166. *Syntheses from Phthalimido-acids. Part VII.* Oxazolones and Other Intermediates in the Synthesis of Phthalylpeptides, and an Investigation of Maleic Acid Derivatives of Amino-acids.*

By F. E. KING, J. W. CLARK-LEWIS, ROY WADE, and (in part) W. A. SWINDIN.

Simple phthalyldipeptides are obtained more conveniently and in higher yields by mixed anhydride coupling than by the use of acid chlorides, and in the former method higher yields are obtained with *iso*butyl chloroformate than with the ethyl ester. Oxazolone coupling of phthalyldipeptides with amino-acid esters may be used with advantage for the preparation of certain phthalyltripeptides, but phthalyl-γ-glutamylamino-acids yield glutarimides instead of oxazolones.

Maleamic acids are readily prepared from amino-acids and maleic anhydride but do not form maleimido-acids; consequently the maleyl group is unsuitable as a protecting device in peptide synthesis.

THE reactions of phthalylglutamic and phthalylaspartic anhydride with alcohols, amines, and amino-acids or their derivatives have been described in earlier Parts of this series, and lead respectively to phthalyl-γ-glutamyl derivatives and to mixtures of the phthalyl-α- and phthalyl-β-aspartyl compounds. The preparation of phthalylpeptides from *mono*-basic phthalimido-acids, however, involves the use of an azide, chloride, or mixed anhydride for reaction with the appropriate amino-acid, ester, or amide. Phthalimido-acid hydrazides must be prepared by indirect methods, *e.g.*, from benzyloxycarbonylhydrazine,[1] or more conveniently from the acid chloride and sodium azide,[2] but this route offers no advantage over direct coupling by means of the acid chloride unless the specificity of the azide method is essential.

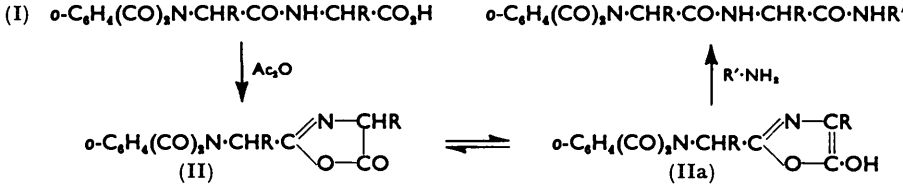
Preparations of phthalylglycylglycine, its ethyl ester, and phthalylglycyl-DL-alanine have been carried out, by various methods; the mixed carbonic anhydride procedure has been found to give better yields than the acid chloride method in each case, and has the further advantages of speed and simplicity. Moreover the use of *iso*butyl chloroformate in this method gave slightly better yields than that of ethyl chloroformate,[3] in agreement with the findings of other investigators.[4,5] Phthalylglycylglycine (55% after purification) was prepared from phthalylglycyl chloride as described by Sheehan and Frank [6] (who claim 89·5%), and it was found that the crude product (*ca.* 90%) contained phthalylglycine.

Oxazolones react with amino-acid esters to give acyldipeptide esters [7] but this method had previously been used only when the protecting group (acetyl, benzoyl) cannot be removed without cleavage of peptides. Unpublished experiments [8] have shown that phthalylglycyl-DL-alanylglycine ethyl ester, and hence by hydrolysis with hydrazine, etc., glycyl-DL-alanylglycine, can be prepared from 4-methyl-2-phthalimidomethyloxazolone, and further examples are now described. Thus reaction of the appropriate base with the oxazolone (II; R = H) from phthalylglycylglycine (I; R = H) gave phthalylglycylglycine anilide (84%) and the ethyl esters (each 80%) of the tripeptides, phthalyldiglycyl-glycine, -L-leucine, and -*S*-benzyl-L-cysteine. The high yields make the oxazolone route useful for the preparation of simple phthalyltripeptides (III) containing a DL-amino-acid or glycine as the central amino-acid. This restriction arises from the ease of racemisation of oxazolones (II), presumably by way of the enol tautomer (IIa). The method is

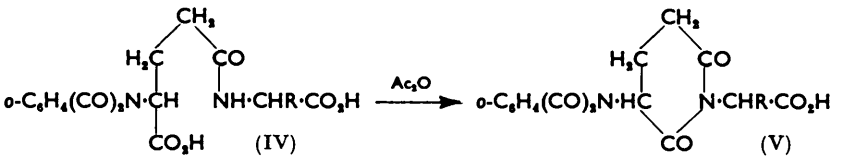
* Part VI, *J.*, 1954, 1046.

[1] Hofmann, Lindenmann, Magee, and Khan, *J. Amer. Chem. Soc.*, 1952, **74**, 470.
[2] King, Clark-Lewis, and Smith, *J.*, 1954, 1046.
[3] Boissonnas, *Helv. Chim. Acta*, 1951, **34**, 874.
[4] Vaughan and Osato, *J. Amer. Chem. Soc.*, 1952, **74**, 676.
[5] Wieland and Bernhard, *Annalen*, 1951, **572**, 190.
[6] Sheehan and Frank, *J. Amer. Chem. Soc.*, 1949, **71**, 1856.
[7] Carter, "Organic Reactions," Wiley, New York, 1946, Vol. III, p. 216.
[8] B. S. Jackson, Thesis, Nottingham, 1951.

applicable in principle to the preparation of higher peptides, a phthalyltripeptide for example being converted into the oxazolone and thence into a phthalyltetrapeptide ester in which only the terminal amino-acids retain their asymmetry. The action of acetic anhydride on phthalyl-γ-glutamylamino-acids (IV; R = H or Me) led to the formation



of substituted glutarimides (V; R = H or Me) instead of oxazolones, the products being stable to alcohols, to aniline, and to amino-acid esters. Phthalyl-DL-glutamine similarly gave 3-phthalimidopiperid-2 : 6-dione with acetic anhydride. Glutarimides have been postulated [9] as intermediates in the conversion of benzyloxycarbonyl-α-glutamylpeptides



into an equilibrium mixture of α- and γ-isomers in which the latter predominate, and similar interconversions have been discussed by Clayton and Kenner [10] and by Battersby and Robinson.[11] Our glutarimides are stable substances however, and it seems more probable that the interconversion of α- and γ-glutamyl derivatives involves a transition-state complex (hydrated glutarimide) in which transfer of OH^- from one carbonyl group to the other occurs simultaneously with the fission of the C–N bond, and that this does not involve formation of a glutarimide, *i.e.*, removal of a proton from the NH group.

Derivatives of Maleamic Acid.—The formal similarity between phthalimide and maleimide suggests that maleimido-acids (VI) might be applied to the synthesis of peptides as with phthalimido-acids. None of the required maleimido-acids (VI) is known however, and the literature records only four maleamic acids (VII) derived from amino-acids.

La Parola [12] prepared maleamic acids (VII; R = *o*-, *m*-, and *p*-$C_6H_4 \cdot CO_2H$) from the benzylidene derivatives of the three aminobenzoic acids, and maleylglycine (VII; R = $CH_2 \cdot CO_2H$) was obtained by Werbin and Spoerri.[13] Maleamic acids have been used recently [14] for the preparation of β-DL-aspartyl derivatives, following work by Fischer and Koenigs [15] on the amination of fumaryldiglycine and fumaryldi-DL-alanine. Maleic anhydride reacts readily with aliphatic amino-acids * or esters dissolved in acetic acid, to give the maleamic acids (VII; R = $CH_2 \cdot CO_2H$, $CH_2 \cdot CO_2Et$, and $CHMe \cdot CO_2H$) and with ethyl

* The ready interaction of amino-acids with maleic anhydride in acetic acid solutions was first demonstrated by Sir Robert Robinson, O.M., F.R.S., in 1939, and the preparation of several maleamic acids by this method has been carried out by A. Bowman (Thesis, Oxford, 1940) and one of us (F. E. K.).

[9] Kovács, Medzihradszky, and Bruckner, *Naturwiss.*, 1954, **41**, 450.
[10] Clayton and Kenner, *Chem. and Ind.*, 1953, 1205; see also Clayton, Kenner, and Sheppard, *J.*, 1956, 371.
[11] Battersby and Robinson, *J.*, 1955, 259.
[12] La Parola, *Gazzetta*, 1934, **64**, 919; see also Snyder, Levin, and Wiley, *J. Amer. Chem. Soc.*, 1938, **60**, 2025.
[13] Werbin and Spoerri, *ibid.*, 1947, **69**, 1681.
[14] Liwschitz and Zilkha, *ibid.*, 1955, **77**, 1265.
[15] Fischer and Koenigs, *Ber.*, 1904, **37**, 4585.

p-aminobenzoate to give the ester (VII; R = *p*-C_6H_4·CO_2Et), but reaction with *p*-aminobenzoic acid in acetic acid led to *p*-acetamidobenzoic acid, whereas in acetone or dioxan the required maleamic acid (VII; R = *p*-C_6H_4·CO_2H) was obtained. No method has been found for cyclising these products to the required maleimido-acids (VI), and maleanilic acid (VII; R = Ph) likewise failed to yield *N*-phenylmaleimide under a variety of conditions, including some described in the patent literature for this cyclodehydration.[16, 17] Inaccessibility of appropriate maleimido-acids (VI) therefore precludes the use of the maleyl group for the protection of amino-acids during peptide synthesis.

Some confusion exists in the early literature on maleamic and fumaramic acid, and the reaction of aniline with maleic anhydride was originally supposed [18, 19] to give fumaranilic acid. Later this conclusion was revised and the product was shown [20] to be maleanilic acid: the subject has been reviewed by Bischoff.[21] Failure to obtain maleimido-acids (VI) appeared to indicate that the primary products were not maleamic acids (VII) but fumaramic acids, but this was disproved by examination of their anthracene adducts. Maleylglycine with anthracene in acetic acid gave anthracene-9 : 10-*endo*-αβ-succinimidoacetic acid [22] (10%), the major product of the reaction being anthracene-9 : 10-*endo*-αβ-succinic anhydride, which had earlier been converted into the glycine derivative. Prolonged heating, however, of maleylglycine and anthracene in acetic acid led to the formation of thermostable anthracene-9 : 10-*endo*-αβ-*trans*-succinic acid.

Experimental

Phthalylglycine and Phthalylglycyl Chloride.—Phthalylglycine (95%), prepared by fusion [23] of phthalic anhydride and glycine at 180—185° for 15 min., crystallised from water in needles, m. p. 192—193° (lit.,[24] 192—193°). The *p*-chlorobenzamidinium and *S*-benzylthiuronium salts melted at 265° (decomp.) and 188—189° respectively.

Phthalylglycyl chloride [25] was obtained by heating phthalylglycine (10 g.) and thionyl chloride (20 c.c.) for 40 min., and evaporation of the thionyl chloride; it crystallised from light petroleum in large prisms (9·3 g., 85%), m. p. 85—86° (lit.,[25, 26] m. p. 84—85°) and was stable for long periods in a desiccator over phosphoric oxide. This method is superior to older methods of preparation.[26, 6]

Phthalylglycylglycine Ethyl Ester.—(a) *Acid chloride method.* Phthalylglycyl chloride (2·25 g.) in pure dry dioxan (10 c.c.) was added dropwise to a stirred mixture of glycine ethyl ester hydrochloride (1·4 g.), triethylamine (3·1 c.c.), and dry dioxan (10 c.c.) at 10°. The solid was collected after 4 hr. and washed with water, aqueous sodium hydrogen carbonate, and water. Phthalylglycylglycine ethyl ester crystallised from ethanol in rods (2·4 g., 80%), m. p. 191—192° (lit.,[27] m. p. 195°) (Found: N, 10·3. Calc. for $C_{14}H_{14}O_5N_2$: N, 10·5%).

(b) *Mixed anhydride method.* A solution of phthalylglycine (2·05 g.) and triethylamine (1·4 c.c.) in dry chloroform (25 c.c.) was cooled to 5° and stirred during the addition of *iso*butyl chloroformate (1·3 c.c.). The mixed anhydride solution was stirred at 5° for 10 min. before the addition of glycine ethyl ester hydrochloride (1·4 g.) dissolved in triethylamine (1·4 c.c.) and chloroform (10 c.c.) (carbon dioxide was evolved). When stirred at room temperature the solution began to deposit solid after 30 min., and the solution was then concentrated to 10 c.c. Next day the solid was collected and, after being washed as described in (a), it crystallised from ethanol in rods (2·6 g., 85%), m. p. 190—192° alone and when mixed with the product obtained by method (a).

[16] Speer, U.S.P. 2,262,262; *Chem. Abs.*, 1942, **36**, 1333.
[17] Searle, U.S.P. 2,444,536; *Chem. Abs.*, 1948, **42**, 7340.
[18] Anschütz, *Ber.*, 1887, **20**, 3214.
[19] Anschütz and Wirtz, *Annalen*, 1887, **239**, 137.
[20] Anschütz, *ibid.*, 1890, **259**, 137.
[21] Bischoff, *Ber.*, 1891, **24**, 2001.
[22] Bachmann and Cole, *J. Org. Chem.*, 1939, **4**, 60.
[23] Reese, *Annalen*, 1887, **242**, 1.
[24] Johnson and Scott, *J. Amer. Chem. Soc.*, 1913, **35**, 1133.
[25] Emerson, U.S.P. 2,498,665: *Chem. Abs.*, 1950, **44**, 4926.
[26] Gabriel, *Ber.*, 1907, **40**, 2648.
[27] Boissonnas and Schumann, *Helv. Chim. Acta*, 1952, **35**, 2229.

(c) *Süs's method.*[28] A mixture of freshly distilled glycine ethyl ester (2·4 g.), phosphorus trichloride (1·4 c.c.), dry benzene (10 c.c.), and dry dioxan (10 c.c.) was shaken in a closed flask for 10 min., whereupon the temperature of the mixture rose to 45° and a gelatinous precipitate formed. Phthalylglycine (3·2 g.) was added to the mixture which was then boiled under reflux for 30 min. before distillation of volatile material under reduced pressure. The residue was digested with saturated aqueous sodium hydrogen carbonate, and crystallisation of the undissolved material from ethanol gave phthalylglycylglycine ethyl ether (2·4 g., 50%), m. p. 191—192° alone and when mixed with the samples already described.

Phthalylglycylglycine.—(*a*) The above ester (2 g.) was boiled for 1 hr. with 4N-hydrochloric acid (5 c.c.) and acetone (5 c.c.), and the acetone was evaporated before collection of crystalline phthalylglycylglycine (1·8 g., *ca.* 100%), m. p. 231—232° (lit.,[6, 25, 29] m. p. 229—231°, 229—230°, and 232°) (Found : N, 12·0. Calc. for $C_{12}H_{10}O_5N_2$: N, 11·8%). The *S*-benzylthiuronium salt had m. p. 173°.

(*b*) *Acid chloride method.* A solution of phthalylglycyl chloride (4·5 g.) in pure dry dioxan (25 c.c.) was added dropwise to a stirred and cooled (5°) suspension of glycine (1·5 g., 1 equiv.) and light magnesium oxide (1·2 g., 1·5 equiv.) in water (75 c.c.), and stirring was continued for 10 min. at room temperature after all the acid chloride had been added (cf. Sheehan and Frank[6]). Acidification of the suspension precipitated a mixture of phthalylglycylglycine and phthalylglycine, and the latter was removed by washing of the filtration residue with several portions of hot ethyl acetate. The residue insoluble in ethyl acetate consisted of phthalylglycylglycine, which crystallised from water in fine needles (2·9 g., 55%), m. p. 231—232° (Sheehan and Frank[6] claimed 89·5%, m. p. 229—231°). Lower yields were obtained when magnesium oxide was replaced by sodium hydroxide or by sodium hydrogen carbonate.

(*c*) *Mixed anhydride method.* Vigorous evolution of carbon dioxide occurred when a solution of glycine (0·75 g.) in N-sodium hydroxide (10 c.c.) was added to a solution at 10° of the mixed anhydride from phthalylglycine (2·05 g.) and *iso*butyl chloroformate, which was prepared as described under phthalylglycylglycine ethyl ester [method (*b*)]. The solution was kept for 20 min. before extraction (thrice) with ether, and the aqueous layer was then acidified to Congo-red with 5N-hydrochloric acid. Crystallisation of the precipitate from water gave phthalylglycylglycine in needles (1·9 g., 73%), m. p. 231—232° alone and when mixed with the products described above. Phthalylglycylglycine (62%) was similarly obtained when the mixed anhydride from ethyl chloroformate and phthalylglycine was used.

Phthalylglycyl-DL-alanine.—(*a*) Phthalylglycyl-DL-alanine (2·2 g., 40%, m. p. 222—224°) (lit.,[25] m. p. 221—222°) was prepared from phthalylglycyl chloride and DL-alanine by the procedure described above for phthalylglycylglycine [method (*b*)]. It crystallised from water in leaflets (Found : N, 11·2. Calc. for $C_{13}H_{12}O_5N_2$: N, 11·1%).

(*b*) Phthalylglycyl-DL-alanine (1·7 g., 62%), leaflets (from water), m. p. 223—224°, was prepared from phthalylglycine and DL-alanine by the mixed anhydride method as described for phthalylglycylglycine [method (*c*)].

Phthalylglycylglycine Anilide from 2-Phthalimidomethyloxazolone.—A mixture of phthalylglycylglycine (2·6 g.) and acetic anhydride (25 c.c.) was boiled for 10 min. and the excess of acetic anhydride was removed by distillation under reduced pressure. 2-Phthalimidomethyloxazol-5-one remained as a viscous, clear yellow residue, which dissolved in organic solvents but did not crystallise. Separation of solid began within a few minutes of the addition of aniline (0·5 c.c.) to a benzene solution (10 c.c.) of the oxazolone from phthalylglycylglycine (1·3 g), and the product (1·4 g., 84%) was collected next day and washed with water, aqueous sodium hydrogen carbonate, and water. The *anilide* crystallised from a small volume of ethanol in needles, m. p. 260—262° (Found : C, 63·8; H, 4·3; N, 12·1. $C_{18}H_{15}O_4N_3$ requires C, 64·1; H, 4·5; N, 12·4%).

Phthalyldiglycylglycine Ethyl Ester.—(*a*) 2-Phthalimidomethyloxazolone, prepared as described above from phthalylglycylglycine (2·6 g.), was dissolved in dry benzene (10 c.c.) and freshly distilled glycine ethyl ester (1·0 g.) was added (a second liquid phase separated). The mixture (pink colour) was kept for 14 hr. before evaporation, and crystallisation of the residue from ethanol gave phthalyldiglycylglycine ethyl ester (2·8 g., 80%) in needles, m. p. 229—230° (lit.,[1] m. p. 228—230°) (Found : C, 55·8; H, 5·1; N, 12·7. Calc. for $C_{16}H_{17}O_6N_3$: C, 55·4; H, 4·9; N, 12·1%).

[28] Süs, *Annalen*, 1951, **572**, 96.
[29] Brigl and Klenk, *Z. physiol. Chem.*, 1923, **131**, 66.

(*b*) Phthalylglycyl chloride (1·2 g.) in dry chloroform (10 c.c.) was added during 15 min. to a stirred solution of glycylglycine ethyl ester hydrochloride [29] (1 g.) and triethylamine (1·6 c.c.) in chloroform (10 c.c.). Solid separated from the solution during 4 hours' stirring and the product was collected next day and washed with water, aqueous sodium hydrogen carbonate, and water. Crystallisation of the residue from ethanol gave phthalyldiglycylglycine ethyl ester in needles (1·0 g., 60%), m. p. 229—230° alone and when mixed with material obtained by method (*a*).

Phthalyldiglycyl-L-leucine Ethyl Ester.—Freshly distilled L-leucine ethyl ester (0·8 g.) was added to a dry benzene solution (20 c.c.) of 2-phthalimidomethyloxazol-5-one prepared, as described, from phthalylglycylglycine (1·3 g.). Next day the solution was washed with aqueous sodium hydrogen carbonate and with water before evaporation. The residue was dissolved in hot ethyl acetate and the solution diluted with light petroleum until slightly turbid and allowed to cool very slowly (lagging). *Phthalyldiglycyl-L-leucine ethyl ester* crystallised in fine needles (1·6 g., 80%), m. p. 154—156° raised to 156—157° by recrystallisation, $[\alpha]_D^{29}$ +21·2° (3·3% in $CHCl_3$) (Found: C, 59·2; H, 6·4; N, 10·1. $C_{20}H_{25}O_6N_3$ requires C, 59·5; H, 6·2; N, 10·4%).

Phthalyldiglycyl-S-benzyl-L-cysteine Ethyl Ester.—A suspension of *S*-benzyl-L-cysteine ethyl ester hydrochloride [30] (1·38 g.) in ethyl acetate (30 c.c.) and triethylamine (0·7 c.c.) was shaken for 3 hr. before addition of an ethyl acetate solution of the oxazolone prepared, as described, from phthalylglycylglycine (1·3 g.). The mixture was kept at room temperature for 24 hr. before extraction with water, aqueous sodium hydrogen carbonate, and water, and the organic layer was then evaporated under reduced pressure to leave a gum which crystallised from hot ethanol. Recrystallisation from a large volume of ethanol gave *phthalyldiglycyl-S-benzyl-L-cysteine ethyl ester* in needles (1·9 g., 80%), m. p. 187—188°, $[\alpha]_D^{20}$ −9·1° (0·8% in $CHCl_3$) (Found: C, 59·5; H, 5·2; N, 8·6. $C_{24}H_{25}O_6N_3S$ requires C, 59·6; H, 5·2; N, 8·7%).

Phthalyl-L-leucylglycine.—Fusion [23] of equimolecular quantities of phthalic anhydride and L-leucine at 180—185° for 10 min. gave phthalyl-L-leucine which crystallised from light petroleum in waxy needles (80%), m. p. 114—116°, $[\alpha]_D^{20}$ −26·8° (3·2% in EtOH). The yield and specific rotation of the product were higher than in previous preparations for which m. p.s 115—116° to 118·5—119·5° and $[\alpha]_D$ −21·9° to −24° are recorded.[23, 31–33] Phthalyl-L-leucine gave the *S*-benzylthiuronium salt, m. p. 149°.

Phthalyl-L-leucyl chloride was prepared by boiling thionyl chloride (10 c.c.) with phthalyl-L-leucine for 45 min., and the excess of thionyl chloride was removed under reduced pressure, the last traces being removed by similar distillations after the addition of benzene (2 × 10 c.c.). The residue of acid chloride was dissolved in dry dioxan (20 c.c.) and coupled with glycine (1·4 g.) by the magnesium oxide procedure [6] as described for phthalylglycylglycine [method (*b*)]. Acidification of the reaction mixture with 2N-hydrochloric acid precipitated an oil which was extracted into ether, and evaporation of the ether left a residue of the dipeptide derivative which dissolved in aqueous sodium hydrogen carbonate. Acidification of this solution (after ether-extraction) with 2N-hydrochloric acid precipitated crystalline *phthalyl-L-leucylglycine dihydrate* (3·9 g., 60%), which crystallised from ethyl acetate–light petroleum (b. p. 60—80°) in rosettes, m. p. 164—165°, $[\alpha]_D^{20}$ −37·8° (2·8% in N-Na_2CO_3) (Found: C, 53·8; H, 6·2; N, 7·9. $C_{16}H_{18}O_5N_2,2H_2O$ requires C, 54·2; H, 6·2; N, 7·9%). An attempt to condense the acid chloride with glycine (2 mol.) in acetic acid led to the formation of glycine hydrochloride and acetylglycine, prisms, m. p. 204—206°.

1-*Carboxymethyl*-3-DL-*phthalimidopiperid*-2 : 6-*dione.*—A mixture of phthalyl-DL-glutamic anhydride [34] (2 g.), glycine (0·58 g.), and glacial acetic acid (15 c.c.) was boiled to effect solution and then heated on a steam-bath for 20 min. before evaporation of the solvent. The residue was boiled with acetic anhydride (10 c.c.) for 4 min. and evaporation under reduced pressure then left a syrup which crystallised from aqueous alcohol in leaflets (1·96 g., 76%), m. p. 202° after sintering at 120°. Recrystallisation from aqueous ethanol gave the *monohydrate* of 1-carboxymethyl-3-DL-phthalimidopiperid-2 : 6-dione in leaflets which sintered at 112—116°, resolidified at *ca.* 138°, and finally melted at 204° (Found: C, 53·5; H, 3·8;

[30] Harington and Pitt-Rivers, *Biochem. J.*, 1944, **38**, 417.
[31] Fling, Minard, and Fox, *J. Amer. Chem. Soc.*, 1947, **69**, 2466.
[32] Billmann and Harting, *ibid.*, 1948, **70**, 1473.
[33] Sheehan, Chapman, and Roth, *ibid.*, 1952, **74**, 3822.
[34] King and Kidd, *J.*, 1949, 3315.

N, 8·8. $C_{15}H_{12}O_6N_2,H_2O$ requires C, 53·9; H, 4·2; N, 8·4%). Crystallisation of the monohydrate from ethanol–light petroleum (b. p. 60—80°) gave 1-*carboxymethyl*-3-DL-*phthalimidopiperid*-2 : 6-*dione* (1·81 g., 74%) in rectangular plates, m. p. 204° (Found : C, 57·0; H, 4·2; N, 9·0. $C_{15}H_{12}O_6N_2$ requires C, 57·0; H, 3·8; N, 8·9%). The glutarimide (85—90%) was also obtained by similar cyclisation of preformed phthalyl-γ-DL-glutamylglycine,[34] although in lower overall yield from phthalyl-DL-glutamic anhydride. The *methyl* ester (1·5 g., 90%), prepared by the action of ethereal diazomethane on the acid (1·58 g.), crystallised from methanol in prisms, m. p. 130—132° (Found : C, 58·3; H, 4·2; N, 8·5. $C_{16}H_{14}O_6N_2$ requires C, 58·2; H, 4·2; N, 8·5%).

1-*Ethoxycarbonylmethyl*-3-DL-*phthalimidopiperid*-2 : 6-*dione*.—A solution of the above anhydrous acid (1 g.) in ethanol (10 c.c.) was saturated with dry hydrogen chloride without cooling, and the solution was boiled for 30 min. before evaporation of the ethanolic hydrogen chloride under reduced pressure. A solution of the residue in ether (35 c.c.) was washed with aqueous sodium hydrogen carbonate and with water, and then dried ($MgSO_4$). Evaporation of the ether and crystallisation of the residue from ethanol–light petroleum gave 1-*ethoxycarbonylmethyl*-3-DL-*phthalimidopiperid*-2 : 6-*dione* (0·37 g., 34%) in rectangular plates, m. p. 100—102° (Found : C, 59·7; H, 4·2; N, 8·4. $C_{17}H_{16}O_6N_2$ requires C, 59·3; H, 4·6; N, 8·1%).

1-*Phenylcarbamoylmethyl*-3-DL-*phthalimidopiperid*-2 : 6-*dione*.—Powdered phosphorus pentachloride (2·19 g.) was added to a solution of the above acid (3·16 g.) in dry chloroform (32 c.c.) at 0° and the cooled mixture was shaken intermittently during 30—40 min. before filtration through glass wool, and the residue was discarded. The filtrate was evaporated under reduced pressure and the residue was washed by decantation with dry light petroleum (2 × 15 c.c.) before being dissolved in dry chloroform (20 c.c.) at 0°. This solution was added during 5 min. to a stirred cold solution of aniline (2·8 c.c., an excess) in chloroform (20 c.c.), and the mixture, after being kept at room temperature for 30 minutes, was washed with 2N-hydrochloric acid, aqueous sodium carbonate, and water. Evaporation of the dried ($MgSO_4$) chloroform solution left a pale yellow gum which crystallised (2·29 g., 58%) readily from aqueous ethanol. Recrystallisation from ethanol (charcoal) gave 1-*phenylcarbamoylmethyl*-3-DL-*phthalimidopiperid*-2 : 6-*dione monohydrate*, m. p. 129—132° (Found : C, 61·2; H, 4·7; N, 10·3. $C_{21}H_{17}O_5N_3,H_2O$ requires C, 61·6; H, 4·7; N, 10·3. Found, in material dried at 130° : C, 64·7; H, 4·6; N, 11·3. $C_{21}H_{17}O_5N_3$ requires C, 64·5; H, 4·3; N, 10·7%).

3-DL-*Phthalimidopiperid*-2 : 6-*dione*.—Phthalyl-DL-glutamine [34] (1 g.) was boiled with acetic anhydride (5 c.c.) for 5 min. before removal of volatile material by evaporation under reduced pressure. The residual clear gum was dissolved in aqueous ethanol and, after the pH had been adjusted to 9 by the addition of aqueous sodium carbonate, crystallisation gave 3-DL-*phthalimidopiperid*-2 : 6-*dione* (0·31 g., 33%) in plates, m. p. 270° raised to 272° (decomp.) by recrystallisation from aqueous ethanol or from aqueous dioxan. The compound darkened progressively above 230° (Found : C, 60·9; H, 3·9; N, 10·4. $C_{13}H_{10}O_4N_2$ requires C, 60·5; H, 3·6; N, 10·8%). The mother-liquors gave an intractable oil, and no improvement in yield resulted when the reaction time was doubled.

1-1′-*Carboxyethyl*-3-DL-*phthalimidopiperid*-2 : 6-*dione*.—A mixture of DL-alanine (1·78 g.) and phthalyl-DL-glutamic anhydride [34] (5·18 g.) in glacial acetic acid (40 c.c.) was heated to boiling and then kept on a steam-bath for 20 min. before evaporation under reduced pressure. The residue of crude phthalyl-γ-DL-glutamyl-DL-alanine was boiled for 5 min. with acetic anhydride (15 c.c.), and the residue, obtained by distillation of the anhydride under reduced pressure, crystallised from aqueous ethanol (charcoal) in plates (2·65 g., 38%), m. p. 206° after sintering at 196°. Recrystallisation from aqueous ethanol gave 1-1′-*carboxyethyl*-3-DL-*phthalimidopiperid*-2 : 6-*dione* in plates, m. p. 216° (Found : C, 58·2; H, 4·4; N, 8·3. $C_{16}H_{14}O_6N_2$ requires C, 58·2; H, 4·2; N, 8·5%).

Maleamic Acids.—Maleylglycine ethyl ester. Anhydrous sodium acetate (8·2 g.) was added to glycine ethyl ester hydrochloride (14 g.) in acetic acid (75 c.c.), and the solution was filtered from sodium chloride (kieselguhr). The filtrate was warmed to 80° and maleic anhydride (10 g.) was added, whereupon it dissolved immediately. The solvent was evaporated under reduced pressure and the residue, a pale brown gum, crystallised when triturated with water (yield, 18 g., 90%). *Maleylglycine ethyl ester* (N-*ethoxycarbonylmethylmaleamic acid*) crystallised from water in prisms, m. p. 89—90° (Found : C, 48·3; H, 5·6; N, 7·05%; *M*, by titration, 199. $C_8H_{11}O_5N$ requires C, 47·8; H, 5·5; N, 7·0%; *M*, 201).

Maleylglycine and maleyl-L-alanine. Glycine (5 g.) in acetic acid (80 c.c.) was added to a cold solution of maleic anhydride (6·5 g.) in acetic acid (30 c.c.), and the product (10 g., 85%), m. p. 180—182°, was collected after 6 hr. Maleylglycine, m. p. 186—187° (lit.,[13] m. p. 189—190°), was obtained by crystallisation from butan-2-ol.

Maleylglycine was heated at 190°/15 mm. for 1 hr. by which time effervescence of the fused mass had ceased, leaving a brown amorphous residue which failed to crystallise from a number of solvents. Maleylglycine (4 g.) was boiled with acetic acid (10 c.c.) and acetyl chloride (20 c.c.) until evolution of hydrogen chloride ceased (1 hr.). Considerable decomposition occurred (tar) and the only product isolated was acetylglycine, prisms, m. p. and mixed m. p. 205—206°.

Maleyl-L-alanine (75%), m. p. 175—176°, was prepared from maleic anhydride and L-alanine by the method described for maleylglycine (Found: C, 45·1; H, 5·0; N, 7·3. $C_7H_9O_5N$ requires C, 44·9; H, 4·8; N, 7·5%).

p-*Ethoxycarbonylmaleanilic acid.* Solutions of ethyl *p*-aminobenzoate (5 g.) and maleic anhydride (3 g.) in acetic acid at 50° were mixed and the yellow product (4 g.) was collected by filtration of the cold suspension. The filtrate was evaporated to dryness under reduced pressure and crystallisation of the residue from aqueous ethanol gave further product (4 g.). Recrystallisation of the combined solids (8 g.) from aqueous ethanol gave p-*ethoxycarbonylmaleanilic acid* in prisms (7 g., 88%), m. p. 189—190° (Found: C, 59·3; H, 5·0; N, 5·6. $C_{13}H_{13}O_5N$ requires C, 59·3; H, 4·9; N, 5·3%).

p-*Carboxymaleanilic acid.* A solution of maleic anhydride (1·8 g.) and *p*-aminobenzoic acid (2·4 g.) in acetone (55 c.c.) was boiled for 30 min. and then concentrated to 25 c.c. before filtration from *p*-carboxymaleanilic acid (2·1 g.), m. p. 210—212°. The filtrate deposited a further crop when kept, and recrystallisation of the combined solids from aqueous ethanol gave *p*-carboxymaleanilic acid in prisms (2·6 g., 63%), m. p. 211—212° (La Parola [12] records m. p. 211—212°). A similar yield was obtained when dioxan was used as the solvent instead of acetone. When the reactants were brought together in acetic acid at 100° *p*-acetamidobenzoic acid (1·3 g., 55%), m. p. and mixed m. p. 254—255°, separated from the cold solution (28 c.c.) (Found: C, 60·7; H, 5·25; N, 8·1%; *M*, by titration, 182. Calc. for $C_9H_9O_3N$: C, 60·3° H, 5·0; N, 7·8%; *M*, 179). When cold solutions of the reactants in acetic acid were mixed *p*-acetamidobenzoic acid and a small amount of *p*-carboxymaleanilic acid, m. p. and mixed m. p. 211—212°, were obtained (Found: *M*, by titration, 237. Calc. for $C_{11}H_9O_5N$: *M*, 235).

Attempts to effect ring closure [16] to the maleimido-acid by heating the acid with thionyl chloride for 3 hr., or with phosphorus trichloride in *o*-dichlorobenzene for 2 hr., led to ill-defined amorphous substances.

Maleanilic acid. The acid was prepared according to Anschütz,[18] and crystallised from ethanol in yellow prisms (16 g., 85%), m. p. 187—188° (lit.,[19] m. p. 187—187·5°; Auwers and Schleicher [35] record m. p. 198°). It failed to yield *N*-phenylmaleimide when treated separately and under a variety of conditions [16,17] with acetic anhydride, acetyl chloride, and thionyl chloride (in benzene and in *o*-dichlorobenzene), and was recovered after being heated at 180—190°/12 mm. for 2 hr. Acetanilide (1 g., 47%) was isolated after maleanilic acid (3 g.) had been boiled with acetic anhydride (6·5 c.c.) for 1 hr., and an unidentified white, crystalline substance (2 g.), m. p. 270°, was obtained when acetyl chloride was used instead of acetic anhydride.

Anthracene-9 : 10-endo-αβ-succinimidoacetic Acid and -succinic Anhydride.—A solution of maleylglycine (2 g.) and anthracene (6 g.) in glacial acetic acid (50 c.c.) was boiled for 3 hr. before evaporation of the solvent under reduced pressure and extraction of the residue with aqueous sodium hydrogen carbonate. Acidification of the extract precipitated anthracene-9 : 10-*endo*-αβ-succinimidoacetic acid, which crystallised from ethyl acetate in prisms (0·4 g., 10%), m. p. 271—272° (lit.,[22] m. p. 270—271°) (Found: C, 71·8; H, 4·9; N, 4·2. Calc. for $C_{20}H_{15}O_4N$: C, 72·1; H, 4·6; N, 4·2%). The residue insoluble in aqueous sodium hydrogen carbonate consisted of anthracene-9 : 10-*endo*-αβ-succinic anhydride [36,37] which, after extraction with aqueous potassium hydroxide (45%) and acidification, crystallised from ethyl acetate in large prisms (1·5 g., 47%), m. p. and mixed m. p. 263—264°. When the reactants were boiled for 48 hr. before evaporation to dryness, separation of the products in the manner described above gave anthracene-9 : 10-*endo*-αβ-succinimidoacetic acid and anthracene-9 : 10-*endo*-αβ-*trans*-succinic acid [37] dihydrate, m. p. and mixed m. p. 240—241° (Found: C, 65·0; H, 5·3. Calc.

[35] Auwers and Schleicher, *Annalen*, 1899, **309**, 347.
[36] Diels and Alder, *ibid.*, 1931, **486**, 191.
[37] Bachmann and Scott, *J. Amer. Chem. Soc.*, 1948, **70**, 1458

for $C_{18}H_{14}O_4,2H_2O$: C, 65·4; H, 5·5%). The *monomethyl ester monohydrate*, m. p. 187—188° was obtained by esterification of the acid with ethereal diazomethane (Found : C, 69·7; H, 5·4. $C_{19}H_{16}O_4,H_2O$ requires C, 69·9; H, 5·5%).

The authors thank the Medical Research Council for maintenance grants (to R. W. and W. A. S.).

THE UNIVERSITY, NOTTINGHAM. [*Received, September 6th*, 1956.]