HYDROGENATION OF BIOPTERIN AND ITS ANALOGUES; APPLICATION FOR THE CONVENIENT PROCEDURE OF BIOPTERIN COFACTOR AND RELATED 5,6,7,8-TETRAHYDROPTERINS

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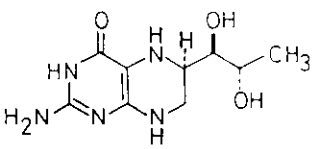
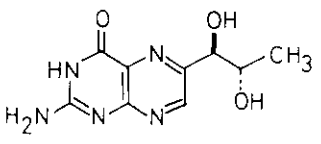
Abstract——A large scale and stereoselective hydrogenation of biopterin to (6R)-5,6,7,8-tetrahydrobiopterin was developed. The procedure was applied for various hydroxyalkylpterins such as neopterin [(1'S,2'R)-6-(1',2',3'-trihydroxypropyl)pterin] and diastereomers of biopterin.

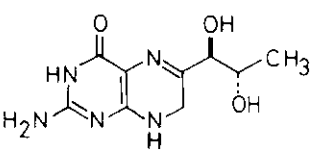
(6R)-5,6,7,8-Tetrahydrobiopterin [1] is the cofactor of aromatic monooxygenases in the metabolism of aromatic amino acids to neurotransmitting catecholamines and serotonin in the human brain.[1] It is proved that the lack of [1] causes several neurological diseases such as Parkinson's disease and atypical phenylketonuria[2] and [1] acts as a potential clinical agent.[3] Many other related 6-substituted tetrahydropterins exhibit the activity as the pseudocofactor because they can replace the natural cofactor *in vitro*.[4] However, it should be noted that the (6S)-isomer of [1] exhibits very different activities against monooxygenases;[5] it can be a "killer" of these enzymes. Thence, a convenient supply of stereochemically pure [1] and the



[1]



[2]



[3]

pseudocofactors has been strongly required for the biochemical and clinical studies. In this paper, we describe about hydrogenation of biopterin[6] [2] and other 6-substituted pterins for the synthesis of [1] and pseudocofactors.

Hydrogenation of Biopterin [2] and 7,8-Dihydrobiopterin [3].——As we reported briefly, hydrogenation of [2] over PtO_2 proceeded stereoselectively in a basic solution to give [1].[7] In order to establish a practical procedure of [1], we examined some effects of pH and metal cations. In acidic or neutral potassium phosphate buffer (pH 2—8) hydrogenation of [2] completed within 2 h, but the selectivities of [1] were unsatisfactory (R:S = about 2:1). Although the rate of hydrogenation was slower (over 20 h), the selectivities of [1] were improved in basic media (pH 10—13). Because the reaction rate was extremely slow and unknown by-products were formed at pH above 13, the optimal pH was about 11.8. Under this pH condition, 7,8-dihydrobiopterin[8] [3] was detected as the intermediate by HPLC analysis.[9] Hydrogenation of [3] gave [1] with the same pH dependent selectivity as the cases of [2], summarized in Table I. In order to examine effects of the metal cation, hydrogenation of [2] was carried out in the presence of various cation instead of K^+. Under the same pH conditions the selectivities, shown in Table II, were unexpectedly all the same even in the presence of Li^+, Zn^{2+}, or $(C_2H_5)_4N^+$ although their abilities of chelation are very different. Alcoholic solutions such as 2-methoxyethanol and methanol or other organic solutions are not suitable for the hydrogenation. For example,

Table I. Stereoselectivity of Hydrogenation of [2] and [3][a]

substrate	pH	selectivity (R:S)
[2]	2.3	2.6:1
[3]	2.3	2.2:1
[2]	5.5	2.8:1
[2]	7.0	3.2:1
[3]	7.0	2.5:1
[2]	8.1	3.3:1
[2]	10.5	4.1:1
[2]	11.5	5.3:1
[3]	11.5	4.5:1
[2]	11.8	7.3:1

[a]The reaction was carried out in a potassium phosphate buffer.

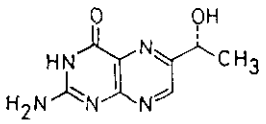
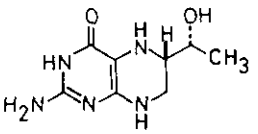
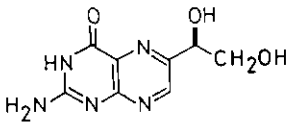
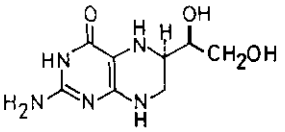
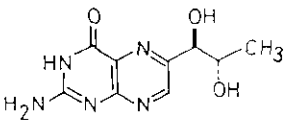
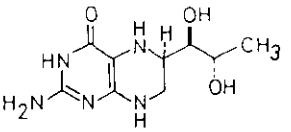
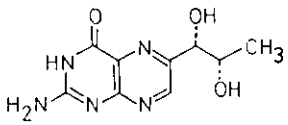
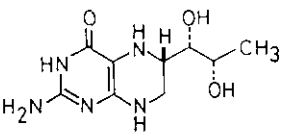
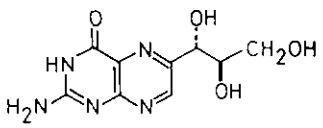
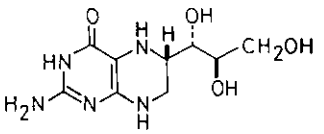
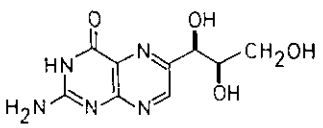
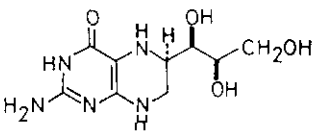
Table II. Hydrogenation of [2] in a H_3PO_4-$M(OH)_n$ Buffer

	selectivity (R:S)		
M^{n+}	pH 2.3	pH 8.8	pH 11.5
Li^+	2.8:1	3.3:1	4.9:1
K^+	2.6:1	3.3:1	5.3:1
Cs^+	2.6:1	3.4:1	5.1:1
Zn^{2+}	2.5:1		
$(C_2H_5)_4N^+$	2.5:1	3.4:1	5.3:1

hydrogenation of [2] in 0.01 M $CH_3OCH_2CH_2ONa$ in dry $CH_3OCH_2CH_2OH$ gave [1] in 2:1 selectivity. Finally, 63 g of the pure dihydrochloride of [1], which possess the activities as the cofactor in vitro and in vivo, was obtained from 60 g of [2] by the optimized condition thus established (see experimental section).

Hydrogenation of Other 6-Substituted Pterins.——The stereoselective hydrogenation was also employable for various kinds of 6-substituted pterins containing the 1′-hydroxyethyl, 1′,2′-dihydroxyethyl, 1′,2′-dihydroxypropyl, or 1′,2′,3′-trihydroxypropyl substituent on C(6).[10] Hydrogenation of these substrates proceeded quantitatively in a potassium phosphate buffer (pH 10.8—

Table III. Hydrogenation of Pterins

substrate	major product	ratio
		2.3 : 1
		2.9 : 1
		7.3 : 1
		3.8 : 1
		2.7 : 1
		2.5 : 1

11.5), and stereochemistries of products were determined by the behavior on the HPLC analysis and CD spectra.[11] Although selectivities were even lower in other starting materials, the products containing 6,1′-erythro[12] relative configurations were produced predominantly, as shown in Table III.

EXPERIMENTAL

A General Procedure for Small Scale Hydrogenation of Pterins.——A mixture of a substrate (2—5 mg) and PtO_2 (1—4 mg) in a potassium phosphate buffer (0.2 ml/mg of the substrate) was stirred vigorously under H_2 atmosphere (1 atm) at room temperature. The mixture (0.10 ml) was centrifuged (6000 rpm, 10 min) in a capillary tube (1 x 50 mm), and the upper clear solution was subjected to the HPLC analysis.[9]

Preparation of (6R)-5,6,7,8-Tetrahydrobiopterin [1].——A mixture of [2] (60 g, 0.25 mol) and PtO_2 (0.5 g) in 0.1 M potassium phosphate buffer (pH 11.8, 900 ml) was shaken under hydrogen (1 atm) at 15 °C for 18 h. The mixture was acidified by addition of conc. HCl (100 ml), and filtered through a 0.45 μm membrane filter. The filtrate (R/S = 7.5) was concentrated to 200 g, and to this was added methanol (100 ml). The resulting precipitate (18.4 g) was removed by filtration. Then the filtrate was reconcentrated to about 250 g, and to this was added conc. HCl (50 ml). Colorless fine needles (R/S = 32.5, 63 g, 79 %) were obtained. Recrystallization from 8 M HCl gave an analytical sample: mp 245—246 °C (dec); $[\alpha]_D^{25}$ -6.81 + 0.05 ° (c = 0.665, 0.1 M HCl), [lit.[13] -6.8 ° (c = 0.18, 0.1 M HCl)]; UV λ_{max} (2 M HCl) 264 nm (ε 16770), (pH 3.5 phosphate buffer) 265 (13900), (pH 7.6) 297 (9500) and 260 (sh, 4690); ^{1}H NMR (D_2O) 3.86—3.70 (4H, m, C_7H_{eq}, C_6H, $C_1'H$, and $C_2'H$), 3.58 (1H, dd, J = 15.5 and 11 Hz, C_7H_{ax}), and 1.16 (3H, d, J = 6.2 Hz, $C_3'H$); ^{13}C NMR (0.1 M DCl in D_2O) 157.88 (C_4), 152.73 (C_2), 149.97 (C_{10}), 86.53 (C_9), 72.28 (C_1'), 69.51 (C_2'), 54.83 (C_6), 38.63 (C_7), and 20.64 (C_3'); FAB MS m/z 242 (M + 1), 223, 185, and 116. Anal. Calcd for $C_9H_{15}N_5O_3 \cdot 2HCl$: C, 34.40; H, 5.45; N, 22.47. Found: C, 34.42; H, 5.44; N, 22.47.

Hydrogenation of (1′R)-6-(1′,2′-Dihydroxyethyl)pterin.——A 2.9:1 mixture of (6R,1′R)- and (6S,1′R)-6-(1′,2′-dihydroxyethyl)-5,6,7,8-tetrahydropterins (0.305 g, 51% yield) was obtained from (1′R)-6-(1′,2′-dihydroxyethyl)pterin (0.446 g) as colorless needles. An analytical sample was prepared by recrystallization from 8 M HCl: mp 236 °C (dec); UV λ_{max} (1 M HCl) 264 nm (ε14850) and 200

(15650), (pH 3.1 phosphate buffer) 265 (11630) and 223 (14830), (pH 8.0) 300 (9350) and 260 (4550); ^{1}H NMR (1 M DCl in D_2O): 4.28 (1H, ddd, J = 6.9, 6.9, and 3.1 Hz, C_6H) and 4.0—3.6 (5H, m, C_7H, $C_{1'}H$, and $C_{2'}H$): ^{13}C NMR (0.1 M DCl in D_2O): 156.63 (C_4), 151.00 (C_2), 147.21 (C_{10}), 85.66 (C_9), 67.89 ($C_{1'}$), 63.07 ($C_{2'}$), 53.64 (C_6), and 39.50 (C_7) for (6R)-isomer; 156.41 (C_4), 151.00 (C_2), 147.37 (C_{10}), 85.06 (C_9), 68.00 ($C_{1'}$), 62.53 ($C_{2'}$), 54.13 (C_6), 37.87 (C_7) for (6S)-isomer. Anal. Calcd for $C_8H_{13}N_5O_3 \cdot 2HCl$: C, 32.01; H, 5.04; N, 23.34. Found: C, 31.93; H, 5.00; N, 24.03.

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(9) HPLC was carried out as follows: pump, JASCO TRIROTOR; column, Partisil 10 SCX 4.5 I.D. x 250 mm; UV detector, JASCO UVIDEC 100III at 270 nm; eluant, 30 mM ammonium phosphate (pH 3.2) containing 3 mM $NaHSO_3$; flow rate, 2 ml/min.

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