Synthesis and Anti-human Immunodeficiency Virus Activity of the Skeleton Isomers of 3′,4′-Di-(*O*)-(−)-camphanoyl-(+)-khellactone

Hiromi Nishioka,[a] Koji Uesugi,[a] Norihiro Ueda,[a] Yuka Kondo,[a] Megumi Tsuji,[a] Hitoshi Abe,[b] Takashi Harayama,[a] Takayuki Hamasaki,[c] Masanori Baba,[c] and Yasuo Takeuchi*,[a]

[a] *Division of Pharmaceutical Sciences, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University; 1–1–1 Tsushima-Naka, Kita-ku, Okayama 700–8530, Japan:* [b] *Faculty of Engineering, Toyama University; 3190 Gofuku, Toyama 930–8555, Japan: and* [c] *Division of Antiviral Chemotherapy Center for Chronic Viral Diseases Graduate School of Medical and Dental Sciences, Kagoshima University; 8–35–1 Sakuragaoka, Kagoshima 890–8544, Japan.*

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Optically active structural isomers (1b—f and *dst*-1b—f) of 3′,4′-di-(*O*)-(−)-camphanoyl-(+)-khellactone (DCK) were synthesized and their anti-human immunodeficiency virus (HIV) activity was investigated. The value of the sensitivity index (SI) of 1b was greater than that of DCK.

Key words anti-human immunodeficiency virus; coumarin; asymmetric dihydroxylation; new Mosher's method; 3′,4′-di-(*O*)-(−)-camphanoyl-(+)-khellactone

3′,4′-Di-(*O*)-(−)-camphanoyl-(+)-khellactone (DCK) is well known as an anti-human immunodeficiency virus (HIV) compound with remarkably high activity. Lee and his coworkers reported the structure–activity relationship (SAR) of the analogue of DCK (**1a**); in particular, they discussed the optimization of substituents on the angular benzo[2,1-*b*:4,3-*b*′]dipyran skeleton.[1—6] We believe that one of the most important studies on the SAR of DCK must involve the arrangement of an angular benzodipyran skeleton. In this paper, we describe the synthesis and anti-HIV activity of the optically active structural isomers (**1b**—**f** and *dst*-**1b**—**f**) of DCK (Fig. 1).

Our desired compounds (**1b**—**d** and *dst*-**1b**—**d**) can be obtained from hydroxycoumarin by the method used to synthesize DCK. 6-, 5-, or 8-hydroxycoumarin (**2b**—**d**) and benzodipyran derivatives (**3b**—**d**) were successfully prepared by mainly using our reported method.[7] The Sharpless asymmetric dihydroxylation of **3b**—**d** with dihydroquinine-2,5-diphenyl-4,6-pyrimidinediyl diether ((DHQ)$_2$PYR) afforded the optically active diols (**4b**—**d**), which exhibited an *R*,*R* configuration. On the other hand, dihydroxylation of **3b**—**d** with dihydroquinidine-2,5-diphenyl-4,6-pyrimidinediyl diether ((DHQD)$_2$PYR) yielded *S*,*S* diols (*ent*-**4b**—**d**). Camphanoylation of **4b**—**d** afforded the final products (**1b**[8]—**d**). Other isomers (**1e**, **f** and *dst*-**1e**, **f**) were prepared in the similar manner (Chart 1). In the case of asymmetric dihydroxylation, even though the values of yield and enantiomeric excess (*ee*) were never high, we could still obtain **4b**—**f** and those enantiomer (*ent*-**4b**—**f**) (Table 1).

The absolute structure of **4b**—**f** was determined by using the modified Mosher's method (Fig. 2).[9] The reaction of **4b**—**f** and *ent*-**4b**—**f** with (*R*)-α-methoxy-α-trifluoromethylaphenylacetic acid ((*R*)-MTPA) afforded monoester (**5b**—**f** and *dst*-**5b**—**f**), where the hydroxy group of the benzylic position was selectively esterified. The difference ($\Delta\delta$) between the chemical shift (ppm) of each peak in the ^{1}H-NMR of **5b**—**f** and *dst*-**5b**—**f** is shown in Fig. 2.

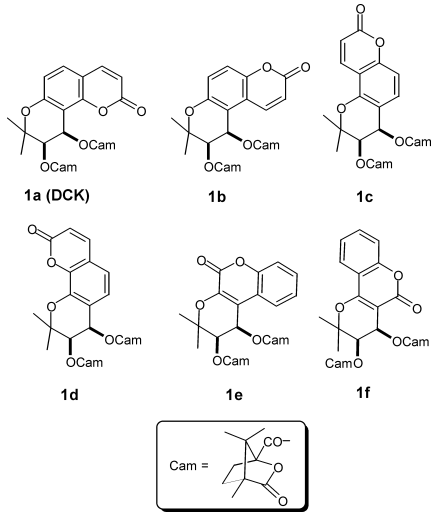

Fig. 1. Structure of DCK and Its Skeleton Isomers

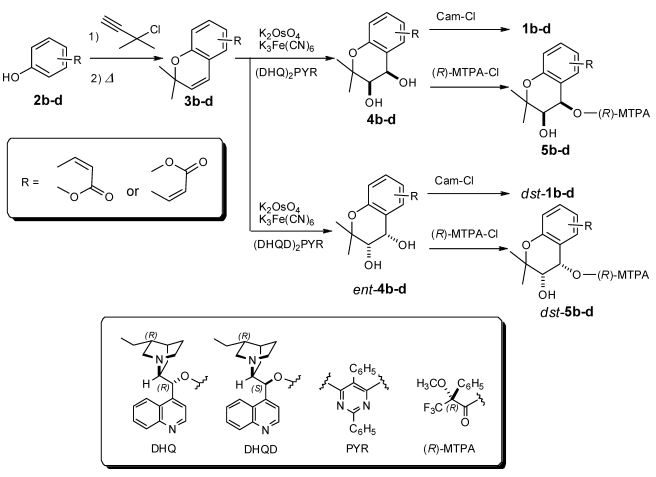

Chart 1

Table 1. Dihydroxylation of **3b**—**f** with (DHQ)$_2$PYR or (DHQD)$_2$PYR Ligand *via* Chart 1

Starting material (**3**)	Ligand	Product (**4**)	Yield %[a)]	*ee* %[b)]
3b	(DHQ)$_2$PYR	**4b**	23	57
3b	(DHQD)$_2$PYR	*ent*-**4b**	43	75
3c	(DHQ)$_2$PYR	**4c**	58	57
3c	(DHQD)$_2$PYR	*ent*-**4c**	66	68
3d	(DHQ)$_2$PYR	**4d**	24	67
3d	(DHQD)$_2$PYR	*ent*-**4d**	54	68
3e	(DHQ)$_2$PYR	**4e**	28	53
3e	(DHQD)$_2$PYR	*ent*-**4e**	61	58
3f	(DHQ)$_2$PYR	**4f**	72	90
3f	(DHQD)$_2$PYR	*ent*-**4f**	41	76

a) After recrystallization. *b*) Determined by chiral HPLC analysis.

* To whom correspondence should be addressed. e-mail: take@pharm.okayama-u.ac.jp

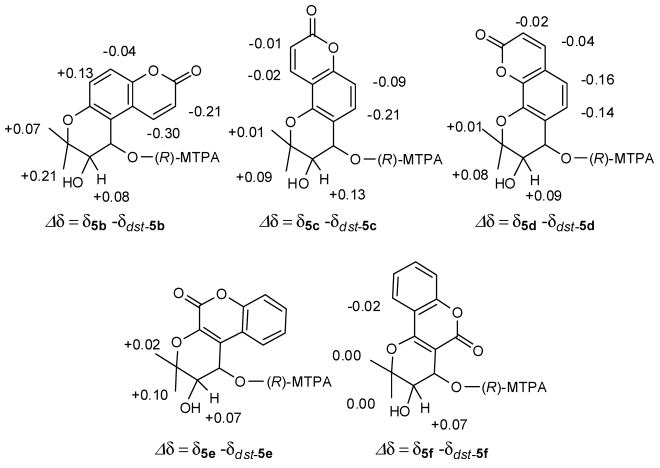

Fig. 2. Analysis Using New Mosher's Method for the Absolute Structure of **5**

Table 2. Anti-HIV Activity of **1**

Conpound (**1**)	EC_{50} (μM)[a]	CC_{50} (μM)[b]	SI (CC_{50}/EC_{50})[c]
1a (DCK)	0.17	>4.0	>23
1b	0.23	52	226
dst-**1b**	>100	>100	—
1c	4.3	>100	>23
dst-**1c**	>100	>100	—
1d	>100	>100	—
dst-**1d**	>100	>100	—
1e	>100	>100	—
dst-**1e**	>100	>100	—
1f	>100	>100	—
dst-**1f**	1.79	30.3	16
AZT[d]	0.076	>20	>263

a) EC_{50}: 50% effective concentration based on the inhibition of HIV-1-induced cell death in virus-infected MT-4 cells. *b*) CC_{50}: 50% cytotoxic concentration based on the reduction of viability in mock-infected MT-4 cells. *c*) Selectivity index: ratio CC_{50}/EC_{50}. *d*) AZT (zidovudine) was used as positive control.

The activity of compound (**1**) against HIV-1 replication was based on the inhibition of virus-induced cytopathogenicity in MT-4 cells. Briefly, the cells (1×10^5 cells/ml) were infected with HIV-1 at a multiplicity of infection (MOI) of 0.1 and were cultured in the presence of various concentrations of the test compounds. After the cells were incubated for 4 d at 37 °C, the number of viable cells was monitored by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. The cytotoxicity of the compounds was simultaneously evaluated with their antiviral activity, on the basis of the viability of mock-infected cells. All experiments were repeated twice.

Among **1b**—**f** and those diastereomers (*dst*-**1b**—**f**), we could not find any derivative whose anti-HIV activity was more potent than that of DCK. However, the selective index (SI) of **1b**, whose anti-HIV activity ($EC_{50}=0.23$ μM) was almost the same as that of DCK, was greater than that of DCK (Table 2). We expect the DCK analogues to also exhibit anti-HIV activity.

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References and Notes

1) Huang L., Kashiwada Y., Cosentino L. M., Fan S., Lee K.-H., *Bioorg. Med. Chem. Lett.*, **4**, 593—598 (1994).
2) Huang L., Kashiwada Y., Cosentino L. M., Fan S., Chen C. H., McPhail A. T., Fujioka T., Mihashi K., Lee K.-H., *J. Med. Chem.*, **37**, 3947—3955 (1994).
3) Xie L., Crimmins M. T., Lee K.-H., *Tetrahedron Lett.*, **36**, 4529—4532 (1995).
4) Xie L., Takeuchi Y., Cosentino L. M., McPhail A. T., Lee K.-H., *J. Med. Chem.*, **44**, 664—671 (2001).
5) Xie L., Takeuchi Y., Cosentino L. M., Lee K.-H., *J. Med. Chem.*, **42**, 2662—2672 (1999).
6) Tang J., Qian K., Zhang B.-N., Chen Y., Xia P., Yu D., Xia Y., Yang Z.-Y., Chen C.-H., Morris-Natschke S. L., Lee K.-H., *Bioorg. Med. Chem.*, **18**, 4363—4373 (2010).
7) Nishita Y., Uesugi K., Satoh Y., Harayama T., *Yakugaku Zasshi*, **118**, 599—608 (1998).
8) **1b**: Colorless needles. mp 210—212 °C [*de*=90.5%] (AcOEt–hexane). IR (KBr) cm^{-1}: 1790, 1730. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.88 (3H, s, 3″-CH_3), 0.98 (3H, s, 3′-CH_3), 1.10, 1.11 (each 3H, each s, 7″-CH_3), 1.13 (6H, s, 7′-CH_3), 1.45, 1.48 (each 3H, each s, 8-CH_3), 1.67—2.54 (8H, m, CH_2), 5.40 (1H, d, *J*=4.5 Hz, 9-H), 6.46 (1H, d, *J*=9.9 Hz, 2-H), 6.67 (1H, d, *J*=4.5 Hz, 10-H), 7.11 (1H, d, *J*=9.3 Hz, 6-H), 7.34 (1H, d, *J*=9.3 Hz, 5-H), 7.50 (1H, d, *J*=9.9 Hz, 1-H). FAB-MS (positive ion mode) *m/z*: 623. *Anal.* Calcd for $C_{34}H_{38}O_{11}$: C, 65.58, H, 6.15. Found: C, 65.50, H, 6.18. $[\alpha]_D^{23.1}$ −60.36 (*c*=0.76, $CHCl_3$). Diastereomer excess was determined by chiral HPLC analysis.
9) Ohtani I., Kusumi T., Kashman Y., Kakisawa H., *J. Org. Chem.*, **56**, 1296—1298 (1991).