Fitzgerald[8] cites some density data for lithium chloride and for sodium nitrate solutions in methylamine at 0°. However, a smooth curve cannot be drawn through his determined points; his values are mostly higher than those of this investigation and were only incidental to some conductivity and viscosity determinations.

The results for the apparent molal volumes of lithium chloride are very interesting when compared with the values obtained in other solvents.

APPARENT MOLAL VOLUME OF LiCl AT 25°

Solvent	Dielectric constant	Φ	Observer
Water	78	17.06	Geffcken[9]
Methyl alcohol	31.2	− 3.8	Vosburgh[10]
Ethyl alcohol	26.5	− 4.4	Vosburgh
Methylamine	<10.5	−19.21	K. & F.

As the dielectric constant decreases, the apparent molal volume also decreases. The value of <10.5 for the dielectric constant for methylamine was determined by Schlundt[11] on an impure sample. The correct value for pure methylamine is probably much lower than 10.5.

(8) Fitzgerald, *J. Phys. Chem.*, **16**, 621 (1912).
(9) Geffcken, *Z. physik. Chem.*, **A155**, 1 (1931).
(10) Vosburgh, Connell and Butler, *J. Chem. Soc.*, 933 (1933).
(11) Schlundt, *J. Phys. Chem.*, **5**, 503 (1901).

Negative values of the apparent and partial molal volumes have been obtained by other investigators for lithium and sodium hydroxides and for magnesium and copper sulfates in water. The negative values for lithium chloride obtained in this investigation seem to point definitely to a solvation of the lithium ions in the methylamine solution. Such solvation would explain, in part, the trends in electrical conductance observed by Anderson,[2] especially in the more concentrated region. The positive values for sodium nitrate would indicate a probable lack of extensive solvation.

Summary

1. The densities of solutions of lithium chloride and of sodium nitrate in liquid monomethylamine have been determined at different temperatures and have been recorded as functions of the concentration.

2. The apparent and partial molal volumes have been calculated and presented.

3. An approximate correlation between the apparent molal volume of lithium chloride and the dielectric constant of the solvent has been presented.

AUSTIN, TEXAS RECEIVED JUNE 16, 1938

[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL CHEMISTRY, COLUMBIA UNIVERSITY]

The Synthesis of Dipeptides from α-Keto Acids[1]

BY DAVID SHEMIN AND ROBERT M. HERBST

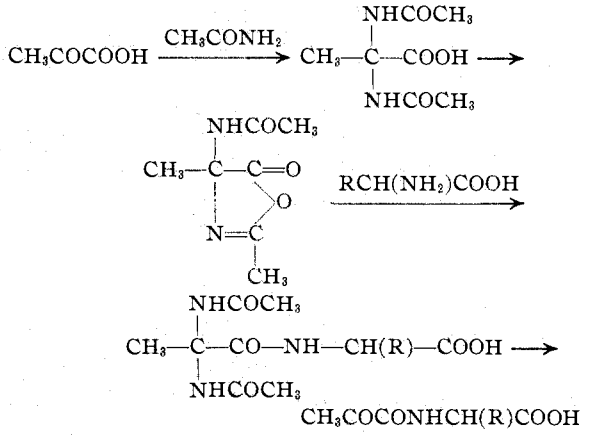
The classical peptide syntheses of Emil Fischer, and the elegant methods of Bergmann have proved to be of great value both practically and theoretically. However, the possibility of synthesizing peptides from α-keto acids appeared attractive since biological materials other than amino acids could be utilized. The methods developed in this report are primarily of theoretical interest, although their further development in some specific instances may prove to be of practical value.

The conversion of α-keto acids into the corresponding amino acids has been accomplished in a variety of ways. The oximes of α-keto acids have been reduced to amino acids either with tin and hydrochloric acid,[2] or with sodium amalgam.[3] Knoop and Oesterlin[4] have succeeded in reducing α-keto acids to amino acids catalytically with hydrogen in the presence of ammonia. Herbst and Engel[5] have been able to convert α-keto acids into the corresponding amino acids by interaction with another α-amino acid, a reaction which has been accomplished recently in biological systems.[6,7] Of biochemical interest are the reduction of the oxime of pyruvic acid to *dl*-alanine by actively fermenting yeast,[8] the synthesis of alanine from pyruvic acid and ammonia by liver tissue slices,[9] and the formation of *l*-aspartic acid from oxalacetic acid and hydroxyl-

(1) This report is from a dissertation submitted by David Shemin in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the Faculty of Pure Science, Columbia University.
(2) Gutknecht, *Ber.*, **13**, 1116 (1880).
(3) Gränacher, *Helv. Chim. Acta*, **5**, 610 (1922).
(4) Knoop and Oesterlin, *Z. physiol. Chem.*, **148**, 294 (1925); **170**, 186 (1927).
(5) Herbst and Engel, *J. Biol. Chem.*, **107**, 505 (1934); Herbst, THIS JOURNAL, **58**, 2239 (1936).
(6) Braunstein and Kritzmann, *Enzymologia*, **2**, 129 (1937).
(7) Virtanen and Laine, *Nature*, **141**, 748 (1938).
(8) Maurer, *Biochem. Z.*, **189**, 216 (1927).
(9) Neber, *Z. physiol. Chem.*, **234**, 83 (1935).

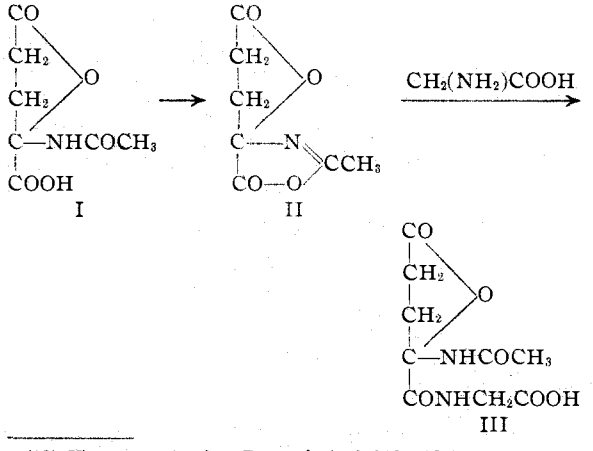
amine by the root nodules of leguminous plants.[10]

Bergmann and Grafe[11,12] have synthesized pyruvyl derivatives of α-amino acids by the following series of reactions



Two methods of converting the pyruvylamino acids into the corresponding dipeptides were tried, the second proving the more satisfactory: (a) catalytic reduction in the presence of ammonia, and (b) catalytic reduction of the oximes of the pyruvylamino acids. Preliminary experiments with the oximes of pyruvic and phenylpyruvic acids, under conditions similar to those employed for simple oximes by Hartung,[13] demonstrated its feasibility for the preparation of amino acids. By this procedure the reduction of the oximes of pyruvylglycine, pyruvylalanine and pyruvylphenylalanine was accomplished. In some cases it was found advantageous to work with the oxime esters.

Application of this synthesis to α-ketoglutaric acid was attempted. Condensation of this keto acid with acetamide led to the formation of the lactone of α-acetamino-α-hydroxyglutaric acid (I). This compound with acetic anhydride formed an azlactone (II) which was coupled with glycine to form the lactone of α-acetamino-α-hydroxyglutarylglycine (III).



The possibility of preparing glutamyl and glutaminyl peptides from the last named compound has not been explored.

Experimental

The melting points, yields and analyses of all compounds prepared are reported in Table I. With three exceptions the yields given are for the reaction leading directly to the product in question; for example, the yield of alanine is for the reduction of pyruvic acid oxime to alanine. The yields of pyruvylglycine oxime ethyl ester, pyruvylalanine ethyl ester and pyruvylphenylalanine oxime are over-all yields based on the amounts of the α,α-diacetaminopropionyl derivatives of glycine, alanine and phenylalanine used, since no intermediates were isolated.

Pyruvylamino Acids.—Pyruvylglycine and pyruvylphenylalanine were prepared as described by Bergmann and Grafe.[12] Pyruvylalanine was prepared by a similar procedure. The mixture obtained by the hydrolysis of α,α-diacetaminopropionylalanine was evaporated to dryness and extracted with dry acetone, from which pyruvylalanine, possessing the properties recorded by Bergmann and his collaborators,[14] was secured on concentration to a small volume and recrystallizing from acetone or ether–petroleum ether.

Ethyl Esters of Pyruvylamino Acids.—Solutions of the pyruvylamino acids, obtained by extracting the above residues with absolute ethyl alcohol instead of acetone, were saturated with hydrogen chloride and boiled under reflux for about half an hour. After the removal of the solvent as completely as possible under reduced pressure, pyruvylglycine ethyl ester was converted into the oxime without further purification; pyruvylalanine ethyl ester was purified by distillation in high vacuum.

Preparation of Oximes.—The oximes of keto acids and pyruvylamino acids were prepared in aqueous solution, those of the ethyl esters of pyruvylamino acids in 50% alcoholic solution by treatment with about one and a half equivalents of hydroxylamine hydrochloride and three equivalents of sodium acetate. The reactions were allowed to proceed for twelve to twenty-four hours at room temperature. The products either crystallized from the reaction mixture before or after acidification to Congo red or were isolated by extraction with ether. Purification was effected either by crystallization from hot water or from ether by addition of petroleum ether or by distillation in high vacuum.

Reduction of Oximes.—The oximes were reduced in alcoholic or aqueous alcoholic solution in the presence of Adams platinum oxide catalyst[15] with hydrogen under 2–3 atmospheres pressure. In some cases, especially with the

(10) Virtanen and Laine, *Enzymologia*, **3**, 266 (1937).
(11) Bergmann and Grafe, *Z. physiol. Chem.*, **187**, 187 (1930).
(12) Bergmann and Grafe, *ibid.*, **187**, 196 (1930).
(13) Hartung, THIS JOURNAL, **50**, 3370 (1928).
(14) Bergmann, Miekeley and Kann, *Z. physiol. Chem.*, **146**, 247 (1925).
(15) Adams, Voorhees and Shriner, *Org. Syntheses*, **8**, 92 (1928).

TABLE I[a]

Compound	Empirical formula	M. p.,[b] °C.	Yield, %	Analyses, % Calculated C	H	N		Found C	H	N	
Alanine	$C_3H_7O_2N$	...	85	..	...	15.7	15.7[c]	..	...	15.6	15.9[c]
Phenylalanine	$C_9H_{11}O_2N$	...	81	65.5	6.8	8.5	8.5[c]	65.6	6.7	8.6	8.7[c]
Pyruvylglycine oxime	$C_5H_8O_4N_2$	202, dec.	78	37.5	5.0	...		37.8	4.9	...[d]	
Pyruvylglycine oxime ethyl ester	$C_7H_{12}O_4N_2$	127	50	44.7	6.4	14.9		44.6	6.3	14.3	
Alanylglycine	$C_5H_{10}O_3N_2$	...	70	..	...	19.2	9.6[c]	..	...	18.9	9.7[c]
Carbethoxyalanylglycine ethyl ester	$C_{10}H_{18}O_5N_2$	72.5–73.5[e]	16	48.8	7.4	11.4		48.9	7.5	11.4	
Carbethoxyalanylglycine	$C_8H_{14}O_5N_2$	120–121	52	44.0	6.5	12.8		43.9	6.6	12.7	
α,α-Diacetaminopropionylalanine	$C_{10}H_{17}O_5N_3$	175–176, dec.	85	46.3	6.6	16.2		46.3	6.7	16.1	
Pyruvylalanine	$C_6H_9O_4N$	143.5	73	45.3	5.7	8.8		45.2	5.8	8.8	
Pyruvylalanine oxime	$C_6H_{10}O_4N_2$	186	96	41.4	5.8	16.1		41.4	5.9	15.5	
Pyruvylalanine ethyl ester	$C_8H_{13}O_4N$	Liquid[g]	16.7	51.3	7.0	7.5		51.1	7.2	7.1	
Pyruvylalanine oxime ethyl ester	$C_8H_{14}O_4N_2$	Liquid[h]	86	47.5	7.0	13.9		46.5	7.0	13.3	
Alanylalanine	$C_6H_{12}O_3N_2$	...	76	..	...	17.5	8.8[c]	..	...	17.4	9.0[c]
Alanylalanine anhydride	$C_6H_{10}O_2N_2$	...	85	50.7	7.1	19.7		50.6	7.3	19.4	
Carbethoxyalanylalanine ethyl ester	$C_{11}H_{20}O_5N_2$	71–72	47	50.8	7.8	10.8		50.7	7.9	10.5	
Pyruvylphenylalanine oxime	$C_{12}H_{14}O_4N_2$	187–188, dec.	54	57.6	5.7	11.2		57.8	5.7	10.8	
Alanylcyclohexylalanine	$C_{12}H_{22}O_3N_2$	..	78	59.5	9.1	11.6	5.8[c]	59.2	8.9	11.3	5.7[c]
Benzoylcyclohexylalanine	$C_{16}H_{21}O_3N$	186–187	71	69.8	7.7	5.1		70.0	7.9	5.1	
Lactone of α-acetamino-α-hydroxyglutaryl-glycine	$C_9H_{12}O_6N_2$	210, dec.	18	44.3	5.0	11.5		43.9	5.0	11.4	

[a] The authors wish to thank Mr. William Saschek for the micro analyses reported in the table. [b] All melting points are corrected. [c] Amino nitrogen, determined according to Van Slyke. [d] Low values for the oximes were obtained by the micro Dumas technique. The micro Kjeldahl process was, of course, not directly applicable. [e] Fischer[16] reports the melting point of carbethoxyalanylglycine ethyl ester as 67.5° and that of carbethoxyalanylglycine as 122°, both corrected. [f] During the preparation of carbethoxyalanylglycine ethyl ester it is partially hydrolyzed to carbethoxyalanylglycine.[16] The combined yield of the two products, obtained in one experiment, amounted to 68%. [g] Distils at 95° bath temperature under 0.4 mm. pressure. [h] Distils at 160–170° bath temperature under 0.7–0.9 mm. pressure.

esters of the pyruvylamino acid oximes, it was found advantageous to carry on the reduction in solutions acidified with a small amount of hydrochloric acid.[13] Free amino acids and dipeptides were isolated from the reduction mixtures by evaporation to dryness after removal of chloride ions, and were purified by crystallization from water by the addition of alcohol. The dipeptide esters obtained by the reduction of the esters of the pyruvylamino acid oximes were isolated as carbethoxy derivatives following the technique of Fischer.[16,17] Phenylalanine was obtained without difficulty by reduction of the oxime of phenylpyruvic acid. However, when the oxime of pyruvylphenylalanine was reduced under the same conditions, alanylcyclohexylalanine was formed. From the latter, cyclohexylalanine was obtained by hydrolysis with dilute hydrochloric acid and isolated as the benzoyl derivative,[18] the identity of which was established by analysis and by mixed melting point determinations.

Lactone of α-Acetamino-α-hydroxyglutarylglycine, (III).—A suspension of 3 g. of α-acetamino-α-hydroxyglutaric lactone (I),[19] in 60 cc. of acetic anhydride was heated on a boiling water-bath until all the material had dissolved. About ten or fifteen minutes were required, during which time the solution became orange-yellow. After removal of the excess acetic anhydride *in vacuo*, the crude azlactone (II), a yellow viscous oil, was suspended in 30 cc. of acetone and treated immediately with a solution of 1.12 g. of glycine in 15 cc. of normal sodium hydroxide. The mixture, containing a trace of undissolved material, was shaken for thirty minutes, neutralized with 15 cc. of normal sulfuric acid, evaporated to dryness *in vacuo*, and extracted thoroughly with cold 95% alcohol. The alcoholic solution was evaporated to dryness; the residue was taken up in a small amount of water and decolorized with charcoal. The aqueous solution was then evaporated to dryness and the residue washed with cold alcohol. The insoluble portion, weighing 1.3 g., was recrystallized by suspension in hot alcohol and dropwise addition of water until solution took place. On cooling 0.7 g. of product separated from the solution.

On direct titration with 0.01 *N* sodium hydroxide, using phenolphthalein as the indicator, the substance immediately consumed somewhat more than one equivalent of alkali, and thereafter behaved like a lactone. The equivalent weight (calcd. 122; found 116, 128) was determined by back-titration with hydrochloric acid after treatment with a slight excess of alkali.

Summary

1. The possibility of synthesizing amino acids and dipeptides from α-keto acids has been demonstrated.

2. The oximes of pyruvic and phenylpyruvic acids were converted into the corresponding amino acids by catalytic reduction.

3. The pyruvyl derivatives of glycine, glycine ethyl ester, alanine, and alanine ethyl ester were converted into the corresponding dipeptides by catalytic reduction of their oximes. In the case of pyruvylphenylalanine oxime, reduction led to alanylcyclohexylalanine.

4. The azlactone of α-acetamino-α-hydroxy-

(16) Fischer, *Ann.*, **340**, 123 (1905).

(17) Fischer, *Ber.*, **35**, 1095 (1902).

(18) Waser and Brauchli, *Helv. Chim. Acta.*, **6**, 199 (1923); *ibid.*, **7**, 740 (1924).

(19) The condensation of α-ketoglutaric acid with acetamide will be described in the succeeding paper.

glutaric lactone was condensed with glycine with the formation of the lactone of α-acetamino-α-hydroxyglutarylglycine.

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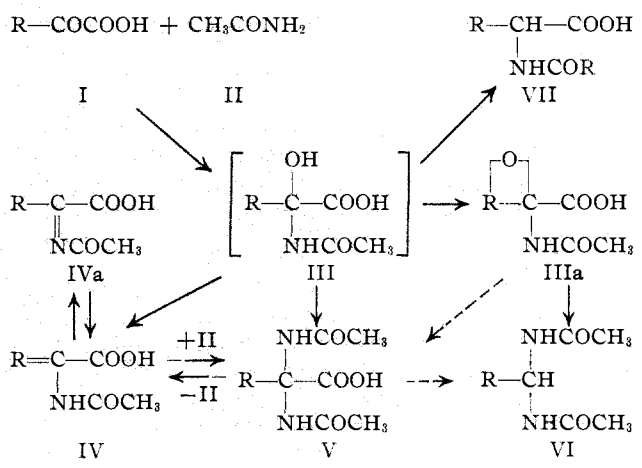
[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL CHEMISTRY, COLUMBIA UNIVERSITY]

The Condensation of α-Keto Acids and Acetamide[1]

BY DAVID SHEMIN AND ROBERT M. HERBST

In order to extend the dipeptide synthesis described in the preceding paper, it was necessary to prepare α,α-diacetamino derivatives from α-keto acids other than the pyruvic acid studied by Bergmann and Grafe.[2] The results obtained in the reaction of α-ketoglutaric acid with acetamide made desirable a more extensive study of this condensation, with a view to obtaining clearer insight into its mechanism.

The results obtained with the several α-keto acids studied are summarized in the following scheme



When α-ketoglutaric acid (I, R = $COOHCH_2CH_2$—) was condensed with acetamide (II) at 70°, the main product was the lactone of α-acetamino-α-hydroxyglutaric acid (IIIa, R = —$COCH_2CH_2$—). If the reaction was carried out at a higher temperature, or if IIIa was heated at a higher temperature with acetamide, a second product, γ,γ-diacetaminobutyric acid (VI, R = $COOHCH_2CH_2$—) was obtained. Whether V is an intermediate in this reaction, as indicated by the dotted arrows, or whether IIIa is decarboxylated, must remain an open question, but the opening of the lactone ring of IIIa by addition of acetamide appears to be a new reaction.

These results suggested that the formation of the diacetamino compounds proceeds in two steps, (a) addition of acetamide to the carbonyl group of the keto acid with the formation of an α-hydroxy-α-acetamino compound (III), and (b) replacement of the hydroxyl group of III by an acetamino group. This hypothesis seemed reasonable in view of the fact that benzaldehyde, on treatment with acetamide under suitable conditions, forms benzylidene-diacetamide.[3,4] However, it should be pointed out that in the preparation of α,α-diacetaminopropionic acid (V, R = CH_3—) from pyruvic acid and acetamide, a small amount of α-acetaminoacrylic acid (IV, R = CH_2=) is always formed.[2] If α-acetamino-α-hydroxypropionic acid (III, R = CH_3—) is formed as the first step in the reaction, loss of water from this intermediate readily explains the formation of α-acetaminoacrylic acid. In the case of α-ketoglutaric acid the intermediate (III) is stabilized by the formation of the lactone (IIIa). On the basis of the above hypothesis α-acetaminoacrylic acid would appear to be a by-product, but the possibility that it is an intermediate must be considered.

To test this point α-acetaminoacrylic acid (IV, R = CH_2=) was heated with acetamide. Surprisingly, almost quantitative conversion to α,α-diacetaminopropionic acid (V, R = CH_3—) resulted, so that α-acetaminoacrylic acid cannot be excluded as an intermediate. In this connection it should be recalled that α-aminoacrylic acid derivatives may be considered as tautomeric substances[2,5] capable of reacting in the forms IV or IVa. Acetamide may therefore add onto either the carbon–carbon double bond of IV or the car-

(1) This report is from a dissertation submitted by David Shemin in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the Faculty of Pure Science, Columbia University.

(2) Bergmann and Grafe, *Z. physiol. Chem.*, **187**, 187 (1930).

(3) Bulow, *Ber.*, **26**, 1972 (1893).

(4) Chattaway and Swinton, *J. Chem. Soc.*, **101**, 1206 (1912).

(5) Bergmann, Miekeley and Kann, *Z. physiol. Chem.*, **146**, 247 (1925).