Biosci. Biotechnol. Biochem., **69** (1), 231–234, 2005

Note

Enantioselective Synthesis of Four Isomers of 3-Hydroxy-4-Methyltetradecanoic Acid, the Constituent of Antifungal Cyclodepsipeptides W493 A and B

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Received August 25, 2004; Accepted October 21, 2004

Four possible stereoisomers of 3-hydroxy-4-methyltetradecanoic acid were enantioselectively synthesized by using Sharpless epoxidation and a subsequent epoxide-ring opening reaction with trimethylaluminum as the key steps. The absolute configuration of the β-oxyacid component of antifungal cyclodepsipeptides W493 A and B was consequently determined as 3*S*,4*R*.

Key words: Sharpless epoxidation; W493 A; W493 B; cyclodepsipeptide; 3-hydroxy-4-methyltetradecanoic acid

By bioassay-guided fractionation, two cyclodepsipeptides, W493 A (**1**) and B (**2**) (Fig. 1), were isolated from the culture broth of *Fusarium* sp. and characterized as potent antifungal substances with unique activity for inducing morphological change to hypha in several fungal species.[1,2] Both compounds contain a novel β-oxyacid component, 3-hydroxy-4-methyltetradecanoic acid (HMTA), which may influence their antifungal potency.[3,4]

In order to further investigate the antifungal mode of action based on derivative synthesis, it was necessary to stereoselectively synthesize the four HMTA isomers, (3*R*,4*R*)-HMTA (**3**), (3*S*,4*S*)-HMTA (**4**), (3*S*,4*R*)-HMTA (**5**), and (3*R*,4*S*)-HMTA (**6**).

The absolute configuration of the naturally occurring HMTA had previously been determined to be 3*S*,4*R* by a comparison of the ^{1}H-NMR spectrum of the corresponding 2-naphthylmethoxyacetic acid (2NMA) ester[5] with those of the four chemically synthesized stereoisomers. A synthetic study on HMTAs has previously been reported by Flippin *et al.*[6] However, no optically pure HMTAs had been obtained so that its chirality remained unclear. We describe here details of a convenient synthesis of the four stereoisomers and their properties.

Epoxy alcohols **7** and **8** were enantioselectively synthesized in a high yield as previously reported, but with slight modifications (Scheme 1).[7] The ring-opening reaction of **7** by Me_3Al proceeded in a regio- and stereoselective manner to produce 1,2-diol **9**.[8] After selectively tosylating the primary hydroxyl group in **9**, the secondary alcohol obtained was converted to β-oxyacid **3** *via* successive steps of cyanide substitution and alkaline hydrolysis. Likewise, **4** was synthesized from epoxy alcohol **8**.

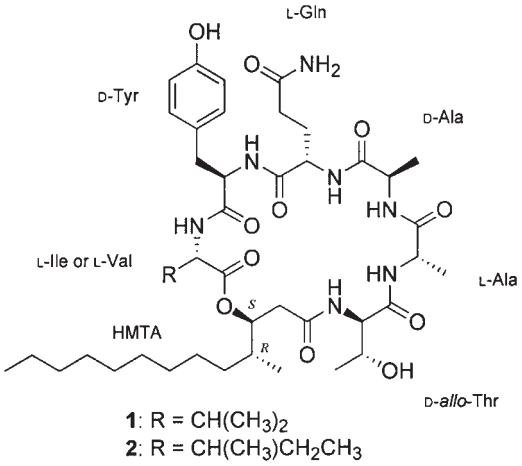

Fig. 1. Structures of W493 A (**1**) and B (**2**).

Selective inversion at C-2 in **9** was needed in order to furnish β-oxyacid **5**. Accordingly, the primary alcohol moiety in **9** was protected as a TBS ether and then the remaining secondary alcohol moiety was tosylated. The removal of the TBS protecting group with excess TBAF at 40 °C provided epoxide **10** in one pot. Desired β-oxyacid **5** was obtained by cyanide substitution and hydrolysis of **10**. Compound **6** was synthesized from **8** by the same steps as those used for obtaining **5** from **7**.

The synthetic HMTAs, **3**, **4**, **5** and **6**, were converted

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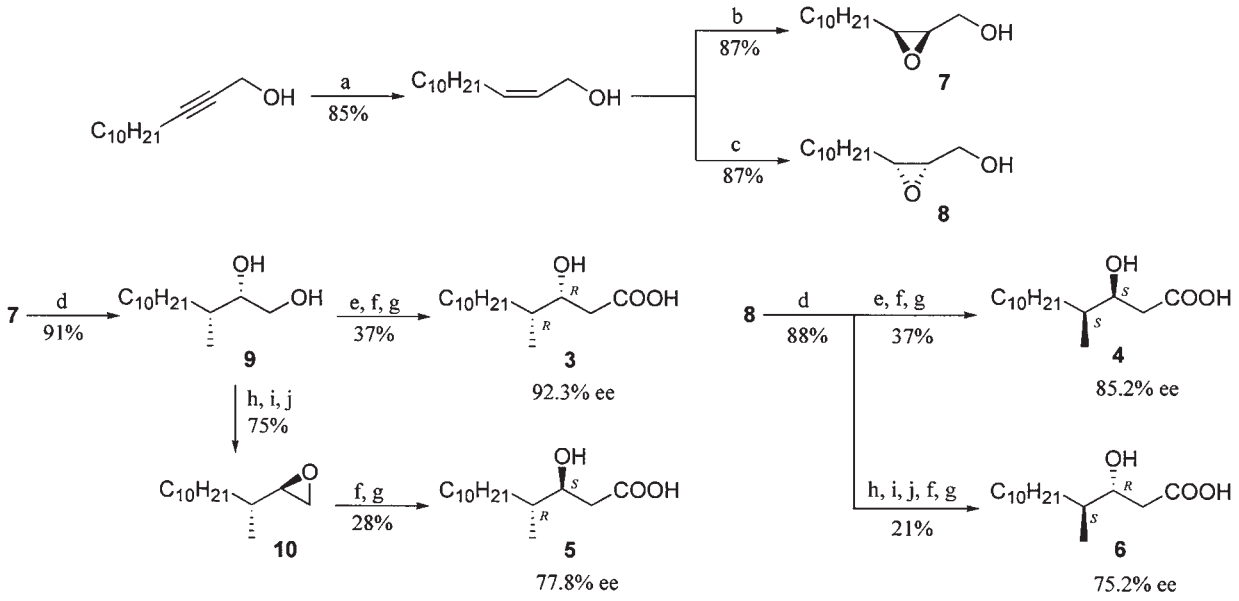
Abbreviations: 2NMA, 2-naphthylmethoxyacetic acid; DET, diethyl tartrate; DMAP, 4-dimethylaminopyridine; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; HMTA, 3-hydroxy-4-methyltetradecanoic acid; TBAF, tetrabutylammonium fluoride; TBSCl, *tert*-butyldimethylsilyl chloride



Scheme 1. Synthesis of (3*R*,4*R*)-HMTA (**3**), (3*S*,4*S*)-HMTA (**4**), (3*S*,4*R*)-HMTA (**5**) and (3*R*,4*S*)-HMTA (**6**).
Reagents: (a) H_2, 5%Pd/$BaSO_4$, quinoline, MeOH; (b) TBHP, Ti(O-*i*-Pr)$_4$, D-DET, CH_2Cl_2; (c) TBHP, Ti(O-*i*-Pr)$_4$, L-DET, CH_2Cl_2; (d) Me_3Al, CH_2Cl_2; (e) TsCl, pyridine/CH_2Cl_2; (f) NaCN, EtOH/H_2O; (g) KOH, EtOH/H_2O; (h) TBSCl, imidazole, DMF; (i) TsCl, pyridine; (j) TBAF, THF.

into the corresponding (*S*)-2NMA esters, **11**, **12**, **13** and **14**, *via* their methyl esters. The ^{1}H-NMR assignment of **13** derived from (3*S*,4*R*)-HMTA (**5**) was in complete agreement with that of the (*S*)-2NMA ester from natural HMTA.[1)] The absolute configuration of natural HMTA was consequently determined to be 3*S*,4*R*.

Experimental

All melting point (mp) values are uncorrected. Optical rotation was measured with a Horiba SEPA-300 high-sensitivity polarimeter. HREI-MS data were measured with a Jeol JMX-AX500 spectrometer, and ^{1}H- and ^{13}C-NMR spectra (400 MHz and 100 MHz) were measured with a Jeol JNM-α400 spectrometer.

(2S,3R)-3-Methyl-1,2-tridecanediol (**9**). To a solution of **7**[7)] (0.50 g, 2.33 mmol) in CH_2Cl_2 (20 ml) at 0 °C was carefully added 6.50 ml of Me_3Al (1.08 M in hexane, 7.02 mmol) under an argon atmosphere. The reaction mixture was stirred at room temperature for 10 h and then quenched with 3 M HCl (10 ml) at 0 °C. After filtering through a pad of Celite, the mixture was extracted with CH_2Cl_2. The organic layer was washed with brine and dried over Na_2SO_4. Concentration *in vacuo* and subsequent silica gel chromatography with EtOAc–hexane (1:4) gave **9** (0.49 g, 91%) as a colorless oil. $[\alpha]_D^{20}$ +8.55° (*c* 1.0, $CHCl_3$). IR ν_{max} (film) cm^{-1}: 3377, 2924, 1467, 1066, 1014. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J = 6.8 Hz), 0.91 (3H, d, J = 6.8 Hz), 1.26 (18H, m), 1.53 (1H, m), 2.05 (1H, bs), 2.68 (1H, bs), 3.55 (3H, m). ^{13}C-NMR ($CDCl_3$) δ: 14.6, 14.6, 22.7, 27.1, 29.4, 29.6, 29.7, 29.9, 31.9, 33.0, 35.8, 65.2, 75.8. HREI-MS m/z (M^+–OH): calcd. for $C_{14}H_{29}O$, 213.2219; found, 213.2185.

(3R,4R)-3-Hydroxy-4-methyltetradecanoic acid (**3**) and *(3S,4S)-3-hydroxy-4-methyltetradecanoic acid* (**4**). To a solution of **9** (0.20 g, 0.87 mmol) in a mixture of pyridine–CH_2Cl_2 (1:6, 7 ml) at 0 °C was added TsCl (0.17 g, 0.89 mmol). After being stirred at 0 °C for 1 h and at 10 °C for 5 h, the mixture was diluted with CH_2Cl_2 (20 ml). The organic layer was successively washed with a saturated $CuSO_4$ solution and brine, and then filtration and concentration gave a crude tosylate. The tosylate in aqueous 40% EtOH (8 ml) was heated to reflux with NaCN (0.74 g, 15.1 mmol) for 8 h. After removing most of the EtOH *in vacuo*, the residue was extracted with ether. The organic layer was washed with brine and dried over Na_2SO_4, filtration and concentration subsequently affording a crude cyanide. The cyanide in aqueous 40% EtOH (8 ml) was heated to reflux with KOH (1.34 g, 23.9 mmol) for 6 h. Most of the EtOH was removed *in vacuo*, and the residue was washed with ether. The aqueous layer was carefully acidified to pH 2 with concentrated HCl while ice-cooling under a hood and then extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was subjected to silica gel chromatography, eluting with EtOAc–hexane (1:4), and crystallization from hexane gave **3** (83 mg, 37%) as colorless needles, mp 68–71 °C. $[\alpha]_D^{20}$ +17.20° (*c* 1.0, $CHCl_3$). IR ν_{max} (film) cm^{-1}: 2916, 1705, 1309, 1066. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J = 6.8 Hz), 0.92 (3H, d, J = 6.8 Hz), 1.26 (18H, m), 1.54 (1H, m), 2.52 (2H, m), 3.97 (1H, dt, J = 8.0, 4.4 Hz). ^{13}C-NMR ($CDCl_3$) δ: 14.1, 14.2, 22.7, 27.2, 29.4, 29.6, 29.7, 29.9, 31.9, 32.7, 38.1, 38.4, 71.3, 177.3. HREI-MS m/z (M^+ + H): calcd. for $C_{15}H_{31}O_3$, 259.2273; found, 259.2287. According to essentially the same procedure, **4** (83 mg, 33%) was synthesized from **8** as colorless needles, mp 67–69 °C.

$[\alpha]_D^{20}$ −17.50° (*c* 1.0, $CHCl_3$). The ^{1}H- and ^{13}C-NMR, HREI-MS, and IR spectral data were in complete agreement with those of **3**.

(2R)-[(1R)-1-methylundecyl]-oxirane (**10**). To a solution of **9** (0.80 g, 3.47 mmol) and imidazole (0.48 g, 7.05 mmol) in DMF (4 ml) at 0 °C was added TBSCl (0.53 g, 3.52 mmol). After being stirred at 0 °C for 0.5 h and then at room temperature for 2 h, the reaction mixture was diluted with CH_2Cl_2 (6 ml). The organic layer was washed with brine and dried over Na_2SO_4, subsequent filtration and concentration giving a crude silyl ether. TsCl (0.63 g, 3.30 mmol) was added to a solution of this silyl ether in pyridine (5 ml) at 0 °C. After being stirred at 0 °C for 1 h and then at room temperature for 5 h, the reaction mixture was diluted with CH_2Cl_2 (10 ml). The organic layer was washed with a saturated $CuSO_4$ solution and dried over Na_2SO_4, subsequent filtration and concentration giving a crude tosylate. To a solution of this tosylate in THF (2 ml) was dropwise added 5.50 ml of TBAF (1.0 M in THF, 5.50 mmol). The reaction mixture was stirred at 40 °C for 6 h and then cooled to room temperature. After being diluted with CH_2Cl_2, the organic layer was washed with brine and dried over Na_2SO_4. Concentration *in vacuo* and subsequent silica gel chromatography with EtOAc–hexane (1:39) gave **10** (0.55 g, 75%) as a colorless oil, $[\alpha]_D^{20}$ +3.10° (*c* 1.0, $CHCl_3$). IR ν_{max} (film) cm^{-1}: 2927, 931, 877, 829. ^{1}H-NMR ($CDCl_3$) δ: 0.81 (3H, d, $J = 6.8$ Hz), 0.85 (3H, d, $J = 6.0$ Hz), 1.19 (18H, m), 1.46 (1H, m), 2.40 (1H, t, $J = 4.0$ Hz), 2.63 (2H, d, $J = 4.0$ Hz). ^{13}C-NMR ($CDCl_3$) δ: 14.1, 15.6, 22.7, 26.9, 29.3, 29.6, 29.9, 31.9, 34.6, 36.1, 45.6, 57.0. HREI-MS *m/z* (M^+): calcd. for $C_{14}H_{28}O$, 212.2140; Found, 212.2158.

(3S,4R)-3-Hydroxy-4-methyltetradecanoic acid (**5**) and *(3R,4S)-3-hydroxy-4-methyltetradecanoic acid* (**6**). According to essentially the same procedure as that used for the preparation of **3** from **9**, except for the tosylation step, **5** (68 mg, 28%) was synthesized as colorless needles, mp 40–42 °C. $[\alpha]_D^{20}$ −3.60° (*c* 1.0, $CHCl_3$). IR ν_{max} (KBr) cm^{-1}: 2928, 1711, 1292, 1045. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, $J = 6.8$ Hz), 0.90 (3H, d, $J = 6.8$ Hz), 1.26 (18H, m), 1.62 (1H, m), 2.47 (1H, dd, $J = 16.4$, 9.6 Hz), 2.54 (1H, dd, $J = 16.4$, 2.8 Hz), 3.90 (1H, ddd, $J = 9.6$, 5.2, 2.8 Hz). ^{13}C-NMR ($CDCl_3$) δ: 14.1, 14.8, 22.7, 27.1, 29.4, 29.6, 29.6, 29.7, 29.9, 31.9, 32.3, 37.6, 38.2, 71.8, 178.1. HREI-MS *m/z* ($M^+ + H$): calcd. for $C_{15}H_{31}O_3$, 259.2273; found, 259.2278. Compound **6** (68 mg, 19%) was synthesized from **8** as colorless needles, mp 41–43 °C. $[\alpha]_D^{20}$ +2.35° (*c* 1.0, $CHCl_3$). The ^{1}H- and ^{13}C-NMR, HREI-MS, and IR spectral data were in complete agreement with those of **5**.

Preparation of the (S)-2NMA esters. Each HMTA ester (2.0 mg, 7.8 μmol) was dissolved in ether (1 ml), and ethereal CH_2N_2 was added until the yellow color was retained. After evaporating, the residue was purified by silica gel chromatography, eluting with EtOAc–hexane (1:4), to give the HMTA methyl ester as a colorless oil, this being used in the next step without further purification.

To each HMTA methyl ester, DMAP (0.4 mg, 3.3 μmol) and (*S*)-2NMA (1.8 mg, 8.3 μmol) dissolved in 0.3 ml of CH_2Cl_2 were added EDC (3.0 mg, 15.6 μmol) and Et_3N (1.5 mg, 14.8 μmol) at 0 °C. After stirring for 12 h, the reaction mixture was diluted with CH_2Cl_2. The organic layer was successively washed with a diluted aqueous citric acid solution, saturated aqueous $NaHCO_3$ solution, water and brine, dried over Na_2SO_4, and evaporated. The crude product was purified by HPLC in a silica gel column (8.0 × 250 nm, Develosil 60-10, Nomura Chemical Co.), eluting with EtOAc–hexane (1:4) at 2.0 ml/min to give the (*S*)-2NMA ester as a colorless oil. The enantiomeric excess (ee) was calculated on the basis of the peak area ratio between the diastereomers in an HPLC analysis at 254 nm.

Methyl (3*R*,4*R*)-3-[(*S*)-2′-naphthylmethoxyacyloxy]-4-methyltetradecanoate (**11**). This was obtained in an 85% yield (t_R 16.2 min, 92.3% ee). ^{1}H-NMR ($CDCl_3$) δ: 0.87 (3H, d, $J = 6.8$ Hz), 0.85 (3H, t, $J = 6.8$ Hz), 1.09–1.30 (18H, m), 1.71 (1H, m), 2.39 (1H, dd, $J = 15.6$, 4.4 Hz), 2.47 (1H, dd, $J = 15.6$, 8.8 Hz), 3.24 (3H, s), 3.46 (3H, s), 4.91 (1H, s), 5.27 (1H, ddd, $J = 8.8$, 4.4, 4.0 Hz), 7.52 (3H, m), 7.83 (3H, m), 7.90 (1H, s).

Methyl (3*S*,4*S*)-3-[(*S*)-2′-naphthylmethoxyacyloxy]-4-methyltetradecanoate (**12**). This was obtained in an 80% yield (t_R 22.9 min, 85.2% ee). ^{1}H-NMR ($CDCl_3$) δ: 0.69 (3H, d, $J = 6.8$ Hz), 0.89 (3H, t, $J = 6.8$ Hz), 0.77–1.31 (18H, m), 1.52 (1H, m), 2.49 (1H, dd, $J = 15.6$, 4.4 Hz), 2.53 (1H, dd, $J = 15.6$, 8.8 Hz), 3.46 (3H, s), 3.60 (3H, s), 4.90 (1H, s), 5.26 (1H, ddd, $J = 8.8$, 4.4, 4.0 Hz), 7.50 (3H, m), 7.82 (3H, m), 7.90 (1H, s).

Methyl (3*S*,4*R*)-3-[(*S*)-2′-naphthylmethoxyacyloxy]-4-methyltetradecanoate (**13**). This was obtained in a 73% yield (t_R 23.1 min, 77.8% ee). ^{1}H-NMR ($CDCl_3$) δ: 0.62 (3H, d, $J = 6.8$ Hz), 0.89 (3H, t, $J = 6.8$ Hz), 0.94–1.30 (18H, m), 1.56 (1H, m), 2.53 (2H, m) 3.46 (3H, s), 3.58 (3H, s), 4.90 (1H, s), 5.26 (1H, td, $J = 6.0$, 5.6 Hz), 7.51 (3H, m), 7.83 (3H, m), 7.91 (1H, s).

Methyl (3*S*,4*S*)-3-[(*S*)-2′-naphthylmethoxyacyloxy]-4-methyltetradecanoate (**14**). This was obtained in a 70% yield (t_R 16.1 min, 75.2% ee). ^{1}H-NMR ($CDCl_3$) δ: 0.85 (3H, d, $J = 6.8$ Hz), 0.88 (3H, t, $J = 6.8$ Hz), 1.18–1.33 (18H, m), 1.81 (1H, m), 2.40 (1H, dd, $J = 15.6$, 4.4 Hz), 2.45 (1H, dd, $J = 15.6$, 8.8 Hz), 3.12 (3H, s), 3.45 (3H, s), 4.90 (1H, s), 5.26 (1H, ddd, $J = 8.8$, 5.2, 4.4 Hz), 7.51 (3H, m), 7.83 (3H, m), 7.90 (1H, s).

Acknowledgment

The authors are indebted to Prof. T. Kusumi of Tokushima University for providing us with chiral 2NMA, and to Dr. E. Fukushi of Hokkaido University for HREI-MS measurements.

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