HETEROCYCLES, Vol. 56, 2002, pp. 387 - 392, Received, 9th August, 2001

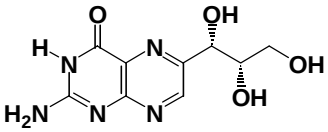
A HIGHLY SENSITIVE AND SPECIFIC FDCD METHOD FOR CHIRALITY ANALYSIS OF NATURALLY OCCURRING PTERIDINES

Ning Chen,[a)] Kazuhisa Ikemoto,[a)] Takashi Sugimoto,[a)] Shizuaki Murata,[*a,b)] Hiroshi Ichinose,[c)] and Toshiharu Nagatsu[c)]

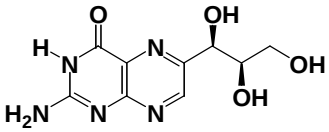
[a)]Graduate School of Environmental Studies, Nagoya University
Chikusa, Nagoya 464-8601, Japan
E-mail: murata@urban.env.nagoya-u.ac.jp
[b)]CREST, JST (Japan Science and Technology)
c/o Graduate School of Environmental Studies, Nagoya University
[c)]Division of Molecular Genetics, Neurochemistry,
Institute for Medical Science, Fujita Health University School of Medicine,
Toyoake, Aichi 470-1192, Japan

Abstract—Absolute configurations of 6-(1-hydroxyalkyl)pterin derivatives are determined by fluorescence detected circular dichroism (FDCD) spectroscopy. This method is at least 10 times more sensitive than CD analysis and specific even existence of 10 times excess amounts of chiral sugars or nucleic acids.

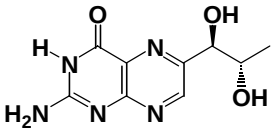
Optically active 6-(1-hydroxyalkyl)pterin[1] is one of the most important nitrogen heterocyclic compounds in living organisms.[2—5] The structural analyses of biopterin, neopterin, and the related pterin derivatives in body fluid, urine, serum, cell extracts, and so on obtained from various kinds of animals and micro organisms have been carried out mainly by using CD and HPLC methodologies,[6,7] and, in particular, only CD analysis was the straightforward technique to determine the absolute configuration of pterins. Although the combination of CD and HPLC is a powerful tool to study structures of tetrahydropterins such as biopterin cofactor,[8] its applications to aromatic pterins were insufficient because of lower and higher sensibilities toward the desired subject and chiral contaminants, respectively. Recently, we found L-*threo*-monapterin (**1**) and D-*threo*-monapterin (**2**) as major pterins in cell extracts of *Escherichia coli*[9] and *Tetrahymena pyriformis*[10] with very low concentrations, respectively, using fluorescence detected circular



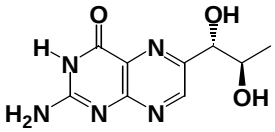
1



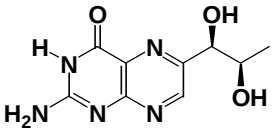
2



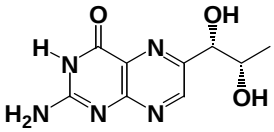
3



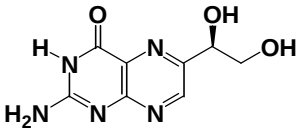
4



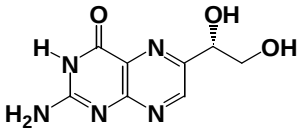
5



6



7



8

dichroism (FDCD) spectroscopy.[11,12] In this paper, we would like to describe the scope and limitation of the FDCD method for structural analyses of biopterin, neopterin, and the related compounds.

EXPERIMENTAL

Materials: The compounds (**1**, **2**), L-*erythro*-biopterin (**3**: the major metabolite of biopterin cofactor), D-*erythro*-biopterin (**4**), D-*threo*-biopterin (**5**), L-*threo*-biopterin (**6**), (*R*)-6-(1,2-dihydroxyethyl)-pterin (**7**), and (*S*)-6-(1,2-dihydroxyethyl)pterin (**8**), were prepared by the condensation of 2,5,6-triamino-4-hydroxypyrimidine with phenylhydrazone derivatives of corresponding sugars as sited in the references.[13,14] Purities of these pterins were confirmed by HPLC analyses.

CD and FDCD Spectra Measurement: CD spectra were recorded on a JASCO J-720 CD spectrometer, and FDCD spectra were detected on a JASCO FDCD-357 module connected to a JASCO J-720 CD spectrometer. Both CD and FDCD spectra were taken in a 1.0 x 1.0 x 5.0 (cm) quartz cell at 22 ºC in a 0.2 *M* potassium phosphate buffer solution (pH 5.3). FDCD spectra were measured by fixed voltage (HT), which DC output voltage from the detector was adjusted to 1.2 V at 275 nm, and represented as excitation spectra.[12] Since the CD analyzer observes absorption of light and FDCD observes fluorescence (emission) intensity, both spectra of the same sample should appear as opposite figures. Therefore observed FDCD spectra were treated to show the same figures as CD spectra.[12]

RESULTS AND DISCUSSION

Relationship between Cotton Sign and Configuration of Pterins: CD and FDCD spectra of **3** and **4** with concentrations equal to 2.11 x 10^{-5} *M* are shown in Figure 1. Positive and negative Cotton effects were observed in the case of **3** which has the 1'*R* and 2'*S* configuration at 238 and 211 nm, respectively. On the contrary, the diastereomer (**5**) with the 1'*R* and 2'*R* configuration exhibited positive and negative Cotton effects at the similar wavelengths, correspondingly. Thence, CD spectra of 6-substituted pterins might be

caused by the influence of the chirality on the C(1') position, and the *R* configuration would afford the positive Cotton effect at 238 nm. The opposite sign in CD spectra would be observed on pterin derivatives with the 1'*S* configuration. Signs in CD spectra of compounds (**1**—**8**) at 300—220 nm in the same concentration are summarized in Table 1 together with those FDCD spectra.

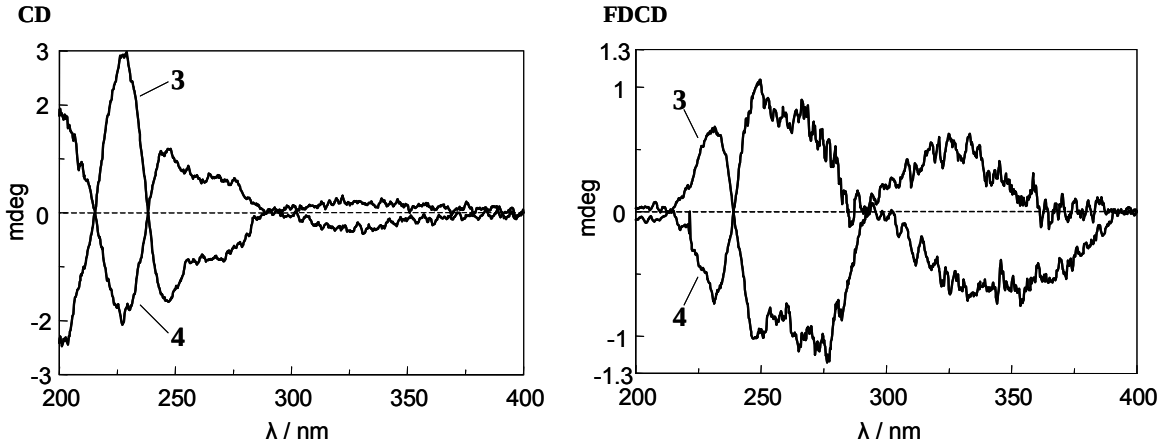


Figure. 1. CD and FDCD spectra of 3 and 4.

Table 1. CD and FDCD Spectra ([θ]/mdeg) of Pterins 1—8.

method	wavelength λ_{max}/nm	compounds[a]/ configuration of C(1')							
		1/S	**2**/R	**3**/R	**4**/S	**5**/R	**6**/S	**7**/R	**8**/S
CD	203	+2.14	-0.75	-2.35	+1.75	-0.78	+0.92	-0.67	+0.52
	211	0	0	0	0	0	0	0	0
	227	-2.81	+3.63	+2.91	-2.06	+2.10	-1.98	+1.92	-1.58
	238	0	0	0	0	0	0	0	0
	245	+1.91	-1.26	-1.58	+1.07	-1.04	+0.84	-0.96	+1.32
FDCD	211	0	0	0	0	0	0	0	0
	231	-0.58	+0.58	+0.64	-0.70	+0.70	-0.50	+0.72	-0.37
	238	0	0	0	0	0	0	0	0
	246	+1.29	-1.29	-0.97	+0.84	-0.96	+1.07	-0.94	+1.10

[a] c = 1.97 x 10^{-5} M (**1** and **2**), 2.11 x 10^{-5} M (**3**—**6**), 2.39 x 10^{-5} M (**7** and **8**).

In Figure 1 and Table 1, it is obvious that FDCD spectra were almost same as CD spectra at the 300—220 nm region. Since both CD and FDCD spectra of those compounds are caused by the same pigment (2-amino-4-hydroxypteridine) and chirality, the hypothesis that 1'*R* and 1'*S* configurations give the positive and negative Cotton effects, respectively, at 238 nm is reasonable. In spite of the optically isomeric structures, FDCD curves of **3** and **4** at 400—300 nm are not symmetric. The phenomenon is well

explained by LD (linear dichroism) of these compounds.[15,16] FDLD (fluorescent detected LD) signals of these compounds are strong enough to interfere with FDCD in the wavelength area over 300 nm, and the FDCD curves overlapping with the FDLD curves are observed. Thus, the optically isomeric structures of those pterins are distinguishable by FDCD spectra at the 220 —300 nm region.

Lower Detection Limit: When sample solutions (c = 2.11 x 10^{-5} M) of **3** and **4** were diluted to the 1/10 concentration, the typical CD curves could be hardly recognized. On the other hand, both enantiomers can be well recognized by FDCD spectra even in the 1/50 concentration (4.2 x 10^{-7} M), as shown in Figure 2. Therefore, the FDCD method is at least 10 times more sensitive than CD in the case of biopterin and the related pterins.

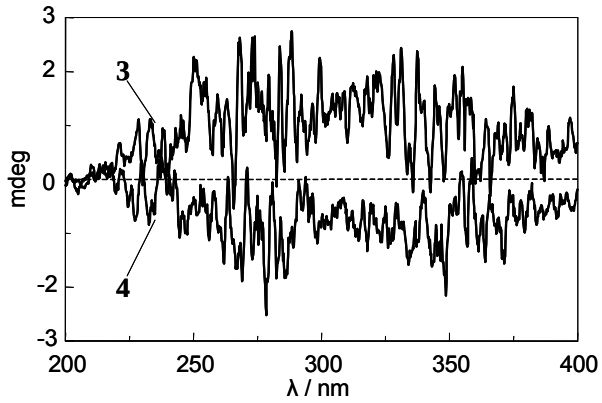


Figure 2. FDCD spectra of 3 and 4 (c = 4.2 x 10^{-7} M).

Effect of Chiral Contaminants: There are many chiral compounds in samples obtained from cell and body fluid of animals and microorganisms. Since almost all of these optically active compounds, such as sugars, amino acids, nucleic acids, and so on, are CD active, structural analyses of naturally occurring pterins using CD spectra sometimes might be interfered by the existence of these chiral contaminants. In order to elucidate the specificity, CD spectra were observed on 9:1, 1:1, and 1:9 mixtures of a solution of **3** (c = 2.11 x 10^{-5} M) and solutions of some chiral molecules, such as D-ribose, L-tyrosine, guanosine, uridine, L-tryptophan, and ascorbic acid (c = 2.11 x 10^{-5} M, respectively), and results are summerized in Table 2. There, "OK" means that the chirality is distinguishable and "NO" means not distinguishable. CD spectra of mixtures of **3** (c = 1.90 x 10^{-5} M) and 1/9 equivalent of D-ribose, L-tyrosine, or guanosine (c = 2.11 x 10^{-6} M) were nearly same as that of original **3**. However, CD spectra of mixtures of **3** and uridine, L-tryptophan, or L-ascorbic acid with the same concentrations (c = 1.06 x 10^{-5} M) were different from **3**. On the contrary, the chiralities of **3** and **4** (c = 2.11 x 10^{-6} M) are distinguishable by FDCD even in the presence of 10 times amounts of D-ribose, L-tyrosine, guanosine,[17] and uridine (c = 2.11 x 10^{-5} M). Because of the fluorescent nature of L-tryptophan and strongly reductive character of L-ascorbic acid, these

Table 2. CD and FDCD Spectra of 3 in the Presence of Chiral Compounds. *a)*

chiral compound	CD spectra			FDCD spectra		
	3/the chiral compound			**3**/the chiral compound		
	10	1	1/10	10	1	1/10
D-ribose	OK	OK	NO	OK	OK	OK
L-tyrosine	OK	NO	NO	OK	OK	OK
guanosine	OK	NO	NO	OK	OK	OK
uridine	NO	NO	NO	OK	OK	OK
L-tryptophan	NO	NO	NO	NO	NO	NO
L-ascorbic acid	NO	NO	NO	NO	NO	NO

a) 10, 1, And 1/10 mean that concentrations of **3** were 1.90 x 10^{-5}, and 1.06 x 10^{-5}, and 2.11 x 10^{-6} *M*, respectively. "OK" means that the the chirality of **3** is distinguishable in the solution, and "NO" means not.

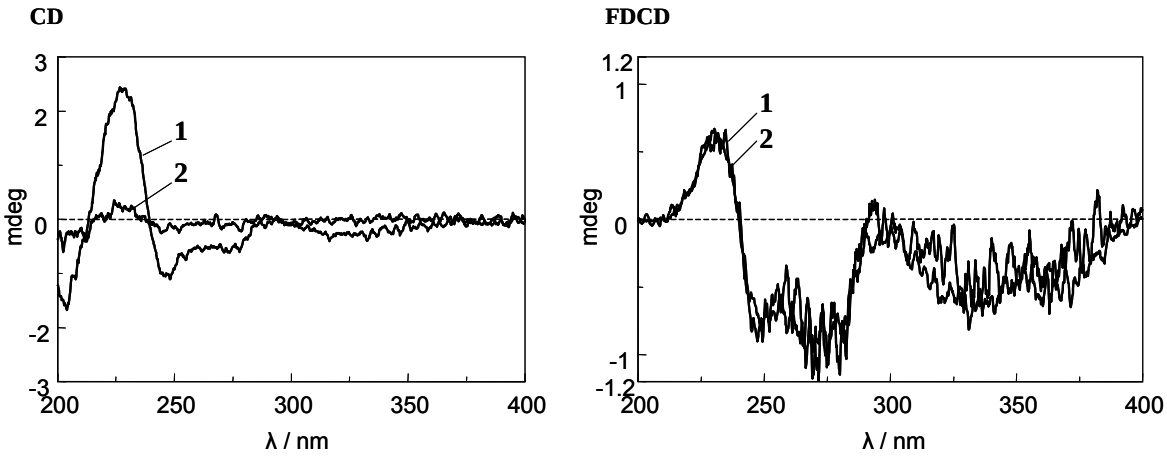


Figure 3. CD and FDCD spectra of a mixture of 3 and D-ribose. Curve 1: 3 (*c* = 1.90 x 10^{-5} *M*) and D-ribose (2.11 x 10^{-6} *M*); curve 2: 3 (1.06 x 10^{-5} *M*) and D-ribose (1.06 x 10^{-5} *M*).

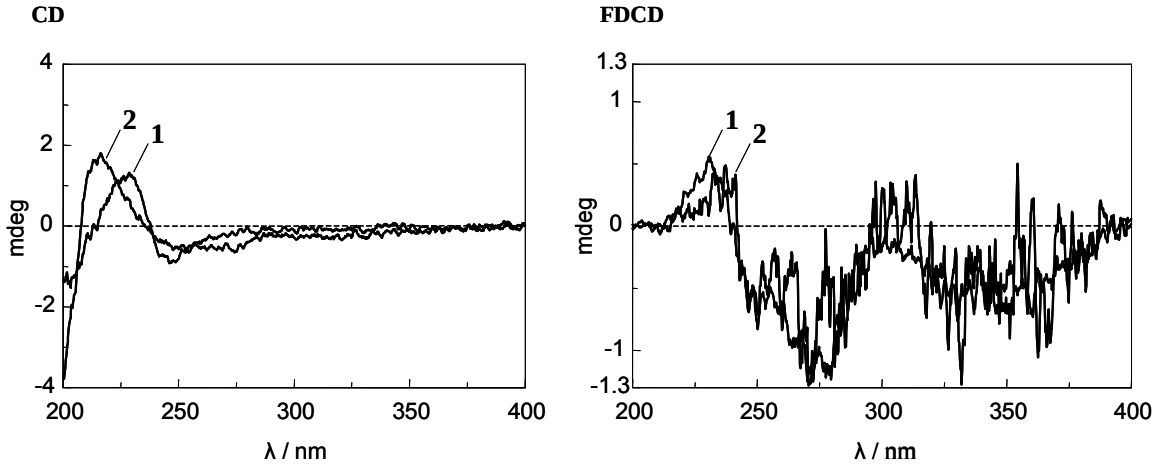


Figure 4. CD and FDCD spectra of a mixture of 3 and guanosine. Curve 1: 3 (*c* = 1.92 x 10^{-5} *M*) and guanosine (2.11 x 10^{-6} *M*); curve 2: 3 (1.06 x 10^{-5} *M*) and guanosine (1.06 x 10^{-5} *M*).

compounds disturbed FDCD observation of pterin. CD and FDCD spectra of **3** in the presence of various concentrations of D-ribose and guanosine are illustrated in Figure 3 and Figure 4, respectively.

ACKNOWLEDGMENTS

We thank Mr. Y. Andoh (School of Information and Sciences, Nagoya University) and Dr. A. Wada and Dr. T. Takakuwa (JASCO Co., LTD.) for their technical support.

REFERENCES AND NOTES

1. 2-Amino-4-hydroxypteridine is abbreviated as pterin.
2. J. C. Nixon, "Folates and Pterins," Vol. 2, ed. by R. L. Blakley and S. J. Benkovic, John Wiley & Sons, New York, 1985, pp. 1–42.
3. M. Sawada and T. Nagatsu, "Topic in neurochemistry and neurobiology," Vol. 2, ed. by W. Lovenberg and R. A. Levine, Talyor & Francis, Lndon, 1987, pp. 131–156.
4. H. Wachter, D. Fuchs, A. Hausen, G. Reibnegger, G. Weiss, and G. W.-Fermayer, "Neopterin," Walter de Gruyter, Berlin, 1991.
5. D. S. Bredt and S. H. Snyder, *Ann. Rev. Biochem.*, 1994, **63**, 175.
6. R. Klein, I. Tatischeff, G. Tham, and C. A. Crolire, *Biochemie*, 1991, **73**, 1281.
7. S. Ogiwara, T. Nagatsu, R. Teradaira, K. Fujita, and T. Sugimoto, *Biol. Chem. Hoppe-Seyler*, 1992, **373**, 1061.
8. S. Matsuura, T. Sugimoto, S. Murata, Y. Sugawara, and H. Iwasaki, *J. Biochem, Tokyo,* 1985, **98**, 1341.
9. T. Sugimoto, K. Ikemoto, S. Murata, M. Tazawa, T. Nomura, Y. Hagino, H. Ichinose, T. Nagatsu, and A. Wada, *Heterocycles*, 2001, **54**, 283.
10. T. Sugimoto, K. Ikemoto, S. Murata, M. Tazawa, T. Nomura, Y. Hagino, H. Ichinose, and T. Nagatsu, *Helv. Chim. Acta*, 2001, **85**, 918.
11. T. Nehira, C. A. Parish, S. Jockusch, N. J. Turro, K. Nakanishi, and N. Berova, *J. Am. Chem. Soc.*, 1999, **121**, 8681.
12. T. Takakuwa and A. Wada, *Jasco Report*, 1998, **40**, 12.
13. T. Sugimoto and S. Matsuura, *Bull. Chem. Soc. Jpn.*, 1975, **48**, 3767.
14. T. Sugimoto and S. Matsuura, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 181.
15. E. W. Lobenstine, W. C. Schaefer, and D. H. Turner, *J. Am. Chem. Soc.*, 1981, **103**, 4936.
16. The effect of LD is significant when the substrate has larger ability to polarize light and smaller values of CD at the corresponding wavelength.
17. Since FDCD spectrum of guanosine is weak at λ = 200 – 300 nm, it does not disturb the FDCD observation of pteridines. Guanosine: FDCD (c = 8.05 x 10^{-4} M), λ/nm ([θ]/m deg) 205 (-0.002); 212 (0); 221 (+0.007); 232 (0); 248 (-0.018).