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A COMPUTATIONAL STUDY ON STABILITIES OF DIHYDROPTERINS

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Abstract – Molecular orbital calculation indicated that the potential energy of 7,8-dihydrobiopterin was at least 30 kJ/mol lower than those of other dihydrobiopterin isomers. The instability and reactivity of quinonoid dihydrobiopterin and 5,6-dihydrobiopterin in aqueous solutions were explained not only by energy values but also by orbital figures of LUMOs.

(6*R*)-Pyruvoyltetrahydropterin (**1**) is an important intermediate in the major biosynthetic process of (6*R*)-tetrahydrobiopterin (**2**), which works as a cofactor in biosynthetic pathways of nitrogen oxide (NO) as well as of catecholamines.[1] The occurrence of a genetic mutation in the biosynthetic route of **2** generally causes a fatal deficit of the cofactor,[2,3] but there is an exceptional case in which the amount of **2** does not decrease so much by virtue of the existence of a salvage path. In that case, (2'*R*)-sepiapterin (**3**), which can be converted to **2** through 7,8-dihyrobiopterin (**4**) by other enzymes, works as a substitution of **1**.[4,5] After completion of enzymatic aromatic amino acid hydroxylation, such as the conversion of tyrosine to L-DOPA, cofactor (**2**) is oxidized to quinonoid dihydrobiopterin (**5**). Then, **5** is reduced back to **2** by another biochemical regeneration process.[1] On the other hand, 5,6-dihydrobiopterin (**6**) has been considered a key intermediate in the practical scale synthesis of **2**.[6,7] Thus, there are several dihydrobiopterin[8] derivatives that occupy transient but important locations in practical as well as biological regulation of cofactor (**2**). However, because these dihydrobiopterin derivatives in general are decomposed quickly by oxidization under aerobic conditions and by tautomeric shift, their chemical characteristics necessary for accurate biochemical understanding have been unclear. Development of a facile methodology to rationalise their characteristics has been required. In this paper, we would like to discuss the stability and reactivity of the dihydrobiopterin derivatives based on molecular orbital calculations.

The structures of several dihydro-6-methylpterin derivatives with a $C_7H_9N_5O$ formula, such as 7,8-dihydro- (**7**), quinonoid dihydro- (**8**), 5,6-dihydro- (**9**), and 5,8-dihydro-6-methylpterin (**10**), were optimized by using an *ab initio* (STO3G) method. Potential energies of these pterins were obtained from calculations

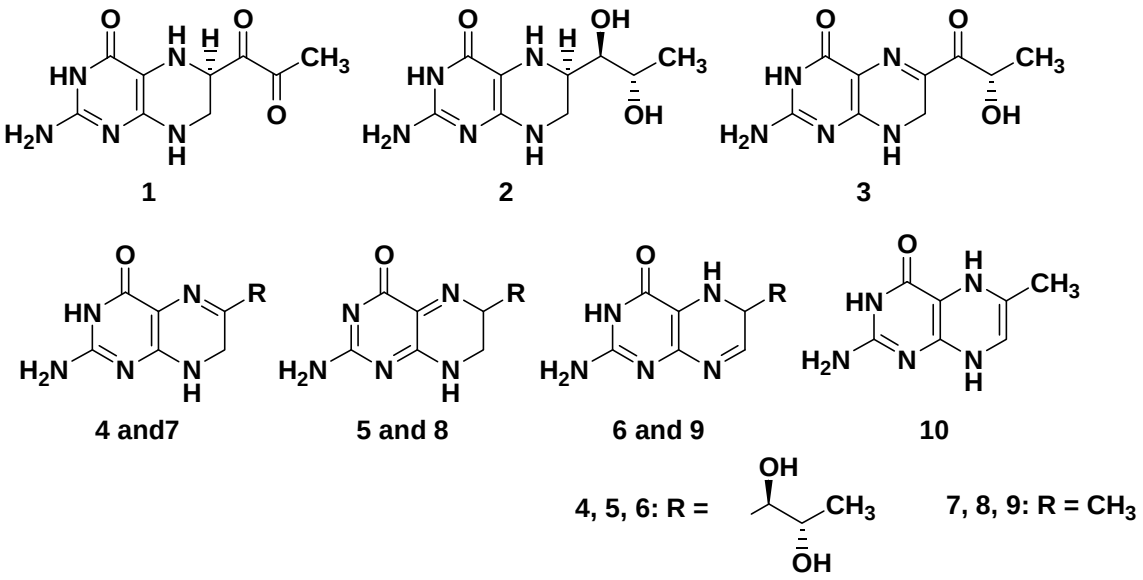


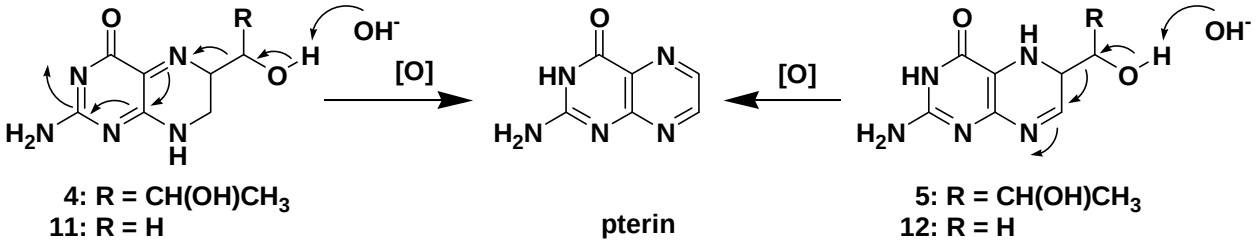
Figure 1. Dihydrobiopterin and related pterin derivatives

Table 1. Potential differences of dihydropterin derivatives

compound	energy difference (kJ mol^{-1}) and calculation method		
	HF/3-21G	HF/6-31G*	B3LYP/6-31G*
4	0		
5	39.205		
6	52.292		
7	0	0	0
8	59.710	59.279	59.325
9	37.949	58.558	35.037
10	36.631	51.277	29.830

more stable than others because its energy is at least 30 kJ/mol lower energy, and the **4** and **7** are stable in both solid and solution phases.

An ammonium salt of **5** is inert in solid phases but decomposes in aqueous solutions in a few minutes.[9,10] Depending on the pH value of the solution, the decomposition proceeds mainly by way of disproportionation to a mixture of **2** and biopterin (pH < 5), isomerization to **4** (pH = 7), or pterin-affording side-chain cleavage (pH > 8). The characteristic C-C bond cleavage under alkaline conditions is used for the quantitative analysis of **5** in biological samples. A similar side-chain cleavage occurred in an alkaline solution of **6** that was produced *in situ* by pterin ring-forming condensation.[11] In the optimized structures of 6-hydroxymethylpterin derivatives (**11** and **12**), the obtained C(6)-C(1') and C(1')-OH bond distances were 1.56 Å and 1.43 Å, respectively, and these values were not different from normal single bond lengths. However, significant elongation of the C-C single bond and shortening of the C-O bond (1.72 Å and 1.31 Å, respectively) were recommended in the optimized structure of the alkoxide anion of **12**. These changes



Scheme 1. Mechanism of side-chain cleavage

indicated the occurrence of retro-aldol-type transformations including weakening of the C-C bond and generation of a double-bond character in the C-O. The structural mutations were more serious in the case of the alkoxide of **11**, and its structure collapsed to pterin and formaldehyde during the structure optimization.

Quinonoid dihydropterin (**5**) can exist in aqueous solutions in the presence of hydrogensulfite ion as the tetrahydropterin-like adduct **13** (X = SO_3^-).[10] Enzymatic dehydration of **14** (X = OH) to **5** is known to be one of the key transformations in the regenerating process of the cofactor (**2**), and the dehydration occurs even non-enzymatically in aqueous solutions.[12] The formal hydrogen peroxide adduct (**15**, X = OOH), although it has not been chemically investigated yet, is considered the active species that carries oxygen atoms into the enzymes for hydroxylation of the aromatic ring.[1,2] The occurrence of a similar nucleophilic addition was known during the chemical syntheses of biopterin, and the initially produced intermediate (**6**) was detected as the tetrahydropterin derivative (**16**).[13,14] These reactions are well understood by considering LUMOs of **8** and **9**, illustrated in Figure 2. Due to the presence of large orbits on the C(4a) position of **8** and the C(7) position of **9**, these compounds are activated for to the nucleophilic attack on the positions.

As described herein, molecular orbital calculation is useful for predicting chemical characteristics of various highly reactive dihydropterin intermediates. In preliminary studies on non-enzymatic conversion of **1** to **3**, the ground state energy differences of several possible tautomeric intermediates existing in the pathway are no more than 30 kJ/mol, and **1** is about 9 kJ/mol more stable than **3**. Further investigations are in progress.

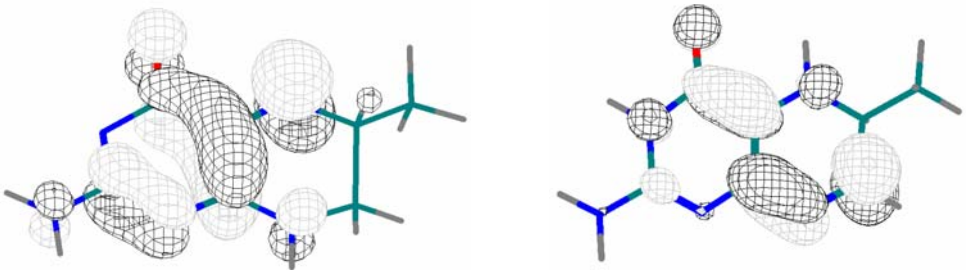
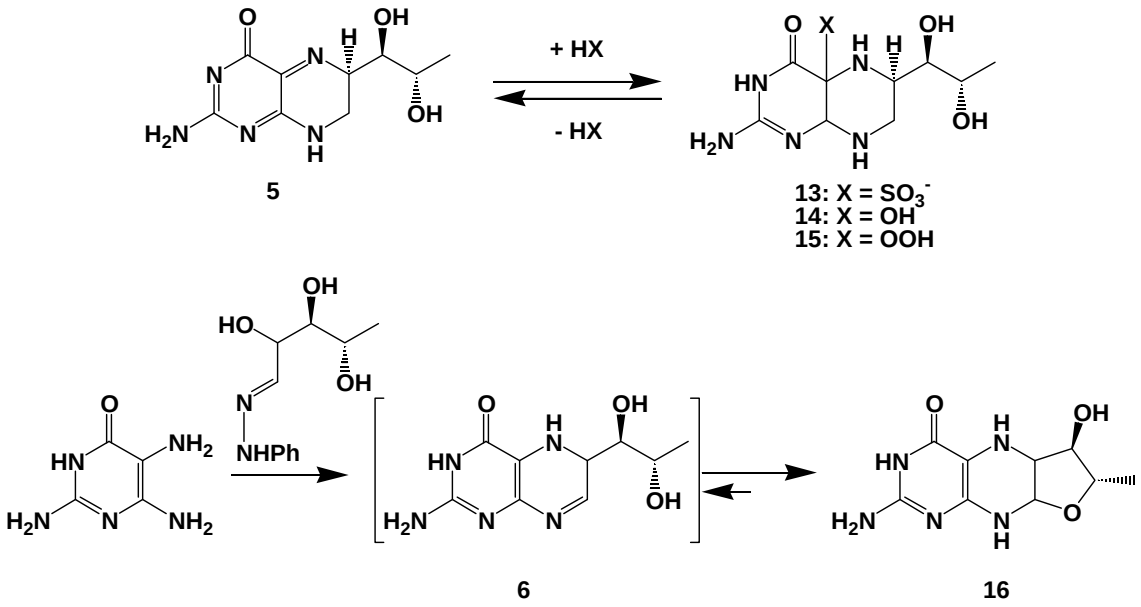


Figure 2. LUMO of **8** (left) and **9** (right)



Scheme 2. Addition of nucleophiles to dihydropterins

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