erally a further precipitation of calcium salts and these are removed by filtration before proceeding with the next step of the process, namely, fermentation of the glucose.

To the cooled (about 30°) concentrated solution, ammonium phosphate is added at the rate of 0.75 g. per liter. A precipitate of calcium phosphate is sometimes formed. It is not necessary to remove this precipitate. The solution is then inoculated with baker's yeast[5] and incubated at approximately 30° Baker's yeast is used to ferment the glucose because this yeast does not attack rhamnose while, as is well known, it ferments glucose.

The solution is allowed to ferment until evolution of gas has ceased and the supernatant liquor begins to clear. This generally requires about seventy-two hours. The fermented liquor is then filtered by means of a filtering aid, the cake washed free of sugar and the filtrate and washings concentrated *in vacuo* until a sample withdrawn and cooled to room temperature shows graining. Caution should be exercised during concentration because of foaming. Sufficient hot 95% ethyl alcohol is added to the sirup to precipitate inorganic impurities and gummy matter still present. The precipitate is filtered off, washed with alcohol and a small amount of acetic acid added to combined filtrate and washings to obtain small crystals. This facilitates subsequent washing of the crystals. The filtrate and washings are again concentrated *in vacuo* until graining starts.

The crystals are filtered by suction to free them from mother liquor and washed three times with cold 95% ethyl alcohol, three times with equal volumes of cold 95% alcohol and ether, and finally twice with ether. The mother liquor is filtered and concentrated *in vacuo* to yield a further crop of crystals. The yield amounts to approximately 20% of the naringin taken, or about 62% of the theoretical.

The product obtained is pure white and may be recrystallized by dissolving in three parts of 80% alcohol by gently warming, filtering through decolorizing carbon and diluting the clear, water-white filtrate with about an equal volume of water. The solution is then concentrated in a vacuum to a thick sirup (about 50% total solids). The cooled sirup quickly grains, especially in the ice box. The sugar is filtered off by suction, washed with a small amount of ice water and the washings and drained mother liquor added to the mother liquor from the first crystallization. The washed crystals are dried at 45 to 50°.

Rhamnose, once crystallized, had an initial reading after three and one-half minutes (4.162 g. in 100 cc. read in a 200-mm. tube) of −1.1°V. with a final rotation $[\alpha]^{20}D$ of +7.5°.[6]

We wish to acknowledge the kindness of the Exchange Orange Products Co., of Ontario, California, who furnished part of the naringin used in this investigation.

Summary

A method is described for preparing rhamnose from naringin, the bitter glucoside of grapefruit. The yield of sugar amounts to about 20% of the naringin taken, or approximately 62% of the theoretical.

(5) Yeast from an ordinary cake of Fleischmann's yeast is satisfactory.

(6) We are indebted to Dr. E. Yanovsky of the Carbohydrate Division for his kindness in making this determination.

WINTER HAVEN, FLA. RECEIVED OCTOBER 24, 1938

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, YALE UNIVERSITY]

The Nitrogen Products Formed by Chlorination of Isothioureas

BY TREAT B. JOHNSON AND JAMES M. SPRAGUE[1]

The correct interpretation of the mechanism of the reaction occurring when an isothiourea is allowed to interact with chlorine in aqueous solution calls for a knowledge of the structures of the characteristic final reaction products that are formed. It has been the experience of the authors that it is a characteristic behavior of most of the isothiourea salts thus far examined to interact with chlorine in aqueous solution at ordinary temperature, to form an alkyl sulfonyl chloride II. This type of reaction product, however, does not account for all the sulfur of the isothiourea, as a part of this element is oxidized in some cases to the sulfate ion. Also, in some isothiourea structures examined, in which the sulfur is attached to groupings as $(CH_3)_3C$—, $C_2H_5OCH_2$—, $HOOCCH_2$—, $C_2H_5OOCCH_2$— and $C_4H_3OCH_2$— (furfuryl), it was impossible to obtain the corresponding sulfonyl chlorides by chlorination. The nitrogen of a simple isothiourea I can be accounted for completely by the formation of the hydrochloride of cyanamide III. These changes in the case of S-ethylisothiourea hydrochloride are expressed as follows[2]

$$\underset{\text{I}}{C_2H_5SC(NH_2)=NH\cdot HCl} + Cl_2 + H_2O = \underset{\text{II}}{C_2H_5SO_2Cl} + \underset{\text{III}}{NH_2CN\cdot 2HCl}$$

(1) Sterling Professorship of Chemistry Research Assistant, 1936–1937.

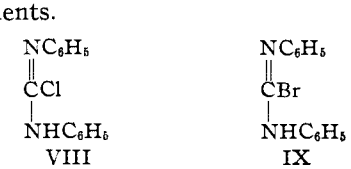
(2) Johnson and Sprague, THIS JOURNAL, **58**, 1348 (1936). Sprague and Johnson, *ibid.*, **59**, 1837 (1937); **59**, 2439 (1937).

In the case of isothioureas substituted on nitrogen by an aliphatic group (IV) we now find that a sulfonyl chloride is also a normal product of the reaction, and the nitrogen is recovered in the form of a substituted cyanamide derivative (V). In the case of phenyl substituted isothioureas (VI) the resulting phenylcyanamide compounds are attacked by the chlorine leading to the formation of chlorine substitution products (VII). These changes are illustrated in equations A and B

$$\underset{\text{IV}}{C_2H_5SC(NHCH_3)=NH\cdot HCl} + Cl_2 + H_2O = C_2H_5SO_2Cl + \underset{\text{V}}{CH_3NHCN\cdot HCl} \quad (A)$$

$$\underset{\text{VI}}{C_2H_5SC(NHC_6H_5)=NH\cdot HCl} + Cl_2 + H_2O = C_2H_5SO_2Cl + \underset{\text{VII}}{Cl_2C_6H_3NHCN} \quad (B)$$

In the case of N,N′-disubstituted isothioureas, the present experimental evidence supports the conclusion that a carbodiimide derivative (XIII) is first formed as an intermediate product on chlorination, which is then changed into a substituted urea (XIV) by addition of water. For example, in the chlorination of S-ethyl- or S-benzyl-N,N′-diphenylisothiourea in dilute acetic acid solution a sulfonyl chloride is formed and the nitrogen is recovered completely in the form of N-2,4-N′-2′,4′-tetrachlorodiphenylurea. It is of especial interest to note here that B. Rathke[3] examined the action of both chlorine and bromine on S-ethyl-N,N′-diphenylisothiourea in 1881. He applied his reaction in aqueous solution and reported the oxidation of the ethylmercapto grouping of the isothiourea to the corresponding sulfonic acid, with formation of chlorine- and bromine-containing nitrogen products. Rathke postulated the formation of the two compounds represented by formulas VIII and IX as primary products of reaction, which then interacted further with the halogens. The authors have been unable to find any further communication in the literature regarding this reaction of Rathke. It seems very probable that this investigator was actually dealing with the same halogenated diphenylurea derivative that we obtained in our experiments.

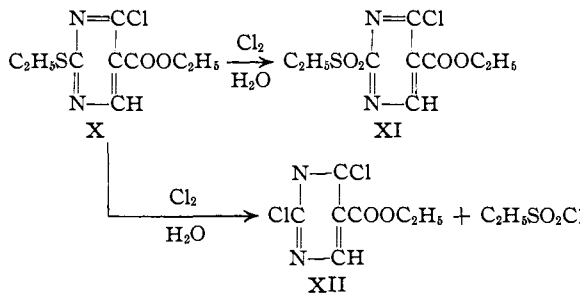


VIII

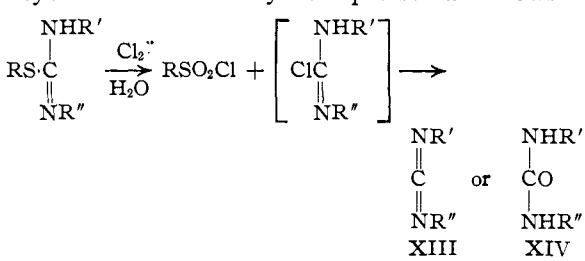
IX

(3) B. Rathke, *Ber.*, **14**, 1774 (188[illegible].

In compounds where the isothiourea structure is incorporated to form an heterocyclic combination as represented in the pyrimidine X it has been shown that the reaction with chlorine may proceed in two ways, depending on conditions, to give either a true sulfon compound XI, or a sulfonyl chloride and a stable chloropyrimidine XII.[4]



Here we have an excellent illustration of a fundamental pyrimidine reaction confined to one reactive zone "A" of the pyrimidine molecule.[5] The analogy between this last transformation XII, and that of the action of chlorine on a simple acyclic isothiourea may be expressed as follows



Experimental Part

Cyanamide Dihydrochloride $NH_2CN\cdot 2HCl$, or Chloroformamidine Hydrochloride, $NH_2CCl{=}NH\cdot HCl$.[6]—This is one of the products of reaction when an S-alkyl-isothiourea is exposed to the action of chlorine in aqueous solution. The salt is obtained easily according to the following procedure. The aqueous solutions from the preparation of *n*-butyl or benzyl-sulfonyl chloride, by chlorination of S-normal-butylisothiourea and S-benzylisothiourea, respectively,[7] was washed thoroughly with ether and finally concentrated under reduced pressure below 60°. Cyanamide dihydrochloride deposited and was separated by

(4) Johnson and Sprague, THIS JOURNAL, **60**, 1622 (1938); Sprague and Johnson, *ibid.*, **57**, 2252 (1935).

(5) See paper by Johnson and Ambelang, *ibid.*, **60**, 2941 (1938).

(6) This hydrochloride was first prepared by Drechsel, *J. prakt. Chem.*, [2] **11**, 315 (1875), and later by Mulder and Smit, *Ber.*, **7**, 1634 (1874). Hantzsch and Vagt, *Ann.*, **314**, 366 (1900), prepared it by passing dry hydrochloric acid gas into an ether solution of cyanamide. Drechsel (p. 317) wrote as follows regarding the structure of this interesting salt: "Die Zusammensetzung des salzsauren Cyanamide ist sehr merkwürdig. Nach Analogie anderer Amide resp. Aminbasen hätte man erwarten sollen, dass das Cyanamide sich nur mit ein mol chlorwasserstoff verbinden wurde." Mulder and Smit (*loc. cit.*) observed that evaporation of an aqueous solution of the salt at 100° leads to the formation of dicyandiamide.

(7) Johnson and Sprague, THIS JOURNAL, **59**, 1839 (1937).

filtration. A second crop of the hydrochloride was obtained by further concentration of the aqueous mother liquor. This salt was washed with cold acetone and preserved in a desiccator. The yield was good and the salt melted at 175–183° with effervescence varying with the rate of heating. It was purified by adding acetone to a concentrated solution of the salt in hydrochloric acid. It melted at 182–183°.

Anal. Calcd. for $CH_4N_2Cl_2$: N, 24.37; Cl, 61.70; NH_2CN, 36.57. Found: N, 24.32; Cl, 61.84; NH_2CN, 36.79.

In order to obtain the free cyanamide, some of the above hydrochloride was dissolved in cold water and the chlorine completely removed by shaking the solution with silver oxide. After concentrating the solution under diminished pressure and cooling, the pure cyanamide was obtained melting at 43–45°.

Preparation of Methylisourea Hydrochloride,[8] $CH_3OC(NH_2)$=$NH\cdot HCl$.—This salt was obtained easily by allowing a methyl alcohol solution of our cyanamide dihydrochloride to stand for several hours at ordinary temperature. The excess of alcohol was removed by distillation when this isourea hydrochloride separated. It was purified by recrystallization from alcohol and melted at 129–130° (McKee reports 132–133°).

Anal. Calcd. for $C_2H_7ON_2Cl$: N, 25.39; Cl, 32.05. Found: N, 25.47; Cl, 32.20.

The benzoyl derivative of this isourea was prepared and melted at 75–76° (McKee reports 76.5°).[8]

Anal. Calcd. for $C_9H_{10}O_2N_2$: N, 17.76. Found: N, 17.78.

Action of Chlorine on S-Benzyl-N-methylisothiourea.[9]—The hydrochloride was chlorinated in aqueous solution below 20°. The benzylsulfonyl chloride was filtered off and the filtrate concentrated under diminished pressure below 65°. The viscous yellow residue was taken up in water, neutralized with sodium hydroxide and the resulting methylcyanamide solution concentrated to dryness under reduced pressure. On evaporating an alcohol extract of this residue, a 70% yield of methylurea was obtained as a viscous oil which soon crystallized, m. p. 101–102° from alcohol. This was identified definitely by the nitrate, m. p. 128–130°, and the acetyl derivative, m. p. 178–179°. The literature gives the melting points as 128–132° and 180–181°, respectively. The yield of sulfonyl chloride was 86%.

Chlorination of S-Benzyl-N-phenylisothiourea Hydrochloride in Aqueous Solution:[10] **The Formation of Benzylsulfonyl Chloride and 2,4-Dichlorophenylcyanamide.** $C_6H_5CH_2SC(NHC_6H_5)$=$NH\cdot HCl \xrightarrow[H_2O]{Cl_2} C_6H_5CH_2SO_2Cl + Cl_2C_6H_3NHCN$.—The isothiourea hydrochloride (27.8 g.) was dissolved in 250 cc. of 50% acetic acid and chlorine gas bubbled into the chilled solution until the above reaction was complete and the benzylsulfonyl chloride had separated. The weight of this chloride was 15.5 g. It melted without further purification at 90–91°. On diluting the acetic acid filtrate with water the 2,4-dichlorophenylcyanamide separated in the form of needles which melted at 154–160°; yield 11.5 g. This compound was purified by crystallization from benzene or dilute alcohol and crystallized in small colorless needles melting at 162–163°. It is a weak acid, dissolves in dilute alkali and is precipitated unchanged by the addition of acids.

Anal. Calcd. for $C_7H_4NCl_2$: N, 14.97; Cl, 37.92. Found: N, 14.92; Cl, 37.70.

Synthesis of 2,4-Dichlorophenylcyanamide. A.—One gram of phenylcyanamide[11] was dissolved in 25 cc. of 50% acetic acid and the solution chlorinated as described above. The dichloro derivative separated in crystalline form and weighed 1 g. This was purified by recrystallization from dilute alcohol and melted at 162°. A mixture of this preparation with that from the previous experiment melted at the same temperature.

Anal. Calcd. for $C_7H_4NCl_2$: N, 14.97; Cl, 37.92. Found: N, 14.96; Cl, 37.79.

B.—The compound was also prepared by interaction of 3.3 g. of cyanogen bromide and 5 g. of 2,4-dichloroaniline in 50 cc. of absolute alcohol and in presence of 3.3 *f.* of potassium bicarbonate. After standing at ordinary temperature for six days, the mixture was heated to boiling and the alcohol evaporated. The residue was triturated with dilute potassium hydroxide solution and the unchanged 2,4-dichloroaniline extracted with ether. On acidifying the alkaline solution, about 1 g. of the dichlorophenylcyanamide was obtained. After recrystallization from benzene it melted at 161–162°.

Anal. Calcd. for $C_7H_4NCl_2$: N, 14.97. Found: N, 14.86.

Chlorination of S-Ethyl-N,N′-diphenylisothiourea and S-Benzyl-N,N′-diphenylisothiourea Hydrochlorides.[12]—Either one of those two isothiourea salts was dissolved in a large volume (400 cc. per 5–10 g.) of 90% acetic acid and the resulting solutions saturated with chlorine gas below 15°. The voluminous precipitate obtained in both experiments was filtered off and dried. The yield was 70–80%. The product was purified easily by crystallization from *n*-butyl alcohol or acetic acid and separated as needles melting at 263–264°.

Anal. Calcd. for $C_{13}H_8ON_2Cl_4$: N, 8.00; Cl, 40.54. Found: N, 7.95; Cl, 40.68.

This compound proved to be identical with 2,4,2′,4′-tetrachlorodiphenylurea prepared from urea and 2,4-dichloroaniline.[13]

In the above chlorination experiment with S-benzyl-N,N′-diphenylisothiourea a 60% yield of benzylsulfonyl chloride was obtained on diluting the cold acetic acid filtrate with water and recrystallizing the precipitated sulfonyl chloride from benzene.

Summary

1. Nitrogen unsubstituted isothiourea salts interact with chlorine in aqueous solution to

(8) McKee, *Am. Chem. J.*, **26**, 244 (1901).

(9) *Hydrochloride*, prepared from benzyl chloride and methylthiourea, was a viscous sirup. *Picrate*, m. p. 182–183. N, found 16.93%, calcd. 17.11%.

(10) Werner, *J. Chem. Soc.*, **57**, 295 (1890).

(11) Hoffmann, *Ber.*, **18**, 3220 (1885).

(12) Rathke, *Ber.*, **14**, 1776 (1881); Werner, *J. Chem. Soc.*, **57**, 297 (1903).

(13) Chattaway and Oxton, *Ber.*, **34**, 1076 (1901).

form a sulfonyl chloride and cyanamide hydrochloride.

2. N-Alkyl substituted isothioureas interact with chlorine under similar conditions to form sulfonyl chloride and the corresponding alkyl cyanamide derivatives.

3. N-Aryl substituted isothioureas interact with chlorine to give cyanamides containing chlorine substituted in the aryl grouping of the cyanamide.

4. N,N'-Diarylisothioureas interact with chlorine to form halogenated diaryl ureas and sulfonyl chlorides.

NEW HAVEN, CONN. RECEIVED NOVEMBER 8, 1938

[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT, FORDHAM UNIVERSITY]

The Determination of Free and Phosphorylated Thiamin by a Modified Thiochrome Assay[1]

BY DOUGLAS J. HENNESSY AND LEOPOLD R. CERECEDO

The assay of thiamin, through its quantitative conversion to thiochrome which can be determined fluorometrically, was proposed by Jansen in 1936.[2] Since that time, several investigators[3-7] have applied the method with little modification. The method is based on the oxidation of thiamin by potassium ferricyanide in an alkaline medium, extraction of the thiochrome formed by isobutanol, and estimation of the intensity of the violet-blue fluorescence in ultraviolet light. Adsorption on Franconite or similar adsorbents has been resorted to in an attempt to separate the thiamin from materials interfering in the assay. There is, however, need for a more efficient purification of the thiamin. Base-exchange, replacing the adsorption technique, allowed of a simpler isolation of thiamin from three natural sources.[8-10] It is also more effective in preparing samples for the thiochrome assay. By means of this step, materials which interfere chemically or physically, are eliminated.

Lohmann and Schuster[11] proved that cocarboxylase is a pyrophosphoric ester of thiamin and that it cures polyneuritis in pigeons. In many yeasts and animal tissues this is the predominant form of vitamin B_1. Although not affecting the conversion to a fluorescent thiochrome, the pyrophosphoric acid group prevents extraction by isobutanol.[12] The presence of interfering materials in the aqueous layer precludes the accurate fluorometric measurement of the nonextractable thiochrome pyrophosphate. Tauber,[13,14] Weijlard and Tauber[15] and Lohmann and Schuster[11] have found that cocarboxylase can be hydrolyzed enzymatically. Tauber, using kidney tissue, measured the decrease in cocarboxylase activity, but did not show the hydrolysis to be a complete removal of the pyrophosphate group. Lohmann and Schuster state that a kidney phosphatase hydrolyzed the pyrophosphate to orthophosphate, and only with an enzyme from prostate were they able, after several days, to obtain thiamin from the hydrolysate. Extraction of beef kidney, clarification of the extract, and fractional precipitation with acetone, has given us a stable preparation, practically free of vitamin B_1, which will in two or three hours quantitatively convert cocarboxylase and thiamin orthophosphate to what is probably thiamin. The thiochrome formed from this hydrolysate is extractable by isobutanol in the usual way.

Experimental

Preparation of the Extracts.—The finely divided sample is refluxed for three minutes with five to twenty parts of 2% acetic acid solution. After cooling and centrifuging, the residue is reëxtracted and the filtrates are combined. In case of gelation, where good separation is not accomplished by centrifuging, the solution is made 30% with

(1) Presented at the Dallas Meeting, April, 1938, and at the Milwaukee Meeting, September, 1938, of the American Chemical Society.

(2) B. C. P. Jansen, *Rec. trav. chim.*, **55**, 1046 (1936).

(3) (a) H. G. K. Westenbrink and J. Goudsmit, *Rec. trav. chim.*, **56**, 803 (1937); (b) W. Karrer and V. Kubli, *Helv. Chim. Acta*, **20**, 369 (1937).

(4) F. Widenbauer, O. Huhn and G. Becker, *Z. ges. exptl. Med.*, **101**, 178 (1937).

(5) K. Ritsert, *Deut. med. Wochschr.*, **64**, 481 (1938).

(6) M. A. Pyke, *Biochem. J.*, **31**, 1958 (1937).

(7) G. Hongo, *J. Pharm. Soc. Japan*, **58**, 361 (1938).

(8) L. R. Cerecedo and D. J. Hennessy, THIS JOURNAL, **59**, 1617 (1937).

(9) L. R. Cerecedo and F. J. Kaszuba, *ibid.*, **59**, 1619 (1937).

(10) L. R. Cerecedo and J. J. Thornton, *ibid.*, **59**, 1621 (1937).

(11) K. Lohmann and P. Schuster, *Biochem. Z.*, **294**, 188 (1937).

(12) H. W. Kinnersley and R. A. Peters, *Biochem. J.*, **32**, 697 (1938).

(13) H. Tauber, *J. Biol. Chem.*, **123**, 499 (1938).

(14) H. Tauber, *ibid.*, **125**, 191 (1938).

(15) J. Weijlard and H. Tauber, THIS JOURNAL, **60**, 2263 (1938).