# Studies on Biologically Active Pteridines. V.[1] Synthesis of (6*S*)-5,6,7,8-Tetrahydro-1,3,5,6-tetramethyllumazine

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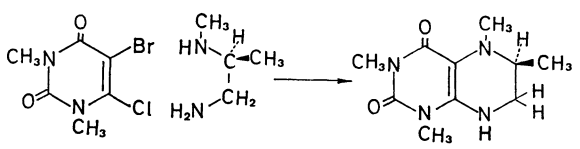
**Synopsis.** (+)-5,6,7,8-Tetrahydro-1,3,5,6-tetramethyllumazine, a compound derived from enzymically reduced (−)-5,6,7,8-tetrahydro-6-methylpterin, was shown to be of (*S*)-configuration at the C-6 chiral center by a synthesis, which was performed by condensation of 5-bromo-6-chloro-1,3-dimethyluracil and (2*S*)-1-amino-2-(methylamino)propane. The structure of the condensation product was determined unequivocally by an independent synthesis using a regioselective methylation of 5,6,7,8-tetrahydro-1,3,6-trimethyllumazine.

(−)-5,6,7,8-Tetrahydrobiopterin[2] is an essential cofactor for phenylalanine,[3] tyrosine,[4] and tryptophan hydroxylases,[5] all of which play important roles in the biosynthesis of neurotransmitting dopamine or serotonine. Thus, deficiency of tetrahydrobiopterin causes severe neurological disturbance such as atypical phenylketonuria.[6] Tetrahydrobiopterin is produced stereospecifically referring to the C-6 chiral center from 7,8-dihydrobiopterin by dihydrofolate reductase and NADPH; chemical reduction gives a mixture of diastereomers. Since the two diastereomers show different biological properties,[7,8] determination of the C-6 configuration of natural (−)-tetrahydrobiopterin is essential for understanding its properties in biological systems. 5,6,7,8-Tetrahydro-6-methylpterin is also produced stereospecifically from the 7,8-dihydro precursor by the action of the same enzyme, and thus formed (−)-tetrahydro-6-methylpterin and (−)-tetrahydrobiopterin were shown to have same L-configuration at C-6 in a rather indirect manner by comparison with 1,2,3,4-tetrahydro-2-methylquinoxaline.[8]

As an alternative and more direct approach to confirm the C-6 configuration of these pterins, we studied the C-6 configuration of (+)-5,6,7,8-tetrahydro-1,3,5,6-tetramethyllumazine, a compound recently derived from (−)-tetrahydro-6-methylpterin by methylation and deamination without altering the configuration.[9]

We synthesized (6*S*)-5,6,7,8-tetrahydro-1,3,5,6-tetramethyllumazine (**3**) from 5-bromo-6-chloro-1,3-dimethyluracil(**1**) and (2*S*)-1-amino-2-(methylamino)propane (**2**). Heating of **1** and **2** in ethanol gave a tetrahydrolumazine as a main product, which showed a weak basicity (p$K_a$ 5.39) and $\lambda_{max}$ at 235(sh.) and 286 (pH 8.0) and at 263 (pH 3.0). Its $^{13}$C-NMR spectrum showed signals at the following chemical shifts (ppm): 160.3(C-4), 150.7(C-2), 144.1(C-8a), 99.5(C-4a), 50.7 (C-6), 40.0(C-7), 41.4, 28.9, 27.9 (three N–$CH_3$), and



**1** **2** **3**

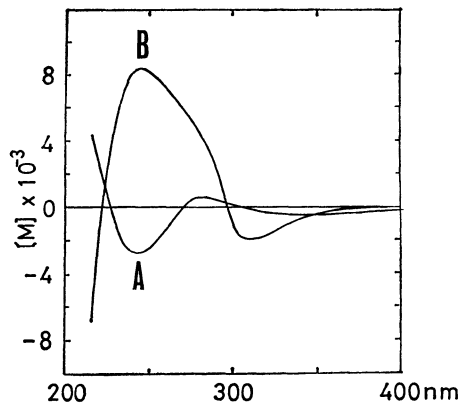



Fig. 1. The ORD curves of (6*S*)-**3**: **A**, at pH 3.0 (cation) and **B**, at pH 8.0 (neutral molecule).

17.1(C–$CH_3$). We tentatively assigned the compound as **3**, because it showed resistance toward air oxidation, an important characteristic of N-5 substituted tetrahydropteridines. However, the observed chemical and physical properties could not exclude the possibility that the compound might have the isomeric structure, *i.e.*, 5,6,7,8-tetrahydro-1,3,7,8-tetramethyllumazine. In order to confirm the above assignment, we synthesized racemic **3** by an independent method: 1,3,6-trimethyllumazine[10] was hydrogenated over platinum oxide into 5,6,7,8-tetrahydro derivative, which was subsequently hydrogenated in the presence of formaldehyde over the same catalyst. A methyl group was introduced regioselectively at N-5 to produce (±)-**3**, whose spectral data allowed to confirm the condensation product as (6*S*)-**3**.

The cation of (6*S*)-**3** showed, as expected, positive Cotton effect in ORD with a peak at 283 nm and trough at 242 nm (Fig. 1), though neutral (6*S*)-**3** gave opposite sign of rotation. The present results confirmed that (+)-**3**, derived from (−)-tetrahydro-6-methylpterin, has (6*S*)-configuration. Consequently, it is concluded that the enzymically reduced (−)-tetrahydro-6-methylpterin has (6*S*)-configuration and the (−)-tetrahydrobiopterin the same (6*R*)-configuration.

## Experimental

The elemental analyses were carried out at the Analytical Section, Meijo University. The p$K_a$ values were determined spectroscopically on a Shimadzu UV-300 spectrometer. The ORD curves were measured on a JASCO ORD/UV-5 spectropolarimeter in appropriate pH buffer solutions and the $^{13}$C-NMR spectra on a JEOL JNM-PFT-60 NMR spectrometer in $CDCl_3$. The chemical shifts are expressed in terms $\delta$ (ppm downfield from the internal TMS).

*(2S)-1-Amino-2-methylaminopropane (2).* L-Alaninamide[11] (60 g) was formylated by acetic formic anhydride

at 20 °C to give (2*S*)-2-(formamino)propanamide (45 g), mp 112—113 °C (from ethanol) (Found: C, 41.59; 7.11; N, 23.84%. Calcd for $C_4H_8N_2O_2$: C, 41.37; H, 6.94; N, 24.13%). The amide (30 g) and $LiAlH_4$ (45 g) in tetrahydrofuran (1.5 l) was refluxed for 24 h and the excess reagent was decomposed by water. The filtered solution was fractionally distilled twice to give **2** (10 g) boiling at 112—122 °C; $^{13}$C-NMR: at $\delta$ 58.0 ($CH_2$), 55.4 (CH), 36.7 (N-$CH_3$), 24.0 (N-$CH_3$), and 18.2 (C-$CH_3$).

*(6S)-5,6,7,8-Tetrahydro-1,3,5,6-tetramethyllumazine (**3**).* A solution of **1**[12] (7.0 g), **2** (2.5 g), and triethylamine (7 g) in ethanol (100 ml) was heated under reflux and nitrogen for 7 h. The solution was evaporated and the residue was dissolved in water (50 ml). The solution was adjusted to pH 1—2 with HCl and extracted continuously with chloroform for 2 h. The aqueous solution was adjusted to pH 7—8 with $NaHCO_3$ and again extracted continuously with chloroform for 1 h. The latter extract was fractionated on a silica gel column (4.5 cm × 40 cm) developed by ethanol and ethyl acetate (1:9). The fraction of the main product was chromatographed once more as above. Evaporation of the eluate gave an oily residue, which was mixed with ethanol (0.5 ml). Dilution with ether (10 ml) and chilling gave colorless prisms (4.85 g) of **3**, mp 164—166 °C (dec), (Found: C, 53.58; H, 7.34; N, 24.90%. Calcd for $C_{10}H_{16}N_4O_2$: C, 53.55; H, 7.19; N, 24.99%); p$K_a$ 5.39±0.01; $\lambda_{max}$ (log $\varepsilon$) in nm at pH 8.0: 235 (sh., 3.72) and 286 (4.11); at pH 3.0: 263 (4.22).

*Racemic **3**.* 1,3,6-Trimethyllumazine (1.5 g) was hydrogenated over platinum oxide (0.5 g) in methanol (100 ml) at atmospheric pressure and temperature for 1 h. Formalin (37%, 2.5 g) was added to the mixture and hydrogenation was continued over night. The filtered solution was evaporated and chromatographed as above to give colorless prisms (1.24 g) of (±)-**3**, mp 200—202 °C (dec), (from ethanol) (Found: C, 53.63; H, 7.42; N, 24.64%. Calcd for $C_{10}H_{16}N_4O_2$: C, 53.55; H, 7.19; N, 24.99%); p$K_a$ 5.36±0.01; $\lambda_{max}$ nm (log $\varepsilon$) at pH 8.0: 235 (sh., 3.73) and 286 (4.10); at pH 3.0: 263 (4.22); $^{13}$C-NMR at $\delta$ 160.3, 150.7, 143.9, 99.6, 50.8, 41.4, 40.1, 28.9, 28.0, and 17.1.

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