Studies on the Structure–Toxicity Relationship of AK-Toxin, a Host-Specific Toxin to Japanese White Pear, Produced by *Alternaria kikuchiana*: Synthesis of Methyl (4*S*,5*R*)-4-(*N*-Acetylphenylalanyl)oxy-5,6-epoxy-5-methylhex-2(*E*)-enoate and Its Stereoisomers

Hideaki KURODA,[a] Masaaki MIYASHITA,[a] Hiroshi IRIE,*[,a] and Tamio UENO[b]

Faculty of Pharmaceutical Sciences, Nagasaki University,[a] Bunkyo-machi, Nagasaki 852, Japan and Faculty of Agriculture, Kyoto University,[b] Sakyo-ku, Kyoto 606, Japan. Received December 8, 1993; accepted February 7, 1994

Methyl (4*S*,5*R*)-4-(*N*-acetylphenylalanyl)oxy-5,6-epoxy-5-methylhex-2(*E*)-enoate, the enantiomeric analogue of AK-toxin, the host-specific toxin, and its stereoisomers have been synthesized and tested for host-specific toxin activity.

Keywords AK-toxin; host-specific toxin; plant pathology; structure–toxicity relationship; enantiomer; Mitsunobu reaction

We have reported the synthesis of the methyl ester (**2**) of AK-toxin II (**1**),[1] a host-specific toxin to Japanese white pear produced by *Alternaria kikuchiana*, and its congeners and revealed that *R* and *S* configurations at C_8 and C_9 in the structure of the toxin are essential for toxicity, while the configuration of the amino acid moiety has no marked influence on the toxicity.[2] From the viewpoint of the structure-toxicity relationship of AK-toxin II, we were interested in whether the enantiomers of the toxin would have similar toxicity to the host plant or not. We prepared the enantiomeric monoene compounds (**7** and **8**) having *S* and *R* configurations at C_4 and C_5 (these centers correspond to C_8 and C_9 of the parent toxin) and investigated their toxicities, since simple monoene compounds **3** and **4** having *R* and *S* configurations at C_4 and C_5 were toxic to the host plant.

Benzoylation of the diol **12**, prepared from the hydroxyester **11**[2] by the Grignard reaction with methyl-

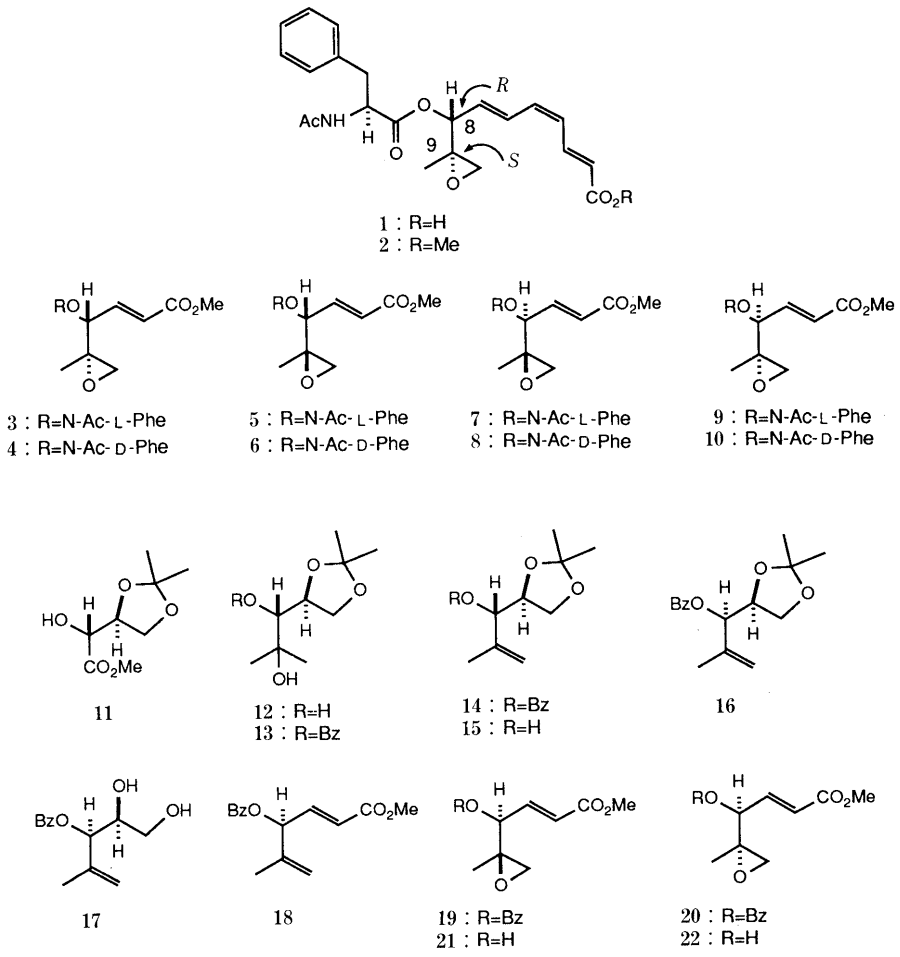

Chart 1

TABLE I. $[\alpha]_D$ Values of the Compounds and Toxicity to Host Plant

Compound	$[\alpha]_D$	Toxicity
3	+6.5° (c=0.28)	+
4	+27.2° (c=0.22)	+
5[a]	+6.4° (c=0.58)	−
6[a]	+27.3° (c=0.41)	−
8	−5.4° (c=0.04)	−
7	−26.0° (c=0.11)	−
10	−5.0° (c=0.36)	−
9	−27.1° (c=0.44)	−

a) These compounds were synthesized by the methods reported previously[2] and the corresponding $[\alpha]_D$ values have been revised. $[\alpha]_D$ is recorded in ethanol.

magnesium iodide, in a conventional way gave the monobenzoate **13** in quantitative yield, and this was easily converted to the olefin **14** with thionyl chloride and pyridine in 82% yield. After removal of the benzoyl group by reduction with lithium aluminum hydride, the resulting allyl alcohol **15** was subjected to the Mitsunobu reaction with diisopropyl azodicarboxylate using benzoic acid as a nucleophile, giving rise to the benzoate **16** diastereomeric to **14** in 77% yield. Removal of the acetonide group in **16** with trifluoroacetic acid in methanol gave the glycol **17** in 91% yield. Oxidation of **17** with periodic acid followed by the Horner–Emmons reaction of the resulting aldehyde with trimethyl phosphonoacetate furnished the diene **18** in 87% overall yield from **17**. Oxidation of **18** with *m*-chloroperbenzoic acid in methylene chloride at room temperature gave two stereoisomeric oxides **19** and **20** in an almost 1 : 1 ratio, as expected,[2] both of which were separable in pure forms by column chromatography. No di- or regioisomeric epoxide was detectable. Methanolysis of **19** and **20** with sodium methoxide gave the alcohols **21** and **22**, respectively. Acylation of **21** with *N*-acetyl-L-phenylalanine by use of dicyclohexyl carbodiimide (DCC) and *N*-hydroxybenzotriazole in the presence of 4-pyrrolidinopyridine gave two stereoisomeric esters **7** and **8** in a 3 : 5 ratio as the result of racemization of the amino acid. The stereoisomeric alcohol **22** gave two stereoisomers **9** and **10** by acylation with the same reagent in the same manner. The structures of these compounds thus obtained were confirmed by comparison of their proton magnetic resonance (^{1}H-NMR) spectra with those of the enantiomeric compounds (**3**—**6**) synthesized previously.[2] The results of toxicity tests of the synthetic monoene compounds (**7**—**10**) and the compounds (**3**—**6**) having the same configurations as the natural product, AK-toxin II, are summarized in the table. Receptors in the Japanese white pear appear to discriminate the stereochemistry as well as the chirality of the host-specific toxins.

Experimental

The ^{1}H-NMR spectra were recorded on a JEOL FX90 spectrometer (90 MHz) using $CDCl_3$ as a solvent and tetramethylsilane as an internal standard, and chemical shifts are given in ppm (δ). Optical rotations were measured with a JASCO DIP-370 digital polarimeter in EtOH. High-resolution mass spectra (HR-MS) were taken with a JEOL JMS-DX303 instrument. Column chromatography was performed with Kiesegel 60 Art. 7734, and flash chromatography with Kieselgel Art. 9385. Merck Kieselgel precoated Silica gel 60F-254 plates were used for preparative thin layer chromatography. $MgSO_4$ was used as a drying agent in the usual manner. Homogeneity of the compounds cited in this report was confirmed by examination of the ^{1}H-NMR spectrum and by thin layer chromatography (TLC).

Grignard Reaction of the Hydroxyester (11) with Methylmagnesium Iodide A solution of the hydroxyester (**11**)[2] (2.43 g, 12.81 mmol) in ether (20 ml) was added to a solution of methylmagnesium iodide prepared from magnesium (1.56 g, 64.05 mmol) and methyl iodide (9.1 g, 14.05 mmol) in ether (23 ml) under gentle reflux for 30 min, and the whole was refluxed for 30 min. After decomposition of the excess reagent by addition of aqueous ammonium chloride, the ethereal solution was washed with water and dried. Removal of the solvent gave the diol (**12**) (2.16 g, 89%) as a colorless oil. IR ($CHCl_3$): 3540 cm^{-1}. ^{1}H-NMR: 1.28 (6H, s), 1.39 (3H, s), 1.43 (3H, s), 2.67 (1H, d, J=3.6 Hz), 3.26 (1H, dd, J=8.0, 3.6 Hz), 3.90—4.50 (4H, m). $[\alpha]_D^{24}$ +3.4° (c=0.9, EtOH).

Benzoylation of the Diol (12) Benzoyl chloride (311 mg, 2.22 mmol) was added dropwise with stirring to a solution of the diol (**12**) (210 mg, 1.11 mmol) in pyridine (2 ml) under ice cooling. The whole was stirred at room temperature for 30 min. After addition of 3% aqueous sodium carbonate and further stirring for 30 min, the solution was extracted three times with ethyl acetate. The organic solution was washed with water, 5% aqueous copper(II) sulfate, and water, and dried. Removal of the solvent gave a residue, which was purified by passing it through a silica gel column in hexane–ethyl acetate (10 : 1) to give the benzoate (**13**) (315 mg, 97%) as colorless crystals, mp 79—80 °C. IR ($CHCl_3$): 1730 cm^{-1}. ^{1}H-NMR: 1.26 (3H, s), 1.38 (3H, s), 1.40 (3H, s), 1.44 (3H, s), 3.66—4.17 (2H, m), 4.63 (1H, dt, J=6.6, 2.8 Hz), 5.05 (1H, d, J=2.8 Hz), 7.33—8.17 (5H, m). $[\alpha]_D^{22}$ −10.1° (c=1.00, EtOH). *Anal.* Calcd for $C_{16}H_{22}O_5$: C, 65.29; H, 7.35. Found: C, 65.06; H, 7.68.

Dehydration of the Benzoate (13) Thionyl chloride (1.74 g, 14.6 mmol) was added dropwise to a solution of the benzoate (**13**) (2.15 g, 7.30 mmol) in pyridine (28 ml) with stirring in an ice bath. After addition of the reagent, the solution was stirred at room temperature for 1 h. Aqueous sodium carbonate was added to the reaction mixture under cooling and the resulting mixture was diluted with ether. The ethereal solution was washed with water, 5% aqueous copper(II) sulfate, and water, dried over potassium carbonate, and concentrated under reduced pressure to give the olefin (**14**) (1.66 g, 82%) as a colorless oil after purification by silica gel column chromatography in hexane–ether (10 : 1). IR ($CHCl_3$): 1730 cm^{-1}. ^{1}H-NMR: 1.38 (3H, s), 1.46 (3H, s), 1.84 (3H, br s), 3.73—4.56 (3H, m), 5.00 (1H, m), 5.12 (1H, m), 5.45 (1H, d, J=6.4 Hz), 7.31—8.16 (5H, m). $[\alpha]_D^{22}$ −15.8° (c=1.1, EtOH).

Reduction of the Benzoate (14) with Lithium Aluminum Hydride A solution of the benzoate (**14**) (2.58 g, 9.34 mmol) and lithium aluminum hydride (780 mg, 20.56 mmol) in ether (50 ml) was stirred under ice cooling for 45 min and the usual work-up gave the alcohol (**15**) (1.31 g, 81%) as a colorless oil. IR ($CHCl_3$): 3560, 1650 cm^{-1}. ^{1}H-NMR: 1.38 (3H, s), 1.46 (3H, s), 1.77 (3H, br. s), 2.49 (1H, br s, OH), 3.65—4.28 (4H, m), 4.90 (1H, m), 5.02 (1H, m). $[\alpha]_D^{22}$ +4.5° (c=1.03, EtOH). *Anal.* Calcd for $C_9H_{16}O_3$: C, 62.76; H, 9.36. Found: C, 62.32; H, 9.39.

Mitsunobu Reaction of the Alcohol (15) A solution of diisopropyl azodicarboxylate (0.23 ml, 1.15 mmol) in tetrahydrofuran (THF, 2 ml) was added to a solution of benzoic acid (141 mg, 1.15 mmol), triphenylphosphine (302 mg, 1.15 mmol), and the alcohol (**15**) (132 mg, 0.77 mmol) in THF (5 ml), and the whole was stirred at room temperature for 2 h and concentrated. The residue was taken up in ethyl acetate. The organic solution was washed with aqueous sodium hydrogen carbonate, aqueous ammonium chloride, and water, and dried. Removal of the solvent gave a residue which was chromatographed on silical gel in hexane–ethyl acetate (8 : 1) to give the benzoate (**16**) (163 mg, 77%) as an oil. IR ($CHCl_3$): 1720 cm^{-1}. ^{1}H-NMR: 1.38 (6H, s), 1.87 (3H, br s), 3.99—4.10 (2H, m), 4.31—4.51 (1H, m), 5.01 (1H, m), 5.10 (1H, m), 5.49 (1H, d, J=5.7 Hz), 7.33—8.11 (5H, m). *Anal.* Calcd for $C_{16}H_{20}O_4$: C, 69.54; H, 7.30. Found: C, 69.27; H, 7.41. $[\alpha]_D^{19}$ +7.0° (c=0.9, EtOH).

Hydrolysis of the Acetonide Group of the Benzoate (16) A solution of the benzoate (**16**) (184 mg, 0.67 mmol) in 30% trifluoroacetic acid in methanol (5 ml) was stirred at room temperature for 3.5 h. Then the mixture was neutralized by addition of sodium hydrogen carbonate under cooling and concentrated under reduced pressure. The residue was diluted with ethyl acetate and passed through a short column of silica gel with the aid of ethyl acetate. The eluate was concentrated to leave an oil which was chromatographed on silica gel in hexane–ethyl acetate (1 : 1). Elution with the same solvent gave the diol (**17**) (144 mg, 91%) as a colorless oil. IR ($CHCl_3$): 3350, 1720 cm^{-1}. ^{1}H-NMR: 1.90 (3H, br s), 3.76—4.03 (3H, m), 5.10 (1H, m), 5.18 (1H, m), 5.44 (1H, d, J=7.2 Hz), 7.44—8.09 (5H, m). HR-MS m/z: Calcd for $C_{13}H_{16}O_4$

(M^+): 236.1049. Found: 236.1053. $[\alpha]_D^{20}$ +30.8° (c=1.37, EtOH).

Methyl 4 (*S*)-Benzoyloxy-5-methyl-2,5-hexadienoate (18) A solution of the foregoing glycol (**17**) (137 mg, 0.58 mmol) and periodic acid (198 mg, 0.87 mmol) in ether (5 ml) was stirred at room temperature for 30 min and diluted with ether. The solution was washed with sodium hydrogen carbonate and brine, dried (K_2CO_3) and concentrated to dryness to leave the aldehyde (113 mg). A solution of the aldehyde in benzene (4 ml) was added to a solution of the Horner–Emmons reagent in benzene prepared from sodium hydride (40 mg 60% in mineral oil, 0.87 mmol) and trimethyl phosphonoacetate (0.14 ml, 0.87 mmol) in benzene (2.5 ml) and the whole was stirred at room temperature for 2 h and diluted with ethyl acetate. The organic solution was washed with water and dried. Removal of the solvent gave a residue which was purified by a column chromatography on silica gel in hexane–ethyl acetate (10 : 1) to give the dienoate (**18**) (132 mg, 87%) as a colorless oil. IR ($CHCl_3$): 1715, 1660 cm^{-1}. ^{1}H-NMR: 1.81 (3H, br s), 3.75 (3H, s), 5.00 (1H, m), 5.17 (1H, m), 6.01—6.19 (2H, m), 6.98 (1H, dd, J=15.0, 5.8 Hz), 7.34—8.13 (5H, m). HR-MS m/z: Calcd for $C_{15}H_{16}O_4$: 260.1049. Found: 260.1055 (M^+). $[\alpha]_D^{20}$ −63.7° (c=0.11, EtOH).

Epoxidation of the Dienoate (18) with *m*-Chloroperbenzoic Acid A solution of the dienoate (**18**) (356 mg. 1.37 mmol) and *m*-chloroperbenzoic acid (354 mg, 2.05 mmol) in methylene chloride (5 ml) was stirred at room temperature for 24 h. After addition of a solution of 5% aqueous sodium thiosulfate, the reaction mixture was extracted with chloroform. The organic solution was washed with 3% aqueous sodium carbonate and water, and dried. Removal of the solvent left a residue which was subjected to flash chromatography on silica gel in hexane–ethyl acetate (10 : 1). Elution with the same solvent gave the *S,R*-oxide (**19**) (192 mg, 51%) as a colorless oil. IR ($CHCl_3$): 1720 cm^{-1}. ^{1}H-NMR: 1.46 (3H, s), 2.76 (1H, d, J=4.6 Hz), 2.93 (1H, d, J=4.6 Hz), 3.76 (3H, s), 5.46 (1H, dd J=5.0, 1.5 Hz), 6.18 (1H, dd, J=15.8, 1.5 Hz), 7.03 (1H, dd, J=15.8, 5.0 Hz), 7.36—8.14 (5H, m). HR-MS m/z: Calcd for $C_{15}H_{16}O_5$: 276.0998. Found: 276.0999 (M^+). $[\alpha]_D^{21}$ −107.6° (c=0.29 EtOH). Subsequent elution with the same solvent gave the *S,S*-oxide (**20**) (176 mg, 47%) as a colorless oil. IR ($CHCl_3$): 1720 cm^{-1}. ^{1}H-NMR: 1.48 (3H, s), 2.73 (1H, d, J=4.6 Hz), 2.89 (1H, d, J=4.6 Hz), 3.74 (3H, s), 5.51 (1H, dd, J=5.0, 1.8 Hz), 6.07 (1H, dd, J=15.8, 1.8 Hz), 6.97 (1H, dd, J=15.8, 5.0 Hz), 7.36—8.14 (5H, m). HR-MS m/z: Calcd for $C_{15}H_{16}O_5$: 276.0998. Found: 276.0991 (M^+). $[\alpha]_D^{21}$ −99.8° (c=0.41, EtOH).

Methyl (4*S*,5*R*)-4-Hydroxy-5,6-epoxy-5-methyl-2-hexenoate (21) A solution of the 4*S*,5*R*-benzoate (**19**) (32 mg, 0.12 mmol) and sodium methoxide generated *in situ* from sodium hydride (5 mg, 0.23 mmol) in methanol (4 ml) was stirred at 0 °C for 2 h. The reaction mixture was diluted with ether and the solution was washed with brine and dried (K_2CO_3). Removal of the solvent gave the 4*S*,5*R*-alcohol (**21**) (15 mg, 74%) as a colorless oil. IR ($CHCl_3$): 1715 cm^{-1}. ^{1}H-NMR: 1.40 (3H, s), 2.62 (1H, d, J=4.6 Hz), 2.88 (1H, d, J=4.6 Hz), 3.76 (3H, s), 4.30 (1H, dd, J=4.8, 1.8 Hz), 6.16 (1H, dd, J=15.6, 1.8 Hz), 6.93 (1H, dd, J=15.6, 4.8 Hz). HR-MS m/z: Calcd for $C_8H_{12}O_4$: 172.0736. Found: 172.0734. $[\alpha]_D^{23}$ −53.2° (c=0.9, EtOH).

Methyl (4*S*,5*S*)-4-Hydroxy-5,6-epoxy-5-methyl-2-hexenoate (22) Treatment of the 4*S*,5*S*-benzoate (**20**) (313 mg, 1.13 mmol) with sodium methoxide in the same way as mentioned above gave the 4*S*,5*S*-alcohol (**22**) (141 mg, 73%) as a colorless oil. IR ($CHCl_3$): 1715 cm^{-1}. ^{1}H-NMR: 1.33 (3H, s), 2.71 (1H, d, J=4.4 Hz), 2.87 (1H, d, J=4.4 Hz), 3.76 (3H, s), 4.03—4.15 (1H, br s), 6.16 (1H, dd, J=15.7, 1.9 Hz), 6.93 (1H, dd, J=15.7, 4.6 Hz). HR-MS m/z: Calcd for $C_8H_{12}O_4$: 172.0736. Found: 172.0723. $[\alpha]_D^{23}$ −45.8° (c=0.27, EtOH).

Acylation of the 4*S*,5*R*-Epoxyalcohol (21) with *N*-Acetyl-L-phenylalanine A solution of *N*-acetyl-L-phenylalanine (54 mg, 0.27 mmol) and DCC (54 mg, 0.27 mmol) in methylene chloride (2 ml) was stirred at room temperature for 1.5 h and filtered through a membrane filter. The filtrate was added to a solution of the 4*S*,5*R*-epoxyalcohol (**21**) (15 mg, 0.09 mmol), 4-pyrrolidinopyridine (26 mg, 0.18 mmol) and *N*-hydroxybenzotriazole (26 mg, 0.18 mmol) in methylene chloride (3 ml) and the whole was stirred at 0 °C for 24 h and diluted with ethyl acetate. The organic solution was passed through a silica gel column and elution with the same solvent gave an oil, which was subjected to preparative thin layer chromatography with hexane–ethyl acetate (1 : 1) to give the 4*S*,5*R*-*N*-acetyl-L-phenylalanine ester (**7**) (4.5 mg, 14%) as colorless crystals, mp 97—99 °C, and 4*S*,5*R*-*N*-acetyl-D-phenylalanine ester (**8**) (6 mg, 19%) as colorless crystals, mp 89—90 °C. **7**: IR ($CHCl_3$): 3450, 1720, 1670 cm^{-1}. ^{1}H-NMR: 1.23 (3H, s), 1.98 (3H, s), 2.57 (1H, d, J=4.8 Hz), 2.72 (1H, d, J=4.8 Hz), 3.12 (2H, d, J=6.4 Hz), 3.76 (3H, s), 4.92 (1H, dt, J=7.7, 6.4 Hz), 5.18 (1H, dd, J=5.3, 1.5 Hz), 6.03 (1H, dd, J=15.8, 1.5 Hz), 6.85 (1H, dd, J=15.8, 5.3 Hz), 7.08—7.33 (5H, m). HR-MS m/z: Calcd for $C_{19}H_{23}NO_6$ 361.1526. Found: 361.1528. $[\alpha]_D^{23}$ −26.0° (c=0.11, EtOH). **8**: IR ($CHCl_3$): 3450, 1720, 1670 cm^{-1}. ^{1}H-NMR: 1.28 (3H, s), 2.00 (3H, s), 2.62 (1H, d, J=4.8 Hz), 2.77 (1H, d, J=4.8Hz), 3.11 (2H, d, J=6.2Hz), 3.77 (3H, s), 4.89 (1H, dt, J=7.7, 6.2 Hz), 5.16 (1H, dd, J=5.7, 1.5 Hz), 5.93 (1H, dd, J=15.8, 1.5 Hz), 6.79 (1H, dd, J=15.8, 5.7 Hz), 7.14—7.31 (5H, m). HR-MS m/z: Calcd for $C_{19}H_{23}NO_6$: 361.1526. Found: 361.1526. $[\alpha]_D^{23}$ −5.4° (c=0.04, EtOH).

Acylation of the 4*S*,5*S*-Epoxyalcohol (22) with *N*-Acetyl-L-phenylalanine The 4*S*,5*S*-epoxyalcohol (**22**) (61 mg, 0.35 mmol) was treated with *N*-acetyl-L-phenylalanine (219 mg, 1.06 mmol) and DCC (219 mg, 1.06 mmol) in the same way as mentioned above to give the 4*S*,5*S*-*N*-acetyl-L-phenylalanine ester (**9**) (36 mg, 28%) as colorless crystals, mp 95.5—96.5 °C, and 4*S*,5*S*-*N*-acetyl-D-phenylalanine ester (**10**) (61 mg, 48%) as colorless crystals, mp 70.5—71.5 °C. **9**: IR ($CHCl_3$): 3450, 1720, 1670 cm^{-1}. ^{1}H-NMR: 1.30 (3H, s), 1.98 (3H, s), 2.67 (1H, d, J=4.4 Hz), 2.77 (1H, d, J=4.4 Hz), 3.17 (2H, d, J=6.2 Hz), 3.75 (3H, s), 4.96 (1H, dt, J=7.7, 6.2 Hz), 5.17 (1H, dd, J=5.1, 1.5 Hz), 6.00 (1H, dd, J=15.8, 5.1 Hz), 6.80 (1H, dd J=15.8, 5.1 Hz), 7.12—7.39 (5H, m). HR-MS m/z: Calcd for $C_{19}H_{23}NO_6$: 361.1526. Found: 361.1526. $[\alpha]_D^{23}$ −27.1° (c=0.44, EtOH). **10**: IR ($CHCl_3$): 3450, 1720, 1670 cm^{-1}. ^{1}H-NMR: 1.30 (3H, s), 1.99 (3H, s), 2.64 (1H, d, J=4.4 Hz), 2.79 (1H, d, J=4.4 Hz), 3.12 (2H, d, J=6.4 Hz), 3.77 (3H, s), 4.91 (1H, dt, J=7.7, 6.4 Hz), 5.24 (1H, dd, J=5.9, 1.5 Hz), 5.89 (1H,dd, J=15.8, 1.5 Hz), 6.72 (1H, dd, J=15.8, 5.9 Hz), 7.06—7.32 (5H, m). HR-MS m/z: Calcd for $C_{19}H_{23}NO_6$: 361.1526. Found: 361.1531. $[\alpha]_D^{23}$ −5.0° (c=0.36, EtOH).

Acknowledgement This work was financially supported in part by a Grant-in-Aid for Scientific Research on Priority Areas (Dynamic Aspects of Natural Product Chemistry) from the Ministry of Education, Science, and Culture of Japan.

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