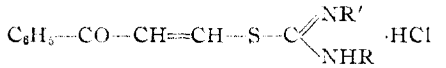
[CONTRIBUTION FROM THE STERLING-WINTHROP RESEARCH INSTITUTE]

Rearrangements of Some Benzoylvinylisothiourea and of Metathiazine Derivatives

BY CHESTER J. CAVALLITO,* CATHERINE M. MARTINI AND FREDERICK C. NACHOD

The reaction of thioureas, $RNH-\overset{S}{\overset{\|}{C}}-NHR'$, with phenyl ethynyl ketone in acid solution yielded benzoylvinylisothiouronium salts. Where R or R' was H, *these rapidly cyclized in dilute solutions by condensation to 2-imino-1,3,2-thiazine* derivatives. The iminometathiazines, in turn, underwent a slower rearrangement to 2-thio-1,2-dihydropyrimidine derivatives. The condensation and rearrangement were both base catalyzed and the latter was a first order reaction. The benzoylvinylisothiouronium salts in which either R or R' was not H could not condense but underwent slow hydrolysis with apparent liberation of benzoylacetaldehyde.

During the preparation of benzoylvinylisothiouronium salts of type I, it was observed that in dilute solutions rearrangements occurred which could be followed readily by changes in ultraviolet light absorption.

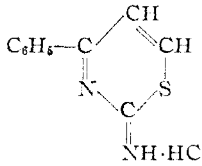


$$C_6H_5-CO-CH{=}CH-S-C\begin{matrix}\diagup NR' \\ \diagdown NHR\end{matrix}\cdot HCl$$

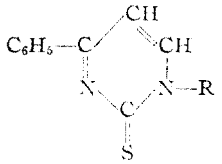
I

a, R = R' = H c, R = R' = $-CH_2C_6H_5$
b, R = $-CH_2C_6H_5$, R' = H d, R—R' = $-CH_2-CH_2-$

Thiourea, monosubstituted, and symmetrical disubstituted thioureas in acid aqueous solutions added readily to phenyl ethynyl ketone to yield compounds of type I under conditions similar to those described by Schubert for the reaction of thiourea with benzoquinone.[1] In aqueous or 95% alcohol solutions Ia underwent intramolecular condensation to the iminometathiazine, II.



II



III

a, R = H
b, R = $-CH_2C_6H_5$

Dilute aqueous or 95% alcohol solutions of Ia or II were found to show identical ultraviolet absorption spectra (Fig. 2–1) and Ib showed a similar absorption curve. This was further complicated by the observation that Ia and II in dilute solutions underwent a further change in absorption spectrum which was complete in about three hours (Fig. 2).

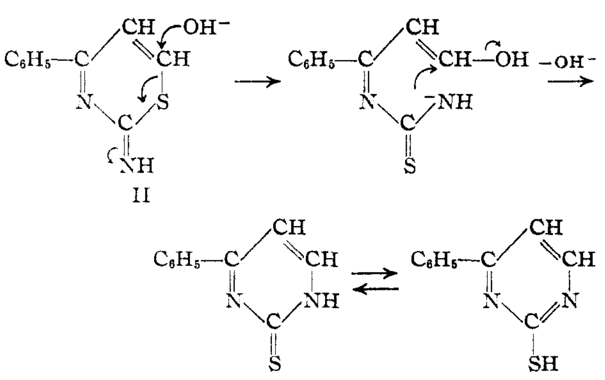
The apparent anomaly of the identity of absorption of Ia and II was resolved by showing that in 0.1 *N* HCl the absorption curve for Ia was quite different from that of II and did not change rapidly (Fig. 1a). This indicated that in very dilute solutions the condensation Ia → II is very rapid and is base catalyzed; the time required for preparation of solutions and making measurements being sufficient to allow completion of the reaction unless inhibited by high hydrogen ion concentrations.

Treatment of Ia or II with mild alkali led to rapid formation of a compound assigned structure IIIa. The ultraviolet absorption curve for IIIa was identical with the final curve obtained when dilute solutions of Ia or II were allowed to stand (Fig. 2). A similar change was shown by Ib to yield IIIb and the absorption curve for IIIb was similar to that of IIIa.

If one calculates the reaction rate for II→IIIa from the optical density figures at $\lambda = 293$ mμ, one finds that the reaction follows a first order law with a k_{25} value of 0.745 ± 0.027 reciprocal hour. Since the half time of a reaction is a useful means of visualizing the velocity at which the reaction proceeds, this figure, ln $2/k$, was calculated as 56 minutes, a value between curves 5 and 6 of Fig. 2. If the reaction is carried out in water containing 10% ethanol for solubility reasons, a similar value, *i.e.*, 55 minutes, is found. In 0.1 *N* HCl the reaction nearly slows to a standstill, while in 0.001 *N* NaOH the half time value is reduced.

The analytical values and physical and chemical properties of I and II are consistent with the structures assigned. The non-basicity and the identical positions of the absorption maxima and minimum of IIIa and IIIb are evidence for structure III rather than that of a 4-phenyl-2-thiolpyrimidine which is not possible for IIIb, but which may exist as a form in equilibrium with IIIa.

The rearrangement of II to IIIa is postulated to proceed by the following mechanism.



II

IIIa

That OH⁻ is involved in the reaction is further evidenced by measuring the rearrangement II→III in "absolute" ethanol. In this solvent, the water concentration is sufficiently low that the rate of the rearrangement is only about one-fifteenth that in 95% ethanol or in water.

The rearrangement of II to IIIa is somewhat analogous to the rearrangement of 5-aminothiazoles to 5-amino-2-mercaptoglyoxalines upon warming

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* Irwin Neisler & Co., Decatur, Ill.

(1) M. Schubert, THIS JOURNAL, **69**, 712 (1947).

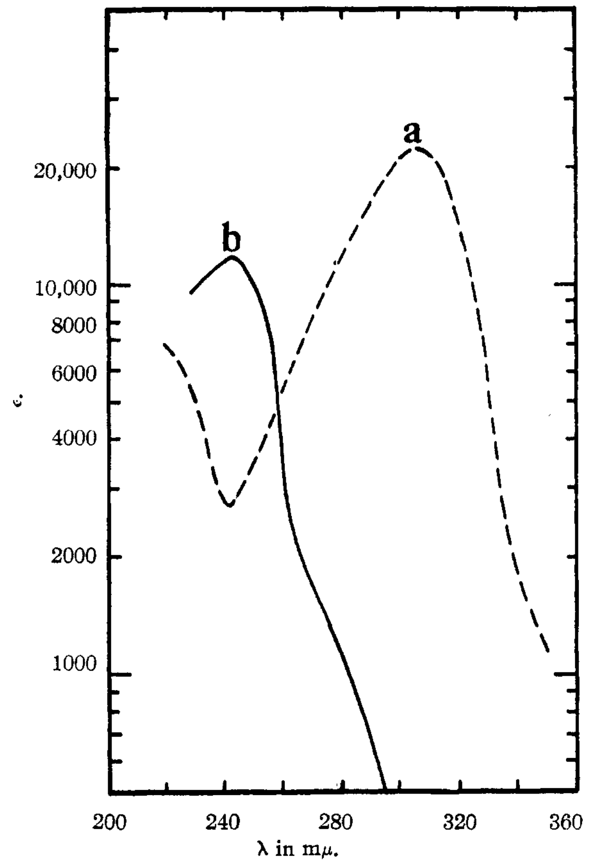
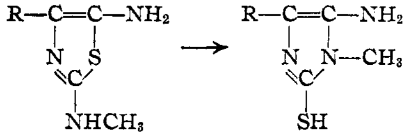


Fig. 1.—Absorption spectra: (a), Ia ($\lambda_{max.}$ = 304 mμ) in 0.1 N HCl; (b), V ($\lambda_{max.}$ = 242 mμ) in 95% EtOH.

with aqueous sodium carbonate[2] excepting that the latter reaction may go with less ease.



Condensation Ia → II should not be possible unless either R or R′ is H. Compounds Ic and Id did not show a spectral shift similar to that of Ia or Ib and did not yield compounds of types II and III. The absorption spectra of Ic and Id (Fig. 3-1) show a maximum at about 300 mμ; this is also the position of the maximum for Ia in acid solution. Derivatives Ic and Id, nevertheless, did show a change in absorption spectrum upon standing (Fig. 3), the change being slower and of different character from that of Ia or Ib. It was suspected that the change in Ic and Id was either a dissociative or hydrolytic breakdown to regenerate phenyl ethynyl ketone or to yield benzoylacetaldehyde, respectively, and the thiourea. Ultraviolet absorption curves of phenyl ethynyl ketone (Fig. 4c), benzoylacetaldehyde (Fig. 4b) and its sodium enolate (Fig. 4a)[3] show that the maximum for benzoylacetaldehyde is at the same position as that appearing in Fig. 3. This indicates that compounds of type I undergo hydrolytic cleavage which is much slower than the OH^- catalyzed ring opening of II intermediate to rearrangement to IIIa. Reactions other than hydrolysis also may be occurring with Ic and Id as evidenced by the deviation of curve 6, Fig. 3 from the isosbestic point.

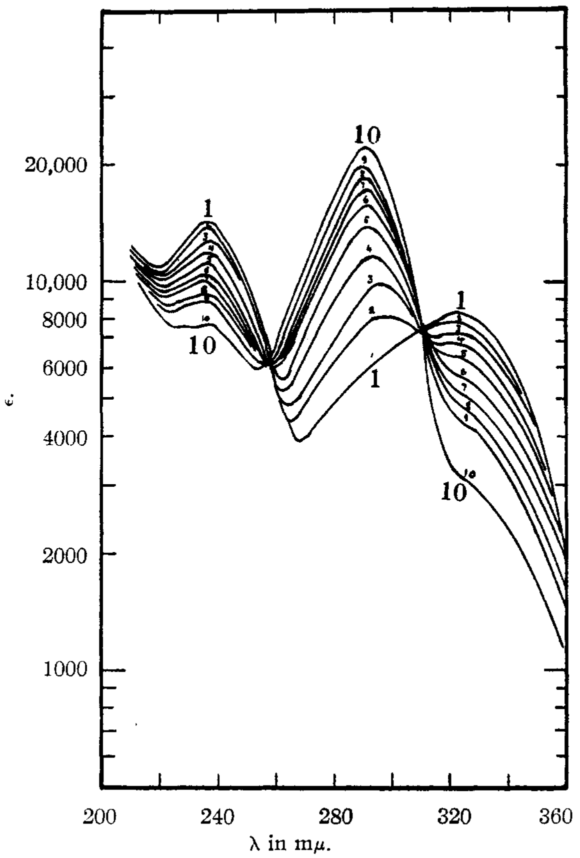


Fig. 2.—Absorption spectra of II → IIIa in 95% ethanol, curve 1 = II: Curve 1 (structure II) after 4.5 min.; curve 2 after 12 min.; curve 3 after 20.5 min.; curve 4 after 31 min.; curve 5 after 45 min.; curve 6 after 60 min.; curve 7 after 1 hr. 15 min.; curve 8 after 1 hr. 30 min.; curve 9 after 1 hr. 45 min.; curve 10 (structure IIIa) after 2 hr. 45 min. (finite curve).

Reaction of thiourea with phenyl vinyl ketone in acid solution yielded S-(2-benzoylethyl)-isothiouronium chloride (IV) the saturated analog of Ia. The absorption curve of IV (Fig. 1b) showed no change upon standing. The rapid ring-closing condensation of Ia compared with the stability of IV under similar conditions might be construed as an indication that the compounds of type I described here have a *cis* configuration.

1,3,2-Thiazines have been virtually uninvestigated. Hale and Brill[4] described a compound assigned the structure, 2-imino-5-nitro-1,3,2-thiazine, which was prepared under alkaline conditions and may possibly have a structure analogous to III.

Compounds Ia and II have sternutatory properties.

Experimental[5]

S-(2-Benzoylvinyl)-isothiouronium Chloride (Ia).—To a solution of 10 g. (0.13 mole) of thiourea in 50 ml. of 2 N hydrochloric acid was added portionwise, with cooling, 13

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[2] I. Heilbron, *J. Chem. Soc.*, 2105 (1949); A. H. Cook and I. Heilbron, *Rec. trav. chim.*, **69**, 351 (1950).

[3] C. Bülow and W. von Sicherer, *Ber.*, **34**, 3891 (1901).

[4] W. J. Hale and H. C. Brill, THIS JOURNAL, **34**, 295 (1912).

[5] All melting points corrected.

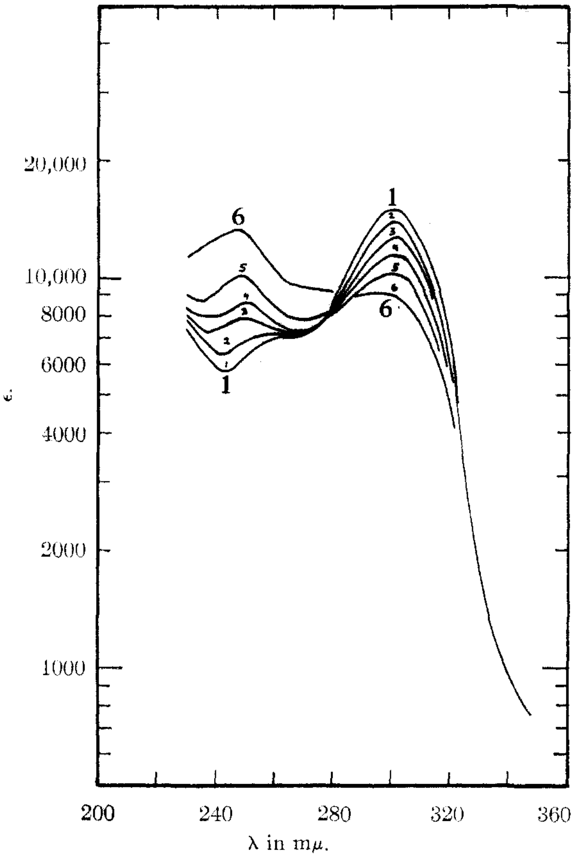

Fig. 3.—Absorption spectra of curve 1 = *Id* in 95% EtOH: Curve 1 after 5.75 min.; curve 2 after 1 hr.; curve 3 after 2 hr.; curve 4 after 3 hr.; curve 5 after 4 hr.; curve 6 after 26 hr.

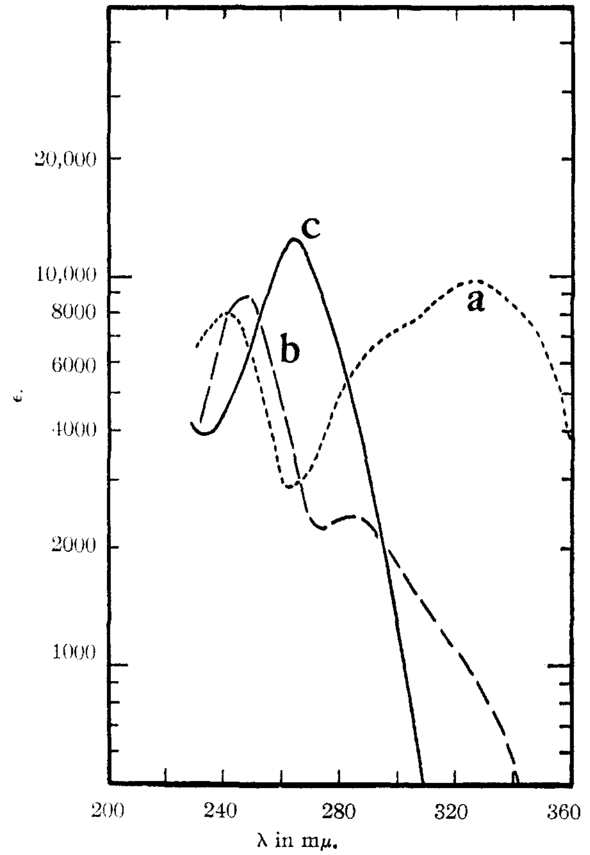

Fig. 4.—Absorption spectra: a, $C_6H_5COCH{=}CHONa$; b, $C_6H_5COCH_2CHO$; c, $C_6H_5COC{\equiv}CH$; solvents: a and c, 95% EtOH; b, 0.01 N HCl; maxima: a, 240 and 327 mμ; b, 247 and 284 mμ; c, 264 mμ.

g. (0.1 mole) of phenyl ethynyl ketone.[6] A white crystalline precipitate appeared within a few minutes. Addition of 25 ml. of concentrated hydrochloric acid to the cooled solution produced more complete precipitation. The product was filtered off, dissolved in 300 ml. of water and 25 ml. of concentrated hydrochloric acid was added to reprecipitate the compound which was filtered off and washed with dilute hydrochloric acid. After drying *in vacuo* over solid sodium hydroxide, then over calcium chloride, the yield was 16.5 g. (63.5%) of white crystalline monohydrate, m.p. 168–169° dec.

Anal. Calcd. for $C_{10}H_{11}ON_2SCl{\cdot}H_2O$: S, 12.29; Cl, 13.60; H_2O, 6.93. Found: S, 12.52; Cl, 13.78; H_2O (Karl Fischer), 6.04.

2-Imino-4-phenyl-1,3,2-thiazine Hydrochloride (II).—A solution of 11 g. of Ia in the least volume of 20% alcohol-water was allowed to stand overnight in a desiccator over calcium chloride. The yellow crystalline residue was dissolved in the least volume of warm 50% alcohol–water, treated with charcoal, filtered, cooled and 10 ml. of concd. hydrochloric acid was added. The lemon-yellow crystalline product was filtered off, washed with dilute hydrochloric acid and dried at 75° to yield 5.5 g. (58%) of II, m.p. 172–177° dec.

Anal. Calcd. for $C_{10}H_9N_2ClS$: C, 53.45; H, 4.04; Cl, 15.78; S, 14.26. Found: C, 53.44; H, 4.39; Cl, 15.51; S, 13.95.

S-(2-Benzoylvinyl)-N-benzylisothiouronium Chloride (Ib).—To a solution of 16.5 g. (0.1 mole) of N-benzylthiourea[7] in 200 ml. of 95% ethanol was added 25 ml. of 2 *N* hydrochloric acid, then 13 g. (0.1 mole) of phenyl ethynyl ketone. After ten minutes the solution was concentrated under reduced pressure to about 100 ml. and diluted with 5% hydrochloric acid to yield a white crystalline precipitate. The product was filtered off, washed with water, then with ether and redissolved in 150 ml. of hot 95% ethanol. After cooling, the solution was diluted with 300 ml. of 1% hydrochloric acid and the precipitate filtered off and dried *in vacuo* over dry sodium hydroxide, then calcium chloride. A crop of 23.7 g. (71%) of cream colored crystals was obtained, m.p. 154.5°.

Anal. Calcd. for $C_{17}H_{17}ON_2SCl$: C, 61.34; H, 5.15; N, 8.90. Found: C, 61.13; H, 5.03; N, 8.71.

S-(2-Benzoylvinyl)-N,N′-dibenzylisothiouronium Chloride (Ic).—To a solution of 4.2 g. (0.0164 mole) of N,N′-dibenzylthiourea in 100 ml. of warm 95% ethanol was added 9 ml. of 2 *N* hydrochloric acid and 2.1 g. (0.0164 mole) of phenyl ethynyl ketone. After 15 minutes, the solution was concentrated under reduced pressure until turbid then diluted with 100 ml. of 1% hydrochloric acid. The solution was decanted from the amorphous precipitate and the latter was washed with ether. The solid was dissolved in the least volume of warm 95% ethanol, cooled, and diluted with ether to yield a white crystalline solid which was filtered off and dried at 75°; yield was 5.5 g. (80%), m.p. 164–165°.

Anal. Calcd. for $C_{24}H_{23}ON_2SCl$: C, 68.39; H, 5.47; Cl, 8.38. Found: C, 68.39; H, 5.47; Cl, 8.43.

S-(2-Benzoylvinyl)-ethyleneisothiouronium Chloride (or Benzoylvinyl-2-thioimidazoline Hydrochloride) (Id).—To a suspension of 10.2 g. (0.1 mole) of ethylenethiourea in 100 ml. of 50% alcoholic, 1 *N* hydrochloric acid solution was added in portions, with stirring, 10.4 g. (0.08 mole) of phenyl ethynyl ketone. The material all dissolved and within a few minutes the solution became a solid mass of crystals. The product was redissolved in water and reprecipitated with hydrochloric acid as with Ia. The product was dried at 75° to yield 11 g. (51%) of white crystals, m.p. 164° dec.

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(6) K. Bowden, I. M. Heilbron, E. R. H. Jones and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946).

(7) A. E. Dixon, *ibid.*, **59**, 553 (1891).

Anal. Calcd. for $C_{12}H_{13}ON_2SCl$: N, 10.43; Cl, 13.20. Found: N, 10.67; Cl, 13.10.

S-(2-Benzoylethyl)-isothiouronium Chloride (IV).—A solution of 7 g. (0.053 mole) of phenyl vinyl ketone in 10 ml. of ethanol was added with shaking to 5 g. (0.066 mole) of thiourea in 30 ml. of 2 *N* hydrochloric acid solution. After ten minutes, 10 ml. of concentrated hydrochloric acid was added, the crystalline precipitate filtered off, dissolved in warm ethanol and reprecipitated by addition of dilute hydrochloric acid. The white crystals were dried at 75°; yield 6 g. (46%), m.p. 143° dec.

Anal. Calcd. for $C_{10}H_{13}ON_2SCl$: S, 13.10; Cl, 14.50. Found: S, 12.91; Cl, 14.30.

4-Phenyl-2-thio-1,2-dihydropyrimidine (IIIa).—When aqueous solutions of Ia or II are allowed to stand for several hours, a yellow solid separates which shows an ultraviolet absorption curve identical with that shown by dilute solutions of Ia or II which have stood for about three hours. The same compound was produced quickly as follows: To a solution of 10 g. of Ia (0.041 mole) in 100 ml. of ethanol and 50 ml. of water was added slowly, with shaking, 3 g. of potassium carbonate. The orange solid which separated was filtered off, washed with water and recrystallized by dissolving in hot ethanol, diluting slowly with water, and cooling. The granular yellow product which separated was filtered off and dried at 75°; yield 5 g. (65%), m.p., dec. > 180°. The compound was insoluble in aqueous acid, but soluble in moderately strong alkali.

Anal. Calcd. for $C_{10}H_8N_2S$: C, 63.80; H, 4.28. Found: C, 64.31; H, 4.07.

1-Benzyl-4-phenyl-2-thio-1,2-dihydropyrimidine (IIIb).—To a solution of 5 g. of Ib in 125 ml. of ethanol was added a solution of 5 g. of potassium carbonate in 15 ml. of water. The two layers were shaken thoroughly, then allowed to stand for one hour at 40 to 50°. The mixture was cooled and the crystalline precipitate was filtered off, recrystallized from hot 95% ethanol and dried at 75°; yield 4 g. (95%), m.p. 160–161°, yellow needles. The compound is insoluble in 1% but soluble in 5% hydrochloric acid solution.

Anal. Calcd. for $C_{17}H_{14}N_2S$: C, 73.35; H, 5.07; N, 10.08. Found: C, 72.91; H, 5.02; N, 10.50.

Ultraviolet Absorption Spectra.—The spectrophotometric studies were carried out with a Cary recording quartz spectrophotometer, model 11, serial no. 37, with a voltage setting of 4 and a slit control setting of 20. Quartz cells of 10 mm. thickness were used and concentrations were adjusted (10 to 30 mg. per liter) so as to give optical density readings in the range of 0.5 to 2.0.

Acknowledgments.—We are indebted to M. E. Auerbach, Kenneth Fleischer and staff for the microanalytical data, and to Drs. C. F. Koelsch and E. J. Lawson for comments on the reaction mechanisms.

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[CONTRIBUTION FROM THE RESEARCH LABORATORIES, JOSEPH E. SEAGRAM & SONS, INC.]

Isolation of a Crystalline Trisaccharide from the Unfermentable Carbohydrate Produced Enzymically from Maltose[1]

BY S. C. PAN, L. W. NICHOLSON AND PAUL KOLACHOV

Submerged cultures of *Aspergillus niger* NNRL 337 contain an enzyme(s) which converts maltose into an unfermentable carbohydrate(s) in addition to glucose. The fermentable sugars (glucose and maltose) are removed from the unfermentable carbohydrate by yeast fermentation; other impurities are removed by basic lead acetate precipitation and by passing the filtrate through ion-exchange resins. A crystalline product is readily obtained from an aqueous methanol solution containing the purified unfermentable carbohydrate. The twice recrystallized product was shown to be a trisaccharide on the basis of its reducing power toward NaIO, its molecular weight, and its paper chromatography. The following evidence shows that the trisaccharide consists of only glucose residues: reducing power of the acid hydrolysate of the trisaccharide toward NaIO; complete fermentability of the hydrolysate; and failure of the hydrolysate to give insoluble phenylhydrazone.

The rate at which the trisaccharide crystallizes from the aqueous methanol solution increases as the temperature is increased (up to the boiling point of the solution). When the fungal culture used in the synthesis has been stored in the refrigerator the rate of synthesis increases but the yield of the crystalline product decreases as the storage time increases.

Introduction

The discovery that submerged cultures of *Aspergillus niger* (NRRL[2] 337) contain an enzyme(s) which can synthesize an unfermentable carbohydrate(s) from maltose has been reported previously.[3] The unfermentable carbohydrate was first obtained as a precipitate; its reducing power showed that it was an oligosaccharide containing not more than three glucose residues per molecule. Further investigation has led to the isolation of a crystalline material from the unfermentable carbohydrate. The purified crystalline product has been shown to be a trisaccharide, containing three glucose residues per molecule.

Norberg and French,[4] using electrophoretic technique, have recently shown that *Bacillus macerans* amylase is capable of converting maltose into a trisaccharide and higher oligosaccharides. The enzyme reaction of *A. niger*, discussed in this paper, will certainly be very similar to that of *B. macerans*. None of the oligosaccharides, however, reported by Norberg and French have been crystallized. It is not known, as yet, whether the trisaccharide described in this paper is one of the products obtained by the action of *B. macerans* amylase acting upon maltose.

The occurrence of trisaccharides composed only of glucose residues as the end products of the enzymic hydrolysis of starch, has been reported by Blom and Rosted[5] and by Myrbäck and associates.[6] The products they describe are not crystalline and, therefore, the exact chemical nature of these sugars has not been fully proved. Recently, Wolfrom and associates[7] have isolated maltotriose as its hendecaacetate in crystalline form from an enzymic

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(1) Presented at the 118th Annual Meeting of the American Chemical Society at Chicago, Ill., on September 6, 1950.

(2) Northern Regional Research Laboratory.

(3) S. C. Pan, A. A. Andreasen and Paul Kolachov, *Science*, **112**, 115 (1950).

(4) E. Norberg and D. French, THIS JOURNAL, **72**, 1202 (1950).

(5) J. Blom and C. O. Rosted, *Acta Chem. Scand.*, **1**, 233 (1947).

(6) K. Myrbäck, *Biochem. Z.*, **297**, 179 (1938); K. Myrbäck, *Advances in Carbohydrate Chem.*, **3**, 251 (1948); B. Ortenblad and K. Myrbäck, *Biochem. Z.*, **303**, 335 (1940).

(7) J. M. Sugihara and M. L. Wolfrom, THIS JOURNAL, **71**, 3357 (1949); M. L. Wolfrom, L. W. Georges, Alva Thompson and I. L. Miller, *ibid.*, **71**, 2873 (1949).