1-Amino-2-nitrocyclopentanecarboxylic Acid. A New Naturally-occurring Nitro-compound

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A plant-growth regulatory compound produced by *Aspergillus wentii* is shown to be 1-amino-2-nitrocyclopentanecarboxylic acid (I; R = NH_2).

When sprayed on pea plants, culture filtrates from fermentations of *Aspergillus wentii* cause marked scorching of the foliage and also produce unusual growth-regulating effects.[1] Apical dominance is broken, lateral buds develop into branches, and chlorophyll synthesis is inhibited; changes in leaf and flower morphology also occur.

The effects are caused by two factors, one of which is responsible for the scorching of the foliage and can be extracted with charcoal; this material has not been examined further. The second, responsible for the more interesting growth-regulating effects, can be extracted from the culture filtrate by sulphonic acid resin and can be recovered from the resin with ammonium hydroxide. After further purification with charcoal and with carboxylic acid resin the active principle crystallises directly from the aqueous concentrate as needles which decompose without melting at temperatures above 150°, and which darken slowly at room temperature.

The active compound, $C_6H_{10}N_2O_4$, can also be adsorbed on a basic ion exchange resin and is therefore amphoteric. No simple derivative of the active compound could be prepared. Its nuclear magnetic resonance (n.m.r.) spectrum, measured in trifluoroacetic acid, shows a one-proton triplet at τ 4·46 and a six-proton multiplet at τ 6·8—8·2.

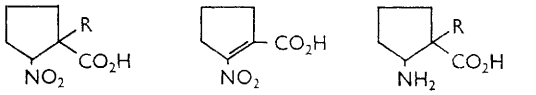
Hydrolysis of the *A. wentii* compound with boiling water yields ammonia and a mixture of carboxylic acids. The major component, m. p. 101—103°, $C_6H_9NO_5$, contains a hydroxyl group ($\nu_{max.}$ 3500 cm.$^{-1}$) which must be tertiary since on acetylation there is no change in the τ 4—6 region of the n.m.r. spectrum, a carboxyl group ($\nu_{max.}$ 2600 and 1710 cm.$^{-1}$) which is readily methylated with diazomethane, and a nitro-group [$\nu_{max.}$ 1550 cm.$^{-1}$, $\lambda_{max.}$ 274 mμ (ε 42)] which is on a secondary carbon (one-proton triplet at τ 4·8). The n.m.r. spectrum of the hydroxy-acid also contains a six-proton multiplet at τ 7·3—8·3.

The minor component, m. p. 102—103°, $C_6H_7NO_4$, has no hydroxyl group (by infrared spectrum) and no magnetically-deshielded proton, but has a carboxyl group ($\nu_{max.}$ 2600 and 1725 cm.$^{-1}$), a nitro-group ($\nu_{max.}$ 1515 cm.$^{-1}$), and a double bond ($\nu_{max.}^{CHBr_3}$ 1668 cm.$^{-1}$) which is conjugated ($\lambda_{max.}$ 220 and 265 mμ) and tetrasubstituted (no olefinic proton in the n.m.r. spectrum). The n.m.r. spectrum of this unsaturated acid shows a four-proton multiplet at τ 7·0 and a two-proton quintuplet at τ 7·8 characteristic of the system $\cdot CH_2 \cdot C\mathit{H}_2 \cdot CH_2 \cdot$ where the flanking methylene groups are magnetically equivalent. The unsaturated acid is obviously a dehydration product of the hydroxy-acid, a relationship confirmed by its formation when the hydroxy-acid is heated in dimethyl

[1] P. W. Brian, G. W. Elson, H. G. Hemming, and M. E. Radley, *Nature*, 1965, **207**, 998.

sulphoxide and by its conversion into the hydroxy-acid with boiling water.

The structure of the hydroxy-acid was deduced from the properties of its hydrogenation product, $C_6H_{11}NO_3$, an amino-acid ($\nu_{max.}$ 1630 and 1545 cm.$^{-1}$). The amino-acid forms an *N*-benzoyl derivative which contains a hydroxyl group ($\nu_{max.}$ 3460 cm.$^{-1}$) which again must be tertiary since there is no change in the τ 4—6 region of the n.m.r. spectrum on formation of an *O*-acetyl-*N*-benzoyl derivative. The proton appearing as a multiplet at τ 5·1 must therefore be on the carbon carrying the amino-group; the n.m.r. spectra of these derivatives also show six protons as a multiplet at τ 7·0—8·5 (as well as the protons of the benzoyl and acetyl groups). The amino-acid reacts with periodic acid, so that the hydroxyl and amino-groups are vicinal, and contains no double bond (ε_{200} 300) so that it must be monocyclic. These properties are consistent only with the structure 2-amino-1-hydroxycyclopentanecarboxylic acid (III; R = OH) for the amino-acid, so that the nitro-acid from which it is formed is 1-hydroxy-2-nitrocyclopentanecarboxylic acid (I; R = OH) and the corresponding unsaturated compound 2-nitrocyclopentenecarboxylic acid (II).

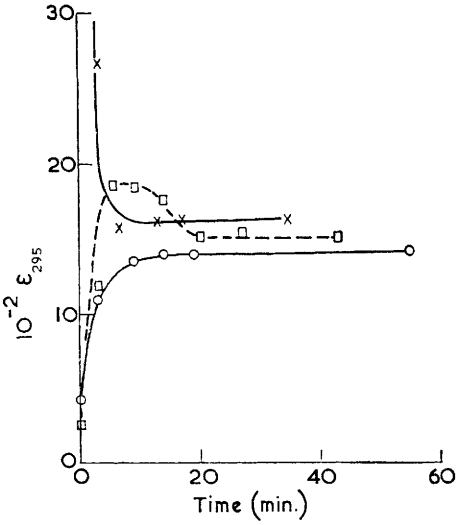


(I) (II) (III)

Reaction of either of the hydrolysis products (I; R = OH) or (II) with ammonia regenerates the *A. wentii* product for which the most likely structure is therefore 1-amino-2-nitrocyclopentanecarboxylic acid (I; R = NH_2). Hydrogenation of the amino-acid (I; R = NH_2) should yield 1,2-diaminocyclopentanecarboxylic acid (III; R = NH_2). In fact the product is an amino-acid with the expected spectroscopic properties but analysing for the diamino-acid plus a molecule of water. The compound could not be obtained anhydrous but was characterised as its crystalline bis-*N*-acetyl derivative.

The structure of the amino-acid (I; R = NH_2) was confirmed by synthesis. Reaction of cyclopent-1-enecarboxylic acid with dinitrogen tetroxide in the presence of iodine (cf. ref. 2) gives 1-iodo-2-nitrocyclopentanecarboxylic acid (I; R = I) as the major product. A by-product, isolated as an oil and not characterised, is presumably 1-iodo-2-nitrocyclopentane since it shows nitro- ($\nu_{max.}^{CHCl_3}$ 1550 cm.$^{-1}$) but not carboxyl group absorption and loses iodine on pyrolysis. The iodo-acid (I; R = I) can be converted into the *A. wentii* product (I; R = NH_2) with ammonia, and into the unsaturated acid (II) with pyridine. A minor product of the latter reaction is cyclopent-1-enecarboxylic acid, possibly formed by *trans*-elimination of I^+ (as iodine) and NO_2^- ions. The amino-group of the acid (I; R = NH_2), activated by the β-nitro-group, undergoes amine exchange on treatment with concentrated aqueous ethylamine to give the amino-acid (I; R = NHEt).

The course of the hydrolysis of the amino-acid (I; R = NH_2) with boiling water has been followed by measuring the appearance of the peak at 295 mμ due to the ammonium salt of the unsaturated compound (II). The results (see Figure) show that the products



Reaction of ×—×—× (*A*) the unsaturated acid (II), —□—□— (*B*) the amino-acid (I; R = NH_2), and O—O—O (*C*) the hydroxy-acid (I; R = OH) in boiling water

are in equilibrium and that the same proportion of the unsaturated compound is obtained from the ammonium salt of the hydroxy-acid (I; R = OH) or of the unsaturated compound (II) itself. The maximum in the curve obtained with the amino-acid (I; R = NH_2), which has been repeated in several experiments, shows that the unsaturated compound (II) is the first product, *i.e.*, that the first step in the reaction is elimination of ammonia.

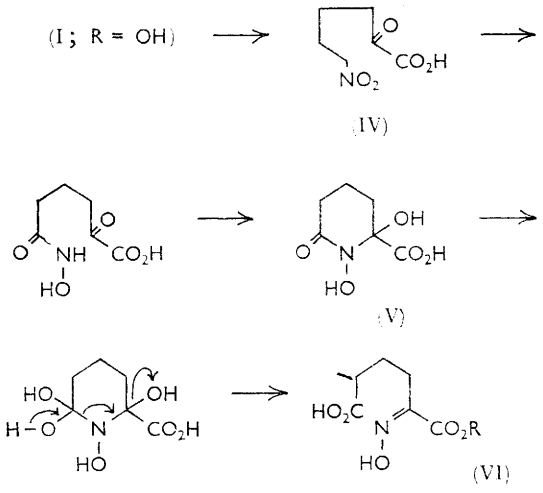
Hydrolysis of the amino-acid (I; R = NH_2), the hydroxy-acid (I; R = OH), or the unsaturated acid (II) with hot mineral acid yields glutaric acid, carbon dioxide, and ammonia. An explanation of this somewhat surprising result was suggested by the observation that treatment of the hydroxy-acid (I; R = OH) with concentrated sulphuric acid at 80° yields α-oximinoadipic acid (VI; R = H). If we assume that this compound is an intermediate in the acid hydrolysis then the products are explained by the fact that α-oximinocarboxylic acids readily decompose to cyanides with one carbon atom less.

The key to the formation of α-oximinoadipic acid is the opening, by a reverse aldol-type reaction, of the cyclopentane ring to give the open-chain compound (IV). Since it is known [3] that primary nitro-compounds are converted into hydroxamic acids by mineral acid we have only to postulate a "*trans*-hydroxylamination," possibly *via* a cyclic intermediate (V), to explain the

[2] T. E. Stevens and W. D. Emmons, *J. Amer. Chem. Soc.*, 1958, **80**, 338.

[3] Cf., *e.g.* S. B. Lippincott and H. B. Hass, *Ind. Eng. Chem.*, 1939, **31**, 118.

formation of α-oximinoadipic acid. In accord with this postulate, treatment of the methyl ester of the hydroxy-acid (I; R = OH) with concentrated sulphuric acid



yields the monomethyl ester (VI; R = Me) of α-oximinoadipic acid. The location of the ester grouping in (VI; R = Me) was proved by the ultraviolet absorption results in the Table.

Ultraviolet absorption spectra

Compound	$\lambda_{max.}$ (ε) in EtOH	in EtOH + NaOH
α-Oximinoadipic acid	218 (8200)	245 (3800)
Dimethyl α-oximinoadipate	220 (7900)	270 (13,800)
Monomethyl ester (VI; R = Me)	220 (8600)	270 (12,000)

There is some evidence that the keto-acid (IV) is present in the mixture of acids formed by the action of boiling water on the amino-acid (I; R = NH_2). The mother-liquor from the first crystallisation of the hydroxy-acid (I; R = OH) contains an oil which cannot be induced to crystallise and which has $\nu_{max.}$ 1720 and 1550 cm.$^{-1}$. Treatment of the hydroxy-acid with alkali leads to the uptake of two equivalents and recovery gives an oil whose infrared spectrum is identical with that of the mother-liquor material, and whose n.m.r. spectrum suggests that it is a mixture of the hydroxy-acid (I; R = OH) and the keto-acid (IV). It is of interest in this context that the hydroxy-acid gives colour-reactions characteristic of a *primary* aliphatic nitro-compound; since both the tests used involve initial conversion to the *aci*-nitro-form with alkali, this is explained by the ring-opening postulated above. While we have not isolated the keto-acid (IV) pure we have obtained supporting evidence for its existence by direct formation of its oxime [$\nu_{max.}$ 3220 (OH), 1687 (C=O), 1650 (C=N), 1542 (NO_2) cm.$^{-1}$, τ 5·30 (CH_2NO_2)] from the hydroxy-acid (I; R = OH).

The 1-amino-2-nitrocyclopentanecarboxylic acid obtained from *A. wentii* is optically inactive either as a result of racemisation during the isolation procedure, which involves contact with aqueous ammonia, or because its biosynthesis, to be the subject of a future communication, involves non-enzymic stages.

EXPERIMENTAL

Unless otherwise stated, infrared spectra were determined for Nujol mulls, ultraviolet spectra were measured for ethanol solutions, and nuclear magnetic resonance (n.m.r.) spectra were for deuterochloroform solutions with tetramethylsilane as internal standard. Silica gel used for chromatography was either B.D.H. chromatographic grade or Hopkin and Williams M.F.C. and light petroleum had b. p. 60—80°. Paper chromatography was on Whatman No. 3 paper in the system butanol–acetic acid–water (100 : 22 : 50) and thin-layer chromatography was on Merck silica gel G in the systems methylene chloride–formic acid (98 : 2) (system 1) or n-butanol–acetic acid–water (60 : 20 : 20) (system 2).

Isolation of 1-*Amino*-2-*nitrocyclopentanecarboxylic Acid*.—A stirred fermentation of *Aspergillus wentii*, Wehm (CMI 49129; No. 1395 in our collection) on Raulin–Thom medium supplemented with 0·1% "Difco" yeast extract was harvested 96 hr. after inoculation, filtered free from mycelium, and clarified by filtration through "Dicalite" diatomaceous earth. The resulting filtrate (65 l.) was passed down a column of Amberlite IR-120 sulphonic acid resin (800 ml.) and the effluent was discarded. The column was washed with distilled water and eluted with 2N-ammonium hydroxide (3·5 l.). The eluate was concentrated *in vacuo*, with bath-temperature not above 40°, to 200 ml. and decolourised with charcoal (10 g.). The resulting solution was passed down a column of Amberlite IRC-50 carboxylic acid resin (100 ml.) and the effluent was further concentrated as above to yield crystals (1·95 g.) of 1-*amino*-2-*nitrocyclopentanecarboxylic acid* (I), containing traces of other amino-acids which can be removed by rapid recrystallisation from water or from aqueous acetone. The pure nitro-acid (I) is obtained in two crystalline modifications, both in the form of fibrous needles decomposing at *ca.* 150° without melting, which are distinguishable by their infrared spectra. Form (*a*) shows $\nu_{max.}$ 3530, 3140sh, 3050br, 2700—2500 (series of bands), 2040w, 1675s, 1620br, 1570s, and 1525 cm.$^{-1}$ (Found: C, 41·2, 41·6; H, 6·1, 5·9; N, 15·7%. $C_8H_{10}N_2O_4$ requires C, 41·4; H, 5·8; N, 16·1%). Form (*b*) shows $\nu_{max.}$ 3500w, 3100—2450, 2000w, 1625br, and 1550br cm.$^{-1}$ (Found: C, 41·2, 41·3; H, 5·9, 5·8; N, 15·7%).

A study (by Dr. J. M. Rowe) of *X*-ray photographs of forms (*a*) and (*b*) reveals that each contains two similar crystal structures one of which predominates in (*a*) while the other predominates in (*b*). The crystal data and densities of the two forms give molecular weights of 191 and 184 for (*a*) and (*b*), respectively ($C_6H_{10}N_2O_4$ requires *M*, 174).

N.m.r. spectrum (in trifluoroacetic acid) (with number of protons in parentheses): τ = 4·46 (1, triplet, *J* = 9 c./sec.), 7·0—8·0 (6, multiplet). R_F 0·45—0·50, detectable by ninhydrin (yellow, fluorescent), ferric chloride (orange-brown), silver chloride–sodium hydroxide (grey), or periodate–Schiff's reagent (faint pink).

Hydrolysis of 1-*Amino*-2-*nitrocyclopentanecarboxylic Acid with Boiling Water*.—(*a*) A solution of the amino-acid (I; R = NH_2) (1·01 g.) in water (60 ml.) was heated under reflux in a stream of nitrogen for 1 hr. The solution was concentrated *in vacuo* and passed down a column of Amberlite

IR-120 (10 ml.). The column was first washed with water (25 ml.), which was combined with the effluent to give the non-basic fraction (*A*), and then eluted with 2N-hydrochloric acid (4 × 25 ml.) to give ammonium *chloride* (*355* mg.; 1·09 equiv.) identified by its infrared spectrum and by paper chromatography.

The non-basic fraction (*A*) was evaporated to give a solid (1·0 g.) which was chromatographed on silica gel (50 g.). Elution with benzene–chloroform (1 : 1) yielded two solid fractions (*B*) (85 mg.) and (*C*) (769 mg.).

Recrystallisation of fraction (*C*) from benzene yielded a solid (*D*) (405 mg.), m. p. 99—102°, and a mother-liquor containing an oil (*E*) which could not be induced to crystallise even after rechromatography. The infrared spectrum (liquid film) of (*E*) was identical with that of the product of alkaline hydrolysis of 1-hydroxy-2-nitrocyclopentanecarboxylic acid (see below).

Further recrystallisation of (*D*) from benzene yielded 1-*hydroxy*-2-*nitrocyclopentanecarboxylic acid* (I; R = OH) as plates (345 g.), m. p. 102—103° [Found: C, 41·2; H, 5·2; N, 8·2%; *M* (*X*-ray), 174. $C_6H_9NO_5$ requires C, 41·1; H, 5·2; N, 8·0%; *M*, 175], $\nu_{max.}$ 3510 and 3495s, 2600s,br, 1710—1700s,br, 1550s cm.$^{-1}$; $\lambda_{max.}$ 274 mμ (ε 42), end absorption ,ε_{205} 4340; n.m.r. spectrum: τ = 4·78 (1, triplet, *J* = 9), 7·3—8·3 (6, multiplet).

Methyl 1-*hydroxy*-2-*nitrocyclopentanecarboxylate*, prepared with diazomethane, occurs in two forms, m. p. 57—59 and 64—65°, with different solid-state infrared spectra (Found: C, 44·7; H, 5·9; N, 7·3%. $C_7H_{11}NO_5$ requires C, 44·4; H, 5·9; N, 7·4%); $\nu_{max.}$, low-melting form, 3495s, 1727vs, 1545vs; high-melting form, 3440s, 1720s, 1538s or, in bromoform, 3550sh, 3510m, 1743vs and 1550vs cm.$^{-1}$; n.m.r. spectrum: τ = 4·73 (1, triplet, *J* = 9 c./sec.), 6·03 (3, singlet), 7·2—8·2 (6, multiplet).

The *acetyl compound* (I; R = OAc), prepared with acetyl chloride in the presence of pyridine, had m. p. 139—141° (Found: C, 44·4; H, 5·2; N, 6·5. $C_8H_{11}NO_6$ requires C, 44·2; H, 5·1; N, 6·5%); $\nu_{max.}$ 1755s, 1725s, 1560s cm.$^{-1}$; n.m.r. spectrum τ = 4·58 (1, triplet, *J* = 5 c./sec.), 7·3—8·3 (6, multiplet), 7·91 (3, singlet).

Fraction (*B*) was rechromatographed on silica gel (3·5 g.) in benzene–chloroform (1 : 1) to give a solid (69·6 mg.) which was recrystallised from benzene to give 2-*nitrocyclopent*-1-*enecarboxylic acid* (II) as buff rods (22 mg.), m. p. 102—103° (Found: C, 45·8; H, 4·5; N, 8·9. $C_6H_7NO_4$ requires C, 45·9; H, 4·5; N, 8·9%); $\nu_{max.}$ 2620m, 2500m, 2350sh, 1725s, 1671m, 1617s, 1515s or, in bromoform, 1745sh, 1715s, 1668m, 1565—1515vs cm.$^{-1}$; $\lambda_{max.}$ 220 (ε 5,100) and 265 mμ (ε 4,200) shifted to 235 (ε 4,300) and 291 mμ (ε 5,300) on addition of ammonium hydroxide; n.m.r. spectrum: τ = 7·0 (4, multiplet), 7·8 (2, quintuplet). With diazomethane, the nitro-acid (II) gives an oil.

(*b*) A solution of 1-amino-2-nitrocyclopentanecarboxylic acid (207 mg.) in water (13 ml.) was heated under reflux for 15 min. and the products were isolated as above to give fractions (*B*) (10 mg.) and (*C*) (131 mg.).

(*c*) A solution of 1-amino-2-nitrocyclopentanecarboxylic acid (3·1 mg.) in water (4·0 ml.), was heated under reflux. Aliquot parts were withdrawn periodically and their ultraviolet absorption was measured. The results are shown in the Figure.

Equilibration of the Hydroxy-acid (I; R = OH) *and the Unsaturated Acid* (II).—(*a*) The acids (I; R = OH) (3·0 mg.) and (II) (3·0 mg.) were separately dissolved in a slight excess of ammonium hydroxide and the solutions were evaporated to dryness. The resulting ammonium salts were treated as in (*c*) above. The results are shown in the Figure.

(*b*) A solution of the ammonium salt of the hydroxy-acid (I; R = OH) in water was heated under reflux for 1 hr., adjusted to pH 3 with hydrochloric acid and extracted with ethyl acetate. Thin-layer chromatography (system 1) of the product showed a small spot corresponding in R_F value to the unsaturated acid (II).

(*c*) A solution of the unsaturated acid (II) (50 mg.) in water (15 ml.) was heated under reflux in a stream of nitrogen for 3 hr., cooled under nitrogen and extracted with ethyl acetate. The product (45 mg.), identified by infrared spectrum and thin-layer chromatogram (system 1) as the hydroxy-acid (I; R = OH), was recrystallised from benzene–light petroleum to give plates, m. p. 99—101°, undepressed on admixture with authentic material.

Dehydration of 1-*Hydroxy*-2-*nitrocyclopentanecarboxylic Acid*.—A solution of the hydroxy-acid (I; R = OH) (250 mg.) in dimethyl sulphoxide (35 ml.) was heated at 90—100° in a stream of dry nitrogen for 5·5 hr. Evaporation to dryness at 80—100° under reduced pressure gave an oil which was chromatographed on silica gel. Elution with benzene–chloroform (7 : 3) gave buff rods (44 mg.) which after recrystallisation from benzene–light petroleum had m. p. 98—100° and were shown by infrared spectrum, thin-layer chromatogram (system 1), and mixed m. p. to be 2-nitrocyclopent-1-enecarboxylic acid (II).

Partial Synthesis of 1-*Amino*-2-*nitrocyclopentanecarboxylic Acid*.—(*a*) A solution of 2-nitrocyclopent-1-enecarboxylic acid (II) (50 mg.) in ammonium hydroxide (5 ml., *d* 0·90) was set aside for 2 days. Evaporation to dryness gave a solid which was redissolved in water and percolated through Amberlite IRC-50 (H^+). Evaporation of the eluate under reduced pressure gave the amino-acid (I; R = NH_2) (52 mg., 96%), identified by infrared spectrum, paper chromatogram, and thin-layer chromatogram (system 2). Recrystallisation from water gave needles decomposing without melting at *ca.* 150°.

(*b*) 1-Hydroxy-2-nitrocyclopentanecarboxylic acid (I; R = OH) (50 mg.), treated with ammonia as above, gave the amino-acid (I; R = NH_2) (47 mg., 94%).

Treatment of 1-*Amino*-2-*nitrocyclopentanecarboxylic Acid with Ethylamine*.—A solution of the amino-acid (I; R = NH_2) (750 mg.) in ethylamine (34 ml.) and water (3·5 ml.) was set aside at 5° for 2 days. The solvents were removed and the residue was dissolved in water and percolated through Amberlite IRC-50 (H^+). Decolourisation with charcoal and crystallisation from water gave microcrystalline 1-*ethylamino*-2-*nitrocyclopentanecarboxylic acid* (I; R = NHEt) (286 mg.), m. p. 131—135° (Found: C, 47·7; H, 7·0; N, 14·0. $C_8H_{14}N_2O_4$ requires C, 47·5; H, 7·0; N, 13·9%); $\nu_{max.}$ 3080w, 2960—2860, 2300w, 1640, 1565 cm.$^{-1}$; n.m.r. spectrum (as the hydrochloride in trifluoroacetic acid): τ = 4·54 (1, triplet, *J* = 8 c./sec.), 6·67 (2, quartet, *J* = 7 c./sec.), 7·2—8·3 (6, multiplet), 8·65 (3, triplet, *J* = 7 c./sec.). Treatment of the ethylamino-compound under the conditions described for the hydrolysis of the amino-compound (I; R = NH_2) was shown by infrared spectroscopy and thin-layer chromatography (system 1) to give the same mixture of acids (I; R = OH) and (II).

Hydrolysis of 1-*Hydroxy*-2-*nitrocyclopentanecarboxylic Acid with Mineral Acid*.—(*a*) A solution of the hydroxy-acid (I; R = OH) (26·7 mg.) in 2N-hydrochloric acid (3 ml.) was heated at 110° for 3 hr. in an autoclave and evaporated

to dryness. Paper chromatography of the residue revealed the presence of ammonium chloride and an acidic (Bromocresol Green) compound. The residue was percolated in water through a small column of Amberlite IR-120 (H^+) and the effluent was evaporated to dryness to give a solid (17 mg.) which was recrystallised from benzene as leaflets (12 mg.), m. p. 99°, with infrared absorption identical with that of glutaric acid (Found: C, 45·5; H, 6·3. Calc. for $C_5H_8O_4$: C, 45·5; H, 6·1%).

(*b*) A solution of the hydroxy-acid (I; R = OH) (70·3 mg.) in 2N-sulphuric acid was heated under reflux in a stream of nitrogen which then passed through a trap containing 0·1N-barium hydroxide. Barium carbonate was precipitated and after 2 hr. the barium hydroxide was titrated against 0·1N-hydrochloric acid (carbon dioxide liberated: 0·98 equiv.).

The reaction mixture was made alkaline with 4N-sodium hydroxide and heated under reflux in a stream of nitrogen which then passed through a trap containing 0·1N-hydrochloric acid. After 2 hr. the hydrochloric acid was titrated against 0·1N-sodium hydroxide (ammonia liberated: 0·74 equiv.).

Hydrolysis of 1-*Amino*-2-*nitrocyclopentanecarboxylic Acid with Mineral Acid.*—A solution of the amino-acid (I; R = NH_2) in 2N-sulphuric acid was treated as described above for the hydroxy-acid. In replicate experiments 0·97, 0·98, equiv. of carbon dioxide and 1·7, 1·7 equiv. of ammonia were liberated.

Hydrolysis of 2-*Nitrocyclopent*-1-*enecarboxylic Acid with Mineral Acid.*—A solution of the unsaturated acid (II) (21 mg.) in 2N-hydrochloric acid (3 ml.) was heated under reflux for 3 hr. and then evaporated to dryness. The residue was percolated in water through Amberlite IR-120 and the effluent was evaporated to dryness to give a solid (14 mg.) with infrared spectrum identical with that of glutaric acid. Recrystallisation from benzene gave leaflets (8·7 mg.), m. p. 90—97°.

Treatment of 1-*Hydroxy*-2-*nitrocyclopentanecarboxylic Acid with Concentrated Sulphuric Acid.*—A solution of the hydroxy-acid (I; R = OH) (43 mg.) in concentrated sulphuric acid (0·1 ml.) was heated during 10 min. to 85° and allowed to cool slowly. The mixture was diluted with ice–water and extracted thoroughly with ethyl acetate to give a solid (29 mg.) which was recrystallised from acetone–benzene to give α-oximinoadipic acid as rocks, m. p. 147—150° (eff.) [Found: C, 41·15; H, 5·2; N, 7·9%; Equiv. 83. Calc. for $C_6H_9NO_5$: C, 41·1; H, 5·2; N, 8·0%; Equiv. 87·5 (dibasic)]. The infrared spectrum was identical with that of an authentic specimen. For ultraviolet absorption, see Table.

α-Oximinoadipic acid forms an oily *dimethyl ester*, b. p. 110—117° (bath)/0·01 mm. (Found: C, 47·0; H, 6·8; N, 6·7%. $C_8H_{13}NO_5$ requires C, 47·3; H, 6·5; N, 6·9%); $\nu_{max.}$ (liquid film): 3350br, 1740s, 1660sh cm.$^{-1}$; for ultraviolet absorption, see Table.

Treatment of Methyl 1-*Hydroxy*-2-*nitrocyclopentanecarboxylate with Concentrated Sulphuric Acid.*—A solution of the methyl ester (33·7 mg.) in concentrated sulphuric acid (0·1 ml.) was treated as above to give a solid (26·8 mg.) which was recrystallised from acetone–benzene to give the *monomethyl ester* (VI; R = Me) of α-oximinoadipic acid as small plates (20·5 mg.), m. p. 112—121° (Found: C, 44·3; H. 6·0; N, 7·2. $C_7H_{11}NO_5$ requires C, 44·4; H, 5·9; N, 7·4%); $\nu_{max.}$ 3200br, 2700—2500br, 1720sh, 1712s, 1645w cm.$^{-1}$; for ultraviolet absorption, see Table.

In one experiment, treatment of α-oximinoadipic acid with diazomethane yielded a monomethyl ester, m. p. 120—123°, with infrared adsorption identical with that of the ester (VI; R = Me) (Found: C, 44·6; H, 5·9; N, 7·1%).

Treatment of 1-*Hydroxy*-2-*nitrocyclopentanecarboxylic acid with Hydroxylamine Hydrochloride.*—A solution of the hydroxy-acid (I; R = OH) (50 mg.), hydroxylamine hydrochloride (100 mg., 5 equiv.) and hydrated sodium acetate (200 mg., 5·5 equiv.) in water (1 ml.) was heated at 100° for 5 min. and then set aside at room temperature for 2 hr. The solution was acidified with dilute sulphuric acid and extracted with ethyl acetate to give a solid (49 mg.), m. p. 102—110° (decomp.), which was recrystallised from ethyl acetate–light petroleum to give 6-*nitro*-2-*oximinohexanoic acid* as needles, m. p. 115—118° (decomp.) (Found: 38·4; H, 5·6; N, 14·3. $C_6H_{10}N_2O_5$ requires C, 38·5; H, 5·7; N, 14·2%); $\nu_{max.}$ 3220, 3080, 1687s, 1650w, 1542s cm.$^{-1}$; n.m.r. spectrum (in a mixture of deuterochloroform and trifluoroacetic acid): τ = 5·30 (2, triplet, J = 7 c./sec., 7·07 (2, triplet, J = 7 c./sec.), 7·5—8·5 (4, multiplet).

Hydrolysis of 1-*Hydroxy*-2-*nitrocyclopentanecarboxylic Acid with Dilute Alkali.*—A solution of the hydroxy-acid (I; R = OH) (9·4 mg.) in 0·05N-sodium hydroxide was set aside at room temperature overnight; back titration with standard acid to phenolphthalein showed that 2 equiv. of alkali had been consumed. Acidification of the solution and isolation of the product with ethyl acetate gave a colourless oil (4·0 mg.); $\nu_{max.}$ 3500s, 2600br, 1720vs, 1630w, 1550vs cm.$^{-1}$; n.m.r. spectrum: τ = 4·79 (triplet, J = 8 c./sec.), 5·06 (triplet, J = 8 c./sec.), 7·2—8·4 (multiplet).

Hydrogenation of 1-*Hydroxy*-2-*nitrocyclopentanecarboxylic Acid.*—A solution of the hydroxy-acid (I; R = OH) (182 mg.) in water was shaken overnight with hydrogen in the presence of platinum (from platinum oxide, 23 mg.). The solution was filtered and concentrated to give crystals which were recrystallised from water to give 2-*amino*-1-*hydroxycyclopentanecarboxylic acid* (III; R = OH) (73 mg.) as prisms which did not melt below 310° (Found: C, 49·6; H, 7·85; N, 9·4. $C_6H_{11}NO_3$ requires C, 49·6; H, 7·6; N, 9·6%); $\nu_{max.}$ 3350sh, 3100br, 2900—2700, 1630s, 1545s cm.$^{-1}$; R_F 0·46—0·56 (purple spot with ninhydrin).

The *hydrochloride* forms small plates (from aqueous ethanol) which decompose without melting at *ca.* 175° (Found: C, 40·1; H, 6·9; N, 7·7; Cl, 19·1. $C_6H_{12}ClNO_3$ requires C, 39·7; H, 6·7; N, 7·7; Cl, 19·5%); $\nu_{max.}$ 3430, 3100—2300, 1742s, 1595m, 1574m cm.$^{-1}$; n.m.r. spectrum (in D_2O): τ = 5·45 (1, triplet, J = 7 c./sec.), 6·8—7·9 (6, multiplet).

The N-*benzoyl derivative*, prepared with benzoyl chloride in excess of alkali, forms elongated plates, m. p. 161—164° (Found: C, 62·4; H, 6·1; N, 5·7. $C_{13}H_{15}NO_4$ requires C, 62·6; H, 6·1; N, 5·6%); $\nu_{max.}$ 3460, 3305, 3100br, 2700—2500, 1725s, 1640s, 1605, 1575, 1548s cm.$^{-1}$; n.m.r. spectrum (in trifluoroacetic acid): τ = 2·0—3·0 (5, multiplet), 5·1 (1, multiplet), 7·0—8·2 (6, multiplet).

The N-*benzoyl*-O-*acetyl derivative* obtained by acetylation of the *N*-benzoyl derivatives forms prisms, m. p. 189—191° (Found: C, 62·1; H, 6·0; N, 5·0. $C_{15}H_{17}NO_5$ requires C, 61·9; H, 5·9; N, 4·8%); $\nu_{max.}$ 3325, 2600—2500, 1735sh, 1720s, 1575m, 1545s cm.$^{-1}$; n.m.r. spectrum (in a mixture of deuterochloroform and trifluoroacetic acid); τ = 2·3—3·0 (5, multiplet), 5·3 (1, multiplet), 7·0—8·5 (6, multiplet), 7·78 (3, singlet).

Reaction of 1-*Hydroxy*-2-*aminocyclopentanecarboxylic Acid with Sodium Periodate.*—A solution of the amino-acid (30·8 mg.) and 0·1M-sodium periodate (4·0 ml.) in water (50 ml.) was set aside in the dark at room temperature. Aliquot parts (2 ml.) were periodically withdrawn and titrated with 0·01N-sodium thiosulphate to a starch end-point. One equiv. of periodate was consumed in 7 hr. and a further 0·6 equiv. in the next 84 hr.

Hydrogenation of 1-*Amino*-2-*nitrocyclopentanecarboxylic Acid.*—A solution of the amino-acid (51 mg.) in water (9 ml.) was shaken overnight with hydrogen and platinum (from platinum oxide, 48 mg.). The solution was filtered and evaporated and the product was recrystallised from aqueous ethanol to give 1,2-*diaminocyclopentanecarboxylic acid* (III; R = NH_2) as needles decomposing at *ca.* 200° (Found: C, 44·9; H, 8·9; N, 17·2. $C_6H_{12}N_2O_2$ requires C, 44·4; H, 8·7; N, 17·3%); $\nu_{max.}$ 3340 and 3290s, 3030, 2800—2400, 2140w, 1655s, 1590s, br, 1532 $cm.^{-1}$; n.m.r. spectrum (in D_2O): τ = 5·98 (1, triplet), 7·0—8·0 (6, multiplet); R_F 0·34—0·44, purple spot with ninhydrin.

The NN-*diacetyl compound*, prepared with acetic anhydride in acetic acid, formed prisms, m. p. 240—243° (decomp.), from methanol–acetone (Found: C, 52·5; H, 7·2; N, 11·3. $C_{10}H_{16}N_2O_4$ requires C, 52·6; H, 7·1; N, 11·5%); $\nu_{max.}$ 3300s, 2700—2500, 1710s, 1653s, 1623s, 1585s, 1545s, $cm.^{-1}$; n.m.r. spectrum (in trifluoroacetic acid): τ = 5·3 (1, multiplet), 7·63 (3, singlet), 7·68 (3, singlet), 7·0—8·3 (6, multiplet).

1-*Iodo*-2-*nitrocyclopentanecarboxylic Acid* (I; R = I).—A solution of dinitrogen tetroxide (31·5 g.) in ether (0·5 l.) was added portionwise to a stirred solution of cyclopent-1-enecarboxylic acid (50·0 g.) and iodine (126·0 g.) in ether (2 l.) cooled in ice and stirring was continued at 0° for a further 3·5 hr. The solvent was evaporated at room temperature and the residue was chromatographed on silica gel (1·25 kg.). The material eluted by chloroform was recrystallised to give 1-*iodo*-2-*nitrocyclopentanecarboxylic acid* (I; R = I) as pale yellow prisms (72·5 g., 57%), m. p. 117—118·5° (Found: C, 25·5; H, 2·9; I, 44·3; N, 4·7. $C_6H_8NO_4I$ requires C, 25·3; H, 2·8; I, 44·5; N, 4·9%); $\nu_{max.}$ 3100—2500, 1695s, 1545s $cm.^{-1}$; n.m.r. spectrum: τ = 4·60 (1, doublet, J = 8 c./sec.), 6·7—8·2 (6, multiplet).

Synthesis of 2-*Nitrocyclopent*-1-*enecarboxylic Acid* (II).—A solution of 1-iodo-2-nitrocyclopentanecarboxylic acid (I; R = I) (4·15 g.) in dry pyridine (125 ml.) was allowed to stand overnight at room temperature, diluted with water (400 ml.), made acid with 6N-hydrochloric acid, and extracted with ether (4 × 200 ml.). The product was dissolved in benzene–chloroform (25 ml., 1 : 1) and chromatographed on silica gel (100 g.). The column was washed with benzene until free from iodine. Subsequent elution with benzene–chloroform (4 : 1) gave cyclopent-1-enecarboxylic acid (172 mg.), which after recrystallisation had m. p. 121—123°. Elution with benzene–chloroform (7 : 3) followed by recrystallisation from benzene–light petroleum gave 2-nitrocyclopent-1-enecarboxylic acid (II) (521 mg.) as buff needles, m. p. 101—102·5°, identified by infrared spectrum, thin-layer chromatogram (system 1) and mixed m. p.

Synthesis of 1-*Amino*-2-*nitrocyclopentanecarboxylic acid.*—A solution of 1-iodo-2-nitrocyclopentanecarboxlyic acid (I; R = I) (200 mg.) in ammonium hydroxide (20 ml., 0·90) was set aside at room temperature for 20 hr. Evaporation to dryness gave a brown crystalline solid which was washed with acetone to remove ammonium iodide, dissolved in water, and passed through Amberlite IRC-50 (H^+). The eluate was concentrated under reduced pressure to give the amino-acid (I; R = NH_2) (61 mg.), identified by infrared spectrum, paper chromatography, and thin-layer chromatography (system 2).

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