Specific Enzyme Inhibitors in Vitamin Biosynthesis. Part I. The Synthesis of 8-Substituted Pyrido[2,3-*d*]Pyrimidines

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Two methods for the synthesis of 8-substituted pyrido[2,3-*d*]pyrimidines are described. These compounds are close structural analogues of the pteridine precursor involved in the biosynthesis of riboflavin, and were required for studies of the inhibition of riboflavin synthetase.

The first method is an adaptation of the Doebner–Miller synthesis of quinolines and is a new route to the pyrido-[2,3-*d*]pyrimidine ring system. It involves condensation of a 6-(substituted amino)uracil with an αβ-unsaturated carbonyl derivative, and leads initially to an unusual tricyclic intermediate. Detailed spectroscopic studies on this intermediate helped to elucidate the structure of the final products.

The second method involved condensation of 6-(substituted amino)uracils with β-dicarbonyl derivatives. The resulting pyrido[2,3-*d*]pyrimidines were different from those expected on the basis of earlier work.

^{1}H N.m.r. studies of the 8-substituted pyrido[2,3-*d*]pyrimidines showed that deuterium exchange of protons on *C*-methyl groups at positions 5 and 7 took place readily in alkaline solution, whereas protons on a 6-methyl group did not exchange. This phenomenon is similar to that observed with the pteridine precursor involved in riboflavin biosynthesis, and an explanation involving a highly delocalised anionic species is offered.

THE enzymes involved in (i) the biosynthesis of vitamins, and (ii) the conversion of vitamins into the metabolically active coenzymes are of particular importance in the rational design of chemotherapeutic agents. Examples of the application of the latter approach are the extensive studies by Hitchings and his colleagues [1] and by Baker [2] of the specific inhibition of dihydrofolate reductase, the enzyme involved in the reduction of dihydrofolate to the metabolically active tetrahydrofolate. A major difficulty in the practical exploitation of such inhibitors is that of achieving the required degree of selective toxicity, since the target enzyme is one which is common to both parasite and host.

Specific inhibition of the enzymatic reactions involved in the biosynthesis (*de novo*) of vitamins by bacteria and related organisms is one way of overcoming this difficulty since such inhibitors affect only the parasite and do not affect the host in which these biosynthetic pathways are absent. This series of papers is concerned with the synthesis of such specific inhibitors. This major field has been explored only tentatively, and detailed studies of the inhibition of selected enzymes of this type should make a significant contribution to a more rational approach to chemotherapy.

The present paper deals with the synthesis of 8-substituted pyrido[2,3-*d*]pyrimidines (13)—(23), which are close structural analogues of the pteridine precursor (1) involved in the biosynthesis of riboflavin.[3] The pyrido-[2,3-*d*]pyrimidine (5-deazapteridine) ring system was selected for study because numerous derivatives have

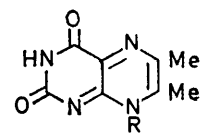
[1] G. H. Hitchings and J. J. Burchall, *Adv. Enzymol.*, 1965, **27**, 417; G. H. Hitchings, *Fed. Proc.*, 1967, **26**, 1078; G. H. Hitchings in 'Chemotherapy of Cancer,' Elsevier, Amsterdam, 1964, p. 77.

[2] B. R. Baker, *Accounts Chem. Res.*, 1969, **2**, 129; B. R. Baker, *Ann. Rev. Pharmacol.*, 1970, **10**, 35; B. R. Baker in 'Design of Active-Site Directed Irreversible Enzyme Inhibitors. The Organic Chemistry of the Active Site,' Wiley, New York, 1967.

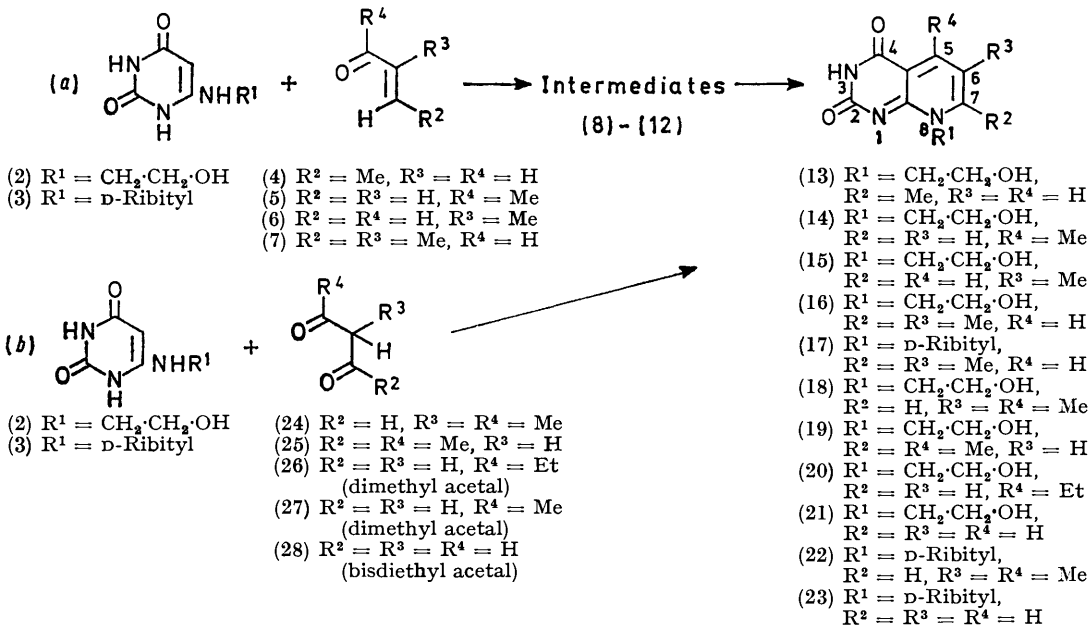
[3] G. W. E. Plaut, *J. Biol. Chem.*, 1960, **235**, PC41; 1963, **238**, 2225; H. Wacker, R. A. Harvey, C. H. Winestock, and G. W. E. Plaut, *J. Biol. Chem.*, 1964, **239**, 3493; R. A. Harvey and G. W. E. Plaut, *J. Biol. Chem.*, 1966, **241**, 2120.

been shown [4] to act as powerful inhibitors of dihydrofolate reductase, and it thus appears that the ring system can replace the normal pteridine substrate with this enzyme. It thus seemed that suitable 8-substituted derivatives might be specific inhibitors of riboflavin synthetase.

We have investigated two synthetic methods of general applicability. The first route (*a*) is an adaptation of the Doebner–Miller synthesis of quinolines [5] and involves replacing the aniline system with a 6-(substituted amino)uracil. The second route (*b*) is based on the synthesis of pyrido[2,3-*d*]pyrimidines from β-dicarbonyl derivatives.[6] These routes have the advantage that they start from 6-aminouracils, which are readily available from the reaction of 6-chlorouracil with the appropriate amine. Suitably substituted pyridines would be difficult to obtain if one were to consider starting from pyridine precursors.[7]



(1) R = D-Ribityl



(2) R^1 = CH_2·CH_2·OH
(3) R^1 = D-Ribityl

(4) R^2 = Me, R^3 = R^4 = H
(5) R^2 = R^3 = H, R^4 = Me
(6) R^2 = R^4 = H, R^3 = Me
(7) R^2 = R^3 = Me, R^4 = H

(2) R^1 = CH_2·CH_2·OH
(3) R^1 = D-Ribityl

(24) R^2 = H, R^3 = R^4 = Me
(25) R^2 = R^4 = Me, R^3 = H
(26) R^2 = R^3 = H, R^4 = Et (dimethyl acetal)
(27) R^2 = R^3 = H, R^4 = Me (dimethyl acetal)
(28) R^2 = R^3 = R^4 = H (bisdiethyl acetal)

(13) R^1 = CH_2·CH_2·OH, R^2 = Me, R^3 = R^4 = H
(14) R^1 = CH_2·CH_2·OH, R^2 = R^3 = H, R^4 = Me
(15) R^1 = CH_2·CH_2·OH, R^2 = R^4 = H, R^3 = Me
(16) R^1 = CH_2·CH_2·OH, R^2 = R^3 = Me, R^4 = H
(17) R^1 = D-Ribityl, R^2 = R^3 = Me, R^4 = H
(18) R^1 = CH_2·CH_2·OH, R^2 = H, R^3 = R^4 = Me
(19) R^1 = CH_2·CH_2·OH, R^2 = R^4 = Me, R^3 = H
(20) R^1 = CH_2·CH_2·OH, R^2 = R^3 = H, R^4 = Et
(21) R^1 = CH_2·CH_2·OH, R^2 = R^3 = R^4 = H
(22) R^1 = D-Ribityl, R^2 = H, R^3 = R^4 = Me
(23) R^1 = D-Ribityl, R^2 = R^3 = R^4 = H

Syntheses Based on Doebner–Miller-type Reactions.— Condensation of 6-(2-hydroxyethylamino)uracil (2) and crotonaldehyde (4) in 20% hydrochloric acid at room temperature gave a single product (8) in good yield. The product ($C_{10}H_{13}N_3O_3$) was readily converted into 8-(2-hydroxyethyl)-7-methylpyrido[2,3-*d*]pyrimidine-2(8*H*),4(3*H*)-dione (13) by heating in diphenyl ether. The conversion could also be carried out by holding the intermediate (8) at its m.p. for a short period or by keeping a solution in dilute acid, at room temperature for a few days.

The n.m.r. spectrum of the 8-substituted pyrido[2,3-*d*]-pyrimidine (13) in trifluoroacetic acid showed a low-field methyl signal at τ 6·83 and an AB quartet (two protons) giving doublets centred at τ 2·32 and 1·33 (Table 1) as expected. In principle, however, condensation of 6-(2-hydroxyethylamino)uracil (2) and crotonaldehyde (4) could have given eventually the 5-methylpyrido[2,3-*d*]pyrimidine (14), and this isomer would have a similar n.m.r. spectrum. The structure of the intermediate (8) proved to be crucial in the eventual assignment of the 7-methyl structure to the product of this reaction.

Treatment of the uracil (2) with methyl vinyl ketone (5) in 20% hydrochloric acid at room temperature gave a similar intermediate (9), which was converted into 8-(2-hydroxyethyl)-5-methylpyrido[2,3-*d*]pyrimidine-2(8*H*),4(3*H*)-dione (14) by refluxing in diphenyl ether. This pyridopyrimidine was different from that obtained from the crotonaldehyde reaction but showed a similar n.m.r. spectrum (Table 1).

A similar reaction with methacraldehyde (6) gave a

[4] B. S. Hurlbert, R. Ferone, T. A. Herrmann, G. H. Hitchings, M. Barnett, and S. R. M. Bushby, *J. Medicin. Chem.*, 1968, **11**, 711.

[5] R. C. Elderfield in ' Heterocyclic Compounds,' Wiley, New York, 1961, vol. 4, p. 1.

[6] G. H. Hitchings and R. K. Robins, *J. Amer. Chem. Soc.*, 1958, **80**, 3449.

[7] W. J. Irwin and D. G. Wibberley, *Adv. Heterocyclic Chem.*, 1969, **10**, 150.

third monomethylpyrido[2,3-*d*]pyrimidine (15), again *via* an intermediate (10). In this case the pyridopyrimidine must be the 6-methyl isomer. This Doebner–Miller type of synthesis is thus an efficient new method for the preparation of pyrido[2,3-*d*]pyrimidines; other examples are reported later. It seems likely that the intermediates (8)—(10) are analogous, and this is confirmed by the u.v. spectra, which are similar (Table 2). Intermediate (8) shows absorption at $\lambda_{max.}$ (pH 1) 282 and (pH 13) 280·5 nm, involving a shift of *ca.* +15 nm from that of the starting material (2).

TABLE 1

^{1}H N.m.r. spectra of pyrido[2,3-*d*]pyrimidines at 60 MHz

Compound	τ Values (*J* in Hz) R^2	R^3	R^4	Solvent
(13)	6·83	2·32 (d, *J* 8)	1·33 (d, *J* 8)	$CF_3{\cdot}CO_2H$
	7·25 [a]	3·27 (d, *J* 6)	2·03 (d, *J* 6)	NaOD
(14)	0·75 (d, *J* 7)	2·05 (d, *J* 7)	6·85 (d, *J* 3) [b]	$CF_3{\cdot}CO_2H$
	1·92 (d, *J* 8)	3·25 (d, *J* 8)		NaOD
(15)	1·72 (or 1·88)	7·68	1·88 (or 1·72)	NaOD
(16)	6·8	7·63	1·29	$CF_3{\cdot}CO_2H$
(18)	1·00	7·32	6·95 (d, *J* 3) [b]	$CF_3{\cdot}CO_2H$
	2·17	7·8		NaOD
(19)	6·92	2·4	7·00	$CF_3{\cdot}CO_2H$
		3·48		NaOD
(20)	0·84 (d, *J* 8)	2·12 (d, *J* 8)	6·8 (q, *J* 7)	$CF_3{\cdot}CO_2H$
			8·4 (t, *J* 7)	
	1·77 (d, *J* 8)	3·12 (d, *J* 8)	8·65	NaOD
(21)	0·7 (d, *J* 7)	2·08 (t, *J* 7)	1·05 (d, *J* 7)	$CF_3{\cdot}CO_2H$
(22)	2·03	7·63	7·23	D_2O
	2·03	7·63		NaOD
(50)	7·53	3·2 (d, *J* 8)	1·92 (d, *J* 8)	NaOD
(51)	7·25 (q, *J* 7)	3·1 (d, *J* 8)	1·81 (d, *J* 8)	NaOD
	8·74 (t, *J* 7)			
(56)	0·85 (d, *J* 8)	2·15 (d, *J* 8)	6·87 (d, *J* 3) [b]	$CF_3{\cdot}CO_2H$

All signals are singlets unless otherwise stated.

[a] Integral <1H. [b] These compounds showed the 5-methyl signal as a doublet (*J* 3 Hz) in trifluoroacetic acid. The relatively small coupling constant suggests some form of long-range coupling, but we were unable to detect any corresponding splitting of the 6- and 7-proton signals.

We formulate the intermediate (8) as the bridged tetrahydropyrido[2,3-*d*]pyrimidine-2,4-dione (40) for the following reasons.

(*a*) Treatment with nitrous acid gave no nitrosopyrimidine, indicating that position 5 of the pyrimidine ring was no longer free.[8]

(*b*) Hydrogenation (uptake 1 mol. equiv.) in acid solution resulted in hydrogenolysis of the benzylic ether-type linkage and formation of the tetrahydropyridopyrimidinedione (29). The latter was also obtained by catalytic reduction (uptake 2 mol. equiv.) of the pyridopyrimidine (13). Similar hydrogenolyses have been reported [9] for 5-hydroxymethyluracil and its ethers and esters, giving 5-methyluracil as the sole pyrimidine product. The possibility that acid-catalysed conversion of (8) into (13) preceded hydrogenation seems unlikely, since hydrogenation took place much more rapidly than the acid-catalysed conversion.

[8] D. J. Brown, 'The Pyrimidines,' Interscience, New York, 1962, p. 19.

[9] R. E. Cline, R. M. Fink, and K. Fink, *J. Amer. Chem. Soc.*, 1959, **81**, 2521.

(*c*) The u.v. spectrum of the intermediate is very similar to that of its reduction product (29), $\lambda_{max.}$ (pH 1) 286 and (pH 13) 283 nm, in keeping with the only chromophoric system present being the diaminouracil ring. Extended conjugation in an alternative 7,8-dihydro-structure (32a) for the intermediate would have led to u.v. absorption at a higher wavelength as is evident from the spectrum [10] of the closely related 7,8-dihydro-8-(2-hydroxyethyl)-6,7-dimethyl-lumazine (35). A second alternative structure (33a) can also be excluded, since its u.v. spectrum would have been similar to that of the dihydropteridine (36) reported by Kaufman [11] [$\lambda_{max.}$ (pH 6·8) 302—304 nm].

TABLE 2

U.v. spectra † of pyrido[2,3-*d*]pyrimidines

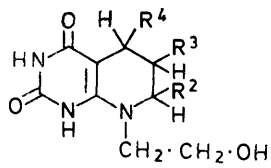
Compound	$\lambda_{max.}$/nm (ε)	pH
(8)	280·5 (18,960)	13
	282 (26,600)	1
(9)	281 (18,350)	13
	283 (21,850)	1
(10)	283 (22,800)	13
	285 (27,000)	1
(11)	281·5 (16,800)	13
	283 (20,200)	1
(13)	257 (13,500), 357 (8700)	13
	275 (4120), 309 (7100), 342 (2280)	1
(14)	257 (18,300), 368 (12,700)	13
	276 (4350), 323 (8760), 358 (4400)	1
(15)	259 (17,050), 376 (9050)	13
	247sh (5140), 276 (3450), 324 (7720), 368sh (1870)	1
(16)	259 (14,400), 365 (8050)	13
	250sh (4900), 277 (4910), 317 (10,900) 354sh (1450)	1
(17)	258 (13,500), 366 (7750)	13
	250sh (5300), 273 (4750), 318 (6850), 358sh (1790)	1
(18)	257 (18,250), 375 (11,500)	13
	255sh (5180), 278 (3070), 329 (11,200), 365sh (3260)	1
(19)	258 (12,100), 357 (8200)	13
	280 (3440), 312 (10,900), 350sh (3160)	1
(20)	258 (17,650), 368 (12,680)	13
	276 (4500), 323 (10,190), 358 (4740)	1
(21)	258 (19,050), 369 (8300)	13
	274 (5720), 317 (6770), 357 (3460)	1
(22)	258 (16,800), 374 (11,000)	13
	255sh (5020), 278 (3140), 329 (9900), 368sh (1790)	1
(23)	256 (17,200), 366 (9250)	13
	273 (6600), 320 (5540), 357 (5420)	1
(29)	283 (18,300)	13
	286 (22,000)	1
(30)	283 (19,100)	13
	287 (23,100)	1
(48 or 49)	270 (13,300), 341 (9000)	13
	271 (10,400), 315 (10,900)	1
(50)	266 (8500), 317 (7300), 337sh (4450)	13
	245 (5680), 307 (9350)	1
(51)	266 (7640), 317 (7200), 337sh (4070)	13
	244 (5380), 308 (9330)	1
(55)	249 (12,150), 273 (7170), 361 (12,400)	13
	240 (8200), 275sh (4730), 323 (10,200), 362 (4100)	1
(56)	249 (11,500), 274 (7030), 361 (11,700)	13
	241 (7970), 275sh (3420), 322 (9150), 360 (4570)	1

† Absorptions below 240 nm are not quoted.

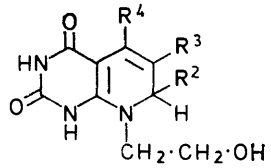
[10] T. Rowan and H. C. S. Wood, *J. Chem. Soc.* (*C*), 1968, 452.

[11] S. Kaufman, *J. Biol. Chem.*, 1964, **239**, 332.

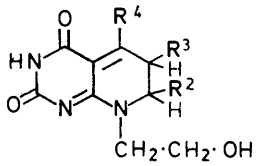
(*d*) High resolution n.m.r. spectra (Table 3) show (i) a chemical shift for H_A consistent with its environment in structure (40), and appearing upfield from the predicted value (τ *ca.* 4) for the alternative structure (33a),



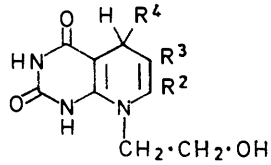
(29) R^2 = Me, R^3 = R^4 = H
(30) R^2 = R^3 = H, R^4 = Me
(31) R^2 = R^4 = H, R^3 = Me



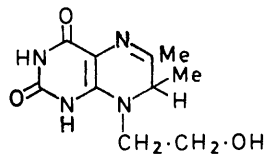
(32) a; R^2 = Me, R^3 = R^4 = H
b; R^2 = R^3 = H, R^4 = Me
c; R^2 = R^4 = H, R^3 = Me



(33)
a; R^2 = Me, R^3 = R^4 = H
b; R^2 = R^3 = H, R^4 = Me
c; R^2 = R^4 = H, R^3 = Me



(34)
a; R^2 = Me, R^3 = R^4 = H
b; R^2 = R^3 = H, R^4 = Me
c; R^2 = R^4 = H, R^3 = Me



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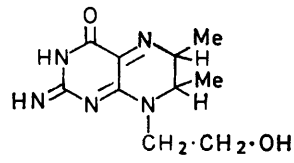


(36)

(ii) that the adjacent methylene groups of the side-chain are less equivalent than would be predicted for a simple 2-hydroxyethyl system, *e.g.* in (33a), and (iii) that the coupling constants J_{AB} and J_{AC} are significantly different, as would be expected in (40), whereas in the alternative formulation (33a) H_A would more or less bisect the dihedral angle H_B–C–H_C.

TABLE 3

^{1}H N.m.r. spectra of compound (8) at 100 MHz (τ values)

In $CF_3 \cdot CO_2H$	In NaOD	Assignment [a]
8·7	8·89	CH_3
8·25	8·56	$CH_2 \cdot CH \cdot CH_3$
7·54	7·8	
6·64	6·98	$CH \cdot CH_3$
6·1	6·3	$CH_2 \cdot CH_2$
5·74	5·95	
5·34	5·68	
4·87	5·13	$CH \cdot O$

Coupling constants [b] (Hz): J_{AB} 3·5; J_{AC} 10·5; J_{BC} 13·0; J_{BD} 2·0; J_{CD} 5·0; J_{DMe} 7·0.

[a] Made from double and triple resonance experiments. [b] *Cf.* structure (40).

(*e*) A 5,8-dihydro-structure (34a), again compatible with the chemical evidence, is excluded since the 7-methyl signal is clearly a doublet (Table 4), and the size of the coupling constant rules out any long-range coupling.

Similar arguments can be elaborated in support of the bridged-ring structures (41) and (42) for the intermediates formed in the methyl vinyl ketone and methacraldehyde reactions. The n.m.r. evidence also establishes that the products obtained from crotonaldehyde and from methyl vinyl ketone must have the methyl groups at positions 7 and 5, respectively (Table 4). Were these assignments to be reversed, the only possible structures for the intermediate compounds would be (34b) and (34a), respectively. Since structure (34c) is conclusively eliminated for the methacraldehyde product (methyl signal is a doublet), and all the intermediates have a similar structure, this possibility can be eliminated.

TABLE 4

^{1}H N.m.r. spectra [a] of reduced pyrido[2,3-*d*]pyrimidines at 60 MHz (τ values; J in Hz)

Compound	5-Me	6-Me	7-Me	Solvent
(8)			9·0 (d, J 7)	NaOD
			8·68 (d, J 5)	$CF_3 \cdot CO_2H$
(9)	8·7(s)			NaOD
	8·43(s)			$CF_3 \cdot CO_2H$
(10)		8·9 (d, J 7)		NaOD
		8·64 (d, J 5)		$CF_3 \cdot CO_2H$
(11)		8·88 (d, J 7)	9·08 (d, J 7)	NaOD
(30)	8·87 (d, J 6)			NaOD
	8·55 (d, J 6)			$CF_3 \cdot CO_2H$

[a] Only the signals due to methyl protons are given: remaining signals were too complex for analysis at 60 MHz.

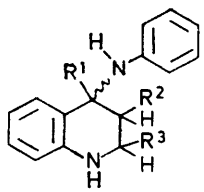
Recent studies [12] of the mechanism of the Doebner–Miller synthesis of quinolines have demonstrated the existence of intermediates (37) formed from two molecules of amine and one molecule of αβ-unsaturated carbonyl derivative. Displacement of one of the amine molecules by water leads to formation of alcohols (38) which are dehydrated and oxidised to give quinolines. Turner [13] has reported that, in the reaction between *N*-methylaniline and hydroxyacetaldehyde, the displacement of amine can occur internally by attack of the hydroxy-group of the side-chain to give a bridged tetrahydroquinoline (39). Our proposals for the bridged-ring structures for the intermediates (8)—(10) are in keeping with these observations.

The new synthesis of pyrido[2,3-*d*]pyrimidines was next applied to the synthesis of 8-substituted 6,7-dimethylpyrido[2,3-*d*]pyrimidines. Thus, condensation of 2-methylbut-2-enal (7) and the hydroxyethylaminouracil (2) gave as one of the main products a pyridopyrimidine (11) with properties similar to those of the intermediates (8)—(10). The n.m.r. spectrum (Table 4) indicated two MeCH< groups, as would be expected from structure (43).

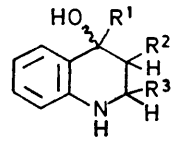
[12] G. M. Badger, H. P. Crocker, B. C. Ennis, J. A. Gayler, W. E. Matthews, W. G. C. Raper, E. L. Samuel, and T. M. Spotswood, *Austral. J. Chem.*, 1963, **16**, 814; L. P. Zalukaev, *Doklady Akad. Nauk S.S.S.R.*, 1956, **110**, 1791; L. P. Zalukaev and L. Y. Spitsina, *Zhur. obshchei Khim.*, 1961, **31**, 3007; 1964, **34**, 3392; G. A. Dauphinee and T. P. Forrest, *Chem. Comm.*, 1969, 327.
[13] A. B. Turner, *Chem. Comm.*, 1968, 1659.

The other major product was the 6,7-dimethylpyridopyrimidine (16), which could also be obtained by heating the intermediate (11) in diphenyl ether or boiling water.

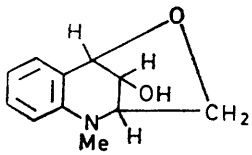
4-D-Ribitylaminouracil (3) was treated in a similar fashion with 2-methylbut-2-enal (7) in acid. A product (12) was obtained but could not be purified satisfactorily and has not been characterised. It did however, exhibit u.v. absorption similar to that of intermediate (11). Treatment in diphenyl ether could not be applied in this case as the product was insoluble in the solvent. Heating the product in 2-ethoxyethanol or water for a few hours gave a mixture. The 6,7-dimethyl-8-D-ribitylpyridopyrimidine (17) was eventually obtained by heating the crude product at its m.p. for a few minutes.



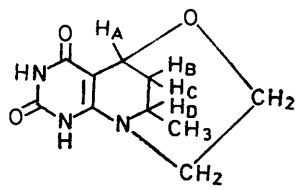
(37)



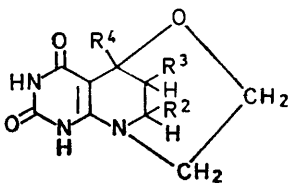
(38)



(39)



(40)



(41) $R^2 = R^3 = H$, $R^4 = Me$
(42) $R^2 = R^4 = H$, $R^3 = Me$
(43) $R^2 = R^3 = Me$, $R^4 = H$

The structure was assigned on the basis of the u.v. spectrum which was almost identical with that of the 8-hydroxyethyl-6,7-dimethyl analogue (16), but different from that of the 8-hydroxyethyl-5,6-dimethyl analogue (18), prepared by an alternative method (See Table 2).

Syntheses from β-Dicarbonyl Derivatives.—Robins and Hitchings [6] have reported that the condensation of 6-aminouracil with β-diketones or β-keto-aldehydes in phosphoric acid at 100° yields pyrido[2,3-*d*]pyrimidine-2,4-diones. We have applied this reaction to the synthesis of 8-substituted pyridopyrimidines from 6-(substituted amino)uracils.

The reaction between 6-(2-hydroxyethylamino)uracil (2) and the sodium salt [14] of 2-methyl-3-oxobutanal (24) in 85% phosphoric acid gave a low yield of a pyrido-[2,3-*d*]pyrimidine. The product differed from the 6,7-dimethylpyrido[2,3-*d*]pyrimidine (16) prepared by the Doebner–Miller-type synthesis from 2-methylbut-2-enal and we therefore formulate it as 8-(2-hydroxyethyl)-5,6-dimethylpyrido[2,3-*d*]pyrimidine-2(8*H*),4(3*H*)-dione (18). The formation of this isomer was unexpected, since Robins and Hitchings,[6] in their extensive study of the reaction of β-keto-aldehydes with 6-aminouracil, found invariably that the sole product of the reaction arose from condensation of the aldehyde function of the β-keto-aldehyde at position 5 of the pyrimidine ring. We have confirmed that this is so for 6-aminouracil, but our experience with 6-(substituted amino)uracils (see later) is that the reverse takes place, the aldehyde function reacting with the 6-(substituted amino)-group of the pyrimidine.

Acetylacetone (25) was condensed in similar fashion with 6-(2-hydroxyethylamino)uracil (2) to give the 5,7-dimethylpyrido[2,3-*d*]pyrimidine (19). The yields in these reactions were low, however, and we found that the use of dilute hydrochloric acid in place of 85% phosphoric acid led to increased yields [this is true only for the 6-(substituted amino)uracils, phosphoric acid remaining the preferred reagent for reactions with 6-aminouracil]. A further increase in yield was obtained by using the acetals of the keto-aldehydes rather than the sodium salts.

In this way the acetals (26)—(28) were condensed with the hydroxyethylaminouracil (2) in dilute hydrochloric acid to give the 8-substituted pyrido[2,3-*d*]pyrimidines (20), (14), and (21) in high yield. The structure of compound (14) was confirmed by direct comparison with a sample prepared by the Doebner–Miller-type synthesis from methyl vinyl ketone. The u.v. spectrum of compound (20) was also in agreement with its formulation as a 5-ethyl, rather than a 7-ethyl, derivative (Table 2). The ribitylaminouracil (3) gave analogous products (22) and (23) on condensation with the keto-aldehyde (24) and the acetal (28), respectively.

Properties of 8-Substituted Pyrido[2,3-d]*pyrimidines.*—Since 8-substituted pyrido[2,3-*d*]pyrimidines have not been reported previously, we have made a preliminary study of some of their properties. The u.v. spectra (Table 2) are characteristic. Typically at pH 13 there are three main regions of absorption, 360—375, 255—260, and 220 nm. At pH 1, however, the long wavelength band collapses to a band of much lower intensity (a shoulder in some cases), with other bands at 315—330, 273—280, and 210—220 nm. Large shifts of the long wavelength band on changing the pH of the solution of an aromatic *N*-heterocycle are often associated with covalent hydration,[15] which involves the addition of a molecule of water across a C=N bond.

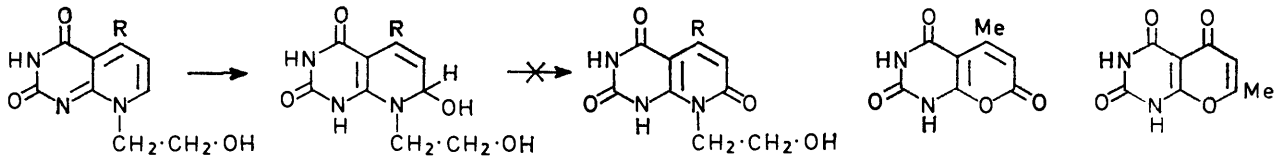
We have been unable to obtain definite evidence in favour of this hypothesis. Thus, attempts to oxidise the pyrido[2,3-*d*]pyrimidines (14) and (21) in acid solution did not give the corresponding 7-oxo-derivatives (46) and (47) which, by analogy with similar work [9,15] in the pteridine series, would be the expected oxidation products of covalent hydrates of type (44) and (45).

[14] E. E. Royals and K. C. Brannock, *J. Amer. Chem. Soc.*, 1954, **76**, 1180.

[15] A. Albert and W. L. F. Armarego, *Adv. Heterocyclic Chem.*, 1965, **4**, 1.

In addition, we have not observed any of the expected steric effects due to methyl groups at the suggested site of hydration. All the monomethyl isomers (13)—(15) have u.v. spectra which are virtually identical at pH 1 with that of the unsubstituted compound (21) (Table 2). The evidence for the formation of covalent hydrates is thus inconclusive, and further investigation would be in order.

An attempt to prepare an authentic pyrido[2,3-*d*]-pyrimidin-7-one (46) by condensation of 6-(2-hydroxyethylamino)uracil (2) with ethyl acetoacetate in hot phosphoric acid led, unexpectedly, to formation of the 7*H*-pyrano[2,3-*d*]pyrimidine (48) or its isomer (49). It seems likely that this arises by preliminary hydrolysis of the 6-aminopyrimidine derivative in acid solution



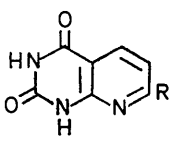
(14) R = Me
(21) R = H

(44) R = Me
(45) R = H

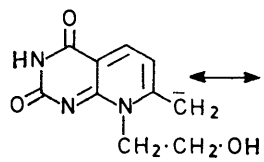
(46) R = Me
(47) R = H

(48)

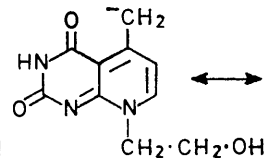
(49)



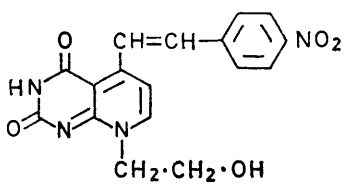
(50) R = Me
(51) R = Et



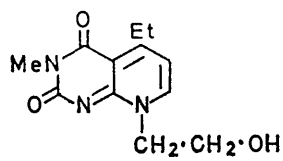
(52)



(53)



(54)



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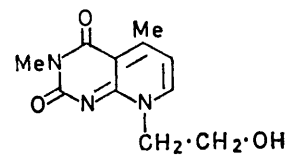


(56)

(*cf.* ref. 16) to give barbituric acid, which is known [17] to react with ethyl acetoacetate in concentrated sulphuric acid to give a pyrano[2,3-*d*]pyrimidine [(48) or (49)].

While examining the ^{1}H n.m.r. spectra of the 8-substituted pyridopyrimidines in sodium deuterioxide, we observed deuterium exchange of protons on *C*-methyl groups at positions 5 and 7 of the pyridopyrimidines (Table 1). The exchange reaction took place on dissolving the product in sodium deuterioxide at pH > 9. Because of the poor solubility of the pyridopyrimidines in acidic media we have not studied the possibility of acid-catalysed deuterium exchange.

Only one methyl group underwent exchange of its protons by deuterium in the 5,6- and 6,7-dimethyl isomers (18) and (16), whereas in the 5,7-dimethyl analogue (19) both sets of *C*-methyl protons were exchanged with deuterium. Of the monomethyl isomers only the 6-methylpyridopyrimidine (15) failed to undergo exchange. Thus both the 5- and 7-methyl groups are capable of undergoing the exchange process.

This exchange phenomenon is not duplicated in the 2,4-dihydroxypyridopyrimidines (50) and (51), at least not to any detectable extent in comparison with the 8-substituted series (Table 1).

The possibility that an oxidative demethylation, or some similar degradation, of the 5- and 7-methyl groups had taken place was ruled out by recovery of unchanged starting material on neutralisation of the sodium deuterioxide.

We have considered two possible mechanisms for the deuteriation of the *C*-methyl groups. The first involves initial covalent hydration at both the 5- and 7-positions, followed by enolisation of the resulting methyl ketones (57) and (58) (see Scheme). This appears unlikely since we have noted that covalent hydration seems to occur in acidic media. Moreover, double hydration of aromatic heterocycles is unusual [18] and normally proceeds in a stepwise manner. Such a mechanism would be impossible if applied to the 5- and 7-positions of the pyridopyrimidines, since the second molecule of water would have no C=N bond to add across.

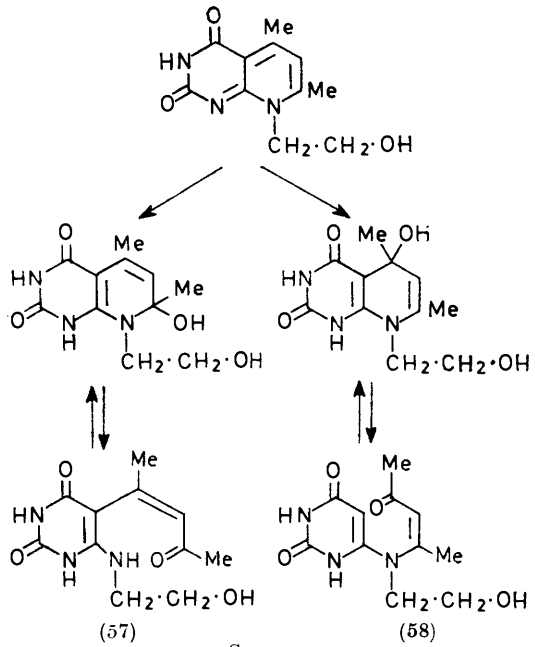
The whole postulate seems unlikely, however, since, even if small amounts of the ring-opened materials (57) and (58) were present, we have shown that under alkaline conditions very little pyridopyrimidine (19) is formed from the substituted aminouracil (2) and a β-dicarbonyl

[16] Ref. 8, p. 229.
[17] M. Ridi, *Gazzetta*, 1950, **80**, 121.
[18] A. Albert, *Angew. Chem. Internat. Edn.*, 1967, **6**, 919.

derivative, and recombination of the ring-opened products thus appears unlikely under these conditions.

A more reasonable mechanism for the deuteriation reaction involves the initial formation of a highly delocalised anionic species (52), illustrated here for the 7-methyl isomer. A similar species (53) can be envisaged for the 5-methylpyridopyrimidine but not for its 6-methyl analogue.

A mechanism of this type has been proposed [19] to explain the deuteriation of the 7-methyl group in riboflavin 5'-phosphate at alkaline pH, and [20] to explain a similar exchange of 7-methyl protons in certain 8-substituted lumazine derivatives. A recent paper [21] confirms this explanation in the case of 8-substituted pteridine derivatives.



SCHEME

The reactivity of the 7-methyl protons in flavins can be demonstrated by the condensation of various benzaldehydes with lumiflavin to give 7-styryl derivatives.[22] The 5-methylpyridopyrimidine (14) undergoes a similar condensation with *p*-nitrobenzaldehyde in sodium hydroxide solution to give a 5-styryl product (54). The n.m.r. spectrum of the product (54) showed no signal corresponding to a methyl group and showed the pattern to be expected from the protons of 1,4-disubstituted benzene derivatives.

Pyrimidines with an ' active ' methyl group have been alkylated on this group under basic conditions, *e.g.* 2,4,6-trimethylpyrimidine on treatment with strong base followed by treatment with methyl iodide gave 4-ethyl-2,6-dimethylpyrimidine.[23] We have carried out a similar sequence of reactions by dissolving the 5-methylpyridopyrimidine (14) in alcoholic potassium hydroxide (giving a red suspension from the colourless starting material) followed by addition of methyl iodide. At least four pyridopyrimidines were formed in the reaction, two of which were identified as the 5-ethyl and 5-ethyl-3-methyl analogues (20) and (55). A third was identified as the 3,5-dimethylpyridopyrimidine (56).

The products (55) and (56) were prepared from 6-(2-hydroxyethylamino)-1-methyluracil and the appropriate keto-aldehyde.

EXPERIMENTAL

U.v. spectra were determined with a Unicam SP 800A spectrophotometer for aqueous solutions of standard pH. N.m.r. spectra were determined with either a Perkin-Elmer R10 spectrometer (60 MHz) or a Varian HA-100 instrument (100 MHz) (tetramethylsilane as standard). I.r. spectra were run with a Perkin-Elmer 257 grating spectrophotometer either for liquid films or for potassium chloride discs. Mass spectra were run with an A.E.I. MS902 instrument.

Paper chromatograms were developed by the ascending technique using Whatman no. 1 paper with (A) butan-1-ol–5N-acetic acid (7 : 3), (B) propan-1-ol–aqueous 1% ammonia (2 : 1), and (C) 3% ammonium chloride as solvents. Spots were located by illumination with filtered u.v. light (254 and 365 nm) and D-ribityl derivatives were further identified by use of a periodate spray.[24]

Reaction between Crotonaldehyde and 6-(2-Hydroxyethylamino)uracil.—6-(2-Hydroxyethylamino)uracil (5·1 g, 0·03 mol) [25] was dissolved in 20% hydrochloric acid (50 ml) and freshly distilled crotonaldehyde (5 ml) was added. The mixture was set aside overnight at room temperature, and the colourless crystals precipitated were filtered off. Careful addition of concentrated ammonium hydroxide to the mother liquor gave a smaller crop of the same product. Recrystallisation from water gave *5,6,7,8-tetrahydro-7-methyl-5,8-(1-oxapropano)pyrido*[2,3-d]*pyrimidine*-2(1H),-4(3H)-*dione* (8) (4·8 g, 72%) as needles, m.p. 249—252° (Found: C, 53·8; H, 6·2; N, 18·5%; M^+, 223·09576. $C_{10}H_{13}N_3O_3$ requires C, 53·8; H, 5·85; N, 18·85%; *M*, 223·09568), which gave no colour [8] when treated with sodium nitrite and acetic acid or sodium nitrite and dilute hydrochloric acid.

8-(2-Hydroxyethyl)-7-methylpyrido[2,3-d]*pyrimidine*-2(8H),4(3H)-*dione* (13).—The intermediate (8) was held at its m.p. for a few minutes to give a new product. The conversion was carried out more satisfactorily by refluxing the intermediate (1 g) in diphenyl ether (50 ml) for 2 h and precipitating the new product with light petroleum or hexane. Recrystallisation from aqueous ethanol gave the *pyridopyrimidine* (0·8 g, 81%) as pale yellow needles, m.p. 295—298° (decomp.) (Found: C, 53·6; H, 5·1; N, 19·05. $C_{10}H_{11}N_3O_3$ requires C, 54·3; H, 5·0; N, 19·0%).

A solution of the product (8) in dilute hydrochloric acid slowly formed the *pyridopyrimidine* (13).

5,6,7,8-Tetrahydro-8-(2-hydroxyethyl)-7-methylpyrido[2,3-d]*pyrimidine*-2(1H),4(3H)-*dione* (29).—A solution of the pyridopyrimidine (13) in 5N-hydrochloric acid was hydrogenated over platinum oxide (uptake 2 mol. equiv.).

[19] F. J. Bullock and O. Jardetzky, *J. Org. Chem.*, 1965, **30**, 2056.
[20] T. Paterson and H. C. S. Wood, *Chem. Comm.*, 1969, 290.
[21] W. Pfleiderer, R. Mengel, and P. Hemmerich, *Chem. Ber.*, 1971, **104**, 2273.
[22] P. Hemmerich, *Helv. Chim. Acta*, 1960, **43**, 1942.
[23] J. C. Roberts, *J. Chem. Soc.*, 1952, 3065.
[24] R. L. Metzenberg and H. K. Mitchell, *J. Amer. Chem. Soc.*, 1954, **76**, 4187.
[25] W. Pfleiderer and G. Nübel, *Annalen*, 1960, **631**, 168.

Work-up gave the *hydrochloride* of (29) as crystals (70%) (Found: C, 45·9; H, 5·9; Cl, 13·7; N, 15·9. $C_{10}H_{15}N_3O_3$,HCl requires C, 45·9; H, 6·1; Cl, 13·55; N, 16·1%). The same product was obtained by catalytic reduction (uptake 1 mol. equiv.) of the intermediate (8).

Reaction between Methyl Vinyl Ketone and 6-(2-*Hydroxyethylamino*)*uracil*.—6-(2-Hydroxyethylamino)uracil (0·34 g, 0·002 mol) and methyl vinyl ketone (0·5 mol) in 20% hydrochloric acid (3·5 ml) were kept at room temperature overnight. Careful addition of concentrated ammonium hydroxide precipitated a white powder which gave 5,6,7,8-*tetrahydro*-5-*methyl*-5,8-(1-*oxapropano*)*pyrido*[2,3-d]*pyrimidine*-2(1H),4(3H)-*dione* (9) (0·14 g, 31%) as crystals, m.p. 266—267° (from water) (Found: C, 53·7; H, 5·9; N, 18·75. $C_{10}H_{13}N_3O_3$ requires C, 53·8; H, 5·85; N, 18·85%).

8-(2-*Hydroxyethyl*)-5-*methylpyrido*[2,3-d]*pyrimidine*-2(8H),4(3H)-*dione* (14).—(*a*) 6-(2-Hydroxyethylamino)-uracil (3·4 g, 0·02 mol) was dissolved in the minimum amount of hot 0·1N-hydrochloric acid, and acetoacetaldehyde dimethyl acetal (4 ml) was added. The mixture was refluxed for 2 h, cooled, and concentrated *in vacuo* until crystallisation commenced. The crystals were filtered off, washed with hot benzene (to remove any 1,3,5-triacetylbenzene), and recrystallised from aqueous ethanol to give the *pyrido*[2,3-d]*pyrimidine* (2·9 g, 67%) as pale yellow needles, m.p. 314—316° (decomp.) (Found: C, 54·7; H, 5·0; N, 19·1. $C_{10}H_{11}N_3O_3$ requires C, 54·3; H, 5·0; N, 19·0%).

(*b*) The intermediate pyridopyrimidine (9) was heated in diphenyl ether as for the 7-methyl isomer. Recrystallisation of the resultant product from aqueous ethanol gave compound (14) (65%), identical with the material prepared in (*a*).

5,6,7,8-*Tetrahydro*-8-(2-*hydroxyethyl*)-5-*methylpyrido*[2,3-d]*pyrimidine*-2(1H),4(3H)-*dione* (30) *Hydrochloride*.—The pyrido[2,3-*d*]pyrimidine (14) (0·74 g, 0·0033 mol) was dissolved in 5N-hydrochloric acid (25 ml) and hydrogenated over platinum oxide (80 mg) (uptake 2 mol. equiv.). The solution was filtered and concentrated *in vacuo* until crystallisation occurred. Recrystallisation from ethanol–acetone gave the *tetrahydropyridopyrimidine hydrochloride* (0·505 g, 58%) as a white powder, m.p. 204—206° (Found: C, 46·0; H, 5·65; Cl, 13·65; N, 15·3. $C_{10}H_{15}N_3O_3$,HCl requires C, 45·9; H, 6·1; Cl, 13·55; N, 16·1%).

The same material was obtained by hydrogenation of the intermediate pyridopyrimidine (9) (uptake 1 mol. equiv.).

Reaction between Methacraldehyde and 6-(2-*Hydroxyethylamino*)*uracil*.—The procedure used for the reaction with methyl vinyl ketone gave 5,6,7,8-*tetrahydro*-6-*methyl*-5,8-(1-*oxapropano*)*pyrido*[2,3-d]*pyrimidine*-2(1H),4(3H)-*dione* (10) (35%) as crystals, m.p. 250—252° (from water) (Found: C, 53·6; H, 6·35; N, 18·55. $C_{10}H_{13}N_3O_3$ requires C, 53·8; H, 5·85; N, 18·85%).

Treatment in diphenyl ether and work-up as before gave 8-(2-*hydroxyethyl*)-6-*methylpyrido*[2,3-d]*pyrimidine*-2(8H),4(3H)-*dione* (15) (60%) as pale yellow needles, m.p. 311—314° (decomp.) (Found: C, 54·3; H, 5·45; N, 18·95. $C_{10}H_{11}N_3O_3$ requires C, 54·3; H, 5·0; N, 19·0%).

Reaction of 2-*Methylbut*-2-*enal and* 6-(2-*Hydroxyethylamino*)*uracil*.—The reaction was carried out in the usual way but was difficult to control. After 24 h at room temperature, the mixture contained a considerable amount of fluorescent material as well as three u.v.-absorbing products. Careful addition of concentrated ammonium hydroxide solution gave a white precipitate containing one u.v.-absorbing product (11) (21%). Recrystallisation from water gave a poor recovery of this material, which had been converted to a blue-fluorescent substance in about 80% yield. 5,6,7,8-*Tetrahydro*-6,7-*dimethyl*-5,8-(1-*oxapropano*)-*pyrido*[2,3-d]*pyrimidine*-2(1H),3(4H)-*dione* (11) was eventually obtained as crystals, m.p. 246—248° (Found: C, 55·2; H, 6·45; N, 17·65. $C_{11}H_{15}N_3O_3$ requires C, 55·7; H, 6·35; N, 17·7%).

The intermediate (11) was converted into 8-(2-*hydroxyethylamino*)-6,7-*dimethylpyrido*[2,3-d]*pyrimidine*-2(8H),4(3H)-*dione* (16) (78%) in the usual way to yield needles, m.p. 317—319° (decomp.) (from ethanol) (Found: C, 56·1; H, 5·5; N, 17·65. $C_{11}H_{13}N_3O_3$ requires C, 56·15; H, 5·55; N, 17·85%), identical with the substance obtained during the attempted recrystallisation of product (11) from water.

The mother liquor of the initial reaction mixture was concentrated *in vacuo* until crystallisation occurred, giving a product (5%) which formed needles, m.p. 243—245° (decomp.) (from water) (Found: C, 53·65; H, 5·9; N, 19·0%).

The mother liquor was then evaporated to dryness *in vacuo*, and the residue (from aqueous ethanol) gave the pyrido[2,3-*d*]pyrimidine (16) (27%).

The mother liquor of the recrystallisation yielded yet another product (2%) as crystals, m.p. 215—216° (Found: C, 50·0; H, 7·8; N, 19·3%).

The u.v. spectra of the two minor products were similar to that of the intermediate pyridopyrimidine (11) but we were unable to assign a structure to either.

Reaction of 6-D-*Ribitylaminouracil and* 2-*Methylbut*-2-*enal*.—2-Methylbut-2-enal (0·4 ml) was added to a solution of 6-D-ribitylaminouracil [26] (0·4 g, 1·53 mmol) in 20% hydrochloric acid and the mixture was left overnight at room temperature. The mother liquor was evaporated to dryness *in vacuo* to give a gum. The product was obtained as an amorphous solid (0·21 g) by dissolution in hot ethanol and precipitation with ether. It slowly became 'tacky' and could not be crystallised, although it appeared to be homogeneous on paper chromatography in systems (A), (B), and (C).

The crude material was refluxed in diphenyl ether but proved to be insoluble; extensive charring occurred.

Heating in 2-ethoxyethanol on a steam bath for 2 h resulted in complete conversion of the product into two blue-fluorescent products and several trace products. The faster running of the former was obtained by heating the crude product at its m.p. for a short period. The melt was crushed in ether and filtered. Attempted purification by dissolving in aqueous ethanol and precipitating with ether gave a gum, which slowly solidified during several days to give 6,7-*dimethyl*-8-D-*ribitylpyrido*[2,3-d]*pyrimidine*-2(8H),4(3H)-*dione* (17) as a tan solid, m.p. 220—222° (Found: M^+, 325·12707. $C_{14}H_{19}N_3O_6$ requires M, 325·12737).

8-(2-*Hydroxyethyl*)-5,6-*dimethylpyrido*[2,3-d]*pyrimidine*-2(8H),4(3H)-*dione* (18).—(*a*) 6-(2-Hydroxyethylamino)-uracil (8·5 g, 0·05 mol) was dissolved with gentle heating in 85% phosphoric acid (60 ml). The sodium salt of 2-methyl-3-oxobutanal [14] (85% pure; 6·1 g, 0·05 mol) was added and the mixture was heated for 3 h on a steam-bath, poured into water (250 ml) and left overnight at 0°. The precipitate was discarded and the mother liquor was adjusted to pH 5 with concentrated ammonium hydroxide solution. Re-

[26] G. F. Maley and G. W. E. Plaut, *J. Biol. Chem.*, 1959, **234**, 641.

frigeration gave a pale brown precipitate which was recrystallised from water (×4) to give the *pyridopyrimidine* (1·2 g, 10·0%) as needles, m.p. 310° (slow decomp.) (Found: C, 56·15; H, 5·85; N, 17·9. $C_{11}H_{13}N_3O_3$ requires C, 56·15; H, 5·55; N, 17·85%).

(*b*) 6-(2-Hydroxyethylamino)uracil (3·4 g, 0·02 mol) and the sodium salt of 2-methyl-3-oxobutanal (85% pure; 2·45 g, 0·02 mol) were refluxed in 0·1N-hydrochloric acid (25 ml); the mixture was adjusted to pH 2 with 1N-hydrochloric acid. After 2 h the hot solution was filtered and cooled. Concentration *in vacuo* until crystallisation occurred, followed by recrystallisation from aqueous ethanol (charcoal), gave the pyridopyrimidine (2·2 g, 47%) as needles, m.p. 310—312° (decomp.).

(*c*) The reaction was repeated in 0·1N-hydrochloric acid but with the dimethyl acetal of the keto-aldehyde in place of the sodium salt. Less solvent was required and an increase in yield (58%) was obtained.

5,6,7,8-*Tetrahydro*-8-(2-*hydroxyethyl*)-5,6-*dimethylpyrido*-[2,3-d]*pyrimidine*-2(1H),4(3H)-*dione*.—The pyrido[2,3-*d*]-pyrimidine (18) (0·235 g, 0·001 mol) was suspended in water (25 ml) and hydrogenated over platinum oxide (100 mg). After uptake of 2 mmol of hydrogen the catalyst was removed and the filtrate was evaporated to dryness *in vacuo*. Recrystallisation from aqueous ethanol gave the *tetrahydropyridopyrimidine* (0·215 g, 90%) as a white powder, m.p. 244—247° (Found: C, 54·8; H, 7·1; N, 17·7. $C_{11}H_{17}N_3O_3$ requires C, 55·25; H, 7·1; N, 18·0%).

8-(2-*Hydroxyethyl*)-5,7-*dimethylpyrido*[2,3-d]*pyrimidine*-2(8H),4(3H)-*dione* (19).—6-(2-Hydroxyethylamino)uracil (3·4 g, 0·02 mol) and acetylacetone (12·0 g, 0·12 mol) were refluxed in 0·1N-hydrochloric acid (100 ml) for 7 days. The solution was concentrated *in vacuo* and refrigerated. The precipitate was filtered off and recrystallised from water (charcoal) to give the *pyridopyrimidine* (0·65 g, 14·0%) as needles, m.p. >360° (Found: C, 55·85; H, 5·7; N, 17·7. $C_{11}H_{13}N_3O_3$ requires C, 56·15; H, 5·55; N, 17·85%).

5-*Ethyl*-8-(2-*hydroxyethyl*)*pyrido*[2,3-d]*pyrimidine*-2(8H),4(3H)-*dione* (20).—6-(2-Hydroxyethylamino)uracil (1·7 g, 0·01 mol) and 3-oxopentanal dimethyl acetal (1·5 g, 0·01 mol) were refluxed in 0·1N-hydrochloric acid for 4 h and cooled at 0° overnight. The precipitate was filtered off but contained very little pyridopyrimidine (the solid was probably 1,3,5-tripropionylbenzene). The mother liquor was evaporated *in vacuo* until precipitation commenced and was then cooled. The product was filtered off and recrystallised from aqueous ethanol to give the *pyridopyrimidine* (0·76 g, 32·5%) as needles, m.p. 249—251° (decomp.) (Found: C, 56·05; H, 5·75; N, 17·85. $C_{11}H_{13}N_3O_3$ requires C, 56·15; H, 5·55; N, 17·85%).

A further crop (0·39 g, 16%) was obtained by concentrating the mother liquor, cooling, and recrystallising from aqueous ethanol.

8-(2-*Hydroxyethyl*)*pyrido*[2,3-d]*pyrimidine*-2(8H),4(3H)-*dione* (21).—(*a*) Malonaldehyde bis(diethyl acetal) (4·4 g, 0·02 mol) and 6-(2-hydroxyethylamino)uracil (3·42 g, 0·02 mol) were heated in 85% phosphoric acid (25 ml) for 1 h. The mixture was poured into water (160 ml) and worked up in the usual way. Recrystallisation from aqueous ethanol (charcoal) gave the *pyridopyrimidine* (0·46 g, 11%) as pale yellow needles, m.p. 280° (decomp.) (Found: C, 51·8; H, 4·5; N, 20·25. $C_9H_9N_3O_3$ requires C, 52·15; H, 4·35; N, 20·3%).

(*b*) The reaction was repeated in refluxing 0·1N-hydrochloric acid (25 ml) for 1 h. The solution was concentrated *in vacuo* and cooled. The product was recrystallised from aqueous ethanol to give the pyridopyrimidine (2·73 g, 66%).

5,6-*Dimethyl*-8-D-*ribitylpyrido*[2,3-d]*pyrimidine*-2(8H),-4(3H)-*dione* (22).—6-D-Ribitylaminouracil (2·6 g, 0·01 mol) and the sodium salt of 2-methyl-3-oxobutanal (85% pure; 1·22 g, 0·01 mol) were refluxed in 0·5N-hydrochloric acid (40 ml) for 2 h. The solution was filtered and concentrated *in vacuo* to *ca.* 5 ml. Ethanol was added until precipitation began and the mixture was refrigerated. The product was collected and recrystallised from aqueous ethanol to give the *pyridopyrimidine* (0·65 g, 20%) as needles, m.p. 247—249° (decomp.) (Found: C, 51·5; H, 6·65; N, 11·55. $C_{14}H_{19}N_3O_6$,EtOH requires C, 51·75; H, 6·75; N, 11·3%).

The mother liquor was again concentrated *in vacuo* and treated with ethanol as before. The precipitate contained several impurities; recrystallisation from aqueous ethanol gave a gel which slowly formed crystals. Repeated recrystallisation gave a further crop of the pure product (0·41 g, 12·6%).

8-D-*Ribitylpyrido*[2,3-d]*pyrimidine*-2(8H),4(3H)-*dione* (23).—6-D-Ribitylaminouracil (2·61 g, 0·01 mol) and malonaldehyde bis(diethyl acetal) (2·2 g, 0·01 mol) were refluxed in 0·1N-hydrochloric acid (10 ml) for 1 h. The red solution was treated with charcoal, filtered, and concentrated *in vacuo* to *ca.* 3 ml. Ethanol was added and the precipitate was recrystallised from aqueous ethanol (charcoal) to give the *ribitylpyridopyrimidine* (1·22 g, 41%) as off-white crystals, m.p. 228—230° (decomp.) (Found: C, 48·5; H, 5·2; N, 14·1. $C_{12}H_{15}N_3O_6$ requires C, 48·5; H, 5·05; N, 14·15%).

Attempted Oxidation of 8-*Substituted Pyrido*[2,3-d]*pyrimidines*.—Various attempts were made to oxidise the pyrido-[2,3-*d*]pyrimidine (21) and its 5-methyl analogue (14). The oxidising agents used were 5% potassium permanganate solution and hydrogen peroxide (100 vol.). Alkaline potassium permanganate gave no reaction even on heating at 70° for 2 or 3 days

The reactions were carried out with various acids (5N-acetic acid, glacial acetic acid, 2N-sulphuric acid, and 5N-sulphuric acid). Permanganate or peroxide was added and the solution was left for a few days at room temperature. No identifiable products were obtained.

5-*Methyl*-7H-*pyrano*[2,3-d]*pyrimidine*-2(1H),4(3H),7-*trione* (48).—6-(2-Hydroxyethylamino)uracil (0·85 g, 0·005 mol) was dissolved in the minimum of 85% phosphoric acid and ethyl acetoacetate (0·5 ml) was added. The solution was heated at 100° for 5 h; little reaction occurred. Excess of ethyl acetoacetate (5 ml) was added and the mixture was kept at 100° for 30 h. A solid was precipitated, filtered off, and recrystallised from water to give the pyranopyrimidine (0·26 g, 22%) as needles, m.p. 350—354° (decomp.) (Found: C, 49·15; H, 3·0; N, 14·85%; M^+, 194·03268. Calc. for $C_8H_6N_2O_4$: C, 49·5; H, 3·05; N, 14·4%; *M*, 194·03275), τ ($CF_3{\cdot}CO_2H$) 7·22 (CH_3) and 3·7 (aromatic H).

7-*Methylpyrido*[2,3-d]*pyrimidine*-2(1H),4(3H)-*dione* (50).—The following procedure is a modification of that reported by Robins and Hitchings.[6]

6-Aminouracil (1·27 g, 0·01 mol) was dissolved in 85% phosphoric acid (10 ml) and the sodium salt of acetoacetaldehyde (1·1 g, 0·01 mol) was added. The mixture was heated at 100° for 3 h and poured into water (50 ml). The solution was cooled at 5° overnight, to give a dark brown precipitate which was discarded. The solution was adjusted to pH 4—5 with concentrated ammonium hydroxide solution and cooled. The crude product was filtered

off and recrystallised from water to give the pyridopyrimidine (0·4 g, 21·0%) as crystals, m.p. 315° (decomp.) (lit.,[27] 314—315°) (Found: C, 54·45; H, 3·95; N, 24·15. Calc. for $C_8H_7N_3O_2$: C, 54·25; H, 3·95; N, 23·8%).

7-Ethylpyrido[2,3-d]*pyrimidine-2*(1H),*4*(3H)*-dione* (51).—6-Aminouracil (3·8 g, 0·03 mol) and 3-oxopentanal dimethyl acetal (4·4 g, 0·03 mol) were heated at 100° in 85% phosphoric acid (25 ml) for 6 h. The brown syrup was poured into water (100 ml) and refrigerated. The precipitate thus obtained was discarded, and the mother liquors were adjusted to pH 5—6 with concentrated ammonium hydroxide solution. The crude product was filtered off and recrystallised from water to give the *pyridopyrimidine* (2·6 g, 45·4%) as crystals, m.p. 218—220° (Found: C, 56·6; H, 4·85; N, 22·2. $C_9H_9N_3O_2$ requires C, 56·55; H, 4·7; N, 22·0%).

8-(2-Hydroxyethyl)-5-(p-nitrostyryl)pyrido[2,3-d]*pyrimidine-2*(8H),*4*(3H)*-dione* (54).—The 5-methylpyrido[2,3-*d*]pyrimidine (14) (0·221 g, 0·001 mol) was dissolved in the minimum of 0·1N-sodium hydroxide and *p*-nitrobenzaldehyde (0·151 g, 0·001 mol) was added. After a short time at 100° a deep yellow precipitate was formed. The solution was cooled and filtered and the product was recrystallised from water to give the *pyridopyrimidine* (0·231 g, 65·5%) as bright orange crystals, m.p. >360° (Found: C, 56·7; H, 3·15; N, 15·5. $C_{17}H_{14}N_4O_5$ requires C, 57·6; H, 3·95; N, 15·8%), τ(CF_3·CO_2H) 4·8—5·4 (4H, m, CH_2·CH_2·OH), and 1·48 (2H, d) and 1·96 (2H, d), (aromatic protons).

Attempted Methylation of the 5-Methylpyrido[2,3-d]*pyrimidine* (14).—The pyridopyrimidine (110 mg) was added to a solution of sodium hydroxide (1 g) in ethanol (10 ml) and the mixture was heated on a steam-bath. A red-brown suspension was obtained; methyl iodide (1 g) was added and the heating was continued for 15 min. The red colour disappeared and an off-white solid was obtained and filtered off. Paper chromatography showed the product to consist mainly of four blue-fluorescent products. One of these was identified as the 5-ethylpyrido[2,3-*d*]pyrimidine (20) by comparison with material prepared before. Two of the others were identified as 5-ethyl-8-(2-hydroxyethyl)-3-methylpyrido[2,3-*d*]pyrimidine-2(8*H*),4(3*H*)-dione (55) and its 3,5-dimethyl analogue (56) by comparison with material prepared later. The fourth product was not identified.

8-(2-Hydroxyethyl)-3,5-dimethylpyrido[2,3-d]*pyrimidine-2*(8H),*4*(3H)*-dione* (56).—6-(2-Hydroxyethylamino)-1-methyluracil [28] (1·85 g, 0·01 mol) was dissolved in refluxing 0·1N-hydrochloric acid (25 ml) and acetoacetaldehyde dimethyl acetal (1·5 g, 0·011 mol) was added. The mixture was refluxed for 3 h, cooled, and filtered to give a precipitate (1·3 g). Concentration of the mother liquor *in vacuo* gave a further crop (0·3 g). Recrystallisation from aqueous ethanol gave the *pyridopyrimidine* (1·5 g, 64%) as needles, m.p. 255—258° (Found: C, 56·15; H, 5·75; N, 17·6. $C_{11}H_{13}N_3O_3$ requires C, 56·15; H, 5·55; N, 17·85%).

5-Ethyl-8-(2-hydroxyethyl)-3-methylpyrido[2,3-d]*pyrimidine-2*(8H),*4*(3H)*-dione* (55).—The foregoing procedure was repeated with 3-oxopentanal dimethyl acetal. The *pyridopyrimidine* (57%) was obtained as needles, m.p. 208—209° (Found: C, 58·05; H, 6·2; N, 16·45. $C_{12}H_{15}N_3O_3$ requires C, 57·85; H, 6·0; N, 16·85%).

Acetoacetaldehyde Dimethyl Acetal (27).—(*a*) The acetal (40%), b.p. 82° at 25 mmHg (lit.,[29] 69° at 20 mmHg) was prepared from the sodium salt of acetoacetaldehyde [30] as described later for 2-methyl-3-oxobutanal dimethyl acetal.

(*b*) The acetal (70%) was prepared from 4-chlorobut-3-en-2-one [29] as described later for 3-oxopentanal dimethyl acetal.

2-Methyl-3-oxobutanal (24) *Dimethyl Acetal.*—The sodium salt of 2-methyl-3-oxobutanal [14] (85% pure; 30·5 g, 0·25 mol) was suspended in dry benzene (100 ml) and the mixture was added slowly to a stirred solution of concentrated sulphuric acid (29 g) and dry methanol (32 g, 1 mol) at 10°. The mixture was stirred for 4 h and neutralised with sodium carbonate. The solids were filtered off and the brown filtrate was distilled *in vacuo* to give the acetal (12·8 g, 35·5%) as a pale yellow oil, b.p. 44—45° at 2 mmHg (lit.,[31] 68—73° at 8 mmHg).

3-Oxopentanal (26) *Dimethyl Acetal.*—A solution of sodium hydroxide (42 g, 1·05 mol) in absolute methanol (350 ml) was added during 2 h to a stirred solution of 1-chloropent-1-en-3-one [29] (118·5 g, 1·0 mol) in absolute methanol (150 ml) at −15° to −10°. The mixture was poured into a saturated solution of sodium chloride (1000 ml) and extracted with ether (4 × 100 ml). The combined extracts were dried ($MgSO_4$) and distilled (with a pinch of potassium carbonate) to give the acetal (91·8 g, 63%) as a pale yellow liquid, b.p. 83° at 10 mmHg (lit.,[32] 80° at 12 mmHg) τ ($CDCl_3$) 8·97 (3H, t, *J* 7 Hz, CH_3·CH_2), 7·52 (2H, q, *J* 7 Hz, CH_3·CH_2), 7·28 (2H, d, *J* 6 Hz, CO·CH_2·CH), 6·62 (6H, s, 2 × OMe), and 5·19 (1H, t, *J* 6 Hz, CO·CH_2·*CH*).

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