PHARMACEUTICAL BULLETIN

Vol. 2 No. 4 December 1954

68. Seizaburo Kano and Shigeshi Toyoshima : Arsenical Chemotherapeutic Drugs. XVI.[1] Syntheses and Antibacterial Activities of 3-Nitro-4-hydroxyphenylarsene Oxide Derivatives.

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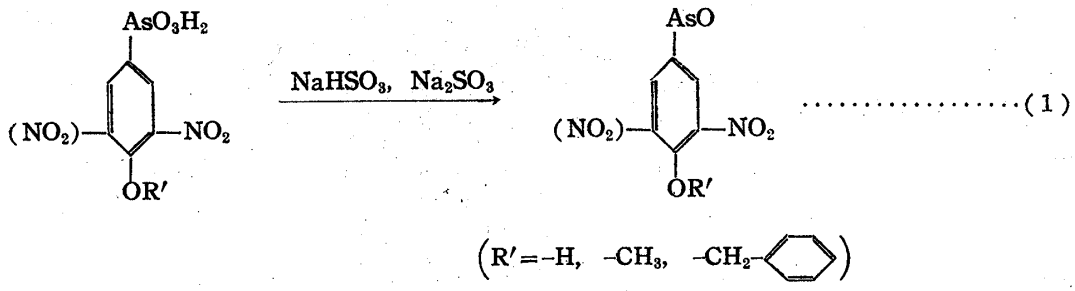
Studies on the experimental chemotherapeutic effect of 3-nitro-4-hydroxyphenylarsene oxide itself against trypanosomiasis, as published by Banks[2], showed that this substance possessed a very strong toxicity. This substance, on the contrary, was observed by the present authors[3] to be effective against *Shigella dysenteriae in vitro*. On the basis of this finding, three substitutes of 3-nitro-4-hydroxyphenylarsene oxide and eleven dithioarsenites derived from 3-nitro-4-hydroxyphenylarsene oxide and its substitutes, were synthesized and examined as to their activities against *Shigella dysenteriae*.

This paper describes the syntheses and antibacterial activities of these 3-nitro-4-hydroxyphenylarsene oxide derivatives.

Syntheses of 3-Nitro-4-hydroxyphenylarsene Oxide and Its Derivatives It was found that 3-nitro-4-hydroxyphenylarsene oxide was not prepared satisfactorily by the usual reduction method from 3-nitro-4-hydroxyphenylarsonic acid. The method of preparation of the arsene oxide was improved by employing aqueous mixture of sodium sulfite and sodium bisulfite instead of sulfur dioxide with mineral acid, or sodium bisulfite with mineral acid. According to this method, 3-nitro-4-methoxyphenylarsene oxide, 3-nitro-4-benzyloxyphenylarsene oxide and 3,5-dinitro-4-hydroxyphenylarsene oxide were synthesized by the reduction of the corresponding arsonic acids respectively with sodium bisulfite.

The dithioarsenites were synthesized by the condensation of four thiol compounds, cysteine, thioglycolic acid, thiomalic acid and thiosalicylic acid with 3-nitro-4-hydroxyphenylarsene oxide and the above substitutes. The whole processes of the above synthetic reactions are illustrated by the following chart.



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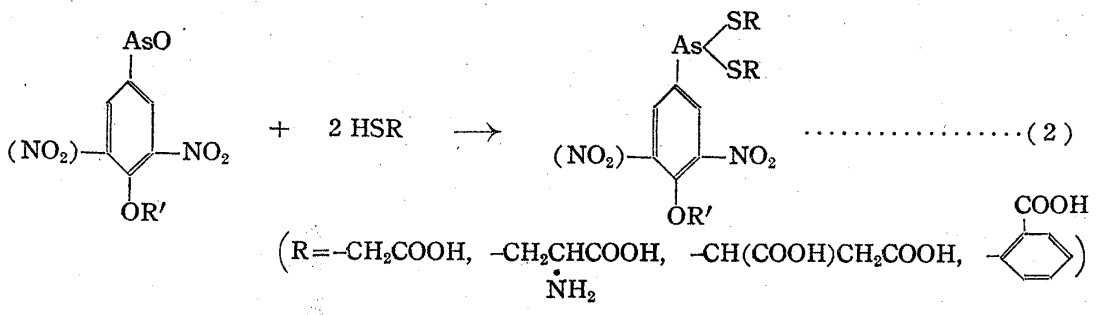
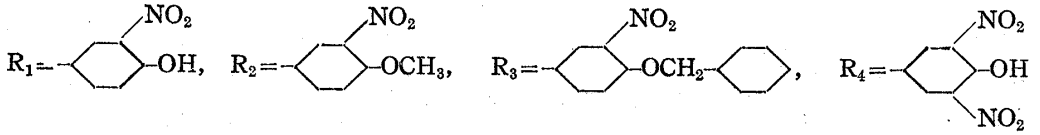
1) Takeo Ueda : Arsenical Chemotherapeutic Drugs. XVI; Part XV : This Bulletin, **2**, 19 (1954).
2) Banks : J. Am. Chem. Soc., **70**, 1762 (1948).
3) T. Ueda, K. Takahashi, S. Toyoshima, K. Kano : This Bulletin, **1**, 16 (1953).



The 3-nitro-4-hydroxyphenylarsene oxide derivatives thus synthesized are summarized in Table I.

TABLE I.



Compound	Mol. formula	m.p., °C. (decomp.)	Appearance	Bacteriostatic concentration against *Shigella dysenteriae* (Komagome BIII) (mole)
R_1–AsO_3H_2				10^{-2}
R_2–AsO_3H_2				10^{-2}
R_3–AsO_3H_2				10^{-2}
R_4–AsO_3H_2				10^{-2}
R_1–AsO	$C_6H_4O_4NAs$	(>250)	Yellow crystals	10^{-7}
R_2–AsO	$C_7H_6O_4NAs$	(>250)	Pale yellow	10^{-6}
R_3–AsO	$C_{13}H_{10}O_4NAs$	(>250)	Yellow powder	10^{-6}
R_4–AsO	$C_6H_3O_6N_2As$	(215)	Orange yellow crystals	10^{-3}
R_1–As($SCH_2CHCOOH$)$_2$ (NH$_2$)	$C_{12}H_{16}O_7N_3AsS_2$	182	Pale yellow powder	10^{-6}
R_2–As($SCH_2CHCOOH$)$_2$ (NH$_2$)	$C_{13}H_{18}O_7N_3AsS_2$	191~193	Pale yellow powder	10^{-6}
R_3–As($SCH_2CHCOOH$)$_2$ (NH$_2$)	$C_{19}H_{22}O_7N_3AsS_2$	(>250)	Yellow powder	10^{-6}
R_1–As(S–C$_6$H$_3$(COOH))$_2$	$C_{20}H_{14}O_7NAsS_2$	(160~162)	Yellow powder	10^{-6}
R_2–As(S–C$_6$H$_3$(COOH))$_2$	$C_{21}H_{16}O_7NAsS_2$	(170~171)	Slightly yellow powder	10^{-6}
R_3–As(S–C$_6$H$_3$(COOH))$_2$	$C_{27}H_{20}O_7NAsS_2$	(198)	Slightly yellow powder	10^{-6}
R_4–As(S–C$_6$H$_3$(COOH))$_2$	$C_{20}H_{13}O_9N_2AsS_2$	(185~187)	Yellow powder	10^{-3}
R_1–As(SCH_2COO)$_2$Ba	$C_{10}H_8O_7NAsBaS_2$	>255	Yellow powder	10^{-6}
R_2–As(SCH_2COO)$_2$Ba	$C_{11}H_{10}O_7NAsBaS_2$	(>260)	Yellow powder	10^{-6}
R_1–As(SCH(COOH)CH$_2$COONa)$_2$	$C_{14}H_{12}O_{11}NAsNa_2S_2$	—	Red plates	10^{-6}

Antibacterial Activities of 3-Nitro-4-hydroxyphenylarsene Oxide The compounds shown in Table I were examined as to their activities *in vitro* against various bacteria and acute toxicities in mice. The bacteriological procedures were the same as those described in Part X of this series[4]. The results obtained are shown in Tables II, III, and IV.

4) T. Ueda, K. Takahashi, S. Toyoshima, S. Kano: This Bulletin, **1**, 17 (1953).

Table II. Acute Toxicity of 3-Nitro-4-hydroxyphenyldithioarsenite

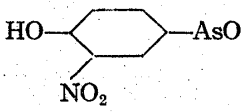
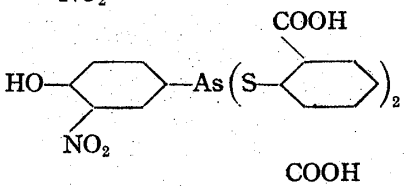
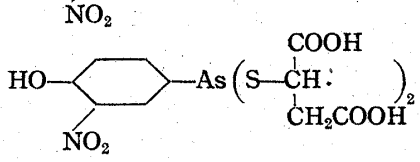
Compound	Max. tolerable dose mg./kg. (intraven.)
	5
	25
	45

Table III. Antibacterial Activity of 3-Nitro-4-hydroxy-bis(α,β-dicarboxyethyl)-thioarsenite against Sulfanilamide-resistant *Shigella dysenteriae*

Bacteria	Min. bacteriostatic concn. (mole)		Bacteria	Min. bacteriostatic concn. (mole)	
	Sulfathiazole	Thioarsenite		Sulfathiazole	Thioarsenite
Komagome	10^{-2}	10^{-6}	Showa	10^{-2}	10^{-6}
Komagome B	10^{-2}	10^{-6}	Oh-izumi	10^{-2}	10^{-6}
Komagome B_{2a}	10^{-2}	10^{-6}	Oh-izumi	10^{-2}	10^{-6}
Komagome B_{2},	10^{-2}	10^{-6}	Nakamura	10^{-2}	10^{-6}
Komagome B_{2b}	10^{-2}	10^{-6}	Kawase	10^{-2}	10^{-6}
Showa	10^{-2}	10^{-6}	Komagome B_{III}	10^{-2}	10^{-6}

Table IV. Antibacterial Activity of 3-Nitro-4-hydroxyphenyl-bis(α,β-dicarboxyethyl)-thioarsenite against Various Bacteria

Bacterium	Min. bacteriostatic concn. (mole)	Bacterium	Min. bacteriostatic concn. (mole)
Shigella dysenteriae	10^{-6}	*Staphylococcus* (209 P)	10^{-7}
Eberthella typhosa	10^{-6}	*B. proteus*	10^{-6}
Salmonella paratyphi A	10^{-6}	*B. pyocyaneus*	10^{-4}
Salmonella paratyphi B	10^{-7}	*Streptococcus haemolyticus*	10^{-10}
Escherichia coli	10^{-6}	*Gonococcus*	10^{-4}
Staphylococcus (Terashima)	10^{-8}		

It is seen in Table I that the compounds of arsonous acid series showed, in general, antibacterial activities stronger than the corresponding compounds of arsonic acid series, and that the activities of the former compounds were not decreased by converting into thioarsenites with thiol compounds. From Tables I and II, it may be said that among the dithioarsenites, 3-nitro-4-hydroxy-bis(α,β-dicarboxyethyl)thioarsenite was the most promising on consideration of both activity and toxicity. This compound was observed, as shown in Tables III and IV, to exert strong activity against sulfanilamide-resistant *Shigella dysenteriae* and a better antibacterial spectrum against various bacteria.

Discussion and Conclusion The antibacterial properties of arsenical compounds have been investigated by Ueda, Toyoshima, and co-workers. Above all, 3-nitro-4-hydroxyphenylarsene oxide was observed to possess the most marked antibacterial activity among the arylarsonous acid series[4]. According to this finding, the derivatives of the arsene oxide were newly synthesized by the authors.

These compounds were examined as to their activities against *Shigella dysenteriae*, according to the findings that arsenical compounds were of interest as invaders into gram-negative bacteria[5]. As shown in Table I, 3-nitro-4-methoxyphenylarsene oxide and

5) S. Toyoshima, S. Kano, T. Ueda: This Bulletin, **1**, 16 (1953).

3-nitro-4-benzyloxyphenylarsene oxide were observed to exert antibacterial activities nearly equal to that of the parent compound, 3-nitro-4-hydroxyphenylarsene oxide, while 3,5-dinitro-4-hydroxyphenylarsene oxide, an activity far weaker than the above three. This fact shows that the parent compound decreased its activity by nitration, but not by etherification. On the other hand, the four arsene oxides were observed to show activities far stronger than those of the corresponding arsonic acids. This fact coincides with the findings of other series of arsenical compounds. Thus, these facts suggest that the arsonoso radical plays the most important role in antibacterial activity of these compounds, while other partial structures might contribute to the activity. Dithioarsenites were shown to possess antibacterial activities nearly equal to those of the original arsene oxides and toxicities lower than the original compounds. Regarding the mode of action of arsenicals, it has been discussed that arsenical drugs decreased their activities in parallel with their toxicities on addition of thiol compounds. The effects of the dithioarsenites with the bacterium observed by the authors are of interest in contrast to the theory with spirocheta and trypanosoma by Ehrlich and others. The above fact with the dithioarsenites coincides with the findings regarding diphenylthioarsinites[6]. Therefore, the discussion on diphenylthioarsinites should hold true for phenyldithioarsenites.

Among the dithioarsenites, 3-nitro-4-hydroxyphenyl-bis(α,β-dicarboxyethyl)dithioarsenite was screened up as the best in balance of effect and toxicity. This compound was observed to show a remarkable effect on sulfanilamide-resistant *Shigella dysenteriae* equal to that on the sulfanilamide-resistant strain. This fact, also, coincides with the finding with diphenylthioarsinites[7]. Therefore, it may be said that this compound is of promise for pharmaceutical use, especially for curative administration against bacterial dysentery, along with 3-amino-4-hydroxydiphenyl-(α,β-dicarboxyethyl)-thioarsinite. The mode of action of this compound will be discussed in detail in the future.

Experimental

Syntheses of Arsene Oxides : 3-Nitro-4-hydroxyphenylarsene Oxide—2.6 g. of 3-nitro-4-hydroxyphenylarsonic acid and 3.4 g. Na_2SO_3 were dissolved in 60 cc. of water, and 4.0 g. $NaHSO_3$ was then added thereto. The solution was bone-blacked, filtered, and allowed to stand for 3 days at a room temp. A precipitate produced was filtered, washed with water, and dried over P_2O_5. Yellow crystals. Yield, 1.3 g. Insoluble in water and in common organic solvents, soluble in alkaline solution with discoloration to orange. *Anal.* Calcd. for $C_6H_4O_4NAs$: As, 32.71. Found : As, 32.70.

3-Nitro-4-methoxyphenylarsene Oxide—2.4 g. of 3-nitro-4-methoxyphenylarsonic acid was treated by the same procedures as above. Pale yellow crystals. Yield, 1.2 g. Insoluble in water and in common organic solvents, soluble in alkaline solution with discoloration to orange. *Anal.* Calcd. for $C_7H_6O_4NAs$: As, 30.85. Found : As, 31.15.

3-Nitro-4-benzyloxyphenylarsene Oxide—3.5 g. of 3-nitro-4-benzyloxyphenylarsonic acid was treated by the same procedures as above. Orange yellow crystals. Yield, 1.6 g. Slightly soluble in water, insoluble in common organic solvents. *Anal.* Calcd. for $C_{13}H_{10}O_4NAs$: As, 23.47. Found : As, 23.43.

3,5-Dinitro-4-hydroxyphenylarsene Oxide—3.1 g. of 3,5-dinitro-4-hydroxyphenylarsonic acid was treated by the same procedures as above. Orange yellow crystals, m.p. 215°(decomp.). Yield, 0.9 g. Slightly soluble in water and in MeOH, soluble in alkaline solution with discoloration to orange red. *Anal.* Calcd. for $C_6H_3O_6N_2As$: As, 27.34. Found : As, 27.60.

Syntheses of Dithioarsenites—1) 0.01 mole of disodium salt of arsene oxide was added into a solution of 0.02 mole of cysteine hydrochloride or thiosalicylic acid in 30 cc. of water, which had been made weakly alkaline with alkali, boiled for several minutes, bone-blacked, and filtered. On cooling, the corresponding dithioarsenite precipitated from the reaction mixture by acidification with AcOH. The precipitate was filtered and washed with water and EtOH. Insoluble in water.

3-Nitro-4-hydroxyphenyl-bis(α-amino-α-carboxyethyl)dithioarsenite—Pale yellow powder, m.p. 182°. *Anal.* Calcd. for $C_{12}H_{16}O_7N_3AsS_2$: As, 16.55. Found : As, 17.05.

3-Nitro-4-methoxyphenyl-bis(α-amino-α-carboxyethyl)dithioarsenite—Pale yellow powder, m.p.

6) T. Ueda, S. Toyoshima, K. Takahashi : This Bulletin, **1**, 26 (1953).

191~193°. *Anal.* Calcd. for $C_{13}H_{18}O_7N_3AsS_2$: As, 16.15. Found : As, 16.75.

3-Nitro-4-benzyloxyphenyl-bis(α-amino-α-carboxyethyl)dithioarsenite—Yellow powder. *Anal.* Calcd. for $C_{19}H_{22}O_7N_3AsS_2$: As, 13.78. Found : As, 13.75.

3-Nitro-4-hydroxyphenyl-bis(2-carboxyphenyl)dithioarsenite—Yellow powder, m.p. 160~162° (decomp.). *Anal.* Calcd. for $C_{20}H_{14}O_7NAsS_2$: As, 14.42. Found : As, 15.01.

3-Nitro-4-methoxyphenyl-bis(2-carboxyphenyl)dithioarsenite—Slightly yellow powder, m.p. 170~171°. *Anal.* Calcd. for $C_{21}H_{16}O_7NAsS_2$: As, 14.01. Found : As, 14.20.

3-Nitro-4-benzyloxyphenyl-bis(2-carboxyphenyl)dithioarsenite—Slightly yellow powder, m.p. 198°(decomp.). *Anal.* Calcd. for $C_{27}H_{20}O_7NAsS_2$: As, 12.25. Found : As, 12.40.

3,5-Dinitro-4-hydroxyphenyl-bis(2-carboxyphenyl)dithioarsenite—Yellow powder, m.p. 185~187°. *Anal.* Calcd. for $C_{20}H_{13}O_9N_2AsS_2$: As, 13.27. Found : As, 13.50.

2) 1.0 cc. of thioglycolic acid was added into a solution of 0.02 mole of arsene oxide dissolved in 40 cc. of water by adding alkali, and boiled for several mins. After cooling, 3.0 cc. of 10% $BaCl_2$ was added cautiously into the reaction mixture. A precipitate, produced by neutralization with ammonia water in the presence of methyl red, was filtered and washed with water and EtOH.

3-Nitro-4-hydroxyphenyl-[barium bis(carboxymethyl)]dithioarsenite—Yellow powder, m.p. > 255°. *Anal.* Calcd. for $C_{10}H_8O_7AsBaS_2$: As, 14.50. Found : As, 15.01.

3-Nitro-4-methoxyphenyl-[barium bis(carboxymethyl)]dithioarsenite—Yellow powder. *Anal.* Calcd. for $C_{11}H_{10}O_7NAsBaS_2$: As, 14.08. Found : As, 14.20.

3-Nitro-4-hydroxyphenyl-(sodium α,β-dicarboxyethyl)dithioarsenite—0.23 g. of 3-nitro-4-hydroxyphenylarsene oxide was mixed with 0.3 g. of thiomalic acid, emulsified by adding 5 cc. of water. Yellow precipitate thus produced was filtered, washed with water, and dried *in vacuo*. The dried compound was redissolved in calculated amount of NaOH solution and dried at 37~40° under a diminished pressure. Red plates. Yield, 0.4 g. *Anal.* Calcd. for $C_{14}H_{12}O_{11}NAsNa_2S_2$: As, 13.54. Found : As, 13.50.

Summary

1. Derivatives of 3-nitro-4-hydroxyphenylarsene oxide were synthesized on the basis that the parent compound exerted a remarkable antibacterial activity among the phenylarsonous series.

2. 3-Nitro-4-hydroxyphenyl-bis(α,β-dicarboxyethyl)dithioarsenite was selected as the most effective against *Shigella dysenteriae*, especially that of sulfa-resistant strains.

(Received June 25, 1954)

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69. Seizaburo Kano and Shigeshi Toyoshima : Arsenical Chemotherapeutic Drugs. XVII.[1]. Resistance Development and Cross-Resistance of *Shigella dysenteriae* to 3-Nitro-4-hydroxyphenyl-bis(α,β-dicarboxyethyl)dithioarsenite and 3-Amino-4-hydroxydiphenyl-(α,β-dicarboxyethyl)-thioarsinite.

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As described in Part XVI and XI of this series[1,2], it was found that 3-nitro-4-hydroxyphenyl-bis(α,β-dicarboxyethyl)dithioarsenite, named Nitrosen, and 3-amino-4-hydroxydiphenyl-(α,β-dicarboxyethyl)-thioarsinite, named Diarsen, possessed strong activities against *Shigella dysenteriae*. Subsequently, resistance development and cross-resistance of these two drugs were investigated by the authors. This paper is concerned with development of bacterial resistance to Nitrosen and Diarsen and cross-resistance between dihydrostreptomycin, terramycin, chloramphenicol, nitrofuracin, and these arsenical drugs.

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1) T. Ueda : Arsenical Chemotherapeutic Drugs. XVII; Part XVI : This Bulletin, **2**, 301(1954).

2) T. Ueda, S. Toyoshima, K. Takahashi : *Ibid.* **1**, 25 (1953).