$$R'RC(OH)ONa \longrightarrow R'RC{:}O + NaOH \qquad (19)$$
$$R'RCHONa + H_2O \longrightarrow R'RCHOH + NaOH \qquad (20)$$

Formation of a pinacone, as observed by Beckmann and Paul, would imply that the sodium compound was itself partially polymerized under the conditions of their experiment and that direct hydrolysis of this polymer occurred to some extent.

The author wishes to acknowledge his deep obligation to Dr. George F. White, at whose suggestion this research was initiated, and to thank the other members of the Faculty of the Department of Chemistry of Clark University for their aid.

Summary

1. Both mono- and disodium derivatives of benzophenone may be prepared readily by the action of sodium in liquid ammonia. Dimetallic derivatives may also result from the action of amides on the corresponding alcohol.

2. The reactions of ethyl bromide and phenyl halides with sodium compounds of benzophenone have been studied. Both substitution and reactions involving the solvent have been observed.

3. Evidence has been presented to show that the occurrence of addition reactions is a more general property of the metal ketyls than has been previously supposed. Even their decomposition by strong acids may be preceded by the formation of addition products.

CAMBRIDGE, MASSACHUSETTS

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HAMLINE UNIVERSITY]

ALIPHATIC-AROMATIC ARSENO COMPOUNDS. II. β-HYDROXYETHYLARSONIC ACID AND SOME ARYL ARSENO-ETHANOLS[1]

BY R. HERBERT EDEE

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A complete series of unsymmetrical aliphatic-aromatic arseno compounds has been described in the literature.[2] In this series it was found that all of the members were soluble because of the presence of a carboxyl group in the aliphatic part of the molecule. Most of the compounds contained additional solubilizing groups in the aromatic part. It was thought desirable to prepare a series of aliphatic-aromatic arseno compounds whose solubilities would be due to the presence of certain groups in the aromatic portion of the molecule. The series to be described contains the hydroxy-ethyl group, $-CH_2CH_2OH$, as the aliphatic portion and various aryl groups as the aromatic part of the arseno molecule.

[1] This paper is the result of an investigation which was begun at the suggestion of Dr. Charles Shattuck Palmer, formerly of Northwestern University.

[2] Palmer and Edee, THIS JOURNAL, 49, 998 (1927).

The first step in this investigation was the preparation of β-hydroxyethylarsonic acid. This acid is mentioned in the literature,[3] but the evidence shows that it has never been prepared in the pure state. By the procedure which was followed a solution containing 85% of β-hydroxyethylarsonic acid was prepared. The only impurities present were found to be water and alcohol. Since neither of these substances influences the reduction of the acid with the subsequent formation of arseno compounds, the preparation of pure aryl arseno-ethanols from this solution was possible. A solution of the acid which had stood in a vacuum over sulfuric acid finally crystallized in large leaflets and gave an analysis which was correct for the pure acid containing one molecule of water of crystallization. It was found impossible to isolate the acid in the form of any of its metallic salts as these are all extremely soluble and decompose on evaporation of their solutions.

In general, the method used in the preparation of the arseno-ethanols consisted in adding the reducing agent, hypophosphorous acid, to $M/50$ portions of the aromatic acid and the β-hydroxyethylarsonic acid in rather strong hydrochloric acid solution. The filtered solution was then placed in the cold for varying lengths of time. As a rule the arseno compound separated and could be washed and dried. Different aromatic acids, however, were found to require variations in this procedure. As in the case of the arseno-acetic acids, the arseno-ethanols are sensitive to heat, and reductions at higher than room temperature result in the formation of products containing high percentages of arsenic. Most of the reductions were carried out at low temperatures.

When phenylglycine-*p*-arsonic acid or 3-amino-4-hydroxyphenylarsonic acid is reduced with β-hydroxyethylarsonic acid, the tetra-arseno compounds result rather than the arseno. This is due to the fact that these acids are very sensitive under the existing conditions, and apparently arsenic is split off in some manner from organic combination. This inorganic arsenic is then reduced simultaneously with the undecomposed acids, resulting in the formation of the tetra-arseno-ethanols. When 4-acetoxyphenylarsonic acid is reduced with β-hydroxyethylarsonic acid, the first product which separates is one which analyzes for three arsenic atoms in the molecule. The formation of this compound is no doubt similar to the formation of the tetra-arseno compounds.

Additional information as to the structure of these arseno compounds is gained from the fact that in alkaline solution they may be oxidized back to the arsonic acids and these acids may be recovered quantitatively. In the case of the arseno compounds from 4-hydroxyphenylarsonic acid and 4-acetoxyphenylarsonic acid, this oxidation was successfully carried out by means of hydrogen peroxide.

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[3] English patent 191,028 (1923); Quick and Adams, THIS JOURNAL, **44,** 809 (1922).

All of the arseno-ethanols are finely divided, orange-yellow powders, the higher arseno compounds being darker colored. None can be crystallized and all are found to be completely soluble in reagents exerting a solubilizing influence upon the particular group present in the aromatic portion of the molecule.

Experimental Part

β-Hydroxyethylarsonic Acid, $H_2O_3AsCH_2CH_2OH$.—One-half mole (100 g.) of arsenic trioxide was dissolved in a solution of three moles (120 g.) of sodium hydroxide in 300 cc. of water. This was sufficient alkali to give the trisodium salt of arsenious acid. The mixture was cooled to room temperature and 1 mole (80 g.) of ethylene chlorohydrin was added dropwise during a period of thirty-five minutes. Vigorous stirring was employed during the whole operation and at the end of the addition the reaction mixture had become quite hot. The solution was allowed to stand overnight at room temperature, and was maintained, with stirring, at 80–90° on a hot water-bath for one hour.

At the end of this time the solution was cooled to 20° and glacial acetic acid was added until the solution reacted acid to litmus, about 65 cc. being required. The mixture was again cooled and the precipitated arsenious oxide was filtered off by suction and washed with 100 cc. of water. The filtrate was made strongly alkaline with ammonium hydroxide and any unreacted arsenious oxide remaining in solution was removed in the following manner. Hydrogen peroxide (3% solution) was added to the alkaline filtrate until the solution liberated iodine from potassium iodide solution. This was an indication that all of the trivalent arsenic had been oxidized by the peroxide solution to the pentavalent state and that the hydrogen peroxide was present in excess. In all about 210 cc. of the peroxide solution was added. The pentavalent arsenic was then precipitated as magnesium ammonium arsenate by the addition of an excess (about 500 cc. in all) of magnesia mixture. After standing overnight the precipitate was filtered off, the filtrate acidified with hydrochloric acid and evaporated on a steam-bath. The inorganic salts which separated were filtered off from time to time.

Absolute alcohol was then added to precipitate more of the inorganic salts and the filtered solution was again evaporated. After several such treatments the acid remained as a yellow oil. Ignition tests showed that no inorganic material was present and direct titration with iodine solution showed the absence of inorganic arsenic. An analysis of the oil made to determine the percentage which could be considered to be pure β-hydroxyethylarsonic acid showed it to be an 84.3% solution of the acid. The only impurities present were water and alcohol. After the acid had stood for a little over a year in a vacuum desiccator over sulfuric acid, it crystallized in large plates which contained one molecule of water of crystallization. This water was completely removed by heating in a vacuum drier at 80°. The anhydrous acid was found to be very hygroscopic and as a result a definite melting point could not be obtained. The compound melted between 157 and 159°.

Anal. Subs., 0.1035, 0.1041: 24.20, 24.35 cc. of I_2 soln. (1 cc. = 0.001884 g. As). Calcd. for $C_2H_7O_4As$: As, 44.11. Found: 44.05, 44.06.

Aryl Arseno-ethanols

4-Hydroxyphenylarseno-ethanol, $(HO)C_6H_4As{=}AsCH_2CH_2OH$.—To a solution of 6.44 g. of a 53% solution of β-hydroxyethylarsonic acid and 4.36 g. of 4-hydroxyphenylarsonic acid in 30 cc. of water and 10 cc. of hydrochloric acid (sp. gr., 1.19), 53 cc. of 50% hypophosphorous acid was added. The mixture was filtered and then placed in an ice-box. The color of the solution changed from a light yellow through orange to red.

At the end of two days an orange precipitate had separated. This was filtered off with suction, washed with dilute hydrochloric acid and water and dried in a vacuum over phosphorus pentoxide. Analysis and solubility tests showed that the compound was not quite pure, and so it was triturated for a short time with fresh portions of warm, dilute (1 to 5) hydrochloric acid. After this treatment the compound analyzed correctly for the unsymmetrical derivative. 4-Hydroxyphenylarseno-ethanol is an orange powder which is completely soluble in dilute aqueous alkalies; yield, 3.86 g., or 67%.

Anal. Subs., 0.1013, 0.1011: 34.60, 34.35 cc. of I_2 soln. (1 cc. = 0.001537 g. of As). Calcd. for $C_8H_{10}O_2As_2$: As, 52.08. Found: 52.49, 52.22.

The above product was further identified by the following experiment wherein the arseno compound was oxidized and the 4-hydroxyphenylarsonic acid recovered. Two grams of the arseno compound was put into solution in about 8 cc. of 12 *N* sodium hydroxide. The solution was warmed with stirring on a water-bath at 50°, and 5% hydrogen peroxide was added to it until all color disappeared. The mixture was stirred and maintained at this temperature for one and one-half hours. The solution was cooled under running water and hydrochloric acid (sp. gr., 1.19) was added until the reaction was acid to congo red. From this acid solution the 4-hydroxyphenylarsonic acid separated. The β-hydroxyethylarsonic acid, being water soluble, remained in solution. Two grams of $C_8H_{10}O_2As_2$ gave 1.501 g. of $C_6H_7AsO_4$; theoretical, 1.513 g.

4-Aminophenylarseno-ethanol Hydrochloride, $(H_2N)C_6H_4As{=}AsCH_2CH_2OH\cdot HCl$.—To a solution of 4.34 g. of arsanilic acid, 4.03 g. of an 84% solution of β-hydroxyethylarsonic acid in 10 cc. of hydrochloric acid (sp. gr., 1.19) and 10 cc. of water, 30 cc. of pyridine and 53 cc. of 50% hypophosphorous acid were added. The mixture was allowed to stand at ice-box temperature for three days. At the end of this time 200 cc. of hydrochloric acid (sp. gr., 1.19) was added and the mixture was again placed in the ice-box overnight. A copious orange-yellow precipitate formed which was filtered off by suction and washed with dilute hydrochloric acid and water. It was dried in a carbon dioxide filled desiccator over phosphorus pentoxide. An analysis of the compound showed that it contained 46.10% of arsenic and 10.49% of chlorine. The theoretical percentages in 4-aminophenylarseno-ethanol hydrochloride are 46.43 and 10.83, respectively. This, then, is the hydrochloride of the unsymmetrical compound.

In order to see the effect of water on this hydrochloride, the orange-yellow powder was triturated with warm water containing only the slightest trace of hydrochloric acid to prevent gumming. Analysis of the filtered and dried compound showed it to be the free base of 4-aminophenylarseno-ethanol.

This reduction was repeated with the exception that the pyridine was omitted. After fourteen days an orange-colored product weighing 1.20 g. had separated. This was filtered off, washed and dried in a vacuum over phosphorus pentoxide. When perfectly dry the compound was completely soluble in warm hydrochloric acid. A sodium fusion showed the absence of halogen. Analysis proved that this was the free base of 4-aminophenylarseno-ethanol, and the compound was identical in every respect with the free base obtained above.

Anal. Subs., 0.1005, 0.1005: 25.20, 25.20 cc. of I_2 soln. (1 cc. = 0.002083 g. of As). Calcd. for $C_8H_{11}ONAs_2$: As, 52.26. Found: 52.23, 52.23. Subs., 0.2014, 0.1963: 6.91, 6.71 cc. of 0.1 *N* HCl. Calcd. for $C_8H_{11}ONAs_2$: N, 4.87. Found: 4.79, 4.82.

Reduction of 4-Arsonophenoxyacetic Acid and β-Hydroxyethylarsonic Acid.—In an attempt to prepare 4-acetoxyphenylarseno-ethanol, 5.52 g. of 4-arsonophenoxyacetic acid and 4.03 g. of an 84% solution of β-hydroxyethylarsonic acid were dissolved in 30 cc. of water and 10 cc. of hydrochloric acid (sp. gr., 1.19). To this was added 53 cc. of 50% hypophosphorous acid and the filtered solution was placed in an ice-box.

After three days there was no evidence of reduction and so the flask and its contents were warmed to 60° for ten minutes. The solution immediately became cloudy and turned a light yellow color. A yellow, alkali soluble precipitate weighing 1.33 g. separated. This was filtered off, washed with dilute hydrochloric acid and water and dried *in vacuo* over sulfuric acid.

Anal. Subs., 0.1020, 0.0984: 26.20, 25.10 cc. of I_2 soln. (1 cc. = 0.002083 g. of As). Calcd. for $C_{10}H_{12}O_4As_3$: As, 53.40. Found: 53.50, 53.15.

An oxidation of 0.8234 g. of this compound gave 0.522 g. of 4-acetoxyphenylarsonic acid; theoretical, 0.539 g. It is probable, then, that this compound is the unsymmetrical triarseno-ethanol.

In order to prepare the arseno compound, the reduction was repeated using the same amounts of the respective arsonic acids and the same amounts of hydrochloric and hypophosphorous acids. The procedure was varied, however, in that the reduction mixture was allowed to stand at room temperature (without heating) for ten days. At the end of that time a yellow, alkali-soluble precipitate similar to the one obtained above had separated. The analysis showed that this was 4-acetoxyphenylarseno-ethanol; yield, 2.34 g., or 36%.

Anal. Subs., 0.1002, 0.1009: 21.30, 21.40 cc. of I_2 soln. (1 cc. = 0.002083 g. of As). Calcd. for $C_{10}H_{12}O_4As_2$: As, 44.62. Found: 44.23, 44.17.

The above compound was further identified by oxidation with hydrogen peroxide and recovery of the 4-acetoxyphenylarsonic acid; 1.5413 g. of $C_{10}H_{12}O_4As_2$ gave 1.228 g. of $C_8H_9O_6As$; theoretical, 1.230 g.

3-Amino-4-hydroxyphenyl-tetra-arseno-ethanol, $3\text{-}(H_2N)\text{-}4\text{-}(HO)C_6H_3As{=}As{-}As{=}As{-}CH_2CH_2OH$.—A solution of 4.66 g. of 3-amino-4-hydroxyphenylarsonic acid and 3.76 g. of β-hydroxyethylarsonic acid monohydrate was made in 15 cc. of water and 15 cc. of hydrochloric acid (sp. gr., 1.19). To this was added 53 cc. of 50% hypophosphorous acid and the mixture was allowed to stand at a temperature of 15° for six days. The orange product which formed was filtered off by suction, washed well with dilute hydrochloric acid and water and dried over phosphorous pentoxide. A sodium fusion showed the absence of halogen. The compound was soluble in dilute aqueous alkalies, and concentrated hydrochloric acid; yield, 1.02 g., or 11.2%.

Anal. Subs., 0.1000, 0.1000: 57.50, 57.55 cc. of I_2 soln. (1 cc. = 0.001208 g. of As). Calcd. for $C_8H_{11}O_2NAs_4$: As, 69.93. Found: 69.46, 69.52. Subs., 0.1297, 0.2041: 28.18, 44.64 cc. of 0.01 N HCl. Calcd. for $C_8H_{11}O_2NAs_4$: N, 3.09. Found: 3.04, 3.05.

The reduction was repeated in an attempt to prepare the arseno compound. In the second reduction the conditions were the same as in the above with the exception that the reduction mixture was diluted with distilled water to a total volume of 450 cc. The orange product which separated was in all respects identical with the one described above.

4-Glycinephenyl-tetra-arseno-ethanol, $(HOOCCH_2NH)C_6H_4As{=}As{-}As{=}As{-}CH_2CH_2OH$.—A solution of 5.5 g. of phenylglycine-*p*-arsonic acid and 3.76 g. of β-hydroxyethylarsonic acid monohydrate was made in 15 cc. of water and 10 cc. of hydrochloric acid (sp. gr., 1.19). In order to insure complete solution, the mixture was warmed gently. After the solution had cooled, it was filtered and 53 cc. of 50% hypophosphorous acid was added. The reduction mixture was allowed to stand at an average temperature of 10°. After two days 1.268 g. of a finely-divided orange precipitate separated. This was completely soluble in dilute aqueous alkalies.

Analysis showed it to be the tetra-arseno compound.

Anal. Subs., 0.1000, 0.1000: 50.00, 50.00 cc. of I_2 soln. (1 cc. = 0.01199 g. of As). Calcd. for $C_{10}H_{18}O_3NAs_4$: As, 60.60. Found: 59.95, 59.95. Subs., 0.1210, 0.1512:

23.78, 30.04 cc. of 0.01 *N* HCl. Calcd. for $C_{10}H_{18}O_3NAs_4$: N, 2.82. Found: 2.75, 2.78.

Summary

1. β-Hydroxyethylarsonic acid has been obtained in a crystalline form.
2. A series of aryl arseno-ethanols has been prepared by the simultaneous reduction of β-hydroxyethylarsonic acid and various aromatic arsonic acids.
3. One member of the previously unknown series of unsymmetrical aliphatic-aromatic tri-arseno compounds has been made.

SAINT PAUL, MINNESOTA

[CONTRIBUTION FROM THE LABORATORY OF PHYSICAL CHEMISTRY OF THE UNIVERSITY OF UPSALA]

THE MOLECULAR WEIGHT OF HEMOCYANIN

BY THE SVEDBERG AND EUGEN CHIRNOAGA[1]

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Hemocyanin is the respiratory blue pigment of the blood of certain lower animals: molusca, crustacea and arachnoides.[2,3,4] Both in physiological function and in composition it shows a certain analogy to hemoglobin, the respiratory pigment of the blood of higher animals. The hemocyanin molecule has a metallic constituent, copper, while hemoglobin contains iron. Hemocyanins from different species seem to differ in their copper content. Griffiths[5] found for *Cancer*, *Homarus* and *Sepia* about 0.33%; Heinze[6] for *Octopus vulgaris* 0.38%; Burdel[7] and Begemann[8] for *Helix pomatia* about 0.27%; Alsberg and Clark[9] for *Limulus polyphemus* 0.28%; while Redfield, Coolidge and Shotts[10] in a very careful investigation found for the same species 0.173%. The differences in the copper content as well as in other properties, for example, the oxygen combining curve, seem to indicate that there exist different kinds of hemocyanin.

Because of the physiological importance of hemocyanin it was thought to be of interest to study its actual molecular weight in solution by means of the new method of ultracentrifuging, already applied to the study of hemoglobin, egg albumin, phycoerythrin and phycocyan.

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[1] Fellow of the International Education Board.

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[4] J. Botazzi, *ibid.*, **18**, 1 (1919).

[5] Griffiths, *Compt. rend.*, **114**, 496 (1892).

[6] Heinze, *Z. physiol. Chem.*, **33**, 370 (1901); **43**, 290 (1904).

[7] Burdel, *Thèses*, Fribourg, 1922.

[8] Begemann, *Proefschrift*, Utrecht, 1924.

[9] Alsberg and Clark, *J. Biol. Chem.*, **8**, 1 (1910).

[10] Redfield, Coolidge and Shotts, *ibid.*, **76**, 185 (1928).