*carboxymethylthiolbenziminazole*) as a bright yellow, amorphous powder (yield, 70%) (Found : As, 25·7; N, 9·5; S, 10·9; atomic ratios As : N : S = 1·00 : 1·98 : 0·99. $C_{18}H_{14}O_4N_4S_2As_2$ requires As, 26·6; N, 9·9; S, 11·3%). It formed a whitish insoluble hydrochloride.

5 : 5′-*Arseno*-(2-*carbamylmethylthiolbenziminazole*) (VII), prepared from 2-carbamylmethylthiolbenziminazole-5-arsinic acid as described under (V) above, was precipitated from the reduction mixture as a bright yellow, amorphous powder (yield, 72%) (Found : As, 26·0; N, 14·5; S, 11·0; atomic ratios As : N : S = 1·00 : 2·99 : 0·99. $C_{18}H_{16}O_2N_6S_2As_2$ requires As, 26·7; N, 15·0; S, 11·4%). It formed a whitish insoluble hydrochloride.

5 : 5′-*Arseno*-(*benziminazole*-2-*sulphonic acid*) (IX) was prepared from 2-sulphobenziminazole-5-arsinic acid as described above under (V). The sodium salt of the arseno-compound was precipitated at first in this case. The acid was obtained as a yellow amorphous powder (yield, 60%) (Found : As, 25·8; N, 9·7; S, 11·2; atomic ratios As : N : S = 1·00 : 2·01 : 1·02. $C_{14}H_{10}O_6N_4S_2As_2$ requires As, 27·6; N, 10·3; S, 11·8%).

Benziminazole 2-*Disulphide* (III).—2-Thiolbenziminazole (5 g.) was dissolved in water (1500 c.c.) by boiling, the solution cooled to 50°, and *N*-iodine solution added (33 c.c.) all at once. *Benziminazole* 2-*disulphide* (4·5 g.) was immediately precipitated in very small, orange prisms, m. p. 198°, insoluble in water and the ordinary organic solvents (Found : N, 18·8; S, 21·4. $C_{14}H_{10}N_4S_2$ requires N, 18·8; S, 21·5%).

The monohydriodide could not be obtained, hydriodic acid causing reduction to 2-thiolbenziminazole with liberation of iodine. The *monohydrochloride* was obtained by stirring the disulphide with cold hydrochloric acid (1 : 10); it crystallised in long yellow needles, decomp. 210°, insoluble in water but soluble in dilute hydrochloric acid (Found when dried at 100° : Cl, 10·8; S, 19·0. $C_{14}H_{10}N_4S_2$,HCl requires Cl, 10·9; S, 19·1%).

Hydrolysis. (*a*) Benziminazole 2-disulphide (3 g.) was boiled for 2 hours with hydrochloric acid (1 : 10; 20 c.c.). The orange solution slowly became colourless, depositing 2-thiolbenziminazole (2·5 g.).

(*b*) Benziminazole 2-disulphide (2 g.) was dissolved in cold sodium hydroxide solution (5%; 40 c.c.). The colourless solution gave 2-thiolbenziminazole (2 g.) on acidification.

The products of the two hydrolyses (*a*) and (*b*) were recrystallised separately from dilute alcohol and proved to be 2-thiolbenziminazole, m. p. 297° (Found : N, 18·7; S, 21·3. Calc. : N, 18·7; S, 21·3%). The mother-liquors contained a small amount of sulphate.

Benziminazole-2-sulphonic Acid (X).—This was obtained from 2-thiolbenziminazole as described under 2-sulphobenziminazole-5-arsinic acid. More water may be used owing to the smaller solubility of the product in cold water (yield, 90%). Recrystallised from water, it formed white prismatic needles, m. p. 365°, moderately easily soluble in cold water and readily soluble in sodium bicarbonate solution (Found in acid dried at 100° : N, 13·5; S, 15·5; loss at 120°, 4·4. $C_7H_6O_3N_2S,\frac{1}{2}H_2O$ requires N, 13·5; S, 15·5; H_2O, 4·3%). It was insoluble in the usual organic solvents. It did not absorb iodine in presence of excess of sodium bicarbonate solution, showing the absence of unchanged 2-thiol compound, and an acidified solution did not decolorise a dilute solution of potassium permanganate, showing that the sulphur was fully oxidised. The sulphonic acid did not react with phosphorus pentachloride and was very resistant to hydrolysis both by acids and by alkalis (compare Lamb and Pyman, *loc. cit.*).

Acid hydrolysis. 1 G. was heated at 170° for 3 hours with hydrochloric acid (10 c.c.). On cooling, unchanged benziminazole-2-sulphonic acid crystallised (0·7 g.; m. p. 365°). Estimation of sulphate in the filtrate gave barium sulphate 0·086 g., representing 7·3% hydrolysis. The filtrate from a separate experiment, when neutralised and treated with picric acid, gave benziminazole picrate, m. p. 222° (0·045 g.; 47·9% of the theoretical).

Alkaline hydrolysis. 1 G. was boiled for two hours with 25% sodium hydroxide solution (20 c.c.). When the liquid was cooled and acidified, unchanged benziminazole-2-sulphonic acid crystallised (0·8 g.; m. p. 365°). The filtrate contained no sulphate, showing that no hydrolysis had occurred.

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[*Received, August 30th*, 1930.]