7. Takeo Ueda, Shigeshi Toyoshima, and Kiyoshi Takahashi: Aresenical Chemotherapeutic Drugs. XII. Antibacterial Properties of Diarylthioarsinites.

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The studies on the antibacterial properties of diarylarsinous acids, as described in the previous paper[1], showed that diphenylarsinous oxide, 4-hydroxydiphenylarsinous acid, and 3-amino-4-hydroxydiphenylarsinous acid exerted remarkable antibacterial activities *in vitro*, but possessed comparatively strong toxicity. Cohen[2] synthesized aryldithioarsenites by combining arylarsonous or arylarsonic acid with SH-compounds in order to decrease the toxicity of arsenical drugs.

For the same purpose, the authors synthesized[3] diarylthioarsinites by condensing SH-compounds with the above three diphenylarsinous acids described in the earlier papers. These diarylthioarsinites are new compounds and are of interest in combatting bacteria, since toxic arsinoso radicals in these compounds have been covered with -SR group.

This paper describes antibacterial properties of diarylthioarsinites so far prepared.

Experimental Procedures The twelve diphenylthioarsinites were synthesized by condensing diphenylarsinous oxide, 4-hydroxydiphenylarsinous acid, and 3-amino-4-hydroxydiphenylarsinous acid with thioglycollic acid, cysteine, thiomalic acid, and thiosalicylic acid, and the three thioarsinites were synthesized by combining 4-aminodiphenlyarsinous acid, 3-nitro-4-hydroxydiphenylarsious acid, and 4,4′-dicarboxydiphenylarsinous acid with thiosalicylic acid. These fifteen compounds, shown in Table I, were tested as to their antibacterial activities.

The bacteriological procedures were the same as those described in Part X[4] of this series. *Escherichia coli*, *Shigella dysenteriae* (Komagome BIII), *Eberthella typhosa* (Kyodai strain), and *Staphylococcus aureus* (Terashima strain) were employed. The compounds were dissolved in aqueous sodium hydroxide solution or organic solvents indifferent to bacteria for the tests. The maximun dilutions in molar concentration necessary for bacteriostasis after 48 hours were measured in media containing complete bouillon by the serial dilution method.

Antibacterial Activities of Diarylthioarsinites The antibacterial activities of 15 thioarsinites are summarized in Table I by the maximum dilutions (molar concentrations) required to inhibit the growths of bacteria.

It is evident from Table I that among the thioarsinites obtained from the three active diphenylarsinous acids, the ten thioarsinites, except 4-hydroxydiphenyl-β-amino-β-carboxyethylthioarsinite and 4-hydroxydiphenyl-α-carboxy-β-carboxyethylthioarsinite, showed remakable antibacterial activities, and the three thioarsinites obtained from the three less active diphenylarsinous acids showed weak activities.

Experimental Toxicity The toxic effects of the active thioarsinites were determined by the single injection method in mice. The results are given in Table I. It is seen from Table I that the four thioarsinites obtained from diphenylarsinous oxide, and 4-hydroxydiphenylcarboxymethylthioarsinite obtained from 4-hydroxydiphenylarsinous acid showed comparatively strong toxicity, while the four thioarsinites from 3-amino-4-hydroxydiphenylarsinous acid possessed comparatively low toxicity.

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1) Part XI: K. Takahashi, S. Toyoshima, T. Ueda: This Bulletin, 1, 21 (1953).
2) A. Cohen: J. Chem. Soc., 1932, 593, 2505, 2866; 1931, 3043, 3236.
3) K. Takahashi: J. Pharm. Soc. Japan, 72, 1148 (1952).
4) T. Ueda, K. Takahashi, S. Toyoshima, S. Kano: This Bulletin, 1, 17 (1953).

Discussion and Conclusion It has been shown by many authors[5] that the activities of arsenicals against spirochaeta and trypanosoma were decreased by combining them with SH-compounds, though, at the same time, the resulting compounds showed lower toxicity than the original compounds. Nevertheless, the observations with diarylthioarsinites, as described above, show that the thioarsinites, with few exceptions, exerted almost equal antibacterial activities as the original diarylarsinous acid and possessed lower toxicity than the original diarylarsinous acids (as shown in Tables I and II).

As shown in Table III, it may be said that the thioarsinites from less active diarylarsinous acid exerted nearly equal activities as the original diarylarsinous acids.

The fact that the toxicities and not their antibacterial effects were decreased in antibacterial diarylarsinous acids by combining them with SH-compounds, is interesting in contrast to the findings by Voegtlin and others[6] that arsenical drugs decreased their activities against trypanosoma and their toxicities on the addition of SH-compounds.

Thus, the thioarsinites from 3-amino-4-hydroxydiphenylarsinous acid are of promise for pharmaceutical use, when considered from their effects and toxicities.

These thioarsinites possess many interesting characteristics: *viz.*, these compounds possess strong effect against pathogenic bacteria, especially gram-negative bacteria and comparatively low toxicity which is further decreased by the addition of *l*-ascorbic acid, glucose, or an antihistamine drug. In addition, it may be suggested that these compounds might be effective against bacteria unsusceptible to other chemotherapeutic drugs, even in so small doses that their toxicity would be negligible.

Studies on other pharmacological properties necessary for the clinical use of these compounds will be described in a medical journal of Japan.

The mode of the actions of effective thioarsinites is not clear at the present stage. However, it may be assumed that the thioarsinites might reach bacteria, without combining easily with SH-enzyme in a host, and exchange easily the SH-components in the compounds with SH-enzyme in bacteria.

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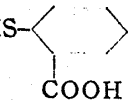
TABLE I

SH-Compound: HS–R (R: A, B, C, or D).

HS–A: $HS-CH_2COOH$

HS–B: $HS-CH_2CH(NH_2)-COOH$

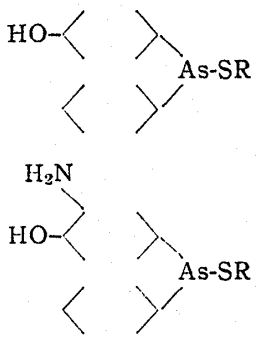
HS–C: $HS-CH(COOH)-CH_2COOH$

HS–D: 

Diarylthioarsinite		Activity against Bacteria				Toxicity (M.T.D.)
	R	*Esch. coli.*	*Staph. aur.*	*Eber. typhosa*	*S. dysenteriae*	mg./kg
	A	10^{-8}	10^{-8}	10^{-8}	10^{-8}	10 (per os)
	B	10^{-7}	10^{-7}	10^{-7}	10^{-7}	20 (per os)
	C	10^{-6}	10^{-6}	10^{-6}	10^{-6}	150 (per os)
	D	10^{-7}	10^{-7}	10^{-7}	10^{-7}	20 (per os)

5) T. Ueda, S. Toyoshima: Papers read before the Annual Meeting of the Pharmaceutical Society of Japan (1949); Recoll, Wilson: J. Pharmacol. Exptl. Therap., 92, 121 (1948); C. Voegtlin, H. Dyer, C. Leonard: Pub. Health Report, 38, 1882 (1923); S. Rosenthal, C. Voegtlin: J. Pharmacol. Exptl. Therap., 39, 347 (1930); H. Eagle: *Ibid.*, 66, 436 (1939); Anderson, Hansen, Peters: *Ibid.*, 91, 112 (1947), etc.

6) C. Voegtlin, *et al.*: *loc. cit.*



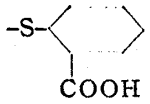
A	10^{-3}	10^{-9}	10^{-9}	10^{-3}	5 (i.v.)
B	10^{-3}	10^{-3}	10^{-3}	10^{-3}	——
C	$>10^{-3}$	$>10^{-3}$	$>10^{-3}$	$>10^{-3}$	——
D	10^{-5}	10^{-6}	10^{-7}	10^{-7}	30 (i.v.)

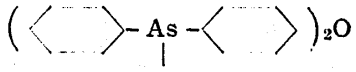
A	10^{-7}	10^{-8}	10^{-3}	10^{-3}	80 (i.v.)
B	10^{-3}	10^{-8}	50^{-8}	10^{-3}	40 (i.v.)
C	10^{-3}	10^{-3}	10^{-3}	10^{-3}	90 (i.v.)
D	10^{-3}	10^{-9}	10^{-7}	10^{-3}	150 (i.v.)

i.v. = intravenous injection
per os = oral administration

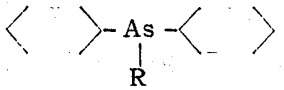
TABLE II

Compound	Activity against Bacteria: *Esch. coli.*	*Staph. aur.*	*S. dysenteriae*	*Eber. typhosa*	Toxicity (M.T.D.) mg./kg.
I	10^{-3}	10^{-3}	10^{-7}	10^{-3}	5 (i.v.)
II	10^{-7}	10^{-7}	10^{-8}	10^{-8}	20 (per os)
III	10^{-3}	10^{-7}	10^{-8}	10^{-3}	2.5 (i.v.)
IV	10^{-5}	10^{-3}	10^{-7}	10^{-3}	80 (i.v.)
V	10^{-5}	10^{-3}	10^{-8}	10^{-3}	20 (i.v.)
VI	10^{-3}	10^{-9}	10^{-3}	10^{-7}	150 (i.v.)

-R: 



(I)



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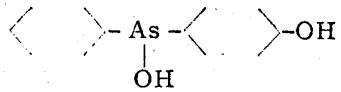

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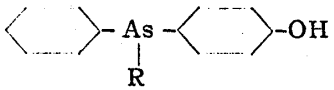

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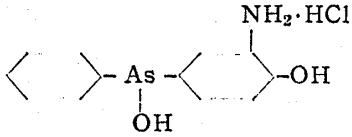

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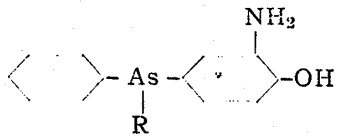
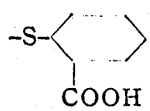
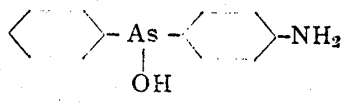


(VI)

TABLE III

Compound	Activity against Bacteria: *Esch. coli.*	*Staph-aur.*	*S. dysenteriae*	*Eber. typhosa*
I	10^{-3}	10^{-5}	10^{-3}	10^{-3}
II	10^{-3}	10^{-4}	$>10^{-3}$	$>10^{-3}$
III	10^{-4}	10^{-7}	10^{-4}	10^{-4}
IV	10^{-3}	10^{-4}	.....	.....
V	10^{-3}	10^{-3}	.....	.....
VI	$>10^{-3}$	10^{-3}	.....	.....

-R: 



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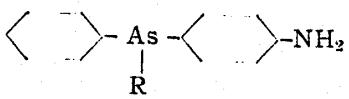

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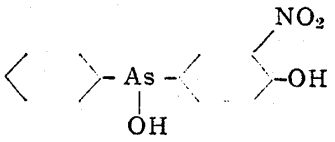

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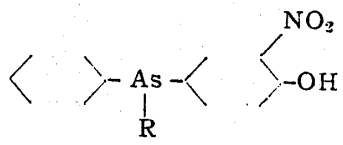

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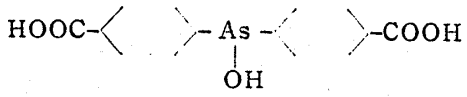

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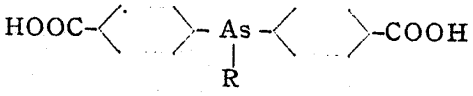

(VI)

Summary

1) Fifteen kinds of diarylthioarsinites were examined as to their antibacterial properties. Among these compounds, 4-hydroxydiphenyl-*o*-carboxyphenylthioarsinite, 3-amino-4-hydroxydiphenylcarboxymethylthioarsinite, 3-amino-4-hydroxydiphenyl-β-amino-β-carboxyethylthioarsinite, 3-amino-4-hydroxydiphenyl-α-carboxy-β-carboxyethylthioarsinite, and 3-amino-4-hydroxydiphenyl-*o*-carboxyphenylthioarsinite exerted marked antibacterial activities *in vitro* and possessed comparatively low toxicity.

2) The above five compounds are of promise for chemotherapeutic use.

3) It was shown that the toxicity of active diarylarsinous acids were decreased intensively but not their antibacterial activity by combining them with SH-compounds.

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