Polymers of Some Basic and Acidic α-Amino-acids.

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The preparation of polymers and copolymers of L-lysine, L-glutamic acid, and L-tyrosine is recorded.

The preparation of some polymers containing acidic and basic amino-acids, by standard and by modified methods, presented minor anomalies.

Digestion of poly(benzyl glutamate) (Hanby, Waley, and Watson, *J.*, 1950, 3239) with hydrogen chloride–acetic acid for 8 hours at 60° (cf. Ben-Ishae and Berger, *J. Org. Chem.*, 1952, **17**, 1564) led to a product which dissolved in bicarbonate solution. Purification and analysis revealed, however, that the polymer was only partially debenzylated. An attempt was consequently made to prepare the γ-methylbenzyl esters of polyglutamic acid in the hope that such compounds might be more readily hydrolysed by the hydrochloric acid treatment. Only small yields of the *m*-methylbenzyl ester resulted when glutamic acid was esterified with *m*-tolylmethanol or when copper glutamate was treated with the appropriate halide (Hanby *et al.*, *loc. cit.*). No better results were obtained by using the *p*-methylbenzyl ester, which proved markedly unstable, decomposing on simple crystallisation. We, therefore, turned again to poly(benzyl glutamate) and found that when the material was suspended in hydrobromic–acetic acid dissolution slowly occurred followed by separation of poly-L-glutamic acid. Van Slyke analyses showed that no decrease in chain length or cyclisation of the *N*-terminal residue had taken place (cf. Hanby *et al.*, *loc. cit.*); racemisation was not observed.

Attempts to prepare poly-L-tyrosine from *ON*-dibenzyloxycarbonyl-L-tyrosine were not pursued as yields of the latter compound were poor. Reaction of tyrosine with one mol. of a cupric salt in the presence of exactly one mol. of alkali hydroxide (larger quantities caused precipitation of the complex) gave a soluble copper complex which afforded *O*-benzyloxycarbonyl-L-tyrosine in satisfactory yield (cf. Kurtz, *J. Biol. Chem.*, 1937, **122**, 477; 1949, **180**, 1253; Neuberger and Sanger, *Biochem. J.*, 1943, **37**, 515), and with carbonyl chloride in dioxan this gave the *N*-carboxyanhydride, smoothly converted into poly-(*O*-benzyloxycarbonyl-L-tyrosine). This polymer was insoluble in acetic acid and unaffected by hydrogen bromide–acetic acid at room temperature. At 50° the benzyloxycarbonyl group was removed without obvious dissolution of the material, to give a poly-L-tyrosine which was slightly discoloured. As the benzyloxycarbonyl-polypeptide was soluble in phenol, attempts were made to effect removal of the blocking group with hydrogen chloride in phenol at 60°; but the quality of the product was not improved.

Better results followed the use of acetyl as the protecting residue. *O*-Acetyl-*N*-benzyloxycarbonyl-L-tyrosine (Bergmann, Zervas, Salzmann, and Schleich, *Z. physiol. Chem.*, 1934, **124**, 22) was converted into *O*-acetyl-L-tyrosine *N*-carboxyanhydride by treatment with thionyl chloride in acetic anhydride (cf. Bailey, *J.*, 1950, 3461) and thence into poly-(*O*-acetyl-L-tyrosine). The last compound dissolved in hydrogen bromide–acetic acid at room temperature with separation, after *ca.* 5 hours, of poly-L-tyrosine, which was not discoloured. Its chain length, as shown by amino-nitrogen determinations, had not been affected by the hydrolytic treatment. The material differed from the poly-L-tyrosine described by Katchalski and Sela (*J. Amer. Chem. Soc.*, 1953, **75**, 5284) (see p. 235).

The benzyloxycarbonyl groups of poly-[1-ε-benzyloxycarbonyl-L-lysine : 1-(*O*-acetyl-L-tyrosine)] amide,* poly-[1-ε-benzyloxycarbonyl-L-lysine : 1-(γ-benzyl L-glutamate)] amide, and poly-[1-*O*-acetyl-L-tyrosine : 3-(γ-benzyl L-glutamate)], prepared *via* the *N*-carboxyanhydrides, were completely removed by hydrogen bromide–acetic acid at room temperature overnight. Poly-[1-*O*-acetyl-L-tyrosine : 1-(γ-benzyl L-glutamate)], after unmasking, was soluble

* The numeral before each component of the polymer indicates the presumed molar proportion of the component in the polymer.

only in caustic alkali : it was, consequently, impossible to show that complete deacetylation had been effected by the acid treatment; that this had occurred was, however, rendered likely by the observation that poly-[1-*O*-acetyl-L-tyrosine : 3-(γ-benzyl L-glutamate)] was fully deacetylated by similar treatment. Poly-(1-L-lysine : 1-L-tyrosine : 1-L-glutamic acid) proved to be completely soluble only in alkali hydroxide.

Copolymers of ε-benzyloxycarbonyl-L-lysine or γ-benzyl L-glutamate with L-cystine gave discoloured products on treatment with hydrogen bromide–acetic acid or with anhydrous hydrogen chloride. Poly-L-histidine could not be prepared. 1'-Benzyl-L-histidine failed to react with carbonyl chloride, whilst its α-benzyloxycarbonyl-derivative gave a crystalline product with phosphorus pentachloride that proved too unstable for identification.

Experimental.

O-Benzyloxycarbonyl-L-tyrosine.—L-Tyrosine (9·0 g., 0·05 mol.) in N-sodium hydroxide (100 ml.; 0·1 mol.) was treated with copper sulphate pentahydrate (6·25 g., 0·025 mol.) in water (25 ml.), and the mixture stirred until the precipitated material had dissolved, then cooled in ice. Benzyl chloroformate (8·6 g., 0·05 mol.) was added in 3 portions during 20 min., with vigorous stirring, the copper benzyloxycarbonyl-complex being precipitated. Stirring was continued for a further 20 min., then the mixture was acidified with concentrated hydrochloric acid in order to dissolve the copper salts from the solids and was stirred for 2 hr., and the product was collected and recrystallised from acetic acid. The analytical sample was crystallised from hot water. *O-Benzyloxycarbonyl-L-tyrosine* had m. p. 215° (decomp.) (Found: C, 65·0; H, 5·4; N, 4·6. $C_{17}H_{17}O_5N$ requires C, 64·8; H, 5·4; N, 4·4%). It was converted into *O*-benzyloxycarbonyl-*N*-carboxy-L-tyrosine anhydride, m. p. 106° (decomp.). Katchalski and Sela (*loc. cit.*) give m. p. 101° (decomp.).

O-Acetyl-N-carboxy-L-tyrosine anhydride (cf. Farthing, *J.*, 1950, 3213).—*O*-Acetyl-*N*-benzyloxycarbonyl-L-tyrosine (8 g.) (Bergmann *et al.*, *loc. cit.*) was suspended in acetic anhydride (20 ml.), and thionyl chloride (11 ml.) added. The mixture was warmed to 40—50° and there maintained until dissolution was complete and effervescence had ceased, then cooled. Dry ether (20 ml.) and light petroleum (150 ml.; b. p. 40—60°) were added, the anhydride crystallising. The product (4·4 g., 79%) had m. p. 119—121° (decomp.) after two crystallisations from ethyl acetate–light petroleum. Bailey (*loc. cit.*) gives m. p. 120°.

Polymerisation of N-*Carboxy-anhydrides.*—(i) In dioxan (3·6 ml. per mol. of anhydride), 0·033 equiv. of ammonia in dioxan was used as initiator, with 16 hours' stirring at room temperature [cf. (*a*) Becker and Stahmann (*J. Amer. Chem. Soc.*, 1952, **74**, 38) whose work indicates that polymers of *ca.* 30 units chain length are formed under these conditions, (*b*) Sela and Berger (*ibid.*, 1953, **75**, 6350)]. One equiv. of 0·16N-hydrochloric acid was then added, the suspension stirred for 6 hr., and the polymer collected, washed with water, and dried *in vacuo*.

(ii) The amino-acid was refluxed overnight in boiling benzene saturated with water, then cooled, and the precipitated polymer was collected, washed with benzene and with ether, and dried *in vacuo*.

(iii) Polymerisation was effected in pyridine at room temperature for 6 days. After addition of ether the precipitated polymer was collected, washed with ether, and dried.

Chain-lengths, when given, are based on van Slyke amino-nitrogen analyses of the polymers obtained after removal of benzyloxycarbonyl-groups. With polymers containing lysine, the assumed chain lengths based on the ratio [anhydride : initiator] are given in parentheses.

Analytical figures for the polymers were seldom satisfactory (cf. Frankel, Cordova, and Breuer, *J.*, 1953, 1991), acetyl determinations being particularly bad.

Poly-(γ-benzyl L-Glutamate)$_{50}$ Amide.—γ-Benzyl *N*-carboxy-L-glutamic anhydride (11·10 g., 0·042 mol.) was polymerised in dioxan to give this *amide* (9·2 g., 100%) [Found: C, 65·1; H, 6·0; N, 6·3. $(C_{12}H_{13}O_3N)_{50},NH_3$ requires C, 65·6; H, 6·0; N, 6·5%].

Poly-(γ-benzyl L-Glutamate)$_{35}$ Amide.—The same anhydride (5·0 g., 0·0189 mol.) in dioxan gave the 35-*amide* (2·7 g., 65%) [Found: C, 64·9; H, 6·0; N, 7·2. $(C_{12}H_{13}O_3N)_{35},NH_3$ requires C, 65·6; H, 6·0; N, 6·6%].

Poly-(O-benzyloxycarbonyl-L-tyrosine)$_{104}$.—This *polymer* was obtained (3·55 g., 92%) from the monomer (4·45 g., 0·013 mol.) in benzene (150 ml.) [Found: C, 68·2; H, 5·1; N, 4·9. $(C_{17}H_{15}O_4N)_{104},H_2O$ requires C, 68·6; H, 5·0; N, 4·7%].

Poly-(O-acetyl-L-tyrosine)$_{79}$.—*O*-Acetyl-*N*-carboxy-L-tyrosine anhydride (3·68 g., 0·015 mol.)

was polymerised in benzene (90 ml.) to give the *poly*-(O-*acetyl*-L-*tyrosine*) (2·84 g., 94%) [Found: C, 63·2; H, 5·6; N, 7·2. $(C_{11}H_{11}O_3N)_{79},H_2O$ requires C, 64·3; H, 5·4; N, 6·8%].

Poly-(O-*acetyl*-L-*tyrosine*)$_{32}$ *Amide*.—The anhydride (2·85 g., 0·011 mol.) was polymerised in dioxan, yielding this *amide* (1·34 g., 57%) [Found: C, 58·3; H, 5·7; N, 7·2; Ac, 27·0. $(C_{11}H_{11}O_3N)_{32},NH_3$ requires C, 64·2; H, 5·4; N, 7·0; Ac, 20·9%].

Poly-(O-*acetyl*-L-*tyrosine*)$_{19}$.—The anhydride (3·35 g., 0·013 mol.) was polymerised in dioxan, giving the 19-*polymer* (1·6 g., 58%) [Found: C, 62·5; H, 5·6; N, 7·1; Ac, 24·3. $(C_{11}H_{11}O_3N)_{19},H_2O$ requires C, 64·1; H, 5·4; N, 6·8; Ac, 20·9%].

Poly-[1-ε-*benzyloxycarbonyl*-L-*lysine*: 1-(γ-*Benzyl* L-*Glutamate*)]$_{30}$ *Amide* (*A*).—γ-Benzyl *N*-carboxy-L-glutamic anhydride (3·70 g., 0·014 mol.) and ε-benzyloxycarbonyl-*N*-carboxy-L-lysine anhydride (4·3 g., 0·014 mol.) in dioxan gave the *copolymer* (6·05 g., 89%) [Found: C, 63·9; H, 6·6; N, 9·0. $(C_{14}H_{18}O_3N_2,C_{12}H_{13}O_3N)_{15},NH_3$ requires C, 64·7; H, 6·5; N, 8·9%].

Poly-(1-ε-*benzyloxycarbonyl*-L-*lysine*: 1-O-*Acetyl*-L-*tyrosine*)$_{30}$ *Amide* (*B*).—*O*-Acetyl-*N*-carboxy-L-tyrosine anhydride (3·63 g., 0·015 mol.) and ε-benzyloxycarbonyl-*N*-carboxy-L-lysine anhydride (4·46 g., 0·015 mol.) in dioxan yielded this 30-*polymer* (6·62 g., 97%) [Found: C, 63·0; H, 6·3; N, 9·1; OAc, 16·8. $(C_{14}H_{18}O_3N_2,C_{11}H_{11}O_3N)_{15},NH_3$ requires C, 64·1; H, 6·3; N, 9·2; OAc, 9·2%].

Poly-[3-(γ-*benzyl* L-*glutamate*): 1-*O*-*acetyl*-L-*tyrosine*]$_{19}$ *amide* (*C*) was obtained (9·05 g., 96%) from the ester anhydride (8·64 g., 0·032 mol.) and the foregoing tyrosine anhydride (2·72 g., 0·011 mol.) by copolymerisation in dioxan [Found: C, 64·5; H, 5·9; N, 6·9; Ac, 13·8. $(C_{12}H_{13}O_3N)_{37},(C_{11}H_{11}O_3N)_{12},NH_3$ requires C, 65·3; H, 5·9; N, 6·6; Ac, 5·0%].

Poly-[1-ε-*benzyloxycarbonyl*-L-*lysine*: 1-(γ-*benzyl* L-*glutamate*): 1-O-*acetyl*-L-*tyrosine*]$_{30}$ *Amide* (*D*).—The foregoing components (4·69 g., 0·018 mol.; and 4·44 g., 0·018 mol., respectively), and ε-benzyloxycarbonyl-*N*-carboxy-L-lysine anhydride (5·45 g., 0·018 mol.) were copolymerised in dioxan, yielding this *polymer* (10·85 g., 89%) [Found: C, 63·6; H, 6·2; N, 8·5; Ac, 15·1. $(C_{14}H_{18}O_3N_2,C_{12}H_{13}O_3N,C_{11}H_{11}O_3N)_{10},NH_3$ requires C, 64·5; H, 6·2; N, 8·3; Ac, 6·25%].

Removal of the Protective Groups.—The following procedure was used for all the polymers except one (see below). The polymer was suspended in glacial acetic acid (5 pt.) and warmed to 50°. Partial dissolution generally occurred, the insoluble fraction swelling so that it could be finely dispersed. The mixture was allowed to cool, dissolved polymer separating as a gel. Then hydrobromic acid in acetic acid (25 pt. of 50% w/v) was added. Dissolution occurred in 5—15 min., after which separation of solids began as follows: polyglutamic acid 1—2 hr.; polytyrosine 4—5 hr.; copolymers of lysine 10—20 min. No separation occurred with copolymers of glutamic acid or of tyrosine except when lysine was also present. Next morning excess of ether was added, and the precipitated material collected by centrifugation, or, if oily, by decantation, and was washed well with ether and dried *in vacuo* over potassium hydroxide.

The 104-polymer did not dissolve in acetic acid. Its suspension in hydrobromic–acetic acid (as above) was warmed at 50° for 4 hr.

During hydrogen bromide treatment of the 35- and of the 19-polymer, samples were periodically assayed for chain-length by van Slyke amino-nitrogen determination. No decrease in chain-length was observed.

Polymers were purified as indicated below.

Poly-L-*glutamic acid*. (i) The crude polymer (from 4·7 g.) was dissolved in 5% potassium hydrogen carbonate solution, charcoal was added, and the mixture filtered through a Sterimat. Acidification precipitated polyglutamic acid which was centrifuged off, and washed well with water containing a trace of hydrochloric acid, and finally with water. The *polymer* was not dialysed to remove salt, as earlier experiments showed that preparations had a tendency to dissolve on dialysis against tap-water. It was dried *in vacuo*, to yield poly-(L-glutamic acid)$_{50}$ amide, $[\alpha]_D^{25}$ −83·3° (*c*, 1·018 in 2% aqueous $KHCO_3$) [Found: C, 43·9; H, 6·0; N, 11·2; amino-N, 0·216. $(C_5H_7O_3N)_{50},NH_3$ requires C, 46·4; H, 5·5; N, 11·0; amino-N, 0·216%].

A sample (0·2779 g.) of this polymer was hydrolysed for 6 hr. with boiling 46—48% hydrobromic acid (10 ml.). The solution was made up to 25 ml. with water, giving α_D^{25} +1·214° (4-dm. tube); the product had N (Kjeldahl) 1·147 mg./ml., hence $[\alpha]_D^{25}$ is +25·19°. L-Glutamic acid, given similar treatment, had $[\alpha]_D^{25}$ +24·52°.

(ii) The 35-polymer (0·75 g.), similarly treated, gave *poly*-(L-*glutamic acid*)$_{35}$ *amide* (0·24 g., 54%), $[\alpha]_D^{18}$ −90·5° (*c*, 1·026 in 2% aqueous $KHCO_3$) [Found: C, 44·7; H, 5·8; N, 9·4; amino-N, 0·311. $(C_5H_7O_3N)_{35},NH_3$ requires C, 36·3; H, 5·5; N, 11·1; amino-N, 0·309%].

Poly-L-*tyrosine*.—(i) The crude benzyloxycarbonyl 104-polymer (3·2 g.) was dissolved in the minimum quantity of N-sodium hydroxide; the solution was immediately treated with charcoal, filtered through a Sterimat, and acidified with dilute hydrochloric acid. The precipitated poly-

mer was centrifuged off, suspended in water, and dialysed against tap-water and finally against distilled water. *Poly*-(L-*tyrosine*)$_{104}$ (730 mg., 42%) was collected by centrifugation and dried *in vacuo*, having $[\alpha]_D^{26}$ +40·9° (*c*, 1·056 in N-NaOH) [Found: C, 63·6; H, 5·9; N, 8·0; amino-N, 0·083. $(C_9H_9O_2N)_{104},H_2O$ requires C, 66·2; H, 5·6; N, 8·6; amino-N, 0·082%].

(ii) The acetyl 79-polymer (2·84 g.) yielded *poly*-(L-*tyrosine*)$_{79}$ (1·0 g., 44%), $[\alpha]_D^{25}$ +32·2° (*c*, 1·014 in N-NaOH) [Found: C, 63·8; H, 5·8; N, 8·1; amino-N, 0·109. $(C_9H_9O_2N)_{79},H_2O$ requires C, 66·1; H, 5·6; N, 8·6; amino-N, 0·109%].

(iii) The acetyl 32-polymer (1·0 g.) yielded *poly*-(L-*tyrosine*)$_{32}$ *amide* (0·4 g., 50%), $[\alpha]_D^{26}$ +33·9° (*c*, 1·02 in N-NaOH) [Found: C, 60·7; H, 5·6; N, 7·4; amino-N, 0·265. $(C_9H_9O_2N)_{32},NH_3$ requires C, 66·0; H, 5·6; N, 8·8; amino-N, 0·267%].

(iv) The acetyl 19-polymer (0·65 g.) gave *poly*-(L-*tyrosine*)$_{19}$ (350 mg., 68%), $[\alpha]_D^{26}$ +17·8° (*c*, 1·01 in N-NaOH) [Found: C, 64·1; H, 5·7; N, 9·0; amino-N, 0·445. $(C_9H_9O_2N)_{19},H_2O$ requires C, 65·9; H, 5·6; N, 8·5; amino-N, 0·449%]. A sample (0·2535 g.) of poly-(L-tyrosine)$_{79}$ was hydrolysed under reflux for 28 hr. with 46—48% hydrobromic acid (15 ml.). The solution was filtered from a trace of insoluble matter and made up to 25 ml. with water. The product had $[\alpha]_D^{23}$ −0·072° (1-dm. tube), and N (Kjeldahl) 0·731 mg./ml., hence $[\alpha]_D^{23}$ −7·6°. L-Tyrosine, given similar treatment, had $[\alpha]_D^{25}$ −7·0°.

All rotations quoted for polytyrosine samples were read 20 min. after the solutions had been made up. It was found that rotations in N-sodium hydroxide at room temperature fell by *ca.* 10% after 5 hr., by 20% after 12 hr., and by 50% after 40 hr.

There is an indication from the values quoted above that rotation varies with chain length. Katchalski and Sela (*loc. cit.*), however, quote $[\alpha]_D^{20}$ +8·7° for a 75-polymer. They list a variety of solvents for this polytyrosine preparation. Our own preparations, in contrast, proved only partly soluble in these, dissolving completely only in caustic alkali.

Poly-[1-L-*lysine hydrobromide* : 1-(L-*glutamic acid*)]$_{30}$ *Amide*.—The crude polymer, obtained by hydrogen bromide treatment (of 4·9 g.), was dissolved in the minimum quantity of water and reprecipitated with excess of ethanol. It was collected and washed thoroughly with alcohol and then with ether. After being dried *in vacuo* the *polymer* (2·5 g., 73%) was dissolved in water, the solution was treated with charcoal, filtered through a Sterimat, and freeze-dried; the product had $[\alpha]_D^{15}$ −37·9° (*c*, 1·012 in H_2O) [Found: C, 37·6; H, 6·6; N, 9·7; Br, 24·7; amino-N, 3·9. $(C_6H_{13}ON_2Br,C_5H_7O_3N)_{15},NH_4Br$ requires C, 38·3; H, 5·9; N, 12·5; Br, 24·7; amino-N, 4·3%].

A solution of the polymer was titrated electrometrically against 0·089N-sodium hydroxide. A smooth sigmoid curve was obtained with neutralisation point at pH 7·2 (CO_2H: found, 13·5; calc., 13·1%).

Poly-(1-L-*lysine hydrobromide* : 1-L-*tyrosine*)$_{30}$ *Amide*.—The crude polymer obtained by hydrogen bromide treatment of the copolymer (B) (5 g.) was purified as for the copolymer (A), giving a *product* (6·62 g., 60%), $[\alpha]_D^{23}$ −19·5° (*c*, 1·046 in H_2O) [Found: C, 38·1; H, 5·6; N, 10·7; Br, 25·5; amino-N, 3·42; Ac, 0. $(C_6H_{13}ON_2Br,C_9H_9O_2N)_{15},NH_4Br$ requires C, 47·6; H, 5·9; N, 11·3; Br, 22·5; amino-N, 3·94%].

Electrometric titration of the polymer with 0·089N-sodium hydroxide did not give a smooth curve, precipitation occurring during the pH range 7·5—10·5. Ultra-violet absorption measurements gave tyrosine residue 31·4 (required 43·1%). It would thus appear that fractionation of the polymer occurred during purification, to give material with the constitution poly-(19-L-lysine hydrobromide : 11-L-tyrosine)$_{30}$ amide (Required: C, 43·7; H, 6·0; N, 11·9; Br, 27·2%).

Poly-[3-(L-*glutamic acid*) : 1-L-*tyrosine*)$_{49}$ *Amide*.—The crude polymer from hydrogen bromide treatment of the copolymer (C) (8·05 g.) was dissolved in 5% potassium hydrogen carbonate solution (100 ml.) and the solution was treated with charcoal, filtered through a Sterimat, and acidified with dilute hydrochloric acid. The precipitated *product* (2·44 g., 47%) was collected, washed well with water, and dried *in vacuo*, then having $[\alpha]_D^{25}$ −40·6° (*c*, 1·014 in 2% aqueous $KHCO_3$) [Found: C, 48·9; H, 5·5; N, 10·3; amino-N, 0·209; Ac, 0·0 $(C_9H_9O_2N)_{12},(C_5H_7O_3N)_{27},NH_3$ requires C, 52·1; H, 5·8; N, 10·3; amino-N, 0·207%]. Ultra-violet absorption measurements gave a tyrosine residue content of 31·8% (required 29·5%).

Poly-[1-L-*lysine* : 1-(L-*glutamic acid*) : 1-L-*tyrosine*)$_{30}$ *Amide*.—The crude polymer from hydrogen bromide treatment of the copolymer (D) (9·0 g.) was dissolved by adding N-sodium hydroxide (35 ml.) to its suspension in water (200 ml.). After treatment with charcoal, the mixture was filtered through a Sterimat and adjusted to pH 5 by hydrobromic acid. The precipitated polymer was collected by centrifugation, washed with water, 75% ethanol, and absolute ethanol, and finally dried *in vacuo* to give a partial *hydrobromide* (3·3 g., 50%), $[\alpha]_D^{20}$

$-35{\cdot}7°$ (*c*, 1·016 in N-NaOH) [Found: C, 53·2; H, 7·3; N, 11·2; Br, 4·2; Ac, 0. $(C_6H_{13}ON_2,C_5H_7O_3N,C_9H_9O_2N)_{10},NH_3,Br_{2\cdot3}$ requires C, 54·4; H, 6·7; N, 13·0; Br, 4·2%]. Tyrosine residue (by ultra-violet spectra) 35·6% (required 36·9%).

1'-Benzyl-α-benzyloxycarbonyl-L-histidine.—1'-Benzyl-L-histidine (1·85 g., 0·0075 mol.) (Du Vigneaud and Behrens, *J. Biol. Chem.*, 1937, **117**, 27), dissolved in N-sodium hydroxide (7·5 ml.), was treated at 0° with benzyl chloroformate (1·3 g., 0·0075 mol.) and N-sodium hydroxide (7·5 ml.) during 20 min. with stirring; a white precipitate separated. After 30 min. the mixture was acidified and the *1'-benzyl-α-benzyloxycarbonyl-L-histidine* (1·9 g., 66%) collected; it had m. p. 216° (decomp.) after crystallisation from pyridine (Found: C, 66·3; H, 5·4; N, 11·3. $C_{21}H_{21}O_4N_3$ requires C, 66·5; H, 5·6; N, 11·1%).

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