The Reaction between Thioamides and Primary Amines

BY MAURICE J. SCHLATTER[1]

The older literature[2] implies that the reaction between thioamides and primary amines proceeds solely with elimination of hydrogen sulfide and resultant formation of amidine. The present studies show that under certain conditions ammonia may be split out between the reacting molecules giving an N-substituted thioamide.[3] The interaction may also result in the simultaneous elimination[4] of hydrogen sulfide and ammonia.

Experimental

Thioacetamide and *n*-Butylamine.—Five grams of thioacetamide (0.077 mole) was mixed with 16.8 g. of *n*-butylamine (0.23 mole) and heated under reflux until the initially brisk gas evolution had almost ceased (about three hours). Fractionation of the product *in vacuo* gave 5.3 g. of light yellow oil,[5] the major portion of which boiled at 131.5° (5 mm.) and gave analytical figures for N-butylthioacetamide.

Anal. Calcd. for $C_6H_{13}NS$: C, 54.91; H, 9.99; N, 10.67. Found: C, 54.91; H, 10.06; N, 10.73.

Thioacetamide and Benzylamine.—Benzylamine (2.14 g. = 0.020 mole) was mixed with thioacetamide (1.43 g. = 0.022 mole) and heated in a bath at 80° for one and one-quarter hours. The initially vigorous gas evolution almost stopped after forty-five minutes. On fractionation, the reaction mixture distilled almost completely at 158–162° at 2 mm. The distillate (2.28 g.) solidified on cooling and on recrystallization from anhydrous ether gave colorless needles, insoluble in water, soluble in alcohol, m. p. 65.1–65.3°[6] (cor.).

Anal. Calcd. for $C_9H_{11}NS$: C, 65.41; H, 6.71; N, 8.48. Found: C, 65.60; H, 6.68; N, 8.27.

Thioacetamide and Ethanolamine.—Ethanolamine (15.3 g. = 0.25 mole) and thioacetamide (18.0 g. = 0.276 mole) were mixed in a flask equipped with stirrer and immersed in a bath. On heating at 60–75° an active gas evolution took place which lasted about one-half hour. The stirrer was then replaced by a distilling head and the temperature of the bath raised slowly to 215° while volatile material was removed *in vacuo* (30 mm.). The residue (17.9 g.) solidified on cooling and was recrystallized from absolute alcohol; colorless rectangular prisms, m. p. 101.0–101.5° (cor.). The analysis indicates that two molecules of each of the reactants have combined with loss of two molecules of ammonia and one of hydrogen sulfide; a possible formula is $[HOCH_2CH_2N{=}C(CH_3)-]_2S$.

Anal. Calcd. for $C_8H_{16}N_2O_2S$: C, 47.03; H, 7.90; N, 13.71; S, 15.69. Found: C, 47.09; H, 7.79; N, 13.64; S, 15.82.

A derivative crystallized from the reaction mixture when equal volumes of saturated solutions of the substance and of picric acid in ethyl acetate were mixed, heated to boiling and then cooled to 0°. The crystals were washed with ether and dried, m. p. 95.0–95.5° (cor.).

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(2) Bernthsen, *Ann.*, **184**, 290 (1877).

(3) Compare Westphal and Andersag, *Chem. Abstr.*, **35**, 1413 (1941); **36**, 1950 (1942); see also Gatewood and Johnson, THIS JOURNAL, **50**, 1423 (1928).

(4) Compare Buchman, Reims, Skei and Schlatter, *ibid.*, **64**, 2696 (1942).

(5) Compare Sakurada, *Chem. Zentr.*, **99**, I, 683 (1928).

(6) Worrall, THIS JOURNAL, **50**, 1459 (1928), gives m. p. 62–63° for N-benzylthioacetamide.

Preparation of *d*-Fructose-1,6-diphosphate by Means of Baker's Yeasts

BY C. NEUBERG AND H. LUSTIG

The preparation of the phosphoric esters of carbohydrates has not been achieved up to now with fresh baker's yeast, as phosphorylation with top yeast was only possible by addition of co-ferment to acetone yeast or alcohol–ether dried yeast (phosphorylation to 100%), or, with the same type of yeast, if dried before in the usual manner (phosphorylation to 20%).[1] By means of the procedure described below, *d*-fructose-1,6-diphosphate is readily obtained by the use of commercial bakers' yeasts without the necessity of treating brewers' yeast by washing, pressing, etc. Nearly equally satisfactory results have been obtained with various brands of fresh bakers' yeast (Atlantic, Federal, Blue Ribbon and National Grain), but not with fresh Fleischmann's yeast.

Procedure

To a solution of 200 g. of sucrose, 42 g. of monosodium phosphate ($NaH_2PO_4{\cdot}2H_2O$) and 11 g. of sodium bicarbonate in 1000 cc. of tap water contained in a 5-l. bottle add 450 g. of fresh bakers' yeast (Atlantic) and 150 cc. of ether.[2] Shake the mixture until homogeneous, stopper the bottle in a manner which will allow the escape of gases, and place in an incubator at 37° until phosphorylation is complete[3] (four and one half hours). The completeness of this process may be judged by adding 3 cc. of 2.5% ammonia and 1 cc. of 10% ammonium chloride to 2 cc. of the filtered fermentation mixture and then adding magnesia mixture. An immediate precipitation does not occur when phosphorylation is complete.

When phosphorylation is complete, add a few cc. of a

(1) C. Neuberg and A. Gottschalk, *Biochem. Z.*, **154**, 492 (1924).

(2) Carbon tetrachloride is an equally satisfactory plasmolytic agent, also for the phosphorylation with brewers' yeast, as our experiments with yeasts obtained from the Piel and Schaefer breweries (bottom yeasts) demonstrate.

(3) The time required for complete phosphorylation varies with different brands of yeast and with the temperature. Similar experiments made with Atlantic and Federal yeasts at room temperature (20–24°) required twenty and seventy-two hours, respectively.

10% solution of octyl alcohol in ethanol to the fermentation mixture and immerse the container in an actively boiling water-bath until the proteins are coagulated. Separate the coagulum by centrifugation or filtration (in a refrigerator), neutralize the clear filtrate to phenolphthalein by the addition of 4 *N* sodium hydroxide and immediately precipitate by the addition of a solution of 55 g. of calcium chloride in 100 cc. of water; *i. e.*, slightly more than one mol of this reagent is required for each mol of monosodium phosphate present in the initial mixture. Complete the precipitation by heating at a boiling water-bath for a short time, and filter the warm mixture with suction. Wash the precipitated calcium *d*-fructose-1,6-diphosphate with warm water; yield, 22 g.

An alternative procedure is the following: When phosphorylation is complete add to the fermentation mixture a solution of 40 g. of picric acid in 200 cc. of hot ethanol. Allow the mixture to stand in a refrigerator for two hours and filter. Add 4 *N* sodium hydroxide to the filtrate until neutral to phenolphthalein, then add calcium chloride as previously described. The precipitated calcium salt contains some picrate but this is removed by washing with hot water and subsequently with alcohol. An experiment conducted in this fashion with the quantities of material stated above yielded 24 g. of crude calcium *d*-fructose-1,6-diphosphate.

To purify the crude calcium salt obtained by either of these procedures, dissolve the moist crude product in 250 cc. of 2 *N* acetic acid, add 125 cc. of water, filter if necessary, and to the clear solution add 2 *N* sodium hydroxide until neutral to phenolphthalein. Heat the resulting mixture in a boiling water-bath for a short time, filter and wash the precipitated salt with warm water; yield about 80% of the crude product.

In analogous manner the barium salt can be prepared, but in this case the use of picric acid for the deproteinization is not advisable because of the difficult solubility of barium picrate.

As mentioned in the introduction, fresh Fleischmann's yeast is not satisfactory for the preparation of *d*-fructose-1,6-diphosphate in the manner described. If, however, this brand of yeast is well dried at room temperature in the usual manner, it may be employed, for with such dried yeast phosphorylation takes place rapidly. When a mixture of 30 g. of dried Fleischmann's yeast, 15 cc. of carbon tetrachloride and 100 cc. of the sugar–phosphate solution of the composition mentioned earlier was incubated at 37°, phosphorylation was found to be complete within one and one-half hours. Similar results were obtained with dried preparations of National Grain and Federal yeasts. The product of phosphorylation contains, as well as in the other cases described, in addition to hexose-diphosphate also sugar-monophosphates.

A purity test can be made using an observation published many years ago.[4] Calcium and barium hexose–diphosphate dissolve readily in solutions of ammonium tri-salts, such as acetate, chloride, nitrate, rhodanide, while calcium and barium phosphate are practically insoluble. Consequently the pure salts of hexose–diphosphate are completely dissolved in the ammonium salt solutions and added magnesia mixture does not cause a precipitate.

(4) C. Neuberg and S. Sabetay, *Biochem. Z.*, **161**, 240 (1925).

We are indebted to the manufacturers of the various brands of yeast mentioned for generous supplies of their respective products.

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The Catalytic Reduction of Cholesterol α-Oxide

BY HOMER E. STAVELY

The catalytic reduction of oxides of the ethylene oxide type has been suggested as a possible approach to certain synthetic problems in the steroid hormone field. Only a few such examples can be found in the literature. Triphenylethylene oxide,[1] ethylene oxide[2] and ethyl stearate oxide[3] have been catalytically reduced to alcohols. Fernholz[4] hydrogenated stigmasterol α-oxide-(5,6) acetate using platinum and acetic acid, and obtained stigmastanediol-3,5 monoacetate in 20% yield. According to a patent[5] 3-acetoxy-pregnadiene-5:6,20:21 can be converted into a dioxide, which can then be reduced in acetic acid with palladium and hydrogen to 3-acetoxypregnanediol-5,20.

Similar treatment of cholesterol α-oxide[6] should yield either 3,5- or 3,6-cholestanediol. The 3,5-diol was obtained by Chinaeva and Ushakov[7] when they treated cholesterol α-oxide with phenyllithium.

Cholesterol α-oxide was hydrogenated in acetic acid with palladium catalyst. The hydrogen uptake was slow, and was therefore allowed to proceed over a period of several days with intermittent shaking. The reaction products were acetylated and chromatographed on alumina. Three substances were isolated in pure form, cholestanediol-3,5 monoacetate,[8] cholestanol-3 acetate, and α-cholestanetriol-3,5,6 diacetate. The latter compound obviously is not a reduction product; it can be prepared by heating the oxide acetate with acetic acid. It is evident that the major part of the oxide is reduced to the 3,5-diol, from

(1) Weill and Kayser, *Bull. soc. chim.*, [5] **3**, 841 (1936).

(2) Ushakov and Mikhailov, *J. Gen. Chem.* (U. S. S. R.), **7**, 249 (1937); *C. A.*, **31**, 4645 (1937).

(3) Pigulevskiĭ and Rubashko, *J. Gen. Chem.* (U. S. S. R.), **9**, 829 (1939); *C. A.*, **34**, 378 (1940).

(4) Fernholz, *Ann.*, **508**, 215 (1934).

(5) Swiss Patent 214,540.

(6) Windaus and Westphalen, *Ber.*, **48**, 1064 (1915).

(7) Chinaeva and Ushakov, *J. Gen. Chem.* (U. S. S. R.), **11**, 335 (1941); *C. A.*, **35**, 5903 (1941).

(8) The melting points of the free diol and the diol acetate are somewhat higher than the figures given by Chinaeva and Ushakov. The two diols may be isomeric at C_5.