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Antibacterial Compounds from *Zanthoxylum rhetsa*

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A new amide, zanthorhetsamide (**1**), along with nine known compounds (**2**-**10**) was isolated from the roots and stem barks of *Zanthoxylum rhetsa*. The structure was characterized by spectroscopic methods. In addition, the antibacterial activity of the isolates was evaluated. Dihydrochelerythrine (**4**) exhibited strong activity against methicillin-resistant *Staphylococcus aureus* SK1 and moderate activity against *Escherichia coli* TISTR 780 with MIC values of 8 and 16 μg/mL, respectively.

Key words: Zanthorhetsamide, *Zanthoxylum rhetsa*, Rutaceae, Antibacterial activity

INTRODUCTION

About 200 species of the plants of the genus *Zanthoxylum* are distributed in pantropical countries. Several species of *Zanthoxylum* are used in traditional medicine and consumed as vegetables, especially in Asian and African countries (Ladino and Suarez, 2010). Among them, *Z. acanthopodium*, *Z. armatum*, *Z. nitidum*, and *Z. rhetsa* are commonly used as spices and condiments in the Northern part of Thailand (Smitinand, 2001). *Z. rhetsa* produces a variety of biologically active metabolites including alkaloids, lignans, coumarins, and terpenoids (Cheng et al., 2005). In our ongoing study on chemical constituents and biological activity of Rutaceae plants, we report herein the isolation and structure elucidation of a new amide (**1**) along with nine known compounds (*N*-(4-methoxyphenethyl)benzamide (**2**) (Vargas et al., 2010), alatamide (**3**) (Maxwell and Ramperad, 1989), dihydrochelerythrine (**4**) (Martin et al., 2005), 6-acetonyldihydrochelerythrine (**5**) (Chen et al., 2011), 8-acetonyldihydronitidine (**6**) (Nissanka et al., 2001), asarinin (**7**) (Gunatilaka et al., 1982), horsfieldine (**8**) (Gunatilaka et al., 1982), 5,7,8-trimethoxycoumarin (**9**) (Cheng et al., 2005) and dictamine (**10**) (Tanaka et al., 1985) from the stem barks and roots of *Z. rhetsa*.

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MATERIALS AND METHODS

General experimental procedure

The $[\alpha]_D$ values were determined with a Bellingham & Stanly ADP440 polarimeter. The infrared (IR) and ultraviolet (UV) spectra were recorded on a Perkin-Elmer FTS FT-IR and a Perkin-Elmer UV-Vis spectrophotometers, respectively. ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded using a 400 MHz Bruker spectrometer. Tetramethylsilane was used as internal reference. A MicroTOF, Bruker Daltonics mass spectrometer was used to correct electrospray ionization time-of-flight mass spectra (ESI-TOF-MS). Quick column chromatography (QCC) and column chromatography (CC) were carried out on silica gel 60 H (Merck, 5-40 μm) and silica gel 100 (Merck, 63-200 μm), respectively. Precoated plates of silica gel 60 F_{254} were used for analytical thin layer chromatography (TLC).

Plant material

The stem barks and roots of *Z. rhetsa* were collected in December 2008 from Chiang Rai Province, Northern Thailand. The plant was identified by Mr. James Maxwell, Chiang Mai University Herbarium and the

specimen (MFU-NPR0026) was deposited at Natural Products Research Laboratory, School of Science, Mae Fah Luang University.

Extraction and isolation

The stem barks and roots of *Z. rhetsa* (6.65 kg) were extracted with acetone over the period of 3 days at room temperature. Removal of solvent under reduced pressure provided an acetone extract (140 g) that was chromatographed by QCC and eluted with a gradient of hexanes-acetone (10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, 0:10) to yield 14 fractions (A-N). QCC of fraction D (9.2 g) with 5% ethyl acetate (EtOAc)-hexanes yielded eight subfractions (DA-DH). Subfraction DC (1.6 g) was purified by CC with 5% EtOAc-hexanes to yield compound **4** (6.2 mg). Fractions G (19.3 g) and H (15.5 g) were combined and subsequently subjected to QCC with 10% EtOAc-hexanes to yield **7** (400.5 mg) and **10** (29.4 mg). Fraction J (7.05 g) was further subjected to QCC with a gradient of EtOAc-hexanes (30% EtOAc-hexanes to 100% EtOAc) to provide 20 subfractions (JA-JT). Subfraction JM (912.7 mg) was further purified by CC with 30% CH_2Cl_2-hexanes to yield four subfractions (JM1-JM4). Subfraction JM3 (274.8 mg) was subsequently separated by CC with 30% EtOAc-hexanes to give **2** (39.5 mg) and **3** (3.0 mg). Subfraction JO (520.2 mg) was further purified by CC using 20% acetone-hexanes to give six subfractions (JO1-JO6). Compound **9** (2.2 mg) was derived from subfraction JO2 (215.4 mg) by repeated CC using 10% EtOAc-hexanes. Subfraction JS (363.0 mg) was subjected to QCC with a gradient of EtOAc-hexanes (30% EtOAc-hexanes to 100% EtOAc) to yield **5** (4.2 mg), **8** (52.0 mg) and 10 subfractions (JS1-JS10). Subfraction JS3 was further purified by CC with 5% CH_2Cl_2-hexanes to provide **6** (15.1 mg). Fraction N (1.11 g) was subjected to CC with 40% hexanes-CH_2Cl_2 to afford five subfractions (NA-NE). Compound **1** (6.2 mg) was obtained from subfraction NA (35.0 mg) by CC with 20% acetone-hexanes as an eluent.

Zanthorhetsamide (1)

White amorphous solid; mp 67-69°C; $[\alpha]_D^{30}$ +55.7 (*c* = 0.01, $CHCl_3$); UV λ_{max} (MeOH) nm (log ε) 228 (4.08), 274 (3.24), 281 (3.16); Fourier transform (FT)-IR (KBr) ν_{max} 1740, 1648 cm^{-1}; ESI-TOF-MS *m/z* 369.3510 $[M]^+$ (Calcd for $C_{22}H_{27}NO_4$, 369.1940); ^{1}H-NMR (400 MHz, in $CDCl_3$) and ^{13}C-NMR (100 MHz, in $CDCl_3$) data, see Table I.

Hydrolysis of zanthorhetsamide

Zanthorhetsamide (2.2 mg), dissolved in methanol (1 mL), was added to K_2CO_3 (20 mg). The resulting mixture was stirred at room temperature for 10 min and filtered through pad column chromatography (30% EtOAc-hexanes) to give *S*-(+)-tembamide (1.3 mg). Yield: 81%; white solid; $[\alpha]_D^{26}$ +45.2 (*c* = 0.01, $CHCl_3$); ^{1}H-NMR (400 MHz, in $CDCl_3$): δ 7.75 (2H, *dd*, *J* = 8.8, 1.6 Hz, H-3", 7"), 7.49 (1H, *tt*, *J* = 7.6, 7.2, 1.6, 1.2 Hz, H-5"), 7.41 (2H, *m*, H-4", 6"), 7.32 (2H, *br d*, *J* = 8.8 Hz, H-2', 6'), 6.90 (2H, *br d*, *J* = 8.8 Hz, H-3', 5'), 6.63 (1H, *br s*, NH), 4.90 (1H, *br dd*, *J* = 5.6, 2.0 Hz, H-2), 3.90 (1H, *s*, HO-2), 3.86 (1H, *m*, H-1), 3.79 (3H, *s*, OMe-4'), 3.50 (1H, *m*, H-1).

Table I. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) data of zanthorhetsamide (**1**) in $CDCl_3$

Position	δ_C	δ_H (mult., *J* in Hz)
1	45.2	3.82 (m)
2	74.1	5.95 (dd, *J* = 8.0, 4.4)
1'	129.8	-
2'	127.9	7.32 (d, *J* = 8.4)
3'	114.1	6.90 (d, *J* = 8.4)
4'	159.8	-
5'	114.1	6.90 (d, *J* = 8.4)
6'	127.9	7.32 (d, *J* = 8.4)
1"	167.5	-
2"	134.3	-
3"	126.8	7.71 (br d, *J* = 7.6)
4"	128.7	7.42 (m)
5"	131.7	7.49 (br t, *J* = 7.2)
6"	128.7	7.42 (m)
7"	126.8	7.71 (br d, *J* = 7.6)
1'"	173.6	-
2'"	34.5	2.34 (t, *J* = 7.6)
3'"	24.9	1.58 (m)
4'"	29.5	1.26 (m)
5'"	22.8	1.27 (m)
6'"	14.1	0.85 (t, *J* = 6.4)
4'-OMe	55.4	3.80 (s)
NH	-	6.42 (br s)

Antibacterial activity testing

Escherichia coli TISTR 780, *Salmonella typhimurium* TISTR 292, and *Staphylococcus aureus* TISTR 1466 were obtained from the Microbiological Resources, Centre of the Thailand Institute of Scientific and Technological Research, and methicillin-resistant *S. aureus* (MRSA) SK1 was obtained from the Department of Microbiology, Faculty of Science, Prince of Songkla University, Thailand. Minimum inhibition concentrations (MICs) were determined by a two-fold serial dilution method using Mueller Hinton broth according to the Clinical and Laboratory Standards Institute recommendations (CLSI, 2002). Vancomycin and genta-

mycin were used as standard antibacterial agents.

RESULTS AND DISCUSSION

Compound **1**, $[\alpha]_D^{30}$ +55.7 ($CDCl_3$), was isolated as a white amorphous solid. ESI-TOF-MS gave an molecular ion peak $[M]^+$ at *m/z* 369.3510, consistent with a molecular formula of $C_{22}H_{27}NO_4$. The UV absorptions at λ_{max} 228, 274, and 281 nm suggested the presence of a conjugated system in the molecule. The IR absorptions showed stretching frequency of carbonyl functionalities at 1740 (ester) and 1648 (amide) cm^{-1}. The ^{1}H-NMR spectrum of **1** showed the presence of a N*H* (δ 6.42, br s), a monosubstituted benzene group [δ 7.71 (2H, br d, *J* = 7.6 Hz, H-3" and H-7"), 7.49 (1H, br t, *J* = 7.2 Hz, H-5") and 7.42 (2H, m, H-4" and H-6")], a 1,4-disubstituted benzene group [δ 7.32 (2H, d, *J* = 8.4 Hz, H-2' and H-6') and 6.90 (2H, d, *J* = 8.4 Hz, H-3' and H-5')], a methoxyl group (δ 3.80, s, 4'-OMe), an oxymethine (δ 5.95, dd, *J* = 8.0, 4.4 Hz, H-2) and methylene protons (δ 3.82, 2H, m, H-1). These signals were similar to the signals described previously for tembamide acetate isolated from *Piper guayranum* (Maxwell and Ramperad, 1989). The ^{1}H- and ^{13}C-NMR spectra of **1** displayed the signals for a hexanoyl moiety (Kanokmedhakul et al., 2007) at δ_H 2.34 (t, 7.6 Hz, H-2'")/δ_C 34.5 (C-2'"), δ_H 1.26 (m, H-3'")/δ_C 29.5 (C-3'"), δ_H 1.58 (m, H-3'")/δ_C 24.9 (C-3'"), δ_H 1.26 (m, H-4'")/δ_C 29.5 (C-4'"), δ_H 0.85 (t, 6.4 Hz, H-6'")/δ_C 14.1 (C-6'") and C=O at δ_C 173.6 (C-1'") instead of the acetyl group of tembamide acetate [δ_H 2.12/δ_C 21.5 (CH_3) and δ_C 172.0 (C=O)] (Maxwell and Ramperad, 1989). These data were suggested by COSY and HMBC correlations as shown in Fig. 2. The mass fragment ion at *m/z* 254.1 $[M\text{-}C_6H_{11}O_2]^+$ confirmed the presence of hexanoyl moiety, which is placed on C-2 due to the correlations of H-2 (δ 5.95), H-2'" (δ 2.34) and H-3'" (δ 1.58) with C-1'" (δ 173.6) in HMBC spectrum. Therefore, the structure **1** was identified to be zanthorhetsamide (2-benzamido-1-(4-methoxyphenyl)ethylhexanoate). Zanthorhetsamide **1** was partially hydrolyzed with K_2CO_3 to yield tembamide, which had a similar ^{1}H-NMR spectrum and specific rotation ($[\alpha]_D^{26}$ +45.2, $CHCl_3$) with synthetic *S*-(+)-tembamide ($[\alpha]_D^{25}$ +56.9, $CHCl_3$) (Kamal et al.,

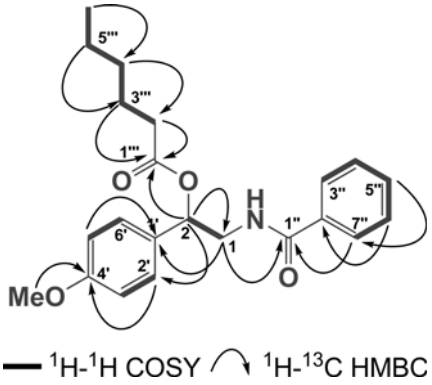

Fig. 2. Key HMBC correlations for zanthorhetsamide (**1**).

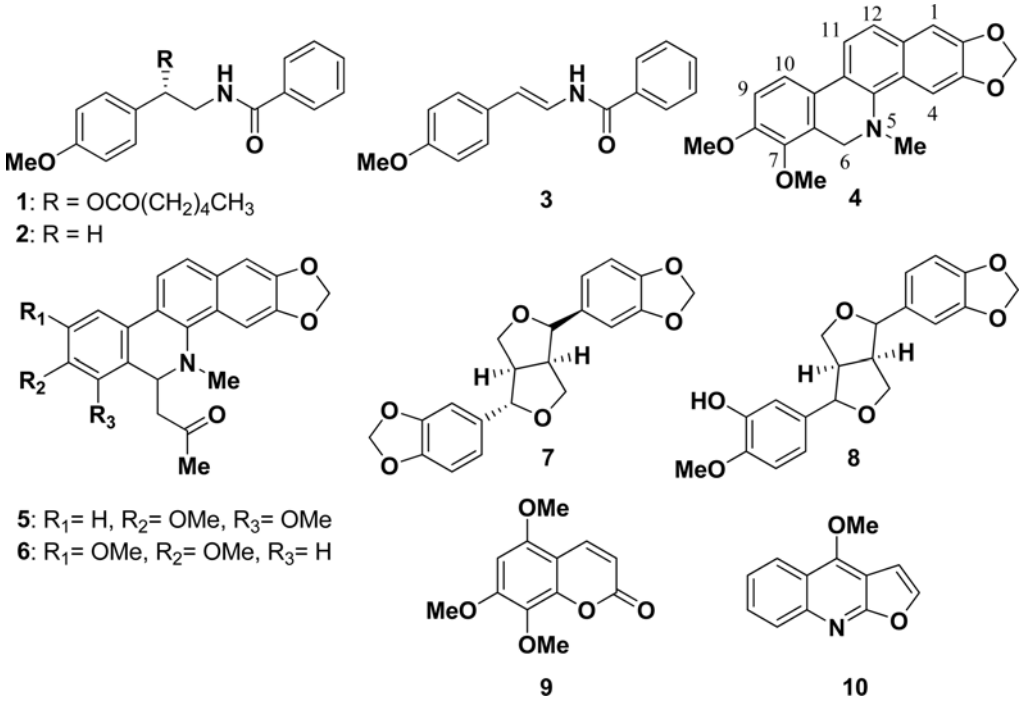

Fig. 1. Structures of compounds **1**-**10**.

2004). This result may imply that zanthorhetsamide should have the same configuration as synthetic *S*-(+)-tembamide. The absolute configuration of zanthorhetsamide was, therefore, proposed to be *2S*-(+)-zanthorhetsamide.

All isolated compounds, except compound **6**, were evaluated for their antibacterial activity against both Gram-positive bacteria (*Staph.* and MRSA SK1) and Gram-negative bacteria (*S. typhimurium* and *E. coli*). The standard drugs were vancomycin (MIC = 1 and 0.25 μg/mL against MRSA SK1 and *Staph.*, respectively) and gentamicin (MIC = 0.25 and 0.125 μg/mL against *E. coli* and *S. typhimurium*, respectively). All isolates displayed weak activity against *S. typhimurium* with the same MIC value of 128 μg/mL, but were inactive against *Staph.* Only compound **4** showed strong activity against MRSA (MIC of 8 μg/mL), while the remaining compounds were inactive. Compound **4** also displayed moderate activity against *E. coli* (MIC = 16 μg/mL), whereas the rest of compounds were weakly active with the same MIC value of 128 μg/mL. It should be noted that the phenanthridine alkaloid **4** without substituent located at C-6 plays an important role in both Gram-positive and Gram-negative antibacterial activity.

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REFERENCES

Chen, J. J., Lin, Y. H., Day, S. H., Hwang, T. L., and Chen, I. S., New benzenoids and anti-inflammatory constituents from *Zanthoxylum nitidum*. *Food Chem.*, 125, 282-287 (2011).

Cheng, M. J., Lee, K. H., Tsai, I. L., and Chen, I. S., Two new sesquiterpenoids and anti-HIV principles from the root bark of *Zanthoxylum ailanthoides*. *Bioorg. Med. Chem.*, 13, 5915-5920 (2005).

CLSI, Method for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Approved Standard M7-A4. http://isoforlab.com/phocadownload/csli/M7-A7.pdf (2006).

Gunatilaka, A. A. L., Jasmin De Silva, A. M. Y., Sotheeswaran, S., and Tellederatne, L. M. V., Horsfieldin, a lignin and other constituents from *Horfieldia iryaghedhi*. *Phytochemistry*, 21, 2719-2723 (1982).

Kamal, A., Shaik, A. A., Sandbhor, M., and Malik, M. S., Chemoenzymatic synthesis of (*R*)- and (*S*)-tembamide, aegeline and denopamine by a one-pot lipase resolution protocol. *Tetrahedron Asymmetry*, 15, 3939-3944 (2004).

Kanokmedhakul, S., Kanokmedhakul, K., and Buayairaksa, M., Cytotoxic clerodane diterpenoids from fruits of *Casearia grewiifolia*. *J. Nat. Prod.*, 70, 1122-1126 (2007).

Ladino, O. J. P. and Suarez, L. E. C., Isoquinoline alkaloids of *Zanthoxylum quinduense* (Rutaceae). *Biochem. Syst. Ecol.*, 38, 853-856 (2010).

Martin, M. T., Rasoanaivo, L. H., and Raharisololalao, A., Phenanthridine alkaloids from *Zanthoxylum madagascariense*. *Fitoterapia*, 76, 590-593 (2005).

Maxwell, A. and Ramperad, D., *β*-Phenylethylamine-derived amides from *Piper guaranum*. *J. Nat. Prod.*, 52, 411-414 (1989).

Nissanka, A. P. K., Karunaratne, V., Bandara, B. M. R., Kumar, V., Nakanishi, T., Nishi, M., Inada, A., Tillekeratne, L. M. V., Wijesundara, D. S. A., and Gunatilaka, A. A. L., Antimicrobial alkaloids from *Zanthoxylum tetraspermum* and *caudatum*. *Phytochemistry*, 56, 857-861 (2001).

Smitinand, T., Thai Plant Names, Rivised ed. Prachachon Co, Bangkok, (2001).

Tanaka, H., Ahn, J. W., Katayama, M., Wada, K., Marumo, S., and Osaka, Y., Isolation of two ovicidal substances against two-spotted spider mite, *Tetranychus urticae* Koch, from *Skimmia repens* Nakai. *Agric. Biol. Chem.*, 49, 2189-2190 (1985).

Vargas, F., Toledo, F. T., and Comasseto, J. V., *N*-Functionalized organolithium compounds via tellurium/lithium exchange reaction. *J. Braz. Chem. Soc.*, 21, 2072-2078 (2010).