ASYMMETRIC DIHYDROXYLATION ONTO THE α,β-UNSATURATED CARBOXYLIC ESTER DERIVATIVES OF CAMPTOTHECIN

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Abstract - Dihydroxyalkanoic ester derivatives (4a,b) and (5a,b) of 20S-camptothecin (1) were diastereoselectively synthesized by exploiting osmium-catalyzed asymmetric dihydroxylation based on the Sharpless procedure. The absolute configuration of the newly formed chiral centers, the 2' and 3' positions of the 20-alkanoyl side chain of 4a,b and 5a,b was determined by the chemical correlation with the known chiral dihydroxyalkanoic acids.

20S-Camptothecin (1), a pentacyclic alkaloid isolated from Camptotheca acuminata by Wall and co-workers in 1966,1 exhibited potent antitumor activity against various cell lines and in animal screens.2 Only the 20S-enantiomer (1) exhibited antitumor activity3 and its mode of action was found to trap a cleavable complex between topoisomerase I and DNA.4 However, several problems such as severe toxicity and poor water solubility prevented its application as a clinical antitumor agent. Therefore, many derivatives of 20S-camptothecin (1) have been synthesized and investigated toward the efficient antitumor agents.5 Among these derivatives, irinotecan hydrochloride showed clinically useful activity against lung, uterine, and ovarian tumors.6 In 1991, we reported a useful information on the development of new podophyllotoxin and epipodophyllotoxin derivatives bearing the ester moiety of long chain fatty acids.6 Hydroxy group at C-20 of 20S-camptothecin (1) should be essential for antitumor activities. In spite of that, there is few attempts to modify the hydroxy group at C-20 of 20S-camptothecin (1). The acyl side chain at C-13 of taxol (2) should also play an important role for the excellent antitumor activity.7 Thus, we have investigated chemical modification of α,β-unsaturated carboxylic esters (3a,b) of 20S-camptothecin (1) and their antitumor activity.
Scheme 1

Reagents and conditions for dihydroxylation:
(a) OsO₄ (0.05 mol eq), NMO (1.5 mol eq), Me₂CO : H₂O = 10 : 1, 0°C;  
(b) (DHQD)₂-PHAL (0.01 mol eq), K₃Fe(CN)₆ (3 mol eq), K₂CO₃ (3 mol eq), K₂OsO₂(OH)₄ (0.002 mol eq),  
MeSO₂NH₂ (1 mol eq), t-BuOH : H₂O = 1 : 1, 0°C;  
(c) (DHQ)₂-PHAL (0.25 mol eq), OsO₄ (0.05 mol eq), NMO (1.5 mol eq), Me₂CO : H₂O = 10 : 1, 0°C

Esterification at the C-20 hydroxy group of 1 was readily done by treatment with E-crotonic acid (3 mol eq) and E-cinnamic acid (3 mol eq) in the presence of dicyclohexylcarbodiimide (DCC, 3 mol eq) and 4-dimethylaminopyridine (DMAP, 0.2 mol eq) in CH₂Cl₂, to give the corresponding esters (3a,b) as colorless needles [3a : mp 215-218°C (MeOH), 3b : mp 285-288°C (CHCl₃-MeOH)] in each 95% yield.

Subsequently, asymmetric dihydroxylation onto 3a,b in the presence of a catalytic amount of chiral ligand (DHQD)₂-PHAL and (DHQ)₂-PHAL,⁸ was efficiently performed on the basis of the Sharpless procedure⁸ [See footnote (reagents and conditions (a)-(c)) in Scheme 1] to furnish the corresponding dihydroxy derivatives (4a,b) and (5a,b) in 57-68% yields (Table 1). Diastereoselectivity of the dihydroxylation products (4a,b) without use of the chiral ligand was very poor (Entries 1 and 4). However, when catalytic (DHQD)₂-PHAL or (DHQ)₂-PHAL was employed, the desired asymmetric dihydroxylation proceeded in a...
fairly good (Entry 3) or an excellent (Entries 2, 5, and 6) diastereoselective manner to give a mixture of 4a and 5a or a mixture of 4b and 5b, respectively (Table 1). Pure compound [4a: mp 178-180°C (MeOH), $[\alpha]_D^{26}$ -74° (c 0.5, CHCl$_3$-MeOH (4 : 1))] was obtained by repeated recrystallization of the mixture of 4a and 5a (97 : 3) in MeOH. Similar recrystallization of other diastereomeric mixtures furnished the corresponding pure compounds, [5a: mp 165-167°C (CHCl$_3$-hexane), $[\alpha]_D^{26}$ -40° (c 0.5, CHCl$_3$-MeOH (4 : 1))], [4b: mp 175-176°C (MeOH), $[\alpha]_D^{26}$ -62° (c 0.5, CHCl$_3$-MeOH (4 : 1))], and [5b: mp 172-174°C (MeOH), $[\alpha]_D^{26}$ +10° (c 0.5, CHCl$_3$-MeOH (4 : 1))], respectively.

Table 1. Asymmetric dihydroxylation of 3a,b.

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>Conditions</th>
<th>Diastereomer ratio</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3a</td>
<td>(a)</td>
<td>4a : 5a = 37 : 63</td>
<td>63</td>
</tr>
<tr>
<td>2</td>
<td>3a</td>
<td>(b)</td>
<td>4a : 5a = 97 : 3</td>
<td>67</td>
</tr>
<tr>
<td>3</td>
<td>3a</td>
<td>(c)</td>
<td>4a : 5a = 11 : 89</td>
<td>68</td>
</tr>
<tr>
<td>4</td>
<td>3b</td>
<td>(a)</td>
<td>4b : 5b = 44 : 56</td>
<td>57</td>
</tr>
<tr>
<td>5</td>
<td>3b</td>
<td>(b)</td>
<td>4b : 5b = 93 : 7</td>
<td>60</td>
</tr>
<tr>
<td>6</td>
<td>3b</td>
<td>(c)</td>
<td>4b : 5b = 8 : 92</td>
<td>57</td>
</tr>
</tbody>
</table>

1) See footnote in Scheme 1. 2) Determined by HPLC analysis. 3) Yield of the diastereomeric mixture. 4) Waters Nova-pak Silica 3.9X150 mm, CH$_2$Cl$_2$-2-propanol (98: 2), flow rate = 1.0 mL/min, UV detector (254 nm). 5) Waters Puresil C$_{18}$ 4.6X150 mm, MeCN-H$_2$O (35 : 65), flow rate = 1.0 mL/min, UV detector (254 nm).

The absolute configuration of the newly formed chiral centers C-2' and C-3' of the dihydroxyalkanoic moiety in all products (4a,b) and (5a,b) was successfully determined by their chemical correlation as shown in Schemes 2 and 3. First of all, known two 2,3-dihydroxybutanoic acids (2S, 3R-9a)$^9$ and (2R, 3S-10a)$^{10}$ and two 2,3-dihydroxy-3-phenylpropanoic acids (2S, 3R-9b)$^{11}$ and (2R, 3S-10b)$^{11}$ were synthesized by exploiting the Sharpless' asymmetric dihydroxylation$^8$ onto the corresponding E-crotonic acid benzyl ester (6a) or E-cinnamic acid benzyl ester (6b) as follows. Dihydroxylation of 6a under the Sharpless procedure$^4$ [See footnote (reagents and conditions (a)) in Scheme 2] in the presence of catalytic (DHQD)$_2$-PHAL (0.01 mol eq) gave chiral dihydroxy compound (7a) in 92% ee$^{12}$ and 76% yield. The similar dihydroxylation onto 6a in the presence of catalytic (DHQD)$_2$-PHAL (0.01 mol eq) gave chiral dihydroxy compound (8a) in 90% ee$^{12}$ and 82% yield. The compound (6b) was also submitted to the same asymmetric dihydroxylation employing a catalytic amount (0.01 mol eq) of (DHQD)$_2$-PHAL or (DHQ)$_2$-PHAL as described above to afford each chiral dihydroxy compound, (7b: 94% ee, 62% yield) or (8b: 90% ee, 71% yield). Hydrogenolysis of all chiral benzyl dihydroxyalkanoates (7a,b) and (8a,b)
on 5% Pd-C in MeOH gave the corresponding known chiral carboxylic acids [2S, 3R-9a : 95% yield, colorless oil, \([\alpha]_D^{25} = -17.00^\circ\) (c 1, H2O), lit.\(^9\) [\alpha]_D^{25} = -17.75^\circ\) (c 1, H2O); 2R, 3S-10a : 90% yield, colorless oil, [\alpha]_D^{25} = +15.0^\circ\) (c 2.0, H2O), lit.\(^{10}\) [\alpha]_D^{20} = +15.9^\circ\) (c 2.1, H2O); 2S, 3R-9b : 95% yield, mp 166-167°C (H2O), [\alpha]_D^{25} = -39.0^\circ\) (c 1, H2O), lit.,\(^l\) [\alpha]_D^{25} = -39.6^\circ\) (c 1, H2O); 2R, 3S-10b : 91% yield, mp 166-167°C (H2O), [\alpha]_D^{25} = +40.0^\circ\) (c 1, H2O), lit.,\(^l\) [\alpha]_D^{25} = +39.6^\circ\) (c 1, H2O)]. The four chiral dihydroxyalkanoic acids (9a,b) and (10a,b) were converted to the corresponding diacetoxyalkanoic methyl esters (2S, 3R-lla,b) and (2R, 3S-12a,b) by their conventional methylation (CH2N2 in MeOH) followed by acetylation (Ac2O in pyridine). Each peak on the HPLC chart\(^1\) of the compounds (2S, 3R-lla,b) and (2R, 3S-12a,b) obtained from alkaline hydrolysis (5% NaOH) of 20S-camptothecin derivatives (2'S, 3'R-4a,b) and (2'R, 3'S-5a,b) followed by neutralization (Amberlite IR 120B(H)) and some other reactions of the resultant dihydroxyalkanoic acids as shown in Scheme 3; was identified with that of the same compounds derived from the authentic chiral dihydroxyalkanoic acids (2S, 3R-9a,b) and (2R, 3S-10a,b) as shown in Scheme 2.

**Scheme 2**

![Scheme 2 Diagram](image)

Reagents and conditions:
- (a) K2Fe(CN)6 (3 mol eq), K2CO3 (3 mol eq), K2OsO2(OH)4 (0.002 mol eq), MeSO2NH2 (1 mol eq), \(\tau\)-BuOH : H2O = 1 : 1, 0 °C; (b) 5% Pd-C, MeOH;
- (c) CH2N2, MeOH; (d) Ac2O (excess), pyridine

**Scheme 3**

![Scheme 3 Diagram](image)

Reagents and conditions:
- (a) 5% NaOH, MeOH; (b) Amberlite IR 120B(H); (c) CH2N2, MeOH;
- (d) Ac2O (excess), pyridine
In the course of the stereochemistry determination of 2S,3R-11a,b and 2R,3S-12a,b as described above, we carried out tentatively diastereoselective dihydroxylation onto 3-crotonyl-4R-isopropyl-1,3-oxazolidin-2-one (13) under the reaction conditions with same reagents systems. Highly diastereoselective dihydroxylation onto 13 was achieved only in the case with catalytic (DHQ)₂-PHAL to give 2R,3S-14 in 90% de and 86% yield (Scheme 4).

Scheme 4

Reagents and conditions:
(a) (DHQ)₂-PHAL (0.01 mol eq), K₂OsO₄(OH)₄ (0.002 mol eq), K₃Fe(CN)₆ (3 mol eq), K₂CO₃ (3 mol eq), MeSO₂NH₂ (1 mol eq), t-BuOH : H₂O = 1 : 1, 0 °C; (b) Ac₂O (excess), pyridine; (c) 5% NaOH, MeOH; (d) Amberlite IR 120B(H); (e) CH₂N₂, MeOH; (f) Ac₂O (excess), pyridine

Figure 1. ORTEP drawing of the crystallographic structure of 16.
Surprisingly, usual acetylation of 14 with Ac₂O in pyridine gave an unexpected diacete [16, 53% yield, mp 95-96°C (MeOH-H₂O)] of which was established by the X-Ray analysis (Figure 1). This diacete (16) could be derived from 14 via a plausible pathway (15). Here, the absolute configuration of 2R, 3S-12a was directly determined by the chemical correlation of 14 to it as shown in Scheme 4.

Interestingly, both diastereomeric 2',3'-dihydroxybutanoyl esters (2'S,3'R-4a) and (2'R,3'S-5a) exhibited fairly strong antitumor activities against p388 lymphocytic leukemia inoculated into mice [4a: T/C = 175 % (200 mg/kg), 175 % (100 mg/kg), and 142 % (50 mg/kg); 5a: T/C = 99 % (250 mg/kg), 210 % (125 mg/kg), and 148 % (62.5 mg/kg)]. However, both diastereomeric 2',3'-dihydroxy-3-phenylpropanoyl esters (2'S, 3'R-4b) and (2'R, 3'S-5b) did not exhibit any antitumor activity.15 The antitumor activities of new compounds (2'S, 3'R-4a) and (2'R, 3'S-5a) must be better than those of 20S-camptothecin [1: T/C = 65 % (100 mg/kg), 88 % (50 mg/kg), and 180 % (25 mg/kg)]. Thus, this convenient asymmetric dihydroxylation method seems to be available for chemical modification of various drugs, which is currently undertaken in our research groups.

REFERENCES AND NOTES
12. HPLC Analysis: Daicel chiralcel OD-H 4.6X150 mm, n-hexane-2-propanol (97 : 3), flow rate = 0.5 mL/min, UV detector 254 nm (11b, 12b and diacetate of 7a,b and 8a,b), 210 nm (11a and
13. HPLC Analysis: Waters Nova pak Silica 3.9X159 mm, n-hexane-2-propanol (95:5), flow rate = 1 mL/min, UV detector 210 nm.

14. The crystallographic data of compound (16) are as follows. C_{14}H_{21}NO_{7}, FW = 315.32, monoclinic, Space Group P2_1 (#4), \( a = 9.361 (3) \text{ Å}, b = 8.064 (1) \text{ Å}, c = 11.445 (4) \text{ Å}, \beta = 106.665 (5)^\circ, Z = 2 \), \( D_{\text{calc}} = 1.265 \text{ g/cm}^3, V = 827.5700 \text{ Å}^3, R = 0.056 \).

15. The authors express their appreciation to Dr. T. Yamori (Cancer Chemotherapy Center, Tokyo, Japan) for his kind antitumor testing. Full detail of the antitumor activity of all 20S-camptothecin esters and the related compounds will be published as part of a forthcoming paper.

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