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 $C_{17}H_{22}N_5O_6S_2As$: 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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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY]

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.

The amylase activities were all measured under the same specified conditions[5] with 1% soluble potato starch adjusted to 0.02 *M* sodium chloride, 0.01 *M* phosphate and *p*H 7.2.[5] The saccharogenic activity refers to the increase in the reducing value (calculated as maltose) of the reaction mixture in thirty minutes at 40° brought about by one milligram of the enzyme preparation when the concentrations of the amylase were selected to give approximately the same (20%) hydrolysis of the starch.

Results

A number of reagents which have been reported to be specific for free sulfhydryl groups[6] were studied for their influence upon the activity of pancreatic amylase. Typical data are summarized in Table I. These results show that the reagents studied had little if any influence upon the activity of pancreatic amylase and lead to the conclusion that free sulfhydryl groups are of little if any importance to the activity of this amylase.

This finding with pancreatic amylase is in marked contrast to the results obtained under similar conditions with these reagents with β-amylase from barley and from malted barley.[3b] In this case, these sulfhydryl reagents caused complete inactivation of the amylase. Moreover, it was possible to restore the amylase activity by treatment of the inactivated enzyme solutions with hydrogen sulfide or with cysteine, a confirmation of the conclusion that sulfhydryl groups are essential to β-amylase activity.

In addition, it is interesting to note that the highly active solutions of pancreatic amylase used here gave no evidence of the presence of free sulfhydryl groups when examined by the nitro prusside reaction, as modified by Anson.[7] This finding is in accord with previous more qualitative indications[2a] and is again in marked contrast to the observations with beta-amylase from barley and from malted barley. With this enzyme, the amylase activities of the solutions were found to be directly proportional to the concentrations of sulfhydryl.[3b,7]

TABLE I

A STUDY OF THE INFLUENCE OF SPECIFIC SULFHYDRYL REAGENTS UPON THE ACTIVITY OF PANCREATIC AMYLASE

Treatment		After treatment Activity	
Reagent	Concn., *M*	Units[d]	% of control
Phenylmercuric-chloride[a]	0	5,200	100
	(satd. soln.)	4,920	95
p-Chloromercuri-benzoic acid[b]	0	5,400	100
	0.0005	4,870	90
Iodoacetamide[c]	0	6,150	100
	0.05	6,350	103

[a] Amylase solutions adjusted to 0.1 *M* phosphate; *p*H 6.8, reacted with reagent for thirty minutes at 0°.

[b] Amylase solutions adjusted to 0.1 *M* phosphate; *p*H 7.0, reacted with reagent for thirty minutes at 0°.

[c] Amylase solutions were adjusted to 0.1 *M* phosphate; *p*H 6.8, treated with the reagent and held at 25° for 120 minutes. The control was treated in the same way except that no iodoacetamide was added.

[d] Milligrams of "maltose" formed in thirty minutes at 40° by one milligram of enzyme acting on 1% soluble potato starch at *p*H 7.2 in the presence of 0.01 *M* phosphate and 0.02 *M* sodium chloride.

Summary

The data presented here confirm and extend previous indications that sulfhydryl groups of the protein are not essential to the activity of pancreatic amylase.

They emphasize another difference between the two typical starch-splitting enzymes, pancreatic amylase and β-amylase from barley and from malted barley.

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(5) H. C. Sherman, M. L. Caldwell and M. Adams, THIS JOURNAL, **50**, 2529, 2535, 2538 (1938).

(6) L. Hellerman and M. E. Perkins, *J. Biol. Chem.*, **107**, 241 (1934); E. D. Schock, J. Jensen and L. Hellerman, *ibid.*, **111**, 553 (1935); L. Hellerman and M. E. Perkins, *ibid.*, **112**, 175 (1935-6); L. Hellerman, *Physiol. Rev.*, **17**, 454 (1937); L. Hellerman, F. P. Chinard and V. R. Dietz, *J. Biol. Chem.*, **147**, 443 (1943).

(7) M. L. Anson, *J. Gen. Physiol.*, **24**, 399 (1940-1941).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, STATE UNIVERSITY OF IOWA]

A New Synthesis of Methyl 3,4,6-Trimethyl-β-D-glucoside and the Preparation of Crystalline 3,4,6-Trimethyl-D-glucose

BY ROBERT L. SUNDBERG, CHESTER M. MCCLOSKEY, DONALD E. REES AND GEORGE H. COLEMAN

In 1934 Haworth, Hirst and Panizzon[1] reported the synthesis of 3,4,6-trimethyl-D-glucose. This represented the synthesis of the last of the four possible trimethyl-D-glucopyranoses which are so important in the determination of the structures of oligo- and polysaccharides. Two of the four have previously been reported as crystalline, 2,3,6-trimethyl-D-glucose[2] and 2,4,6-trimethyl-D-glucose.[3]

(1) Haworth, Hirst and Panizzon, *J. Chem. Soc.*, 154 (1934).

(2) Irvine and Hirst, *ibid.*, 1213 (1922).

(3) Haworth and Sedgwick, *ibid.*, 2573 (1926).

A sample of 3,4,6-trimethyl-D-glucose which had been prepared in this Laboratory by the hydrolysis of methyl 3,4,6-trimethyl-β-D-glucoside crystallized spontaneously on standing. This leaves, therefore, 2,3,4-trimethyl-D-glucose as the only one of the four which has not been obtained crystalline.

Samples of 3,4,6-trimethyl-D-glucose subsequently prepared in this Laboratory by the same method crystallized readily at room temperature. The crystals were thick plates (m. p. 97–98° (cor.) after recrystallization) and proved to be the