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(+)-Cystothiazole G: isolation and structural elucidation

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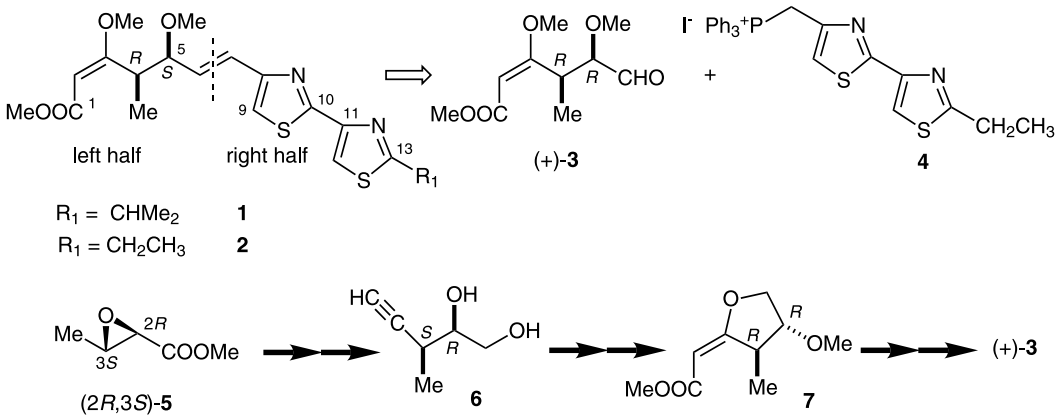
Abstract—Palladium-catalyzed cyclization-methoxycarbonylation of (2*R*,3*S*)-3-methylpent-4-yne-1,2-diol (**6**) derived from (2*R*,3*S*)-epoxybutanoate **5** followed by methylation gave the tetrahydro-2-furylidene acetate (−)-**7**, which was converted to the left-half aldehyde (+)-**3**. A Wittig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

1. Introduction

Recently, we reported the structural elucidation and the total synthesis of an antifungal substance named cystothiazole A (**1**)[1,2] from the myxobacterium *Cystobacter fuscus* strain AJ-13278 by using an inhibition assay against the phytopathogenic fungus, *Phytophthora capsici*. Further investigation of a large-scale culture of this strain resulted in the isolation of additional cystothiazole analogs, cystothiazole G (**2**).[3] This compound showed also inhibitory activity against *P. capsici*. More recently, further close analogues of cystothiazole G (**2**) named melithiazol H (**2**) have been isolated by a German group from another myxobacterium, *Myxococcus stipitatus*, strain Mx s64, although the absolute structure of melithiazol H (**2**) was not determined.[4] This paper describes the isolation, determination of the absolute structure based on the total synthesis, and biological activity of cystothiazole G (**2**) (Scheme 1).

A further search for additional cystothiazoles afforded 0.5 mg of a new member, cystothiazole G (**2**) ($[\alpha]_D^{24}=+108$ ($c=0.039$, $CHCl_3$)) from the extracts of a large-scale fermentation. The structure of cystothiazole G (**2**) was



Scheme 1.

Keywords: (+)-Cystothiazole G; Cyclization–methoxycarbonylation; Chiral synthesis.
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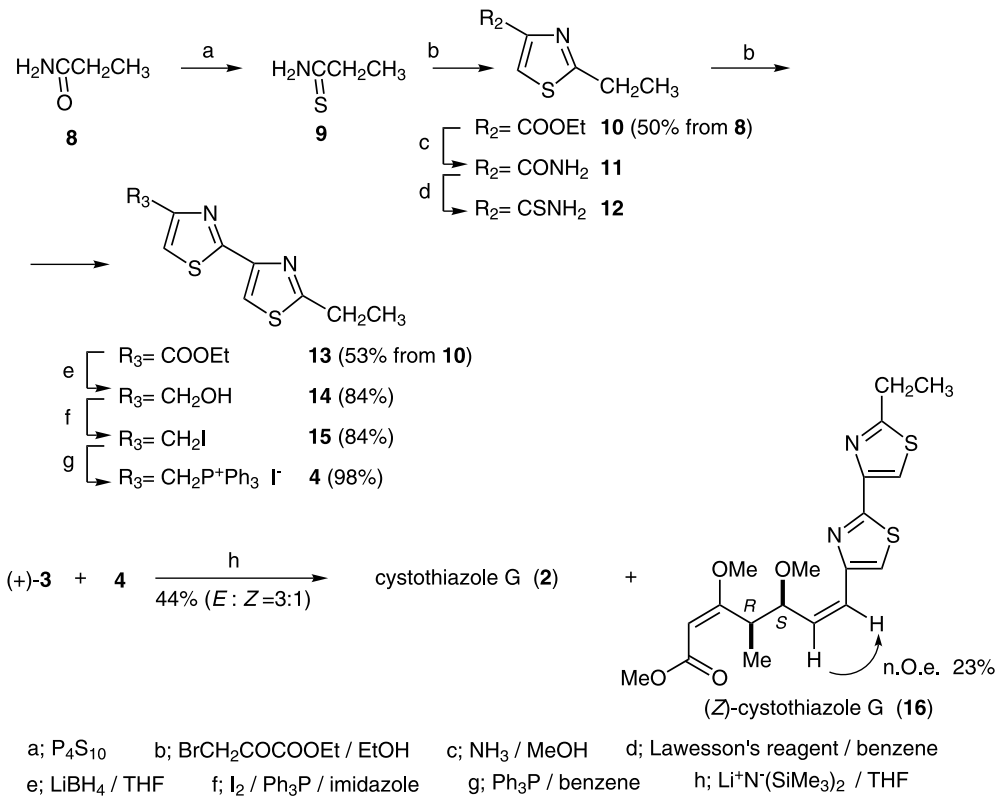

Scheme 2.

elucidated by comparison with the spectral data for cystothiazole A (**1**). The ^{1}H NMR data were similar to those for cystothiazole A (**1**) except for the presence of the signals due to an ethyl group observed at δ 1.43 (t, J=7.6 Hz, 3H) and 3.09 (q, J=7.6 Hz, 2H) and for the absence of the signals due to the isopropyl group of cystothiazole A (**1**). This NMR information as well as the molecular formula that is less than that of cystothiazole A (**1**) by CH_2 suggest that cystothiazole G (**2**) is 14-demethylcystothiazole A. A similar specific rotation to that of cystothiazole A (**1**) suggests that the absolute stereochemistry of cystothiazole G (**2**) is the same as that of cystothiazole A (**1**). 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] Palladium-catalyzed cyclization–methoxycarbonylation of (2*R*,3*S*)-3-methylpent-4-yne-1,2-diol (**6**) derived from (2*R*,3*S*)-epoxybutanoate (**5**) followed by methylation gave the tetrahydro-2-furylideneacetate **7**, which was converted to the left-half aldehyde (+)-**3**.[2] The synthesis of the right part **4** is shown in Scheme 2.

Treatment of propionamide (**8**) with P_4S_{10} gave a propionthioamide (**9**), which was reacted with α-bromopyruvate to afford a mono-thiazole ester **10** in 50% overall yield from **8**. Treatment of **10** with NH_3/MeOH followed by thioamidation with Lawesson's reagent yielded a thioamide **12**, which was reacted with α-bromopyruvate to afford a bithiazole ester **13** in 53% overall yield from **10**. $LiBH_4$ reduction (alcohol **14**: 84% yield) of **13** followed by treatment with I_2/Ph_3P/imidazole provided an iodide **15** in 84% yield. The reaction of **15** and triphenylphosphine gave a phosphonium salt **4** in 98% yield, which was condensed with (+)-**3** in the presence of lithium bis(trimethylsilyl)amide in THF to afford a mixture ((+)-(*E*)-**2**/(+)-(*Z*)-**16**=3:1) of olefins in 44% yield. Both isomers were isolated by preparative silica-gel thin-layer chromatography to provide (+)-**2** (colorless needles from *n*-hexane/AcOEt (20:1), mp 121–122 °C, $[\alpha]_D^{21}$=+108.8 (c=1.025, $CHCl_3$)) and (+)-**16** ($[\alpha]_D^{24}$=+215.9 (c=1.09, $CHCl_3$)). The (*Z*)-geometry of (+)-**16** was confirmed by the NOE enhancement for the olefinic protons (23%). The physical data of the synthetic (+)-**2** were identical with those ($[\alpha]_D^{24}$=+108 (c=0.039, $CHCl_3$), ^{1}H NMR($CDCl_3$)) of the isolated cystothiazole G (+)-**2**. Thus, the stereochemistry of cystothiazole G (**2**) was proved to be (4*R*,5*S*,6(*E*)). ^{1}H NMR (CD_3OD) data of the synthetic (+)-**2** were also identical with those of Melithiazole H (**2**), while the absolute structure of Melithiazole H (**2**) was not determined because of no information with respect to the specific rotation. The antifungal activity of the natural cystothiazole G (**2**) against the phytopathogenic fungus, *P. capsici*, was evaluated by using a paper disc assay method as reported previously.[1] The minimum dose applied on a paper disc to inhibit the fungal growth was 1 μg/disc. The synthetic cystothiazole G (**2**) also showed the activity at a similar level of dosage (0.2 μg/disc). According to our recent studies on antifungal tests using the phytopathogenic fungus, *P. capsici*, synthetic cystothiazole A (**1**) ((4*R*,5*S*)-**1**) showed activity up to a dose of 0.04 μg/disc. However, not only the enantiomer ((4*S*,5*R*)-**1**) but also the two diastereomers ((4*S*,5*S*)-**1** and (4*R*,5*R*)-**1**) did not show any antifungal activity up to 100 μg/disc. This result was not

expected at all, because all the stereoisomers possess the β-methoxyacrylate unit that is regarded as the binding site to the target molecules.[5]

In conclusion, palladium-catalyzed cyclization–methoxycarbonylation of (2*R*,3*S*)-3-methylpent-4-yne-1,2-diol (**6**) derived from (2*R*,3*S*)-epoxybutanoate **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 synthetic (+)-cystothiazole A (**2**), whose spectral data were identical with those of the natural product (+)-**2**. The stereochemistry of cystothiazole G (**2**) was proved to be (4*R*,5*S*,6(*E*)).

2. Experimental

2.1. 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 by DEPT pulse sequence. High-resolution mass spectra (HR-MS) and the fast atom bombardment mass spectra (FAB-MS) were obtained with a JEOL JMS 600H spectrometer. IR spectra were recorded with a JASCO FT/IR-300 spectrometer. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. All evaporations were performed under reduced pressure. For column chromatography, silica gel (Kieselgel 60) was employed.

2.2. Isolation of cystothiazole G (2)

A large-scale fermentation (150 L culture) and fractionation to obtain the fractions of the first silica gel column chromatograph were reported in the literature.[1] The most polar part (950–1150 mL) of the hexane/EtOAc (4:1) fractions and the EtOAc fraction (400 mL) were combined (2.5 g), and a portion (1.35 g) was subjected to silica gel medium-pressure liquid chromatography (Develosil Lop 60, Nomura Chemical; 128 min linear gradient from 1 to 33% acetone in toluene, 5 mL/min) to give seven fractions. The first fraction eluted from 1 to 12% acetone (98.2 mg) was separated by preparative HPLC (Develosil ODS-10 (20 i.d.×250 mm), Nomura Chemical; 60% MeCN, 7 mL/min, detected at 254 nm) to give cystothiazole G (**2**) (0.5 mg, t_R=62.5 min) as a solid. Cystothiazole G (**2**): $[\alpha]_D^{24}$=+108 (c=0.039, $CHCl_3$); UV (MeOH) λ_{max} 226 (ε 33,400), 244 (ε 34,000), 312 (ε 12,500) nm, IR ($CHCl_3$): 1717, 1700, 1624, 1150, 1094 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.22 (3H, d, J=6.9 Hz), 1.43 (3H, t, J=7.6 Hz), 3.09 (2H, q, J=7.6 Hz), 3.33 (3H, s), 3.60 (3H, s), 3.66 (3H, s), 3.81 (1H, t, J=7.6 Hz), 4.17 (1H, dq, J=7.6, 6.9 Hz), 4.97 (1H, s), 6.41 (1H, dd, J=7.6, 15.7 Hz), 6.58 (1H, d, J=15.7 Hz), 7.09 (1H, s), 7.84 (1H, s). ^{13}C NMR ($CDCl_3$): δ 14.1, 14.1, 26.9, 39.8, 50.8, 55.5, 57.0, 84.4, 91.1, 115.0, 115.2, 125.6, 131.7, 148.9, 154.5, 162.4, 167.7, 173.6, 176.7. HR-MS (FAB) (m/z): calcd for $C_{19}H_{25}O_4N_2S_2$ (M^++1): 409.1256. Found: 409.1238.

2.3. 2-Ethylthiazole-4-carboxylic acid ethyl ester (10)

To a solution of phosphorus pentasulfide (P_4S_{10}; 5.1 g, 11.47 mmol) in ether (40 mL) was added propionamide **8** (8.39 g, 114.7 mmol) and the whole mixture was stirred for 2 h at room temperature. The reaction mixture was diluted with brine and extracted with ether. The organic layer was dried over $MgSO_4$ and evaporated to give a crude **9**. A mixture of the crude **9** and ethyl α-bromopyruvate (22.39 g, 114.5 mmol) in EtOH (100 mL) was stirred at reflux for 15 min. The reaction mixture was evaporated, diluted with AcOEt, and washed with 7% aqueous $NaHCO_3$. The organic layer was dried over $MgSO_4$ and evaporated to give a crude oil, which was chromatographed on silica gel (200 g, *n*-hexane/AcOEt=15:1) to afford colorless compound **10** (10.81 g, 51% overall yield from **8**).

Compound **10**. IR (KBr): 1719 cm^{-1}; ^{1}H NMR: δ 1.41 (3H, t, J=7.2 Hz), 1.41 (3H, t, J=7.6 Hz), 3.10 (2H, q, J=7.6 Hz), 4.42 (2H, q, J=7.2 Hz), 8.06 (1H, s). ^{13}C NMR: δ 14.3, 14.4, 27.1, 61.4, 126.8, 146.8, 161.5, 173.8. MS (FAB) m/z: 186 (M^++1).

2.4. 2′-Ethyl[2,4′]bithiazolyl-4-carboxylic acid ethyl ester (13)

A mixture of **10** (6.0 g, 32.38 mmol) and NH_3 saturated EtOH (100 mL) in a sealed tube was stood for 3 days at room temperature. After cooling, the reaction mixture was evaporated to afford a crude amide **11**. To a solution of crude **11** in benzene (100 mL) was added Lawesson's reagent (6.47 g, 16 mmol) and the whole mixture was stirred for 1 h at reflux. 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 thioamide **12**. To a solution of the crude thioamide **12** and ethyl α-bromopyruvate (6.05 g, 31 mmol) in absolute EtOH (200 mL) was stirred for 1 h at reflux. The reaction mixture was evaporated, diluted with 7% aqueous $NaHCO_3$ 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 (60 g, *n*-hexane/AcOEt=15:1) to afford **13** (4.6 g, 53%). Recrystallization of **13** from *n*-hexane provided colorless needles **13**.

Compound **13**. Mp 82–83 °C; IR (KBr): 1712 cm^{-1}; ^{1}H NMR: δ 1.43 (3H, t, J=7.2 Hz), 1.44 (3H, t, J=6.8 Hz), 3.09 (2H, q, J=7.6 Hz), 4.45 (2H, q, J=7.2 Hz), 8.03 (1H, s), 8.16 (1H, s). ^{13}C NMR: δ 14.0, 14.4, 26.9, 61.5, 116.6, 127.6, 147.9, 147.9, 161.5, 163.6, 173.7. Anal. Calcd for $C_{11}H_{12}N_2O_2S_2$: C, 49.23; H, 4.51; N, 10.44. Found: C, 49.28; H, 4.49; N, 10.45. MS (FAB) m/z: 269 (M^++1).

2.5. 2′-Ethyl[2,4′]bithiazolyl-4-methanol (14)

A mixture of **13** (2.0 g, 7.45 mmol) and $LiBH_4$ (0.492 g, 22.6 mmol) in THF (30 mL) was stirred for 50 min at room temperature. The reaction mixture was diluted with H_2O (10 mL) and the whole was stirred for 5 h at the same temperature. The reaction mixture was extracted with AcOEt and 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=5:1) to afford **14** (1.43 g, 84%). Recrystallization of **14** from *n*-hexane–AcOEt provided colorless needles **14**.

Compound **14**. Mp 94–96 °C; IR (KBr): 3223 cm^{-1}; ^1H NMR: δ 1.43 (3H, d, J=7.6 Hz), 3.08 (2H, q, J=7.6 Hz), 4.82 (2H, s), 7.20 (1H, s), 7.87 (1H, s). ^{13}C NMR: δ 14.1, 26.9, 60.8, 115.2, 115.6, 148.4, 157.1, 163.5, 173.8. Anal. Calcd for $C_9H_{10}N_2OS_2$: C, 47.76; H, 4.45; N, 12.38. Found: C, 47.68; H, 4.51; N, 12.33. MS (FAB) m/z: 227 (M^++1).

2.6. 2′-Ethyl[2,4′]bithiazolyl-4-methyleneiodide (15)

To a mixture of **14** (1.22 g, 5.39 mmol), triphenylphosphine (1.56 g, 5.95 mmol) and imidazole (0.550 g, 8.1 mmol) in THF (25 mL) was added I_2 (1.51 g, 5.94 mmol) under 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 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=30:1) to afford **15** (1.53 g, 84%). Recrystallization of **22** from *n*-hexane provided colorless needles **15**.

Compound **15**. Mp 119–121 °C; IR (KBr): 1533, 1498, 1432 cm^{-1}; ^1H NMR: δ 1.43 (3H, t, J=7.6 Hz), 3.08 (2H, q, J=7.6 Hz), 4.56 (2H, s), 7.25 (1H, s), 7.86 (1H, s). ^{13}C NMR: δ −1.53, 14.1, 26.9, 115.6, 116.7, 148.5, 154.0, 163.1, 173.7. Anal. Calcd for $C_9H_9IN_2S_2$: C, 32.15; H, 2.70; N, 8.33. Found: C, 32.15; H, 2.90; N, 8.00. MS (FAB) m/z: 337 (M^++1).

2.7. 2′-Ethyl[2,4′]bithiazolyl-4-methylenetriphenylphosphonium iodide (4)

A mixture of **15** (1.40 g, 4.16 mmol) and triphenylphosphine (2.74 g, 10.4 mmol) in benzene (14 mL) was stirred for 8 h at reflux. After cooling, the resulting colorless powder **4** (2.46 g, 98%) was obtained by filtration.

Compound **4**. Mp 265–267 °C; ^1H NMR: δ 1.40 (3H, t, J=7.6 Hz), 3.04 (2H, q, J=7.6 Hz), 5.51 (2H, d, J=14 Hz), 7.26 (1H, s), 7.63–7.84 (15H, m), 8.08 (1H, d, J=3.2 Hz). Anal. Calcd for $C_{27}H_{24}IN_2PS_2$: C, 54.18; H, 4.04; N, 4.68. Found: C, 54.35; H, 4.11; N, 4.57. MS (FAB) m/z: 471 (M^+−I).

2.8. Wittig condensation of (+)-3 and 4

To a solution of **4** (0.527 g, 0.88 mmol) in THF (5 mL) was added lithium bis(trimethylisilyl)amide (1 M solution in THF, 0.88 mL, 0.88 mmol) at 0 °C under argon atmosphere and the whole mixture was stirred for 20 min at the same temperature. A solution of (+)-**3** (0.095 g, 0.44 mmol) in THF (2 mL) was added to the above reaction mixture at 0 °C and the whole mixture was stirred for 15 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 (10 g, *n*-hexane/AcOEt=20:1) to give a mixture ((*E*)/(*Z*)=3:1) of **2**. This mixture was subjected to thin-layer chromatography (silica-gel, *n*-hexane/AcOEt=5:1) to afford (+)-**2** (59.3 mg, 33%) and a colorless oil (+)-**16** (19.8 mg, 11%). Recrystallization of (+)-**2** from *n*-hexane/AcOEt (20:1) provided colorless needles **2**.

Compound (+)-**2**. Mp 121–122 °C; $[\alpha]_D^{21}$=+108.8 (c=1.025, $CHCl_3$); IR (KBr): 3110, 2977, 1710, 1618, 1144, 1079 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.22 (3H, d, J=6.8 Hz), 1.43 (3H, t, J=7.6 Hz), 3.09 (2H, q, J=7.6 Hz), 3.32 (3H, s), 3.60 (3H, s), 3.66 (3H, s), 3.81 (1H, t, J=7.8 Hz), 4.17 (1H, dq, J=7.6, 6.8 Hz), 4.97 (1H, s), 6.41 (1H, dd, J=7.7, 15.7 Hz), 6.57 (1H, d, J=15.7 Hz), 7.08 (1H, s), 7.84 (1H, s). ^1H NMR (CD_3OD): δ 1.26 (3H, d, J=6.8 Hz), 1.46 (3H, t, J=7.6 Hz), 3.12 (2H, q, J=7.6 Hz), 3.37 (3H, s), 3.66 (3H, s), 3.66 (3H, s), 3.83 (1H, t, J=8.4 Hz), 4.23 (1H, dq, J=8.4, 6.8 Hz), 5.08 (1H, s), 6.39 (1H, dd, J=8.4, 15.6 Hz), 6.62 (1H, d, J=15.6 Hz), 7.40 (1H, s), 8.04 (1H, s). ^{13}C NMR (CD_3OD): δ 14.6, 15.1, 27.6, 41.2, 51.2, 56.2, 57.1, 85.8, 92.0, 116.7, 117.0, 126.8, 132.3, 149.4, 155.2, 163.8, 169.3, 175.1, 177.5. $C_{19}H_{24}N_2O_4S_2$: C, 55.86; H, 5.92; N, 6.86. Found: C, 55.85; H, 6.03; N, 6.69. MS (FAB) m/z: 409 (M^++1).

Compound (+)-**16**. $[\alpha]_D^{24}$=+215.9 (c=1.09, $CHCl_3$); IR ($CHCl_3$): 1705, 1620 cm^{-1}; ^1H NMR (CD_3OD): δ 1.22 (3H, d, J=6.8 Hz), 1.41 (3H, t, J=7.6 Hz), 3.08 (2H, q, J=7.6 Hz), 3.29 (3H, s), 3.34 (3H, s), 3.62 (3H, s), 4.16 (1H, dq, J=6.8, 9.6 Hz), 4.96 (1H, s), 5.20 (1H, t, J=9.6 Hz), 5.49 (1H, dd, J=9.6, 12.0 Hz), 6.57 (1H, d, J=12.0 Hz), 7.42 (1H, s), 7.93 (1H, s). ^{13}C NMR (CD_3OD): δ 14.5, 15.2, 27.6, 40.7, 51.2, 55.7, 56.8, 80.1, 91.8, 116.5, 119.7, 126.4, 133.3, 149.8, 154.6, 162.9, 169.5, 175.4, 177.8. HR-MS (FAB) (m/z): calcd for $C_{19}H_{25}O_4N_2S_2$ (M^++1): 409.1256. Found: 409.1276.

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