Synthesis and Reactions of α-Thioformyl Dipeptides, Possible Biogenetic Precursors of Penicillin

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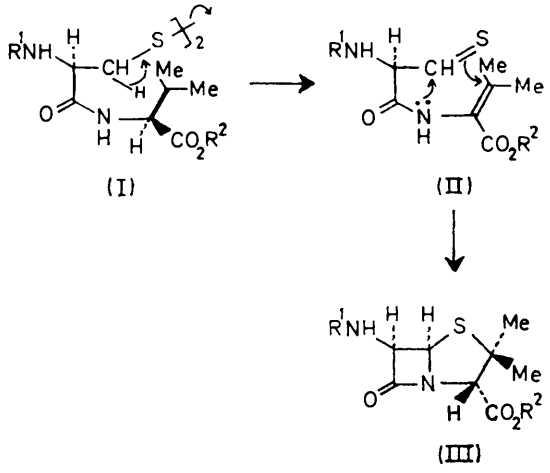
Thioformyl dipeptides (II) have been synthesised by various routes and the existence of these unstable compounds has been verified by trapping experiments. At room temperature the thioaldehydes polymerise readily and at lower temperatures the thioenol forms are stabilised. When thioenolisation is prevented by the presence of an α-methyl substituent as in (XX), polymerisation is the only observable reaction. Cyclisation to β-lactam derivatives on a preparative scale has not been observed.

THE antibiotic penicillin (III) is known to be derived in nature from the amino-acids L-cystine [1] and L-valine,[1,2] and the α-hydrogen atom and one of the β-hydrogen atoms in cystine are retained when this amino-acid is incorporated into penicillin.[3] Various larger precursors have been fed to *Penicillium chrysogenum* but there is no positive experimental evidence on the later stages of penicillin biosynthesis. The finding [4] of 5-amino-5-carboxypentanoylcysteinyl-valine in the mycelium of *P. chrysogenum* implies that a dipeptide is formed from cystine and valine and the change in stereochemistry of the valine unit, which is D in penicillin, suggests the intermediacy of a didehydrovaline unit, since didehydro-amino-acids are thought to be intermediates in the biosynthesis of peptide antibiotics containing D-amino-acid residues.[5] Arnstein [3,6] has suggested a mechanism for the biosynthesis of penicillin in which the peptide intermediate achieves the oxidation level of penicillin by successive oxidation to a thioaldehyde and dehydrogenation to a didehydrovaline peptide. This scheme might also involve development of the thioaldehyde functionality by heterolytic cleavage of a disulphide bond [(I) ⟶ (II)] as in Scheme 1. The thioaldehyde mechanism has been criticised by Birch,[7] who acknowledged that the thioaldehyde would be expected to be very electrophilic, but felt that the amide nitrogen atom would not be nucleophilic enough to allow cyclisation. There are, however, many instances of amide nitrogen atoms acting as nucleophilic centres.[8]

In order to test the chemical feasibility of the thioaldehyde mechanism, we determined to synthesise the thioaldehydes (II), which we anticipated would be unstable and which we hoped would serve as substrates for cyclisation to the penicillins (III). Since there have been reports [9] of heterolytic cleavage of a disulphide bond to yield an unstable thioaldehyde and a thiol, generation of the thioaldehyde (II) from the disulphide (I) by photolysis, pyrolysis, or treatment with base [10] seemed especially attractive, since this reaction might mimic a second step in the biosynthetic sequence.



SCHEME 1

An attractive potential synthesis of dipeptides of didehydrovaline would involve condensation of primary amides with dimethylpyruvic acid. This condensation has been achieved by Ziegler [8b] using acetamide as the primary amide, but we were not successful in applying the method with the more complex primary amides necessary for our synthesis. We were, therefore, obliged to examine peptide condensations using methyl α-amino-ββ-dimethylacrylate [11] (IV) (methyl didehydrovalinate). This didehydro-amino-ester proved to have the enamine structure (IV) although the hydro-

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[1] H. R. V. Arnstein and P. T. Grant, *Biochem. J.*, 1954, **57**, 353; H. R. V. Arnstein and P. T. Grant, *ibid.*, p. 360; C. M. Stevens, P. Vohra, E. Inamine, and D. A. Roholt, *J. Biol. Chem.*, 1953, **205**, 1001.

[2] C. M. Stevens P. Vohra and C. W. DeLong *J. Biol. Chem.*, 1954, **211**, 297; H. R. V. Arnstein and M. E. Clubb, *Biochem. J.*, 1957, **65**, 618; H. R. V. Arnstein and M. Margreiter, *ibid.*, 1958, **68**, 339.

[3] H. R. V. Arnstein and J. C. Crawhall, *Biochem. J.*, 1957, **67**, 180.

[4] H. R. V. Arnstein, D. Morris, and E. J. Toms, *Biochim. Biophys. Acta*, 1959, **35**, 561; H. R. V. Arnstein, M. Artman, D. Morris, and E. J. Toms, *Biochem. J.*, 1960, **76**, 353; H. R. V. Arnstein and D. Morris, *ibid.*, p. 357.

[5] B. W. Bycroft, *Nature*, 1969, **224**, 595.

[6] H. R. V. Arnstein, *Ann. Reports*, 1957, **54**, 339,

[7] A. J. Birch and H. Smith, 'Amino Acids and Peptides with Antimetabolic Activity, Ciba Foundation Symposium,' eds. G. E. W. Wolstenholme and C. M. O'Connor, Little Brown, Boston 1958 p. 247.

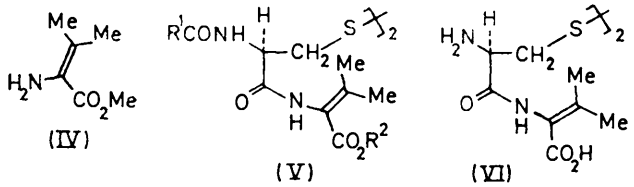
[8] See for example (*a*) H. E. Baumgarten J. F. Fuerholzer, R. D. Clark, and R. D. Thompson, *J. Amer. Chem. Soc.*, 1963, **85**, 3303; (*b*) T. Wieland, G. Ohnacker and W. Ziegler, *Chem. Ber.*, 1957, **90**, 194; (*c*) H. Böhme, A. Dick, and G. Driesen, *ibid.* 1961, **94**, 1879.

[9] A. Schoberl and H. Grafje, *Naturwiss.*, 1956, **43**, 445.

[10] See for example J. A. R. Coope and W. A. Bryce, *Canad. J. Chem.*, 1954, **32**, 768; K. J. Rosengren, *Acta Chem. Scand.*, 1962, **16**, 1401; A. Schoberl and H. Eck, *Annalen*, 1936, **522**, 97; A. J. Parker and N. Kharasch, *Chem. Rev.*, 1959, **59**, 583.

[11] C. Shin, M. Masaki, and M. Ohta, *J. Org. Chem.*, 1967, **32**, 1860.

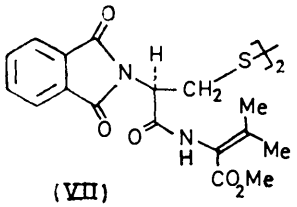
chloride appeared from the n.m.r. spectrum [12] to be mainly either the olefin–hydrogen chloride adduct or the imine. Direct synthesis of the disulphide 'precursor' (V; $R^1 = PhCH_2$, $R^2 = H$) of penicillin G proved



(IV)

(V)

(VI)



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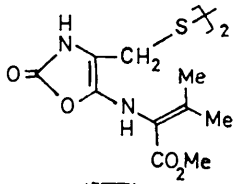

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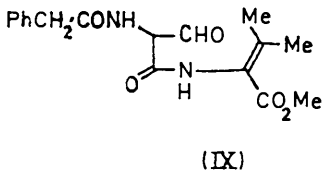

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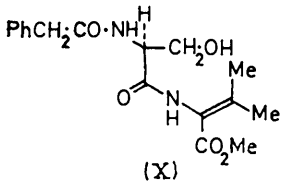
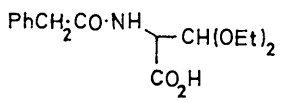
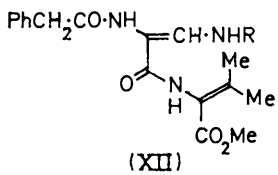


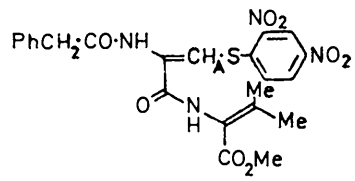
(X)



(XI)



(XII)



(XIII)

difficult, but both this compound and the 'precursor' (V; $R^1 = PhO{\cdot}CH_2$, $R^2 = H$) of penicillin V could readily be made by first synthesising the trifluoroacetyl dipeptide (V; $R^1 = CF_3$, $R^2 = Me$) by condensation of *NN'*-bistrifluoroacetyl-L-cystyl chloride [13] with methyl didehydrovalinate (IV). Hydrolysis of this compound in base yielded the amino-acid (VI), which reacted with phenylacetyl chloride to give (V; $R^1 = PhCH_2$, $R^2 = H$) and with phenoxyacetyl chloride to give (V; $R^1 = PhO{\cdot}CH_2$, $R^2 = H$). Methyl esters of the acids could be made by reaction with diazomethane.

The benzyloxycarbonyl dipeptide (V; $R^1 = PhCH_2{\cdot}O$, $R^2 = Me$) and the phthaloyl dipeptide (VII) could be made by direct condensation of methyl didehydrovalinate (IV) with the acid chlorides of *NN'*-bisbenzyloxycarbonyl-L-cystine [14] and *NN'*-bisphthaloyl-L-cystine,[15] respectively.

Treatment with base and pyrolysis of the various disulphides were equally unsuccessful in generating useful products, although pyrolysis of the bisbenzyloxycarbonyl dipeptide (V; $R^1 = PhCH_2{\cdot}O$, $R^2 = Me$) in refluxing dimethyl sulphoxide, did yield one product with a carbonyl absorption in the β-lactam region of the i.r. spectrum. It was evident from the n.m.r. spectrum, however, that the benzyloxycarbonyl group was no longer present, and since no other acyl dipeptide gave a similar product, and the mass spectrum of the compound did not show the breakdown pattern associated with β-lactams,[16] the most likely formulation for the compound appeared to be (VIII), which would arise by loss of benzyl alcohol from (V; $R^1 = PhCH_2{\cdot}O$, $R^2 = Me$) to yield an intermediate isocyanate.

When we investigated the photolysis of the various dipeptides under a large variety of experimental conditions, we were encouraged by the presence in some of the reaction mixtures of a material having antibacterial properties which could be negated by application of penicillinase. The results of this very sensitive test were not always reproducible, however, and when we synthesised the dipeptide (V; $R^1 = PhCH_2$, $R^2 = H$) from phenyl[1-^{14}C]acetic acid and, after photolysis, diluted the mixture with penicillin G, crystallisation of the *N*-ethylpiperidinium salt to constant activity showed that yields were extremely low and an exhaustive search for conditions which would yield isolable amounts of penicillin G proved fruitless.

Thus, attractive as disulphide heterolysis might be as a possible biogenetic model, there was little evidence for the formation of synthetically useful amounts of thioaldehyde from it. When we began our work, the only known monomeric thioaldehydes, pyrrole thioaldehydes, had been prepared from aldehyde imines or enamine salts,[17] and so the aldehyde (IX) seemed to be a suitable starting material for the preparation of the corresponding thioaldehyde. The seryl dipeptide (X) was synthesised by condensation of *N*-phenylacetylserine [18] with methyl didehydrovalinate (IV), but the yield was small, and oxidation to the aldehyde (IX) proved difficult. The diethyl acetal of the aldehyde (IX) was prepared by low-temperature condensation of the mixed anhydride of benzylpenaldic acid diethyl acetal (XI) [19a] and ethyl chloroformate with methyl

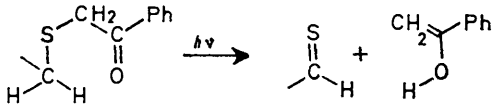
[12] For early speculation on this tautomeric equilibrium see E. P. Kohler and N. L. Drake, *J. Amer. Chem. Soc.*, 1923, **45**, 2381.
[13] F. Weygand and R. Geiger, *Chem. Ber.*, 1956, **89**, 647.
[14] F. W. Holly, E. W. Peel, E. L. Luz, and K. Folkers, *J. Amer. Chem. Soc.*, 1952, **74**, 4539.
[15] G. H. L. Nefkins, G. I. Tesser, and R. J. F. Nivard, *Rec. Trav. Chim.*, 1960, **79**, 688.
[16] P. V. DeMarco and R. Nagarajan in 'Cephalosporins and Penicillins,' ed. E. H. Flynn, Academic Press, New York, 1972, p. 320.
[17] (*a*) R. B. Woodward, W. A. Ayer, J. M. Beaton, F. Bickelhaupt, R. Bonnett, P. Buchschacher, G. L. Closs, H. Dutler, J. Hannah, F. P. Hauk, S. Ito, A. Langemann, E. LeGoff, W. Leimgruber, W. Lwowski, J. Sauer, Z. Valenta, and H. Volz, *J. Amer. Chem. Soc.*, 1960, **82**, 3800; (*b*) S. McKenzie and D. H. Reid, *Chem. Comm.*, 1966, 401; *J. Chem. Soc.* (*C*), 1970, 145.
[18] W. Baker and W. D. Ollis, *J. Chem. Soc.*, 1951, 446.
[19] 'The Chemistry of Penicillin,' eds. H. T. Clarke, J. R. Johnson, and R. Robinson, Princeton University Press, Princeton, 1949. (*a*) p. 506; (*b*) p. 507; (*c*) p. 510.

didehydrovalinate (IV), and the unstable aldehyde could be generated from this compound by hydrolysis as it was required.

The next step towards synthesis of a thioaldehyde was formation of an imine or an enamine salt. Reaction of the aldehyde (IX) with ethylamine gave the enamine (XII; R = Et) which was shown to be the enamine tautomer by the u.v. spectrum, and which, according to the n.m.r. spectrum, was a mixture of the two possible geometrical isomers. These isomers could not be separated. In the hope of generating an imine by addition of a conjugating group to the amine, the aldehyde (IX) was treated both with aniline and with urea,* but the spectra of the products showed them to be enamines (XII; R = Ph or CO·NH_2), and not imines. Both enamines existed as geometric isomers which could be separated chromatographically. The predominant isomer in each case was the one which absorbed at the lower wavelength in the u.v. spectrum and so presumably was the *trans*-isomer.[21] The individual geometrically isomeric *N*-phenyl enamines (XII; R = Ph) slowly reverted to the original mixture.

In neutral solution, the enamine dipeptides (XII) were stable to hydrogen sulphide, but when a small amount of acid was present, reaction occurred. When hydrogen sulphide was passed into a solution of the *N*-ethyl enamine (XII; R = Et) containing 1 equiv. of hydrogen chloride, a product was obtained with no u.v. absorption, but which developed absorption at 315 nm on addition of alkali. In the hope that this absorption denoted the presence of an enethiolate, the product was treated with sodium methoxide in methanol followed by 1-chloro-2,4-dinitrobenzene, to yield 2,4-dinitroanisole and two isomeric compounds $C_{23}H_{22}N_4O_8S$ which were evidently the geometrically isomeric unsaturated thioethers (XIII). One of these ($\lambda_{max.}$ 280 and 368 nm) was identical with a sample prepared from the tetrahydrothiazepine (XVI) by Leonard's method,[22] and the other ($\lambda_{max.}$ 268 and 354 nm) differed in the n.m.r. spectrum mainly in the position of the signal due to the olefinic proton H_A. There was no evidence for β-lactam products in any quantity from any of the reactions used to generate the thioaldehyde (II; R^1 = $PhCH_2$·CO, R^2 = CH_3) from the enamines (XII) or directly from the aldehyde (IX).

The foregoing trapping experiment showed that the thioaldehyde (II; R^1 = $PhCH_2$·CO, R^2 = Me) can be synthesised, but the method of synthesis would not necessarily produce this as the thioaldehyde rather than the thioenol tautomer. In 1970 Woodward[23] announced the synthesis of a thioaldehyde by the use of a Norrish type II photolysis as outlined in Scheme 2. This method must lead, in the first instance, to the thioaldehyde tautomer and so it seemed appropriate to synthesise a dipeptide thioether (XIV) which should yield the required thioaldehydes (II) on photolysis.



SCHEME 2

Synthesis of the peptide (XIV; R^1 = $PhCH_2$·CO, R^2 = H) by reduction of the disulphide (V; R^1 = $PhCH_2$, R^2 = H) and reaction of the resultant product with a phenacyl halide proved very difficult to achieve in good yield, and a more lengthy procedure was adopted. Reduction of *NN'*-bisphenylacetyl-L-cystine with sodium in liquid ammonia followed by *in situ* reaction of the resultant thiolate with phenacyl chloride gave *N*-phenylacetyl-*S*-phenacyl-L-cysteine. This could be condensed with methyl didehydrovalinate (IV) in good yield to give the desired peptide (XIV; R^1 = $PhCH_2$·CO, R^2 = Me). The peptide (XIV; R^1 = Bu^tO_2C, R^2 = Me) was obtained in a similar manner from *NN'*-bis-t-butoxycarbonyl-L-cystine,[24] and (XIV; R^1 = phthaloyl, R^2 = Me) was obtained in small yield by reduction of the disulphide (VII) followed by *in situ* reaction with phenacyl chloride.

Photolysis of the peptide (XIV; R^1 = $PhCH_2$·CO, R^2 = Me) at room temperature under a variety of conditions yielded acetophenone and oligomeric material with spectral properties similar to those reported by Leonard[22] for the polythioaldehyde (XV; R = H) derived from treatment of the tetrahydrothiazepine (XVI) with base followed by acidification of the resultant sodium salt. Photolysis in pyridine yielded five oligomers which, according to their t.l.c. behaviour, were probably not homologous, but which had molecular weights compatible with structures (XV; R = H, *n* = 2—4, 8, or 9). Photolysis in benzene–methanol seemed to yield only one oligomer (XV; R = H, *n* = 10), presumably the polythioaldehyde reported by Leonard.[22]

When photolysis was conducted at low temperatures, samples showed a weak absorption at *ca.* 300 nm, shifting to 335 nm on addition of base. The 335 nm absorption was irreversibly removed on acidification and the 300 nm absorption slowly decreased when samples were allowed to warm to room temperature. The absorption at 335 nm is close to that reported by Leonard for the thioenolate (XVII) (330 nm), and we were able to prove the presence of the thioenolate in our reaction by adding sodium methoxide to the product of a reaction run in pyridine at −30 °C. Subsequent addition of 1-chloro-2,4-dinitrobenzene gave a mixture of 2,4-dinitroanisole and the two geometrically isomeric thioethers (XIII). Photolysis in sodium methoxide–methanol at −78 °C followed by addition of benzyl bromide to the reaction mixture yielded the thioether

* The ureide of ethyl benzylpenaldate has been shown to exist as either the imine or the enamine tautomeric form depending on the solvent used in the crystallisation.[20]

[20] B. W. Bycroft, D. Cameron, A. Hassanali-Walji, and A. W. Johnson, *Tetrahedron Letters*, 1969, 2539.

[21] See H. P. Schad, *Helv. Chim. Acta*, 1955, **38**, 1117.

[22] N. J. Leonard and R. Y. Ning, *J. Org. Chem.*, 1967, **32**, 677.

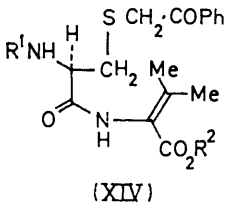
[23] R. B. Woodward, Hanbury Memorial Lecture of the Royal Pharmaceutical Society 1970; *cf.* K. Heusler in ref. 16, p. 274.

[24] E. Schnabel, *Annalen*, 1967, **702**, 188.

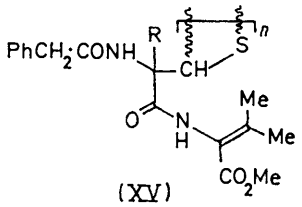
(XVIII), $\lambda_{max.}$ 299 nm, reported by Leonard,[22] and its geometrical isomer, $\lambda_{max.}$ 285 nm.

We have thus succeeded in synthesis of the thioaldehyde (II; R^1 = $PhCH_2$·CO, R^2 = Me), and the mode of synthesis requires that this be generated as the thioaldehyde tautomer. There was, however, no evidence of any β-lactam products from the many photolysis reactions conducted using the peptides (XIV; R^1 = $PhCH_2$·CO, R^2 = Me), (XIV, R = Bu^tO_2C, R^2 = Me), and (XIV; R^1 = phthaloyl, R^2 = Me) as substrates. Since the major reaction of the thioaldehyde at low temperatures seemed to be tautomerism to the thioenol, replacement of the hydrogen atom α to the thioaldehyde by a protecting group to prevent enolisation might allow the desired condensation to a β-lactam. Reiner and Zeller [25] have successfully and reversibly blocked C-6 of 6-aminopenicillanic acid by treatment with formaldehyde, but in view of the lability of the protecting group it was felt that the possibility of cyclisation could best be tested by use of a methyl substituent, and synthesis of the peptide (XIX) was attempted.

N-Phenylacetyl-*S*-benzyl-α-methylcysteine was readily prepared from *S*-benzyl-α-methylcysteine,[26] and reduction with sodium in liquid ammonia followed by reaction *in situ* with phenacyl chloride yielded *N*-phenylacetyl-*S*-phenacyl-α-methylcysteine (XXI). It is well



(XIV)



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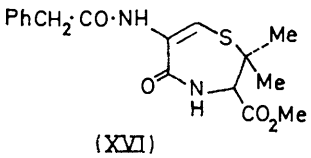

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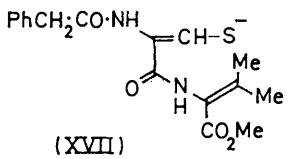
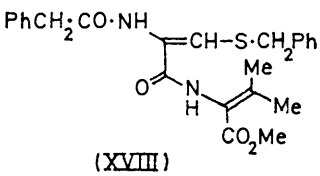


(XVII)



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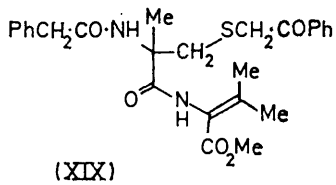
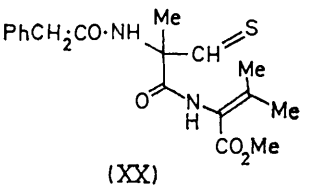
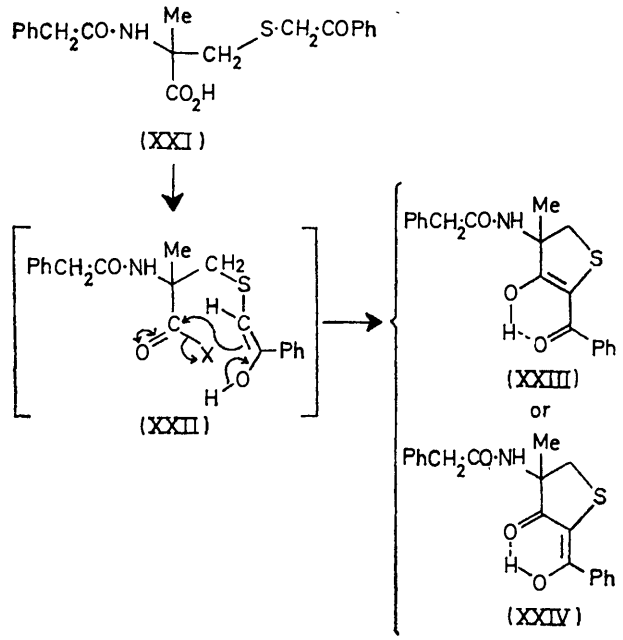


(XIX)



(XX)

known [27] that steric factors in α-methyl amino-acids make peptide condensation difficult, and most of our attempts to achieve condensation of *N*-phenylacetyl-*S*-phenacyl-α-methylcysteine (XXI) with methyl didehydrovalinate (IV) were unsuccessful. When the



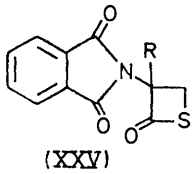
SCHEME 3

acid was activated by formation of the mixed anhydride with pivalic acid or the acid chloride, the main product was a yellow solid, $C_{20}H_{19}NO_3S$, $\lambda_{max.}$ 392 nm, with an n.m.r. spectrum compatible with the β-diketone structures (XXIII) and (XXIV). This compound formed a mixture of acetates on treatment with acetic anhydride–pyridine, and one acetate, $\lambda_{max.}$ 357 nm, could be obtained pure. The blue shift (392 ⟶ 357 nm) is expected on acetylation of conjugated enols.[28] The formation of the β-diketone from the acid (XXI) could occur as outlined in Scheme 3, and since no similar cyclisation was observed in the absence of the methyl group, this reaction may be a consequence of steric compression aiding the cyclisation and/or steric hindrance preventing attack of the weakly nucleophillic enamine (IV). It is of interest in this context that when we prepared the thiolactone (XXV; R = Me), it was extremely unreactive towards amine nucleophiles, although the thiolactone (XXV; R = H) is reported [29] to be reactive towards such reagents.

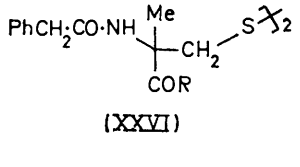
The disulphide (XXVI; R = OH) prepared from *N*-phenylacetyl-*S*-benzyl-α-methylcysteine, could be converted into the activated ester (XXVI; R = O·CH_2·CN) and, although this compound proved to be unreactive towards the enamine (IV), reaction with n-propylamine gave the corresponding amide (XXVI; R = $NHPr^n$). It therefore seemed appropriate to activate the methyl didehydrovalinate unit in the condensation reaction, and the required dipeptide (XIX) was finally

[25] R. Reiner and P. Zeller, *Helv. Chim. Acta*, 1968, **51**, 1905.
[26] H. R. V. Arnstein, *Biochem. J.*, 1958, **68**, 333.
[27] M. T. Leplawy, D. S. Jones, G. W. Kenner, and R. C. Sheppard, *Tetrahedron*, 1960, **11**, 39.
[28] A. I. Scott, 'Interpretation of the Ultra Violet Spectra of Natural Products,' Pergamon, Oxford, 1964, p. 58, Table 2.
[29] D. Fles, A. Markovac-Prpic, and V. Tomasic, *J. Amer. Chem. Soc.*, 1958, **80**, 4654.

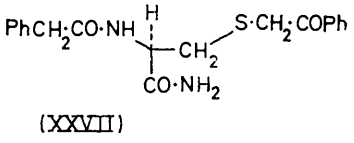
synthesised, albeit in low yield, by reaction of *N*-phenylacetyl-*S*-phenacyl-α-methylcysteine (XXI) with methyl didehydrovalinate in the presence of phosphorus trichloride. Photolysis of this compound, under a large variety of conditions, including the basic conditions used by Baumgarten [8a] to synthesise α-lactams by nucleophilic attack of an amide nitrogen atom, yielded acetophenone and polythioaldehydes as the only recognisable products.



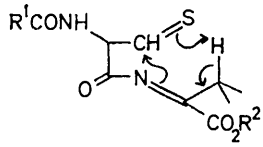
(XXV)



(XXVI)



(XXVII)



(XXVIII)

Pyrolysis of the various polythioaldehydes (XV; R = H or Me) under a variety of conditions failed to yield β-lactam products, and when the primary amide (XXVII) was prepared, photolysis again gave no useful products.

Recently Baldwin [30] has formulated an alternative scheme for penicillin biosynthesis in which thioaldehydes (XXVIII), the imine tautomers of the enamines (II) synthesised by us, are cyclised to β-lactam products by an ' ene ' reaction. Since, under the various conditions to which we have subjected our thioaldehydes, the possibility of tautomerism has been available, we may consider that we have been able to test Baldwin's mechanism *in vitro*. In our hands the *in vitro* conversion of the key intermediates in Arnstein's scheme [3,6] and in Baldwin's scheme [30] has failed to yield isolable amounts of β-lactams.

The synthesis of possible penicillin and cephalosporin precursors developed in these experiments is currently being adapted to synthesis of labelled compounds so that the mechanistic suggestions of Arnstein and Baldwin can be tested *in vivo*.

EXPERIMENTAL

M.p.s were determined on a Kofler hot-stage apparatus. I.r. spectra were recorded on Perkin-Elmer 237 and 257 instruments and u.v. spectra on a Unicam SP 800 spectrophotometer. N.m.r. spectra were recorded on Varian T60, A60, and HA 100 instruments and mass spectra on Hitachi RMU-6 and A.E.I. MS9 instruments. Optical rotations were determined on a Perkin-Elmer 141 polarimeter. Molecular weights were determined on a Hewlett–Packard 302B vapour pressure osmometer with ethyl acetate as solvent and naphthalene as standard. Radioactive compounds were counted by the liquid scintillation method using a solution of naphthalene (60 g), 2,5-diphenyloxazole (4 g), 1,4-bis-(5-phenyloxazol-2-yl)benzene (0·2 g), ethylene glycol (20 ml), and dioxan (880 ml) and a Beckmann LS-100 counter. Microanalyses were performed by Mr. and Mrs. A. G. Olney.

Methyl α-Amino-ββ-dimethylacrylate (*Methyl Didehydrovalinate*) (IV).—This compound was prepared by the method of Shin.[11] Aluminium foil was used in place of aluminium turnings and this was activated by treatment with 2N-sodium hydroxide before use. It was essential *not* to allow the amalgam to become dry since the reaction mixture could then spontaneously catch fire. No attempt was made to purify the ester, which was usually prepared as it was needed. It could be stored, for short periods only, in a refrigerator. The ester was a yellow liquid, $\nu_{max.}$ (film) 3450, 3360 (NH), 1725, and 1695 cm^{-1}, $\lambda_{max.}$ 263 nm (log ε 3·9), τ ($CDCl_3$) 8·27br (3H, s, CH_3·C=), 7·97br (3H, s, CH_3·C=), 6·53br (<2H, NH), and 6·24 (3H, s, CO_2Me).

Didehydrovaline Methyl Ester Hydrochloride.—Methyl didehydrovalinate (1·7 g) was dissolved in dry ether (200 ml) and a stream of dry hydrogen chloride was passed through the solution at room temperature for 3—4 min. The initial oily precipitate was collected by decantation and solidified on trituration with benzene. The amorphous solid was recrystallised from methanol–ether; yield 0·59 g, $\lambda_{max.}$ (MeOH) 217 nm; τ [imine or HCl adduct in $(CD_3)_2SO$] 9·05 (d, *J* 7 Hz, CH_3·CH), 6·93 (septet, *J* 7 Hz, CH), and 6·31 (CO_2Me); τ [enamine in $(CD_3)_2SO$] 8·05 (s, CH_3·C=), 7·94 (s, CH_3·C=), and 6·34 (CO_2Me). The ratio of ' imine ' to enamine as shown by integration was 4 : 1.

NN′-*Bistrifluoroacetyl-*L*-cystyl Chloride*.—*NN′*-Bistrifluoroacetyl-L-cystine [13] (3·0 g) was added to powdered phosphorus pentachloride (4·5 g) in anhydrous ether (100 ml) at room temperature. The mixture was shaken for 15 min and left for a further 45 min before evaporation to leave a solid product. This was washed thoroughly with dry petroleum (3 × 15 ml) to give the crude acid chloride (2·8 g), $\nu_{max.}$ (KBr) 1775 cm^{-1} (COCl).

Dimethyl NN′-*Bistrifluoroacetyl-*L*-cystylbisdidehydrovalinate* (V; $R^1 = CF_3$, R^2 = Me).—A solution of freshly prepared methyl didehydrovalinate (IV) (1·7 g) in anhydrous ether (3 ml) was added dropwise to a stirred solution of the crude acid chloride from the foregoing reaction (3·0 g) in anhydrous ether (100 ml). Triethylamine (1·2 ml) was added and the suspension was stirred overnight at room temperature and filtered. The solid product was washed with water and dried *in vacuo*. Recrystallisation from methanol yielded a white *solid* (3·09 g), m.p. 254—255° (decomp.), $[\alpha]_D^{23}$ −125° (MeOH) (Found: C, 40·35; H, 4·15; N, 8·7. $C_{22}H_{28}F_6N_4O_8S_2$ requires C, 40·35; H, 4·38; N, 8·55%), *m/e* 654 (parent), $\nu_{max.}$ (KBr) 3300 (NH), 1725 (ester), 1710, and 1665 cm^{-1} (amide), τ [$(CD_3)_2SO$] 8·21 (6H, s, CH_3·C=), 7·95 (6H, s, CH_3·C=), 6·82 (4H, m, CH_2S), 6·39 (6H, s, CO_2Me), 5·30 (2H, m, CH), and 0·5—0·3 (NH).

L-*Cystylbisdidehydrovaline* (VI).—Methyl *NN′*-bistrifluoroacetyl-L-cystylbisdidehydrovalinate (1·725 g) was stirred vigorously with N-sodium hydroxide (30 ml) for 2 h. The solution was washed with chloroform, neutralised to pH 2 with 6N-hydrochloric acid, and again washed with chloroform. The aqueous solution was evaporated to dryness and the white solid residue was extracted with methanol. The extract was filtered and evaporated to yield an oil which slowly crystallised, m.p. 250—253° (from methanol), $[\alpha]_D^{23}$ −43·5° (MeOH) $\nu_{max.}$ (Nujol) 1690 cm^{-1}

[30] J. E. Baldwin, S. B. Haber, and J. Kitchin, *J.C.S. Chem. Comm.*, 1973, 790.

(CO_2H), τ (D_2O) 8·17 (6H, s, CH_3·C=), 7·96 (6H, s, CH_3·C=), 6·55 (4H, m, CH_2·S), and 5·7 (2H, m, CH).

NN′-*Bisphenylacetyl*-L-*cystylbisdidehydrovaline* (V; R^1 = $PhCH_2$, R^2 = H).—Crude L-cystylbisdidehydrovaline (1·77 g) obtained from evaporation of the foregoing aqueous hydrolysis mixture was dissolved in 0·5N-sodium hydroxide (50 ml) and cooled to 0 °C. Phenylacetyl chloride (3 ml) was added dropwise with stirring and more 0·5N-sodium hydroxide (50 ml) was added so that the solution remained alkaline throughout. The solution was stirred at 0 °C for 30 min and then washed with ether, acidified to pH 1, and left at 4 °C overnight. The precipitate was filtered off and washed with ether. Recrystallisation from methanol gave a *solid* (1·05 g), m.p. 206—208°, $[\alpha]_D^{22}$ −42° (MeOH) (Found: C, 57·1; H, 5·75; N, 8·3. $C_{32}H_{38}N_4O_8S_2$ requires C, 57·3; H, 5·7; N, 8·35%), $\nu_{max.}$ (Nujol) 1715 (acid) and 1645 cm^{-1} (amide), τ [$(CD_3)_2SO$] 8·31 (6H, s, CH_3·C=), 7·99 (6H, s, CH_3·C=), 6·94 (4H, m, CH_2·S), 6·47 (4H, s, $PhCH_2$·CO), 5·37 (2H, m, CH), 2·72 (10H, s, Ph), and 1·7br (2H, d, *J* 8 Hz, NH).

Dimethyl NN′-*Bisphenylacetyl*-L-*cystylbisdidehydrovalinate* (V; R^1 = $PhCH_2$, R^2 = Me).—*NN′*-Bisphenylacetyl-L-cystylbisdidehydrovaline (10 mg) was dissolved in methanol (3 ml) and treated with ethereal diazomethane until no further effervescence was observed and the solution turned pale yellow. The solution was left for 30 min at room temperature and an oily solid was precipitated. The excess of diazomethane was destroyed with glacial acetic acid and the solution was filtered. Recrystallisation of the resultant solid from methanol gave the *diester* (8 mg), m.p. 225—228° (decomp.) (Found: C, 58·25; H, 6·3; N, 8·2. $C_{34}H_{42}N_4O_8S_2$ requires C, 58·5; H, 6·0; N, 8·05%, τ [$(CD_3)_2SO$] 8·22 (6H, s, CH_3·C=), 7·92 (6H, s, CH_3·C=), 6·8 (4H, m, CH_2·S), 6·38 (s) and 6·36 (s) (10H, CO_2Me and $PhCH_2$), 5·2 (2H, m, C*H*), 2·64 (10H, aromatic), and 1·54 and 1·05 (NH).

NN′-*Bisphenoxyacetyl*-L-*cystylbisdidehydrovaline* (V; R^1 = PhO·CH_2, R^2 = H).—This was prepared from L-cystylbisdidehydrovaline (VI) and phenoxyacetyl chloride by the procedure described for the preparation of *NN′*-bisphenylacetyl-L-cystylbisdidehydrovaline. The yield of *product* from the trifluoroacetate (V; R^1 = CF_3, R^2 = H) was 55%; m.p. 230° (decomp.), $[\alpha]_D^{23}$ −319° (MeOH) (Found: C, 53·95; H, 5·25; N, 7·6. $C_{32}H_{38}N_4O_{10}S_2$ requires C, 54·7; H, 5·4; N, 8·0%), $\nu_{max.}$ (Nujol) 1690 (CO_2H), 1650, and 1630 cm^{-1} (amide), τ [$(CD_3)_2SO$], 8·16 (6H, s, CH_3·C=), 7·86 (6H, s, CH_3·C=), 6·7 (4H, m, CH_2·S), 5·32 (4H, s, O·CH_2·CO), 5·12 (2H, s, CH), 2·6—3·0 (10H, m, aromatic), and 1·55 and 0·80 (NH).

Dimethyl NN′-*Bisbenzyloxycarbonyl*-L-*cystylbisdidehydrovalinate* (V; R^1 = $PhCH_2O$, R^2 = Me).—A solution of methyl didehydrovalinate (1·54 g) in anhydrous ether (3 ml) was added dropwise to a stirred suspension of *NN′*-bisbenzyloxycarbonyl-L-cystyl chloride [14] (1·54 g) in anhydrous ether (20 ml) at room temperature. Triethylamine (distilled from acetic anhydride; 0·6 ml) was added and the mixture was stirred overnight at room temperature. The precipitate was filtered off and washed well with ether and water. Recrystallisation from methanol gave white *crystals* (1·19 g), m.p. 220—221° (Found: C, 55·35; H, 5·9; N, 7·7. $C_{34}H_{42}N_4S_2O_{10}$ requires C, 55·7; H, 5·75; N, 7·65%) $\nu_{max.}$ (KBr) 1722 (ester), 1690, and 1660 cm^{-1} (amide), τ ($CDCl_3$) 8·17 (6H, s, CH_3·C=), 7·87 (6H, s, CH_3·C=), 6·96 (4H, m, CH_2·S), 6·28 (6H, s, CO_2Me) 4·90 (4H, s, $PhCH_2$·O), 4·15 (2H), and 2·65 (aromatic, s)

Dimethyl NN′-*Bisphthaloyl*-L-*cystylbisdidehydrovalinate* (VII).—*NN′*-Bisphthaloyl-L-cystine [15] (9 g) was added to thionyl chloride (100 ml) and the mixture was heated under reflux for 30 min. The excess of thionyl chloride was removed *in vacuo* to leave a yellow oil which was dissolved in chloroform (150 ml). Methyl didehydrovalinate (6·6 g) and triethylamine (6·0 ml) were added to this solution, which was then stirred overnight and washed with saturated aqueous sodium hydrogen carbonate, N-hydrochloric acid, and water, and dried ($MgSO_4$). Removal of the solvent *in vacuo* gave a pale yellow oil which solidified on trituration with ether. The *solid* was recrystallised from methanol; yield 8·1 g, m.p. 105—106°, $[\alpha]_D^{23}$ −159° (MeOH) (Found: C, 56·4; H, 5·0; N, 7·85. $C_{34}H_{34}N_4O_{10}S_2$ requires C, 56·5; H, 4·7; N, 7·75%), $\lambda_{max.}$ (MeOH) 295 nm (log ε 3·53), $\nu_{max.}$ (Nujol) 1780 (phthaloyl), 1725 (ester), and 1660 cm (amide), τ ($CDCl_3$) 8·15 (6H, s, CH_3·C=), 7·87 (6H, s, CH_3·C=), 6·34 and 6·25 (10H, 2 × s, CH_2·S and CO_2Me), 4·7br (2H, t, *J* 8 Hz), and 2·17 and 2·05 (8H, aromatic).

Pyrolysis of the Dipeptide (V; R^1 = $PhCH_2$·O, R^2 = Me).—Dimethyl *NN′*-bisbenzyloxycarbonyl-L-cystylbisdidehydrovalinate (V; R^1 = $PhCH_2$·O, R^2 = Me) (200 mg) was dissolved in dimethyl sulphoxide (50 ml) and benzene (50 ml) and refluxed for a total of 11 h. Removal of the solvent *in vacuo* gave an oil, $\nu_{max.}$ (film) 1775 cm^{-1}, which was subjected to t.l.c. on silica gel with ethyl acetate–chloroform (1 : 1) as eluant. One product appeared as an oily solid, $\nu_{max.}$ ($CHCl_3$) 1784 and 1725 cm^{-1}, τ ($CDCl_3$) 8·12 (s, CH_3·C=), 7·62 (s, CH_3·C=), 7·53 (s, CH_2·S?), and 6·25 (s, CO_2Me). The oxazolone structure (VIII) was tentatively assigned to this compound.

NN′-*Bisphenyl*[1-^{14}C]*acetyl*-L-*cystylbisdidehydrovaline*.—Phenyl[1-^{14}C]acetic acid (0·25 mCi; Radiochemical Centre, Amersham), was made up to 800 mg with unlabelled acid, the mixture was dissolved in dry benzene (50 ml), and thionyl chloride (10 ml) was added. The solution was stirred at room temperature for 18 h and the solvent was removed *in vacuo* to yield a brown liquid, $\nu_{max.}$ (film) 1785 cm^{-1}, which was dissolved in dry dioxan (5 ml). Dimethyl *NN′*-bistrifluoroacetyl-L-cystylbisdidehydrovalinate (650 mg) was hydrolysed to L-cystylbisdidehydrovaline with N-sodium hydroxide (20 ml) during 2 h at room temperature. The pH was adjusted to 9·8 with N-hydrochloric acid and the solution was cooled to 5 °C. The dioxan solution of the crude acid chloride was added slowly, keeping the pH constant at 9·8 by addition of N-sodium hydroxide with an automatic titrimeter. Non-radioactive *NN′*-bisphenylacetyl-L-cystylbisdidehydrovaline (100 mg) was added and the solution was washed with ether (2 × 50 ml), acidified to pH 1·0 with 5N-hydrochloric acid, and left overnight at 5 °C. Ether was added and the solid product (550 mg) was washed thoroughly with ether and recrystallised to constant activity (5·83 × 10^7 disint. min^{-1} $mmol^{-1}$) from methanol (4×).

Photolysis of NN′-*Bisphenyl*[1-^{14}C]*acetyl*-L-*cystylbisdidehydrovaline*.—The labelled peptide (65 mg) was spread in a thin layer over the bottom of a Pyrex flask and irradiated with water cooling for 6 h with a Hanovia 500 W medium-pressure mercury lamp. The product was dissolved in 0·1M, pH 7·0 phosphate buffer (100 ml) containing sodium hydrogen carbonate (0·5 mg), and potassium penicillin G (40·4 mg) was added. The mixture was shaken, covered with ether (50 ml), and acidified to pH 2·8 with 10% phosphoric acid. A white precipitate of unchanged peptide

was filtered off, and the aqueous layer was extracted with ether. The combined extracts were washed with water (50 ml), dried ($MgSO_4$), and evaporated to yield an oil. This was dissolved in anhydrous acetone (10 ml), an excess of *N*-ethylpiperidine was added, and the mixture was left overnight at 0 °C. A solid salt was obtained which was crystallised to constant activity from acetone and chloroform.

Experiment 1. Weight of peptide photolysed 65 mg; initial activity of peptide (corrected) $5{\cdot}83 \times 10^7$ disint. min^{-1} $mmol^{-1}$; weight of potassium penicillin G added 40 mg.

	Background (d.p.m.) †	Activity (corrected for background) (d.p.m. mg^{-1})	Activity (corrected for background) (d.p.m. $mmol^{-1} \times 10^{-3}$)
1st crystallisation	15·5	87·1	38·9
2nd	17·2	27·0	12·1
3rd	10·7	11·3	5·04
4th	12·6	10·2	4·55
5th	12·2	10·0	4·47
6th	10·8	10·3	4·58

Apparent yield of penicillin G, $1{\cdot}76 \times 10^{-2}$%

† Disintegrations per minute

Experiment 2. Weight of peptide photolysed 18 mg; initial activity of peptide (corrected) $2{\cdot}808 \times 10^8$ disint. min^{-1} $mmol^{-1}$; weight of potassium penicillin G added 47 mg.

	Background (d.p.m.)	Activity (corrected for background) (d.p.m. mg^{-1})	Activity (corrected for background) (d.p.m. $mmol^{-1} \times 10^{-3}$)
1st crystallisation	12·4	12·0	5·36
2nd	10·8	10·0	4·47
3rd	13·2	9·0	4·02
4th	10·3	9·0	4·02
5th	9·8	9·0	4·02
6th	12·3	9·0	4·02

Apparent yield of penicillin G, $1{\cdot}34 \times 10^{-2}$%

Experiment 3. Weight of peptide photolysed 25 mg; initial activity of peptide $2{\cdot}55 \times 10^8$ disint. min^{-1} $mmol^{-1}$; weight of potassium penicillin G added 52 mg.

	Background (d.p.m.)	Activity (corrected for background) (d.p.m. mg^{-1})	Activity (corrected for background) (d.p.m. $mmol^{-1} \times 10^{-3}$)
1st crystallisation	12·2	76·5	34·10
2nd	13·3	32·6	14·55
3rd	10·7	10·2	4·55
4th	13·1	8·3	3·72
5th	9·7	8·3	3·72
6th	10·3	8·2	3·67

Apparent yield of penicillin G = $1{\cdot}08 \times 10^{-2}$%

Control experiment. The peptide (20 mg; activity $2{\cdot}808 \times 10^8$ disint. min^{-1} $mmol^{-1}$) was dissolved in saturated aqueous sodium hydrogen carbonate (10 ml) and diluted with potassium penicillin G (50 mg). The solution was acidified to pH 2·8 with N-hydrochloric acid and extracted with ether. The extracts were washed with water, dried (Na_2SO_4), and evaporated. The residue was isolated as the *N*-ethylpiperidinium salt and crystallised to constant activity.

	Background (d.p.m.)	Activity (corrected for background) (d.p.m. mg^{-1})	Activity (corrected for background) (d.p.m. $mmol^{-1} \times 10^{-3}$)
1st crystallisation	9·5	15·1	6·74
2nd	10·5	7·11	3·17
3rd	11·3	3·45	1·54
4th	10·2	1·82	0·81
5th	9·8	1·85	0·83
6th	11·6	1·91	0·85

Methyl N-*Phenylacetyl*-L-*seryldidehydrovalinate* (X).—*N*-Phenylacetyl-L-serine [18] (0·5 g) was dissolved in acetonitrile (5 ml) and didehydrovaline methyl ester (0·5 g) was added, followed by dicyclohexylcarbodi-imide (0·4 g) in acetonitrile (5 ml). The mixture was stirred at 0 °C for 4 h and filtered. The solvent was removed *in vacuo* from the filtrate and the resultant mixture was dissolved in chloroform (20 ml) and washed successively with N-hydrochloric acid, aqueous N-potassium carbonate, and water, dried (Na_2SO_4), and evaporated. The resultant solid was washed with ether and recrystallised from methanol giving *methyl* N-*phenylacetyl*-L-*seryldidehydrovalinate*, m.p. 172—175 °C, $[\alpha]_D^{23}$ −30° (MeOH) (Found: C, 61·7; H, 6·35; N, 8·5. $C_{17}H_{22}N_2O_5$ requires C, 61·1; H, 6·6; N, 8·4%), $\nu_{max.}$ (Nujol) 1720 (ester) and 1645 cm^{-1} (amide), τ ($CDCl_3$) 8·26 (3H, s, CH_3·C=), 7·90 (3H, s, CH_3·C=) 6·8 (m), 6·42 (2H, s, PhCH_2), 6·33 (3H, s, CO_2Me), 5·4 (m), 3·2 (m), and 2·78 (5H, s, aromatic).

Ethyl Benzylpenaldate.—This was made by the literature procedure [19a] as a crude oil, $\lambda_{max.}$ (OH^-) 267 nm, characterised as the dinitrophenylhydrazone, m.p. 190—192 °C (lit.,[19c] 190·5—192°) (Found: C, 53·15; H, 4·45; N, 16·35. Calc. for $C_{19}H_{19}N_5O_7$: C, 53·15; H, 4·45; N, 16·3%).

Ethyl Benzylpenaldate Diethyl Acetal.—Ethyl benzylpenaldate (crude; 26 g) was dissolved in ethanol (65 ml) and triethyl orthoformate (65 ml) and conc. hydrochloric acid (0·4 ml) were added. The mixture was refluxed until the u.v. absorption, $\lambda_{max.}$ (OH^-) 267 nm (log ε *ca.* 4·0) was negligible, and the solvents were removed *in vacuo*. The residue was dissolved in chloroform (100 ml) and washed with aqueous sodium hydrogen carbonate and with water, and dried (Na_2SO_4). Removal of the solvent yielded a brown oil (24 g) which was chromatographed on alumina (grade III neutral). Elution with benzene–petroleum (1 : 1) gave an oil, $\nu_{max.}$ (film) 1740 (ester) and 1660 cm^{-1} (amide), τ ($CDCl_3$) 9·02, 8·98, and 8·90 (9H, three overlapping triplets, *J* 7 Hz), 6·50 (s) and 6·3—6·8 (m) (6H, PhCH_2 and CH_2·CH_3), 5·94 (2H, q, *J* 7 Hz, CH_2·CH_3), 5·15—5·40 (2H, m), 3·5br (<1H, d, *J* 7·5 Hz, NH), and 2·78 (5H, s, aromatic).

Benzylpenaldic Acid Diethyl Acetal (XI).—Ethyl benzylpenaldate diethyl acetal was saponified by the procedure used for the methyl ester dimethyl acetal [19b] to yield a solid, m.p. 106—108° (lit.,[19b] 112°), $\nu_{max.}$ (Nujol) 1720 (acid) and 1674 cm^{-1} (amide), τ($CDCl_3$) 8·88br (6H, t, *J* 7 Hz, CH_3·CH_2), 6·45br (q, *J* 7 Hz, 2 × CH_2·CH_3) and 6·35 (s, PhCH_2) (total 6H), 5·2 (2H, m), 3·4br (<1H, d), 2·69 (5H, s, aromatic), and 0·07br (1H, s, NH).

Methyl Benzylpenaldyldidehydrovalinate Diethyl Acetal.—Benzylpenaldic acid diethyl acetal (1·0 g) was dissolved in toluene (10 ml) and triethylamine (0·47 ml; distilled from CaH_2), and the solution was cooled to −50 °C. Ethyl chloroformate (0·32 ml) was added and the mixture was stirred at −50 °C for 3 h. Didehydrovaline methyl ester (0·47 g) in chloroform (5 ml) was added and the

mixture was stirred at −50 °C for a further 3 h, allowed to warm to −30 °C for 1 h, and left to reach room temperature overnight. Chloroform (10 ml) was added and the solution was washed with 0·5N-hydrochloric acid, saturated aqueous sodium hydrogen carbonate, and water, dried (Na_2SO_4), and evaporated. The resulting yellow *solid* was washed with ether and recrystallised from ethanol; yield 0·4 g, m.p. 186° (Found: C, 62·1; H, 7·6; N, 6·9. $C_{21}H_{30}N_2O_6$ requires C, 62·05; H, 7·4; N, 6·9%), $\nu_{max.}$ (Nujol) 1727 (ester) and 1645 cm^{-1} (amide), τ ($CDCl_3$) 8·91 and 8·76 (6H, 2 overlapping triplets, J 7 Hz, $CH_3{\cdot}CH_2$), 8·19 (3H, s, $CH_3{\cdot}C=$), 7·86 (3H, s, $CH_3{\cdot}C=$), 6·38 (s), 6·28 (s), and 6·25 (q) (9H, CO_2Me, $PhCH_2$, and 2 × $CH_2{\cdot}CH_3$), 5·2 (2H, m), and 2·67 (5H, s, aromatic). Acidification of the hydrogen carbonate washings followed by extraction with chloroform gave unchanged benzylpenaldic acid diethyl acetal.

When the reaction was carried out at −5 °C yields were much lower and a compound thought to be 2-benzyl-4-*N*-(1-methoxycarbonyl-2,2-dimethylvinyl)aminomethylene-5(4*H*)-oxazolone was obtained as a by-product.

Methyl Benzylpenaldyldidehydrovalinate (IX).—Methyl benzylpenaldyldidehydrovalinate diethyl acetal (2·0 g) was dissolved in acetonitrile (150 ml) and heated to reflux. 0·4N-Hydrochloric acid (50 ml) was added and the solution was refluxed for 15 min, cooled for 5 min, and added to chloroform (200 ml). The chloroform layer was separated and the aqueous layer was extracted with chloroform. The combined extracts were dried (Na_2SO_4) and the solvent was removed *in vacuo* to yield a yellow gum which solidified on trituration with ether. The compound crystallised from ethanol; m.p. 100—110°, $\lambda_{max.}$ (OH^-) 280 nm (log ε 4·0), $\nu_{max.}$ ($CHCl_3$) 1720 (ester) and 1660br cm^{-1} (amide), τ ($CDCl_3$) 8·26 (3H, s, $CH_3{\cdot}C=$), 7·87 (3H, s, $CH_3{\cdot}C=$), 6·40 (s) and 6·33 (s) (*ca.* 6H, CO_2Me and $PhCH_2$), 2·70 (*ca.* 6H, aromatic), and 0·5 (<1H, s, CHO). Analytical figures for the aldehyde were poor but the *dinitrophenylhydrazone* had m.p. 230° (decomp) (Found: C, 54·1; H, 5·0; N, 16·55. $C_{23}H_{24}N_6O_8$ requires C, 53·9; H, 4·7; N, 16·4%).

Methyl β-Ethylamino-α-phenylacetamidoacryloyldidehydrovalinate (XII; R = Et).—Methyl benzylpenaldyldidehydrovalinate (0·5 g) was dissolved in chloroform (20 ml) and cooled to 10 °C. Ethylamine (2 ml) was added and an exothermic reaction ensued. After 30 min at room temperature the solvent was removed to yield a white *solid* which was recrystallised from ethanol; yield 0·22 g, m.p. 138—146° (Found: C, 63·7; H, 7·3; N, 12·15. $C_{19}H_{25}N_3O_4$ requires C, 63·5; H, 7·0; N, 11·7%), $\lambda_{max.}$ (MeOH) 286 nm (log ε 4·04), τ ($CDCl_3$) 8·94 and 8·92 (6H, 2 × t, J 7 Hz, $CH_3{\cdot}CH_2$), 8·40 and 8·08 (large singlets, $CH_3{\cdot}C=$), 8·28 and 7·96 (smaller singlets, $CH_3{\cdot}C=$), 7·0 (m), 6·50 and 6·42 (5H, $PhCH_2$ and CO_2Me), and 2·82 (5H, aromatic).

Methyl β-Anilino-α-phenylacetamidoacryloyldidehydrovalinate (XII; R = Ph).—Methyl benzylpenaldyldidehydrovalinate (500 mg) was dissolved in aniline (10 ml); the solution was left at room temperature for 6 h and warmed to 60 °C for 10 min. The aniline was removed *in vacuo* and the residue was triturated with ether to give a white *solid* (410 mg), which crystallised from ethanol; m.p. 165—175° (Found: C, 67·15; H, 6·2; N, 10·3. $C_{23}H_{25}N_3O_4$ requires C, 67·8; H, 6·2; N, 10·3%), *m/e* 407, $\lambda_{max.}$ (MeOH) 319 and 290 nm (log ε 4·15 and 3·81); $\nu_{max.}$ (Nujol) 1720 (ester), 1675, and 1660 cm^{-1} (amide), τ ($CDCl_3$) 8·26 (3H, s, $CH_3{\cdot}C=$), 7·93 (3H, s, $CH_3{\cdot}C=$), 6·28 (3H, s, CO_2Me), 6·33 (2H, s, $PhCH_2$), and 2·65—3·0 (10H, m, aromatic).

A small amount of the enamine was separated by preparative t.l.c. on silica gel with chloroform–ethyl acetate (1 : 1) as eluant. The upper spot had $\lambda_{max.}$ (MeOH) 290sh and 318 nm.

Reaction of Methyl Benzylpenaldyldidehydrovalinate (IX) *with Urea.*—Methyl benzylpenaldyldidehydrovalinate diethyl acetal (100 mg) was hydrolysed to the aldehyde by heating under reflux for 15 min in 0·1N-hydrochloric acid in acetonitrile–water (3 : 1). After the solution had cooled, urea (200 mg) was added, and the solution was left at room temperature for 48 h. The solvents were removed *in vacuo* and the residue was washed with ether to yield a white solid which crystallised from methanol; m.p. 230—235° (decomp.), $\lambda_{max.}$ (MeOH) 272 nm (log ε 4·15), $\nu_{max.}$ (Nujol) 1720 and 1665 cm^{-1}. This could be separated into the two geometrically isomeric ureides (XII; R = $CONH_2$) by preparative t.l.c. with ethyl acetate–acetic acid (20 : 1) as eluant. The upper band, $\lambda_{max.}$ (MeOH) 276 nm (log ε 4·11), $\nu_{max.}$ (Nujol) 1730, 1720 (ester), 1690, and 1665 cm^{-1} (amide), had τ (CD_3OD) 8·33 (3H, s, $CH_3{\cdot}C=$), 8·1 (5H, m, NH?), 7·98 (3H, s, $CH_3{\cdot}C=$), 6·32 (4—5H, s, $PhCH_2$ and CO_2Me), and 2·8 (5H, aromatic). The lower band, $\lambda_{max.}$ 267 nm (log ε 4·15), $\nu_{max.}$ (Nujol) 1720 (ester) and 1660 cm^{-1} (amide), had τ (CD_3OD) 8·30 (3H, s, $CH_3{\cdot}C=$), 8·0 (4H, m, NH?), 7·92 (3H, s, $CH_3{\cdot}C=$), 6·36 (3H, s, CO_2Me), 6·25 (2H, s, $PhCH_2$), and 2·67 (5H, aromatic), *m/e* 374.

Reaction of Methyl β-Ethylamino-α-phenylacetamidoacryloyldidehydrovalinate (XII; R = Et) *with Hydrogen Sulphide.*— β-Ethylamino-α-phenylacetamidoacryloyldidehydrovaline methyl ester (200 mg) was dissolved in dry benzene (500 ml), and 0·177M-hydrogen chloride in benzene (3·19 ml) was added. Hydrogen sulphide was bubbled through the solution for 30 min and the mixture was left overnight at room temperature. The solvent was removed *in vacuo* and the resultant material was dissolved in chloroform; the solution was filtered and evaporated. The gummy solid was washed with ether, the ether-insoluble portion having $\lambda_{max.}$ (OH^-) 315 nm, irreversibly destroyed with acid.

Reaction of the Enethiolate (XVII) *with 1-Chloro-2,4-dinitrobenzene.*—The product from the above reaction (from 100 mg of ester), after removal of the benzene, was dissolved in dry methanol (10 ml), and 0·29M-sodium methoxide in methanol (1·91 ml) was added. After 15 min the solvent was removed *in vacuo* and the residue was partitioned between chloroform and water. The aqueous layer was separated and evaporated *in vacuo* to yield a solid (40 mg), which was dissolved in methanol (5 ml) and treated with a small excess of 1-chloro-2,4-dinitrobenzene. The mixture was left overnight at room temperature and the solvent was removed to yield a yellow solid which was subjected to preparative t.l.c. with chloroform–ethyl acetate (1 : 1) as eluant. 2·4-Dinitroanisole, identical with an authentic specimen, and methyl β-(2,4-dinitrophenylthio)-α-phenylacetamidoacryloyldidehydrovalinate (XIII), identical with a sample prepared by the method of Leonard,[22] were obtained together with a third compound which was recrystallised from methanol; m.p. 170—171° (Found: C, 53·45; H, 4·85; N, 10·35. Calc. for $C_{23}H_{22}N_4O_8S$: C, 53·7; H, 4·3; N, 10·9%), $\lambda_{max.}$ (MeOH) 268 and 354 nm, $\nu_{max.}$ ($CHCl_3$) 1720 (ester) and 1650 cm^{-1} (amide), τ ($CDCl_3$) 8·2 (3H, s, $CH_3{\cdot}C=$), 7·91 (3H, s, $CH_3{\cdot}C=$), 6·36 (5H, s, $PhCH_2$ and CO_2Me), 2·86 (5H, aromatic), and 1·47 (1H, s, H_A).

N-*Phenylacetyl*-S-*phenacyl*-L-*cysteine*.— *NN'*-Bisphenylacetyl-L-cystine [31] (60 g) was added to liquid ammonia (1 l) and clean sodium (*ca.* 12 g) was added to the stirred solution until a permanent blue colour was obtained. Ammonium chloride was added in portions until the blue colour was just dispersed and phenacyl chloride (39·4 g) was added. The pale yellow solution was stirred for 20 min and solid ammonium chloride (20 g) was added. The solution was allowed to evaporate overnight and final traces of ammonia were removed *in vacuo*. The residue was dissolved in water and acidified to pH 1·0 with 6N-hydrochloric acid. The thick brown oil was separated and the remainder of the solution was extracted with ethyl acetate. The combined organic layers were evaporated *in vacuo* and dissolved in an excess of saturated aqueous sodium hydrogen carbonate. This solution was washed with chloroform, acidified to pH 1·0 with 6N-hydrochloric acid, and extracted with ethyl acetate. The combined extracts were washed with water and saturated aqueous sodium chloride and dried (Na_2SO_4). Removal of the solvent yielded an oil (88 g) which crystallised slowly. The solid product was triturated with ether and purification by precipitation from methanol with ether to give an off-white *solid*, m.p. 143—155° (Found: C, 65·8; H, 5·0; N, 3·6. $C_{19}H_{19}NO_4S$ requires C, 63·95; H, 5·3; N, 3·95%), *m/e* 357 (parent), $\nu_{max.}$ (Nujol) 1720 (acid), 1670 (ketone), and 1625 cm^{-1} (amide), $\lambda_{max.}$ (MeOH) 246 and 280 nm, τ ($CDCl_3$) 7·02br (2H, m, CH_2·S), 6·4 (2H, s, PhCH_2), 6·22 (2H, s, CH_2·COPh), 5·23br (1H, m, CH), and 2·0—2·8 (10H, m, aromatic). The 2,4-*dinitrophenylhydrazone* had m.p. 120—121° (from methanol) (Found: C, 54·55; H, 4·8; N, 12·65. $C_{25}H_{23}O_7N_5S,CH_3OH$ requires C, 54·85; H, 4·8; N, 12·3%).

Methyl N-*Phenylacetyl*-S-*phenacyl*-L-*cysteinyldidehydrovalinate* (XIV; R^1 = $PhCH_2$·CO, R^2 = Me).—(i) *Mixed anhydride method*. *N*-Phenylacetyl-*S*-phenacyl-L-cysteine (1·785 g) was dissolved in dry chloroform (15 ml), triethylamine (0·94 ml) was added, and the solution was cooled to −30 °C. Ethyl chloroformate (0·64 ml) was added and the solution was stirred at −30 °C for 3 h. Methyl didehydrovalinate (0·94 g) in chloroform (5 ml) was added and the solution was stirred at −30 °C for a further 6 h, allowed to warm to room temperature, washed with N-hydrochloric acid, saturated aqueous sodium hydrogen carbonate, water, and saturated aqueous sodium chloride, and dried (Na_2SO_4). The solvent was removed to yield a yellow oil (2·1 g), which afforded a solid on trituration with ether. The *product* was recrystallised from chloroform–ether; m.p. 163—164°, yield 0·98 g (42%), $[\alpha]_D$ −9·56° (*c* 2·25 in HOAc) (Found: C, 64·15; H, 6·35; N, 6·1. $C_{25}H_{28}N_2O_5S$ requires C, 64·1; H, 6·0; N, 6·0%), $\nu_{max.}$ (Nujol) 1720 (ester), 1695 (ketone), and 1635 cm^{-1} (amide), $\lambda_{max.}$ (MeOH) 241 and 275 nm (log ε 4·303 and 3·469), τ ($CDCl_3$) 8·18 (3H, s, CH_3·C=), 7·88 (3H, s, CH_3·C=), 7·10 (2H, m, CH_2·S), 6·42 (2H, s, PhCH_2), 6·33 (3H, s, CO_2Me), 5·92 (2H, s, CH_2·COPh), 5·40 (1H, m, CH), 3·10 (1H, m, NH), 2·0—2·8 (10H, m, aromatic), and 1·76br (1H, s, NH).

(ii) *Activated amine method*. *N*-Phenylacetyl-*S*-phenacyl-L-cysteine (357 mg) was dissolved in dry dioxan (5 ml) and dry benzene (5 ml). Methyl didehydrovalinate (194 mg) in benzene (5 ml) was added, followed by redistilled phosphorus trichloride (103 mg). The temperature rose to 50 °C and the solution was refluxed for 30 min and stirred at room temperature for 15 h. The solvent was removed and the residue was dissolved in ethyl acetate and washed with 2N-sodium hydroxide, N-hydrochloric acid, water, and saturated aqueous sodium chloride. The solution was dried (Na_2SO_4) and concentrated *in vacuo*; white crystals then began to appear. The mixture was cooled and filtered and the residue was washed with ether yielding a white solid (330 mg, 71%) which was recrystallised from ethyl acetate. The product had m.p. 162—165° and was identical with the product obtained by the mixed anhydride method.

N-*t-Butoxycarbonyl*-S-*phenacyl*-L-*cysteine*.— *NN'*-Bis-t-butoxycarbonyl-L-cystine [24] (1·10 g) was dissolved in liquid ammonia (120 ml) with stirring, and clean sodium metal was added until a permanent blue colour was obtained. Solid ammonium chloride was added until the blue colour was just dispersed. Phenacyl chloride (777 mg) was then added and the solution was stirred for 45 min. Ammonium chloride (300 mg) was added, the solution was stirred for 1 h, and the ammonia was allowed to evaporate overnight. The residue was dissolved in water, washed with ethyl acetate, acidified to pH 4·0 with aqueous 10% citric acid, and extracted with ethyl acetate. The extracts were washed with water, dried (Na_2SO_4) and evaporated to yield a yellow *powder* (1·2 g), which was recrystallised from ethyl acetate–diethyl ether–petroleum (b.p. 60—80°); m.p. 154—155°, $[\alpha]_D$ +39·5° (*c* 1·4 in $CHCl_3$) (Found: C, 56·65; H, 6·3; N, 4·05. $C_{16}H_{21}NO_5S$ requires C, 56·65; H, 6·25; N, 4·15%), $\nu_{max.}$ (Nujol) 1740br (acid) and 1695—1660 cm^{-1} (ketone and amide), $\lambda_{max.}$ (MeOH) 244 and 277 nm (log ε 4·08 and 3·34), τ [$(CD_3)_2SO$] 8·73 (9H, s, Bu^t), 7·22 (2H, m, CH_2·S), 6·01 (2H, s, CH_2·COPh), 5·91 (1H, m, CH), 2·10—2·51 (5H, m, aromatic), and 3·07br (1H, NH).

Methyl N-*t-Butoxycarbonyl*-S-*phenacyl*-L-*cysteinyldidehydrovalinate* (XIV; R^1 = Bu^tO_2C, R^2 = Me).—*N*-t-Butoxycarbonyl-*S*-phenacyl-L-cysteine (339 mg) was dissolved in dry methylene chloride (50 ml) and cooled to −30 °C. Triethylamine (0·14 ml) was added, the solution was stirred for 10 min, and ethyl chloroformate (109 mg) was added. Stirring was continued at −30 °C for 1 h, the solution was allowed to warm to −20 °C, and a solution of methyl didehydrovalinate (195 mg) in dry methylene chloride (15 ml) was added. The solution was stirred for a further 1 h at −20 °C and left overnight at room temperature. The resulting solution was washed with aqueous 10% citric acid, saturated aqueous sodium hydrogen carbonate, and water, dried (Na_2SO_4), and evaporated to yield a yellow oil (360 mg) which could be crystallised with diethyl ether–petroleum (b.p. 60—80°). The product (295 mg) was recrystallised from diethyl ether; m.p. 111—112°, $[\alpha]_D$ −31·4° (*c* 1·0 in $CHCl_3$) (Found: C, 58·4; H, 6·7; N, 6·1. $C_{22}H_{30}N_2O_6S$ requires C, 58·65; H, 6·65; N, 6·2%), $\nu_{max.}$ (Nujol) 1720 (ester) and 1660—1690 (ketone and amide), $\lambda_{max.}$ (MeOH) 241 and 271 nm (log ε 4·28 and 3·45), τ ($CDCl_3$) 8·50 (9H, s, Bu^t), 8·09 (3H, s, CH_3·C=), 7·82 (3H, s, CH_3C=), 7·00 (2H, m, CH_2·S), 6·22 (3H, s, CO_2Me), 5·90 (2H, s, CH_2·CO), 5·60 (1H, m, CH), 4·30 (1H, m, NH), and 1·90—2·70 (5H, m, aromatic).

Methyl N-*Phthaloyl*-S-*phenacyl*-L-*cysteinyldidehydrovalinate* (XIV; R^1 = phthaloyl, R^2 = Me).—Methyl *NN'*-bisphthaloyl-L-cystylbisdidehydrovalinate (722 mg) was dissolved in glacial acetic acid (20 ml) and powdered zinc (500 mg) was added with stirring. Vigorous stirring was continued for 30 min, the temperature being kept at

[31] Z. Foldi, *Acta Chim. Acad. Sci. Hung.*, 1954, **5**, 187 (*Chem. Abs.*, 1956, **50**, 981i).

20 °C by cooling with ice. The excess of zinc was filtered off and the filtrate was diluted to 100 ml with water and extracted with ethyl acetate. The extracts were washed with saturated aqueous sodium hydrogen carbonate and saturated aqueous sodium chloride, dried ($MgSO_4$), and evaporated to yield an oil which was dissolved in dry methanol (50 ml). Sodium methoxide (119 mg) was added to the solution, which was stirred under nitrogen for 30 min. Phenacyl chloride (340 mg) was added and the mixture was stirred at room temperature overnight. The solvent was removed *in vacuo* and the residue was partitioned between chloroform and water. The chloroform layer was separated, washed with saturated aqueous sodium chloride, dried ($MgSO_4$), and evaporated and the residue was separated into three fractions by preparative t.l.c. on silica gel with ethyl acetate–chloroform (1 : 1) as eluant. The middle fraction was *methyl* N-*phthaloyl*-S-*phenacyl*-L-*cysteinyldidehydrovalinate* (335 mg), m.p. 124—125° (from diethyl ether), $[\alpha]_D$ −86° (*c* 1·05 in $CHCl_3$) (Found: C, 62·7; H, 5·25; N, 5·85. $C_{25}H_{24}N_2O_6S$ requires C, 62·5; H, 5·05; N, 5·85%), $\nu_{max.}$ (Nujol) 1780 (imide), 1720 (ester), 1680 (ketone), and 1640 cm^{-1} (amide), $\lambda_{max.}$ (MeOH) 239sh, 272sh, and 281 nm (log ε 4·48, 3·69, and 3·66), τ ($CDCl_3$) 8·13 (3H, s, CH_3·C=), 7·83 (3H, s, CH_3·C=), 6·49br (2H, d, *J* 8 Hz, CH·CH_2·S), 6·33 (3H, s, CO_2Me), 6·09 (2H, s, CO·CH_2S), 4·80br (1H, t, *J* 8 Hz, CH), and 2·0—2·8 (10H, m, aromatic and NH).

Photolysis of Methyl N-*Phenylacetyl*-S-*phenacyl*-L-*cysteinyldidehydrovalinate* (XIV; R^1 = $PhCH_2$·CO, R^2 = Me). —(a) *In pyridine at* 35—40 °C. The ester (1 g) was dissolved in redistilled pyridine (500 ml) and the solution was degassed and irradiated under nitrogen with a Hanovia 125 W medium-pressure lamp with a Pyrex filter. Samples (3 ml) were evaporated *in vacuo* at room temperature. The u.v. absorption at 241 nm decreased and a small band at 285 nm was just apparent. T.l.c. indicated that there was little of the starting peptide after 25 h and the solvent was removed *in vacuo* to yield a yellow oil (970 mg), which could be separated into seven fractions by preparative t.l.c. [ethyl acetate–chloroform (1 : 1)]. A fraction of R_F 0·90 proved to be acetophenone (57 mg) (spectral comparison with an authentic sample). A fraction of R_F 0·73 (35 mg) was subjected to further preparative t.l.c. to yield a pale yellow solid, m.p. 132—155°, $\nu_{max.}$ (Nujol) 1720 (ester) and 1650 cm^{-1} (amide); osmometric *M* 1010 [(XV; R = H, *n* = 3) requires 1044], τ ($CDCl_3$) 8·28br (3H, s, CH_3·C=), 7·92br (3H, s, CH_3·C=), 6·32br (5H, s, CO_2Me and $PhCH_2$), and 2·68br (5H, s, aromatic).

A fraction of R_F 0·51 proved to be the starting peptide and a fraction of R_F 0·425 (149 mg) was further purified by preparative t.l.c. to yield a yellow solid which could be precipitated from chloroform by addition of ether; m.p. 117—124°, $\nu_{max.}$ (Nujol) 1720 (ester) and 1645 cm^{-1} (amide); osmometric *M* 890 [(XV; R = H, *n* = 2) requires 696], τ ($CDCl_3$) 8·26br (3H, s, CH_3·C=), 7·91br (3H, s, CH_3·C=), 6·33br and 6·30br (5H, singlets, $PhCH_2$ and CO_2Me), 5·10br (*ca.* 1H, m, C), and 2·70br (5H, s, aromatic). A fraction of R_F 0·35 (202 mg) was further purified by preparative t.l.c. and trituration with ether; m.p. 177—189°, $\nu_{max.}$ (Nujol) 1720 (ester) and 1642 cm^{-1} (amide); osmometric *M* 2700 [(XV; R = H, *n* = 8) requires 2784], τ ($CDCl_3$) 8·35 (3H, s, CH_3·C=), 7·96 (3H, s, CH_3·), 6·44 (2H, s, $PhCH_2$), 6·34 (3H, s, CO_2Me), 3·38 (1H, NH), 2·80 (5H, s, aromatic), and 0·90br (1H, s, NH). A fraction of R_F 0·31 was further purified by t.l.c. and trituration with ether; m.p. 115—130°, $\nu_{max.}$ (Nujol) 1720 (ester) and 1645 cm^{-1} (amide), osmometric *M* 1230 [(XV; R = H, *n* = 4) requires 1392], τ ($CDCl_3$) 8·35br (3H, s, CH_3·C=), 7·90br (3H, s, CH_3·C=), 6·35br (5H, s, $PhCH_2$ and CO_2Me), and 2·70br (5H, s, aromatic). The base-line spot was purified by further preparative t.l.c. and trituration with ether to yield a yellow solid, m.p. 178—202°, $\nu_{max.}$ (Nujol) (ester), and 1642 cm^{-1} (amide) osmometric *M* 3000 [(XV; R = H, *n* = 9) requires 3132].

(b) *In pyridine at* −30 °C. The ester (100 mg) was dissolved in pyridine and the solution was degassed and irradiated under nitrogen with a Hanovia 125 W medium-pressure lamp with a Pyrex filter. A weak u.v. absorption at 300 nm appeared, shifting to 335 nm on addition of base. After irradiation for 15 h at −30 °C, the solvent was removed and the residue was separated into six fractions by preparative t.l.c. The fractions were identified as acetophenone and polymeric thioaldehydes from their spectra.

(c) *Trapping experiment.* Irradiation of the peptide (300 mg) in pyridine (500 ml) as in (b) for 18 h was followed by removal of the solvent at 5 °C, addition of sodium methoxide (35 mg) in methanol (20 ml), and further removal of the solvent. The residue was washed by decantation with chloroform and redissolved in ethanol (10 ml). 1-Chloro-2,4-dinitrobenzene (131 mg) was added and the mixture was stirred at room temperature for 10 min. Addition of water (2 ml) yielded a gum which was washed by decantation with water and dried *in vacuo*. Preparative t.l.c. on silica gel [ethyl acetate–chloroform (1 : 1)] yielded 2,4-dinitroanisole, m.p. 192—194 °C, identical (spectra) with an authentic sample; methyl β-(2,4-dinitrophenylthio)-α-phenylacetamidoacryloyldidehydrovalinate (XIII), identical with a sample prepared by the method of Leonard; [22] and the geometrical isomer of (XIII), identical with the sample isolated from the trapping experiment on the reaction of methyl β-ethylamino-α-phenylacetamidoacryloyldidehydrovalinate with hydrogen sulphide.

(d) *In benzene–methanol.* Methyl *N*-phenylacetyl-*S*-phenacyl-L-cysteinyldidehydrovalinate (400 mg) was dissolved in benzene (450 ml) and methanol (50 ml). The solution was degassed and irradiated under nitrogen through a Pyrex filter with a Hanovia medium-pressure 125 W lamp. After 9 h, reaction seemed complete and the solvent was removed to yield a yellow oil which was triturated with ether to give a brown solid (210 mg). The ether-soluble material seemed to be mainly acetophenone and the ether-insoluble material, m.p. 129—141 °C, had spectra similar to those of the polythioaldehydes isolated from the photolysis in pyridine, and an osmometric molecular weight of 3400 [(XV; R = H, *n* = 10) requires 3480].

(e) *In sodium methoxide–methanol and reaction of the product with benzyl bromide.* The ester (468 mg) was dissolved in redistilled methanol, sodium methoxide (1·8 g) was added, and the solution was degassed and irradiated at −78 °C under nitrogen through a Pyrex filter with a Hanovia 125 W medium-pressure lamp for 10 h. The mixture was warmed to room temperature overnight and benzyl bromide (3·42 g) was added. The solution was stirred for 3 h and the solvent was removed. The residue was partitioned between ethyl acetate (50 ml) and water (50 ml) and the organic layer was washed with water and saturated aqueous sodium chloride, dried ($MgSO_4$) and concentrated *in vacuo* to 5 ml. The solution was diluted with diethyl ether and petroleum (b.p. 60—80°) and left overnight at room temperature. The gummy

precipitate was a mixture of mainly two components (t.l.c.) and could be triturated with ethyl acetate at 5 °C to yield a solid which on repeated recrystallisation from ethyl acetate yielded white needles, m.p. 183—184 °C $\lambda_{max.}$ (MeOH) 285 nm, identical with an authentic sample of (XVIII).[32] The mother liquors were purified by preparative t.l.c. to give a still impure compound, $\lambda_{max.}$ 299 nm. This was presumably the geometric isomer of (XVIII) reported by Leonard.[22]

N-Phenylacetyl-S-benzyl-α-methylcysteine.— *S*-Benzyl-α-methylcysteine [26] (5·23 g) was dissolved in 0·5N-sodium hydroxide (80 ml) and cooled to *ca.* 0 to 5 °C. Phenylacetyl chloride (10 ml) was added dropwise with stirring over 1 h, the pH being kept constant at 9·5 by addition of 2N-sodium hydroxide from an automatic titrimeter. The solution was allowed to warm to room temperature over 1 h and acidified to pH 1·0 with 6N-hydrochloric acid. The white suspension was stirred under ether (100 ml) for 1 h and filtered, and the *product* was washed with ether (yield 4·0 g) and recrystallised from ethyl acetate; m.p. 172—174° (Found: C, 66·4; H, 6·3; N, 3·9; $C_{19}H_{21}NO_3S$ requires C, 66·45; H, 6·15; N, 4·1%), $\nu_{max.}$ (Nujol) 1720 (CO_2H) and 1610 cm^{-1} (amide), τ [$(CD_3)_2SO$] 8·83 (3H, s, Me), 7·36 and 6·95 (2H, AB, J_{AB} 14 Hz, CH_2·S), 6·70 (2H, s, CH_2), 6·56 (2H, s, CH_2), 2·90br (10H, s, aromatic), and 1·88 (CO_2H).

N-Phenylacetyl-S-phenacyl-α-methylcysteine (XXI).— *N*-Phenylacetyl-*S*-benzyl-α-methylcysteine (3·11 g) was added to liquid ammonia (120 ml) and clean sodium pieces were added with stirring until a permanent blue colour was obtained. Solid ammonium chloride was slowly added until the blue colour was just dispersed and phenacyl chloride (1·55 g) was then added. The solution was stirred for a further 40 min and an excess of ammonium chloride was added. The ammonia evaporated off at room temperature overnight and the suspension was acidified to pH 2 with 6N-hydrochloric acid; the precipitate was redissolved by addition of 2N-sodium hydroxide. The solution was washed with the chloroform, acidified to pH 1 with 6N-hydrochloric acid, and extracted with ethyl acetate. The extracts were washed with saturated aqueous sodium chloride, dried (Na_2SO_4), and evaporated to yield a pale yellow *powder* which was recrystallised from ethyl acetate; yield 2·95 g, m.p. 148—149° (Found: C, 64·55; H, 5·85; N, 3·55. $C_{20}H_{21}NO_4S$ requires C, 64·7; H, 5·65; N, 3·8%), $\nu_{max.}$ (Nujol) 1715 (CO_2H), 1685 (ketone), and 1610 cm^{-1} (amide), $\lambda_{max.}$ (MeOH) 244 and 273 nm (log ε 4·05 and 3·31), τ [$(CD_3)_2SO$] 8·78 (3H, s, Me), 7·20 and 6·77 (2H, AB, J_{AB} 14 Hz, CH_2·S), 6·70 (2H, s, PhCH_2), 6·18 (2H, s, S·CH_2·CO), 2·20—2·90 (10H, m, aromatic), and 1·86 (1H, s, CO_2H).

The β-Diketone (XXIII) *or* (XXIV).—*N*-Phenylacetyl-*S*-phenacyl-α-methylcysteine (371 mg) was stirred as a suspension in anhydrous toluene (10 ml) containing triethylamine (101 mg). When the solid had dissolved, the solution was cooled to −5 °C and pivaloyl chloride (120 mg) was added. The solution was stirred for 1 h at −5 °C and for 1 h at room temperature and the solvent was removed *in vacuo* to yield the anhydride as a solid, $\nu_{max.}$ (Nujol) 1810 (anhydride), 1670 (ketone), and 1640 cm^{-1} (amide). The anhydride was dissolved in toluene (10 ml) and methyl didehydrovalinate (130 mg) was added. The solution was heated at 60 °C for 1 h and evaporated. The residue was dissolved in ethyl acetate, washed with N-hydrochloric acid, saturated aqueous sodium hydrogen carbonate, and water, and dried (NA_2SO_4). Removal of the solvent yielded a yellow solid (120 mg), which was crystallised from methanol; m.p. 208—209° (Found: C, 68·05; H, 5·7; N, 4·0. Calc. for $C_{20}H_{19}NO_3S$: C, 68·0; H, 5·4; N, 3·95%), $\nu_{max.}$ ($CHCl_3$) 1670 (ketone) and 1630 cm^{-1} (amide), $\lambda_{max.}$ (MeOH) 251 and 394 nm (log ε 4·15 and 4·04), τ [$(CD_3)_2SO$] 8·52 (3H, s, Me), 7·10 and 6·51 (2H, AB, J_{AB} 10 Hz, CH_2·S), 6·50 (2H, s, PhCH_2), 2·0—2·9 (10H, aromatic), and 1·11br (1H, s, OH).

Acetylation of the β-Diketone (XXIII) *or* (XXIV).— The β-diketone (XXIII) or (XXIV) (100 mg) was dissolved in anhydrous pyridine (20 ml) and acetic anhydride (5 ml) and left at room temperature for 48 h. The solvent was removed *in vacuo* to yield a dark oil which was dissolved in ethyl acetate, washed with N-hydrochloric acid and saturated aqueous sodium hydrogen carbonate, and dried ($MgSO_4$). The solvent was removed to yield an oily solid (110 mg), which appeared to be a mixture of two acetates from the n.m.r. spectrum: τ ($CDCl_3$) 8·40 (6H, s, 2 × Me), 7·72 (3H, s, MeCO), 7·62 (3H, s, MeCO), 6·82, 6·57, 6·37, and 6·25 (8H, 4s, 2 × PhCH_2, 2 × CH_2·S), 3·71br (2H, s, NH), and 2·0—2·7br (20H, m, aromatic). One of the two acetates was obtained pure by preparative t.l.c. on silica gel [chloroform–ethyl acetate (1 : 1)]; m.p. 120—121° (from ether) (Found: C, 67·1; H, 5·25; N, 3·5. Calc. for $C_{22}H_{21}NO_4S$: C, 66·9; H, 5·3; N, 3·55%), $\nu_{max.}$ ($CHCl_3$) 1770 (enol acetate), 1710 (ketone), and 1670 cm^{-1} (amide), $\lambda_{max.}$ (MeOH) 246 and 357 nm (log ε 4·07 and 3·99), $\lambda_{max.}$ (OH^-) 253 and 384 nm (log ε 4·07 and 3·33), τ ($CDCl_3$) 8·40 (3H, s, Me), 7·62 (3H, s, MeCO), 6·57br (2H, s, CH_2·S), 6·38 (2H, s, PhCH_2), 3·74 (1H, s, NH), and 2·0—2·60br (10H, m, aromatic).

3-Methyl-3-phthalimidothietan-2-one (XXV; R = Me).— Dry *S*-Benzyl-α-methylcysteine [26] (1·125 g) was ground intimately with phthalic anhydride (1·63 g) and the mixture was heated to 180 °C for 20 min and allowed to cool. The residue was dissolved in methanol (20 ml) and starting material (175 mg) was filtered off. The solvent was removed *in vacuo*, the residue heated with water (50 ml), and the solvent once more removed. The residue was dissolved in ether and left at −15 °C overnight; phthalic acid separated out. The solvent was removed *in vacuo* and the product was triturated with water to give *N-phthaloyl-S-benzyl-α-methylcysteine* as a white solid, which was recrystallised from benzene; yield 1·15 g, m.p. 123—124° (Found: C, 64·4; H, 5·15; N, 3·75. $C_{19}H_{17}NO_4S$ requires C, 64·45; H, 4·8; N, 3·95%), $\nu_{max.}$ (Nujol) 1775sh (imide), 1740, 1710br (CO_2H), and 1680 cm^{-1}, $\lambda_{max.}$ (MeOH) 293 nm (log ε 3·38), τ ($CDCl_3$) 7·97 (3H, s, Me), 6·87 and 6·40 (2H, AB, J_{AB} 14 Hz, CH_2·S), 6·33 (2H, s, PhCH_2), 2·81 (5H, s, aromatic), 2·27br (4H, s, aromatics), and −0·38br (1H, s, CO_2H).

N-Phthaloyl-*S*-benzyl-α-methylcysteine (6·1 g) was dissolved in dry benzene (150 ml) and redistilled thionyl chloride (20 ml) and stirred at room temperature for 15 h. The solvent was removed to yield the acid chloride as an oil, $\nu_{max.}$ (film) 1780—1720br cm^{-1}. The acid chloride (2·8 g) was dissolved in dry benzene (20 ml) and, with vigorous shaking, aluminium chloride (3·2 g) in dry benzene (10 ml) was added. The mixture was stirred at room temperature for 3 h and the resulting solution was poured on a mixture of crushed ice (20 g) and conc. hydrochloric acid (4 ml). The aqueous layer was extracted with benzene, and the combined organic layers were washed with water and

[32] C. J. Veal and D. W. Young, unpublished observations.

saturated aqueous sodium hydrogen carbonate and dried ($MgSO_4$). The solvent was removed to yield a *compound* (XXV; R = Me) which crystallised from ethyl acetate at −15 °C and was recrystallised from ethyl acetate; yield 0·87 g, m.p. 113—114 °C (Found: C, 58·55; H, 4·0; N, 5·9. $C_{12}H_9NO_3S$ requires C, 58·3; H, 3·65; N, 5·65%); $\nu_{max.}$ (Nujol) 1805sh, 1780, and 1700 cm^{-1}, $\lambda_{max.}$ (MeOH) 294 nm (log ε 3·34), τ ($CDCl_3$) 7·78 (3H, s, Me), 6·84 and 6·42 (2H, AB, J_{AB} 8 Hz, CH_2·S), and 2·20 (4H, m, aromatic).

NN′-*Bisphenylacetyl-α-methylcystine* (XXVI; R = OH).—*N*-Phenylacetyl-*S*-benzyl-α-methylcysteine (8·2 g) was dissolved in anhydrous liquid ammonia (250 ml) and clean sodium was added until a permanent blue colour was obtained. An excess of solid ammonium chloride was added, and the ammonia was evaporated off by stirring at room temperature overnight. The residue was dissolved in water (250 ml) and washed with ethyl acetate. The pH of the aqueous layer was adjusted to 8·0 with acetic acid, a crystal of iron(III) chloride was added and oxygen was passed through the solution for 18 h. The solution was acidified to pH 1 with 6N-hydrochloric acid and the white *precipitate* was filtered off, washed with ether, and recrystallised from ethyl acetate; yield 5·2 g, m.p. 212—213° (Found: C, 57·05; H, 5·6; N, 5·55. $C_{24}H_{28}N_2O_6S_2$ requires C, 57·15; H, 5·55; N, 5·55%), $\nu_{max.}$ (Nujol) 1715 (acid) and 1610 cm^{-1} (amide), τ [$(CD_3)_2SO$] 8·65 (3H, s, Me), 6·88 and 6·53 (2H, AB, J_{AB} 14 Hz, CH_2·S), 6·60 (2H, s, PhCH_2), 2·78 (5H, s, aromatic), and 1·78 (1H, s, CO_2H).

NN′-*Bisphenylacetyl-α-methylcystine Biscyanomethyl Ester* (XXVI; R = O·CH_2·CN).—*NN*′-Bisphenylacetyl-α-methylcystine (504 mg) was stirred as a suspension in chloroacetonitrile (10 ml). Triethylamine (202 mg) was added and the solution was stirred for 30 min at room temperature. The solvent was removed to yield an oil which was dissolved in chloroform and washed with N-hydrochloric acid, saturated aqueous sodium hydrogen carbonate and water, and dried (Na_2SO_4). The solvent was removed to yield a white *solid* (485 mg) which was recrystallised from chloroform–diethyl ether; m.p. 166—167° (Found: C, 57·55; H, 5·2; N, 9·6. $C_{28}H_{30}N_4O_6S_2$ requires C, 57·7; H, 5·15; N, 9·6%), $\nu_{max.}$ (Nujol) 1750 (ester) and 1640 cm^{-1} (amide), τ [$(CD_3)_2SO$] 8·64 (3H, s, Me), 6·93 and 6·77 (2H, AB, J_{AB} 14 Hz, CH_2·S), 6·61 (2H, s, PhCH_2), 5·15 (2H, s, CH_2·CN), and 2·82 (5H, s, aromatic).

Reaction of the Diester (XXVI; R = OCH_2·CN) *with n-Propylamine.*—The ester (200 mg) was dissolved in acetonitrile containing imidazole (100 mg). n-Propylamine (1 ml) was added and the solution was refluxed overnight. The solvent was removed and the resulting oil was dissolved in ethyl acetate (10 ml), washed with N-hydrochloric acid, water, and saturated aqueous sodium chloride, and dried (Na_2SO_4). The solvent was removed and the residue was crystallised from chloroform–diethyl ether; yield 105 mg, m.p. 174—175° (Found: C, 61·3; H, 7·0; N, 9·45. $C_{30}H_{42}N_4O_4S_2$ requires C, 61·45; H, 7·15; N, 9·55%), $\nu_{max.}$ (Nujol) 1670 and 1645 cm^{-1} (amide), τ ($CDCl_3$) 9·15 (3H, t, J 7 Hz, CH_3·CH_2), 8·62 (2H, m, CH_2), 8·47 (3H, s, Me), 6·74br (2H, m, CH_2·N), 6·42 (2H, s, PhCH_2), 6·40 (2H, m, CH_2·S), 3·41 and 2·88 (NH), and 2·69 (5H, aromatic).

Methyl N-*Phenylacetyl*-S-*phenacyl-α-methylcysteinyldidehydrovalinate* (XIX).—*N*-Phenylacetyl-*S*-phenacyl-α-methylcysteine (1·113 g) was dissolved in dry dioxan (50 ml) and the solution was diluted with dry benzene (50 ml) and heated to reflux. Methyl didehydrovalinate (426 mg) was added, followed immediately by redistilled phosphorus trichloride (310 mg). The solution was refluxed for 1 hour, cooled, and stirred at room temperature overnight. The solvent was removed and the residue was dissolved in ethyl acetate, washed with N-hydrochloric acid, N-sodium hydroxide, and saturated aqueous sodium chloride, and dried ($MgSO_4$). Removal of the solvent yielded an oil which solidified (0·48 g) on trituration with ether. The *product* was recrystallised from diethyl ether; m.p. 132—133° (Found: C, 64·4; H, 6·4; N, 5·7. $C_{26}H_{30}N_2O_5S$ requires C, 64·7; H, 6·25; N, 5·8%), $\nu_{max.}$ ($CHCl_3$) 1720 (ester), 1680sh (ketone), and 1660 cm^{-1} (amide), $\lambda_{max.}$ (MeOH) 241 and 273 nm (log ε 4·28 and 3·50), τ ($CDCl_3$) 8·28 (6H, s, Me), 7·90 (3H, s, CH_3·C=), 6·29 (3H, s, CO_2Me), 6·27 (2H, s, PhCH_2), 6·02 (2H, s, S·CH_2·CO), 2·0—2·8 (10H, m, aromatic), and 1·57br (1H, s, NH).

Photolysis of Methyl N-*Phenylacetyl*-S-*phenacyl-α-methylcysteinyldidehydrovalinate* (XIX).—The peptide (100 mg) was dissolved in pyridine (500 ml) and the solution was degassed and irradiated at −30 °C under nitrogen with a 125 W Hanovia medium-pressure lamp and a Pyrex filter. After 9 h, the solution was allowed to warm to room temperature and the solvent was removed at 30 °C. The residue was dissolved in chloroform (5 ml) and diluted with diethyl ether; a white solid was obtained. This was purified by repeated precipitation from chloroform with ether; yield 25 mg, m.p. 118—127°, osmometric M 1920 [(XV; R = Me, n = 5) requires 1810], $\nu_{max.}$ ($CHCl_3$) 1720 (ester) and 1670 cm^{-1} (amide), τ ($CDCl_3$) 8·34br (6H, m, Me), 7·92br (3H, s, CH_3·C=), 6·34br (5H, s, PhCH_2 and CO_2Me), 2·70br (5H, s, aromatic).

The ether-soluble material was concentrated *in vacuo* to *ca.* 5 ml; dilution with petroleum (b.p. 60—80°) gave a white precipitate which was purified by repeated precipitation from ether with petroleum; yield 12 mg, m.p. 65—77°, osmometric M 1030 [(XV; R = Me, n = 3) requires 1086], $\nu_{max.}$ ($CHCl_3$) 1720 (ester) and 1670 cm^{-1} (amide), τ ($CDCl_3$) 8·36br (3H, s, Me), 8·22br (3H, s, CH_3·C=), 7·86br (3H, s, CH_3·C=), 6·4br and 6·3br (5H, m, PhCH_2 and CO_2Me), and 2·70br (5H, s, aromatic). The petroleum-soluble material proved to be mainly acetophenone, identified by comparison (spectra) with an authentic sample.

N-*Phenylacetyl*-S-*phenacyl*-L-*cysteinamide* (XXVII).—*N*-Phenylacetyl-*S*-phenacyl-L-cysteine (1·07 g) was dissolved in methanol (20 ml) and treated with an excess of ethereal diazomethane. The solution was left for 30 min at room temperature and the solvent was removed to yield a yellow oil which was dissolved in chloroform and washed with saturated aqueous sodium hydrogen carbonate and water and dried (Na_2SO_4). The solvent was removed to yield the oily ester, $\nu_{max.}$ (film) 1740 (ester), 1680, and 1650 cm^{-1} (ketone and amide), τ ($CDCl_3$) 6·92br (2H, d, J 5 Hz, CH·CH_2), 6·38 (2H, s, PhCH_2), 6·27 (3H, s, CO_2Me), 6·17 (2H, s, PhCOCH_2), 5·08br (1H, m, CH·CH_2), and 2·0—2·8 (10H, m, aromatic).

The crude ester (742 mg) was dissolved in dry ethanol (50 ml) and the solution was saturated with anhydrous ammonia and stirred for 48 h at room temperature. The solvent was removed and the residual oil was dissolved in chloroform (10 ml). Dilution with diethyl ether (200 ml) yielded a pale yellow *solid* which was recrystallised from ethyl acetate (yield 420 mg), and from acetone–petroleum; m.p. 150—151° (Found: C, 64·0; H, 5·75; N, 7·85.

$C_{19}H_{20}N_2O_3S$ requires C, 64·05; H, 5·65; N, 7·85%), $\nu_{max.}$ (Nujol) 1675 (ketone) and 1640 cm^{-1} (amide), τ ($CDCl_3$) 7·03br (2H, d, J 5 Hz, $CH{\cdot}CH_2$), 5·25br (1H, m, $CH{\cdot}CH_2$), 6·33 (2H, s, $PhCH_2$), 5·95 (2H, s, $CO{\cdot}CH_2$), 5·25br (1H, m, $CH{\cdot}CH_2$), 3·90br (1H, m, NH), 2·90br (*ca.* 2H, s, NH_2), and 2·0—2·8 (10H, m, aromatic).

We thank Mr. T. A. Holland and Miss C. Battrick for technical assistance, Mrs. B. Brown and Mrs. B. O'Connor for bioassays, and Bristol Laboratories, Syracuse, New York, for financial support.

[3/2464 *Received*, 3*rd December*, 1973]