276. *The Preparation of* dl-*Asparagine and* dl-*Aspartic Acid.*

By Wesley Cocker.

dl-Asparagine and *dl*-aspartic acid have been prepared as follows: An ethereal solution of the oxime of ethyl oxaloacetate prepared from its sodio-derivative was reduced by means of aluminium amalgam to ethyl aspartate. The latter was converted into asparagine by heating with concentrated aqueous ammonia in a sealed tube or alternatively was hydrolysed with water under pressure to yield aspartic acid. The overall yields of the products were 30% and 45% respectively.

The synthetic methods for the preparation of asparagine are largely due to Piutti (*Gazzetta*, 1887, **17**, 126; 1888, **18**, 472), who reduced the oxime of ethyl oxaloacetate with sodium amalgam in dilute acetic acid, thus obtaining a mixture of isomeric monoethyl aspartates, from which β-monoethyl aspartate was isolated through its copper salt. From this ester he obtained asparagine by heating with alcoholic ammonia. This procedure has been repeated and found to be tedious and expensive, and for the same reason a second method due to Piutti (*Ber.*, 1896, **29**, 2070) cannot be recommended to other workers. In the latter method maleic anhydride is heated with aqueous ammonia under pressure.

It has now been shown that ethyl aspartate of the highest purity can be obtained in 60% yield by reduction of an ethereal solution of the oxime of ethyl oxaloacetate with aluminium amalgam. No other materials were discovered in the reduction product and it is probable that the losses are due to incomplete reaction during oximation. The yield stated is much higher than that obtained by Enkvist (*Ber.*, 1939, **72**, 1930) by the reaction of fumaric or maleic acid with ammonia, followed by esterification of the product.

Many attempts were made to reduce the oxime catalytically with palladium charcoal in methyl alcohol, the time and the pressure of hydrogen being varied. Acid conditions did not assist and neither Raney nickel nor platinum effected reduction.

The conversion of ethyl aspartate into asparagine was performed with hot aqueous ammonia under pressure, and during this reaction the α-ester group was hydrolysed and the β-ester group was converted into the amido-group. This is in accordance with the work of E. Fischer on the ready hydrolysis of α-amino-esters. The yield of asparagine was 44%, calculated on the ester used, and the product was so pure that repeated crystallisation only raised the m. p. by 1°.

Aspartic acid has been prepared by heating ethyl aspartate with water under pressure at 150°. The aqueous solution thus obtained contained only aspartic acid, which was isolated in 70% yield. This method compares favourably with the method of Dunn and Fox (*J. Biol. Chem.*, 1933, **101**, 493) in which aspartic acid is produced in 60% yield by heating ethyl fumarate with alcoholic ammonia. The method now described is simpler.

Experimental.

Ethyl Oxaloacetate Oxime.—The sodio-derivative of ethyl oxaloacetate (5 g.) was added to a solution of hydroxylamine hydrochloride (2·4 g.; 1·5 mols.) in 5 c.c. of water, and the mixture shaken until solution of the sodio-derivative was complete. Barium carbonate (0·5 g.) was then added, and the mixture shaken for 1 hour. The liquid was filtered, and the precipitate washed with ether. The filtrate, containing oily drops of the oxime, was extracted thrice with ether and the combined extracts and washings (about 30 c.c.) were dried over sodium sulphate.

Ethyl Aspartate.—Aluminium foil (1·3 g.), cut into pieces about ½″ square and loosely folded, was amalgamated by Vogel's method (J., 1927, 597; see also Baker, this vol., p. 459) and covered with commercial " not for anæsthesia " ether. The ethereal solution of the oxime (above), decanted from the sodium sulphate, was added rapidly to the reducing agent contained in a flask fitted with a reflux condenser. The flask was immersed in cold water, and the mixture kept for 12—15 hours with periodical shaking during the first two or three hours. Cooling was necessary during reduction, since otherwise the reaction became violent and quantities of ammonia were produced. At the end of the reduction the liquid was filtered (pump), the sludge of aluminium hydroxide washed three times with ether, and the combined ethereal solutions freed from solvent on the water-bath. The residue was distilled under reduced pressure. After removal of traces of alcohol ethyl aspartate distilled as a colourless oil (2·7 g., 70% of the theoretical), b. p. 97—98°/1 mm. (Found: C, 50·5; H, 7·5; N, 7·7. Calc. for $C_8H_{15}O_4N$: C, 50·8; H, 7·9; N, 7·4%). The *phenylcarbamido*-derivative was prepared by adding phenyl *iso*cyanate (0·3 g.) to the ester (0·5 g.), stirred and cooled under running water. The white solid produced was collected, washed with a little cold benzene, and recrystallised from that solvent, forming clusters of colourless pointed prisms, m. p. 104° (Found: C, 58·5; H, 6·1; N, 8·9. $C_{15}H_{20}O_5N_2$ requires C, 58·4; H, 6·5; N, 9·1%).

Acetyl derivative. A mixture of ethyl aspartate (2 g.) and acetic anhydride (1 g.) was warmed on the water-bath for ½ hour and then distilled under reduced pressure. A colourless viscous oil was collected at 143—145°/4—5 mm., and this set to a colourless glass which refused to crystallise after standing for 8 weeks (Found: C, 51·2; H, 7·6; N, 6·0. $C_{10}H_{17}O_5N$ requires C, 52·0; H, 7·4; N, 6·0%).

Asparagine.—A mixture of ethyl aspartate (2·8 g.) and aqueous ammonia (40 c.c.; *d*, 0·880) was heated in a sealed tube immersed in boiling water for 24 hours. The clear liquid was then heated over a free flame to remove the excess of ammonia, evaporated on the water-bath to 15 c.c., and set aside to crystallise. Asparagine was collected in a series of crops (m. p. 296°), leaving a syrupy mother-liquor. Each crop was washed with a little cold dilute alcohol; the united crops crystallised from hot dilute alcohol in large hexagonal prisms (0·8 g., 44% of the theoretical), m. p. 297° (Piutti, *Gazzetta*, 1887, **17**, 126, gives m. p. 275°). For analysis the asparagine was dried for 4 hours at 110° (Found: C, 36·6; H, 6·4; N, 21·5. Calc. for $C_4H_8O_3N_2$: C, 36·4; H, 6·1; N, 21·2%). The presence of further quantities of asparagine in the syrupy mother-liquor was demonstrated by benzoylation as described below. Practically pure benzoyl-asparagine (about 0·5 g.) was obtained.

Benzoylasparagine. This was obtained by shaking a mixture of asparagine in dilute sodium bicarbonate solution with a slight excess of benzoyl chloride. The clear solution was then acidified, and the precipitate collected, dried, and freed from benzoic acid by repeated extraction with light petroleum (b. p. 40—60°). The residue crystallised from hot water in long colourless prisms, m. p. 190—191° (decomp.) (Found: C, 55·4; H, 5·0; N, 11·5. Calc. for $C_{11}H_{12}O_4N_2$: C, 55·9; H, 5·1; N, 11·9%) (Cherbuliez and Chambers, *Helv. Chim. Acta*, 1925, **8**, 398, give m. p. 190—196°, but state that benzoylasparagine prepared from asparagine in aqueous solution contains some benzoylaspartic acid).

Benzenesulphonylasparagine. Prepared according to the method of Berlingozzi and Carobbi (*Gazzetta*, 1930, **60**, 573), this crystallised from hot water (charcoal) in large, colourless, pointed prisms, m. p. 174—175° (Found: C, 44·35; H, 4·05; N, 10·8. Calc. for $C_{10}H_{12}O_5N_2S$: C, 44·1; H, 4·4; N, 10·3%).

Aspartic Acid.—Ethyl aspartate (1·0 g.) was dissolved in water (20 c.c.) and heated in a sealed tube for 2 hours at 115—120° and then at 140—150° for a further 5 hours. The mixture was evaporated until crystallisation commenced and was then set aside to cool slowly. Large colourless rectangular prisms were deposited (0·5 g., 70% of the theoretical), m. p. 338—339° (decomp.) (Dunn and Smart, *J. Biol. Chem.*, 1930, **89**, 41, give m. p. 326°; Enkvist, *loc. cit.*, gives m. p. 227—228°. The latter may be a misprint). On recrystallisation from dilute alcohol no further rise in m. p. was observed (Found: C, 36·3; H, 5·7; N, 10·9. Calc. for $C_4H_9O_4N$: C, 36·1; H, 5·3; N, 10·5%).

The *phenylhydantoin* was obtained by shaking a solution of aspartic acid with a slight excess of phenyl *iso*cyanate. The liquid was then filtered to remove carbanilide and was acidified and warmed on the water-bath for a short time. On cooling, the required compound was deposited; it crystallised from hot water in long colourless needles, m. p. 225—225·5° (Found: C, 56·1; H, 4·7. $C_{11}H_{10}O_4N_2$ requires C, 56·4; H, 4·3%).

The benzoyl derivative was prepared in presence of sodium bicarbonate; the alkaline solution was then acidified, the precipitate collected, washed with water, dried, and extracted with light petroleum to remove benzoic acid, and the residue (m. p. 166°) repeatedly crystallised from boiling water. The m. p. fell to 160° and then to 158° (compare Fischer, *Ber.*, 1899, **32**, 2460, who gives m. p. 164—165°, and Karrer and Schneider, *Helv. Chim. Acta*, 1930, **13**, 1286, who give m. p. 175° and m. p. 119° for the monohydrate). For analysis the compound was dried at 110° for 4 hours; it then had m. p. 165—166° (Found: C, 55·7; H, 4·8; N, 6·3. Calc. for $C_{11}H_{11}O_5N$: C, 55·7; H, 4·6; N, 5·9%).

The *benzenesulphonyl* compound crystallised from water in large colourless prisms, m. p. 181—182° (Found: C, 44·3; H, 4·2. $C_{10}H_{11}O_6NS$ requires C, 44·0; H, 4·0%).

KING'S COLLEGE, UNIVERSITY OF DURHAM. [*Received, September 26th*, 1940.]