The relative stabilities of dihydropterins; a comment on the structure of Moco, the cofactor of the oxomolybdoenzymes

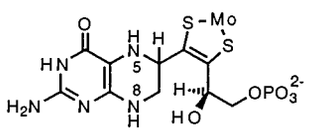
Stephen P. Greatbanks, Ian H. Hillier,* C. David Garner and John A. Joule

Chemistry Department, The University of Manchester, Manchester, UK M13 9PL

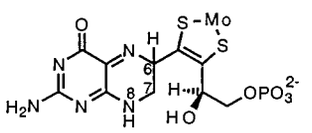
Ab initio **calculations have been performed on 7,8-dihydro-2-aminopteridin-4(3*H*)-one and on 39 dihydropteridine tautomers of it leading to an assessment of their relative energies and thence the likelihood of their involvement in the operation of the cofactor of the oxomolybdoenzymes, Moco. The effect of a polar environment on the tautomer energetics has been explored using a continuum model. At the most advanced level of calculation, and in the presence of water, the 7,8-dihydro-3*H*- (T1) and 5,6-dihydro-3*H*- (T4) tautomers are essentially isoenergetic, with 7,8-dihydro-6*H*- (T13) and 5,8-dihydro-3*H*- (T3) tautomers only 8.0 and 15.5 kJ mol^{-1} more energetic than T1. Any of these four tautomers can reasonably be implicated in the mode of action of Moco. The *ortho* quinonoid tautomer (T2) is of considerably higher energy and seems much less likely to be implicated.**

Introduction

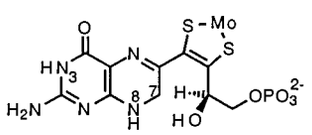
The oxomolybdoenzymes have a cofactor, known as Moco, which, with minor variations, is common to all.[1] Extensive degradative studies by Johnson, Rajagopalan and others, extending over a period of some twenty years, culminated in a proposal for the structure of the organic moiety (molybdopterin, MPT) of the cofactor, as an ene-1,2-dithiolate on a C-6-side-chain on a 2-aminopteridin-4-one (pterin).[2] Most of the degradative studies produced fully aromatic pteridine products[3] but spectroscopic measurements on the whole enzyme were taken as a clear demonstration that, in the native enzyme, the bicyclic heterocycle is in a partially reduced state. Initially, a 5,6,7,8-tetrahydropterin **1** structure was suggested for Moco;[4,5] this was later modified to a dihydropterin, for which the 7,8-dihydro-6*H*-pteridine tautomer **2** was suggested as the native form of Moco.[6] Other workers have pointed out[7] that the role of the molybdenum centre cannot be ignored and postulated that Moco involves a Mo^{VI}–*tetra*hydropterin complex rather than a Mo^{IV}–*di*hydropterin complex.



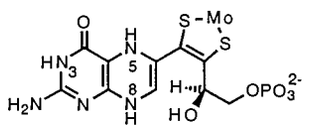
1



2

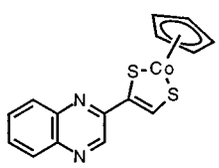


3



4

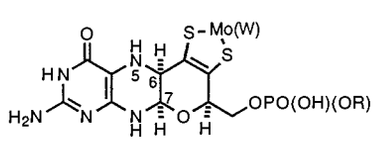
In electrochemical experiments designed to model possible co-operativity between metal centre and pteridine moiety in the operation of molybdenum enzymes, we clearly showed that protonation of a heterocyclic nitrogen modifies the redox properties of the metal in simple model systems such as **5**.[8]



5

We are inclined to believe that a related interaction operates for Moco. For there to be a role for the pteridine moiety, over and above its involvement in hydrogen bonding interactions with the surrounding protein, there must be some mechanism for electronic communication between the metal centre and the pteridine and/or the nitrogen(s) of the pyrazine ring. Accordingly, we have favoured[1] a postulate for the active form of MPT in which C-6 is sp^2 hybridised, *i.e.* if Moco involves MPT at a dihydro level in its enzymatically active form, we take the view that it is more likely to be either a 7,8-dihydro-3*H*-pterin **3** or a 5,8-dihydro-3*H*-pterin **4** rather than a 7,8-dihydro-6*H*-pterin **2**.

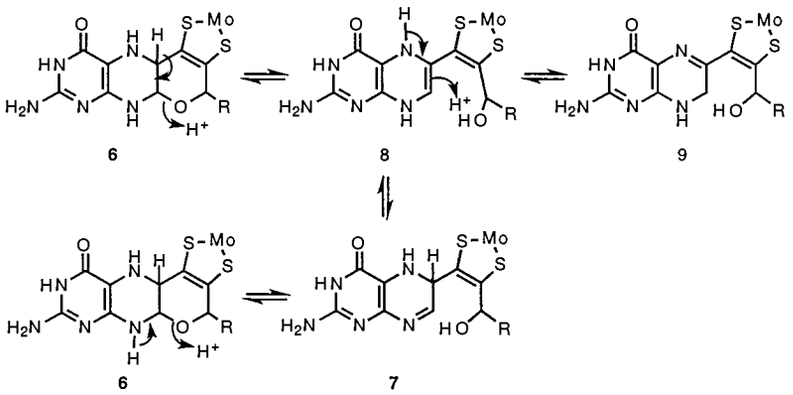
Recently, three X-ray crystallographic determinations have shed new light on the nature of MPT. In the aldehyde oxidase from *Desulfovibrio gigas*,[9] in dimethyl sulfoxide (DMSO) reductase from *Rhodobacter sphaeroides*,[10] and in the hyperthermophilic tungsten enzyme, ferredoxin aldehyde oxidoreductase from *Pyrococcus furiosus*,[11] the situation around the molybdenum (tungsten) was as shown in **6**. MPT has a metal-chelating ene-1,2-dithiolate, attached at C-6 of a pteridine ring, just as originally proposed by Rajagopalan. However, MPT also has a tetrahydropyran ring, which can be viewed formally as the result of a cyclisation of a side-chain hydroxy group at C-7 of a 5,6-dihydropteridine. It is not yet established whether the form of MPT found in samples prepared for crystallography represents a catalytically active form. Certainly, simple proton-catalysed processes could lead from the structure shown in **6** to forms in which the N(8)C–O bond has been



6
(R = H or cytosine)
(other ligands on metal not shown)

cleaved, introducing a double bond into the pyrazine ring and thus, by simple prototropy any of the three dihydropteridine tautomers, **7**, **8** or **9** could be generated (Scheme 1). Conversely, the tetrahydropyran-containing form found in the crystal structure determinations could, by the reverse of these simple processes, have been produced during the isolation and preparation of samples for X-ray analysis.

Reduced pterins are involved in other enzyme-catalysed processes, notably in the recycling of the coenzyme 5,6,7,8-tetrahydrofolic acid, which is responsible for biosynthetic one-



Scheme 1

carbon atom transfers; an intermediate is 7,8-dihydrofolic acid, subsequently reduced by dihydrofolate reductase.[12] A 5,6,7,8-tetrahydropterin is a cofactor in aromatic amino acid hydroxylases.[13] This cofactor is believed to operate by the initial formation of a 4a-hydroperoxy-5,6,7,8-tetrahydro-4a*H*-pterin, then used in conjunction with iron, for the aromatic hydroxylation, leaving a 4a-hydroxy-5,6,7,8-tetrahydro-4a*H*-pterin to be recycled. This, it is believed, involves loss of water to generate a *para* quinonoid 7,8-dihydro-6*H*-pterin, comparable to **2**, which is subsequently reduced.[13] The operation of this cycle has provoked considerable experimentation and attempts to model the processes, for example a 7,8-dihydro-6*H*-pterin was claimed as an intermediate in a multi-step conversion of a pyrimidine into 5,6,7,8-tetrahydrofolic acid; it was suggested that the traces of 7,8-dihydro-3*H*-folic acid in the product arose *via* rapid isomerisation of the 6*H*-isomer *in situ*.[14] Treatment of 6,7-dimethyl-5,6,7,8-tetrahydropterin with $MoO_2(detc)_2$ (detc = diethyl dithiocarbamate), monitored by ^{1}H NMR, appeared to show the fomation of a dihydropterin, which was shown not to be the 7,8-dihydro-3*H*-isomer and was assigned a 7,8-dihydro-6*H*-pterin structure.[15]

The partial reduction[16,17] of aromatic pterins produces isolable 7,8-dihydro-derivatives as does the partial oxidation of 5,6,7,8-tetrahydropterins, suggesting that the 7,8-dihydro-3*H*-tautomer is the most stable.[16,18] 2-Amino-6,7-diphenyl-5,6-dihydropteridin-4(3*H*)-one isomerises to its 7,8-dihydro isomer in hot acetic acid.[19] The initial formation of a 'quinonoid' dihydropterin during oxidation, which then isomerises rapidly to the 7,8-dihydro-3*H*-product, has been described.[20] Quinonoid forms, assigned 7,8-dihydro-6*H*-pterin structures, have been produced by bromine oxidation of 6,6-dimethyl-5,6,7,8-tetrahydropterin or oxidative ring closure of 6-[(2-amino-2-methylpropyl)amino]-2,5-diaminopyrimidin-4(3*H*)-one,[21] and by oxidation of 6-methyl-5,6,7,8-tetrahydropteridin-4(3*H*)-one and 6,7-dimethyl-5,6,7,8-tetrahydropteridin-4(3*H*)-one with hydrogen peroxide.[22]

Thus, the chemical evidence above suggests that 7,8-dihydro-3*H*-pterin is the most stable form of a dihydropterin. However, alternative tautomers, though of higher energy, may be involved in the operation of the oxomolybdoenzymes and their tungsten relatives.

In the absence of direct experimental data on the relative stabilities of most of the various tautomers of di- and tetrahydropterins, we investigate here the theoretical predictions of these quantities. The accuracy of *ab initio* treatments of tautomer energetics is well documented; at the highest levels of theory, including correlation effects, relative energies can confidently be predicted within *ca.* 10 kJ mol^{-1}.[23] However, even with quite modest basis sets (6-31G**) and modest levels of electron correlation (MP2) useful results can be obtained. Furthermore, the use of continuum models has been successful in predicting the effect of solvent on tautomer energetics often leading to important changes in the relative stabilities of different tautomeric species.[24,25]

Calculations of the structure and energetics of a number of pteridines using minimal (STO-3G) and split valence (3-21G) basis sets have been reported.[26] Although such calculations can give useful indications of structure and stability, they lack the accuracy of higher basis sets such as are used here.

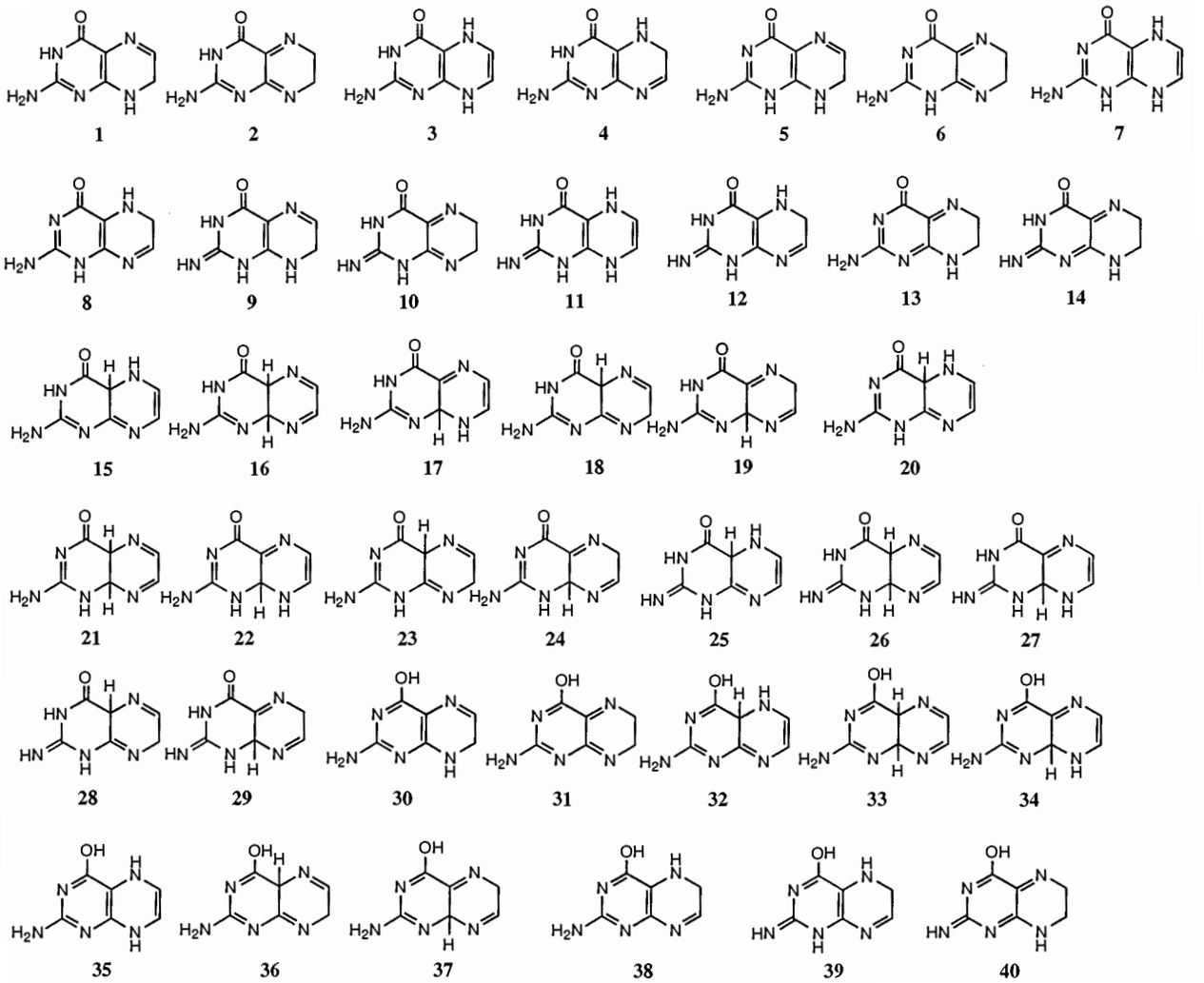
Computational details

Ab initio calculations were carried out using the program GAUSSIAN94.[27] Full geometry optimization of 40 structures **T1**–**40** was carried out at the Hartree–Fock level using a 6-31G** basis. The ten most stable structures were confirmed to be energy minima by calculations of the harmonic vibrational frequencies, which were all real. It is assumed that the remaining structures are also energy minima. To include the effect of electron correlation on the relative energies, MP2 calculations were carried out on the low energy structures. To go some way to modelling the relative stability of the tautomers in an enzyme environment, predictions of tautomer energetics in a polar environment may be of more value than gas-phase values alone. We have therefore used the polarisable continuum model (PCM) at the Hartree–Fock level, as implemented in GAUSSIAN94 (ISOPCM) to predict the relative energies of a number of tautomers, including the lower energy gas phase species, in water (ε = 78.4), taking as molecular geometries the optimised gas-phase values, to yield relative energies [RHF(l)] in water. Solvation energies calculated at the Hartree–Fock level were also used, in conjunction with gas phase MP2 energy differences [MP2(g)] to yield the relative energies, in water [MP2(l)], given in Table 1. This is, of course, a quite crude approximation to the actual environment of the enzyme, where specific structural details of the substrate–residue interactions such as hydrogen-bonding, must be considered in a more realistic model.

Computational results

The calculated energies and dipole moments of the 40 tautomers **T1**–**40** studied are given in Table 1. From these we have selected 11 of lowest energy to study at the MP2 level, the results being shown in Table 2. Although the precise ordering of those species which are quite close in energy at the Hartree–Fock level may be altered when electron correlation is included, the overall picture of the relative stabilities of the tautomers at the Hartree–Fock and MP2 levels is similar.

Echoing the trend well-recognised in simpler heterocyclic systems,[28] hydroxy tautomers tend to be more stable than their carbonyl counterparts in the gas phase; one may illustrate this with the pair **T1**/**T30** where the latter form, having the hydroxy group, is calculated to be more stable than the former, carbonyl



tautomer (17.8 and 11.7 kJ mol^{-1}, respectively, at the RHF and MP2 levels). However, this is not always the case. Thus, for the **T3/T35** pair in the gas phase, the carbonyl form **T3** is less stable than the hydroxy form **T35**, at the RHF level, but the inclusion of electron correlation renders both structures essentially isoenergetic. As in the pyridin-2-one/pyridin-2-ol pair,[28] the results of the PCM calculations (Table 2) predict that the carbonyl form is more strongly solvated than the hydroxy form so that the carbonyl tautomers **T1** (*versus* **T30**) and **T3** (*versus* **T35**) are preferred by 9.2 and 29.9 kJ mol^{-1} at the RHF level and by 5.3 and 44.7 kJ mol^{-1} at MP2. For the pair **T4/T38**, even in the gas phase the carbonyl tautomer (**T4**) is favoured (by 1.2 or 3.1 kJ mol^{-1}) and this becomes 33.5 or 35.4 kJ mol^{-1} in favour of the carbonyl form when solvated. Although it is difficult to know how best to model the active site of an enzyme *in vivo*, we assume that the biological environment is better modelled by the calculations in the presence of water. Therefore, the results presented in Table 2 lead to a clear preference for the carbonyl tautomers as more likely candidates for the real situation. This conclusion is likely to hold irrespective of the level of theory employed, particularly so, since increasing the level of electron correlation beyond MP2 has been found to preferentially stabilise the carbonyl form in some smaller heterocycles.[29,30]

Further aspects of the importance of different tautomeric structures must be considered. Addressing the question of the possible importance of 1*H*- *versus* 3*H*-tautomers, it is fruitful to compare the relative energies of the 3*H*-/1*H*- pairs **T1/T5**, **T4/T8** and **T3/T7**: in each case the RHF calculations predict the 3*H*-tautomer to be considerably more stable than its 1*H*-isomer, by 85.5, 21.7 and 55.1 kJ mol^{-1}, respectively, in the gas phase and by 27.5, 12.6 and 34.0 kJ mol^{-1} in water. We therefore conclude that 1*H*-tautomers are probably not important (however, see below).

Turning to the possible importance of 3-imino tautomers in these systems, one can usefully compare the amino–imino pairs **T1/T9 T4/T12** and **T3/T11**; in each case the RHF calculations predict that the amino-tautomer is significantly more stable than the imino-tautomer, by 66.1, 24.2 and 47.2 kJ mol^{-1}, respectively in the gas phase, and by 35.3, 24.1 and 30.1 kJ mol^{-1} in water. Thus, it seems probable that imino tautomers are not involved in Moco enzyme chemistry.

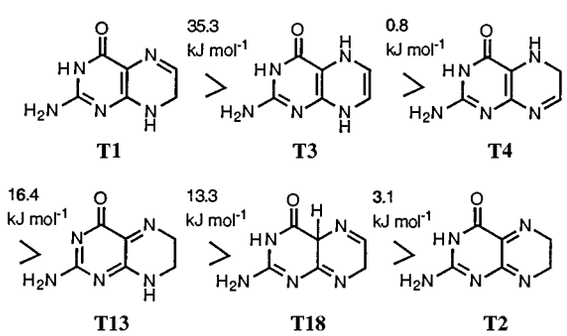
With regard to the possible structure of Moco, and setting aside hydroxy, imino and 1*H*-tautomers (see above), it is most relevant to compare the 7,8-dihydro-3*H*- (**T1**), 5,8-dihydro-3*H*- (**T3**) and 5,6-dihydro-3*H*- (**T4**) tautomers with the higher energy 6,7-dihydro-3*H*- (**T2**), 7,8-dihydro-6*H*- (**T13**) and 4a,7-dihydro-3*H*- (**T18**) tautomers. RHF calculations for gas phase and aqueous species predict the *ortho* quinonoid **T2** to be the least stable of this group, though somewhat surprisingly there is only a small difference between the calculated energy for **T2** and that for **T18** (the latter being 3.1 and 5.0 kJ mol^{-1} more stable in gas phase and water, respectively). The tautomers **T17**, **T16** and **T19** are considerably more highly energetic (64.0, 64.9 and 29.4 kJ mol^{-1}, respectively, less stable in water than **T2**) and discountable. Schemes 2 and 3 summarise these theoretical results, showing the dihydropterin tautomer candidates in order of decreasing calculated stabilities in gas phase at the RHF level and water, respectively.

Notwithstanding the conclusions drawn above regarding the significance of 3-imino-tautomers, one must pay regard to the effect that combinations of functional groups can have on relative stabilities: several of the imino tautomers have RHF

Table 1 Relative energies (kJ mol^{-1}) of tautomers **T1**–**T40** at 6-31G**(RHF)//6-31G**(RHF) level, and dipole moment (D)

Tautomer	Relative Energy/ kJ mol^{-1}	Dipole moment/D
T1	0.0[a]	6.9
T2	68.9	3.3
T3	35.3	4.8
T4	36.1	1.6
T5	85.5	10.2
T6	73.9	7.1
T7	90.4	7.6
T8	57.8	6.5
T9	66.1	8.6
T10	63.2	4.4
T11	82.5	5.3
T12	60.3	5.4
T13	52.5	8.5
T14	50.9	6.9
T15	79.1	4.4
T16	114.9	2.7
T17	103.6	5.4
T18	65.8	3.3
T19	104.0	1.9
T20	67.6	4.8
T21	124.9	7.6
T22	137.9	9.4
T23	68.5	7.0
T24	109.6	7.5
T25	59.8	2.4
T26	112.7	5.4
T27	127.3	7.2
T28	59.5	4.7
T29	100.9	5.6
T30	−17.8	3.0
T31	102.9	3.1
T32	124.3	4.8
T33	154.4	1.7
T34	137.0	2.0
T35	21.4	2.4
T36	102.4	2.7
T37	147.0	2.2
T38	37.3	2.7
T39	137.3	6.6
T40	121.8	6.5

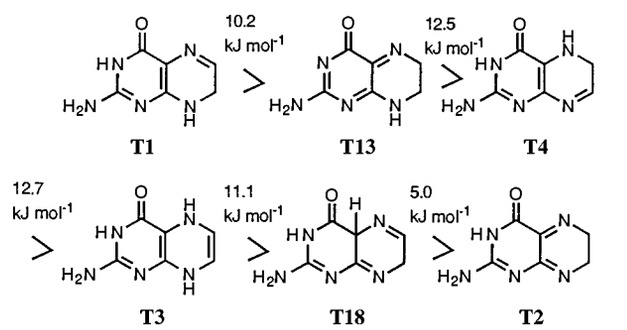
[a] Total energy = −578.428 952 au.



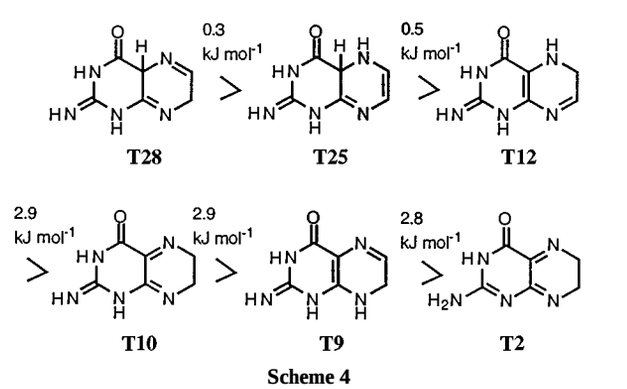
Scheme 2 Gas phase, RHF relative stabilities

Table 2 Relative energies (kJ mol^{-1}) of tautomers in the gas phase (g) and aqueous solvent (l) at 6-31G** level, using 6-31G** (RHF) structures; dipole moments (μ/D) in parentheses

Tautomer	Relative energy/kJ mol^{-1} RHF (g) (μ/D)	RHF (l)	MP2 (g)	MP2 (l)
T1	0.0 (6.9)	0.0	0.0	0.0
T30	−17.8 (3.0)	9.2	−11.7	15.3
T13	52.5 (8.5)	10.2	50.3	8.0
T14	50.9 (6.9)	15.7	38.6	3.4
T4	36.1 (1.6)	22.7	13.2	−0.2
T5	85.5 (10.2)	27.5		
T28	59.5 (4.7)	33.1	51.9	25.5
T8	57.8 (6.5)	35.3	40.3	17.8
T9	66.1 (8.6)	35.3		
T3	35.3 (4.8)	35.4	15.4	15.5
T10	63.2 (4.4)	39.0		
T18	65.8 (3.3)	46.5		
T12	60.3 (5.4)	46.8		
T2	68.9 (3.3)	51.5		
T38	37.3 (2.7)	56.2	16.3	35.2
T25	59.8 (2.4)	57.8	44.3	42.3
T35	21.4 (2.4)	65.3	16.3	60.2
T11	82.5 (5.3)	65.5		
T7	90.4 (7.6)	69.4		
T20	67.6 (4.8)	72.0		
T19	104.0 (1.9)	80.9		
T17	103.6 (5.4)	115.5		
T16	114.9 (2.7)	116.4		



Scheme 3 Water, RHF relative stabilities



Scheme 4

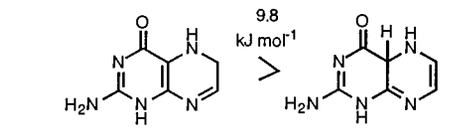
gas phase energies lower than that calculated for **T2**; these are summarised in Scheme 4.

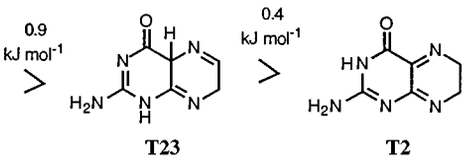
Furthermore, and again notwithstanding conclusions drawn above, one must note the relatively low RHF gas phase energy calculations for 1*H*-tautomers, compared in Scheme 5, with **T2**.

At the most advanced level of calculation and in the presence of water (Table 2; Scheme 6) there is virtually no difference in the energies of 5,6-dihydro-3*H*- (**T4**) and 7,8-dihydro-3*H*- (**T1**) tautomers. Further, the *para* quinonoid form, **T13**, a tautomer of the type invoked in the catalytic cycle of aromatic hydroxylases,[13] is shown to be only marginally more energetic than **T1** and **T4**. Even the 5,8-dihydro-3*H*-isomer (**T3**), for which there is little chemical precedent, is calculated to be a reasonable tautomer to consider, from the point of view of its stability. Therefore, one may conclude that any of these tautomeric forms can reasonably be considered as a tautomeric form for MPT in the operation of Moco, *in vivo*, in a polar environment.

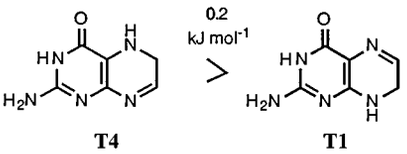
Finally, we have addressed the question of the relative changes in energy attendant upon (partial) reduction of the pyrazine ring of a pterin. This question is central to a consideration of the possible role of fully aromatic *versus* dihydropterin *versus* tetrahydropterin units in the mechanism of action of Moco and of other enzymes in which pterins or reduced pterins are implicated.

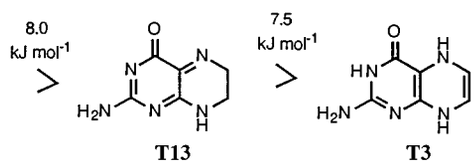
For the isodesmic[31] reaction (1), the energy change is calcu-





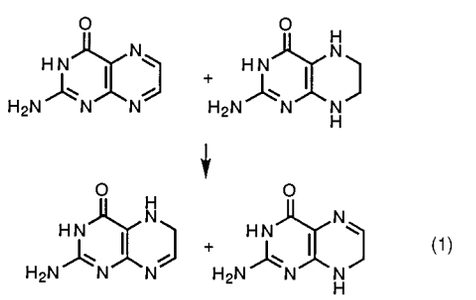
Scheme 5



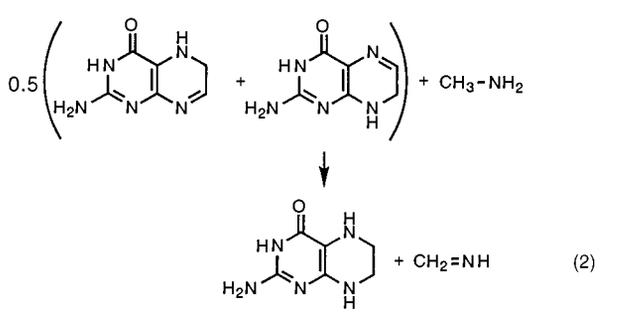


Scheme 6

lated to be 91 kJ mol^{-1}, a value associated with the delocalisation energy of the aromatic form of pterin, compared with 5,6,7,8-tetrahydropterin.

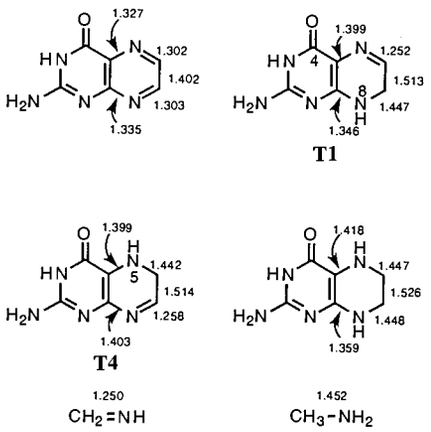


This point is reinforced by considering the isodesmic reaction (2), for which there is less than 4 kJ mol^{-1} between left- and



right-hand sides, showing the imine units in 5,6- and 6,7-dihydropteridines to be essentially localised—*i.e.* no stabilisation is achieved by the placement of the imine unit within the bicyclic framework.

The optimised bond lengths, shown in Scheme 7, clearly illustrate the points discussed above. Thus, in the fully aromatic pteridine, all C–N bonds in the pyrazine ring are nearly the same length—between 1.302 and 1.335 Å. The vinylogous amide conjugation between N-8 and the 4-carbonyl in **T1** is expressed in the shorter bond (1.346 Å) linking the nitrogen to the pyrimidine ring, compared with 1.399 for the N-5-ring bond in **T4**, and must contribute to the stability of this tautomer.



Scheme 7 Bond lengths in Å

Conclusions

We conclude that the calculations discussed above lend support to the view that in the catalytically active form of Moco: (*a*) the pteridine has a 2-amino-4(3*H*)-one tautomeric form, (*b*) that if there is a double bond in the pyrazine ring in the catalytically active state, then on energetic grounds it could reasonably be located at the 7,8-position (**T4**), the 5,6- (**T1**) or the 6,7- (**T3**) positions (in the last two cases it would be able to transmit electronic information between the metal centre and the dihydropterin) or that the *para*-quinonoid tautomer (**T13**) could be implicated. Scheme 1 showed how such tautomers could be easily formed from the tetrahydropyran-containing structures which have been revealed by X-ray studies of molybdenum and tungsten enzymes involving MPT.

Acknowledgements

We thank the EPSRC for support of this research.

References

1 For a review: *The structure and mode of action of the cofactor of the oxomolybdoenzymes* see D. Collison, C. D. Garner and J. A. Joule, *Chem. Soc. Rev.*, 1996, 25.
2 Pterin = 2-aminopteridin-4(3*H*)-one; for reviews of pterin chemistry see *Fused pyrimidines. Part three; Pteridines*, ed. D. J. Brown, *The Chemistry of Heterocyclic Compounds*, ed. E. C. Taylor, Wiley Interscience, New York, 1988, vol. 24, Part 3; W. Pfleiderer, *J. Heterocycl. Chem.*, 1992, **29**, 583.
3 For an exception in which a 5,6,7,8-tetrahydropterin derivative was obtained see S. P. Kramer, J. L. Johnson, A. A. Ribeiro, D. S. Millington and K. V. Rajagopalan, *J. Biol. Chem.*, 1987, **262**, 16 357.
4 Other molybdenum ligands are omitted for clarity.
5 K. V. Rajagopalan, S. Kramer and S. Gardlik, *Polyhedron*, 1986, **5**, 573.
6 S. Gardlik and K. V. Rajagopalan, *J. Biol. Chem.*, 1990, **265**, 13 047.
7 S. J. N. Burgmayer, M. R. Arkin, L. Bostick, S. Dempster, K. M. Everett, H. L. Layton, K. E. Paul, C. Rogge and A. L. Rheingold, *J. Am. Chem. Soc.*, 1995, **117**, 5812.
8 E. M. Armstrong, M. S. Austerberry, J. H. Birks, R. L. Beddoes, M. Helliwell, J. A. Joule and C. D. Garner, *Heterocycles*, 1993, **35**, 563.
9 M. J. Romão, M. Archer, I. Moura, J. J. G. Moura, J. LeGall, E. Engh, M. Schneider, P. Hof and R. Huber, *Science*, 1995, **270**, 1170.
10 H. Schindelin, C. Kisker, J. Hilton, K. V. Rajagopalan and D. C. Rees, *Science*, 1996, **272**, 1615.
11 M. K. Chan, S. Mukund, A. Kletzin, M. W. W. Adams and D. C. Rees, *Science*, 1995, **267**, 1463.

12 For a review see D. W. Young, *Chem. Soc. Rev.*, 1994, **23**, 119.
13 For a review see T. A. Dix and S. J. Benkovic, *Acc. Chem. Res.*, 1988, **21**, 101.
14 S. W. Bailey, R. Y. Chandrasekaran and J. E. Ayling, *J. Org. Chem.*, 1992, **57**, 4470; see also S. W. Bailey, I. Renbrin, S. R. Boerth and J. E. Ayling, *J. Am. Chem. Soc.*, 1995, **117**, 10 203.
15 S. J. N. Burgmayer, A. Baruch, K. Kerr and K. Yoon, *J. Am. Chem. Soc.*, 1989, **111**, 4982.
16 W. Pfleiderer and H. Zondler, *Chem. Ber.*, 1966, **99**, 3008; R. Prewo, J. H. Bieri, S. N. Ganguly and M. Viscontini, *Helv. Chem. Acta*, 1982, **65**, 1094; P. A. Charlton and D. W. Young, *J. Chem. Soc., Perkin Trans. 1*, 1982, 1363.
17 B. Schirks, J. H. Bieri and M. Viscontini, *Helv. Chim. Acta*, 1978, **61**, 2731.
18 J. H. Bieri and M. Viscontini, *Helv. Chim. Acta*, 1974, **57**, 1651; W. Pfleiderer, *Chem. Ber.*, 1979, **112**, 2750.
19 M. Viscontini and S. Huwyler, *Helv. Chim. Acta*, 1965, **48**, 764.
20 M. C. Archer and K. G. Scrimgeour, *Can. J. Biochem.*, 1970, **48**, 278; M. C. Archer, D. J. Vonderschmitt and K. G. Scrimgeour, *Can. J. Biochem.*, 1972, **50**, 1174; S. Kaufman, *J. Biol. Chem.*, 1964, **239**, 332.
21 S. W. Bailey and J. E. Ayling, *Biochemistry*, 1983, **22**, 1790.
22 S. Matsuura, T. Sugimoto and S. Murata, *Tetrahedron Lett.*, 1985, **26**, 4003.
23 I. R. Gould, N. A. Burton, R. J. Hall and I. H. Hillier, *J. Mol. Struct.* (*THEOCHEM*), 1995, **331**, 147.
24 M. W. Wong, K. B. Wiberg and M. J. Frisch, *J. Am. Chem. Soc.*, 1992, **114**, 1645.
25 P. E. Young, D. V. S. Green, I. H. Hillier and N. A. Burton, *Mol. Phys.*, 1993, **80**, 503.
26 J. E. Gready, *J. Comput. Chem.*, 1985, **6**, 377; *J. Mol. Struct.* (*THEOCHEM*), 1985, **25**, 1; *J. Am. Chem. Soc.*, 1985, **107**, 6689.
27 M. J. Frisch, G. W. Trucks, H. B. Schlegel, P. M. W. Gill, B. G. Johnson, M. A. Robb, J. R. Cheeseman, T. A. Keith, G. A. Petersson, J. A. Montgomery, K. Raghavachari, M. A. Al-Laham, V. G. Zakrzewski, J. V. Ortiz, J. B. Foresman, J. Cioslowski, B. B. Stefanov, A. Nanayakkara, M. Challacombe, C. Y. Peng, P. Y. Ayala, W. Chen, M. W. Wong, J. L. Andres, E. S. Replogle, R. Gomperts, R. L. Martin, D. J. Fox, J. S. Binkley, D. J. Defrees, J. Baker, J. J. P. Stewart, M. Head-Gordon, C. Gonzalez and J. A. Pople, *GAUSSIAN94*, Revision A.1, Gaussian, Inc., Pittsburgh, PA, 1995.
28 R. J. Hall, N. A. Burton, I. H. Hillier and P. E. Young, *Chem. Phys. Lett.*, 1994, **220**, 129.
29 O. G. Parchment, I. H. Hillier and D. V. S. Green, *J. Chem. Soc., Perkin Trans. 2*, 1991, 799.
30 O. G. Parchment, D. V. S. Green, P. J. Taylor and I. H. Hillier, *J. Am. Chem. Soc.*, 1993, **115**, 2352.
31 *Exploring Chemistry with Electronic Structure Methods: A Guide to using Gaussian*, J. B. Foresman and A. Frisch, Gaussian Inc., Pittsburgh, PA15213, USA.

Paper 7/00330G
Received 14*th January* 1997
Accepted 25*th March* 1997