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Antibacterial carbazole alkaloids from *Clausena harmandiana* twigs

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ABSTRACT

Three new carbazole alkaloids, harmandianamines A-C (**1**–**3**), together with fifteen known compounds (**4**–**18**) were isolated from the twigs of *Clausena harmandiana*. The structures were elucidated by spectroscopic methods, including UV, IR, NMR, and MS. The antibacterial activity against *Escherichia coli* TISTR 780, *Salmonella typhimurium* TISTR 292, *Staphylococcus aureus* TISTR 1466 and methicillin-resistant *S. aureus* (MRSA) SK1 of some isolated compounds was also evaluated. Compound **6** exhibited significant antibacterial activity against MRSA SK1 with an MIC value of 0.25 μg/mL which higher than that of standard drug, vancomycin (MIC value = 1 μg/mL) whereas compounds **14** and **5** showed strong activity with MIC values of 4 and 8 μg/mL, respectively. Only compound **14** showed strong antibacterial activity against *S. aureus* TISTR 1466 with an MIC value of 4 μg/mL.

1. Introduction

Clausena harmandiana (Pierre) belonging to the family of Rutaceae, is distributed in northeastern Thailand. The young leaves are edible [1] and some parts of the plant have been used as traditional medicines for the treatment of illness, stomachache, and headache [2]. Many of carbazole alkaloids as well as coumarins were recognized as major compounds of *C. harmandiana* and some of them displayed interesting biological activities including antimalaria, anti-TB, cytotoxicity, and stimulate glucose uptake in L6 myotubes [1–4]. In a continuing search for bioactive metabolites from *Clausena* plants [5–9], we report herein the isolation and identification of three new carbazole alkaloids (**1**–**3**), harmandianamines A-C, along with fifteen known compounds including clausevatine D (**4**) [11], clausamine A (**5**) [12], clausamine B (**6**) [12], clausine S (**7**) [13], girinimbine (**8**) [14], *O*-demethylmurrayanine (**9**) [15], clauszoline I (**10**) [16], clausine Z (**11**) [17], clauszoline N (**12**) [18], clausine D (**13**) [19], clausine F (**14**) [19], clausemine D (**15**) [20], heptaphylline (**16**) [21], dectamine (**17**) [22], and γ-fagarine (**18**) [22] (Fig. 1) from *C. harmandiana* twigs. Also, the antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus* TISTR1466 and methicillin-resistant *S. aureus* SK1) and Gram-negative bacteria (*Escherichia coli* TISTR 780 and *Salmonella typhimurium* TISTR 292) was also reported.

2. Experimental

2.1. General

Melting points were determined on a Buchi, B-540 visual thermometer. The optical rotation $[\alpha]_D$ values were determined with a Bellingham & Stanley ADP400 polarimeter. UV–vis spectra were recorded with a Perkin-Elmer UV–vis spectrophotometer. The IR spectra were recorded using Perkin-Elmer FTS FT-IR spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded by 400 MHz Bruker or 500 MHz Varian UNITY INOVA spectrometers. Tetramethylsilane (TMS) are used as internal reference. The EI-MS and HR-EI-MS data were used MAT 95 XL mass spectrometer. Quick column chromatography (QCC) and column chromatography (CC) were carried out on

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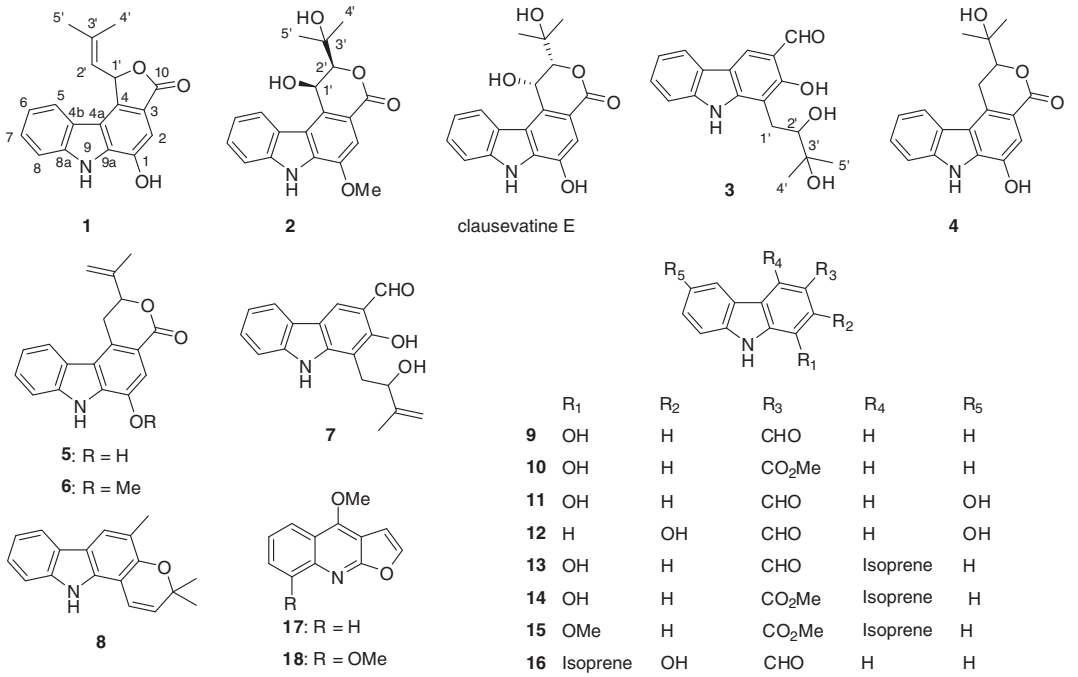

	R_1	R_2	R_3	R_4	R_5
9	OH	H	CHO	H	H
10	OH	H	CO_2Me	H	H
11	OH	H	CHO	H	OH
12	H	OH	CHO	H	OH
13	OH	H	CHO	Isoprene	H
14	OH	H	CO_2Me	Isoprene	H
15	OMe	H	CO_2Me	Isoprene	H
16	Isoprene	OH	CHO	H	H

Fig. 1. Structures of alkaloids from *C. harmandiana* twigs.

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 purposes.

2.2. Plant material

The *C. harmandiana* twigs were collected from Chiang Rai Province, northern Thailand, in March 2010. The plant was identified by Dr. Monthon Norsaengsri and Mr. James Maxwell and a voucher specimen number QBG 45334 was deposited at the herbarium collection of Queen Sirikit Botanic Garden, Mae Rim, Chiang Mai, Thailand.

2.3. Extraction and isolation

Air-dried twigs of *C. harmandiana* (4.91 Kg) were extracted successively with hexanes and acetone over a period of 3 days at room temperature. The hexanes (14.39 g) and acetone (29.22 g) extracts were combined (43.61 g) and subjected to QCC over silica gel using a gradient of hexanes-EtOAc (100% hexanes to 100% EtOAc) to provide twelve fractions (A-L). Fraction C (1.10 g) was separated by CC with 30% CH_2Cl_2-hexanes to afford compounds **8** (2.1 mg) and **16** (19.9 mg). Fraction D (1.05 g) was performed by CC using 50% CH_2Cl_2-hexanes to yield compound **15** (46.0 mg). Fraction E (1.16 g) was subjected to CC with 60% CH_2Cl_2-hexanes and followed by Sephadex LH-20 eluting with 100% MeOH to give compound **7** (4.6 mg). Compounds **6** (18.3 mg), **14** (13.8 mg), and **17** (40.0 mg) were obtained from fraction G (1.75 g) by repeated Sephadex LH-20 with 100% MeOH and followed by CC using 30% EtOAc-hexanes. Fraction H (2.98 g) was separated by CC with 30% EtOAc-hexanes to provide four subfractions (H1-H4). Subfraction H2 (234.1 mg) was subjected to Sephadex LH-20 using 100% MeOH to afford compound **13** (5.8 mg) and four subfractions (H2a-H2d). Subfraction H2b (52.7 mg) was purified by CC with 5% acetone-CH_2Cl_2 to give compounds **9** (30.3 mg), **10** (1.4 mg), and **12** (1.9 mg). Fraction H4 (160.3 mg) was subjected to Sephadex LH-20 using 100% MeOH to give compound **5** (22.3 mg). Purification of fraction J (2.84 g) by QCC with 5% EtOAc-CH_2Cl_2 and followed by Sephadex LH-20 using 100% MeOH yielded compound **18** (2.4 mg). Fraction K (4.42 g) was separated by QCC with a gradient of 20% EtOAc-hexanes to 100% EtOAc to afford seven subfractions (G1-G7). Subfraction G2 (394.6 mg) was purified by Sephadex LH-20 using 100% MeOH and followed by CC with 10% acetone-hexanes to give compounds **1** (1.1 mg) and **3** (1.6 mg). The purification of subfraction K4 (534.5 mg) by Sephadex LH-20 using 100% MeOH gave compound **11** (11.5 mg). Compounds **2** (1.8 mg) and **4** (7.1 mg) were derived from subfraction G6 (442.2 mg) by Sephadex LH-20 with 100% MeOH and followed by CC using 30% EtOAc-CH_2Cl_2.

Harmandianamine A (**1**): Yellow solid; mp 203.6-204.0 °C; $[\alpha]_D^{28} = +6.46$ ($c = 0.008$, MeOH); UV (MeOH) λ_{max} 204, 207, 240, 250, 267, 310, 323, 337; IR (neat) ν_{max} 3250, 2923, 2852, 1735, 1708; ^1H and ^{13}C NMR spectroscopic data see Table 1; EI-MS *m/z* 292 (94), 277 (56), 250 (18), 233 (12), 209 (100), 181 (15); HR-EI-MS (*m/z*): $[M]^+$ 293.1054 (calc. for $C_{18}H_{15}NO_3$, 293.1046).

Harmandianamine B (**2**): Yellow solid; mp 216.7-217.1 °C; $[\alpha]_D^{29} = +35.2$ ($c = 0.012$, MeOH); UV (MeOH) λ_{max} 204, 212, 237, 249, 269, 279, 323, 335; IR (neat) ν_{max} 3340, 2921, 2830, 1695, 1584, 1361; ^1H and ^{13}C NMR spectroscopic data see Table 1; EI-MS *m/z* 340 (88), 264 (100), 236 (46), 224 (22), 209 (20), 153, (14); HR-EI-MS (*m/z*): $[M]^+$ 341.1266 (calc. for $C_{19}H_{19}NO_5$, 341.1258).

Harmandianamine C (**3**): Yellow solid; mp 228.3-228.6 °C; $[\alpha]_D^{29} = +18.6$ ($c = 0.011$, MeOH); UV (MeOH) λ_{max} 204,