Studies on Biologically Active Pteridines. VII.[1] Absolute Configuration of (−)-6-Methyltetrahydropterin Produced by Enzymic Reduction

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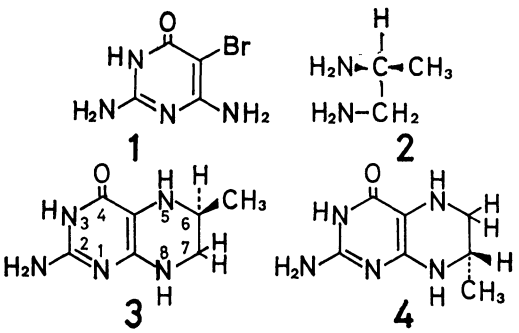
Synopsis. The C-6 configuration of (−)-6-methyltetrahydropterin, produced by enzymic reduction of the 7,8-dihydro precursor, is shown to be *S* by a synthesis. Condensation of 2,4-diamino-5-bromo-6-hydroxypyrimidine with (*S*)-1,2-propanediamine gave (*S*)-6-methyltetrahydropterin. Examination of CD spectra of the 6-methyltetrahydropterins from the two origins led to the above conclusion.

The reduction of 7,8-dihydrobiopterin and 7,8-dihydro-6-methylpterin to the 5,6,7,8-tetrahydro derivatives by the action of dihydrofolate reductase is stereospecific and the (−)-tetrahydropterins thus produced are shown to possess the same configuration at the C-6 chiral center.[2,3] (−)-Tetrahydrobiopterin is the natural cofactor for aromatic amino acid hydroxylases, whereas the C-6 diastereoisomeric (+)-tetrahydrobiopterin shows different cofactor characteristics.[3-5] Because of the indispensable contribution of (−)-tetrahydrobiopterin to the biosynthesis of neurotransmitting serotonin and dopamine, the determination of the C-6 configuration is highly desired and has been studied in several ways.[2,3,6,7] In these studies, the C-6 configuration of the enzymically reduced (−)-6-methyltetrahydropterin was shown to be *S* (and then, by analogy, that of (−)-tetrahydrobiopterin to be *R*), by comparison with a tetrahydroquinoxaline or by transformation into a tetrahydrolumazine or a piperazine. This paper describes a straightforward proof for the C-6 configuration of (−)-6-methyltetrahydropterin as *S* by its synthesis.

Since N-5 unsubstituted tetrahydropterins are notoriously unstable to air oxidation, we first attempted to synthesize a N-5 alkylated derivative of 6-methyltetrahydropterin, such as 5,6,8-trimethyl- or 5,8-dibenzyl-6-methyl-tetrahydropterin. In contrast to the ready formation of 5,8-dimethyl-5,6,7,8-tetrahydropterin[8] by condensation of 2,4-diamino-5-bromo-6-hydroxypyrimidine (**1**) with 1,2-bis(methylamino)ethane, an analogous condensation of **1** with 1,2-bis(methylamino)propane or 1,2-bis(benzylamino)ethane gave a very complex mixture, from which we could detect no tetrahydropterins. Heating of **1** with (*S*)-1,2-propanediamine (**2**) at about 115 °C, however, gave 6- and 7-methylpterins as predominant products. These compounds were



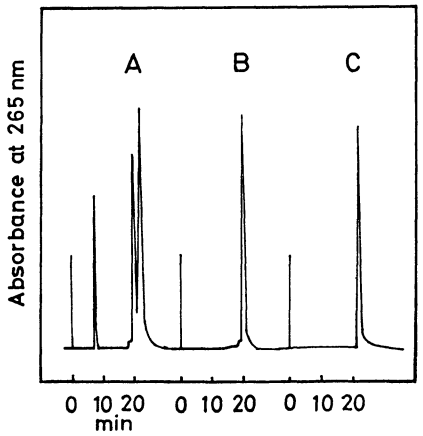


Fig. 1. HPLC of a reaction mixture after CM-Sephadex column separation (A), and authentic (*RS*)-6-methyl- (B) and (*RS*)-7-methyl-tetrahydropterin (C). Column: Whatman Partisil-10 SCX (8.0 mm × 250 mm). Eluant, 30 mM† aqueous $NH_4H_2PO_4$ adjusted to pH 3.0 with H_3PO_4. Flow rate 4.0 ml/min.

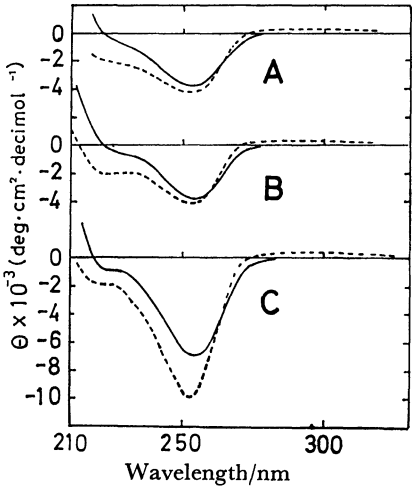


Fig. 2. CD spectra of the enzymically reduced tetrahydro-6-methylpterin[2] (A), synthetic (*S*)-6-methyl- (B), and (*S*)-7-methyl-tetrahydropterin (C). The data were obtained at pH 3.0 (——, monocation) and at H_0 −1.0 (----, dication).

undoubtedly produced by oxidation of the initially formed (*S*)-6-methyl- and (*S*)-7-methyl-5,6,7,8-tetrahydropterins by atmospheric oxygen. This oxidation could be prevented effectively when the condensation was carried out under hydrogen atmosphere. The 6- and 7-methyltetrahydropterins were isolated as a mixture (15% yield, estimated from UV spectra) by chromatography on a Florisil column and subsequently

† 1 M=1 mol dm^{-3}.

on a CM-Sephadex column. Separation of 6- and 7-methyltetrahydropterin from each other was achieved by means of HPLC on a preparative Partisil-10 SCX column (8 mm × 250 mm) using an ammonium phosphate buffer (30 mM, pH 3.0) as the solvent. Under the conditions, (*S*)-6-methyl-5,6,7,8-tetrahydropterin (**3**) was eluted a little, but sufficiently, faster than the isomeric (*S*)-7-methyltetrahydropterin (**4**) as shown in Fig. 1. The structure of these compounds were confirmed by comparing their chromatograms (HPLC) and UV spectra with those of the authentic racemates.[9,10]

The CD spectra of (*S*)-6-methyltetrahydropterin (**3**) were found superimposable with those of the enzymatically reduced (−)-6-methyltetrahydropterin[2] as shown in Fig. 2 at two different pH values, *i. e.* pH 3.0 (monocation) and H_0 −1.0 (dication). (*S*)-7-Methyltetrahydropterin (**4**) also showed a negative Cotton effect in CD spectra (Fig. 2) with a trough at a wavelength slightly longer than the isomer (**3**).

The results described here clearly prove that the C-6 configuration of (−)-6-methyltetrahydropterin is *S*, and accordingly that of (−)-tetrahydrobiopterin is *R*, which is consistent with the previous conclusions.[2,3,6,7]

Experimental

The UV spectra were measured on a Shimadzu UV-300 spectrometer, and the CD spectra on a JASCO J-40A recording spectropolarimeter equipped with a JASCO J-PRY data processor. The high-performance liquid chromatography was carried out using a JASCO TRI ROTAR on a Partisil-10 SCX column (8.0 mm × 250 mm), which was eluted with a 30 mM ammonium phosphate (pH 3.0) buffer (flow rate 4.0 ml/min) and detected on a JASCO UVIDEC 100-II spectrometer. The retention time was determined by means of a SYSTEM INSTRUMENTS model 500E integrator.

(S)-6-Methyl-5,6,7,8-tetrahydropterin (3) and the *(S)-7-Methyl Isomer (4).* A mixture of 2,4-diamino-5-bromo-6-hydroxypyrimidine[11] (1.0 g), (*S*)-1,2-propanediamine[12] (4.0 g), and acetic acid (0.7 g) was heated at 115 °C under hydrogen atmosphere for 7 h. The excess amine was removed by distillation under diminished pressure. The residue was dissolved in water (5 ml) and made acid with acetic acid. The solution was fractionated on a Florisil column (20 mm × 80 mm), eluted by 0.3 M acetic acid. The eluate was concentrated to about 10 ml and then passed through a CM-Sephadex column (20 mm × 250 mm). The column was washed with water (1000 ml). The tetrahydropterins were eluted gradiently by 0—0.1 M hydrochloric acid (100 ml). The yield of the tetrahydropterins, as a mixture, was estimated to be 15% from the UV absorbance at 265 nm of the pooled eluate. The eluate was concentrated to about 6 ml under reduced pressure. A 0.5 ml aliquot of the concentrate was injected to a preparative Partisil-10 SCX column (8 mm × 250 mm) and eluted with the above mentioned ammonium phosphate buffer (4.0 ml/min) to give (*S*)-6-methyltetrahydropterin (retention time 21.5 min) and the (*S*)-7-methyl isomer (retention time 22.7 min). The pooled eluates of each fraction were used for measuring the UV and CD spectra.

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