Determination of the Absolute Structure of (+)-Cystothiazole B

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Palladium-catalyzed cyclization-methoxycarbonylation of (2*R*,3*S*)-3-methylpenta-4-yne-1,2-diol (6) derived from (2*R*,3*S*)-epoxy butanoate 5, followed by methylation, gave the tetrahydro-2-furylidene acetate (−)-7, which was converted to the left-half aldehyde (+)-3. A Wittig reaction between (+)-3 and the phosphoranylide derived from the bithiazole-type phosphonium iodide 4 using lithium bis(trimethylsilyl)amide afforded (+)-cystothiazole B (2), the spectral data of which were identical to those of the natural product (+)-2. Thus the stereochemistry of cystothiazole B (2) was confirmed to be [4*R*, 5*S*, 6(*E*)].

Key words (+)-cystothiazole B; chiral synthesis; structure elucidation

Antifungal substances called cystothiazoles A (**1**) and B (**2**) were isolated from the myxobacterium *Cystobacter fuscus* strain AJ-13278 using an inhibition assay against the phytopathogenic fungus *Phytophthora capsici*.[1] The structure of cystothiazole B (**2**) was deduced based on the similar ^{1}H-NMR data (H-4 and H-5) and specific rotations of cystothiazole A (**1**) (Chart 1).[1] This paper describes the determination of the absolute structure of cystothiazole B (**2**) based on the total synthesis of the chiral form of **2**.

Retrosynthetically, the synthesis of **2** can be achieved by Wittig condensation of the left-half aldehyde (+)-**3** and the right-half phosphonium iodide **4**. The synthesis of chiral aldehyde (+)-**3** was achieved in the total synthesis of cystothiazole A (**1**).[2,3] Palladium-catalyzed cyclization-methoxycarbonylation of (2*R*,3*S*)-3-methylpenta-4-yne-1,2-diol (**6**) derived from (2*R*,3*S*)-epoxy butanoate (**5**), followed by methylation, gave the tetrahydro-2-furylidene acetate (**7**), which was converted to the left-half aldehyde (+)-**3**.[2,3] The synthesis of the right-half **4** is shown in Chart 2.

Treatment of the reported bithiazole ester (**8**)[2,3] with *N*-bromosuccinimide (NBS) in the presence of 2,2′-azobisisobutyronitrile (AIBN) gave a bromo compound (**9**), which was used without further purification for the next reaction. Treatment of crude **9** with saturated $NaHCO_3$ aqueous solution yielded an alcohol (**10**) in 91% overall yield from **8**. Diisobutylaluminium hydride (Dibal-H) reduction (alcohol **11**: 70% yield) of **10** followed by treatment with I_2/Ph_3P/imidazole provided an iodide (**12**) in 76% yield. The reaction of **12**

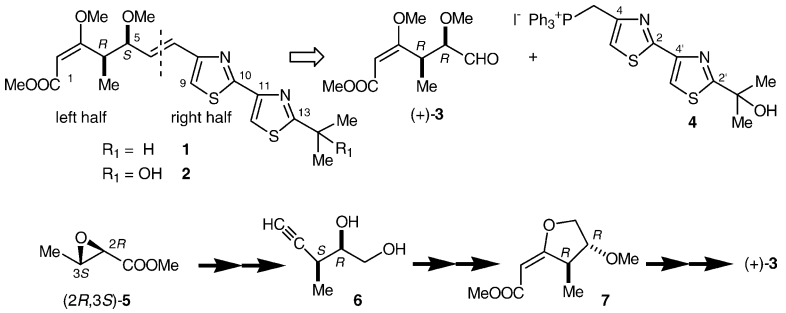

Chart 1

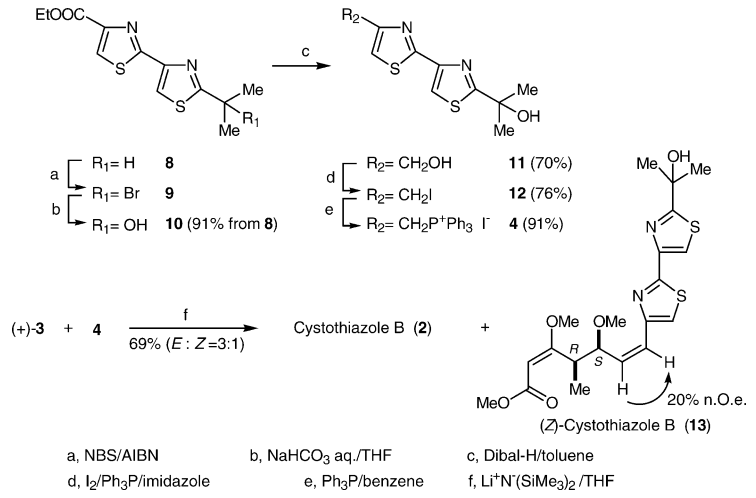

Chart 2

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and triphenylphosphine gave a phosphonium salt (**4**) in 91% yield, which was condensed with (+)-**3** in the presence of lithium bis(trimethylsilyl)amide in THF to afford a mixture ((+)-(*E*)-**2**/(+)-(*Z*)-**13**=3/1) of olefins in 69% yield. The condensation reaction conditions were not optimized at this stage. This mixture was subjected to the preparative thin-layer chromatography (silica gel) to provide a colorless oil (+)-**2** ($[\alpha]_D^{26}$ +135.0° (*c*=0.46, $CHCl_3$)) and a colorless oil (+)-**13** ($[\alpha]_D^{23}$ +201.4° (*c*=0.5, $CHCl_3$)). The physical data of the synthetic (+)-**2** were identical to those ($[\alpha]_D^{25}$ +139° (*c*=0.086, $CHCl_3$), ^{1}H-NMR ($CDCl_3$)) of the natural cystothiazole B (+)-**2**.[1] The (*Z*)-geometry of (+)-**13** was confirmed by both the NOE enhancement for the olefinic protons (20%) and the coupling constant (*J*=12.0 Hz) between C_6-H and C_7-H.

In conclusion, palladium-catalyzed cyclization-methoxycarbonylation of (2*R*,3*S*)-3-methylpenta-4-yne-1,2-diol (**6**) derived from (2*R*,3*S*)-epoxy butanoate **5**, followed by methylation, gave the tetrahydro-2-furylidene acetate (−)-**7**, which was converted to the left-half aldehyde (+)-**3**. A Wittig reaction between (+)-**3** and the phosphoranylide derived from the bithiazole-type phosphonium iodide **4** using lithium bis(trimethylsilyl)amide afforded the (+)-cystothiazole B (**2**), the spectral data of which were identical to those of the natural product (+)-**2**. The stereochemistry of cystothiazole B (**2**) was confirmed to be (4*R*, 5*S*, 6(*E*)).

Experimental

General All melting points were measured on a Yanaco MP-3S micro melting point apparatus and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were recorded on JEOL AL 400 spectrometer in $CDCl_3$. Carbon substitution degrees were established based on DEPT pulse sequence. High-resolution mass spectra (HR-MS) and fast atom bombardment mass spectra (FAB-MS) were obtained with a JEOL JMS 600H spectrometer. IR spectra were recorded on a JASCO FT/IR-300 spectrometer. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. All evaporations were performed under reduced pressure. For column chromatography, silica gel (Kieselgel 60) was employed.

2′-[2″-Hydroxyisopropyl(2,4′)bithiazolyl]-4-carboxylic Acid Ethyl Ester (10) i) A mixture of **8** (2.0 g, 7.08 mmol), *N*-bromosuccinimide (NBS, 1.5 g, 8.43 mmol) and 2,2′-azobisisobutyronitrile (AIBN, 232 mg, 1.41 mmol) in CCl_4 (50 ml) was refluxed with stirring for 2 h. After cooling, the generated precipitate was filtered off, and the filtrate was washed with 5% $Na_2S_2O_3$ aqueous solution. The organic layer was dried over $MgSO_4$ and evaporated to give a crude product, which was used without further purification for the next reaction. ii) A mixture of the above-mentioned crude product and saturated $NaHCO_3$ aqueous solution (15 ml) in THF (15 ml) was stirred for 12 h at room temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. The organic layer was evaporated to give a crude residue, which was chromatographed on silica gel (40 g, *n*-hexane : AcOEt=5 : 1) to afford **10** (1.91 g, 91%). Recrystallization of **10** from *n*-hexane–AcOEt provided the colorless prism **10**. **10**: mp 150—151 °C; IR (KBr): 3482, 1718 cm^{-1}; ^{1}H-NMR: δ 1.43 (3H, t, *J*=7.2 Hz), 1.73 (6H, s), 3.08 (1H, br s), 4.42 (2H, q, *J*=7.2 Hz), 8.08 (1H, s), 8.17 (1H, s). ^{13}C-NMR: δ 14.4, 30.9, 30.9, 61.6, 73.4, 117.4, 127.7, 147.9, 147.9, 161.5, 163.5, 180.0. *Anal.* Calcd for $C_{12}H_{14}N_2O_3S_2$: C, 48.30; H, 4.73; N, 9.39. Found: C, 48.40; H, 4.71; N, 9.27. MS (FAB) *m*/*z*: 299 (M^++1).

2′-[2″-Hydroxyisopropyl(2,4′)bithiazolyl]-4-methanol (11) To a solution of **10** (200 mg, 0.67 mmol) in toluene (10 ml) was added 1 M-HAl (*i*-Bu)$_2$ toluene solution (1.7 ml, 1.7 mmol) in toluene (10 ml) with stirring at −78 °C and the mixture was stirred for 3 h at 0 °C. The reaction mixture was diluted with H_2O (10 ml) and the generated precipitate was filtered off with the aid of Celite. The filtrate was extracted with AcOEt, washed with brine, and dried over $MgSO_4$. The organic layer was evaporated to give a crude residue, which was chromatographed on silica gel (10 g, *n*-hexane : AcOEt=2 : 1) to afford **11** (120 mg, 70%). Recrystallization of **10** from *n*-hexane–AcOEt provided the pale yellow prism **11**. **11**: mp 124—126 °C; IR (KBr): 3436, 3218 cm^{-1}; ^{1}H-NMR (CD_3OD): δ 1.65 (3H, s), 4.73 (2H, s), 7.39 (1H, s), 7.97 (1H, s). ^{13}C-NMR (CD_3OD): δ 30.8, 30.8, 61.2, 74.1, 116.5, 117.2, 150.0, 159.0, 165.0, 183.4. *Anal.* Calcd for $C_{10}H_{12}N_2O_2S_2$: C, 46.85; H, 4.72; N, 10.93%. Found: C, 46.67; H, 4.66; N, 10.77. MS (FAB) *m*/*z*: 257 (M^++1).

2′-[2″-Hydroxyisopropyl(2,4′)bithiazolyl]-4-methyleneiodide (12) To a mixture of **11** (481 mg, 1.87 mmol), triphenylphosphine (492 mg, 1.87 mmol), and imidazole (19 mg, 2.8 mmol) in THF (10 ml) was added a solution of I_2 (476 mg, 1.87 mmol) in THF (5 ml) under an argon atmosphere and the whole mixture was stirred for 30 min at room temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with 1% aqueous $Na_2S_2O_3$ solution, washed with brine, and dried over $MgSO_4$. The organic layer was evaporated to give a crude residue, which was chromatographed on silica gel (20 g, *n*-hexane : AcOEt=20 : 1) to afford **12** (522 mg, 76%). Recrystallization of **12** from *n*-hexane–AcOEt provided pale yellow needles **12**. **12**: mp 142—144 °C; IR (KBr): 3237 cm^{-1}; ^{1}H-NMR (CD_3COCD_3): δ 1.66 (6H, s), 4.68 (2H, s), 7.62 (1H, s), 8.02 (1H, s). ^{13}C-NMR (CD_3COCD_3): δ −0.5, 31.1, 31.1, 73.6, 117.0, 117.8, 149.6, 155.3, 163.7, 183.1. *Anal.* Calcd for $C_{10}H_{11}IN_2OS_2$: C, 32.79; H, 3.03; N, 7.65. Found: C, 32.88; H, 3.07; N, 6.91. MS (FAB) *m*/*z*: 367 (M^++1).

2′-[2″-Hydroxyisopropyl(2,4′)bithiazolyl]-4-methylenetriphenylphosphonium Iodide (4) A mixture of **12** (522 mg, 1.43 mmol) and triphenylphosphine (411 mg, 1.57 mmol) in benzene (14 ml) was stirred for 8 h at reflux. After cooling, the resulting colorless powder **4** (850 mg, 91%) was obtained by filtration. **4**: mp 271—273 °C; ^{1}H-NMR: δ 1.70 (6H, s), 5.56 (2H, d, *J*=14 Hz), 7.29 (1H, s), 7.63—7.86 (15H, m), 8.17 (1H, d, *J*=3.6 Hz). *Anal.* Calcd for $C_{28}H_{26}IN_2OPS_2$: C, 52.77; H, 3.94; N, 4.56. Found: C, 53.36; H, 4.13; N, 4.23. MS (FAB) *m*/*z*: 502 (M^++H−I).

Wittig Condensation of (+)-3 and 4 To a solution of **4** (554 mg, 0.84 mmol) in THF (5 ml) was added lithium bis(trimethylsilyl)amide (1 M solution in THF, 2.2 ml, 1.76 mmol) at 0 °C under an argon atmosphere and the whole mixture was stirred for 20 min at the same temperature. A solution of (+)-**3** (90 mg, 0.42 mmol) in THF (2 ml) was added to the above reaction mixture at 0 °C and the whole mixture was stirred for 30 min at the same temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was dried over $MgSO_4$ and evaporated to afford a crude product which was chromatographed on silica gel (15 g, *n*-hexane : AcOEt=5 : 1) to give a mixture of (**2** and **13**; **2** : **13**=3 : 1). It was subjected to preparative thin-layer chromatography (silicagel, *n*-hexane : AcOEt=2 : 1) to give a colorless oil (+)-**2** (96 mg, 52%) and a colorless oil (+)-**13** (31 mg, 17%). (+)-**2**: $[\alpha]_D^{26}$ +135.0° (*c*=0.46, $CHCl_3$); IR ($CHCl_3$): 3682, 3615, 1706, 1623 cm^{-1}; ^{1}H-NMR: δ 1.22 (3H, d, *J*=6.8 Hz), 1.72 (6H, s), 3.33 (3H, s), 3.60 (3H, s), 3.66 (3H, s), 3.81 (1H, t, *J*=7.8 Hz), 4.18 (1H, dq, *J*=6.8, 7.2 Hz), 4.97 (1H, s), 6.41 (1H, dd, *J*=7.6, 15.8 Hz), 6.57 (1H, d, *J*=15.8 Hz), 7.10 (1H, s), 7.90 (1H, s). ^{13}C-NMR: δ 14.1, 30.9, 30.9, 39.8, 50.8, 55.5, 57.0, 73.3, 84.4, 91.1, 115.1, 116.0, 125.5, 131.7, 148.9, 154.5, 162.3, 167.8, 176.7, 179.7. HR-MS (FAB) (*m*/*z*): Calcd for $C_{20}H_{26}N_2O_5S_2$: 439.1351 (M^++1). Found: 439.1415. (+)-**13**: $[\alpha]_D^{23}$ +201.4° (*c*=0.5, $CHCl_3$); IR ($CHCl_3$): 3683, 3619, 1707, 1620 cm^{-1}; ^{1}H-NMR: δ 1.25 (3H, d, *J*=6.8 Hz), 1.73 (6H, s), 2.90 (3H, s), 3.33 (3H, s), 3.35 (3H, s), 3.67 (3H, s), 4.23 (1H, dq, *J*=6.4, 7.2 Hz), 4.92 (1H, s), 5.10 (1H, t, *J*=9.4 Hz), 5.60 (1H, dd, *J*=9.4, 12 Hz), 6.58 (1H, d, *J*=12 Hz), 7.23 (1H, s), 7.88 (1H, s). ^{13}C-NMR: δ 14.7, 31.0, 31.0, 39.2, 50.8, 55.1, 56.2, 73.4, 78.5, 91.1, 115.7, 118.0, 125.3, 132.7, 149.0, 153.6, 161.3, 167.8, 176.6, 179.7. HR-MS (FAB) (*m*/*z*): Calcd for $C_{20}H_{26}N_2O_5S_2$: 439.1351 (M^++1). Found: 439.1362.

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