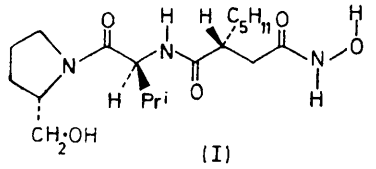
Studies concerning the Antibiotic Actinonin. Part II.[1] Total Synthesis of Actinonin and Some Structural Analogues by the Isomaleimide Method†

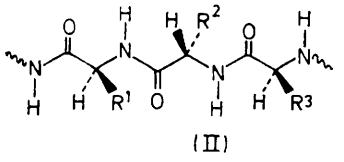
By **Nicholas H. Anderson, W. David Ollis,* John E. Thorpe,** and **A. David Ward,** Department of Chemistry, The University, Sheffield S3 7HF

A general method for the synthesis of actinonin (I) and several structural analogues is described. L-Valyl-L-prolinol (III) and the isomaleimide (XIV) yield the intermediate (XVII), which gives actinonin (I) on hydrogenation. Corresponding routes yield compounds (XXV) (L-alanylpyrrolidine and DL-alanylpyrrolidine analogues), (XXVI) (L-valylpyrrolidine analogue), and (XXVII) (L-valyl-L-prolinol analogue), which are actinonin analogues lacking the pentyl side-chain.

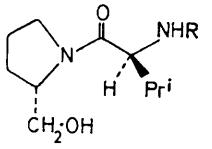
THE constitution (I) [1-3] of the antibiotic actinonin is of interest in several respects. It is the first natural product to be recognised as a simple hydroxamic acid of the type RCO·NH·OH,[4a] and it is also the first known naturally occurring derivative of L-prolinol. Actinonin (I) may be compared with polypeptide antibiotics [4b] and could be described as having a pseudopeptide structure; this is indicated by comparison of its stereoformula (I) with the polypeptide stereoformula (II) containing L-α-amino-acid residues. The isosteric correspondence of the side-chain of the D-pentylsuccinic acid residue with the side-chain (R^2) of the corresponding L-α-amino-acid is particularly



(I)



(II)



(III) R=H

(IV) R=$PhCH_2O·CO$

striking.[4c] These aspects of the actinonin structure could well be related [4,5] to the biological properties [1,2] of actinonin, which include activity against various Gram-positive, Gram-negative, and acid-fast bacteria as well as some antiphage activity. In order to explore possible structure–activity relationships involving actinonin, a synthetic approach was required which could be used for the synthesis of actinonin, its diastereoisomers, and structural analogues. Progress in these directions is now reported.

The total synthesis of actinonin (I) was first considered in relation to the possibility of achieving a useful reaction between L-valyl-L-prolinol (III) and a suitable derivative of pentylsuccinic acid. The synthesis of L-valyl-L-prolinol (III) was straightforward: L-prolinol was obtained [6] by reduction, with lithium aluminium hydride, of L-2-ethoxycarbonyl-5-pyrrolidone, prepared from L-glutamic acid. L-Prolinol and *N*-benzyloxycarbonyl-L-valine *p*-nitrophenyl ester yielded, by the standard coupling procedure,[7] *N*-benzyloxycarbonyl-L-valyl-L-prolinol (IV), which on catalytic hydrogenation gave L-valyl-L-prolinol (III).

Possible ways of discriminating synthetically between the two carboxy-groups of pentylsuccinic acid were now considered. The pentyl substituent was not expected to provide a useful source of discrimination on *electronic* grounds but it could be associated with a significant *steric* effect. It was then appreciated that this steric effect upon the relative reactivity of two appositely placed carbonyl groups would be more pronounced in pentylmaleic anhydride than in pentylsuccinic anhydride. Thus pentylmaleic anhydride would be expected to undergo preferential reaction with nucleophilic amines at the carbonyl group remote from the pentyl substituent. However, having recognised this, we had still to consider how each of the two carbonyl groups of pentylmaleic acid could be selectively incorporated into the two different peptide linkages associated with the L-valyl-L-prolinol and hydroxylamine residues. The solution to this problem was provided by a route involving isomaleimide

† Preliminary communication, N. H. Anderson, W. D. Ollis, J. E. Thorpe, and A. D. Ward, *J.C.S. Chem. Comm.*, 1974, 420.

[1] Part I, J. J. Gordon, J. P. Devlin, A. J. East, W. D. Ollis, I. O. Sutherland, D. E. Wright, and L. Ninet, preceding paper.

[2] J. J. Gordon, B. K. Kelly, and G. A. Miller, *Nature*, 1962, **195**, 701.

[3] A. J. East, W. D. Ollis, and I. O. Sutherland, in 'Chemistry of Microbial Products,' Institute of Applied Microbiology Symposium No. 6, University of Tokyo, 1964, p. 204.

[4] (*a*) J. B. Bapat, D. St. C. Black, and R. F. C. Brown, *Adv. Heterocyclic Chem.*, 1969, **10**, 199; H. Maehr, *Pure Appl. Chem.*, 1971, **28**, 603; (*b*) R. O. Studer, *Progr. Medicin. Chem.*, 1967, **5**, 1; (*c*) M. M. Shemyakin, Yu. A. Ovchinnikov, and V. T. Ivanov, *Angew. Chem. Internat. Edn.*, 1969, **8**, 492.

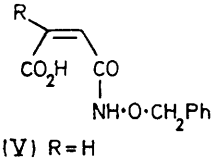
[5] H. R. V. Arnstein, *Ann. Reports*, 1957, **54**, 347.

[6] (*a*) P. Karrer and P. Portmann, *Helv. Chim. Acta*, 1948, **31**, 2088; (*b*) F. F. Blicke and C.-J. Lu, *J. Amer. Chem. Soc.*, 1955, **77**, 29; (*c*) F. P. Doyle, M. O. Mehta, G. S. Sach, and J. L. Pearson, *J. Chem. Soc.*, 1958, 4458; (*d*) R. Buyle, *Chem. and Ind.*, 1966, 195.

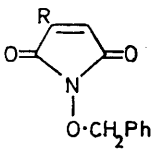
[7] M. Bodanzky and V. du Vigneaud, *J. Amer. Chem. Soc.*, 1959, **81**, 5688.

intermediates, in which an *N*-benzyloxy-group was incorporated as a potential hydroxyamino-group.

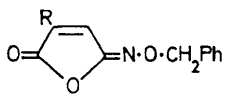
Dehydration of *N*-benzyloxymaleamic acid (V) with thionyl chloride followed by treatment with pyridine is reported to give a product, m.p. 80—81° [$\lambda_{max.}$ 288 nm (ε 14,000); $\nu_{max.}$ 1733 and 1721 cm^{-1}], formulated as the maleimide (VI).[8] In our hands this reaction gave an anhydro-derivative, m.p. 80° [$\lambda_{max.}$ 288 nm (ε 11,500); $\nu_{max.}$ 1795 and 1640 cm^{-1}], which, in spite of differences in the i.r. spectral maxima, was obviously identical with the compound described by Ames and Grey.[8] However, their proposed constitution (VI) is clearly excluded by



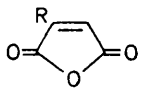
(V) R = H
(IX) R = Me
(XII) R = C_5H_{11}



(VI) R = H
(XIII) R = C_5H_{11}



(VII) R = H
(X) R = Me
(XIV) R = C_5H_{11}



(VIII) R = Me
(XI) R = C_5H_{11}

the n.m.r. spectrum of the anhydro-derivative, which showed an isolated AB system (τ_A 2·70, τ_B 3·62; J_{AB} 5·7 Hz) together with signals assignable to the benzyloxy-group. This showed conclusively that the anhydro-derivative of *N*-benzyloxymaleamic acid (V) is the unsymmetrical isoimide (VII) rather than the previously proposed [8] symmetrical maleimide (VI).

The selectivity of ring-opening of monosubstituted maleic anhydrides by *O*-benzylhydroxylamine has now been demonstrated and the constitutions of the derived maleamic acids have been firmly established in the following manner. *O*-Benzylhydroxylamine and methylmaleic anhydride (VIII) reacted together rapidly in ethereal solution at room temperature to give one product, shown to be *N*-benzyloxy-2-methylmaleamic acid (IX). This acid (IX) with diazomethane gave a methyl ester which on ozonolysis gave methyl pyruvate, isolated as its 2,4-dinitrophenylhydrazone.[9]

Dehydration of pentylfumaric acid [10] with phosphoric anhydride gave pentylmaleic anhydride (XI). This anhydride also gave one product (XII) on treatment with *O*-benzylhydroxylamine. This constitution (XII) was similarly confirmed by ozonolysis and identification of the final product as the 2,4-dinitrophenylhydrazone of methyl 2-oxoheptanoate. An authentic sample of this hydrazone was prepared by ozonolysis of dimethyl pentylfumarate and treatment of the derived methyl 2-oxoheptanoate with 2,4-dinitrophenylhydrazine.

NN'-Dicyclohexylcarbodi-imide has been shown [11,12] to dehydrate maleamic acids to give isoimides, and with this reagent the maleamic acid (V) gave the same product (VII) as was obtained by the thionyl chloride–pyridine procedure.[8] The maleamic acid (IX) similarly gave the isoimide (X) [$\lambda_{max.}$ 287 nm; $\nu_{max.}$ 1795 and 1640 cm^{-1}; τ_{Me} 8·01 (d), τ_H 3·11 (q) (*J* 1·5 Hz)]. However, in the pentyl series, the maleamic acid (XII) gave two anhydro-derivatives which could be formulated as the maleimide (XIII) [$\lambda_{max.}$ 216 nm; $\nu_{max.}$ 1730 and 1625 cm^{-1}; τ_H 3·94 (t, *J* 0·9 Hz)] and the isomaleimide (XIV) [$\lambda_{max.}$ 285 nm; $\nu_{max.}$ 1795 and 1640 cm^{-1}; τ_H 3·11 (t, *J* 0·9 Hz)].

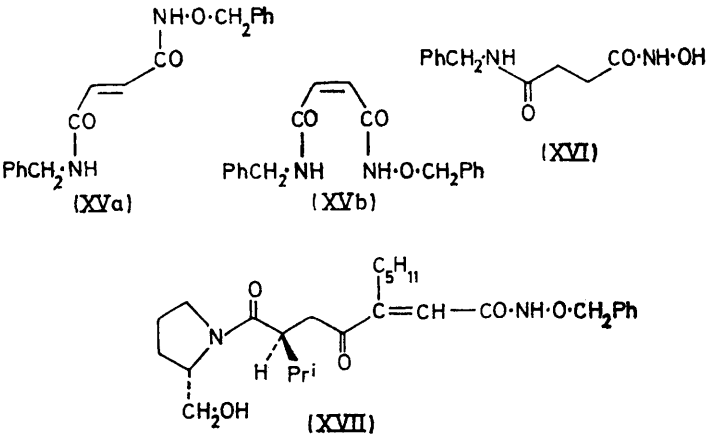
Isoimides [11–15] undergo nucleophilic attack [12,14,15] at the carbonyl group as the favoured process and as a model for the application of this reaction in the synthesis of actinonin the following experiments were carried out. *N*-Benzyloxyisomaleimide (VII) and benzylamine in boiling chloroform yielded *N*-benzyl-*N'*-benzyloxyfumaramide (XVa), m.p. 238°, as the sole product which, on catalytic hydrogenation, was debenzylated and reduced giving the carbamoyl-hydroxamic acid (XVI) directly. The product of m.p. 238° was identified as the fumaric acid derivative (XVa) because the corresponding reaction of *N*-benzyloxyisomaleimide (VII) and benzylamine at room temperature gave the isomer (XVb).[16] The isomerisation (XVb) ⟶ (XVa) occurs when the maleic acid derivative (XVb) is boiled in chloroform with a trace of benzylamine.[16]

Reaction between L-valyl-L-prolinol (III) and the isoimide (XIV) gave *O*-benzyldidehydroactinonin (XVII) which, on catalytic hydrogenation,[17] gave a mixture of two diastereoisomers. Actinonin (I) was isolated from this mixture by fractional crystallisation.

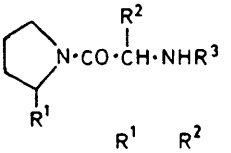
This synthetic route has also been used for the synthesis of several analogues of actinonin (I), involving replacement of the L-prolinol, L-valine, or D-pentylsuccinic acid residue by a pyrrolidine, alanine, or succinic acid residue.

Coupling of the *N*-benzyloxycarbonyl-amino-acid *p*-nitrophenyl esters [7] derived from L-alanine, DL-alanine, and L-valine with either pyrrolidine or L-prolinol gave the intermediate *N*-benzyloxycarbonyl derivatives (XVIII) (L-alanine or DL-alanine residue), (XIX) (L-valine residue), and (IV) (L-prolinol and L-valine residues), which were hydrogenated giving the aminoacyl compounds

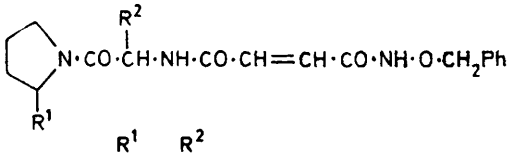
[8] D. E. Ames and T. F. Grey, *J. Chem. Soc.*, 1955, 631.
[9] W. D. Ollis, M. V. J. Ramsay, I. O. Sutherland, and S. Mongkolsuk, *Tetrahedron*, 1965, **21**, 1453.
[10] Method of W. R. Vaughan and K. S. Anderson, *J. Amer. Chem. Soc.*, 1955, **77**, 6702; *J. Org. Chem.*, 1956, **21**, 673.
[11] R. J. Cotter, C. K. Sauers, and J. M. Whelan, *J. Org. Chem.*, 1961, **26**, 10.
[12] R. Paul and A. S. Kende, *J. Amer. Chem. Soc.*, 1964, **86**, 4162.
[13] M. M. S. Hoggewerff and W. A. van Dorp, *Rec. Trav. chim.*, 1893, **12**, 12; 1895, **14**, 252; P. H. van der Meulen, *ibid.*, 1896, **15**, 282, 323; K. C. Tsou, R. J. Barrnett, and A. M. Seligman, *J. Amer. Chem. Soc.*, 1955, **77**, 4613; D. Y. Curtin and L. L. Miller, *Tetrahedron Letters*, 1965, 1869; *J. Amer. Chem. Soc.*, 1967, **89**, 637.
[14] W. R. Roderick and P. L. Bhatia, *J. Org. Chem.*, 1963, **28**, 2018.
[15] C. K. Sauers and R. J. Cotter, U.S. Pat. 3,133,070 (*Chem. Abs.*, 1964, **61**, 7026); E. Hedaya, R. L. Hinman, and S. Theodoropulos, *J. Org. Chem.*, 1966, **31**, 1317.
[16] M. T. W. Hearn and A. D. Ward, in preparation.
[17] M. Masaki, J. Ohtake, M. Sugigama, and M. Ohta, *Bull. Chem. Soc., Japan*, 1965, **38**, 1802.



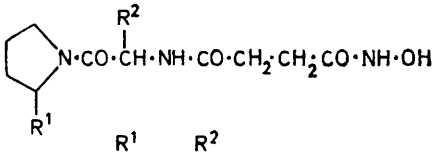
(XX), (XXI), and (III). These aminoacyl compounds when heated with *N*-benzyloxyisomaleimide (VII) in chloroform gave the *O*-benzyldidehydro-compounds



	R^1	R^2	R^3
(XVIII)	H	Me	$PhCH_2·O·CO$
(XIX)	H	Pr^i	$PhCH_2·O·CO$
(XX)	H	Me	H
(XXI)	H	Pr^i	H



	R^1	R^2
(XXII)	H	Me
(XXIII)	H	Pr^i
(XXIV)	CH_2OH	Pr^i



	R^1	R^2
(XXV)	H	Me
(XXVI)	H	Pr^i
(XXVII)	CH_2OH	Pr^i

(Table 1), which were reduced to the actinonin analogues listed in Table 2. The debenzylation was assisted by addition of a trace of pyridine [17] during the hydrogenation step.

The biological activity of these actinonin analogues and the mass spectral fragmentation patterns of the compounds described here will be discussed in later papers in this series.

EXPERIMENTAL

General experimental procedures are given in Part I.[1]

L-*Prolinol*.—Modification of the published procedure,[6] by the use of purified lithium aluminium hydride and continuous extraction with ether, was necessary. Lithium aluminium hydride (15 g) was stirred with anhydrous ether (400 ml) and insoluble material was removed. This solution was then slowly added to a gently boiling stirred solution of L-2-ethoxycarbonyl-5-pyrrolidone (18 g) in ether (400 ml). After heating (24 h) under reflux, water (14 ml) was carefully added to the cooled solution followed by aqueous sodium hydroxide (10N; 500 ml). Continuous extraction (24 h) with ether and drying (Na_2SO_4) and evaporation of the extract gave L-prolinol (7·1 g) as an oil, b.p. 88—90° at 15 mmHg (lit.,[6c] 89° at 6 mmHg), characterised as its oxalate, m.p. 156° (from ethanol), $[\alpha]_D^{21}$ +19° (*c* 0·2 in H_2O) {lit.,[6a] m.p. 159°, $[\alpha]_D$ +21·5 (H_2O)}.

N-*Benzyloxycarbonyl*-L-*valyl*-L-*prolinol* (IV).—A solution of L-prolinol (2·95 g) in ethyl acetate (20 ml) was added to *N*-benzyloxycarbonyl-L-valine *p*-nitrophenyl ester [7] (9·20 g) in ethyl acetate (20 ml) at room temperature. After 48 h, chloroform (40 ml) was added and, after shaking with 2N-hydrochloric acid (20 ml), 2N-ammonium hydroxide (30 ml portions to remove *p*-nitrophenol), and water (20 ml), evaporation gave *N*-benzyloxycarbonyl-L-valyl-L-prolinol (8·2 g) as a gum, $[\alpha]_D^{25}$ −0·41° (*c* 0·2 in EtOH), *m/e* 334 (M^+, $C_{18}H_{26}N_4O_9$); $\nu_{max.}$ 1700 and 1610 cm^{-1}; τ 2·70 (s, Ph), 4·25br (d, NH), 4·95 (s, $PhCH_2$), 6·20 (d, *J* 5·5 Hz, CH_2·OH), and 9·02 (H_A, d) and 9·09 (H_B, d) (*J* 6·5 Hz, Me_AMe_BCH).

L-*Valyl*-L-*prolinol* (III).—Hydrogenation (24 h) at room temperature of *N*-benzyloxycarbonyl-L-valyl-L-prolinol (8·2 g) in ethyl acetate (100 ml) over 10% palladium–charcoal (1·0 g), followed by filtration and evaporation, gave the intermediate carbamic acid ($\nu_{max.}$ 1700 cm^{-1}). Thermal decarboxylation (20 min; 100°; 15 mmHg) gave L-valyl-L-prolinol (4·75 g) as a gum, *m/e* 200 (M^+, $C_{10}H_{20}N_2O_2$), $\nu_{max.}$ 1610 cm^{-1}, characterised [18] as its N-2,4-*dinitrophenyl derivative*, yellow needles, m.p. 125° [from benzene–light petroleum (b.p. 60—80°)] (Found: C, 52·7; N, 5·8; N, 15·1%; M^+, 366. $C_{16}H_{22}N_4O_6$ requires C, 52·5; H, 6·1; N, 15·3%; *M*, 366); $[\alpha]_D^{24}$ +74° (*c* 0·2 in EtOH), and its *picrate*, m.p. 193° (from ethyl acetate–ether) (Found: C, 44·9; H, 4·9; N, 16·4. $C_{10}H_{20}N_2O_2$,$C_6H_3N_3O_7$ requires C, 44·7; H, 5·4; N, 16·3%).

[18] K. R. Rao and H. A. Sober, *J. Amer. Chem. Soc.*, 1954, **76**, 1328.

O-Benzylhydroxylamine.—*O*-Benzylacetoxime [19] (20 g) was added to concentrated hydrochloric acid (25 ml) and the stirred mixture was boiled (30 min). After cooling (0°) the crystalline precipitate was collected and treated with an excess of aqueous 2N-sodium hydroxide. Extraction with ether and distillation gave *O*-benzylhydroxylamine (7·2 g) as an oil, b.p. 90° at 15 mmHg (lit.,[20] 118—119° at 30 mmHg), τ 2·76 (s, Ph), 4·82 (s, NH_2), and 5·45 (s, $PhCH_2$), characterised as its N-2,4-*dinitrophenyl* derivative, yellow needles, m.p. 141° (from ethanol) (Found: C, 54·0; H, 3·9. $C_{13}H_{11}N_3O_5$ requires C, 54·0; H, 3·8%).

Pentylfumaric Acid.—Bromine (32 g) was added dropwise to a stirred solution of ethyl 2-pentylacetoacetate [21] (10 g) in dry ether (15 ml) at 0°. The mixture was then heated under reflux (5 h), water (100 ml) was added, and the *lower* ethereal layer was separated and added dropwise to a stirred slurry of potassium hydroxide (27 g) in ethanol (30 ml) at 0°. This mixture was heated during 30 min to 100°, kept at this temperature for a further 30 min, then steam-distilled, and the residue was acidified. Extraction with ether followed by crystallisation from benzene–light petroleum (b.p. 60—80°) gave *pentylfumaric acid* (6·0 g), m.p. 171° (Found: C, 58·2; H, 7·6. $C_9H_{14}O_4$ requires C, 58·0; H, 7·6%).

Pentylmaleic Anhydride (XI).—Pentylfumaric acid (10 g) was intimately mixed with phosphoric anhydride (9·0 g) and the mixture was heated during 1 h to 180°. Distillation gave *pentylmaleic anhydride* (7·5 g), b.p. 125—130° at 15 mmHg (Found: C, 64·7; H, 7·1. $C_9H_{12}O_3$ requires C, 64·3; H, 7·2%), $\nu_{max.}$ (film) 1840, 1780, and 1640 cm^{-1}; τ 3·36 (t, *J* 1·5 Hz, vinylic H), 7·47br (t, CH_2·C=C), 8·0—8·9 (m, $[CH_2]_3$), and 9·09 br (t, Me).

N-*Benzyloxy*-2-*pentylmaleamic Acid* (XII).—A solution of *O*-benzylhydroxylamine (1·4 g) in dry ether (20 ml) was added dropwise to a solution of pentylmaleic anhydride (2·0 g) in dry ether (20 ml) at 0°. After stirring for a further 30 min at 20°, light petroleum (b.p. 40—60°) was added until the solution became cloudy. After 24 h at 0° the precipitate was collected; crystallisation from ether–light petroleum (b.p. 40—60°) at 0° gave N-*benzyloxy*-2-*pentylmaleamic acid* (2·62 g) as prisms, m.p. 77° (Found: C, 66·2; H, 7·0; N, 4·9. $C_{16}H_{21}NO_4$ requires C, 66·0; H, 7·3; N, 4·8%), $\nu_{max.}$ (Nujol) 1690 and 1640 cm^{-1}; τ −1·5br (s, CO_2H and NH), 2·69br (s, Ph), 3·73br (s, vinylic H), 5·09 (s, $PhCH_2$), 7·65br (t, CH_2·C=C), 8·1—8·9br (m, $Me[CH_2]_3$), and 9·13br (t, Me).

N-*Benzyloxy*-2-*pentylmaleimide* (XIII) *and* N-*Benzyloxy*-2-*pentylisomaleimide* (XIV).—A solution of *NN'*-dicyclohexylcarbodi-imide (730 mg) in anhydrous ethyl acetate (5 ml) was added at 0° to a solution of *N*-benzyloxy-2-pentylmaleamic acid (1·0 g) in ethyl acetate (5 ml). After 30 min the precipitated *NN'*-dicyclohexylurea was removed and the filtrate evaporated. Fractionation of the residue by thick-layer chromatography (benzene) gave two fractions which were extracted with chloroform. The faster-moving band gave N-*benzyloxy*-2-*pentylisomaleimide* (610 mg) as an oil (Found: C, 70·1; H, 6·9; N, 5·1%; M^+, 273. $C_{16}H_{19}NO_3$ requires C, 70·3; H, 7·0; N, 5·1%; *M*, 273); $\lambda_{max.}$ 285 nm (ε 11,300); $\nu_{max.}$ 1795 and 1640 cm^{-1}; τ 2·65 (s, Ph), 3·11 (t, *J* 0·9 Hz, vinylic H), 4·81 (s, $PhCH_2$), 7·62br (m, CH_2·C=C), 8·62 (m, $[CH_2]_3$), and 9·11br (t, Me). The slower-moving fraction gave N-*benzyloxypentylmaleimide* (160 mg) as plates, m.p. 34° [from light petroleum (b.p. 40—60°) at −20°] (Found: C, 70·5; H, 7·1; N, 5·1. $C_{16}H_{19}NO_3$ requires C, 70·3; H, 7·0; N, 5·1%); $\lambda_{max.}$ 216 nm (ε 16,600); $\nu_{max.}$ 1730 and 1625 cm^{-1}; τ 2·65 (s, Ph), 3·94 (t, *J* 0·9 Hz, vinylic H), 4·78 (s, $PhCH_2$), 7·62br (m, CH_2·C=C), 8·62 (m, $[CH_2]_3$), and 9·11br (t, Me).

O-*Benzyldidehydroactinonin* (XVII).—A solution of *N*-benzyloxy-2-pentylisomaleimide (630 mg) and L-valyl-L-prolinol (450 mg) in chloroform was set aside at room temperature for 4 days. Evaporation and column chromatography (elution with chloroform followed by chloroform–ethyl acetate) of the residue yielded O-*benzyldidehydroactinonin* (635 mg) as an amorphous solid (Found: C, 65·8; H, 8·2; N, 9·1. $C_{26}H_{39}N_3O_5$ requires C, 66·0; H, 8·3; N, 8·9%), $\nu_{max.}$ 1660 and 1620 cm^{-1}.

Actinonin (I).—Hydrogenation (1 atm; room temperature; 12 h) of a solution of *O*-benzyldidehydroactinonin (360 mg) in ethanol (10 ml) containing pyridine [17] (0·2 ml) over palladium–charcoal (10%; 200 mg), followed by filtration and evaporation, gave a residue. Recrystallisation from ethanol–ether gave actinonin (30 mg) identical with the natural product.

N-*Benzyloxyisomaleimide* (VII).—(*a*) Dehydration of *N*-benzyloxymaleamic acid (200 mg), as in the preparation of compounds (XIII) and (XIV), gave N-*benzyloxyisomaleimide* as needles (130 mg), m.p. 80° [from light petroleum (b.p. 60—80°)] (Found: C, 65·0; H, 4·8; N, 7·1. $C_{11}H_9NO_3$ requires C, 65·0; H, 4·5; N, 6·9%), $\lambda_{max.}$ 288 nm (ε 15,000); $\nu_{max.}$ (Nujol) 1795 and 1640 cm^{-1}; τ 2·63 (s, Ph), 2·70 (H_A, d), and 3·62 (H_B, d) (J_{AB} 5·7 Hz, CH_A=CH_B), and 4·78 (s, $PhCH_2$); literature data [8] for '*N*-benzyloxymaleimide': m.p. 80—81°, $\lambda_{max.}$ 288 nm (ε 14,000), $\nu_{max.}$ (Nujol) 1733 and 1721 cm^{-1}.

(*b*) Dehydration of *N*-benzyloxymaleamic acid with thionyl chloride following the published procedure [8] gave *N*-benzyloxyisomaleimide, identical with the product from method (*a*).

N-*Benzyl*-N'-*benzyloxyfumaramide* (XVa).—A solution of *N*-benzyloxyisomaleimide (167 mg) and benzylamine (88 mg) in chloroform (10 ml) was heated (24 h) under reflux, then cooled, and the crystalline precipitate was collected. Recrystallisation from chloroform gave N-*benzyl*-N'-*benzyloxyfumaramide* (190 mg), m.p. 238° (Found: C, 69·5; H, 6·1; N, 9·2. $C_{18}H_{18}N_2O_3$ requires C, 69·7; H, 5·9; N, 9·0%), $\nu_{max.}$ (Nujol) 1630 cm^{-1}.

N-*Benzyl*-N'-*hydroxysuccinamide* (XVI).—Hydrogenation (1 atm; room temperature; 12 h) of a solution of *N*-benzyl-*N'*-benzyloxyfumaramide (120 mg) in ethyl acetate (5 ml) over palladium–charcoal (10%; 10 mg) yielded N-*benzyl*-N'-*hydroxysuccinamide* (85 mg), m.p. 149° (from ethyl acetate) (Found: C, 59·8; H, 6·6; N, 12·8. $C_{11}H_{14}N_2O_3$ requires C, 59·5; H, 6·4; N, 12·6%).

N-*Benzyloxy*-2-*methylmaleamic Acid* (IX).—A solution of *O*-benzylhydroxylamine (7·8 g) in dry ether (20 ml) was added slowly to a stirred cooled solution of methylmaleic anhydride (7·0 g) in dry ether (20 ml) at 20°. After stirring for a further 30 min the product was removed; recrystallisation from chloroform–ether gave N-*benzyloxy*-2-*methylmaleamic* acid (10·8 g), m.p. 114° (Found: C, 61·1; H, 5·3; N, 6·2. $C_{12}H_{13}NO_4$ requires C, 61·1; H, 5·6; N, 6·0%), $\nu_{max.}$ (Nujol) 1690 and 1645 cm^{-1}; τ (C_5D_5N) −4·00br (s, CO_2H and NH), 2·75br (s, Ph), 3·67br (s, vinylic H), 4·78 (s, $PhCH_2$), and 7·86 (d, *J* 1·5 Hz, Me).

N-*Benzyloxy*-2-*methylisomaleimide* (X).—A solution of *N*-benzyloxy-2-methylmaleamic acid (4·4 g) in dry ethyl acetate (150 ml) was added to a solution of *NN'*-dicyclohexylcarbodi-imide (3·8 g) in ethyl acetate (50 ml). After

[19] A. Janny, *Ber.*, 1883, **16**, 174.
[20] R. Behrend and K. Leuchs, *Annalen*, 1890, **257**, 207.
[21] P. Centerick, *Bull. Soc. chim. belges*, 1936, **45**, 545.

24 h the precipitated *NN'*-dicyclohexylurea was removed and the filtrate evaporated. Recrystallisation from ether–light petroleum (b.p. 40—60°) at 0° gave *N*-benzyloxy-2-methylisomaleimide (2·8 g), m.p. 50° (Found: C, 66·4; H, 5·1; N, 6·8. $C_{12}H_{11}NO_3$ requires C, 66·4; H, 5·1; N, 6·5%), $\lambda_{max.}$ 287 nm (ε 12,300); $\nu_{max.}$ 1795 and 1640 cm^{-1}; τ 2·67 (s, Ph), 3·11 (q, *J* 1·5 Hz, vinylic H), 4·83 (s, PhCH_2), and 8·01 (d, *J* 1·5 Hz, Me).

Methyl N-*Benzyloxy*-2-*methylmaleamate*.—A solution of *N*-benzyloxy-2-methylmaleamic acid (1·0 g) in methanol was treated with an excess of ethereal diazomethane and after 30 s a few drops of acetic acid were added. The solvent was evaporated off and the residue recrystallised from light petroleum (b.p. 60—80°) giving *methyl* N-*benzyloxy*-2-*methylmaleamate*, m.p. 72°; $\nu_{max.}$ 1720, 1690sh, and 1650 cm^{-1}; τ 0·38br (s, NH), 2·68 (s, Ph), 4·12 (m, vinylic H), 5·15 (s, PhCH_2), 6·25 (s, CO_2Me), and 8·03 (d, *J* 1·6 Hz, CH_3·C=CH).

Ozonolysis of Methyl N-*Benzyloxy*-2-*methylmaleamate*.—A solution of the methyl ester (100 mg) in dry ethyl acetate (100 ml) at −40° was treated with a slight excess of ozonised oxygen. The solvent was evaporated off and the ozonide warmed with water (10 ml) for a few minutes. The water was then evaporated off and the residue treated with a methanolic solution of 2,4-dinitrophenylhydrazine in sulphuric acid. Purification by t.l.c. (benzene) gave methyl pyruvate *trans*-2,4-dinitrophenylhydrazone (6 mg), m.p. 151°, and the *cis*-isomer (11 mg), m.p. 187°, both identical with authentic samples.[9]

Ozonolysis of Dimethyl Pentylfumarate; Formation of Methyl 2-*Oxoheptanoate* 2,4-*Dinitrophenylhydrazone*.—Pentylfumaric acid with diazomethane in methanol gave the corresponding dimethyl ester. This ester (160 mg) was ozonised in dry ethyl acetate (10 ml) at −80° and the intermediate ozonide was reduced at 0° over Adams platinic oxide. Removal of the catalyst and evaporation of the filtrate gave a residue which was treated with a methanolic solution of 2,4-dinitrophenylhydrazine in concentrated sulphuric acid. Fractionation by t.l.c. (chloroform) gave *methyl* 2-*oxoheptanoate* 2,4-*dinitrophenylhydrazone* (70 mg) as yellow needles, m.p. 145° [from benzene–light petroleum (b.p. 60—80°)] (Found: C, 49·4; H, 5·5; N, 16·9. $C_{14}H_{18}N_4O_6$ requires C, 49·7; H, 5·3; N, 16·6%); $\nu_{max.}$ 1710, 1620, and 1600 cm^{-1}; τ −3·50br (s, NH), 0·94 (H_X, d), 1·66 (H_B, double d), and 1·92 (H_A, d) [J_{AB} 10, J_{BX} 3, J_{AX} 0 Hz, $C_6H_3(NO_2)_2$], 6·02 (s, CO_2Me), 7·33br (t, CH_2–C=N), 8·0—8·9 (m, $[CH_2]_3$), and 9·07br (t, Me).

Ozonolysis of N-*Benzyloxy*-2-*pentylmaleamic Acid; Formation of Methyl* 2-*Oxoheptanoate* 2,4-*Dinitrophenylhydrazone*.—As in the preceding experiment, ozonolysis of *N*-benzyloxy-2-pentylmaleamic acid (200 mg), reduction of the intermediate ozonide, and treatment with diazomethane followed by Brady's reagent gave methyl 2-oxoheptanoate 2,4-dinitrophenylhydrazone (18 mg), m.p. 145°.

(N-*Benzyloxycarbonylaminoacyl*)*pyrrolidines* (XVIII) *and* (XIX).—These compounds were prepared [7] from various *p*-nitrophenyl esters (*N*-benzyloxycarbonyl-L-alanine *p*-nitrophenyl ester,[22a] *N*-benzyloxycarbonyl-DL-alanine *p*-nitrophenyl ester,[22b] and *N*-benzyloxycarbonyl-L-valine *p*-nitrophenyl ester [22c]) and pyrrolidine following the procedure described above for the synthesis of *N*-benzyloxycarbonyl-L-valyl-L-prolinol (IV).

TABLE 1

O-Benzyldidehydro-compounds

RNH·CO·CH=CH·CO·NH·O·CH_2Ph

Compound	Base residue RNH– derived from	Yield (%)	M.p. (°C)	Found (%) C	H	N	*M*	Formula	Required (%) C	H	N	*M*
(XXII)	L-Alanylpyrrolidine	90	115—116 (Amorphous solid)	62·9	7·0	12·4	345	$C_{18}H_{23}N_3O_4$	62·6	6·7	12·2	345
(XXII)	DL-Alanylpyrrolidine	93	117—118 (Amorphous solid)	62·5	6·4	12·2	345	$C_{18}H_{23}N_3O_4$	62·6	6·7	12·2	345
(XXIII)	L-Valylpyrrolidine	63	Gum	64·2	7·1	11·2	373	$C_{20}H_{27}N_3O_4$	64·3	7·3	11·3	373
(XXIV)	L-Valyl-L-prolinol	84	Gum	62·4	7·3	10·5	403	$C_{21}H_{29}N_3O_5$	62·5	7·3	10·4	403

TABLE 2

Carbamoyl-hydroxamic acids

RNH·CO·CH_2·CH_2·CO·NH·OH

Compound	Base residue RNH– derived from	Yield (%)	Found (%) C	H	N	*M*	Formula	Required (%) C	H	N	*M*
(XXV)	L-Alanylpyrrolidine	88 (gum)				257	$C_{11}H_{19}N_3O_4$				257
(XXV)	DL-Alanylpyrrolidine	85 (m.p. 76°)	51·3	7·8	16·1	257	$C_{11}H_{19}N_3O_4$	51·4	7·5	16·4	257
(XXVI)	L-Valylpyrrolidine	91 (gum)			14·8	285	$C_{13}H_{23}N_3O_4$			14·7	285
(XXVII)	L-Valyl-L-prolinol	93 (gum)	52·9	7·8		315	$C_{14}H_{25}N_3O_5$	53·2	7·7		315

1-(N-*Benzyloxycarbonyl*-L-*alanyl*)*pyrrolidine* (XVIII) had m.p. 131° [from ether–light petroleum (b.p. 40—60°)], $[\alpha]_D^{25}$ −1·1 (*c* 0·1 in $CHCl_3$) (Found: C, 65·4; H, 7·4; N, 10·1%; M^+, 276. $C_{15}H_{20}N_2O_3$ requires C, 65·2; H, 7·3; N, 10·1%; *M*, 276).

1-(N-*Benzyloxycarbonyl*-DL-*alanyl*)*pyrrolidine* (XVIII) had m.p. 110° [from ether–light petroleum (b.p. 40—60°)] (Found: C, 65·6; H, 7·4; N, 10·4%; M^+, 276. $C_{15}H_{20}N_2O_3$ requires C, 65·2; H, 7·3; N, 10·1%; *M*, 276).

The preparation of 1-(*N*-benzyloxycarbonyl-L-valyl)-pyrrolidine (XIX) is described in Part III.[23]

Aminoacylpyrrolidines (XX) *and* (XXI).—Catalytic hydrogenation of the preceding (*N*-benzyloxycarbonyl-aminoacyl)pyrrolidines was carried out by the method described above for the synthesis of L-valyl-L-prolinol (III).

1-(L-Alanyl)pyrrolidine (XX) was an oil, *m/e* 142 (M^+,

[22] (*a*) M. Goodman and K. C. Stueben, *J. Amer. Chem. Soc.*, 1959, **81**, 3980; (*b*) Th. Wieland and B. Heinke, *Annalen*, 1958, **615**, 184; (*c*) B. Iselin, W. Rittel, P. Sieber, and R. Schwyzer, *Helv. Chim. Acta*, 1957, **40**, 373.

[23] Part III, J. P. Devlin, W. D. Ollis, J. E. Thorpe, R. J. Wood, B. J. Broughton, P. J. Warren, K. R. H. Wooldridge, and D. E. Wright, following paper.

$C_7H_{14}NO$), characterised as its N-2,4-*dinitrophenyl derivative*, yellow needles, m.p. 153° (from ethanol) (Found: C, 50·6; H, 5·2; N, 18·2%; M^+, 308. $C_{13}H_{16}N_4O_5$ requires C, 50·6; H, 5·2; N, 18·2%; *M*, 308).

1-(DL-*Alanyl*)*pyrrolidine* (XX) was an oil, *m/e* 142 (M^+, $C_7H_{14}NO$), characterised as its N-2,4-*dinitrophenyl derivative*, m.p. 203° (from ethanol) (Found: C, 50·8; H, 5·5; N, 18·5%; M^+, 308. $C_{13}H_{16}N_4O_5$ requires C, 50·6; H, 5·2; N, 18·2%; *M*, 308).

The preparation of 1-(L-valyl)pyrrolidine and its 2,4-dinitrophenyl derivative are given in Part III.[23]

O-*Benzyldidehydro-compounds* (XXII)—(XXIV).—These compounds were prepared by heating under reflux equimolecular amounts of the amine (RNH_2) and *N*-benzyloxyisomaleimide (VI) in chloroform (12 h), followed by evaporation of solvent and purification by column chromatography on silica. Yields and analytical data are given in Table 1.

Carbamoyl-hydroxamic Acids (XXV)—(XXVII).—The *O*-benzyldidehydro-compounds (XXII)—(XXIV) were hydrogenated in the same way as *O*-benzyldidehydroactinonin (XVII). After removal of the catalyst and the solvent, the product was purified by chromatography on a polyamide column (elution with ethanol). The yields and analytical data are given in Table 2.

We gratefully acknowledge the award of an S.R.C. Studentship (to N. H. A.) and two Research Fellowships (to J. E. T. and A. D. W.) supported by the National Research Development Corporation.

[4/1142 *Received*, 12*th June*, 1974]