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The Synthesis of 6-Aminomethyl-5,6,7,8-tetrahydropterin

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Abstract

6-Aminomethyl-5,6,7,8-tetrahydropterin has been prepared by reduction of 2-acetamido-6-cyanopteridin-4(3*H*)-one* to 2-acetamido-6-aminomethyl-5,6,7,8-tetrahydropteridin-4(3*H*)-one followed by acid hydrolysis. The hitherto undescribed 6-cyanopterin was prepared by careful hydrolysis of the 2-acetamido compound prepared by dehydration of the oxime derived from 2-acetamido-6-formylpteridin-4(3*H*)-one. The latter was prepared by selenium dioxide oxidation of the methyl compound. Oxidation of 6-aminomethyl-5,6,7,8-tetrahydropterin at neutral pH appears to proceed with significant side-chain loss in Tris buffer but not in phosphate buffer.

Tetrahydrofolic acid (1a) plays a key role in one-carbon metabolism.[1] It is produced in mammals by enzymic reduction of folic acid or 7,8-dihydrofolic acid by dihydrofolate reductase (DHFR).[1] It is important that tetrahydrofolate be kept at the biologically active tetrahydro level and it has been suggested that one function of the high concentrations of the enzyme dihydropteridine reductase (DHPR) in brain is to maintain folate at the tetrahydro level.[2] Dihydropteridine reductase reduces quinonoid dihydro pteridines to the tetrahydro form (2) → (1).[3] Quinonoid dihydropteridines are 7,8-dihydro 6*H*-isomers (2) and are the first readily identified product of oxidation of simple tetrahydro pterins.[3] Simple quinonoid dihydropterins rearrange quickly to the thermodynamically more stable 7,8-dihydro-3*H*-pterins (3) which are not substrates for dihydropteridine reductase.[3] Thus 6-methyl-5,6,7,8-tetrahydropterin (1b) readily oxidizes in Tris buffer at pH 7·6 to the quinonoid from (2a) which rearranges to the 7,8-dihydro 3*H*-form (3; R = Me). This rearrangement is first order in pterin and is general acid/base catalysed.[4] In 6-hydroxymethyl-5,6,7,8-tetrahydropterin (1c) and tetrahydrobiopterin which possesses a dihydroxypropyl side chain in the 6-position,

* Pterin is the trivial name for 2-aminopteridin-4(3*H*)-one. Pteridinone names have been used for convenience in this paper, in spite of the presence of functional groups, e.g. amide, nitrile, which should take preference as suffix over the keto group, e.g. 2-acetamido-4-oxo-3,4-dihydropteridin-6-carbonitrile.

1 Blakely, R. L., and Benkovic, S. J., (Ed.) in 'Folates and Pterins' Vol. 1 (John Wiley: New York 1984).

2 Pollock, R. J., and Kaufman, S., *J. Neurochem.*, 1978, **31**, 115.

3 Armarego, W. L. F., Randles, D., and Waring, P., *Med. Res. Rev.*, 1984, **4**, 267.

4 Archer, M. C., and Scrimgeour, K. G., *Can. J. Biochem.*, 1970, **48**, 278.

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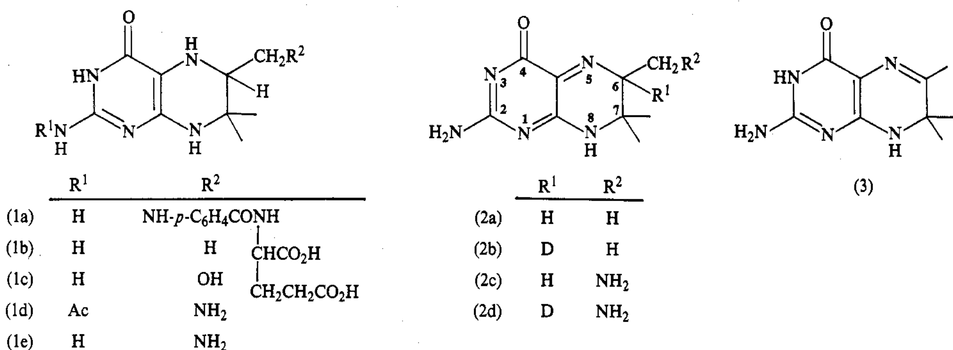
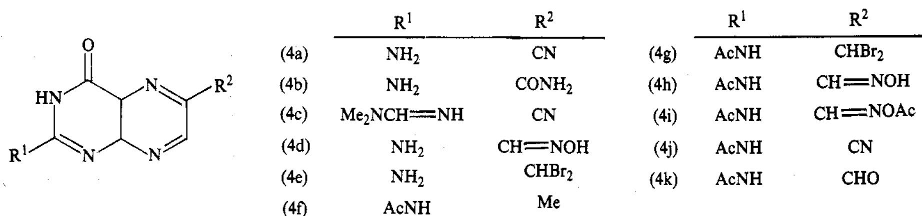
however, the side chain at the 6-position is lost by a reverse Prins-type reaction[5] after formation of the quinonoid species. The same loss of side chain appears to occur in tetrahydrofolic acid,[6] although the kinetic details have not been studied. Because of the importance of the aminomethyl fragment of tetrahydrofolic acid in its biologically important reactions,[1] and possibly in its mode of oxidative decomposition, we decided to synthesize the hitherto unknown 6-aminomethyl-5,6,7,8-tetrahydropterin (1e) as a simple model for tetrahydrofolate.



	R^1	R^2
(1a)	H	NH-*p*-C_6H_4CONH–$CHCO_2H$–$CH_2CH_2CO_2H$
(1b)	H	H
(1c)	H	OH
(1d)	Ac	NH_2
(1e)	H	NH_2

	R^1	R^2
(2a)	H	H
(2b)	D	H
(2c)	H	NH_2
(2d)	D	NH_2



	R^1	R^2
(4a)	NH_2	CN
(4b)	NH_2	$CONH_2$
(4c)	$Me_2NCH{=}NH$	CN
(4d)	NH_2	CH=NOH
(4e)	NH_2	$CHBr_2$
(4f)	AcNH	Me

	R^1	R^2
(4g)	AcNH	$CHBr_2$
(4h)	AcNH	CH=NOH
(4i)	AcNH	CH=NOAc
(4j)	AcNH	CN
(4k)	AcNH	CHO

6-Aminomethyl-6-methyl-5,6,7,8-tetrahydropterin has been prepared by reduction of the corresponding 6-cyano-6-methyl-5,6,7,8-tetrahydropterin available by nucleophilic addition of cyanide ion to 6-methyl-7,8-dihydropterin.[7] As a target molecule for preparation of the title compound we chose the previously unknown 6-cyanopterin (4a) reasoning that catalytic reduction would reduce both the pyrazine ring and the nitrile function to give the desired product. One approach was the dehydration of 6-carbamoylpterin (4b) by phosphorus oxychloride. Several attempts at this approach and after quenching in ice water gave only intractable mixtures. Treatment of the amide in dimethylformamide at 0° with thionyl chloride gave the 2-dimethylaminomethyleneamino derivative (4c) of the nitrile in moderate yield. This compound could not be cleanly hydrolysed to the required 6-cyanopterin under various conditions because of accompanying hydrolysis of the nitrile substituent. Dehydration of the oxime (4d) presented an alternative route to the nitrile. The oxime is known and is prepared by treatment of the crude dibromide (4e) with hydroxylamine

[5] Armarego, W. L. F., Randles, D., and Taguchi, H., *Eur. J. Biochem.*, 1983, **135**, 393.
[6] Chippel, D., and Scrimgeour, K. G., *Can. J. Biochem.*, 1970, **48**, 999.
[7] Armarego, W. L. F., and Waring, P., *Aust. J. Chem.*, 1981, **34**, 1921.

hydrochloride.[8] Preparation of 6-dibromomethylpterin (4e) from 6-methylpterin by published procedures[8] gave, in our hands, very low yields of a product contaminated with the mono- and tri-bromomethylpterin. Acetylated 6-methylpterin (4f) is more soluble in acetic acid than 6-methylpterin and is readily available isomerically pure and in good yield.[9] Bromination of this compound giving (4g) by using bromine/acetic acid is cleaner than bromination of 6-methylpterin with Br_2/HBr, but a product free from mono- or tri-bromide could still not be obtained under a variety of conditions. It was, however, possible to prepare the acetylated oxime (4h) from the crude acetylated dibromide by treatment with aqueous hydroxylamine hydrochloride. Treatment of the acetylated oxime with acetic anhydride gave the *O*-acetylated product (4i) under mild conditions followed by the acetylated nitrile (4j) after prolonged heating. The *O*-acetylated product could not be isolated cleanly by direct acetylation of the oxime (4d). The oxime (4h) would be better prepared from N^2-acetylated 6-formylpterin (4k). 6-Formylpterin has been prepared by a number of routes including oxidative cleavage of neopterin[10] or by Taylor's method.[11] The former requires a complex pterin as starting point and the latter, while elegant, is time-consuming. We investigated the direct oxidation of 6-methylpterin with selenium dioxide as a source of 6-formylpterin. 6-Methylpterin was found to be resistant to SeO_2 oxidation, probably because of its low solubility in acetic acid. However, the N^2-acetylated 6-methylpterin (4f) which is very soluble in hot acetic acid gave the acetylated aldehyde (4k) in moderate to good yield. Since acetylated 6-methylpterin can be made in good yield and in high isomeric purity,[9] this is a useful and efficient route to (4k). The latter compound is only prepared by acetylation of 6-formylpterin by using large volumes of acetic anhydride;[12] the presence of acetic acid results in the formation of the diacetal.[13] We repeated this work but the latter could not be hydrolysed to the 2-acetamido-6-formylpteridine in our hands because of the accompanying *N*-deacetylation. Treatment of the acetylated aldehyde with hydroxylamine gave the acetylated oxime (4h) quantitatively.

6-Cyanopterin (4a) could be prepared by careful hydrolysis of 2-acetamido-6-cyanopteridin-4(3*H*)-one (4j) in aqueous ethanol. The nitrile function appears to activate the acetate on the 2-amino group to hydrolysis, as would be expected by a powerful electron-withdrawing group. Too vigorous conditions, such as boiling, results in partial hydrolysis to the amide. For solubility reasons it was found easier to reduce the acetylated nitrile in trifluoroacetic acid to the 2-acetamido-6-aminomethyl-5,6,7,8-tetrahydropteridin-4(3*H*)-one (1d) which could be isolated as the hydrochloride. The latter was then hydrolysed in aqueous acid to the required 6-aminomethyl-5,6,7,8-tetrahydropterin (1e).

Hydrolysis of (1d) to (1e) could be monitored by proton n.m.r. (at 90 MHz) following the decrease in the N^2-methyl signal (Fig. 1). This unequivocally establishes the remaining presence of the acetyl group on the nitrogen following reduction. The signals due to H 6, H 7 (*ax*) and H 7 (*eq*) become complex due to the two species as the reaction proceeds, but assume the same pattern of the starting material at completion

[8] Waller, C. W., Goldman, A. A., Angier, R. B., Boothe, J. H., Hutchings, B. L., Mowat, J. H., and Semb, J., *J. Am. Chem. Soc.*, 1950, **72**, 4630.
[9] Waring, P., and Armarego, W. L. F., *Aust. J. Chem.*, 1985, **38**, 629.
[10] Viscontini, M., and Bieri, J. H., *Helv. Chim. Acta*, 1971, **54**, 2291.
[11] Taylor, E. C., Henrie, R. N., and Portnoy, R. C., *J. Org. Chem.*, 1978, **43**, 736.
[12] Bieri, J. H., and Viscontini, M., *Helv. Chim. Acta*, 1973, **56**, 2905.
[13] Boyle, P. H., and O'Mahony, M. J., *J. Heterocycl. Chem.*, 1984, **21**, 909.

of the hydrolysis. The high-field spectrum of the title compound (1e) is discussed below.

High-Field ^{1}H N.M.R. Spectrum of 6-Aminomethyl-5,6,7,8-tetrahydropterin (1e)

The 200-MHz ^{1}H n.m.r. spectrum of 6-aminomethyl-5,6,7,8-tetrahydropterin in 2 M DCl is shown in Fig. 2. The two quartets centred on 3·60 ppm and 3·87 ppm are due to the H 7 axial and H 7 equatorial protons respectively. The H 6 proton appears as a broadened signal at 4·08 ppm. This pattern is qualitatively the same as that seen for 6-methyl-5,6,7,8-tetrahydropterin,[14] except in that case the H 6 signal is almost coincident with that for H 7 (*eq*). The first-order coupling constants for H 6, H 7 (*eq*) and H 7 (*ax*) are shown in Fig. 2 and are comparable with those observed for 6-methyl-5,6,7,8-tetrahydropterin.[14] The methylene fragment at C 6 now appears as

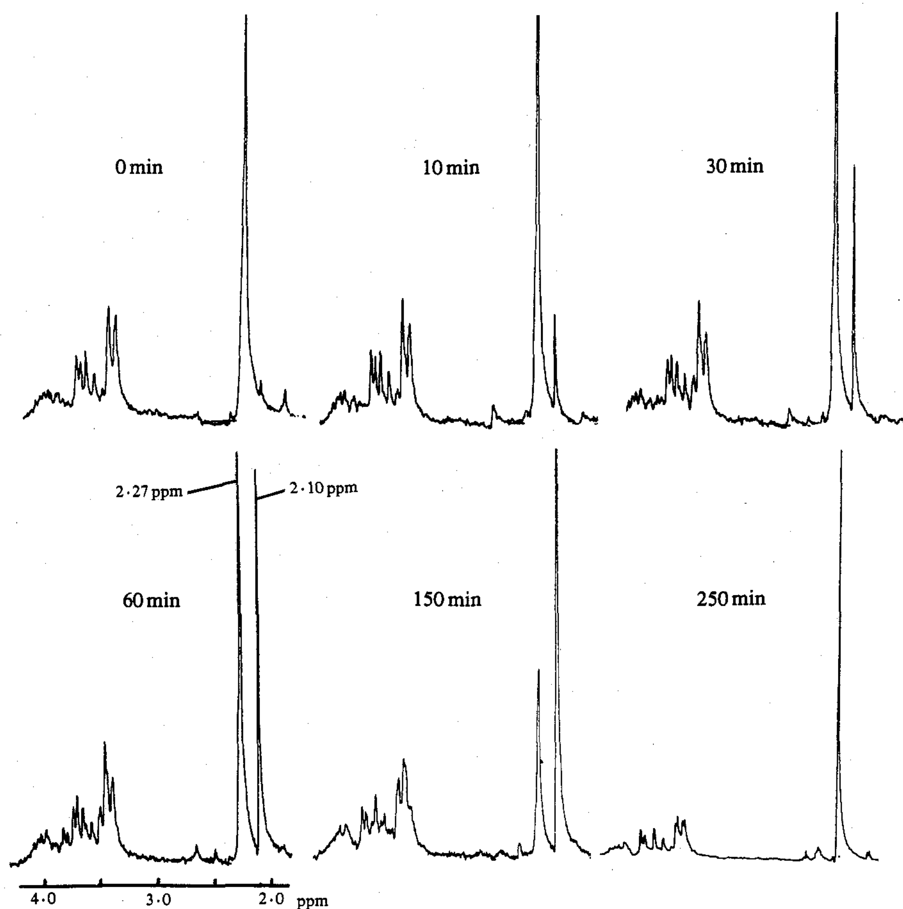


Fig. 1. Hydrolysis of 2-acetamido-6-aminomethyl-5,6,7,8-tetrahydropteridin-4(3*H*)-one in 3 M DCl as followed by ^{1}H n.m.r.

[14] Armarego, W. L. F., and Schou, H., *J. Chem. Soc., Perkin Trans. 1*, 1977, 2529.

an apparent quartet centred on 3·45 ppm. Since there is likely to be restricted rotation about the C6–CH_2NH_2 bond in strong acid because of protonation, the methylene protons and H6 formally constitute an ABX system. It is likely that the four lines centred on 3·45 ppm constitute part of a deceptively simple ABX pattern[15] with three of the other four lines just discernible as marked in Fig. 2. Line 8 is obscured by the signal at 3·55 ppm. The value of J_{AB} is given by E_3-E_1, E_4-E_2, E_7-E_5 or E_8-E_6 [15] and is 14·0 Hz. Further analysis is not possible because of the complexity of the X portion due to further coupling with the protons at C7.

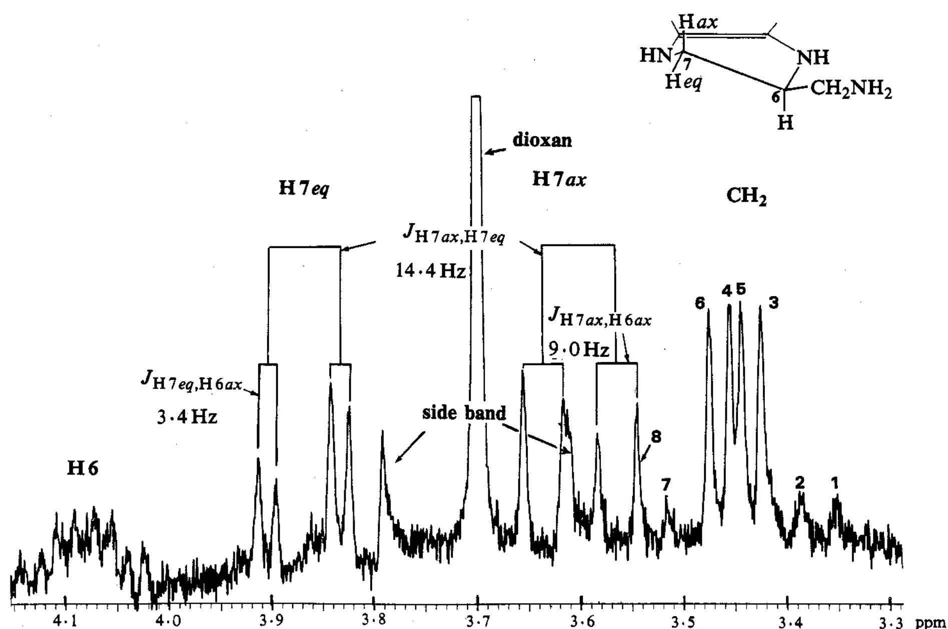


Fig. 2. 200-MHz ^1H n.m.r. spectrum of 6-aminomethyl-5,6,7,8-tetrahydropterin in 2 M DCl.

The Formation and Rearrangement of Quinonoid 6-Aminomethyl-7,8-dihydro-6*H*-pterin (2c)

Quinonoid 6-aminomethyl-7,8-dihydro-6*H*-pterin (2c) formed by bromine oxidation at pH 7·5 of the tetrahydro form had a typical[3] quinonoid spectrum with λ_{max} 300–302 nm and ϵ approximately $1·2\times10^4$. The tetrahydro form has λ_{max} 295 nm at pH 7·5. The quinonoid form also had the typical[3] broad shoulder at 350 nm. The rates of rearrangement (decay) of (2c) and the deuterated form (2d) measured at 30° in Tris and phosphate buffer at pH 7·5 are shown in Table 1. This table also shows the corresponding rates for quinonoid 6-methyl-7,8-dihydro-6*H*-pterin (2a) and the deutero compound (2b), determined in the same way. The final products of oxidation and rearrangement of all compounds in Table 1 in either buffer have spectra characteristic of 7,8-dihydro-3*H*-pterins (3), i.e. λ_{max} at 280 nm and

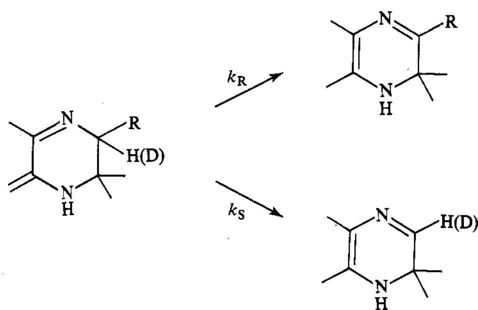
[15] Abraham, R. J., in 'The Analysis of High-Resolution Spectra' p. 62 (Elsevier: Amsterdam 1971).

324–326 nm with $\epsilon_{280}/\epsilon_{325} = 2$. This is expected since either rearrangement or side-chain loss gives 7,8-dihydro-3*H*-pterins; in the former case substituted, and in the latter unsubstituted, at C 6 (Scheme 1). 7,8-Dihydro-3*H*-pterins unsubstituted at the 6-position may covalently hydrate[16] (see later). Such covalently hydrated species may further oxidize to yield xanthopterin as the final product in the presence of excess oxidant,[5] e.g. peroxidase, since a small but finite amount of the hydrated species may be present even at neutral pH. However, since only one mole of oxidant is used in this instance, the reaction stops at the first product of rearrangement (or side-chain loss).

Table 1. Rate data at pH 7·5

Compound	0·1 M buffer	Observed first-order rate of decay[A] (min^{-1})	$t_{1/2}$ (min)	k^H/k^D
(2a)	phosphate	0·0651	10·6	8·7
(2b)	phosphate	0·00752	92	
(2c)	Tris	0·088	7·9	1·4
(2d)	Tris	0·061	11·3	
(2c)	phosphate	0·63	1·1	4·5
(2d)	phosphate	0·14	4·9	

[A] Apparent first-order rate constant.



Scheme 1

The side-chain loss following oxidation of tetrahydrobiopterin is characterized by little or no kinetic deuterium isotope effect since this is not accompanied by loss of 6-H or 6-D.[5] Thus it appears from Table 1 that in Tris buffer significant side-chain loss occurs in compound (2c) and (2d). This is supported by the observation that following acidification (pH *c.* 0) of the final product of oxidation of (2c) and (2d) in Tris buffer the observed spectrum is qualitatively similar to a 5,6,7,8-tetrahydropterin with λ_{max} 260 nm and with the disappearance of the maxima at 326 nm. This is due to covalent hydration across the 5,6-double bond following protonation of N 5. This hydration is sterically hindered by a 6-substituent.[16] However, acidification of the product from the oxidation of (2c) in phosphate buffer exhibits a long wavelength shift to 360–370 nm characteristic of a protonated (non-hydrated) 6-substituted 7,8-dihydropterin;[17] this suggests a larger proportion of 'normally' rearranged product, (3; R = CH_2NH_2).

[16] Albert, A., and Armarego, W. L. F., *Adv. Heterocycl. Chem.*, 1965, **4**, 1.
[17] Pfleiderer, W., and Mengel, R., in 'Chemistry and Biology of Pteridines' (Eds I. Iwai, M. Akino, M. Goto and Y. Iwanami) p. 43 (International Academic Printing: Tokyo 1970).

The sum of the pseudo-first-order processes, side-chain loss and rearrangement is also a first-order process[18]—Scheme 1.

The normal deuterium isotope effect for rearrangement of (2a) in 0·1 M phosphate buffer is 8·7. If this is also true for k_R in Scheme 1, then

$$k_R{}^D = k_R{}^H/8\cdot7$$

where $k_R{}^H$ and $k_R{}^D$ are the corresponding pseudo-first-order rates of *rearrangement* of (2c) and (2d) respectively. The value of k_s, the pseudo-first-order rate constant for loss of side chain should be unaffected by deuterium at C6, hence

$$0\cdot63 = k_s + k_R{}^H$$

and

$$0\cdot14 = k_s + k_R{}^D = k_s + k_R{}^H/8\cdot7$$

that is,

$$0\cdot49 = (7\cdot7/8\cdot7)k_R{}^H$$

or

$$k_R{}^H = 0\cdot55 \text{ min}^{-1}$$

and

$$k_s = 0\cdot08 \text{ min}^{-1}$$

Since the ratio of rate constants determines the ratio of products,[18] decay of (2c) in 0·1 M phosphate results in 85% rearrangement and 15% side-chain loss by the above analysis. This is consistent with observed spectral changes. The deutero compound (2d) would show a 40% rearranged product and 60% side-chain loss in phosphate buffer. The significant partial isotope effect of 4·5 is clearly due to comparable values for the rates of rearrangement and side-chain loss. This may be due to a relative increase in the rate of rearrangement due to protonation of the primary amino group and consequent increasing of the acidity of H6.

The corresponding amounts of side-chain loss of (2c) and (2d) in Tris buffer are 66% and 95% respectively, assuming a kinetic isotope effect of 9 in k_R.[4] These values are relatively insensitive to changes in the kinetic isotope effect between 7 and 12.

Experimental

Analyses were performed by the Australian National University Analytical Service. Ultraviolet spectra were run on a Carey 219 instrument. The ^{1}H n.m.r. and ^{13}C spectra were run on a JEOL FX90Q or a Varian XL200 instrument and chemical shifts are in ppm downfield from tetramethylsilane. Mass spectra were measured on an MS-9 mass spectrometer.

Quinonoid 7,8-dihydro-6*H*-pterins were generated *in situ* by the addition to the tetrahydropterin at pH 7·5 of exactly 1 mol equiv. of bromine freshly prepared in the appropriate

[18] Pilling, M. J., in 'Reaction Kinetics' p. 99 (Clarendon Press: Oxford 1975).

buffer. In all cases the rate of rearrangement or side-chain loss was determined by monitoring the absorbance change at 300 nm. First-order rates were determined by plotting $\log[A_0/(A_0 - A_t)]$ against t, where A_0 is the initial absorbance and A_t the absorbance at time t. The slope of this plot as determined by a least-squares fit was taken as the pseudo-first-order rate constant. All correlation coefficients were >0·99 over 85% of the reactions. 6-Methyl-5,6,7,8-tetrahydropterin was prepared as previously described.[14]

6-Carbamoylpterin (4b)

6-Carboxypterin methyl ester[19] (1 g) was stirred in ammonium hydroxide solution (40 ml, 8 M) for 16 h at 25°. The slurry was separated from the supernatant by centrifuging, washed in water (3×50 ml) and ethanol (3×50 ml) and dried 100°/0·1 mm, m.p. >300° (dec.) (Found: C, 36·5; H, 3·7; N, 36·2. Calc. for $C_7H_6N_6O_2.1{\cdot}4H_2O$: C, 36·3; H, 3·85; N, 36·3%). ^1H n.m.r. $[(CD_3)_2SO]$: δ 9·13, s.

6-Cyano-2-dimethylaminomethyleneaminopteridin-4(3H)-one (4c)

The dry amide (450 mg) was suspended in dry dimethylformamide (15 ml) and cooled to 0°. Thionyl chloride (1·3 g) was added over 20–25 min with vigorous stirring and the solution was allowed to come slowly to room temperature. Stirring was continued for a further 36 h. After this period, ether (100 ml) was added to the clear yellow solution and the resulting oil washed with ether. The oil was dried by nitrogen stream and dissolved in ice-cold water (15 ml). The pH was adjusted to 7 with saturated bicarbonate. The yellow crystalline solid was collected and dried at 100°/0·1 mm giving crystals (250 mg, 47%), m.p. 290–295° (Found: C, 46·7; H, 4·1; N, 39·0. Calc. for $C_{10}H_9N_7O.0{\cdot}65H_2O$: C, 47·1; H, 4·1; N, 38·5%). ν_{max} 2250 cm^{-1} (CN stretch). ^1H n.m.r. $[(CD_3)_2SO]$: δ 3·15, s, 3H; 3·28, s, 3H; 8·91, br s, 1H; 9·14, s, 1H.

2-Acetamido-6-dibromomethylpteridine-4(3H)-one (4g)

2-Acetamido-6-methylpteridine-4(3*H*)-one (2 g) was dissolved in warm glacial acetic acid (120 ml) and refluxed with stirring. Bromine (7·2 g, 5 molar excess) was added over 5 min in a little acetic acid and the solution was refluxed for 40 min. The solution was cooled and poured into ether (300 ml) and the resulting solid removed, washed well with cold ether and dried at 60°/0·1 mm. ^1H n.m.r. indicated 75% of the dibromomethylpterin, together with the mono- and the tri-bromo compound. ^1H n.m.r. $[(CD_3)_2SO]$: δ 2·25, s, 3H; 7·57, s, 1H; 9·09, s, 1H.

2-Acetamido-6-formylpteridin-4(3H)-one (4k)[12] *by Selenium Dioxide Oxidation of the 6-Methyl Compound*

2-Acetamido-6-methylpteridine-4(3*H*)-one (1 g, dried 100°/2 h) was dissolved in glacial acetic acid (40 ml) and the solution stirred under nitrogen. The clear solution was treated with freshly sublimed selenium dioxide (0·5 g) and the solution stirred vigorously for 2 h. After this time a further 0·5 g of selenium dioxide was added and the solution stirred a total of 8·5 h at 100°. The reaction was monitored by t.l.c. (Merck silica, propanol/acetic acid 5:1). The solution was cooled to room temperature and ether (40 ml) added. The gelatinous selenium which precipitated was removed by centrifugation and the orange supernatant evaporated to dryness at 0·5 mm in a rotary evaporator (bath temp. 60°). The oily product was triturated with light petroleum (40–60°) and the resulting solid dried at 60°/0·1 mm giving 1·3 g (95%) of crude aldehyde with one mole of acetic acid. A small amount was recrystallized from boiling ethanol for mass spectra. Mass spectrum: m/z 233 (M), 218 (M – Me), 190 (M – Ac); high-resolution measured mass 233·0553, $C_9H_7N_5O_3$ requires 233·0549 (1·7 ppm). ^1H n.m.r. $[(CD_3)_2SO]$: δ 2·25, s, 3H; 9·25, s, 1H; 10·09, s, 1H.

2-Acetamido-6-(hydroxyiminomethyl)pteridin-4(3H)-one (4h)

(A) *From the 6-formylpterin.*—The aldehyde (1 g) was dissolved in water (30 ml) and the pH adjusted to 7. The solution was treated with hydroxylamine hydrochloride (220 mg) at 20° with stirring. The white precipitate was removed by centrifuging and washed with water (3×20 ml), ethanol (3×20 ml) and ether (3×20 ml) and dried, giving a solid (800 mg) (75%) (Found: C, 42·4;

[19] Pfleiderer, W., Zondler, H., and Mengel, R., *Justus Liebigs Ann. Chem.*, 1970, 741, 64.

H, 3·9; N, 31·2. Calc. for $C_9H_8N_6O_3.0·5H_2O$: C, 42·0; H, 3·5; N, 32·7%). The determined value for nitrogen was consistently low. ^{1}H n.m.r. [$(CD_3)_2SO$]: δ 2·23, s, 3H; 8·23, s, 1H; 9·22, s, 1H.

(B) *From 2-acetamido-6-dibromomethylpteridin-4(3*H*)-one.*—The dibromomethylpteridine (3·9 g, 75%) was dissolved in acetone (25 ml). Water (130 ml) containing sodium acetate (16 g) and hydroxylamine hydrochloride (7·2 g) was warmed to 55–60° and the dibromomethyl compound added in acetone over 15–20 min with vigorous stirring. The slurry was stirred overnight at 60°, then cooled in ice. The dark solid was removed by centrifuging and washed with water (5×50 ml), ethanol (5×25 ml) and dried at 80° for 3 h giving 1·2 g (80% based on available dibromide). Identical by ^{1}H n.m.r. as the material above.

*2-Acetamido-6-(acetoxyiminomethyl)pteridin-4(3*H*)-one (4i)*

The oxime (1·25 g) was refluxed in acetic acid (20 ml) and acetic anhydride (20 ml) until complete dissolution (approx. 10 min). The clear solution was cooled to room temperature, giving a crystalline solid which was washed with diethyl ether and dried 60°/0·1 mm (800 mg). A further crop of 330 mg was obtained by adding excess diethyl ether to the supernatant. Total yield 80%, recrystallized from methanol, m.p. 229–231° (Found: C, 43·9; H, 3·6; N, 27·8. Calc. for $C_{11}H_{10}N_6O_4.0·5H_2O$: C, 44·1; H, 3·7; N, 28·0%). ^{1}H n.m.r. [$(CD_3)_2SO$]: δ 2·24, s, 3H; 2·26, s, 3H; 8·77, s, 1H; 9·27, s, 1H.

*2-Acetamido-6-cyanopteridin-4(3*H*)-one (4j) from*
*2-Acetamido-6-(hydroxyiminomethyl)pteridin-4(3*H*)-one*

The oxime (3·2 g) was refluxed in a 1 : 1 mixture of acetic acid and acetic anhydride (50 ml) for 1·5 h. The solution was cooled and poured into cold ether, and left overnight at 0°. The solid was removed and washed in ether (2·7 g) (70%). The material isolated in this way is the acetate salt. The material was freed from acetic acid by washing in cold water and drying at 20°/0·5 mm. Recrystallized from ethanol, m.p. 230–233° (Found: C, 46·4; H, 2·7; N, 35·3. Calc. for $C_9H_6N_6O_2.0·25H_2O$: C, 46·1; H, 2·8; N, 35·8%). ^{1}H n.m.r. [$(CD_3)_2SO$]: δ 2·24, s, 3H; 9·29, s, 1H. ν_{max} 2240 cm^{-1} (moderate) (CN stretch). Mass spectrum: *m/z* 230 (M), 215 (M − Me), 204 (M − CN). The acetylated nitrile could be obtained from heating the *N*-acetylated (*O*-acetylated) oxime in acetic anhydride/acetic acid as above. The product from this reaction (86%) is cleaner. A sample of the solid precipitating from ether, m.p. 253–255° (dec.), analysed for 0·8AcOH (Found: C, 45·5; H, 3·2; N, 30·0. Calc.: C, 45·8; H, 3·3; N, 30·2%). ^{1}H n.m.r. confirmed approximately one mole of acetic acid.

Heating of the *N*-acetylated (*O*-acetylated) oxime dry at 220–225° for 5–10 min also gives 80–85% yield of the crude nitrile, but contaminated with polar pyrolysis products. The acetylated nitrile could also be prepared directly from 6-oximinopterin by a procedure similar to that for 2-acetamido-6-(hydroxyiminomethyl)pteridin-4(3*H*)-one, but the overall yields were much lower (40–50%).

6-Cyanopterin (4a)

2-Acetamido-6-cyanopteridin-4(3*H*)-one containing approximately one mole of acetic acid (60 mg) was suspended in ethanol (10 ml). Water (5 ml) was added and the suspension stirred at 80°. The solution clears after 2–5 min and then slowly deposits a white solid. Stirring at 80° was continued for 4–6 h, the solution cooled to 20°, the solid removed by centrifuging, and washed in cold ethanol (2×20 ml) and dried 110°/0·1 mm/4 h, giving 40 mg (75%) of a cream *solid*, m.p. >300° (dec.) (Found: C, 44·6; H, 2·3; N, 44·1. $C_7H_4N_6O$ requires C, 44·7; H, 2·1; N, 44·7%). ^{1}H n.m.r. [$(CD_3)_2SO$]: δ 9·03, s. ν_{max} 2240vw cm^{-1} (CN).

*2-Acetamido-6-aminomethyl-5,6,7,8-tetrahydropteridin-4(3*H*)-one (1d)*

2-Acetamido-6-cyanopteridin-4(3*H*)-one (350 mg) was shaken under hydrogen at 1 atm in trifluoroacetic acid (15 ml) containing prereduced platinum oxide (150 mg) for 12 h. The solution was filtered under nitrogen and 0·8 M HCl in aqueous ethanol (50 ml) added to the solution at 0°. After standing for 1 h, the solid was separated by centrifugation, washed in diethyl ether (4×20 ml) and dried at 20°/0·5 mm for 10 h, giving 300 mg of a dark solid. A portion was dissolved in methanol and diethyl ether added until the solution was faintly turbid. The solution was cooled in ice and the cream solid isolated by centrifuging and washing in diethyl ether.

Dried at 100° for 3 h, m.p. >300° (Found: C, 36·0; H, 5·1; Cl, 15·3; N, 27·3. Calc. for $C_9H_{14}N_6O_2.1\cdot27HCl.1\cdot0H_2O$: C, 35·7; H, 5·7; Cl, 14·9; N, 27·7%).

6-Aminomethyl-5,6,7,8-tetrahydropterin (1e)

2-Acetamido-6-aminomethyl-5,6,7,8-tetrahydropteridin-4(3*H*)-one (250 mg) was allowed to stand in 3 M HCl (3 ml) for 2 days at room temperature. The solution was then evaporated to *c.* 1 ml (Rotovap, bath temperature 60°) and cooled in ice. The crystals which deposited (30 mg) were removed, washed in ice-cold ethanol and dried (0·5 mm/100°) for 2 h, m.p. >300° (dec.) (Found: C, 27·8; H, 5·0; Cl, 32·1; N, 27·1. Calc. for $C_7H_{12}N_6O.2\cdot74HCl.0\cdot52H_2O$: C, 27·5; H, 5·2; Cl, 31·8; N, 27·5%). λ_{max} (1·5 M HCl) 266 nm (ϵ $1\cdot22\times10^4$). Addition of ethanol to the aqueous supernatant gave a further crop of material (60–80 mg). Evaporation of the supernatant to dryness gives a further batch of crude product contaminated by oxidized material as determined by ultraviolet spectroscopy.

For the study of the tetrahydropterins and their oxidation products with deuterium in the 6-position, 6-methylpterin was reduced by using deuterium gas as previously described,[14] and the 6-aminomethyl-6-deutero-tetrahydropterin was prepared as above in the same way. The presence of deuterium in the 7-position and for the latter compound in the methylene protons of the 6-side chain as well will not significantly affect the observed rates.

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