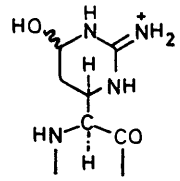
Viomycin. Part II.[1] The Structure of the Chromophore

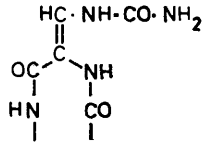
By B. W. Bycroft,* D. Cameron, L. R. Croft, A. Hassanali-Walji, A. W. Johnson,† and T. Webb, Department of Chemistry, University of Nottingham, Nottingham NG7 2RD

The chromophoric unit of viomycin is shown to be the monoureide of *C*-formylglycine, which is present in peptide combination. Hydrogenolysis of viomycin followed by hydrolysis yields alanine, which is not present in viomycin hydrolysates and which is derived from the chromophore. Other reactions of viomycin which involve the chromophore are discussed. The rate of evolution of urea during mild hydrolyses of viomycin is shown to correspond to the rate of loss of the chromophore; the unstable product, deureidoviomycin, can be recombined with urea to reconstitute the antibiotic.

In the preceding paper,[1] the detailed chemical evidence which led to the elucidation of the nature of the guanidine-containing fragment (I) of viomycin was presented, together with a review of chemical literature on the antibiotic. In this paper the structure of the chromophore (II) of viomycin is deduced. Both the guanidine-carbinol fragment (I) and the chromophore are very sensitive groupings and the reactions of the antibiotic designed to modify one of these units frequently affect the other.



(I)



(II)

Viomycin sulphate absorbs at 268 nm (ε 24,000) in acidic or neutral media and at 285 nm (ε 15,000) in aqueous 0·1N-sodium hydroxide. These spectral changes are reversible and the pK_a of the ionising group responsible for the alkaline bathochromic shift as calculated from the isosbestic point is 12·6. The similarity of the positions of the u.v. absorption maxima of viomycin to those of certain simple pyrimidines, *e.g.* thymine [$\lambda_{max.}$ in acidic or neutral solution, 265 nm (ε 9500); in alkaline solution, 291 nm (ε 5440)] led early workers to conclude that the chromophoric unit and the high pK_a value were in some way associated with the guanidine unit.[2-4] This conclusion received a measure of support from the observations that sodium hypochlorite–phenol, which was known to react with cytosine, uracil, and thymine, gave a positive reaction (sky-blue colouration) with viomycin. Furthermore, viomycin reacted readily with bromine, with consequent loss of the u.v. spectrum, behaviour which is also shown by pyrimidines and related compounds. However, the large discrepancy between the extinction coefficients of the u.v. maxima of viomycin and pyrimidines, the instability of the chromophore under hydrolytic conditions, and our evidence that the guanidine fragment, the precursor of viomycidine, is not a part of the chromophore,[1] necessitated a complete revision of ideas on the nature of this grouping.

Attempts to isolate the intact chromophore by degradative methods were unsuccessful because of its instability under hydrolytic conditions, and this proved to be a major obstacle to structural investigations. Mild hydrolysis of viomycin with 0·1N-hydrochloric acid at 95° for 5 h led to complete loss of the u.v. absorption and liberated 1 equiv. of urea. Further hydrolysis of the resulting peptide, deureidoviomycin,[2-4] ‡ afforded all the usual products of a similar hydrolysis of viomycin with the exception of urea. Viomycin in D_2O solution shows a low-field signal in the n.m.r. spectrum at τ 1·9 (1H, broad s) which is not present in deureidoviomycin. Both Mason [2] and Jones [3] had attempted to correlate the loss of the chromophore of viomycin, when it is treated with hot dilute acid, with the liberation of urea, but their results were inconclusive because the methods employed for the determination of the urea were inaccurate, as no

† *Present address:* School of Molecular Sciences, University of Sussex, Falmer, Brighton BN1 9QJ.

‡ Previously referred to as ' desureaviomycin.'

[1] Part I, B. W. Bycroft, L. R. Croft, A. W. Johnson, and T. Webb, preceding paper.

[2] L. H. Mason, Ph.D. Thesis, University of Illinois, 1953.

[3] W. A. Jones, Ph.D. Thesis, University of London, 1963.

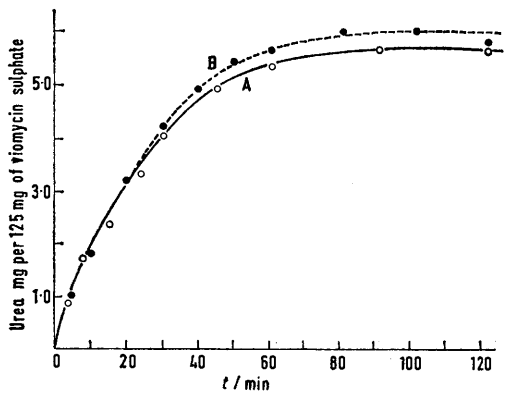
[4] J. R. Dyer, C. K. Kellogg, R. F. Nassar, and W. E. Streetman, *Tetrahedron Letters*, 1965, 585.

account was taken of the breakdown of urea under the reaction conditions:

$$\text{viomycin} \xrightarrow{k_1} \text{deureidoviomycin} + \text{urea}$$

$$\text{urea} + H_2O \xrightarrow{k_2} 2NH_3 + CO_2$$

It is necessary to determine the rate constant k_1 and k_2 before the rates of decay of the chromophore and production of urea can be compared directly, and a kinetic investigation was therefore undertaken. The rate of decay of the chromophore in 0·1N-hydrochloric acid at 100° was determined spectrophotometrically and the reaction was shown to obey first-order kinetics with a rate constant (k_1) of $2{\cdot}46 \times 10^{-2}$ min^{-1}. Urea was determined by a slight modification of the method of Allen and Luck [5] (see Experimental section). Examination of urea alone under the same conditions showed that its decomposition also followed first-order kinetics with a rate constant (k_2) of $4{\cdot}04 \times 10^{-3}$ min^{-1}. From these data it was possible to compute a curve for the overall production of urea from viomycin, assuming that



Production of urea by hydrolysis of viomycin with 0·1N-hydrochloric acid; A, experimental valves; B, calculated values

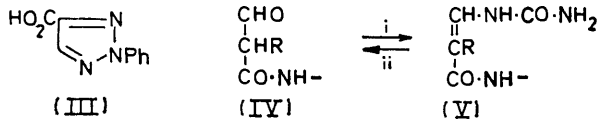
the rate of decay of the chromophore was identical with the rate of production of urea. A comparison of this calculated curve with that observed experimentally from the hydrolysis of viomycin is shown in the Figure. The agreement between the two curves provided strong evidence that urea was part of the viomycin chromophore and suggested that it was linked to the rest of the molecule by a single covalent bond.

By use of the above information it was possible to obtain the optimum conditions for the production of deureidoviomycin, which proved to be a very unstable compound. This accounts for the earlier discrepancies in descriptions of its properties. It was not possible to obtain satisfactory analytical data; chemical and spectral properties were determined on freshly prepared material. Amino-acid analyses of the total hydrolysis product of deureidoviomycin showed that it possessed the same amino-acid content, including the small quantities of glycine, as viomycin. Appreciable amounts of ammonia and carbon dioxide (*ca.* 1 mol equiv. of each) were released in the course of the hydrolysis, indicating that the source of these products in the viomycin hydrolyses was not only urea. The significance of the small quantities of glycine in the hydrolysates of viomycin and deureidoviomycin tended to be ignored in the early work, especially as microbial peptides frequently occur as families of closely related compounds differing only in minor variations of the amino-acid content.

Deureidoviomycin in acidic or neutral media exhibited no u.v. absorption, but in 0·1N-sodium hydroxide solution it showed a strong maximum at 272 nm. Its n.m.r. spectrum (D_2O) lacked any low-field signal, but on addition of NaOD a signal (1H, s) appeared at τ 1·5. In contrast to viomycin, deureidoviomycin gave a deep red colour with ferric ions, and readily reduced solutions of ammoniacal silver nitrate, and copper(II) acetate, as well as Tillman's reagent.[6] Even more important was the observation that deureidoviomycin could be converted smoothly at room temperature in the presence of urea and 2N-hydrochloric acid into a product identical with viomycin in all its properties including biological activity. This reaction established that no deep-seated rearrangement had occurred in the formation of deureidoviomycin, and confirmed that the urea was associated with the chromophore, as had been deduced from kinetic data. It also provided an extremely simple route to a series of semi-synthetic antibiotics containing substituted urea derivatives. However, it is noteworthy that 1,3-dimethylurea reacts readily with deureidoviomycin under the conditions described to give a product showing a u.v. spectrum similar to that of viomycin. This observation, together with the isolation of *N*-formylurea from mild potassium permanganate oxidation of viomycin (an observation made initially by Jones [3] and confirmed by ourselves) endorsed the tentative conclusion drawn from the kinetic data that the urea is linked in viomycin by a single covalent bond.

It was apparent that some fragments, other than urea, liberated on hydrolysis of the chromophore, were so far undetected, and the combined molecular weights of the known fragments [1] of viomycin still left a discrepancy of about 100. In a search for carbonyl compounds, we hydrolysed viomycin with acid in the presence of 2,4-dinitrophenylhydrazine; among the products glyoxal bis-2,4-dinitrophenylhydrazone was identified. Jones [3] had shown that when viomycin was subjected to the sequence of reactions: (i) oxidation with aqueous iodine, (ii) formation of an osazone with phenylhydrazine, (iii) cyclisation of the osazone with dilute acid, (iv) total acid hydrolysis, a crystalline degradation product was obtained which was identified as 2-phenyl-1,2,3-triazole-4-carboxylic acid (III). Jones concluded that this observation established the presence of a potential 1,2,3-tricarbonyl system in viomycin. Furthermore, the pK_a value of 12·6 for the chromophoric unit is compatible with an acyclic β-dicarbonyl function.

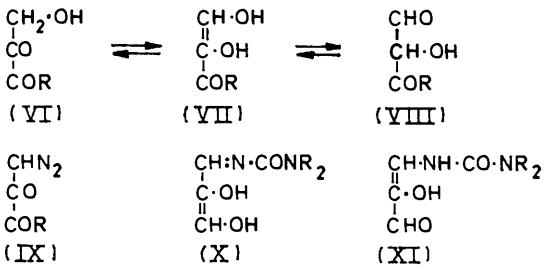
[5] F. W. Allen and J. M. Luck, *J. Biol. Chem.*, 1929, **82**, 693.
[6] H. von Euler, H. Hasselquist, and O. Ceder, *Annalen*, 1953, **581**, 198.



(III) (IV) (V)

Reagents: i, H^+, $NH_2 \cdot CO \cdot NH_2$; ii, H^+,H_2O

The above chemical and spectral evidence initially led us to consider the partial structures (IV; R = OH) (or a tautomeric equivalent) and (V; R = OH) for the chromophores of deureidoviomycin and viomycin, respectively, and in order to provide support for this formulation, we endeavoured to synthesise simple models of this system. Sprinson and Chargaff [7] have shown that hydroxypyruvic acid (VI; R = OH) can be converted in solution into the tautomer (VII; R = OH) *via* the enediol (VII; R = OH). Accordingly we prepared solutions of ethyl hydroxypyruvate (VI; R = OEt) and the corresponding *N*-methylamide (VI; R = NHMe) in aqueous ethanol by treatment of the diazo-compounds (IX; R = OEt or NHMe) with acid. Solutions of compounds (VI; R = OEt or NHMe) gave red colours with ferric ions and reduced ammoniacal silver nitrate, copper(II) acetate, and Tillman's reagent.[6] The u.v. spectra of these compounds in acidic or neutral media showed no absorption, but in 0·1N-sodium hydroxide showed maxima at 272 and 283 nm, respectively. However, these absorptions had half-lives of only 2—3 min, in contrast to deureidoviomycin which was relatively stable under these conditions. Furthermore, attempts to condense compounds (VI; R = OEt or NHMe) with urea in presence of acid under a variety of conditions failed to yield products with the characteristic u.v. absorption of viomycin.



(VI) (VII) (VIII)

(IX) (X) (XI)

Reductone (VII; R = H) has been reported [8] to form a ureide easily, which was formulated as (X; R = H). Purification of reductone ureide was difficult owing to the ease with which it cyclises to 2,4-dihydroxypyrimidine, but the *NN*-dimethylureide (X; R = Me) crystallised readily from a solution of reductone and *NN*-dimethylurea in 1,2-dimethoxyethane containing a few drops of 2N-hydrochloric acid. The n.m.r. spectrum of the dimethylureide (X; R = Me) in [$^2H_6$]dimethyl sulphoxide showed two low-field signals, a singlet at τ 1·18 and a doublet centred at τ 3·10 (J 11·8 Hz), and on addition of D_2O the latter collapsed to a singlet. The i.r. spectrum showed a strong band at 1685 cm^{-1} and similar spectral properties were also observed for a sample of reductone ureide (X; R = H) which had not been purified completely. These properties are inconsistent with the previously proposed structure (X) but are in accord with that of the isomer (XI). This was therefore considered as a more acceptable model for the viomycin chromophore but again there were a number of inconsistencies. Thus the u.v. spectra of compounds (XI; R = H or Me) showed strong absorption at *ca.* 295 nm, which shifted in base to 392 nm. Furthermore, as already stated, the olefinic proton in the spectrum of (XI; R = Me) appeared at τ 3·10 whereas in viomycin in D_2O solution, it appeared at τ 1·9; even allowing for solvent variation this was too great a divergence. In addition, it was difficult to reconcile the partial structure (V; R = OH) with the analytical data and the evidence relating to the peptide sequence,[9] and it was clear that further degradative evidence concerning the elusive chromophore fragment was required. Although previous attempts [2,3] to reduce viomycin had been unsuccessful, we examined the hydrogenation of viomycin trihydrochloride in 4N-hydrochloric acid in the presence of an excess of platinum catalyst; a perhydro-derivative was obtained which showed no u.v. absorption in acid, neutral, or alkaline media. This product was not isolated but subjected to total acid hydrolysis, and the hydrolysate was then subjected to quantitative amino-acid analysis. All the usual amino-acids of an acidic hydrolysate of viomycin were present in the hydrolysate except that β-lysine was retained on the column and capreomycidine was observed instead of viomycidine; the conversion of the guanidine system of viomycin into capreomycidine by hydrogenation and hydrolysis has been reported already.[1] In addition alanine (*ca.* 1 mol. equiv.), predominantly the D-isomer, was obtained which must have been derived by reduction, partially stereospecific, of the chromophore. Similarly, reduction of deureidoviomycin under the same conditions, gave a perhydro-derivative which on acid hydrolysis afforded alanine (0·3 mol. equiv.) and serine (0·7 mol. equiv.) in addition to the expected amino-acids. These facts, taken with the earlier evidence, are explicable only in terms of the partial structures (IV; R = NH·COR′) and (II ≡ V; R = NH·COR′) for the chromophores of deureidoviomycin and viomycin, respectively. The production of all the observed hydrolysis products can then be rationalised as shown in Scheme 1.[9]

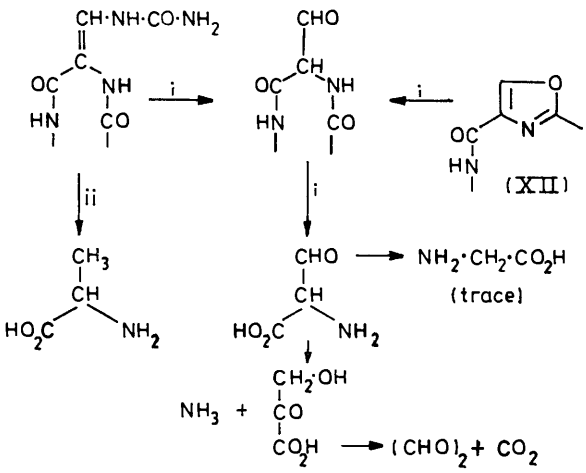
Vigorous acidic hydrolysis of the chromophore would be expected, on this basis, to result in the breakdown of deureidoviomycin to yield mainly ammonia and hydroxypyruvic acid, from which glyoxal would be readily produced. In addition the traces of glycine might well arise by deformylation of the initially formed *C*-formylglycine, and it is noteworthy that glycine was not detected in the acid hydrolysate of the perhydro-compounds. It is known that derivatives of oxazole-4-carboxylic acid (XII) undergo hydrolytic degradation in an

[7] D. B. Sprinson and E. Chargaff, *J. Biol. Chem.*, 1946, **164**, 417.

[8] H. von Euler and C. Martius, *Arkiv Kemi*, 1934, **11***B*, 3.

[9] B. W. Bycroft, D. Cameron, L. R. Croft, A. Hassanali-Walji, A. W. Johnson, and T. Webb, *Tetrahedron Letters*, 1968, 5901; *Experientia*, 1971, **27**, 501.

analogous manner,[10,11] but a basic ring nitrogen atom was not present in viomycin on the evidence of titration.[1] However, the course of the hydrogenation of viomycin is also paralleled by the behaviour of the antibiotic griseoviridin,[12] where the unit (XIII) is readily reduced by hydrogenation over Raney nickel to give a compound which afforded alanine on total hydrolysis. It is also significant that the olefinic proton associated with this

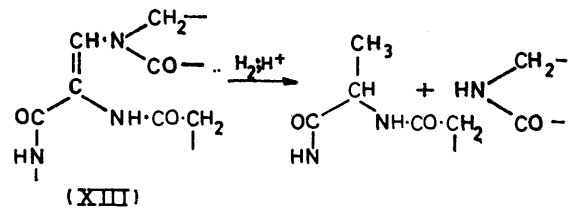


Reagents: i, H^+; ii, H_2,H^+

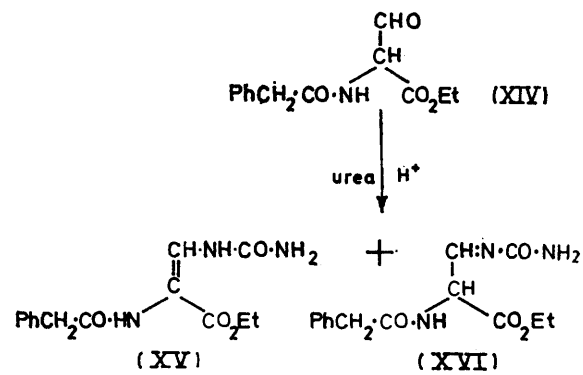
SCHEME 1

system corresponded to a singlet at τ 1·9 in the n.m.r. spectrum.

Reduction of the partial structure (IV; R = NH·COR′) in deureidoviomycin to give a product which on hydrolysis yielded serine rather than alanine is a reasonable supposition on the grounds that (IV; R = NH·COR′) can be regarded as a derivative of *C*-formyl-



glycine. The lack of the low-field signal in the n.m.r. spectrum of deureidoviomycin (see above) is attributed to hydration of the formyl group. The chemistry of *C*-formylglycine derivatives had been investigated extensively in the early work on penicillin and the reported chemical and spectral properties of several penicillin intermediates [13] agreed closely with those of deureidoviomycin. In particular, ethyl benzylpenaldate (XIV) proved to be a convenient model for deureidoviomycin. It was readily prepared by formylation of ethyl phenaceturate with sodium and ethyl formate in ether, and was characterised as its benzoate and benzylamine derivatives.[14] The u.v. spectra of compound (XIV) in acidic and basic media were remarkably similar to those of desureaviomycin, and, like deureidoviomycin, compound (XIV) gave an intense red-purple colour with ferric ions and reduced aqueous silver nitrate and Tillmann's reagent.[6] Acid-catalysed condensation of (XIV) with urea at room temperature in 1,2-dimethoxyethane afforded, in moderate yield, a crystalline ureide which could be isolated in either of two forms. Crystallisation from aqueous methanol afforded the enamine (XV), m.p. 218—219°, $\nu_{max.}$ 1655, 1690, and 1705 cm^{-1}, whereas crystallisation from chloroform afforded a product with the same m.p. but with $\nu_{max.}$ 1655, 1698, and 1739 cm^{-1}, which has been tentatively assigned the imine structure (XVI). On dissolving the latter in polar solvents, it reverted to the enamine structure (XV), the u.v. spectrum of which was similar to that of viomycin. The n.m.r. spectrum of the enamine (XV) [$(CD_3)_2SO$–D_2O] showed a low-field signal at τ 2·2 associated with the olefinic proton. The pK_a value associated with the change in the u.v. spectrum caused by addition of base was found to be 12·6 and the chemical properties of (XV) were also similar to those observed for the chromophore of viomycin. Thus the enamine (XV) was fairly labile to mild acidic or basic hydrolysis; catalytic reduction followed by acid hydrolysis gave alanine in good yield, and permanganate oxidation afforded *N*-formylurea.



The remaining structural problem associated with the chromophore was the possibility of geometrical isomerism about the ureide double bond, *i.e.* the existence of the isomers (II) and (XVII). Only one isomer occurred in the natural antibiotic, and it was this isomer which was obtained from the partial synthesis of viomycin from deureidoviomycin. As this reaction was carried out under equilibrating conditions, it could be assumed that the thermodynamically more stable isomer was formed. The same arguments also apply to the model system (XV). Of the two possibilities we favour (II) rather

[10] G. R. Delpierre, F. W. Eastwood, G. E. Gream, D. G. I. Kingston, P. S. Sarin, Lord Todd, and D. H. Williams, *J. Chem. Soc.* (*C*), 1966, 1653.

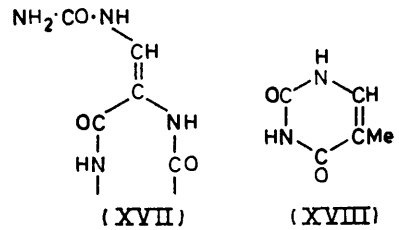
[11] G. A. Snow, *Biochem. J.*, 1965, **97**, 166.

[12] M. C. Fallona, P. de Mayo, T. C. McMorris, T. Money, and A. Stoessl, *Canad. J. Chem.*, 1964, **42**, 371.

[13] 'The Chemistry of Penicillin,' ed. H. T. Clarke, J. R. Johnson, and Sir Robert Robinson, Princeton Univ. Press, New Jersey, 1949, pp. 486, 501, 716.

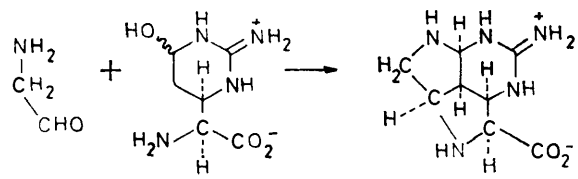
[14] B. W. Bycroft, D. Cameron, A. Hassanali-Walji, and A. W. Johnson, *Tetrahedron Letters*, 1969, 2539.

than (XVII) because of the comparison of the u.v. spectrum of viomycin with that of thymine (XVIII) (see earlier), which can be regarded as a cyclic form of (XVII) in which the carbonyl function is *cis*-oriented with respect to the urea. It is probable that the *trans*-isomer (II) would have markedly enhanced extinction coefficients compared with those of (II), as is found to be the case. These arguments are supported by the observation that neither viomycin nor the ureide (XV) yields hydroxypyrimidines on aqueous acid treatment, as might be expected for the corresponding *cis*-isomers.



(XVII) (XVIII)

It is also pertinent that our view of the acid-catalysed degradation of the viomycin chromophore provides an explanation of the formation of the minor product, viocidic acid,[1,15,16] which may be obtained formally by the condensation of aminoacetaldehyde (decarboxylation product of formylglycine) with the guanidine-carbinol fragment (Scheme 2).



SCHEME 2

Alternatively if the dehydroserine unit should prove to be adjacent to the guanidine-carbinol unit, as is suggested for tuberactinomycin O by *X*-ray examination,[17] and also for viomycin by preliminary *X*-ray results,[18] then the production of viocidic acid can be formulated as an intramolecular process.

EXPERIMENTAL

Spectra and m.p.s were determined and amino-acid mixtures analysed as described in the preceding paper.

Kinetic Studies on Hydrolysis of Viomycin Sulphate in Dilute Acid.—Viomycin sulphate (125 mg) was dissolved in 0·1N hydrochloric acid (20 ml); samples of the solution were heated under reflux for specified times and then cooled rapidly to room temperature. Fresh solutions were prepared for each determination, and the equipment and methods were standard throughout. Samples of the cooled solutions were taken for optical density measurements at 268 nm and urea determinations; the latter were carried out by a modification of the method of Allen and Luck [5] whereby the urea is converted into its dixanthydryl derivative, which is then estimated by chromic acid titration. No urea was found in unheated solutions of viomycin in 0·1N-hydrochloric acid. The rate of decay of the chromophore was found to obey first-order kinetics with a rate constant (k_1) of $2{\cdot}46 \times 10^{-2}$ min^{-1}. Experiments were carried out to determine the rate at which urea (40 mg) in 0·1N-hydrochloric acid (150 ml) was destroyed under reflux conditions. Samples were heated for varying times, and the decomposition was found to approximate to first-order kinetics with a rate constant (k_2) of $4{\cdot}04 \times 10^{-3}$ min^{-1}. Ammonia in the acid hydrolysates of viomycin was determined by the microdiffusion method of Conway and Byrne.[19]

Hydrolysis of Viomycin by Acid in the Presence of 2,4-Dinitrophenylhydrazine.—6N-Hydrochloric acid (250 ml) was added to viomycin sulphate (750 mg) and a solution of 2,4-dinitrophenylhydrazine in ethanolic hydrochloric acid (20 ml) and the mixture was heated under reflux in an atmosphere of nitrogen for 24 h (*cf.* ref. 10). The cooled solution was filtered and the dark brown residue, containing a mixture of 2,4-dinitrophenylhydrazones and unchanged reagent, was extracted with boiling chloroform. The residue from the extract was purified further by precipitation with ethanol from a solution in *NN*-dimethylformamide. Crystallisation from ethanol–nitrobenzene then gave glyoxal bis-2,4-dinitrophenylhydrazone, m.p. and mixed m.p. 332—335° (decomp.).

Oxidation of Viomycin by Permanganate (cf. *ref.* 3).—Viomycin sulphate (2·5 g) was dissolved in distilled water and dilute sulphuric acid was added until the solution had pH 2. A saturated aqueous solution of potassium permanganate was added, along with dilute sulphuric acid to maintain the pH below 7, until the purple colour persisted for 5 min. The mixture was warmed to 50° to coagulate the manganese dioxide, cooled, and filtered. The filtrates were pale yellow in colour; more permanganate solution was added until the purple colour persisted for 5 min, although when it was heated to 50°, cooled, and filtered, the solution was again pale yellow. The filtrates (45 ml) were acidified to pH 4—5 with dilute sulphuric acid and added to acetone (450 ml). The resulting milky suspension was kept at room temperature overnight and then filtered through diatomite. The solvent was removed at 35—40° *in vacuo*; a white solid (224 mg) remained, which was dissolved in ethanol. The hot solution was filtered and concentrated on a steam-bath, and cooled. The crystals were separated, washed with a little ethanol, then ether, and dried *in vacuo* at room temperature (99 mg); they then had m.p. 140—160° although the i.r. spectrum was similar to that of synthetic *N*-formylurea. Two further crystallisations from ethanol produced colourless crystals (33 mg), m.p. 164—167° (lit.,[20] 168—169°).

Isolation of Alanine by Hydrogenolyses of Viomycin and Deureidoviomycin.—Viomycin hydrochloride (1·152 g) was dissolved in 4N-hydrochloric acid (30 ml) containing a suspension of Adams catalyst (530 mg) and hydrogenated for 24 h. The catalyst was separated and washed with

[15] B. W. Bycroft, D. Cameron, L. R. Croft, A. W. Johnson, T. Webb, and P. Coggon, *Tetrahedron Letters*, 1968, 2925.
[16] P. Coggon, *J. Chem. Soc.* (*B*), 1970, 838.
[17] H.Yoshioka, T. Aoki, H. Goko, K. Nakatsu, T, Noda, H. Sakakibara, T. Take, A. Nagata, J. Abe, T. Wakamiya, T. Shiba, and T. Kaneko, *Tetrahedron Letters*, 1971, 2043.
[18] B. W. Bycroft, unpublished results.
[19] E. J. Conway and A. Byrne, *Biochem. J.*, 1933, **27**, 419.
[20] E. W. Maynert and E. Washburn, *J. Org. Chem.*, 1950, **15**, 259.

water (10 ml), and hydrochloric acid (10 ml) was added to the combined filtrates, which were then heated under reflux for 5 h. The acid was then removed *in vacuo* and the residue dissolved in water (10 ml). Paper chromatography indicated the presence of alanine, serine, αβ-diaminopropionic acid, β-lysine, α-(2-iminohexahydro-4-pyrimidyl)glycine, and possibly a peptide. The solution was added to a column (3 × 24 cm) of Dowex 50 W × 8 (H^+ form) and eluted with N-hydrochloric acid at room temperature. The column yielded L-serine (fractions 22—36; 105 mg), isolated as the hydrochloride, and identical with an authentic sample; and alanine (fractions 42—70), isolated as the hydrochloride (42 mg), $[\alpha]_D^{23.5}$ −4·7° (*c* 0·3 in H_2O), the i.r. spectrum of which showed only small differences from that of L-alanine hydrochloride. The n.m.r. spectra of the two samples were identical, as was their chromographic behaviour on paper [in t-butyl alcohol–acetic acid–water (2 : 1 : 1)] and on silica (thin layer; potassium dihydrogen phosphate buffer at pH 6).

Freshly prepared deureidoviomycin (500 mg) was hydrogenated under the same conditions as viomycin. The product showed only end absorption in the u.v. in acid, neutral, or alkaline solution. Amino-acid analysis of the total acid hydrolysate of the hydrogenated product (6N-hydrochloric acid; sealed tube overnight) gave viomycidine, αβ-diaminopropionic acid, β-lysine, serine, and alanine in the ratios of 1 : 1 : 1 : 2·5 : 0·5.

Deureidoviomycin and its Reaction with Urea.—Viomycin sulphate (1·0 g) was dissolved in 0·1N-hydrochloric acid (200 ml) which had been previously deaerated by bubbling nitrogen through it for 1 h, and the solution was heated in an atmosphere of nitrogen for 4½ h. The hydrolysate was cooled and concentrated under reduced pressure at 30° to *ca.* 40 ml, and the concentrate was poured into ethanol–acetone (1 : 1; 400 ml). The precipitated deureidoviomycin was separated and dried *in vacuo* (P_2O_5) to give a colourless amorphous solid (0·78 g), $\lambda_{max.}$ (0·1N-NaOH) 272 nm.

Deureidoviomycin (250 mg) and urea (2·9 g) were dissolved in 2N-hydrochloric acid (5 ml) and the solution was kept at room temperature under nitrogen for 24 h. Precipitation of the product from ethanol–acetone (1 : 1; 100 ml) yielded a white solid, which was collected and dried (P_2O_5) *in vacuo* (yield 110 mg); $\lambda_{max.}$ (pH 2) 267 nm ($E^{1\%}_{1\,cm}$ 270), $\lambda_{max.}$ (pH 14) 290 nm ($E^{1\%}_{1\,cm}$ 174), τ (D_2O) 1·9 (s), identical with the values for viomycin. However, t.l.c. (silica; pyridine–acetic acid buffer, pH 3·4) revealed the presence of two products, the main product having R_F 0·69 (corresponding to viomycin) and the minor product with R_F 0·65.

Ethyl Diazopyruvate and the Corresponding N-*Methylamide* (IX; R = OEt or NHMe).—An ethereal solution of diazomethane (0·12 mol) was added dropwise to an ice-cold, stirred solution of ethyl chloroglyoxylate (2 g) in dry ether (100 ml) and the mixture was stirred overnight at room temperature. Aqueous 2N-acetic acid (50 ml) was then added to decompose the residual diazomethane and the resulting solution was extracted with chloroform (50 ml × 5). The extract was washed with sodium carbonate (2N; 100 ml × 2), dried ($MgSO_4$), and evaporated to dryness to give an orange oil (1·8 g) which crystallised on cooling and scratching. Recrystallisation from benzene–light petroleum (b.p. 60—80°) gave *ethyl diazopyruvate* as needles (1·4 g, 59%), m.p. 169—173° (Found: C, 42·1; H, 4·25; N, 19·65. $C_5H_6N_2O_3$ requires C, 42·25; H, 4·25; N, 19·7%), $\nu_{max.}$ ($CHCl_3$), 2080, 1727, and 1650 cm^{-1}, $\lambda_{max.}$ (EtOH) 256 (ε 5640) and 303 nm (8030), τ ($CDCl_3$) 3·58 (1H, s), 5·68 (2H, q, *J* 8 Hz), and 8·62 (3H, t, *J* 8 Hz).

The foregoing ester (1 g) was dissolved in 25% (w/w) methylamine in ethanol (5 ml) and the mixture was stirred at room temperature for 18 h. Removal of the solvent left a solid (0·8 g) which on crystallisation from benzene–light petroleum (b.p. 60—80°) gave N-*methyldiazopyruvamide* (0·72 g, 90%) as colourless needles, m.p. 111—125° (Found: C, 37·8; H, 4·05; N, 32·85. $C_4H_5N_3O_2$ requires C, 37·8; H, 3·95; N, 33·05%), $\nu_{max.}$ ($CHCl_3$) 3460, 2070, 1690, and 1635 cm^{-1}, $\lambda_{max.}$ (EtOH) 256 and 300 nm (ε 11,160 and 6280), τ ($CDCl_3$) 3·62 (1H, s) and 7·08 (3H, d, *J* 5 Hz).

Reductone N′N′-*Dimethylureide* (XI; R = Me).—Reductone was prepared by a modification of the method of von Euler and Martius.[8] A solution of glucose (50 g) in deionised water (2 l) was heated under nitrogen at 88° for 1 h and lead acetate (30 g) was added, followed by sodium hydroxide (17·5 g) in water (50 ml). The resulting mixture was left at about 90° for 3 min, and aqueous acetic acid (4N; 42 ml) was then added, causing precipitation of the lead salt of reductone. The mixture was then cooled and rapidly filtered, and the lead salt (19·5 g) was washed with methanol and then ether and dried (P_2O_5) *in vacuo* for 24 h. It was then suspended in dry acetone (500 ml) and hydrogen sulphide was bubbled through the suspension rapidly for 2½ h. The solution was filtered rapidly under reduced pressure and the filtrate was evaporated to dryness to give a light tan-coloured solid, which on crystallisation from acetic acid gave reductone (1·6 g, 6·5%) as colourless crystals, m.p. 200—215° (decomp.) (lit.,[21] 200—220°).

Reductone (400 mg) and 1,1-dimethylurea (322 mg) were dissolved in a mixture of 1,2-dimethoxyethane (25 ml) and aqueous hydrochloric acid (2N; 0·25 ml), and the solution was stirred in an atmosphere of nitrogen for 18 h. Removal of the solvents at 30° under reduced pressure gave a solid which on crystallisation from ethanol gave *reductone* N′N′-*dimethylureide* as tiny prisms (255 mg, 36%), m.p. 154—155° (decomp.) (Found: C, 45·7; H, 6·65; N, 17·5%; M^+, 158·06889. $C_6H_{10}N_2O_3$ requires C, 45·55; H, 6·35; N, 17·7%; *M*, 158·069137), $\nu_{max.}$ (KBr) 3400, 1685, 1662, and 1610 cm^{-1}, $\lambda_{max.}$ (H_2O) 297 nm (ε 25,100) at pH 2 and 392 nm (ε 19,300) at pH 13, τ [$(CD_3)_2SO$] 1·08 (1H, s), 1·5br (1H, disappears on addition of D_2O), 3·04 (1H, d, *J* 10·6 Hz, coalesces into a singlet in D_2O), 6·6 (1H, s, disappears in D_2O), and 7·05 (6H, s).

N-*Methylaceturamide.*—Methyl aceturate (89·6 g, 0·685 mol) was dissolved in ethanol (120 ml) and a solution of methylamine in ethanol (33%; 330 ml, *ca.* 3 mol) was added. The yellow solution became steadily warmer and was kept for 1½ h; the colour had then faded and crystallisation had started. The mixture was kept at −5° for 1 h and filtered. The solid was dried by suction, washed with dry ether, and dried at room temperature *in vacuo* (H_2SO_4). A second crop had been deposited from the filtrates after 12 h at −5°. The methylamide (66·3 g, 74·6%) had m.p. 158—160° (lit.,[22] 158°), and sublimed at 100—110° and 0·1 mmHg to give white crystals, m.p. 158·5—159·5° (Found: C, 46·1; H, 7·65; N, 21·95. Calc. for $C_5H_{10}N_2O_2$: C, 46·1; H, 7·7; H, 21·55%).

N-*Methylphenaceturamide.*—Ethyl phenaceturate (22·1 g, 0·1 mol) was added to ethanol (30 ml), followed by a solution of methylamine in ethanol (33%; 50 ml, *ca.* 0·4 mol). The mixture was gently warmed until a clear solution was

[21] H. von Euler and C. Martius, *Annalen*, 1933, **505**, 73.
[22] S. Mizushima, T. Shimanouchi, M. Tsuboi, T. Sugita, E. Kato, and E. Kondo, *J. Amer. Chem. Soc.*, 1951, **73**, 1330.

obtained; crystallisation began after 1½ h. The mixture was kept for 2 h at −5° and filtered. The solid was dried by suction, washed with dry ether, and dried *in vacuo* at room temperature (H_2SO_4). A second crop had been deposited from the filtrates after 12 h at −5°. The *methylamide* (18·1 g, 90%) had m.p. 162—163° (Found: C, 64·2; H, 6·3; N, 13·5. $C_{11}H_{14}N_2O_2$ requires C, 64·0; H, 6·8; N, 13·6%), $\nu_{max.}$ 700, 745, 780, 837, 887, 910, 1028, 1070, 1102, 1160, 1200, 1255, 1280, 1322, 1350, 1375, 1418, 1445, 1455, 1500, 1570, 1640, 1655, 1970, 2580, 2810, 2950, 3100, and 3320 cm^{-1}.

Ethyl Benzylpenaldate.—Ethyl phenaceturate (11·05 g, 0·05 mole) was dissolved in sodium-dried ether (65 ml) containing a suspension of sodium (1·15 g, 0·05 g atom) and the mixture was cooled in ice to 0°. Freshly distilled ethyl formate (15 ml) was then added and the mixture was stirred at 0—4° for 19 h. The light tan-coloured sodio-derivative was separated (12 g) and mixed with crushed ice (100 g); the aqueous solution was washed with chloroform (100 ml × 3). The aqueous layer was then acidified with hydrochloric acid and extracted with chloroform (100 ml × 3). Evaporation to dryness of the dried ($MgSO_4$) chloroform extract, gave ethyl benzylpenaldate (5·2 g, 45%) as a colourless, viscous oil, $\nu_{max.}$ (film) 3400s and 1760s cm^{-1}, $\lambda_{max.}$ (0·1N-NaOH) 272 nm (ε 15,500), τ (CHCl$_3$) 2·2 (1H, broad s), 2·68 (5H, s), 5·87 (2H, q, *J* 8 Hz), 6·32 (2H, s), and 8·8 (3H, t, *J* 8 Hz). The *benzoate* had m.p. 153—154° (Found: C, 68·05; H, 5·35; N, 3·95. $C_{20}H_{19}NO_5$ requires C, 68·0; H, 5·4; N, 3·95%), $\nu_{max.}$ (KBr), 3260, 1735, 1715sh, 1665, 1655, and 1609 cm^{-1}, $\lambda_{max.}$ (EtOH) 241 (ε 14,600) and 258 nm (13,500), τ ($CDCl_3$) 1·64 (1H, s), 2—2·5 (5H, m), 2·64 (5H, s), 5·75 (2H, q, *J* 8 Hz), 6·28 (2H, s), and 8·6 (3H, t, *J* 8 Hz); the benzylamine derivative had m.p. 104—105° (lit.,[13] 105—106°) (Found: C, 71·25; H, 6·35; N, 8·3. Calc. for $C_{20}H_{22}N_2O_3$: C, 71·0; H, 6·5; N, 8·3%), $\nu_{max.}$ (KBr) 3350, 1690, 1640, and 1620 cm^{-1}, $\lambda_{max.}$ (EtOH) 281 nm (ε 21,000) (no shift in alkali), τ ($CDCl_3$) 2·72 (aromatic protons plus $CHCl_3$ peak), 2·9 (½ doublet of olefinic proton), 3·7 (1H, broad), 5·68 (2H, d, *J* 6 Hz), 5·94 (2H, q, *J* 8 Hz), 6·36 (2H, s), and 8·82 (3H, t, *J* 8 Hz). These data are consistent with the enamine structure.

A sample of this derivative (25 mg) in ethanol (10 ml) was hydrogenated over Adams catalyst for 47 h. The solution was filtered and the filtrate evaporated to dryness. The residue was hydrolysed with hydrochloric acid (2N; 4 ml) at reflux temperature for 2 h. Examination of the hydrolysate by paper chromatography [Whatman no. 3; t-butyl alcohol–acetic acid–water (2 : 1 : 1)] revealed the presence of mainly alanine with a trace of glycine.

Ethyl Benzylpenaldate Ureide.—Ethyl benzylpenaldate (3 g) and urea (9·5 g) were dissolved in 1,2-dimethoxyethane (75 ml) containing aqueous hydrochloric acid (10N; 5 ml) and the mixture was left at room temperature for 48 h. It was then diluted with water (100 ml) and extracted with chloroform (75 ml × 5). The extract was washed with water (50 ml × 3) and evaporated to dryness to give a white solid. Chromatography on silica gel, eluting with chloroform–methanol (9 : 1), gave the pure ureide (1·69 g, 46%). Crystallisation from aqueous methanol gave the *enamine form*, m.p. 218—219° [Found: C, 57·75; H, 5·8; N, 14·35%; *M* (mass spec.), 291. $C_{14}H_{17}N_3O_4$ requires C, 57·75; H, 5·85; N, 14·45%; *M*, 291], $\lambda_{max.}$ (0·1N-HCl in 50% aqueous ethanol) 266 nm (ε 22,100), $\lambda_{max.}$ (N-NaOH in 50% aqueous ethanol) 308 nm (ε 24,000), τ [$(CD_3)_2SO$] 1·02 (1H, s) 1·45 (1H, d, *J* 12 Hz, disappears on addition of D_2O), 2·2 (1H, d, *J* 12 Hz), 2·7 (5H, s), 3·42 (2H, s, disappears on addition of D_2O), 5·9 (2H, q, *J* 8 Hz), 6·38 (2H, s), and 8·82 (3H, t, *J* 8 Hz). Crystallisation from chloroform gave the *imine form*, m.p. 218—219° [Found: C, 57·55; H, 5·9; N, 14·3%; *M* (mass spec.), 291]. No u.v. absorption was observed for freshly prepared solutions, but a band slowly appeared at 266 nm because of tautomerisation to the enamine form. Attempts to obtain a satisfactory n.m.r. spectrum of the imine form failed owing to its ready conversion into the enamine form. The pK_a value of the ureide was 12·64 and was obtained by first determining the u.v. spectra at pH 7, 12, 12·5, and 14 and showing the existence of an isosbestic point at 35,600 cm^{-1}, and then applying the method of Bates and Bower [23] to optical density data at 31,500 and 33,800 cm^{-1}.

Hydrogenation of Ethyl Benzylpenaldate Ureide.—Ethyl benzylpenaldate ureide (25 mg) was dissolved in ethanol (7·5 ml), platinum oxide (10 mg.) was added, and the mixture was shaken vigorously in an atmosphere of hydrogen for 2 days. At the end of this period the u.v. absorption at 266 nm had virtually disappeared. The ethanolic solution was filtered and evaporated, giving a white solid which was hydrolysed with hydrochloric acid (2N; 5 ml) for 2 h at reflux temperature. Examination of the hydrolysate by paper chromatography [Whatman no. 3; butan-1-ol–acetic acid–water (12 : 1 : 1) or t-butyl alcohol–acetic acid–water (2 : 1 : 1)] revealed the presence mainly of alanine.

Oxidation of Ethyl Benzylpenaldate Ureide with Permanganate.—Ethyl benzylpenaldate ureide (410 mg) was dissolved in a warm mixture of 1,2-dimethoxyethane (50 ml) and 0·1N-sulphuric acid (50 ml). Saturated aqueous potassium permanganate was added dropwise with vigorous stirring during 2 h until the purple colour of permanganate persisted. The mixture was warmed to about 50° for 15 min, cooled, and filtered. The pale yellow filtrate was evaporated to dryness; the solid residue was then suspended in hot ethanol (50 ml) and filtered off. Concentration of the filtrate, followed by cooling, deposited a crystalline material (16 mg) which after several crystallisations from ethanol gave *N*-formylurea (4 mg), m.p. 165—168° (lit.,[19] 168—169°), identical with an authentic sample (i.r. and mixed m.p.).

Ethyl Benzylpenaldate N'N'-Dimethylureide.—Ethyl benzylpenaldate (0·87 g) and 1,1-dimethylurea (3 g) were dissolved in 1,2-dimethoxyethane (50 ml) containing hydrochloric acid (10N; 1 ml) and the mixture was stirred at 40° in an atmosphere of nitrogen for 24 h. It was then cooled, diluted with water (50 ml), and extracted with chloroform (60 ml × 4). The extract was washed with water (75 ml × 3), dried ($MgSO_4$), filtered, and evaporated to dryness to yield an oily product, which on chromatography on a column of silica gel (25 × 3 cm) [eluting with chloroform–methanol (19 : 1)] afforded a colourless, viscous oil (0·82 g, 74%). This solidified on scratching into a waxy substance, m.p. 97—106°. Repeated attempts to obtain a crystalline form of the ureide failed. A sample was purified by rechromatography on a column of silica gel [Found: C, 60·2; H, 6·6; N, 13·15%; *M* (mass spec.), 319. $C_{16}H_{21}N_3O_4$ requires C, 60·0; H, 6·3; N, 12·85%; *M*, 319], $\nu_{max.}$ ($CHCl_3$) 3400, 1685, and 1655 cm^{-1}, $\lambda_{max.}$ (0·1N-HCl in 50% aqueous ethanol) 277 nm (ε 17,000), $\lambda_{max.}$ (N-NaOH in 50% aqueous ethanol) 317 nm (ε 19,700), τ [$(CD_3)_2SO$] 0·65 (1H, s, disappears on addition of D_2O), 1·67 (1H, d, *J* 12 Hz, disappears

[23] R. G. Bates and V. E. Bower, *Analyt. Chem.*, 1956, **28**, 1322.

on addition D_2O), 2·30 [1H, d, J 12 Hz, singlet in D_2O–$(CD_3)_2SO$], 1·62 (5H, s), 5·78 (2H, q, J 8 Hz), 6·28 (2H, s), 7·17 (6H, s), and 8·76 (3H, t, J 8 Hz).

Ethyl Benzylpenaldate N-Methylureide.—Ethyl benzylpenaldate (985 mg) and 1-methylurea (3 g) were dissolved in 1,2-dimethoxyethane (50 ml) containing some dry hydrogen chloride and the mixture was stirred at 48° for 18 h. The u.v. spectrum of the mixture at this stage showed that little reaction had occurred, whereupon more of the urea (15 g) in water (5 ml) was added and the mixture was stirred in nitrogen for 3 days at room temperature. Water (100 ml) was then added and the aqueous solution was extracted with chloroform (75 ml × 5). The chloroform extract was washed with water (75 ml × 3), dried ($MgSO_4$), filtered, and evaporated to yield a brown, semi-solid material (820 mg). T.l.c. (silica; chloroform) of the product revealed the presence of one main component and several minor ones. The mixture was separated by using a column of silica gel (35 × 3 cm) and eluting with chloroform. The main component was obtained as colourless, silky needles (305 mg, 21%), m.p. 175—177°, identified as *ethyl benzylpenaldate N′-methylureide* (Found: C, 59·3; H, 6·05; N, 13·75. $C_{15}H_{19}N_3O_4$ requires C, 59·0; H, 6·25; N, 13·75), $\nu_{max.}$ (KBr), 3400, 3310, 1712, 1670, and 1540 cm^{-1}, $\lambda_{max.}$ (0·1N-HCl in 50% aqueous ethanol) 270 nm (ε 22,000), $\lambda_{max.}$ (N-NaOH in 50% aqueous ethanol) 312 nm (ε 24,000), τ ($CDCl_3$) 0·9 (1H, d, J 12 Hz, disappears on addition of D_2O), 2·14 (1H, d, J 12 Hz, appears as a singlet on addition of D_2O), 2·35br (1H, absent in D_2O–$CDCl_3$), 5·82 (2H, q, J 8 Hz), 6·36 (2H, s), 7·13 (3H, d, J 4 Hz, singlet in D_2O–$CDCl_3$), and 8·76 (3H, t, J 8 Hz).

Ethyl Benzylpenaldate NN′-Dimethylureide.—Ethyl benzylpenaldate (1·5 g) and a purified sample of 1,3-dimethylurea (5 g) were dissolved in a mixture of 1,2-dimethoxyethane (50 ml) and aqueous hydrochloric acid (2N; 20 ml) and the mixture was stirred at room temperature, in an atmosphere of nitrogen, for 5 days. The solution was then diluted with water (100 ml) and the aqueous solution was extracted with chloroform (75 ml × 4). The chloroform extract was washed with water (100 ml × 3), dried ($MgSO_4$), filtered, and evaporated to give an oily product (1·83 g). This was chromatographed on a column of silica gel (30 × 3 cm) (chloroform as eluant), affording *ethyl benzylpenaldate NN′-dimethylureide* (900 mg, 50%) as colourless needles (from aqueous methanol), m.p. 129·5—130° [Found: C, 60·25; H, 6·15; N, 13·05%; M (mass spec.), 319. $C_{16}H_{21}N_3O_4$ requires C, 60·2; H, 6·6; N, 13·15%; M, 319], $\nu_{max.}$ (KBr) 3400, 3330, 1705, 1670, 1655, and 1535 cm^{-1}, $\lambda_{max.}$ (0·1N-HCl in 50% aqueous ethanol) 275 nm (ε 21,000), τ [$(CD_3)_2SO$] 1·0 (1H, s, disappears on addition of D_2O), 2·08 (1H, s), 2·8 (7H, s, broad base, integrates for 5H after addition of D_2O), 5·9 (2H, q, J 8 Hz), 6·45 (2H, s), 7·06 (3H, s), 7·35 (3H, d, J 3·5 Hz, coalesces into a singlet on addition of D_2O), and 8·82 (3H, t, J 8 Hz).

Ethyl Benzylpenaldate Thioureide.—Ethyl benzylpenaldate (700 mg) and thiourea (1·2 g) were dissolved in a mixture of 1,2-dimethoxyethane (25 ml) and aqueous hydrochloric acid (10N; 2 ml) and the mixture was stirred at room temperature in nitrogen for 24 h. The solution was then diluted with water (100 ml) and extracted with chloroform (100 ml × 3). The chloroform extract was washed with water (100 ml × 2), dried ($MgSO_4$), filtered, and evaporated to yield a colourless solid (629 mg). Crystallisation from aqueous methanol afforded *ethyl benzylpenaldate thioureide* (440 mg, 51%) as colourless needles, m.p. 144—146° [Found: C, 54·8; H, 5·35; N, 13·5%; M (mass spec.), 307. $C_{14}H_{17}N_3O_3S$ requires C, 54·7; H, 5·6; N, 13·7%; M, 307], $\nu_{max.}$ (KBr) 3500, 3180, 3090, 1698, 1660, 1637, and 1600 cm^{-1}, $\lambda_{max.}$ (pH 1) 258 and 300 nm (ε 15,000 and 20,000), $\lambda_{max.}$ (pH 14) 268 and 339 nm (ε 10,870 and 28,100), τ [$(CD_3)_2SO$] 0·52 (1H, d, J 12 Hz, disappears on addition of D_2O), 0·86 (1H, s, disappears on addition of D_2O), 1·75 (3H, d, J 12 Hz, broad base, probably due to NH_2 of the thiourea, which disappears on addition of D_2O leaving a sharp singlet), 2·69 (5H, s), 5·88 (2H, q, J 8 Hz), 6·28 (2H, s), and 8·85 (3H, t, J 8 Hz).

1,3-Bis(ethyl benzylpenaldyl)urea.—Ethyl benzylpenaldate (800 mg) and urea (2·9 g) were dissolved in 1,2-dimethoxyethane (50 ml) containing dry hydrogen chloride and the stirred mixture was warmed at 45° for 48 h in an atmosphere of nitrogen. The solution was cooled, diluted with water (50 ml), and extracted with chloroform (100 ml × 3). The chloroform extract was washed with water (50 ml × 2), dried ($MgSO_4$), filtered, and evaporated to dryness to give a white solid (680 mg). T.l.c. of the product (silica; 50 : 1 chloroform–methanol) revealed the presence of two products, one corresponding to the monoureide (above), and the other a faster-moving product in smaller amount. Separation was achieved by chromatography on a column of silica gel (20 × 3 cm), eluting with chloroform–methanol (50 : 1). The product was obtained (85 mg, 11·8%) as colourless needles (from aqueous ethanol), m.p. 231·5° [Found: C, 61·7; H, 5·75; N, 10·65%; M (mass spec.), 522. $C_{27}H_{30}N_4O_7$ requires C, 62·05; H, 5·75; N, 10·75%; M, 522], $\nu_{max.}$ (KBr) 3380, 1750, 1705, and 1655 cm^{-1} (the peak at 1750 cm^{-1} suggests that at least one half of the molecule is in the imine form in the solid state), $\lambda_{max.}$ (0·1N-HCl in 50% aqueous ethanol) 295 nm (ε 38,130), $\lambda_{max.}$ (N-NaOH in 50% aqueous ethanol) 343 nm (ε 48,000), τ ($CDCl_3$; 100 MHz) −0·05 (2H, d, J 11 Hz, disappears on addition of D_2O), 2·26 (2H, d, J 11 Hz, becomes a singlet on addition of D_2O), 2·4 (2H, s, disappears on addition of D_2O), 2·64 (10H, m), 5·18 (4H, q, J 7·8 Hz), 6·28 (4H, s), and 8·76 (6H, t, J 7·8 Hz).

The monoureide was obtained (331 mg, 33%) as needles (from aqueous methanol), m.p. 221—221·5°, identical with the sample obtained previously (i.r., u.v., and n.m.r. spectra).

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