HETEROCYCLES, Vol. 56, 2002, pp. 227-234, Received, 28th May, 2001

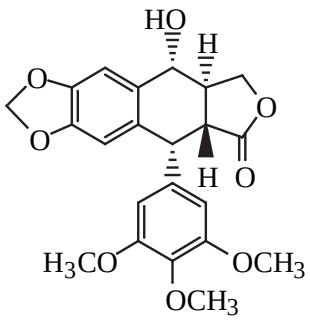
ENZYME-CATALYZED DEDIASTEREOMERIZATION OF DIBENZYLBUTANOLIDES BY PLANT CELL CULTURES

Masumi Takemoto[a]*, Yuki Matsuoka[a], Kiyoshi Tanaka[a], Kazuo Achiwa[a], Nikolay Stoynov[b] and James Peter Kutney[b]*

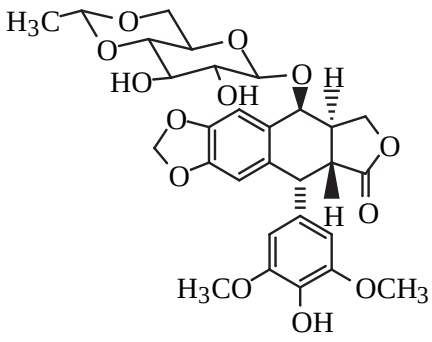
[a] School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Shizuoka 422-8526, Japan
[b]Department of Chemistry , The University of British Columbia, 2036 Main Mall, Vancouver, B. C., Canada V6T 1Y6

Abstract- We have been developed a novel biocatalytic dediastereomerization method, i. e., reactions allowing the transformation of two diastereomers into one diastereomer in quantitative yield. When a mixture of two diastereomers (4*R**-**5**) and (4*S**-**5**) (1:1 ratio) was subjected to *Catharanthus roseus* cells in B5 medium, dediastereomerization took place to give a single diastereomer (4*R**-**5**) in 80 % chemical yield with 100% diastereomeric excess and 0 % enantiomeric excess.

Lignans have attracted much attention among many organic chemists due to their variation of structure together with the important pharmacological activities they possess.[1] Among all lignans, the podophyllotoxin family has been extensively studied over the years.[2,3] Podophyllotoxin is an antitumor lignan, and its derivatization led to a glucoside analog etoposide which is now used clinically as an antitumor agent.[3]
Recently, Kutney *et al.* reported enzyme-catalyzed cyclization of synthetic dibenzylbutanolies to members of the podophyllotoxin family with horseradish peroxiase and with enzymes produced from the cell culture of *Nicotiana sylvestris*.[4]



Podophyllotoxin



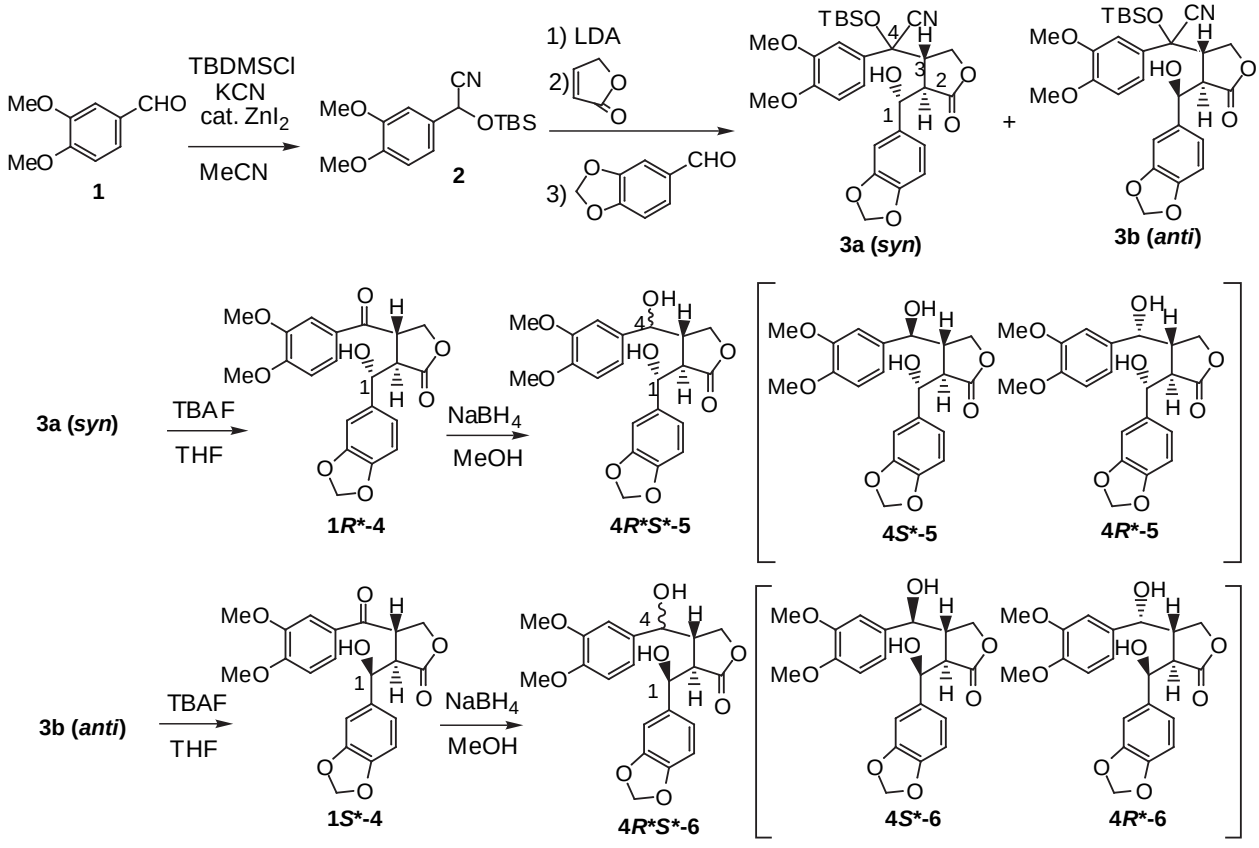
Etoposide

On the other hand, much effort has been devoted to developing an efficient method for the asymmetric synthesis of lignans. Morimoto and Achiwa *et al.* reported efficient asymmetric synthesis of naturally occurring lignan lactones using asymmetric catalytic hydrogenation as a crucial step.[5] Enzymes are known to be versatile and are widely used as catalyst in asymmetric syntheses.[6] In recent

years, much attention has been paid to the ability of cultured cells in which enantioselective transformation of not only secondary metabolites but also organic foreign substrates is possible.[7] We have reported synthetic methods using plant cell cultures so far as follows: 1) asymmetric reduction of benzoyl pyridines,[8-10] 2) asymmetric hydrolysis of (α-acetoxybenzyl)pyridines,[8-10] 3) deracemization[11-13] of racemic alcohols, i.e. 100% conversion of racemic alcohols to the corresponding optically active alcohols, 4) decarboxylation of *trans*-cinnamic acids to the corresponding styrenes or 2-furan.[14,15]

Very recently, we developed a novel dediastereomerization method of dibenzylbutanolides, i.e., reactions allowing the transformation of two diastereomers into one diastereomer in quantitative yield with plant cell cultures.[16] It is quite interesting that the plant cell culture can discriminate the two diastereomers. In this paper, we would like to report in detail this novel dediastereomerization by plant cell cultures.

Racemic dibenzylbutanolides (**5**) and (**6**) were synthesized as shown in Scheme 1 according to the procedure of H. Ohmizu and T. Iwasaki *et al.* [17,18] The synthesized dibenzylbutanolides (**5** and **6**), which are the key intermediates for the synthesis of lignans, were utilized as substrates for the following enzyme-catalyzed dediastereomerization.



Scheme 1

t-Butyldimethylsilyl protected cyanohydrine (**2**) was prepared by the synthetic method of Cava *et al.*[19] Thus, veratraldehyde (**1**) was reacted directly with t-butyldimethylsilyl chloride (TBDMSCl) (1.2 eq) and KCN (4 eq) in acetonitrile containing a catalytic amount of ZnI_2 to afford **2** in 93 % yield. The Michael addition reaction of the anion generated by treatment of **2** with lithium diisopropylamide (LDA) to 2-butenolide in THF at -78℃, followed by treatment of the resulting lithium enolate with piperonal at the same temperature afforded the condensation products (**3a**) and (**3b**). The mixture of **3a** and **3b** was subjected to

silica gel column chromatography using a 2 : 1 mixture of hexane-AcOEt as the eluent to separate **3a** (43%) and **3b** (33%). The isomer (**3a**) was treated with tetrabutylammonium fluoride (TBAF) in THF at room temperature to afford 1*R**-**4** in 90% yield. The ketone (1*R**-**4**) was reduced to 4*R***S**-**5** with $NaBH_4$ in MeOH in 83% yield. The mixture (4*R***S**-**5**) was separated by silica gel column chromatography using a 50 : 1 mixture of CH_2Cl_2-MeOH as the eluent to give 4*S**-**5** (23 %) and 4*R**-**5** (60 %). In the same way, the diastereomers (4*S**-**6**) and (4*R**-**6**) were obtained from **3b** in good overall yield. In this work, we used suspension-cultured cells which had originally been isolated from *Nicotiana tabacum* " Bright Yellow -2", *Daucus carota*, *Camellia sinensis* , and *Catharanthus roseus*. These cell cultures (*N. tabacum*, *D. carota*, *C. sinensis*, and *C. roseus*) were prepared as described in our previous papers.[8-10,15] The dediastereomerization of the compounds (4*R***S**-**5**, 4*S**-**5**, 4*R**-**5**, and 1*R***S**-**4**) was performed with freely suspended plant cell culture in the stationary phase after 10 days of incubation [30-40 g of cells in Murashige and Skoog's (MS) medium or Gamborg' s B5 (B5) medium 80 mL]. A substrate (50 mg) was added to the freely suspended plant cell culture and the mixture was shaken on a rotary shaker (110 rpm) at 25℃. When a mixture of two diastereomers (4*R**-**5**) and (4*S**-**5**) (1:1 ratio) was subjected to *C. roseus* cells in B5 medium, only 4*R**-**5** was isolated in 80% chemical yield with 100% diastereomeric excess and 0% enantiomeric excess. (Table 1). We named this novel reaction dediastereomerization. It is interesting that plant cell culture can discriminate the two diastereomers.

Table 1. Dediastereomerization of a mixture of two diastereomers (4*R**-**5**) and (4*S**-**5**) (1:1 ratio)

4*R***S**-**5** $\xrightarrow{\text{plant cell culture}}$ 4*R**-**5**

			4*R**-**5**		
Entry	Plant cell culture	Time	yield(%)	de(%)	ee(%)
1	*C. roseus*	5 days	80	100	0
2	*C. sinensis*	18 hours	75	100	0
3	*N. tabacum*	6 hours	80	100	0
4	*D. carota*	3 days	80	100	0

We next surveyed a variety of plant cell cultures to find the optimal conditions, and these results are shown in Table 1. Dediastereomerization of 4*R***S**-**5** proceeded with *C. sinensis*, *N. tabacum*, and *D. carota* cell cultures to afford 4*R**-**5** in 100% de. Among the plant cell cultures tested, *N. tabacum* cells gave the best result of conversion in 6 h.

Next we tried the dediastereomerization of 1*R***S**-**4** as shown in Table 2. 1*R***S**-**4** was converted into 1*S**-**4** in 8 days incubation with *C. roseus* cells in 70% chemical yield with 100% de. This dediastereomerization was unsuccessful with other plant cell cultures [*C. sinensis*, *N.tabacum*, *and D. carota*]. To confirm the mechanism of these dediastereomerizations, we carried out experiments using single diastereomers (4*R**-**5**) and (4*S**-**5**) as a substrate with *C. roseus* cells as shown in Scheme 2. 4*S**-**5** was inverted to 4*R**-**5** in 80% chemical yield without any other isomerizations. On the other hand, 4*R**-**5** was resistant to the reaction and was recovered unchanged. From these experiments, the course of dediastereomerization was confirmed by the stereoinversion of 4*S**-**5** to 4*R**-**5**. However, the precise mechanism for this stereoinversion is presently unclear.

Table 2. Dediastereomerization of a mixture of two diastereomers (1*R**-**4**) and (1*S**-**4**) (1:1 ratio)

1*R***S**-**4** —plant cell culture→ 1*S**-**4**

Entry	Plant cell culture	Time (days)	1*S**-**4** yield(%)	de(%)	ee(%)	Recovery yield(%)
1	*C. roseus*	8	70	100	0	-
2	*C. sinensis*	28	-	-	-	80
3	*N. tabacum*	14	-	-	-	75
4	*D. carota*	17	-	-	-	90

Scheme 2

4*S**-**5** —*C. roseus*, 5 days→ 4*R**-**5** (80%, 100% de)

4*R**-**5** —*C. roseus*, 5 days→ 4*R**-**5**

Thus, we have developed a novel dediastereomerization method, i. e., reactions allowing transformation of a mixture of two diastereomers into one diastereomer, utilizing plant cell cultures in almost quantitative yield.

EXPERIMENTAL

^{1}H-NMR spectra were measured at 270 MHz on a JEOL JNM-EX 270 FT NMR spectrometer. Chemical shifts are quoted in ppm with tetramethylsilane as an internal standard, and coupling constants (*J*) are given in Hz. FAB-MS was taken on a JEOL JMS-SX 102 mass spectrometer.

Preparation of *t*-butyldimethylsilyl protected cyanohydrine (2)

To a flame-dried flask, maintained under a positive pressure of argon, were added successively veratraldehyde (**1**) (1.66 g, 10 mmol), acetonitrile (50 mL), KCN (2.6 g, 40 mmol), zinc iodide (50 mg), and TBDMSCl (1.8 g, 12 mmol). The mixture was stirred vigorously. Upon disappearance of the starting material, the solvent was removed *in vacuo*, and the residue was resuspended in ether (50 mL). Precipitates were removed by filtration and rinsed thoroughly with ether (25 mL). The filtrate and washings were combined and the whole was washed with water (25 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to silica gel column chromatography using hexane/AcOEt (4:1) to afford **2** (2.8 g, 93 %) as white powders.

2: mp 59-60℃. ^{1}H NMR ($CDCl_3$) δ : 0.02 (6H, s, $SiMe_2$), 0.72 (9H, s, *t*-Bu), 3.68 (3H, s, OMe), 3.69 (3H, s, OMe), 5.24 (1H, s, CH(OTBS)CN), 6.67- 6.95 (3H, m, aromatic H). *Anal.* Calcd for $C_{16}H_{25}NO_3Si$: C, 62.50; H, 8.20; N, 4.56. Found: C, 62.81; H, 8.17; N, 4.68.

Preparation of 3a and 3b

LDA was prepared by addition of butyllithium (1.6 M in hexane, 2 mL) to a solution of diisopropylamine (254 mg, 2.5 mmol) in THF (5 mL) at -78℃. To the mixture were added succesively solutions of cyanohydrin (**2**) (430 mg, 1.4 mmol) in THF (5 mL), 2-butenolide (141 mg, 1.7 mmol) in THF (2 mL), and piperonal (294 mg, 2.0 mmol) in THF (5 mL) at the same temperature for 1 h. The mixture was quenched by addition of water (20 mL) containing AcOH (290 mg, 4.8 mmol). The organic layer was separated and the aqueous layer was extracted with AcOEt (50 mL). The combined organic layer was washed with brine (10 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to silica gel column chromatography using hexane/AcOEt (2:1) to afford **3a** (syn) (320 mg, 43 %) and **3b** (anti) (245 mg, 33 %) as white powders.

3a (syn): mp 183-185℃. ^{1}H NMR($CDCl_3$) δ : 0.01 (3H, s, SiMe), 0.14 (3H, s, SiMe), 0.90 (9H, s, *t*-Bu) 0.97 (9H, s, *t*-Bu), 2.21 (1H, s, OH), 2.68 (1H, m), 3.71 (3H, s, OMe), 3.88 (3H, s, OMe), 4.61 (2H, m), 5.16(1H, m), 5.80 (2H, s, -OCH_2O-), 6.13 (1H, m, aromatic-H), 6.40-6.80 (5H, m, aromatic-H). *Anal*. Calcd for $C_{28}H_{35}NO_8Si$: C, 62.09; H, 6.51; N, 2.59. Found: C, 61.86; H, 6.47; N, 2.61.

3b (anti): mp 145-146℃. ^{1}H NMR($CDCl_3$) δ : 0.02 (3H, s, siMe), 0.20-0.30 (3H, s, SiMe), 0.99 (9H, s, *t*-Bu), 2.78 (1H, m), 2.93 (1H, m), 3.82 (3H, s, OMe), 3.92 (3H, s, OMe), 4.21 (1H, m), 4.55-4.67 (2H, m), 5.97 (2H, s, -OCH_2O-), 6.49-7.03 (6H, m, aromatic-H). *Anal.* Calcd for $C_{28}H_{35}NO_8Si$: C, 62.09; H, 6.51; N, 2.59. Found: C, 62.29; H, 6.53; N, 2.66.

Preparation of 1*R-4**

To a solution of **3a** (syn) (270 mg, 0.5 mmol) in THF (3 mL) was added TBAF (180 mg) in THF (5 mL) at rt. After 30 min, the solution was washed with water (10 mL) and brine (10 mL) and dried over $MgSO_4$, and concentrated *in vacuo*. The residue was subjected to silica gel column chromatography using hexane/AcOEt (1:1) to afford 1*R**-**4** (180 mg, 90 %) as white powders.

1*R**-**4**: mp 176-178℃. ^{1}H-NMR($CDCl_3$) δ : 2.70 (1H, s, OH), 3.60 (1H, dd, *J*=7.9, 2.9 Hz), 3.89 (3H, s, OMe), 3.95 (3H, s, OMe), 4.15 (1H, m), 4.48-4.61 (2H, m), 5.40 (1H, d, *J*=2.7 Hz), 5.81(2H, d, *J*= 16.2 Hz, -OCH_2O-), 6.59 (1H, d, *J*=8.1 Hz, aromatic-H), 6.72 (2H, d, *J*=8.5 Hz, aromatic-H), 6.81 (1H, d, *J*=8.1 Hz, aromatic-H), 7.23-7.29 (2H, m, aromatic-H). *Anal.* Calcd for $C_{21}H_{20}O_8$: C, 62.99; H, 5.04. Found: C, 63.33; H, 5.06. FAB-MS (m/z): 400 [M^+]

Preparation of 4*S-5 and 4*R**-5**

To a solution of 1*R**-**4** (100 mg, 0.25 mmol) in MeOH (5 mL) was added in one portion sodium borohydride (18.9 mg, 0.5 mmol) at 0℃. The mixture was stirred for 30 min at rt. The solvent was removed *in vacuo*. The residue was disolved in CH_2Cl_2 (in 20 mL) and the solution was washed with brine (10 mL), dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was subjected to silica gel column chromatography using CH_2Cl_2/MeOH (50:1) to afford 4*S**-**5** (23 mg, 23 %) and 4*R**-**5** (60 mg, 60 %) as white powders.

4*R**-**5**: mp 157-159°C. ^{1}H NMR($CDCl_3$) δ : 2.33 (1H, br s, OH), 2.73 (1H, m), 2.92 (1H, t, *J*=4.3 Hz), 2.95 (1H, br s, OH), 3.78 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 4.20 (1H, dd, *J*=4.3, 8.2 Hz), 4.33 (1H, t, *J*=8.6 Hz), 4.66 (1H, d, *J*=4.9 Hz), 5.14 (1H, d, *J*=3.6 Hz), 5.94 (2H, d, *J*=8.1 Hz, -OCH_2O-), 6.44-6.47 (2H, d, *J*=8.2 Hz, aromatic-H), 6.61-6.70 (4H, m, aromatic-H). *Anal.* Calcd for $C_{21}H_{22}O_8$: C, 62.68; H, 5.51. Found : C, 62.50; H, 5.41. FAB-MS (m/z): 402 [M^+]

4*S**-**5**: mp 67-70 ℃. ^{1}H NMR ($CDCl_3$) δ : 2.65 (1H, m), 2.95 (1H, m, OH), 3.26 (1H, dd, *J*=4.6 Hz, 8.6 Hz), 3.27(1H, s, OH), 3.79 (1H, m), 3.85 (1H, m), 3.88 (3H, s, OMe), 3.90 (3H, s, OMe), 5.41(1H, t, *J*=4.6 Hz), 5.52 (1H, d, *J*=3.6 Hz), 5.97 (2H, s, -OCH_2O-), 6.78 -6.91 (6H, m, aromatic-H). *Anal*. Calcd for $C_{21}H_{22}O_8$: C, 62.68; H, 5.51. Found : C, 62.34; H, 5.59. FAB-MS (m/z): 402 [M^+]

Preparation of 1*S-4**

To a solution of **3b** (anti) (270 mg, 0.5 mmol) in THF (3 mL) was added TBAF (180 mg) in THF (5 mL) at rt. After 30 min, the solution was washed with water (10 mL) and brine (10 mL) and dried over $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to silica gel column chromatography using hexane/AcOEt (1:1) to afford 1*S**-**4** (160 mg, 80 %) as white powders.

1*S**-**4**: mp 157-159℃. ^{1}H-NMR ($CDCl_3$) δ : 3.64 (1H, dd, *J*=7.9, 8.9 Hz), 3.83 (1H, s, OH), 3.91 (3H, s, OMe), 3.96(3H, s, OMe), 4.16 (2H, m), 4.41 (1H, m), 4.90 (1H, d, *J*=7.6 Hz), 5.81 (2H, d, *J*= 8.9 Hz, -OCH_2O-), 6.56 (1H, d, *J*=8.8 Hz, aromatic-H), 6.74 (2H, m, aromatic-H), 6.82 (1H, d, *J*=8.8 Hz, aromatic-H), 7.27 (2H, m, aromatic-H). *Anal.* Calcd for $C_{21}H_{20}O_8$: C, 62.99; H, 5.03. Found : C, 63.30; H, 5.09. FAB-MS (m/z): 400 [M^+]

Preparation of 4*S-6 and 4*R**-6**

To a solution of 1*S**-**4** (100 mg, 0.25 mmol) in MeOH (5 mL) was added sodium borohydride (18.9 mg, 0.5 mmol) at 0℃. The mixture was stirred for 30 min at rt. The solvent was removed *in vacuo*. The residue was mixed with CH_2Cl_2 (20 mL) and the mixture was washed with brine (10 mL), dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was subjected to silica gel column chromatography using CH_2Cl_2/MeOH (50:1) to afford 4*S**-**6** (20 mg, 20 %) and 4*R**-**6** (59 mg, 59 %) as white powders.

4*R**-**6**: mp 61-63℃. ^{1}H NMR ($CDCl_3$) δ : 2.74 (1H, m), 3.14 (1H, dd, *J*=4.1, 9.1 Hz), 3.46 (1H, br s, OH), 3.66-3.79 (2H, m), 3.87 (3H, s, OMe), 3.88 (3H, s, OMe), 4.11 (1H, s, OH), 4.53(1H, d, *J*=8.6 Hz), 5.33 (1H, d, *J*=4.0 Hz), 5.97(2H, d, *J*=4.0 Hz, -OCH_2O-), 6.75 -6.95 (6H, m, aromatic-H). *Anal.* Calcd for $C_{21}H_{22}O_8$: C, 62.68; H, 5.51. Found : C, 63.00; H, 5.35. FAB-MS (m/z): 402 [M^+]

4*S**-**6**: mp 62-64℃. ^{1}H NMR ($CDCl_3$) δ : 1.98 (1H, s, OH), 2.51 (1H, m), 2.94 (1H, t, *J*= 8.3 Hz), 3.81 (3H, s, OMe), 3.87 (3H, s, OMe), 4.02-4.05 (2H, m), 4.03 (1H, s, OH), 4.41 (1H, dd, *J*= 7.3, 9.2 Hz), 4.78 (1H, d, *J*=8.6 Hz), 5.95 (2H, d, *J*= 8.9 Hz, -OCH_2O-), 6.50-6.90 (6H, m, aromatic-H). *Anal.* Calcd for $C_{21}H_{22}O_8$: C, 62.68; H, 5.51. Found : C, 62.83; H, 5.60. FAB-MS (m/z): 402 [M^+]

Cultivation of *C. roseus* cells

Suspension cells of *C. roseus* were subcultured every 7 day by transferring a 1-week culture (8 mL) into B5 medium (80 mL) containing 2,4-dichlorophenoxyacetic acid (2,4-D) (1 mg/L) and 2% sucrose (pH 5.5) on a rotary shaker (110 rpm) at 25℃ in the dark.

Cultivation of *D. carota* cells

Suspension cells of *D. carota* were subcultured every 7 day by transferring a 1-week culture (8 mL) into MS medium (80 mL) containing 2,4-D (2 mg/L) and 3% sucrose (pH 5.8) on a rotary shaker (110 rpm) at 25℃ in the dark.

Cultivation of *N. tabacum* cells

Suspension cells of *N. tabacum* were subcultured every 7 day by transferring a 1-week culture (1.3 mL) into MS medium (80 mL) containing 2,4-D (2 mg/L) and 3% sucrose (pH 5.8) on a rotary shaker (110 rpm) at 25℃ in the dark.

Cultivation of *C. sinensis* cells

Suspension cells of *C. sinensis* were subcultured every 10 day by transferring a 1-week culture (10 mL) into B5 medium (80 mL) containing 2,4-D (1.25 mg/L) and 5% sucrose (pH 5.8) on a rotary shaker (110 rpm) at 25℃ in the dark.

Biotransformation of Substrates (4*RS**-5, 4*R**-5, 4*S**-5, or 1*R**S**-4) with plant cell cultures**

A substrate (4*R**S**-**5**, 4*R**-**5**, 4*S**-**5**, or 1*R**S**-**4**) (50 mg) was added to the freely suspended *C. roseus* (B-5 medium, pH 5.5), *N. tabacum* ' Bright Yellow-2' (MS medium, pH 5.8), *D. carota* (MS medium, pH 5.8), and *C. sinensis* (B-5 medium, pH 5.8). The mixture was shaken at 25℃ on a rotary shaker (110 rpm) in the dark. At the termination of the reaction, the incubation mixture was filtered, and the filtered cells were washed with CH_2Cl_2. The filtrates and washings were combined and extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to silica gel column chromatography. The reaction time and the chemical yield are listed in Tables 1, 2 and Scheme 2.

Analysis of optical yield of 4*R-5, 4*S**-5 and 1*S**-4**

Optical purity of the products 4*R**-**5**, 4*S**-**5** and 1*S**-**4** were checked by HPLC analysis (Chiralpak AD hexane /IPA =5 / 1).

ACKNOWLEDGEMENTS

This work was supported in part by the Research Foundation for Pharmaceutical Sciences.

REFERENCES

1. a) W. D. MacRae and G. H. N. Towers, *Phytochemistry*, 1984, **23**, 1207. b) W. D.

MacRae, J. B. Hudson, and G. N. H. Towes, *Planta Med*., 1989, **55**, 531. For reviews, see: b) R. S. Ward, *Nat. Prod. Repts*, 1997, **14**, 43. c)R. S. Ward, *Nat. Prod. Repts,* 1995, **12**, 183.

2. D. C. Ayres and J. D. Loike, Lignans: Chemical, biological and clinical properties; Cambridge University Press: Cambridge, 1990.
3. B. F. Issel, A. R. Rudolph, A. C. Lewis, and T. W. Doyle,"Etoposide (VP-16): Current Status and New developments,"ed. by B. F. Issel, F. M. Muggia, and S. K. Carter, Academic Press, New York, 1984, Chaps. 1 and 2.
4. J. P. Kutney, X. Du, R. Naidu, N. M. Stoynov, and M. Takemoto, *Heterocycles*, 1996, **42**, 479.
5. a) T. Morimoto, M. Chiba, and K. Achiwa, *Heterocycles*, 1992, **33**, 435. b) T. Morimoto, M. Chiba, and K. Achiwa, *Tetrahedron*, 1993, **49**, 1793.
6. E. Santaniello, P. Ferraboshi, P. Gsenti, and A. Manzo, Chem. Rev., 1992, **92**, 1071.
7. J. P. Kutney, *Synlett*, 1991, 11.
8. M. Takemoto, Y. Moriyasu, and K. Achiwa, *Chem. Pharm. Bull*., 1995, **43**, 1458.
9. M. Takemoto, K. Achiwa, N. Stoynov, D. Chen, and J. P. Kutney, *Phytochemistry*, 1996, **49**, 423.
10. M. Takemoto, Y. Yamamoto, and K. Achiwa, *Chem. Pharm. Bull*., 1998, **46**, 419.
11. M. Takemoto and K. Achiwa, *Tetrahedron Asymmetry* , 1995, **12**, 2925.
12. M. Takemoto and K. Achiwa, *Chem. Pharm. Bull*., 1998, **46**, 577.
13. M. Takemoto and K. Achiwa, *Phytochemistry*, 1998, **49**, 1627.
14. M. Takemoto and K. Achiwa, *Tetrahedron Lett*., 1999, **40**, 6595.
15. M. Takemoto and K. Achiwa, *Chem. Pharm. Bull*., 2001, **49**, 639.
16. M. Takemoto, Y. Matsuoka, K. Achiwa, and J. P. Kutney, *Tetrahedron Lett*., 2000, **41**, 499.
17. T. Ogiku, S. Yoshida, M. Takahashi, T. Kuroda, H. Ohmizu, and T. Iwasaki, *Tetrahedron Lett*., 1992, **33**, 4473.
18. T. Ogiku, M. Seki, M. Takahashi, H. Ohmizu, and T. Iwasaki, *Tetrahedron Lett*., 1990, **31**, 5487.
19. V. H. Rawel, J. A. Rao, and M. P. Cava, *Tetrahedron Lett*., 1985, **26**, 4275.