ing α-*l*-arabinose, although Simon[8] reported a value of $[\alpha]^{20}_{D} < +76.0°$ for a modification of this sugar, which he termed "gamma." The studies of Hudson and Yanovsky[7c] supplied a separate calculation of the rotation of α-*l*-arabinose, $[\alpha]_D$ +54.0°, which is 21° lower than that given above by the authors. The reasons for this large divergence are not yet clear, for the presence of the calcium chloride in a solution of the compound described here has not greatly influenced either the velocity of the mutarotation or the rotation at equilibrium of the carbohydrate constituent.

Experimental

A mixture of 16.7 g. of *l*-arabinose, 32 g. of $CaCl_2 \cdot 2H_2O$, and 28 cc. of water was heated on the steam-bath in a gentle stream of air until crystals of hexagonal tablets began to form. The reaction product was refrigerated overnight, the crystals filtered out, washed quickly with alcohol, and dried at 50° *in vacuo* to 16.7 g. (37% of the calcd. for a substance of the formula later determined). The substance was recrystallized to constant properties by concentration of a solution of 9.5 g. in 7.5 cc. of water *in vacuo* over calcium chloride at 25°. The purified substance softened at 106–108°, slowly liquefied at 112–115°, and decomposed at 214–215°. A freshly prepared 4% aqueous solution of the substance was observed to give, at 20°, the following values of $[\alpha]^{20}_{D}$: +36.41°, 3 minutes after solution; +40.44°, 9.6 minutes after solution; +44.48°, 22.2 minutes after solution; and +47.65°, constant, 18 hours after solution. Samples of the substance, dried *in vacuo* at room temperature for twelve hours over calcium chloride, were found to contain 12.15% Ca; 21.20% Cl; and 21.45% H_2O (from loss in weight at 106° and 127° at 0.12 mm. Hg). For a substance of the accepted formula, $C_5H_{10}O_5 \cdot CaCl_2 \cdot 4H_2O$, the calculated values are 12.02% Ca; 21.28% Cl and 21.70% H_2O.

Summary

The preparation of a crystalline compound of calcium chloride and *l*-arabinose, α-*l*-arabinose·$CaCl_2 \cdot 4H_2O$, the first known substance containing α-*l*-arabinose as the carbohydrate constituent, has been described. The observations and calculations on the mutarotation of the substance have afforded a new determination of the rotation of α-*l*-arabinose.

CHICAGO, ILLINOIS RECEIVED DECEMBER 13, 1933

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[8] Simon, *Compt. rend.*, **132**, 487 (1901).

[CONTRIBUTION FROM NORTHWESTERN UNIVERSITY MEDICAL SCHOOL]

Studies on Proteins in Liquid Ammonia. II. Reaction of Sodium in Liquid Ammonia with Amino Acids and Related Substances[1]

BY C. O. MILLER AND R. G. ROBERTS

In the previous article[2] of this series, it was shown that proteins are acidic in liquid ammonia and react readily with sodium to form hydrogen and ammonolytic products containing sodium. We have investigated quantitatively the amount of hydrogen which is liberated by given amounts of proteins, peptones, dipeptides, diketopiperazine, amino acids and related substances, and have correlated the amount of hydrogen evolved with the nitrogen content of the sample and the amount of sodium used. Preliminary reports have been given on investigations in which proteins and peptones were used.[3] Complete reports will be given on these later.

Experimental

The substances used in the analyses were dried in an oven at 80° for several days and kept over concentrated sulfuric acid in a vacuum desiccator. Approximately one gram samples were used. One hundred and fifty cc. of dried liquid ammonia was placed in the vacuum flask.[4] The apparatus was arranged so that small pieces of sodium could be dropped into the liquid ammonia, a piece at a time, without opening the flask. The hydrogen was collected in a gasometer over 10% sodium hydroxide. Rubber stoppers and a glass delivery tube were used. The air in the reaction flask was displaced by allowing the ammonia to boil for fifteen minutes. The vacuum flask was then connected to the gasometer and sodium was dropped into the liquid ammonia a piece at a time. The ammonia was allowed to boil until the gas bubbles were completely absorbed. From one-half to three hours were required for this, depending upon the substance being used. The sodium hydroxide solution in the gasometer was slowly replaced by distilled water and then by 2 *N* hydrochloric acid, which was sprayed into the gas until the solution remained acid. Portions of the gas were then mixed with oxygen and the hydrogen was determined in the Hempel apparatus. Tests for oxygen, carbon monoxide, carbon dioxide and hydrocarbons were negative.

A given weight of each substance was treated with varying quantities of sodium, and the amount of hydrogen

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[1] A part of the material in this paper was presented before the Organic Division of the meeting of the American Chemical Society, Indianapolis, March, 1931.

[2] McChesney and Miller, THIS JOURNAL, **53**, 3888 (1931).

[3] C. O. Miller and R. G. Roberts, *Proc. Soc. Exptl. Biol. Med.*, **29**, 533 (1932); R. G. Roberts and C. O. Miller, *ibid.*, **30**, 821 (1933).

[4] The liquid ammonia was dried by the method described by Fernelius and Johnson, *J. Chem. Ed.*, **6**, 20 (1929).

evolved was measured. The volume of hydrogen liberated is corrected for the hydrogen liberated by the decomposition of the ammoniated sodium atom into sodium and amide ions. The data are given in Tables I and II. Table I gives the ratio of the moles of hydrogen evolved to the moles of substance used when the various substances and mixtures are treated with a slight excess of sodium. Table II gives the ratio of the moles of hydrogen evolved to the moles of diketopiperazine used when various amounts of sodium are added.

TABLE I

Substance	Moles of H_2/moles of substance	
Glycine	0.50	
Alanine	.48	0.48
Tyrosine	.65	.67
Glycolic acid	.94	.96
l-Cystine	.53	.55
l-Leucine	.64	
Diketopiperazine	.32	
Glycylglycine	.51	
Glycyl-*dl*-alanine	.51	.52
N-Methylacetamide	.53	.54
Acetanilide	.05	.06
Glycylglycine and diketopiperazine mole ratio 1:1	.02	.02
Glycylglycine, 2 moles and diketopiperazine, 1 mole	.05	.05
Glycine and tyrosine, mole ratio 1:1	.39	
Glycine 53.3%, alanine 31.1%, tyrosine 15.6%	.36	

TABLE II

Substance	Atomic wts. of Na/moles of substance	Moles of H_2/moles of substance
Diketopiperazine	0.50	0.04
Diketopiperazine	1.00	.06
Diketopiperazine	2.00	.32
Diketopiperazine	3.00	.32
Diketopiperazine	4.00	.32

Discussion

Two types of chemical reaction are possible under these conditions, salt formation with liberation of hydrogen and reduction. Both types may take place simultaneously. As is to be expected, the data for glycine and alanine, given in Table I, indicate that the carboxyl group is acidic in liquid ammonia and reacts almost quantitatively with sodium to form hydrogen and the sodium salt. Voss and Guttman[5] have shown that the sodium salts of the monoaminomonocarboxylic acids can be conveniently prepared in this way.

In the liquid ammonia system of acids and bases, the peptide link is an acid aquo-ammono ester, and should therefore react with sodium to form hydrogen. The data in Table I for glycylglycine and glycyl-*dl*-alanine indicate that they react as monobasic acids and that the peptide link of dipeptides is not appreciably acidic. In contrast to the action of the peptide link of dipeptides, N-methylacetamide and acetanilide react with sodium. N-Methylacetamide liberates hydrogen almost quantitatively, as shown in Table I. Acetanilide is reduced by sodium and does not liberate hydrogen quantitatively.

It is to be expected that diketopiperazine would react as a dibasic acid in liquid ammonia. The imide groups of pyrrole, indole and carbazole have been shown to be slightly acidic in liquid ammonia. Four atoms of sodium are oxidized in the reaction for each molecule of diketopiperazine present. Hydrogen is not liberated quantitatively as shown in Table II. Diketopiperazine is probably reduced. McChesney and Miller showed that diketopiperazine under these conditions reacts to form an unstable basic substance. They were unable to isolate it as a salt.

The data in Table I for tyrosine show that the phenol group is slightly acidic in liquid ammonia. For comparison, data are given for glycolic acid which show that it liberates hydrogen in quantities slightly less than would a dibasic acid. The reaction product of tyrosine turned to a brown-black melanin-like material on standing in contact with air for a month, indicating that some of the tyrosine had been reduced. The reaction product from glycolic acid was stable toward air.

Du Vigneaud, Audrieth and Loring[6] have reduced cystine to cysteine by sodium in liquid ammonia by using 4 atoms of sodium for each molecule of cystine. Theoretically, 2 gram atoms of sodium are sufficient to effect the reduction if the hydrogen is quantitatively absorbed. The product would be the sodium salt of cysteine. Since three-fourths of a mole of hydrogen is evolved when 4 gram atoms of sodium is used, as shown in Table I, it indicates that reduction is effected essentially by the sodium acting directly on the disulfide bond and not by active hydrogen acting upon it.

Leucine liberates more hydrogen when an excess of sodium is used than is expected for a monoamino monocarboxylic acid. It was prepared from blood corpuscle paste and was free from tyrosine, its most likely contaminant, by the Millon test.

When various mixtures of amino acids, dipeptides and diketopiperazine were treated with

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(5) Voss and Guttman, *Ber.*, **63B**, 1726 (1930).

(6) Du Vigneaud, Audrieth and Loring, THIS JOURNAL, **52**, 4500 (1930).

sodium, the liberation of hydrogen was not additive. For example, an equimolecular mixture of glycine and tyrosine liberated 67% of the amount of hydrogen that they would liberate if taken separately; a mixture of glycine, alanine and tyrosine in the proportions in which they are present in silk liberated 72%; an equimolecular mixture of glycylglycine and diketopiperazine liberated about 2%, and a mixture of 2 moles of glycylglycine and 1 mole of diketopiperazine liberated 5% of the calculated amount. This unexpected decrease in the quantity of hydrogen liberated may be due to a more complete reduction of diketopiperazine by sodium and an acid than by sodium alone. We have no explanation to offer for the anomalous behavior of the mixtures of amino acids. If partial condensation of amino acids took place with formation of dipeptides and water or some diketopiperazine and water, they would act in this way.

Summary

1. A study of the reaction of sodium in liquid ammonia with certain amino acids, dipeptides, diketopiperazine, and type compounds has been made.

2. Glycine and alanine react as monobasic acids. The phenol group of tyrosine is slightly acidic. Some tyrosine seems to be reduced in the treatment. Leucine liberates more hydrogen than does a monoaminomonocarboxylic acid. Cystine is reduced by sodium, in part, by sodium acting directly upon the disulfide bond.

3. The peptide linkage in dipeptides is not acidic in liquid ammonia.

4. Diketopiperazine is reduced by sodium in liquid ammonia.

5. Mixtures of amino acids and of amino acids and diketopiperazine do not liberate hydrogen, additively, as is to be expected.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF CALIFORNIA]

The Dissociation Constants of the Chlorophenyl and Phenetyl Boric Acids[1]

BY G. E. K. BRANCH, DAVID L. YABROFF AND BERNARD BETTMAN

The idea that a group possesses a single characteristic, called variously its negativity, polarity and electron-sharing ability, or group electric dipole, whereby it influences the dissociation of an acid, is quite old. This theory could not have persisted for so long a time without having a large element of truth, but it gives an incomplete picture of the effects of groups on acidic strength. That other influences exist is shown by the fact that even the sign of the effect upon substituting one group for another is often dependent on the rest of the molecule. For example, the dissociation constant of chloroacetic acid is greater than that of acetoacetic acid, but acetic acid is stronger than hypochlorous acid. Also cyanacetic acid is stronger than chloroacetic acid but hydrogen cyanide is much weaker than hydrogen chloride. The effects of groups on dissociation constants should therefore be studied in all possible types of acids. We propose to obtain and compare the dissociation constants of a variety of substituted boric acids. That these constants have not been investigated by other workers is sufficient excuse for the work. The dissociation constants of these substances, however, are especially interesting, since they depend on the possibilities of molecular resonances that arise through the lack of a completed octet in the valence shell of the trivalent boron atom.

Since many substituted boric acids are but sparingly soluble in water, we have used a 25% solution of ethyl alcohol (by volume) in water as the solvent. The results are therefore not directly comparable with those obtained in water but only among themselves. Some of the more soluble acids have also been measured in water solution. The dissociation constants are all smaller in the alcohol solution than in water. The ratio of the constants, in water and in alcohol solution, is about five for boric acid and about seven for phenylboric and *o*-tolylboric acids.

The ionization of boric acid and probably of its derivatives can be complex,[2] involving poly-

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(1) It is suggested by the Referee of this paper that the name aryl boric acid, as applied to $ArB(OH)_2$, should be changed to aryl boronic acid in analogy to the sulfonic acids. We agree with this suggestion since the "aryl boric" acids are actually misnamed. We have retained the old nomenclature, however, since many of these compounds have been known for a long time and are catalogued under that name. It might be advisable that the Referee's suggestion be adopted by contributors to THIS JOURNAL.

(2) Kolthoff, *Rec. trav. chim.*, **45**, 501 (1926).