that the ester and acid reacted in the tautomeric forms IA and IIA.

4. The reaction between a completely methylated quinone and sodium enolates appears to be a general method for preparation of 6-hydroxy methylated coumarins with substituents in the 3-position.

MINNEAPOLIS, MINNESOTA RECEIVED DECEMBER 9, 1935

[CONTRIBUTION FROM NORTHWESTERN UNIVERSITY MEDICAL SCHOOL]

Studies on Proteins in Liquid Ammonia. III. Reaction of Sodium in Liquid Ammonia with Proteins and Related Substances

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In the previous paper[1] of this series, we reported the results of our investigation of the reactions of certain amino acids, dipeptides, diketopiperazine and related substances with sodium in liquid ammonia. Some of these substances are reduced and all are acidic in liquid ammonia. We have made a similar study using several proteins.

Experimental

The analytical procedure was essentially the same as described in the previous paper. The proteins were dried by heating in an electric oven at 85° for forty-eight hours, followed by transfer to a vacuum desiccator containing concentrated sulfuric acid for an additional forty-eight hours. Drying experiments were carried out with casein (Pfanstiehl) in which the casein was dried by the following methods: (1) air drying by exposure to atmosphere at room temperature for three months, (2) drying in a vacuum desiccator over concentrated sulfuric acid for three months, (3) drying at 85° in an electric oven for forty-eight hours followed by drying in a vacuum desiccator for forty-eight hours, (4) drying in an electric oven at 85° for six weeks, (5) drying in an electric oven at 85° for six weeks followed by additional drying in a vacuum desiccator over concentrated sulfuric acid for six weeks, (6) drying for six weeks in an electric oven at 105° followed by additional drying for six weeks in a vacuum desiccator over concentrated sulfuric acid and (7) drying at 115° in an electric oven for six weeks followed by an additional six weeks in a vacuum desiccator over concentrated sulfuric acid. Effectiveness of drying was determined by the amount of hydrogen liberated by casein in liquid ammonia when excess sodium was added. Casein dried by methods 3, 4, 5, 6 and 7 gave the smallest volumes of hydrogen and in equivalent amounts within the limits of experimental error.

Drying experiments with silk fibroin, edestin (Pfanstiehl), egg albumin (Merck) and meat peptone (Armour) showed that either drying them for forty-eight hours in a vacuum desiccator over concentrated sulfuric acid or heating them in an electric oven at 75 to 115° for forty-eight hours removed all of the water, but that a combination of the two was more effective and gave minimum hydrogen liberation.

Whether this method of drying removes all of the water from the protein, we do not know. When traces of water are present in a protein, there is a brief period of rapid reaction with sodium in liquid ammonia, which subsides to a slow rate of reaction characteristic of proteins. If the proteins, after the drying treatment, still contain water, it must be bound water or water of constitution which cannot be removed without decomposition of the protein. While bound water would give slightly higher figures for hydrogen evolved, it will not alter the shape of the curves. Each curve will have a slightly higher position on the ordinate axis.

In general, proteins react with sodium in liquid ammonia to give hydrogen more slowly than either amino acids or dipeptides. The reaction of sodium with amino acids and dipeptides is complete in a few minutes, with diketopiperazine in about one hour, and with most proteins in two or three hours.

The drying of certain proteins presents some difficulties. When zein and gliadin, freshly precipitated from absolute alcohol, were kept in a vacuum desiccator over sulfuric acid for a few days, they were found to be quite soluble or dispersed in liquid ammonia. This is in agreement with a previous observation made by Taft[2] that zein and gliadin are soluble or dispersed in liquid ammonia. However, after drying zein and gliadin at 80° for forty-eight hours, they were found to be practically insoluble in liquid ammonia.

The data are given graphically in Fig. 1. Moles of hydrogen evolved are plotted against atomic weights of sodium used, the nitrogen content of the sample portions of proteins being the same in all cases. An excess of sodium was considered to have been used when the liquid ammonia solution remained blue for three hours. Sodium reacts with liquid ammonia very slowly. The quantity of hydrogen liberated when sodium reacts with a protein is corrected for this. The ratios of moles of sodium to gram atoms of nitrogen necessary to use to have an excess of sodium for each of the proteins were: edestin, 1.25; silk fibroin, 1.50; casein, 1.00; and egg albumin, 1.25. No attempt was made to determine the end-point exactly.

(1) C. O. Miller and R. G. Roberts, THIS JOURNAL, **56**, 935 (1934).

(2) Taft, *Trans. Kansas Acad. Sci.*, **32**, 38 (1929); *J. Phys. Chem.*, **34**, 2722 (1930).

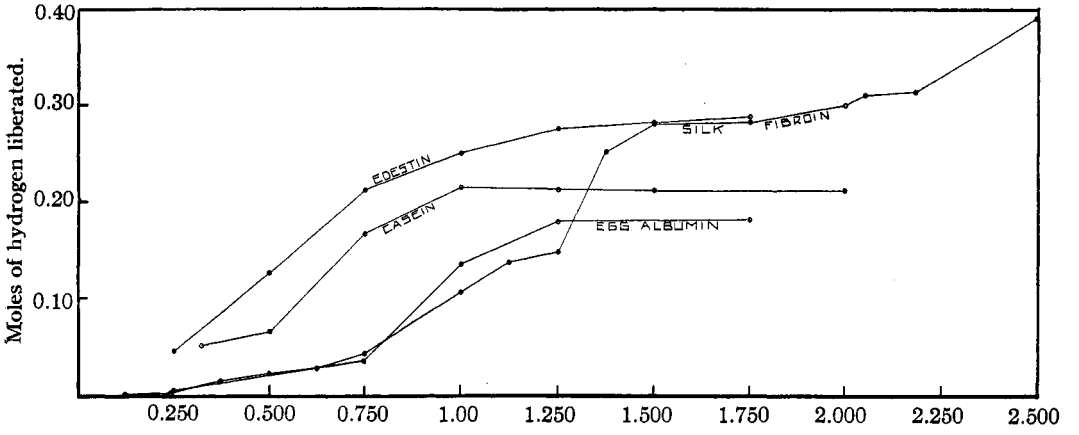

Fig. 1.—Atoms of sodium used.

When zein and gelatin are treated with a slight excess of sodium in liquid ammonia, the ratio of moles of hydrogen liberated to gram atoms of nitrogen in the substances was 0.09 and 0.350, respectively. Hemoglobin and hematin liberate hydrogen continuously when excess sodium is present and in quantities disproportionate to the acid groups present, indicating that they catalyze the reaction between sodium and ammonia. Hematoporphyrin, hemocyanin from lobster's blood, chlorophyll, glutathione and methylene blue do not act in this way.

Calculations have been made for four proteins to determine how much hydrogen would be liberated by the monoaminodicarboxylic acids, glutamic acid, aspartic acid and hydroxyglutamic acid, contained in the proteins when an excess of sodium is used. The content of these amino acids in the proteins was taken from a review by Cohn.[3] The data are given in Table I. These four proteins were chosen because their composition is known up to 90% or more.

TABLE I

Protein	% of amino acid present Glutamic	Aspartic	Hydroxy-glutamic	Moles H_2/g. atoms N Calcd.	Found
Casein	21.77	4.10	10.5	0.1210	0.215
Edestin	19.16	10.19	0.0	.0775	.285
Gelatin	5.80	3.40	0.0	.0255	.350
Zein	31.30	1.80	2.50	.1049	.09

The hydroxyl groups of the amino acids, tyrosine, serine, oxyproline and hydroxyproline would also react with sodium in liquid ammonia. We found previously that approximately one-fourth of a mole of sodium reacts with the hydroxyl group of 1 mole of tyrosine. We do not have data for the other hydroxy amino acids. In the cases of casein, edestin and gelatin, more hydrogen is liberated than can be accounted for by the second carboxyl group of the dicarboxylic acids, and in the case of zein, the amount of hydrogen liberated is slightly less than the amount that might come from the dicarboxylic acids.

Summary

1. A study of the reaction of sodium in liquid ammonia with certain proteins has been made.

2. Proteins are acidic in liquid ammonia, and liberate hydrogen when sodium is added to them.

3. Hemoglobin and hematin catalyze the reaction of sodium and ammonia.

CHICAGO, ILLINOIS RECEIVED DECEMBER 12, 1935

(3) Cohn, *Ergebnisse Physiol.*, **33**, 870 (1931).