Synthesis of *trans*-4,5-Didehydro-DL-lysine (2,6-Diaminohex-4-enoic Acid) and of 4-Oxo-L-lysine (2,6-Diamino-4-oxohexanoic Acid)

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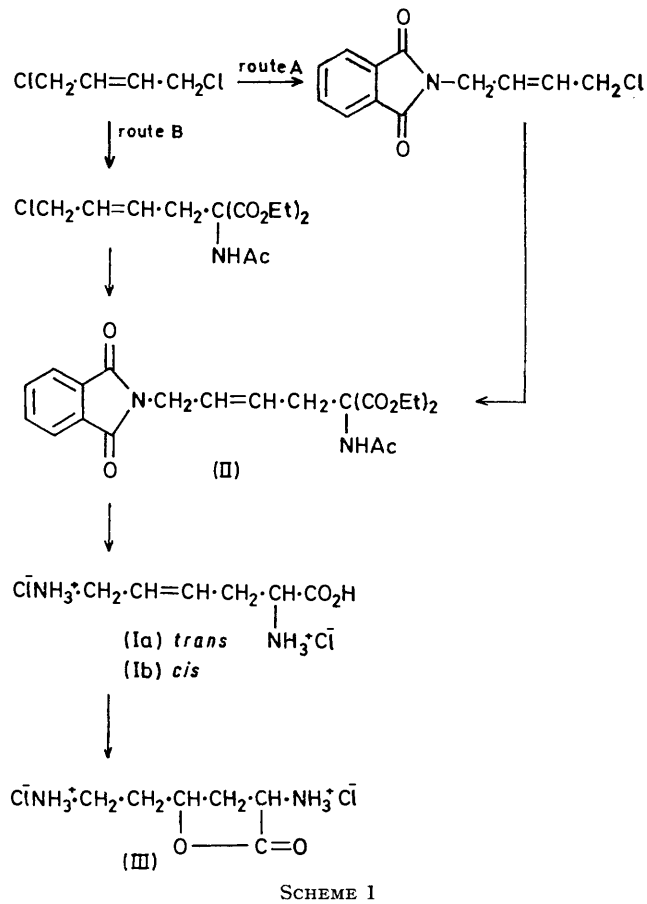
The synthesis of two new amino-acids, *trans*-4,5-didehydro-DL-lysine and 4-oxo-L-lysine is described. Corrections are made concerning a previous report on the synthesis of *cis*- and *trans*-4,5-didehydrolysine. A cyclic hemiacetal structure is proposed for *threo-NN*-diphthaloyl-4-hydroxy-L-lysineamide.

DURING biosynthetic studies involving the cyclisation of 4-substituted homarginines [1] it became necessary to undertake the synthesis of the lysine analogues (I) and (VII). We report details of various synthetic routes utilised to obtain these compounds.

trans-4,5-*Didehydro*-DL-*lysine*.—The syntheses of both *trans*- and *cis*-4,5-didehydro-DL-lysine (2,6-diaminohex-4-enoic acid) dihydrochloride (Ia and b) have been previously reported by Davis, Skinner, and Shive.[2] The synthesis of the former (Ia) involved the condensation of *trans*-1-chloro-4-phthalimidobut-2-ene (obtained from *trans*-1,4-dichlorobut-2-ene) with diethyl acetamidomalonate to produce the substituted acetamidomalonate (II). Compound (II) was subsequently hydrolysed with concentrated hydrochloric acid to yield a white solid which Davis *et al.* claimed to be *trans*-didehydrolysine dihydrochloride (Ia) (Scheme 1, route A). When we utilised this procedure we obtained a crystalline product showing many of the characteristic properties reported earlier.[2] The i.r. spectrum, however, showed a strong γ-lactone carbonyl absorption (1765 cm^{-1}), whereas the expected carbonyl absorption (1720 cm^{-1}) was absent. Thus the reported procedure did not produce compound (Ia) but instead gave the isomeric products resulting from lactonisation, namely a mixture of racemic *threo*- and *erythro*-diastereoisomers of 4-hydroxylysine lactone dihydrochloride (III). Since we expected such a mixture of diastereoisomers to prove difficult to separate, as witnessed by the constancy of the m.p., we resorted to confirming the structure of the product obtained under the conditions of Davis *et al.* by comparison with an equivalent diastereoisomeric mixture of 4-hydroxy-DL-lysine lactone dihydrochloride (III) produced by an independent synthesis.

6-Aminohexan-4-olide (IV), readily obtained from furfural by a modification of the method of Grundmann

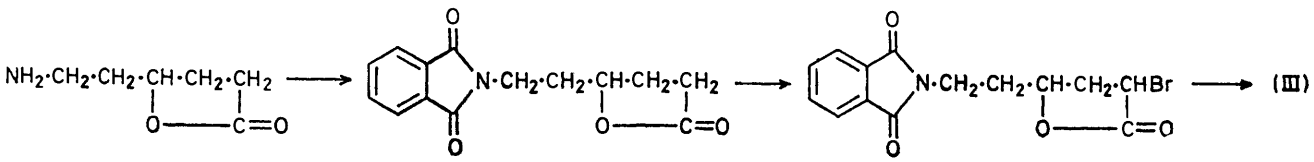


SCHEME 1

and Ruske,[3] was converted into its *N*-phthaloyl derivative (V) with *N*-ethoxycarbonylphthalimide, and then

[1] R. C. Hider and D. I. John, in preparation.
[2] A. L. Davis, G. G. Skinner, and W. J. Shive, *J. Amer. Chem. Soc.*, 1961, **83**, 2279.
[3] C. Grundmann and W. Ruske, *Chem. Ber.*, 1953, **86**, 939.

brominated to yield the 1-bromo-lactone (VI). Treatment of the bromo-lactone with liquid ammonia followed by acidic hydrolysis gave 4-hydroxy-DL-lysine lactone dihydrochloride (III) as the mixture of diastereoisomers. This diamino-lactone hydrochloride mixture was identical (i.r. spectrum, chromatographic R_F values, and m.p.) with that obtained from the hydrolysis of the phthalimido-malonate derivative (II). Further comparison



(IV) (V) (VI)

SCHEME 2

with authentic *threo*-4-hydroxy-L-lysine lactone dihydrochloride,[4] obtained *via* photochlorination of lysine,[5] showed that the n.m.r. spectrum and chromatography R_F values of this pure diastereoisomer were identical with those of the product from the synthetic procedure of Davis *et al.* A simple rate study of the hydrolysis of compound (II) in 20% hydrochloric acid was followed by ionophoresis. Under these hydrolytic conditions the relatively unstable diamino-acid was formed initially, reaching a maximum concentration after 4 h, but was gradually converted into 4-hydroxylysine acetone dihydrochloride (III). After 18 h under reflux (the conditions used by Davis *et al.*[2]) the product was almost entirely the lactone (III), with just a slight trace of the unstable diamino-acid. By using the optimum reaction time *trans*-4,5-didehydrolysine dihydrochloride (Ia) was isolated in high yield. The i.r. and n.m.r. spectra together with results of elemental analysis confirmed the unsaturated structure. A sample of pure *trans*-4,5-didehydro-DL-lysine (Ia) when refluxed with concentrated hydrochloric acid for 20 h was shown by ionophoresis to be almost entirely converted into 4-hydroxylysine lactone (III).

By rearranging the order of condensations with *trans*-1,4-dichlorobut-2-ene, we have further modified the previously reported synthesis (Scheme 1, route B). The conditions for condensation of 1,4-dichlorobut-2-ene with sodiomalonate derivatives are more easily controlled than those where potassiophthalimide is condensed with the dichloro-compound. Thus by adopting route B the extent of disubstitution of the chlorine atoms can be limited. This modification, together with the optimal hydrolytic conditions, gave an increase from 3·5 to 32% in the overall synthetic yield.

We have not investigated the synthesis of *cis*-4,5-didehydro-DL-lysine (Ib) which Davis *et al.* also reported, but since this utilised a similar procedure to that reported for the *trans*-isomer (Ia), we suspect that once again the actual product obtained would have been the mixture of 4-hydroxy-DL-lysine lactones. The confirmatory evidence which these workers reported for the structure of their synthetic products was based on the reduction of the suspected didehydrolysine to lysine, which was not isolated but merely identified by comparative chromatography. The fact that lysine and 4-hydroxylysine in the solvents systems reported have almost the same R_F values makes this type of confirmation dubious and probably accounts for the previous misinterpretation.*

4-Oxo-L-lysine (VII).—By analogy with previously reported syntheses of 4-oxo-2-amino-acids, such as 2-acetylalanine and 2-benzoylalanine,[7] the synthesis of 4-oxolysine was initially thought to hold no outstanding difficulties. The only problems that were foreseen were that the susceptibility of this polyfunctional amino-acid towards β-elimination under alkaline conditions, coupled with its sensitivity towards reduction, would limit the choice of protective groups for amino- and carbonyl functions in any synthetic intermediate. However additional difficulties arose from neighbouring group interactions. Several promising synthetic routes utilising general procedures for the synthesis of amino acids as well as procedures based on the synthesis of closely related diamino-acids did not succeed; eventual success was only achieved through a highly specific pathway.

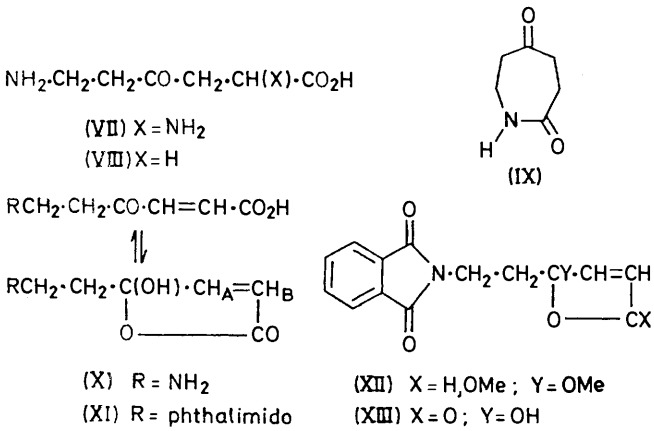
Initial approaches involving the condensation of 4-phthalimido-, 4-chloro-, and 4-nitro-derivatives of 1-halogenobut-2-enes with sodioacetamido- and sodiophthalimido-malonates consistently gave rise to complex mixtures which appeared to arise from cleavage of the newly formed carbon skeleton. Furthermore, attempts to obtain 2-halogeno-derivatives of *N*-acylated 6-amino-4-oxohexanoic acids (VIII) and 4-oxohexanelactam (IX) by specific halogenation of the available parent compounds or their carbonyl protected derivatives only led to mixtures of undesirable halogenation products.

To avoid these difficulties the addition of ammonia to *N*-acyl derivatives of 6-amino-4-oxohex-2-enoic acid

* It has recently been reported [6] that *trans*-4,5-didehydrolysine can be incorporated into collagen. The resulting abnormal collagen possessed much less 4-hydroxylysine and glycosylated 4-hydroxylysine; furthermore it had different biochemical properties. As these workers used the method of Davis *et al.* it seems highly likely that the synthetic amino-acid they used was 4-hydroxylysine.

[4] N. Izumiya, S. Fujita, S. Irreverre, and B. Witkop, *Biochemistry*, 1965, **4**, 2501.
[5] J. Kollonitsch, A. Rosegay, and G. A. Doldouras, *J. Amer. Chem. Soc.*, 1964, **86**, 1857.
[6] P. J. Christner and J. Rosenbloom, *J. Biol. Chem.*, 1971, **246**, 7551.
[7] O. Wiss and H. Fuchs, *Helv. Chim. Acta*, 1952, **35**, 407.

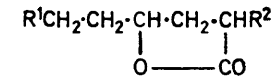
(X) was attempted. In an analogous synthesis Marei and Raphael [8] converted 5-benzamido-4-oxopent-2-enoic acid into 4-oxo-DL-ornithine. Phthaloylation of 2-furylethylamine followed by electrolytic methoxylation



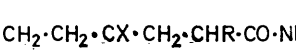
furnished the two diastereoisomers of 2,5-dihydro-2,5-dimethoxy-2-(2-phthalimidoethyl)furan (XII). Oxidation and hydrolysis of this diastereoisomeric mixture yielded 4-oxo-6-phthalimidohex-2-enoic acid as the cyclic tautomer (XIII) [$\nu_{max.}$ 1755 cm^{-1} ($\alpha\beta$-unsaturated γ-lactone CO)]. Treatment of this pseudo-acid with aqueous ammonia yielded an oil which on acid hydrolysis *in situ* yielded a hygroscopic solid. Ionophoretic examination showed this to consist of a mixture of amino-acids. The major product gave brown colour with ninhydrin and was indistinguishable from authentic 4-oxolysine (VII) and 4-oxo-ornithine in two chromatographic systems (Table). The mixture also contained a substantial quantity of glycine with smaller quantities of basic amino-acids. We were unable to isolate the oxo-acid from the mixture because of its unstability. 4-Oxoproline has also been reported to be unstable.[9]

Concurrently with these total synthetic methods, the conversion of lysine into 4-oxolysine was attempted. 4-Hydroxylysine is easily obtained by photochlorination followed by hydrolysis of the resulting 4-chlorolysine.[5] We therefore tried to prepare 4-oxolysine by oxidisng *N*-acyl derivatives of 4-hydroxylysine. Various *NN'*-diacyl derivatives of 4-hydroxy-L-lysine were prepared and subjected to oxidation with permanganate in alkali, in an analogous manner to that reported by Dey [10] for the oxidation of *NN'*-diphthaloyl-4-hydroxy-DL-ornithine to the corresponding 4-oxo-DL-ornithine derivative. Oxidation of the protected 4-hydroxylysine lactones (XIV)—(XVII) by this procedure gave up to 70% recovery of unchanged lactones; the remainder of the reaction mixture on hydrolysis yielded a mixture of more than ten ninhydrin-positive compounds (as shown by ionophoresis and chromatography). The use of excess of permanganate made little difference to the amount of lactone isolated. The four major compounds contained in the hydrolysis mixture were shown to be 4-hydroxylysine, glycine, aspartic acid, and 4-oxolysine, all present in approximately equal amounts. Similar results were obtained when bromine in alkali was used as oxidising agent.

The identification of glycine and aspartic acid as oxidation products in addition to 4-oxolysine, indicated that the 4-oxolysine derivative formed initially was highly susceptible to further oxidation, with preferential fission occurring between C-4 and C-5. β-Alanine, the expected product from fission between C-3 and C-4, was not detected. As the tendency for oxidation of a ketonic carbonyl group is directly related to its ease of enolisation, these results indicated that preferential assistance of enolisation of the C-5 hydrogen atoms occurs in the case of *NN'*-diacyl-4-oxolysine. The interaction of the 6-amide carbonyl group with this hydrogen atom could account for this preferential enolisation (XXI).



(XIV) $R^1 = R^2$ = phthalimido
(XV) R^1 = phthalimido, R^2 = benzamido
(XVI) $R^1 = R^2$ = benzamido
(XVII) $R^1 = R^2$ = benzyloxycarbonylamino



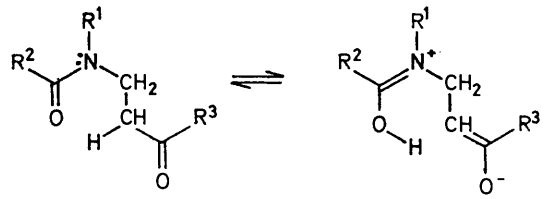
(XVIII) R = benzyloxycarbonylamino, X = H,OH
(XIX) R = benzyloxycarbonylamino, X = O
(XX) R = phthalimido, X = H,OH

In view of this carbon–carbon fission under alkaline conditions it was considered that oxidation of 4-hydroxylysine derivatives under acidic conditions might prove more fruitful. Protection of the carboxy-group by formation of an ester or amide was essential in order to prevent lactonisation under the acidic conditions. Although the *N*-acylated lactones (XIV)—(XVII) were stable to alcoholysis, a report by Witkop *et al.*[4] describes the successful ring opening of *NN'*-di(benzyloxycarbonyl)-4-hydroxylysine (XVII) by ammonia to yield the corresponding primary amide (XVIII). Subsequent oxidation of (XVIII) by chromic acid–acetone yielded *NN'*-di(benzyloxycarbonyl)-4-oxo-L-lysineamide (XIX). Repeating the reaction under these conditions we confirmed Witkop's finding; furthermore treatment of (XIX) with mild acid yielded 4-oxo-L-lysine dihydrochloride (VII) with minimal side reactions.

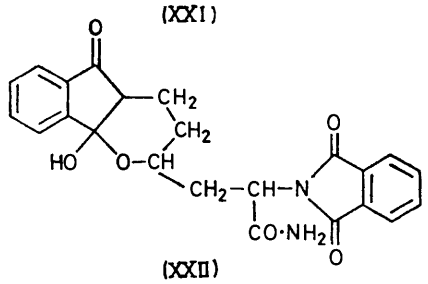
Unfortunately the *NN'*-di(benzyloxycarbonyl) lactone (XVII) can only be prepared with difficulty, in contrast to the corresponding *NN'*-diphthaloyl derivative (XIV).[11] Ammonolysis of (XIV) was therefore studied and eventually the corresponding amide derivative (XX) was obtained. Treatment of this 4-hydroxy-amide with chromic acid–acetone, however, resulted in over 80% recovery of starting material, with no evidence

[8] A. A. Marei and R. A. Raphael, *J. Chem. Soc.*, 1958, 2624.
[9] A. A. Patchett and B. Witkop, *J. Amer. Chem. Soc.*, 1957, **79**, 185.
[10] A. N. Dey, *J. Chem. Soc.*, 1937, 1166.
[11] S. Clarke, R. C. Hider, and D. I. John, *J.C.S. Perkin I*, submitted.

of any oxidation having occurred. Further attempted oxidation with pyridine–chromium trioxide and dimethyl sulphoxide–pyridine–trifluoroacetic acid–dicyclohexylcarbodi-imide [12] once again resulted in almost total recovery of the diphthaloyl hydroxy-amide (XIX) and indicated the total resistance of this compound to oxidation. This exceptional stability of the hydroxy-group of *NN'*-diphthaloyl-*threo*-4-hydroxy-L-lysineamide towards oxidation can be explained by the existence of a



(XXI)



(XXII)

cyclic hemiacetal tautomer (XXII), an analogous hydroxy-amide tautomer has been invoked to explain the resistance of *N*-(hydroxyacyl) dioxopiperazines towards oxidation with chromic acid–acetone.[13] The tendency towards acetal formation found with this system would also explain why ketonic oxidation occurs in preference to the oxidation of the corresponding hydroxy-group of *NN'*-diacyl-4-hydroxylysines under alkaline conditions. Cyclic hemiacetals are extremely stable in alkaline media owing to the formation of stable anions.[14] The specific ease of oxidation in the case where this was achieved (*i.e.* with the benzoxycarbonyl protective group) further supports the concept of interference due to acetal formation. Bodanszky and Ondetti have previously attributed the optical stability of *N*-benzyloxycarbonyl amino-acids to the oxygen–carbonyl resonance within that group.[15] For the same reasons the acetal structure would be less stable in (XVIII) and hence the hydroxy-group would be susceptible to oxidation.

EXPERIMENTAL

N.m.r. spectra were determined at 60 MHz with tetramethylsilane as an internal standard. I.r. spectra were determined for Nujol mulls, unless otherwise stated. M.p.s were determined with a Kofler hot-stage apparatus. Dimethyl sulphoxide was redistilled (72·5°, 12 mmHg) from and stored over 4 Å molecular sieves.

The preparations of the various *N*-acyl derivatives utilised in this paper have been previously reported.[11]

1-Bromo-6-phthalimidohexan-4-olide (VI).—A vigorously stirred suspension of 6-phthalimidohexan-4-olide (V) (1·04 g, 0·0025 mol) and red phosphorus (0·06 g, 0·003 mol) in carbon tetrachloride (20 ml) was cooled to 0°. Bromine (0·385 ml) was added during 1 h. The mixture was then heated to 70°, and bromine (0·385 ml) was added during 30 min. The temperature was maintained between 70 and 80° for 3 h. Air was passed through the cooled mixture for 30 min and water (0·15 ml) was added. When the resulting vigorous reaction ceased, water (1·5 ml) was added, and the mixture was refluxed for 4 h. On cooling, methylene chloride (50 ml) and water (30 ml) were added, and the organic layer was washed with sodium carbonate solution (5%; 50 ml), sodium hydrogen sulphite solution (5%; 50 ml), and water (10 ml), and dried ($MgSO_4$). Removal of the solvent yielded an oil, which solidified when scratched to give the *bromo-lactone* (VI), (1·1 g, 84%), m.p. 122—123° (from ethyl acetate) (Found: C, 49·9; H, 3·6; N, 4·2. $C_{14}H_{12}BrNO_4$ requires C, 49·7; H, 3·9; N, 4·1%); $\nu_{max.}$ 1720 and 1770 (phthalimido CO) and 1785 cm^{-1} (γ-lactone CO); τ 2·1 (4H, phthalimido), 6·1 (2H, CO·N·CH_2·C), 7·85 (2H, CO·N·CH_2·CH_2), 5·5 (1H, C*H*·O·COR), 7·5, (2H, CH_2·CHBr), and 5·1, (1H, C*H*Br).

4-Hydroxy-DL-lysine Lactone Dihydrochloride (III).—The bromo-lactone (VI) (0·338, 0·001 mol) dissolved in saturated ethanolic ammonia was stored at room temperature for 12 h. Evaporation left an oil, which was directly hydrolysed by hydrochloric acid (20%; 10 ml) for 4 h. The cooled hydrolysate was extracted with methylene chloride and the aqueous fraction was evaporated to yield a hygroscopic solid. Trituration with ethanol–ether (5 : 1) and cooling to 0° for 12 h yielded a solid. Recrystallisation from ethanol and ether gave the *lactone dihydrochloride* (III) (0·148 g, 66%), m.p. 177—187° (Found: C, 32·9; 6·5; N, 12·75. $C_6H_{14}Cl_2N_2O_2$ requires C, 33·15; H, 6·5; N, 12·9%); $\nu_{max.}$ 1765 (γ-lactone CO), and 3350—2200 and 1990 cm^{-1} (NH_3^+); τ (CF_3·CO_2H) 6·4 (2H, NH_3^+·CH_2·C), 7·3—7·5 (4H, CH_2·CHO·COR), 4·9 [1H, C*H*(NH_3^+)CO_2R], 5·2 (1H, C*H*·O·COR), 1·9 (3H, ε-NH_3^+), and 1·9 (3H, α-NH_3^+).

*Ethyl 2-Acetamido-6-chloro-2-ethoxycarbonyl-*trans*-hex-4-enoate*.—An ethanolic solution (50 ml) of diethyl acetamidosodiomalonate (2·4 g, 0·01 mol) was added dropwise during 2 h to a refluxing ethanolic solution (100 ml) of 1,4-dichloro-*trans*-but-2-ene (1·25 g). The mixture was refluxed for 1 h, cooled, filtered, and evaporated to yield an oil. The bulk of this oil distilled between 130 and 140° (0·1 mmHg) and the distillate solidified on cooling. Recrystallisation from ethanol and water yielded the *diester* (1·66 g, 55%), m.p. 63—63·5° (Found: C, 51·2; H, 6·6; N, 4·3. $C_{11}H_{20}ClNO_5$ requires C, 51·1; H, 6·55; N, 4·2%); $\nu_{max.}$ 3240 (amide NH), 1640 and 1515 (amide I and II), and 1740 cm^{-1} (ester CO); τ 5·85 (2H, Cl·CH_2·C), 7·0 (2H, CH_2·CH·C), 7·8 (3H, CH_3·CO·NH), 2·9 (1H, amide NH), 4·15, (2H, olefinic), and 5·5 (4H) and 8·6 (6H) (2 × OEt).

*Ethyl 2-Acetamido-2-ethoxycarbonyl-6-phthalimido-*trans*-hex-4-enoate* (II).—The foregoing malonate (3·05 g, 0·01 mol) and potassiophthalimide (1·85 g, 0·01 mol) were ground together, and the mixture was protected from moisture and heated at 150° for 2 h. Methylene chloride (100 ml) and water (50 ml) were added to the cooled

[12] K. E. Pfitzner and J. G. Moffatt, *J. Amer. Chem. Soc.*, 1963, **85**, 3027.
[13] H. Ott, A. J. Frey, and A. Hofmann, *Tetrahedron*, 1963, **19**, 1675.
[14] R. G. Groit and A. J. Frey, *Tetrahedron*, 1963, **19**, 1661.
[15] M. Bodansky and M. Ondetti, 'Peptide Synthesis,' Interscience, London, 1966.

crystalline mass. The organic layer was washed with water (2 × 50 ml), dried ($MgSO_4$), and evaporated to yield a solid. Recrystallisation from benzene and light petroleum gave the phthalimidomalonate (II) (3·65 g, 85%), m.p. 130—131° (lit.,[2] 126—127°).

trans-4,5-*Didehydrolysine* (2,6-*Diaminohex*-4-*enoic Acid*) *Dihydrochloride* (Ia).—The finely ground substituted malonate (II) (4·16 g, 0·01 mol) was added to hydrochloric acid (20%; 60 ml). The suspension was refluxed for 4 h. The cooled solution was extracted with methylene chloride (3 × 100 ml), and the aqueous fraction was freeze-dried to yield a crystalline solid. Recrystallisation from water and ethanol yielded trans-4,5-*didehydrolysine dihydrochloride* (Ia) (2·0 g, 92%), m.p. 170—200° (Found: C, 33·2; H, 6·5; N, 12·6. $C_6H_{14}Cl_2N_2O_2$ requires C, 33·15; H, 6·5; N, 12·9%); $\nu_{max.}$ 3000—2500 and 1970 (NH_3^+), and 1710 cm^{-1} (carboxylic CO); τ ($CF_3{\cdot}CO_2H$) 6·85 (2H, $NH_3^+{\cdot}CH_2{\cdot}C$), 3·75 (2H, olefinic), 6·0 (2H, $CH_2{\cdot}C{\cdot}C$), 4·35 [1H, $CH(NH_3^+){\cdot}CO_2H$], 2·65 (3H, ε-NH_3^+), and 2·2 (3H, α-NH_3^+).

4-*Oxohexanelactam* (IX).—4-Hydroxyhexanelactam [16] was purified by absorption on alumina and eluted with methanol. Chromium trioxide (3 g, 0·003 mol) was added to dry pyridine (50 ml), and the resulting suspension cooled to 0°. A solution (30 ml) of 4-hydroxyhexanelactam (0·645 g, 0·005 mol) in pyridine–dimethyl sulphoxide (5 : 1) was added to the complex, and the mixture was set aside at room temperature for 24 h. Ethyl acetate (200 ml) was added and the mixture was filtered through Hiflosupercel. The filtrate was passed down an alumina column (3 × 25 in), and elution was continued with ethyl acetate until removal of pyridine was complete. Subsequent elution with methanol–ethyl acetate (1 : 9) yielded a yellow band. Evaporation yielded 4-*oxohexanelactam* (IX) (0·41 g, 60%), m.p. 138—138·5° (from chloroform and light petroleum) (Found: C, 56·6; H, 7·1; N, 10·7. $C_6H_9NO_2$ requires C, 56·8; H, 7·1; N, 11·0%); $\nu_{max.}$ 3050—3300 (amide NH), 1715 (ketonic CO), and 1680 cm^{-1} (amide CO); τ 2·2 (1H, NH), 6·35 (2H, $CO{\cdot}NH{\cdot}CH_2{\cdot}C$), 7·2 (2H, $NH{\cdot}CO{\cdot}CH_2{\cdot}C$), and 7·2 (4H, $C{\cdot}CH_2{\cdot}CO{\cdot}CH_2{\cdot}C$). Treatment of the lactam (IX) with ethane-1,2-dithiol gave 4,4-*ethylenedithiohexanelactam* (0·23 g, 55%), m.p. 165—166° (Found: C, 47·2; H, 6·3; N, 6·8. $C_8H_{13}NOS_2$ requires C, 47·4; H, 6·4; N, 6·9%); $\nu_{max.}$ 3050 and 3280 (amide NH) and 1640 and 1690 cm^{-1} (amide CO); $\nu_{max.}$ ($CHCl_3$) 3410 (amide NH) and 1665 cm^{-1} (amide CO); τ 2·8 (1H, NH), 6·7 (2H, $CO{\cdot}NH{\cdot}CH_2{\cdot}C$), 7·5 (2H, $NH{\cdot}CO{\cdot}CH_2{\cdot}C$), 7·7—8·0 [4H, $CH_2{\cdot}C(SCH_2)_2$], and 6·7 (4H, $S{\cdot}CH_2CH_2{\cdot}S$).

1-(2-*Furyl*)-2-*phthalimidoethane*.— 2-Furylethylamine [17] (4·14 g, 0·04 mol) and *N*-ethoxycarbonylphthalimide (8·8 g, 0·044 mol) were dissolved in acetonitrile (50 ml), triethylamine (4 ml, 0·05 mol) was added, and the mixture was set aside for 12 h. The precipitated triethylamine hydrochloride was filtered off and the solvent was removed to yield 1-(2-*furyl*)-2-*phthalimidoethane* (6·2 g, 65%), m.p. 76—77° (from methanol) (Found: C, 69·8; H, 4·7; N, 5·8. $C_{14}H_{11}NO_3$ requires C, 69·7; H, 4·6; N, 5·8%); $\nu_{max.}$ 1790 and 1720 cm^{-1} (phthalimido CO); τ 2·3 (furyl 5-H), 3·4 (furyl 3-H), 2·0 (4H, phthalimido), 6·0 (2H, $CO{\cdot}N{\cdot}CH{\cdot}C$), and 7·0 (2H, $ArCH_2{\cdot}C$).

2,5-*Dihydro*-2,5-*dimethoxy*-2-(2-*phthalimidoethyl*)*furan* (XII).—1-(2-Furyl)-2-phthalimidoethane (2·42 g, 0·02 mol) in methanol (35 ml) and ether (20 ml) at −25° was added dropwise to a cold solution of bromine (1·2 ml) in methanol (7 ml) at such a rate that the temperature remained below −20°. The mixture was stirred for 1 h at −20°, then cooled to −40°, and treated with gaseous ammonia, the temperature being maintained below −5°. The mixture was allowed to attain room temperature, and then stirred for a further 30 min. Ether (150 ml) was added and the precipitated ammonium bromide was filtered off. The filtrate was evaporated to yield the *product* (XII) (4·9 g, 85%). A sample obtained by distillation (200—210° at 0·1 mmHg) was analysed (Found: C, 63·2; H, 5·4; N, 5·0. $C_{16}H_{17}NO_5$ requires C, 63·4; H, 5·6; N, 4·9%); $\nu_{max.}$ 1710 and 1770 cm^{-1} (phthalimido CO); τ 2·0 (4H, phthalimido), 6·1 (2H, $CO{\cdot}N{\cdot}CH_2{\cdot}C$), 7·75 (2H, $CO{\cdot}N{\cdot}CH_2{\cdot}CH_2$), 6·45 (3H, OMe), 6·75 (3H, OMe), 3·75 (2H, olefinic), and 4·3 (1H, $O{\cdot}CH{\cdot}O$).

4-*Oxo*-6-*phthalimidohex*-2-*enoic Acid* (XI).—To a stirred solution of the mixed isomers (XII) (3·3 g, 0·011 mol) in acetone (50 ml) at 0 °C was added a cold solution of chromium trioxide (2·5 g) in water (30 ml) and concentrated sulphuric acid (8·5 ml) at such a rate that the temperature did not exceed 10°. After 1 h, a mixture of methylene chloride (100 ml) and ice (100 g) was added to the green suspension. The organic extract was washed with water (2 × 100 ml), dried ($MgSO_4$), and evaporated to yield an oil (2·5 g) which solidified. Recrystallisation from chloroform (20 ml) yielded 4-*oxo*-6-*phthalimidohex*-2-*enoic acid* (XI) (1·3 g, 44%), m.p. 151—152° (Found: C, 61·3; H, 4·05; N, 5·0. $C_{14}H_{11}NO_5$ requires C, 61·5; H, 4·05; N, 5·1%); $\nu_{max.}$ 3400 (OH), 1710 and 1775 (phthalimido CO), and 1755 cm^{-1} (αβ-unsaturated γ-lactone CO); τ [$(CD_3)_2SO$] 2·2 (4H, phthalimido), 2·4 (1H, H_A), 3·75 (1H, H_B), 6·4 (2H, $CO{\cdot}N{\cdot}CH_2{\cdot}CH_2$), 7·8 (2H, $CO{\cdot}N{\cdot}CH_2{\cdot}CH_2$), and 2·4 (1H, OH).

Ammonolysis of 4-*Oxo*-6-*phthalimidohex*-2-*enoic Acid* (XI).—The acid (0·5 g) was dissolved in ammonia (*d* 0·88; 3 ml) and stored at room temperature for 16 h. The solvent was removed under reduced pressure to yield a hygroscopic solid, which was hydrolysed by hydrochloric acid (20%; 10 ml) for 4 h. After extraction with methylene chloride the aqueous solution was freeze-dried to give an oil, which was shown by ionophoresis to be a mixture of several amino-acids (Table).

	R_F Values	
Amino-acid	Butan-1-ol–acetic acid–water (3 : 1 : 1)	Pyridine–water (65 : 35)
4-Hydroxy-L-lysine lactone (III)	0·10	0·50
4-Oxo-L-lysine (VII)	0·15	0·45
4-Oxo-DL-ornithine	0·15	0·45

Oxidation of NN′-*Diphthaloyl*-4-*hydroxylysine Lactone* (XIV).—The lactone (XIV) [11] (0·1 g, 0·00025 mol) was dissolved in aqueous potassium hydroxide (0·25N; 1 ml) at room temperature. This solution was cooled to 0°. On addition of a dilute solution of potassium permanganate (0·027 g in 0·5 ml), a deep green colour developed. Continuous stirring for 3 h at 0° caused precipitation of manganese dioxide. The mixture was filtered and acidified. Extraction with chloroform yielded an oil. Trituration with benzene initiated crystallisation. Recrystallisation from benzene yielded starting material (XIV) (0·045 g, 45%). The mother liquor from the trituration was hydrolysed by hydrochloric acid, and the hydrolysate was studied by ionophoresis.

[16] G. Boffa, *Gazzetta*, 1956, 646.
[17] A. Dornow and M. Gellrich, *Annalen*, 1955, **594**, 177.

Identical treatment of *NN'*-dibenzoyl-4-hydroxy-L-lysine lactone (XVI) [11] and *N*(α)-benzoyl-*N*(ε)-phthaloyl-4-hydroxy-L-lysine lactone (XV) [11] resulted in the isolation of starting material, and the mother liquors also yielded a similar ionophoretic distribution of amino-acids on hydrolysis.

4-Oxo-L-lysine (2,6-Diamino-4-oxohexanoic Acid) Dihydrochloride (VII).—*NN'*-Di(benzyloxycarbonyl)-4-oxo-L-lysineamide (XVIII) [4] (1·62 g) was refluxed in concentrated hydrochloric acid–acetone (5 : 1 v/v; 10 ml) for 3 h. After extraction with methylene chloride the aqueous fraction was evaporated to give an oil (0·8 g), which failed to crystallise on trituration in various solvents. The oil was dissolved in ethanol–ether (5 : 2) and maintained at 0° for several days. Crystallisation yielded an extremely hygroscopic solid, *4-oxo-L-lysine dihydrochloride* (VII) (0·150 g), m.p. 160—180° (Found: C, 30·7; H, 6·1; N, 12·4. $C_6H_{14}Cl_2N_2O_3$ requires C, 30·9; H, 6·05; N, 12·1%); $\nu_{max.}$ (KBr) 1720 (ketonic CO), 1710 (carboxylic CO), and 3100—2200 cm^{-1} (NH_3^+).

threo-NN'-*Diphthaloyl-4-hydroxy-L-lysineamide* (XX).—*NN'*-Diphthaloyl-4-hydroxy-L-lysine lactone (XIV)[11] (0·2 g, 0·0005 mol) was dissolved in acetonitrile (50 ml) previously saturated with ammonia. The flask was stoppered and left at room temperature for 24 h. The solvent was evaporated off under reduced pressure to yield an oil. Trituration with ether at 0° initiated crystallisation. Recrystallisation from acetone, benzene, and light petroleum (2 : 2 : 1) gave the *amide* (XX) (0·15 g, 72%), m.p. 175—177° (decomp) (Found: C, 63·2; H, 4·1; N, 9·95. $C_{22}H_{17}N_3O_6$ requires C, 63·0; H, 4·1; N, 10·0%); $\nu_{max.}$ 1770 and 1710 (phthalimido CO), 1650 (amide CO), and 3500—3200 cm^{-1} (OH and amide NH); τ [$(CD_3)_2SO$] 2·05 and 2·1 (8H, aromatic), 2·4 (2H, CO·NH_2), 6·2 (2H, CO·N·CH_2·C), 5·3 (1H, CO·N·C*H*R·CO·NH_2), 6·1 (1H, C*H*OH), and 7·5—8·0, (4H, CH_2·CH·OH).

Attempted Oxidation of the Hydroxy-amide (XX).—(i) *With aqueous chromium trioxide.* To a solution of the lysineamide (XX) (0·22 g, 0·0005 mol) in acetone (40 ml), was added 8N-chromium trioxide (0·5 ml) in sulphuric acid during 5 min. Triethylamine (0·4 ml) was added, and the mixture was concentrated under reduced pressure to a small volume, diluted with water, and extracted with methylene chloride (100 ml). The extract was dried ($MgSO_4$) and evaporated to yield a crystalline residue. Recrystallisation from ethyl acetate gave starting material (0·18 g, 82%), m.p. 140°.

(ii) *With pyrimidine–chromium trioxide.* The lysineamide (XX) (0·22 g, 0·0005 mol) was dissolved in pyridine and added to a solution of chromium trioxide (0·3 g) in pyridine (5 ml). The mixture was stored at room temperature for 24 h. The resulting paste was treated as described for the preparation of 4-oxohexanelactam (IX). Elution of the alumina column with ethyl acetate yielded *NN'*-diphthaloyl-4-hydroxy-L-lysine lactone (XIV) (0·12 g, 59%) (recrystallised from chloroform and light petroleum).

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