STUDIES ON UROTHION. DETERMINATION OF THE ABSOLUTE CONFIGURATION OF THE DEPHOSPHO FORM B DERIVED FROM UROTHION

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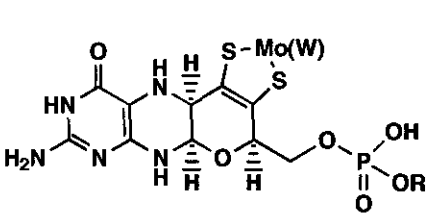
Abstract - In order to determine the absolute configuration of urothion (**4**), the CD spectrum of the dephospho form B (**3b**) derived from **4** was compared with those of both enantiomers of the dephospho form B ((***R***)-**3b** and (***S***)-**3b**) which were prepared by asymmetric synthesis. *R*-Configuration was concluded for the secondary hydroxyl group on the side chain of **3b**, as well as **4**, which is the same configuration as that of molybdopterin (**1**). This supports the view that **4** might be a urinary metabolite of **1**.

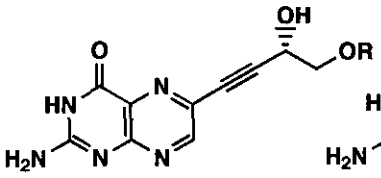
The pioneering analytical studies on representative of the oxomolybdoenzymes by Rajagopalan[1] showed the presence of a pteridine (molybdopterin) having two sulfur atoms which ligate molybdenum. X-ray crystallographic determinations on the oxomolybdoenzymes clearly define the nature of molybdopterin and its mode of ligation to Mo and W **1**.[2] Several oxidative degradation products of **1** have been isolated. Structure (**2**) has been assigned to form A derived from **1**, including the absolute configuration of *S* at the chiral center on the side chain.[3-5] On the other hand, form B, which possesses a fused thiophene ring and retains one of the original sulfur atoms, has been assigned to planar structure (**3a**).[3,4,6]

The planar structure of urothion,[7] a pteridine pigment in urine, has been determined as **4** by total synthesis.[8,9] Furthermore, on the basis of the metabolic study of the molybdenum cofactor, urothion (**4**) was presumed to be a urinary metabolite of **1**.[4] However, the biological activity and the biochemical significance of **4** are unknown.

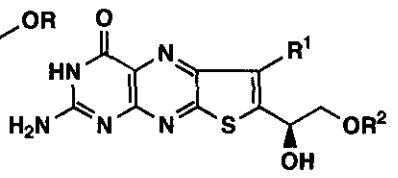
We reported in the previous papers on the structure determination of **4** that the desulfurization of **4** with Raney Ni afforded 2-amino-6-(3,4-dihydroxybutyl)pteridin-4(3*H*)-one (**5**) and 7-amino-2-(1,2-dihydroxyethyl)-2,3-dihydrothieno[3,2-*g*]pteridin-5(6*H*)-one (**6**).[7] Furthermore, the absolute configuration at the



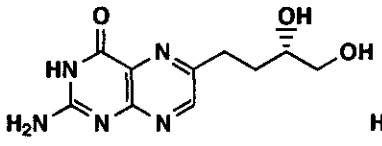
1 : R=H or nucleoside



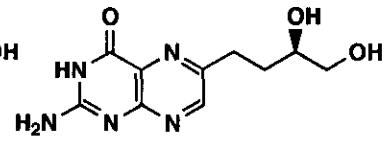
2 : R =PO_3H_2



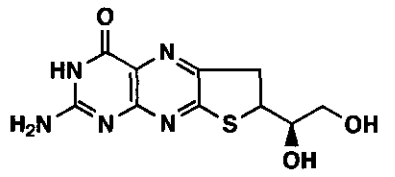
3a : R^1=H R^2=PO_3H_2
3b : R^1=R^2=H
4 : R^1=SCH_3 R^2=H



***(S)*-5**



***(R)*-5**



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side chain of **5** has been determined to have *S*-configuration, on the basis of the asymmetric syntheses of (*S*)-**5** and (*R*)-**5**, which suggested for **4** to have *R*-configuration at the chiral center.[10]

On the other hand, clear evidence for the chirality of the dephospho form B (**3b**) would be given by the asymmetric synthesis of **3b** and by the comparison with **3b** derived by selenium dioxide oxidation of **6**. For this purpose, we achieved the syntheses of 7-amino-2-[(1*R*)-1,2-dihydroxyethyl]thieno[3,2-*g*]pteridin-5(6*H*)-one ((*R*)-**3b**) and its (1*S*)-enantiomer ((*S*)-**3b**), and reported the results in our previous paper.[12] This paper deals with CD spectral determination of the absolute configuration of **3b**.

EXPERIMENTAL

^{1}H NMR spectra were recorded with a Bruker AC300P (300 MHz) spectrometer. CD spectra were measured with a JASCO J-500A CD spectrophotometer. UV spectra were measured with a Shimazu UV-3100 UV sepctrophotometer.

7-Amino-2-(2,3-dihydroxyethyl)-2,3-dihydrothieno[3,2-*g*]pteridin-5(6*H*)-one (6). Compound (**6**) was prepared according to the method described in our previous paper.[7] Urothion (**4**) (9 mg) which was isolated from human urine by a method newly devised by us, afforded **6** (3 mg) by reduction with hydrogen in the presence of Raney Ni, followed by purification by ion exchange chromatography (Dowex 1×8, 200~400 mesh, $CH_3CO_2^-$ form, 2×20 cm, eluent: 0.05 mol L^{-1} $CH_3CO_2NH_4$).

Oxidation of 6 derived from 4 by SeO_2. To a solution of **6** (3 mg, 0.011mmol) in acetic acid (3 mL) was added SeO_2 (2.2 mg, 0.020 mmol). The mixture was stirred for 2 h at 80 °C under an argon atmosphere, followed by dilution with water (25 mL) and adsorption on a column of Frorisil (5 g). After washing with water (500 mL), elution with acetone/water (1:1, 100 mL) and evaporation of the solvent,

the residue was purified by the reverse phase column chromatography (Merck, LiChroprep RP-8, size B, eluent: aqueous 20% MeOH), to afford 2.8 mg (yield 93%) of 7-amino-(1,2-dihydroxyethyl)thieno-[3,2-*g*]*pteridin-5(6H)-one* (**3b**) *as yellowish crystals:* mp 198~205 °C, decomp (from water). UV (0.1 mol L^{-1} NaOH) 242 (ε 23800), 268 (28600), and 395 nm (12700); ^{1}H NMR (DMSO-d_6) δ =3.61 (2H, m, $C\underline{H}_2OH$), 4.90 (1H, br q, J=5 Hz, $C\underline{H}OH$), 5.04 (1H, br t, J=6 Hz, $CH_2O\underline{H}$), 6.10 (1H, br d, J=5 Hz, $CHO\underline{H}$), 6.95 (2H, br s, NH_2), 7.38 (1H, s, =CH), and 8.45 (1H, br s, NHCO); CD (1% NH_3) λ ext 260 nm (Δ ε -1.3).

7-Amino-2-[(1*R*)-1,2-dihydroxyethyl]thieno[3,2-*g*]pteridin-5(6*H*)-one ((*R*)-3b) and Its (1*S*)-Enantiomer ((*S*)-3b). Compounds (***R***)-**3b** and (***S***)-**3b** were synthesized, respectively, in the same method as described in our previous paper.[11] Their UV and ^{1}H NMR spectra were identical with those of **3b** derived from **4**. (***R***)-**3b**: CD (1% NH_3) λ ext 260 nm (Δ ε -1.3). (***S***)-**3b**: CD (1% NH_3) λ ext 260 nm (Δ ε +1.3).

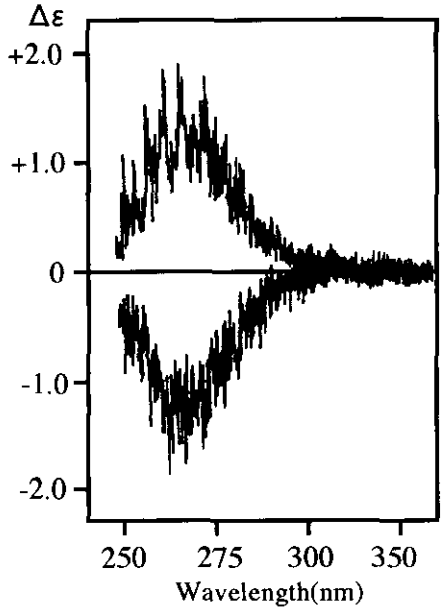

Figure 1. The CD spectra of the synthesized (*S*)-**3b** (upper) and (*R*)-**3b** (bottom) in 1% NH_3.

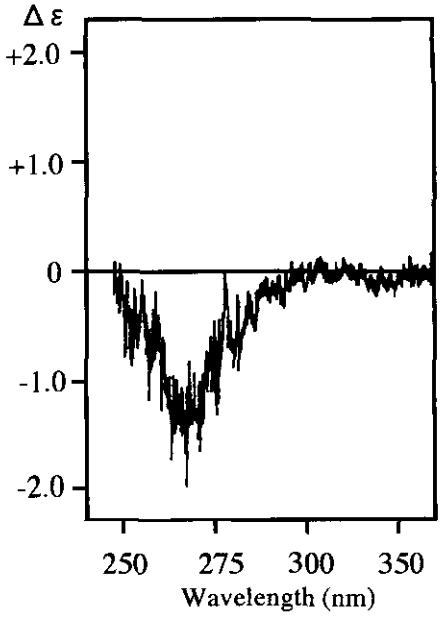

Figure 2. The CD spectrum of **3b** derived from **4** in 1% NH_3.

RESULTS AND DISCUSSION

The UV and ^{1}H NMR spectra of **3b** derived from the natural **4** were identical with those of the asymmetrically synthesized (***R***)-**3b** and (***S***)-**3b**.

For the determination of the absolute configuration, CD spectra in 1% aqueous ammonia of (***R***)-**3b** and (***S***)-**3b** were compared with those of **3b** derived from **4**. The reults are shown in Figures 1 and 2, respectively. The spectrum of **3b** was found superimposable with that of (***R***)-**3b**. From these

observations, **3b** was confirmed to have the *R*-configuration at the chiral center on the side chain. This finding also led to the *R*-configuration of **4**, as was suggested from the asymmetric syntheses of the desulfurizaton products ((***R***-**5**) and (***S***)-**5**).[10]

From discovery that children suffering from biochemical abnormalities due to combined deficiencies of sulfite oxidase and xanthine dehydrogenase lacked the active molybdenum cofactor (molybdopterin), and that their urine was devoid of urothion, Johnson and Rajagopalan proposed the view that urothion was a urinary metabolite of the molybdenum cofactor.[4]

In conclusion, these results demonstrate that **4** has *R*-configuration, which is identical with the configuration in **1**. This supports the view that **4** might be a urinary metabolite of **1**, as well as the results concluded in our previous reports.[10]

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