SHORT REPORT

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Rec. Nat. Prod. 6:4 (2012) 386-389

records of natural products

Chemical Constituents from the Roots of *Clausena excavata* and Their Cytotoxicity

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(Received May 23, 2011; Revised April 30, 2012; Accepted June 15, 2012)

Abstract: Six coumarins (**1-6**) and twelve alkaloids (**7-18**) were isolated from the roots of *Clausena excavata*. Their structures were elucidated on the basis of spectroscopic methods. This is the first report on the isolation of compounds **1**, **7** and **17** from *C. excavata.* Compound **1** is also the first example of an unsymmetrical dimer coumarin isolated from *Clausena* species. The completed assignment of ^{13}C NMR spectral data of **1** as well as HMBC spectral data is also reported here for the first time. Compounds **2-7**, **11-16** and **18** were evaluated for their cytotoxicity against three human cancer cell lines, oral cavity cancer (KB), breast cancer (MCF7) and small cell lung cancer (NCI-H187). The results showed that compounds **4**, **11** and **18** exhibited highest cytotoxicity against KB, MCF7 and NCI-H187 cell lines with IC_{50} values of 5.95, 3.76 and 5.65 µg/mL, respectively.

Keywords: *Clausena excavata*; Rutaceae; coumarins; alkaloids; cytotoxicity.

1. Plant Source

Clausena excavata, commonly known as “San Soak” in Thai, is a wild shrub of the Rutaceae family which is widely distributed in South and South East Asia [1, 2, 3]. Several parts of the plant have been used as a traditional medicine for the treatment of cold, malaria, AIDS, dermatopathy, abdominal pain, snake-bite and detoxification agents in Thailand [4, 5]. The roots of *C. excavata* were collected from Suratthani Province, Southern of Thailand, in June 2010. Botanical identification was achieved through comparison with a voucher specimen number QBG 6277 in the herbarium collection of Queen Sirikit Botanic Garden, Mae Rim District, Chiang Mai, Thailand.

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The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 7/20/2012 EISSN: 1307-6167

2. Previous Studies

Carbazole alkaloids and coumarins are major components in this plant, so far, approximately sixty carbazole alokaloids and fifty coumarins were isolated from various parts of the plant. [1, 3-7] Furthermore, a small group of tetranortriterpenoids, [8] flavonoids, [1] and essential oils [9] was reported.

3. Present Study

The air-dried roots of *C. excavata* were extracted with acetone over the periods of 3 days at room temperature. Removal of the solvent under reduced pressure provided acetone extract (288.02 g) which was purified by chromatographic techniques to give compounds **1**-**18** (Figure 1). Detailed isolations of compounds **1**-**18** are shown in supplementary material.

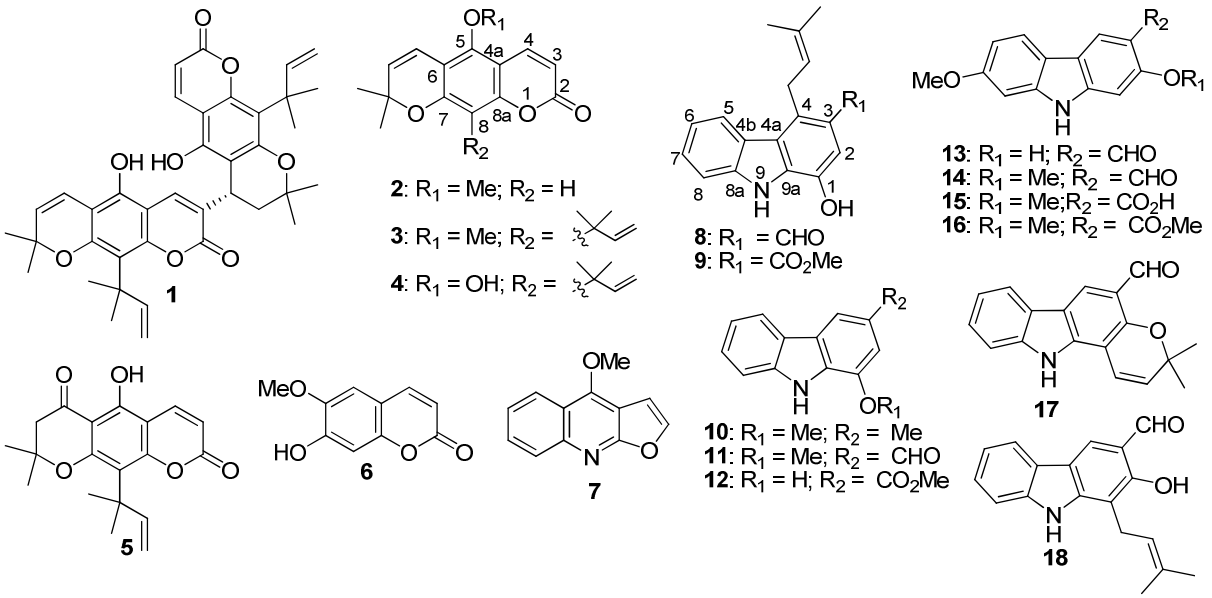

Figure 1. The structures of compounds **1**-**18**.

All compounds were characterized by spectroscopic methods as well as comparison of their spectroscopic data with those reported in the literatures and identified as binorponcitrin (**1**) [10], xanthoxyletin (**2**) [11], dentatin (**3**) [12], nordentatin (**4**) [1], clausenidin (**5**) [1], scopoletin (**6**) [13], dictamine (**7**) [14] clausine D (**8**) [5], clausine F (**9**) [5], murrayafoline A (**10**) [15], murrayanine (**11**) [16], clauszoline I (**12**) [17], 2-hydroxy-3-formyl-7-methoxycarbazole (**13**) [18], 3-formyl-2,7-dimethoxycarbazole (**14**) [18], clauszoline J (**15**) [17], clausine H (**16**) [5], murrayacine (**17**) [19] and heptaphylline (**18**) [18]. To the best of our knowledge, this is the first report on the isolation of compounds **1**, **7** and **17** from *C. excavata*. Compound **1** is also the first example of an unsymmetrical dimer coumarin which was isolated from *Clausena* species. The completed assignment of ^{13}C NMR spectral data of **1** as well as HMBC spectral data is also reported here for the first time (see supplementary material).

As summarized in Table 1, compounds **2**-**7**, **11**-**16** and **18** were evaluated for their cytotoxicity against three human cancer cell lines, oral cavity cancer (KB), breast cancer (MCF7) and small cell lung cancer (NCI-H187). The results showed that most of them had cytotoxicity against all cell lines except compounds **14** and **16** were found to be inactive. Compounds **4** and **18** exhibited the highest cytotoxicity against KB and NCI-H187 cell lines with the IC_{50} values of 5.95 and 5.64 μg/mL, respectively, whereas compounds **5** and **6** were found to be selectively active against NCI-H187 (**5**, IC_{50} 8.63 μg/mL) and MCF7 (**6**, IC_{50} 17.09 μg/mL) cell lines. Compound **2** was also specific against NCI-H187 cell line but it was weak active (35.54 μg/mL). Only compounds **7** and **11** exhibited

significant cytotoxic effect against MCF7 cell line with IC_{50} values of 5.68 and 3.76 μg/mL, recpectively, which are stronger than standard drug, doxorubicin (IC_{50} 7.62 μg/mL).

It should be note that the pyranocoumarin (**3**) with substituent at C-8 exhibited strong activity than **2**. The cytotoxicity is increased when the methoxyl group at C-5 in **3** was replaced by the hydroxyl group in **4**. Furthermore, a double bond at C-11 and C-12 in **4** plays an important role for cytotoxicity comparing with compound **5** but carbonyl group at C-12 in **5** exhibits selectively active against NCI-H187 cell line. These results implied that the methoxyl group at C-5 and 2-methylbut-3-en-2-yl unit at C-8 is important for the cytotoxiciy. Structural variation of **14**-**16** also corresponds to the remarkably different activity. The carboxyl substituent in **15** appears to be particularly responsible for the cytotoxicity against all three human cancer cell lines whereas formyl (**14**) and methyl ester (**16**) groups were found to be reduced activity. In addition, the hydroxyl group at C-2 in **13** seems to be much more effective with all cancer cell lines when compared to the methoxyl group in **14**.

Table 1. Cytotoxicity of compounds **2-7**, **11-16** and **18**.

Compounds	Cytotoxicity (IC_{50}, μg/mL)		
	KB[a]	MCF7[b]	NCI-H187[c]
2	>50	>50	35.54
3	33.16	26.72	15.92
4	5.95	15.28	7.10
5	>50	>50	8.63
6	>50	17.09	>50
7	36.60	5.68	21.66
11	19.34	3.76	10.72
12	17.76	15.43	9.38
13	43.74	16.67	11.07
14	>50	>50	>50
15	39.56	24.74	30.07
16	>50	>50	>50
18	26.31	47.75	5.65
Doxorubicin	0.483	7.62	0.087
Ellipticine	1.76	Not tested	1.06

[a]KB = Oral cavity cancer. [b]MCF7 = Breast cancer. NCI-H187 = Small cell lung cancer. Activity: <5, strong; 5–20, moderate; 20–50, weak; >50, inactive.

Acknowledgments

We would like to thank Thailand Research Fund (TRF) through the Royal Golden Jubilee PhD program for a scholarship to T.S. and S.L. (grant no. PHD/0004/2553) and Mae Fah Luang University for financial supports. We are grateful to Ms. Nareerat Thongtip, Phuket Rajabhat University, for recording NMR spectral data. Special thanks are addressed to Dr. Nawong Boonnak for collecting plants material. The Bioassay Research Facility of BIOTEC (Thailand) is also acknowledged for the cytotoxicity tests.

Supporting Information:

The following Supporting Information is available for this article: http://www.acgpubs.org/RNP

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