VI. PREPARATION OF α -AMINO-ACIDS THROUGH α -OXIMINO-ESTERS

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AMINO-ACIDS free from biologically important impurities which may accompany those prepared from natural sources have been required in this department for the study of bacterial nutrition. Synthesis of simple amino-acids presents little difficulty by the usual routes, but serviceable methods are still required in certain cases, particularly if ring closure of intermediate derivatives is apt to occur. We have therefore extended to new compounds the ethyl acetoacetate synthesis of Bouveault & Locquin [1904; 1906] and Schmidt & Widman [1909] via α -oximino-esters, since this method has also the merit of accessible starting materials. Difficulties in the earlier work have been minimized by using the convenient catalytic reduction of oximino-esters applied by Harington & Randall [1931] to hydroxyglutamic acid. This reduction may have considerable biological interest, since oximinosuccinic acid has been isolated by Virtanen [1938] as an intermediary in the production of aspartic acid during nitrogen assimilation by legume bacteria, of which process it probably constitutes the first stage.

Glutamic acid has been successfully synthesized in an overall yield of 39% as compared with 18.5% in the best method hitherto available from ethyl malonate [Dunn *et al.* 1931], and the even smaller yields from other sources [cf. Dunn *et al.* 1931]. Hydroxyproline has been prepared, but the yield was not superior to that of Leuchs [1905]. The synthesis of threonine has been attempted, but was discontinued following the publication by Adkins & Reeve [1938] of a satisfactory synthesis of that acid by an analogous method. Other preparations have been deferred in accordance with the needs of our work.

EXPERIMENTAL

Glutamic acid

Diethyl- α -acetylglutarate was prepared by the following modification of the method of Clemo & Welch [1928]. To a cooled solution of sodium (4.03 g.; 0.175 mol.) in absolute alcohol (53 ml.) ethyl acetoacetate (44 ml.; 0.35 mol.) was gradually added, followed by ethyl β -chloropropionate (21 ml.; 0.175 mol.). The mixture was kept 3 hr. at room temperature and refluxed for 2 hr.; during the last $\frac{1}{2}$ hr. the pH of an aqueous solution of a drop of the mixture remained at 9. Alcohol was removed at 100°, NaCl which separated during the process dissolved in the minimum quantity of water and the aqueous solution extracted with ether. The ethereal extracts were washed with very dilute HCl, dried with CaCl_2 , evaporated and the residue distilled. Excess ethyl acetoacetate distilled first, and the glutaric ester (31.8 g.; 79% theory) at 146–150°/12 mm.

Diethyl- α -oximinoglutarate was prepared from the acetyl compound according to Wislicenus & Grutzner [1909].

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Glutamic acid. The freshly prepared oximino-ester (16 g.; 0.075 mol.), platinum oxide (0.4 g.; Adams *et al.* [1932]) and anhydrous Na_2SO_4 (15 g.) in acetic acid (60 ml.; redistilled) were shaken in hydrogen at a little over atmospheric pressure until the theoretical uptake had taken place (3 days). The solution was filtered, water added, the further precipitate of catalyst filtered off and the solution taken to dryness. The amino-ester remaining was immediately hydrolysed by refluxing with 5 *M* aqueous HCl (50 ml.), the product evaporated to 25 ml. under reduced pressure, cooled in ice and saturated with HCl; the glutamic acid hydrochloride was collected after keeping for 12 hr. at 0°. Yield 9.0 g. A further crop of about 1 g. was obtained from mother liquors.

The salt was dissolved in water, freshly distilled aniline (20 g.) added, the solution warmed and ethyl alcohol added till crystallization began. The glutamic acid was crystallized from water and washed with alcohol; m.p. 197°. Yield 7.8 g., i.e. 39% of the theoretical from the β -chloropropionic ester used. (Found: amino-N, 9.5%; $\text{C}_5\text{H}_9\text{O}_4\text{N}$ requires 9.5%. *Hydrochloride*, m.p. 198°; *picrolonate*: found, amino-N 3.4%; $\text{C}_5\text{H}_9\text{O}_4\text{N} \cdot \text{C}_{10}\text{H}_8\text{O}_5\text{N}_4$ requires 3.4%.)

Hydroxyproline

α -Oximino- δ -chloro- γ -valerolactone. α -Acetyl- δ -chloro- γ -valerolactone [Leuchs, 1911; b.p. 135–137°/1 mm.] (17.6 g.; 0.1 mol.) was added with vigorous stirring to conc. H_2SO_4 (20 ml.) cooled in ice-salt. Nitrosylsulphuric acid (12.7 g.; 0.1 mol.) in conc. H_2SO_4 (12 ml.) was added during $\frac{1}{2}$ hr., the whole poured on to crushed ice, the product extracted with ether, the ethereal solution washed quickly with water followed by aqueous Na_2CO_3 , dried and evaporated. The oxime remained as an oil, decomposing violently at 170° on attempted distillation but separating from benzene as pale yellow crystals. Yield, 11 g. (67%) m.p. 118°. (Found: N, 8.6%; $\text{C}_5\text{H}_8\text{O}_3\text{NCl}$ requires N, 8.6%.)

α -Amino- δ -chloro- γ -valerolactone salts. The oxime (8.2 g.; 0.05 mol.), platinum oxide (0.4 g.), anhydrous Na_2SO_4 (5 g.) and glacial acetic acid (50 ml.) were shaken in hydrogen for 3 days; the solution was filtered and evaporated and the residue rubbed with alcohol. The amine acetate separated and was recrystallized from aqueous alcohol, yielding 2.9 g. (0.014 mol.) of colourless prisms m.p. 177°. (Found: amino-N, 7.0%; $\text{C}_5\text{H}_8\text{O}_2\text{NCl} \cdot \text{C}_2\text{H}_4\text{O}_2$ requires 6.7%.) A similar yield of hydrochloride was obtained on reducing the oxime in alcoholic HCl.

Hydroxyproline. α -Amino- δ -chloro- γ -valerolactone acetate (4 g.) with saturated aqueous ammonia (80 ml.) was warmed at 30°. Titration of small portions indicated the halogen to be completely eliminated as Cl^- after 2 hr. Hydroxyprolines *a* and *b* were isolated according to Leuchs [1905]. Yields: *a*, 0.1 g., m.p. (d.) 254°. (Found: N, 10.4%; $\text{C}_5\text{H}_9\text{O}_3\text{N}$ requires 10.7%); *b*, 0.7 g., m.p. (d.) 244°. (Found: N, 10.5%.)

Threonine

Reduction of ethyl- α -oximinoacetoacetate. Reduction and hydrolysis under the conditions used by Harington & Randall [1931] yielded only α -aminobutyric acid; yield, 80%. (Found: N, 13.4%; $\text{C}_4\text{H}_9\text{O}_2\text{N}$ requires 13.6%.) It was not found possible to arrest the reduction at the hydroxy-acid stage.

Ethyl- α -amino- β -ketobutyrate hydrochloride. This intermediary in the above reduction was isolated in order to attempt its reduction by other methods. Ethyl isonitrosoacetoacetate (8.0 g.) palladium charcoal (3.7 g.) and alcoholic HCl (50 ml. 2*N*) were shaken in hydrogen. Theoretical uptake occurred in 5 hr. The solution was filtered and evaporated under reduced pressure to a few ml., when the hydrochloride separated in white prisms. It was recrystallized from

alcohol with the addition of light petroleum. Yield, 62%, M.P. 125°. (Found: N, 7.85%; $C_6H_{11}O_3N \cdot HCl$ requires 7.7%.) The salt exhibited the properties described by Gabriel & Posner [1894] and by Cherchez & Cherbuliez [1931] but was obtained in better yield and melted much higher than described by those authors. Attempted further reduction yielded only α -aminobutyric acid or diethyl-2:5-dimethylpyrazine-3:6-dicarboxylate.

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