Heterocyclic Studies. Part III.[1,2] Hydrates of Ethyl Pteridine-4-carboxylate and Some Methyl Derivatives

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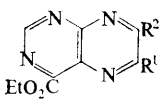
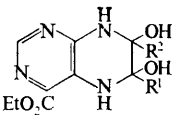
Ethyl pteridine-4-carboxylate, and its 7-methyl- and 6,7-dimethyl-derivatives have been converted into covalently dihydrated species (V)—(VII) in which two molecules of water are added across the 5,6- and 7,8-bonds. Dihydrates of this type have been obtained in pure solid form from the anhydrous pteridines (I)—(III) in two ways and also directly from ethyl 5,6-diaminopyrimidine-4-carboxylate without going through the anhydrous pteridine. In at least one case different stereoisomers of a hydrate have been isolated. The u.v., i.r., ^{1}H n.m.r., and mass spectroscopic data are recorded.

THE previous paper in this series [1] described the preparation of ethyl pteridine-4-carboxylate and some of its methyl derivatives (I)—(IV). All the compounds reacted reversibly with water to give, in solution, a proportion of covalently dihydrated species (V)—(VIII). Only in the case of ethyl pteridine-4-carboxylate (I) was the dihydrated species isolated and characterised. The present paper describes methods of obtaining solid dihydrates (VI) and (VII) of the methyl derivatives (II) and (III), as well as new methods for preparing dihydrates of (I).



(I) $R^1 = R^2 = H$
(II) $R^1 = H$, $R^2 = Me$
(III) $R^1 = R^2 = Me$
(IV) $R^1 = Me$, $R^2 = H$

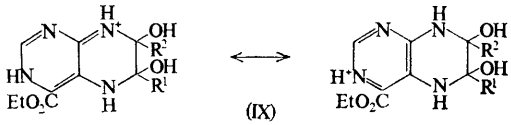


(V) $R^1 = R^2 = H$
(VI) $R^1 = H$, $R^2 = Me$
(VII) $R^1 = R^2 = Me$
(VIII) $R^1 = Me$, $R^2 = H$

Three ways of preparing hydrated compounds were used in this investigation.

Method (a).—The anhydrous pteridine [*e.g.*, (I)] was shaken with water until equilibrium was attained. If no precipitate resulted the solution was frozen by immersion in acetone and solid carbon dioxide and freeze-dried. Ether extraction of the residue removed unchanged pteridine. These conditions ensured little or no reversion to anhydrous species during the isolation process since the desired hydrate remained as a solid throughout. This method was not applicable to the dimethyl derivative (III) which is only slightly hydrated at equilibrium.[1]

Method (b).—A cold, concentrated, aqueous acidic solution of the pteridine (I)–(III) was rapidly neutralised and buffered to a pH value near 6. In each case a dihydrated compound (V)—(VII) separated. This method depends on the fact that the anhydrous compounds are rapidly converted, in acid solution, into very water-soluble, resonance-stabilised, dihydrated cations (IX).[1,3] Neutralisation then gives, almost instantaneously, a high concentration of dihydrated neutral molecules. Even if, as in the case of the dimethyl derivative (VII), the hydrated species is the less stable form, reversion to the more stable anhydrous form (III) is slow since hydration and dehydration reactions show a rate minimum near neutrality.[4]



(IX)

Method (c).—Ethyl 5,6-diaminopyrimidine-4-carboxylate (X) was shaken at room temperature in aqueous solution with polyglyoxal, pyruvaldehyde, or diacetyl to yield (V), (VI), or (VII), respectively. The products could not have arisen by hydration of first-formed pteridines (I—III). In the case of the 6,7-dimethyl derivative (III) hydration would not occur to any considerable extent in neutral conditions [1] and in fact the yield of dihydrate diminished, owing to dehydration, if the reaction time was extended. In the other cases (I) and (II) hydration would be much too slow under the reaction conditions to account for the products. Several reaction sequences are possible and these are illustrated for the dimethyl derivative. The first step almost certainly involves the more reactive [5] 5-amino-group of the diamine (X) and the product (XI) may cyclise to give the dihydrate (VII) directly. However the sequence (X) ⟶ (XI) ⟶ (XII) ⟶ (XIII) ⟶ (VII) is also a possibility. Similar schemes may be written for the formation of the other hydrates (V) and (VI) but in these cases the aldehyde groups of the carbonyl reagents and intermediates are probably hydrated or combined in some other way.

Each of the structures (V)—(VII) represents several isomers. The two hydroxy-groups may be on the same or opposite sides of the pyrazine ring and each geometrical isomer has several possible optical and, if the pyrazine ring is non-planar, conformational isomers. In addition there are numerous possibilities of inter- and intramolecular hydrogen bonding. It seems highly probable that covalent hydration is involved in some biochemical processes [6] so the stereochemical features of the hydration reactions are of great interest. Methods (b) and (c)

[1] Part II, J. Clark, *J. Chem. Soc.* (*C*), 1967, 1543.
[2] Preliminary communication, J. Clark, *Tetrahedron Letters*, 1967, 1099.
[3] A. Albert and W. L. F. Armarego, *Adv. Heterocyclic Chem.*, 1965, **4**, 34.
[4] D. D. Perrin, *Adv. Heterocyclic Chem.*, 1965, **4**, 70.
[5] D. J. Brown, 'The Pyrimidines,' ed. A. Weissberger, Interscience, New York and London, 1962, p. 322 *et seq.*; A. Albert and S. Matsuura, *J. Chem. Soc.*, 1961, 5131; 1962, 2162; W. Wilson, *ibid.*, 1948, 1157.
[6] F. Bergmann, H. Kweitny, G. Levin, and D. J. Brown, *J. Amer. Chem. Soc.*, 1960, **82**, 598; Y. Inoue and D. D. Perrin, *J. Chem. Soc.*, 1962, 2600.

always gave the same product and the three compounds (V)—(VII) obtained in these ways are probably the most stable stereoisomers of dihydrates. Where hydrates are referred to subsequently, without qualification, these are the isomers concerned.

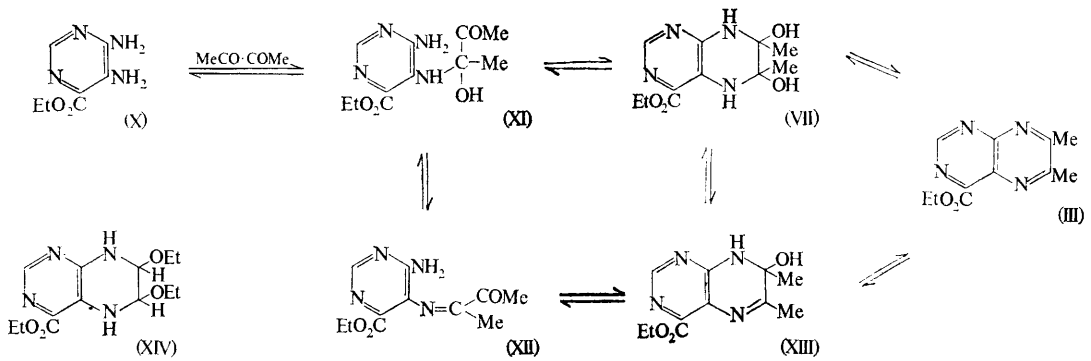


TABLE 1

^{1}H N.m.r. spectroscopy [a]

Pteridine	Solvent [b]	Ester CH_3 [c]	Ester CH_2 [d]	Chemical shifts (τ) 6-H	7-H	2-H	6-Me	7-Me	5-NH	8-NH	6-OH	7-OH	Remarks
(V) [e]	DMSO	8·71	5·74	5·24 [f]		2·12			2·25 [g]	1·60 [g]	4·27 [h]	4·19 [i]	OH signals collapse to 2 singlets and NH signals sharpen on irradiation of 6,7-proton signal
(V) [j]	DMSO	8·71	5·75	5·25 [f]		2·15			2·29 [k]	1·63 [k]	4·3 [m]		NH signals sharpened by irradiation of 6,7-proton signal, OH signals hardly affected
(V) [j]	D_2O	8·70	5·67	4·92 [n]	4·85 [n]	2·08							Irradiation of 6-proton signal causes 5-NH and 6-OH signals to become singlets
VI [e]	DMSO	8·72	5·75	5·40 [l]				8·57	2·24 [g]	1·74	4·26 [p]	4·58	Coupling constants determined by irradiating 5-NH and 6-OH signals successively
(VI) [q]	DMSO	8·71	5·73	5·38 [l]				8·57	2·27 [g]	1·77	4·25 [l]	4·61 [l]	
(VII)	DMSO	8·71	5·73			2·14	8·56	8·54	2·59	1·77	4·60	4·49	OH signals very sharp and NH signals fairly sharp singlets

[a] Measured on Varian HA 100 and A60A spectrometers. [b] DMSO = deuteriated dimethylsulphoxide with tetramethylsilane as internal standard. Spectra in D_2O measured with dioxan (τ 6·32) as internal standard. [c] Triplet, J = 7·2 c./sec. [d] Quartet, J = 7·2 c./sec. [e] More stable isomer. [f] Partly resolved multiplet. [g] Partly resolved doublet, $J \simeq 4$ c./sec. [h] Doublet, $J \simeq 4$ c./sec. [i] Doublet, $J \simeq 6$ c./sec. [j] 'Second isomer.' [k] Broadened by coupling with CH but not resolved. [l] Broad. [m] Only one broad OH peak which integrates for less than 2 protons, solvent water peak also broadened (τ 6·7). [n] Half of AB quartet (J = 2·3 c./sec.). [p] Doublet, $J \simeq 5$ c./sec. [q] May contain 'second isomer' (see text).

Hydration of ethyl pteridine-4-carboxylate (I) by simple dissolution in water [method (a)] has already been described.[1] The compound prepared in this way was lower-melting and much more water-soluble than that prepared by the new methods. Both compounds were, however, clearly hydrated across the 5,6- and 7,8-bonds as shown by ^{1}H n.m.r. (Table 1) and u.v. spectroscopy (Table 2). The lower-melting 'second isomer' was distinguished by its lower thermal stability (see discussion of mass spectra below). Treatment of the 7-methylpteridine (II) with water gave approximately 40% of the same stable hydrate (VI) as that obtained by methods (b) and (c). Removal of this isomer and freeze-drying of the filtrate gave a product which could not be purified and fully characterised but was probably largely the 'second isomer' of (VI). (See discussion of n.m.r. and mass spectra below). It has not yet proved possible to state with certainty which isomers are *cis*-diols and which are *trans*.

Ultraviolet Spectra and pK_a *Values.*—Comparison of the u.v. spectra of the pteridines (I)—(III)[1] and their dihydrates (V)—(VII) (Table 2) shows that hydration causes a considerable bathochromic shift although two double bonds are eliminated in the process. The spectra of the hydrates closely resemble those of corresponding ionic species of the diamine (X). Introduction of a 4-ethoxycarbonyl group into the strongly electron deficient molecule of pteridine ($\lambda_{max.}$ 233, 298, and 308)[7] to give ethyl pteridine-4-carboxylate ($\lambda_{max.}$ 245, 304, and 311)[1] has little effect, but introduction of the same group into 4,5-diaminopyrimidine ($\lambda_{max.}$ 246 and 280)[8] to give ethyl 5,6-diaminopyrimidine-4-carboxylate (X) ($\lambda_{max.}$ 228, 253, and 337)[1] where through-conjugation between the 5-amino- and 4-ethoxycarbonyl-groups is possible, causes a bathochromic shift of 48 mμ in the longest wavelength peak.

Spectra of the cations of hydrates (V)—(VII) were

[7] D. D. Perrin, *J. Chem. Soc.*, 1962, 645.
[8] S. F. Mason, *J. Chem. Soc.*, 1954, 2071.

almost indistinguishable from corresponding spectra obtained after dissolving pteridines (I)—(III) in aqueous acid,[1] confirming that the latter compounds are rapidly and almost completely hydrated in aqueous acidic solution. However spectra obtained by standing the hydrates (V) and (VI) in pH 6 buffer at 20° until near equilibrium was reached (Table 2) differed appreciably from those obtained after standing the anhydrous pteridines (I) and (II) under the same conditions. The nature of the differences suggests that slow hydrolysis of the ester group may occur, particularly in the anhydrous species, so that true equilibrium between anhydrous and hydrated esters is never reached. In the case of the dimethyl derivatives (III) and (VII) almost identical spectra were obtained as dehydration of (VII) was rapid compared with hydrolysis. Revised estimates, based on near equilibration of preformed hydrates (V) and (VI) rather than anhydrous compounds, suggest that ethyl pteridine-4-carboxylate would be at least 98% and its 7-methyl derivatives at least 90% hydrated at equilibrium. pK_a Values of the hydrates (Table 2) are consistent with the suggested structures.

TABLE 2

Ultraviolet spectra and ionisation constants

Pteridine	Ionisation (H_2O, 20°) pK_a and spread	Spectroscopy [a] Species [b]	$\lambda_{max.}$ (mμ)	log $\epsilon_{max.}$	pH
(V) [c]		○	235, 257, 347	3·84, 3·57, 3·98	5·9
	3·73 ± 0·04 [d]	+	240, 284, 346	3·74, 3·59, 4·08	1·6
(V) [e]		○	233, 258, 346	3·86, 3·62, 3·97	5·9
	3·64 ± 0·04 [d]	+	238, 284, 346	3·72, 3·58, 4·06	1·6
(VI)		○	236, 259, 347	3·80, 3·55, 3·95	5·9
	3·88 ± 0·03 [d]	+	240, 284, 346	3·65, 3·53, 4·06	1·6
(VII)		○	235, 259, 347	3·80, 3·54, 3·98	6·0
	~3·9 [f]	+	243, 288, 345	3·64, 3·56, 4·04	1·8
Spectra after standing with pH 6 buffer at 20° for 3—14 days (see text).					
(I) [1]		○	233, 258, 342	3·85, 3·62, 3·88	6·0
(V) [c]		○	234, 258, 345	3·85, 3·57, 3·98	6·0
(II) [1]		○	233, 259, *318*, 344	3·80, 3·56, *3·72*, 3·90	6·0
(VI)		○	234, 258, 345	3·81, 3·56, 3·95	6·0
(III) [1]		○	*255*, 307, 316, *347*	*3·43*, 3·97, 3·98, *3·34*	6·0
(VII)		○	*254*, 308, 317, *347*	*3·42*, 3·96, 3·98, *3·34*	6·0

[a] Inflexions and shoulders in italics. [b] ○ = neutral molecule. + = cation. [c] More stable isomer. [d] By titration of M/250 solution. [e] Second isomer. [f] Approximate pK_a by rapid partial titration.

^{1}H *N.m.r. spectra.*—The n.m.r. spectrum of (VI), in deuteriated dimethylsulphoxide, is representative of the spectra of the more stable forms of all the hydrates (Table 1). The peaks due to OH protons, as well as CH protons, were very sharp. The signals at low field (τ 1·7 and 2·2) which disappear on deuteriation could be associated with either NH or OH groups. However these resonances are relatively broad which suggests that the protons concerned are attached to nitrogen and this is confirmed by comparison with the spectrum of the diethanol adduct (XIV) in which similar peaks still appear. The peak at τ 1·7 is split by the 6-proton and is therefore due to the 5- and not the 8-NH group. Similarly, of the two OH signals at τ 4·3 and 4·6, the former is a doublet and is due to the 6-OH group. The coupling constants were determined by observing the effect on the 6-proton signal of irradiating, successively, the 5-NH and 6-OH signals and finally both 5-NH and 6-OH signals simultaneously. The results were confirmed by measurements at both 60 and 100 Mc./sec.

The spectrum of (V) was similar to that of its 7-methyl derivative except that both OH and both NH groups were now coupled to adjacent (6- or 7-) protons. The 6- and 7-protons had very similar chemical shifts in deuteriated dimethyl sulphoxide since the multiplet at τ 5·2 collapsed to a singlet on addition of a little deuterium oxide. However, in a mixture of deuterium oxide and pyridine the 6- and 7-protons were observed as an AB quartet, J = 2·2 c./sec., showing that chemical shifts of the 6- and 7-protons were no longer identical. This may be due to the fact that under these conditions the 5-deuteron was exchanging quite slowly with the solvent while the 8-deuteron exchanged much more rapidly. Deuteriation of the 5-NH group, which is suitably placed for hydrogen bonding with the ester group, is demonstrably slower than deuteriation of the 8-NH group in all the hydrates discussed and in the ethanolate (XIV).

The ^{1}H n.m.r. spectrum of the ' second isomer ' of (V) differed from that of its more stable isomer mainly in the OH signals. These were broad and did not appear to be split by coupling with adjacent protons. Integration of the broad OH signal near τ 4·3 accounted for less than two protons. This and considerable broadening of the solvent water peak near τ 6·7 suggested that much more rapid exchange with solvent water was occurring, than in the case of the stable isomer of (V). A rather similar spectrum but with two broad OH peaks was given by material thought to contain a second isomer of (VI) (see above). Spectra of cations of (V)—(VII) measured in N-DCl in D_2O, were indistinguishable from those obtained after dissolving the corresponding anhydrous

pteridines in the same solvent.[1] The different stereoisomers of each hydrate also gave identical spectra in acid conditions. The chemical shifts for particular protons were closely similar to those reported for other 5,6:7,8-dihydrated pteridines.[9]

Mass Spectra.—Ethyl pteridine-4-carboxylate fragmented in the mass spectrometer mainly by loss of the ester group in two steps, to yield pteridine ion presumably, and then by break up of the pyrimidine ring. Its methyl derivatives (II) and (IV) and its 6,7-dimethyl derivative broke up in analogous fashion. Mass spectra of the hydrates (V—VII) showed all the peaks due to corresponding anhydrous pteridines which were formed by thermal dehydration during vaporisation. In addition however, the stable forms of the hydrates gave a series of low intensity peaks corresponding to the molecular ions of hydrates and their fragmentation products. The second isomer of (V) was completely dehydrated before ionisation. No molecular ion peak corresponding to the hydrated species was observed and the spectrum was identical with that of the anhydrous pteridine (I). Similarly the material believed to be largely the second isomer of (VI) gave a mass spectrum identical with that of the 7-methylpteridine (II) (mass spectra data in Experimental section).

Simple vacuum sublimation experiments confirmed the above conclusions on thermal stability. Thus the higher melting form of (V) was fairly stable at 100° *in vacuo* and up to 20% of the sublimate obtained at 153°/10^{-4} mm. was unchanged (V), the remainder being pteridine (I). The second isomer, however, gradually decomposed at 77°/10^{-4} mm. and gave a sublimate which was entirely anhydrous (I). The other stable hydrates also gave sublimates containing some unchanged dihydrate but the crude second isomer of (VI) gave almost entirely anhydrous pteridine (II).

The fact that high yields of covalent hydrates (V) and (VI) could be obtained from the diamine (X) and a dicarbonyl compound at room temperature [method (c)] led to useful alternative syntheses of the pteridines (I) and (II). Hydrates prepared in this way were converted to anhydrous pteridines in refluxing tertiary butanol, a solvent chosen to obviate the problem of adduct formation encountered previously.[1] Evidently 4,5-diaminopyrimidines condense with dicarbonyl compounds under milder conditions than those normally employed in pteridine syntheses. This was confirmed by the synthesis of pteridine itself from 4,5-diaminopyrimidine and polyglyoxal in water at 20° although none of the compound had been obtained from a similar reaction at (presumably) higher temperature.[10]

EXPERIMENTAL

Ethyl 6,7-Dihydroxy-5,6,7,8-tetrahydropteridine-4-carboxylate (V).—(*a*) Ethyl pteridine-4-carboxylate (I) (0·1 g.) in water (5 ml.) was protected from light, kept for 48 hr., cooled to −30° and freeze-dried at 0·2 mm. pressure. The residue was shaken with ether (15 ml.) for 2 hr. and filtered to yield the dihydrate (0·107 g.) as a solid which gradually decomposed above 100°; m. p. 112° with moderate rate of heating. This hydrate was identical with that described previously.[1] The i.r. spectrum (KBr disc) showed one well defined NH peak at 3370 and broad absorption at 3020—3370 cm.$^{-1}$.

(*b*) Ethyl pteridine-4-carboxylate (0·2 g.) was dissolved in N-hydrochloric acid (2·0 ml.). After 2 min. a mixture of 0·1M-potassium dihydrogen phosphate (1 ml.) and 0·1M-disodium hydrogen phosphate (1 ml.) was added, immediately followed by N-sodium hydroxide solution (2·0 ml.). The *dihydrate* separated as a solid (0·18 g.), m. p. 167—168°, which, on crystallisation from water gave pure material, m. p. 181° [Found: C, 45·2; H, 4·9; N, 23·3%; *M* (mass spectrum), 240. $C_9H_{12}N_4O_4$ requires C, 45·0; H, 5·0; N, 23·3%; *M*, 240]. The i.r. spectrum showed NH bands at 3370 and 3290 cm.$^{-1}$. [The diethanol adduct (XIV)[1] had NH bands at 3420 and 3370 cm.$^{-1}$ in $CHCl_3$ solution.]

(*c*) Ethyl 5,6-diaminopyrimidine-4-carboxylate (0·5 g.), 30% aqueous glyoxal solution (1 ml.), and water (15 ml.) were shaken together for 3 hr. A solid (0·52 g.), m. p. 177—178°, was filtered off and crystallised from water to yield pure dihydrate, m. p. 181°.

The products prepared by methods (b) and (c) were shown to be identical by comparison of i.r., ^{1}H n.m.r., u.v., and mass spectra and by t.l.c. in 3 solvent systems. Method (a) yielded a less stable hydrate referred to as the 'second isomer' of (V) throughout this paper.

Mass spectra. Major peaks in spectra of hydrates prepared by methods (a), (b), and (c) and in the spectrum of ethyl pteridine-4-carboxylate were at *m/e* 204 [molecular ion of (I)], 160, 132 (base peak), 105, 104, and 77. The hydrate prepared by methods (b) and (c) gave a spectrum with additional small peaks at *m/e* 240, 223, and 222. Metastable peaks at *m/e* 125·5 and 109 confirm fragmentation path 204 ⟶ 160 ⟶ 132.

Ethyl 6,7-Dihydroxy-7-methyl-5,6,7,8-tetrahydropteridine-4-carboxylate (VI).— (*a*) Ethyl 7-methylpteridine-4-carboxylate (0·05 g.) was protected from light and shaken with water (3 ml.) for 90 hr. A *dihydrate* (VI) (0·022 g.) was filtered off and washed successively with a little water and ethanol. The solid was identical with that prepared by methods (b) and (c) (below). Refrigeration for 3 hr. of the filtrate from which this hydrate separated yielded a small amount of precipitate which was discarded. The solution was freeze dried and the residue shaken with ether (5 ml.). Filtration gave a solid which gradually darkened above 100°. Spectroscopy (see text) showed that it was substantially an *ethyl 6,7-dihydroxy-7-methyl-5,6,7,8-tetrahydropteridine-4-carboxylate* (VI) but not identical with that isolated above. The i.r. spectrum (KBr disc) had one NH band at 3345 and broad absorption at 3200—3340 cm.$^{-1}$.

(*b*) Ethyl 7-methylpteridine-4-carboxylate (0·05 g.) in N-hydrochloric acid (1·0 ml.) was kept for 2 min. and rapidly mixed with N-sodium hydroxide (1·0 ml.), 0·1M-disodium hydrogen phosphate (0·5 ml.), and 0·1M-potassium dihydrogen phosphate (0·5 ml.). Refrigeration yielded the *dihydroxypteridine* (VI) in highly crystalline form. Recrystallisation from a little water or ethanol with minimum heating gave pure material, m. p. 142—143° (decomp.) (Found: C, 44·3; H, 5·8; N, 20·4%; *M* (mass spectrum), 254. $C_{10}H_{14}N_4O_4,H_2O$ requires C, 44·1; H, 5·9; N, 20·6%;

[9] T. J. Batterham, *J. Chem. Soc.* (*C*), 1966, 999; A. Albert, T. J. Batterham, and J. J. McCormack, *J. Chem. Soc.* (*B*), 1966, 1105.

[10] A. Albert, D. J. Brown, and G. Cheeseman, *J. Chem. Soc.*, 1951, 474.

M, 254). The i.r. spectrum had NH bands at 3405 and 3355 and broad absorption at 3100—3300 cm.$^{-1}$. Mass spectrum: main peaks include *m/e* 218, 174, 146 (base peak), 119, 118, and 91. These peaks were also present in the spectrum of (II). Small peaks at *m/e* 240, 222, and 223 are present in the spectrum of the stable form of hydrate only [sole product of (*b*) and (*c*) and first product from (*a*)]. Metastable peaks at 139 and 122·5 confirm that early stages of fragmentation of (II) are analogous to those of (I).

(*c*) Ethyl 5,6-diaminopyrimidine-4-carboxylate (0·5 g.), 40% aqueous pyrraldehyde (1 ml.), and water (15 ml.) were shaken together for 15 min. The solid (0·5 g.) which had m. p. 137—139°, raised to 142—143° (decomp.) on recrystallisation from water or ethanol, was identical with that prepared by method (b) and the first product of method (a) (shown by comparison of u.v., i.r., ^{1}H n.m.r., and mass spectra and by t.l.c. in 3 solvent systems).

Ethyl 6,7-Dihydroxy-6,7-dimethyl-5,6,7,8-tetrahydropteridine-4-carboxylate (VII).—(*a*) Ethyl 6,7-dimethylpteridine-4-carboxylate (0·06 g.) in N-hydrochloric acid (1·0 ml.) was neutralised and buffered as described for its 7-methyl analogue [method (b) above]. Refrigeration yielded the *dihydroxypteridine* (VII) (0·03 g.), m. p. 145—146°. The m. p. was unchanged after recrystallising the compound by dissolving it in hot tetrahydrofuran and adding a little light petroleum (b. p. 60—80°) (Found: C, 49·5; H, 6·3%; *M* (mass spectrum), 258. $C_{11}H_{16}N_4O_4$ requires C, 49·2; H, 6·0%; *M*, 258). The i.r. spectrum had NH bands at 3345 and 3405 and a broad band around 3230 cm.$^{-1}$. Mass spectrum: main peaks include *m/e* 232, 188, 160 (base peak), 133, and 132. These peaks were also present in the spectrum of (III). Small peaks at *m/e* 268 and 250 were present in the spectrum of (VII) but not of (III). Metastable peaks at *m/e* 152·5 and 136 confirm that first stages of fragmentation of (III) are analogous to those of (I).

(*b*) Ethyl 5,6-diaminopyrimidine-4-carboxylate (0·1 g.), diacetyl (0·1 ml., freshly distilled), and water (3 ml.) were shaken together for 15 min. and the mixture was then refrigerated. A dihydroxypteridine (0·085 g.), m. p. 140—142° (decomp.) was filtered off and washed 3 times with small volumes of water. The compound was shown to be identical with that prepared by method (a) by comparison of u.v., i.r., ^{1}H n.m.r., and mass spectra, and by t.l.c. in 3 solvent systems.

Ethyl Pteridine-4-carboxylate (I).—Ethyl 6,7-dihydroxy-5,6,7,8-tetrahydropteridine-4-carboxylate (V), prepared by method (c) (0·2 g.), was heated under reflux with t-butanol (10 ml.) for 8 hr. Evaporation of the solution was followed by thorough extraction of the residue with boiling light petroleum (b. p. 60—80°). The extract, after treatment with charcoal and magnesium sulphate, yielded the pteridine (0·105 g.), m. p. 75° (lit.,[1] 76°) in 2 crops. A similar reaction in diglyme at 145° for ½ hr. also yielded the pteridine (0·07 g.), m. p. 75°.

Ethyl 7-Methylpteridine-4-carboxylate (II).—Ethyl 6,7-dihydroxy-7-methyl-5,6,7,8-tetrahydropteridine-4-carboxylate (VI), prepared by method (c), (0·2 g.) and t-butanol were heated under reflux for 2 hr. Evaporation of the solution followed by crystallisation of the residue from light petroleum (b. p. 80—100°) gave the methylpteridine (II) (0·09 g.), m. p. 141—143° (decomp.) [lit.,[1] 142—144° (decomp.)].

Pteridine.—4,5-Diaminopyrimidine (0·5 g.), polyglyoxal (0·38 g.), and water (10 ml.) were shaken together for 1 hr. The solution was extracted with chloroform and the extract dried and evaporated. Crystallisation of the residue from light petroleum (b. p. 60—80°) gave pteridine (0·15 g.), m. p. 137° (lit.,[10] 137—138·5°). The u.v. spectrum of the compound was identical with that published.[7]

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