[CONTRIBUTION FROM THE AVERY LABORATORY OF CHEMISTRY OF THE UNIVERSITY OF NEBRASKA]

Arsenic Derivatives of Phenoxy-*s*-triazines

BY IVAN H. WITT[1] AND CLIFF S. HAMILTON

For a number of years various workers in this Laboratory have been interested in benzene arsonic acids, containing a substituted alkoxyl group in the molecule, as possible therapeutic agents. In view, therefore, of the recent report of Friedheim[2] on the successful treatment of African trypanosomiasis with 2-(4'-arsonoanilino)-4,6-diamino-*s*-triazine, it seemed advisable to study compounds in which a substituted *s*-triazine residue is united through oxygen to the benzene molecule containing the arsono group.

The condensation of 2-chloro-4,6-diamino-*s*-triazine with 4-hydroxyphenylarsonic acid in the presence of two or more equivalents of alkali resulted in poor yields. However, a better approach involved the reaction of 2-chloro-*s*-triazine derivatives with the sodium salts of 2- and 4-nitrophenol. The nitro compounds thus obtained were reduced catalytically by hydrogen to the corresponding amines and the benzenoid amino group in each was then replaced by the arsono group by means of the Bart[3] reaction. Under the experimental conditions used, the primary and secondary amino groups in the triazine nucleus did not react with nitrous acid. Thioglycolate derivatives of the arsonic acids were also investigated.

Experimental

2-(4'-Nitrophenoxy)-4,6-diamino-*s*-triazine (I).—A mixture of 2-chloro-4,6-diamino-*s*-triazine[4] (10 g., 0.069 mole), the sodium salt of 4-nitrophenol (22 g., 0.138 mole) and water (130 ml.) was stirred and refluxed for twelve hours. The excess sodium phenolate was removed from the alkali-insoluble product by extraction with a dilute sodium hydroxide solution and recrystallized from 95% ethanol; yield, 16 g. (94%); m. p. > 250°.

Anal. Calcd. for $C_9H_8N_6O_3$: C, 43.55; H, 3.25. Found: C, 43.43; H, 3.30.

2-(4'-Aminophenoxy)-4,6-diamino-*s*-triazine (II).—I (16 g.) was reduced catalytically with Raney nickel, hydrogen at fifty pounds pressure and 95% ethyl alcohol. After removal of the solvent, the amine was extracted from the catalyst with dilute hydrochloric acid. The product precipitated when the filtrate was made basic with ammonium hydroxide; yield, 13 g. (92%); m. p. > 250°.

Anal. Calcd. for $C_9H_{10}N_6O$: C, 49.53; H, 4.62. Found: C, 49.44; H, 4.77.

2-(4'-Arsonophenoxy)-4,6-diamino-*s*-triazine (III).—This arsonic acid was prepared in two ways.

(A) II (45 g., 0.206 mole) was dissolved in water (315 ml.) which contained hydrochloric acid (0.618 mole). The solution of the amine hydrochloride was diazotized at 0° by the dropwise addition of sodium nitrite (14.22 g., 0.206 mole in 50 ml. of water). The arsenite solution was prepared by treating arsenic trioxide (24.75 g., 0.248 eq.) with sodium hydroxide (19.8 g., 0.495 mole) and sodium bicarbonate (17.33 g., 0.206 mole) in water (630 ml.). This arsenite solution, to which had been added copper sulfate (2.4 g.), was introduced rapidly into the cold stirred solution of the diazonium salt. Stirring was continued for three hours, and the solution was allowed to stand in the refrigerator overnight. The reaction mixture was filtered, the filtrate neutralized to litmus paper, the mixture filtered and the filtrate made just basic to congo red paper. The pale yellow crystalline product which separated was dissolved in a sodium bicarbonate solution and filtered. Upon acidification of the filtrate with hydrochloric acid, 37 g. of the arsonic acid precipitated; yield 55%; m. p. > 250°. This product was recrystallized from dilute acetic acid.

Anal. Calcd. for $C_9H_{10}N_6O_4As$: As, 22.90. Found: As,[5] 22.77.

(B) 2-Chloro-4,6-diamino-*s*-triazine (5 g., 0.035 mole), 4-hydroxyphenylarsonic acid (22.7 g., 0.104 mole) and sodium hydroxide (8.3 g., 0.207 mole) were refluxed in water (120 ml.). The reaction mixture was made acid to congo red paper and the excess 4-hydroxyphenylarsonic acid was filtered off. When the filtrate was carefully made basic to congo red paper, a white precipitate formed (1.0 g., 9% yield). This compound was found to be identical with that prepared by the Bart reaction (A).

2-(2'-Nitro-4'-arsonophenoxy)-4,6-diamino-*s*-triazine (IV).—III (1 g.) was added slowly to a mixture of red fuming nitric acid (3 ml., sp. gr. 1.6) and concentrated sulfuric acid (3 ml.) at 0°. After the reaction had stood at room temperature for forty hours, the reaction mixture was poured onto crushed ice, and the acid solution was slowly neutralized to litmus paper with sodium hydroxide. The precipitate of inorganic salts was filtered off and upon making the solution just basic to congo red paper a yellow solid appeared which was recrystallized from dilute acetic acid; yield, 0.15 g. (13%).

Anal. Calcd. for $C_9H_9N_6O_6As$: As, 20.13. Found: As 20.39.

2-[4'-Di-(carboxymethylene-thio)-arsenosophenoxy]-4,6-diamino-*s*-triazine (V).—An aqueous suspension of III (18 g., 0.055 mole) was added to a sodium thioglycolate solution[6] (0.22 mole in 210 ml. of water). After standing for twenty-four hours at 25°, the reaction mixture was acidified with acetic acid, whereupon a solid material separated. The precipitate was washed with ethyl alcohol and ethyl acetate, and finally recrystallized from 20% acetic acid; yield, 14 g. (56%); m. p. 226–228°.

Anal. Calcd. for $C_{13}H_{14}N_5O_6S_2As$: As, 16.31; N, 15.25. Found: As, 16.23; N, 15.00.

2-(2'-Nitrophenoxy)-4,6-diamino-*s*-triazine (VI) was prepared in a manner similar to compound I; m. p. 249–250°; yield, 64%.

Anal. Calcd. for $C_9H_8N_6O_3$: C, 43.55; H, 3.25. Found: C, 43.64; H, 3.33.

2-(2'-Aminophenoxy)-4,6-diamino-*s*-triazine (VII) was obtained by the reduction of VI using the procedure given for the synthesis of II with the exception of the separation of the amine from the catalyst. This isolation was accomplished by dissolving the amine in hot ethyl alcohol, and upon cooling the solvent small plates separated; m. p. 220–222°; yield, 83%.

Anal. Calcd. for $C_9H_{10}N_6O$: C, 49.53; H, 4.62. Found: C, 49.50; H, 4.73.

2,4-Dichloro-6-ethylamino-*s*-triazine and **2-chloro-4-amino-6-ethylamino-*s*-triazine** were prepared according to the method of Diels.[7]

(1) Parke, Davis and Company Fellow.

(2) Friedheim, *Schweiz. Med. Wochschr.*, **71**, 116 (1941) [*C. A.* **36**, 1676 (1942)].

(3) Bart, *Ann.*, **429**, 55 (1922).

(4) Banks, *et al.*, THIS JOURNAL, **66**, 1771 (1944).

(5) See Cislak and Hamilton, *ibid.*, **52**, 638 (1930).

(6) Barber, *J. Chem. Soc.*, 2727 (1930).

(7) Diels, *Ber.*, **32**, 699 (1899).

2-(4'-Nitrophenoxy)-4-amino-6-ethylamino-*s*-triazine (VIII).—2-Chloro-4-amino-6-ethylamino-*s*-triazine was converted into VIII by the procedure used for I. The compound was crystallized from an absolute ethanol–benzene mixture; m. p. 211–213°; yield, 90%.

Anal. Calcd. for $C_{11}H_{12}N_6O_3$: C, 47.82; H, 4.37. Found: C, 47.64; H, 4.48.

2-(4'-Aminophenoxy)-4-amino-6-ethylamino-*s*-triazine (IX) was prepared by the procedure described for II in 77% yields; m. p. 204–206°.

Anal. Calcd. for $C_{11}H_{14}N_6O$: C, 53.64; H, 5.73. Found: C, 53.60; H, 5.71.

2-(4'-Arsonophenoxy)-4-amino-6-ethylamino-*s*-triazine (X) was synthesized in 25% yields from IX according to the directions given in III.

Anal. Calcd. for $C_{11}H_{14}N_5O_4As$: As, 21.09; N, 19.72. Found: As, 20.97; N, 19.50.

2-Chloro-4,6-diethylamino-*s*-triazine[8] (XI) was prepared by a procedure similar to that for 2,4-dichloro-6-ethylamino-*s*-triazine.

2-(4'-Nitrophenoxy)-4,6-diethylamino-*s*-triazine (XII) was synthesized by the method of Otto.[9] A mixture of XI (34 g., 0.17 mole), 4-nitrophenol (80 g., 0.58 mole) and the sodium salt of 4-nitrophenol (28 g., 0.17 mole) was fused in a casserole at 130–140° for fifteen minutes, followed by another fifteen minutes at 150°. The reaction mixture was extracted several times with dilute sodium hydroxide, and the residue recrystallized twice from ethanol to give a white product; yield, 39 g. (76%); m. p. 210–211°.

Anal. Calcd. for $C_{13}H_{18}N_6O_3$: C, 51.31; H, 5.30. Found: C, 51.25; H, 5.33.

2-(4'-Aminophenoxy)-4,6-diethylamino-*s*-triazine (XIII).—The procedure employed for the reduction of XII was that previously described in VII; yield, 77%; m. p. 226–228°.

Anal. Calcd. for $C_{13}H_{18}N_6O$: C, 56.91; H, 6.61. Found: C, 56.81; H, 6.61.

(8) Hofmann, *Ber.*, **18**, 2755 (1885).

(9) Otto, *ibid.*, **20**, 2236 (1887).

2-(4'-Arsonophenoxy)-4,6-diethylamino-*s*-triazine (XIV) was synthesized from XIII in 9% yields according to directions given for III (A).

Anal. Calcd. for $C_{13}H_{18}N_5O_4As$: As, 19.55. Found: As, 19.84.

2-[4'-Di-(carboxymethylene-thio)-arsenosophenoxy]-4,6-diethylamino-*s*-triazine (XV).—The reaction was carried out as previously described for V. The product was recrystallized from methanol; yield, 45%; m. p. 170–173°.

Anal. Calcd. for $C_{17}H_{22}N_5O_5S_2As$: As, 14.53. Found: As, 14.52.

2-Chloro-4,6-dimorpholino-*s*-triazine (XVI) was obtained in almost quantitative yields in a manner similar to that used to produce XI; m. p. 175–176°.

Anal. Calcd. for $C_{11}H_{16}N_5O_2Cl$: C, 46.23; H, 5.65. Found: C, 46.40; H, 5.71.

2-(4'-Nitrophenoxy)-4,6-dimorpholino-*s*-triazine (XVII).—The procedure employed was the same as that described above for XII. Recrystallization from a benzene–absolute ethanol (3:1) solution gave a flocculent white compound; m. p. 227–229°; yield, 77%.

Anal. Calcd. for $C_{17}H_{20}N_6O_5$: C, 52.57; H, 5.19. Found: C, 52.55; H, 5.35.

2-(4'-Aminophenoxy)-4,6-dimorpholino-*s*-triazine (XVIII).—The reduction was carried out as described for VII except that benzene served as the solvent and it was necessary to warm the reaction mixture to 50–60°; yield, almost quantitative; m. p. 227–229°.

Anal. Calcd. for $C_{17}H_{22}N_6O_3$: C, 56.97; H, 6.19. Found: C, 56.80; H, 6.09.

Summary

Several 2-(4'-arsonophenoxy)-4,6-amino- and alkylamino-*s*-triazines are reported for the first time.

The preparation of certain intermediates, leading to the syntheses of these arsonic acids, is described.

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Further Studies of the Essential Groups of Pancreatic Amylase

BY M. L. CALDWELL, C. EDWIN WEILL AND RUTH S. WEIL[1]

Studies of the inactivation of pancreatic amylase[2a,b] and of β-amylase from barley and from malted barley[3a,b] by the use of special reagents have shown that certain free groups of these proteins are essential to their amylase activities and have brought to light additional differences in the properties of these two types of starch-splitting enzymes.

Free primary amino groups of the protein are essential to the activity of pancreatic amylase[3a,b] but appear to be of little if any importance to the activity of β-amylase from barley or from malted barley.[3a] On the other hand, free sulfhydryl and free tyrosine groups of the protein are essential to the activity of β-amylase from barley or from malted barley but appear to be of little if any importance to the activity of pancreatic amylase.[2a,b]

This latter conclusion regarding free sulfhydryl groups, suggested by the work of Little and Caldwell,[2a] is confirmed and extended by the observations reported here.

Experimental

Highly purified preparations of pancreatic amylase[4] were used. The influence of each reagent upon the amylase activity was judged by direct comparisons of the activity of the amylase solution under observation with that of an aliquot of the same amylase solution which had been treated in an otherwise identical manner except for the reagent concerned. These aliquots are referred to as controls in Table I. None of the reagents was found to influence the activity measurements in the concentrations usually employed. Exceptions with larger concentrations were taken care of by the use of suitable blanks.

(1) We are greatly indebted to the Takamine Laboratory, Inc., for a grant in aid of this investigation.

(2) (a) J. E. Little and M. L. Caldwell, *J. Biol. Chem.*, **142**, 585 (1942); (b) J. E. Little and M. L. Caldwell, *ibid.*, **147**, 229 (1943).

(3) (a) C. E. Weill and M. L. Caldwell, THIS JOURNAL, **67**, 212 (1945); (b) C. E. Weill and M. L. Caldwell, *ibid.*, **67**, 214 (1945).

(4) H. C. Sherman, M. L. Caldwell and M. Adams, *J. Biol. Chem.*, **88**, 295 (1930), and unpublished work.