100. DL-β-*Methylasparagines.*

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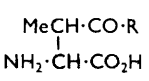
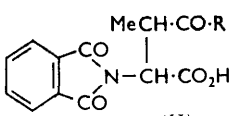
DL-*threo*- and DL-*erythro*-β-Methylasparagine have been prepared from the corresponding DL-β-methylaspartic acids, and the base-catalysed interconversion of the phthaloyl derivatives of these acids and their anhydrides has been studied. Evidence is adduced that the catalytic effect of acetic anhydride on this interconversion is to be ascribed to acetate ion formed by dissociation, and not to the acetic anhydride molecule as a whole.

A RECENT account by Barker, Smyth, Wilson, and Weissbach [1] of the isolation of both racemic forms of β-methylaspartic acid (I; R = OH) makes it worth while to record the conversion of these, obtained independently in this laboratory, into their β-amides (I; R = NH_2) (β-methylasparagines) which were required for other work, and also some observations on the interconversion of the phthaloyl derivatives of the acids. The racemic acids, obtained essentially by the method described by Barker *et al.*,[1] differed markedly in their solubility in water: the less soluble form (obtained in 30—35% yield; decomp. *ca.* 270°), previously obtained by Dakin [2] and designated by Barker *et al.*[1] as the DL-*threo*-acid, dissolved only to the extent of 4·3 g./l. at 25°, whereas the DL-*erythro*-acid (obtained in *ca.* 20% yield; decomp. 250—255°) had a solubility of 190 g./l. at this temperature. Following the procedure whereby King and Kidd [3] obtained DL-asparagine from DL-aspartic acid, we caused the former to react with phthalic anhydride in boiling pyridine, and converted the resulting phthaloyl derivative (II; R = OH) into its anhydride from which, by the action of ammonia, a good yield of a phthalimido-amide (II; R = NH_2) was obtained. Removal of the phthaloyl group by the action of hydrazine gave an amide subsequently established, as discussed below, as being the β-amide, DL-*threo*-β-methylasparagine. Although, considerable evidence is recorded [3-6] that the direction of opening of acylated aspartic anhydride rings by ammonia is sensitive to conditions, the nature of the acyl group, etc., the replacement of a β-hydrogen atom by a small alkyl group, such as a methyl group, had therefore apparently little, if any, effect on the course of the reaction under the same conditions.



(I)



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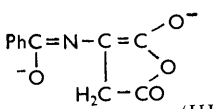


(III)

When DL-*erythro*-β-methylaspartic acid, the soluble isomer, was subjected to the same series of reactions a phthaloyl-β-methylaspartic anhydride resulted of the same melting point as, and indistinguishable from, that obtained from the *threo*-acid and, moreover, the phthaloyl-β-methylasparagine given by it as above was identical with that from the *threo*-acid. A configurational change, analogous to the racemisation observed when L-glutamic acid is converted in the same way into phthaloyl-DL-glutamic anhydride,[7] had clearly occurred in the reaction either of the *threo*- or of the *erythro*-acid. Since hydrolysis by boiling water of the phthaloyl-anhydride from the soluble *erythro*-acid, followed by removal of the phthaloyl group, gave the sparingly soluble *threo*-acid, the structural change, presumably at C_α in view of the observed racemisation of L-glutamic

[1] Barker, Smyth, Wilson, and Weissbach, *J. Biol. Chem.*, 1959, **234**, 320.
[2] Dakin, *J. Biol. Chem.*, 1941, **141**, 945.
[3] King and Kidd, *J.*, 1951, 2976.
[4] Bergmann and Zervas, *Ber.*, 1932, **65**, 1192.
[5] Tanenbaum, *J. Amer. Chem. Soc.*, 1953, **75**, 1754.
[6] Chambers and Carpenter, *J. Amer. Chem. Soc.*, 1955, **77**, 1522.
[7] King and Kidd, *J.*, 1949, 3315.

acid [7] in which this is the only asymmetric carbon atom, had clearly occurred in the reaction of the *erythro*-acid with phthalic anhydride in pyridine. The phthaloyl-anhydride and -amide given by DL-*threo*-β-methylaspartic acid were therefore unchanged in configuration. This was confirmed by the subsequent isolation of the true DL-*erythro*-β-methylasparagine which crystallised as a hydrate (unlike the DL-*threo*-amide) and decomposed at a somewhat lower temperature.

Repeated attempts to obtain DL-*erythro*-β-methylasparagine by the route used by King and Kidd [7] to obtain unracemised phthaloyl derivatives of L-aspartic and L-glutamic acid were unsuccessful. Condensation of diethyl DL-*erythro*-β-methylaspartate with phthalic anhydride in ether at room temperature and ring closure of the resulting phthalamic acid by thionyl chloride appeared to proceed normally, but it proved impossible to find conditions under which the ester groups of the diethyl phthaloyl-DL-*erythro*-β-methylaspartate so obtained could be hydrolysed without simultaneously splitting off phthalic acid. Although the phthaloyl derivative of the *erythro*-acid was subsequently obtained in relatively small amount, as described below, the amide of this acid was eventually obtained * by the classical route of Bergmann, Zervas, and Salzman [8] by way of the benzyloxycarbonyl derivative in which it has been shown that structural change can be avoided.[9] DL-*erythro*-β-Methylasparagine was more soluble in water than the *threo*-isomer and crystallised from aqueous alcohol with 1·5 molecules of water of crystallisation. On hydrolysis by boiling dilute hydrochloride acid no trace of the sparingly soluble *threo*-acid could be detected, confirming the view that the *erythro*-structure had been retained.

Both amides were shown to be β-amides by establishing that they retained the α-amino-acid structure, many tests for which have been employed from time to time. Thus, Waser [10] showed that in 50% aqueous pyridine certain α-amino-acids, but not β-amino-acids, gave characteristic colours when warmed with *p*-nitrobenzoyl chloride, in some cases alone and in others in the presence of dilute sodium carbonate; Van Slyke, MacFadyean, and Hamilton [11] showed that only α-amino-acids evolved one mol. of carbon dioxide on being heated with ninhydrin in buffered solution—aspartic acid, the sole exception, evolved two mol.; King and Kidd [7] established the structure of phthaloyl-DL-glutamine from the fact that the action of sodium hypobromite followed by hydrolysis gave αγ-diaminobutyric acid; Crumpler and Dent [12] were able to distinguish α-amino-acids from others by two-dimensional paper chromatography in which the path of the acids in the first run was sprinkled with fine copper carbonate to retain the α-amino-acids, only the rest then migrating at right angles in the second solvent; more recently, Swan [13] utilised the fact that only α-amino-acids gave rise to crystalline acetylthiohydantoins when heated with ammonium thiocyanate and acetic anhydride. Since both carboxyl groups of aspartic acid are decarboxylated on deamination with ninhydrin, this reagent is unreliable for differentiating α- and β-amides of this acid and its homologues, and the remaining reactions presented a confusing picture when applied to the β-methylasparagines. Thus, whilst DL-*threo*-β-methylasparagine behaved in Waser's test like asparagine, it gave no crystalline acetylthiohydantoin (for which a possible reason subsequently emerged) and far from convincing results in Crumpler and Dent's procedure. Both compounds were established as β-amides by a modification of the method used by Pope and Stevens [14] to estimate the amino-acid content of protein hydrolysates, aliquot parts of which were

* At the time the very useful properties of *N*-ethoxycarbonylphthalimide (Nefkens, *Nature*, 1960, **185**, 309) had not been disclosed.

[8] Bergmann, Zervas, and Salzman, *Ber.*, 1933, **66**, 1288.
[9] Fruton, *Adv. Protein Chem.*, 1949, **5**, 41.
[10] Waser, *Mitt. Lebensm. Hyg.*, 1929, **20**, 260.
[11] Van Slyke, MacFadyean, and Hamilton, *J. Biol. Chem.*, 1941, **141**, 671.
[12] Crumpler and Dent, *Nature*, 1949, **164**, 442.
[13] Swan, *Nature*, 1952, **169**, 826; cf. Johnson and Guest, *Amer. Chem. J.*, 1912, **48**, 103.
[14] Pope and Stevens, *Biochem. J.*, 1939, **33**, 1070.

allowed to react with an excess of an alkaline copper phosphate reagent, prepared in a specified manner, and the quantities of copper taken into solution as amino-acid complex then estimated volumetrically. This reagent has now been shown to respond, not only to α-amino-acids, two molecules of which combine per copper ion, but also to some extent to β-amino-acids, though in the latter case the number of molecules required per copper ion is higher and varies considerably with the alkalinity of the reagent. However, by reducing the alkalinity a reagent has been devised towards which α-amino-acids behave as above, whereas β-amino-acids are virtually inert. Since towards this reagent both β-methylasparagines behaved as α-amino-acids their structure was unequivocally established.

The change in configuration of the *erythro*-acid when heated with phthalic anhydride in pyridine was clearly potentially reversible and, providing the difference of energy content of the phthaloyl derivatives was not too great, these should both be present in the reaction product irrespective of whether the *threo*- or *erythro*-acid was used. The difference in energy content resulting from the presence of the β-methyl group in such a molecule is unlikely to be large and the yield of phthaloyl-DL-*threo*-β-methylaspartic anhydride was such as to suggest that a considerable amount of the *erythro*-isomer might also be present. This has been shown to be the case. Each acid was condensed with phthalic anhydride in pyridine, the product remaining after the removal of pyridine was dissolved in water without previous anhydridisation with acetic anhydride, and the resulting solution was acidified with hydrochloric acid; in each case phthaloyl-DL-*threo*-β-methylaspartic acid crystallised rapidly in prisms; also in each case the mother-liquor, when left at 0°, slowly deposited tufts of needles (together with a little more prismatic material) which were established as phthaloyl-DL-*erythro*-β-methylaspartic acid. The yield of phthaloyl-DL-*threo*-β-methylaspartic acid was somewhat smaller than of the corresponding anhydride, possibly because any phthalamic acid formed in the reaction would be converted into phthaloyl derivative by the action of acetic anhydride in the latter case but lost in the former. Roughly seven parts of phthaloyl-*threo*-acid were obtained to two parts of the *erythro*-isomer.

Barker reported [15] that *N*-benzoyl-L-aspartic anhydride dissolved in N-sodium hydroxide at room temperature and was completely racemised in **3** hours owing, he suggested, to the formation of an ion (III) in which the asymmetry at C_α is lost. The reaction of phthaloyl-DL-*threo*-β-methylaspartic anhydride with cold aqueous alkali was therefore examined in the hope that it might provide a simple means of obtaining further quantities of the phthaloyl-*erythro*-acid from the *threo*-anhydride as a result of a similar equilibration. Whilst, however, this anhydride dissolved easily in cold N-sodium hydroxide, no crystalline product separated on acidification. Indeed, even if phthaloyl-DL-*threo*-β-methylaspartic acid itself were allowed to stand in alkaline solution (NaOH; 6 equiv.) for some time (18 hr.) at room temperature, none was recoverable on acidification, whereas at least 90% crystallised unchanged from a carefully neutralised solution of the acid or from its solution in an excess of sodium hydrogen carbonate when treated in the same way. Since in the first case it was possible to show that three equivalents of alkali were neutralised by the anhydride, it appears that even under these mild conditions the phthaloyl group undergoes partial hydrolysis to give the phthalamic acid. Heating phthaloyl-DL-*threo*-β-methylaspartic acid with acetic anhydride and pyridine resulted, as was to be expected, in its equilibration (indeed phthaloyl-DL-*erythro*-β-methylaspartic acid equilibrated even when kept for some time at room temperature with a mixture of acetic anhydride and pyridine), and simply heating the acid with pyridine alone gave some 15% of the *erythro*-isomer though in this case the recovery of residual phthaloyl-*threo*-acid was relatively poor. Sheehan and Bolhofer [16] reported racemisation of phthaloyl-L-glutamic acid (of unknown optical purity) when it was heated for **30** minutes with acetic anhydride in xylene and it

[15] Barker, *J.*, 1953, 453.
[16] Sheehan and Bolhofer, *J. Amer. Chem. Soc.*, 1950, **72**, 2470.

has now been shown that heating phthaloyl-DL-*threo*-β-methylaspartic acid with acetic anhydride alone causes equilibration. After hydrolysis of the resulting anhydrides, some 20% of the phthaloyl-*erythro*-acid was obtained and, as in this case the remaining *threo*-isomer was easily recoverable, this provided a simple, if somewhat protracted, means of preparation of the *erythro*-isomer.

Equilibration of the phthaloyl-acids or their anhydrides by hot pyridine is in accordance with the views expressed in the literature [17-19] that the process of racemisation of acylamino-acids and their derivatives, as also that of the related hydantoins, involves primarily ionisation of the hydrogen attached to C_α and is therefore base-catalysed; yet the part played by acetic anhydride in bringing about such a change has been discussed hitherto only with respect to acylamino-acids of the type R·CO·NH·CHR'·CO_2H (free amino-acids being acetylated as a first step) in which it has been shown to occur through the corresponding azlactones.[20] Even here, under the conditions employed, virtually no racemisation occurs in the absence of a base such as acetate ion. The racemisation of acetyl-L-proline,[21] acetyl-*N*-methyl-D-phenylalanine,[20] and phthaloyl-L-glutamic acid [16] and the equilibration of the compounds at present under discussion, caused by heating the compounds with acetic anhydride alone or in non-basic solvents without any additional base, call therefore for some other explanation, since in these cases azlactones cannot be formed. The equilibration of the phthaloyl-β-methylaspartic acids by acetic anhydride was therefore investigated further.

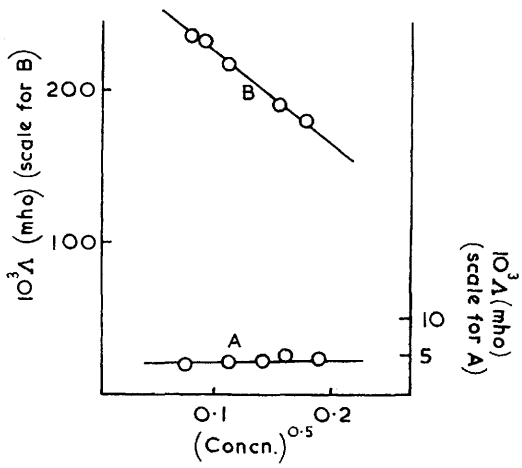
Now, acetate ion has a marked catalytic effect on the racemisation of azlactone-forming amino-acids on acetylation with acetic anhydride; and acetic anhydride has a small but definite electrical conductivity which Jander, Rüsberg, and Schmidt [22] attributed to a slight ionisation, $(CH_3{\cdot}CO)_2CO \rightleftharpoons CH_3{\cdot}CO^+ + CH_3{\cdot}CO_2^-$. Thus, acetate ion seemed the most likely basic catalyst in our system.

Phthaloyl-DL-*erythro*-β-methylaspartic acid was converted by boiling acetyl chloride into its anhydride, in which no *threo*-isomer could be detected, and the effect was examined of heating this anhydride with acetate ion in an appropriate solvent of the same order of polarity as acetic anhydride. In such solvents, apart from dimethylformamide with which the anhydride apparently reacted, no metallic acetate was readily soluble. Ultimately, from an examination of the phthaloyl-acids obtained by hydrolysis subsequent to reaction, it was shown that, whereas this anhydride could be boiled for an hour in anhydrous methyl cyanide, alone or in the presence of tetraethylammonium bromide, without sign of equilibration, the addition of a little tetraethylammonium acetate caused equilibration even though the boiling point of this solvent was some 50° below that of acetic anhydride. Even sodium acetate, in spite of its limited solubility in methyl cyanide, had the same effect. Whilst, however, the catalytic effect of acetate ion was thereby established, the possibility was not precluded that the acetic anhydride molecule as a whole, which Wolf [23] has hinted might act as a proton acceptor, could also act catalytically, or that intramolecular change could occur in the anhydride at the boiling point of acetic anhydride and involve migration of the α-hydrogen atom.

Evidence of proton-binding capacity of the intact acetic anhydride was sought from conductance measurements of (+)-camphor-β-sulphonic acid in acetic anhydride. This acid, available as the monohydrate, was easily obtained in the anhydrous form by recrystallisation from hot acetic anhydride. For conductivity measurements " AnalaR " acetic anhydride was fractionated under reduced pressure and the conductivities of each fraction determined as described in the Experimental section. The best anhydride so obtained

[17] Bovarnick and Clarke, *J. Amer. Chem. Soc.*, 1938, **60**, 2428.
[18] Bailey and Neurath, " The Proteins," Academic Press Inc., New York, 1953, Vol. IA, 60.
[19] Neuberger, *Adv. Protein Chem.*, 1948, **4**, 350.
[20] Carter and Stevens, *J. Biol. Chem.*, 1940, **133**, 117.
[21] Carter and MacMillan, *J. Amer. Chem. Soc.*, 1952, **74**, 2863.
[22] Jander, Rüsberg, and Schmidt, *Z. anorg. Chem.*, 1948, **255**, 238.
[23] Wolf, *Ann. Chim.* (*France*), 1953, **8**, 238.

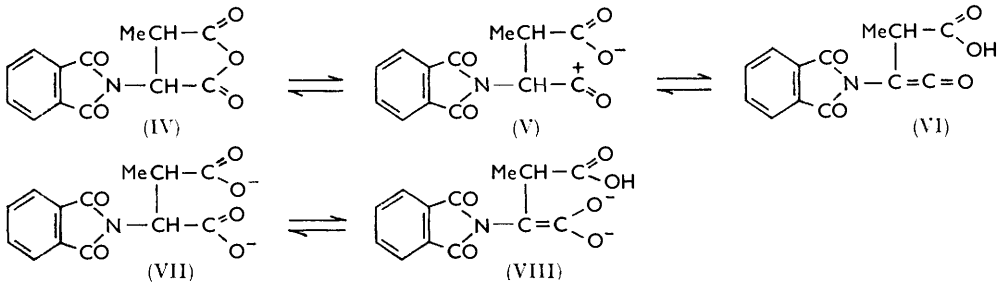
had a specific conductivity of 10^{-7} mho. The conductivities of anhydrous (+)-camphor-β-sulphonic acid (A) and its monohydrate (B) in this solvent are summarised in the Figure, in which the equivalent conductivity is plotted against the square root of the concentration. In the range of concentrations employed the monohydrated acid behaved as a strong electrolyte with equivalent conductivity of the order of 0·2 mho, whilst the equivalent conductivity of the anhydrous acid was only about one-fiftieth of this over the same concentration range. Acetic anhydride therefore shows virtually no ability to accept protons from anhydrous camphor-β-sulphonic acid, and the conductivity of the hydrated acid must consequently be due to its presence in solution as $C_{10}H_{15}O{\cdot}SO_3^-H_3O^+$, *i.e.*, at room temperature the camphorsulphonate ion and water have base strengths considerably greater than that of acetic anhydride. Whilst it was not possible to examine the catalytic effect of water on the equilibrium of phthaloyl-DL-*erythro*-β-methylaspartic anhydride, that of the (+)-camphor-β-sulphonate ion was open to investigation.



Plot of equivalent conductivity, $10^3\Lambda$, of (+)-camphor-β-sulphonic acid (A) and its monohydrate (B) against square root of the molar concentration in acetic anhydride.

When phthaloyl-DL-*erythro*-β-methylaspartic anhydride was heated with tetraethylammonium (+)-camphor-β-sulphonate in n-butyl cyanide at 130° for one hour a slight, but definite, trace of the isomeric *threo*-isomer was always formed. This was also the case when tetraethylammonium toluene-*p*-sulphonate was used, and it seemed that these sulphonic anions had a very small but definite catalytic influence. However, when the *erythro*-anhydride was heated alone under the same conditions in n-butyl cyanide or in the much less polar *p*-xylene, or when the corresponding acid was converted into its anhydride by heating it with hexanoyl chloride at 130° for one hour, similar traces of the *threo*-isomer resulted. In no case did this amount to more than a quite minor proportion of the product, whereas with acetate the less soluble *threo*-isomer formed the bulk of the recovered phthaloyl acids. It is concluded (*a*) that the catalytic effect, if any, of the (+)-camphor-β-sulphonate ion must be extremely small compared with that of acetate ion and, since acetic anhydride was shown to be even less basic than the (+)-camphor-β-sulphonate ion, the catalytic effect of molecular acetic anhydride must be insignificant compared with that of acetate ion, and (*b*) that very slow equilibration of the *erythro*-phthaloyl anhydride is possible even in the absence of added catalysts. In view of the conclusion regarding the manner of action of acetic anhydride as catalyst, this very much slower change is most simply ascribed to a similar " dissociation " of the phthaloyl-anhydride itself to give a dipolar ion (V). This would lead to equilibration if followed either by an intramolecular shift of H_α to give (VI) (an overall reaction analogous to the thermal dissociation of acetic anhydride to keten and acetic acid), or by an intermolecular shift of H_α from another molecule, the intermediate (V) thus acting in the same way as acetate ion.

To some extent this view was supported by observations that the free phthaloyl-*erythro*-acid also underwent equilibration when heated for an hour in dimethylformamide with tetraethylammonium acetate or in acetic acid with sodium acetate. In either solvent alone, or in dimethylformamide containing tetraethylammonium bromide, no change was detectable. Further, when phthaloyl-*erythro*-DL-β-methylaspartic acid was carefully neutralised with tetraethylammonium hydroxide and the salt isolated and heated as before in dimethylformamide, equilibration occurred just as though acetate ion had been present. Inter- or intra-molecular migration of H_α of the ion (VII) seems to be the only



possible mechanism in this case, and the slow reaction of the anhydride in the absence of other catalyst is therefore not unreasonably explained by a similar process.

More concrete evidence for this and for the direction of opening of the anhydride ring is being sought, as this may have some bearing on the differences in the behaviour of such cyclic anhydrides under different conditions (cf. King and Kidd [3]).

Experimental

Phthaloyl-DL-threo-β-*methylaspartic Anhydride.*—Ground DL-*threo*-β-methylaspartic acid (3·1 g.) was refluxed with redistilled phthalic anhydride (3·1 g.) in dry pyridine as described by King and Kidd.[3] The acid dissolved readily at first but usually, even after prolonged boiling, a little remained insoluble and was filtered off before the pyridine was removed under reduced pressure. The residual gum was boiled with acetic anhydride (4 ml.) for a few minutes and then rapidly cooled. Recrystallisation of the product from glacial acetic acid gave *phthaloyl*-DL-threo-β-*methylaspartic anhydride*, m. p. 197—198° (4·2 g., 73%) (Found: C, 60·3; H, 3·7; N, 5·5. $C_{13}H_9NO_5$ requires C, 60·3; H, 3·5; N, 5·4%).

Repetition with DL-*erythro*-β-methylaspartic acid (1·5 g.) gave an anhydride (1·92 g., 73%), m. p. 198—199° alone or mixed with the preceding anhydride. Part of this (0·65 g.), hydrolysed by boiling with water and treated with 50% hydrazine (0·28 g.) in a solution of sodium carbonate (0·32 g.), readily gave sparingly soluble prisms of DL-*threo*-β-methylaspartic acid. A further portion, treated with ammonia, as described below, gave a product (0·36 g., 84%), m. p. 143° (softening at 140°), not depressed on admixture with phthaloyl-DL-*threo*-β-methylasparagine.

Phthaloyl-DL-threo- *and* -erythro-β-*methylaspartic Acid.*—The residual gum obtained from DL-*threo*-β-methylaspartic acid (2 g.), after removal of pyridine in the previous preparation, was dissolved in water and re-evaporated under reduced pressure, then redissolved in water and acidified to Congo Red with concentrated hydrochloric acid. *Phthaloyl*-DL-threo-β-*methylaspartic acid* (2·16 g., 57%) which slowly crystallised was filtered off, and the mother-liquor was placed in a refrigerator; clusters of slender needles (0·6 g., 16%) of *phthaloyl*-DL-erythro-β-*methylaspartic acid*, containing a little of the isomer, slowly separated. Phthaloyl-DL-*threo*-β-methylaspartic acid (1·7 g.) recrystallised from water in prisms, m. p. 202—204°, softening at 194° (Found: C, 55·9; H, 4·1; N, 5·3. $C_{13}H_{11}NO_6$ requires C, 56·3; H, 4·0; N, 5·1%); the DL-*erythro*-isomer (0·4 g.) in tufts of needles, m. p. 189—190°, softening at 184° (Found: C, 56·3; H, 4·0; N, 5·1%).

Equilibration of the threo- *and* erythro-*Forms of the Acid by Acetic Anhydride.*—Phthaloyl-DL-*threo*-β-methylaspartic acid (1·7 g.) was refluxed for 1 hr. with acetic anhydride (20 ml.) which was then distilled off under reduced pressure from a water-bath. The residual anhydrides

were hydrolysed by boiling water, and the resulting phthaloyl-DL-*threo*- (1·2 g., 70%) and -*erythro*-acid (0·35 g., 20%) separated and purified as above.

Action of Alkali on Phthaloyl-DL-threo-β-*methylaspartic Acid.*—(*a*) The acid (100 mg.) in a little water was neutralised with N-sodium hydroxide (phenolphthalein). After 18 hr. the solution was acidified with concentrated hydrochloric acid; the original acid (90·4 mg.; m. p. 196—197°) slowly crystallised. Repetition with three times the amount of sodium hydroxide gave no crystalline product on acidification.

(*b*) The acid (127·8 mg.), dissolved in 5 ml. of N-sodium hydroxide, required on titration after 18 hr. 7·11 ml. of 0·5N-hydrochloric acid, whence 1 mol. had neutralised 3·1 mol. of alkali.

Phthaloyl-DL-erythro-β-*methylaspartic Anhydride.*—Phthaloyl-DL-*erythro*-β-methylaspartic acid (0·3 g.) was boiled with acetyl chloride (10 ml.) until completely dissolved. The acetyl chloride was then distilled off and the oily residue, which solidified, was freed from acetic acid over solid sodium hydroxide and recrystallised from dry benzene, to give *phthaloyl*-DL-erythro-β-*methylaspartic anhydride* (0·2 g.), m. p. 189—190° (Found: C, 60·4; H, 3·5; N, 5·4. $C_{13}H_9NO_5$ requires C, 60·3; H, 3·5; N, 5·4%).

This anhydride, when hydrolysed by boiling water, gave on cooling only the clusters of needles characteristic of the phthaloyl-*erythro*-acid (m. p. 189—190°, depressed on admixture with the *threo*-isomer).

Phthaloyl-DL-threo-β-*methylasparagine.*—Phthaloyl-DL-*threo*-β-methylaspartic anhydride, when treated in dioxan solution with dry ethereal ammonia as described by King and Kidd,[3] gave *phthaloyl*-DL-threo-β-*methylasparagine* (3·7 g., 90%), which crystallised from aqueous alcohol in plates containing one molecule of water of crystallisation (Found: C, 53·4; H, 5·1; N, 9·4; loss at 100°, 6·2%; equiv., 293. $C_{13}H_{12}N_2O_5,H_2O$ requires C, 53·1; H, 4·8; N, 9·5; H_2O, 6·1%; equiv., 294). Even after several recrystallisations the compound sintered (132—139°) and melted indefinitely with effervescence at temperatures (140—145°) depending on the rate of heating. This was due to slow dehydration since at 125° the compound lost weight equivalent to two molecules of water (Found: loss at 125°, 12·6. Calc.: $2H_2O$, 12·2%) to give a product which crystallised from water in needles, m. p. 235—236°. This was insoluble in cold sodium hydrogen carbonate, but evolved ammonia when heated with sodium hydroxide and was believed to be the related DL-α-phthalimido-β-methylsuccinimide.

DL-threo-β-*Methylasparagine.*—Phthaloyl-DL-*threo*-β-methylasparagine (2·8 g.) was dissolved in N-aqueous sodium carbonate (13 ml.) and treated with 50% hydrazine (1 g.), and the resulting DL-threo-β-*methylasparagine* (1·0 g., 71%) was isolated as described by King and Kidd.[3] It crystallised from aqueous alcohol in anhydrous prisms, decomp. *ca.* 245° (Found: C, 41·2; H, 6·9; N, 19·0. $C_5H_{10}N_2O_3$ requires C, 41·1; H, 6·8; N, 19·2%). Adding solid *p*-nitrobenzoyl chloride to a warm solution of the amide in 50% aqueous pyridine [10] with or without dilute sodium carbonate caused a reddish-violet colour to appear rapidly. Asparagine behaved in the same way but β-alanine gave no response. Towards copper phosphate reagent the amide reacted as an α-amino-acid, as discussed below.

N-*Benzyloxycarbonyl*-DL-erythro-β-*methylaspartic Anhydride.*—DL-*erythro*-β-Methylaspartic acid (4 g.) in water (25 ml.) was treated with magnesium oxide (2·1 g.) and benzyl chloroformate (6 g.), and the product was extracted with ethyl acetate as described by Bergmann *et al.*[8] Evaporation of the washed, dried (Na_2SO_4), and filtered extract left an oily acid (7·1 g., 93%) which was extremely soluble in water and a number of organic solvents and separated from others as an oil. The crystalline acid (m. p. 122—123°) was most conveniently obtained by boiling the crude material with dry benzene until no more benzene–water azeotrope distilled off, and allowing the solution to cool. The crystalline acid retained benzene somewhat tenaciously and was not analysed. The crude material (from 4 g. of β-methylaspartic acid) was heated on the water-bath for 10 min. with acetic anhydride (10 ml.) which was then removed as rapidly as possible under reduced pressure. The residue, which solidified on cooling, gave needles (4·9 g., 69%) of N-*benzyloxycarbonyl*-DL-erythro-β-*methylaspartic anhydride*, m. p. 119—120° (from dry chloroform) (Found: C, 59·1; H, 5·1; N, 5·2. $C_{13}H_{13}NO_5$ requires C, 59·3; H, 4·9; N, 5·3%).

α-*Benzyl* N-*Benzyloxycarbonyl*-DL-erythro-β-*methylaspartate.*—*N*-Benzyloxycarbonyl-DL-*erythro*-β-methylaspartic anhydride (3·0 g.) and freshly distilled benzyl alcohol (1·9 g.) were heated together for 3½ hr. at 100°, and the resulting benzyl ester was isolated as described by Bergmann *et al.*[8] The ester remaining when the dried (Na_2SO_4) and filtered ether extract was evaporated solidified and was recrystallised by dissolving it in ethyl acetate and adding light

petroleum (b. p. 60—80°) to turbidity. Needles (3·4 g., 80%) of *α-benzyl* N-*benzyloxycarbonyl*-DL-erythro-β-*methylaspartate*, m. p. 111—112°, separated (Found: C, 64·8; H, 5·4; N, 4·0. $C_{20}H_{21}NO_6$ requires C, 64·8; H, 5·7; N, 3·8%).

α-Benzyl N-*Benzyloxycarbonyl*-DL-erythro-β-*methylasparagine*.—Finely divided phosphorus pentachloride (1 g.) was shaken with an ice-cold solution of the preceding benzyl ester (1·35 g.) in dry ether (10 ml.) until virtually all had dissolved. The solution was decanted at once into stirred ice-cold aqueous 6M-ammonia (25 ml.), and the precipitated *amide* (1·28 g., 94%) was filtered off as soon as it had flocculated, washed with cold water, and dried *in vacuo* over calcium chloride. When recrystallised from methanol, it had m. p. 158—160° (1·08 g.) (Found: C, 64·6; H, 5·6; N, 7·8. $C_{20}H_{22}N_2O_5$ requires C, 64·9; H, 5·9; N, 7·6%).

DL-erythro-β-*Methylasparagine*.—α-Benzyl *N*-benzyloxycarbonyl-DL-*erythro*-β-methylasparagine (1 g.) in methanol (30 ml.) and water (8 ml.) was hydrogenated (3 hr.) in the presence of palladium black (from 1 g. of chloride), with occasional warming to dissolve deposited crystals, and the product was isolated as described by Bergmann *et al.*[8] Hydrated DL-erythro-β-*methylasparagine* (0·4 g., 93%) crystallised from aqueous ethanol. It was more soluble in water than the *threo*-isomer and decomposed at *ca.* 233° after shrinking at 105° (Found: C, 34·6; H, 7·6; N, 15·3; loss at 105°, 16·0. $C_5H_{10}N_2O_3,1\frac{1}{2}H_2O$ requires C, 34·7; H, 7·5; N, 16·2; H_2O, 15·6%).

The amide was hydrolysed by boiling dilute hydrochloric acid, and the solution concentrated and adjusted to pH 3 by addition of aqueous ammonia. No sparingly soluble prisms of DL-*threo*-β-methylaspartic acid separated (as when the *threo*-amide was similarly hydrolysed), even on long storage. The behaviour towards the copper phosphate reagent, described below, established that it was the β-amide.

Differentiation of α- and β-Amino-acids: Copper Phosphate Method.—A shaken mixture of 0·16M-cupric chloride (10 ml.) and 0·16M-trisodium phosphate solution (10 ml.) was added to a solution of a known weight of amino-acid (50—100 mg.) in a 100 ml. standard flask. The mixture was diluted to the mark, left for 5 min., and then filtered through a dry filter into a dry flask (the first runnings were rejected), and 50 ml. of the filtrate were titrated with 0·025N-sodium thiosulphate after addition of 25% acetic acid (10 ml.) and potassium iodide (1 g.). The following figures show that, under these conditions 1 g.-ion of copper was dissolved for every 2 moles of α-amino-acid, whereas β-amino-acids dissolved very little copper indeed. Both β-methylasparagines, behaving in this way as α-amino-acids, were therefore β-amides.

Number of g.-ions of copper dissolved by 2 moles of amino-acid.

Glycine	0·99	L-Asparagine	1·03	DL-*threo*-β-Methylasparagine	0·99
DL-Alanine	0·98	β-Alanine	0·04	DL-*erythro*-β-Methylasparagine ...	1·00

Equilibration Studies.—Portions of DL-*erythro*-β-methylaspartic acid (100 mg.), which had been recrystallised until the residue obtained on slow evaporation of the mother-liquor was completely free from *threo*-isomer, its pure anhydride, or, in one case, its tetraethylammonium salt, were dissolved in the relevant solvent (5 ml.) and allowed to react under the conditions summarised in the Table. Where salts were also added these were in quantities of 10 mg. The solvent was then removed under reduced pressure on the water-bath, and the residue dissolved in the minimum amount of boiling water. When the product was the anhydride, boiling was prolonged a little to ensure complete hydrolysis to the acid. The cooled solution, acidified with a few drops of dilute hydrochloric acid, was set aside to crystallise and the crystals examined under the microscope for the readily identifiable prisms of the *threo*-isomer among the tufts of needles of unchanged *erythro*-form. Further evidence was unnecessary as the observations fell into three distinct groups as shown.

Conductivity Experiments.—"AnalaR" acetic anhydride was fractionated under reduced pressure through a 22-in. column packed with glass rings. The distillate was collected in fractions of about 20 ml. in a special receiver, fitted with plain platinum electrodes so that the conductivity of each fraction could be determined *in situ* by means of a Mullard Bridge, type E7566 and the fraction could then be rejected if necessary without breaking the vacuum. The constant of the cell was determined by using potassium chloride solution. The conductivity of the anhydride (b. p. 58°/30 mm.) fell progressively to $0·1 \times 10^{-6}$ mho, at which it remained constant. Such a fraction was used for the conductivity measurements with (+)-camphor-β-sulphonic acid and its hydrate.

Equilibrating effect of 10 mg. of various reagents on 100 mg. portions of (*a*) phthaloyl-DL-*erythro*-β-methylaspartic anhydride, (*b*) the free acid, and (*c*) its tetraethylammonium salt.

N.C. = No *threo*-isomer detected in product.
E. = Product mainly *threo*-isomer.
Tr. = Product contained trace of *threo*-isomer.

	Solvent	Reagent	Conditions	Effect
(*a*)	Methyl cyanide	—	81°/1 hr.	N.C.§
	,,	NEt_4Br	,,	N.C.
	,,	NEt_4OAc *	,,	E.
	,,	NaOAc	,,	E.
	n-Butyl cyanide	—	130°/1 hr.	Tr.
	,,	NEt_4 (+)-camphorsulphonate *	,,	Tr.
	,,	NEt_4 toluene-*p*-sulphonate *	,,	Tr.
	p-Xylene	—	,,	Tr.
(*b*)	Acetic acid	—	120°/1 hr.	N.C.§
	,,	NaOAc	,,	E.
	Dimethylformamide	—	100°/1 hr.	N.C.§
	,,	NEt_4Br	,,	N.C.
	,,	NEt_4OAc^-	,,	E.
	Acetic anhydride	—	130°/1 hr.	E.
	Acetyl chloride	—	55°/1 hr.	N.C.§
	Hexanoyl chloride	—	130°/1 hr.	Tr.
(*c*)	Dimethylformamide	—	100°/1 hr.	E.

* Prepared as described by Stiegmann and Hammett, *J. Amer. Chem. Soc.*, 1937, **59**, 2536.
§ Confirmed by m. p.

The monohydrated acid, recrystallised several times from ethyl acetate, had m. p. 193° (decomp.) (agreement with lit.) and an equivalent, by titration, of 250·5 (Calc. for $C_{10}H_{16}SO_4,H_2O$: equiv., 250), and from this the anhydrous acid was obtained by crystallisation from hot acetic anhydride. The crystals were washed with dry benzene and dried *in vacuo* over anhydrous calcium chloride (Found: equiv., 231·5. Calc. for $C_{10}H_{16}SO_4$: equiv., 232). Successive weighed portions of acid were introduced into the selected fraction of distillate, after release of the vacuum, for conductivity determination. It was established that this procedure had no effect on the conductivity of the anhydride itself. The volume of solvent was found from the weight of the conductivity-receiver before and after the series of determinations, and the density of acetic anhydride after allowance for the weight of solute added. The results are summarised in the Figure.

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