drate was prepared by dehydration of the hexahydrate at 115–120°. Compositions near that of the trihydrate were prepared by adding water with a micropipet to a sample of the dihydrate. The mixture was then finely ground and a sample taken for analysis simultaneously with the sealing of the material in the capillary tube.

Discussion.—The solubility and solid phase data given in Table I and shown graphically in Fig. 2 are the results of five separate experimental runs which overlap. Some of the values were obtained going up the temperature scale and some by proceeding from a higher to a lower temperature. No decomposition of uranyl nitrate was detected below 184° either by visual observation or by titration of the standard ceric sulfate solution contained in the gas train. Above 184° the dihydrate decomposes giving oxides of nitrogen.

The melting point data for the dihydrate and the trihydrate are given in Table II. These values check those obtained by extrapolation of the solubility curves.

TABLE II

MELTING POINT DETERMINATIONS

Weight %, $UO_2(NO_3)_2$	87.2	87.5	89.1	89.9	91.63
First observed melting, *t*., °C.	113	113	113	112	184

Data available from the literature[2,3,4,5] are also shown in Fig. 2 and serve to indicate the amount of deviation from our values. Wasilieff[5] gives 121.5° as the trihydrate melting point compared to our value of 113° (incongruent point). Benrath[2] gives 187°, and Wasilieff[5] 179.3°, as the dihydrate melting point for which we have determined the temperature to be 184°.

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RECEIVED OCTOBER 5, 1950

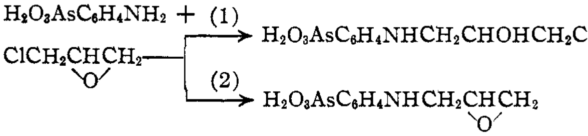
Some Substituted *p*-Arsanilic Acids[1,2]

BY ROBERT L. MCGEACHIN AND ROBERT E. COX

The reactions of 4-hydroxyphenylarsonic acid and *p*-arsanilic acid with various aliphatic chlorides and bromides have been studied by Hamilton[3,4,5,6] and his co-workers. In this investigation, we have extended the study, carrying out the reactions of *p*-arsanilic acid with 1,3-dichloropropanol-2, epichlorohydrin, β-chloro-β′-hydroxydiethyl ether (diglycolchlorohydrin) and trimethylene dibromide.

In the reaction of *p*-arsanilic acid with 1,3-dichloropropanol-2 using sodium bicarbonate as a buffer, yields were comparable to those obtained in similar reactions using unbuffered media (30%), with the bis-compound, N,N′-di-(4-arsonophenyl)-1,3-diaminopropanol-2, the only compound that could be isolated. However, when disodium phosphate was used as a buffer, both the bis-compound and N-(3-chloro-2-hydroxypropyl)-*p*-arsanilic acid were found.

With epichlorhydrin, unsatisfactory results were obtained in the buffered medium but when the reaction was run without a buffer characterizable products were obtained, although in low yields. Of the two possible mechanisms for the first stage of this reaction, (1) is the more probable



$$H_2O_3AsC_6H_4NH_2 + ClCH_2\underset{\diagdown O \diagup}{CHCH_2} \xrightarrow{(1)} H_2O_3AsC_6H_4NHCH_2CHOHCH_2Cl$$

$$\xrightarrow{(2)} H_2O_3AsC_6H_4NHCH_2\underset{\diagdown O \diagup}{CHCH_2}$$

since we were able to isolate N-(3-chloro-2-hydroxypropyl)-*p*-arsanilic acid from the reaction mixture. This could then react with another molecule of *p*-arsanilic acid to give the bis-compound which was found to be the principal product.

β-N-(4-Arsonophenyl)-amino-β′-hydroxydiethyl ether, formed by the reaction of diglycolchlorhydrin on *p*-arsanilic acid, proved to be so water-soluble that the free acid could not be isolated in the pure form so that it was necessary to isolate it as the sodium salt. This was unexpected since β-(β′-hydroxy)-ethoxyethoxyphenylarsonic acid,[6] a compound closely resembling our product in structure, was easily isolated as the free acid.

In the reaction of trimethylene dibromide with *p*-arsanilic acid only the bis-compound, N,N′-di-(4-arsonophenyl)-1,3-diaminopropane, could be isolated as was the case in the reaction of ethylene dibromide with *p*-arsanilic acid.[3]

Experimental

Reaction of *p*-Arsanilic Acid with 1,3-Dichloropropanol-2.—To a solution of 2 g. of *p*-arsanilic acid (I), 3.7 g. of sodium hydroxide and 9.5 g. of sodium bicarbonate in 60 ml. of water, 7 g. of 1,3-dichloropropanol-2 (II) was added and the mixture boiled under reflux for six hours. Acidification to congo red with concd. hydrochloric acid precipitated a white solid, N,N′-di-(4-arsonophenyl)-1,3-diaminopropanol-2. This product was purified by dissolving in 10% sodium hydroxide, reprecipitating with acid, washing with water and drying over calcium chloride *in vacuo*. It does not melt below 250°; yield of 6.8 g. (30%).

Anal.[7] Calcd. for $C_{15}H_{20}O_7N_2As_2$: As, 30.6. Found: As, 30.4.

Sixty-seven grams of I was dissolved in 100 ml. of 2.3 *N* sodium hydroxide, 22 g. of disodium phosphate added and the mixture heated to boiling. Fifteen ml. (20 g.) of II was added, 1 ml. every ten minutes with each addition followed after five minutes by the addition of 1 ml. of 40% sodium hydroxide. Acidification of the cooled reaction mixture to congo red with concd. hydrochloric acid precipitated the bis-compound which was purified as described above; yield of 22 g. (29%).

Anal. Calcd. for $C_{15}H_{20}O_7N_2As_2$: As, 30.6. Found: As, 30.3.

The filtrate from above, when adjusted to a *p*H of 5 with 10% sodium hydroxide, deposited white crystals on standing in the ice-box. This product was washed with ice-cold water, dried at 120° for several hours and then *in vacuo* over calcium chloride. Qualitative tests showed it contained chlorine. It does not melt below 250°; yield of 3 g. (6%).

Anal. Calcd. for $C_9H_{13}O_4NClAs$: As, 24.2. Found: As, 24.0.

Reaction of I with Epichlorhydrin.—To 67.3 g. of I dissolved in 75 ml. of 4 *N* sodium hydroxide, 28.7 g. of epichlorhydrin was added and the mixture boiled under reflux for one hour. Acidification in the usual manner precipitated the bis-compound which was purified in the manner described above; yield of 10 g. (14%).

Anal. Calcd. for $C_{15}H_{20}O_7N_2As_2$: As, 30.6. Found: As, 30.4.

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(1) Taken in part from the M.S. thesis of Robert E. Cox.

(2) This work was aided by a grant to the University of Louisville from the Kentucky State Medical Research Commission.

(3) C. S. Hamilton, THIS JOURNAL, **45**, 2751 (1923).

(4) L. A. Sweet and C. S. Hamilton, *ibid.*, **56**, 2409 (1934).

(5) P. O. Bare and C. S. Hamilton, *ibid.*, **59**, 2444 (1937).

(6) J. Parker, Ph.D. thesis, University of Nebraska, 1940.

(7) A modification of the method of F. E. Cislak and C. S. Hamilton, THIS JOURNAL, **52**, 638 (1930), was used in the arsenic analyses.

The filtrate from above, when adjusted to a *p*H of 5 with 10% sodium hydroxide, deposited a brown gum. This dissolved in 95% alcohol, precipitated by the addition of several volumes of ether, dried at 120 for three hours and then *in vacuo* over concd. sulfuric acid. Qualitative tests showed it contained chlorine. It did not melt below 250°; yield of 2 g. (4%).

Anal. Calcd. for $C_9H_{13}O_4NClAs$: As, 24.2. Found: As, 23.9.

Reaction of I with β-Chloro-β'-hydroxydiethyl Ether.—To 5 g. of I dissolved in 25 ml. of *N* sodium hydroxide was added 4.1 g. of β-chloro-β'-hydroxydiethyl ether and the mixture boiled under reflux for 5 hours. The cooled mixture was made acid to congo red with concd. hydrochloric acid and then evaporated to dryness on a steam-bath. The residue was extracted with two 50-ml. portions of boiling 95% alcohol, the extract charcoaled and filtered. To the filtrate was added 200 ml. of ether, precipitating a brown oily gum. The gum was dissolved in 10 ml. of 2.5 *N* sodium hydroxide and 100 ml. of cold 95% alcohol added, precipitating a white solid. This was washed with cold alcohol, dried at 120° for 2 hours and then *in vacuo* over calcium chloride; yield of 3.2 g. (43%).

Anal. Calcd. for $C_{10}H_{15}O_6NNaAs$: As, 22.9. Found: As, 23.1.

Reaction of I with Trimethylene Dibromide.—To 21.7 g. of I dissolved in 100 ml. of *N* sodium hydroxide was added 13 g. of trimethylene dibromide and the mixture boiled under reflux for three hours, a white solid gradually separating. The mixture was made acid to congo red with concd. hydrochloric acid, the white solid filtered off and washed with 95% alcohol and water. This product was redissolved in 5% sodium hydroxide, reprecipitated by addition of concd. hydrochloric acid, washed with 95% alcohol, hot water, and dried at 120°. This compound does not melt below 250°; yield of 6 g. (25%).

Anal. Calcd. for $C_{15}H_{20}O_6As_2$: As, 31.6. Found: As, 31.3.

The sodium salt was formed in the usual manner by dissolving in sodium hydroxide solution and precipitating by addition of alcohol.

Anal. Calcd. for $C_{15}H_{18}O_6N_2Na_2As_2$: As, 29.0. Found: As, 29.0.

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RECEIVED OCTOBER 14, 1950

Water Absorption of Proteins. VI. Effect of Guanidino Groups in Casein[1]

BY EDWARD F. MELLON, ALFRED H. KORN, ELSIE L. KOKES AND SAM R. HOOVER

The vapor phase water absorption isotherms[2] of the amino groups in casein and of the peptide groups of some long polyglycine molecules show that these polar groups are specific sorptive sites over the entire range of relative humidities. The basic nature of the guanidino group indicated that it might also be a specific site for water absorption. Therefore, guanidino groups have been introduced into casein by the reaction of S-methylisothiourea with the free amino groups in alkaline solution. Several casein derivatives containing various amounts of substituted guanidino groups have been prepared in this manner.

The vapor phase water absorption isotherms of these samples were obtained as in the previously described studies.[2] The absorptions were calculated for a 1000-g. content of casein to eliminate the dilution effect due to the varying weight of guanidino group in each sample. The absorption of the substituted samples were subtracted from the control sample and the results listed in Table I.

TABLE I

DECREASE IN ABSORPTION DUE TO SUBSTITUTION

Relative humidity, %	Sorption of control, g./1000 g.	Decreased absorption, g./1000 g. casein, by samples 1	2	2A[a]
11.8	35	0	0	9
31.4	69	1	−1	16
50.9	97	0	0	23
75.1	146	2	1	32
83.6	174	6	5	40

[a] Calculated values for decreased absorptions due to the removal of the same number of amino groups as were substituted in sample 2.

The data listed for sample 2A are the calculated water absorption values for the same number of free amino groups[2] in casein as have been substituted in derivative 2. The absorption of the control sample was equal to the absorption of the same original casein which had not been treated with the alkali. This shows that there was no significant change in the casein due to this treatment. These data show that the vapor phase water absorption of the guanidino groups produced from the amino groups is not significantly different from the absorption of the free amino groups themselves.

These introduced guanidino groups differ from the naturally occurring guanidino groups in proteins only in that they are one methylene group further removed from the peptide chain of which they are side groups. This, however, would not be expected to cause any great difference in the polarity or water absorbing capacity of these groups. The 4.1 g. of arginine naturally present in 100 g. of casein[3] contains 0.0235 mole of guanidino groups. Therefore, the guanidino groups naturally present in casein will account for an average of 8.7% of the total water absorption of casein between 30 and 85% relative humidity and may account for as high as 12% at lower humidities.

The almost constant fractions of the total water absorption at all relative humidities which have been found to be due to the amino, peptide and guanidino groups show that these polar groups which comprise only a few per cent. of the total protein must be considered in any theoretical analysis of the sorption phenomena. It is consistent with the polarization theory of Bradley which we have shown fits the data for the water absorption of proteins and other high polymers.[4]

Experimental

Preparation of Guanidinated Caseins.[5]—Twenty-five grams of high nitrogen casein was swelled for one hour in 100 ml. of water. Then 200 ml. of water and 210 ml. of concd. ammonium hydroxide were added, and the mixture was stirred until the casein dissolved. S-Methylisothiourea sulfate (0.0, 4.0 or 12.0 g.)[6] in a small amount of water was added and the mixture was stored at 5° for three days. The solution was then transferred to a viscose tube and dialyzed

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[1] Presented before the 117th Meeting of the American Chemical Society at Philadelphia, April, 1950. Article not copyrighted.

[2] E. F. Mellon, A. H. Korn and S. R. Hoover, THIS JOURNAL, **69**, 827 (1947); *ibid.*, **70**, 3040 (1948).

[3] W. G. Gordon, W. S. Semmett, R. S. Cable and M. Morris, *ibid.*, **71**, 3293 (1949).

[4] S. R. Hoover and E. F. Mellon, *ibid.*, **72**, 2562 (1950).

[5] E. Schütte, *Z. physiol. Chem.*, **279**, 59 (1943).

[6] Control and samples 1 and 2, respectively.