DOI 10.5012/bkcs.2011.32.8.3161

Structural Elucidations of Pyrano[3,2-*g*]pteridine Derivatives by 2D NMR Spectroscopy†

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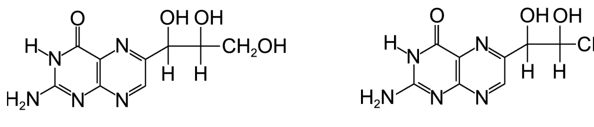
Received March 14, 2011, Accepted April 20, 2011

Key Words : Neopterin, Biopterin, Pyrano[3,2-*g*]pteridine

Pterin derivatives are natural materials biosynthesized from guanosinetriphosphate (GTP) in biological systems and generated from all living things in the nature. Biopterin was reported as a 6-(D-*erythro*-1,2-dihydroxypropyl)pterin isolated from human's urine.[1] Its biosynthetic pathway has been paid attention as they play an important role in amino acid hydroxylation as a tetrahydroform. Atypical phenylketouria disease is caused by the defect of biosynthetic process for tetrahydrobiopterin.[2,3] Neopterin was identified as a 6-(D-*erythro*-1,2,3-trihydroxypropyl)pterin from larva of bee, royal jelly[4,5] and human's urine.[6]



Neopterin was synthesized by Viscontini Reaction of 2,5, 6-triamino-pyrimidin-4(3*H*)-one and D-arabinose phenylhydrazone, with more than 80% of yield,[7] while biopterin was obtained from the reaction of 5-deoxy-L-arabinose phenylhydrazone with 40% yield.[8] In general, high yield of sugar hydrazone was produced from the simple reaction of sugar and phenylhydrazine, however, 5-deoxy-L-arabinose phenylhydrazone was obtained through the several steps.

Both neopterin and biopterin are formed by the addition of 1,2,3-trihydroxypropyl group and 1,2-dihydroxypropyl group to the 6-position of the pterin, respectively. Therefore, the substitution of terminal 3-OH group with methyl group in neopterin is strongly prospected to improve the synthetic process of biopterin. The selective protection to the 1,2-dihydroxyl group, the secondary alcohols, is necessary for the conversion of neopterin into biopterin. There were few attempts to protect the two secondary alcohols of neopterin derivatives.[9-11] However, the trials failed because the primary 3-OH in 1,2,3-trihydroxypropyl group has higher chemical reactivity than the others.

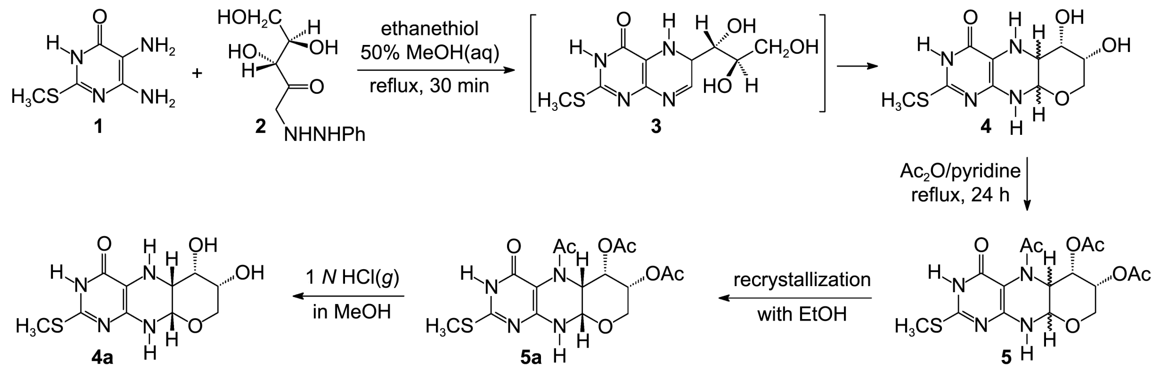
In general, 2-aminopteridin-4(3*H*)-one (pterin) derivatives are insoluble in water or organic solvents. 2-Alkylthiopterin derivatives which provide better solubility than ordinary pterins and can be converted easily to the corresponding pterins by replacement reaction of 2-alkylthio group with amines[12] were synthesized.

For the synthesis of 6-(D-*erythro*-1,2,3-trihydroxypropyl)-2-methylmercapto-4-oxo-3,4,5,6-tetrahydropteridine (**3**), the analogous of neopterin, the condensation of 4,5-diamino-2-methylmercapto-6-oxopyrimidine (**1**) with and D-arabinose phenylhydrazone (**2**) in acidic medium by a method similar to the reported procedure[7] provided two compounds on TLC (R_f = 0.31 and 0.4, SiO_2, EtOAc:*n*-PrOH:H_2O=4:1:2, *v/v*, upper phase). From ^1H NMR spectrum, the isolated precipitate was expected to be a diastereomeric mixture, 3*R*,4*S*-dihydroxy-8-methylmercapto-6-oxo-3,4,4a(*R*,*S*), 5,6,7,10,10a(*S*,*R*)-octahydro-5,10*H*-pyrano[3,2-*g*]pteridine (**4**). It was assumed that the compound **3** was converted in situ to diastereomeric mixture **4** in acidic medium (Scheme 1). Compound **4** is predicted to be a key compound in converting neopterin to biopterin because it can be converted to neopterin derivative with free primary 3'-OH group by introducing protecting group to the secondary 3- and 4-OH groups of its pyran ring followed by oxidation. Because of the difficult chromatographical separation of this mixture due to the poor solubility in organic solvent, compound **4** was acetylated with acetic anhydride in pyrimidine and triacetylated derivatives **5** were obtained. By the recrystallization of **5** with ethanol, a portion of 3*R*,4*S*-diacetoxy-5-acetyl-8-methylmercapto-6-oxo-3,4, 4a(*R*),5,6,7,10,10a(*S*)-octahydropyrano[3,2-*g*]pteridine (**5a**) was selectively separated and the rest remained in filtrate as a mixture with the other diastereomer. From ^1H-NMR spectrum of **5a**, trimethyl group of acetyls appeared at 2.2, 1.9, and 1.8 ppm as singlets. In NOESY spectrum of **5a**, C10a-H at 5.6 ppm was correlated with the N-H peak at 7.1 ppm and the C4a-H peak at 5.0 ppm. The deacetylation of **5a** by the treatment with 1*N* methanolic HCl solution gave 3*R*,4*S*-8-methylmercapto-6-oxo-3,4,4a(*R*),5,6,7,10,10a(*S*)-octahydro-pyrano[3,2-*g*]pteridine (**4a**) (Scheme 1).

In the COSY spectrum of **4a**, the proton on C4a is clearly coupled to the protons on C4, C10a, and N5. Additionally, the proton C10a-H correlates to proton C4a-H (Figure 1).

The spectra presented in Figure 2 illustrates NOE difference analysis of **4a**. The bottom spectrum in Figure 2 shows the normal proton spectrum of **4a**. The top spectrum was determined with the simultaneous irradiation of **4a** at 3.1 ppm. The peaks in the spectrum that appear as the positive

†This paper is dedicated to Professor Eun Lee on the occasion of his honourable retirement.



Scheme 1

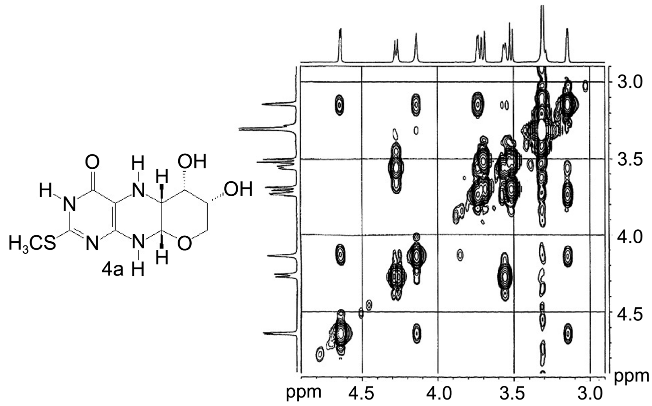


Figure 1. The COSY spectrum of 3*R*,4*S*-8-methylmercapto-6-oxo-3,4,4a(*R*),5,6,7,10,10a(*S*)-octahydropyrano[3,2-*g*]pteridine (**4a**) in DMSO-d_6.

peaks are the 4-H peak at 3.7 ppm, the 10a-H peak at 4.6 ppm, and the N10-H peak at 7.7 ppm. Finally, the middle spectrum was determined with the simultaneous irradiation of **4a** at 4.6 ppm. The peaks that appear as the positive peaks are the 4a-H peak at 3.2 ppm, the upper proton of the 2-H peak at 3.5 ppm, and the N10-H peak at 7.7 ppm, as expected.

With the results so far achieved from various NMR spectroscopic analysis, the configuration of **4a** was identified as 3*R*,4*S*-8-methylmercapto-6-oxo-3,4,4a(*R*),5,6,7,10,10a(*S*)-octahydropyrano[3,2-*g*]pteridine as shown in Figure 3.

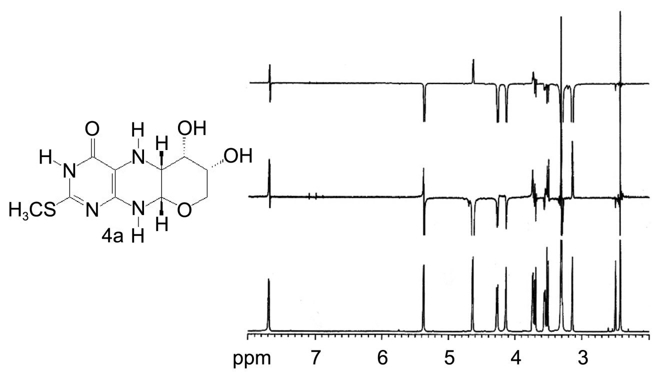


Figure 2. NOE difference spectrum of **4a**. Top spectrum: NOE difference spectrum with irradiation at 3.1 ppm. Middle spectrum: NOE difference spectrum with irradiation at 4.6 ppm: Bottom spectrum: proton NMR spectrum of **4a** without decoupling.

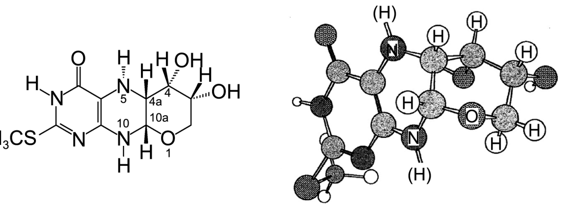


Figure 3. The configuration of 3*R*,4*S*-8-methylmercapto-6-oxo-3,4,4a(*R*),5,6,7,10,10a(*S*)-octahydropyrano[3,2-*g*]pteridine (**4a**).

Experimental Section

Diastereomeric Mixture of 3*R*,4*S*-dihydroxy-8-methylmercapto-6-oxo-3,4,4a(*R,S*),5,6,7,10,10a(*S,R*)-octahydro-5,10*H*-pyrano[3,2-*g*]pteridine (4). To 360 mL of 50% aqueous methanol which was saturated with N_2 gas, 4,5-diamino-2-methylmercapto-6-oxo-pyrimidine (**1**, 6.5 g, 27 mmol), D-arabinose phenylhydrazone (**2**, 6.5 g, 27 mmol), and ethanethiol (1 mL) were added. The reaction mixture was heated under reflux for 30 min. After cooling, the reaction mixture was filtered, and the yellow solid was washed with ethanol and diethyl ether repeatedly. The solid was dried under vacuum to obtain compound **4** in 66% yield. The product **4** was acetylated without further purification.

3*R*,4*S*-Diacetoxy-5-acetyl-8-methylmercapto-6-oxo-3,4,4a(*R*),5,6,7,10,10a(*S*)-octahydropyrano[3,2-*g*]pteridine (5a). The reaction mixture of **4** (2.5 g, 8.7 mmol) and acetic anhydride (50 mL) in anhydrous pyridine (30 mL) was heated under reflux for 24 h. After the addition of methanol (50 mL) to the hot reaction mixture carefully, the insoluble precipitate was removed by hot filtration. The filtrate was evaporated to the dryness under vacuum. The recrystallization of the remained residue with ethanol provided the compound **5a** in 35% yield as a colorless crystal. mp 228 °C; UV (MeOH) λ_{max} (logε) 236 (4.33), 295 (3.98); ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.8, 2.0, 2.2 (s, CH_3 in acetyl, 3 × 3H), 2.5 (s, SCH_3, 3H), 3.7 (q, C2-H, 1H), 3.5 (q, C2-H, 1H), 5.0 (q, C3-H, 1H), 5.1 (t, C4a-H, 1H), 5.5 (q, C4-H, 1H), 6.2 (d, C10a-H, 1H), 12.1 (bs, N7-H, 1H); ^{13}C-NMR (300 MHz, DMSO-d_6) δ 12.7 (SCH_3), 22.0, 20.6, 20.3 (CH_3 in acetyl), 169.1, 169.8, 170.7 (C=O in acetyl), 42.8 (C4a), 60.7 (C2), 64.3 (C3), 68.1 (C4), 77.0 (C10a), 94.1 (C5a), 152.2 (C9a), 157.8 (C8), 158.6 (C6).

3*R*,4*S*-8-Methylmercapto-6-oxo-3,4,4a(*R*),5,6,7,10,10a (*S*)-octahydropyrano[3,2-*g*]pteridine (4a). The compound **5a** (1 g, 2.5 mmol) was stirred in 1 *N* methanolic HCl solution (150 mL) at room temperature for 2 d. The reaction mixture was evaporated to the dryness under vacuum. The recrystallization of the remaining residue with methanol twice gave the compound **4a** in 72% yield as a slightly yellow powder. mp 226 °C; UV (pH 4) λ_{max} (logε) 229 (4.32), 305 (3.94); ^{1}H-NMR (300 MHz, DMSO-d_6) δ 2.4 (s, SCH_3, 3H), 3.2 (d, C4a-H, 1H), 3.5 (q, C2-H, 1H), 3.6 (q, C3-H, 1H), 3.7 (q, C2-H, 1H), 3.7 (q, C4-H, 1H), 4.1 (bs, N5-H, 1H), 4.2 (d, 3-OH, 1H), 4.6 (d, C10a-H, 1H), 5.4 (d, 4-OH, 1H), 7.7 (bs, N10-H, 1H); ^{13}C-NMR (300 MHz, DMSO-d_6) δ 12.8 (SCH_3), 53.3 (C4a) 67.9 (C3), 68.1 (C2), 68.4 (C4), 79.0 (C10a), 105.0 (C5a), 146.3 (C8), 151.4 (C9a), 156.3 (C6); Anal Calcd For $C_{10}H_{14}N_4O_4S\cdot 1/2H_2O$: C, 40.67; H, 5.12; N, 18.97. Found: C, 40.87; H, 5.32; N, 18.94.

Acknowledgments. This work was supported by Hanyang University Research Grant, 2006.

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