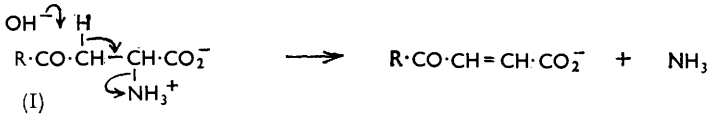
569. *Amino-acids and Peptides. Part IV.* Alkaline Hydrolysis of α-Acetylalanine (2-Amino-4-oxopentanoic Acid), N-Glycyl-α-acetylalanine, and Related Compounds.*

By C. H. Hassall, D. I. John, T. G. Martin, and J. A. Schofield.

β-Acetylalanine (2-amino-4-oxopentanoic acid) and its *NN*-dimethyl derivative undergo rapid elimination of the amino-group under mildly alkaline conditions whereas *N*-acyl derivatives of β-acetylalanine do not. Elimination of glycine from *N*-glycyl-β-acetylalanine occurs in 0·1N-sodium carbonate at 70°. *N*-Acyl derivatives of the dipeptide do not undergo this ready cleavage. The mechanism of the reaction is discussed.

α-Amino-γ-oxo-acids such as β-isobutyrylalanine (2-amino-5-methyl-4-oxohexanoic acid) and β-acetylalanine (2-amino-4-oxopentanoic acid) (I; R = Me) decompose exceptionally readily in the presence of mild alkali to give ammonia and the corresponding unsaturated acid or its decomposition products:[1]



(I)

A similar base-catalysed elimination has been reported to occur with asparagine,[2] which gives fumaramic acid when heated at 100° at pH 6·5—7·5; and formation of kynurenic acid by the action of sodium hydrogen carbonate on kynurenine probably involves the same process. The kinetics of the decomposition of β-acetylalanine and its *NN*-dimethyl derivative have been studied. The rate of elimination in the pH range 7·3—9·7 was followed by measuring the change of optical density at 220 mμ resulting from the release of β-acetylacrylic acid, which was stable under these conditions. The study of β-acetylalanine showed that at constant pH there was good agreement with the first-order rate equation:

$$\mathrm{d[\beta\text{-Acetylacrylic acid}]}/\mathrm{d}t = k_1[\beta\text{-Acetylalanine}].$$

Moreover, the increase in the rate of reaction with increasing pH followed second-order kinetics, if the ionised form (I; R = Me) of β-acetylalanine was regarded as the reacting species. The results were in good agreement with the equation:

$$\mathrm{d[\beta\text{-Acetylacrylic acid}]}/\mathrm{d}t = k_2[\mathrm{OH^-}][\beta\text{-Acetylalanine}].$$

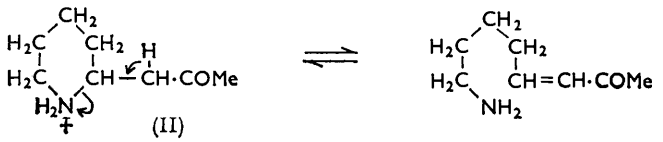
The value of k_2 for this reaction at 55° was 35·9 l. mole^{-1} sec.$^{-1}$. As might be expected from the greater nucleophilic character of the nitrogen function, the *NN*-dimethyl derivative reacted considerably faster, k_2 being 14×10^3 l. mole^{-1} sec.$^{-1}$. However, for the methyl ester of β-acetylalanine at pH 8·51, the pseudo-unimolecular rate constant $2{\cdot}1 \times 10^{-5}$ sec.$^{-1}$ was similar to that obtained with β-acetylalanine under the same conditions ($5{\cdot}2 \times 10^{-5}$ sec.$^{-1}$). Evidently the carboxyl group does not play an important role. The reaction may then be represented as a typical elimination initiated by attack of the hydroxyl ion at the β-position, leading either to carbanion formation followed by elimination of the nitrogen group or, as represented above, to elimination through a concerted reaction (*E*2). The evidence does not distinguish between the two possibilities.[3] The formation of *trans*-β-acetylacrylic acid as the product of reaction is in accord with

* Part III, *J.*, 1960, 4112.

[1] Ellington, Hassall, Plimmer, and Seaforth, *J.*, 1959, 80.
[2] Talley, Fitzpatrick, and Porter, *J. Amer. Chem. Soc.*, 1959, **81**, 174.
[3] Hine, "Physical Organic Chemistry," McGraw-Hill Co., New York, 2nd edn., p. 187.

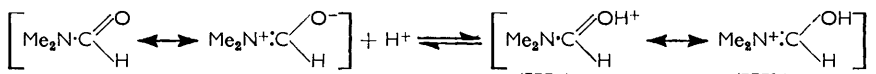
either interpretation. It deserves mention that the racemisation of hygrine and isopelletierine (II) in mild alkali probably involves a similar process although an alternative explanation has been offered.[4]

N-*Glycyl*-β-*acetylalanine and Related Compounds.*—The results obtained with β-acetylalanine and its simple derivatives indicate that the elimination depends on the existence of an ionised nitrogen group in the reactive species. There is good evidence from recent investigations on the hydrolysis of simple amides in alkaline solution for the occurrence of a " tetrahedral intermediate " involving an $-NH_2R^+$ group.[5] Moreover, the nuclear



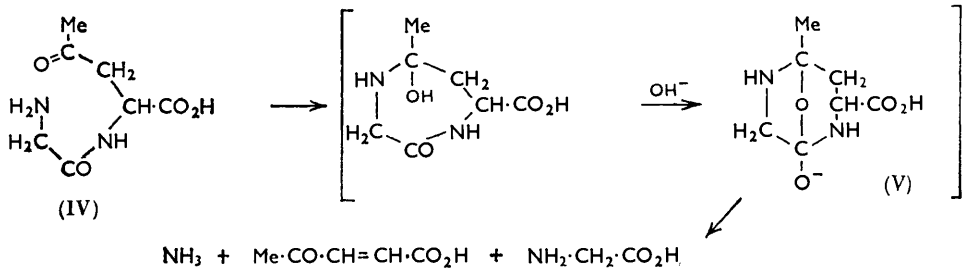
(II)

magnetic resonance spectrum of dimethylformamide in acid solution [6] and of its complex with iodine [7] have been interpreted as indicating that (IIIb) is an important contributing form. It seemed possible that the process which has been shown to take place with β-acetylalanine might influence the rate of hydrolysis of an *N*-acyl derivative with mild alkali. This could provide a means of preferential cleavage of the amide link in a peptide incorporating β-acetylalanine. When it was found that *N*-glycyl-β-acetylalanine was



(IIIa) (IIIb)

hydrolysed completely at 70° and pH 11·2 in 24 hr., whereas peptides such as glycylleucine and glycylaspartic acid were not hydrolysed to any extent in these conditions, there seemed to be support for this view. However, the preferential cleavage of the peptide bond in *N*-glycyl-β-acetylalanine cannot be attributed to activation by the β-acetyl function. If this were the case, the related compounds *N*-(glycylglycyl)-β-



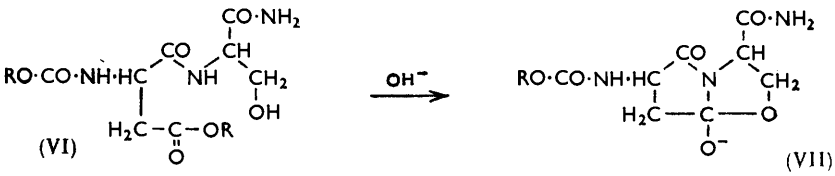
(IV) (V)

acetylalanine, *N*-(*N*-benzoylglycyl)-β-acetylalanine, and *N*-formyl- or *N*-benzoyl-β-acetylalanine should also be hydrolysed in conditions similar to those that were effective for *N*-glycyl-β-acetylalanine; in fact, the tripeptide undergoes a small amount of random cleavage, and the remaining three derivatives appear to be quite stable in these conditions.

Any explanation for the increased rate of hydrolysis of the amide link in *N*-glycyl-β-acetylalanine (IV) must involve both the unsubstituted terminal amino-group and the β-acetyl function. We suggest that the increase arises through formation of a complex (V) by interaction of the amino, carbonyl, and amide groups. This complex would be

[4] Galinovsky, Bianchetti, and Vogl, *Monatsh.*, 1953, **84**, 1221.
[5] Bender and Ginger, *J. Amer. Chem. Soc.*, 1955, **77**, 348; Bender and Thomas, *ibid.*, 1961, **83**, 4183.
[6] Fraenkel and Franconi, *J. Amer. Chem. Soc.*, 1960, **82**, 4478.
[7] Schmulbach and Drago, *J. Amer. Chem. Soc.*, 1960, **82**, 4484.

expected to be hydrolysed readily to glycine, ammonia, and β-acetylacrylic acid. In several other cases,[8] including the base-catalysed " amino-acid insertion " reaction of *O*-glycylsalicylamide,[9] similar cyclic intermediates have been postulated. It has been suggested[10] that the base-catalysed hydrolysis of aspartylserine derivatives such as



β-benzyl-*N*-benzyloxycarbonylaspartylserine amide (VI; R = CH_2Ph) proceeds through an intermediate (VII). A somewhat similar mechanism underlies the acceleration of hydrolysis of amides in which there is a hydroxyl group in γ-, δ-, or ε-relation to the carbonyl portion of the amide group.[11]

Experimental

M. p.s were determined on a Kofler block. In paper chromatography Whatman No. 1 paper was used. Ultraviolet spectra were determined on a Unicam S.P. 500 spectrophotometer.

β-Acetylalanine Methyl Ester Hydrochloride (Methyl 2-Amino-4-oxopentanoate Hydrochloride).—This was prepared from β-acetylalanine[12] (3·19 g.) by the method of Boissonnas *et al.*[13] Recrystallisation from methanol–ether gave the *ester hydrochloride* (2·32 g., 77%), m. p. 132—133°, $\lambda_{max.}$ 265 mμ (ε 110) (Found: C, 39·8; H, 6·7; Cl, 19·8; N, 7·9. $C_6H_{12}ClNO_3$ requires C, 39·7; H, 6·7; Cl, 19·5; N, 7·7%).

2-Formamido-4-oxopentanoic Acid.—Formylation of β-acetylalanine (0·33 g.) was carried out according to the procedure of Waley and Watson.[14] Crystallisation of the product from ethanol gave the *formyl derivative* (0·21 g., 77%), m. p. 139·5—140·5°, $\lambda_{max.}$ 212 (ε 1230), 270 mμ (ε 63) (Found: C, 45·9; H, 6·1; N, 9·1. $C_6H_9NO_4$ requires C, 45·3; H, 5·7; N, 8·8%).

2-Benzamido-4-oxopentanoic Acid.—β-Acetylalanine hydrochloride (1·68 g.) was treated by the Schotten–Baumann procedure. The *product* (1·97 g., 65%) extracted by ether and crystallised from methanol–benzene, had m. p. 138—139°, $\lambda_{max.}$ 228 mμ (ε 8800) (Found: C, 61·2; H, 5·5; N, 5·9. $C_{12}H_{13}NO_4$ requires C, 61·3; H, 5·6; N, 6·0%).

2-(2,4-Dinitrophenylamino)-4-oxopentanoic Acid.—β-Acetylalanine hydrochloride (0·167 g.) was treated by the procedure of Rao and Sober.[15] Recrystallisation of the crude product (0·28 g., 94%) from aqueous methanol gave the *dinitrophenyl derivative*, m. p. 82—84°, $\lambda_{max.}$ 360 mμ (ε 15,900) (Found: C, 44·0; H, 4·2; N, 14·1. $C_{11}H_{11}N_3O_7$ requires C, 44·5; H, 3·7; N, 14·1%).

NN-Dimethyl-β-acetylalanine Hydrochloride.—Prepared from β-acetylalanine hydrochloride (1·0 g.) by the method of Bowman and Stroud[16] and recrystallised from ethanol–ether, this *hydrochloride* (0·89 g., 76%) had m. p. 121—122°, $\lambda_{max.}$ 270 mμ (ε 80) (Found: C, 42·9; H, 7·1; N, 7·1. $C_7H_{14}ClNO_3$ requires C, 43·0; H, 7·2; N, 7·2%).

Methyl 4-Oxo-2-(N-tritylglycylamino)pentanoate.—A solution of *N*-tritylglycine[17] (2·12 g.) in dry chloroform (20 ml.) containing triethylamine (1·34 g.) was cooled to 0°, and ethyl chloroformate (0·72 g.) was added. After 15 min. a solution of β-acetylalanine methyl ester hydrochloride (1·21 g.) in chloroform (10 ml.) containing triethylamine (0·67 g.) was added and the mixture was kept at room temperature for 30 min. It was then washed successively with

[8] Bender, *Chem. Rev.*, 1960, **60**, 53.
[9] Brenner, Zimmerman, Wehrmüller, Quitt, Hartman, Schneider, and Beglinger, *Helv. Chim. Acta*, 1957, **40**, 1497.
[10] Bernhard, Berger, Carter, Katchalski, Sela, and Shalitin, *J. Amer. Chem. Soc.*, 1962, **84**, 2421.
[11] Zürn, *Annalen*, 1960, **631**, 56; Witkop, *Adv. Protein Chem.*, 1961, **16**, 221.
[12] Wiss and Fuchs, *Helv. Chim. Acta*, 1952, **35**, 407.
[13] Boissonnas, Guttman, Jacquenoud, and Waller, *Helv. Chim. Acta*, 1956, **39**, 1421.
[14] Waley and Watson, *Biochem. J.*, 1954, **57**, 529.
[15] Rao and Sober, *J. Amer. Chem. Soc.*, 1954, **76**, 1328.
[16] Bowman and Stroud, *J.*, 1950, 1342.
[17] Zervas and Theodoropoulos, *J. Amer. Chem. Soc.*, 1956, **78**, 1359.

dilute acetic acid, dilute aqueous diethylamine, and water, dried (Na_2SO_4), and evaporated to dryness. The residual oil crystallised from ethanol, to give *methyl 4-oxo-2-(N-tritylglycylamino)pentanoate* (1·8 g., 61%), m. p. 166—168° (Found: C, 72·4; H, 6·4; N, 6·4. $C_{27}H_{28}N_2O_4$ requires C, 72·9; H, 6·4; N, 6·3%).

4-Oxo-2-(N-tritylglycylamino)pentanoic Acid.—The protected dipeptide ester (0·8 g.) was treated with a mixture of dioxan (3·2 ml.) and N-alcoholic potassium hydroxide (2·8 ml.) for 5 min. at room temperature. The solution was diluted with water (3 vol.) and acidified with acetic acid, giving an oily precipitate which crystallised. Recrystallisation from ethanol gave the *acid* (0·49 g., 67%), m. p. 156° (Found: C, 72·7; H, 6·2; N, 6·1. $C_{26}H_{26}N_2O_4$ requires C, 72·5; H, 6·1; N, 6·5%).

2-(Glycylamino)-4-oxopentanoic Acid.—The protected dipeptide (0·16 g.) was heated with 50% aqueous acetic acid (3 ml.) at 100° for 3 min. The mixture was diluted with water, and the precipitate was removed. Evaporation of the filtrate to dryness yielded a residue which crystallised from aqueous ethanol to give the *dipeptide* (0·06 g., 86%), m. p. 220° (decomp.) (Found: C, 44·1; H, 6·7; N, 14·6. $C_7H_{12}N_2O_4$ requires C, 44·7; H, 6·4; N, 14·9%).

N-Ethanolic hydrogen chloride (0·7 ml.) was added to a solution of methyl 4-oxo-2-(*N*-tritylglycylamino)pentanoate (0·3 g.) in hot anhydrous ethanol (4 ml.), and the solution was heated on a water-bath for 4 min. Evaporation to dryness gave a colourless oil which gave crystals (0·14 g., 89%) on trituration with ether. Recrystallisation from ethanol–ether gave the *dipeptide methyl ester hydrochloride*, m. p. 154—156° (Found: C, 40·4; H, 6·2; Cl, 14·6; N, 11·5. $C_8H_{15}ClN_2O_4$ requires C, 40·3; H, 6·3; Cl, 14·9; N, 11·7%).

2-(N-Benzoylglycylamino)-4-oxopentanoic Acid.—The glycylamino-acid (0·155 g.) was suspended in dry ethyl acetate (10 ml.) and benzoyl chloride (0·12 ml.) which was refluxed for 2 hr. Removal of unchanged dipeptide followed by evaporation to dryness yielded white crystals. Recrystallisation from ethyl acetate gave the *benzoyl dipeptide* (0·09 g., 38%), m. p. 158—159° (Found: C, 57·1; H, 5·7; N, 9·2. $C_{14}H_{16}N_2O_5$ requires C, 57·5; H, 5·5; N, 9·6%.

Methyl 4-Oxo-2-(N-trityldiglycylamino)pentanoate.—A solution of tritylglycylglycine [17] (1·57 g.) in chloroform (30 ml.) containing triethylamine (0·85 g.) was cooled to 0° and ethyl chloroformate (0·46 g.) was added. A solution of β-acetylalanine methyl ester hydrochloride (0·76 g.) in chloroform (15 ml.) containing triethylamine (0·43 g.) was added immediately and the mixture was kept for 30 min. at room temperature. It was then washed successively with dilute acetic acid, dilute aqueous diethylamine, and water, dried ($MgSO_4$), and evaporated to dryness. The residual gum crystallised from ethanol, to give the protected *tripeptide ester* (1·81 g., 88%), m. p. 135—136° (Found: C, 69·5; H, 6·5; N, 8·3. $C_{29}H_{31}N_3O_5$ requires C, 69·4; H, 6·2; N, 8·4%).

2-Glycylglycylamino-4-oxopentanoic Acid.—The protected tripeptide ester (1·5 g.) was treated with dioxan (10 ml.) and N-ethanolic potassium hydroxide (8 ml.) for 10 min. at 50°. The solution was diluted with water (3 vol.), and the small amount of insoluble material removed. Acidification with acetic acid precipitated a gum. This was heated in 50% aqueous acetic acid (20 ml.) for 3 min. at 100°. Evaporation to dryness followed by addition of ethanol then yielded crystals. Recrystallisation from aqueous ethanol gave the *tripeptide* (0·21 g., 28%), m. p. 230° (decomp.) (Found: C, 44·1; H, 6·3; N, 17·4. $C_9H_{15}N_3O_5$ requires C, 44·1; H, 6·1; N, 17·1%).

4-Oxopent-2-enoic Acid.—A solution of β-acetylalanine hydrochloride (0·84 g.) in 3·3% sodium hydrogen carbonate solution (75 ml.) was heated for 50 min. at 80°, acidified with concentrated sulphuric acid, and extracted with ether (3 × 50 ml.). The extract was dried and evaporated, yielding crystals (0·21 g., 37%) which on sublimation gave 4-oxopent-2-enoic (β-acetylacrylic) acid, m. p. 125—126° (lit.,[18] m. p. 122—125°), $\lambda_{max.}$ 220 mμ (ε 13,200 in 1% aq. $NaHCO_3$).

Preliminary Studies on the Elimination.—(i) *Derivatives of β-acetylalanine.* 0·02% Solutions of the *N*-formyl, *N*-benzoyl, and *N*-dimethyl derivative and of the methyl ester of β-acetylalanine in 1% aqueous sodium hydrogen carbonate were heated at 40°, 55°, and 70°. The ultraviolet spectra of the resulting solutions were then examined. With the methyl ester, the absorption at 218—220 mμ characteristic of β-acetylacrylic acid or its ester was obtained after treatment at 70°. The *N*-formyl and *N*-benzoyl derivatives, however, gave only their own characteristic absorptions at 212 and 228 mμ.

[18] Hellström, *Kgl. Lantrukshogskolans Ann.*, 1955, **22**, 297.

The dimethyl derivative showed the absorption at 220 mμ immediately after dissolution in the bicarbonate solution.

When a solution of 2-(2,4-dinitrophenylamino)-4-oxopentanoic acid (5 mg.) in 1% aqueous sodium hydrogen carbonate (10 ml.) was heated at 70°, then cooled and extracted with ether, 2,4-dinitroaniline could not be detected in the extract. Acidification of the aqueous solution followed by extraction gave the unchanged 2,4-dinitrophenyl derivative, m. p. 84—86°.

(ii) *Peptides containing* β-*acetylalanine.* Sealed tubes containing peptide (5—10 mg.) in 0·1N-sodium carbonate (5—10 ml.) were heated in a thermostat-bath at 70°. Samples were withdrawn progressively and deionised by passage through a column of Amberlite CG-50 resin. Eluates were then concentrated and analysed by paper chromatography or paper electrophoresis.

(*a*) 2-*Glycylamino*-4-*oxopentanoic acid.* Paper chromatography with butan-1-ol–acetic acid–water (4 : 1 : 5) showed a gradual increase in the amount of glycine (R_F 0·24) and a corresponding decrease in the amount of dipeptide (R_F 0·29) present in the mixture. The presence of β-acetylalanine could not be detected at any time during the reaction. After 20 hr. the intensity of the peptide spot had decreased to about 10—20% of its original intensity. In a further experiment, with 0·2N-sodium hydroxide instead of sodium carbonate, hydrolysis appeared to be complete after 1 hr.

(*b*) 2-*Glycylglycylamino*-4-*oxopentanoic acid.* Paper electrophoresis (1% acetic acid buffer) of a sample that had been treated for 20 hr. at 70° showed the main component to be unchanged tripeptide along with some glycine and a trace of glycylglycine.

Treatment of the tripeptide with 0·1N-sodium hydroxide for 30 min. at 100° gave a similar result.

(*c*) 2-(N-*Benzoylglycylamino*)-4-*oxopentanoic acid.* Paper chromatography showed only unchanged starting material after 20 hr.

(iii) *Control experiments.* Control experiments were carried out with glycyl-DL-aspartic acid and glycyl-DL-leucine. No evidence of hydrolysis of either was detected after treatment with 0·1N-sodium carbonate for 20 hr. at 70° although trial experiments confirmed that 2·5% or more of amino-acid could be detected by the paper-chromatographic procedure employed.

Quantitative Studies.—(i) β-*Acetylalanine.* Runs were carried out with borate buffers of pH ranging from 7·97 to 9·67. In each case the preparation of the buffer and the initiation of the reaction were brought about simultaneously in a flask fitted with a side-tube. The pH of the buffer solutions at 55° was measured by using an E.I.L. pH meter fitted with a temperature compensator.

A portion (25 ml.) of a stock solution of β-acetylalanine hydrochloride (100 mg.) in 0·2M-boric acid (1 l.) containing potassium chloride (0·2 mole) was introduced into the side-tube while the appropriate quantities of 0·2N-sodium hydroxide, diluted to 75 ml., filled the bulb of the flask. After equilibration in the thermostat-bath for 1 hr. the contents of the side-tube were mixed with those of the bulb of the stoppered flask, *via* the side arm. A sample of the resulting solution was quickly transferred to the reaction cell by means of a jacketed pipette through which water from the thermostat-bath was circulated. The buffer solutions for the control cell were made up in an identical manner. The optical densities at 220 mμ were plotted against time. The first-order rate constant k_1 was obtained from the slope of conventional plots of $\log_{10}[a/(a-x)]$ against time by using the integrated first-order expression $k_1 t = \log_{10}[a/(a-x)]$ where a is either the observed or calculated maximum optical density corresponding to the final concentration of β-acetylacrylic acid in the reaction mixture, and x is the optical density corresponding to the concentration of β-acetylacrylic acid after time t.

The rate of reaction increased with increasing hydroxyl-ion concentration. However, a plot of k_1 against $[OH^-]$ could be applied only to the second-order equation, when the ionised form of the amino-group was regarded as the reacting species. A plot of the corrected first-order rate constant k_1' against $[OH^-]$ gave a straight line, the slope of which gave $k_2 = 39{\cdot}5$ l. mole^{-1} sec.$^{-1}$.

From the plot of $\log_{10} k_1'$ against $\log_{10}[OH^-]$, a slope of 0·98 was obtained, confirming the finding that the reaction was of first order with respect to $[OH^-]$ and obeyed overall second-order kinetics.

The results are given in Table 1.

(ii) NN-*Dimethyl*-β-*acetylalanine.* M/15-Potassium phosphate buffer and experimental procedures similar to the preceding were used. The second-order rate constant ($1{\cdot}4 \times 10^4$ l. mole^{-1} sec.$^{-1}$) was much larger than that of β-acetylalanine. The order of reaction with respect

TABLE 1.

Rate of decomposition of β-acetylalanine in borate buffer at 55°.

pH	$[OH^-]$ (10^{-7} mole/l.)	1st-order rate constant (k_1) (10^{-5} sec.$^{-1}$)	Factor A $[NH_2 + NH_3^+]/[NH_3^+]$	$k_1' (= k_1A)$ (10^{-5} sec.$^{-1}$)
7·95	8·91	2·91	1·36	3·95
7·95	8·91	2·79	1·36	3·78
8·43	25·7	5·22	2·07	10·8
8·43	25·7	4·69	2·07	9·7
8·97	93·3	7·68	4·11	36·2
8·97	93·3	7·74	4·11	36·4
9·67	468·0	9·48	19·5	184·7
9·67	468·0	9·22	19·5	180·0

to $[OH^-]$ obtained from the plot of $\log_{10} k_1'$ against $\log_{10} [OH^-]$ was 0·95. The results are given in Table 2.

(iii) *2-Glycylamino-4-oxopentanoic acid.* The dipeptide (55·4 mg.) was dissolved in 0·1N-sodium carbonate solution (50 ml.) in a stoppered flask which was immersed in a thermostat-bath at 70°. Samples (2 ml.) were withdrawn periodically and mixed with 0·1N-sodium carbonate (8 ml.). An excess of 1-fluoro-2,4-dinitrobenzene was added, and the mixture stirred vigorously for 1 hr. at 40° and then extracted with benzene. The aqueous solution was then transferred to a column (21 × 1·5 cm.) of ECTEOLA cellulose (chloride form) which was next washed with water. Elution with 0·05M-lithium chloride gave the 2,4-dinitrophenyl-dipeptide and 2,4-dinitrophenylglycine as successive fractions. The optical density of each fraction was measured at 350 mμ.

After reaction for 24 hr. no 2,4-dinitrophenyl-dipeptide could be detected although cleavage as indicated by the amount of 2,4-dinitrophenylglycine had occurred only to the extent of 78%. Other examples of incomplete 2,4-dinitrophenylation have been observed.

A straight-line plot of $\log_{10} [a/(a - x)]$ against time was obtained only by assuming that the amount of 2,4-dinitrophenylglycine obtained after 24 hr. corresponded to 100% cleavage of the dipeptide. The straight-line plot gave a value of $k_1 = 6{\cdot}25 \times 10^{-5}$ sec.$^{-1}$.

TABLE 2.

Rate of decomposition of *NN*-dimethyl-β-acetylalanine at 55°.

pH	$[OH^-]$ (10^{-7} mole/l.)	1st-order rate constant (k_1) (10^{-3} sec.$^{-1}$)	Factor A $[NMe_2 + NHMe_2^+]/[NHMe_2^+]$	$k_1' (= k_1 A)$ (10^{-3} sec.$^{-1}$)
7·8	6·31	8·15	1·16	9·51
7·8	6·31	8·22	1·16	9·60
7·59	3·89	5·46	1·10	6·02
7·59	3·89	5·22	1·10	5·76
7·39	2·45	4·0	1·06	4·26
7·39	2·45	3·9	1·06	4·16
7·28	1·91	3·23	1·05	3·38
7·28	1·91	3·34	1·05	3·51

(iv) *2-Benzamido-4-oxopentanoic acid.* The benzoyl derivative (7·9 mg.) was dissolved in 0·1N-sodium carbonate (10 ml.) in a stoppered flask which was immersed in a thermostat-bath at 70°. After 20 hr. the solution was transferred to a column (21 × 1·5 cm.) of ECTEOLA cellulose (chloride form) which was then washed with water. Elution was carried out with 0·05M-lithium chloride, the optical density (270 mμ) of each fraction (10 ml.) being measured. The presence of benzoic acid could not be detected although the benzoyl derivative and benzoic acid could be separated by this procedure, 4% of benzoic acid being easily detected.

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