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Allelochemicals of the tropical weed *Sphenoclea zeylanica*

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Abstract

Nine plant growth inhibitors were isolated from the tropical weed *Sphenoclea zeylanica*, which shows allelopathic properties. Those compounds hitherto not reported from any plant source were the isomers of cyclic thiosulfinate, (1*S*,3*R*,4*R*)-(+)- and (1*R*,3*R*,4*R*)-(−)-4-hydroxy-3-hydroxymethyl-1,2-dithiolane-1-oxides, and (2*R*,3*R*,4*R*)-(−)- and (2*S*,3*R*,4*R*)-(+)-4-hydroxy-3-hydroxymethyl-1,2-dithiolane-2-oxides. These were named zeylanoxide A, *epi*-zeylanoxide A, zeylanoxide B and *epi*-zeylanoxide B, respectively. The absolute configurations at C-3 and C-4 were elucidated by chemical synthesis of both enantiomers from L- and D-glucose. Two of the inhibitors were secologanic acid and secologanoside, and three other inhibitors were by known secoiridoid glucosides formed as artifacts during extraction with methanol. The cyclic thiosulfinates and secoiridoid glucosides completely inhibit the root growth of rice seedlings at 3.0 mM. While the specific activity of the inhibitors was not high, since they accumulated to circa 0.61% *S. zeylanica* by dry weight, this suggests that the inhibitors are nervertheless potent allelochemicals in this weed.

Keywords: *Sphenoclea zeylanica*; Sphenocleaceae; Gooseweed; Allelopathy; Plant growth inhibitor; Secoiridoid glucoside; Thiosulfinate; 4-Hydroxy-3-hydroxymethyl-1,2-dithiolane-1-oxide; 4-Hydroxy-3-hydroxymethyl-1,2-dithiolane-2-oxide

1. Introduction

Sphenoclea zeylanica Gaertn. is an annual weed belonging to the Family Sphenocleaceae. The weed is distributed in the tropics, not only in Africa where the weed is a native species, but also in Asia and America, where it is called "phak pot" in Thailand and "gooseweed" in the USA (Noda et al., 1994). It grows in seasonal swamps or depressions, and thus paddy fields are ideal for its growth (Holm et al., 1977). The life cycle of the weed is coincident with that of rice plants. In Thailand, when rice fields are irrigated in March, seeds of the weed germinate in the water under anaerobic conditions similar to rice seeds (Pons, 1982). The weed is at a flowering stage in May, and the seeds are mature in June. The seeds are only 0.5 mm in length, and remain in dried soil until the next irrigation. When *S. zeylanica* begins to invade paddy fields, it grows between rice plants, but the size of the colony increases each year. Thus, this weed has been designated as one of the most serious weeds of rice (Holm et al., 1977).

Domination of rice fields by *S. zeylanica* may be caused by its rapid growth and tolerance against 2,4-D (Mercado et al., 1990). However, the growth of rice and other weeds was depressed in soil where the weed had grown (Premasthira and Zungsontiporn, 1996b), and the methanol extracts from the weed inhibited growth of rice seedlings (Premasthira and Zungsontiporn, 1996a). These observations suggested the involvement of allelopathy in this domination. Therefore, investigated plant growth inhibitors of *S. zeylanica*, which might be allelochemicals, as a first step in an ecological study of the weed, and found new inhibitors along with secoiridoid glucosides. Here, we describe their identification, chemical synthesis and contents in the weed.

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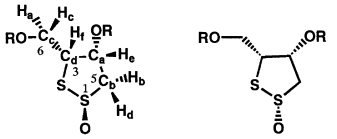
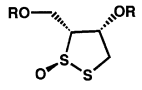
2. Results and discussion

Materials extracted from dried *S. zeylanica* with 80% aqueous methanol were partitioned between water and ethyl acetate. The aqueous layer showed higher inhibitory activity on rice seedling growth than the organic layer. The aqueous materials were purified with active charcoal, and four inhibitors (**1–4**) were isolated from fractions eluted with 10 and 20% aqueous acetone. The fraction eluted with 50% aqueous acetone yielded five inhibitors (**5–9**) by silica gel chromatography. All inhibitors were water-soluble.

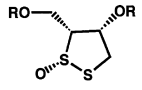


(1*S*,3*R*,4*R*)-**1**: R = H (Zeylanoxide A)
(1*S*,3*R*,4*R*)-**11**: R = Ac

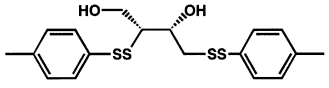
(1*R*,3*R*,4*R*)-**2**: R = H (*epi*-Zeylanoxide A)
(1*R*,3*R*,4*R*)-**13**: R = Ac



(2*R*,3*R*,4*R*)-**3**: R = H (Zeylanoxide B)
(1*R*,3*R*,4*R*)-**14**: R = Ac



(2*S*,3*R*,4*R*)-**4**: R = H (*epi*-Zeylanoxide B)
(1*R*,3*R*,4*R*)-**15**: R = Ac



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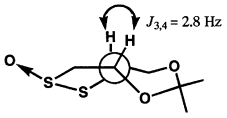

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The ^{1}H-NMR spectrum of **1** showed signals of four methylene protons at δ 3.42 (H_a), 3.82 (H_b), 3.90 (H_c) and 3.97 (H_d) ppm, and of two methine protons at δ 4.34 (H_e) and 5.20 (H_f) ppm. This suggested that all protons were bound to carbons bonding to heteroatoms such as oxygen and sulfur. The presence of a cyclic thiosulfinyl group in **1** was suggested by absorption maxima at 210 and 248 nm in the UV spectrum, and at 1060 cm^{-1} in the IR spectrum (Lindberg and Bergson, 1965). Reaction of **1** with *p*-thiocresol gave a (−)-bis(*p*-tolyldithio) derivative (**10**), which confirmed the presence of a cyclic thiosulfinyl group (Singh et al., 1988). Acetylation of **1** gave a diacetate (**11**), indicating that **1** had two hydroxyl groups. The high-resolution mass spectrum of **11** gave the molecular formula of **11** $C_8H_{12}O_5S_2$, and the molecular formula of **1** was determined to be $C_4H_6O_3S_2$.

The ^{13}C-NMR spectrum of **1** showed four carbons at δ 61.0 (C_a), 61.4 (C_b), 70.4 (C_c) and 76.4 (C_d) ppm. The HMQC spectrum of **1** showed that these carbons bonded to H_e, $H_{b,d}$, $H_{a,c}$ and H_f, respectively. In the HMBC spectrum of **1**, correlations were observed between C_a, and $H_{a,c}$ and $H_{b,d}$, between C_b, and H_e and H_f, between C_c and H_e, and between C_d, and $H_{a,c}$ and $H_{b,d}$. These correlations suggested that compound **1** had a partial structure of X-$C_bH_{b,d}$-C_aH_e(X)-C_dH_f(X)-$C_cH_{a,c}$-X where X represent hydroxyl groups or sulfur atoms. The ^{1}H-NMR spectrum of **11** showed that $H_{b,d}$ and H_f of **1** were shifted to δ 4.24 and 4.39 ppm, and to δ 6.16 ppm, respectively, by acetylation. This indicated that C_b and C_d bonded to primary and secondary hydroxyl groups, respectively. The partial structure, and the presence of the cyclic thiosulfinyl group suggested that the plane structure of **1** was 4-hydroxy-3-hydroxymethyl-1,2-dithiolane-1-oxide or its 2-oxide. In the ^{13}C-NMR spectrum of **1**, the chemical shift of C-5 (C_c) was lower than that of C-3 (C_a), so the oxide bonded to S-1. The relative configuration at C-3 and C-4 could not be determined by the coupling constant between H-3 and H-4 in the ^{1}H-NMR spectrum due to flexibility of the five-membered ring. To fix the ring, compound **1** was converted to its 4,6-*O*-isopropylidene derivative (**12**). If the relationship between H-3 and H-4 is *cis*, its coupling constant should be less than 8 Hz, and if it is *trans*, the coupling constant should be larger than 9 Hz. The observed coupling constant was 2.8 Hz, indicating that the relationship between H-3 and H-4 was *cis*.

Compounds **2–4** had a molecular weight of 168, and showed ^{1}H- and ^{13}C-NMR spectra similar to those of **1**, but optical rotations different from that of **1**. The HMQC and HMBC spectra of **2–4**, and the ^{1}H-NMR spectra of their diacetates (**13–15**, respectively) showed that these compounds were also 4-hydroxy-3-hydroxymethyl-1,2-dithiolane-1-oxide or its 2-oxide. In the ^{13}C-NMR spectrum of **2**, the chemical shift (69.2 ppm) of C-5 was lower than that (66.5 ppm) of C-3, indicating that compound **2** was 4-hydroxy-3-hydroxymethyl-1,2-dithiolane-1-oxide. The ^{13}C-NMR spectra of **3** and **4** showed that the chemical shifts (88.6 and 81.6 ppm, respectively) of C-3 were lower than those (45.0 and 48.5 ppm, respectively) of C-5, so compounds **3** and **4** were identified as 4-hydroxy-3-hydroxymethyl-1,2-dithiolane-2-oxide. 4,6-*O*-Isopropylidene derivatives of **2–4** showed coupling constants of 4.5, 4.6 and 6.6 Hz between H-3 and H-4, respectively, indicating that the relationships between H-3 and H-4 of **2–4** were also *cis*. Reaction of **2–4** with *p*-thiocresol gave the same (−)-bis(*p*-tolyldithio) derivative (**10**) as that of **1**. This indicated that C-3 and C-4 of compounds **2–4** had the same absolute configuration as of **1**. The relative configurations at the sulfinyl sulfurs of **1–4** were elucidated by the chemical shifts of H-3 and H-4 in their ^{1}H-NMR spectra. In a cyclic thiosulfinate, a proton in the *syn* position to a sulfinyl oxygen showed lower chemical shift than that in the *anti* position (Wudl et al., 1969). This deshielding effect was attributed to a proximity effect and/or acetylenic-type anisotropy of a sulfinyl group (Buck et al., 1966; Foster et al., 1967). The chemical shift (4.34 ppm) of H-3 of **1** was lower than that (4.15

ppm) of **2**, so H-3 of **1** was in a *syn* position to the 1-*S*-oxide, and H-3 of **2** was in an *anti* position. The chemical shift (5.20 ppm) of H-4 of **3** was lower than that (5.14 ppm) of **4**, showing that H-4 of **3** and **4** were in *syn* and *anti* positions to the 2-*S*-oxides, respectively.

We attempted to elucidate the absolute configuration at C-3 and C-4 using the Mosher method (Dale and Mosher, 1973) and X-ray analysis, but could not obtain α-methoxy-α-trifluoromethylphenylacetate monoesters and good crystals of **1–4** for these experiments. The absolute configuration was finally identified by chemical synthesis of both enantiomers. The starting material for the synthesis was determined by retrosynthesis (Fig. 1). Cleavage of the disulfide bond of (3*R*,4*R*)-**1–4** gives 1,3-dithiothreitol. Substitution of sulfhydryl groups of 1,3-dithiothreitol with hydroxyl groups gives L-erythritol, accompanying inversion of the configuration at C-1 by an S_N2 reaction. Oxidation at C-3 gives L-erythrose, which can be converted from L-glucose by oxidation.

Compounds (3*R*,4*R*)-**1–4** were synthesized from L-glucose by the route shown in Fig. 2. Hydroxyl groups at C-4 and C-6 of L-glucose were protected by an ethylidene group to give derivative **16** (Hall and Stamm, 1970), and oxidative removal of C-2 and C-3 of **16** by sodium periodate gave a mixture of an aldehyde (**17**) and its formate (**18**) (Kiso et al., 1986). The mixture was reduced with lithium borohydride, and the diol (**19**) thus produced was tosylated. The ditosyl derivative (**20**) was treated with potassium thiocyanate to give a dithiocyanate (**21**) (Corey and Mitra, 1962). In the ^{1}H-NMR spectra, the coupling constant between H-2 and H-3 of **21** was 1.8 Hz corresponding to a *cis* relationship, while that of **20** was 9.4 Hz corresponding to a *trans* relationship. This confirmed that the configuration at C-3 of **20** was inverted by the S_N2 reaction. Alkaline hydrolysis and deprotection of **21** gave a dithiolane (**23**) via **22**. Oxidation of **23** with hydrogen peroxide gave a mixture of (3*R*,4*R*)-**1–4** (Allen and Brook, 1962). The mixture was separated into each isomer by chromatography. Enantiomeric isomers (3*S*,4*S*)-**1–4** were synthesized from D-glucose by the same method as used for (3*R*,4*R*)-**1–4**. Specific optical rotations of natural **1–4** were in good accordance with those of synthetic (3*R*,4*R*)-**1–4**, respectively. Thus, the absolute configurations at C-3 and C-4 of natural **1–4** were identified as *R* and *R*, respectively, and the absolute configurations at S-1 of natural **1–4** were determined to be 1*S*, 1*R*, 2*R* and 2*S*, respectively, based on the relative configuration between the sulfinyl group and methine protons described above. Compounds **1–4** were new cyclic thiosulfinates, and were named zeylanoxide A, *epi*-zeylanoxide A, zeylanoxide B and *epi*-zeylanoxide B, respectively. Asparagusic acid *S*-oxide (Yanagawa et al., 1973) and brugierol (Kato and Numata, 1972) are other natural cyclic thiosulfinates occurring in *Asparagus officinalis* and *Brugiera conjugata*, respectively.

Compounds **3** and **4** isomerized to each other in aqueous solution and gave an equilibrium mixture in a ratio of 2:3 by heating at 50°C for 1 day, whereas **1** and **2** did not. This indicated that inversion of the 2-sulfinyl sulfur occurred, but inversion of the 1-sulfinyl sulfur, and migration of the sulfinyl oxygen did not. The inversion was not observed in methanol solution, suggesting that the oxygen atom at S-2 was exchanged with an oxygen atom from water. However, compound **3** incubated in $H_2{}^{18}O$ solution did not show $[MH+2]^+$ ions in its FAB mass spectrum (data not shown). This result was in agreement with the catalytic inversion at sulfinyl sulfur by contaminating radical species (Ishii et al., 1997), which probably attack sulfenyl sulfur. The more sterically hindered sulfenyl sulfur of **1** and **2** as compared to those of **3** and **4** might, therefore, depress inversion of the 1-sulfinyl sulfur. Disulfide **23** is a potent biosynthetic precursor of **1–4** in *S. zeylanica*. The presence of the four isomers suggests that oxidation of the disulfide may be non-stereoselective. It is unclear whether the oxidation is catalyzed by an enzyme(s).

Compound **5** was identified as vogeloside (equals 7α-methoxysweroside) by comparison of its spectral data with those reported in the literature (Kawai et al., 1988). Compounds **6–9** were also identified as known secoiridoid glucosides, 7-*epi*-vogeloside (**6**) (Kawai et al., 1988; Boros and Stermitz, 1991), secologanic acid (**7**) (Damtoft et al., 1994), secologanin dimethyl acetal (**8**) (Kawai et al., 1988) and secologanoside (**9**) (Calis and Sticher, 1984). The methylated compounds **5** and **6** and **8** would be artifacts derived from **7** and secologanin, respectively, during extraction with methanol (Tomassini et al., 1995). In fact, compounds **5**, **6** and **8** were not detected in the acetone extracts from *S. zeylanica*, and compound **7** easily formed **5** and **6** after dissolution in

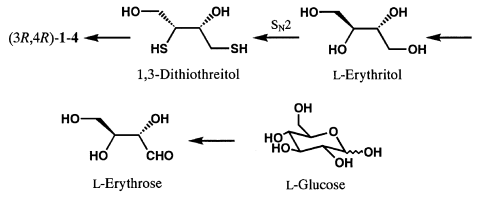

Fig. 1. Retrosynthesis of (1*S*,3*R*,4*R*)-**1**.

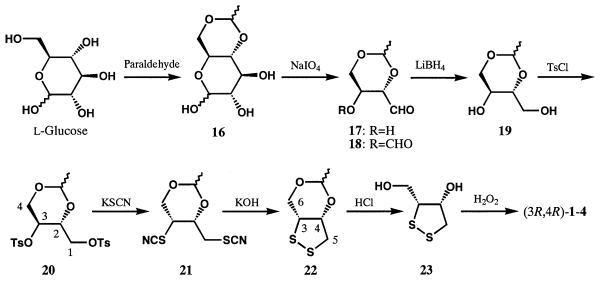

Fig. 2. Synthesis of (3*R*,4*R*)-**1–4**.

methanol. Steamed young plants of *S. zeylanica* are used as a slightly bitter vegetable in Java (Soerjani et al., 1987). The secoiridoid glucosides are probably responsible for the bitter taste (Harborne et al., 1999). In the privet tree, iridoid glucosides function as antifeedants against insects (Konno et al., 1999); the secoirdoid glucosides might, therefore, protect *S. zeylanica* from insects.

Compounds **1–7** showed higher inhibitory activities on root growth of rice seedlings than on shoot growth. The natural cyclic thiosulfinates, (3*R*,4*R*)-**1–4** completely inhibited root growth at 3 mM (Fig. 3a). Secoiridoid glucosides **5–7** showed inhibitory activities similar to those of the cyclic thiosulfinates (Fig. 3b). These compounds elongated roots at low concentrations, but the root shape was abnormal. Inhibitory activities of unnatural enantiomers (3*S*,4*S*)-**1–4** were similar to those of the natural isomers (data not shown). The sulfinyl group seemed to be more important for expression of activity than the absolute configuration at C-3 and C-4. In contrast to **1–4**, inhibitory activities of dithiolane **23** were different between the enantiomers. Dithiolane (3*R*,4*R*)-**23** completely inhibited root growth of rice seedlings at 1mM, but (3*S*,4*S*)-**23**, which has an unnatural configuration at C-3 and-4, required 3 mM for complete inhibition (Fig. 3c). Inhibition of root growth by the dithiolanes might be caused by a mechanism different from that by the cyclic thiosulfinates. Compounds (3*R*,4*R*)-**1–4** almost completely inhibited germination of lettuce seeds at 3 mM (Fig. 3d). This indicated that the cyclic thiosulfinates did not have selective activities between monocotyledons or dicotyledons. Compounds **5–7** did not show inhibitory effects on lettuce seed germination at 3 mM. These secoiridoid glucosides may have selective activities although their activities on rice seedlings were not high. Compounds (3*R*,4*R*)-**1–4** did not inhibit growth of fungi or bacteria, whereas thiosulfinates such as allicin posses antimicrobial activity (Cavallito and Bailey, 1944). Compounds (3*R*,4*R*)-**1–4** would not function in protection of the weed from microbial infection.

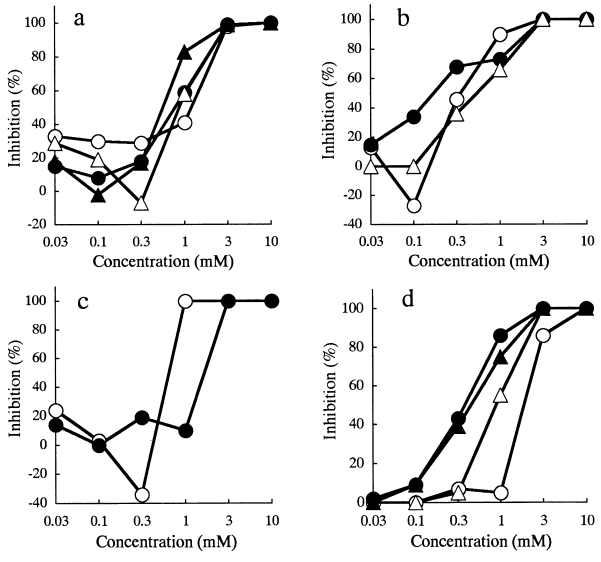

Fig. 3. Inhibitory activities of (3*R*,4*R*)-**1–4** (a), of **5–7** (b), and of (3*R*,4*R*)- and (3*S*,4*S*)-**23** (c) on root weight of rice seedlings, and of (3*R*,4*R*)-**1–4** (d) on lettuce seed germination. (a) and (d) ○: **1**, ●: **2**, △: **3**, ▲: **4**; (b) ○: **5**, ●: **6**, △: **7**; (c) ○: (3*R*,4*R*)-**23**, ●: (3*S*,4*S*)-**23**.

Contents of **1–7** in fresh and dried *S. zeylanica* at the flowering stage were analyzed using an ODS HPLC approach. Compounds **8** and **9** were not analyzed due to their small contents. Methanol was used to extract the plant materials since this solvent showed higher efficiency for extracting the compounds than acetone. Contents of **5** and **6**, that were artifacts derived from **7** as described above, were included in those of **7**. Compounds **1** and **3**, and **2** and **4** were not separated from each other with the column, and contents of each pair are shown in Table 1 along with the contents of **7**. The secoiridoid glucoside **7** showed higher contents than the cyclic thiosulfinates **1–4** in fresh and dried plants. The content of **7** in the dried plant corresponded to 23% of that in the fresh plant considering the loss of water. The secoiridoid glucoside seemed to be hydrolyzed by β-glucosidase during drying, and the formed aglycone may conjugate with amino acid and proteins through the aldehyde group of the aglycone (Harborne et al., 1999; Konno et al., 1999). Decreases in the contents of **1–4** by drying were less than that of **7**, showing stability of the cyclic thiosulfinates. The total content of the cyclic thiosulfinates **1–4** and the secoiridoid glucoside **7** was 0.48% by weight in fresh plants, and 0.61% after drying. Inhibitory activities of many allelochemicals on plant growth are low, but their contents in plants and amounts released from plants or residues into the environment are high (Rice, 1984). The high contents and low activities of **1–7** suggested that these inhibitors are potent allelochemicals of *S. zeylanica*. The cyclic thiosulfinates and secoiridoid glucosides may leach from decomposed plant residues into the soil by rain and irrigation after withering of the weed, and affect the growth of rice.

Table 1
Contents of compounds **1–4** and **7** in fresh and dried *S. zeylanica*

Compound	Fresh plant (mg/g)	Dried plant (mg/g)
1 and **3**[a]	0.14	0.43 (0.09)[c]
2 and **4**[a]	0.11	0.42 (0.08)
7[b]	4.51	5.26 (1.05)
Total	4.76	6.11 (1.22)

[a] Compounds **1** and **3**, and **2** and **4** were not separated from each other by HPLC.

[b] Contents of **7** included those of **5** and **6**.

[c] Values in parentheses show the contents in fresh plants estimated by considering that fresh plant lost 80% of its weight by drying.

3. Experimental

3.1. General

^{1}H- and ^{13}C-NMR, and HMQC and HMBC spectra were recorded with TMS as an internal standard using Bruker AC 300 (300 MHz for ^{1}H) and ARX 500 (500 MHz for ^{1}H) spectrometers. Mass spectra were obtained with JEOL DX-300 and JMS-600 mass spectrometers. UV and IR spectra, and optical rotations were measured with Shimadzu UV-2200AI and FTIR-8100AI spectrometers, and a Jasco DIP-1000 digital polarimeter, respectively.

3.2. Extraction and isolation of compounds *1–9*

Whole plants of *S. zeylanica* at the flowering stage were collected from paddyfields in Ang Thong, Thailand, on 25–31 May 1995 and 1996. Voucher specimens of *S. zeylanica* have been deposited at the Herbarium, Botany Section, Botany and Weed Science Division, Department of Agriculture, Bangkok 10900, Thailand. Isolation of the compounds was guided by rice seedling assays.

Dried and pulverized *S. zeylanica* (700 g) was extracted with 2.8 l of MeOH–H_2O (80:20, v/v) at room temperature for 6 days. The extract was filtered, concentrated under reduced pressure, and partitioned between H_2O and EtOAc. Aqueous materials (49 g) were subjected to charcoal (154 g) chromatography using mixtures of H_2O and various amounts of Me_2CO as eluant to give materials that eluted with 10, 20 and 50% Me_2CO.

Materials (9.0 g) eluted with 10 and 20% Me_2CO were combined, and subjected to silica gel (120 g) chromatography using a mixture of $CHCl_3$ and MeOH containing 1% HOAc as eluant. Materials (405 mg) eluted with 20% MeOH were subjected to silica gel (80 g) chromatography using a mixture of $CHCl_3$–MeOH–HOAc (195: 5: 2). The eluate was collected in 15 ml fractions. Fractions 79–95 and 100–120 were concentrated to give compound **1** (104 mg) and compound **3** (55 mg) as colorless oils, respectively. Materials of fractions 40–60 were subjected to silica gel (30 g) chromatography using a mixture of toluene–Me_2CO–MeOH (20: 2: 1), with the eluate collected in 15 ml fractions. Fractions 16–18 and 19–22 were concentrated to give compound **4** (77 mg) as colorless needles (mp 94°C) and compound **2** (99 mg) as colorless needles (mp 112°C), respectively.

Materials (2.5 g) eluted with 50% Me_2CO were subjected to silica gel (30 g) chromatography using a mixture of $CHCl_3$ and MeOH containing 1% HOAc as the eluant to give materials that eluted with 20 and 30% MeOH. Materials (467 mg) eluted with 20% MeOH were subjected to silica gel (80 g) chromatography using a mixture of $CHCl_3$, MeOH and HOAc (95 : 5 : 1) as the eluant, and the eluate was collected in fractions of 16 ml. Fractions 60–62, 65–72 and 76–96 were concentrated separately to give compounds **8** (5 mg), **5** (160 mg) and **6** (150 mg) as colorless powders, respectively. Materials (905 mg) eluted with 30% MeOH were subjected to silica gel (80 g) chromatography using a mixture of $CHCl_3$, MeOH and HOAc (90 : 10 : 1) as the eluant, and the eluate was collected in fractions of 16 ml. Fractions 59–76 were concentrated to give compound **7** (242 mg) as a colorless powder. Materials eluted in fractions 77–128 were purified with an ODS HPLC column (YMC AQ-311, 6 i. d.×100 mm) by elution with 20% MeOH in 0.1% aqueous HOAc at 1.0 ml min^{-1}, with the eluate being monitored at 254 nm. A material eluted at t_R 10.0 min was collected, and concentrated to give compound **9** (4.8 mg).

3.3. Spectral data of compounds *1–4*

Compound **1** [(1*S*,3*R*,4*R*)-(+)-4-hydroxy-3-hydroxymethyl-1,2-dithiolane-1-oxide; zeylanoxide A]. $[\alpha]_D^{26}$ +526° (MeOH; *c* 0.094); UV λ_{max}^{MeOH} nm (log ϵ): 210 (3.26), 248 (2.95); IR ν_{max}^{KBr} cm^{-1}: 3350, 2920, 1660, 1400, 1300, 1210, 1170, 1120, 1060, 500, 470, 455; FABMS (matrix: glycerol) *m*/*z* (rel. int.): 169 $[MH]^+$ (100); ^{1}H-NMR (500 MHz, CD_3OD): δ 3.42 (1H, *dd*, *J* = 13.2 and 4.4 Hz, H-5), 3.82 (1H, *dd*, *J* = 11.6 and 7.1 Hz, H-6), 3.90 (1H, *dd*, *J* = 13.2 and 6.7 Hz, H-5), 3.97 (1H, *dd*, *J* = 11.6 and 5.2 Hz, H-6), 4.34 (1H, *ddd*, *J* = 7.1, 5.2 and 4.8 Hz, H-3), 5.20 (1H, *ddd*, *J* = 6.7, 4.8 and 4.4 Hz, H-4); ^{13}C-NMR (125 MHz, CD_3OD): δ 61.0 (C-3), 61.4 (C-6), 70.4 (C-5), 76.4 (C-4).

Compound **2** [(1*R*,3*R*,4*R*)-(−)-4-hydroxy-3-hydroxymethyl-1,2-dithiolane-1-oxide; *epi*-zeylanoxide A]. $[\alpha]_D^{29}$ −283° (MeOH; *c* 0.10); UV λ_{max}^{MeOH} nm (log ϵ): 209 (3.13), 250 (2.99); IR ν_{max}^{KBr} cm^{-1}: 3440, 3320, 2920, 2870, 1630, 1470, 1410, 1380, 1310, 1280, 1250, 1170, 1080, 1060, 1020, 960, 890, 760, 610, 560, 460; FABMS (matrix: glycerol) *m*/*z* (rel. int.): 169 $[MH]^+$ (100); ^{1}H-NMR (500 MHz, CD_3OD): δ 3.39 (1H, *dd*, *J* = 13.5 and 3.5 Hz, H-5), 3.88 (1H, *dd*, *J* = 13.5 and 2.3 Hz, H-5), 3.99 (1H, *dd*, *J* = 11.1 and 7.6 Hz, H-6), 4.15 (1H, *ddd*, *J* = 7.6, 6.1 and 4.0 Hz, H-3), 4.26 (1H, *dd*, *J* = 11.1 and 6.1 Hz, H-6), 5.18 (1H, *ddd*, *J* = 4.0, 3.5 and 2.3 Hz, H-4); ^{13}C-NMR (125 MHz, CD_3OD): δ 62.7 (C-6), 66.5 (C-3), 69.2 (C-5), 79.5 (C-4).

Compound **3** [(2*R*,3*R*,4*R*)-(−)-4-hydroxy-3-hydroxymethyl-1,2-dithiolane-2-oxide; zeylanoxide B]. $[\alpha]_D^{29}$ −253° (MeOH; *c* 0.12); UV λ_{max}^{MeOH} nm (log ϵ): 210 (3.25), 247 (2.83); IR ν_{max}^{KBr} cm^{-1}: 3350, 2900, 1720, 1570, 1410, 1320, 1260, 1240, 1160, 1040, 880, 760, 490, 460; FABMS (matrix: glycerol) *m*/*z* (rel. int.): 169 $[MH]^+$ (100); ^{1}H-NMR (500 MHz, CD_3OD): δ 3.44 (1H, *dd*, *J* = 11.3 and 5.3 Hz, H-5), 3.67 (1H, *ddd*, *J* = 9.5, 4.8 and 4.7 Hz, H-3), 3.85 (1H, *dd*, *J* = 12.2 and 9.5 Hz, H-6), 3.96 (1H, *dd*, *J* = 11.3 and 4.4 Hz, H-5), 4.17 (1H, *dd*, *J* = 12.2 and 4.8 Hz, H-6), 5.20 (1H, *ddd*, *J* = 5.3, 4.7 and

4.4 Hz, H-4); ^{13}C-NMR (125 MHz, CD_3OD): δ 45.0 (C-5), 57.6 (C-6), 76.7 (C-4), 88.6 (C-3).

Compound **4** [(2*S*,3*R*,4*R*)-(+)-4-hydroxy-3-hydroxymethyl-1,2-dithiolane-2-oxide; *epi*-zeylanoxide B]. $[\alpha]_D^{29}$ +487° (MeOH; *c* 0.12); UV λ_{max}^{MeOH} nm (log ϵ): 209 (3.26), 248 (2.98); IR ν_{max}^{KBr} cm^{-1}: 3330, 3270, 2850, 1630, 1410, 1300, 1220, 1150, 1060, 1050, 1020, 990, 970, 910, 860, 750, 460; FABMS (matrix: glycerol) *m*/*z* (rel. int.): 169 $[MH]^+$ (100); ^{1}H-NMR (500 MHz, CD_3OD): δ 3.32 (1H, *ddd*, *J* = 9.6, 4.7 and 1.4 Hz, H-3), 3.70 (1H, *dd*, *J* = 11.1 and 4.3 Hz, H-5), 3.83 (1H, *dd*, *J* = 11.1 and 1.1 Hz, H-5), 4.22 (1H, *dd*, *J* = 11.8 and 4.7 Hz, H-6), 4.34 (1H, *dd*, *J* = 11.8 and 9.6 Hz, H-6), 5.14 (1H, *ddd*, *J* = 4.3, 1.4 and 1.1 Hz, H-4); ^{13}C-NMR (125 MHz, CD_3OD): δ 48.5 (C-5), 57.4 (C-6), 79.5 (C-4), 81.6 (C-3).

3.4. Derivatives of compounds *1–4*

3.4.1. Bis(p-tolyldithio) derivative (*10*) of *1*.

To a soln of **1** (10 mg) in MeOH (1 ml) was added *p*-thiocresol (15 mg), and stirred for 1 h at room temperature under N_2 in the dark. The mixture was concentrated and subjected to silica gel (2.4 g) chromatography using a mixture of toluene and Me_2CO (9:1) as the eluant to give **10** (9 mg). $[\alpha]_D^{27}$ −32° (MeOH; *c* 0.10); FABMS (matrix: glycerol) *m*/*z* (rel. int.): 398 $[M]^+$ (10); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.336 (3H, *s*, 4′-CH_3), 2.342 (3H, *s*, 4″-CH_3), 2.94 (2H, *d*, *J* = 6.5 Hz, H-1), 3.13 (1H, *ddd*, *J* = 5.4, 4.7 and 3.1 Hz, H-3), 3.79 (1H, *dd*, *J* = 11.7 and 4.7 Hz, H-4), 3.92 (1H, *dd*, *J* = 11.7 and 5.4 Hz, H-4), 4.25 (1H, *td*, *J* = 6.5 and 3.1 Hz, H-2), 7.13 (4H, *d*, *J* = 8.0 Hz, H-3′,-5′,-3″ and-5″), 7.43 (2H, *d*, *J* = 8.0 Hz, H-2′ and-2″, or H-6′ and-6″), 7.46 (2H, *d*, *J* = 8.0 Hz, H-6′ and-6″, or H-2′ and -2″); ^{13}C-NMR (75 MHz, CD_3OD): δ 21.0, 21.1, 44.3, 62.0, 62.9, 69.4, 129.9, 130.5, 130.9, 134.9, 135.4, 138.7, 139.0. Compounds **2–4** (5 mg each) were treated by the same method as used for compound **1** to give the bis(*p*-tolyldithio) derivatives (6 mg each). The derivatives of **2–4** showed $[\alpha]_D^{27}$ −31°, $[\alpha]_D^{27}$ −33° and $[\alpha]_D^{27}$ −30° (MeOH; *c* 0.10), respectively, and their FAB mass, ^{1}H-NMR and ^{13}C-NMR spectra were the same as those of **10**.

3.4.2. Diacetate (*11*) of *1*

Acetic anhydride (2 ml) was added to a pyridine solution (3 ml) of **1** (2.0 mg), and left at room temperature for 3 h. Ice chips were added to the solution, followed by concentration to give **11** (2.0 mg). EIMS (probe) 70 eV, *m*/*z* (rel. int.): 252 $[M]^+$ (5), 192 (12), 172 (7), 150 (8), 132 (34), 112 (100), 70 (82); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.08 (3H, *s*), 2.13 (3H, *s*), 3.64 (1H, *dd*, *J* = 13.4 and 4.8 Hz, H-5), 3.75 (1H, *dd*, *J* = 13.4 and 7.3 Hz, H-5), 4.24 (1H, *dd*, *J* = 11.7 and 6.8 Hz, H-6), 4.39 (1H, *dd*, *J* = 11.7 and 6.0 Hz, H-6), 4.65 (1H, *dt*, *J* = 6.8 and 6.0 Hz, H-3), 6.16 (1H, *ddd*, *J* = 7.3, 6.0 and 4.8 Hz, H-4).

3.4.3. Diacetates (*13–15*) of *2–4*

Compounds **2–4** (2 mg each) were treated by the same method as used for **1** to give the diacetates (2 mg each). Diacetate (**13**) of **2**. EIMS (probe) 70 eV, *m*/*z* (rel. int.): 252 $[M]^+$ (7), 192 (10), 172 (8), 150 (11), 132 (32), 112 (100), 70 (85); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.08 (3H, *s*), 2.14 (3H, *s*), 3.41 (1H, *dd*, *J* = 14.0 and 4.2 Hz, H-5), 3.92 (1H, *dd*, *J* = 14.0 and 3.2 Hz, H-5), 4.29 (1H, *ddd*, *J* = 7.4, 6.8 and 4.7 Hz, H-3), 4.48 (1H, *dd*, *J* = 11.4 and 6.8 Hz, H-6), 4.69 (1H, *dd*, *J* = 11.4 and 7.4 Hz, H-6), 6.02 (1H, *ddd*, *J* = 4.7, 4.2 and 3.2 Hz, H-4). Diacetate (**14**) of **3**. EIMS (probe) 70 eV, *m*/*z* (rel. int.): 252 $[M]^+$ (20), 172 (10), 150 (13), 112 (53), 84 (39), 70 (100); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.12 (6H, *s*), 3.50 (1H, *dd*, *J* = 11.8 and 5.8 Hz, H-5), 4.01 (1H, *ddd*, *J* = 9.3, 5.4 and 5.1 Hz, H-3), 4.11 (1H, *dd*, *J* = 11.8 and 5.1 Hz, H-5), 4.25 (1H, *dd*, *J* = 12.3 and 9.3 Hz, H-6), 4.57 (1H, *dd*, *J* = 12.3 and 5.4 Hz, H-6), 6.14 (1H, *dt*, *J* = 5.8 and 5.1 Hz, H-4). Diacetate (**15**) of **4**. EIMS (probe) 70 eV, *m*/*z* (rel. int.): 252 $[M]^+$ (32), 172 (15), 144 (10), 132 (4), 112 (70), 84 (45), 70 (100); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.12 (3H, *s*), 2.14 (3H, *s*), 3.52 (1H, *dt*, *J* = 9.4 and 5.1 Hz, H-3), 3.79 (1H, *dd*, *J* = 12.4 and 5.4 Hz, H-5), 3.88 (1H, *dd*, *J* = 12.4 and 2.0 Hz, H-5), 4.59 (1H, *dd*, *J* = 12.0 and 9.4 Hz, H-6), 4.70 (1H, *dd*, *J* = 12.0 and 5.1 Hz, H-6), 6.14 (1H, *ddd*, *J* = 5.4, 5.1 and 2.0 Hz, H-4).

3.4.4. 4,6-O-Isopropylidene derivative (*12*) of *1*

To a solution of **1** (3 mg) in DMF (0.5 ml) was added Me_2CO (1 ml) and *p*-toluenesulfonic acid monohydrate (5 mg), and stirred for 5 h at room temperature. The mixture was concentrated and subjected to silica gel (8 g) chromatography using a mixture of toluene and Me_2CO (7:3) as the eluant to give **12** (2 mg, 56% yield). EIMS (probe) 70 eV, *m*/*z* (rel. int.): 208 $[M]^+$ (13), 193 (33), 150 (100), 126 (58); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.39 (3H, *s*, CH_3), 1.49 (3H, *s*, CH_3), 3.47 (1H, *dd*, *J* = 13.9 and 4.8 Hz, H-5), 3.97 (1H, *dd*, *J* = 13.9 and 1.8 Hz, H-5), 4.04 (1H, *dd*, *J* = 15.2 and 4.3 Hz, H-6), 4.45 (1H, *ddd*, *J* = 4.3, 3.4 and 2.8 Hz, H-3), 4.47 (1H, *dd*, *J* = 15.2 and 3.4 Hz, H-6), 5.18 (1H, *ddd*, *J* = 4.8, 2.8 and 1.8 Hz, H-4).

3.4.5. 4,6-O-Isopropylidene derivatives of *2–4*

Compounds **2–4** (8 mg each) were treated by the same method as used for **1** to give the 4,6-*O*-isopropylidene derivatives (8 mg each, 80% yield). 4,6-*O*-Isopropylidene derivative of **2**. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.469 (3H, *s*, CH_3), 1.474 (3H, *s*, CH_3), 3.20 (1H, *dd*, *J* = 13.7 and 4.5 Hz, H-5), 3.90 (1H, *dd*, *J* = 13.7 and 1.0 Hz, H-5), 4.05 (1H, *dd*, *J* = 11.1 and 6.0 Hz, H-6), 4.17 (1H, *ddd*, *J* = 6.0, 5.3 and 4.5 Hz, H-3), 4.24 (1H, *dd*, *J* = 11.1 and 5.3 Hz, H-6), 5.33 (1H, *td*, *J* = 4.5 and 1.0 Hz, H-4). 4,6-*O*-Isopropylidene derivative of **3**. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.35 (3H, *s*, CH_3), 1.47 (3H, *s*, CH_3), 3.63 (1H, *ddd*, *J* = 8.1, 5.1 and 4.6 Hz, H-3), 3.64 (1H, *br. d*, *J* = 12.7 Hz, H-5), 4.18 (1H, *d*, *J* = 12.7 Hz, H-5),

4.19 (1H, *dd*, $J=12.8$ and 8.1 Hz, H-6), 4.40 (1H, *dd*, $J=12.8$ and 5.1 Hz, H-6), 5.13 (1H, *br. dd*, $J=4.6$ and 2.7 Hz, H-4). 4,6-*O*-Isopropylidene derivative of **4**. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.428 (3H, *s*, CH_3), 1.433 (3H, *s*, CH_3), 3.42 (1H, *ddd*, $J=8.1$, 6.8 and 6.6 Hz, H-3), 3.89 (1H, *dd*, $J=12.3$ and 6.6 Hz, H-5), 3.96 (1H, *dd*, $J=12.3$ and 5.5 Hz, H-5), 4.23 (1H, *dd*, $J=12.0$ and 6.8 Hz, H-6), 4.31 (1H, *dd*, $J=12.0$ and 8.1 Hz, H-6), 5.00 (1H, *td*, $J=6.6$ and 5.5 Hz, H-4).

3.5. Synthesis of natural (3R,4R)-1–4

3.5.1. 4,6-O-Ethylidene-α- and β-L-glucose (16)

To a finely powdered and dried mixture of α- and β-L-glucose (10.0 g) was added Et_2O (5.6 ml), paraldehyde (5.4 ml) and conc. H_2SO_4 (0.16 ml). The mixture was stirred for 1 h, and kept at room temperature for 24 h. To the mixture was added Et_2O until a slurry was formed, and then K_2CO_3 (0.28 g). The mixture was kept at room temperature overnight, and filtered. The residue was extracted with hot Me_2CO containing 0.1% NH_4OH, and the extract was filtered. The filtrate was concentrated, and crystallized to give **16** as colorless needles (3.9 g, mp 149°C). The mother liquor was concentrated and subjected to silica gel (100 g) chromatography using a mixture of $CHCl_3$ and MeOH (4:1) as the eluant to give **16** (5.0 g). Total yield of **16**, a mixture of α- and β-anomers (1 : 1), was 78% (8.9 g). $[\alpha]_D^{23}$ −54° (MeOH; *c* 0.10); FABMS (matrix: glycerol) *m/z* (rel. int.): 207 $[MH]^+$ (16); HR-FABMS (matrix: glycerol): $[MH]^+$ at *m/z* 207.0866 ($C_4H_9O_3S_2$ requires 207.0869); ^{1}H-NMR (300 MHz, CD_3OD): δ 1.30 (6H, *d*, $J=5.0$ Hz, CH_3 of α- and β-anomers), 3.15–3.55 (8H, *m*, H-2, -3,-4 and-5 of α- and β-anomers), 3.78 (2H, *m*, H-6 of α- and β-anomers), 3.99 (1H, *dd*, $J=10.1$ and 4.9 Hz, H-6 of α-anomer), 4.07 (1H, *dd*, $J=10.3$ and 4.5 Hz, H-6 of β-anomer), 4.53 (1H, *d*, $J=7.7$ Hz, H-1 of β-anomer), 4.73 (2H, *q*, $J=5.0$ Hz, H-7), 5.09 (1H, *d*, $J=3.8$ Hz, H-1of α-anomer).

3.5.2. 2,4-O-Ethylidene-L-erythrose (17) and its 3-O-formate (18)

To a solution of **16** (8.0 g) in dry MeOH (350 ml) was added $NaIO_4$ (23 g), and the mixture was stirred at room temperature for 24 h. The mixture was filtered, and the filtrate was concentrated and subjected to silica gel (220 g) chromatography using a mixture of $CHCl_3$ and MeOH (9:1) as the eluant to give **17** and **18** (total 7.7 g, 99% yield). Compound **17**; EIMS (probe) 70 eV, *m/z* (rel. int.): 146 $[M]^+$ (2), 145 (20), 131 (9), 117 (100); HR-EIMS: $[M]^+$ at *m/z* 146.0584 ($C_6H_{10}O_4$ requires 146.0579); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.40 (3H, *d*, $J=5.0$ Hz), 3.43 (1H, *m*), 4.16 (2H, *m*), 4.67 (1H, *q*, $J=5.0$ Hz), 4.98 (1H, *d*, $J=4.9$ Hz), 9.77 (1H, *s*). Compound **18**; EIMS (probe) 70 eV, *m/z* (rel. int.): 173 $[M-H]^+$ (4), 145 $[M-CHO]^+$ (85), 101 (100); HR-EIMS: $[M-H]^+$ at *m/z* 173.0471 ($C_7H_9O_5$ requires 173.0450); ^{1}H-NMR (500 MHz, $CDCl_3$) of the major isomer: δ 1.42 (3H, *d*, $J=5.0$ Hz), 4.04 (1H, *dd*, $J=9.8$ and 1.2 Hz), 4.28 (1H, *dd*, $J=9.8$ and 5.5 Hz, H-4), 4.53 (1H, *dd*, $J=12.0$ and 0.9 Hz), 4.80 (1H, *q*, $J=5.0$ Hz), 5.09 (1H, *m*), 8.02 (1H, *s*), 9.61 (1H, *s*).

3.5.3. 2,4-O-Ethylidene-L-erythritol (19)

To a THF solution (50 ml) of $LiBH_4$ (2.3 g) was added a mixture (7.2 g) of **17** and **18** in THF (60 ml), and the mixture was stirred at room temperature for 1 h. A THF–H_2O (60:40) solution was added to the reaction mixture. The mixture was filtered, and the filtrate was concentrated and subjected to silica gel (120 g) chromatography using a mixture of $CHCl_3$ and MeOH (7 : 3) as the eluant to give **19** (4.2 g, 69% yield). $[\alpha]_D^{21}$ +49° (MeOH; *c* 0.10); FABMS (matrix: glycerol) *m/z* (rel. int.): 149 $[MH]^+$ (65); HR-FABMS (matrix: glycerol): $[MH]^+$ at *m/z* 149.0801 ($C_6H_{13}O_4$ requires 149.0814) ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.34 (3H, *d*, $J=5.1$ Hz), 3.41 (1H, *t*, $J=10.4$ Hz), 3.46 (1H, *m*), 3.78 (1H, *m*), 3.85 (2H, *m*), 4.13 (1H, *dd*, $J=10.7$ and 5.3 Hz), 4.72 (1H, *q*, $J=5.1$ Hz).

3.5.4. 2,4-O-Ethylidene-1,3-O-ditosyl-L-erythritol (20)

To a solution of **19** (4.2 g) in pyridine (50 ml) was added *p*-toluenesulfonyl chloride (15.8 g), and the mixture was stirred at room temperature overnight. The mixture was concentrated and subjected to silica gel (200 g) chromatography using a mixture of toluene and Me_2CO (19:1) as eluant to give **20** (9.7 g, 76% yield). $[\alpha]_D^{23}$ +35° (MeOH; *c* 0.10); UV λ_{max}^{MeOH} nm (log ϵ): 225 (4.38); EIMS (probe) 70 eV, *m/z* (rel. int.): 456 $[M]^+$ (15), 441 (10), 369 (15), 285 (20), 227 (28), 155 (100); HR-EIMS: $[M]^+$ at *m/z* 456.1000 ($C_{20}H_{24}O_8S_2$ requires 456.0912); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.19 (3H, *d*, $J=5.0$ Hz, CH_3), 2.44 (3H, *s*, CH_3), 2.48 (3H, *s*, CH_3), 3.48 (1H, *t*, $J=10.5$ Hz, H-4), 3.67 (1H, *ddd*, $J=9.4$, 5.6 and 2.0 Hz, H-2), 3.80 (1H, *dd*, $J=11.2$ and 5.6 Hz, H-1), 4.01 (1H, *dd*, $J=11.2$ and 2.0 Hz, H-1), 4.13 (1H, *dd*, $J=10.5$ and 5.4 Hz, H-4), 4.23 (1H, *ddd*, $J=10.5$, 9.4 and 5.4 Hz, H-3), 4.55 (1H, *q*, $J=5.0$ Hz), 7.33 (2H, *d*, $J=8.3$ Hz), 7.39 (2H, *d*, $J=8.3$ Hz), 7.74 (2H, *d*, $J=8.3$ Hz), 7.78 (2H, *d*, $J=8.3$ Hz).

3.5.5. (2R,3R)-2,4-O-Ethylidene-1,3-dithiocyanato-1,3-dithiothreitol (21)

To a solution of **20** (9.4 g) in DMSO (50 ml) was added KSCN (30 g) and stirred at 120°C for 4 days. The mixture was diluted with H_2O (300 ml), and partitioned four times with 200 ml of EtOAc. The organic layer was dried (Na_2SO_4), filtered, concentrated and subjected to silica gel (200 g) chromatography using a mixture of *n*-hexane and EtOAc (1:1) to give **21** (2.3 g, 49% yield). $[\alpha]_D^{21}$ +6° (MeOH; *c* 0.10); EIMS (probe) 70 eV, *m/z* (rel. int.): 230 $[M]^+$ (10), 229(10), 186 (100), 178 (29), 144 (14), 128 (23); HR-EIMS: $[M]^+$ at *m/z* 230.0169

($C_8H_{10}N_2O_2S_2$ requires 230.0184); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.38 (3H, *d*, $J=5.1$ Hz, CH_3), 3.13 (1H, *dd*, $J=13.9$ and 5.6 Hz, H-1), 3.29 (1H, *dd*, $J=13.9$ and 7.8 Hz, H-1), 3.58 (1H, *ddd*, $J=1.9$, 1.8 and 1.7 Hz, H-3), 4.21 (1H, *dd*, $J=12.6$ and 1.9 Hz, H-4), 4.28 (1H, *ddd*, $J=7.8$, 5.6 and 1.8 Hz, H-2), 4.40 (1H, *dd*, $J=12.6$ and 1.7 Hz, H-4), 4.83 (1H, *q*, $J=5.1$ Hz).

3.5.6. (3R,4R)-4,6-O-Ethylidene-4-hydroxy-3-hydroxymethyl-1,2-dithiolane (**22**)

Compound **21** (2.3 g) was dissolved in 60 ml of a mixture of EtOH and 1 N KOH (3:1), and heated under reflux for 5 h with stirring. The solution was diluted with 60 ml of H_2O and partitioned with 40 ml of EtOAc four times. The organic layer was dried (Na_2SO_4), filtered, concentrated and subjected to silica gel (60 g) chromatography using a mixture of toluene and Me_2CO (9:1) as the eluant to give **22** (1.2 g, 69% yield). $[\alpha]_D^{25}$ -80° ($CHCl_3$; *c* 0.10); EIMS (probe) 70 eV, *m/z* (rel. int.): 178 $[M]^+$ (100), 134 (55), 104 (32); HR-EIMS: $[M]^+$ at *m/z* 178.0118 ($C_6H_{10}O_2S_2$ requires 178.0122); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.39 (3H, *d*, $J=5.0$ Hz, CH_3), 3.27 (1H, *dd*, $J=12.2$ and 1.6 Hz, H-5), 3.37 (1H, *dd*, $J=12.2$ and 5.0 Hz, H-5), 3.44 (1H, *m*, H-3), 4.14 (2H, *m*, H-4), 4.27 (2H, *br. s*, H-6), 4.79 (1H, *q*, $J=5.1$ Hz).

3.5.7. (3R,4R)-4-Hydroxy-3-hydroxymethyl-1,2-dithiolane (**23**)

A solution of **22** (1.10 g) was dissolved in 18 ml of a mixture of 1 N HCl and MeOH (5:4) and heated under reflux for 8 h with stirring. The solution was neutralized with 1 N KOH, concentrated and subjected to silica gel (40 g) chromatography using a mixture of $CHCl_3$ and MeOH (9:1) as the eluant to give **23** (0.61 g, 65% yield). $[\alpha]_D^{23}$ $+18^\circ$ (MeOH; *c* 0.10); EIMS (probe) 70 eV, *m/z* (rel. int.): 152 $[M]^+$ (100), 134 (14), 121 (11); HR-EIMS: $[M]^+$ at *m/z* 151.9965 ($C_4H_8O_2S_2$ requires 151.9966); ^{1}H-NMR (500 MHz, CD_3OD): δ 3.14 (1H, *dd*, $J=11.6$ and 3.0 Hz, H-5), 3.33 (1H, *dd*, $J=11.6$ and 4.3 Hz, H-5), 3.65 (1H, *ddd*, $J=8.0$, 5.9 and 4.5 Hz, H-3), 3.75 (1H, *dd*, $J=11.3$ and 8.0 Hz, H-6), 4.03 (1H, *dd*, $J=11.3$ and 5.9 Hz, H-6), 4.78 (1H, *ddd*, $J=4.5$, 4.3 and 3.0 Hz, H-4).

3.5.8. (3R,4R)-4-Hydroxy-3-hydroxymethyl-1,2-dithiolane-1 and 2-oxides [(3R,4R)-**1–4**]

To a solution of **23** (0.61 g) in HOAc (5 ml) was added 30% H_2O_2 aqueous solution (0.91 ml), and the mixture was stirred for 1 h at room temperature. The mixture was concentrated and subjected to silica gel (80 g) chromatography using a mixture of $CHCl_3$ and MeOH (39:1). The eluate was collected in fractions of 16 ml. Fractions 65–71 were combined and concentrated to give a mixture (85 mg) of (3*R*,4*R*)-**2** and-**4**. The mixture was subjected to silica gel (15 g) chromatography using a mixture of toluene, Me_2CO and MeOH (40 : 2 :1) as eluant to give (1*R*,3*R*,4*R*)-**2** (16 mg) and (2*S*,3*R*,4*R*)-**4** (9 mg). Fraction 112 was purified with an ODS HPLC column (YMC SH-342-5, 20 i.d. $\times$150 mm) with 30% MeOH in 0.1% aqueous HOAc at 3.0 ml min^{-1} with detection at 254 nm to give (1*S*,3*R*,4*R*)-**1** (t_R 11.4 min, 7 mg). Fraction 115 was purified with an ODS HPLC column according to the same method as used for **1** to give (2*R*,3*R*,4*R*)-**3** (t_R 11.5 min, 3 mg). (1*S*,3*R*,4*R*)-(+)-4-Hydroxy-3-hydroxymethyl-1,2-dithiolane-1-oxide [(1*S*,3*R*,4*R*)-(+)-**1**]. $[\alpha]_D^{25}$ $+497^\circ$ (MeOH; *c* 0.04); HR-FABMS (matrix: glycerol): $[MH]^+$ at *m/z* 169.0003 ($C_4H_9O_3S_2$ requires 168.9993). (1*R*,3*R*,4*R*)-(−)-4-Hydroxy-3-hydroxymethyl-1,2-dithiolane-1-oxide [(1*R*,3*R*,4*R*)-(−)-**2**]. $[\alpha]_D^{25}$ -302° (MeOH; *c* 0.10); HR-FABMS (matrix: glycerol): $[MH]^+$ at *m/z* 169.0012 ($C_4H_9O_3S_2$ requires 168.9993). (2*R*,3*R*,4*R*)-(−)-4-Hydroxy-3-hydroxymethyl-1,2-dithiolane-2-oxide [(2*R*,3*R*,4*R*)-(−)-**3**]. $[\alpha]_D^{25}$ -213° (MeOH; *c* 0.04); HR-FABMS (matrix: glycerol): $[MH]^+$ at *m/z* 168.9990 ($C_4H_9O_3S_2$ requires 168.9993). (2*S*,3*R*,4*R*)-(+)-4-Hydroxy-3-hydroxymethyl-1,2-dithiolane-2-oxide [(2*S*,3*R*,4*R*)-(+)-**4**]. $[\alpha]_D^{25}$ $+459^\circ$ (MeOH; *c* 0.04); HR-FABMS (matrix: glycerol): $[MH]^+$ at *m/z* 168.9993 ($C_4H_9O_3S_2$ requires 168.9993). The UV, FAB mass and ^{1}H-NMR spectra of the synthesized **1–4** were the same as those of natural **1–4**, respectively, isolated from the weed.

3.6. Synthesis of (3S,4S)-**1–4**

Isomers, (3*S*,4*S*)-**1–4**, were synthesized from 4,6-*O*-ethylidene-D-glucose (5.0 g) by the same method as used for natural (3*R*,4*R*)-**1-4**. (1*R*,3*S*,4*S*)-(−)-4-Hydroxy-3-hydroxymethyl-1,2-dithiolane-1-oxide (10 mg) [(1*R*,3*S*,4*S*)-(−)-**1**]. $[\alpha]_D^{26}$ -566° (MeOH; *c* 0.10); HR-FABMS (matrix: glycerol): $[MH]^+$ at *m/z* 168.9992 ($C_4H_9O_3S_2$ requires 168.9993). (1*S*,3*S*,4*S*)-(+)-4-Hydroxy-3-hydroxymethyl-1,2-dithiolane-1-oxide (29 mg) [(1*S*,3*S*,4*S*)-(+)-**2**]. $[\alpha]_D^{26}$ $+292^\circ$ (MeOH; *c* 0.10); HR-FABMS (matrix: glycerol): $[MH]^+$ at *m/z* 168.9993 ($C_4H_9O_3S_2$ requires 168.9993). (2*S*,3*S*,4*S*)-(+)-4-Hydroxy-3-hydroxymethyl-1,2-dithiolane-2-oxide (18 mg) [(2*S*,3*S*,4*S*)-(+)-**3**]. $[\alpha]_D^{26}$ $+273^\circ$ (MeOH; *c* 0.10); HR-FABMS (matrix: glycerol): $[MH]^+$ at *m/z* 168.9996 ($C_4H_9O_3S_2$ requires 168.9993). (2*R*,3*S*,4*S*)-(−)-4-Hydroxy-3-hydroxymethyl-1,2-dithiolane-2-oxide (14 mg) [(2*R*,3*S*,4*S*)-(−)-**4**]. $[\alpha]_D^{26}$ -447° (MeOH; *c* 0.11); HR-FABMS (matrix: glycerol): $[MH]^+$ at *m/z* 168.9992 ($C_4H_9O_3S_2$ requires 168.9993). The UV, FAB mass and ^{1}H-NMR spectra of the synthesized **1–4** were the same as those of natural **1–4**, respectively, isolated from the weed.

3.7. Isomerization of **1–4**, and incubation of **3** in an $H_2{}^{18}O$ solution

A D_2O solution (0.5 ml) of **1** (2.5 mg) was added to an NMR tube (5 mm i.d.), and heated at 50°C for 10 days.

Isomerization was monitored by measurement of its ^{1}H-NMR spectra every 24 h. Ratios of **1–4** in the solution were calculated from signal integrals of each isomer. Isomerization of **2–4** was examined by the same method as used for **1**.

Compound **3** (2.8 mg) was dissolved in 0.1 ml of $H_2{}^{18}O$ (95 atom% ^{18}O, Aldrich), heated in a sealed tube at 50°C for 24 h, and its FAB mass spectrum was measured.

3.8. Bioassays

For rice seedling assay, seeds of rice (*Oryza sativa* L. cv. Nihonbare) were soaked in EtOH for 5 min, sterilized with 1% antiformin ($NaClO_4$) for 1 h, and washed with running tap water for 3 h. The seeds were allowed to germinate in H_2O for 2 days at 30°C. The resulting seedlings were placed in a glass tube containing 2 ml of test solution, and grown with the tube sealed with a sheet of polyethylene film under continuous illumination at 30°C. The weight of the root was measured after 7 days, and the inhibition ratio was calculated. The inhibition ratio was defined as $[(A-B)/A]\times 100\%$, where A = the mean weight of the root when H_2O was used, and B = the mean weight of the root when test compound was used.

For lettuce seed germination assay, 50 seeds of lettuce (*Lectuca sativa* L. cv. Cisco) were placed on two sheets of No. 2 filter paper (5.5 cm in diameter, Toyo Roshi Ltd.) soaked in 3 ml of a test solution, and allowed to germinate under illumination at 25°C. After 48 h, the inhibition ratio was calculated. The inhibition ratio was defined as $[(A-B)/A]\times 100\%$, where A = the number of seeds that germinated when H_2O was used, and B = the number of seeds that germinated when test compound was used.

For antimicrobial assay against *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans*, *Saccharomyces cerevisiae*, *Aspergillus niger*, *Fusarium oxysporum*, *Pyricularia oryzae*, a pulp disk (5 mm in diameter) soaked in MeOH solution (1000 ppm) of test compound was dried, put on potato–dextrose–agar medium containing the microorganism, and incubated at 37°C for the bacteria and at 25°C for the fungi in the dark. The diameter of the zone showing growth inhibition was measured after 3 days for the bacteria, and after 7 days for the fungi. For antimicrobial assay against *Pythium debaryanum*, *Phytophthora infestans*, *Pyricularia oryzae*, *Botrytis cinerea*, *Rhizoctonia solani*, and *Septoria tritici*, the fungi on an agar disk (5 mm in diameter) were incubated on potato–dextrose–agar medium containing test compound at 100 ppm at 25°C in the dark. The diameters of fungal colonies were measured after 5 days, and compared with those of the respective organisms grown on potato–dextrose–agar medium without the compound.

3.9. Contents of 1–7 in S. zeylanica

The MeOH extracts of the fresh and dried *S. zeylanica* were analyzed with an ODS HPLC column (YMC AQ-311, 6 i.d.×100 mm) by eluting with 100% H_2O for **1–4**, and with 30% MeOH in 0.1% HOAc aqueous solution for **5–7** at 1.0 ml min^{-1}, and the eluate was monitored at 254 nm. Compounds **1** and **3**, **2** and **4**, **5**, **6** and **7** were eluted at t_R 5.7 min, 6.7 min, 12.5 min, 9.6 min and 6.7 min, respectively. The amounts of **1** and **3**, **2** and **4**, **5**, **6**, and **7** were calculated from the calibration curves between peak area and amount of authentic samples. The contents in the dried plant were corrected by H_2O content (80%) of the fresh plant.

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