Preparation of 2-Carboxymethylpyrimidines Analogous to the Pyrimidine of Thiamine and Some of their Pyridine Isomers

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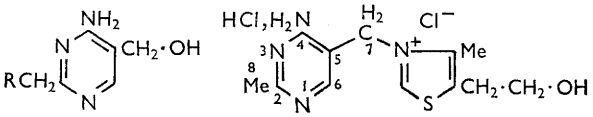
Basic hydrolysis of 4-amino-5-cyano-2-2′-hydroxyethylpyrimidine (IV) yields 4-amino-5-carboxy-2-2′-hydroxyethylpyrimidine (V) and catalytic hydrogenation in aqueous hydrochloric acid yields 4-amino-5-formyl-2-2′-hydroxyethylpyrimidine (VI) which is reduced by sodium borohydride to 4-amino-2-2′-hydroxyethyl-5-hydroxymethylpyrimidine (VII). Attempts to oxidise the alcohol function of (IV) were without success. Condensation of ethoxymethylenemalononitrile with ethoxycarbonylamidine yields 2,6-diamino-3-cyano-5-ethoxycarbonylpyridine (IX) which is hydrolysed to 2,6-diamino-3,5-dicarboxypyridine (X) and catalytically hydrogenated to 2,6-diamino-3-aminomethyl-5-ethoxycarbonylpyridine (XI). The latter compound yields 2,6-diamino-5-carboxy-3-aminomethyl-pyridine (XII) after basic hydrolysis. However condensation of malonamide amidine with ethoxymethylenemalononitrile does yield 2-acetamido-4-amino-5-cyanopyrimidine (XIII) which is easily hydrolysed to the unstable 4-amino-5-carboxy-2-carboxymethylpyrimidine (XIV) and catalytically hydrogenated 2-acetamido-4-amino-5-formylpyrimidine (XVI). The latter is reduced by sodium borohydride to 2-acetamido-4-amino-5-hydroxymethylpyrimidine (XVII) which on basic hydrolysis yields 4-amino-2-carboxymethyl-5-hydroxymethylpyrimidine (I) the carboxymethyl analogue of the pyrimidine of thiamine.

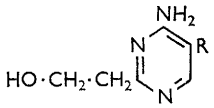
KNOWN pyrimidine compounds with a methylcarboxy-group in position 2, 4, or 6 are only referred to twice in the literature.[1] These three positions are particularly electron deficient [2] thus any carboxymethyl group attached to them should decarboxylate easily. A compound such as 4-amino-2-carboxymethyl-5-hydroxymethylpyrimidine (I) is of particular interest for biosynthetic studies of thiamine (II) since on decarboxylation it would yield the recognised precursor, 4-amino-5-hydroxymethyl-2-methylpyrimidine (III) (pyramine). This view seemed to be reinforced by Tomlinson *et al.*[3] who reported that only the methyl group of acetic acid was incorporated into the methyl group attached to position 2 of pyramine in the case of *Bacillus subtilis*. However this result is not easily reconciled with the more recent publication by Newell and Tucker,[4] indicating that in the case of *Salmonella typhimurium*, C-2 and the methyl group could have another origin. Nevertheless compound (I) is biologically interesting as a permanent source of pyramine in tissues even though the decarboxylation may be purely chemical.

The known compound, 4-amino-5-cyano-2-2′-hydroxyethylpyrimidine [5] (IV) was prepared in order to have a function on position 5 that could easily be modified and a two carbon chain at position 2 which should be easily



(I) R = CO_2H
(III) R = H

(II)



(IV) R = CN (VI) R = CHO
(V) R = CO_2H (VII) R = CH_2·OH

oxidised. The nitrile group attached to position 5 of (IV) was easily hydrolysed to a carboxy-group by N-sodium hydroxide at 50° for 15 hours to give 4-amino-5-carboxy-2-2′-hydroxyethylpyrimidine (V). Catalytic hydrogenation of (IV) in aqueous 2N-hydrochloric acid yields 4-amino-5-formyl-2-2′-hydroxyethylpyrimidine (VI). This type of hydrogenation has been observed for other 4-amino-5-cyanopyrimidines,[6] and confirms the

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[1] F. L. Rose, *J. Chem. Soc.*, 1954, 4116; F. L. Rose and D. J. Brown, *ibid.*, 1956, 1953.

[2] D. J. Brown, 'The Pyrimidines,' Interscience, New York and London, 1962, p. 7.

[3] R. V. Tomlinson, D. P. Kuhlman, P. F. Torrence, and H. Tiecklmann, *Biochim. Biophys. Acta*, 1967, **148**, 1.

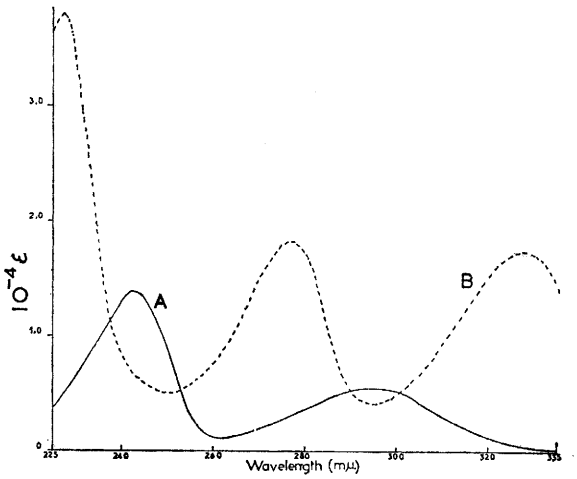
[4] P. C. Newell and R. G. Tucker, *Biochem. J.*, 1968, **106**, 279.

[5] T. Matsukawa and S. Yarugi, *J. Pharm. Soc. Japan*, 1952, **72**, 1585.

[6] (*a*) G. Arpad, F. Odön, and J. Oskar, *Magyar Kém. Folyòirat*, 1955, **61**, 112; (*b*) H. Tieckelmann, R. Guthrie, J. Nairn, *J. Org. Chem.*, 1960, **25**, 1257; (*c*) H. Bredereck, G. Simchen, and H. Traut, *Chem. Ber.*, 1967, **100**, 3664.

pyrimidine structure of (IV). The aldehyde (VI) reacts normally with sodium borohydride to form 4-amino-2-2′-hydroxyethyl-5-hydroxymethylpyrimidine (VII), which is the hydroxymethyl analogue of pyramine (II). Attempts to oxidise (IV) by potassium permanganate under varying conditions and nickel peroxide [7] yielded a solution which had no significant absorption in the ultraviolet above 210 mμ. Oxidation by ruthenium tetroxide [8] and by platinum catalyst [9] at pH 9 had no effect.

Ethoxycarbonylacetamidine was condensed with ethoxymethylenemalononitrile with the idea of preparing a pyrimidine compound (VIII) having a side chain already oxidised. Instead of (VIII) an isomeric compound was isolated after purification by sublimation. The elemental analysis of this compound corresponds to that of (VIII), the mass spectrum had a mass peak of 206 and the peak 207 was 11·49% that of 206 corresponding to the formula $C_9H_{10}N_4O_2$. The ultraviolet spectrum of the compound was totally different from that of (IV) (Figure); the nuclear magnetic resonance spectrum



U.v. spectra (MeOH) of (A) 4-amino-5-cyano-2-2′-hydroxyethylpyrimidine and (B) 2,6-diamino-3-cyano-5-ethoxycarbonylpyridine

indicated the presence of an ethyl group but did not indicate an isolated methylene group (Table 1); and the infrared spectrum indicated the presence of nitrile and amino-groups. The catalytic hydrogenation of this compound showed that 2 mol. of hydrogen were absorbed instead of 1 as was expected. All these results could be interpreted by admitting that the condensation took place on the methylene group and one of the nitrogen atoms of ethoxycarbonylacetamidine to form 2,6-diamino-3-cyano-5-ethoxycarbonylpyridine (IX) instead of (VIII).

This structure was confirmed by decarboxylating at 250°, 2,6-diamino-3,5-dicarboxypyridine (X) obtained after basic hydrolysis of (IX), to give 2,6-diaminopyridine which was identical with a commercial sample.

[7] K. Nakagawa, P. Konaka, and T. Nakata, *J. Org. Chem.*, 1962, **27**, 1597.
[8] V. M. Parikh and J. K. N. Jones, *Canad. J. Chem.*, 1965, **43**, 3452.

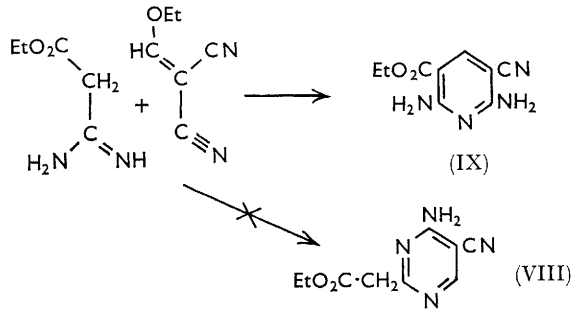
TABLE 1

N.m.r. spectral results of the pyridine compounds

Compound (R^1 and R^2)	Solvent [a]	δ (p.p.m.) a	b	c	d	e	f	g [c]	(J_{H-H}, c./sec.[b]) No. of protons
(IX) ($CO_2C_2H_5$ and CN)	CF_3CO_2H	1·49 (7t) 3	4·52 (7q) 2	—	—	8·70 (s) 1	7·60 (w) 2		
(XI) ($CO_2C_2H_5$ and CH_2NH_2)	$CDCl_3$	1·32 (7t) 3	4·23 (7q) 2	3·73 (s) 2	1·28 (s) 2	7·69 (s) 1	5·92 (w) 2	6·24 (w) 2	
(XI) ($CO_2C_2H_5$ and CH_2NH_2HCl)	D_2O	1·29 (7t) 3	4·26 (7q) 2	4·04 (s) 2	—	8·30 (s) 1	—	—	
(XII) (CO_2H and CH_2NH_2)	D_2O	—	—	4·04 (s) 2	—	8·21 (s) 1	—	—	

[a] $SiMe_4$ as internal reference for organic solvents, and external reference for D_2O. [b] s = Singlet, t = triplet, q = quadruplet, w = wide. [c] Protons shown: a, methyl of ester; b, methylene of ester; c, methylene of amine; d, NH_2 of side chain; e, ring at C-4; f and g, NH_2 on positions 2 and 6.

The hydrogenation product of (IX) was 2,6-diamino-3-aminomethyl-5-ethoxycarbonylpyridine (XI) which was easily hydrolysed to 2,6-diamino-3-aminomethyl-5-carboxypyridine (XII).

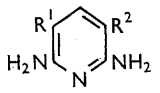


Matsukawa [10] prepared 2-acetamido-4-amino-5-cyanopyrimidine (XIII) by condensing malonamidoamidine with ethoxymethylenemalononitrile. The structure of (XIII) was suspect since malonamidoamidine is the amide of ethoxycarbonylamidine and it is known to condense with other ethoxymethylene compounds to form pyridines.[11] However the ultraviolet spectrum is similar to that of (IV), and the ^1H nuclear magnetic resonance spectrum indicates the presence of a methylene group at δ 4·42 p.p.m. The methylene group of malonamidoamidine is thus less nucleophilic than the two amidine nitrogens during the condensation with ethoxymethylenemalononitrile.

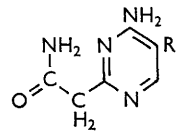
The pyridine isomer was not found during the reaction and was probably eliminated by the purification procedure. Basic hydrolysis of (XIII) yielded three products, 4-amino-5-carboxy-2-carboxymethylpyrimidine (XIV) (60%), the decarboxylation product 4-amino-5-carboxy-2-methylpyrimidine (XV) (10%), and the pyridine compound (X) (24%). There was probably ring opening between N-1 and C-6 of the pyrimidine ring and recyclisation to the pyridine compound during the

[9] K. Heyne and L. Blajewicz, *Tetrahedron*, 1960, **9**, 67.
[10] T. Matsukawa, *J. Pharm. Soc. Japan*, 1942, **62**, 417.
[11] A. Durnow and E. Neuse, *Chem. Ber.*, 1951, **84**, 296.

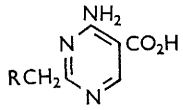
reaction. The pyrimidine diacid (XIV) was unstable especially in solutions below pH 5 decarboxylating to the methyl derivative (XV). These two compounds are easily separated by preparative paper chromatography



(X) $R^1 = R^2 = CO_2H$
(XI) $R^1 = CO_2Et$, $R^2 = CH_2{\cdot}NH_2$
(XII) $R^1 = CO_2H$, $R^2 = CH_2{\cdot}NH_2$



(XIII) R = CN
(XVI) R = CHO
(XVII) R = $CH_2{\cdot}OH$



(XIV) R = CO_2H
(XV) R = H

on Whatman No. 3 using a basic solvent mixture. The purified diacid (XIV) indicated the presence of (XV) after a second chromatographic run, the decomposition probably taking place during the elution of the product from the paper. The plotting of the neutralization curve of (XIV) showed that two acid groups were present in the molecule; the neutralization point of one was found at 0·64 equiv. and the second was found 1·0 equiv. later which was further evidence of the instability of (XIV). Catalytic hydrogenation of (XIII) gave 2-acetamido-4-amino-5-formylpyrimidine (XVI), the ultraviolet spectrum of which was very similar to that of (VI) and that of 4-amino-5-formylpyrimidine.[6c] The aldehyde (XVI) was normally reduced by sodium borohydride to 2-acetamido-4-amino-5-hydroxymethylpyrimidine (XVII). This compound was readily hydrolysed to give the required acid (I), which can be obtained pure by ion-exchange chromatography on Dowex 2 (acetate). An attempt to purify (I) by ion-exchange chromatography on Dowex 50 (ammonium) yielded two pyrimidine compounds (I), and its decarboxylation product (III). Revelation of a chromatogram by an auto-biogram on a pyrimidine requiring mutant of *Saccharomyces cerevisiae* of an analytically pure sample of (I) indicated after a few hours that (III) was present, whereas (I) only showed up after a few days.[12] Thus (I) can decarboxylate in a physiological medium to give pyramine, but whether this decarboxylation is enzymic or chemical remains to be verified.

The nuclear magnetic resonance spectra of the pyrimidine compounds are given on Tables 2 and 3 and present some interesting characteristics. The two protons of the amino-group on position 4 of the two formylpyrimidines (VI) and (XVI), are split since in each case a hydrogen bond forms between the oxygen of the aldehyde function and one of the protons. This is not, observed in the case of the other substituents on position 5. The two protons of the methylene group between the ring and carbonyl function for (XVII) appear at δ 4·20 p.p.m. in trifluoroacetic acid, but completely disappear in deuterium oxide which indicates that this methylene group has similar properties to that of the methylene group of malonic acid. The formation of the pyridine diacid (X) during the hydrolysis of (XIII) is thus plausible.

[12] B. Estramareix, unpublished result.
[13] K. Fink and W. S. Adams, *J. Chromatog.*, 1966, **22**, 118.

TABLE 2

N.m.r. spectral results for the 2-2′-hydroxyethylpyrimidines

Compound (R)	Solvent [a]	δ (p.p.m.) (J_{H-H}, c./sec.[b]) a	b	c	d	e	f [c]	No. of protons
(IV) (CN)	CF_3CO_2H	3·17 (6q)	4·66 (6t)	8·76 (s)	—	8·08 (w)	4·94 (6t)	2 2 1 — 2 1
(V) (CO_2H)	D_2O	3·05 (6t)	4·05 (6t)	8·53 (s)	—	—	—	2 2 1 — — —
(VI) (CHO)	D_2O	3·50 (6t)	4·66 (6t)	9·2 (s)	10·38 (s)	—	—	2 2 1 1 — —
(VI) (CHO)	CF_3CO_2H	3·49 (6q)	4·56 (6t)	8·93 (s)	10·00 (s)	8·22 (w) 9·46 (w)	5·00 (6t)	2 2 1 1 1 1
(VII) (CH_2OH)	D_2O	2·86 (6t)	3·95 (6t)	8·05 (s)	4·52 (s)	—	—	2 2 1 2 — —

[a] $SiMe_4$ as internal reference for organic solvents and external reference for D_2O. [b] s = Singlet, t = triplet, q = quintuplet, w = wide. [c] Protons shown: a, β-methylene; b, C-8; c, ring at C-6; d, C-7; e, NH_2; f, OH.

TABLE 3

N.m.r. spectral results for the 2-acetamidopyrimidines

Compound (R)	Solvent [a]	δ (p.p.m.). (No. of protons) a	b	c	d	e	f
(XIII) (CN)	CF_3CO_2H	4·24 (2)	7·42 (2)	8·05 (2)	8·76 (1)	—	—
(XVI) (CHO)	CF_3CO_2H	4·26 (2)	7·50 (2)	7·95 (1) 9·46 (1)	8·96 (1)	10·05 (1)	—
(XVII) (CH_2OH)	CF_3CO_2H	4·20 (2)	7·76 (4)	7·76 (4)	8·30 (1)	4·96 (2)	5·50 (1)
(XVII) (CH_2OH)	D_2O	Exchanged	—	—	8·10 (1)	4·56 (2)	—

[a] $SiMe_4$ as internal reference for CF_3CO_2H and external reference for D_2O. [b] Protons show: a, C-8; b, amide NH_2; c, NH_2 on C-4; d, ring at C-6; e, C-7; f, OH of (XVII).

EXPERIMENTAL

All evaporations were carried out using a rotary evaporator. M.p.s were determined on a Kofler hot-stage microscope. U.v. spectra were determined at dilutions of approx. 5×10^{-5}M with a Jobin and Yvon MarocIV apparatus. I.r. spectra were determined with a Perkin-Elmer Infracord 237 apparatus as Nujol mulls. N.m.r. spectra were determined at 35° with a Varian A60 apparatus.

Paper chromatography was carried out on Whatman No. 1 paper in two different solvent systems: Solvent A: n-butyl alcohol–water–acetic acid (4 : 1 : 1); solvent B:[13] t-butyl alcohol–ethyl methyl ketone–water–ammonia (4 : 3 : 2 : 1), and the spots were visible in the u.v. (254 mμ), dark colour for the pyrimidine compounds and light colour for the pyridine compounds.

4-Amino-5-cyano-2-2′-hydroxyethylpyrimidine (IV).—This compound was prepared according to Matsukawa and

Yarugi,[5] $\nu_{max.}$ 2225 cm.$^{-1}$ (C≡N), $\lambda_{max.}$ (MeOH) 295 and 242·5 mμ (ε 4700 and 11,500), R_F 0·68 (solv. A) and 0·83 (solv. B).

4-*Amino*-5-*carboxy*-2-2′-*hydroxyethylpyrimidine* (V).—The pyrimidine nitrile (IV) (1·64 g.) was left for 15 hr. at 50° in N-NaOH (20 ml.). The soln. was neutralized by Amberlite 120 (H^+; 4·7 g.) ion-exchange resin until it was at pH 7. The resin was filtered off and washed with water (2 × 10 ml.). The filtrate and washings were evaporated to dryness and the residue was crystallized from water (10 ml.) to yield a white *powder* (1·83 g., 98%), m.p. 270—272° with decarboxylation (Found: C, 45·6; H, 4·95; N, 23·0. $C_7H_9N_3O_3$ requires C, 45·9; H, 4·95; N, 22·95%), pK_a 5·9 and *M* by titration 186 (requires 190), $\nu_{max.}$ 1640 cm.$^{-1}$ (C=O), $\lambda_{max.}$ (0·1N-NaOH) 287·5 and 240 mμ (ε 5600 and 11,200), (0·1N-HCl) 242·5 (ε 12,500) and λ_{sh} 280 (ε 4000), R_F 0·33 (solv. A) and 0·28 (solv. B).

4-*Amino*-5-*formyl*-2-2′-*hydroxyethylpyrimidine* (VI).—The pyrimidine nitrile (IV) (1·64 g.) was dissolved in 2N-HCl (13·5 ml.) and hydrogenated for 15 hr. in the presence of 5% Pd–C catalyst (135 mg.). The catalyst was filtered off and the filtrate cooled to 10° was brought to pH 7 by cautious addition of conc. ammonia. The precipitate was crystallized in water (10 ml.) to give white needle-like *crystals* (0·7 g., 42%), m.p. 162—163° with a change of crystalline form at 153° (Found: C, 50·1; H, 5·2; N, 25·4. $C_7H_9N_3O_2$ requires C, 50·3; H, 5·4; N, 25·1%), $\nu_{max.}$ 1650 cm.$^{-1}$ (C=O), $\lambda_{max.}$ (MeOH) 307·5 and 248·5 mμ (ε 5600 and 8600), R_F 0·61 (solv. A) and 0·80 (solv. B).

4-*Amino*-2-2′-*hydroxyethyl*-5-*hydroxymethylpyrimidine* (VII).—$NaBH_4$ (60 mg.) dissolved in water (10 ml.) was added to the formylpyrimidine (VI) (234 mg.) dissolved in water (10 ml.) and the reaction solution was left at room temperature for 24 hr. The solution was brought to pH 5 by N-acetic acid and then passed over a Dowex 50 (H^+; 10 mequiv.) ion-exchange column. The column was washed with water (15 ml.) and the product eluted by 2M-ammonia (50 ml.). The eluate was evaporated to dryness and the residue crystallized from absolute alcohol (3 ml.) to yield white *crystals* (200 mg., 84%), m.p. 167—168° (Found: C, 49·4; H, 6·35; N, 24·7. $C_7H_{11}N_2O_3$ requires C, 49·7; H, 6·35; N, 24·85%), $\lambda_{max.}$ (MeOH) 271 and 235 mμ (ε 5300 and 10,500), R_F 0·44 (solv. A) and 0·66 (solv. B).

2,6-*Diamino*-3-*cyano*-5-*ethoxycarbonylpyridine* (IX).—Pure ethoxycarbonylacetamidine hydrochloride [14] (23·5 g.) and pure ethoxymethylenemalononitrile [15] (17·3 g.) were dissolved in absolute alcohol (1500 ml.) at −5° under continual mechanical stirring. Once this solution was homogenous, a solution of sodium (3·26 g.) in absolute alcohol (200 ml.) was added dropwise during 3 hr. The yellow precipitate was collected and washed thoroughly in water (2 × 500 ml.) with stirring. The orange filtrate was concentrated to approx. 200 ml. and then diluted by water (1000 ml.) when a further precipitate formed. The combined precipitates were dried *in vacuo* (P_2O_5). The crude yellow powder indicated two spots in thin layer fluorescent silica-gel chromatography with ethyl acetate–dioxan (2 : 1) at R_F 0·8 and 0·9. This substance was sublimed (oil bath 220°/18 mm.) to yield a white *powder* (12 g., 41%) which could be crystallized in ethyl acetate (4% solubility at the b.p.), m.p. 248—249° (Found: C, 52·45; H, 4·95; N, 27·05; O, 15·4. $C_9H_{10}N_4O_2$ requires C, 52·4; H, 4·9; N, 27·2; O, 15·5%), $\nu_{max.}$ 2206 cm.$^{-1}$ (C≡N), $\lambda_{max.}$ (MeOH) 328, 276, and 227·5 mμ (ε 17,300, 18,200, and 37,900), R_F 0·86 (solv. A) and 0·89 (solv. B). Significant peaks in the mass spectrum (MS 9 apparatus at 180° and 70 ev) occurred at *m/e* 207 (11·49%), 206 (parent peak), 178 (38%), 161 (93%), 133 (92%), 106 (37%), 79 (51%), and 43 (40%). Metastable ions indicated that the following transformations occurred: 206 ⟶ 178, 206 ⟶ 161, 178 ⟶ 133, 161 ⟶ 133, 133 ⟶ 106, and 106 ⟶ 79.

2,6-*Diamino*-3,5-*dicarboxypyridine* (X).—The pyridine nitrile (IX) (106 mg.) was heated under reflux for 20 hr. in N-NaOH (15 ml.). The cooled solution was filtered to remove the slight turbidity and glacial acetic acid (1 ml.) was added to the filtrate. The precipitate formed was collected by centrifuging and was purified by dissolution in 0·5M-ammonia (2 × 10 ml.) and reprecipitation by glacial acetic acid (2 × 1 ml.). The white *powder* (75 mg., 76%) was dried *in vacuo* (KOH), had no m.p. <350° (Found: C, 42·2; H, 3·6; N, 21·2. $C_7H_7N_3O_4$ requires C, 42·6; H, 3·6; N, 21·3%), $\lambda_{max.}$ (0·01N-HCl) 333 and 275 mμ (ε 23,900 and 19,700), (0·01N-NaOH) 325, 277, and 223 mμ (ε 15,900, 15,900, and 24,400), R_F drags (solv. A) and 0·05 (solv. B).

This compound (98·2 mg.) was heated in a sublimating apparatus (oil bath 250°/11 mm.) to yield 2,6-diaminopyridine (42·7 mg., 78%) which was identical with a commercial sample (Koch-Light) by its undepressed mixed m.p. 118° (lit.,[16] 119—120°), u.v. and i.r. spectra.

2,6-*Diamino*-3-*aminomethyl*-5-*ethoxycarbonylpyridine* (XI).—The cyanopyridine (IX) (512 mg.) was completely dissolved in dioxan (50 ml.) by gentle warming, and the solution was then diluted with 2N-HCl (50 ml.). This solution then fixed hydrogen (2 mol.) in the presence of 5% Pd–C catalyst (100 mg.) after 10 hr. The catalyst was filtered off and the filtrate evaporated to dryness. The last traces of HCl were removed *in vacuo* (KOH). The residue was dissolved in hot water (5 ml.) and the undissolved particles were filtered off. Absolute alcohol (50 ml.) was added to the filtrate and the solution was left until the product crystallized. This solution yielded white crystals (373 mg.) and a further crop (172 mg.) was obtained from concentrating the mother liquor (92·5% total). These crystals had the analytical composition of 2,6-*diamino*-3-*aminomethyl*-5-*ethoxycarbonylpyridine dihydrochloride*, m.p. 228—229° (Found: C, 38·1; H, 5·75; N, 19·9; HCl, 25·2. $C_9H_{16}Cl_2N_4O_2$ requires C, 38·4; H, 5·65; N, 19·9; HCl, 25·2%).

The free amine was obtained by dissolving the dihydrochloride (2·85 g.) in N-ammonia solution (20 ml.) and extracting it by chloroform (3 × 20 ml.). The chloroform solution was dried (Na_2SO_4) and then evaporated to dryness. The residue was crystallized from benzene (50 ml.) followed after complete dissolution by light petroleum (10 ml.) to yield white *crystals* (1·8 g., 85%), m.p. 116—117° (Found: C, 51·15; H, 6·4. $C_9H_{14}N_4O_2$ requires C, 51·4; H, 6·7%), $\lambda_{max.}$ (0·01N-HCl) 333 and 267 mμ (ε 19,900 and 14,000), R_F 0·42 (solv. A) and 0·75 (solv. B).

2,6-*Diamino*-3-*aminomethyl*-5-*carboxypyridine* (XII).—The dihydrochloride of (XI) (285 mg.) was heated on a steam bath with N-NaOH (4 ml.) for 1 hr. After cooling, the solution was passed over a Dowex 2 ($CH_3CO_2^-$; 15 mequiv.) ion-exchange column, which was then washed with water (20 ml.). The product was eluted by 0·5N-acetic acid (40 ml.) and the eluate evaporated to dryness. The last traces of acetic acid were removed *in vacuo* (KOH). The

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residue was then dissolved in hot water (5 ml.) and absolute alcohol (10 ml.) was added to the solution. The product crystallized on cooling (73·7 mg.) and a further amount (29·1 mg.) was recovered from the mother liquor (51%). The white powder had the analytical composition of *2,6-diamino-3-aminomethyl-5-carboxypyridine monohydrate* and decomposed during a second crystallization and on heating, thus no defined m.p. found (Found: C, 42·2; H, 6·05; N, 27·8. $C_7H_{10}N_4O_2,H_2O$ requires C, 42·0; H, 6·05; N, 28·0%), $\lambda_{max.}$ (0·01N-HCl) 335 and 267·5 mμ (ε 20,800 and 12,600), (0·01N-NaOH) 321 and 263 mμ (ε 13,000 and 11,000), R_F 0·25 (solv. A) and 0·17—0·37 (solv. B).

2-Acetamido-4-amino-5-cyanopyrimidine (XIII).—This compound was prepared according to Matsukawa,[10] $\nu_{max.}$ 2220 cm.$^{-1}$ (C≡N), $\lambda_{max.}$ (MeOH) 297 and 242·5 mμ (ε 4900 and 11,700), R_F 0·51 (solv. A) and 0·65 (solv. B).

4-Amino-5-carboxy-2-carboxylmethylpyrimidine (XIV).—The above pyrimidine (XIII) (350 mg.) was heated under reflux with N-NaOH (5 ml.) for 5 hr. After cooling the solution was brought to pH 5 by 3N-acetic acid (1·5—2·0 ml.) and then centrifuged. The precipitate was dried *in vacuo* (KOH) and identified chromatographically and spectroscopically as (X) (95 mg., 24%). The supernatant liquid was passed over IR 120 (pyridinium; 25 mequiv.) ion-exchange resin and the product was eluted by water (35 ml.). The eluate was evaporated to dryness and the residue redissolved in N-ammonia (4 ml.). This solution was equally deposited on 4 Whatman No. 3 sheets (40 × 40 cm.) and chromatographed with solvent B for 20 hr. Two bands (7·5 ± 2 and 20 ± 2 cm.) were cut out and eluted by N-ammonia. The product isolated from the second band after eluting and evaporating to dryness was identified as 4-amino-5-carboxy-2-methylpyrimidine (XV) (33·5 mg., 10%) by mixed m.p. (278—280°), comparative chromatography, and u.v. spectroscopy. The eluate of the first band was evaporated to dryness and the residue (270 mg., 62%) was kept *in vacuo* (KOH) at −18°. The titration curve gave two neutralization points with pK_{a_1} approx. 4·0, and pK_{a_2} 6·1. The mol. wt. calculated from the difference of the two neutralization points was 206 (requires 207) which corresponds to *4-amino-5-carboxy-2-carboxymethylpyrimidine*. The compound was very unstable and decarboxylated easily in aqueous solution below pH 5·5, m.p. 258° (decomp.), λ_{sh} (0·01N-HCl) 278 mμ (ε 3200), $\lambda_{max.}$ (0·01N-HCl) 246 mμ (ε 10,000), (0·01N-NaOH) 290 and 241 mμ (ε 4100 and 8400), R_F drags from 0·0 to 0·4 (solv. A) and 0·1 (solv. B).

2-Acetamido-4-amino-5-formylpyrimidine (XVI).—The acetamidocyanopyrimidine (XIII) (1·75 g.) was dissolved in boiling water (90 ml.), when the dissolution was complete 4N-HCl (10 ml.) was added. This hot solution was immediately placed under an atmosphere of hydrogen in the presence of 5% Pd–C catalyst (150 mg.) and stirred for 16 hr. to fix H_2 (1 mol.). The catalyst and a little starting material were filtered off. The filtrate was concentrated to 70 ml. and then carefully adjusted to pH 6·5—7·0 at a pH meter by dropwise addition of concentrated ammonia. The precipitate formed was dissolved in water (20 ml.), the solution charcoaled and filtered hot. The white *crystals* (0·82 g., 46%), m.p. 249—250° (decomp.) were dried *in vacuo* (P_2O_5) (Found: C, 46·8; H, 4·4; N, 31·3. $C_7H_8N_4O_2$ requires C, 46·7; H, 4·5; N, 31·1%), $\nu_{max.}$ 1680 cm.$^{-1}$ (C=O of CHO), $\lambda_{max.}$ (MeOH) 310 and 248·5 mμ (ε 6000 and 8400), R_F 0·31 (solv. A) and drags 0·20 to 0·72 (solv. B).

2-Acetamido-4-amino-5-hydroxymethylpyrimidine (XVII).—Sodium borohydride (190 mg.) was added to a solution of the acetamidoformylpyrimidine (XVI) (360 mg.) in water (100 ml.). The reaction mixture was left at room temperature overnight. The excess of borohydride was destroyed by 3N-acetic acid (2 ml.) and this acidified solution was passed over IR 120 (H^+; 25 mequiv.) ion-exchange column. The column was washed with water (25 ml.) and the product eluted by M-pyridine (25 ml.). The eluate was evaporated to 5 ml. and the precipitate formed collected. This precipitate was chromatographically pure and for analysis was crystallized from n-propanol to give a white *powder*, m.p. 230—231° (decomp.) (Found: C, 46·3; H, 5·5; N, 30·9. $C_7H_{10}N_4O_2$ requires C, 46·15; H, 5·5; N, 30·8%), $\nu_{max.}$ large envelope 3380—3460 cm.$^{-1}$ (OH), $\lambda_{max.}$ (MeOH) 273 and 236 mμ (ε 4500 and 9000), R_F 0·15 (solv. A) and 0·0—0·64 (solv. B).

4-Amino-2-carboxymethyl-5-hydroxymethylpyrimidine (I).—The above pyrimidine (XVII) (183 mg.) was heated under reflux for 3 hr. with N-NaOH (2 ml.). After cooling, the reaction mixture was diluted and then passed over Dowex 2 ($CH_3CO_2^-$; 20 mequiv.) ion-exchange column. The column was washed with water (30 ml.) and the product was eluted by 0·5N-acetic acid (50 ml.). The eluate was rapidly evaporated to 4 ml. and then absolute alcohol (25 ml.) was added. The precipitate was dissolved in water (4 ml.) and allowed to crystallise. The white crystals *4-amino-2-carboxymethyl-5-hydroxymethylpyrimidine* (141 mg., 74%) had m.p. 167° (shrinking) and finally at 193° [m.p. of 4-amino-5-hydroxymethyl-2-methylpyrimidine (III)] (Found: C, 45·9; H, 5·05; N, 22·9. $C_7H_9N_3O_3$ requires C, 45·9; H, 4·95; N, 22·95%). Determination of mol. wt. by titration against 0·1N-NaOH gave 184 (requires 183), pK_a 6·05, $\lambda_{max.}$ (0·01N-HCl) 248 mμ (ε 11,700), (0·2N-NH_4OH) 274 and 235 mμ (ε 4900 and 9000), R_F 0·0 (solv. A) and 0·36 (solv. B). When the reaction mixture was demineralized over Dowex 50 (NH_4^+) ion-exchange column, the eluate showed a second spot in paper chromatography at R_F 0·51 (solv. A) and 0·86 (solv. B), which corresponds to that of (III) used as both an internal and external reference. However (I) was much more stable then (XIV) and decarboxylated less rapidly.

[8/1001 *Received, July 15th,* 1968]