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Original paper

The dihydropteridine reductase (human brain) activity of some lipophilic quinonoid dihydropterins

Paul WARING and Wilfred L. F. ARMAREGO *

Department of Biochemistry, John Curtin School of Medical Research, Australian National University, P.O. Box 334, Canberra City A.C.T. 2601 Australia

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Summary — The syntheses of 6-ethyl **2c**, 6-*n*-propyl **2d**, 6-*n*-hexyl **2e**, 6-phenethyl **2f**, 6-neopentyl **2g** and 7-neopentyl **2h** 5,6,7,8-tetrahydropterins are described. The oxidation of these gave the corresponding quinonoid species **3c**—**3h** all of which were good substrates for dihydropteridine reductase from human brain when compared with the natural cofactor quinonoid dihydrobiopterin **3i**, the quinonoid 6- and 7-methyl dihydropterins **3a** and **3b**, and quinonoid dihydrofolic acid **3j**. In contrast, 5-2′-propylimino and 5-2′-octylimino 2,4-diaminopyrimidin-6 (1*H*)-ones **7a** and **7b** were devoid of substrate or inhibitor activities. The potential use of these lipophilic pterins in the therapy of diseases where there is a deficiency of tetrahydrobiopterin is discussed.

Résumé — **Activité vis-à-vis de la dihydroptéridine réductase (cerveau humain) de quelques dihydroptérines quinoïdiques lipophiles.** *Ce travail décrit les synthèses des éthyl-6 **2c**, n-propyl-6 **2d**, n-hexyl-6 **2e**, phénéthyl-6 **2f**, néopentyl-6 **2g** et néopentyl-7 **2h** tétrahydro-5,6,7,8 ptérines. L'oxydation de ces ptérines donne les analogues quinonoïdiques **3c**—**3h** qui sont de bons substrats de la dihydroptéridine réductase, d'origine humaine, comparés au cofacteur naturel quinonoïdique dihydrobioptérine **3i**, aux méthyl-6 (et -7)dihydroptéridines quinonoïdiques **3a** et **3b**, et la forme quinonoïdique de l'acide dihydrofolique **3j**. Au contraire les propylimino-5-2′ et octylimino-5-2′ diamino-2,4 pyrimidin(1H)-ones-6 **7a** et **7b** ne conviennent pas comme substrat et n'inhibent pas cet enzyme. On discute la possibilité d'utiliser ces ptérines lipophiles dans les cas de carence de tétrahydrobioptérine.*

lipophilic quinonoid dihydropterins / dihydropteridine reductase activity / structure—activity relationships

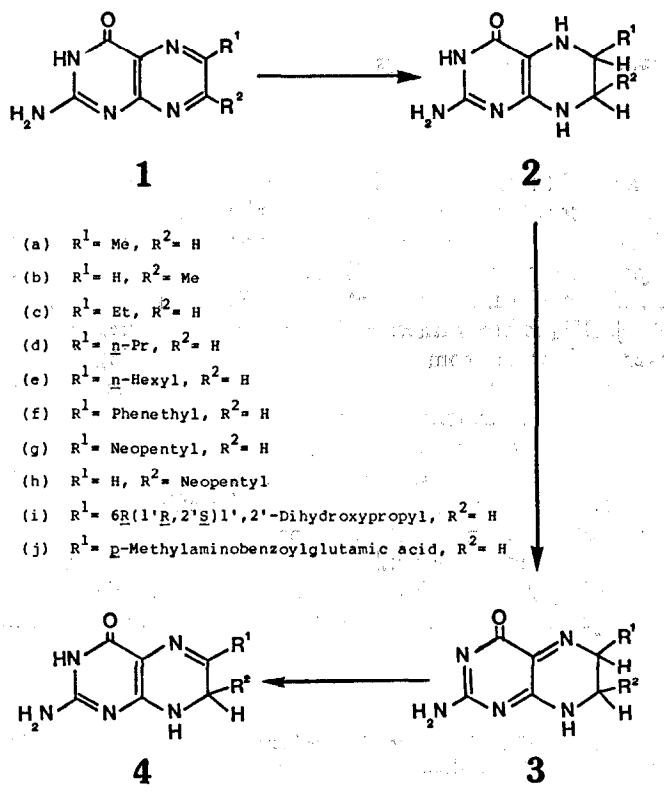
Introduction

Tetrahydrobiopterin (BH_4, **2i**) is the natural cofactor in mammalian metabolism for the enzymatic hydroxylation of phenylalanine, tyrosine and tryptophan [1]. The hydroxylation of phenylalanine (to tyrosine) is essential for the removal of phenylalanine, and the hydroxylation of tyrosine (to 3,4-dihydroxyphenylalanine, dopa) and tryptophan (to 5-hydroxytryptophan) are necessary for the production of catecholamines and serotonin which are obligatory neurotransmitters for the proper function of the nervous system [2]. The cofactor BH_4 is oxidized to the quinonoid species **3i** in these hydroxylations which are recycled *in vivo* back to BH_4 by enzymatic reduction with dihydropteridine reductase DHPR, (EC 1.6.99.7) [3]. Deficiencies in any of these enzymes lead to decreased levels of neurotransmitters (or increased levels of phenylalanine in phenylalanine hydroxylase deficiency) resulting in severe neurological disorders such as those observed in the inherited metabolic disease phenylketonuria (PKU) and its variants [4, 5]. BH_4 is biosynthesized in mammalian tissues including liver and brain from guanosine triphosphate [6], and any deficiencies in the enzymes in this biosynthesis also lead to reduced neurotransmitter levels with neurological disturbances as observed in malignant hyperphenylalaninemia (MHPA) [7—10]. Danks *et al.* [8, 11] showed that intravenous administration of BH_4 could be effective in variant forms of PKU and continuous oral administration of BH_4 has been used successfully in patients with defective BH_4 synthesis [12, 13], although the advantages of BH_4 therapy were questioned [14]. Low values of BH_4 have been found

* *Author to whom correspondance should be addressed.*
Abbreviations: **BH_4**: 6*R*(1′*R*,2′*S*),1′,2′-dihydroxypropyl-5,6,7,8-tétrahydrobiopterin; **DHPR**: dihydropteridine reductase; **pterin**: 2-aminopteridin-4(3H)-one; **TMS**: tetramethylsilane; **TPS**: sodium 3-(trimethylsilyl)-propionate; **DMSO**: dimethyl sulfoxide.

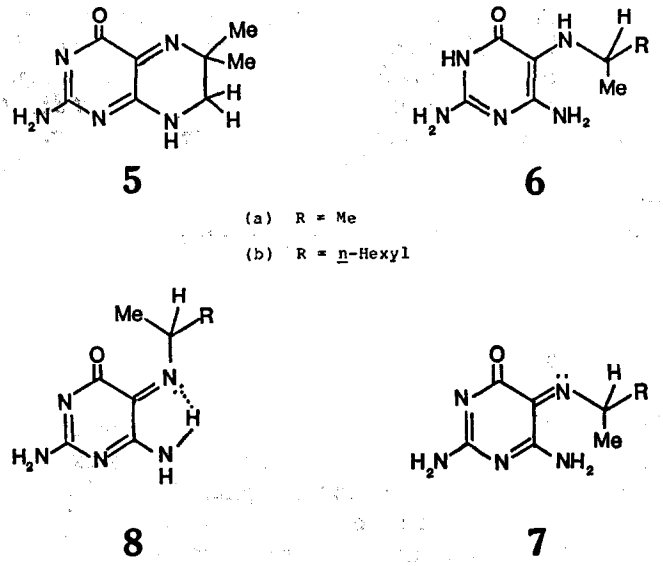
in the cerebrospinal fluid of patients suffering from Parkinson's disease [15, 16], Shy—Drager and Steel—Richardson syndromes, essential tremor, Huntington's chorea and Alzheimer's disease [15] and may reflect the levels in the brain. The concentrations of BH_4 in post-mortem human brain (caudate nucleus) were considerably reduced in Parkinson patients [17]. BH_4 therapy of patients with Parkinson's disease and endogenous depression has met with some success [18] but it is unlikely that it will be adopted for general use [19]. The main reason for the inefficiency of BH_4 therapy is attributed to the poor ability of BH_4 to penetrate the blood—brain barrier and is due to the hydrophilic nature of the cofactor **2i**, probably caused by the dihydroxypropyl side chain to a large extent [20, 21].

Kapatos and Kaufman [20] have shown that BH_4 has a limited ability to penetrate the blood—brain barrier, whereas the more liposoluble 6-methyl-5,6,7,8-tetrahydropterin **3a** enters the brain much more readily (*ca.* 10 times). Other disadvantages in using BH_4 therapeutically are its high cost and its instability. It has a very short half-life at physiological pH and decomposes with loss of the side chain [22]. We have consequently synthesized a variety of 5,6,7,8-tetrahydropterins with lipophilic side chains **3a**—**3h** with the aim of obtaining a drug which has much more favorable blood—brain barrier penetrating properties. Another prerequisite for an effective drug is that it must also be a good cofactor for recycling the enzyme dihydropteridine reductase and for the aromatic amino acid hydroxylases. In the present study, we report the synthesis and properties of these more lipophilic tetrahydropterins and show that they are all very good substrates for dihydropteridine reductase.



Chemistry

Compounds **2a** [23], **2b** [24], **2f** [25] and **5** [26] were prepared by published procedures. The pterins **1e** and **1d** were prepared by the procedure of Rosowsky and Chen [27] and gave, in our hands, small amounts of the 7-isomer (~ 15%) detected by NMR. These were removed by recrystallization from 50% formic acid. The previously undescribed compounds **1c**, **1g** and **1h** were made by procedures similar to those in reference [27] except that in the case of the neopentyl compound, the 7-isomer was the major product. The 6-isomer could be prepared in only small amounts. The tetrahydropterins were prepared by catalytic reductions of the pterins in 1 M HCl over platinum oxide. The correct position of the side chain in compounds **1g** and **1h** was established by ^{13}C NMR coupling constants as described under experimental protocols. The solubility of the tetrahydropterin hydrochlorides in dilute acid decreased with the increase in the length of the side chain. A saturated solution of the 6-*n*-hexylpterin **2e** in 1.5 M HCl at 25°C was 1.23 mM. This pterin hydrochloride is quite soluble in chloroform. Compounds **6a** and **6b** were prepared by reductive alkylation of 2,4,5-triaminopyrimidin-6(1*H*)-one using acetone and 1-octanone, respectively. Acetylation of the pterins **1a** and **1b** was carried out as before [28].



Biological Results and Discussion

Previous studies have shown that substitution of the 2-amino group by hydrogen [29] or replacing the 2-amino group by a 2-methylamino group of pterins greatly reduces DHPR activity [30, 31], and that a dimethylamino group at position 2 almost completely abolishes activity [31]. Substitution at N-8 likewise reduces DHPR activity [30, 31]. A methyl group on N-3 or N-5 prevents the formation of any significant concentration of quinonoid dihydropterin species [32] and may result in the decomposition of the pterin molecule [33].

We therefore chose to locate the lipophilic side chain at C6. The second possibility is at C7, however quinonoid dihydropterins without substituents at C6 tend to be shorter lived (see below). We have examined the DHPR activity of seven quinonoid dihydropterins which would be expected to be more lipophilic than either the parent quinonoid 6-methylhydropterin **3a** or the natural cofactor, quinonoid dihydrobiopterin **3i**. These are the 6-substituted ethyl **3c**, *n*-propyl **3d**, *n*-hexyl **3e**, phenethyl **3f** and neopentyl **3g** pterins, the 7-neopentyl **3h** and the 6,6-geminal-dimethyl pterin **5**. In addition, we measured the substrate activity of the 6- and 7-methylpterins **3a** and **3b** respectively for comparison purposes. Because of the importance of tetrahydrofolate in brain [34], we also examined the DHPR activity of the quinonoid form of folic acid **3j**. Following a report that the quinonoid form of 2,4,5-triaminopyrimidin-6(1*H*)-one is a substrate for DHPR [35], we prepared the pyrimidines **6a** and **6b** for testing the respective quinonoid forms with DHPR. Note that the pyrimidines **6a** and **6b** have features in common with the pterins **2a** and **2e**.

Table I. Kinetic parameters of quinonoid dihydropteridine reductase (human brain) in 0.1 M Tris-HCl buffer (pH 7.2, 30°C)[a].

7,8-Dihydro(6*H*)-pterin	K_m(μM)	V_{max}[b]	V/K
3a 6-Methyl	11.0	491	45
3b 7-Methyl	14.2	800	56
3c 6-Ethyl	3.7	382	103
3d 6-*n*-Propyl[c]	2.1	267	127
3e 6-*n*-Hexyl	3.5	527	150
3f 6-Phenethyl	4.8	455	95
3g 6-Neopentyl	3.6	260	72
3h 7-Neopentyl	5.7	366	64
3i 6*R*(1′*R*, 2′*S*)2′-dihydroxypropyl[d]	1.3	27	21
3j 6-*p*-Methylaminobenzoylglutamic acid[e]	—	~ 218	—
5 6,6-Dimethyl	271	444	1.6

[a] NADH at ~ 100 μM, standard errors were ⩽ ± 5%.
[b] μM NADH oxidized/min/mg of enzyme.
[c] Quinonoid species were formed by *in situ* oxidation with $K_3Fe(CN)_6$.
[d] From natural tetrahydrobiopterin, at 25°C (*cf.* ref. [22]).
[e] Determination of this value was different from the others, see Experimental protocols.

Table I lists the DHPR substrate activities of compounds **3a**—**3j** and **5**. The quinonoid forms of the pyrimidines **6a** and **6b** showed no detectable substrate activities and were not inhibitors when tested at concentrations up to 0.5 mM (see below). Results are presented as apparent V_{max}, K_{max} and V/K values with saturating NADH concentrations. A K_m value for the pterin **3j** could not be determined because this substrate did not obey Michaelis—Menten kinetics (see below), although a V_{max} could be estimated. A literature value [22] for the natural cofactor with the human liver enzyme has been included in Table I. All of the compounds studied except **3j** obeyed Michaelis—Menten kinetics over the concentration range studied (0.1—5 K_m).

The stability of the quinonoid dihydropterins **3** was next examined. These rearrange to the 7,8-dihydro-isomers **4** in a general acid—base catalyzed reaction [32]. The rates of rearrangement of the quinonoid dihydropterins **3a**—**h** and **3j** to the 7,8(3*H*)-dihydro isomers, *i.e.*, **3** → **4**, at pH 7.2 are shown in Table II. The half-life for decomposition of the natural cofactor has been determined as 1.3 min. The rearrangements are usually studied by oxidizing the tetrahydropterin to the quinonoid species in Tris buffer with peroxidase and hydrogen peroxide, and following the UV spectral changes with time. The UV spectral changes are very characteristic [3] and are an indication that the process is proceeding smoothly. When we were examining the 6-*n*-propyl derivative **3d** we did not obtain the characteristic spectral changes, but the spectra were similar to those observed after the oxidation of 5,6,7,8-tetrahydropteridin-4-ones and 6,6-dimethyl-7,8-dihydro(6*H*)pterin **5** (which cannot rearrange) which are known to undergo degradation together with some rearrangement and degradation alone respectively [29]. Consequently when the 6-*n*-propyl derivative **2d** was oxidized with peroxidase and hydrogen peroxide it underwent both rearrangement and degradation reactions. On the other hand, if peroxidase alone in aqueous buffer was used to oxidize **2d** to **3d**, the characteristic spectral changes were observed showing that rearrangement mainly was occurring. This was further confirmed by comparing the rearrangement of **3d** with the corresponding 6-*n*-propyl-6-deutero-7,8-dihydropterin. Normally, the rearrangement of **3** to **4** is considerably retarded when 6H is replaced by 6D, resulting in large deuterium isotope effects [36, 37]. The deuterium isotope effect k_H/k_D when the oxidant was peroxidase and hydrogen peroxide was 2.6 compared with 6.2 when the oxidant was peroxidase alone. Since the degradation does not involve cleavage of the 6C—D bond and is not subject to an isotope effect [29], then in the oxidation with peroxidase and hydrogen peroxide, only 42% of the reaction proceeds *via* rearrangement to **4d**. In the 6-alkylpterins **3a**—**c** and **3e**—**h** the rearrangement reactions appear to be predominantly favored over degradation reactions, but in the *n*-propyl derivative **3d**, it is not clear why the degradation reaction occurs so readily.

Table II. Rates of rearrangement of quinonoid dihydropterins in 0.1 M Tris-HCl buffer (pH 7.2, 30°C)[a].

7,8-Dihydro(6*H*)-pterin	1st order rate constant (min^{-1})	Half-life (min)
3a 6-Methyl	0.030	23.1
3b 7-Methyl	0.103	6.7
3c 6-Ethyl	0.0215	32.2
3d 6-*n*-Propyl[b]	0.027	34.3
3e 6-*n*-Hexyl	0.031	22.2
3f 6-Phenethyl	0.028	24.8
3g 6-Neopentyl	0.015	44.7
3h 7-Neopentyl	0.129	5.4
3j 6-*p*-Methylaminobenzoyl-glutamic acid[c]	~ 0.18	~ 4

[a] The quinonoid pterins **3** were formed by oxidation of the corresponding pterins **2** with peroxidase, and the analytical wavelengths were 300 nm.
[b] The analytical wavelength was 305 nm.
[c] Value measured at 2°C, it was too fast at 30°C.

Clearly the quinonoid dihydropterins **3a**—**3h** are all excellent substrates for human brain DHPR and **3a** and **3e**—**3g** are relatively stable (Table II). Both the 6- and 7-methyl quinonoid dihydropterins are, as expected substrates for human brain DHPR. The 6,6-disubstituted pterin **5** shows greatly reduced activity as indicated by the V_{max}/K_m value, although the turnover is comparable with that of the 6-methylpterin. This is consistent with the previously measured activity of **5** with the enzyme from other sources [38]. Comparison of the kinetic parameters of **3b**—**3h** with the values determined for the natural cofactor **3i** shows that the presence of a lipophilic substituent at 6C (or 7C) in the pyrazino ring has no unfavorable effect on enzyme activity, in fact activity as measured by V/K is enhanced by a factor of up to *ca.* 7.2 in the case of the *n*-hexyl pterin. The reduced affinity of **5** for the human enzyme has also been observed for partially purified monkey liver and sheep liver DHPR and has been explained in terms of steric crowding about 5N, the probable site of hydride transfer [3]. It has been argued elsewhere that the presence of both an axial and an equatorial substitutent at C6 will cause an encroachment on the NADH binding site [38]. Examination of space filling models for **3g** and **3h** shows the *t*-butyl group to be at least as bulky as the geminal dimethyl group **5**, but is some two bonds removed from C6.

Two explanations may be advanced for the apparent insensitivity of DHPR to the bulky substrates **3g** and **3h**. Either the active site at this position is large enough to accommodate a bulky group (*i.e.*, the side chain can move freely around, or protrudes from the active site and is exposed to the solvent), or the side chain of the pterin substrate (at a distance of two bonds from 6C) plays some significant role in binding to DHPR. In either case, it follows that even bulky lipophilic substitutents at 6C, one carbon atom removed from the pyrazine ring do *not interact unfavorably* with residues on the enzyme—NADH complex. It is generally held that binding of NADH to DHPR precedes binding of pterin, and recent detailed kinetic studies confirm this [39, 40]. The work also suggests that a conformational change following pterin binding may be the rate limiting step during enzyme turnover.

The effect of the 6,6-dimethyl substituent is mainly on the K_m which affects seriously the V/K value, a measure of enzyme—substrate interaction at low substrate concentration [41]. Once the enzyme substrate complex is formed, turnover procedes 'normally' as shown by the almost identical V_{max} values for **3a** and **5**. Thus the rate limiting step in the turnover is not affected at pH 7.2, but binding of substrate to the enzyme probably is. The high K_m value for **5** is consistent with this. A measure of the binding constants of these compounds to DHPR would be necessary to confirm this. Although the V_{max} value for the 6-neopentylpterin **3g** is lower than that of the 7-isomer **3h**, the V/K values for these sterically bulky pterins show that the 6-isomer is perhaps a marginally better substrate. This may be due to the smaller K_m value probably attributable to better binding and is also reflected in the lower K_m values of the more lipophilic 6-substituted substrates **(3c—3f)**. The possibility that the lipophilic portions of the side chains are interacting with lipophilic residues on the enzyme cannot be discounted. It should be noted that the substrate activity of 6,7,7-trimethyl-7,8(6*H*)-dihydropterin in which the 7 position is disubstituted has greatly diminished activity when tested with human liver enzyme [42].

When the kinetic data for the reduced folate **3j** were plotted in a double reciprocal form, significant non-linearity was observed. The plot curved upwards suggesting positive co-operativity [43]. The data could be fitted to a parabola of the general form $Y = A + Cx^2$ successfully using a computer program kindly supplied by Dr. J. F. Morrison. This gave $A = 4.59 \times 10^{-3}$ and $C = 2.20$.

This enabled a value of V_{max} to be determined since $A = 1/V_{max}$. The presence of 200 μM *p*-aminobenzoylglutamic acid did not affect the kinetic behavior of quinonoid-6-methyl-7,8-dihydro(6*H*)pterin **(3a)**, although the possibility of a separate binding site for *p*-aminobenzoylglutamic acid on DHPR had been discussed [44, 45].

Of particular significance is the comparison of the natural cofactor **3i** with the substrate **3d** in which the two lipophobic (hydrophilic) OH groups on 1′C and 2′C are absent. Removal of the hydroxyl groups results in the doubling of the K_m value (possibly decreased binding) and increasing the turnover by one order of magnitude. The high turnover of quinonoid dihydrofolic acid **3j** by human brain DHPR also shows the ability of this enzyme to accommodate a side chain at 6C which is equivalent to *ca.* 12 carbon atoms in length.

Together with the known loss in DHPR activity of quinonoid pterins when substituted at 2-NH_2 or 8-N, we propose that these data support the notion that the basic quinonoid structure defined by carbon atoms 2, 4, 4a, 6, 7 and 8a in structure **3** binds intimately to the enzyme, and that side chains at 6C and 7C, greater than 2 bonds in length, do not significantly interact with the active site. Thus quite dramatic changes in structure in the side chain are possible with retained activity. This may give DHPR the ability to catalyze the reduction of any naturally occurring 6-substituted quinonoid pterins to the tetrahydro form, and could act in a general way to maintain tetrahydropterins in their active form. It should be pointed out that like quinonoid dihydrobiopterin **3i**, the pterins **3a**—**h**, also have an asymmetric center at 6C. Whereas **3i** is a diastereoisomer, because of the chiral side chain, the pterins **3a**—**h** are enantiomers. The pterins **3a**—**h** in this study are all racemates and in the final evaluation the pure enantiomers of the most promising substrates will have to be tested. However, earlier work had shown that with the 6-methyl-7,8-dihydro(6*H*)pterin **3a** there was virtually no difference between the DHPR activities of the 6*R*, 6*S* and 6*RS* compounds [42].

The peroxidase/H_2O_2 oxidation of the pyrimidines **6a** and **6b** gave compounds formally analogous to quinonoid dihydropterins. The ultraviolet spectral changes accompanying oxidation show a small (282 nm—290 nm) change to longer wavelength when the neutral species are oxidized. The new spectrum has a long wavelength shoulder at 350 nm analogous to that observed in quinonoid dihydropterins [3]. In addition, compounds **6a** and **6b** are reduced

back to the parent pyrimidines within seconds upon the addition of a 10-fold excess of dithiothreitol (data not shown). The quinonoid forms are also reduced non-enzymatically by NADH at a rate twice that of **5** and approaching 40% of that of the parent 6-methylpterin **3a** (see Table III). These data support the formation of an analogous quinonoid species. Nevertheless, they show no substrate activity at concentrations up to 0.5 mM and no inhibition at this concentration. This is despite the substrate activity of the parent 2,4,5-triaminopyrimidin-6(1*H*)-one (K_m 76 μM with human liver DHPR [35]). At concentrations in excess of this, non-enzymatic reduction by NADH interferes. The absence of activity is no doubt due to the greater conformational mobility of the side chain, and two explanations are possible for inactivity. If the geometrical isomer **7a** is formed exclusively, unhindered rotations about the 5'N—1'C bond may allow the quinonoid form to adopt a conformation of **7** analogous to that of the 6-methylpterin **3a**, or at worse, analogous to the 6,6-dimethylpterin **5** if the positions of H and Me are exchanged. This latter compound does however show significant activity (at 270 μM see Table I), whereas oxidized **6a** is completely inactive (at 500 μM). An alternative explanation is the formation of the other geometrical isomers **8a** and **8b**. This seems the more likely product of the oxidation of **6a**, since it minimizes unfavorable *peri* interaction of the lone pair on 5'N with the 4-carbonyl group and allows hydrogen bonding between 5'N and the amino group at 6C. Quinonoid forms of the type **8a** and **8b** now present sterically bulky isopropyl and isooctyl groups to a region of the enzyme likely to be involved in NADH binding and reduce the activity dramatically.

Table III. Second order rate constants for the non-enzymatic reduction of quinonoid dihydropterins and pyrimidines by NADH in 0.1 M Tris-HCl buffer (pH 7.2, 30°C)[a].

7,8-Dihydro(6*H*)-pterin	$k \times 10^3$ mM^{-1} · min^{-1}
3a 6-Methyl	1.38
3g 6-Neopentyl	1.17
5 6,6-Dimethyl	0.28
2,4-Diaminopyrimidin-6(1*H*)-one	
7a 5-(2'-Propylimino)	0.55
7b 5-(2'-Octylimino)	0.27

[a] Analytical wavelength was 340 nm.

The conformational mobilities of the side chains in the quinonoid forms **7a** (or **7b**) and **8a** (or **8b**) can be compared by noting the difference in the non-enzymatic rates of reduction by NADH. There is almost a factor of 2 between them (see Table III). By comparison, the neopentyl group in the pterin **3g** has only a marginal effect on reduction by NADH when compared with **3a**. In contrast, the two methyl groups at 6C in the geminal pterin **5** which are in close proximity to 5N, the site of hydride attack, reduce the non-enzymatic rate by 80%. Thus, modifications of 2,4,5-triaminopyrimidin-6(1*H*)-one at 5'N will not prove to be useful in the development of alternate lipophilic substrates for DHPR.

All the 6-alkyl substituted quinonoid dihydropterins are good substrates for DHPR compared with the natural cofactor **3i**, and are more stable *in vitro*. They are, therefore, unlikely to have deleterious effects on this enzyme *in vivo*. As judged by the *V*/*K* values, the 6-*n*-hexylpterin **3e** is the best of these substrates, but it is only marginally better than the 6-*n*-propylpterin **3d**. The final choice of pterin for the purposes described in the introduction must await the results of studies of their activities towards phenylalanine, tyrosine and tryptophan hydroxylases, and their abilities to penetrate the mammalian blood—brain barrier which are in progress in our laboratory. Ozawa and Suzuki [46] had already shown that racemic 6-*n*-propyl-5,6,7,8-tetrahydropterin was a good substrate for tyrosine hydroxylase from bovine adrenal medulla.

Experimental protocols

NMR spectra at 90 MHz, 200 MHz and 300 MHz were measured on Jeol FX90Q, Varian XL200E and XL300 spectrometers. UV spectra were measured on PYE Unicam 1800 and Varian 219 spectrometers and maxima were read on a single beam Perkin—Elmer Lambda 1 spectrometer at 25°C. All kinetic runs were carried out on the Varian 219 spectrometer using a 0—0.1 absorbance scale, unless otherwise stated, with cells thermostated at 30°C. Kinetic parameters were calculated from at least seven concentrations of substrates and computed using a program kindly supplied by Dr. Cornish-Bowden [47].

Syntheses of pterins

6-n-Hexylpterin ***1e*** *and 6-phenethylpterin* ***1f***

These were prepared according to a reported method [27], except that in our hands we detected up to 15% of the 7-isomers (by NMR) which were completely removed by a single recrystallization from 50% aqueous formic acid. The ^{1}H NMR spectrum of **1e** had δ (DMSO-d_6, 90 MHz, TMS δ = 0) 0.86 (br t, *J* = 8 Hz, CH_3); 1.30 (br s, (CH_2)$_3$); 1.63 (br m, CH_2); 2.78 (br t, *J* = 8 Hz, 1'CH_2) and 8.71 (s, 7*H*). The 7-isomer had chemical shifts indistinguishable from the above except for δ 8.45 (s, 6*H*). The 6-phenethylpterin **1f** had δ (DMSO-d_6, 90 MHz, TMS δ = 0) 3.67 (br m, CH_2CH_2); 7.78 (s, C$_6$H$_5$) and 9.20 (s, 7*H*); and the 7-isomer had δ 9.14 (s, 6*H*). The pterins were only slightly soluble in DMSO, but addition of a drop of trifluoroacetic acid facilitated dissolution. However, this had the effect of bringing the chemical shifts of 6H and 7H of the respective isomers closer together.

6-Ethylpterin ***1c***

1-Methylthio-1-butanol-2-one (1 g, prepared by the method in ref. [27] and used crude) was stirred with 2,4,5-triaminopyrimidin-6(1*H*)-one sulfate monohydrate (1.8 g) in water (160 ml) containing sodium sulfite (21 g) and sodium hydrogen sulfite (0.9 g) for 5 days at 20°C. The pH remained at 7. The solid was removed by centrifugation and washed with water (3 × 100 ml), ethanol (3 × 50 ml) and dried at 20°C for 6 h in a vacuum. The pterin (1 g) was estimated by ^{1}H NMR to be a 86/14 mixture of 6- and 7-ethylpterin isomers.

Separation was achieved by recrystallization from 2 M sodium hydroxide. The ^{1}H NMR spectrum of the 6-isomer had δ (2 M HCl, 90 MHz, TPS δ = 0) 1.34 (t, *J* = 8 Hz, CH_3); 3.00 (q, *J* = 8 Hz, CH_2) and 8.81 (s, 7*H*), and the 7-isomer had δ 1.34 (t, *J* = 8 Hz, CH_3), 3.02 (q, *J* = 8 Hz, CH_2) and 8.69 (s, 6*H*).

6- and 7-Neopentylpterins ***1g*** *and* ***1h***

1-Methylsulfinyl-4,4-dimethylheptan-2-one was prepared from ethyl 3,3-dimethylbutanoate by the method in ref. [27], except that the

reaction time was extended to 2 days. The crude product (90% yield) was > 95% pure by ^{1}H NMR [δ ($CDCl_3$, 90 MHz, TMS δ = 0) 1.05 (s, $(CH_3)_3$); 2.50 (s, CH_2), 2.69 (s, SCH_3) and 3.77 (s, CH_2)]. The sulfinyl compound (1.76 g) was refluxed with the triaminopyrimidinone sulfate monohydrate (2.6 g) in acetic acid (60 ml) containing sodium acetate (1.6 g) for 1.5 h. The solution was cooled and the yellow solid was washed with water (3 × 50 ml), cold ethanol (3 × 50 ml) and dried at 20°C for 6 h in a vacuum. The crude material (0.5 g) consisted of 65/35 mixture of the 6- and the 7-neopentyl isomers. The 6-isomer can be obtained in small amounts by repeated recrystallization from 50% aqueous formic acid. Its ^{1}H NMR spectrum had δ (DMSO-d_6, 90 MHz, TMS δ = 0) 0.91 (s, CH_3); 2.69 (s, CH_2) and 8.52 (s, 7*H*). 1-Methylthio-4,4-dimethyl-1-heptanol-2-one [0.75 g, prepared from the sulfinyl compound in 70% yield as above, ^{1}H NMR spectrum had δ ($CDCl_3$, 90 MHz, TMS δ = 0) 1.05 (s, CH_3); 1.92 (s, SCH_3); 2.59 (s, CH_2); 5.20 (d, *J* = 6.4 Hz, C*H*) and 4.20 (d, *J* = 6.4 Hz, O*H*)] was stirred with the triaminopyrimidinone sulfate monohydrate (1 g) in water (100 ml) and ethanol (30 ml) containing sodium sulfite (12 g) and sodium hydrogen sulfite (0.5 g) at 5—10°C for 2 h, and stirred at 25°C for 5 days. The solid was collected by centrifugation, washed with water (3 × 50 ml), ethanol (3 × 50 ml) and was dried in a vacuum to give 7-neopentylpterin **1h** (400 mg). Its ^{1}H NMR spectrum had δ (DMSO-d_6, 90 MHz, TMS δ = 0) 0.94 (s, CH_3); 2.69 (s, CH_2) and 8.25 (s, 6*H*); and the ^{13}C NMR spectrum had δ (DMSO-d_6, 90 MHz, TMS δ = 0) 30.0 (CH_3); 32.6 (—$C(CH_3)_3$); 48.9 (CH_2); 127.9 (*C*4a); 139.2 (*C*6); 157.3 (*C*8a); 159.8 (*C*7); 165.1 (*C*2) and 172.8 (*C*4). Assignments followed from the chemical shifts and from the signal multiplicity in the coupled spectra (data not shown). The value of $J_{C4a,H6}$ was 9.8 Hz (see below).

The orientation of the side chain alkyl groups in 6- and 7-neopentyl pterins

The problem of the orientation of the side chains in the pyrazine ring of a pterin can be solved in principle with ^{13}C NMR by measuring the coupling between either 4aC and 6H (for the 7-isomer) or 8aC and 7H (for the 6-isomer). This is only possible if enough material is available to obtain a coupled spectrum and the material is sufficiently soluble to give a suitably high concentration to overcome the loss of signal-to-noise during accumulation of a coupled spectrum. This was possible in the case of the 7-neopentylpterin and a value of 9.8 Hz for $J_{C4a,H6}$ was obtained (see above). It was necessary, however, to examine model compounds with known orientations for comparison (see also ^{1}H NMR spectra of the 5,6,7,8-tetrahydro derivatives below). The logical choice was 6- and 7-methylpterins, but these proved too insoluble in DMSO to provide a noise decoupled spectrum. The 2-acetamido derivatives were sufficiently soluble to provide spectra. 2-Acetamido-7-methylpteridin-4(3*H*)-one had δ (DMSO-d_6, 90 MHz, TMS δ = 0) 22.1 (CH_3), 24.0 (CH_3CON); 129.1 (*C*4a); 142.7 (*C*6); 149.6 (*C*8a); 134.8 (*C*2); 159.6 (*C*4); 160.7 (*C*7) and 174.2 (NCO) and 2-acctamido-6-methylpteridin-4(3*H*)-one had δ 20.9 (CH_3); 23.7 ($COCH_3$); 130.2 (*C*4a); 145.5 (*C*8a); 148.7 (*C*2); 150.3 (*C*7); 151.3 (*C*6); 159.3 (*C*4) and 173.8 (NCO). The value of $J_{C4a,H6}$ in the 7-methylpterin was 10.3 Hz, whereas the signal due to 4aC in the 6-isomer was a sharp singlet. This confirms the orientation of the neopentyl side chain in the 7-isomer (see above). Conversely, the signal due to 8aC in the 7-methyl isomer was, as suspected, a singlet, but the coupling between 8aC and 7H in the 6-methyl isomer could not be resolved because of unsatisfactory signal-to-noise levels. It should be noted that in the ^{1}H NMR spectra of the pterins the signals from 7H in the 6-isomers are always downfield from those of 6H in the 7-isomers. On the basis of this, the major product in the preparation of the ethylpterins was assigned the 6-ethyl orientation. The formation of larger amounts of the 7-isomer in the synthesis of the neopentylpterin at lower temperatures appears to be a special case and is not doubt related to the steric bulk of the *t*-butyl side chain during the initial condensation on the 5-amino group of the pyrimidine ring.

5,6,7,8-Tetrahydropterin hydrochlorides

These were prepared in all cases by reduction of the corresponding pterin in ethanol—2 M HCl at 20°C 720 mmHg by shaking with hydrogen using pre-reduced platinum oxide. In a typical experiment, the pterin (50 mg) was added to 2 M HCl (5 ml) and ethanol (1 ml) and shaken with platinum oxide (25 mg). Shaking was carried out overnight, although 3—4 h were sufficient. More ethanol was added to solubilize the more lipophilic pterins. The solution was filtered and evaporated to dryness at 50°C/18 mmHg in a rotary evaporator. The solid residue was dried and redissolved in a minimum volume of dry methanol. The tetrahydropterin hydrochloride was precipitated by adding ether until the solution was slightly turbid and allowed to stand at 0°C. It was collected by centrifugation, washed with ether and dried (100°C/3 h). They analyzed for more than one molecule of HCl and usually contained some water. The amounts of HCl and water varied with the extent of drying.

6-Ethyl-5,6,7,8-tetrahydropterin ***2c*** *hydrochloride.* m.p. > 300°C (decomp.); $C_8H_{13}N_5O$, 1.03 HCl, 0.17 H_2O (CHNCl) had UV λ_{max} 266 nm; ε 11000 M^{-1} cm^{-1} (4 mM HCl); and ^{1}H NMR (2 M DCl, 200 MHz, TPS δ = 0) δ 1.08 (t, *J* = 7.5 Hz, CH_3); 1.85 (m, CH_2); 3.45 (q, J_{vic} = 9 Hz, J_{gem} = — 13 Hz, $7H_{ax}$); 3.6 (br m, 6*H*) and 3.89 (q, J_{vic} = 3 Hz, J_{gem} = — 13 Hz, $7H_{eq}$).

*6-*n*-Propyl-5,6,7,8-tetrahydropterin* ***2d*** *hydrochloride.* m.p. > 285°C (decomp); $C_9H_{15}N_5O$, 2.8 HCl, 4.4 H_2O (CHNCl) had UV λ_{max} 268 nm; ε 14474 M^{-1} cm^{-1} (6.3 mM HCl); and ^{1}H NMR (2 M DCl, 200 MHz, TPS δ = 0) δ 0.97 (t, *J* = 7.1 Hz, 3′CH_3); 1.48 (m, 2′CH_2); 1.78 (m, 1′CH_2); 3.43 (q, J_{vic} = 9.2 Hz, J_{gem} = — 14 Hz, $7H_{ax}$); 3.66 (m, 6*H*) and 3.85 (q, J_{vic} = 3.2, J_{gem} = — 14 Hz, $7H_{eq}$).

*6-*n*-Hexyl-5,6,7,8-tetrahydropterin* ***2e*** *hydrochloride.* m.p. > 300°C (decomp); $C_{12}H_{21}N_5O$, 1.67 HCl (CHNCl) had UV λ_{max} 266 nm; ε 10600 M^{-1} cm^{-1} (4 mM HCl); and ^{1}H NMR (1 M DCl, 300 MHz, TPS δ = 0) δ 0.85 (t, 6′CH_3); 1.30 (m, 2′, 3′, 4′, 5′$(CH_2)_4$); 1.74 (m, 1′CH); 1.86 (m, 1′CH); 3.42 (q, J_{vic} = 9.5 Hz, J_{gem} = — 14.1 Hz, $7H_{ax}$); 3.63 (br m, 6*H*) and 3.85 (q, J_{vic} = 3.0 Hz, J_{gem} = — 14.1 Hz, $7H_{eq}$).

6-Phenethyl-5,6,7,8-tetrahydropterin ***3f*** *hydrochloride.* m.p. > 300°C (decomp); $C_{14}H_{17}N_5O$, 1.05 HCl, 0.8 H_2O (CHNCl) had UV λ_{max} 266 nm; ε 10500 M^{-1} cm^{-1} (4 mM HCl) and ^{1}H NMR (1 M DCl, 300 MHz, TPS δ = 0) δ 2.08 (m, 1′CH); 2.25 (m, 1′CH); 2.86 (m, 2′-CH_2); 3.49 (q, J_{vic} = 8.9 Hz, J_{gem} = — 14.3 Hz, $7H_{ax}$); 3.65 (m, 6*H*); 3.87 (q, J_{vic} = 3.5 Hz, J_{gem} = — 14.3 Hz, $7H_{eq}$) and 7.38 (m, C_6H_5).

6-Neopentyl-5,6,7,8-tetrahydropterin ***2g*** *hydrochloride.* m.p. > 300°C (decomp); $C_{11}H_{19}N_5O$, 1.66 HCl, 0.55 H_2O (CHNCl) had UV λ_{max} 266 nm; ε 12100 M^{-1} cm^{-1} (4 mM HCl) and ^{1}H NMR (2 M DCl, 200 MHz, TPS δ = 0) δ 0.86 (br s, ~ CH_3); 1.32 (br s, ~ $(CH_3)_2$); 1.80 (br m, 1′CH_2); 3.44 (q, J_{vic} = 9.3 Hz, J_{gem} = — 14.1 Hz, $7H_{ax}$); 3.65 (m, 6*H*) and 3.87 (q, J_{vic} = 3.1 Hz, J_{gem} = — 14.1 Hz, $7H_{eq}$).

7-Neopentyl-5,6,7,8-tetrahydropterin ***2h*** *hydrochloride.* m.p. > 300°C (decomp); $C_{11}H_{19}N_5O$, 1.47 HCl, 0.15 H_2O (CHNCl) had UV λ_{max} 265 nm; ε 11500 M^{-1} cm^{-1} (4 mM HCl) and ^{1}H NMR (2 M DCl, 200 MHz, TPS δ = 0) δ 1.01 (s, 2′, 2′, 2′, $(CH_3)_3$); 1.63 (t, 1′CH_2); 3.78 (q, J_{vic} = 7.1 Hz, J_{gem} = — 12.5 Hz, $7H_{ax}$); 3.61 (q, J_{vic} = 3.3 Hz, J_{gem} = — 12.5 Hz, $7H_{eq}$) and 3.98 (br m, 6*H*).

It should be noted that the pattern of signals and the magnitude of the coupling constants of all the above tetrahydropterin hydrochlorides in aqueous solution indicate that the predominant conformer in each case is the one in which the dihydropiperazine ring is puckered and the side chain at 6C (or 7C) is equatorial with 6H (or 7H) axial as in 6-methyl-5,6,7,8-tetrahydropterin hydrochloride [23]. The spectra of the 6- and 7-neopentyl compounds **2g** and **2h** show an interesting feature. The signals from the *t*-butyl group of the 6-isomer appear as two broad singlets in which the high field signal integrates for one methyl group and the low field signal integrates for two methyl groups. The three methyl groups of the 7-isomer, on the other hand, appear as one sharp signal. The explanation for this may be that the neopentyl group of the 6-isomer is close to the protonated 5N group and allows for restricted conformers. In the 7-isomer the neopentyl group is close to the unprotonated 8N group which is perhaps undergoing nitrogen inversion and allows freer rotation of the *t*-butyl group. The 1′CH_2 resonances are also affected. In the 6-isomer the CH_2 protons appears as many peaks due to the coupling in various conformers, whereas in the 7-isomer the CH is made up of three main signals (together

with minor satellites on either side) as part of the ABX pattern involving the $1'CH_2$ and 7H. The ^1H NMR spectra of the tetrahydropterins can also be used to distinguish between the 6- and 7-substituted isomers. In the 6-isomers, the chemical shifts of 6H are always between those of $7H_{eq}$ and $7H_{ax}$, whereas in the 7-isomers the shifts of 6H are at a lower field than those of $7H_{eq}$ and $7H_{ax}$ (compare the 6- [23] and 7-methyltetrahydropterins [24]).

2,4-Diamino-5-2'-propylaminopyrimidin-6 (1H)-one **6a** *and 2,4-diamino-5-2'-octylaminopyrimidin-6(1H)-one* **6b**

They were prepared by reducing a mixture of 2,4,5-triaminopyrimidin-6(1*H*)-one sulfate monohydrate (0.55 g) and acetone (0.54 g) or octan-2-one (0.54 g) in 50% aqueous methanol together with pre-reduced platinum oxide and hydrogen at 25°C/720 mmHg for 12 h. The catalyst was filtered off and the residue evaporated to dryness. The residue was recrystallized from methanol—ether to give the hydrochloride of **6a** (80%): m.p. > 300°C (decomp); $C_7H_{13}N_5O$, 1.87 HCl, 0.87 H_2O (CHNCl) and the hydrochloride of **6b** (80%): m.p. > 300°C (decomp); $C_{12}H_{23}N_5O$, 1.28 HCl (CHNCl).

Kinetic measurements

Dihydropteridine reductase was purified from human brain using naphthoquinone affinity chromatography as described previously [48] and gave only one band on sodium dodecyl sulfate—polyacrylamide gel electrophoresis. The apparent kinetic parameters K_m and V_{max} for human brain DHPR in Table I were determined as before [35], using the following solutions (final concentrations in parentheses): Tris-HCl buffer (0.1 M), peroxidase (20 μg), H_2O_2 (220 μM), NADH ($\sim$ 100 μM), tetrahydropterin hydrochloride ($\sim$ 0.5—3 K_m, prepared in 4 mM HCl and the concentrations determined from the respective ϵ values) and water to make 1 ml, in cuvettes of a double beam spectrometer, and the reaction initiated by injection of 5 μl (0.055 μg) of enzyme. The first three ingredients in the requisite volume of water were added together as a stock solution. The initial rates of oxidation of NADH (ϵ_{340} 6200 M^{-1} cm^{-1}) were measured by the rate of change of absorbance at 340 nm. In the case of the 6-*n*-propyl compound **2d** the peroxidase and H_2O_2 solutions were replaced by $K_3Fe(CN)_6$ solution (2 mM) because of excessive decomposition of **3d** by H_2O_2. Unlike the conjugated pterins **3a**—**3i**, the folate compound **3j** rearranged very rapidly (half-life *ca.* 2—3 min) to the 7,8-dihydro derivative **4j**. Consequently, the analytical wavelength used was 330 nm which is the isosbestic point for the rearrangement **3j** to **4j**. The corresponding ϵ_{330} used for NADH was 5800 M^{-1} cm^{-1}. The rates of NADH oxidation were measured in duplicate as quickly as possible using the single beam mode. Non-enzymatic rates were similarly measured in the absence of the quinonoid substrate and the enzymatic rates were obtained by the difference. At least seven points were determined.

The first order rate constants for the rearrangement of the quinonoid species **3** to the corresponding 7,8-dihydropterins **4** in Table II were carried out using a previous method [32], *i.e.*, the oxidation of species **2** to **3** was performed in 0.1 M Tris-HCl buffer at pH 7.2 with peroxidase (20 μg) (oxidation was complete in less than 1 min), and the rate of change of absorbance at a suitable wavelength was used to determine the rate constants.

For the determination of the second order rate constants for the non-enzymatic oxidation of NADH in Table III, a series of concentrations of quinonoid dihydropterin (1—30 μM) were generated as above in 1 ml cuvettes. A stock solution of NADH (final concentration 0.15 mM) was added to each cuvette and the rate of decrease in absorbance at 340 nm was measured. An absorbance scale of 0—0.02 was used. This gave the *initial rates* of reduction of quinonoid dihydropterins at various concentrations. By plotting log(initial rates) against log(concentration of quinonoid pterins) straight lines were obtained (fitted by least squares). Extrapolation to log(concentration) = 0 provided $\log k_{app}$, where k_{app} is the apparent first order rate constant. The second order rate constant k is then equal to k_{app}/[NADH], *i.e.*, k_{app}/0.15 mM^{-1} min^{-1}.

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References

1 Kaufman S. & Miller D. B. (1974) *in: Molecular Mechanisms of Oxygen Activation* (Hayaishi O., ed.), Academic Press, New York, 285—369
2 Kaufman S. (1976) *Biochem. Med.* 15, 42—45
3 Armarego W. L. F., Randles D. & Waring P. (1984) *Med. Res. Rev.* 4, 67—321
4 Usdin E. & Snyder S. H. (1973) *in: Frontiers in Catecholamine Research*, Pergamon Press, New York, pp. 1219
5 Haber B., Gabay S., Issidorides M. R. & Alvisatos S. G. A. (1979) *in: Serotonin: Current Aspects of Neurochemistry and Function*, Pergamon Press, New York, pp. 824
6 Curtius H.-Ch., Heintel D., Gisla S., Kuster Th., Leimbacher W. & Niederwieser A. (1985) *J. Inher. Metab. Dis.* 8 suppl. 1, 28—33
7 Kaufman S. (1985) *J. Inher. Metab. Dis.* 8 suppl. 1, 20—27
8 Niederwieser A., Ponzon A. & Curtius H.-Ch. (1985) *J. Inher. Metab. Dis.* 8 suppl. 1, 34—38
9 Danks D. M., Cotton R. G. H. & Schlesinger B. (1975) *Lancet* ii, 1043
10 Dhondt J.-L. (1984) *J. Pediatr.* 104, 501—508
11 Danks D. M. & Cotton R. G. H. (1980) *J. Pediatr.* 96, 854—856
12 Curtius H.-Ch., Niederwieser A., Viscontini M., Otten A., Schaub J., Scheibenritter S. & Schmidt H. (1979) *Clin. Chim. Acta* 93, 251—262
13 Enders W., Niederwieser A., Curtius H.-Ch., Wang M., Ohrt B. & Schaub J. (1982) *Helv. Paediatr. Acta* 37, 489—498
14 Smith I., Hyland K., Kendall B. & Leeming R. (1985) *J. Inher. Metab. Dis.* 8 suppl. 1, 39—45
15 Williams A. C., Levine R. A., Chase T. N., Lovenberg W. & Calne D. B. (1980) *J. Neurol. Neurosurg. Psych.* 43, 735—738
16 Lovenberg W., Levine R. A., Dobson D. S., Ebert M., Williams A. C. & Calne D. B. (1979) *Science* 204, 624—626
17 Nagatsu T., Yamaguchi T., Kato T., Sugimoto T., Matsuura S., Akino M., Nagatsu I., Iizuka R. & Narabayashi H. (1981) *Clin. Chim. Acta* 109, 305—311
18 Curtius H.-Ch., Muldner H. & Niederwieser A. (1982) *in: Biochemical and Clinical Aspects of Pteridines* (Wachter H., Curtius H.-Ch. & Pfleiderer W., eds.), Vol. 1, W. de Gruyter, Berlin, pp. 285—292
19 Moore A. P., Behan P. O., Jacobson W. & Armarego W. L. F. (1987) *J. Neurol. Neurosurg. Psych.* 50, 85—87
20 Kapatos G. & Kaufman S. (1980) *Science* 212, 955—956
21 Kettler R., Bartolini G. & Pletscher A. (1974) *Nature* 249, 476—477
22 Armarego W. L. F., Randles D. & Taguchi H. (1985) *Eur. J. Biochem.* 146, 467—474
23 Armarego W. L. F. & Schou H. (1977) *J.C.S. Perkin Trans. 1* 2529—2536
24 Ganguly A. N., Sengupta P. K., Bieri J. H. & Viscontini M. (1980) *Helv. Chim. Acta* 63, 395—401
25 Bobst A. & Viscontini M. (1966) *Helv. Chim. Acta* 49, 875—884
26 Armarego W. L. F. & Waring P. (1981) *Aust. J. Chem.* 34, 1921—1933
27 Rosowsky A. & Chen K. K. N. (1973) *J. Org. Chem.* 38, 2073—2077
28 Waring P. & Armarego W. L. F. (1985) *Aust. J. Chem.* 38, 629—631
29 Armarego W. L. F., Ohnishi A. & Taguchi H. (1986) *Biochem. J.* 234, 335—342
30 Armarego W. L. F., Ohnishi A. & Taguchi H. (1986) *Aust. J. Chem.* 39, 31—41
31 Kaufman S. (1964) *J. Biol. Chem.* 239, 332—338
32 Armarego W. L. F. & Waring P. (1982) *J.C.S. Perkin Trans. 2*, 1227—1233
33 Mager H. I. X. (1975) *in: Chemistry and Biology of Pteridines* (Pfleiderer W., ed.), W. de Gruyter, Berlin, pp. 753—773
34 Botez M. I. & Reynolds E. H. (1979) *in: Folic Acid in Neurology, Psychiatry and Internal Medicine*, Raven Press, New York
35 Armarego W. L. F., Randles D. & Taguchi H. (1983) *Biochem. J.* 211, 357—361
36 Archer M. C. & Scrimgeour K. G. (1970) *Can. J. Biochem.* 48, 278—287

37 Armarego W. L. F. (1984) *Lect. Heterocycl. Chem.* 7, 121—130
38 Armarego W. L. F. & Waring P. (1983) *in: Chemistry and Biology of Pteridines* (Blair J. A., ed.), W. de Gruyter, Berlin, pp. 429—433
39 Poddar S. & Henkin J. (1984) *Biochemistry* 23, 3143—3148
40 Randles D. (1986) *Eur. J. Biochem.* 55, 301—304
41 Fersht A. (1985) *in: Enzyme Structure and Mechanism*, 2nd ed., W. H. Freeman & Co., New York, pp. 105—106
42 Armarego W. L. F. (1979) *in: Chemistry and Biology of Pteridines* (Brown G. M. & Kisliuk R. L., eds.), Elsevier, New York, pp. 1—6
43 Dixon M. & Webb E. C. (1979) *in: Enzymes*, 3rd edn, Academic Press, New York, pp. 400—402
44 Scrimgeour K. G., Tippak A. & Chauvin M. M. (1979) *in: Chemistry and Biology of Pteridines* (Brown G. M. & Kisliuk R. L., eds.), Elsevier, New York, pp. 207—219
45 Chauvin M. M., Korri K. K., Tippak A., Simpson R. C. & Scrimgeour K. G. (1979) *Can. J. Biochem.* 57, 178—187
46 Ozawa H. & Suzuki K. (1971) *Yakugaku Zasshi* 91, 1250—1252
47 Cornish-Bowden A. & Endrenyi L. (1981) *Biochem. J.* 193, 1005—1008
48 Armarego W. L. F. & Waring P. (1983) *Biochem. Biophys. Res. Commun.* 113, 895—899