was mixed with 1,2,3,4-tetrahydrocarbazole prepared by the conversion with sulfuric acid according to the method reported by Hoshino and Takiura[3], no melting point depression was observed.

Ethyl α-Acetamido-α-carbethoxy-β-(3-indole)-propionate (IV)—γ-Acetamido-γ,γ-dicarbethoxybutyraldehyde phenylhydrazone[4] (5 g.) was mixed with Amberlite IR-120 (10 g.) and 30 cc. of water. The reaction mixture was heated to the reflux temperature with vigorous stirring. The phenylhydrazone liquified at first, and after approximately 2 hours the suspended liquid solidified. The refluxing was continued for 4 hours. After cooling, the reaction mixture was treated as in the previous case (III). This gave colorless plates of m.p. 157~158°; yield, 3.4 g. or 73%. *Anal.* Calcd. for $C_{18}H_{22}N_2O_5$: C, 62.44; H, 6.40; N, 8.09. Found: C, 62.21; H, 6.44; N, 8.25.

When the cyclization reaction was carried out in aqueous alcohol, the cyclized product was obtained in approximately the same yield. The use of toluene in this reaction lowered the yield to 35.3%. Cyclization of the phenylhydrazone using the phenolic sulfonic acid resin (Diaion K) as the catalyst gave the cyclized product in a very poor yield. For caution's sake, this compound was also prepared by a reaction between gramine and ethyl acetamidomalonate, as described by Howe, Zambito, Snyder, and Tishler[5]. When resulting substance was mixed with the above-described sample, no melting point depression was observed.

Summary

The authors carried out the Fischer indole synthesis by employing a cation exchanger as the condensing agent. As a result, cation exchangers were found to be an effective and convenient agent for this reaction. 2,3-Dimethylindole, 2-phenyl-3-methylindole, 1,2,3,4-tetrahydrocarbazole, and ethyl α-acetamido-α-carbethoxy-β-(3-indole)-propionate were prepared from corresponding phenylhydrazone by this method.

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5. Takeo Ueda, Kiyoshi Takahashi, Shigeshi Toyoshima, and Seizaburo Kano: Arsenical Chemotherapeutic Drugs. X.* Antibacterial Properties of Arylarsonic Acids and Arylarsonous Acids.

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Arsenical chemotherapeutic drugs have, hitherto, been investigated mainly as to their efficacies against spirochaetosis and trypanosis, but hardly tested as to their activities against pathogenic bacteria. Drugs of the arsphenamine series[1] were studied as to their activities against gonococcus, streptococcus, and staphylococcus, and about 60 compounds belonging to the arylarsonic acid series and a few compounds belonging to the diarylarsinic acid series[2] were tested as to their activities against tuberculosis. However, there still remain systematic studies to be carried out on antibacterial properties of arsenical drugs.

Since arsenical compounds containing poisonous arsenic atom exert more or less toxicity, chemotherapeutic utilization of these compounds should be limited to a short range. Accordingly, it may be noted that arsenical compounds should be non-beneficial in several respects, compared with antibiotics possessing extremely small toxicities. However, it may be expected that arsenical compounds could be clinically utilized, if these compounds would

3) Hoshino, Takiura : Bull. Chem. Soc. Japan, 11, 218 (1936).
4) Moe, Warner : J. Am. Chem. Soc., 70, 2763 (1948).
5) Howe, Zambito, Snyder, Tishler : J. Am. Chem. Soc., 67, 38 (1945).
* Part IX: J. Pharm. Soc. Japan, 72, 1148 (1952).
** Shinano-machi, Shinjuku-ku, Tokyo. (上田武雄, 高橋 廉, 豊島 滋, 加納晴三郎).
1) T. Ueda, S. Toyoshima: Papers read before the Annual Meeting of the Pharmaceutical Society of Japan (1950).
2) M. Matsui, N. Hirano : Saikingaku-zasshi, 567 (1943), (Japan).

exert efficacies against pathogenic bacteria unsusceptible to such drugs as antibiotics, sulfanilamides, and furacin in so small doses as to make their toxicities negligible.

Modes of antimicrobial actions of arsenical chemotherapeutic drugs have not, hitherto, been clearly determined since antagonists to these drugs have not been so sufficiently discussed as those of sulfanilamides. However, it may be assumed from discussions on spirochaeta and trypanosoma[3] that arsenical compounds would exert activities in the form of -As=O or -As(OH)$_2$, by combining with SH-enzymes in microbes, and toxicities by combining with SH-enzymes in hosts. These assumptions could, therefore, be applied to the cases of bacteria with arsenical compounds.

According to these assumptions, arsenical compounds should exert antibacterial activities, based on mechanisms other than those of known chemotherapeutic drugs, and it may be expected that new types of chemotherapeutic drugs might be found among arsenical compouds.

However, there still remain the questions of residues necessary for carrying -As=O or -As(OH)$_2$ to bacteria to be solved. In order to solve such questions, the authors investigated many of arylarsonic acids and arylarsonous acids as to their antibacterial properties. This paper describes the antibacterial properties of arylarsonic acids and arylarsonous acids.

Arsenical Compounds employed to test Antibacterial Properties The 20 compounds of the arylarsonic acid series shown in Table I and the 11 compounds of the arylarsonous acid series shown in Table II were prepared. Among these compounds, bis-[*p*-(1-arsono-4-aminonaphthylazo)]-diphenyl is a new compound, which was synthesized by coupling bis-diazonium compound of benzidine with 4-aminonaphthalene arsonic acid[4].

Bacteriological Procedures

Strains—(1) *Escherichia coli*, (2) *Shigella dysenteriae* (Komagome BIII), (3) *Eberthella typhosa* (Kyodai strain), and (4) *Staphylococcus aureus* (Terashima strain) were employed.

Media—All experiments with *Esch. coli*, *S. dysenteriae*, and *Eber. typhosa*, *in vitro*, were carried out in synthetic media as follows: (1) For *Esch. coli*: 6 g. of sodium chloride, 0.2 g. of magnesium sulfate, 1 g. of potassium phosphate, 2 g. of sodium citrate, 0.5 g. of asparagine, and 1 g. of glucose dissolved in 1000 cc. of distilled water, and pH adjusted to 7.4. (2) For *S. dysenteriae* (Komagome BIII): 0.5 g. of sodium chloride, 0.1 g. of magnesium sulfate, 1 g. of potassium phosphate, 0.5 g. of asparagine, 1 g. of glucose, 0.5 g. of tryptophane, and 10^{-5} mole of nicotinamide dissolved in 1000 cc. of distilled water, and pH adjusted to 7.2. (3) For *Eber. typhosa* (Kyodai strain): 0.5 g. of sodium chloride, 0.2 g. of potassium phosphate, 0.5 g. of ammonium sulfate, 0.1 g. of glucose, and 0.05 g. of tryptophane dissolved in 100 cc. of distilled water, and pH adjusted to 7.0. (4) For *Staph. aureus* (Terashima strain): no synthetic medium was used.

Experimental Procedures—4.5 cc. of each medium was placed in test tubes of series and autoclaved at 10 lb. for 10 minutes, 0.5 cc. of one of the arsenicals in solution was added to the medium, and diluted by tenfold steps. These test tubes were inoculated with one drop of the solution, made to a dilution of 1:10,000, of each test organism of 18-hour culture in the same medium as mentioned. After incubation at 37° for 48 hours, the extent of bacterial growth was examined visually. The endpoint was assessed on a serial dilution scale in molar concentration required to inhibit the growth of the organism. As a control, sulfathiazole was employed in each experiment.

Antibacterial Activities of Arsenical Compounds Antibacterial activities of arylarsonic acids and arylarsonous acids are summarized in Tables I and II, respectively, by the maximum dilutions (molar concentrations) required to inhibit the growths of bacteria. It can be seen from Table I that among arylarsonic acids, *p*-arsonoacetophenone phenylhydrazone alone showed efficacy and all other compounds did not exert any activity. It can also be seen from Table II that, among arylarsonous acids, 3-nitro-4-hydroxyphenylarsene oxide showed a remarkable activity and all other compounds exerted weak activities.

Discussion and Conclusion As described above, none of the compounds of the arylars-

3) Voegtlin, Smith: J. Pharmacol. Exptl. Therap., 15, 475 (1920); Voegtlin, Dyer, Leonard; Publ. Health Report, 38, 1882 (1923); Rosenthal, Voegtlin; J. Pharmacol. Exptl. Therap., 39, 347 (1930); Cohn, King, Strangway: J. Chem. Soc., 1931, 3043; Eagle: J. Pharmacol. Exptl. Therap., 66, 346 (1939).

4) Red crystalline powder. *Anal.* Calcd. for $C_{32}H_{26}O_6H_6As_2$: As, 20.25. Found: As, 20.14.

onic acid series, except *p*-arsonoacetophenone phenylhydrazone, showed any positive activity. Though *p*-arsonoacetophenone phenylhydrazone showed an activity approximately equal to that of sulfanilamide, it was not of promise for a pharmaceutical use, because its toxicity (M.T.D.) was found to be 5 mg./kg.

It may be assumed from these results that arsono radical combined with aryl residue does not show any antibacterial activity *in vitro* and that the activity of *p*-arsonoacetophenone phenylhydrazone may be due to chemical structure other than the arsono radical.

According to the Ehrlich's theory on arsenical drugs, arylarsonic acids were assumed to be activated by being reduced to arsonoso compounds in living bodies. However, it may be concluded that none of arylarsonic acids was of promise, when criticized from the analogy[5] that 3-amino-4-hydroxyphenylarsonic acid was weaker in antispirochaetal and antitrypanosomal activities than the corresponding arsonoso compound, *i.e.* 3-amino-4-hydroxyphenylarsonous acid.

Among the arylarsonous acids, *p*-nitrophenylarsonous acid showed a weak activity, 3-nitro-4-hydroxyphenylarsene oxide, a more remarkable activity than sulfathiazole, and all the other compounds showed activities approximately equal to sulfanilamide.

It may be said from these results that arsonoso radical combined with aryl residue exerted antibacterial activities *in vitro*. However, they were not of promise for chemotherapeutic use, because their toxicities were comparatively strong.

Oxophenarsine hydrochloride has lately been used for diseases caused by microbes unsusceptible to antibiotics, sulfanilamides, etc. It is suggested from these findings that arsonoso compounds could be used clinically if their toxicity could be diminished. In connection with this problem, the authors made an extensive survey on 3-nitro-4-hydroxyphenylarsene oxide and its derivatives. The results will be described in another paper.

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TABLE I

Arsono Compouds	*Esch. coli*	*Staph. aur.*	*Eber. typhosa*	Arsono Componds	*Esch. coli*	*Staph. aur.*	*Eber. typhosa*
(I)	$>10^{-2}$	$>10^{-2}$	$>10^{-2}$	(XII)	10^{-2}	$>10^{-2}$	$>10^{-2}$
(II)	$>10^{-2}$	$>10^{-2}$	$>10^{-2}$	(XIII)	10^{-4}	10^{-3}	10^{-4}
(III)	10^{-2}	10^{-2}	10^{-2}	(XIV)	10^{-3}	10^{-3}	10^{-3}
(IV)	10^{-2}	$>10^{-2}$	10^{-2}	(XV)	10^{-3}	...	10^{-2}
(V)	$>10^{-3}$	$>10^{-3}$	$>10^{-3}$	(XVI)	10^{-2}	...	10^{-2}
(VI)	10^{-2}	10^{-2}	10^{-2}	(XVII)	$>10^{-2}$	10^{-1}	$>10^{-2}$
(VII)	10^{-2}	$>10^{-1}$	10^{-2}	(XVIII)	10^{-2}	...	10^{-2}
(VIII)	10^{-2}	$>10^{-2}$	10^{-2}	(XIX)	10^{-1}	$>10^{-2}$	10^{-2}
(IX)	10^{-2}	$>10^{-2}$	10^{-2}	(XX)	10^{-2}	10^{-2}	10^{-2}
(X)	$>10^{-2}$	10^{-3}	10^{-3}	Sulfathiazole (Control)	10^{-6}	10^{-6}	10^{-5}
(XI)	10^{-2}	$>10^{-2}$	10^{-2}				

R: -AsO(OH)

(I) R-C$_6$H$_4$-

(II) R-C$_6$H$_3$(OH)-

(III) R-C$_6$H$_4$-NH$_2$

(IV) R-C$_6$H$_4$-NO$_2$

(V) R-C$_6$H$_3$(NO$_2$)-OH

(VI) R-C$_6$H$_3$(NH$_2$)-OH

(VII) R-C$_6$H$_4$-COCH$_3$

(VIII) R-C$_6$H$_4$-NHCONH$_2$

(IX) R-C$_6$H$_4$-NHCH$_2$CONH$_2$

(X) R-C$_6$H$_4$-C(CH$_3$)=N·NHCSNH$_2$

(XI) R-C$_6$H$_4$-C(CH$_3$)=N·NHCONH$_2$

5) Voegtlin, Smith: *loc. cit.*; Tatum, Cooper; J. Pharmacol. Exptl. Therap., 56, 198 (1934).

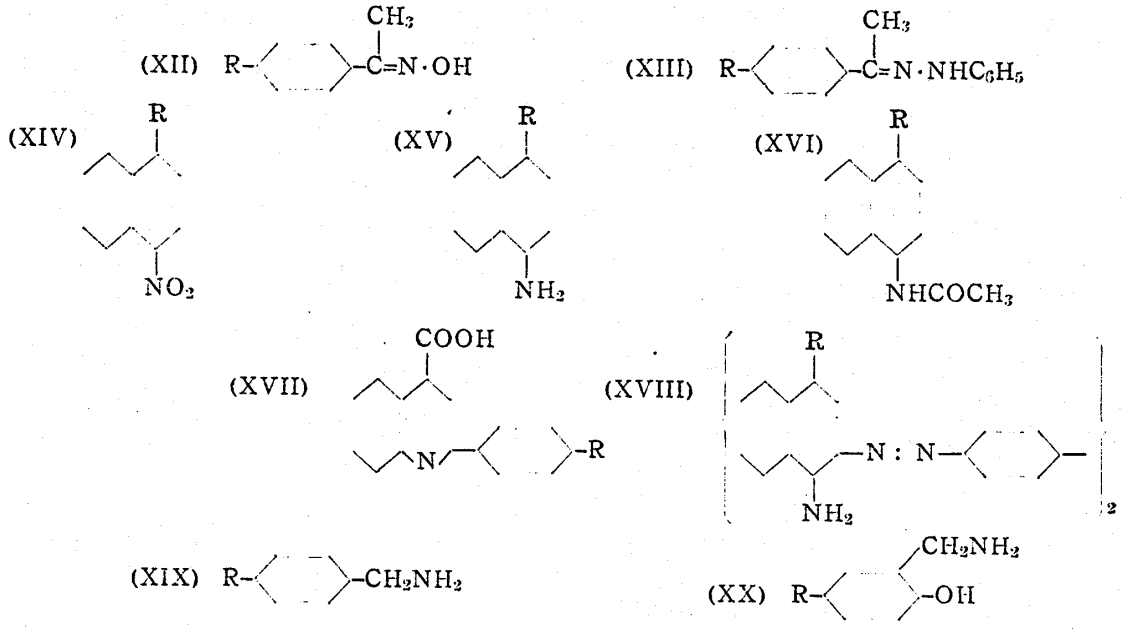
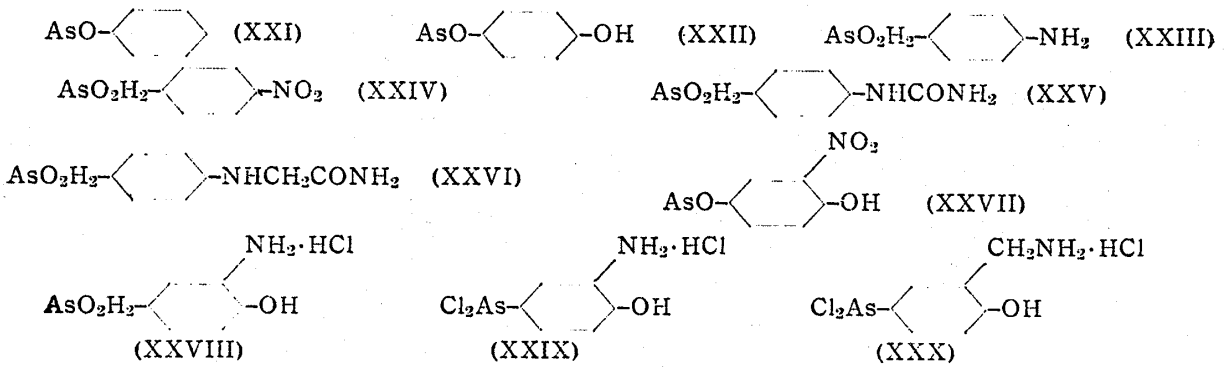


TABLE II

Arsonoso Compounds	*Esch. coli*	*Staph. aur.*	*Eber. typhosa*	*S. dysenteriae*
(XXI)	10^{-4}	—	10^{-4}	—
(XXII)	10^{-4}	—	10^{-5}	—
(XXIII)	10^{-4}	10^{-7}	—	—
(XXIV)	10^{-3}	—	10^{-3}	—
(XXV)	10^{-3}	—	10^{-4}	—
(XXVI)	10^{-3}	—	10^{-4}	—
(XXVII)	10^{-3}	10^{-10}	10^{-3}	10^{-7}
(XXVIII)	10^{-4}	10^{-4}	10^{-4}	10^{-4}
(XXIX)	10^{-3}	10^{-4}	10^{-4}	10^{-4}
(XXX)	10^{-3}	10^{-3}	10^{-4}	10^{-4}
Sulfathiazole (Control)	10^{-3}	10^{-4}	10^{-5}	10^{-5}
Neoarsphenamine (Control)	10^{-5}	10^{-5}	10^{-5}	10^{-4}

Arsonoso Compounds



Summary

1) Twenty compounds of the arylarsonic acid series and ten compounds of the arylarsonous acid series were examined as to their antibacterial activities.

2) Among the arylarsonic acid series, none of the compounds, except *p*-arsonoacetophenone phenylhydrazone, showed any positive activity. The hydrazone, however, was not of promise for pharmaceutical use, on account of its high toxicity.

3) Among the arylarsonous acid series, *p*-nitrophenylarsonous acid showed a weak activity, 3-nitro-4-hydroxyphenylarsene oxide, a more remarkable activity than sulfathiazole, and all other compounds, activities approximately equal to sulfanilamide. However, they were not of promise for chemotherapeutic use, because their toxicities were comparatively strong.

4) It may be concluded that arsonoso radical might be favorable for the antibacterial property, compared with arsono radical.

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6. Kiyoshi Takahashi, Shigeshi Toyoshima, and Takeo Ueda: Arsenical Chemotherapeutic Drugs. XI.[1] Antibacterial Properties of Diarylarsinic Acids and Diarylarsinous Acids.

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As described in the previous paper[1], the authors could not find any drug possessing a remarkable antibacterial activity and a low toxicity among primary arsenical compounds. As described in the previous papers[2], the authors synthesized many compounds belonging to the diarylarsinous acid series.

These types of arsenical compounds, although of interest for chemotherapeutic use, have scarcely been investigated as to their antibacterial activities. Only three compounds of dihydroxydiphenylarsinic acid were examined as to their effects on tuberculosis[3].

This paper describes the antibacterial activities of diarylarsinic acids and diarylarsinous acids.

Experimental Procedures Twenty-three compounds of the diarylarsinic acid series and 17 compounds of the diarylarsinous acid series were prepared according to the methods described in the previous papers[2]. These compounds, as shown in Tables I and II, were examined for their antibacterial activities.

Bacteriological procedures were the same as described in the foregoing paper[1].

Escherichia coli, *Shigella dysenteriae* (Komagome BIII), *Eberthella typhosa* (Kyodai strain) and *Staphylococcus aureus* (Terashima strain) were employed. The maximum dilution in molar concentration necessary for bacteriostasis after 48 hours were measured in media containing complete buillon by the serial dilution methods.

Antibacterial Activities of the Arsenical Compounds The antibacterial activities of the diarylarsinic acids are given in Table I by the maximum dilution in molar concentration required to inhibit the growths of bacteria.

It is evident from Table I that none of the diarylarsinic acids exerted a positive antibacterial activity *in vitro*.

The antibacterial activities of the diarylarsinous acids are given in Table II by the maximum dilution in molar concentration required to inhibit the growths of bacteria.

It is seen from Table II that among the diarylarsinous acids three compounds of diphenylarsinous oxide, 4-hydroxydiphenylarsinous acid, and 3-amino-4-hydroxydiphenylarsinous acid hydrochloride possessed more marked activities than sulfathiazole, seven compounds of 2-carboxydiphenylarsinous acid anhydride, 4-carboxydiphenylarsinous acid, 4-aminodiphenylarsinous acid, 4-nitrodiphenylarsinous acid, 4,4′-dihydroxydiphenylarsinous acid, 4,4′-diaminodiphenylarsinous acid, and 3-nitro-4-hydroxydiphenylarsinous acid posses-

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1) Part X: T. Ueda, K. Takahashi, S. Toyoshima, S. Kano: This Bulletin, 1, 17 (1953).
2) K. Takahashi: J. Pharm. Soc. Japan, 72, 529, 533, 1144 (1952).
3) M. Matsui, N. Hirano: Saikingaku-zasshi, 567 (1943), (Japan).