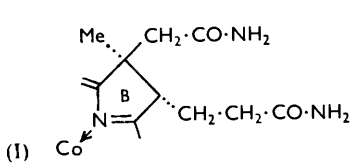
670. *Chemistry of the Vitamin B_{12} Group. Part VII.* The Products of Chromic Acid Oxidation.*

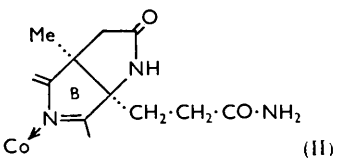
By V. M. Clark, A. W. Johnson, I. O. Sutherland, and Sir Alexander Todd.

Chromic acid oxidation of dehydrovitamin B_{12} and the hexacarboxylic acid (V), but not of vitamin B_{12} or its acid degradation products, yields a new imide-lactam shown to have structure (VI). The isolation of this product confirms the presence of an extra ring both in dehydrovitamin B_{12} and in the products of vigorous alkaline hydrolysis of the vitamin.

In the elucidation[1,2] of the structure of vitamin B_{12} oxidative studies have been of importance, for, depending on the conditions used for oxidation, it is possible either (i) to modify the macrocyclic system in the vitamin, *e.g.*, in the formation of dehydrovitamin B_{12} (cf. I ⟶ II)[2] wherein the acetamido-residue of ring B has undergone cyclisation to give the *cis*-fused pyrrolidone, or (ii) to disrupt the molecule, whereby a variety of aliphatic acids are obtained together with the succinimide (III; R = NH_2) and the related lactone[3] (IV). The substituted succinimides (III; R = OH) and (IV) were first obtained as optically inactive products from the oxidation of acid-hydrolysed vitamin B_{12} using sodium dichromate in acetic acid; however, the vitamin itself yields optically active (III; R = NH_2) in addition to (IV).



(I)



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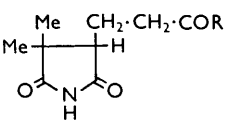
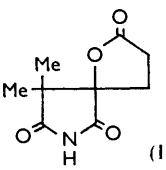


(III)



(IV)

We now report an extended study of this type of oxidation using chromic acid in aqueous acetic acid, the substrates being vitamin B_{12}, dehydrovitamin B_{12}, and the hexabasic acid (V) arising from vigorous alkaline hydrolysis of the vitamin.[4] Both the vitamin and the hexabasic acid yield the lactone (IV) whereas, under the same conditions, it has proved impossible to convert amide or acid (III; R = NH_2 or OH) into this lactone (IV).[5] Formation of the *spiro*-lactone by oxidation is presumably akin to that of the lactone produced by halogenation of vitamin B_{12},[2] *i.e.*, cyclisation occurs at an early stage in the oxidative process.

Initial experiments on oxidation of the hexabasic acid (V) yielded both the acid (III; R = OH) and the lactone (IV), together with a third product, $C_{10}H_{12}O_5N_2$, which also appeared to be an imide. The products (III) and (IV) are obviously derived from ring C of the macrocycle, and, whilst it would not be expected that rings A and D would readily yield succinimides, ring B might be the source of similar oxidation products.[6] The

* Part VI, *J.*, 1957, 1168.

[1] Hodgkin, Pickworth, Robertson, Trueblood, Prosen, and White, *Nature*, 1955, **176**, 325; Bonnett, Cannon, Johnson, Sutherland, Todd, and Lester Smith, *ibid.*, p. 328.

[2] Bonnett, Cannon, Clark, Johnson, Parker, Lester Smith, and Todd, *J.*, 1957, 1158.

[3] Kuehl, Shunk, and Folkers, *J. Amer. Chem. Soc.*, 1955, **77**, 251; Kuehl, Shunk, Moore, and Folkers, *ibid.*, p. 4418.

[4] Bonnett, Cannon, Johnson, and Todd, *J.*, 1957, 1148.

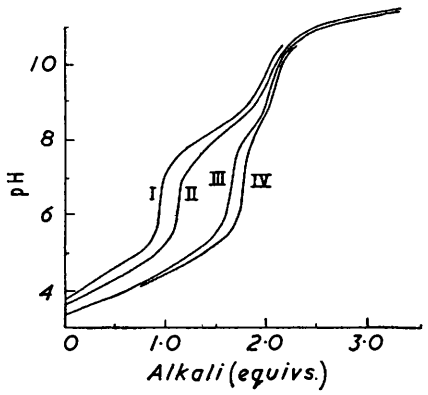
[5] Dr. K. Folkers, personal communication.

[6] Cf. Ficken, Johns, and Linstead, *J.*, 1956, 2272, 2280.

presence of two nitrogen atoms in this new oxidation product derived from the hexacarboxylic acid (V) led us to believe that the product had structure (VI) and, in this paper, we present evidence in support of this view. As might be expected, this new material has also been observed among the products of oxidation of dehydrovitamin B_{12} (II) and other alkaline hydrolysis products of the vitamin, but not from the vitamin itself or from its acid degradation products: these observations are in accordance with our previously postulated dependence of the ring B cyclisation upon alkaline oxidative conditions.

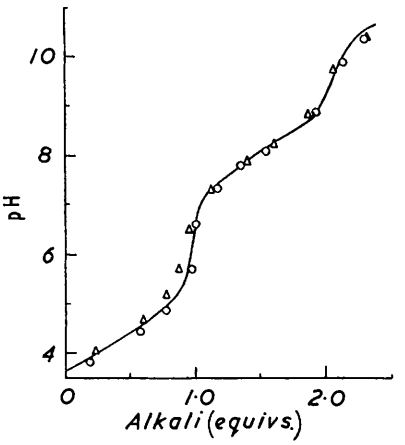
The infrared spectrum of the new product contained bands in the carbonyl region at

FIG. 1. *Alkaline hydrolysis of the oxidation product.*



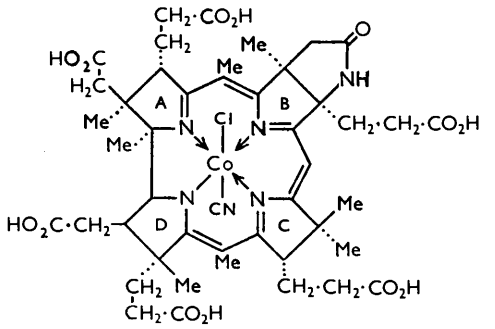
I, Initial titration; II, III, and IV after exposure to 3 equivs. of 0·003N-alkali for 2, 9, and 22 days.

FIG. 2. *Titration of the oxidation product.*



△ *Back-titration against acid.*
○ *Re-titration against alkali.*

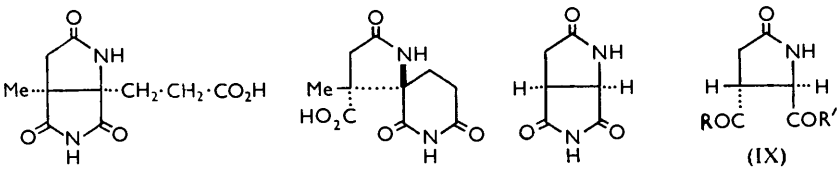
1775, 1723, and 1700 cm.$^{-1}$ (Nujol mull) closely analogous to the bands at 1776 and 1709 cm.$^{-1}$ (Nujol mull) of the succinimide (III; R = OH), although in the former case there was an additional intense band at 1655 cm.$^{-1}$. The spectrum in dioxan solution did not contain the 1655 cm.$^{-1}$ band though the other carbonyl bands remained at 1780 and 1727 cm.$^{-1}$. In this solution spectrum we assign these bands to the carbonyl groups of the imide, with the lactam-carbonyl absorption superposed upon the 1727 cm.$^{-1}$ band. That the spectrum of the new degradation product was consonant with structure (VI) rather than (VII) was clear from a comparison of its infrared spectrum with that of α-acetamidoglutarimide which exhibited bands at 1747, 1664, and 1623 cm.$^{-1}$ (KBr disc).



(V)

On treatment with aqueous barium hydroxide, the oxidation product slowly liberated one mole of ammonia: the course of hydrolysis was also followed by electrometric titration

and after three weeks in the presence of three equivalents of 0·003N-sodium hydroxide solution the compound was almost completely converted into a dicarboxylic acid (Fig. 1), a change corresponding to the cleavage of the succinimide to the succinamic acid. Electrometric titration of a dilute aqueous solution of the oxidation product gave a completely reversible curve (Fig. 2), showing the presence of two acidic groups within the molecule, the apparent pK_a values being 4·4 and 8·1.* Treatment with diazomethane gave a neutral dimethyl compound: this new compound shows only the lactam N–H stretching frequency in the infrared region (original product, 3340, 3180, 3080 cm.$^{-1}$; dimethyl derivative 3200, 3090 cm.$^{-1}$) and gives, on alkaline hydrolysis, methylamine, indicating that the original oxidation product contained an acidic >N–H group. This sequence of methylation, followed by the liberation of methylamine on alkaline hydrolysis, was also observed with



(VI) (VII) (VIII) (IX)

succinimide,[7] and with the lactone-imide (IV). The *cis*-fused pyrrolidone-succinimide (VIII) could also be methylated with diazomethane though lack of material prevented study of the subsequent hydrolysis.

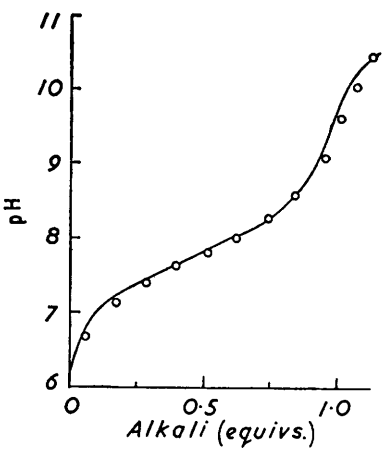


FIG. 3. *Titration of* 4-*hydroxy*-3 : 3-*dimethyl*-2 : 5-*dioxopyrrolidine*-4-*propionic lactone.*

Continuous line—forward-titration against alkali. ○ *Back-titration against acid.*

Of the two pK_a values, that of 4·4 evidently corresponds to the carboxyl group in structure (VI), whilst that of 8·1 must relate to the >NH of the succinimide. The pK_a values of succinimides usually lie within the range 8·8—9·7 (see Table 1), being approximately two units more acidic than glutarimide (pK_a' = 11·2).[8]

Comparison of the values for compounds (IV) and (VIII) with the others suggests that the enhancement of acidity is related (i) to substitution by a more electronegative atom and (ii) to a specific geometrical disposition of the carbonyl dipoles in the imide and lactone or lactam system, since neither acyclic amido-substitution (compound 3) nor a fused carbocyclic ring (compounds 5, 6, 7) has an analogous effect.

The close similarity of the oxidation product and compound (VIII) on electrometric

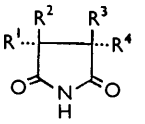
* pK_a values refer to the pH recorded at 20° by a Pye glass electrode and meter of an aqueous solution at the half-neutralisation point. The meter was standardised with aqueous buffer at pH 7.

[7] Labruto, *Gazzetta*, 1933, **63**, 266.

[8] Kornfeld, Jones, and Parke, *J. Amer. Chem. Soc.*, 1949, **71**, 150.

titration is paralleled in their ultraviolet spectra. Succinimides usually exhibit an absorption maximum at ~240 mμ (ε ~120),[9] but the oxidation product has $\lambda_{max.}$ 254 mμ (ε 364) and (VIII) has $\lambda_{max.}$ 252 mμ (ε 233). We therefore feel justified in concluding that our oxidation product $C_{10}H_{12}O_5N_2$ is correctly represented by structure (VI).

TABLE 1. pK_a *values for succinimides*



No.	R^1	R^2	R^3	R^4	pK_a
1	H	H	H	H	9·70 [a]
2	H	H	H	NH_2	9·0 [b]
3	H	H	H	NHAc	8·85
4	Me	Me	Me	Me	9·7
5	H	*cis*-$[CH_2]_2$		H	9·63
6	H	*cis*-$[CH_2]_3$		H	9·53
7	H	*cis*-$[CH_2]_4$		H	9·72
Compound (IV)	Me	Me	*spiro*-Butyrolactone		7·9 *
Compound (VIII)	H	*cis*-2 : 3-Pyrrolid-5-one		H	8·15

All values refer to aqueous solution and are reproducible to ±0·05 unit.

* The reversible nature of the curves obtained in the titration of this compound (Fig. 3) shows the acidity attributable to the loss of a proton rather than the opening of the lactone ring.

[a] Schwarzenbach and Lutz, *Helv. Chim. Acta*, 1940, **23**, 1162. [b] Sondheimer and Holley, *J. Amer. Chem. Soc.*, 1954, **76**, 2467.

The closest analogue to the oxidation product so far synthesised is compound (VIII). Triethyl 1-aminoprop-1-ene-1 : 2 : 3-tricarboxylate,[10] on reduction with hydrogen and Raney nickel, gave diethyl *trans*-5-oxopyrrolidine-2 : 3-dicarboxylate (IX; R = R′ = OEt). That intramolecular acylation (*i.e.*, lactam formation) had occurred in preference to formation of the dioxopiperazine was confirmed by a molecular-weight determination. Hydrolysis by ethanolic sodium hydroxide gave the sodium salt of the monoester (IX; R = OEt, R′ = OH),* which, on treatment with ammonia gave the ammonium salt of the monoamide; conversion into the acid and esterification by diazomethane then gave the *trans*-amido-ester (IX; R = NH_2, R′ = OMe).

The cyclisation of succinamic esters to succinimides under alkaline conditions proceeds readily; thus Sondheimer and Holley [11] obtained a 90% yield in three minutes using an aspartic acid (*i.e.*, acyclic) derivative. However, compounds (IX) were *trans*, the diacid giving no anhydride even under forcing conditions, and cyclisation to the succinimide must occur after inversion at the ring carbon atom bearing the methoxycarbonyl group. In the event, by using sodium methoxide in methanol, a 57% yield of the imide (VIII) was obtained after 24 hours at room temperature.

It is of interest that the new oxidation product (VI), 1-2′-carboxyethyl-5-methyl-2 : 7-diaza*bicyclo*[3 : 3 : 0]octane-3 : 6 : 8-trione, should be optically active. A 5% solution showed no detectable rotation in sodium light but at 365 mμ it had [α] −68·6°.

EXPERIMENTAL

Oxidation of the Hexacarboxylic Acid Degradation Product (V) *of Vitamin* B_{12}.—To a well-stirred solution of the hexabasic acid (3·972 g.) in acetic acid (250 ml.) at 20° was added, during 45 min., a solution of chromic acid (12·03 g.) in water (100 ml.). After 1 hr. at room temperature the solution was slowly heated during 4 hr. to 100° and kept at that temperature for

* Assignment of direction of preferential hydrolysis based on the behaviour of *N*-acylaspartic acids [11] and the observed pK_a values.[12]

[9] Cf. Saidel, *Nature*, 1953, **172**, 955.
[10] Wislicenus and Waldmüller, *Ber.*, 1911, **44**, 1566.
[11] Sondheimer and Holley, *J. Amer. Chem. Soc.*, 1954, **76**, 2467.
[12] Clayton, Kenner, and Sheppard, *J.*, 1956, 371.

2 hr. Excess of chromic acid was then reduced by ethanol (40 ml.), the solution evaporated to dryness under reduced pressure, and the residue dissolved in 0·5N-sulphuric acid (350 ml.). The acid solution was continuously extracted with ether for two successive periods of 5 days to give extracts I and II respectively.

Extract I was evaporated and an aqueous solution of the residue allowed to percolate through a column of Amberlite I.R.-120 resin (H^+ form) in order to remove small quantities of inorganic cations. Removal of the solvent gave a colourless gum (1·122 g.) which was further purified by counter-current distribution between ether and water. After 31 transfers at 20° the following fractions were obtained, the contents of each tube being examined by paper chromatography, by the ascending method with ethanol–ammonia (*d* 0·88)–water (20 : 1 : 4) as solvent on Whatman no. 1 paper; the chromatograms were developed with Universal indicator. Fraction *A* (455 mg.; tubes 1—2), gum, R_F 0·23, 0·05; *B* (134 mg.; tubes 3—8), gum, R_F 0·20; *C* (325 mg.; tubes 9—16), gum R_F 0·46; *D* (146 mg.; tubes 17—31), colourless non-acidic crystals. Fraction *A* was combined with the product from extract II (see below). Fraction *B* did not crystallise, contained neither an amide nor an imide, and was not further examined. After 3 recrystallisations from water, fraction *C* had m. p. 140—141°, alone or mixed with synthetic DL-3 : 3-dimethyl-2 : 5-dioxopyrrolidine-4-propionic acid (Found: C, 54·5; H, 6·0; N, 7·0. Calc. for $C_9H_{13}O_4N$: C, 54·3; H, 6·6; N, 7·0%). The infrared spectra of the oxidation product and the synthetic compound were identical over the range 4000—650 cm.$^{-1}$.

After two crystallisations from water, fraction *D* had m. p. 150—151°, undepressed on admixture with synthetic DL-4-hydroxy-3 : 3-dimethyl-2 : 5-dioxopyrrolidine-4-propionic lactone (Found: C, 54·8; H, 5·7; N, 7·05. Calc. for $C_9H_{11}O_4N$: C, 54·8; H, 5·6; N, 7·1%). The infrared spectra of the oxidation product and the synthetic compound were identical over the range 4000—650 cm.$^{-1}$. Methylation of the lactone in methanolic solution with ethereal diazomethane (1 mol.) gave *4-hydroxy-1 : 3 : 3-trimethyl-2 : 5-dioxopyrrolidine-4-propionic lactone*, m. p. 104° after recrystallisation from benzene–light petroleum (b. p. 40—60°) and sublimation at 100°/0·2 mm. (Found: C, 57·1; H, 6·1; N, 6·7. $C_{10}H_{13}O_4N$ requires C, 56·9; H, 6·2; N, 6·65%).

Extract II was combined with fraction *A* from extract I and subjected to 33 transfers at 25° between ethyl acetate and water. The contents of each tube were evaporated and examined by paper chromatography as before. The following fractions were taken: *E* (137 mg.; tubes 1—2), green gum, R_F 0·21, 0·05; *F* (370 mg.; tubes 3—6), colourless crystals, R_F 0·23; *G* (133 mg.; tubes 7—10), R_F 0·16, 0·23, 0·48. Fraction *E* contained inorganic material and was not examined further. Fraction *F* was recrystallised twice from water; then it had m. p. 213—214° (Found: C, 49·8; H, 5·2; N, 11·65%; equiv., 250. $C_{10}H_{12}O_5N_2$ requires C, 50·0; H, 5·05; N, 11·65%; equiv., 240), main infrared bands (Nujol mull) 3340, 3180, 3080, 2920, 2750, 2550, 2500, 1775, 1723, 1706, 1655, 1432, 1390, 1348, 1330, 1317, 1282, 1268, 1225, 1157, 1127, 1103, 967, 807, 755; (dioxan solution) 3170, 3080, 1780, 1727 cm.$^{-1}$.

Fraction *G* was combined with the residue obtained from the mother-liquors of fraction *F* and subjected to a further counter-current distribution with the system water–acetic acid–ethyl acetate (25 : 8 : 25). After 39 transfers at 20° a substance (115 mg.; *K* 0·55) was obtained in tubes 8—20 which was identical with that obtained from fraction *F*.

Comparison of Oxidation of Dehydrovitamin B_{12}, and Alkaline and Acid Hydrolysis Products of Vitamin B_{12}.—Chromic acid (67·3 mg.) in water (1·5 ml.) was added to a stirred solution of the substance (21·7 mg.) in acetic acid (5 ml.) at 20°, and after 2 hr. the solution was slowly heated to 100° during 4 hr. and kept at that temperature for 2 hr. Excess of chromic acid was reduced by ethanol (0·5 ml.) and, after evaporation, the residue was continuously extracted with ether from 0·5N-sulphuric acid (30 ml.) for 48 hr. The ethereal solution, on evaporation, gave a green gum (11·8 mg.) which was examined by paper chromatography with the solvent systems: (*a*) *tert.*-butyl alcohol–acetic acid–water (5 : 2 : 3); (*b*) ethanol–ammonia (*d* 0·88)–water (20 : 1 : 4).

Dehydrovitamin B_{12} and the hexacarboxylic acid from the alkaline hydrolysis of the vitamin gave products A, B, C, and D; the heptacarboxylic acid from the acid hydrolysis of the vitamin gave products A, C, D, and E.

Product A, R_F 0·05 in system (*b*), was an unidentified acid.

Product B, R_F 0·71 in system (*a*), 0·25 in (*b*), was an acid and an amide or imide,[13] identifiable as the compound in fraction *F* above.

[13] Rydon and Smith, *Nature*, 1952, **169**, 922; Reindel and Hoppe, *Chem. Ber.*, 1954, **87**, 1103.

Product C, R_F 0·84 in system (*a*), 0·48 in (*b*), was an acid and an amide or imide, identified as DL-3 : 3-dimethyl-2 : 5-dioxopyrrolidine-4-propionic acid.

Product D, R_F 0·18 in system (*b*), was an unidentified acid.

Product E, R_F 0·77 in system (*a*), 0·31 in (*b*), was an acid and an amide or imide, unidentified.

Reactions of the compound from fraction F. (*a*) Methylation. A methanolic solution of the product (10 mg. in 1 ml.) from the foregoing experiment reacted rapidly with ethereal diazomethane and consumed 2·05 mols. of the reagent. After evaporation of the solvent, the residue was recrystallised from hot ethyl acetate (0·5 ml.) by adding light petroleum (1 ml.; b. p. 40—60°); the *product* had m. p. 149° (hot stage) (Found: N, 10·2. $C_{12}H_{16}O_5N_2$ requires N, 10·4%).

(*b*) Hydrolysis. The compound (39·8 mg.) from fraction *F* was heated in 10% aqueous barium hydroxide (10 ml.) on the water-bath, in a stream of nitrogen, and the ammonia evolved was collected in an excess of standard sulphuric acid. After 78 hr. evolution of ammonia (0·92 mol.) had ceased.

The above methylated product (3 mg.) was heated in 20% aqueous sodium hydroxide (5 ml.) on the water-bath. The evolved gases were collected, as before, in excess of hydrochloric acid and after 12 hr. the experiment was discontinued and the acidic solution evaporated to dryness. Examination of the residue by paper chromatography with the system butan-1-ol–acetic acid–water (4 : 1 : 5) revealed a spot (R_F 0·28) corresponding to methylamine (R_F of authentic sample 0·28) after development with ninhydrin.

L-N-*Acetylaspartimide.*—L-*N*-Benzyloxycarbonylaspartimide [11] (302 mg.) in acetic anhydride (15 ml.) was hydrogenated at room temperature over palladium black (50 mg.). The product (201 mg.) recrystallised from ethanol, to give L-N-*acetylaspartimide*, m. p. 162—164° (decomp.), $[\alpha]_D^{20}$ −56·6° (Found: C, 45·8; H, 5·1; N, 17·9. $C_6H_8O_3N_2$ requires C, 46·15; H, 5·15; N, 17·95%), main infrared bands (KBr disc) 3425, 3240, 3070, 1792, 1724, 1634, 1575, 1555, 1376, 1362, 1333, 1302, 1276, 1236, 1199, 1170, 1130, 1040, 1018, 926, 893, 810 cm.$^{-1}$.

DL-α-*Acetamidoglutarimide.*—L-*N*-Acetylglutamic acid [14] (3·0 g.) was dissolved in acetic anhydride at 100° and after 30 min. the solvent was evaporated; the residual colourless gum, in chloroform solution, was treated with gaseous ammonia whereupon crystals of the ammonium salts of *N*-acetylglutamine and *N*-acetyl*iso*glutamine separated. The mixed salts (2·0 g.) were dissolved in acetic anhydride at 100° and after 20 min. the solvent was evaporated and the residue extracted into ethyl acetate from an aqueous solution brought to pH 7 by means of sodium hydrogen carbonate. Removal of the ethyl acetate gave the *product* (300 mg.) which crystallised as needles, m. p. 180°, from ethyl acetate–*n*-hexane (Found: C, 49·2; H, 5·7; N, 16·3. $C_7H_{10}O_3N_2$ requires C, 49·4; H, 5·9; N, 16·5%), main infrared bands (KBr disc) 3390, 3195, 3067, 1745, 1681, 1623, 1408, 1381, 1337, 1305, 1274, 1242, 1138, 1047, 1026, 990, 954, 873, 844 cm.$^{-1}$.

Triethyl 1-*Aminoprop*-1-*ene*-1 : 2 : 3-*tricarboxylate.*—Dry ammonia was passed through a solution of diethyl ethoxalylsuccinate (50 g.) in dry ether (250 ml.). The precipitated ammonium salt was separated and kept in a vacuum-desiccator over solid potassium hydroxide for 7 days. Recrystallisation of the product from ethanol gave the amino-ester, m. p. 68° (32 g., 65%) as colourless needles (Found: C, 52·7; H, 7·1; N, 5·15. Calc. for $C_{12}H_{19}O_6N$: C, 52·7; H, 7·0; N, 5·15%), main infrared bands (Nujol mull) 3405, 3300, 2980, 1723, 1665, 1613, 1538, 1487, 1466, 1448, 1408, 1392, 1369, 1343, 1293, 1233, 1185, 1162, 1093, 1024, 955, 915, 864, 837, 777, 725; ($CHCl_3$ solution) 3470, 3310, 1739, 1673, 1600 cm.$^{-1}$.

Diethyl trans-5-*Oxopyrrolidine*-2 : 3-*dicarboxylate.*—The foregoing ester (15 g.) in ethanol (50 ml.) was hydrogenated at 120°/100 atm. for 7 hr. over Raney nickel (*ca.* 7 g.). After removal of catalyst and solvent, the residue was distilled under reduced pressure and the fraction of b. p. 170—180°/0·5 mm. representing the *pyrrolidine ester* was collected. The viscous oil (7 g., 57%) crystallised after several weeks and after 2 recrystallisations from ethyl acetate–light petroleum (b. p. 40—60°) had m. p. 48·5—50° [Found, on a fraction of b. p. 177°/0·5 mm.: C, 52·1; H, 6·7; N, 5·9%; *M* (Rast), 217. $C_{10}H_{15}O_5N$ requires C, 52·4; H, 6·6; N, 6·1%; *M*, 229], main infrared bands (Nujol mull) 3180, 3090, 1739, 1726, 1703, 1673, 1488, 1355, 1291, 1268, 1233, 1217, 1200, 1176, 1117, 1083, 1039, 1027, 862, 799, 763, 730, 680; (dioxan solution) 3250, 1737, 1721 cm.$^{-1}$.

This diester (1·14 g.) was dissolved in 4% aqueous sodium hydroxide (10 ml.) at 100°. After 45 min., the solution was cooled and percolated through a column of Dowex 50 (H^+ form);

[14] Nicolet, *J. Amer. Chem. Soc.*, 1930, **52**, 1192.

evaporation of the acidic eluate gave a colourless gum which slowly crystallised. From ethanol–benzene the *diacid* separated as colourless prisms (700 mg., 81%), m. p. 210° (Found: C, 41·8; H, 4·2; N, 8·3%; equiv., 89. $C_6H_7O_5N$ requires C, 41·6; H, 4·1; N, 8·1%; equiv., 86·5), main infrared bands (Nujol mull) 3220, 1765, 1715, 1645, 1327, 1248, 1170, 1084, 1020, 959, 752, 703, 658 cm.$^{-1}$.

trans-3-*Ethoxycarbonyl*-5-*oxopyrrolidine*-2-*carboxylic Acid*.—The above pyrrolidine diester (1·02 g.) was dissolved in cold ethanolic sodium hydroxide (0·2 g., in 10 ml.); the monosodium salt was precipitated at room temperature in 15 min. The mixture was diluted with water (20 ml.) and percolated through Dowex 50 (H^+ form). The percolate was evaporated and the residue recrystallised from ethyl acetate, to give the *monocarboxylic acid* (630 mg., 70%) as colourless prisms, m. p. 153°, raised to 160—161° by 2 recrystallisations from water (Found: C, 47·5; H, 5·6; N, 7·05%; equiv., 195. $C_8H_{11}O_5N$ requires C, 47·75; H, 5·5; N, 7·0%; equiv., 201). Titration in aqueous solution indicated an apparent pK_a 3·5. Main infrared bands were at (Nujol mull) 3190, 3060, 2970, 2900, 2600, 2520, 1733, 1652, 1475, 1455, 1417, 1377, 1357, 1270, 1244, 1220, 1187, 1117, 1086, 1040, 1014, 957, 920, 876, 857, 756, 720, 692; (dioxan solution) 3250, 1737, 1721 cm.$^{-1}$.

trans-2-*Methoxycarbonyl*-5-*oxopyrrolidine*-3-*carboxyamide*.—The acid ester (630 mg.) from the previous experiment was dissolved in methanol (25 ml.) saturated with ammonia and kept for 5 days at room temperature. Removal of the solvent gave a crystalline ammonium salt which was converted into the free acid by allowing its aqueous solution to percolate through Dowex 50 (H^+ form). After evaporation of the eluate, the *amic acid* was obtained as a colourless gum which crystallised on being scratched. Two recrystallisations from 95% ethanol gave the acid (400 mg., 70%) as prisms, m. p. 191° (Found: C, 42·0; H, 5·0; N, 16·4%; equiv., 175. $C_6H_6O_4N_2$ requires C, 41·85; H, 4·7; N, 16·3%; equiv., 172). Titration in aqueous solution indicated an apparent pK_a 3·0; main infrared bands (Nujol mull) were at 3395, 3300, 3180, 1725, 1661, 1615, 1299, 1255, 1224, 1172, 1096, 1027, 910, 835, 669 cm.$^{-1}$.

The corresponding *methyl ester*, obtained in 46% yield by use of diazomethane in ether–methanol and recrystallised from acetone, had m. p. 159—160° (Found: C, 45·6; H, 5·4; N, 15·35. $C_7H_{10}O_4N_2$ requires C, 45·15; H, 5·4; N, 15·05%), main infrared bands (Nujol mull) 3385, 3290, 3175, 3070, 1739, 1699, 1665, 1614, 1384, 1294, 1263, 1230, 1204, 1115, 1038, 1016, 996, 909, 799, 777, 659; (dioxan solution) 3270, 1746, 1714, 1701 cm.$^{-1}$.

cis-5-*Oxopyrrolidine*-2 : 3-*dicarboxyimide*.—The above *trans*-amide ester (465 mg.) was kept in a solution of sodium methoxide (270 mg.) in dry methanol (25 ml.) for 24 hr. at room temperature, then diluted with water (25 ml.) and percolated through Dowex 50 (H^+ form). Electrometric titration of the eluate indicated that it consisted of 55% of the required imide, together with free carboxylic acid. The solution was evaporated, and the residual gum dissolved in water (10 ml.) and percolated through Dowex 1 × 2 (acetate form). The solvent was removed from the eluate to give colourless crystals of the required *imide* (220 mg., 57%), m. p. 240—241° (decomp.) after recrystallisation from ethanol (Found: C, 46·6; H, 4·15; N, 18·25%; equiv., 152. $C_6H_6O_3N_2$ requires C, 46·75; H, 3·9; N, 18·2%; equiv., 154), main infrared bands (Nujol mull) 3270, 3130, 3060, 2980, 2760, 1775, 1725, 1708, 1687, 1672, 1436, 1425, 1395, 1357, 1324, 1250, 1202, 1168, 1107, 1088, 1044, 1004, 995, 955, 860, 807, 758, 717, 670; (dioxan solution) 3240, 1730 cm.$^{-1}$.

cis-1-*Methyl*-5-*oxopyrrolidine*-2 : 3-*dicarboxyimide*.—The imide (50 mg.) from the foregoing experiment was dissolved in a minimum of methanol and an excess of ethereal diazomethane was added. After 30 min., solvent was removed to give the N-*methyl compound* (25 mg., 46%), m. p. 185—186° (decomp.) after 2 recrystallisations from ethyl acetate (Found: C, 50·0; H, 5·5; N, 16·45. $C_7H_8O_3N_2$ requires C, 50·0; H, 4·8; N, 16·65%), main infrared bands (Nujol mull) 3335, 2980, 2940, 2860, 1779, 1710, 1474, 1442, 1420, 1387, 1321, 1286, 1254, 1127, 1094, 1060, 1034, 976, 882, 826, 777, 731, 700; (dioxan solution) 3250, 1787, 1720 cm.$^{-1}$.

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