Sulfur-Containing Compounds from *Clinacanthus siamensis*

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Two new sulfur-containing compounds, *trans*-3-methylsulfonyl-2-propenol (1) and *trans*-3-methylsulfinyl-2-propenol (2) were isolated together with *trans*-3-methylthioacrylamide (3), entadamide A (4) and entadamide C (5) from the leaves of *Clinacanthus siamensis*. The structures were established on the basis of the spectroscopic data. The compounds were tested for antimalarial and antimycobacterial activity.

Key words *Clinacanthus siamensis*; Acanthaceae; leaf; sulfur-containing compound

Clinacanthus siamensis BREM. (Thai name: lin nguu hao) (Acanthaceae) is an endemic plant of Thailand. *C. siamensis* has been used in Thailand as a traditional medicine for the treatment of insect-bite and skin rashes. *C. siamensis* is often confused with *C. nutans* (Thai name: phaya yo or phaya plongtong) in Thailand. Phytochemical and bioactivity investigations of *C. siamensis* have not been previously reported. From the *n*-BuOH-soluble fraction of the ethanolic extract of the fresh leaves of *C. siamensis*, two new sulfur-containing compounds, *trans*-3-methylsulfonyl-2-propenol (**1**) and *trans*-3-methylsulfinyl-2-propenol (**2**) have been isolated together with *trans*-3-methylthioacrylamide (**3**), entadamide A (**4**) and entadamide C (**5**). The structures were identified by spectroscopic data. Their antimalarial and antimycobacterial activity have been tested.

The fresh leaves of *C. siamensis* were extracted with EtOH at room temperature. After evaporation, the residue obtained was dissolved in water and partitioned with EtOAc and then *n*-BuOH. The *n*-BuOH-soluble fraction was subjected to extensive separation by chromatography to give the sulfur-containing compounds **1**—**5**.

Compound **1** was obtained as pale yellow needles and had a molecular formula of $C_4H_8O_3S$. The IR spectrum showed strong absorption bands at 3376 (broad) cm^{-1} (OH), 1634 cm^{-1} (C=C) and 1273 and 1130 cm^{-1} (SO_2). The ^{1}H- and ^{13}C-NMR spectra of **1** (Experimental and Table 1) showed the signals of a 1,2-*trans*-disubstituted double bond at δ_H 7.02 (dt, J=15.0, 3.0 Hz) and 6.74 (dt, J=15.0, 2.4 Hz) which corresponded to the carbon signals at δ 148.4 and 128.5, respectively, an oxymethylene group at δ_H 4.34 (dd, J=3.0, 2.4 Hz), δ_C 60.5 and a methylsulfonyl signal at δ_H 2.95 (s), δ_C 43.1. These data led to the identification of compound **1** as *trans*-3-methylsulfonyl-2-propenol.

The acetate derivative (**1a**) of **1** had spectral data (Experimental and Table 1) in good agreement with the structure.

Compound **2** was obtained as a pale yellow oil. The HR-MS and ^{13}C-NMR spectral data (Table 1) of **2** indicated that **2** had the molecular formula of $C_4H_8O_2S$. The IR spectrum of **2** had bands at 3374 (broad) cm^{-1} (OH), 1647 cm^{-1} (C=C) and 1016 (strong) cm^{-1} (SO, OH). The ^{13}C-NMR spectrum of **2** (Table 1) contained signals for two olefinic carbons at δ 132.5 and 139.8, a methylene carbon at δ 61.2 and a methylsulfinyl carbon at δ 40.3. The ^{1}H-NMR spectrum of **2** showed a singlet of the methylsulfinyl group at δ 2.65 and broadered signals from two olefinic protons centered at δ 6.60 and a prochiral CH_2 group at δ 4.34. A 2D-COSY spectrum confirmed that the two groups were coupled. A high resolution spectrum (32 K data points and Loretz–Gaussian multiplication of the fid) converted these two signals into two AB quartet patterns with strong central lines and weak wings because of tight coupling and extra lines due to vicinal and allylic coupling. The olefinic protons gave one signal at δ 6.63 (J=14.8, 1.0 Hz) and another at δ 6.58 (J=14.8, 2.6 Hz). The prochiral CH_2 group gave a doublet of doublets of doublets patern centered at δ 4.36 (J=18.4, 2.6, 1.0 Hz) and a doublet of doublets centered at δ 4.32 (J=18.4, 2.0 Hz). The latter J (2.0 Hz) could not be observed in the patern for the olefinic signal presumably because of 2nd order effects. The molecular formula of **2** differed from that of **1** by one oxygen. Compound **2** was thus identified as the sulfoxide related to **1**, *trans*-3-methylsulfinyl-2-propenol.

The acetate derivative (**2a**) of **2** showed spectral data (^{1}H- and ^{13}C-NMR) (Experimental and Table 1) in good agreement with the structure.

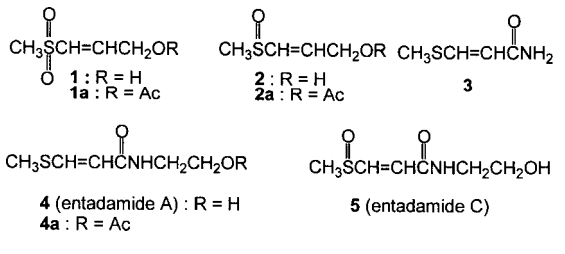

Table 1. ^{13}C-NMR Spectral Data of **1** ($CDCl_3$–DMSO-d_6, 3 : 1), **2** ($CDCl_3$), **3** ($CDCl_3$–DMSO-d_6, 3 : 1), **1a** ($CDCl_3$), **2a** ($CDCl_3$), and **4a** ($CDCl_3$)

Carbon	**1**	**1a**	**2**	**2a**	**3**	**4a**
1	60.5	61.3	61.2	62.4	166.4	164.6
2	128.5	130.3	132.5	132.2	115.4	115.3
3	148.4	141.2	139.8	135.7	42.5	143.6
SCH_3	—	—	—	—	14.0	14.6
$SOCH_3$	—	—	40.3	40.5	—	—
SO_2CH_3	43.1	42.8	—	—	—	—
CH_2OAc	—	—	—	—	—	63.3
CH_2NH	—	—	—	—	—	38.9
$CO\underline{CH_3}$	—	20.6	—	20.7	—	20.8
$\underline{CO}CH_3$	—	169.9	—	170.2	—	171.1

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Compound **3** was determined to be *trans*-3-methylthioacrylamide by comparison its spectral data (UV, IR, ^{1}H-NMR and MS) with those previously reported.[1] The ^{13}C-NMR of **3** was assigned by a combination of DEPT, HMQC and HMBC experiments (Table 1). The methylthioacrylamide derivative **3** was previously isolated as a metabolic product from methionine in *Streptomyces*.[1—3]

Compounds **4** and **5** were identified as entadamide A and entadamide C, respectively by comparison of their spectral data with those previously reported.[4,5] The ^{1}H- and ^{13}C-NMR spectral data for the acetate derivative (**4a**) are provided in the Experimental and Table 1, respectively. Entadamide A (**4**) and entadamide C (**5**) were previously isolated from the seeds of *Entada phaseoloides* (Leguminosae).[4,5] Entadamide A was found to inhibit 5-lipoxygenase activity of RBL-1 cells, suggesting that entadamide A may be an example of a new type of antiinflammatory drug.[6] It is important to note that the glucosides of compounds **1**, **3** and entadamide C (**5**) have been isolated from the leaves of the plant said to be *Clinacanthus nutans*.[7] In our hands *C. nutans* leaves yielded not sulfur-containing compounds, but cerebrosides.[1]

trans-3-Methylthioacrylamide (**3**) possessed antimycobacterial activity with the MIC value of 200 μg/ml, while compounds **1**, **2**, **4** and **5** did not exhibit any activity. All of the isolated compounds (**1**—**5**) (EC_{50} >20 μg/ml) did not show activity in an *in vitro* screening against *Plasmodium falciparum*.

Experimental

Melting points are uncorrected. Optical rotations were determined with a Jasco digital polarimeter. UV spectra were recorded with a Shimadzu UV-240 spectrophotometer. IR spectra were recorded with a Jasco A-302 spectrophotometer. ^{1}H- and ^{13}C-NMR were measured in $CDCl_3$ or $CDCl_3$–DMSO-d_6 on a Bruker Avance 400 (400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR) spectrometer. Chemical shifts are given in δ (ppm) with tetramethylsilane as an internal standard. MS were recorded on a VG 7070 mass spectrometer operating at 70 eV or with a VG Quattro triple quadrupole mass spectrometer for the electrospray mass spectra. Column chromatography was carried out on Kieselgel 60 (Merck 70—230 mesh or 230—400 mesh). TLC and PLC were performed on precoated silica gel 60 F_{254} plates (Merck); spots were detected by UV or spraying with 1% $CeSO_4$ in 10% aq. H_2SO_4 following by heating. A voucher specimen (Bansiddhi 9764) of the plant material has been deposited at the Herbarium, the Division of Medicinal Plant Research and Development, Department of Medical Science, Nonthaburi, Thailand.

Extraction and Isolation The fresh leaves of *Clinacanthus siamensis* (3.4 kg) were extracted with 95% EtOH at room temperature. After filtration, the filtrate was evaporated to give a dark green thick oil (291.5 g) which was partitioned between water (700 ml) and EtOAc (3×500 ml) and the water layer then extracted with *n*-BuOH (4×300 ml). Removal of the solvent of each fraction gave the EtOAc-soluble fraction as a dark green oil (46.3 g), the *n*-BuOH-soluble fraction as a brown thick oil (51.9 g) and the water fraction as a brown solid (196.8 g).

The *n*-BuOH-soluble fraction (51.9 g) was separated by flash column chromatography using silica gel 60 (230—400 mesh, diameter 13 cm× height 5.0 cm) and the column was eluted with (500 ml each) EtOAc, gradient of EtOAc–MeOH (100 : 1, 80 : 1, 60 : 1, 40 : 1 and 30 : 1) and EtOAc–MeOH–H_2O (lower phase) (30 : 1 : 1, 25 : 1 : 1, 20 : 1 : 1, 15 : 1 : 1, 10 : 1 : 1, 7 : 1 : 1 and 5 : 1 : 1) to give 17 fractions.

Fractions 2 and 3 were combined (1.94 g) and chromatographed on a column of silica gel 60 (70—230 mesh, 190 g) using CH_2Cl_2–MeOH–H_2O (lower phase) (100 : 3 : 1, 70 : 3 : 1, 50 : 3 : 1, 30 : 3 : 1, 20 : 3 : 1 and 15 : 3 : 1) to give compound **1** (not pure) as a pale yellow solid (899 mg). Further purification of this material on a column of silica gel (70—230 mesh, 55 g) using CH_2Cl_2–MeOH–H_2O (lower phase) (100 : 3 : 1, 70 : 3 : 1, 50 : 3 : 1) gave **1** as pale yellow needles (745 mg).

Fraction 4 (647 mg) was separated on a column of silica gel (70—230 mesh, 65 g) using EtOAc, gradient of EtOAc–MeOH (100 : 1, 80 : 1, 60 : 1) as the eluent to give **2** as a pale yellow solid (133 mg). A portion of the pale yellow solid (76 mg) was separated by PLC (silica gel 60 F_{254}, 1 mm) using CH_2Cl_2–MeOH (10 : 1) as the developing solvent to give **2** as a pale yellow solid (13 mg).

Fractions 7 and 8 were combined (688 mg) and stirred with MeOH. A white precipitate which formed was removed by filtration and the filtrate was evaporated to give a brown oil (567 mg). The oil was separated on a column of silica gel (70—230 mesh, 55 g) using a gradient of CH_2Cl_2–MeOH–H_2O (lower phase) (100 : 3 : 1, 70 : 3 : 1, 50 : 3 : 1, 40 : 3 : 1, 30 : 3 : 1, 20 : 3 : 1 and 10 : 3 : 1) to give entadamide A (**4**) as a pale yellow oil (235 mg). A portion of the oil (93 mg) was further purified by PLC (silica gel 60 F_{254}, 1 mm) using CH_2Cl_2–MeOH–H_2O (lower phase) (20 : 3 : 1, 2 runs) as the developing solvent to give **4** as a pale yellow oil (54 mg).

Fraction 12 (7.4 g) was separated on a column of silica gel (70—230 mesh, 450 g) using CH_2Cl_2–MeOH–H_2O (50 : 3 : 1, 40 : 3 : 1, 30 : 3 : 1, 20 : 3 : 1, 10 : 3 : 1, 7 : 3 : 1 and 6 : 4 : 1) to give crude **3** as a brown oil (1.35 g). A portion of the brown oil (48 mg) was further purified by PLC (silica gel 60 F_{254}, 1 mm) using EtOAc–MeOH (10 : 1, 2 runs) as the developing solvent to give **3** as a pale yellow oil (36 mg).

Fraction 13 (1.69 g) was purified by column chromatography using silica gel (70—230 mesh, 170 g) and CH_2Cl_2–MeOH–H_2O (lower phase) (50 : 3 : 1, 40 : 3 : 1, 30 : 3 : 1, 20 : 3 : 1, 10 : 3 : 1, 8 : 3 : 1, 7 : 3 : 1 and 6 : 4 : 1) as the eluent to give compound **3** as a pale yellow oil (174 mg), entadamide C (**5**) as a pale yellow solid (86 mg) and compound **5** (not pure) as a yellow solid (71 mg).

***trans*-3-Methylsulfonyl-2-propenol (1)** Pale yellow needles (MeOH), mp 57—60 °C. IR ν_{max}^{Nujol} cm^{-1}: 3376 (broad), 3065, 1634, 1273, 1130, 1086, 940, 827, 779. ^{1}H-NMR ($CDCl_3$–DMSO-d_6, 3 : 1): δ: 2.95 (3H, s, CH_3SO_2), 4.34 (2H, dd, J=3.0, 2.4 Hz, CH_2), 5.75 (1H, br s, OH), 6.74 (1H, dt, J=15.0, 2.4 Hz, SO_2CH=C<u>H</u>CH_2), 7.02 (1H, dt, J=15.0, 3.0 Hz, SO_2C<u>H</u>=$CHCH_2$); EI-MS m/z (rel. int. %): 136 $[M]^+$ (1), 107 (100), 81 (30), 63 (30), 57 (46). HR-MS m/z: Calcd for $C_4H_8O_3S$: 137.0272. Found: 137.0268. ^{13}C-NMR data: Table 1.

Acetylation of 1 A mixture of **1** (54 mg), pyridine (0.5 ml) and Ac_2O (0.5 ml) was heated at 80 °C for 2 h. After the usual work up, the crude acetate derivative (**1a**) was obtained as a brown oil (119 mg), which was purified by a column of silica gel (70—230 mesh, 3 g) with hexane–EtOAc (1 : 1, 1 : 2, 1 : 3) as the eluent to give **1a** as a pale yellow oil (44 mg). IR ν_{max}^{neat} cm^{-1}: 3060, 3010, 2921, 1737, 1653, 1426, 1378, 1304, 1276, 1224, 1132, 967. ^{1}H-NMR ($CDCl_3$): δ: 2.16 (3H, s, CH_3CO), 2.79 (3H, s, CH_3SO_2), 4.83 (2H, dd, J=3.9, 2.0 Hz, CH_2), 6.63 (1H, dt, J=15.2, 2.0 Hz, SO_2CH=C<u>H</u>CH_2), 6.97 (1H, dt, J=15.2, 3.9 Hz, SO_2C<u>H</u>=$CHCH_2$). ESI-MS m/z (rel. int. %): 179 $[M+H]^+$ (72), 136 (100), 101 (20), 107(39), 99 (79), 81 (44), 73 (17), 63 (50), 58 (48); HR-MS m/z: Calcd for $C_6H_{11}O_4S$: 179.0377. Found: 179.0374. ^{13}C-NMR data: Table 1.

***trans*-3-Methylsulfinyl-2-propenol (2)** A pale yellow oil. $[\alpha]_D^{25}$ +22° (c=0.69, MeOH). UV λ_{max}^{MeOH} nm (log ε): 226 (3.34). IR ν_{max}^{neat} cm^{-1}: 3374 (broad), 1647, 1413, 1093, 1016 (strong), 943. ^{1}H-NMR ($CDCl_3$): δ: 2.65 (3H, s, CH_3S), 3.42 (1H, s, OH), 4.32 (1H, dd, J=18.4, 2.0 Hz), SOCH=CH<u>CH$_a$</u>OH), 4.36 (1H, ddd, J=18.4, 2.6, 1.0 Hz, SOCH=CH<u>CH$_b$</u>OH), 6.58 (1H, dd, J=14.8, 2.6 Hz, SOCH=<u>CH</u>CH_2OH), 6.63 (1H, dd, J=14.8, 1.0 Hz, SO<u>CH</u>=$CHCH_2$OH). EI-MS m/z (rel. int. %): 120 $[M]^+$ (34), 105 (3), 103 (4), 91 (17), 87 (12), 77 (17), 73 (81), 71 (12), 64 (78), 59 (100), 55 (27), 51 (7). HR-MS m/z: Calcd for $C_4H_8O_2S$: 120.0245. Found : 120.0245. ^{13}C-NMR data: Table 1.

Acetylation of 2 A mixture of **2** (49 mg), pyridine (0.5 ml) and Ac_2O (0.5 ml) was refluxed for 1 h. After the usual work up, the crude acetate derivative (**2a**) was obtained as a yellow oil (16 mg) which was purified by column chromatography using silica gel (70—230 mesh, 3 g) and hexane–EtOAc (4 : 1 and 2 : 1) as the eluent to give **2a** as a light yellow oil. $[\alpha]_D^{25}$ +27° (c=0.22, MeOH). UV λ_{max}^{MeOH} nm (log ε): 229 (3.24). IR ν_{max}^{neat} cm^{-1}: 1739, 1651, 1234, 1028, 964. ^{1}H-NMR ($CDCl_3$): δ: 2.12 (3H, s, CH_3CO), 2.65 (3H, s, CH_3S), 4.77 (1H, dd, J=17.2, 2.8 Hz, SOCH=CH<u>CH$_a$</u>OAc), 4.80 (1H, ddd, J=17.2, 3.6, 0.8 Hz, SOCH=CH<u>CH$_b$</u>OAc), 6.52 (1H, dd, J=14.8, 3.6 Hz, SOCH=<u>CH</u>CH_2OAc), 6.57 (1H, dd, J=14.8, 0.8 Hz, SO<u>CH</u>=$CHCH_2$OAc). ESI-MS m/z (rel. int. %): 163 $[M+H]^+$ (100), 145 (40), 103 (98), 87 (26), 59 (14). HR-MS m/z: Calcd for $C_6H_{10}O_3S$: 162.0350. Found : 162.0343. ^{13}C-NMR data: Table 1.

***trans*-3-Methylthioacrylamide (3)** A pale yellow solid, mp 116—118 °C (lit.[1] 117—118 °C). Its spectral data (UV, ^{1}H-NMR and MS) were identical to those previously reported.[1] ^{13}C-NMR data: Table 1.

Entadamide A (4) A pale yellow oil. Its ^{1}H- and ^{13}C-NMR spectral data were identical to those previously reported.[4]

Acetylation of 4 A mixture of **4** (175 mg), pyridine (0.5 ml) and Ac_2O (0.5 ml) was refluxed for 1 h. After the usual work up, the crude acetate derivative (**4a**) was obtained as a yellow oil (47 mg), which was chromatographed on a column of silica gel (70—230 mesh, 5 g) with hexane–EtOAc (4 : 1, 2 : 1 and 1 : 1) as the eluent to give **4a** as a colorless oil (28 mg). UV λ_{max}^{MeOH} nm (log ε): 271 (4.25). IR ν_{max}^{neat} cm^{-1}: 1740, 1641, 1574, 1233, 1052, 948. ^{1}H-NMR ($CDCl_3$): δ: 2.08 (3H, s, CH_3CO), 2.33 (3H, s, CH_3S), 3.59 (2H, q, J=5.4 Hz, $NH\underline{CH_2}CH_2OAc$), 4.19 (2H, t, J=5.4 Hz, $NHCH_2\underline{CH_2}OAc$), 5.64 (1H, d, J=13.6 Hz, $CH_3SCH{=}\underline{CH}CO$), 5.84 (1H, br s, NH), 7.63 (1H, d, J=13.6 Hz, $CH_3S\underline{CH}{=}CHCO$). EI-MS m/z (rel. int. %): 203 $[M]^+$ (11), 188 (5), 143 (5), 128 (11), 101 (100), 73 (25), 58 (21). ^{13}C-NMR data: Table 1.

Entadamide C (5) Colorless needles from acetone; mp 141—142 °C (lit.[5] 144—145 °C); $[\alpha]_D^{25}$ +185° (c=0.20, MeOH) (lit.[5] +186°). Its ^{1}H- and ^{13}C-NMR spectral data were identical to those previously reported.[5]

Antiplasmodial Assay The *Plasmodium falciparum* (K1, multidrug resistant strain) was cultured continuously according to the method of Trager and Jensen.[8] The quantitative assessment of the antiplasmodial activity *in vitro* was performed by mean of the microculture radioisotope technique based upon the method described by Desjardins *et al.*[9] Standard sample, chloroquin diphosphate (IC_{50} value of 0.16 μg/ml, 0.31 μM) was used as reference compound for the assay.

Antimycobacterial Assay The antimycobacterial activity was assessed against *Mycobacterium tuberculosis* H37Ra using the Microplate Alamar Blue Assay (MABA).[10] Standard drugs, isoniazide (MIC of 0.040—0.090 μg/ml) and kanamycin sulfate (MIC of 2.0—5.0 μg/ml) were used as reference compounds for the assay.

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