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HIGHLY STEREOSELECTIVE PROCEDURE FOR (6R)-TETRAHYDROBIOPTERIN COFACTOR

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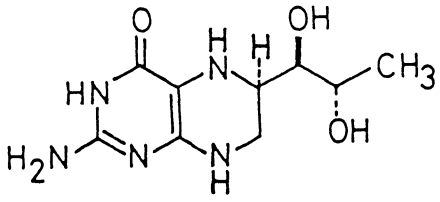
(6R)-Tetrahydrobiopterin cofactor for aromatic amino acid hydroxylases was synthesized stereoselectively by catalytic hydrogenation of biopterin over platinum oxide at pH 11.4 and subsequent crystallization from hydrochloric acid and ethanol.

The biosynthesis of the neurotransmitting catecholamines from phenylalanine requires tetrahydrobiopterin cofactor, (6R)-2-amino-4-hydroxy-6-[(1R,2S)-1,2-dihydroxypropyl]-5,6,7,8-tetrahydropteridine (1), at the monooxygenation step of phenylalanine[1] and tyrosine.[2] It is supposed that the catecholamine biosynthesis is regulated in a great extent by tetrahydrobiopterin cofactor,[3] and thus a decrease of the cofactor in central nerve systems causes several neurological disorders like parkinsonism[4] and atypical phenylketonuria.[5]



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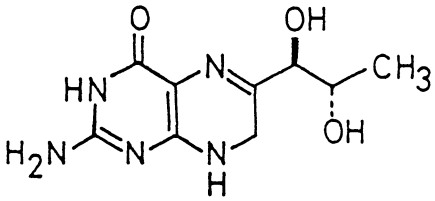

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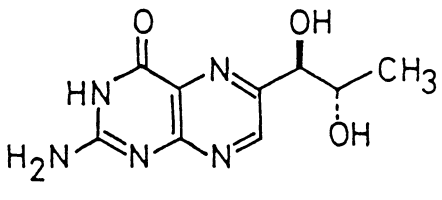

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Indeed, we have shown that the concentration of the cofactor in the brain of parkinsonian patients was much lower as compared to healthy control,[4] and demonstrated that the symptoms of parkinsonian patients were improved without any side effects by oral administration of tetrahydrobiopterin.[6] Accordingly, an effective synthesis of the cofactor has become particularly important to develop the basic and clinical studies on parkinsonism and other neurological diseases. We describe here a convenient procedure suitable for a large scale synthesis of the cofactor, which was hithero quite difficult to access.

The enzymatic reduction of 7,8-dihydrobiopterin (2) by dihydrofolate reductase gives (6R)-tetrahydrobiopterin (1),[7,8,9] but this method is impractical for a large scale preparation. In contrast, the most frequently employed catalytic hydrogenation of biopterin (3) in an acidic media suffered from low stereoselectivity, and produced (6R)-tetrahydrobiopterin only a little more than the (6S)-isomer (about 30% de).[9] However, the ratio of (6R)-tetrahydrobiopterin/(6S)-isomer (hereafter shown as R/S ratio) depended on the pH of the solution, as shown in Fig. 1. It should be noted that the relative yield of the (6R)-isomer is higher in basic solutions than in acidic or neutral solutions, and the extent of the change is largest around pH 11.

The catalytic hydrogenation of biopterin (3) (pK_a 7.7) to tetrahydrobiopterin undergoes via 7,8-dihydrobiopterin (2) (pK_a 10.8) intermediate. Since the curve of R/S ratio changes most steeply at the pH value equivalent to the pK_a value of 2, it is most probable that the anion rather than the neutral molecule of 2 exists in a more suitable conformation for producing (6R)-tetrahydrobiopterin (1). However, hydrogenation at pH 13 or higher, where more than 99% of 2 are ionized to the anion, was found not optimal for the following reasons; the reaction was extremely slow and an additional unidentified compound was formed without improving the R/S ratio. Because of these reasons, we employed the hydrogenation at pH 11.4 where no byproduct was detected. The required (6R)-tetrahydrobiopterin (>99% de) was obtained as dihydrochloride in 67% yield simply by recrystallization from a mixture of hydrochloric acid and ethanol without any chromatographic purification. The following example is representative.

A mixture of biopterin (3) (5.00 g, 21 mmol) and K_2HPO_4 (1.8 g, 10 mmol) in water (500 ml) was adjusted to pH 11.4 by addition of 10 M KOH solution. The

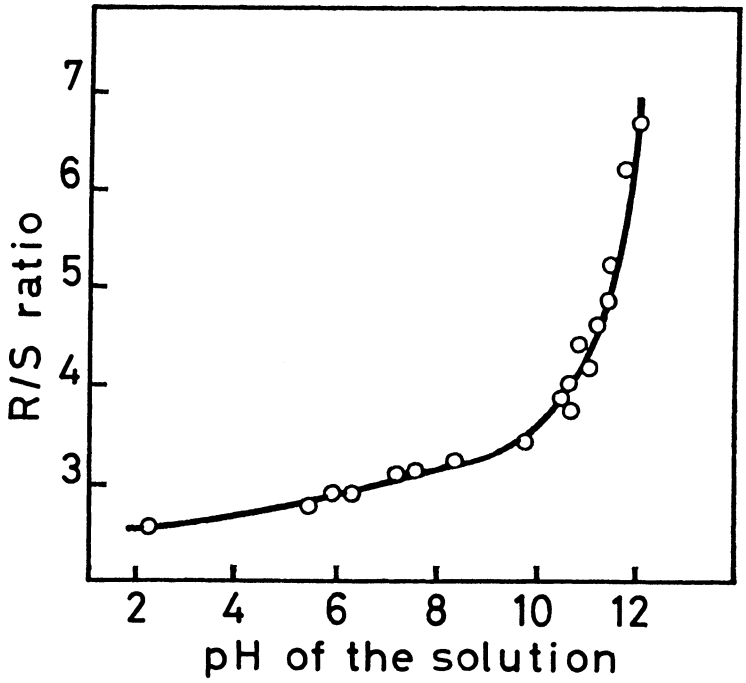

Fig. 1. The influence of pH upon the formation of (6R)-and (6S)-tetrahydrobiopterin on catalytic hydrogenation of biopterin.[10]

resulting solution was shaken vigorously with PtO_2 powder (0.5 g) under hydrogen atmosphere (1 kg/cm^2) at 20 °C for 4.5 h. After addition of concd HCl (20 ml), the catalyst was removed by filtration through a 0.45 μm membrane filter. On evaporation of the filtrate in vacuo to 50 ml and dilution with methanol (140 ml), inorganic salts precipitated, which were removed by filtration. The filtered solution was further concentrated to 30 ml, to which concd HCl (6 ml) and then ethanol (100 ml) were added in this order to give colorless needles (5.80 g; 88% de). The products were dissolved in concd HCl (15 ml) under gentle warming. To the warm solution, ethanol (30 ml) was added dropwise, then chilled to give colorless needles. These procedures were repeated once more to give (6R)-tetrahydrobiopterin dihydrochloride as colorless needles (4.69 g, 67% yield; over 99% de), mp 245—246 °C (decomp.);$[\alpha]_D^{25}$ -6.81° (c 0.67, 0.1 M HCl).[11]

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10) Biopterin (25 mg) was hydrogenated over PtO_2 catalyst (4 mg) in 20 mM potassium phosphate buffer (5 ml) adjusted to an appropriate pH at 20 °C under atmospheric pressure. The products were analyzed by HPLC under the following conditions: column: Partisil-10 SCX, 4.5 x 250 mm; eluant: 30 mM ammonium phosphate buffer (pH 3.0) containing 3 mM $NaHSO_3$; flow rate: 2 ml/min; detector: UV at 275 nm. The peak areas of (6R)-tetrahydrobiopterin (retention time: 5.87 min) and the (6S)-isomer (8.45 min) were calculated on a SIC 5000E integrator.

11) H. -J. Furrer, J. H. Bieri, and M. Viscontini [Helv. Chim. Acta, 62, 2577 (1979)] reported $[\alpha]_{589}^{25}$ -6.8° (c 0.68, 0.1 M HCl).

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