folded forms of hog thyroglobulin, thymus nucleohistone and diphtheria antitoxin under particular conditions, in the ultracentrifuge along with the native folded forms. These so-called X-proteins seemed to be intermediate between the native and denatured forms.[20a]

Steinhardt's work on the heat-denaturation of pepsin[21] and the re-examination of the activation energies of denaturation by Eyring and Stearn[22] have modified the conception of the denatured state as one of such great configurative variety as suggested by the earlier calculations of Mirsky and Pauling.[23] The definite differences noted between alkali-DnEa and the other forms, as well as the antigenicity and characteristic specificity of DnEa, are incompatible with the characterization of the denatured state as a "debris of peptide chains."[19] Even the size of the aggregates of DnEa in aqueous solution is now shown to be controllable, so that the manner of aggregation, also, would appear to be an orderly process.

(20a) *Cf.* also H. Neurath, J. P. Greenstein, F. W. Putnam and J. O. Erickson, *Chem. Rev.*, **34**, 157 (1944).

(21) J. Steinhardt, *Kgl. Danske Videnskab. Selskab. Math. fys. Medd.*, **14**, 11 (1937).

(22) H. Eyring and A. E. Stearn, *Chem. Rev.*, **24**, 253 (1939).

(23) A. E. Mirsky and L. Pauling, *Proc. Nat. Acad. Sci.*, **22**, 439 (1936).

Summary

1. Serological studies of DnEa prepared in a variety of ways showed that all preparations behaved alike in that part of the reaction range characterized by antibody excess.

2. Alkali-DnEa, in the reaction region of antigen excess, showed higher antibody-antigen ratios than did acid- or heat-DnEa. Within any one type the results were modified by aggregation and degradation. Decrease in the size of DnEa aggregates on aging lessened the amount of nitrogen precipitated from anti-Ea serum.

3. The quantitative immunochemical technique has served to supplement information gained by parallel chemical and physical studies of DnEa. Definite structural entities are indicated for DnEa, rather than a disordered state.

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Substituted Diphenylarsinic Acids and their Reduction Products

BY HUGO BAUER

Organic derivatives of arsenic hitherto investigated for their action against streptococci proved to be inactive. The strong bactericidal activity of bis-(4-aminophenyl)-sulfone[1] and of 4-nitro-4'-aminodiphenylsulfone[2] suggested the preparation of arsenicals of analogous structure. Diphenylarsinic acids, substituted in para position by amino and nitro groups, and their reduction products, were synthesized and tested for antistreptococcal action.[3] For starting material, 4-nitro-4'-aminodiphenylarsinic acid was prepared by action of 4-nitrodiazobenzene upon 4-acetylaminophenylarsine oxide, using the Sakellarios modification of the Bart method.[4] Reduction of the nitro group served as a convenient method for preparing bis-(4-aminophenyl) arsinic acid which hitherto was obtained in small yield only as a by-product in preparing arsanilic acid.[5] A series of reduction products was prepared employing the usual procedures. Secondary arsyl oxides and hydroxides were obtained by reduction of the corresponding arsinic acid with sulfur dioxide in presence of iodine; they were amorphous, but one of them (V) could be obtained in crystalline form containing benzene of crystallization. For the preparation of secondary diarsyls, hypophosphorous acid in presence of potassium iodide was employed. They were obtained as crystalline powders.

The bis-(4-aminophenyl)-arsyl hydroxide (X) and the corresponding arsyl chloride (IX) decompose readily in acid solution with formation of the tertiary tris-(4-aminophenyl)-arsine (XIII). This reaction is analogous to the formation of XIII from 4-aminophenyl-arsine oxide, described by Ehrlich and Bertheim.[6] From the arsine XIII, the corresponding arsine oxide (XIV) could be obtained by oxidation with iodine.

The description of 4,4',4'',4'''-tetraaminotetraphenylarsyl oxide given in the earlier publication[3] should be disregarded because we were then dealing with a decomposition product.

The compounds tested by S. M. Rosenthal[3] against hemolytic streptococci in mice, are shown in the table. 4-Nitro-4'-aminodiphenylarsinic acid showed some activity which was increased by acetylation. The activity of the acetyl derivative (I) was approximately the same as that of sulfanilamide, but curative effects were obtained only when the drug was administered in amounts close to the tolerated dose. The corresponding arsyl oxide and hydroxide (V and VI)

(1) G. A. H. Buttle, *et al.*, *Lancet*, **232**, 1331 (1937); E. Fourneau, *et al.*, *Compt. rend.*, **204**, 1763 (1937).

(2) E. Fourneau, *et al.*, *Bull. Acad. Med.*, **118**, 210 (1937); G. A. H. Buttle, *et al.*, *Biochem. J.*, **32**, 1101 (1938).

(3) S. M. Rosenthal, H. Bauer and E. Elvove, *Pub. Health Repts.*, **54**, 1317 (1939).

(4) E. Sakellarios, *Ber.*, **57**, 1514 (1924).

(5) F. L. Pyman and W. C. Reynolds, *J. Chem. Soc.*, **93**, 1180 (1908); L. Benda, *Ber.*, **41**, 2367 (1908).

(6) P. Ehrlich and A. Bertheim, *ibid.*, **43**, 917 (1910).

were approximately twice as active and twice as toxic as the pentavalent compound. Of the arsines, the acetylated compound (VII) was more active than the deacetylated compound (VIII), the former showing a therapeutic index of about 2.

When tested against *trypanosoma equiperdum* infection in mice, the compounds I, II, IV and V proved to be inactive.

The activity of some secondary organic arsenicals upon streptococcic infections, even though of no practical value because of their toxicity, shows that the activity of the sulfones cannot be attributed to the presence of sulfur alone. The central sulfur atom may be replaced by arsenic or other elements. In this connection, a secondary phosphorus compound of anti-streptococcic activity was previously reported by us.[7]

Experimental

I. 4-Nitro-4'-acetylaminodiphenylarsinic Acid.—*p*-Nitroaniline (16 g.) was dissolved in a mixture of 60 cc. of concentrated hydrochloric acid and 30 cc. of water with heating, and cooled. To the suspension of the hydrochloride thus formed, ice was added and, with stirring, a concentrated aqueous solution of sodium nitrite containing 8.9 g. was added. The diazo solution was filtered and diluted to a volume of 2 liters. 4-Acetylaminobenzenearsine oxide[8] (31 g.) was dissolved in water with addition of 25 cc. of 10 *N* sodium hydroxide, diluted to 2 liters, and 120 g. of crystalline sodium acetate was dissolved in this solution. The mixture was added with stirring to the diazo solution through a dropping funnel with the stem submerged under the surface of the liquid containing ice. Following the evolution of nitrogen, a yellow precipitate of a by-product separated which, after standing overnight in the cold room, was filtered off. Upon addition of concentrated hydrochloric acid to the filtrate, more of the by-product separated, which was removed. The clear yellow solution was carefully acidified with small amounts of hydrochloric acid, allowing the precipitate to change from the amorphous to the crystalline state. After filtering, washing with water and drying in the vacuum desiccator, the yellowish fine crystals weighed 22 g. (52% of the calcd.).

From 36% acetic acid cream-colored fine needles were obtained which softened between 245 and 250° and melted at 262° with decomposition. The sodium salt, obtained by dissolving the acid in sodium carbonate, is sparingly soluble in an excess of sodium carbonate solution.

II. 4-Nitro-4'-aminodiphenylarsinic Acid $O_2NC_6H_4AsO(OH)C_6H_4NH_2$.—Compound I (20 g.) was heated with 100 cc. of 25% hydrochloric acid until solution was complete. The solution was diluted with water and neutralized with sodium carbonate solution. After removing a first fraction of impurities, yellow needles of m. p. 239° were obtained; yield 15.3 g.

III. 4-Amino-4'-acetylaminodiphenylarsinic Acid.—A solution of 30 g. of I in 700 cc. of 2 *N* sodium carbonate solution was added to a hot solution of 160 g. of ferrous sulfate in 600 cc. of water. The mixture was heated to boiling for five minutes, the iron sludge was filtered off and washed with hot water. To the filtrate, hydrochloric acid was added until the reaction was just acid to congo paper. The crystalline precipitate thus obtained (23 g. = 83.6% of the calcd.) was purified by dissolving in 2 *N* hydrochloric acid, stirring with charcoal and reprecipitating with sodium acetate solution. Colorless crystals separated which melted at 279° with decomposition.

IV. Bis-(4-aminophenyl)-arsinic Acid.—Twenty-five grams of III was boiled with 125 cc. of 5 *N* hydrochloric acid for thirty minutes. The deacetylated compound was precipitated by addition of 325 cc. of 2 *N* sodium acetate: colorless microscopic prisms of m. p. 248° (Pyman,[5] 248–249°).

$$\left[\begin{matrix} O_2NC_6H_4 \\ CH_3COHNC_6H_4 \end{matrix}\!\!>\!As-\right]_2O$$

V. 4,4''-Dinitro-4',4'''-diacetylaminotetraphenylarsyl Oxide.—To a solution of 5 g. of I in 20 cc. of glacial acetic acid, a drop of hydriodic acid and 10 cc. of 25% hydrochloric acid was added. A stream of sulfur dioxide was passed through the solution for thirty minutes. Upon addition of water, a sticky precipitate separated which was washed first with a solution of sodium hydrogen carbonate, then with water. After drying in the vacuum desiccator, the amorphous material crystallized when triturated with benzene. From hot benzene in which the substance is moderately soluble, colorless clusters of needles were obtained which contained two molecules of benzene of crystallization. A melting point of 133° was obtained by slowly heating the melting point tube in order to evaporate gradually one molecule of benzene. When submerged in a bath of 105°, the substance melted immediately. One molecule of benzene could be accounted for by heating the substance in the vacuum oven at 98° for two hours. The crystals did not melt and retained their form. The melting point was 133°. The second molecule of benzene is firmly bound and could be removed only at higher temperature, 180° for three hours being adequate. After this time, the loss of weight corresponded to two molecules of benzene, while the substance had decomposed to a dark melted mass. However, decomposition with loss of weight continued upon further heating. The presence of benzene in the crystallized substance was proved by sealing a small sample in an evacuated glass tube and heating until melted. Benzene was condensed in the capillary tube and was detected by its odor and by burning with a smoky flame. This test was positive also for the second molecule of benzene, using the substance dried at 98°.

Anal. The substance was heated in the vacuum oven to 98° for 180 minutes, subsequently to 180° for 150 minutes. Calcd. for $C_{28}H_{24}As_2N_4O_7 + 2C_6H_6$: 1 mole C_6H_6, 9.36; 2 moles C_6H_6, 18.72. Found: 1 mole C_6H_6, 9.15; 2 moles C_6H_6, 18.72. The substance was titrated in acetic acid solution with 0.1 *N* iodine solution. Calcd. for $C_{28}H_{24}As_2N_4O_7 + 2C_6H_6$: As, 17.96. Found: As, 18.12. Calcd. for $C_{28}H_{24}As_2N_4O_7 + C_6H_6$: As, 19.81. Found: As, 19.88.

VI. 4-Nitro-4'-acetylaminodiphenylarsyl Hydroxide, $O_2NC_6H_4As(OH)C_6H_4NHCOCH_3$.—By dissolving V in alcohol and adding sodium hydroxide solution, an orange solution was formed. Upon acidifying the dilute solution with acetic acid, a milky suspension was obtained from which colorless crystals deposited. The substance softened at 70° without showing a melting point and was not soluble in benzene.

$$\left[\begin{matrix} O_2NC_6H_4 \\ CH_3COHNC_6H_4 \end{matrix}\!\!>\!As-\right]_2$$

VII. 4,4''-Dinitro-4',4'''-diacetylaminotetraphenyl Diarsyl.—To a solution of 3 g. of I in 50 cc. of acetone, 50 cc. of 30% hypophosphorous acid and 1 cc. of *N* potassium iodide was added. Soon a yellow, crystalline product separated which, after standing for three hours, was filtered, washed with water and dried in the vacuum desiccator; yield 2.7 g. The substance was insoluble in water, alcohol, glacial acetic acid, sodium hydroxide, but soluble in acetone.

$$\left[\begin{matrix} O_2NC_6H_4 \\ H_2NC_6H_4 \end{matrix}\!\!>\!As-\right]_2$$

VIII. 4,4''-Dinitro-4',4'''-diaminotetraphenyldiarsyl.—To a solution of 3 g. of II in 100 cc. of 30% hypophosphorous acid, 2 cc. of *N* potassium iodide solution was added. After standing in a carbon dioxide atmosphere overnight, the yellow precipitate was washed with water. The color changed to a deep yellow, owing to the hydrolysis of the hypophosphorous salt. The hydrolysis was completed by washing with *N* sodium

(7) H. Bauer and S. M. Rosenthal, *Pub. Health Repts.*, **54**, 2093 (1939); H. Bauer, THIS JOURNAL, **63**, 2137 (1941).

(8) A. Bertheim, *Ber.*, **44**, 1070 (1911).

TABLE I

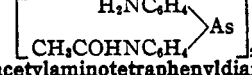
	Compound[a]	Appearance	M. p., °C.	Formula	Analyses, % As Calcd.	Found	Therapeutic index[b]
I	4-Nitro-4'-acetylaminodiphenylarsinic acid	Cream col. needles	262	$C_{14}H_{13}AsN_2O_5$	20.58	20.43[c]	1 subc. 2 oral
II	4-Nitro-4'-aminodiphenylarsinic acid	Yellow needles	239	$C_{12}H_{11}AsN_2O_4$	23.26	23.61[c]	<1
III	4-Amino-4'-acetylaminodiphenylarsinic acid	Colorless crystals	279	$C_{14}H_{15}AsN_2O_4$	22.43	22.47[d]	
IV	Bis-(4-aminophenyl)-arsinic acid[g]	Colorless micr. prisms	248				0
V	4,4''-Dinitro-4',4'''-diacetylaminotetraphenylarsyl oxide	Colorless needles	133	$C_{28}H_{24}As_2N_4O_7 + 2C_6H_6$	17.96	18.12[f]	1
VI	4-Nitro-4-acetylaminodiphenylarsyl hydroxide	Colorless crystals	70[g]	$C_{14}H_{13}AsN_2O_4$	21.53	20.89	1
VII	4,4''-Dinitro-4',4'''-diacetylaminotetraphenyl diarsyl	Yellow crystals	...	$C_{28}H_{24}As_2N_4O_6$	22.63	22.65[h]	1-2
VIII	4,4''-Dinitro-4',4'''-diaminotetraphenyl diarsyl	Orange powder	...	$C_{24}H_{20}As_2N_4O_4$	25.92	24.72	<1
IX	Bis-(4-aminophenyl)-arsyl chloride dihydrochloride	Colorless crystals	...	$C_{12}H_{14}AsCl_3N_2$	20.38	20.06	
					Cl 28.94	28.39	
X	Bis-(4-aminophenyl)-arsyl hydroxide	Amorphous	70–72[g]	$C_{12}H_{13}AsN_2O$	27.13	26.93	
XI	4,4''-Diamino-4',4'''-diacetylaminotetraphenyl diarsyl	Amorphous	147–152	$C_{28}H_{28}As_2N_4O_2$	24.87	25.08	
XII	Tetra-(4-aminophenyl)-diarsyl	Colorless powder	155–160	$C_{24}H_{24}As_2N_4$	28.91	28.91	
XIII	Tris-(4-aminophenyl)-arsine[i]	Crystalline powder	174–175	$C_{18}H_{18}AsN_3$	21.33	21.63	
XIV	Tris-(4-aminophenyl)-arsine oxide	Colorless crystals	No m. p.	$C_{18}H_{18}AsN_3O$	20.40	20.65[d]	

[a] Compounds I, II, V, VI, VII and VIII were described previously in a preliminary manner (ref. 3). [b] The therapeutic index is the ratio of the maximum tolerated dose to the minimum effective dose (ref. 3). [c] R. G. Fargher, *J. Chem. Soc.*, **115**, 982 (1919). [d] Carius method. [e] See ref. 5. [f] Titrated in acetic acid solution with 0.1 *N* iodine. [g] Softened without a definite m. p. [h] Oxidized by 0.1 *N* iodine in presence of sodium bicarbonate and the excess titrated back with 0.1 *N* sodium thiosulfate. [i] See reference 6.

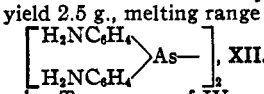
carbonate solution, followed by water; 2.8 g. of an orange product was obtained.

IX. Bis-(4-aminophenyl)-arsyl Chloride Dihydrochloride, $(HCl{\cdot}H_2NC_6H_4)_2AsCl$.—To a solution of 5 g. of IV in 50 cc. of hydrochloric acid (sp. gr. 1.12), 5 drops of concentrated hydriodic acid was added. A stream of sulfur dioxide was passed through the solution. Immediately a white crystalline precipitate was formed which was filtered, washed with hydrochloric acid and dried in the vacuum desiccator; yield 5.9 g. (calcd. 6.3 g.). It decomposes with acid as described under XIII.

X. Bis-(4-aminophenyl)-arsyl Hydroxide, $(H_2NC_6H_4)_2AsOH$.—To a solution of 2 g. of IX in 200 cc. of water, a 1% ammonium hydroxide solution was added. A white, sticky precipitate was formed which solidified after some time; yield 1 g. It softened at about 70–72° without giving a sharp melting point. It was soluble in alcohol, not soluble in ether and petroleum ether.



XI. 4,4''-Diamino-4,4'''-diacetylaminotetraphenyldiarsyl.—To a solution of 3 g. of III in 20 cc. of 50% hypophosphorous acid, 2 cc. of *N* potassium iodide solution was added. After standing for four hours, the reduction product was precipitated by 2 *N* sodium acetate solution as a sticky mass which soon became solid; yield 2.5 g., melting range 147–152°.



XII. Tetra-(4-aminophenyl)-diarsyl.—Two grams of IV was reduced as described for compound XI. A powdery white substance (1.6 g.) was obtained which softened at 150° and melted between 155 and 160°.

XIII. Tris-(4-aminophenyl)-arsine, $(H_2NC_6H_4)_3As$.—This compound, formerly described by Ehrlich and Bertheim,[6] is formed by decomposition of compound IX or X with acids. A solution of 2 g. of IX in 25 cc. of water was heated to 90° for five minutes. Upon addition of 10% ammonium hydroxide, an oil precipitated which solidified to a white crystalline powder (0.75 g.). It was recrystallized from dilute alcohol: m. p. 174–175° (E. and B. 173–174°).

XIV. Tris-(4-aminophenyl)-arsine Oxide, $(H_2NC_6H_4)_3AsO$.—A solution of 3.3 g. of IX in 30 cc. of water was heated to 90° for five minutes. After cooling a slight excess of *N* iodine solution was added and the excess removed with 0.1 *N* sodium thiosulfate. Upon addition of sodium hydrogen carbonate, colorless crystals separated which were filtered and washed with water; yield 1.25 g. (calcd. 1.65 g.); sparingly soluble in hot alcohol, moderately in hot methyl alcohol, insoluble in ether and petroleum ether. It can be recrystallized from hot methyl alcohol with addition of ether and petroleum ether and did not melt up to 315°, but became gradually black. A compound of the same composition, but of different properties, was reported by Morgan and Micklethwait.[9]

Summary

The preparation of 4-nitro-4'-aminodiphenylarsinic acid, bis-(4-aminophenyl)-arsinic acid and their reduction products has been described. Some of these secondary arsenic compounds have been found to be active against streptococcic infections in mice.

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(9) G. T. Morgan and F. G. Micklethwait, *J. Chem. Soc.*, **95**, 1473 (1909).