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Quinonoid Dihydrobiopterin, an Important Metabolic Intermediate of Biopterin Cofactor in the Aromatic Hydroxylation of Amino Acids

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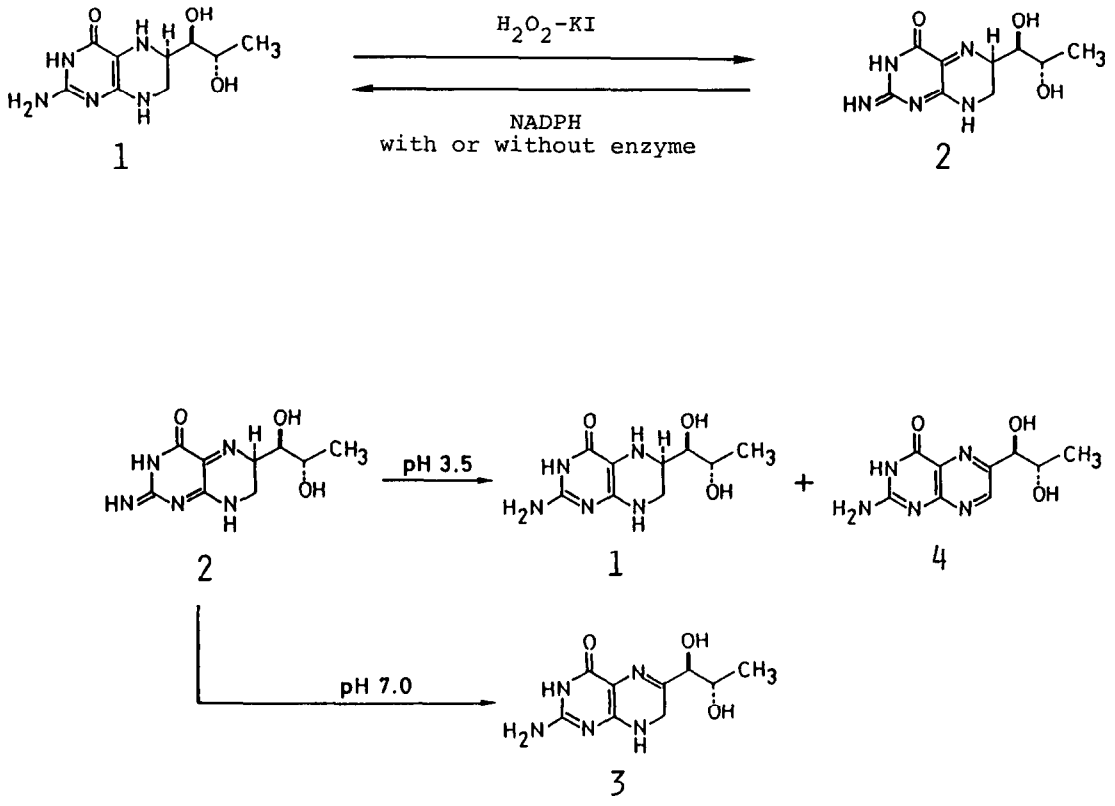
Abstract: p-Quinonoid (6R)-dihydrobiopterin hydrochloride was synthesized from (6R)-5,6,7,8-tetrahtdrobiopterin dihydrochloride by hydrogenperoxide in the presence of potassium iodide. Several characters of quinonoid dihydrobiopterin were examined.

In the biosynthesis of neurotransmitting catecholamines, the hydroxylation of phenylalanine and tyrosine requires (6R)-5,6,7,8-tetrahydrobiopterin (**1**) as the natural cofactor.[1] During the enzymatic reactions, **1** is oxidized to quinonoid (6R)-dihydrobiopterin (**2**), which is reduced back to **1** by dihydropterin reductase and NADPH (or NADH).[1a] In the absence of such recycling pathway, **2** will spontaneously isomerize to thermodynamically more stable 7,8-dihydrobiopterin (**3**) which is no more active towards the reductase.[1a] Although the existence of **2** has been deduced from the kinetic study of the enzymatic hydroxylation reaction, preparation of **2** has been unsuccessful because of its instability and unavailability of **1**. Thence biological and spectroscopic studies have been performed on quinonoid 6-methyl- and 6,7-dimethyldihydropterins, both of which are the unstable oxidation products of the respective tetrahydropterin pseudocofactors.[2,3] In this communication we would like to describe a chemical synthesis and some properties of **2**.

(6R)-Tetrahydrobiopterin (**1**) dihydrochloride[4] (0.630 g, 2.0 mmol) and KI (0.033 g, 0.20 mmol) were dissolved into ice chilled water (5 ml), to which was added 30% H_2O_2 (0.2 ml, 2.0 mmol) at 0 °C. After stirring for 5 min, the precipitate was collected by filtration and washed with cold water and ethanol. By this washing procedure, iodine was completely removed, leaving 0.354 g (64% yield) of quinonoid dihydrobiopterin (**2**) hydrochloride as ivory



needles.[5] The IR spectrum (KBr disk) of the compound exhibited a typical band at 1740 cm^{-1} for the quinonoid dihydropterin structure.[3]

The obtained crystals of **2** could be stored without significant decomposition at 5 °C for more than two months in a tightly sealed bottle. On contrary, **2** decomposed very rapidly in any kind of solutions examined except in a neutral or weak acidic aqueous solution containing $NaHSO_3$. This is probably because the quinonoid dihydro compound may form some semi stable adducts with $NaHSO_3$. In fact, when $NaHSO_3$ is destroyed by oxidation after standing in air, the dihydro compound **2** starts to decompose. Thus, all the chromatographic analyses were performed using a pH 3.2 buffer containing $NaHSO_3$ as the eluant on a strong cation exchange HPLC column.[6] A single and sharp peak corresponding to **2** was observed on chromatograms indicating that no decompositions had proceeded during the chromatography. In a pH 3.5 solution without $NaHSO_3$, the compound decomposed spontaneously with a half life 3.5 min.[7] The main decomposition products were unexpectedly (6R)-tetrahydrobio-

pterin (**1**) and fully oxidized biopterin (**4**) in nearly equal amounts. As the decomposition processed, amounts of **1** and **4** increased proportionally to the decreased amount of **2**. The expected 7,8-dihydrobiopterin (**3**) was detected as a minor component. Since (6R)-tetrahydrobiopterin (**1**) was formed stereospecifically and in an equal amount with **4**, it is most rational to consider that these two compounds were generated directly from quinonoid dihydrobiopterin by a disproportionation reaction. A similar, but slower, disproportionation was also observed with quinonoid 6-methyldihydropterin.[3] Thus the ^1H NMR analysis[8] of the target compound inevitably suffered from the complication by the disproportionation products. Quinonoid dihydrobiopterin (**2**) exhibited two double doublets at 3.64 ppm for C(7)H_{ax} and at 4.03 ppm for C(7)H_{eq}, and a ddd signal at 4.52 ppm for C(6)H. The two tertiary protons on the side chain appeared as a quintet like dt at 3.89 ppm (C(2′)H) and a dd at 4.01 ppm (C(1′)H). The low field resonance of the proton on C(6) is due to its location adjacent to the imino nitrogen atom. The two protons on C(7) resonance at higher fileds, suggesting that there is no imino nitrogen atom nearby. These results confirmed the structure of the oxidation product to be p-quinonoid (6R)-dihydrobiopterin (**2**) rather than the ortho isomer same as the 6-methyl and 6,7-dimethyl analogues.[2a,2g,3]

Isomerization to 7,8-dihydrobiopterin (**3**), a reaction typical for quinonoid dihydropterins,[2b,2c,2d,2f] was the main decomposition pathway of **2** at pH 7.0. The decomposition of **2** proceeded with a half life 0.9 min in a 0.1 M phosphate buffer[7] and 1.9 min in a 0.1 M Tris buffer,[7] indicating the acceleration effect of the phosphate anion.[2c,2f] Other decomposition products were again **4** and **1**, though in small quantities. The isomerization was virtually diminished by a large excess NADPH, which non enzymatically and stereospecifically afforded **1**. Under the same conditions, **3** remained intact.

References and Notes

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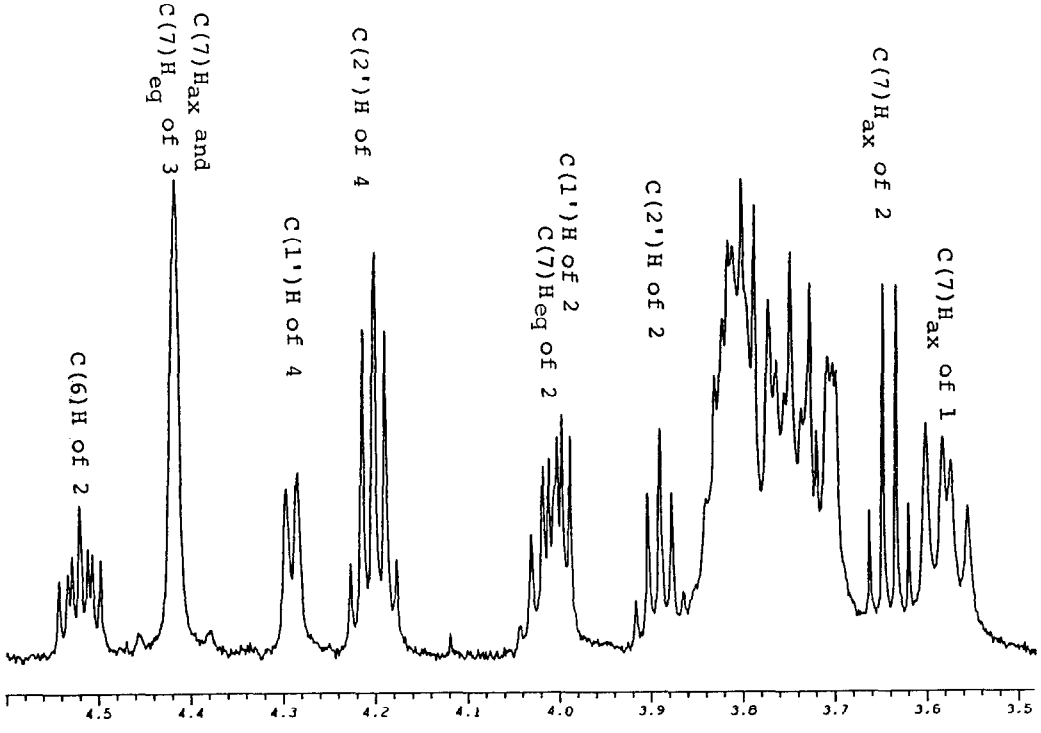
5. mp 190 °C (dec). Anal. Calcd for $C_9H_{13}N_5O_3 \cdot HCl$: C, 39.21; H, 5.12; N, 25.40. Found: C, 39.08; H, 5.06; N, 26.53. Further purification of the sample were unsuccessful due to the spontaneous decompositions (see text).
6. HPLC analysis was performed as follows: column, Partisil-10 SCX 4.5 x 250 mm; detector, UV at 265 nm; eluant, 30 mM ammonium phosphate buffer, pH 3.2, containing 3 mM $NaHSO_3$; flow rate 2 ml/min. Retention volumes of pterins concerned were as follows: **2**, 3.62 ml; **4**, 4.36 ml; **3**, 5.02 ml; **1**, 7.26 ml; (6S)-tetrahydrobiopterin, 8.92 ml.
7. The values were calculated based on the decrease of the absorbance at 370 nm in 0.1 mM solution at 18 °C.
8. The 500 MHz ^1H NMR spectrum was measured on a JEOL GX-500 spectrometer at Chemical Instrument Center of Nagoya University. The sample (3.95 mg) was dissolved in D_2O (1.0 ml) containing $(CH_3)_3SiCD_2CD_2COOD$ (0.1%) and the spectrum was observed at 18 °C for 7 min. After the observation completed, about 25% of **2** was still alive. The ^1H NMR spectrum, in which all individual signals were assigned by the comparison with the spectra of authentic **1**, **3**, and **4**, was shown below.



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