p-**Aminophenol.**—After 5 g. of benzylphenylhydroxylamine had been added to 200 cc. of 5% aqueous sulfuric acid, the mixture was heated until solution was complete. After cooling, the mixture was made basic with sodium carbonate. The green oil which separated was removed by extraction with ether and the ether was dried with sodium sulfate and then distilled. The residue was crystallized from an alcohol–ether mixture giving 1 g. (36%) of *p*-aminophenol, m. p. 183–184° (184°). A mixed melting point with an authentic sample showed no depression.

Summary

Several reactions of N-benzylphenylhydroxylamine have been examined. Sulfuric acid cleaves the molecule giving first phenylhydroxylamine and then rearranging the latter to *p*-aminophenol.

URBANA, ILLINOIS RECEIVED JULY 10, 1941

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF WISCONSIN]

The Chemistry of the Rye Germ. VI. Allantoin and Other Acetone-Extractives[1]

BY AARON J. IHDE AND H. A. SCHUETTE

Several years ago in this Laboratory it was observed that the oil which had been extracted from rye germs with acetone soon deposited a small amount of crystalline material upon standing. This is apparently a characteristic of a rye oil so recovered, for a similar behavior was never noted in the oil extracted from the same mother substance with any of the other common fat solvents. Further investigations revealed that the crystalline material could be removed in fairly pure form by hot acetone extraction of the germs previously defatted with petroleum ether. A positive Molisch test, together with a sweet taste, indicated a sugar. Recently, upon resumption of this study, it was observed that, when this material was dissolved in hot water, a turbid solution, from which tasteless crystals separated on cooling, resulted. The turbidity was shown to be due to phospholipids. The tasteless, insoluble crystals proved to be allantoin; the sugar was found to be sucrose.

Experimental

Rye germs, which had been made substantially fat-free by extraction with benzene, were exhausted with hot acetone in a Soxhlet-type extractor which was so designed that the rising vapors kept the germs and surrounding solvent near the boiling point of acetone. A 72-hour treatment removed approximately 1% of material which deposited as a light brown, crystalline sediment in the heating flask. Longer extraction failed to remove significantly larger amounts.

Identification of Phospholipids.—Sufficient fat-free rye germ was treated with acetone to obtain 500 g. of solvent-free extract. This extract was refluxed with three 500-ml. portions of ethyl ether. The ether-insoluble portion was transferred to a Büchner funnel and washed with ether until the washings were colorless. The ether solutions and washings were combined and concentrated to approximately one-fourth their original volume to give a brownish, slightly opalescent solution. On addition of four volumes of acetone a white precipitate settled out. On removal of the clear supernatant liquid a gummy white solid remained which quickly turned brown on exposure to air. It was partially, but not entirely, soluble in 95% ethanol and was precipitated by ethanolic cadmium chloride solution. Tests for phosphorus, nitrogen and glycerol were positive. Saponification with ethanolic potassium hydroxide solution yielded typical fatty acids with a neutralization equivalent of 293.1. The Rosenheim bismuth test for choline was positive.

Identification of Allantoin.—The phospholipid-free portion of the extract, amounting to 290 g., was dissolved in 500 ml. of hot water. On cooling, several grams of prism-like crystals separated from solution. The supernatant liquid was removed and concentrated to approximately two-thirds of its original volume and set aside overnight. A second crystal crop was thus obtained. This concentration–crystallization process was twice repeated. The last concentration gave only a few small crystals and a sirupy mother liquor. The total crystal yield was 14.2 g., equivalent to 0.03% of the original rye germ.

The crystals were fairly soluble in hot water but very insoluble in cold water. After several recrystallizations from water the compound melted with decomposition at 231°, though browning began at 220°. Nitrogen was present. Solubility behavior and classification reactions indicated an amide, but it was not possible to hydrolyze it and to isolate a parent acid. Because the compound gave a negative murexide test, it was not a purine. A determination of its nitrogen content indicated that the compound might be allantoin. This was confirmed when the behavior toward Fehling solution and Nessler reagent was typical; when the Schiff furfural test for allantoin was positive; and when a mixed melting point determination with pure allantoin showed no depression. The optical inactivity was also characteristic of allantoin.

Anal. Calcd. for $C_4H_6O_3N_4$: C, 30.39; H, 3.81; N, 35.44. Found: C, 30.44, 30.21; H, 3.81, 3.71; N, 35.31, 35.40.

Identification of Sucrose.—The sirupy mother liquor from which the allantoin had been removed was further

(1) For communications I, II and V in this series, see THIS JOURNAL, **54**, 3298 (1932), **56**, 210 (1934), and **61**, 1901 (1939); for III, see *Oil and Soap*, **14**, 295 (1937), and for IV see *Cereal Chem.*, **15**, 445 (1938).

concentrated after which crystallization was induced by dilution with ethanol. An aqueous solution of the crystals was decolorized with charcoal and set aside to crystallize. The crystals had a sweet taste, were readily soluble in water and gave a positive Molisch test, and melted at 178° with decomposition. Tests with Fehling solution were negative before hydrolysis and positive after heating with acid. The osazone prepared from the hydrolysis products melted at 206°. The Seliwanoff test was positive; $[\alpha]^{20}D$ +66.45. The hydrolyzed solution showed a levorotation which became zero at 87°.

Discussion

The solubilities of allantoin, sucrose and phospholipids in acetone are generally considered insignificant, but these compounds made up the acetone extractives of fat-free rye germs. It appears that in certain natural mixtures the normal solubilities are altered. Thus, mixtures of compounds are removed by solvents in which each of the pure compounds is insoluble. We have here an instance in which phospholipids, in large part, have resisted extraction with benzene but are removed by acetone, which is extensively used to precipitate phospholipids from fatty oils.

The simultaneous removal of phospholipids and carbohydrates is not new. McKinney, Jamieson and Holton[2] obtained phospholipids and a hexose sugar by alcohol extraction of fat-free soy beans. They postulated that the phospholipids were combined with the sugar in a glycosidic linkage which was hydrolyzed during precipitation with cadmium chloride. Rewald[3] also observed that carbohydrates were present in phospholipid preparations. He was able to make a separation by purely physical methods involving solvent extraction.

Results of this study again raise the question as to whether there is not a close association of phospholipid and carbohydrate in many seeds. It is possible that the carbohydrate accompanying the lipid material may sometimes have been overlooked or ignored. The possibility of chemical combination is remote in the case of rye, however, since the accompanying sugar is sucrose and glycoside formation is obviously impossible.

The role of allantoin in rye germ is speculative. Although an end-product of purine metabolism in most animals it is also known as a cell proliferant. Macalister[4] showed it to be the active ingredient of the rhizome of comphrey (*Symphtum officinale*) which was widely used in Europe in promoting the healing of open sores. It was shown by Robinson[5] to be the healing agent functioning in maggot therapy. Greenbaum[6] has also reported an increased growth rate in shoots and flowers when allantoin was injected into the bulbs of hyacinths, tulips and chrysanthemums. Allantoin has been reported present in plants in areas of active metabolism, such as bark and leaf buds,[7,8] and Richardson and Crampton[9] found it in wheat germ. This raises the question as to whether allantoin in plants may be not merely an end-product of metabolism but a stimulatory agent in regions of active growth.

Summary

It has been shown that the material extracted in amounts of 1% by hot acetone from defatted rye germ is made up of phospholipids (42%), sucrose (55%) and allantoin (2.8%).

MADISON, WISCONSIN RECEIVED JUNE 27, 1941

(2) R. S. McKinney, G. S. Jamieson and W. B. Holton, *Oil and Soap*, **14**, 126 (1937).

(3) B. Rewald, *Food*, **6**, 7 (1936); *J. Soc. Chem. Ind.*, **56**, 77T (1937).

(4) C. J. Macalister, *Brit. Med. J.*, **1**, 10 (1912).

(5) W. Robinson, *J. Bone and Joint Surgery*, **17**, 267 (1935).

(6) F. R. Greenbaum, *Am. J. Pharm.*, **112**, 205 (1940).

(7) E. Schultze and J. Barbieri, *Ber.*, **14**, 1602 (1881).

(8) E. Schultze and E. Bosshard, *Z. physiol. Chem.*, **9**, 20 (1885).

(9) C. Richardson and C. A. Crampton, *Ber.*, **19**, 1180 (1886).