

## XV.—*Butyl Esters of the Simpler Amino-acids.* *Part I.*

By WALTER THOMAS JAMES MORGAN.

HITHERTO, with few exceptions, only the ethyl esters of the amino-acids have been prepared and examined (Curtius, *Ber.*, 1883, **16**, 753; 1884, **17**, 953; *J. pr. Chem.*, 1888, **37**, 150; Fischer, *Ber.*, 1901, **34**, 433). Butyl esters have now been prepared. These are more stable than the corresponding ethyl esters—inasmuch as quantities up to 20 g. distil almost completely, whereas ethyl esters distilled under similar conditions invariably leave a brown residue—and are much less volatile in ether vapour under diminished pressure. They should be, therefore, the more suitable form in which the monoamino-acids produced by the hydrolysis of proteins may be estimated. Since the application of this method for the separation of the monoamino-acids of protein is now unavoidably postponed, it seems desirable to publish the work so far completed.

### EXPERIMENTAL.

*n-Butyl Glycine Hydrochloride.*—Glycine (5 g.) suspended in 100 g. of dried and carefully fractionated butyl alcohol was esterified by the method of Curtius (*Ber.*, *loc. cit.*). Several similar preparations of the hydrochloride were made using the dry lead salt of the acid. The finely divided lead chloride that separates has, according to Foreman (*Biochem. J.*, 1919, **13**, 378), a favourable catalytic effect

on the rate of esterification. The mixture saturated with hydrogen chloride was heated on a boiling water-bath until the amino-acid dissolved, the excess of butyl alcohol was then distilled under reduced pressure, and the pale yellow syrup of the *ester hydrochloride* crystallised from ethyl acetate. The solid so obtained was quickly ground, kept for 2 days over solid potassium hydroxide, and crystallised from ether-ethyl acetate (1 : 2), separating in flat laminæ, m. p. 69—71°. (All melting-point determinations were carried out in sealed capillary tubes owing to the hygroscopic nature of the ester hydrochloride.) It dissolved readily in water, alcohol, acetone, or ethyl acetate, but was only sparingly soluble in ether or chloroform (Found : N, 8.2; Cl, 21.1.  $C_6H_{14}O_2NCl$  requires N, 8.3; Cl, 21.2%).

*n-Butyl Glycine*,  $NH_2 \cdot CH_2 \cdot CO \cdot O \cdot C_4H_9$ .—To a vigorously shaken suspension of the hydrochloride (21.5 g.) in dry ether (250 c.c.) at 0°, anhydrous baryta was added, in portions of about 1 g., until it was in excess; even so, some hydrochloride remained, but this decomposed on addition of 5 c.c. of 70% potassium hydroxide solution. The whole was immediately shaken and filtered and the residue, consisting of baryta, barium chloride and a little undissolved ester, was shaken with two further quantities of ether. The combined ethereal filtrates were concentrated under reduced pressure to about 40 c.c., the temperature being kept below 30° to prevent volatilisation of the ester with the ether vapour. The ester distilled at 81—81.5°/18 mm. as a colourless oil having the characteristic odour of all amino-acid esters (yield, 14 g. or 83%;  $d^{16}$  0.9703). The ester is soluble in water, ether, or alcohol (Found : N, 10.6.  $C_6H_{13}O_2N$  requires N, 10.7%).

In a sealed glass tube, the freshly distilled ester (5 g.) changed completely in 24 hours into a white, crystalline solid. This substance gave an intense biuret reaction, charred without melting at 240°, and was insoluble in alcohol, chloroform, glacial acetic acid, or benzene but fairly easily soluble in hot water (Found in material washed free from butyl alcohol : C, 48.6; H, 7.7. Triglycylglycine butyl ester requires C, 47.7; H, 7.3%). A further preparation of this compound is being carried out, when it is hoped to recrystallise the substance and determine its molecular weight.

The *picrate* was prepared by warming together at 60° for  $\frac{1}{4}$  hour an alcoholic solution of the ester (3 g.) and a solution of picric acid (5.2 g.) in 50% alcohol. On cooling, the *picrate* separated in pale yellow laminæ, m. p. 113°, which were very soluble in acetone, somewhat soluble in chloroform or ether, and insoluble in light petroleum or ligroin (Found : N, 15.6.  $C_{12}H_{16}O_9N_4$  requires N, 15.5%).

The *benzoquinone* derivative,  $(C_4H_5 \cdot O \cdot CO \cdot CH_2 \cdot NH)_2 C_6H_2O_2$ ,

separated in scarlet needles from the dark red solution produced by the interaction of the ester (3 g.) and benzoquinone (4 g.) in ether at 0°. The crystals were washed with ether and freed from quinol by dissolution in chloroform; the quinone compound, m. p. 179—180°, was precipitated from the solution by light petroleum (Found: N, 7.6.  $C_{18}H_{26}O_6N_2$  requires N, 7.6%).

*n-Butyl carbamidoacetate*,  $H_2N \cdot CO \cdot NH \cdot CH_2 \cdot CO \cdot O \cdot C_4H_9$ , separated in tufts of colourless needles, m. p. 119°, when butyl glycine hydrochloride (2.8 g.), dissolved in a minimum of water, was warmed with a solution of potassium cyanate (1.7 g.) on a water-bath. It was soluble in hot ethyl alcohol, sparingly soluble in cold acetone, and insoluble in light petroleum or ligroin (Found: N, 16.1.  $C_7H_{14}O_3N_2$  requires N, 16.2%).

*isoButyl Glycine Hydrochloride*.—A suspension of glycine (15 g.) in dry *isobutyl* alcohol (250 c.c.) was saturated with hydrogen chloride, and solution completed by heating on a boiling water-bath for  $\frac{1}{2}$  hour. The subsequent procedure was as described in the case of the *n*-butyl ester hydrochloride. The *hydrochloride*, m. p. 84—88°, is very hygroscopic and quickly liquefies on exposure to air. It is readily soluble in alcohol or ethyl acetate, but only sparingly soluble in ether or chloroform (Found: N, 8.4; Cl, 21.2.  $C_6H_{14}O_2NCl$  requires N, 8.4; Cl, 21.2%).

*isoButyl glycine* was obtained from its hydrochloride (37 g., recrystallised from ether—ethyl acetate [2:1] and dissolved in 750 c.c. of dry ether) in the same way as the normal ester. The ether was dried over anhydrous potassium sulphate. The pure *ester* is a colourless, oily liquid with a characteristic odour (yield, 73%), b. p. 79.5—80.5°/18 mm.,  $d^{20}$  0.9609,  $d^{17}$  0.9618. It is readily soluble in water, alcohol, or chloroform (Found: C, 54.9; H, 9.9.  $C_6H_{13}O_2N$  requires C, 55.1; H, 9.9%).

The *picrate* was obtained by warming together an alcoholic solution of the ester (2 g.) and an aqueous-alcoholic solution of picric acid (1 mol.) and pouring the mixture into water. The *picrate* was crystallised from benzene, dried in a vacuum desiccator, and, as the melting point was not sharp, recrystallised from alcohol and was thus obtained in needles, m. p. 82—87°. It was only slightly soluble in ether or light petroleum (b. p. 40—60°) and insoluble in ligroin (Found: N, 15.5.  $C_{12}H_{16}O_9N_4$  requires N, 15.5%).

The *benzoquinone* derivative was prepared and purified as was the preceding isomeride. The scarlet needles obtained, m. p. 171—172°, were insoluble in hot or cold water or ligroin, somewhat soluble in ether, and readily soluble in cold chloroform or 50% aqueous alcohol (Found: C, 59.2; H, 7.3; N, 7.7.  $C_{18}H_{26}O_6N_2$  requires C, 59.0; H, 7.1; N, 7.7%).

Unless stated otherwise, the following substances were prepared in essentially the same ways as the corresponding compounds already described.

*n-Butyl dl-Alanine Hydrochloride*.—Prepared from racemic alanine (15 g.) and recrystallised from ethyl acetate, this compound melted at 58—61° (sealed tube), rapidly deliquesced in moist air, and was only slightly soluble in ligroin or ether but readily soluble in ethyl alcohol, benzene, chloroform, or acetone (Found : N, 7.7; Cl, 19.5.  $C_7H_{16}O_2NCl$  requires N, 7.7; Cl, 19.6%).

*n-Butyl dl-alanine* was obtained as a colourless, oily liquid, b. p. 81.5—82°/16 mm.,  $d^{20}$  0.9342 (yield 66%), having the usual characteristic odour. It is soluble in water, ether, alcohol, or chloroform. After being kept in a sealed glass tube for 3 weeks, the ester showed no signs of anhydride formation (Found : N, 9.7.  $C_7H_{15}O_2N$  requires N, 9.6%).

The *picrate*, m. p. 124°, crystallised in needles from 30% and 50% alcohol and in opaque, dendritic crystals from 5% alcohol (Found : N, 14.7.  $C_{13}H_{18}O_9N_4$  requires N, 14.9%).

Several attempts were made to isolate the benzoquinone derivative. A solution of the ester and benzoquinone in dry ether developed a bright scarlet colour, but after cooling for several hours in a freezing mixture nothing separated; on cooling in solid carbon dioxide and ether, crystals of quinol were deposited. Evaporation of the ether solution gave only a red syrup which could not be crystallised. Precipitation with light petroleum gave only a dark red oil. The isolation of this compound is being further studied.

*isoButyl dl-Alanine Hydrochloride*.—The syrup obtained was very difficult to crystallise even after drying in a vacuum desiccator over phosphoric oxide. Precipitation with ether of a solution of the hydrochloride in ethyl acetate gave a syrupy liquid which crystallised in flat, soft, soapy plates, m. p. 60—63° (sealed tube), which changed into a syrup on being exposed to air for a few minutes (Found : N, 7.6.  $C_7H_{16}O_2NCl$  requires N, 7.7%).

*isoButyl dl-alanine*, prepared from the ester hydrochloride (31 g.) and distilled at 77—78°/19 mm., was a colourless, oily liquid soluble in water, alcohol, ligroin, or chloroform (yield 95%);  $d^{20}$  0.9330 (Found : N, 9.7.  $C_7H_{15}O_2N$  requires N, 9.6%).

As in the case of the *n*-butyl ester, all attempts to isolate the quinone derivative failed.

The *picrate* was obtained quantitatively in small, flat plates, m. p. 132—133°, by warming the ester (2.5 g.) with a solution of picric acid (3.6 g.) in 50 c.c. of 50% alcohol at 70—80° for  $\frac{1}{2}$  hour and cooling over-night. It was soluble in alcohol, ether, or acetone,

sparingly soluble in chloroform, and insoluble in light petroleum or ligroin (Found : N, 15.1.  $C_{13}H_{18}O_9N_4$  requires N, 15.0%).

*n-Butyl dl-Valine Hydrochloride*.—Prepared from *dl*-valine (5 g.) and crystallised from ethyl acetate, the *hydrochloride* was obtained in colourless, deliquescent needles, m.p. 59—60°, which were readily soluble in alcohol or acetone and sparingly soluble in dry ether or light petroleum (Found : N, 6.7.  $C_9H_{20}O_2NCl$  requires N, 6.7%).

*n-Butyl dl-valine* was obtained from the hydrochloride (3.5 g.) in the usual way; the combined ethereal filtrates were dried with anhydrous sodium sulphate and the ether was evaporated below 25°. The *ester*, b. p. 98—98.5°/17 mm.,  $d^{14}$  0.9266, was a colourless, syrupy liquid (yield 97.5%) having the usual characteristic odour. It was soluble in water, alcohol, ether, or chloroform (Found : N, 8.3.  $C_9H_{19}O_2N$  requires N, 8.1%).

The *picrate* formed long, yellow needles, m. p. 91—92°, which were soluble in acetone, ether, or chloroform, somewhat soluble in benzene, and insoluble in light petroleum (Found : N, 13.9.  $C_{15}H_{22}O_9N_4$  requires N, 13.9%).

*n-Butyl dl-Leucine Hydrochloride*.—Leucine (6 g.) was esterified in the usual manner. After standing in the ice-chest for 48 hours, the alcoholic solution deposited flat, colourless plates, which were recrystallised from ether and dried in a vacuum desiccator; m. p. 110—111°. The *hydrochloride* was exceedingly hygroscopic, and very soluble in alcohol but only slightly soluble in chloroform or ether. It crystallised from ethyl acetate in stellate clusters of colourless needles (Found : N, 6.4; Cl, 16.0.  $C_{10}H_{22}O_2NCl$  requires N, 6.3; Cl, 15.9%).

*n-Butyl dl-leucine*, prepared from the hydrochloride (4 g.), was a clear, colourless, oily liquid (yield 69%), b. p. 113.5°/18 mm.,  $d^{15.5}$  0.8733, which was soluble in water, alcohol, ether, or chloroform (Found : N, 7.4.  $C_{10}H_{21}O_2N$  requires N, 7.5%).

The *picrate* was obtained in yellow needles, m. p. 139°, which were soluble in alcohol, ether, or acetone and insoluble in ligroin or benzene (Found : C, 46.1; H, 13.6.  $C_{16}H_{24}O_9N_4$  requires C, 46.2; H, 5.8; N, 13.5%).

*isoButyl dl-Leucine Hydrochloride*.—This compound was a clear, colourless syrup which slowly crystallised on standing for 2 or 3 days over solid caustic potash in a vacuum desiccator; it was recrystallised from ethyl acetate, washed with ether, and again dried in a vacuum desiccator (yield 81%); m. p. 97—99° (Found : N, 6.3; Cl, 15.7.  $C_{10}H_{22}O_2NCl$  requires N, 6.3; Cl, 15.9%).

*isoButyl dl-leucine*, b. p. 110°/19 mm.,  $d^{18.4}$  0.9066, was obtained as a colourless, oily liquid (yield 88%) which was soluble in alcohol,

chloroform, or ether (Found : C, 64.1; H, 11.0; N, 7.4.  $C_{10}H_{21}O_2N$  requires C, 64.2; H, 11.2; N, 7.5%).

The *picrate* crystallised from dilute alcohol in flat plates, m. p. 135—136°, which were soluble in hot water, chloroform, or ethyl alcohol, slightly soluble in cold water or ether, and insoluble in light petroleum (Found : C, 46.0; H, 6.0; N, 13.6.  $C_{16}H_{24}O_9N_4$  requires C, 46.2; H, 5.8; N, 13.5%).

The author wishes to express his gratitude to Dr. G. Dean for his kind interest at all times, and to the Chemical Society for assistance in the purchase of materials.

WEST HAM MUNICIPAL COLLEGE.

[Received, August 6th, 1925.]

---