639. *Purines, Pyrimidines, and Glyoxalines. Part XV.*[1] *Acetyluracils from Amines and Amino-acids. A New Method for the Study of* N-*Terminal Residues in Proteins.*

By J. H. Dewar and G. Shaw.

α-Acetyl-β-ethoxy-*N*-ethoxycarbonylacrylamide reacts with amines and amino-acids, forming acid-stable 5-acetyluracils. The reaction has been applied to the determination of the *N*-terminal residues in insulin and ribonuclease.

Part IV [2] of this series described the preparation of 5-cyanouracils by reaction of amines, amino-acids, and glycylglycine with the ethoxyacrylamide derivative (II). Development of this reaction for use in *N*-terminal assay studies of peptides and proteins was limited by the lability of the 5-cyano-group in the uracils to acid-hydrolysis, which may give rise

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[1] Part XIV, Shaw and Butler, *J.*, 1959, 4040.

[2] Atkinson, Shaw, and Warrener, *J.*, 1956, 4118.

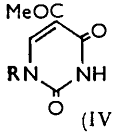
under different conditions to mixtures of the corresponding carboxyamide, carboxylic acid, and decarboxylated material.

We now report the preparation of analogous 5-acetyluracils which are completely immune to hydrolysis by strong acid and may be used to advantage in *N*-terminal studies of proteins.

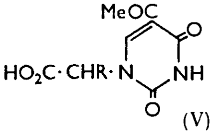
N-Acetoacetylurethane (from diketen and urethane; cf. Part XII [3]) with ethyl orthoformate and acetic anhydride readily gave a good yield of the crystalline ethoxyacrylamide (I; R = Et). This with ammonia or methylamine gave the 5-acetyluracils (IV; R = H or Me), and with aniline gave the linear anilinoacrylamide (III; R = Ph) which cyclised to the acetyluracil (IV; R = Ph) when heated at its melting point or warmed with aqueous alkali. Hydrolysis of the ethoxyacrylamide (I; R = Et) under mild conditions with sodium hydroxide also gave the hydroxyacrylamide (I; R = H).

(I) $\mathrm{Me{\cdot}CO{\cdot}C(\dot{:}CH{\cdot}OR){\cdot}CO{\cdot}NH{\cdot}CO_2Et}$ $\mathrm{CN{\cdot}C(\dot{:}CH{\cdot}OEt){\cdot}CO{\cdot}NH{\cdot}CO_2Et}$ (II)

$\mathrm{Me{\cdot}CO{\cdot}C(\dot{:}CH{\cdot}NHR){\cdot}CO{\cdot}NH{\cdot}CO_2Et}$ (III)



(IV)



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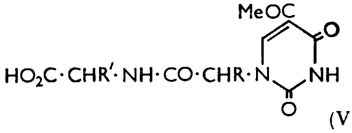

(VI)

Similar reaction of the ethoxyacrylamide (I; R = Et) with most of the L-amino-acids normally found in proteins, in dilute sodium hydroxide solution or, in some cases, in aqueous triethylamine, for short periods, gave corresponding 5-acetyl-1-uracilacetic acids (V) in substantially quantitative yields and, except for the derivatives from L-cysteine and L-tryptophan, as crystalline solids. The acetyluracils are useful derivatives of amino-acids and may be separated, identified, and estimated by paper chromatography, ion-exchange, ultraviolet absorption spectrophotometry, and by reactions of the exocyclic ketone group, *e.g.*, with 2,4-dinitrophenylhydrazine. L-Aspartic and L-glutamic acid were exceptional in forming linear derivatives (III) in sodium hydroxide solution, but the corresponding uracils were readily prepared by acid hydrolysis of the easily cyclised L-asparagine and L-glutamine derivatives. Other linear derivatives were also obtained from the urethane (I; R = Et) and L-cysteine, L-arginine, and L-proline in sodium hydrogen carbonate solution or aqueous triethylamine when the reaction was not prolonged. Further treatment of the linear derivative from arginine with warm sodium hydrogen carbonate solution gave the cyclic product. The linear acrylamide from L-cysteine with dilute sodium hydroxide rapidly gave the cyclic product, and with sodium hydroxide and iodine it gave the uracil derived from L-cystine. The last compound was also prepared in the normal manner from the ethoxyacrylamide (I; R = Et) and L-cystine. In all these reactions there appears to be no combination of the ethoxyacrylamide (I; R = Et) with any of the other functional groups present in the amino-acids, *e.g.*, alcoholic or phenolic hydroxyl, thiol, the NH group in histidine, or the guanidine group of arginine.

Acetyluracil derivatives (VI) of several dipeptides have also been prepared as readily crystallising solids from the ethoxyacrylamide (I; R = Et) in aqueous triethylamine or sodium hydroxide without rupture of the peptide bond. In these reactions cyclisation appears to occur even more readily than with the amino-acids, and linear acrylamides were not isolated. Acid hydrolysis of the peptide derivatives gave the uracil derived from the *N*-terminal amino-acid, together with a free amino-acid, as the sole products and these were readily characterised by paper chromatography.

To establish the stability of the pyrimidine ring to acid hydrolysis under conditions comparable to those required for protein hydrolysis, samples of acetyluracil-1-acetic acids

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[3] Ralph, Shaw, and Naylor, *J.*, 1959, 1169.

(V) derived from glycine, L-leucine, L-aspartic acid, and L-histidine were boiled with 6N-hydrochloric acid for 7 hr. Ultraviolet absorption spectra of the hydrolysates indicated a quantitative recovery of the uracils.

The reactions have also been applied to the determination of the *N*-terminal residues in insulin and ribonuclease. Reactions of the proteins with the ethoxyacrylamide (I; R = Et) were carried out in aqueous triethylamine for 1 hr. at room temperature. The resulting solid derivatives were hydrolysed and the evaporated solutions extracted with ethyl acetate. Paper chromatograms showed in the case of insulin two absorbing spots only, which corresponded exactly with the uracils derived from glycine and L-phenylalanine; and from ribonuclease a single spot corresponding to the bisuracil derivative of L-lysine was the sole absorbing material. Also chromatograms of the aqueous-hydrolysis solutions showed a single absorbing spot which corresponded to the ε-monouracil derivative of L-lysine. The spots were further characterised as from ketones by spraying them with a solution of 2,4-dinitrophenylhydrazine. In these reactions removal of the excess of ethoxyacrylamide (I; R = Et) appears to be unnecessary since it is completely hydrolysed by acid to simple volatile products.

EXPERIMENTAL

Paper chromatography (ascending) was carried out on Whatman No. 1 paper at room temperature with the following solvents (ratios in volume proportions): (1) butan-1-ol–acetic acid–water (4 : 1 : 5), (2) propan-2-ol–ammonia (*d* 0·88)–water (7 : 1 : 3), (3) butan-1-ol saturated with water, (4) propan-2-ol–10N-hydrochloric acid–water (17 : 5 : 3). The acetyluracils were detected on paper as dark absorbing spots under an ultraviolet lamp (Hanovia Chromatolight) or as yellow spots by spraying them with a solution of 2,4-dinitrophenylhydrazine hydrochloride in water or methanol. Spectral measurements were made on an Optica double-beam recording CF4 spectrophotometer.

*α-Acetyl-β-ethoxy-*N*-ethoxycarbonylacrylamide.*—A mixture of *N*-acetoacetylurethane[3] (70 g.), ethyl orthoformate (61 g.), and acetic anhydride (101 ml.) was boiled under reflux for 1 hr. The red solution was evaporated *in vacuo* to an oil which rapidly crystallised. The solid was washed with ether which removed most of the colour. The *acrylamide* (58 g.) recrystallised from light petroleum (b. p. 60—80°) containing a little benzene as needles, m. p. 90° (Found: C, 51·9; H, 6·3; N, 6·4. $C_{10}H_{15}NO_5$ requires C, 52·2; H, 6·6; N, 6·1%). This compound is referred to below as the " ethoxyacrylamide."

*α-Acetyl-β-hydroxy-*N*-ethoxycarbonylacrylamide.*—The ethoxyacrylamide (1 g.) was shaken for a few minutes with 2N-sodium hydroxide (3 ml.), a clear solution being obtained. This was cooled and acidified to precipitate the *hydroxyacrylamide* (0·2 g.), which separated from a little water as needles, m. p. 57—58° (Found: C, 47·75; H, 5·35; N, 6·3. $C_8H_{11}NO_5$ requires C, 47·7; H, 5·5; N, 6·95%), giving a red colour with ferric chloride solution.

5-Acetyluracil.—The ethoxyacrylamide (2 g.) was warmed with 3N-ammonia (50 ml.) until a clear solution was obtained. This was evaporated to remove the excess of ammonia, cooled, and acidified with acetic acid, to give after a short time a crystalline precipitate. *5-Acetyluracil* (1·2 g.) separated from ethanol (*ca.* 250 ml.) as prisms, m. p. 283—285° (decomp.). Bergmann and Johnson[4] give m. p. 294° (decomp.) (Found: C, 46·8; H, 3·65; N, 18·0. Calc. for $C_6H_6N_2O_3$: C, 46·75; H, 3·95; N, 18·2%). The *2,4-dinitrophenylhydrazone* crystallised from acetic acid as orange-red needles, m. p. 276° (decomp.) (Found: C, 43·3; H, 3·2; N, 24·7. $C_{12}H_{10}N_6O_6$ requires C, 43·15; H, 3·0; N, 25·15%).

5-Acetyl-1-methyluracil.—A suspension of the ethoxyacrylamide (0·3 g.) in water was warmed for a few minutes with a little aqueous methylamine: a clear solution was rapidly obtained. This was cooled, and acidified with acetic acid to precipitate *5-acetyl-1-methyluracil* (0·22 g.) which recrystallised from ethanol as plates, m. p. 234° (Found: C, 50·05; H, 4·85; N, 16·95. $C_7H_8N_2O_3$ requires C, 50·0; H, 4·8; N, 16·7%).

5-Acetyl-1-phenyluracil.—A solution of the ethoxyacrylamide (1·15 g.) and aniline (0·5 ml.) in ethanol (5 ml.) was warmed for 30 min., then set aside overnight whereupon crystals separated. *α-Acetyl-β-anilino-*N*-ethoxycarbonylacrylamide* (0·96 g.) separated from ethanol as

[4] Bergmann and Johnson, *Ber.*, 1933, **66**, 1492.

prisms, m. p. 118° (Found: C, 60·7; H, 5·75; N, 10·4. $C_{14}H_{16}N_2O_4$ requires C, 60·85; H, 5·85; N, 10·15%). This linear derivative (0·5 g.) was heated at 170° (bath), effervescing and then solidifying. The residue, *5-acetyl-1-phenyluracil* (0·41 g.), crystallised from ethanol as needles, m. p. 269° (decomp.) (Found: C, 62·3; H, 4·2; N, 12·4. $C_{12}H_{10}N_2O_3$ requires C, 62·6; H, 4·4; N, 12·2%). The same compound was also obtained by warming the linear acrylamide with sodium hydroxide solution until a clear solution was obtained. Acidification of this then precipitated the phenyluracil.

Aminoacrylamides from α-Amino-acids and the Ethoxyacrylamide.—(a) L-*Cysteine.* A solution of L-cysteine hydrochloride (1·95 g.) in water (5 ml.) and triethylamine (4·3 ml.) was warmed with the ethoxyacrylamide (2·76 g.) for 3 min. The cooled solution was acidified with 10N-hydrochloric acid to precipitate a gum which rapidly crystallised. The *acrylamide* (2·5 g.) recrystallised from ethyl acetate–ether as needles, m. p. 146° (Found: C, 43·05; H, 5·2; N, 8·75. $C_{11}H_{16}N_2O_6S$ requires C, 43·45; H, 5·3; N, 9·2%). The compound gave an intense purple colour with sodium nitroprusside in alkaline solution.

(b) L-*Aspartic acid.* A solution of L-aspartic acid (1·12 g.) in 2N-sodium hydroxide (7·5 ml.) and water (2·5 ml.) was warmed with the ethoxyacrylamide (1·84 g.) for 10 min. The cooled solution was acidified with 10N-hydrochloric acid (2·5 ml.) to give after a short time a crystalline precipitate. The *acrylamide* (2·5 g.) separated from water as needles, m. p. 200° (decomp.) (Found: C, 45·55; H, 5·15; N, 8·8. $C_{12}H_{16}N_2O_8$ requires C, 45·6; H, 5·1; N, 8·85%). The same compound was obtained when the reaction was carried out in aqueous triethylamine or sodium hydrogen carbonate solution.

(c) L-*Glutamic acid.* L-Glutamic acid (1·84 g.) in 2N-sodium hydroxide (7·5 ml.) was warmed with the ethoxyacrylamide (1·84 g.) for 10 min. The cooled solution was acidified with hydrochloric acid and continuously extracted with ether for 3 hr. The extract was evaporated and the dried residue crystallised from acetone–light petroleum (b. p. 40—60°), to give the *acrylamide* (3 g.) as needles, m. p. 158° (Found: C, 47·35; H, 5·6; N, 8·8. $C_{13}H_{18}N_2O_8$ requires C, 47·3; H, 5·5; N, 8·5%). The same compound may be similarly prepared in aqueous triethylamine or sodium hydrogen carbonate solution.

(d) L-*Proline.* A mixture of L-proline (0·24 g.), water (1 ml.), triethylamine (0·8 ml.), and the ethoxyacrylamide (0·46 g.) was warmed for 15 min. on a water-bath. The cooled solution was acidified with 10N-hydrochloric acid (0·6 ml.) to give a solid precipitate. The *acrylamide* (0·6 g.) crystallised from a small quantity of water as needles, m. p. 75° (Found: C, 48·4; H, 6·6; N, 8·8. $C_{13}H_{18}N_2O_6,1\frac{1}{2}H_2O$ requires C, 48·0; H, 6·6; N, 8·6%).

(e) L-*Arginine.* A mixture of L-arginine (0·72 g.) in water (10 ml.) containing sodium hydrogen carbonate (0·5 g.) and the ethoxyacrylamide (0·92 g.) in ethanol (10 ml.) was shaken at room temperature for 4 hr., a clear solution being obtained. This was evaporated to a small volume and acidified with 10N-hydrochloric acid (1 ml.): a gel separated. A portion of this, when boiled with water and then cooled, gave a crystalline precipitate. The gel soon crystallised when seeded with the crystalline sample. The *acrylamide hydrochloride hydrate* (1·5 g.) recrystallised from water as needles, m. p. 145° (Found: C, 41·1; H, 6·35; N, 17·7. $C_{14}H_{23}N_5O_6,HCl,H_2O$ requires C, 40·8; H, 6·35; N, 17·0%).

Cyclisation of α-Acetyl-β-carboxymethylaminoacrylamides.—*Cysteine and cystine.* The linear acrylamide from L-cysteine (0·915 g.) was warmed for 10 min. with 2N-sodium hydroxide (3·6 ml.) in an atmosphere of hydrogen. The cooled solution was acidified with 10N-hydrochloric acid to precipitate a little gum. The mixture was diluted with water and continuously extracted with ether. Evaporation of the extract gave a semi-solid residue from which the *acetyluracil* (0·5 g.) was obtained as an amorphous solid by precipitation of an acetone solution with light petroleum (b. p. 40—60°). It gave an intense purple colour with an alkaline solution of sodium nitroprusside. The compound also gave only one absorbing spot on paper chromatograms. The foregoing acrylamide from L-cysteine (0·59 g.) in N-sodium hydroxide (6·9 ml.) was treated with a solution of iodine (4·95 ml. of a 5·6% solution in 20% potassium iodide). After a few minutes the solution was covered with ethyl acetate and acidified with 10N-hydrochloric acid. The solution was extracted once more with ethyl acetate. Evaporation of the combined extracts gave the crystalline *acetyluracil* derivative of L-cystine (0·5 g.) which gave no colour with sodium nitroprusside in alkaline solution. The same compound was detected on paper chromatograms from the reaction of the ethoxyacrylamide with L-cystine in dilute sodium hydroxide solution. M. p.s, analyses, and other physical data are recorded in Table 1.

*5-Acetyluracil-*1*-acetic Acids from* α*-Amino-acids and the Ethoxyacrylamide.*—M. p.s, analyses, and other data are recorded in Table 1. (a) *Glycine.* The ethoxyacrylamide (0·5 g.) was warmed for 5 min. with a solution of glycine (0·18 g.) in 2N-sodium hydroxide (2·5 ml.). The cooled solution was acidified with 10N-hydrochloric acid (0·5 ml.) to precipitate the *uracil* (0·5 g.).

TABLE 1. 5-*Acetyl*-1-*uracilacetic acids* (V).

No.	R	Generating acid [a, q]	M. p.[b]	R_F of ketone in solvent 1	2	3	R_F of 2,4-dinitrophenylhydrazone in solvent 1	2	3
1	H [c]	Glycine	200°	0·48	0·59	0·04	0·73	0·70	0·24
2	Me [c]	Alanine	174	0·66	0·66	0·07	0·82	0·78	0·28
3	Pr^i [c]	Valine	196	0·84	0·77	0·21	0·89	0·86	0·42
4	Bu^i [d]	Leucine	153	0·85	0·80	0·31	0·90	0·92	0·47
5	Bu^s [d]	Isoleucine	187	0·87	0·82	0·27	0·90	0·91	0·45
6	$Ph{\cdot}CH_2$ [c]	Phenylalanine	214	0·83	0·79	0·25	0·89	0·88	0·41
7	*p*-$HO{\cdot}C_6H_4{\cdot}CH_2$ [c]	Tyrosine	183	0·78	0·71	0·17	0·86	0·83	0·34
8	$HO{\cdot}CH_2$ [g, j]	Serine	216	0·51	0·64	0·04	0·72	0·76	0·20
9	$HS{\cdot}CH_2$ [j, p]	Cysteine	143	0·65	0·55	0·08	0·74	0·68	0·30
10	$C_9H_9N_2O_5S{\cdot}S{\cdot}CH_2$ [d]	Cystine	197	0·52	0·56	0·01	0·89	0·70	0·04
11	$Me{\cdot}CH(OH)$ [h, j]	Threonine	96	0·63	0·70	0·08	0·81	0·80	0·30
12	$MeS{\cdot}CH_2{\cdot}CH_2$ [f]	Methionine	159	0·79	0·79	0·16	0·88	0·83	0·34
13	$C_8H_6N{\cdot}CH_2$ [n]	Tryptophan	166	0·82	0·74	0·20	0·89	0·79	0·37
14	$HO_2C{\cdot}CH_2$ [f, j, k]	Aspartic acid	209	0·58	0·57	0·01	0·75	0·68	0·09
15	$H_2N{\cdot}CO{\cdot}CH_2$ [c]	Asparagine	157	0·51	0·66	0·04	0·65	0·70	0·13
16	$HO_2C{\cdot}CH_2{\cdot}CH_2$ [h, j, l]	Glutamic acid	180	0·62	0·63	0·01	0·79	0·71	0·03
17	$H_2N{\cdot}CO{\cdot}CH_2{\cdot}CH_2$ [e]	Glutamine	187	0·39	0·65	0·03	0·68	0·74	0·14
18	$H_2N{\cdot}C(:NH){\cdot}NH{\cdot}[CH_2]_3$ [c]	Arginine	>300	0·51	0·74	0·06	0·74	0·76	0·25
19	$C_6H_5N_2O_3{\cdot}[CH_2]_4$ [c, o]	Lysine	152	0·62	0·70	0·06	0·75	0·73	0·20
20	$C_3H_3N_2{\cdot}CH_2$ [i, m]	Histidine	236	0·25	0·69	0·06	0·51	0·73	0·23

No.	Found (%) C	H	N	Formula	Required (%) C	H	N
1	41·55	4·5	12·75	$C_8H_8N_2O_5,H_2O$	41·75	4·4	12·2
2	41·65	5·6	11·15	$C_9H_{10}N_2O_5,1\frac{3}{4}H_2O$	41·95	5·3	10·9
3	51·65	5·7	10·8	$C_{11}H_{14}N_2O_5$	51·95	5·55	11·0
4	51·2	6·0	10·05	$C_{12}H_{16}N_2O_5,\frac{3}{4}H_2O$	51·15	6·25	9·95
5	53·75	5·95	10·4	$C_{12}H_{16}N_2O_5$	53·7	6·0	10·45
6	60·05	4·6	9·15	$C_{15}H_{14}N_2O_5$	59·6	4·65	9·25
7	53·1	4·9	8·15	$C_{15}H_{14}N_2O_6,H_2O$	53·55	4·8	8·35
8	42·35	4·3	10·9	$C_9H_{10}N_2O_6,\frac{3}{4}H_2O$	42·25	4·55	10·95
9	42·8	5·0	10·4	$C_9H_{10}N_2O_5S,\frac{1}{4}C_3H_6O$	42·95	4·25	10·3
10	39·65	3·45	10·55	$C_{18}H_{18}N_4O_{10}S_2,1\frac{1}{2}H_2O$	39·95	3·9	10·35
11	44·05	5·4	10·3	$C_{10}H_{12}N_2O_6,H_2O$	43·8	5·15	10·2
12	46·0	4·8	9·35	$C_{11}H_{14}N_2O_5S$	46·15	4·95	9·8
13	57·7	5·6	11·9	$C_{17}H_{15}N_3O_5,\frac{3}{4}H_2O$	57·55	4·7	11·85
14	44·35	3·7	10·4	$C_{10}H_{10}N_2O_7$	44·45	3·75	10·35
15	42·4	4·65	14·9	$C_{10}H_{11}N_3O_6,H_2O$	41·85	4·55	14·65
16	43·75	4·6	9·45	$C_{11}H_{12}N_2O_7,H_2O$	43·7	4·65	9·25
17	45·15	4·95	14·1	$C_{11}H_{13}N_3O_6,\frac{1}{2}H_2O$	45·2	4·85	14·4
18	40·35	5·6	19·0	$C_{12}H_{17}N_5O_5,HCl,\frac{1}{2}H_2O$	40·4	5·35	19·65
19	46·6	5·25	12·45	$C_{18}H_{20}N_4O_8,2\frac{1}{2}H_2O$	46·45	5·4	12·05
20	41·6	4·55	16·5	$C_{12}H_{12}N_4O_5,HCl,H_2O$	41·55	4·35	16·15

[a] The amino-acids all have the L-configuration. [b] With decomp. [c] Needles from water. [d] Prisms from water. [e] Plates from water. [f] Needles from ethyl acetate–light petroleum (b. p. 40—60°). [g] Needles from acetone. [h] Needles from ethyl acetate. [i] Needles from ethanol. [j] Isolated from the aqueous solution by continuous extraction with ether. [k] Prepared by boiling the asparagine derivative with 6N-hydrochloric acid for 2 hr. [l] Prepared by boiling the glutamine derivative with 6N-hydrochloric acid for 2 hr. [m] The hydrochloride crystallised from the aqueous solution after evaporation to a small volume. [n] Amorphous solid from acetone–ether. [o] The bis-uracil derivative. [p] The compound retained a little acetone. [q] Compounds were dried over phosphoric oxide at room temperature and 0·5 mm. before analysis.

(b) L-*Arginine.* A mixture of L-arginine (0·72 g.) in water (10 ml.) containing sodium hydrogen carbonate (0·5 g.), and the ethoxyacrylamide (0·92 g.) in ethanol (10 ml.) was set aside at 40—50° over a week-end. The cooled solution was acidified with hydrochloric acid and extracted with ether. The aqueous phase was evaporated to dryness *in vacuo*, and the residue extracted with dimethylformamide. The extract was added to ether to produce a solid precipitate of the *uracil hydrochloride hemihydrate* (1 g.) which was purified by repeating the last process.

Other 5-acetyluracil-1-acetic acids prepared in yields of 75—100% as under (a) above are described in Table 1. The uracils derived from L-alanine, L-serine, and L-tyrosine were also similarly prepared in aqueous triethylamine. Compounds which did not separate from the acidified reaction mixture were isolated by continuous extraction with ether.

Preparation of 2,4-*Dinitrophenylhydrazones of* 5-*Acetyluracil*-1-*acetic Acids.*—Small quantities of the derivatives were prepared for paper chromatography from each of the acetyluracils derived from amino-acids, by addition of a solution of 2,4-dinitrophenylhydrazine in 2N-hydrochloric acid or methanolic sulphuric acid to solutions of the uracils in hot water or methanol. The derivatives were centrifuged off and recrystallised, generally from water, ethanol, or aqueous ethanol, and finally collected by centrifugation. The products were not analysed. R_F values are recorded in Table 1.

5-*Acetyluracils from Peptides and the Ethoxyacrylamide.*—M. p.s, analyses, and other data are recorded in Table 2. (a) *Glycyl*-L-*tyrosine.* A solution of the peptide (0·25 g.) in N-sodium hydroxide (2·5 ml.) was warmed with the ethoxyacrylamide (0·23 g.) for a few minutes. The cooled solution was acidified with hydrochloric acid to precipitate the uracil (0·31 g.). (b) *Glycyl*-L-*tryptophan.* The peptide (0·07 g.) in water (1 ml.) and triethylamine (0·2 ml.) was warmed for a few minutes with the ethoxyacrylamide (0·07 g.). The cooled solution was acidified with hydrochloric acid to precipitate a gum which rapidly crystallised to give the *uracil* (0·1 g.). Other peptide derivatives prepared in the same way in yields of 80—100% are recorded in Table 2.

TABLE 2. 5-*Acetyluracil peptides* (VI).

					R_F in solvent		
No.	R	R′	Generating acid	M. p.[c]	1	2	3
1	H	H[a]	Glycylglycine	271°	0·48	0·63	0·02
2	H	Me[a]	Glycyl-DL-alanine	258	0·84	0·85	0·04
3	H	Bu^i[a]	Glycyl-DL-leucine	237	0·82	0·78	0·15
4	H	Ph·CH$_2$[a]	Glycyl-DL-phenylalanine	251	0·79	0·77	0·12
5	H	*p*-HO·C$_6$H$_4$·CH$_2$[a]	Glycyl-L-tyrosine	239	0·70	0·68	0·06
6	H	C$_8$H$_6$N·CH$_2$[b]	Glycyl-L-tryptophane	275	0·77	0·72	0·09
7	Bu^i	H[a]	DL-Leucylglycine	240	0·83	0·83	0·16

	Found (%)				Required (%)		
No.	C	H	N	Formula	C	H	N
1	45·05	4·25	15·5	$C_{10}H_{11}N_3O_6$	44·6	4·1	15·6
2	45·0	4·3	14·65	$C_{11}H_{13}N_3O_6,\frac{1}{2}H_2O$	45·2	4·85	14·4
3	51·35	5·85	12·7	$C_{14}H_{19}N_3O_6$	51·7	5·9	12·9
4	56·0	4·6	11·65	$C_{17}H_{17}N_3O_6$	56·8	4·75	11·7
5	52·1	4·5	11·25	$C_{17}H_{17}N_3O_7,H_2O$	51·9	4·85	10·7
6	54·95	4·7	13·75	$C_{19}H_{18}N_4O_6,H_2O$	54·8	4·85	13·45
7	49·9	5·9	12·6	$C_{14}H_{19}N_3O_6,\frac{1}{2}H_2O$	50·3	6·05	12·6

[a] Needles from water. [b] Needles from ethanol. [c] With decomp.

Hydrolysis of Peptide Derivatives.—The acetyluracil derived from glycyl-L-tyrosine (2—3 mg.) was heated at 100° with 6N-hydrochloric acid for 7 hr. The solution was evaporated to dryness, and the residue chromatographed on paper. A single absorbing spot was observed with R_F values 0·6 (solvent 2) and 0·84 (solvent 4) corresponding to the acetyluracil derived from glycine, R_F 0·6 (solvent 2) and 0·84 (solvent 4), which was run as a control. Also when the paper chromatograms from the hydrolysate were sprayed with dinitrogen tetroxide and ammonia, or with diazotised sulphanilic acid and sodium hydroxide solution, a coloured spot (R_F 0·65 in solvent 2 and 0·63 in solvent 4) was observed which corresponded with L-tyrosine (R_F 0·65 in solvent 2 and 0·64 in solvent 4). Hydrolysis of other peptide derivatives gave analogous results.

Stability of 5-*Acetyluracil*-1-*acetic Acids to Acid Hydrolysis.*—Stock solutions of the acetyluracil-1-acetic acids derived from glycine, L-leucine, L-histidine, and L-aspartic acid containing 20 mg. in 100 ml. of 6N-hydrochloric acid were prepared. Portions of each of these were diluted to a concentration of 1 mg. per 100 ml. of 0·5N-hydrochloric acid, and the absorption spectra of these solutions were measured. Portions of the stock solutions were boiled under reflux for 7 hr., then diluted to the same concentration as the standards, and the absorption spectra were measured. The results, recorded in Table 3, indicated quantitative retention of the uracil structure in all cases.

TABLE 3. *Stability of* 5-*acetyluracil*-1-*acetic acids* (V) *to acid hydrolysis.*

		Absorption spectra			
		Before hydrolysis		After hydrolysis	Recovery [a]
R	Generating acid	$\lambda_{\max.}$ (mμ)	ε	$\lambda_{\max.}$ (mμ)	(%)
H	Glycine	229	11,350	228	101
		283	14,500	283	
Bu^i	L-Leucine	230	9,300	230	102
		286	12,650	286	
$C_3H_3N_2{\cdot}CH_2$	L-Histidine	223	12,650	223	100
		284	14,300	284	
$HO_2C{\cdot}CH_2$	L-Aspartic acid	230	9,100	230	99·5
		282	12,600	282	

[a] An average of the yields calculated from measurements of the extinction coefficients at both maxima.

Determination of the N-*Terminal Residues in Insulin and Ribonuclease.*—(a) *Insulin.* A solution of insulin (0·09 g.) in water (1 ml.) and triethylamine (0·05 ml.) with the ethoxyacrylamide (0·025 g.) was set aside at room temperature with occasional shaking for 1 hr. The solution was then acidified with hydrochloric acid to give a solid precipitate. This was centrifuged off and washed successively with small amounts of water, acetone, and ether, and collected by centrifugation. The derivative (0·02 g.) was boiled under reflux with 6N-hydrochloric acid (4 ml.) for 7 hr. The solution was evaporated to dryness, and the residue dissolved in water and extracted with ethyl acetate. The extract was evaporated and the residue chromatographed on paper. Two absorbing spots only were observed with R_F values in solvent 1 0·45 and 0·83, and in solvent 2 0·58 and 0·75 which corresponded with the acetyluracils derived from glycine (R_F 0·45 in solvent 1 and 0·58 in solvent 2), and L-phenylalanine (R_F 0·83 in solvent 1 and 0·75 in solvent 2).

(b) *Ribonuclease.* A solution of ribonuclease (0·05 g.) in water (1 ml.) and triethylamine (0·05 ml.) with the ethoxyacrylamide (0·025 g.) was set aside at room temperature with occasional shaking for 1 hr. The solution was acidified to give a solid precipitate which was centrifuged off and washed with water, acetone, and ether. The dried material weighed 0·05 g. The derivative (0·05 g.) was boiled under reflux with 6N-hydrochloric acid (5 ml.) for 7 hr., then evaporated to dryness. The residue was dissolved in water and the solution extracted with ethyl acetate. Chromatography of the extract on paper showed one absorbing spot (R_F 0·59 and 0·7 in solvents 1 and 2 respectively) which corresponded to the bisacetyluracil derivative of L-lysine (R_F 0·59 and 0·7 in solvents 1 and 2). The aqueous solution from the hydrolysis was also chromatographed on paper; a single absorbing spot (R_F 0·34 in solvent 1) was observed which corresponded to the ε-monouracil derivative of L-lysine (R_F 0·34 in solvent 1). A solution of the last-mentioned uracil admixed with the bisuracil was obtained as follows. A solution of L-lysine monohydrochloride (1·8 g.) in water (5 ml.) was treated with cupric carbonate (2 g.). After 5 min., 2N-sodium hydroxide (5 ml.) was added, giving a deep blue solution. This was filtered, then treated with portions of 2N-sodium hydroxide (total 12 ml.) and the ethoxyacrylamide (total 2·3 g.) alternately during 30 min. with ice-cooling. The solution was set aside overnight at 0°, filtered from a small amount of precipitate, and acidified with 10N-hydrochloric acid (5 ml.). The solution was again filtered, boiled, and treated with hydrogen sulphide. The cupric sulphide was removed, and the solution evaporated to a gum. A portion of this was chromatographed on paper; two absorbing spots were observed (R_F 0·58 and 0·34 in solvent 1); the first of these corresponded to the bisuracil from L-lysine (R_F 0·59), and the second to the ε-monouracil derivative. The formation of the latter material from ribonuclease confirms the structure.

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