Theoretical Studies on Pteridines. 3. Geometries, Tautomer and Ionization Energies, and Rearrangement and Reduction Mechanisms of the Quinonoid Dihydropterin Substrates of Dihydropteridine Reductase

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Abstract: Ab initio SCF calculations with STO-3G and 3-21G basis sets are reported for tautomeric, protonated, deprotonated, and 6-methylated forms of quinonoid dihydropterin, the pteridine substrate for dihydropteridine reductase. Full geometry optimizations at the SCF/STO-3G level were performed for most of the molecules studied. The three 4-oxo tautomers, 2-amino-6,7-dihydropteridin-4(8*H*)-one (**1**), 2-imino-6,7-dihydropteridin-4(3,8*H*)-one (**2**), and 2-amino-6,7-dihydropteridin-4(3*H*)-one (**3**), were found to be approximately equally stable but ~26 kcal/mol higher in energy than the most stable dihydropterin tautomer, 2-amino-7,8-dihydropteridin-4(3*H*)-one (**5**). Theory predicts that **1–3** should be readily interconvertible at neutral pH via the preferred cation **7** which has a relatively high calculated pK_a of ~5.3 due to stabilization by the extended-guanidinium resonance. Theory also predicts ready acid- and base-catalyzed rearrangements of **1–3** to 7,8-dihydropterin via **7** and the 7,8-dihydropterin anions **8–10**. The most likely mechanism for both enzymic and non-enzymic reductions is suggested to be that involving preprotonation of quinonoid dihydropterin to form **7** followed by hydride ion transfer to N5 to form directly 2-amino-5,6,7,8-tetrahydropteridin-4(3*H*)-one (**6**).

Biological hydroxylations of aromatic amino acids require a reduced pteridine cofactor (L-*erythro*-5,6,7,8-tetrahydrobiopterin) and produce a dihydropteridine which evidence[1] suggests is a 6,7-(quinonoid) rather than 7,8-, 5,6-, or 5,8-dihydro tautomer. Tetrahydrobiopterin is regenerated from quinonoid dihydrobiopterin in a reaction catalyzed by dihydropteridine reductase (DHPR) requiring, usually, NADH: the DHPR literature has been reviewed recently.[1] In addition to the natural substrate (6-R ≡ (1′*R*,2′*S*)-1′,2′-dihydroxypropyl), 6- and 6,7-methyl derivatives of quinonoid dihydropterin (pterin ≡ 2-amino-4-oxopteridine) are good substrates for DHPR and are routinely used in enzyme studies. This choice is convenient as quinonoid dihydropterins rapidly rearrange to the more stable 7,8-dihydropterin tautomer form in a reaction subject to general acid or base catalysis[2] and which for quinonoid dihydrobiopterin also involves side-chain cleavage.[1] As the rate-limiting step in this rearrangement is cleavage of the C6–H bond,[2] deuteration[2,3] or dimethylation[4,5] at C6 impedes the reaction.

Due to its instability, identification of the preferred quinonoid tautomeric species has been difficult with most of the early work being indirect kinetic studies:[1] theoretically there are eight possible neutral tautomers of the 6,7-dihydropterins of which three of the 4-oxo forms (**1–3**) have been considered most likely. Recent ^{1}H[6] and ^{15}N[7] NMR studies have suggested that the predominant tautomer is one of the N8(*H*) forms (**1** or **2**)[6] with at least ~70% in the endocyclic form **1**.[7] Bailey and Ayling[5] determined a pK_a of 5.15 for quinonoid 6,6-dimethyldihydropterin and suggested that if the protonated form were the structure containing the extended-guanidinium resonance (**7**), then **1–3** could be rapidly equilibrated via small concentrations of the cation.

Study of the DHPR reaction with NADP^{3}H[8] showed loss of label in the tetrahydropterin product, implying hydride ion transfer to a nitrogen not a carbon atom: arguments[9] have been presented for hydride ion transfer to N5. The results of recent kinetic experiments[10] are consistent with a mechanism in which a rate-limiting isomerization in the ternary complex precedes hydride ion transfer. Unlike the 7,8-dihydropterins, quinonoid dihydropterins are readily reduced non-enzymically to tetrahydropterins by NADH or NADPH[1] and have higher basic pK_a's.[5] Also, there is no evidence of similarity in the enzymic mechanisms of dihydropteridine reductase and dihydrofolate reductase,[11] the enzyme which catalyzes the reduction of oxidized and 7,8-dihydropterins, including 7,8-dihydrobiopterin,[12] to the tetrahydropterins.

This paper is part of a series[13,14] aimed at studying the structures and properties of pteridines of biological interest, particularly aspects which have proved difficult to study experimentally. A reliable and economical computational protocol for this purpose has been developed and tested against experimental data.[14] In the present work, we have determined the geometries of four quinonoid dihydropterin tautomers, the preferred cation, and several anions by using gradient techniques[15] at the ab initio SCF/STO-3G level: on account of their lability, there are no X-ray structures of quinonoid dihydropterins. In addition, we have investigated relative tautomer, protonation, and deprotonation energies with both STO-3G and 3-21G[16] basis sets and used these results to study the pathways for interconversion of quinonoid dihydropterins and rearrangement to the more stable 7,8-dihydropterin form. The most likely mechanism for the non-enzymic and enzymic reductions is also discussed.

Computational Procedure

Full details of the multistage computational protocol used in this study together with a critical assessment against pteridine experimental data of the level of theory required for reliable prediction of given chemical properties have already been given.[14] This selective procedure is necessary because of the large size of the molecules and, hence, inherent high cost of the calculations. Briefly the stages are as follows: (i) SCF/STO-3G calculations with full geometry optimization for neutral tautomers; (ii) SCF/STO-3G calculations for possible forms of (i) involving nitrogen protonation and deprotonation using neutral molecule geometries

(1) Armarego, W. L. F.; Randles, D.; Waring, P. *Med. Res. Rev.* **1984**, *4*, 267–321.
(2) Archer, M. C.; Scrimgeour, K. G. *Can. J. Biochem.* **1970**, *48*, 278–287.
(3) Armarego, W. L. F.; Waring, P. *J. Chem. Soc., Perkin Trans. 2* **1982**, 1227–1233.
(4) Armarego, W. L. F.; Waring, P. *Aust. J. Chem.* **1981**, *34*, 1921–1933.
(5) Bailey, S. W.; Ayling, J. E. *Biochemistry* **1983**, *22*, 1790–1798.
(6) Lazarus, R. A.; De Brosse, C. W.; Benkovic, S. J. *J. Am. Chem. Soc.* **1982**, *104*, 6871–6872.
(7) Benkovic, S. J.; Sammons, D.; Armarego, W. L. F.; Waring, P.; Inners, R. *J. Am. Chem. Soc.* **1985**, *107*, 3706–3712.
(8) Kaufman, S. *J. Biol. Chem.* **1964**, *239*, 332–338.
(9) Armarego, W. L. F.; Waring, P. In "Chemistry and Biology of Pteridines"; Blair, J. A. Ed.; de Gruyter: Berlin, 1983; pp 57–61 and 429–433.
(10) Poddar, S.; Henkin, J. *Biochemistry* **1984**, *23*, 3143–3148.
(11) Gready, J. E. *Adv. Pharmacol. Chemother.* **1980**, *17*, 37–102.
(12) Kaufman, S. In "Pteridine Chemistry", Pfleiderer, W.; Taylor, E. C., Eds.; Pergamon Press: New York, 1964; pp 307–322.
(13) Gready, J. E. *J. Mol. Struct.: THEOCHEM* **1984**, *109*, 231–244.
(14) Gready, J. E. *J. Comput. Chem.*, in press.
(15) Pulay, P. *Mol. Phys.* **1967**, *17*, 197–204.
(16) Binkley, J. S.; Pople, J. A.; Hehre, W. J. *J. Am. Chem. Soc.* **1980**, *102*, 939–947.

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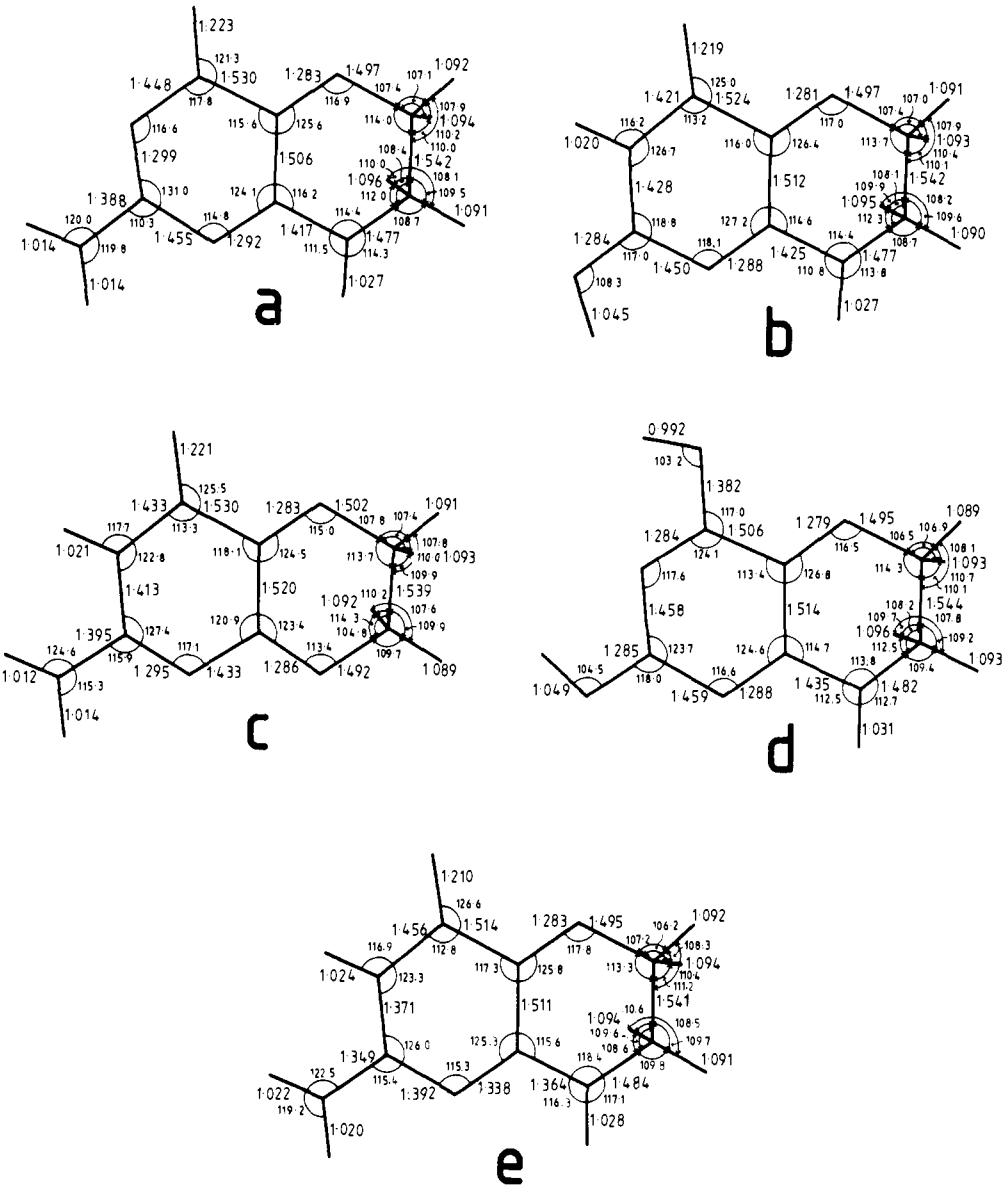


Figure 1. STO-3G optimized geometries. (a) *p*-Quinonoid dihydropterin (**1**), (b) *p*-iminoquinonoid dihydropterin (**2**), (c) *o*-quinonoid dihydropterin (**3**), (d) *p*-hydroxyiminoquinonoid dihydropterin (**4**), (e) protonated quinonoid dihydropterin (**7**). For the nonplanar pyrazine rings, torsion angles with respect to three given atoms and the positions of atoms above (+) or below (−) the pyrimidine ring plane are as follows: (a) N5 (−177.91 [C8A, C4A, N1], −0.038 Å), N8 (−0.15 [C8A, C4A, N5], −0.050 Å), C6 (−0.82 [N5, C4A, C8A], −0.067 Å), C7 (29.31 [N8, C8A, C4A], 0.566 Å), H6 (0.453 Å), H6′ (−1.115 Å), H7 (0.360 Å), H7′ (1.654 Å), H8 (0.265 Å). (b) N5 (−178.85 [C8A, C4A, N1], −0.021Å), N8 (−2.01 [C8A, C4A, N5], −0.072 Å), C6 (−0.61 [N5, C4A, C8A], −0.033 Å), C7 (32.05 [N8, C8A, C4A], 0.584 Å), H6 (0.506 Å), H6′ (−1.075 Å), H7 (0.396 Å), H7′ (1.668 Å), H8 (0.222 Å). (c) N5 (10.05 [C8A, C4A, N8], −0.084 Å), N8 (−174.5 [C8A, C4A, C4], 0.103 Å), C6 (−2.22 [N5, C4A, C8A], −0.135 Å), C7 (12.11 [N8, C8A, C4A], 0.506 Å), H6 (0.370 Å), H6′ (−1.185 Å), H7 (0.283 Å), H7′ (1.592 Å). (d) N5 (179.10 [C4A, C8A, N1], −0.016 Å), N8 (−2.76 [C8A, C4A, N5], −0.083 Å), C6 (−0.08 [N5, C4A, C8A], −0.035 Å), C7 (32.29 [N8, C8A, C4A], 0.574 Å), H6 (0.505 Å), H6′ (−1.079 Å), H7 (0.383 Å), H7′ (1.660 Å), H8 (0.211 Å). (e) N5 (−176.26 [C4A, C8A, N1], −0.068 Å), N8 (2.76 [C8A, C4A, N5], −0.021 Å), C6 (−0.27 [N5, C4A, C8A], −0.149 Å), C7 (23.16 [N8, C8A, C4A], 0.472 Å), H6 (0.361 Å), H6′ (−1.211 Å), H7 (0.202 Å), H7′ (1.564 Å), H8 (0.127 Å).

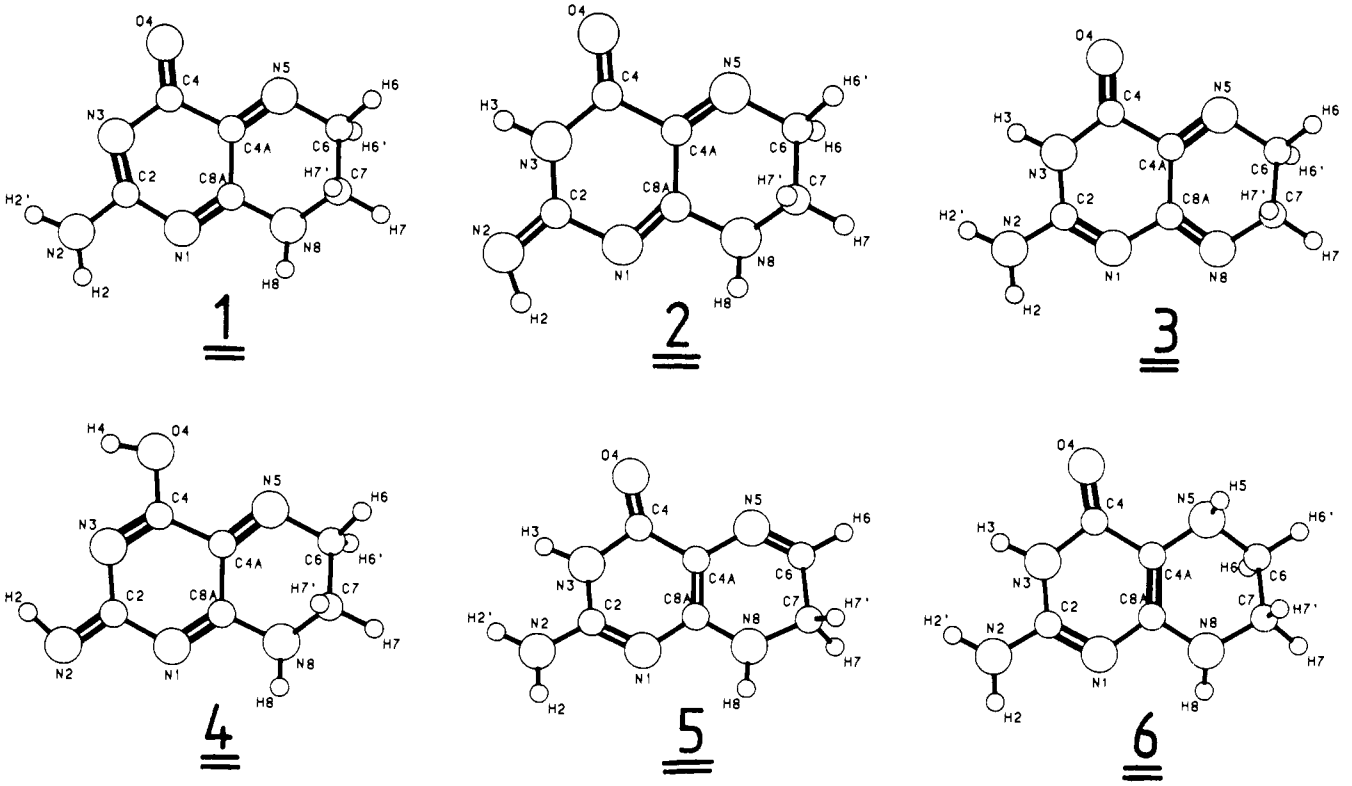
and standard geometric parameters for added protons (the aim of this step was to gain an overall idea of the order of preferred protonation and deprotonation for *N* sites in given tautomers): (iii) SCF/STO-3G calculations with full geometry optimization for protonated and deprotonated forms suggested to be the most favoured by (ii) and/or likely intermediates in the acid- or base-catalyzed interconversions of quinonoid dihydropterins and rearrangements to 7,8-dihydropterin; (iv) SCF/3-21G calculations for stage (i) and (iii) species using the STO-3G optimized geometries; and (v) SCF/STO-3G calculations for stage (i) and (iii) species with a 6-methyl group using 6-H STO-3G optimized geometries and standard methyl-group geometric parameters. The aim of this step was to check the effects of 6-substitution without significantly increasing the computational cost.

SCF/STO-3G (6-H), SCF/STO-3G (6-CH_3), and SCF/3-21G (6-H) calculations for the quinonoid dihydropterins require 68, 74, and 122 basis functions, respectively, with CPU times in the ratio 1:1.3:6. In the geometry optimizations bond lengths and angles have been converged to ~0.002 Å and 0.2°. Results for the quinonoid dihydropterin tautomers **1**–**4** have been obtained in this work while those for 7,8-dihydropterin (**5**) and 5,6,7,8-tetrahydropterin (**6**) have been taken from the earlier work.[13,14] Except for **5** which has a fully planar pteridine ring (heavy atoms), all these molecules contain nonplanar pyrazine rings but essentially planar pyrimidine rings with in-plane NH_2 or OH substituents.

Results and Discussion

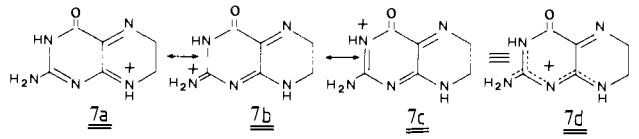
Geometries. The STO-3G optimized geometries for four quinonoid dihydropterins and the preferred protonated form are given in Figure 1. Overall the structures for the neutral molecules show strong alternation of single and double bonds following the patterns predicted by **1**–**4**, thus suggesting that these molecules are essentially nonaromatic. By contrast, the optimized structures[13] for 7,8-dihydropterin and 5,6,7,8-tetrahydropterin exhibited intermediate single/double bond structure in the pyrimidine ring, indicating considerable π-electron delocalization. π-Bond order calculations[17] confirmed these differences in relative aromaticity.

Chart I



The similarity of the pyrazine ring structures for **1**, **2**, and **4** indicates little effect on the pyrazine ring due to tautomeric changes in the pyrimidine ring.

The structure (Figure 1e) for the protonated species **7** is, however, markedly different. **7** can be formed by protonation of **1** on N3 (**7c**), **2** on N2′ (**7b**), or **3** on N8 (**7a**). Here the lower



fragment of the molecule (N3C2(N2′)N1C8AN8) displays intermediate single/double bond lengths consistent with the resonance description **7d** which defines the extended-guanidinium group. The structure of the remainder of the molecule resembles that for the neutral species **1–3**, with the C4A-N5 bond, in particular, being still essentially a pure double bond. The effect of the participation of the C8AN8H8 group in the delocalized structure **7d** is also seen in the flattening of this group with respect to the pyrimidine ring plane: i.e., N8 and H8 are −0.02 and 0.13 Å out-of-plane compared with ∼−0.06 and ∼0.24 Å for **1** and **2**. Note also, however, that the C8AN8H8 group is flatter in **1**, **2**, and **4** than would be expected if N8 were purely sp^3-hydridized. In general, the structure of **7** more closely resembles those of the quinonoid dihydropterins **1–3** rather than that of 7,8-dihydropterin from which it can be formed by protonation on C6 (see Figure 2).

Although the pyrazine rings for all structures in Figure 1 are nonplanar, they contain only one heavy atom (C7) which is significantly out-of-plane. A similar result was obtained for the pyrazine ring conformation of 5,6,7,8-tetrahydropterin[13] and some of its tautomeric and ionized forms,[14] except that in these cases C6 was the heavy atom which deviated significantly from the pyrimidine ring plane.

The structures of the anions **8–11** (Figure 2) obtained for the study of the preferred base-catalyzed rearrangement pathways are not reported here.[18] The structure of **8**, the N3-deprotonated anion of 7,8-dihydropterin, has been given previously.[14] The structures of **8–10** resemble more closely the 7,8-dihydropterin-derived structures, as drawn in Figure 2, rather than their respective quinonoid-derived structures. In particular, the pyrazine rings (heavy atoms) of **8–10** are planar and contain essentially double N5–C6 bond lengths. The structure of **11** is, as drawn, closer to that for **3** than **1**. The structure of the N3-deprotonated anion of tetrahydropterin **15** (Figure 3) has been reported previously.[14]

Table I. Quinonoid Dihydropterin Tautomer Energies[a]

	STO-3G		3-21G	
	total (H)	ΔE, kcal/mol	total (H)	ΔE, kcal/mol
p-imine (**2**)	−571.0065	0.0	−575.1134	0.0
p-amine (**1**)	−571.0041	1.5	−575.1117	1.0
o-amine (**3**)	−570.9991	4.6	−575.1104	1.8
p-hydroxyimine (**4**)	−570.9937	8.0	−575.0756	23.7
7,8-dihydropterin (**5**)	−570.0106	−2.6	−575.1540	−25.5
6-methyl-*p*-imine	−609.5888	0.0		
6-methyl-*p*-amine	−609.5863	1.6		
6-methyl-*o*-amine	−609.5812	4.8		
6-methyl-7,8-dihydropterin	−609.5970	−5.1		

[a] STO-3G optimized geometries or 6-H STO-3G optimized geometries plus standard methyl parameters.

Energies: Tautomers. The total and relative energies for the four O–H/N–H quinonoid dihydropterin tautomers including some 6-methylated species and for the C–H/N–H tautomer 7,8-dihydropterin[14] are given in Table I for both STO-3G and 3-21G basis sets.

Both the STO-3G and 3-21G results indicate similar stabilities for the N–H/N–H tautomers **1–3**: energy differences at the 3-21G level of 1–2 kcal/mol are not significant. The effect of 6-methylation on the relative stabilities of **1–3** is minor. For the N–H/O–H tautomers **2** and **4**, the 3-21G and STO-3G relative energies differ greatly: the inadequacy of the STO-3G basis for representing relative enol/oxo tautomer energies has been docu-

(17) Gready, J. E. *J. Comput. Chem.* **1984**, *5*, 411–426.

(18) Available from the author on request.

Table II. Quinonoid Dihydropterin Protonation Energies

		STO-3G[a]		STO-3G[b]		3-21G[b]	
	site	total (H)	ΔE, kcal/mol[c]	total (H)	ΔE, kcal/mol[c]	total (H)	ΔE, kcal/mol[c]
p-imine (**2**)	N1	−571.4350	−268.9				
	N2′(**7**)	−571.4674	−289.3	−571.4888	−302.7	−575.5301	−261.5
	N5	−571.4175	−257.9				
p-amine (**1**)	N1	−571.4253	−264.3				
	N3(**7**)	−571.4672	−290.6	−571.4888	−304.2	−575.5301	−262.5
	N5	−571.4215	−261.9				
o-amine (**3**)	N1(**14**)	−571.4565	−287.0				
	N5	−571.4201	−264.2				
	N8(**7**)	−571.4632	−291.2	−571.4888	−307.3	−575.5301	−263.3
7,8-dihydropterin (**5**)	C6(**7**)			−571.4888	−300.0	−575.5301	−236.0
6-methyl-*p*-imine	N2′			−610.0729	−303.7		(−262.5)[d]
6-methyl-*p*-amine	N3			−610.0729	−305.3		(−263.6)[d]
6-methyl-*o*-amine	N8			−610.0729	−308.5		(−264.5)[d]
6-methyl-7,8-dihydropterin	C6			−610.0729	−298.6		(−234.6)[d]

[a] Neutral ring geometries. [b] STO-3G optimised geometries or 6-H STO-3G optimized geometries plus standard methyl parameters. [c] Compared with neutral molecule energies of Table I. [d] Estimate based on 3-21G 6-H protonation energies with STO-3G correction for the effects of 6-methylation.

Table III. Quinonoid Dihydropterin Deprotonation Energies

		STO-3G[a]		STO-3G[b]		3-21G[b]	
	site	total (H)	ΔE, kcal/mol[c]	total (H)	ΔE, kcal/mol[c]	total (H)	ΔE, kcal/mol[c]
p-imine (**2**)	N3(**12**)	−570.2710	461.5				
	N8(**13**)	−570.2730	460.2				
	C6(**9**)	−570.2920[d]	448.3	−570.3300	424.5	−574.5736	338.7
p-amine (**1**)	N2′(**12**)	−570.2710[e]	460.0				
	N8(**11**)	−570.2899[f]	448.2	−570.3148	432.5	−574.5328	363.3
	C6(**8**)	−570.2997[d]	442.0	−570.3235	427.1	−574.5671	341.7
o-amine (**3**)	N2′(**13**)	−570.2730[e]	455.6				
	N3(**11**)	−570.2899	445.0	−570.3148	429.4	−574.5328	362.4
	C6(**10**)	−570.2738[d]	455.1	−570.3006	438.3	−574.5459	354.3
7,8-dihydropterin (**5**)	N2′(**9**)	−570.2920	450.9	−570.3300	427.1	−574.5736	364.2
	N3(**8**)	−570.2997	446.1	−570.3235	431.2	−574.5671	368.3
	N8(**10**)	−570.2738	462.4	−570.3006	445.5	−574.5459	381.6
6-methyl-*p*-imine	C6			−608.9141	423.4		(337.6)[g]
6-methyl-*p*-amine	N8			−608.8962	443.0		(363.8)[g]
	C6			−608.9082	425.5		(340.1)[g]
6-methyl-*o*-amine	N3			−608.8962	429.8		(362.8)[g]
	C6			−608.8854	436.6		(352.6)[g]
6-methyl-7,8-dihydropterin	N2′			−608.9141	428.5		(365.6)[g]
	N3			−608.9082	432.2		(369.3)[g]
	N8			−608.8854	446.5		(382.6)[g]

[a] Neutral ring geometries except as indicated. [b] STO-3G optimized geometries or 6-H STO-3G optimized geometries plus standard methyl parameters. [c] Compared with neutral molecule energies of Table I. [d] Neutral geometry of **5**. [e] Neutral geometry of **2**. [f] Neutral geometry of **3**. [g] Estimate based on 3-21G 6-H deprotonation energies with STO-3G correction for the effects of 6-methylation.

mented,[19] and basis set discrepancies of similar magnitude were found for other pterin enol/oxo tautomer pairs.[14] However, while the enol forms of the N3(*H*) pterins (cf. **5** or **6**) were found[14] to be less stable than their oxo forms by only ~2–9 kcal/mol, **4** is clearly a very disfavored tautomer. As discussed previously,[14] the 7,8-dihydropterin is considerably more stable than the quinonoid dihydropterin forms, and 6-methylation increases this relative stability by ~2.5 kcal/mol.

Recent ^{15}N NMR studies[7] have suggested that **1** is the predominant tautomer with estimates of its contribution ranging from ~67 to 100%, while ^{1}H NMR studies[6] provided no evidence for **3**. A ratio of 67% **1** to 33% **2** at 298 K corresponds to a ΔG value of only 0.4 kcal/mol, while a hypothetical 95:5 ratio implies a ΔG of only 1.7 kcal/mol. Assuming $\Delta H \simeq \Delta G$ for this tautomerization, this latter energy difference is comparable with the theoretical maximum energy difference between the tautomers **1**, **2**, and **3**. Hence, if **3** is present at all in the solution mixture, it must be present in very small concentrations (≤1%) not detectable by the NMR experiments.

Energies: Protonation. The N-protonation energies for tautomers **1**–**3** at the STO-3G and 3-21G levels, calculated with both optimized and nonoptimized protonated-molecule geometries are given in Table II. Results for C6 protonation of 7,8-dihydropterin are also shown.

The first set of results (stage (ii)) indicates that protonations on N2′ in **2**, N3 in **1**, and N8 in **3** are the most favored: the same species **7** is formed. N5 is the least basic nitrogen in all three tautomers. The relatively high basicity of N1 in **3**, as in the other N3(*H*) pterins,[14] is due to formation of the guanidinium group encompassing N3C2(N2′)N1. Previous experience for pterins (Figure 8, ref 14) indicates that N1-protonation energies at the STO-3G level are slightly overestimated compared with other N sites, and thus the difference in basicity between N1 and N8 in **3** is probably greater than these results suggest.

Higher level stage (iii)–(v) calculations were restricted to the most stable protonated form **7**. The 3-21G and second set of STO-3G results simply reflect the relative tautomer energy differences of Table I. The effect of 6-methylation on the protonation energies is minimal. When the theoretical (3-21G)/experimental correlation curve compiled from pterin data in ref 14 is used, a basic p*K* value for 6-methylquinonoid dihydropterin of 5.1–5.4 is predicted from the theoretical protonation energies (−262.5 to −264.5 kcal/mol). This compares well with the experimental value[5] for 6,6-dimethylquinonoid dihydropterin of 5.15 ± 0.05.

Comparison with other pterin calculations (Figure 8, ref 14) indicates that the basis set correction of ~−22 kcal/mol (Table I) between the STO-3G and 3-21G relative tautomer energies for 7,8-dihydropterin and the quinonoid dihydropterins appears in the C6-protonation energies not the N-protonation energies. This is consistent with the structure of **7** resembling more of **1**–**3** rather than **5**. The p*K* for C-6 protonation of 6-methyl-7,8-dihydropterin

(19) Schlegel, H. B.; Gund, P.; Fluder, E. M. *J. Am. Chem. Soc.* **1982**, *104*, 5347–5351.

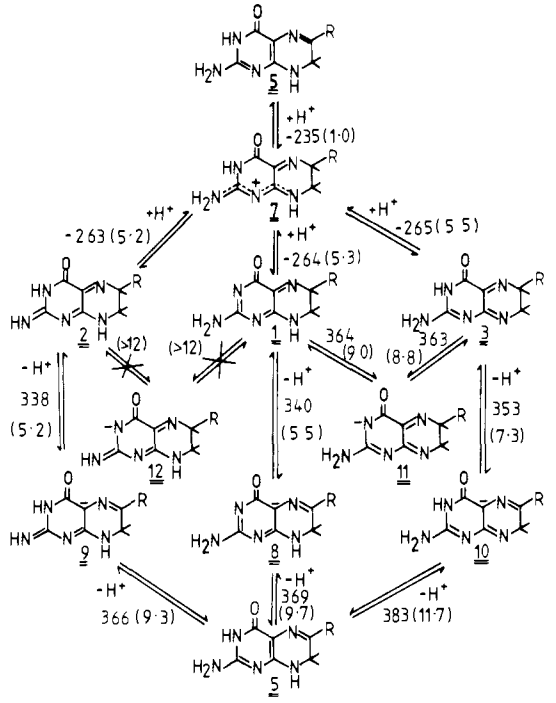


Figure 2. Acid- and base-catalyzed rearrangement pathways for quinonoid dihydropterins. The numbers given are the 3-21G protonation or deprotonation energies and theoretically estimated pK_a's for 6-methylated species.

is predicted to be ~1.0: this is less than the predicted pK of 4.2 for N5 protonation (cf. experiment:[20] 4.17) but comparable with a predicted value of 1.2 for N1 protonation (cf. experiment:[14,21] 1.27).

No good correlation was found between the calculated protonation energies and the nitrogen electron populations[22] although the N5 populations are markedly lower in **1–3**. Average π and total populations are as follows: for N1, 1.22, 7.31 (STO-3G) and 1.29, 7.76 (3-21G); for N5, 0.98, 7.19 (STO-3G) and 0.92, 7.52 (3-21G). The N3, N2′, and N8 populations in **1**, **2**, and **3**, respectively, are as follows: N3, 1.27, 7.34 (STO-3G) and 1.35, 7.77 (3-21G); N2′, 1.15, 7.33 (STO-3G) and 1.21, 7.70 (3-21G); N8, 1.07, 7.23 (STO-3G) and 1.11, 7.58 (3-21G). As the pyrazine ring is nonplanar, the π populations for N5 and N8 are approximate only.

Energies: Deprotonation. The N- and C-deprotonation energies for tautomers **1–3** at the STO-3G and 3-21G levels, calculated with both optimized and nonoptimized deprotonated-molecule geometries are given in Table III. Relevant results for N deprotonation of 7,8-dihydropterin are also given. These deprotonated forms represent all possible intermediates on the base-catalyzed rearrangement pathways in Figure 2.

The stage (ii) results indicate that N3 and N8 deprotonations of **2**, N2′ deprotonation of **1**, and N2′ deprotonation of **3** which produce forms **12** and **13** are very disfavored (effective pK_a >12[14]). Higher level stage (iii)–(v) calculations were restricted to forms **8–11**. The effect of 6-methylation is, again, minimal.

Correlation with the earlier pterin results[14] indicates that the STO-3G/3-21G basis set discrepancy of ~−22 kcal/mol for the relative tautomer energies of 7,8-dihydropterin/quinonoid dihydropterin appears in the C6-deprotonation energies not the N-deprotonation energies. This is consistent with the structures of **8–10** resembling that of **5** rather than **1–3**. This basis set defect is crucial in predicting the relative stabilities of the C6-deprotonated anions **8–10** compared with the N-deprotonated anion **11**. At both STO-3G levels, these appear to be of comparable stability, whereas at the 3-21G level **11** is seen to be much less

(20) Pfleiderer, W.; Zondler, H. *Chem. Ber.* **1966**, *99*, 3008–3021.
(21) Pfleiderer, W. *Chem. Ber.* **1979**, *112*, 2750–2755.
(22) Gready, J. E., unpublished results.

Table IV. Relative Stabilities toward Reduction of Dihydropterins

	ΔE, kcal/mol			
	STO-3G		3-21G	
	6-H	6-CH_3	6-H	6-CH_3[a]
p-iminequinonoid dihydro-(**2**) → tetrahydropterin	−750.6	−750.6	−759.7	(−759.7)
7,8-dihydro → tetrahydropterin	−748.0	−745.5	−734.2	(−731.7)

[a] Estimated as for Tables II and III.

stable. Using the theoretical (3-21G)/experimental correlation curve of ref 14, the pK's for C6 deprotonation of the 6-methylated forms of **1**, **2**, and **3** are predicted to be 5.5, 5.2, and 7.3, while that for formation of the 6-methylated form of **11** is ~9.

As formation of the C6-deprotonated anion(s) is found[2] to be the rate-limiting step in the base-catalyzed rearrangement of quinonoid dihydropterin to 7,8-dihydropterin, the intermediate transient forms **8–10** would not be expected to be observable at around neutral pH. However, the predicted N deprotonation of quinonoid dihydropterin to form **11** should be observable at pH's >~9, particularly in the 6,6-dimethyl form where the C6 deprotonation is absent. No spectral or pK results in basic solution are reported for 6,6-dimethylquinonoid dihydropterin.[4,5] Armarego and Waring[3] reported that there are no significant UV spectral shifts in the bands of the transient spectra of quinonoid dihydropterin and its 6,7-dimethyl- and 6,7-dimethyl-2-dimethylamino derivatives in buffers between pH 6.8 and 11.6, while the highest pH at which the ^{15}N NMR spectrum of 6,7-dideuterio-6,7-dimethylquinonoid dihydropterin was obtained[7] was 8.6.

Rearrangement Pathways. The theoretical ionization energies and predicted pK_a's from Tables II and III are summarized schematically in Figure 2.

Under the assumption that the formation of the intermediate ionized forms is the rate-limiting step in these rearrangements, then Figure 2 predicts that both acid- and base-catalyzed tautomerizations of quinonoid to 7,8-dihydropterin and the acid-catalyzed interconversion of the quinonoid species should be rapid at neutral pH. By the same argument, base-catalyzed interconversions of **1** and **2** via **12** and **2** and **3** via **13** are extremely unlikely, while the pathway from **1** ⇌ **3** via **11** is disfavored, at about neutral pH, relative to the acid-catalyzed pathway via **7**.

However, these assumptions are not entirely correct. For the quinonoid to 7,8-dihydropterin rearrangement, it was found from 6-^{2}H-quinonoid dihydropterin labeling experiments that the cleavage of the C–H bond at position 6 is the rate-limiting step.[2,3] Thus, while this finding is consistent with formation of the intermediate anions being the rate-limiting step in base catalysis, cation (**7**) formation is not rate-limiting in acid catalysis.

Archer and Scrimgeour[2] also found that the quinonoid to 7,8-dihydropterin rearrangement was subject to general acid/base catalysis by buffer with the relative contributions of acid to base catalysis dependent on the buffer pK_a. There was no evidence for spontaneous catalysis by water or for either specific hydronium or hydroxide ion catalysis. In acetate buffer below pH 4.8 and glycine buffer above pH 10.0, buffer catalysis decreases.[2] At high pH, this finding is consistent with the trapping of the quinonoid species in the anionic form **11**, while at low pH the quinonoid species might similarly be partly trapped in a protonated form such as **14** (see Table II), the N1-protonated form of **3**.

Under conditions of predominantly base catalysis (Tris chloride buffer at pH 7.6), Armarego and Waring[3] found that the rearrangement of 3,6,7-trimethylquinonoid dihydropterin was greatly enhanced compared with the rate for the 6,7-dimethyl compound, while the results for the 6,7,8-trimethylated form were essentially unchanged. An alternative interpretation of these results to that advanced in ref 3 is given below. It is based on the theoretical geometry results which suggest greater differences in the pyrazine-ring conformation of **3** compared with **1** or **2** than between **1** and **2**.

Taking into account that the rate-limiting step for the rearrangement is C6 deprotonation, that the proton is abstracted by the buffer base, and that 3- and 8-methylations are unlikely to

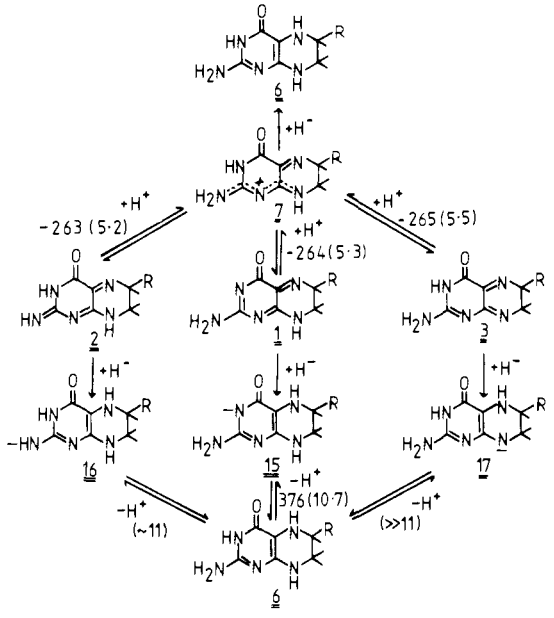


Figure 3. Quinonoid dihydropterin reduction pathways involving hydride ion transfer to N5. The numbers given are the 3-21G protonation or deprotonation energies and theoretically estimated pK_a's for 6-methylated species.

affect substantially the pK_a's for this deprotonation, the theoretical results suggest that the differences in the kinetic results[3] may be due to differences in the conformation of the pyrazine ring which make the C6 proton more accessible in the 3,6,7-trimethyl compound than in the 6,7,8- or 6,7-methylated compounds. There is ample NMR evidence that the conformation of the pyrazine ring in tetrahydro-[23-27] and quinonoid dihydropterins[28,29] depends on the nature of the ring substituents although on general grounds one might expect an 8-methyl group to have a greater effect on the pyrazine-ring conformation of the 6,7-dimethyl compound than a 3-methyl group. However, if the predominant structure of the 3,6,7-trimethyl compound is of the form **3** while the predominant structures of the 6,7,8-trimethyl and 6,7-dimethyl compounds are of the forms **1** or **2**, then the theoretical results provide a basis for rationalizing the kinetic results in terms of differing pyrazine-ring conformations. Kinetic results for a more complete range of both *N*-methyl and 6-substituted derivatives in a range of buffers could provide further information on this question.

Reduction Mechanisms. The calculated energy differences between dihydro- and tetrahydropterins[14] at the STO-3G and 3-21G levels are presented in Table IV. The basis set and 6-methylation effects for the individual reductions have been discussed previously.[14] The difference in the ΔE values for the two reductions simply reflects the relative tautomer stabilities for the two dihydro forms: as the STO-3G result does not accurately reflect the tautomer energy, the STO-3G relative reduction energies are also inaccurate. Also, as the quinonoid dihydropterins are less stable than the 7,8-dihydropterin tautomer, they are necessarily more easily reducible *thermodynamically*.

Quinonoid dihydropterins are reduced non-enzymically to tetrahydropterins at physiological pH by a variety of reducing agents including NADPH and NADH, although these rates are generally slow when compared with the enzymic reductions.[1] The pH dependence of the non-enzymic reduction has not been studied. The pH optimum for DHPR activity for the enzyme from several sources varies from ~6.3 to 7.5.[1] It is generally assumed that the tetrahydropterin formed in the reduction is the most stable N3(*H*) tautomer.

Both the enzymic and non-enzymic reductions could proceed either (i) by a protonation preceding the hydride ion transfer or (ii) by a hydride ion transfer followed by protonation: there is no experimental evidence distinguishing these two mechanisms. Also, as both hydride ion and proton additions are to nitrogen atoms radioactive labeling experiments cannot establish which nitrogen atom receives the hydride ion.[8] The following theoretical evidence, however, suggests that N5 receives the hydride ion. The protonation energy results of Table II clearly indicate that **7**, and not one of the N5-protonated quinonoid species, is the preferred protonated form, while the significantly lower electron populations on N5 in **1–3** support the view that hydride ion transfer is to N5. Also, were hydride ion transfer via mechanism (ii) to take place on N3, N2′, or N8 of **1**, **2**, or **3**, respectively, then the extremely unstable[14] N5 anion of tetrahydropterin would be formed as an intermediate.

The theoretical results for type (i) and (ii) reduction pathways involving hydride ion transfer to N5 are summarized in Figure 3.

The N3–(**15**), N2′–(**16**), and N8–(**17**) anions of tetrahydropterin are all less stable than their 7,8-dihydropterin counterparts shown in Figure 2: the pK_a values for **16** and **17** have been estimated from stage (ii) results of ref 14 and the 7,8-dihydropterin anion results of Table III, while the results for **15** are taken from ref 14. The relatively low stability of these intermediate anions on pathway (ii) suggests (i) is the more likely mechanism especially in the non-enzymic reduction where no mechanism for stabilization of the anion exists. Also, the relatively high pK for formation of **7** implies a concentration of the intermediate cation in mechanism (i) of ~2% at neutral pH.

As the positive charge in **7** is delocalized within the extended-guanidinium resonance, this protonation would not be expected to cause a very large electron depletion at N5: the 3-21G total and (approximate) π-electron depletions on N5 on formation of **7** from **1**, **2**, or **3** are ~0.07 and 0.09 electrons. These values can be compared with analogous total and π-electron depletions of 0.17 and 0.26 electrons at C6 following N5 protonation of 7,8-dihydropterin, the proposed[11,30] intermediate step in the reduction of 7,8-dihydropterin to tetrahydropterin.

In agreement with earlier suggestions,[9] the theoretical results support the preprotonation/N5 hydride ion transfer mechanism for the enzymic reduction, although a N5 hydride ion transfer/postprotonation mechanism involving enzyme stabilization of the intermediate anions **15** and **16** is still a possibility. Given the theoretical predictions that the three tautomer forms **1–3** are of comparable stability and are readily interconvertible at neutral pH via the cation **7**, it is possible that any of **1–3** could be the quinonoid form of the DHPR substrate irrespective of which tautomer is solution-preferred or which tautomer is formed directly by the amino acid hydroxylation reactions. Alternatively, the cation **7** could be the substrate form, in which case no enzyme-assisted protonation would be necessary.

Summary. The present calculations indicate that the three 4-oxo forms of quinonoid dihydropterin **1–3** are approximately equal in energy and should be readily interconvertible at neutral pH via the preferred cation **7**. The pK_a of **7** is predicted to be ~5.3 in agreement with an experimental value[5] of 5.15 for 6,6-dimethylquinonoid dihydropterin. A 4-enol tautomer **4** is predicted to be much less stable than **1–3**. The theoretically optimized geometries of the neutral tautomers **1–4** show strong alternation of single and double bonds, suggesting that they are essentially nonaromatic, while the structure of **7** displays intermediate single/double bond lengths consistent with a substantial contribution by the extended-guanidinium resonance **7d**. Theory predicts a quinonoid dihydropterin anion **11** with a pK of ~9 which, however, has not been observed experimentally.[3]

(23) Weber, R.; Viscontini, M. *Helv. Chim. Acta* **1975**, *58*, 1772–1780.
(24) Armarego, W. L. F.; Schou, H. *J. Chem. Soc., Perkin Trans. 1* **1977**, 2529–2534.
(25) Poe, M.; Hoogsteen, K. *J. Biol. Chem.* **1978**, *253*, 543–546.
(26) Furrer, H.-J.; Bieri, J. H.; Viscontini, M. *Helv. Chim. Acta* **1978**, *61*, 2744–2751.
(27) Armarego, W. L. F.; Waring, P. *J. Chem. Res., Synop.* **1980**, 318–319; *J. Chem. Res., Miniprint* **1980**, 3911–3926.
(28) Armarego, W. L. F.; Waring, P.; Paal, B. *Aust. J. Chem.* **1982**, *35*, 785–793.
(29) Armarego, W. L. F.; Randles, D.; Taguchi, H. *Eur. J. Biochem.* **1983**, *135*, 393–403.
(30) Gready, J. E. *Biochemistry* **1985**, *24*, 4761.

The theoretical results indicate ready acid- and base-catalyzed rearrangements of all three quinonoid forms **1–3** to 7,8-dihydropterin at neutral pH, in agreement with experiment.[2] The quinonoid forms are predicted to be ~26 kcal/mol higher in energy than 7,8-dihydropterin.

The reduction mechanism most consistent with the theoretical results involves preprotonation of quinonoid dihydropterin to form the cation **7** followed by hydride ion transfer to N5 to form directly the most stable N3(*H*) 5,6,7,8-tetrahydropterin. However, for the DHPR enzymic reduction, hydride ion transfer from NADH to N5 as the initial step to form the enzyme-stabilized tetrahydropterin anions **15** or **16** is also a possibility.

Acknowledgment. I thank Dr W. L. F. Armarego (ANU) for stimulating my interest in DHPR and for access to results prior to publication and the National Health and Medical Research Council for financial support. The calculations were performed by using the Cyber-720, Cyber-825, and VAX 11/780 computers of the University of Sydney, the UNIVAC-1108 computer of the Australian National University, and the VAX 11/780 computer of TAFENET (Sydney).

Registry No. 1 (R = H), 98482-79-2; **1** (R = CH_3), 83650-48-0; **2** (R = H), 98482-81-6; **2** (R = CH_3), 83650-46-8; **3** (R = H), 98482-80-5; **3** (R = CH_3), 98482-90-7; **4** (R = H), 98482-89-4; **5** (R = H), 98482-82-7; **5** (R = CH_3), 98509-13-8; **6** (R = H), 98509-14-9; **7** (R = H), 98482-84-9; **8** (R = H), 98482-83-8; **9** (R = H), 98482-85-0; **10** (R = H), 98482-86-1; **11** (R = H), 98482-87-2; **12** (R = H), 98482-88-3; **15** (R = H), 98482-93-0; **16** (R = H), 98482-92-9; **17** (R = H), 98482-91-8; dihydropteritine reductase, 9074-11-7.

Cartesian Correlation Times for NMR AX_2 Spin Systems

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Abstract: The spectral densities derived from the analysis of NMR relaxation in AX_2 spin systems are reexpressed in a Cartesian tensor basis. Four orientational correlation times are derived from the Cartesian spectral densities. For rigid spherical, symmetric, and asymmetric tops, abiding by a rotational diffusion model appropriate to the symmetry, there are one, two, and three distinct correlation times, respectively. Flexible molecules require more than three correlation times to describe the rotational dynamics. The Cartesian correlation times are model independent and provide a convenient reduced form for reporting experimental data.

In recent years dipolar spin relaxation has been used to study the dynamics of molecules in condensed phases. At first the relaxation behavior was observed for several pairs of dipolar coupled spins whose orientations in the molecule are linearly independent.[1] Later, as the relaxation behavior of several coupled spins became well understood,[2,3] coupled spin systems such as AX_2 (e.g., an $^{13}CH_2$ group) became useful probes of the dynamics.[4-10]

There is a wealth of information available in the relaxation of even a simple coupled spin system. The dipolar relaxation of an AX_2 spin system is characterized by four spectral densities, $J_{AX,AX}$, $J_{AX,AX'}$, $J_{AX,XX'}$, and $J_{XX',XX'}$ (where pairs of subscripts refer to a particular dipole–dipole interaction). Other contributions to the relaxation are treated as random magnetic fields. For molecules that rotate as rigid bodies and that obey a rotational diffusion equation, the NMR spectral densities yield the principal components of the diffusion tensor and an angle, specifying the geometry of the spin system. This kind of interpretation for rigid molecules is not possible for flexible molecules. The dynamics of nonrigid molecules are complex and can be represented by several models.[11-15] Because of this complexity, it is desirable that the dynamical information obtained from the NMR relaxation measurements is not biased toward any model. Such a model-free representation facilitates the comparison of the predictions of theory with experimental data.

In this paper, we convert the dipolar spectral densities describing AX_2 relaxation to a Cartesian basis, yielding orientational correlation times that have a simple physical interpretation and that can be compared directly with theory of molecular dynamics in fluids. As a simple illustration, the orientational correlation times for a rigid asymmetric top are calculated and discussed.

Relationship of Spectral Densities to Cartesian Correlation Times

The dipolar Hamiltonian for a pair of spins, ij, is:[16]

$$H^{ij}(t) = \frac{\gamma_i\gamma_j\hbar}{r_{ij}^3}\left\{(\vec{\mathbf{I}}^i\cdot\vec{\mathbf{I}}^j) - \frac{3}{r_{ij}^2}(\vec{\mathbf{I}}^i\cdot\vec{\mathbf{r}}_{ij})(\vec{\mathbf{I}}^j\cdot\vec{\mathbf{r}}_{ij})\right\} \quad (1)$$

where γ_i is the magnetogyric ratio of spin i, $\vec{\mathbf{r}}_{ij}$ the internuclear vector connecting i and j, and $\vec{\mathbf{I}}^i$ the spin operator. Equation 1 can be rewritten as

$$H^{ij}(t) = \frac{\gamma_i\gamma_j\hbar}{r_{ij}^3}(\vec{\mathbf{I}}^i\cdot\tilde{\mathbf{D}}_{ij}\cdot\vec{\mathbf{I}}^j) \quad (2)$$

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(1) Grant, D. M.; Pugmire, R. J.; Black, E. P.; Christensen, K. A. *J. Chem. Soc.* **1973**, *95*, 8465.
(2) Werbelow, L. G.; Grant, D. M. *Adv. Magn. Reson.* **1977**, *9*, 189.
(3) Vold, R. L.; Vold, R. R. *Prog. NMR Spectrosc.* **1978**, *12*, 79.
(4) Bovee, W.M.M.J. *Mol. Phys.* **1975**, *29*, 1673.
(5) Mayne, C. L.; Grant, D. M.; Alderman, D. W. *J. Chem. Phys.* **1976**, *65*, 1684.
(6) Chenon, M. T.; Bernassau, J. M.; Mayne, C. L.; Grant, D. M. *J. Phys. Chem.* **1982**, *86*, 2733.
(7) Fuson, M. M.; Prestegard, J. H. *J. Chem. Phys.* **1982**, *76*, 1539.
(8) Fuson, M. M.; Prestegard, J. H. *J. Am. Chem. Soc.* **1983**, *105*, 168.
(9) Fuson, M. M.; Prestegard, J. H. *Biochemistry* **1983**, *22*, 1311.
(10) Brown, M. S.; Mayne, C. L.; Grant, D. M.; Chou, T. C.; Allred, E. L. *J. Phys. Chem.* **1984**, *88*, 2708.
(11) Weber, T. A. *J. Chem. Phys.* **1978**, *69*, 2347.
(12) Ryckaert, J. P.; Bellemans, A. *Faraday Discuss. Chem. Soc.* **1978**, *66*, 95.
(13) Rebertus, D. W.; Berne, B. J.; Chandler, D. *J. Chem. Phys.* **1979**, *70*, 3395.
(14) Pear, M. R.; Weiner, J. H. *J. Chem. Phys.* **1979**, *71*, 212.
(15) Fixman, M. *J. Chem. Phys.* **1978**, *69*, 1527, 1538.
(16) Abragam, A. "The Principles of Nuclear Magnetism"; Clarendon Press: Oxford, 1961.

0002-7863/85/1507-6695$01.50/0