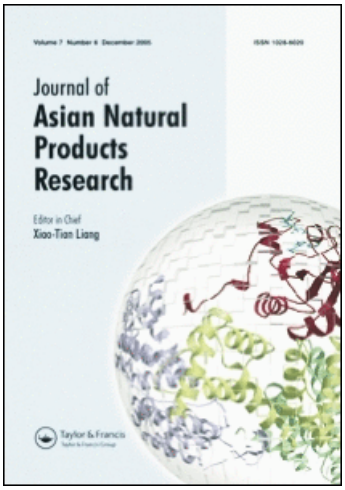

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Carbazole alkaloids from the stems of *Clausena excavata*

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NOTE

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A new carbazole alkaloid, sansoakamine (**8**), together with 11 known compounds, was isolated from the stems of *Clausena excavata*. All structures were elucidated by spectroscopic methods. Compound **7** showed moderate anti-malarial activity against *Plasmodium falciparum* with a MIC value of 6.74 μg/ml.

Keywords: *Clausena excavata*; carbazole alkaloids; Rutaceae; sansoakamine

1. Introduction

Clausena excavata or 'San Soak' in Thailand is a wild shrub of the Rutaceae plant family, which is widely distributed in southern and south-eastern Asian countries. The plant has been used as a traditional medicine for the treatment of cold, malaria, AIDS, dermatopathy, abdominal pain, and snake-bite, and also used as a detoxification agent [1,2]. The main constituents of this plant are coumarins [3,4] and carbazole alkaloids [2,4], nevertheless some benzenoids [5], terpenoids [6], flavonoids [5] and steroids [3] have also been reported. In our continuing study on chemical constituents from Thai medicinal plants, we describe herein the isolation and structural elucidation of a new carbazole alkaloid, sansoakamine (**8**) (Figure 1), together with 11 known compounds (**1**–**7**, **9**–**12**) from the stems of *C. excavata* collected from Satoon Province, in the southern part of Thailand.

2. Results and discussion

Sansoakamine (**8**) was obtained as a light brown solid. Its molecular formula, $C_{14}H_{11}NO_4$, was determined by HR-EI-MS at *m/z* 257.0683 $[M]^+$. The UV, IR, ^1H and ^{13}C NMR spectral data of compound **8** (see Section 3) were similar to those of compounds isolated from the stem bark of *C. excavata* by Wu *et al.* [4], which indicated that compound **8** possessed a 3-methyl ester carbazole alkaloid skeleton [4]. The ^1H NMR spectrum of **8** showed a signal of methoxyl at δ 3.98 which is located on C-3, as well as a hydroxyl proton at δ 11.03 on C-2. The downfield shift of 2-OH suggested the presence of intramolecular hydrogen bonding. The existence of 3-methyl ester and 2-OH caused the chemical shift of H-1 (δ 6.85) shifted to the higher field, whereas H-4 (δ 8.52) moved to the lower field in the aromatic region. These results were also supported by ^2J and ^3J HMBC correlations of H-1 with C-2 (δ 160.4), C-3 (δ 116.9), H-4 with C-5a (δ 124.2), C-1a (δ 146.3), and the carbonyl carbon of methyl ester (δ 171.3) and 2-OH with C-1 (δ 96.5), C-2 (δ 160.4). In addition, ABX coupling aromatic protons of ring A were also observed at δ 6.92 (dd, $J = 8.8$, 2.4 Hz),

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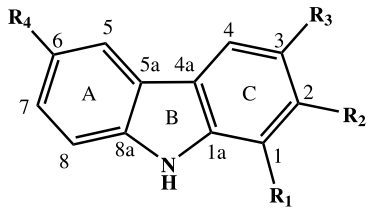

	R_1	R_2	R_3	R_4
1	OMe	H	CO_2Me	H
2	H	H	CO_2Me	H
3	H	OH	CHO	OMe
4	OMe	H	CHO	H
5	H	H	CHO	H
6	H	OH	CO_2Me	H
7	H	OMe	CHO	H
8	H	OH	CO_2Me	OH
9	OH	H	CO_2Me	H
10	OH	H	CHO	H
11	OH	H	CO_2Me	OH
12	OH	H	CHO	OH

Figure 1. Structure of compounds **1**–**12**.

7.28 (d, $J = 8.8$ Hz) and 7.50 (d, $J = 2.4$ Hz) assignable to H-7, H-8, and H-5, respectively, on the basis of COSY and HMBC correlations (Figure 2). The remaining signals at δ 8.06 and 10.26 were identified as N*H* and 6-O*H* protons, respectively. From the above evidences, the structure of **8** was elucidated as shown in Figure 1.

The remaining 11 known carbazole alkaloids including mukonine (**1**) [7], methyl carbazole-3-carboxylate (**2**) [7], lansine (**3**) [8], murrayanine (**4**) [7], 3-formylcarbazole (**5**) [9], mukonidine (**6**) [10], *O*-methylmukonal (**7**) [11], clauszoline-I (**9**) [12], *O*-demethylmurrayanine (**10**) [13], methyl 1,6-dihydroxy-9*H*-carbazole-3-carboxylate (**11**) [14], and clausine-Z (**12**) [15] were identified by 1D and 2D NMR spectral data and by comparison of their reported physical and spectroscopic data with those reported in the literature.

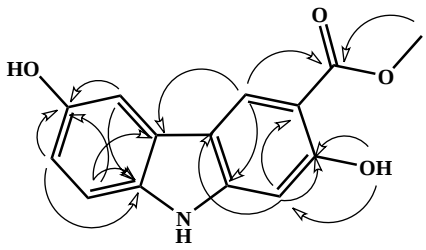

Figure 2. Key HMBC correlations of sansoakamine (**8**).

Compounds **1**, **7**–**10**, and **12** were evaluated for their anti-malarial activity. Unfortunately, only compound **7** showed moderate anti-malarial activity against *Plasmodium falciparum* K1 strain with a MIC value of 6.74 μg/ml.

In fact, a number of carbazole alkaloids have been isolated from *C. excavata*. Herein, except for the new compound sansoakamine (**8**), compound **11** was also isolated from the natural product for the first time.

3. Experimental

3.1 General experimental procedures

Melting points were determined on the Buchi melting point B-540 apparatus. The UV spectra were recorded with a Perkin-Elmer UV–vis spectrophotometer. The IR spectra were recorded with a Perkin-Elmer FTS FT-IR spectrophotometer. The NMR spectra were recorded using a 400 MHz Bruker FTN-MR Ultra Shield spectrometer. Chemical shifts were recorded in parts per million (δ) in $CDCl_3$ or acetone-d_6 with tetramethylsilane as an internal reference. The HR-MS was obtained from a MAT 95 XL spectrometer. Quick column chromatography (QCC) and column chromatography (CC) were carried out on silica gel 60 H (5–40 μm; Merck, Darmstadt, Germany) and silica gel 100 (63–200 μm; Merck), respectively. The gel filtration was carried out on Sephadex™ LH-20 (Uppsala, Sweden). Precoated plates of silica gel 60 F_{254} (Merck) were used for analytical purposes.

3.2 Plant material

The stems of *C. excavata* were collected from Satoon Province, in the southern part of Thailand, in May 2008. Botanical identification was carried out by compari-

son with a voucher specimen number QBG 6277 in the herbarium collection of Queen Sirikit Garden, Mae Rim District, Chiang Mai, Thailand.

3.3 Extraction and isolation

The stems of *C. excavata* were extracted with ethyl acetate (EtOAc) over a period of 3 days at room temperature. Removal of the solvent under reduced pressure provided the EtOAc extract (70.50 g). This extract was chromatographed by QCC over silica gel and eluted with a gradient of hexane–acetone (100% hexane to 100% acetone) to afford 26 fractions (A–Z). Fraction I (297.3 mg) was subjected to QCC with 27% CH_2Cl_2–hexane to yield 12 subfractions (I1–I12). Subfraction I4 (27.0 mg) was recrystallized with hexane to give compound **1** (4.4 mg). Fractions K and L (1.15 g) were combined and subjected to Sephadex LH-20 with 60% CH_2Cl_2–MeOH to give five subfractions. Subfraction KL4 (829.5 mg) was fractionated by repeated QCC with 25% CH_2Cl_2–hexane and gave six subfractions (KL4.1–4.6). Subfraction KL4.3 (80.7 mg) was purified by CC with 60% CH_2Cl_2–hexane to give compound **2** (7.3 mg), whereas compound **3** (2.6 mg) was derived from subfraction KL4.5 (55.4 mg) by repeated CC using 80% CH_2Cl_2–hexane as the eluent. Subfraction KL4.6 (130.0 mg) was further purified by CC with 40% CH_2Cl_2–hexane to give compound **4** (5.0 mg), along with three subfractions (KL4.6.1–4.6.3). Compound **5** (17.6 mg) was obtained from subfraction KL4.6.3 by repeated CC eluted with 70% CH_2Cl_2–hexane. Fractions P and Q (842.0 mg) were combined and subjected to QCC with 75% CH_2Cl_2–hexane as the eluent to afford eight subfractions (PQ1–8). Subfraction PQ2 (103.9 mg) was purified by CC with 80% CH_2Cl_2–hexane as the eluent to give compounds **6** (6.0 mg) and **7** (51.0 mg). Subfraction PQ5 (65.8 mg) was recrystallized with CH_2Cl_2 to give compound **8** (8.0 mg). Compound **9** (12.0 mg) was obtained from subfraction PQ8 (73.0 mg) by repeated CC with 23% EtOAc–CH_2Cl_2, while compound **10** (10.0 mg) was obtained from subfraction PQ13 (40.4 mg) by CC with 18% EtOAc–CH_2Cl_2. Fraction S (445.0 mg) was subjected to QCC with 10% EtOAc–CH_2Cl_2 to afford four subfractions (S1–S4). Subfraction S2 (20.0 mg) was further purified by CC with 30% acetone–hexane to give compound **11** (2.4 mg). Fraction U (561.0 mg) was subjected to Sephadex LH-20 CC with 60% CH_2Cl_2–MeOH to obtain three subfractions (U1–U3). Subfraction U3 (145.1 mg) was recrystallized with 50% acetone–CH_2Cl_2 to give compound **12** (62.0 mg).

3.3.1 Sansoakamine (8)

A light brown solid; mp 243.8–245.6°C; UV λ_{max} (log ε) (MeOH): 223 (1.47), 243 (1.46), 256 (1.46), 278 (1.59), 297 (1.54), 356 (1.25) nm; IR (neat) ν_{max}: 3357, 1660, 1598, 1458, 1435, 1363, 1266, 1163, 1089 cm^{-1}; ^1H NMR (400 MHz, acetone-d_6): δ 11.03 (1H, s, 2-OH), 10.26 (1H, s, 6-OH), 8.52 (1H, s, H-4), 8.06 (1H, s, *NH*), 7.50 (1H, d, $J = 2.4$ Hz, H-5), 7.28 (1H, d, $J = 8.8$ Hz, H-8), 6.92 (1H, dd, $J = 8.8$, 2.4 Hz, H-7), 6.85 (1H, s, H-1), 3.98 (3H, s, 3-OMe); ^{13}C NMR (100 MHz, acetone-d_6): δ 171.3 (C=O), 160.4 (C-2), 151.8 (C-6), 146.3 (C-1a), 134.9 (C-8a), 124.2 (C-5a), 122.5 (C-4), 116.9 (C-3), 114.4 (C-7), 111.3 (C-8), 105.3 (C-5), 104.8 (C-4a), 96.5 (C-1), 51.6 (3-OMe). HR-EI-MS *m/z*: 257.0683 $[M]^+$ (calcd for $C_{14}H_{11}NO_4$, 257.0688).

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References

[1] T.S. Wu, S.C. Huang, P.L. Wu, and K.H. Lee, *Bioorg. Med. Chem. Lett.* **4**, 2395 (1994).
[2] N. Kongkathip and B. Kongkathip, *Heterocycles* **79**, 121 (2009).
[3] T.T. Thuy, H. Ripperger, A. Porzel, T.V. Sung, and G. Adam, *Phytochemistry* **52**, 551 (1999).
[4] T.S. Wu, S.C. Huang, P.L. Wu, and C.S. Kuoh, *Phytochemistry* **43**, 1427 (1996).
[5] T.S. Wu, S.C. Huang, J.S. Lai, C.M. Teng, F.N. Ko, and C.S. Kuoh, *Phytochemistry* **32**, 449 (1993).
[6] H.P. He, J.X. Zhang, Y.M. Shen, Y.N. He, C.X. Chen, and X.J. Hao, *Helv. Chim. Acta* **85**, 671 (2002).
[7] T.S. Wu, S.C. Huang, and P.L. Wu, *Phytochemistry* **43**, 133 (1996).
[8] D. Prakash, K. Raj, R.S. Kapil, and S.P. Popli, *Indian J. Chem.* **19B**, 1075 (1980).
[9] W.S. Li, J.D. McChesney, and F.S. El-Feraly, *Phytochemistry* **30**, 343 (1991).
[10] H.J. Knolker and M. Wolpert, *Tetrahedron* **59**, 5317 (2003).
[11] B. Kongkathip, N. Kongkathip, A. Sunthitikawinsakul, C. Napaswat, and C. Yoosook, *Phytother. Res.* **19**, 728 (2005).
[12] C. Ito, S. Katsuno, H. Ohta, M. Omura, I. Kajiura, and H. Furukawa, *Chem. Pharm. Bull.* **45**, 48 (1997).
[13] B.T. Ngadjui, J.F. Ayafor, B.L. Sondengam, and J.D. Connolly, *Phytochemistry* **28**, 1519 (1989).
[14] C. Borger and H.J. Knolker, *Synlett* **11**, 1698 (2008).
[15] O. Potterat, C. Puder, W. Bolek, K. Wagner, C. Ke, Y. Ye, and F. Gillardon, *Pharmazie* **58**, 346 (2005).