form. After refrigerating overnight a nearly quantitative yield of ester was obtained; m. p. 143–150°. A mixture of this ester and the synthetic tosyl DvLv–LvDv methyl ester (m. p. 149–151°) melted at 148–151°. The melting points of other mixtures were depressed, *e. g.*, a mixture of the natural ester and synthetic tosyl DvDv–LvLv methyl ester melted over the range 125–145°.

"Natural" Benzoylvalylvaline.—This compound was prepared by benzoylation of the valylvaline from gramicidin according to the method reported previously.[3a] Recrystallization from acetone yielded tiny diamond-shaped crystals which melted at 231.5–232° alone and on admixture with synthetic benzoyl DvLv–LvDv.

"Natural" Benzoylvalylvaline Methyl Ester.—Methylation of a few mg. of the above acid was accomplished using ethereal diazomethane and a chloroform solution of the benzoyl compound. Recrystallization of the methyl ester from ethanol and water yielded fine glossy needles; m. p. 186–187°. When mixed with synthetic benzoyl DvLv–LvDv methyl ester the melting point was not depressed, but admixture with synthetic benzoyl DvLv methyl ester (m. p. 187–187.5°) depressed the melting point to 172–176°. Comparison of the infrared spectra and the X-ray diffraction patterns (Figs. 1 and 2) confirmed the identity of the natural benzoylvalylvaline methyl ester as the DL–LD racemic modification.

Acid Hydrolysis of Isomeric Dipeptides.—The four free, isomeric, valylvalines were subjected, in solutions of 0.021 to 0.024 *M* concentration (by weighing) to the action of 6 *N* hydrochloric acid at 100° in sealed tubes for thirty hours. After removal of the hydrochloric acid *in vacuo*, duplicate aliquots of the hydrolysates were analyzed for α-amino nitrogen by the manometric ninhydrin method at *p*H 2.5[23,24] and the degree of hydrolysis calculated.

Discussion

The dilemma presented by the appearance of similar quantities of two enantiomorphic dipeptides upon the hydrolysis of gramicidin still remains. Racemization, unless it occurred as an epimerization, as suggested by Neuberger,[25] would not appear to be an adequate explanation. The differences in stability to hydrolysis of the isomers are in the wrong direction to account for the isomers found. The incomplete homogeneity of gramicidin[8] does not provide any obvious explanation. The racemic dipeptide isolated might conceivably arise by the hydrolysis of a structure of the type R-D-valyl-L-valyl-D-valyl-R'. Synge has isolated two peptides, alanylvaline and valylglycine, containing excesses of D- and L-valine, respectively.[9,26] It is probably significant that valylvaline, despite its great stability, apparently is not formed when the acid hydrolysis is performed under at least two other sets of conditions.

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Summary

1. Paper chromatography of the unfractionated gramicidin hydrolysate revealed the presence of valylvaline of R_f corresponding to that of the racemic modification D-valyl-L-valine plus L-valyl-D-valine or its component isomers. The other two isomers could not be detected.

2. Comparison of several derivatives of the valine dipeptide separated from gramicidin hydrolysates with corresponding synthetic products as to melting point, infrared absorption and X-ray diffraction studies indicated that the isolated dipeptide consisted of the above racemic modification.

3. The isomeric dipeptides of valine and a number of their derivatives have been synthesized.

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[CONTRIBUTION FROM THE VENABLE CHEMICAL LABORATORY OF THE UNIVERSITY OF NORTH CAROLINA]

The Pyridine Acylation of Sarcosine and Esters of Alpha-Acylamino Acids[1]

BY RICHARD H. WILEY[1b] AND OLIN H. BORUM[1a]

Previous communications[2,3] have described the conversion of α-amino acids to acylamido ketones on refluxing with acid anhydrides in pyridine.

$$RCH(NH_2)CO_2H + 2(R'CO)_2O \xrightarrow{\text{pyr.}} RCH(NHCOR')COR' + 2RCO_2H + CO_2$$

The present paper describes the course of the reaction when applied to the α-acylamino acid esters and to the N-acetyl derivative of sarcosine.

Acylation of Acetylsarcosine.—Acetylsarcosine reacts on refluxing with acetic anhydride in the presence of pyridine to form a mixture of N-methylacetamidoacetone and its acetyl derivative.

$$CH_3NHCH_2CO_2H + (CH_3CO)_2O \longrightarrow CH_3CON(CH_3)CH_2COCH_3 + CH_3CON(CH_3)CH{=}C(OCOCH_3)CH_3\ (?)$$

The two products, which are obtained as a mixture boiling at 99–109° (3–4 mm.), are separated

(1) Taken from the thesis submitted by Olin H. Borum to the Graduate School of the University of North Carolina in partial fulfillment of the requirements for the Ph.D. degree. Presented before the Division of Organic Chemistry of the American Chemical Society, Atlantic City, September, 1949. (a) du Pont Company, Philadelphia, Pa. (b) University of Louisville, Louisville, Ky.

(2) Wiley, *J. Org. Chem.*, **12**, 43 (1947).

(3) Wiley and Borum, THIS JOURNAL, **70**, 2005 (1948).

by ether extraction of the acetyl derivative from an aqueous solution of the mixture. The acetyl derivative of the N-methyl acylamidoketone is not hydrolyzed by steam as is the acetyl derivative of acetamidoacetone,[3] although it is hydrolyzed to N-methylacetamidoacetone in 65% yield by heating at 70–90° with sodium bicarbonate solution. The structure of the acetyl derivative given above is tentative. The acylamidoketone was converted to the simple 2,4-dinitrophenylhydrazone.

The conversion of acetylsarcosine to N-methylacetamidoacetone in this reaction is significant in showing that an oxazolone (azlactone) is not a necessary intermediate. Recent discussions[4,5] of the mechanism of this reaction have concentrated attention on the oxazolone as an intermediate. The observation[6] that phenylacetic acid is converted to methyl benzyl ketone in this reaction also indicates that an oxazolone is not an essential intermediate.

A mechanism sufficiently comprehensive to include the sarcosine reaction is desirable and the following mechanism is accordingly suggested. The acylamino acid is first decarboxylated by the base to a carbanion. This reaction is to be expected of a carboxylic acid carrying an electrophilic group in the beta position.

$$CH_3CON(CH_3)CH_2COOH \xrightarrow{\text{base}} [CH_3CON(CH_3)CH_2]^- + CO_2 + H^+$$

Both the acylamido and N-methylacylamido groups are apparently sufficiently electrophilic to promote this reaction. The carbanion formed in this reaction then adds to the carbonyl group of the anhydride (or acyl halide) to give the anion shown

$$[CH_3CON(CH_3)CH_2]^- + CH_3CO{-}O{-}COCH_3 \longrightarrow \left[CH_3CON(CH_3)CH_2\underset{\displaystyle CH_3}{\overset{\displaystyle O}{\overset{|}{C}}}{-}O{-}COCH_3\right]^-$$

This addition anion then loses the acetate ion to form the ketone or combines with a proton and

$$\left[CH_3CON(CH_3)CH_2\underset{\displaystyle CH_3}{\overset{\displaystyle O}{\overset{|}{C}}}{-}O{-}COCH_3\right]^- \longrightarrow CH_3CON(CH_3)CH_2COCH_3 + CH_3CO{-}O^-$$

loses acetic acid. The addition of a carbanion, formed by decarboxylation, to a carbonyl group has been previously observed.[7]

An alternative mechanism regarded as less likely in view of the expected ease of loss of carbon dioxide postulated as the first step in the preceding mechanism, is that the acylamino acid undergoes C-acylation as an active methylene compound. This can be visualized as proceeding through the intermediate $[CH_3CON(CH_3)CH{-}CO_2H]^-$ in which the carboxyl group may have been converted to a mixed anhydride, an ion, or an azlactone. This is merely a generalization of the previously stated azlactone mechanism. C-Acylation of active methylene compounds has long been known and is illustrated by the formation of di-acetoacetic ester from aceto-acetic ester and acetyl chloride in the presence of sodium[8] or by the rearrangement of β-acetoxy-crotonic ester.[9]

Acetylation of Acylamino Acid Esters.—The reaction of acetylglycine ethyl ester or acetylleucine ethyl ester with acetic anhydride on refluxing in pyridine gives, from each, a diacetyl derivative characterized as the N,N-diacetyl ester. Acetylglycine ethyl ester yields 94% of N,N-diacetylglycine ethyl ester. This ester has been reduced to diethylethanolamine by means of lithium aluminum hydride to identify the N,N-diacetyl structure.[10] Comparison with an authentic sample[11] eliminated the possibility that this product was ethyl acetamidoacetoacetate formed by C-acetylation. Similarly, the pyridine acetylation of acetylleucine ethyl ester gives N,N-diacetylleucine ethyl ester. Ultraviolet absorption data of these diacetyl derivatives compared to those of similar compounds are consistent with the N,N-diacetyl structure. Comparison of the absorption curves (Fig. 1) shows that the diacetyl esters and diacetylbutylamine absorb in the 245–300 mμ range. Ethyl α-ethoxyethylideneaminoacetate[12,13] and acetylglycine ethyl ester do not absorb as strongly but the absorption curves for these two compounds resemble each other sufficiently to indicate presence of the enol form in the latter. These absorption curves show no peaks but only what

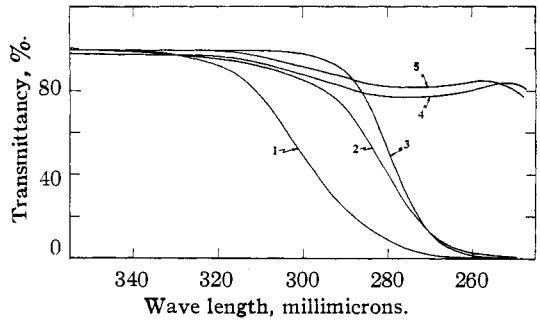

Fig. 1.—Transmittancy–wave length curves for 0.01 *M* solutions in chloroform; 1, diacetylglycine ethyl ester; 2, diacetylleucine ethyl ester; 3, diacetylbutylamine; 4, acetylalanine ethyl ester; 5, ethyl α-ethoxyethylideneaminoacetate.

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appears to be end absorption and are not, therefore, highly significant.

Experimental

Acetylsarcosine.—This compound was prepared from a 13% aqueous alkaline solution of sarcosine supplied by the General Aniline and Film Corporation.[14] Two moles of acetic anhydride per mole of sarcosine were added to the solution at 20° with stirring and cooling. After standing one hour the solution was acidified to thymol blue by adding hydrochloric acid, filtered from a small amount of gelatinous precipitate, saturated with salt, and extracted with nitromethane. The nitromethane was removed from the extract at reduced pressure and the residue recrystallized from a mixture of ethyl acetate and absolute alcohol to give a 52% yield of a solid, m. p. 134–134.5°. The reported melting point is 134–135°.[15]

N-Methylacetamidoacetone.—A mixture of 131.1 g. (1 mole) of acetylsarcosine, 460 ml. of pyridine (5.7 moles) (J. T. Baker, C.P.) and 1150 ml. (12.2 moles) of acetic anhydride (95% minimum assay) was refluxed with stirring for 6.5 hours. Low boiling materials were removed at reduced pressure and the residue refractionated to give 95.4 g., b. p. 99–109° at 3–4 mm. The product was steam distilled until the distillate no longer turned blue litmus to red. The residue was fractionated to give 67.5 g., b. p. 101–103°, at 2 mm. This product yielded the bisphenylhydrazone of methylglyoxal identified by melting point and mixed melting point with a known sample. Nitrogen analysis indicated a value between that for N-methylacetamidoacetone and its acetyl derivative in the ratio of 0.69 mole per cent. of the acylamido ketone to 0.31 mole per cent. of the acetyl derivative. The acetyl derivative was separated from an aqueous solution of 59.5 g. of the mixture by twenty-hour continuous ether extraction. The ether extracts were dried and the ether removed. The residue, amounting to about 35% of the mixture subjected to extraction was fractionated to give 21.3 g. of the acetyl derivative of N-methylacetamidoacetone. A center cut, $n^{25}D$ 1.4656, b. p. 98–98.5° at 4 mm., was analyzed.

Anal. Calcd. for $C_8H_{13}O_3N$: C, 56.13; H, 7.65; N, 8.18. Found: C, 56.23; H, 7.95; N, 8.39.

N-Methylacetamidoacetone was obtained by fractionation of the extracted aqueous solution after removal of the water by distillation; yield 24.2 g., b. p. 99–101° at 4 mm., $n^{25}D$ 1.4582. A center cut was analyzed.

Anal. Calcd. for $C_6H_{11}C_2N$: C, 55.78; H, 8.58; N, 10.84. Found: C, 55.53; H, 8.74; N, 10.76.

The acetyl N-methylacetamidoacetone was hydrolyzed to N-methylacetamidoacetone by stirring with about 1.2 equivalents of aqueous sodium bicarbonate at 70–90° on the steam-bath for one hour. The acetamido ketone was isolated by acidifying with acetic acid, adding anhydrous sodium sulfate to absorb the water of the solution, and extracting the solid hydrate in a continuous extractor with ether. A 65% hydrolysis yield of N-methylacetamidoacetone was obtained. A center cut, $n^{25}D$ 1.4584, b. p. 101° at 5 mm., was analyzed.

Anal. Calcd. for $C_6H_{11}O_2N$: N, 10.84. Found: N, 10.89.

The simple 2,4-dinitrophenylhydrazone was prepared as follows. A solution of 1.98 g. of 2,4-dinitrophenylhydrazine in 200 ml. of 96% alcohol containing 0.60 ml. of acetic acid was prepared by heating at gentle reflux. After addition of 1.29 g. of the N-methylacetamidoacetone the mixture was refluxed on the steam-bath for three hours. The solution was then filtered after cooling and the alcohol removed at reduced pressure. The residue was recrystallized from absolute alcohol to give 1.54 g. of the 2,4-dinitrophenylhydrazone, m. p. 148.5–149.5°.

Anal. Calcd. for $C_{12}H_{15}O_5N_5$: C, 46.60; H, 4.89; N, 22.65. Found: C, 46.60; H, 5.00; N, 22.69.

A much higher yield of the 2,4-dinitrophenylhydrazone was obtained when one equivalent of concentrated hydrochloric acid was used as the condensation catalyst. One sample of the 2,4-dinitrophenylhydrazone was prepared which had a m. p. 155.5–156.5°. A mixture with the 148.5–149.5° derivative melted at 156–157°. The high melting derivative was analyzed.

Anal. Calcd. for $C_{12}H_{15}O_5N_5$: N, 22.65. Found: N, 22.88.

Acetylglycine Ethyl Ester.—The ester was prepared from acetylglycine[16] in 85% yield by an azeotropic distillation technique. The ester is a hygroscopic solid, m. p. 47–49°. The reported m. p. is 48°[17] and the reported b. p. is 106° at 2 mm. and 145° at 11 mm.[18]

Acetylleucine Ethyl Ester.—This compound was prepared in an 87.5% yield by a procedure similar to that above. A center cut, b. p. 126–129° at 6 mm., $n^{25}D$ 1.4460, was analyzed. *Anal.* Calcd. for $C_{10}H_{19}O_3N$: N, 6.96. Found: N, 6.96. Reported[18] b. p. 114° at 2 mm. and 101° at 1 mm.

N,N-Diacetylglycine Ethyl Ester.[10]—A mixture of 145 ml. (1.8 moles) of pyridine (J. T. Baker C. P.), 305 ml. (3.0 moles) of acetic anhydride (95% minimum assay) and 43.7 g. (0.3 mole) of acetylglycine ethyl ester was heated with stirring at a gentle reflux for six and one-half hours. The solvent was removed at reduced pressure and the product isolated by vacuum distillation. Refractionation yielded 49.6 g., 88% of the theoretical amount, b. p. 106–110° at 2 mm., d^{25}_{25} 1.1254, $n^{25}D$ 1.4525.

N,N-Diacetylleucine Ethyl Ester.—This compound was prepared in 91% yield by the pyridine acetylation of acetylleucine ethyl ester following the procedure given for the glycine derivative. A center cut, b. p. 106–110° at 2–3 mm., $n^{25}D$ 1.4522, was analyzed.

Anal. Calcd. for $C_{12}H_{21}O_4N$: C, 59.23; H, 8.70; N, 5.76. Found: C, 59.15; H, 8.92; N, 6.14.

Ethyl α-Ethoxyethylideneaminoacetate.—This compound was prepared by the procedure of Schmidt[9] as modified by Cornforth.[10]

Summary

N-Methylacetamidoacetone and its acetyl derivative have been synthesized by the pyridine acetylation of acetylsarcosine and characterized. The fact that an acylamido ketone is obtained in this reaction shows that an azlactone is not a necessary intermediate in the synthesis of acylamido ketones by means of pyridine acylation of α-amino acids.

An investigation of the reaction as applied to the esters of α-acylamino acids has shown that the reaction pursues a different course. The products, obtained in over 90% yield, have been characterized as N,N-diacetylamino acid esters.

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(14) The authors appreciate the generous gift of this material.

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