Absolute Stereostructure Determination of Cladospolide A Using MTPA Ester Method[1)]

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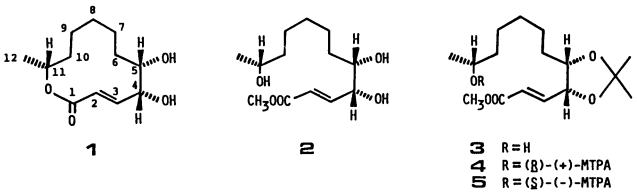
Synopsis. The absolute configuration of cladospolide A, a phytotoxic macrolide produced by a fungus *Cladosporium cladosporioides*, was determined as (4*R*,5*S*, 11*R*,2*E*)-4,5-dihydroxy-2-dodecen-11-olide by means of MTPA method.

Cladospolide A (**1**), a novel fungal metabolite, was isolated from culture filtrate of *Cladosporium cladosporioides* FI-113 and showed the inhibitory activity to lettuce seedlings. In the previous papers, we reported cladospolide A was a novel macrolide and its planar structure was shown as (*E*)-4,5-dihydroxy-2-dodecen-11-olide.[2)] The relative stereochemistry of three asymmetric centers of **1** was also established as (4*R*,5*S*,11*R*) or (4*S*,5*R*,11*S*) by X-ray single crystal structure elucidation.[1,3)] We now wish to report here the determination of the absolute configuration of cladospolide A by means of the MTPA method.[4,5)]

The lactone moiety of **1** was transformed into hydroxy ester, methyl (*E*)-4,5,11-trihydroxy-2-dodecenoate (**2**), by treatment with methanolic hydrogen chloride. Isopropylidenation of 1,2-diol part of **2** with acetone in acidic medium gave methyl (*E*)-4,5-(isopropylidenedioxy)-11-hydroxy-2-dodecenoate (**3**), which possesses an acyclic and secondary alcohol structure without steric congestion. To this alcohol (**3**), the MTPA method was applied.[4)] The alcohol (**3**) was transformed into the corresponding (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid [(*R*)-(+)-MTPA] ester (**4**) and (*S*)-(−)-MTPA ester (**5**) according to Mosher's method.[6)] ^{1}H NMR spectra of **4** and **5** were taken with various molar ratio (0—0.6) of Eu(fod)$_3$-d_{27} to MTPA ester in $CDCl_3$, and the magnitudes of induced chemical shifts of each signal were plotted *versus* molar ratio (Eu(fod)$_3$-d_{27}/MTPA ester). In this range the induced shifts were essentially linear with respect to molar ratio of the reagent and MTPA ester. Lanthanoid induced shift (LIS) values for the selected proton signals of **4** and **5** were given in Table 1. The ΔLIS_{OMe} value for the methoxyl group of MTPA was largely positive (+1.67), while other ΔLIS values were very small. This indicates that the chiral center at $C_{(11)}$ of **3** is in an *R*-configuration according to the generality for the MTPA method.[4)] Then, the stereostructure of cladospolide A was determined as (4*R*,5*S*,11*R*,2*E*)-4,5-dihydroxy-2-dodecen-11-olide (**1**).[7)]

Table 1. Lanthanoid induced shift (LIS) values for the selected proton signals of (*R*)-(+)-MTPA ester (**4**) and (*S*)-(−)-MTPA ester (**5**)

	LIS values of **4**	ΔLIS	LIS values of **5**
$C_{(1)}OOCH_3$	2.85	+0.17	2.68
$C_{(2)}$-H	3.08	+0.25	2.83
$C_{(3)}$-H	2.91	+0.15	2.76
$C_{(4)}$-H	0.63	+0.01	0.62
$C_{(5)}$-H	0.26	+0.05	0.21
$C_{(11)}$-H	1.17	+0.32	0.85
$C_{(11)}$-CH_3	0.63	+0.34	0.29
MTPA-OCH_3	5.05	+1.67	3.38



Experimental

General Procedures. Infrared (IR) spectra were measured on JASCO IRA-2 and Hitachi 260-30 spectrometers. Proton and carbon-13 nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectra were taken using Varian EM390 (90 MHz for ^{1}H), JEOL FX-90Q (90 MHz for ^{1}H), JEOL FX-100 (100 MHz for ^{1}H and 25 MHz for ^{13}C), and JEOL MH-100 (100 MHz for ^{1}H) spectrometers. Chemical shifts were expressed in δ (ppm) downfield from tetramethylsilane as an internal standard and coupling constants in Hz. Mass (MS) spectra were run on a JEOL D-300 mass spectrometer operating at 70 eV. Wakogel C-200 (Wako) were used for column chromatography.

Methyl (4R,5S,11R,2E)-4,5,11-Trihydroxy-2-dodecenoate (2). Cladospolide A (**1**; 88 mg) was dissolved in 20 ml methanol saturated with hydrogen chloride. After standing for 1 h, the reaction mixture was concentrated *in vacuo* and extracted with ethyl acetate. The extract was subjected to separation by silica gel (20 g) column chromatography and was eluted with ethyl acetate to afford **2** as an oil (91 mg). **2**: IR (neat) 3400, 1715, and 1665 cm^{-1}; ^{1}H NMR (100 MHz; $(CD_3)_2CO$) δ=1.24 (3H, d, *J*=6 Hz), 3.75 (3H, s), 3.4—4.1 (3H including 2×−OH, m),[8)] 4.2—4.5 (3H including 1×−OH, m),[8)] 6.05 (1H, br d, *J*=15 Hz), 7.05 (1H, dd, *J*=15 and 4.5 Hz); ^{13}C NMR (25 MHz; $(CD_3)_2CO$) δ=24.42 (CH_3), 26.94 (CH_2), 27.02 (CH_2), 30.94 (CH_2), 33.96 (CH_2), 40.60 (CH_2), 52.00 (CH_3), 67.98 (CH), 75.14 (CH), 75.29 (CH), 121.62 (CH), 150.20 (CH), 167.63 (C=O);[9)] MS *m/z* 261 (M+H$^+$; 0.001),[10)] 116 (100).

Methyl (4R,5S,11R,2E)-4,5-(Isopropylidenedioxy)-11-hydroxy-2-dodecenoate (3). Trihydroxy ester (**2**; 80 mg) was

dissolved in dry acetone (20 ml) containing 0.5% 6 M* hydrochloric acid and the mixture was allowed to stand at room temperature for 45 h. The reaction mixture was neutralized with 0.1 M sodium methoxide and evaporated *in vacuo*. The residue was subjected to silica gel (20 g) column chromatographic separation and was eluted with benzene–ethyl acetate (9:1) to afford **3** as an oil (33 mg). **3**: IR (neat) 3450, 1720, 1630 cm^{-1}; ^1H NMR (100 MHz; $CDCl_3$) δ=1.16 (3H, d, J=6 Hz), 1.36 (3H, s), 1.48 (3H, s), 3.70 (3H, s), 4.16 (1H, m), 4.60 (1H, br t, J=4.5 Hz), 6.00 (1H, br d, J=15 Hz), 6.76 (1H, dd, J=15 and 4.5 Hz); ^{13}C NMR (25 MHz; $CDCl_3$) δ=23.60, 25.62, 25.62, 26.32, 28.13, 29.57, 30.53, 39.31, 51.74, 68.18, 77.48, 78.39, 108.93, 122.67, 144.23, 166.55; MS m/z 285 ($M-15^+$; 24), 193 (10), 156 (27), 125 (17), 98 (100). No molecular ion was observed.

(R)-(+)-MTPA Ester (4). A mixture of dry pyridine (0.3 ml), (*R*)-(+)-MTPA chloride (0.05 ml), the substrate alcohol (**3**; 13 mg), and carbon tetrachloride (0.3 ml) was stirred at room temperature for 3 d. Excess amount of *N,N*-dimethyl-1,3-propanediamine (*ca.* 0.1 ml) was added and the mixture allowed to stand for 10 min. It was then extracted with ether, washed (with dil. HCl ×2, saturated aqueous solution of sodium carbonate, and saturated brine ×2), and dried over magnesium sulfate. The filtered ether solution was concentrated, and the reaction product was purified by passing through a silica-gel (*ca.* 5 g) column. Elution with benzene–acetone(5:1) gave 14 mg of (*R*)-(+)-MTPA ester (**4**; 63% yield): an oil, IR (neat) 1740, 1725, and 1660 cm^{-1}; ^1H NMR (90 MHz; $CDCl_3$) δ=1.25 (3H, d, J=6 Hz), 1.38 (3H, s), 1.51 (3H, s), 3.57 (3H, s, OCH_3 at MTPA part), 3.75 (3H, s, $-COOCH_3$), 4.22 (1H, m, $C_{(5)}$–H), 4.64 (1H, td, J=6 and 1 Hz, $C_{(4)}$–H), 5.13 (br q, J=6 Hz, $C_{(11)}$–H), 6.07 (1H, dd, J=16 and 1 Hz, $C_{(2)}$–H), 6.84 (1H, dd, J=16 and 6 Hz, $C_{(3)}$–H), and 7.3—7.6 (5H, m); MS m/z 516 (M^+; 2), 501 (43), 484 (3), 225 (58), 193 (40), 189 (60), 156 (98), and 98 (100); Found: m/z 516.2352. Calcd for $C_{26}H_{35}O_7F_3$: M, 516.2334.

(S)-(−)-MTPA Ester (5). Alcohol (**3**; 14 mg) was treated with (*S*)-(−)-MTPA chloride by the same procedure as above to transform into (*S*)-(−)-MTPA ester (**5**; 15 mg; 62% yield): an oil, IR (neat) 1740, 1725, and 1660 cm^{-1}; ^1H NMR (90 MHz; $CDCl_3$) δ=1.28 (3H, diffused d), 1.38 (3H, s), 1.51 (3H, s), 3.57 (3H, s, $-OCH_3$ at MTPA part), 3.75 (3H, s, $-COOCH_3$), 4.21 (1H, m, $C_{(5)}$–H), 4.64 (1H, br t, J=6 Hz, $C_{(4)}$–H), 5.15 (1H, br q, J=6 Hz, $C_{(11)}$–H), 6.07 (1H, br d, J=15 Hz, $C_{(2)}$–H), 6.84 (1H, dd, J=15 and 6 Hz, $C_{(3)}$–H), and 7.3—7.6 (5H, m); MS m/z 516 (M^+; 2), 501 (36), 484 (6), 225 (60), 193 (38), 189 (91), 156 (96), and 98 (100). Found: m/z 516.2299. Calcd for $C_{26}H_{35}O_7F_3$: M, 516.2334.

* 1 M=1 mol dm^{-3}.

References

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2) A. Hirota, A. Isogai, and H. Sakai, *Agric. Biol. Chem.*, **45**, 799 (1981); A. Hirota, H. Sakai, and A. Isogai, *ibid.*, **49**, 731 (1985).

3) A detail description on the X-ray analysis of cladospolide A (**1**) will be reported elesewhere.

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7) CD spectrum of bis(*p*-chlorobenzoate) of **1** was also taken, and $[\Theta]_{245}$=−23500 was observed, which was coincident with the result from MTPA method; as cladospolide A (**1**) posesses two conformations even in solid state (see Ref. 1 and 3), we estimate it very dangerous to apply dibenzoate chirality rule easily to a derivative of this compound in solution state.

8) Signals of -OH protons were assigned by addition of D_2O in the sample tube.

9) INEPT (1/4J, 2/4J, and 3/4J) techniques were applied to clarify the multiplicities of these signals.

10) While the molecular ion was not observable, the $(M+H)^+$ ion was abundant, which was given by an ion-molecular reaction: F. W. McLafferty, *Anal. Chem.*, **29**, 1782 (1957).