Synthesis of Capreomycidine and Epicapreomycidine, the Epimers of α-(2-Iminohexahydropyrimid-4-yl)glycine

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Capreomycidine (II) and epicapreomycidine (VIII) have been synthesised by hydrogenation and hydrolysis of 2-amino-4-(α-methoxycarbonyl-α-hydroxyimino)methylpyrimidine (VII; R = CO_2Me, R^1 = H). A similar sequence of reactions with the 6-methoxy- or 6-hydroxy-analogues failed to yield viomycidine.

THE antitubercular antibiotic capreomycin was isolated in 1960 from *Streptomyces capreolus* in the laboratories of Eli Lilly by Herr and his colleagues.[1] It was later shown [2] to be a mixture of four closely related substances, designted capreomycins 1A, 1B, 2A, and 2B, of which the first two were the major components. A review of the work which has led to the determination of the complete structure (I) of capreomycin 1B has been presented recently.[3] Acid hydrolysis of capreomycin 1B[2] gave αβ-diaminopropionic acid (2 mol.), alanine (1 mol.), β-lysine (1 mol.), and a guanidine-containing fragment, capreomycidine (1 mol.); in

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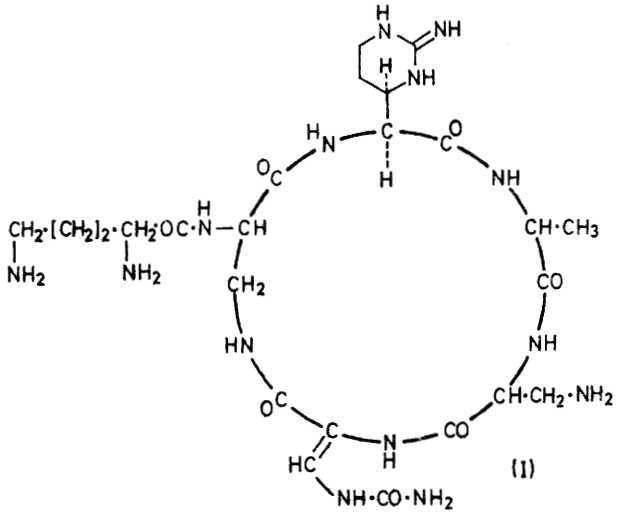
[1] E. B. Herr, M. E. Haney, G. E. Pittenger, and C. E. Higgens, *Proc. Indiana Acad. Sci.*, 1960, **69**, 134.

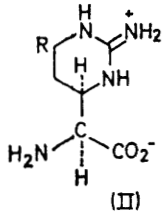
[2] E. B. Herr and M. Redstone, *Ann. New York Acad. Sci.*, 1966, **135**, 940.

[3] B. W. Bycroft, D. Cameron, L. R. Croft, A. Hassanali-Walji, A. W. Johnson, and T. Webb, *Nature*, 1971, **231**, 301.

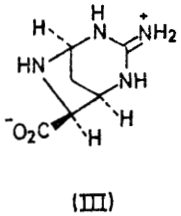
addition we have observed [3] that urea (1 mol.), carbon dioxide, and traces of glycine are formed during the hydrolysis. Acid hydrolysis of capreomycin 1A gave similar products except that alanine was replaced by serine. An interpretation of the results of titration, elemental analysis, n.m.r. spectra, and chemical tests led Herr [4] to propose structure (II; R = H), but without defining the stereochemistry, for capreomycidine. This was in accord with the molecular formula, $C_6H_{12}N_4O_2$, and the observed $pK_{a'}$ values (66% aqueous dimethylformamide) of 3·0, 7·6, and 13·8. Later studies have shown that capreomycidine is one of a family of cyclic guanido amino-acids, probably derived biogenetically from arginine and which include the precursor (II; R = OH) [5] of viomycidine (III) from



(I)



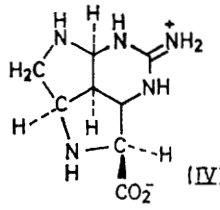
(II)



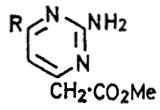
(III)

viomycin,[6] and several other examples.[7] The amino-acid (II; R = OH) has also been isolated from tuberactinomycin.[8] The relative stereochemistry of the asymmetric centres of (II) was established by interpretations of the n.m.r. spectra of viomycidine [9] and later the absolute stereochemistry was established by *X*-ray examinations of viocidic acid (IV),[10] another acid hydrolysis product from viomycin, and viomycidine.[11,12] Hydrogenolysis of viomycin, followed by hydrolysis gave capreomycidine and thus provided evidence that the configurations at the asymmetric centres of the two degradation products capreomycidine and viomycidine were identical.[13]

For confirmation of the structure of capreomycidine (II; R = H) by synthesis it was our intention to devise a method which might be adapted also to a synthesis of viomycidine (III) or its precursor (II; R = OH). The basic intermediate for our scheme was methyl 2-amino-6-hydroxy-pyrimid-4-ylacetic acid (V; R =



(IV)



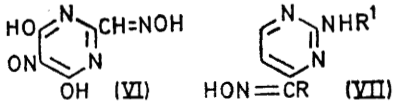
(V)

OH), prepared by condensation of guanidine carbonate with diethyl acetonedicarboxylate.[14] The reported properties of analogues of the pyrimidine (V; R = OH), *e.g.* the oximation of 2-hydroxy-4,6-dimethylpyrimidine at the C-4 methyl group,[15] led us to believe that the C-4 methylene substituents would be sufficiently active to permit C-nitrosation. However, nuclear nitrosation at C-5 was clearly a further possibility and one which had to be avoided; thus, for example, the conversion of 4,6-dihydroxy-2-methylpyrimidine into the nitroso-oxime derivative (VI) has been recorded.[16]

Methyl 2-amino-6-hydroxypyrimid-4-ylacetate (V; R = OH) was readily converted into (V; R = Cl) with phosphorus oxychloride but attempts to remove chlorine with zinc and boiling water resulted in good yields of 2-amino-4-methylpyrimidine, *i.e.* hydrolysis and decarboxylation had occurred as well as dechlorination. The ease of decarboxylation of the related 2,6-dihydroxypyrimid-4-ylacetic acid has been referred to by Russian authors.[17] However, hydrogenolysis of the chloro-derivative (V; R = Cl) in presence of palladium–charcoal and magnesium oxide gave a good yield of methyl 2-aminopyrimid-4-ylacetate (V; R = H), which did not melt sharply; it was converted into the 2-acetylamino-derivative. Attempts to oximate this product using King and King's method [16] which involved dissolution in alkali as a first step, again caused hydrolysis and decarboxylation with the formation of

[4] E. B. Herr, *Antimicrobial Agents and Chemotherapy*, 1962, 201.
[5] B. W. Bycroft, D. Cameron, L. R. Croft, A. W. Johnson, T. Webb, and P. Coggon, *Tetrahedron Letters*, 1968, 2925.
[6] B. W. Bycroft, D. Cameron, L. R. Croft, A. Hassanali-Walji, A. W. Johnson, and T. Webb, *Experientia*, 1971, **27**, 501.
[7] B. W. Bycroft, L. R. Croft, A. W. Johnson, and T. Webb, paper in preparation.
[8] T. Wakamiya, T. Shiba, T. Kaneko, H. Sakakibara, T. Take, and J. Abe, *Tetrahedron Letters*, 1970, 3497.
[9] Professor G. Büchi, private communication. J. A. Raleigh, Ph.D. Thesis, M.I.T., 1966.
[10] P. Coggon, *J. Chem. Soc.* (*B*), 1970, 838.
[11] J. C. Floyd, J. A. Bertrand, and J. R. Dyer, *Chem. Comm.*, 1968, 998.
[12] T. Takita and K. Maeda, *J. Antibiotics*, 1969, **22**, 34.
[13] B. W. Bycroft, L. R. Croft, A. W. Johnson, and T. Webb, *J. Antibiotics*, 1969, **22**, 133.
[14] D. E. Worrall, *J. Amer. Chem. Soc.*, 1943, **65**, 2053.
[15] A. J. Boulton, D. T. Hurst, J. F. W. McOmie, and M. S. Tute, *J. Chem. Soc.* (*C*), 1967, 1202.
[16] F. E. King and T. J. King, *J. Chem. Soc.*, 1947, 943.
[17] Y. P. Shvachkin, M. K. Berestenko, and G. P. Mishin, *J. Gen. Chem., U.S.S.R.*, 1964, **34**, 1687.

2-acetylamino-4-hydroxyiminomethylpyrimidine (VII; R = H, R^1 = Ac). However, when an aqueous solution of methyl 2-acetylaminopyrimid-4-ylacetate was treated with sodium nitrite and acidified to pH 2—3 with dilute hydrochloric acid, an excellent yield of the desired hydroxyimino-derivative (VII; R = CO_2Me, R^1 = Ac) was obtained. Attempts to substitute the hydroxy-



group of methyl 2-acetylamino-6-hydroxypyrimid-4-ylacetate by chlorine failed in an attempt to carry out similar transformations in the 2-acetylamino-series, but this intermediate [*N*-acetyl derivative of (V; R = OH)] was used to establish the best conditions for the removal of the *N*-acetyl group while retaining the ester. Refluxing the *N*-acetyl derivative with methanolic hydrogen chloride proved to be satisfactory and gave (V; R = OH) in high yield. Accordingly (VII; R = CO_2Me, R^1 = Ac) was treated in a similar manner when the corresponding amino-derivative (VII; R = CO_2Me, R^1 = H) was obtained (78%). N.m.r. evidence was obtained for the existence of the amine–imine isomers in this product, and similar evidence for the existence of *syn*- and *anti*-forms of the various oximes was also found. The oximes all gave sensitive and intense blue ferrous reactions. Hydrogenation of (VII; R = CO_2Me, R^1 = H) in acidified aqueous methanol in presence of 5% palladium–charcoal, followed by acid hydrolysis gave a solution which had chromatographic properties similar to those of natural capreomycidine. The acid solution was passed through a column of basic ion exchange resin and saturated picric acid solution was added to bring the pH to 6—7. The picrate so obtained was fractionally crystallised to give two picrates which had identical chromatographic properties but markedly different i.r. spectra particularly in the region 650—800 cm^{-1}. These i.r. spectral differences were used as a guide to the separation. Both picrates, after purification, had analyses consistent with the formula $C_{12}H_{15}N_7O_8,H_2O$ and it appeared that they were the picrates of the racemic forms of capreomycidine and epicapreomycidine. They were initially termed racemate A picrate and racemate B picrate and each was converted into the corresponding base by treatment of hot aqueous solutions with basic ion exchange resin. The i.r. spectra of the two bases (potassium bromide discs; solubility properties precluded measurements in chloroform or carbon tetrachloride solution) also differed between themselves and that of natural capreomycidine (Figure 1) although the chromatographic properties were identical. However, the n.m.r. spectra of the monohydrochlorides of racemate A and racemate B in deuterium oxide at both 60 and 100 MHz also showed differences, particularly in the τ 5—7 region, but they also indicated (Figure 2) that racemate A hydrochloride corresponded to the salt of natural capreomycidine and thus racemate B is epicapreomycidine (VIII).

After our work had been completed,[18] Takita and Maeda [19] reported that reduction of viomycin with sodium borohydride followed by hydrolysis gave a

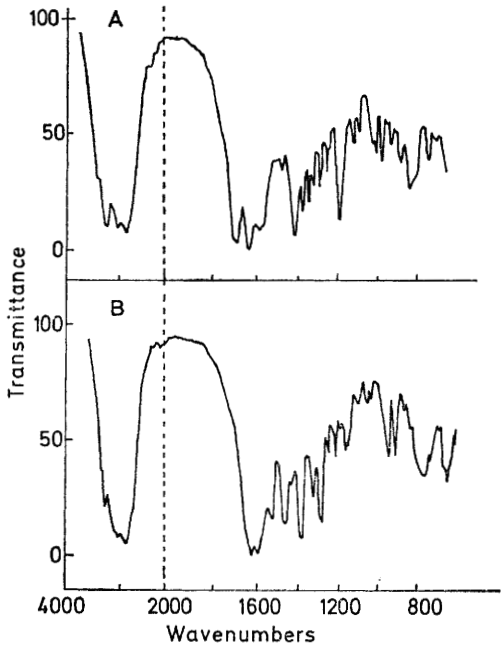


FIGURE 1 I.r. spectra (KBr discs) of synthetic, racemic capreomycidine and epicapreomycidine

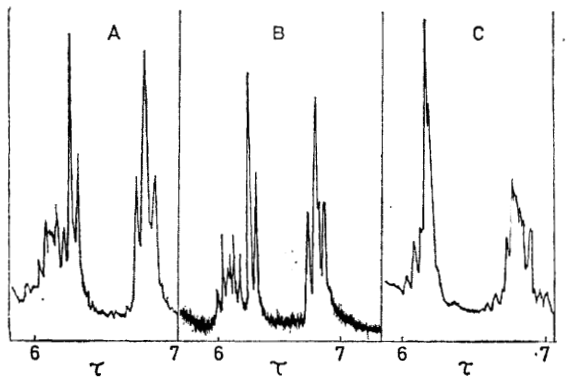


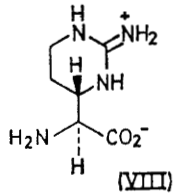
FIGURE 2 N.m.r. spectra (100 MHz D_2O) of the hydrochlorides of synthetic capreomycidine, epicapreomycidine, and capreomycidine from capreomycin

compound which they called dihydroviomycidine hydrochloride, $C_6H_{12}N_4O_2,\frac{3}{2}H_2O,HCl$, $[\alpha]_D^{25}$ +25°, with an equivalent of 241 and showing positive ninhydrin and Sakaguchi tests. Comparison of the n.m.r. spectrum of their product with that of capreomycidine hydrochloride led them to conclude that 'dihydroviomycidine' and capreomycidine were diastereoisomers, *i.e.* 'dihydroviomycidine' was epicapreomycidine (VIII).

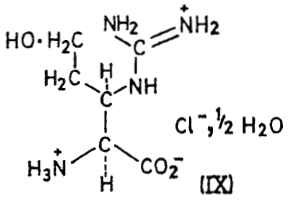
However the n.m.r. spectrum of 'dihydroviomycid-

[18] B. W. Bycroft, D. Cameron, L. R. Croft, and A. W. Johnson, *Chem. Comm.*, 1968, 1301.
[19] T. Takita and K. Maeda, *J. Antibiotics*, 1968, **21**, 512.

ine ' hydrochloride differed from that of our synthetic racemic epicapreomycidine hydrochloride and a reconsideration of the Japanese work led us to the conclusion that ' dihydroviomycidine ' hydrochloride was



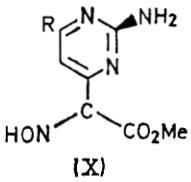
(VIII)



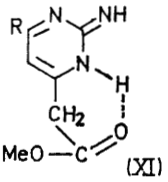
(IX)

more likely to have structure (IX), *i.e.* it was formed by reduction of the open-chain tautomer of the guanidine-carbinol system of the viomycidine precursor; it was also noted that this structure agreed closer with the quoted analytical results.[7,13] Further work by Takita and Maeda [12] confirmed these conclusions. They also prepared an *O*-acetate of ' dihydroviomycidine ', the structure of which was confirmed by i.r. and n.m.r. spectroscopy, and they accepted our views on the structure of their product.

In attempts to extend the above synthetic approach to a preparation of viomycidine (III), the reduction of 6-methoxypyrimidine oximes of type (X; R = OMe) was examined, when it was hoped that the 6-methoxy-substituent of the reduced pyrimidine ring might be subject to nucleophilic substitution by the developing amino-group of the side-chain. 2-Amino-6-methoxy-4-methoxycarbonylmethylpyrimidine (XI; R = OMe) was prepared from the 6-chloro-compound with sodium methoxide in methanol. It showed an ester carbonyl band at 1700 cm^{-1}, indicative of fairly strong hydrogen bonding between the ester carbonyl and the nuclear 3-imino-group [as in (XI)], whereas the corresponding 6-chloro-compound, in contrast, showed the ester carbonyl group at 1738 cm^{-1}.



(X)



(XI)

On one occasion, a metastable isomer of (XI; R = OMe) with a lower m.p. was observed and this may have contained the guanidine group in a tautomeric form. The lower-melting form readily reverted to the stable form. *N*-Acetyl and *N*-benzoyl derivatives of (XI; R = OMe) were prepared and although reaction of the former with nitrous acid, liberated from sodium nitrite with hydrochloric acid, gave a mixture of isomeric products, similar reaction with nitrous acid, liberated by acetic acid, gave a single isomer [*N*-acetyl derivative of (X)]. This material was deacetylated and then hydrogenated but no viomycidine could be isolated from the product.

A parallel series of reactions was carried out with the 6-hydroxypyrimidines and the compound (X; R = OH) was prepared as a single isomer although evidence of isomerism was obtained when it was dissolved in trifluoroacetic acid. The product was deacetylated and hydrogenated but once again it was not possible to isolate any viomycidine from the product. The pyrimidine α-hydroxyimino-esters (X) offer many possibilities for tautomerism, *e.g.* the oxime may hydrogen bond with either the ester carbonyl group, or the nuclear imino-group and the cyclic guanidine may exist with an exocyclic imino- or amino-grouping. Evidence for the existence of isomeric forms of certain of these derivatives particularly (X; R = H), was obtained from n.m.r. spectra and details are given in the Experimental section.

EXPERIMENTAL

M.p.s were determined with a Kofler block. I.r. spectra, except where otherwise stated, are for potassium bromide discs using a Unicam SP200 spectrophotometer. N.m.r. spectra were measured with a Perkin-Elmer R10 instrument at 60 MHz and with a Varian HA100 instrument at 100 MHz using D_2O solutions.

2-Amino-4-methoxycarbonylmethyl-6-hydroxypyrimidine (V; R = OH).—Prepared from dimethyl acetonedicarboxylate, (86%), by the method of Worrall,[14] this compound had m.p. 194—197° (lit., 192—193°). The *N-acetyl* derivative formed colourless needles, m.p. 187—188·5° (from methanol) (Found: C, 48·05; H, 4·8; N, 18·5. $C_9H_{11}N_3O_4$ requires C, 48·0; H, 4·9; N, 18·7%). The n.m.r. spectrum contained signals at τ 3·89 (1H, s, 5-H), 6·29 (3H, s, ester methyl protons), 6·53 (2H, s, methylene protons), and 7·74 (3H, s, acetylamino methyl protons); $\lambda_{max.}$ 235·5, 243·5 (infl.), and 289·5 nm. (ε 12,600, 8870, and 8170). No evidence of isomerism was detected in this compound. Deacetylation of the *N*-acetyl derivative with methanolic hydrogen chloride gave the original aminopyrimidine.

2-Amino-4-methoxycarbonylmethyl-6-chloropyrimidine (V; R = Cl).—Phosphorus oxychloride (283 ml., 3·08 mol.) was added to 2-amino-4-methoxycarbonylmethyl-6-hydroxypyrimidine (100 g., 0·546 mol.; m.p. 194—197°, prepared by the method of Worrall [14]) with vigorous stirring. The resulting mixture was heated on a steam-bath for *ca.* 10 min. when a turbid orange solution was obtained. Heating was continued for a further 10—15 min. after which the reaction mass was allowed to cool with stirring for *ca.* 1½ hr.; finally it was cooled to room temperature with external cooling if necessary. The dark red solution (usually slightly turbid) was then added cautiously and portionwise to ice (2½ kg.), stirring being started as soon as sufficient ice had melted. Ammonia (*d*, 0·88; 550 ml.) was added along with ice to keep the temperature below 30° and the deep red strongly acid solution was filtered to remove tar. The filtrates were treated with more ammonia (*d*. 0·88; *ca.* 200 ml.) at less than 30° and refiltered to remove more tar. Tar was removed in this manner until the solution was pale yellow in colour and still strongly acidic. Two treatments usually sufficed. The pale orange filtrates were then stirred and made alkaline (pH 7—8) with ammonia (*d*. 0·88; 200 ml.), causing precipitation of the chloro-compound; occasionally this came down as an oil and seeding was necessary

to effect solidification. The suspension was separated and the filtrates were concentrated and then extracted portionwise with chloroform (total 900 ml.). The wet filter cake was then added to the chloroform solution along with a further 200 ml. of chloroform; the resulting solution was dried (Na_2SO_4). Removal of the solvent yielded the crude product (79·7 g., 72·5%), m.p. 94—103°. A purer product (m.p. 100—103°, yield 55—60%) was obtained when only the filter cake was extracted with chloroform. The crude product was purified by sublimation (100°/0·1 mm.) or by crystallisation from water. Material crystallised from water showed n.m.r. signals (Me_4Si τ 10·04) at τ 3·32 (1H, s, 5-H), 6·29 (3H, s, ester methyl protons) 6·42 (2H, s, methylene protons) 4·0—4·6 (2H, broad s, amino protons). Sublimed material had m.p. 103·5—104·5° (Found: C, 42·0; H, 4·05; Cl, 17·5; N, 21·1. $C_7H_8ClN_3O_2$ requires C, 41·7; H, 3·95; Cl, 17·15; N, 20·9%), $\lambda_{max.}$ 233 and 299 nm. ($\varepsilon_{max.}$ 15,160 and 4940). No evidence of isomerism (i.r.) was observed with this compound, $\upsilon_{max.}$ 1738 cm^{-1} (ester carbonyl).

2-Amino-4-methoxycarbonylmethylpyrimidine (V; R = H).—2-Amino-4-methoxycarbonylmethyl-6-chloropyrimidine (30 g., 0·149 mol.) was dissolved in methanol (560 ml.). 5% Palladium–charcoal catalyst (7·5 g.), magnesium oxide (7·5 g.), and water (560 ml.) were added and the mixture was shaken vigorously until the theoretical quantity of hydrogen (0·149 mol.) had been absorbed. The rate of hydrogenation was erratic, the time required varying from *ca.* 17 hr. to *ca.* 30 hr. The suspension was separated and the residue was extracted with chloroform (200 ml.). The filtrates were also extracted with chloroform (4 × 200 ml.) and the combined chloroform extracts were dried ($NaSO_4$) and filtered; the solvent was removed to yield a pale yellow solid (16·6 g.). Concentration of the once-extracted filtrates followed by further chloroform extraction, yielded a further crop (1·26 g.). The crude material was purified by crystallisation from benzene (charcoal) yielding large rhombohedral *crystals*, m.p. 120—142° (with previous softening) (Found: C, 49·95; H, 5·35; N, 24·9. $C_7H_9N_3O_2$ requires C, 50·2; H, 5·4; N, 25·2%). The n.m.r. spectrum contained signals at (Me_4Si τ 10·01): τ 1·75 (1H, d, J = 5·4 Hz, 6-H), 3·39 (1H, d, J 5·4 Hz, 5-H), 4·3—4·8 (2H, broad s, amino protons), 6·29 (3H, s, ester methyl protons), and 6·4 (2H, s, methylene protons); $\lambda_{max.}$ 228·5 and 296 nm. ($\varepsilon_{max.}$ 13,640 and 3920). On occasions material was isolated from the hydrogenation reaction mixture which had an i.r. spectrum different from that of the above product. However, the n.m.r. spectra of the two products were identical. Thus, this compound may exhibit isomerism in the solid state (which may not be detectable in chloroform solution). The physical data quoted above refers to the material normally isolated and purified. The i.r. and n.m.r. spectra of this material did not change after crystallisation but the wide melting point range suggests that heating may cause some isomerisation.

If ethanol instead of methanol is used during the reduction, some ester interchange occurs (n.m.r.).

The N-*acetyl* derivative, prepared by reaction of the amino-compound with acetic anhydride, was sublimed at 125°/0·1 mm. to yield colourless crystals, m.p. 118·5—120° (Found: C, 51·9; H, 5·35; N, 20·45. $C_9H_{11}N_3O_3$ requires C, 51·7; H, 5·25; N, 20·05%). The n.m.r. spectrum contained signals at τ 0·3—0·6 (1H, broad s, NH group), 1·26 (1H, d, J 5·4 Hz, 6-H), 2·8 (1H, d, J 5·4 Hz), 6·16 (2H, s, methylene protons), 6·2 (3H, s, ester methyl protons), and 7·42 (3H, s, acetylamino methyl protons); $\lambda_{max.}$ 236, 266, 310 (infl.), and 335·5 nm. (infl.) ($\varepsilon_{max.}$ 18,400, 4860, 935, and 635). No evidence of isomerism was observed in this derivative.

2-Amino-4-methylpyrimidine.—2-Amino-6-chloro-4-methoxycarbonylmethylpyrimidine (2·58 g., 0·0128 mol.) was added to water (250 ml.). Zinc dust (20 g.) was added and the mixture was stirred, and heated under reflux for 4 hr. The hot mixture was then separated and the pale yellow filtrates were cooled and extracted with chloroform (3 × 100 ml.). The chloroform extracts were dried (Na_2SO_4), filtered, and the solvent removed to yield a yellow solid (0·95 g., 68%), m.p. 154—156°. After sublimation at 100°/0·1 mm., the product formed colourless crystals, m.p. 159—160° (lit.,[20] 159—160°). The n.m.r. spectrum showed signals at τ 1·76 (1H, d, J 5·4 Hz, 6-H), 3·46 (1H, d, J 5·4 Hz, 5-H), 4·4—4·9 (2H, broad s, amino protons), and 7·69 (3H, s, C-4 methyl protons). Treatment of the chloro-compound with zinc dust and water at either 50—55°/2 hr. or 100°/15 min. caused little reaction.

2-Acetamido-4-(α-methoxycarbonyl-α-hydroxyimino)-methylpyrimidine (VII; R = CO_2Me, R^1 = COMe).—2-Acetamido-4-methoxycarbonylmethylpyrimidine (above; 18 g., 0·086 mol.) was dissolved in water (1 l.). Sodium nitrite (8·3 g.; 0·12 mol.) was added and the solution was acidified (pH 2—3) by the addition of concentrated hydrochloric acid. The solution was kept in the cold room (−5°) for 3 hr. by which time the product had precipitated. The buff-coloured oxime was separated, washed with water, methanol, and ether, and dried *in vacuo* at room temperature (18·2 g., 88·5%). Crystallisation from water (charcoal) yielded colourless needles, m.p. 229—231·5° (Found: C, 45·2; H, 3·95; N, 23·4. $C_9H_{10}N_4O_4$ requires C, 45·3; H, 4·2; N, 23·5%). The n.m.r. spectrum showed signals (i) in dimethyl sulphoxide (DMSO), at τ −2·8 to −3·4 (1H, broad, oxime proton), −0·67 (1H, s, imino-proton), 1·20 (1H, d, J 5·4 Hz; 6-H), 2·41 (1H, d, J 5·4 Hz, 5-H), 6·02 (3H, s, methyl ester protons), and 7·62 (*ca.* 3H, s, acetylamino methyl protons; integration uncertain because of DMSO peaks); (ii) in CF_3CO_2H at τ 1·17 (1H, d, J 6 Hz, 6-H), 1·77 (1H, d, J 6 Hz, 5-H), 5·83 (3H, s, ester methyl protons), and 7·38 (3H, s, acetylamino methyl protons). In addition small doublets were observed at τ 0·9 (1H, J 6 Hz) and 1·85 (1H, J 6 Hz) and a shoulder was observed on the signal at τ 5·83. The presence of these small signals may suggest that some isomerisation is caused by trifluoroacetic acid; $\lambda_{max.}$ 240, 298, and 352 (infl.) nm. (ε 27,800, 4590, and 558). Addition of ferrous sulphate to an aqueous solution of the product gave an intense blue colour which was discharged on addition of acid but regenerated by the further addition of sodium hydrogen carbonate.

2-Acetamino-4-hydroxyiminomethylpyrimidine (VII; R = H, R^1 = COMe).—2-Acetamino-4-methoxycarbonylmethylpyrimidine (*ca.* 300 mg. as a paste in acetic anhydride) was dissolved in 2N-aqueous potassium hydroxide (10 ml.) giving a yellow solution. Sodium nitrite (200 mg.) was added and the solution was cooled to 0° followed by concentrated hydrochloric acid dropwise until pH 3—4 was obtained. The off-white precipitate was separated, washed with water and then acetone and ether, and finally

[20] S. Gabriel and J. Colman, *Ber.*, 1898, **32**, 2921; E. Benary, *Ber.*, 1930, **63**, 2601.

dried *in vacuo* at room temperature. The product (174 mg.) was crystallised from water to yield colourless crystals, m.p. 228—229·5° (Found: C, 46·95; H, 4·75; N, 31·1. $C_7H_8N_4O_2$ requires C, 46·6; H, 4·4; N, 31·1%). $\lambda_{max.}$ 216·5 (infl.) 283·5, and 293 nm. ($\varepsilon_{max.}$ 11,600, 30,200, and 5480). The n.m.r. spectrum (i) (in $CD_3{\cdot}SO{\cdot}CD_3$) showed signals at τ 1·23 (1H, broad d, J 5·4 Hz, 6-H), 1·89 (1H, s, side-chain methine proton), 2·54 (1H, sharp d, J 5·4 Hz, 5-H), and 7·7 (3H, s, acetylamino methyl protons) (ii) in DMSO: signals at τ −0·52 (broad s, 1H, imino proton) 1·33 (1H, broad d, J 5·4 Hz, 6-H), 1·99 (1H, s, side-chain methine proton), 2·5 (1H, broad d, J 5·4 Hz, 5-H), and 7·79 (3H, s, acetylamino methyl protons); $\nu_{max.}$ 1658, 2990, 3100sh, and 3200 cm.$^{-1}$. No evidence of isomerisation was observed. The product gave an intense purple ferrous reaction, the colour being discharged by acid but reappeared on addition of sodium hydrogen carbonate.

2-*Amino*-4-(α-*methoxycarbonyl*-α-*hydroxyimino*)*methylpyrimidine Hydrochloride* (VII; R = CO_2Me, R^1 = H).—(i) 2-Acetamido-4-(α-methoxycarbonyl-α-hydroxyimino)-methylpyrimidine (15 g., 0·063 mol.) was added to methanol (150 ml.) which had been saturated with dry hydrogen chloride. The mixture was heated under reflux for 1½ hr., the odour of methyl acetate soon being detected at the top of the condenser. The resulting solution was cooled, seeded, and then kept at −5° when yellow crystals slowly appeared. The product was separated, washed with a little methanol and then dry ether, and then dried *in vacuo* at room temperature (9 g.). A second crop (2·4 g.) was obtained from the filtrates after several days at −5°. Crystallisation from methanol (charcoal) gave pale yellow crystals, m.p. 190—192° (dec.) (Found: C, 36·35; H, 4·05; Cl, 15·6; N, 24·25. $C_7H_9ClN_4O_3$ requires C, 36·1; H, 3·85; Cl, 15·3; N, 24·1%). The n.m.r. spectrum (D_2O) contained signals at τ 1·72 (1H, d, J 7·2 Hz, 6-H), 2·63 (1H, d, J 7·2 Hz, 5-H), and 6·05 (3H, s, ester methyl protons); $\lambda_{max.}$ 238 and 330 nm. (ε 22,390 and 3820); $\nu_{max.}$ 670, 722, 740, 763, 799, 845, 905, 963, 983, 1019, 1052, 1082, 1199, 1255, 1330, 1438, 1470, 1518, 1620, 1675, 1730, 2820, 3050, 3150, and 3390 cm.$^{-1}$. The salt gave an intense ferrous reaction in presence of sodium hydrogen carbonate.

(ii) 2-Amino-4-methoxycarbonylmethylpyrimidine (0·5 g.) was dissolved in water (40 ml.), and sodium nitrite (slight excess) was added to the solution followed by concentrated hydrochloric acid to pH 2. Precipitation then commenced and after the mixture had been kept for 1¾ hr., the yellow product was separated, and was washed with water, methanol, and ether; it was then dried *in vacuo* at room temperature, to give a product, isomer A (138 mg.), m.p. 210·5—213·5° (d); $\upsilon_{max.}$ 678, 718, 740, 778, 799, 838, 952, 1030, 1098, 1143, 1218, 1251, 1310, 1355, 1450, 1475, 1490, 1570, 1630, 1650, 1738, 2720, 2810, 3020, 3380, and 3470 cm.$^{-1}$.

The filtrates from the above reaction were basified (pH 8) with sodium hydrogen carbonate and the resulting yellow precipitate was separated after 1 hr., washed with water and a little methanol and ether, and dried at room temperature *in vacuo* (173 mg.), $\nu_{max.}$ 682, 721, 780, 800, 820, 840, 892, 905, 970, 1042, 1095, 1140, 1222, 1250, 1310, 1338, 1352, 1470, 1520, 1581, 1630, 1738, 2650 (very broad), 3250, 3410, and 3480 cm.$^{-1}$. This material is termed isomer B. Crystallisation from water (charcoal) yielded colourless plates which were dried *in vacuo* (Found: C, 42·35; H, 4·15; N, 28·65. $C_7H_8N_4O_3$ requires C, 42·8; H, 4·1; N, 28·6%). The n.m.r. spectrum (DMSO) showed signals at τ 1·53 (1H, d, J 5·4 Hz, 6-H), 2·91 (1H, d, J 5·4 Hz, 5-H), 3·16 (2H, broad s, amino protons), and 6·03 (3H, s, ester methyl protons). When heated, isomer B underwent crystal change at 165—175° and finally melted at 210—213° (dec.); a sample heated at 180°/2 hr. reverted to isomer A (i.r. spectrum). The n.m.r. spectrum of isomer A was similar to that of isomer B except that the signals were shifted slightly (τ *ca.* 0·04) to higher field. Basification of an aqueous solution of the hydrochloride with sodium hydrogen carbonate precipitated isomer B (i.r. spectrum).

α-(2-*Iminohexahydropyrimid*-4-*yl*)*glycine Isomers*.—(i) *Picrates*. 2-Amino-4-(α-methoxycarbonyl-α-hydroxyimino)methylpyrimidine hydrochloride (8 g., 0·036 mol.) was dissolved in a mixture of distilled water (400 ml.) methanol (400 ml.), and concentrated hydrochloric acid (5 ml.). 5% Palladium–charcoal catalyst (6 g.) was added and the mixture was shaken vigorously in an atmosphere of hydrogen until no further hydrogen was absorbed. The reduction period varied from *ca.* 20 hr. to *ca.* 50 hr. The mixture was filtered and the colourless filtrate was concentrated (to *ca.* 60 ml.) *in vacuo*; the aqueous acidic solution was used without further treatment for the preparation of α-(2-iminohexahydro-4-pyrimidyl)glycine. The acid solution was heated on a steam-bath for 5 hr. and cooled. Paper chromatographic examination of this solution using t-butyl alcohol–acetic acid–water (2 : 1 : 1) as solvent showed only one ninhydrin-positive spot (R_F 0·3—0·33), which corresponded to that obtained from a solution of natural capreomycidine in dilute hydrochloric acid. The cooled solution was then passed down a column of Amberlite resin 6400 (OH^-) which had been washed alkali free (pH 7) with distilled water just prior to use. The eluant was discarded until it became strongly alkaline (>pH 11) when collection was started. Distilled water was added to the column as necessary and the eluant collected until the alkalinity began to decrease (pH 9). Elution and collection of a second fraction was continued until the eluant had pH 7. Fraction 1 from the Amberlite 6400 (OH^-) column (above) was filtered to remove a small amount of flocculent material which was deposited when the fraction was kept. Saturated aqueous picric acid solution was added to the filtrates until the pH was 6—7 when crystallisation soon commenced. The mixture was kept for 2 hr. after which the picrate was filtered off and washed with water (2 × 20 ml.), methanol, and dry ether; it was then dried *in vacuo* at room temperature (5·13 g.). The washings were added to the mixed filtrates and by gradual concentration of the solution, 3 further crops were obtained. The second fraction from the Amberlite column was treated similarly. Fractional crystallisation of the picrates (total 7·831 g., 54·2%) from water was carried out, the i.r. spectra, expecially in the region 650—800 cm.$^{-1}$, being used as a criterion of identity and purity. By this means material (2·916 g.) of predominantly racemate A picrate and material (2·046 g.) of predominantly racemate B picrate was obtained. The former was again crystallised from water to give the pure derivative (2·218 g.), m.p. 152—155° (not sharp) (Found: C, 34·5; H, 4·05; N, 23·3. $C_{12}H_{15}N_7O_8,H_2O$ requires C, 34·3; H, 4·05; N, 23·4%). $\upsilon_{max.}$ 650, 685, 710, 725, 745, and 785 cm.$^{-1}$ in the 650—800 cm.$^{-1}$ region. A similar crystallisation of the racemate B

picrate from water gave the pure derivative (1·489 g.) m.p. 166—169° (not sharp) (Found: C, 34·25; H, 4·01; N, 23·0%), $\nu_{max.}$ 650, 702, 740, and 785 cm.$^{-1}$ in the 650—800 cm.$^{-1}$ *region*. Attempted chromatographic separation of the picrates on paper was unsuccessful.

(ii) *Hydrochlorides*.—The racemate A picrate of α-(2-iminohexahydro-4-pyrimidyl)glycine (2 g., 0·00475 mol.) was dissolved in boiling water (200 ml.) and the hot solution was passed down a column of Amberlite resin 6400 (OH^-) which had been washed alkali free (pH 7) just prior to use. When the eluant became alkaline (pH 9) collection was started. Hot water was added to the column as necessary and the eluant was collected until the alkalinity decreased (pH 7). The bulk of the water was removed *in vacuo* and the remaining small volume was either seeded or scratched until crystallisation commenced; when this appeared complete, the mushy mixture was dried on the rotary evaporator at 50—60°/15 mm. for ½—1 hr. to yield the solid product (0·78 g., 90·6% assuming the base is not hydrated). Racemate B base was prepared from the picrate in an identical manner. Rapid evaporation of solutions of either base produced a glass. In chromatography on paper using t-butyl alcohol–acetic acid–water (2 : 1 : 1) as solvent, both racemates as well as natural capreomycidine had identical R_F values (0·3—0·33). The hydrochlorides were prepared from solutions of the bases in the minimum quantity of distilled water, the theoretical quantity of 0·1N-hydrochloric acid being added and the solution being concentrated to low volume *in vacuo*. Crystallisation soon commenced after seeding or scratching, and when this appeared complete the mixture was dried on a rotary evaporator at 50—60°/15 mm. for ½—1 hr. The 100 MHz n.m.r. spectra (D_2O) of the two isomeric hydrochlorides is shown as well as that of natural capreomycidine hydrochloride in Figure 2. Racemate A hydrochloride (Found: C, 31·95; H, 6·7; N, 24·4. $C_6H_{13}ClN_4O_2,H_2O$ requires C, 31·8; H, 6·6; N, 24·75%), $\nu_{max.}$ 690, 715, 792, 852, 879, 924, 972, 1110, 1125, 1181, 1218, 1278, 1321, 1339, 1368, 1390, 1445, 1507, 1572 (shoulder), 1595 (shoulder), 1630, 1670, 2950, 3100, 3210, and 3380 cm.$^{-1}$; τ 5·9 (1H, m, 2-H), 6·09 (1H, d, J 4·8 Hz 1-H), 6·6 (t, J 6·0 Hz, C-4, methylene protons), and 7·95 (m, C-3 methylene protons). Racemate B hydrochloride (Found: C, 31·7; H, 6·7; N, 24·6%). $\nu_{max.}$ 692, 718, 775, 814, 839, 893, 932, 980, 1060, 1098, 1162, 1177, 1218, 1238, 1262, 1318, 1343, 1399, 1518, 1585, 1618, 1634, 1663, 2310 (shoulder), 2490, 2550, 2860, 2950, 3110, 3270, and 3380 cm.$^{-1}$. τ 5·97 (1H, m, 2-H) superimposed on 5·98 (1H, d, J 4·8 Hz, 1-H), 6·65 (m, C-4 methylene protons), and 8·07 (m, C-3 methylene protons).

(iii) 2,4-*Dinitrophenyl derivative*. Prepared from the mixed isomers of the base (500 mg.) the product formed yellow crystals m.p. 255—257° (dec.) (from aqueous methanol) (Found: C, 42·4; H, 4·3; N, 25·05. $C_{12}H_{14}N_6O_6$ requires C, 42·5; H, 4·15; N, 24·8%).

2-*Amino*-6-*methoxy*-4-*methoxycarbonylmethylpyrimidine* (V; R = OMe).—2-Amino-4-carbomethoxymethyl-6-chloropyrimidine (13·1 g., 0·065 mol.; above) was added to a solution of sodium (2·04 g., 0·088 g.-atom) in methanol (AnalaR; 190 ml.) and the mixture was heated under reflux for 2 hr. The chloropyrimidine soon dissolved and sodium chloride slowly deposited. The mixture was cooled to room temperature and the salt separated. The filtrates were normally brown/amber in colour at this stage; the solvent was removed from the filtrates *in vacuo* and water (10—20 ml.) was added to the yellow residue. Aqueous hydrochloric acid (50%) was added to bring the pH to 2—3 and the mixture was concentrated. Decolourising charcoal was added and the hot solution was filtered. The filtrate was cooled to room temperature and solid sodium hydrogen carbonate was added until the pH was 7—8, when the product precipitated. The mixture was kept for 1 hr., separated, and the product washed with a little water, dried by suction, and then dried at 60—70° for 2 hr. The yellow solid (8 g., 62%) had m.p. 103—106°. The n.m.r. spectrum of this material ($CDCl_3$) showed signals at τ 3·92 (1H, s, 5-H), 4·2—4·8 (2H, broad s, amino protons), 6·12 (3H, s, methoxy protons), 6·29 (3H, s, ester methyl protons), and 6·5 (2H, s, methylene protons). After sublimation at 110—120°/0·1 mm., colourless crystals, m.p. 107·5—109·5° were obtained (Found: C, 48·75; H, 5·65; N, 21·0. $C_8H_{11}NO_3$ requires C, 48·7; H, 5·6; N, 21·3%). $\lambda_{max.}$ 231 and 279 nm. ($\varepsilon_{max.}$ 12,800 and 5660); $\nu_{max.}$ 1700 (ester carbonyl) cm.$^{-1}$. On one occasion, crystalline material of m.p. 88·5—90·5° was obtained, the spectral properties of which were identical to those quoted above. When kept it slowly reverted to material of m.p. 107·5—109·5° and the lower-melting form is probably metastable.

The N-*acetyl* derivative was purified by sublimation at 90—100°/1 mm. when it formed colourless cyrstals, m.p. 89—91° (Found: C, 50·3; H, 5·55; N, 18·05. $C_{10}H_{13}N_3O_4$ requires C, 50·1; H, 5·45; N, 17·55%); $\lambda_{max.}$ 238·5 and 259 (infl.) nm. ($\varepsilon_{max.}$ 19,500 and 9950). The n.m.r. spectrum showed signals at τ 1·6—2·0 (1H, broad s, imino proton), 3·55 (1H, s, 5-H), 6·03 (3H, s, methoxy protons), 6·25 (3H, s, ester protons), 6·31 (2H, s, methylene protons), and 7·42 (3H, s, acetyl methyl protons).

2-*Acetamino*-6-*methoxy*-4-(α-*methoxycarbonyl*-α-*hydroxyimino*)*methylpyrimidine* (X; R = OMe).—2-Acetamino-6-methoxy-4-methoxycarbonylmethylpyrimidine (6 g.; above) was dissolved in water (300 ml.) and the solution was decanted from a small amount of oil. Sodium nitrite (4·5 g., 0·065 mol.) was added followed by glacial acetic acid until the pH of the solution was 3—4. Precipitation was induced by gentle scratching and the reaction mixture was kept for ½ hr., when the yellow product was separated; this was washed with water, a little methanol, and finally ether, and then dried *in vacuo* at room temperature (4·97 g.), m.p. 171—174°. A second crop (1·37 g.) was deposited with time, m.p. 169—174°. Crystallisation from water (charcoal) gave colourless needles, which were dried at 78°/15—20 mm. when they had m.p. 173—176° (Found: C, 44·7; H, 4·45; N, 21·3. $C_{10}H_{12}N_4O_5$ requires C, 44·7; H, 4·45; N, 20·9%), $\lambda_{max.}$ 239 and 295 nm. ($\varepsilon_{max.}$ 29,500 and 5150); $\nu_{max.}$ 680, 698, 740, 763, 790 (sh), 810, 828, 855, 875, 950, 982, 1031, 1060, 1097, 1150 (sh), 1183, 1203, 1250 (sh), 1293, 1321, 1382, 1437, 1463, 1518, 1567, 1596, 1650, 1736, 2890, 2980, 3080 (sh), 3120, and 3280 cm.$^{-1}$.

In certain preparations of this compound, particularly when hydrochloric rather than acetic acid was used to liberate the nitrous acid (acidifying to pH 1 rather than pH 3—4), material was obtained which showed additional i.r. bands at 1120, 1698, and 1718 cm.$^{-1}$ which persisted throughout the purification process. This is ascribed to isomerism which is induced by mineral acids or boiling water. Further evidence for isomerism was provided by the n.m.r. spectrum of material showing the additional i.r. bands (above). This product was also crystallised

from water, and dried when it had m.p. 173—176° (Found: C, 44·65; H, 4·4; N, 20·9%). The n.m.r. spectrum in dimethyl sulphoxide (main solvent peak τ 7·15) showed signals at τ 2·91, 2·95 (total 1H, both s, ratio 2/1, 5-H), 5·79, 5·89 (integration obscured by solvent peaks, both s, ratio 1/1, methoxy protons), 5·95, 6·02 (integration obscured by solvent peaks, both s, ratio 1/1, ester protons), 7·46, 7·5 (total 3H, both s, ratio 2/1, acetyl protons). Below τ 0, signals were observed at −0·55, −0·73 (total 1H, 2 broad s, ratio 2/1, imino proton) and −2·4 to −3·5 (very broad, oxime proton). Double peaks were also observed using CF_3CO_2H as solvent: singlets at τ 2·23, 2·79 (5-H), 5·68, 5·69 (methoxy protons), 5·79, 5·83 (ester protons), 7·40, 7·42 (acetyl protons) as well as a broad singlet at τ −0·65 (imino proton). This splitting was not observed with material lacking the additional i.r. bands. The product gave an intense purple ferrous reaction, discharged by acids but restored by sodium hydrogen carbonate.

2-Acetylamino-6-hydroxy-4-(α-methoxycarbonyl-α-hydroxyimino)methylpyrimidine (X; R = OH).—2-Acetylamino-6-hydroxy-4-methoxycarbonylmethyl-pyrimidine (above; 4·5 g., 0·02 mol.) was dissolved in water (400 ml.) at 40° with stirring. The solution was cooled to 15° and sodium nitrite (1·7 g., 0·0247 mol.) was added. Concentrated hydrochloric acid was then added until the pH of the solution was 1. The side of the vessel was scratched and the solution kept in the cold room (−5°) for 5 hr. The off-white product was then separated, washed with water, methanol, and ether and dried *in vacuo* at room temperature (4·29 g., 84·2%).

Crystallisation from water yielded colourless plates, m.p. 225·5—228° (Found: C, 42·3; H, 4·05; N, 21·5. $C_9H_{10}N_4O_5$ requires C, 42·5; H, 3·95; N, 22·0%); $\lambda_{max.}$ 242 and 317 nm.; $\varepsilon_{max.}$ 25000, and 4900. The n.m.r. spectrum showed signals (i) in DMSO (main solvent peak τ 7·35) at τ 3·6 (1H, s, 5-H), 6·11 (3H, s, ester protons), and 7·75 (3H, s, acetyl protons) and below τ 0 at −1·4 to −1·8 (1H, broad s, imino proton), and −2·8 (1H, s, oxime proton) (ii) in CF_3CO_2H at 2·53, 3·0 (total 1H, both s, 5-H), 5·81, 5·85 (total 3H, both s, ester protons), and 7·43 (3H, s, acetyl protons), *i.e.* in CF_3CO_2H solution there is evidence of isomeric forms. The compound gave an intense purple ferrous colouration.

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