[CONTRIBUTION FROM THE DERMATOLOGICAL RESEARCH LABORATORIES]

N-ACYL DERIVATIVES OF 3-AMINO-4-HYDROXY-PHENYLARSONIC ACID

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The importance of 3-amino-4-hydroxy-phenylarsonic acid lies in its close relationship to arsphenamine. The difficulties of preparing this acid, due to the sensitiveness of the amino group (in *ortho* position to the hydroxyl group) to oxidation,[1] have been overcome by careful purification.

3-Amino-4-hydroxy-phenylarsonic acid can be recrystallized from water without oxidation, in a nitrogen atmosphere. It thus forms an abundance of almost colorless to light brown prisms, free from water of crystallization and chemically pure.

In preparing N-acyl derivatives, since oxidation of the parent compound is promoted by alkali, a better yield of purer products results by working in a neutral or slightly acid medium. 3-Amino-4-hydroxy-phenylarsonic acid is treated with lower-member fatty acid anhydrides which in aqueous suspension react distinctly acid. To effect condensation, the intermediates are heated for a few hours at moderate temperature. In case of the formyl derivatives, formic acid can replace water as a medium. The formation of the propionyl and butyryl derivatives is facilitated by the addition of copper turnings which shorten the time of heating.

The N-acyl derivatives, except the propionyl, do not crystallize with water of crystallization; the propionyl derivative forms with one molecule of water of crystallization, while the salts of these N-acyl derivatives contain water of crystallization in varying amounts.

Modifications for the preparation of 3-acetylamino-4-hydroxy-phenylarsonic acid, known as Stovarsol, have given a better yield of purer product. Chemotherapeutic tests, reported elsewhere, indicate that the new derivatives (except the formyl), though less toxic, are therapeutically of no more value than the acetyl derivative. In fact, the latter, when given internally, has been found superior as a prophylactic in spirochetal infections.

Experimental Part

3-Amino-4-hydroxy-phenylarsonic Acid, $H_2O_3AsC_6H_3(OH)NH_2$.—This product was prepared by reducing 3-nitro-4-hydroxy-phenylarsonic acid with sodium hydrosulfite, using the method modified by Raiziss and Gavron.[1c]

Ten g. of 3-amino-4-hydroxy-phenylarsonic acid, suspended in 500 cc. of water and kept under nitrogen to prevent oxidation, is dissolved by boiling; after the addition of 2 g. of charcoal the solution is filtered. The filtrate is cooled (under nitrogen), the

[1] (a) Ger. pat. 224,953 (1910). (b) Fargher, *J. Chem. Soc.*, **115**, 990 (1919). (c) Raiziss and Gavron, THIS JOURNAL, **43**, 583 (1921).

crystals are filtered off and washed with a little methyl alcohol, then with ether. The yield is about 8 g. The pure compound crystallizes in very light brown, short prisms of the monoclinic system.

3-Formylamino-4-hydroxy-phenylarsonic Acid, $H_2O_3AsC_6H_3(OH)NH.OCH$.—Ten g. of 3-amino-4-hydroxy-phenylarsonic acid is suspended in 40 cc. of 85% formic acid, the mixture refluxed on a free flame for three hours, cooled to room temperature, diluted with ten volumes of water and the precipitate filtered off. The compound is washed free from excess of acid with distilled water, a little methyl alcohol and ether, and is dried in a vacuum. The product is purified by dissolving in 10% sodium hydroxide solution; Nuchar "W" (a form of carbon with very high decolorizing properties) is added and the whole allowed to stand for 15 minutes. It is then filtered, and the filtrate cooled by keeping the flask in ice water; glacial acetic acid is added during stirring until a test to congo red paper indicates an excess of acid; yield, 10.7 g.

3-Formylamino-4-hydroxy-phenylarsonic acid, when recrystallized from water, forms long, colorless prisms in the monoclinic system. It decomposes on long boiling with water or dil. sodium hydroxide solution. It is soluble in dil. sodium, potassium or ammonium hydroxides, sparingly soluble in boiling water, almost insoluble in cold water and insoluble in acids and the usual organic solvents. It decomposes slowly on heating and does not melt at 275°.

Anal. Calcd. for $C_7H_8O_5NAs$: N, 5.37; As, 28.72. Found: N, 5.24; As, 28.29.

SODIUM SALT, $Na_2O_3AsC_6H_3(ONa)NH.OCH$.—3-Formylamino-4-hydroxy-phenylarsonic acid, plus a small amount of water containing 3 molecules of sodium hydroxide, is poured into 10 volumes of ethyl alcohol (95%), the mixture allowed to stand for several hours at room temperature and the crystalline precipitate filtered off. The sodium salt is recrystallized from a small amount of distilled water, filtered off, washed with a little ethyl alcohol and then with ether.

It forms colorless prisms, and is soluble in water giving a colorless solution. It decomposes slowly but does not melt when heated to 275°.

Anal. Calcd. for $C_7H_8O_5NAsNa_2 + 7H_2O$: N, 3.09; As, 16.55; H_2O of cryst., 27.81. Found: N, 2.93; As, 16.52; H_2O of cryst., 27.52.

3-Acetylamino-4-hydroxy-phenylarsonic Acid, $H_2O_3AsC_6H_3(OH)NH.OCCH_3$.—The preparation of this substance has found brief mention in a United States patent,[2] and was further elaborated by Raiziss and Gavron[3] and referred to by Christiansen.[4] The value of the method described in this paper lies in the better yields obtained with it, and in the pure and entirely colorless product obtained.

To a suspension of 10 g. of 3-amino-4-hydroxy-phenylarsonic acid in 40 cc. of water is added 9 cc. (2 molecular equivalents) of acetic anhydride. The mixture is mechanically stirred for two hours, and the temperature kept at 50–55°. At the end of the reaction the mixture is filtered and the filtrate discarded. The product, containing some unchanged amino compound, is suspended in 100 cc. of 10% hydrochloric acid and well stirred for 15 minutes. It is filtered off and washed with a little 10% hydrochloric acid.

This product is repurified to free it from coloring matter and to remove the last traces of amino compound. It is suspended in 100 cc. of water to which is added 10% aqueous sodium hydroxide until everything dissolves; 5 g. of Nuchar "W" is added and

[2] U. S. pat. 1,077,462 (1913).

[3] Ref. 1 c, p. 582.

[4] Christiansen, THIS JOURNAL, **44**, 2340 (1922).

the whole mechanically stirred for one-half hour, then filtered. The filtrate is treated in the cold with glacial acetic acid until a small excess is present. The acetyl compound begins to crystallize, forming colorless, microscopic prisms. After the crystallization is completed, the compound is filtered off and washed with water until free from sodium chloride, sodium acetate and acetic acid. It is then washed with a little methyl alcohol and finally ether. The yield is 9.7 g. This product can be recrystallized from boiling water.

3-Acetylamino-4-hydroxy-phenylarsonic acid crystallizes from water in short, colorless prisms in the monoclinic system. It slowly decomposes on long boiling in water or dilute alkalies. It is soluble in cold dilute alkalies and insoluble in dilute acids and the usual organic solvents. It melts with much decomposition between 240° and 250°. It forms colorless, crystalline salts (very easily soluble in water) of sodium, potassium, lithium, ammonium and strontium and sparingly soluble salts of barium and calcium.

Anal. Calcd. for $C_8H_{10}O_5NAs$: C, 34.90; H, 3.63; N, 5.09; As, 27.27. Found: C, 34.27; H, 3.42; N, 5.06; As, 26.98.

SODIUM SALT, $Na_2O_3AsC_6H_3(ONa)NH.OCCH_3$.—Forty g. of 3-acetylamino-4-hydroxy-phenylarsonic acid is dissolved in 100 cc. of water containing 17.4 g. (3 molecular proportions) of sodium hydroxide, 5 g. of Nuchar "W" is added, and the whole allowed to stand for 15 minutes; it is then filtered, and diluted with 1000 cc. of ethyl alcohol (95%). After some time in the cold, the sodium salt crystallizes. It is filtered off and washed with a little alcohol and finally with ether; yield, 45 g.

It appears as short, colorless prisms, soluble in cold water. When heated, it effervesces at about 113°, again at 150° and apparently melts with decomposition at about 210°.

Anal. Calcd. for $C_8H_7O_5NAsNa_3 + 4H_2O$: N, 3.39; As, 18.16; H_2O of cryst., 17.43. Found: N, 3.68; As, 18.88; H_2O of cryst., 17.40.

3-Propionylamino-4-hydroxy-phenylarsonic Acid, $H_2O_3AsC_6H_3(OH)NH.OCCH_2CH_3$.—A suspension of 11.6 g. of 3-amino-4-hydroxy-phenylarsonic acid in 50 cc. of water, mixed with 3 g. of copper turnings and 13 g. (2 molecular proportions) of propionic anhydride, is placed in a small flask connected with a reflux condenser. The mixture is refluxed for four hours and cooled; 2 cc. of hydrochloric acid (d., 1.18) is added and the whole filtered. The crystalline precipitate is washed with water until free from chlorides.

The product is purified by solution in 20 cc. of water containing enough sodium hydroxide to complete the solution, and treatment with 3 g. of Nuchar "W;" the whole is allowed to stand for 15 minutes, then filtered and to the filtrate concd. hydrochloric acid is added until an excess is present. The precipitate formed is filtered off, washed with water until free from chlorides, then with a little acetone and finally with ether; yield, 10 g. It is further purified by being recrystallized thrice from boiling water, using fresh charcoal each time. Finally, the product is washed with a little cold water, acetone and ether and dried in a vacuum.

When recrystallized from water it occurs as colorless, diamond- and hexagonal-shaped plates which contain one molecule of water of crystallization. It is soluble in dilute alkalies and in methyl alcohol, sparingly soluble in boiling water and almost insoluble in cold water, dilute acids and the usual organic solvents. It melts with much decomposition at 228–229°.

Anal. Calcd. for $C_9H_{12}O_5NAs + 1H_2O$: N, 4.56; As, 24.43; H_2O of cryst., 5.85. Found: N, 4.16; As, 24.52; H_2O of cryst., 6.10.

SODIUM SALT, $Na_2O_3AsC_6H_3(ONa)NH.OCCH_2CH_3$.—3-Propionylamino-4-hydroxy-phenylarsonic acid is dissolved in a small amount of water containing 3 molecular pro-

portions of sodium hydroxide. Nuchar is added and the solution allowed to stand for 15 minutes. The mixture is then filtered and the filtrate diluted with ten volumes of 95% ethyl alcohol. After the solution has stood in a cool place for several hours the sodium salt is filtered off and washed with a little alcohol and with ether and then is air dried.

It forms colorless needles, soluble in cold water. When heated it decomposes slowly, but does not melt at 275°.

Anal. Calcd. for $C_9H_9O_5NAsNa_3 + 2H_2O$: N, 3.58; As, 19.18; H_2O of cryst., 9.20. Found: N, 3.68; As, 19.39; H_2O of cryst., 10.08.

3-Butyrylamino-4-hydroxy-phenylarsonic Acid, $H_2O_3AsC_6H_3(OH)NH.OCCH_2CH_2CH_3$.—A suspension of 10.6 g. of 3-amino-4-hydroxy-phenylarsonic acid, in 100 cc. of water, mixed with 15.9 g. of *n*-butyric anhydride and 6 g. of copper turnings is heated to 100° in a flask provided with a reflux condenser. After ten minutes the mixture is cooled, diluted with 200 cc. of water containing 16 cc. of hydrochloric acid (d., 1.18), the whole well stirred and filtered. The compound is washed with a small amount of 10% hydrochloric acid, then with water until free from chloride ions. It is obtained dry by washing with acetone, then ether and keeping in a vacuum; yield, 10 g.

3-Butyrylamino-4-hydroxy-phenylarsonic acid must be crystallized quickly from hot water and occurs as colorless crystals in the form of microscopic hexahedrons of the isometric system. When continuously boiled in water this substance soon splits into butyric acid and 3-amino-4-hydroxy-phenylarsonic acid. It is soluble in alkalies, very sparingly so in methyl alcohol, almost insoluble in cold water and insoluble in dilute acids and the usual organic solvents. It melts with decomposition at 218–219°.

Anal. Calcd. for $C_{10}H_{14}O_5NAs$: N, 4.62; As, 24.75. Found: N, 4.72; As, 24.84.

The sodium salt is prepared in the same manner as the sodium salts of the preceding compounds. It crystallizes in clumps of colorless needles containing ten molecules of water of crystallization. The crystals are very soluble in cold water and methyl alcohol and insoluble in ether. When heated they effervesce and lose water at 108–115°. At a higher temperature the product decomposes, but does not melt at 275°.

Anal. Calcd. for $C_{10}H_{11}O_5NAsNa_3 + 10H_2O$: N, 2.55; As, 13.66. Found: N, 2.53; As, 13.55.

3-Chloro-acetylamino-4-hydroxy-phenylarsonic Acid, $H_2O_3AsC_6H_3(OH)NH.OCCH_2Cl$.—To a solution of 23.3 g. of 3-amino-4-hydroxy-phenylarsonic acid in 200 cc. of water containing 2 molecular proportions of sodium hydroxide is at once added 22.6 g. (2 molecular proportions) of chloro-acetyl chloride. The whole is stirred for 15 minutes and cooled by surrounding the flask with ice; 10 cc. of hydrochloric acid (d., 1.18) is added and the whole stirred for 15 minutes longer. The precipitate is filtered off, suspended in 100 cc. of 10% hydrochloric acid, again filtered off, washed with a little 10% hydrochloric acid, then with water until free from chlorides. It is further purified by recrystallization from boiling water, using Nuchar; yield of the recrystallized substance, 19 g.

If forms colorless prisms in the monoclinic system, easily soluble in dilute alkalies and methyl alcohol, quite soluble in boiling 30% acetic acid and sparingly soluble in boiling water; it is almost insoluble in cold water, dilute acids and the usual organic solvents. When heated, the crystals begin to darken at 230° and melt sharply with decomposition at 238–239°.

Anal. Calcd. for $C_8H_9O_5NAsCl$: N, 4.52; As, 24.23. Found: N, 4.39; As, 24.68.

The sodium salt is made in the same way as the salts of previous compounds. It crystallizes in clumps of colorless, twisted, hair-like needles and is very easily soluble in cold water. It slowly decomposes on heating, but does not melt at 275°.

Anal. Calcd. for $C_8H_6O_5NAsClNa_3 + 8H_2O$: N, 2.69; As, 14.43; H_2O of cryst., 27.71. Found: N, 3.12; As, 14.27; H_2O of cryst., 27.84.

Summary[5]

Improvements have been suggested for the preparation and purification of 3-amino-4-hydroxy-phenylarsonic acid and its acetyl derivative. New compounds have been described which result from the interaction of this acid with lower member fatty acid anhydrides such as formyl, propionyl, butyryl and chloro-acetyl derivatives and their salts.

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THE ABSORPTION SPECTRA OF RESORCINOL-BENZEIN[1]

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A brief outline of our experimental method and procedure has already been given in an article on the Absorption of Benzaurin.[3] As there explained, it is not always feasible to plot all of the observed points, but in drawing the curves, we have been guided as in the previous paper by all of the observations available.

As resorcinol-benzein[4] is the mother substance of fluorescein, sulfonefluorescein and of the other phthaleins containing a pyrone ring, it was thought highly desirable to study its absorption spectrum in order to compare it with that of fluorescein itself. A preliminary report upon the absorption of resorcinol-benzein in the visible region only has been made by Medhi and Watson.[5] Since they did not make quantitative measurements and since they report contradictory evidence of the existence of certain absorption bands, it is difficult to compare their data with those given below. Moir[6] has also published some results obtained in the visible region,

[5] This paper was presented at the meeting of the American Chemical Society, at Baltimore, April, 1925.

[1] The assistance of a grant made to the first two authors from the Heckscher Research Foundation of Cornell University which enabled us to make the measurements described in this report is gratefully acknowledged.

[2] Heckscher Research Assistant in Physics and Chemistry, Cornell University, 1923–25.

[3] THIS JOURNAL, **47**, 2767 (1925). Unfortunately an error appears in our previous article. The curves for Figs. 4 and 5, pp. 2774 and 2775, should be interchanged leaving the caption beneath each on the same page as it there appears.

[4] Kehrmann and Loth, *Ber.*, **47**, 2271 (1914). F. G. Pope, *J. Chem. Soc.*, **105**, 251 (1914).

[5] Medhi and Watson, *J. Chem. Soc.*, **107**, 1579 (1915).

[6] Moir, *Trans. Roy. Soc. S. Africa*, **7**, 5 (1918).