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Enantioselective synthesis of 20(*S*)-camptothecin using an enzyme-catalyzed resolution

Akihiro Imura,* Motohiro Itoh and Akihiko Miyadera

Chemical Technology Research Laboratories, Daiichi Pharmaceutical Co. Ltd, 16-13, Kitakasai 1-chome, Edogawa-ku, Tokyo 134, Japan

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Abstract

The important key intermediate of a 20(*S*)-camptothecin synthesis was prepared enantioselectively using an enzyme-catalyzed resolution. A commercially available papain was found to exhibit the highest enantioselectivity with moderate activity, and the (*S*)-enantiomer of 99% ee was obtained as the remaining substrate.

1. Introduction

20(*S*)-Camptothecin **1** is a pentacyclic alkaloid with potent antitumor activity, and was isolated from *Camptotheca acuminata* by Wall et al. in 1966.[1] Only the (*S*)-enantiomer **1** exhibits antitumor activity, and its mechanism was found to inhibit topoisomerase I.[2]

Many successful syntheses of this alkaloid have been reported,[3] but most such syntheses have been racemic. Corey et al. reported the first total synthesis of optically active (*S*)-**1** using a resolution process.[4] Tagawa et al. reported another new efficient route by which the non racemic key intermediate **2** and amine **3** yield **1** (Scheme 1).[5] Recently, Jew et al. reported the synthesis of the hydroxylactone **2** by using a catalytic asymmetric dihydroxylation.[6] However, the expensive chiral auxiliary is required stoichiometrically[5] and the enantiomeric excess of **2** is not particularly high (82% ee).[6] Therefore, a practical access of the key intermediate **2** is limited in the above two syntheses.

In this paper, we describe the first practical asymmetric synthesis of the key intermediate **2** using an enzyme-catalyzed resolution process and the recycling of the unnatural compound **6**.

* Corresponding author. E-mail: fwih8621@mb.infoweb.or.jp

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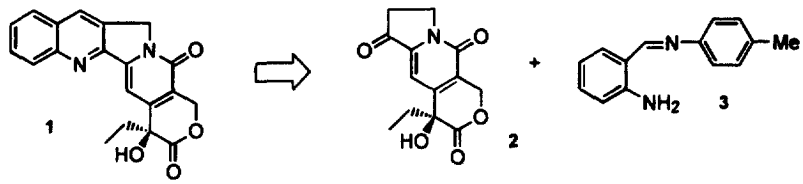
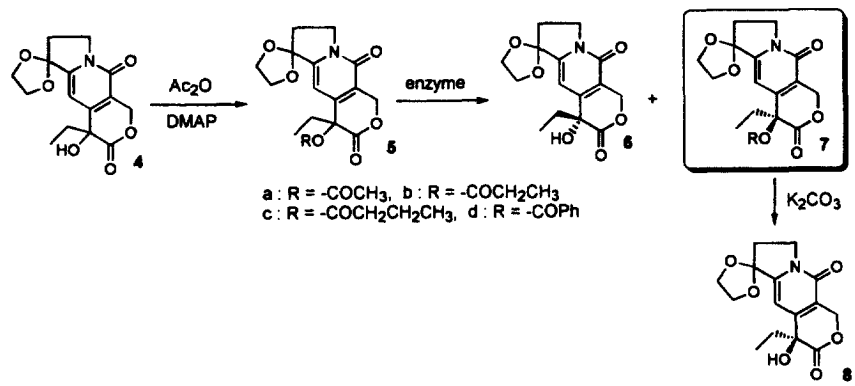


Scheme 1.

2. Results and discussion

The synthesis of racemic ethyl-6,6-(ethylenedioxy)-7,8-dihydro-1*H*-pyrano[3,4-*f*]indolizine-3,10(4*H*)-dione **4** was achieved by Tagawa's route.[7] Conversion of **4** to acetate **5a** was readily accomplished by treatment with acetic anhydride and dimethyaminopyridine in dichloromethane in the usual fashion (Scheme 2). We selected the racemic acetate **5a** as the substrate for enzymes and attempted resolution by enantioselective hydrolysis.



Scheme 2.

About one hundred commercially available enzymes including esterases, lipases and proteases from various sources were examined to screen for enzymes which were highly active and enantioselective towards **5a**. Many esterases and lipases did not exhibit deacetylating activity due to the bulkiness around the hydroxyl group compared to that in the primary and secondary alcohols, but a few plant proteases did exhibit deacetylating activity (Table 1). The enantioexcess of **6** determined by HPLC was moderately high in general. In particular a commercially available papain was found to exhibit high enantioselectivity and yielded optically active **6** in 98% ee, but with it the chemical yield of **6** was only 20%.

We first examined the reaction in a phosphate buffer (pH 6.5) at room temperature employing papain as a catalyst. The low yield of **6** appeared to be due to the insolubility of the substrate **5a** in the phosphate buffer, and the addition of organic solvents to the reaction mixture was therefore attempted. Dichloromethane, acetone and acetonitrile gave poor results due to the inactivity of the enzyme. Among

Table 1
Screening of enzymes catalyzing hydrolysis of racemate **5a**

protease[a]	source	reaction time(hr)	*R*-alcohol **6**		*S*-acetate **7a**	
			yield(%)	*ee*(%)	yield(%)	*ee*(%)
papain	*papaya*	40	20	98	76	28
ficin	*fig*	48	4	95	94	-
bromeline	*pineapple*	48	2	95	97	-

[a] 1mg **5a**, 5mg enzyme, 0.1M phosphate buffer pH 6.5 (1ml)-40°C.

Table 2
3Enantioselective hydrolysis of **5** by papain in different reaction conditions using co-solvent

co-solvent[a]	*R*-alcohol **6**		*S*-acetate **7a**		E^b
	yield(%)	*ee*(%)	yield(%)	*ee*(%)	value
acetone	1	-	98	-	
dichloromethane	1	-	98	-	-
acetonitrile	2	-	96	-	-
ethyl acetate	49	98	50	99	>400
n-hexane	19	96	76	30	52.6
benzene	7	96	92	9	48.0

[a] 0.5g **5a**, 1.0g papain - 0.1M phosphate buffer pH 6.5 / co-solvent = 40ml / 10ml.
[b] The enantiomeric ratio E is calculated from the equation: $E = \ln\{(1-c)[1-ee(s)]\}/\ln\{(1-c)[1+ee(s)]\}$.[8]

Table 3
Effect of chain length of the alkyl group on enzymatic hydrolysis by papain

substrate[a]	alkyl chain	reaction time(hr)	*R*-alcohol (6)		*S*-acetate (7a-d)		E^b
			yield(%)	*ee*(%)	yield(%)	*ee*(%)	value
5a	R = -$COCH_3$	40	49	98	50	99	>400
5b	-$COCH_2CH_3$	48	24	96	75	34	85.3
5c	-$COCH_2CH_2CH_3$	48	7	94	92	8	38.7
5d	-COPh	48	<2	-	-	-	-

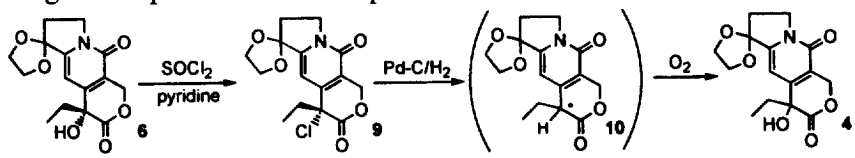
[a] 5g **5a-d**, 1.0g papain - 0.1M phosphate buffer pH 6.5 / ethyl acetate = 40ml / 10ml (40℃).
[b] The enantiomeric ratio E is calculated from the equation: $E = \ln\{(1-c)[1-ee(s)]\}/\ln\{(1-c)[1+ee(s)]\}$.[8]

the solvent systems screened, as shown in Table 2, the yield of **6** in the phosphate buffer with ethyl acetate was surprisingly different from that in the phosphate buffer alone.

The chain length of the alkyl group was varied, and the effect of this variation on the activity of papain was investigated. As shown in Table 3, **5a** was the most reactive among the four substrates examined, and reaction rate decreased with increasing chain length.

The enzymatic hydrolysis of acetate **5a**, the best substrate of papain, was carried out in 20% ethyl acetate containing 0.1 M phosphate buffer (pH 6.5) at 40°C. The pH was maintained at 6.5 by the addition of 10% aqueous sodium hydroxide with an autotitrator. After termination of the reaction, a mixture of optically active **6** and **7a** was isolated by extraction, and chromatographed on silica to obtain **6** (45%, 98% ee) and **7a** (48%, 99% ee). The optically active acetate **7a** was deacetylated by aqueous potassium carbonate to obtain alcohol **8**. From the specific rotation, we determined the absolute configuration of **8** to be (*S*).[9] The hydroxylactone **8** was converted to **2** by hydrolysis with aqueous trifluoroacetic acid. The Friedlander condensation of **2** and amine **3**[10] was performed in the presence of *p*-toluenesulfonic acid by refluxing in toluene, to obtain **1**. Synthetic compound **1** was identical to authentic material in all respects.

In this resolution system with papain, an equal amount of the unwanted compound **6** was obtained. Therefore, recycling of compound **6** was attempted.



Conversion of **6** to chloride **8** was accomplished by treatment with thionylchloride and pyridine in dichloromethane to obtain the expected **9** with 86% yield. Reduction of this chloride **9** with Pd–C under hydrogen gave **10**, which was directly oxidized to **4** in the usual fashion. The ^{1}H-NMR and IR spectra of

4 were identical to those of **8**. Thus, we were able to utilize the unnatural compound **6** for the synthesis of natural camptothecin with a total yield of 70% in three steps.

3. Conclusion

A commercially available papain was screened as the best enzyme for the synthesis of the key intermediate **8**. Enzymatic resolution was performed in a biphasic system (water–ethyl acetate) to obtain **8** with high enantiomeric purity (>99% ee) and a chemical yield of 44%.

4. Experimental section

4.1. General procedures

Melting points were determined on a Yanagimoto apparatus and are uncorrected. Infrared spectra were recorded on a FT-720 spectrometer (Horiba). ^{1}H-NMR spectra were recorded on a JEOL JNM-EX270 (270 MHz) instrument. Coupling constants are reported in hertz (Hz) and chemical shifts in ppm downfield from internal TMS. Mass spectra were recorded on a JEOL JMS-HX110 or JMS-AX505W mass spectrometer. Optical rotations were measured with a SEPA-300 polarimeter (Horiba). All chemicals were obtained from commercial sources and were used without further purification. A Merck Kieselgel 60 Art.7744 was used for column chromatography. Papain from *papaya* was obtained from Merck Co., Ltd. Ficin from *figs* was obtained from Sigma Co., Ltd. Bromeline from *pineapple* was obtained from Merck Co., Ltd. Determination of enantiomeric excesses of optically active compounds was by analytical HPLC [ULTRON ES-OVM column (Shinwa Kako Co., Ltd) 4.6×150 mm; eluent 2% ethanol containing 20 mM phosphate buffer (pH 6.0); flow rate 1.0 ml/min; UV detection 300 nm].

*4.1.1. 4-Acetoxy-4-ethyl-6,6-(ethylenedioxy)-7,8-dihydro-1*H*-pyrano[3,4-f]indolizine-3,10(4*H*)-dione* ***5a***

Acetic anhydride (40 ml, 425 mmol) and dimethylaminopyridine (1.50 g, 12.0 mmol) were added to a solution of **4** (65.0 g, 208 mmol) in dichloromethane (250 ml). The solution was stirred at 40°C for 4 h, and then the solvent was removed *in vacuo* and the residue was crystallized from 2-propanol to obtain **5a** as colorless crystals (71.3 g, 98.1%). Mp 208–209°C; ^{1}H-NMR ($CDCl_3$) δ: 0.91 (t, J=7.6 Hz, 3H, CH_3CH_2), 1.89–2.20 (m, 2H, CH_3CH_2), 2.14 (s, 3H, $COCH_3$), 2.38 (t, J=6.9 Hz, 2H, C_7–H), 3.92–4.10 (m, 6H, C_8–H and OCH_2CH_2O), 5.25, 5.53 (ABq, J=16.4 Hz, 2H, C_1–H), 6.10 (s, 1H, C_5–H); m/z 350 (M^++1); IR (KBr) 1745, 1666 and 1614 cm^{-1}; anal. calcd for $C_{17}H_{19}NO_7$: C, 58.45; H, 5.48; N, 4.01. Found: C, 58.25; H, 5.54; N, 3.90.

*4.1.2. 4-Propanoyloxy-4-ethyl-6,6-(ethylenedioxy)-7,8-dihydro-1*H*-pyrano[3,4-f]indolizine-3,10(4*H*)-dione* ***5b***

Yield 92.1%; mp 148–149°C; ^{1}H-NMR ($CDCl_3$) δ: 0.91 (t, J=7.6 Hz, 3H, CH_3CH_2), 1.15 (t, J=7.6 Hz, 3H, CH_3CH_2CO), 1.96–2.24 (m, 2H, CH_3CH_2), 2.34–2.53 (m, 4H, C_7–H and CH_3CH_2CO), 4.08–4.18 (m, 6H, C_8–H and OCH_2CH_2O), 5.25, 5.54 (ABq, J=17.1 Hz, 2H, C_1–H), 6.08 (s, 1H, C_5–H); m/z 363 (M^+).

4.1.3. 4-Butanoyloxy-4-ethyl-6,6-(ethylenedioxy)-7,8-dihydro-1H-pyrano[3,4-f]indolizine-3,10(4H)-dione 5c

Yield 94.8%; mp 128–129°C; ^{1}H-NMR ($CDCl_3$) δ: 0.89–0.99 (m, 6H, CH_3CH_2 and $CH_3CH_2CH_2CO$), 1.63–1.73 (m, 2H, $CH_3CH_2CH_2CO$), 1.92–2.31 (m, 2H, CH_3CH_2), 2.35–2.46 (m, 4H, C_7–H and $CH_3CH_2CH_2CO$), 4.08–4.17 (m, 6H, C_8–H and OCH_2CH_2O), 5.26, 5.53 (ABq, J=16.8 Hz, 2H, C_1–H), 6.09 (s, 1H, C_5–H); m/z 377 (M^+).

4.1.4. 4-Benzoyloxy-4-ethyl-6,6-(ethylenedioxy)-7,8-dihydro-1H-pyrano[3,4-f]indolizine-3,10(4H)-dione 5d

Yield 89.6%; mp 179–180°C; ^{1}H-NMR ($CDCl_3$) δ: 1.03 (t, J=7.3 Hz, 3H, CH_3CH_2), 2.08–2.29 (m, 2H, CH_3CH_2), 2.36 (t, J=6.9 Hz, 2H, C_7–H), 3.94–4.14 (m, 6H, C_8–H and OCH_2CH_2O), 5.32, 5.63 (ABq, J=17.2 Hz, 2H, C_1–H), 6.12 (s, 1H, C_5–H), 7.42–8.14 (m, 5H, Ar–H); m/z 411 (M^+).

4.2. Enzymatic hydrolysis of 5a

The acetate **5a** (35.0 g, 100 mmol) was suspended in ethyl acetate (180 ml) and 0.1 M phosphate buffer pH 6.5 (720 ml). L-Cysteine hydrochloride (7.7 g) and papain (70 g) were added and the mixture was stirred at 40°C for 40 h at pH 6.4–6.6 with an autotitrater and 10% aqueous sodium hydroxide. The reaction mixture was filtered through Celite and extracted with dichloromethane (3×250 ml) to obtain the mixture of **6** and **7a**. The residue was chromatographed on silica. Elution with chloroform/acetone (20/1) gave acetate **7a**. After the fraction was evaporated *in vacuo*, the residue was dissolved in 50% methanol, and potassium carbonate (18.0 g, 130 mmol) was added, and the mixture was left at room temperature. The reaction mixture was evaporated by a half volume, and adjusted to pH 2 with 10% hydrochloric acid, extracted with dichloromethane. The organic layer was evaporated *in vacuo*, and the residue was crystallized from ethyl acetate to obtain colorless crystals of (*S*)-4-ethyl-6,6-(ethylenedioxy)-7,8-dihydro-1*H*-pyrano[3,4-*f*]indolizine-3,10(4*H*)-dione **8** (13.5 g, 44.0%, >99% ee). Mp 169–170°C; $[\alpha]_D^{25}$ +104.3 (*c*=0.55, $CHCl_3$); H-NMR ($CDCl_3$) δ: 0.98 (t, J=7.3 Hz, 3H, CH_3CH_2), 1.62 (m, 2H, CH_3CH_2), 2.42 (t, J=6.9 Hz, 2H, C_7–H), 4.13 (m, 6H, C_8–H and OCH_2CH_2O), 5.16, 5.61 (ABq, J=16.4 Hz, 2H, C_1–H), 6.57 (s, 1H, C_5–H); m/z 307 (M^+); IR (KBr) 3311, 1755, 1658 and 1590 cm^{-1}; anal. calcd for $C_{15}H_{17}NO_6$: C, 58.63; H, 5.58; N, 4.56. Found: C, 58.61; H, 5.66; N, 4.55. Elution with chloroform/acetone (10/1) gave an alcohol which was crystallized from ethyl acetate to afford colorless crystals of (*R*)-4-ethyl-6,6-(ethylenedioxy)-7,8-dihydro-1*H*-pyrano[3,4-*f*]indolizine-3,10(4*H*)-dione **6** (13.8 g, 45.0%, 98.2% ee). Mp 168–169°C; $[\alpha]_D^{25}$ −103.1 (*c*=0.55, $CHCl_3$). The ^{1}H-NMR and IR spectra of compound **6** obtained here were identical to those of **8**. Anal. calcd for $C_{15}H_{17}NO_6$: C, 58.63; H, 5.58; N, 4.56. Found: C, 58.71; H, 5.69; N, 4.54.

4.3. 4-Chloro-4-ethyl-6,6-(ethylenedioxy)-7,8-dihydro-1H-pyrano[3,4-f]indolizine-3,10(4H)-dione 9

Pyridine (7.3 ml, 90.0 mmol) and thionylchloride (4.0 ml, 54.6 mmol) were added to the solution of **6** (12.0 g, 39.0 mmol) in dichloromethane (100 ml), and the mixture was refluxed for 7 h. After cooling, the reaction mixture was dropped in 10% aqueous sodium hydrogen carbonate solution (300 ml). The organic layer was washed with water, dried, and evaporated *in vacuo*. The residue was crystallized from 2-propanol, yielding yellow crystals (10.9 g, 85.9%). Mp 125°C (dec.); ^{1}H-NMR ($CDCl_3$) δ: 0.95 (t, J=7.3 Hz, 3H, CH_3CH_2), 2.08–2.31 (m, 4H, CH_3CH_2 and C_7–H), 4.01–4.14 (m, 6H, C_8–H and OCH_2CH_2O), 5.38, 5.44 (ABq, J=16.8 Hz, 2H, C_1–H), 6.44 (s, 1H, C_5–H); m/z 326 (M^+); IR (KBr) 1751, 1666 and

1616 cm^{-1}; anal. calcd for $C_{15}H_{16}NO_5Cl$: C, 55.30; H, 4.95; N, 4.30; Cl, 10.90. Found: C, 55.33; H, 5.01; N, 4.21; Cl, 10.91.

4.4. 4-Ethyl-6,6-(ethylenedioxy)-7,8-dihydro-1H-pyrano[3,4-f]indolizine-3,10(4H)-dione 4

A solution of **9** (9.0 g, 27.7 mmol) in dimethylformamide (50 ml) was hydrogenated in the presence of 10% Pd–C (1.4 g) at room temperature for 4 h at a pressure of 1 atm. After the catalyst was removed by filtration, a dimethylformamide solution of **10** was obtained. Triethylphosphite (9.7 ml, 57.0 mmol) and sodium *t*-butoxide (8.2 g, 85.1 mmol) were added to the solution at −40°C. Dry oxygen had been bubbled into the solution for 2 h at the same temperature and the reaction mixture was poured into water (250 ml). Then 10% hydrochloric acid was added to the solution and the mixture was extracted with dichloromethane (3×100 ml). The combined organic layer was washed with water and dried. Removal of the solvent resulted in a brown solid which was crystallized from ethyl acetate to afford **4** as a white solid (6.8 g, 79.5% from **9**). Mp 180–181°C; $[\alpha]_D^{25}$ +0.1 (*c*=0.52, $CHCl_3$). The ^{1}H-NMR and IR spectra of compound **6** obtained here were identical with those of **8**. Anal. calcd for $C_{15}H_{17}NO_6$: C, 58.63; H, 5.58; N, 4.56. Found: C, 58.69; H, 5.58; N, 4.49.

4.5. (S)-4-Ethyl-7,8-dihydro-4-hydroxy-1H-pyrano[3,4-f]indolizine-3,6,10(4H)-trione 2

Compound **8** (2.0 g, 6.5 mmol) was dissolved in 80% aqueous trifluoroacetic acid (40 ml), and the mixture was left at room temperature for 3 h under a nitrogen atmosphere. After the solvent was removed, the residue was diluted with water and the aqueous mixture was extracted with dichloromethane (3×20 ml). The combined organic layer was washed with water, and dried. The solvent was removed *in vacuo*, leaving a yellow oil, which was crystallized from ethanol to afford **2** as slightly yellow needles (1.52 g, 88.9%). Mp 176–177°C; $[\alpha]_D^{25}$ +123.0 (*c*=0.50, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ: 0.98 (t, J=7.6 Hz, 3H, CH_3CH_2), 1.75–1.91 (m, 2H, CH_3CH_2), 2.98 (t, J=7.6 Hz, 2H, C_7–H), 4.34 (t, J=7.6 Hz, 2H, C_8–H), 5.23, 5.66 (ABq, J=16.8 Hz, 2H, C_1–H), 7.22 (s, 1H, C_5–H); m/z 263 (M^+); IR (KBr) 3423, 1736, 1655 and 1606 cm^{-1}; anal. calcd for $C_{13}H_{13}NO_5$: C, 59.31; H, 4.98; N, 5.32. Found: C, 59.30; H, 5.02; N, 5.21.

4.6. 20(S)-Camptothecin 1

A solution of **2** (400 mg, 1.52 mmol) and **3** (320 mg, 1.52 mmol) in toluene (80 ml) was refluxed in a nitrogen atmosphere. The reaction was continued for 30 min, then *p*-toluenesulfonic acid monohydrate (10 mg) was added, and the mixture was refluxed for another 3 h. The precipitate obtained after cooling was filtered and crystallized from acetonitrile:methanol (4:1), yielding **1** (433 mg, 82.0%). Mp 264–266°C (dec.); $[\alpha]_D^{25}$ +42.1 (*c*=0.50, $CHCl_3$:MeOH=4:1), {lit., $[\alpha]_D$ +40.7[11], +42.0[5] ($CHCl_3$:MeOH=4:1)}; ^{1}H-NMR (DMSO-d_6) δ: 0.97 (t, J=7.6 Hz, 3H, CH_3CH_2), 1.91–2.09 (m, 2H, CH_3CH_2), 5.37 (s, 2H, CH_2), 5.52 (s, 2H, CH_2), 6.61 (s, 1H, OH), 7.43 (s, 1H, Ar–H), 7.70–8.31 (m, 4H, Ar–H), 8.78 (s, 1H, Ar–H); m/z 348 (M^+); IR (KBr) 1740, 1660 and 1590 cm^{-1}.

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