In the present study we have calculated a $\Delta F^{\circ}_{298}$ value for one or more representatives of each of the four important classes of hydrocarbons. The numerical results serve to illustrate that the order of decreasing thermodynamic stability (*i. e.*, increasing free energy of formation) at room temperature is: (1) paraffin, (2) naphthene, (3) olefin, (4) aromatic hydrocarbons.

Before concluding, the authors wish to thank the Shell Development Company, Dr. Albert L. Henne, Professor G. Chavanne and Professor Lee Irvin Smith for the valuable hydrocarbons which made this research possible.

## Summary

1. The specific heats of twenty hydrocarbons have been measured over a wide range of temperatures. Heats of fusion and of transition have also been determined in the case of seventeen of these compounds.

2. The entropies of the twenty hydrocarbons have been calculated from these heat capacity data. In general the results are in good agreement with the corresponding values calculated by an empirical equation developed in preceding papers.

3. The corresponding free energies for fourteen of these hydrocarbons have also been calculated. The order of decreasing thermodynamic stability at 298° K. is (1) paraffin, (2) naphthene, (3) olefin, (4) aromatic hydrocarbons.

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[CONTRIBUTION FROM THE CHEMISTRY LABORATORY OF NORTHWESTERN UNIVERSITY MEDICAL SCHOOL]

# STUDIES ON PROTEINS IN LIQUID AMMONIA. I[1,2]

BY EVAN W. McCHESNEY AND CLEMMY O. MILLER

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During the last few years there has been an increasing interest in the study of proteins in non-aqueous media. Granacher[3] has studied the reactions of proteins and polypeptides in ethyl alcohol at 170°; Fodor and Epstein[4] have studied the decomposition of gelatin by acetic anhydride.

[1] This article is taken from the dissertation presented by Evan W. McChesney to the Graduate School of Northwestern University in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

[2] This is an abstract of two papers, one of which was presented before Section C of the American Association for the Advancement of Science, Cleveland, Ohio, and the other before the Organic Division of the American Chemical Society in Indianapolis, Indiana, April, 1931.

[3] Granacher, *Helv. Chim. Acta*, **8**, 784 (1925).

[4] Fodor and Epstein, *Z. physiol. Chem.*, **171**, 222 (1927): *Biochem. Z.*, **200**, 211 (1928); **228**, 310 (1930).

Abderhalden and his co-workers[5] have studied the reduction of peptones, peptides and diketopiperazines by sodium in ethyl or amyl alcohol; and Troensegaard and Mygind[6] have recently reported a study of the hydrogenation of proteins by sodium in amyl alcohol. A correlation of the results of investigations carried out in non-aqueous media with the results of investigations carried out in water will help to elucidate the problem of protein structure. Taft[7] has studied the solubility and colloidal properties of proteins in liquid ammonia. He found that zein, gliadin, nucleic acid, proteose, peptone and bactopeptone are dispersed in liquid ammonia, glutenin is dispersed fairly well, gelatin and albumin swell slightly and silk neither swells nor disperses. Our observations on the proteins that we used agree with this. We can add that blood albumin and casein slightly swell in ammonia. Voss and Guttman[8] have shown that the sodium salts of the amino acids can be prepared conveniently by adding the amino acid to a solution of sodium in ammonia until the blue color of the solution is discharged. Du Vigneaud, Audrieth and Loring[9] have reduced cystine by sodium in liquid ammonia and have prepared benzylcysteine in liquid ammonia.

Liquid ammonia offers interesting possibilities as a medium for studying the chemistry of proteins. The characteristic properties of proteins are more dependent upon the nitrogen atoms of the protein molecule than upon the oxygen atoms. The peptide link is an aquo-ammono ester group, having a hydrogen atom that might be acidic. Ammonolysis of a peptide link would give a free amino group and a free amide group. The ammonolytic product, when dissolved in water, would be a stronger base than the analogous hydrolytic product and might lend itself to isolation by crystallization methods better than the analogous hydrolytic product. Since the alkali metals are readily soluble in liquid ammonia, a solution of an alkali metal in liquid ammonia might be a more satisfactory medium for the reduction of proteins than is sodium and alcohol, which has generally been used.

**Reactions of Proteins with Ammono-Bases.**—Proteins are partially ammonolyzed by ammono-bases in liquid ammonia, the extent of ammonolysis being dependent upon the base and the temperature. Ammonolysis by potassium amide proceeds slowly at $-33.5°$, but when sodamide is used it is not appreciable below 40°. Water-insoluble proteins,

[5] Abderhalden and Stix, *Z. physiol. Chem.*, **132**, 238 (1925); Abderhalden, Klarmann and Schwab, *ibid.*, **134**, 180 (1924); Abderhalden and Schwab, *ibid.*, **152**, 230 (1926).

[6] Troensegaard and Mygind, *ibid.*, **193**, 171 (1930).

[7] Taft, *Trans. Kansas Acad. Sci.*, **32**, 38 (1929); *J. Phys. Chem.*, **34**, 2792 (1930).

[8] Voss and Guttman, *Ber.*, **63B**, 1726 (1930).

[9] Du Vigneaud, Audrieth and Loring, THIS JOURNAL, **52**, 4500 (1930).

like fibrin and silk[10] rendered water soluble by treatment with an ammonobase in liquid ammonia. The derived protein is partly precipitated by half-saturation with ammonium sulfate and completely precipitated with full-saturation with ammonium sulfate. The filtrate does not give a biuret test. When fibrin is heated to 80–90° with sodamide for four days, only a slight precipitate occurs on full saturation of the ammonium sulfate, but the filtrate gives a positive biuret test and a relatively large precipitate with phosphotungstic acid. Proteins treated with ammonia alone do not effect this change.

A quantitative analysis of various ammonolytic products was made. The material was prepared for the analysis as follows: the protein (40 g.) and sodamide (40 g.) were placed in a 1500-cc. steel cylinder[11] and sufficient liquid ammonia was added to fill the cylinder two-thirds full. It was cooled in a mixture of carbon dioxide and acetone before the ammonia was added. After the heating was completed, the cylinder was cooled in a mixture of carbon dioxide snow and acetone, opened, and 55 g. of ammonium chloride was added to neutralize the sodamide. The ammonia solution was colored a pale yellow. To facilitate solution of the precipitated material, the cylinder was re-sealed and heated in a boiling water-bath for two hours with frequent shaking. The solution was cooled and emptied into a vacuum flask. It had changed in color to a dark brown. The cylinder was washed several times with ammonia and the washings were combined with the solution. This solution was filtered from the heavy precipitate of sodium chloride that had formed during neutralization. The ammonia was allowed to evaporate through a mercury trap and the solid residue was pulverized and dried in a vacuum desiccator, over sulfuric acid. The product was soluble in water and was neutral to litmus. It was usually soluble in 95% alcohol. The ammonolytic substances obtained at 110–120° gave, at most, only a slight precipitate upon saturation of their water solution with ammonium sulfate. They were precipitated by phosphotungstic acid but not by picric acid.

**Method of Analysis.**—An exactly 1.00% solution was prepared. Aliquot portions were analyzed for the following: (1) total nitrogen by micro-Kjeldahl; (2) amino nitrogen by Van Slyke method; (3) ammonia nitrogen by distillation with calcium hydroxide solution; (4) amide nitrogen by digesting with 20% hydrochloric acid for four hours, addition of slight excess of 10% suspension of calcium hydroxide and vacuum distillation.[12] This gives amide nitrogen and ammonia nitrogen. The amide nitrogen is the difference between this value and the value for the ammonia nitrogen. The analytical data are given in Table I.

The increase in amino nitrogen and amide nitrogen indicates that some ammonolysis has taken place. Since the ratio of the increase in amide nitrogen and amino nitrogen is not 1, some reaction or reactions other than ammonolysis must also be taking place. In general, there is an increase in total nitrogen, but in some cases there is a decrease, indicating

[10] The silk fibroin used was silk noils, kindly furnished to us by Cheney Brothers, South Manchester, Conn. It is free from sericin.

[11] The cylinder was a hydrogen cylinder obtained from Matheson Co., North Bergen, N. J. The valve was removed and a threaded steel plug used to stopper the cylinder. A lead gasket was used.

[12] Van Slyke, *J. Biol. Chem.*, **10**, 15 (1911).

TABLE I

ANALYTICAL DATA

| Substance | Base | Temp, °C. | Time, hrs. | N analysis in % Total | Amide | Amino | Ratio $\frac{\text{Amide N}}{\text{Total N}} \times 100$ | Soly. | Biuret reaction |
|---|---|---|---|---|---|---|---|---|---|
| Silk | .... | ... | .. | 17.98 | 0.47 | 0.0 | 2.60 | ..... | ... |
| Casein | .... | ... | .. | 15.62 | 1.60 | .. | 10.20 | ..... | ... |
| Witte's peptone | .... | ... | .. | 16.08 | 1.40 | 2.30 | 8.70 | ..... | ... |
| Silk | .... | 90 | 48 | 18.22 | 0.77 | ..[a] | 4.36 | Unchanged | ... |
| Silk | $NaNH_2$ | 90 | 72 | 19.06 | 0.56 | ..[a] | 2.93 | Sl. sol. $H_2O$ | + |
| Silk | $KNH_2$ | 90 | 40 | 19.29 | 0.56 | ..[a] | 2.90 | Sl. sol. $H_2O$ | + |
| Silk | $NaNH_2$ | 110 | 72 | 15.50 | 3.04 | 4.00 | 19.7 | Sol. $H_2O$, $NH_3$, alc. | Neg. |
| Silk | $NaNH_2$ | 110 | 72 | 13.55 | 2.48 | 4.35 | 18.8 | Sol. $H_2O$, $NH_3$, alc. | Neg. |
| Silk | $NaNH_2$ | 120 | 96 | 20.29 | 6.20 | 1.60 | 30.6 | Sol. $H_2O$, $NH_3$, alc. | Neg. |
| Silk | $NaNH_2$ | 120 | 68 | 18.74 | 4.14 | 2.50 | 22.10 | Sol. $H_2O$, $NH_3$, alc. | Neg. |
| Casein | $NaNH_2$ | 120 | 72 | 10.87 | 2.22 | ..[a] | 20.6 | Partly sol. $H_2O$ | Neg. |
| Witte's peptone | $NaNH_2$ | 120 | 48 | 12.36 | 2.09 | ..[a] | 16.8 | Sol. $H_2O$, $NH_3$, alc. | Neg. |

[a] Not analyzed because of incomplete solubility in water.

that the protein or its ammonolytic products were decomposed to give volatile nitrogen compounds.

**Reactions of Proteins with Ammono-Acids.**—Blood fibrin, edestin and silk fibroin were placed in liquid ammonia with ammono-acids like ammonium chloride and ammonium bromide, and warmed to temperatures varying from 35–115° for two days. The ammonolytic products were partly soluble in water. A slight precipitate was formed when a water solution was half-saturated with ammonium sulfate. It was completely precipitated by saturation with ammonium sulfate. In the case of silk, the ammonolytic product was partly soluble in alcohol. These substances were not analyzed for increase in total nitrogen nor for change in the distribution of nitrogen.

**Reactions of Proteins with Alkali Metals Dissolved in Liquid Ammonia.**—When sodium (3.5 g.) was added in 1-g. pieces to silk fibroin (10 g.) suspended in 300 cc. of liquid ammonia, a vigorous reaction took place. A considerable quantity of hydrogen was liberated. The silk fibers were disintegrated in about three minutes and after an hour sufficient ammonium chloride (0.4 g.) was added to just discharge the blue color of the sodium. A massive white precipitate formed. When more ammonium chloride was added, an amount chemically equivalent to the sodium, the white precipitate went into solution. Some sodium chloride remained as a precipitate. The liquid ammonia solution was decanted and the precipitate of sodium chloride was washed with liquid ammonia by decantation. The washings were combined with the solution and the liquid ammonia was allowed to evaporate. The solid residue was brown; it was dried, powdered and kept in a desiccator over sulfuric acid. It is soluble in water and the water solution is alkaline to litmus and gives a biuret test.

In one experiment potassium was used instead of sodium, but the silk fibers remained essentially intact.

Similar experiments were carried out with casein and edestin. Casein (Pfanstiehl,

5 g.) was suspended in liquid ammonia and sodium (5 g.) was added piece by piece. A vigorous reaction took place and large volumes of hydrogen were liberated. It was allowed to stand for three hours, during which time a part of the casein dissolved. Sufficient ammonium chloride was then added just to discharge the blue color of the solution. The ammonia was allowed to evaporate. The solid residue was extracted with 95% alcohol. The extracts were evaporated to dryness over a water-bath. The residue was a yellow gum. It was soluble in water and the solution was alkaline to litmus. The solution gave a rose-red biuret test.

Edestin swelled immediately in liquid ammonia, forming a tough lump. When sodium was added, a reaction occurred, but not as violent as in the case of casein and silk. The excess sodium was removed by ammonium chloride. The residue from the evaporation of ammonia was extracted with 95% alcohol. The alcohol was evaporated and the residue was extracted with 95% alcohol. It was soluble in water. The solution was strongly alkaline and gave a rose-red biuret test.

Quantitative analyses were made on six preparations. Aliquot portions of a 1.00% solution were used. The same methods were used as for the data given in Table I. Sodium was determined by ashing and titrating with standard acid. Ammonia and sodamide were determined by aeration of a water solution into standard acid. Negligible amounts of these substances were present. The analytical data are given in Table II.

TABLE II

ANALYTICAL DATA

| Preparation | Reaction time, hrs. | Na, % | N, % | Ratio Na/N | % Amino N | Ratio $\frac{\text{Amino N}}{\text{Total N}} \times 100$ |
|---|---|---|---|---|---|---|
| I | 1 | 21.4 | 11.1 | 1.18 | 2.04 | 18.5 |
| II | 1 | 22.3 | 12.1 | 1.13 | 1.52 | 12.6 |
| III | 1 | 20.3 | 11.6 | 1.06 | 1.55 | 13.4 |
| IV | 1 | 21.1 | 12.7 | 1.01 | 1.80 | 14.1 |
| Average | | 21.3 | 11.9 | 1.09 | 1.73 | 14.6 |
| Calculated | | 23.9 | 13.7 | 1.06 | | |
| V | 2 | 23.6 | 10.4 | 1.38 | 1.40 | 13.5 |
| VI | 5 | 25.1 | 11.0 | 1.39 | 1.75 | 15.9 |

Analysis was also made for amide nitrogen but it proved not to have increased significantly.

These salts in acid solution decolorized bromine water instantaneously. Their reducing power was most conveniently obtained by treating them in acid solution with an excess of bromine water at room temperature. The excess of bromine was then determined by adding potassium iodide and titrating with standard sodium thiosulfate. Fifty milligrams of the sodium salt reduced about 1.75 cc. of 0.100 *N* bromine water.

NOTE.—The calculated values were obtained assuming that one mole of sodium enters the protein molecule for each mole of nitrogen and also one for each mole of tyrosine present. Silk fibroin contains 18% nitrogen and about 11% tyrosine.

There are three possible reactions that may take place when protein is treated with sodium in liquid ammonia: salt formation, ammonolysis and reduction. The marked evolution of hydrogen and the absence of sodamide in the product indicates that salt formation takes place. Since the ratio of the equivalents of sodium to nitrogen is approximately 1, it indicates that the acidity of the protein bears some relation to the nitrogen content. This suggests that the imide hydrogen is sufficiently acidic

in liquid ammonia to form stable salts. Since the ammonolytic product, dissolved in acid solution, decolorizes bromine water instantaneously, the presence of a reduced product is indicated. It seems likely that some reduction would take place under these conditions. Since the ammonolytic product was somewhat brown, the reducing action in bromine water may be due to decomposition products rather than to reduced derived proteins. At present it is not possible to say definitely whether or not the protein is reduced. Our further experiments were inconclusive. The increase in amino nitrogen indicates that some ammonolysis took place. However, there is also a decrease in total nitrogen, which indicates that the protein was decomposed to give a volatile nitrogen compound. This will be discussed more fully later.

Portions of two preparations were digested with pancreatin at $P_H$ 8 and 37°. Both preparations showed definite and steady increases in amino nitrogen. After forty-eight hours, the increase in the amino nitrogen amounted to 50 and 70%. Another portion was ethylated by refluxing with ethyl iodide in benzene for two hours. Over 90% of the sodium reacted as indicated by the titration for iodide. The ethylated product still gave the biuret reaction.

The analytical results given in Table II indicate that there is a decrease in total nitrogen during the reaction, even though there is an increase in amino nitrogen. This observation was confirmed by the following analyses.

(a) Silk (1.000 g. containing 180 mg. of nitrogen) was suspended in liquid ammonia and treated with about 0.4 g. of sodium. After one hour the excess sodium was neutralized with ammonium chloride and the ammonia was allowed to evaporate. The residue was taken up in water and made up exactly to 100 cc. Five cubic centimeter portions were analyzed for total nitrogen and ammonia nitrogen, the latter being subtracted to give the total nitrogen coming from the protein.

(b) Similar preparations were made except that 1 g. of sodium was used and the time of reaction was three hours.

(c) Controls: silk (1.000 g.) was suspended in 20% hydrochloric acid and refluxed

TABLE III

RESULTS OF ANALYSES

| Group a | Total N mg./5 cc. | Ammonia N, mg./5 cc. | Protein N, mg./100 cc. |
|---|---|---|---|
| I | 8.76 | 0.70 | 161.2 |
| II | 9.33 | 1.11 | 164.4 |
| III | 9.56 | 1.26 | 166.0 |
| | | | Average, 163.9 |
| Group b | | | |
| I | 11.49 | 3.53 | 159.2 |
| II | 17.64 | 9.52 | 162.4 |
| | | | Average, 160.8 |
| Group c | | | |
| I | 8.97 | | 179.4 |
| II | 9.04 | | 180.8 |
| III | 9.00 | | 180.0 |
| | | | Average, 180.1 |

for two hours. The solution was then made up to 100 cc. and portions of 5 cc. were removed for analysis. Results of analysis are given in Table III.

The amino nitrogen of one preparation was determined by three methods: Van Slyke's, Foreman's and Sorensen's formol method. The results obtained for the same amount of protein material (800 mg.) was 14.64 mg., 7.84 mg. and 4.26 mg. of amino nitrogen, respectively. There is no change in the amino nitrogen of the preparation following oxidation with bromine water or dilute alkaline potassium permanganate. This leads us to conclude that the Van Slyke method gives the amount of amino nitrogen most accurately.

**Reactions and Decomposition Products of Type Compounds with Sodium in Liquid ammonia.** 1. A dipeptide, glycyl-*dl*-alanine (5 g., 1 mol) was dissolved in 300 cc. of liquid ammonia and sodium (5 g., 6.3 mols) was added. After the reaction had continued for five hours, sufficient ammonium chloride was added to discharge the color of the sodium, and the ammonia was evaporated and the residue was dried as usual. The residue was suspended in absolute alcohol and a rapid stream of dry hydrogen chloride was passed into the solution until it became saturated. Large quantities of sodium chloride were removed by filtration and the product, presumably in solution as an ester hydrochloride, was obtained by evaporating the alcoholic solution *in vacuo* to a sirup. The sirup was dissolved in a small amount of water and the free base was released by adding solid sodium carbonate until the whole mass became pasty. An ether extract of this paste yielded a base. The picrate of this base was prepared and crystallized three times from water. The melting point was 97–98°. Glycyl-*dl*-alanine ethyl ester picrate was prepared similarly by esterification of glycyl-*dl*-alanine and crystallization from water. It also melted at 97–98°, and the mixed melting point of the two products was 97–98°. This compound has not been reported previously as far as we know.

*Anal.* (by Research Service Laboratories). 4.271 mg. subs. gave 5.995 mg. $CO_2$; 1.707 mg. $H_2O$. 4.517 mg. subs. gave 0.665 cc. $N_2$ at 25° and 762 mm. Calcd. for $C_{13}H_{17}N_5O_{10}$: C, 38.71; H, 4.22; N, 17.37. Found: C, 38.27; H, 4.47; N, 16.91.

The recovery of glycyl-*dl*-alanine as the picrate of the ester indicates that the imide link of the peptide is not quantitatively reduced by sodium in liquid ammonia.

2. A diketopiperazine, 2.5 piperazinedione (5 g., 1 mol) was suspended in 300 cc. of liquid ammonia and sodium (5 g., 5.7 mols) was added. Very little hydrogen was liberated. The reaction mixture was allowed to stand for three hours, then ammonium chloride (11.6 g., 5.7 mols) was added. After evaporation of the ammonia and drying of the residue, the whole was extracted with 300 cc. of cold absolute alcohol. The extract was intensely alkaline to litmus. The basic substance could be precipitated from the alcohol with ether, hydrochloric acid, picric acid, tartaric acid, malonic acid and 3,5-dinitrobenzoic acid, but not by *p*-aminobenzoic acid. Attempts were made to purify these salts by crystallization from warm water, but they invariably were decomposed. This base manifests the properties (with regard to instability) that we would expect of an hydroxypiperazine. Further studies will be made on this base in an effort to identify it.

3. An amino acid ester, glycine ethyl ester hydrochloride (42 g., 1 mol) was dissolved in 500 cc. of liquid ammonia and sodium (6.9 g., 1 mol) was added in small pieces with stirring. As soon as the reaction was finished, anhydrous ether was added to the ammonia as rapidly as possible without having the mixture boil over. Then the solution was filtered and evaporated *in vacuo* until both ammonia and ether had boiled off. Glycine ester (18 g.) was obtained and was identified by converting to diketopiperazine by addition of 11 g. of water; yield, 5 g. of diketopiperazine.

Another portion of glycine ethyl ester hydrochloride (14 g., 1 mol) was dissolved

in 300 cc. of liquid ammonia and sodium (10 g., 4.3 mols) was added in small pieces. After two hours, ammonium chloride (16.8 g., 3.3 mols) was added and anhydrous ether in small portions until the solution was about half ether and half ammonia. It was then filtered and allowed to stand at room temperature until most of the ammonia had evaporated. During this evaporation a brown solid separated out. This was collected on a filter and washed with ether; it was then dissolved in water and proved to be intensely alkaline in reaction. A saturated aqueous solution of picric acid was added and a crystalline product separated. After three crystallizations from water the product was obtained as light yellow elongated plates melting at 121° (corr.). The nature of the compound has not been established and will require further study.

4. A substituted acid amide. N-methyl acetamide (5 g., 1 mol) was dissolved in 200 cc. of liquid ammonia and sodium (5 g., 3.2 mols) was added. The reaction was allowed to continue for five hours, then solid ammonium chloride (11.6 g., 3.2 mols) was added and the ammonia was allowed to evaporate. The residue was extracted several times with ether. After evaporation of the ether, about four grams of an oil, b. p. 201–203°, was obtained. It is apparent, therefore, that methyl acetamide is not completely reduced or decomposed by sodium in liquid ammonia.

## Chemical Nature of the Products Formed by Sodium Acting upon Protein

1. **Complete Hydrolysis.**—Reduced silk fibroin (10 g., as sodium salt) was dissolved in 20% hydrochloric acid and the solution was boiled under a reflux condenser for four hours. The solution was then evaporated to a sirup *in vacuo* and esterified by the standard method of Fischer. The alcoholic solution of the ester hydrochlorides was concentrated *in vacuo* to a small volume and after it had stood in the refrigerator for two days, needle-like crystals of glycine ethyl ester hydrochloride separated out. These were identified by converting to the free ester and preparing the picrate; yellow needles, m. p. 157°.

2. **Direct Esterification of the Reduced Protein.**—Silk fibroin (20 g.) was reduced by suspending in 600 cc. of liquid ammonia and adding sodium (20 g.) as previously described, except that after five hours' reaction the excess sodium was neutralized with ammonium chloride. Then the ammonia was allowed to evaporate. The dry residue, after being freed of ammonia *in vacuo*, was extracted with 500 cc. of absolute ethyl alcohol and filtered. Most of the protein material, but not all, went into solution. The pale yellow alcoholic solution gave a strong biuret test and was intensely alkaline in reaction. The alcohol was saturated with dry hydrogen chloride and in the course of this reaction a heavy precipitate consisting of sodium chloride and some substances giving the biuret reaction (possibly by occlusion) was obtained. This precipitate was filtered off and the filtrate was a red sirup. It was evaporated *in vacuo* to about 200 cc. (if to dryness, some of the material precipitated out and did not dissolve completely again), then treated with the calculated amount of sodium ethylate in absolute alcohol. The filtrate from this treatment was alkaline. Solid picric acid was added until it reacted neutral to litmus. The solution was concentrated to about 100 cc. Water (100 cc.) was added and the solution was again concentrated to 150 cc., then cooled in the refrigerator. Crystalline picrates separated out and these were filtered off and crystallized from hot water. Beautiful elongated plate-like crystals separated out. These were collected and dried. They decomposed gradually at temperatures above 200°, but had not melted at 250°. By further evaporation and crystallization, other types of crystals were obtained but the type mentioned above far outnumbered any others. Apparently the products have a high molecular weight.

The products obtained by treating silk fibroin with the smaller amounts of sodium (3 g. sodium to 10 g. protein) gave similar alcoholic extracts and crystalline picrates

except that the amount of protein which went into solution in the alcohol was much smaller, possibly not more than five to ten per cent. of the total.

3. **Methylation.**—Reduced protein (10 g.) was treated with benzene (100 cc.) (in which it appears to be slightly soluble) and an excess of methyl iodide (10 g.) was added. This mixture was shaken vigorously, then warmed slightly on the water-bath until it just began to boil. Vigorous shaking was continued. In the course of the reaction, the white sodium salt disappeared and a brown substance separated out. The benzene was poured off and the solid was washed several times with ether, then dissolved in 95% alcohol. The addition of an equal volume of ether precipitated the methylated derivative as an oil. This was thoroughly extracted with ether to remove traces of alcohol, and dried *in vacuo*. It gave a biuret test. It was analyzed for nitrogen and methoxyl.

*Anal.* (by Research Service Laboratories). 8.855 mg. subs.; 5.595 mg. of AgI; 4.870 mg. subs.: 0.241 cc. $N_2$ at 26°, 758 mm. Found: $OCH_3$, 8.34; N, 5.63. (Product contained some sodium iodide.) Ratio $OCH_3$/N, 0.669.

## Summary

1. Proteins are partially ammonolyzed by ammono-bases and ammono-acids in liquid ammonia. The extent of ammonolysis is dependent upon the base and the temperature.

2. Proteins decolorize solutions of sodium and potassium in liquid ammonia, indicating that they are acidic in liquid ammonia. A preliminary study of the reaction or reactions has been made.

3. The reaction of glycyl-*dl*-alanine, glycine ethyl ester hydrochloride, and diketopiperazine with sodium in liquid ammonia has been studied. Other investigations are in progress which we hope will help to throw light on this problem.

CHICAGO, ILLINOIS

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[CONTRIBUTION FROM THE INSECTICIDE DIVISION, BUREAU OF CHEMISTRY AND SOILS]

# ROTENONE. XV. THE STRUCTURE OF DERRIC ACID

BY F. B. LAFORGE

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Derric acid, which is obtained by peroxide oxidation of derrisic acid, is represented by the formula $C_{12}H_{14}O_7$. It is a dibasic acid containing two methoxyl groups, and it is converted by permanganate into its next lower homolog, which Takei has named rissic acid. Rissic acid readily loses carbon dioxide when heated, yielding decarboxyrissic acid, $C_{10}H_{12}O_5$.

These facts lead us tentatively to consider derric acid to be a dimethoxyphenylmalic acid, and rissic acid a dimethoxyphenyltartronic acid.[1]

Since our last publication on this subject Takei has claimed to have substantiated our formulas and in addition to have placed the methoxy groups in the 2,5-positions.[2]

[1] LaForge and Smith, THIS JOURNAL, **52**, 2878 (1930).

[2] Takei, *Ber.*, **64**, 248 (1931).