Chemistry of Natural Compounds, Vol. 43, No. 4, 2007

XYLOGRANATININ, A NEW PYRIDO[1, 2-*a*]PYRAZINE ALKALOID FROM THE FRUIT OF A CHINESE MANGROVE *Xylocarpus granatum*

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UDC 547.944/945

*9-Hydroxy-3-methoxy-6H-pyrido[1,2-*a*]pyrazin-6-one, a new pyrido[1,2-*a*]pyrazine alkaloid named xylogranatinin, was isolated from the fruit of a Chinese mangrove* Xylocarpus granatum. *Its structure was elucidated on the basis of spectroscopic data, especially 2D NMR techniques including HSQC and HMBC. To the best of our knowledge, this is the first time that a pyrido[1,2-*a*]pyrazine alkaloid was found as a natural product.*

Key words: 9-hydroxy-3-methoxy-6*H*-pyrido[1,2-*a*]pyrazin-6-one, *Xylocarpus granatum.*

The mangrove *Xylocarpus granatum* is distinguished for producing antifeedant limonoids, especially mexicanolides and phragmalins. Previous investigations on the seeds of two meliaceae plants of mangrove, *X. granatum* and *X. moluccensis*, uncovered an obacunol, two phragmalins, three andirobins, and 14 mexicanolides, including xyloccensins A-K [1–5]. Recently, we have reported the isolation and identification of eight new mexicanolides and 11 novel phragmalins, named xyloccensins L-Z [6–13], from the stem bark and fruit of a Chinese mangrove *Xylocarpus granatum*. Further investigation of the fruit of the same plant resulted in the discovery of a new alkaloid, 9-hydroxy-3-methoxy-6*H*-pyrido[1,2-*a*]pyrazin-6-one, which was given the name xylogranatinin. Its structure was elucidated on the basis of spectroscopic data, especially 2D NMR techniques including HSQC and HMBC.

The ethanolic extract of the fruit of *X. granatum* was subjected to sequential extraction with *n*-hexane and ethyl acetate. The resulting ethyl acetate extract was chromatographed on silica gel followed by preparative reverse-phase C_{18} HPLC to yield xylogranatinin.

Xylogranatinin, a white powder, has the molecular formula $C_9H_8N_2O_3$ as established by its HR-ESIMS spectrum (*m/z* 215.0430, calcd for $[M+Na]^+$ 215.0433). Consequently, xylogranatinin has seven degrees of unsaturation. From the ^1H and ^{13}C NMR data (Table 1), it was clear that five of the seven elements of unsaturation come from three carbon-carbon double bonds (including a carbonyl). Therefore, the molecule is bicyclic.

The ^1H and ^{13}C NMR data (Table 1) indicated that xylogranatinin has a methoxy group, four methines, including two coupled ones and two non-coupled ones, and four quaternary carbons. An α,β-unsaturated lactam of the pyridine ring, characterized by the NMR data, was confirmed by the HMBC correlations from H-7 to C-6, C-8 and H-8 to C-6, C-9, C-10, respectively. A pyrazine ring, coalesced with the above pyridine ring as shown in Fig. 1, was confirmed by HMBC correlations between H-2/C-3, H-2/C-6, H-2/C-10, H-5/C-3, H-5/C-8, H-5/C-9, H-5/C-10 (Fig. 1). Consequently, xylogranatinin was identified as a pyrido[1,2-*a*]pyrazine alkaloid. The proton (δ_H = 3.90 s) of the methoxy group showed an HMBC correlation with C-3 (δ_C 147.4 s), indicating that it was attached to C-3. A hydroxyl group, whose existence was deduced from the molecular formula of xylogranatinin, was found to be located at the last oxygenated quaternary C-9 (δ_C 151.7 s). Therefore, xylogranatinin was characterized as 9-hydroxy-3-methoxy-6*H*-pyrido[1,2-*a*]pyrazin-6-one. To the best of our knowledge, this is the first time that a pyrido[1,2-*a*]pyrazine alkaloid was found as a natural product.

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Published in Khimiya Prirodnykh Soedinenii, No. 4, pp. 351-352, July-August, 2007. Original article submitted May 11, 2006.

0009-3130/07/4304-0426

TABLE 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Data for Xylogranatinin in Methanol-d_4

Atom	δ_H (J/Hz)	δ_C	Atom	δ_H (J/Hz)	δ_C
2	6.75 s	104.1 d	8	7.84 (d, 9.5)	146.2 d
3		147.4 s	9		151.7 s
5	7.09 s	109.9 d	10		153.7 s
6		164.2 s	OCH_3	3.90 s	56.8 q
7	6.17 (d, 9.5)	112.3 d			

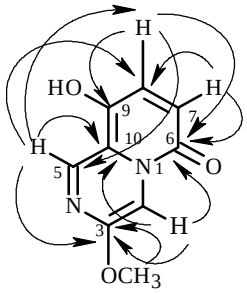

Fig. 1. HMBC correlations for xylogranatinin.

EXPERIMENTAL

General Procedure. NMR spectra were recorded in methanol-d_4 using a Bruker AV-500 spectrometer (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR) with tetramethylsilane as the internal standard. UV spectra were obtained on a Beckman DU-640 UV spectrophotometer. ESI-MS spectra were measured on a Bruker APEX II spectrometer in the positive or negative ion mode. Preparative HPLC was carried out on ODS columns (250 × 10 mm i.d., YMC) with a Waters 996 photodiode array detector. For CC, silica gel (200–300 mesh) (Qingdao Mar. Chem. Ind. Co. Ltd.) and octadecylsilyl silica gel (80–100 mm) (Unicorn) were used.

Plant Material. The fruits of *Xylocarpus granatum* were collected in June 2004 from Hainan island, southern China. The identification of the plant was performed by Prof. Yongshui Lin, Laboratory of Marine Biology, South China Sea Institute of Oceanology, Chinese Academy of Sciences. A voucher sample (No. GKLMMM-002-2-2) is maintained in the Herbarium of the South China Sea Institute of Oceanology.

Extraction and Isolation. The dried fruit (4.0 kg) of *X. granatum* was extracted with hot 95% ethanol for three times. The extract was concentrated under reduced pressure, followed by suspension in water. After defatting with petroleum ether, the aqueous layer was further extracted with ethyl acetate. The ethyl acetate extract (60 g) was chromatographed on silica gel column and eluted using chloroform-methanol system (100:0~2:1) to yield 120 fractions. Fractions 50 to 78 (4.2 g) were combined, and further purification with preparative HPLC (YMC-Pack ODS-5-A, 250 × 20 mm i.d., methanol–water 15:85 to 20: 80) yielded xylogranatinin (4 mg).

Xylogranatinin. A white powder. UV (MeCN) λ_{max} 204, 227, 296, 343 nm. ^{1}H (500 MHz, methanol-d_4) and ^{13}C NMR (125 MHz, methanol-d_4): see Table 1. MS (HR-ESI): *m/z* 215.0430 $[M+Na]^+$. ($C_9H_8N_2O_3Na$ requires 215.0433).

ACKNOWLEDGMENT

Support for this work from the Natural Science Foundation of Guangdong Province (04100729) is gratefully acknowledged.

REFERENCES

1. K. A. Alvi, P. Crews, B. Aalbersberg, R. Prasad, J. Simpson, and R. T. Weavers, *Tetrahedron*, **47**, 8943 (1991).
2. U. Kokpol, W. Chavasiri, S. Tip-pyang, G. Veerachato, and F. L. Zhao, *Phytochemistry*, **41**, 903 (1995).
3. I. Kubo, I. Miura, and K. Nakanishi, *J. Am. Chem. Soc.*, **98**, 6704 (1976).
4. D. A. Mulholland, B. Parel, and P. H. Coombes, *Curr. Org. Chem.*, **4**, 1011 (2000).
5. A. S. Ng and A. G. Fallis, *Can. J. Chem.*, **57**, 3088 (1979).
6. J. Wu, S. Zhang, Q. Xiao, Q. X. Li, J. S. Huang, Z. H. Xiao, and L. J. Long, *Z. Naturforsch.*, **58B**, 1216 (2003).
7. J. Wu, S. Zhang, Q. Xiao, Q. X. Li, J. S. Huang, L. J. Long, and L. M. Huang, *Tetrahedron Lett.*, **45**, 591593 (2004a).
8. J. Wu, Q. Xiao, J. S. Huang, Z. H. Xiao, S. H. Qi, Q. X. Li, and S. Zhang, *Org. Lett.*, **6**, 1841 (2004b).
9. J. Wu, Q. Xiao, S. Zhang, X. Li, Z. H. Xiao, H. X. Ding, and Q. X. Li, *Tetrahedron*, **61**, 8382 (2005a).
10. J. Wu, Z. H. Xiao, Y. Song, S. Zhang, Q. Xiao, C. Ma, H. X. Ding, and Q. X. Li, *Magn. Reson. Chem.*, **44**, 87 (2005b).
11. J. Wu, S. Zhang, Y. Song, Z. H. Xiao, Q. Xiao, and Q. X. Li, *Z. Naturforsch.*, **60B**, 1291 (2005c).
12. Y. Zhou, F. Cheng, J. Wu, and K. Zou, *J. Nat. Prod.*, **69**, 1083 (2006).
13. F. Cheng, Y. Zhou, J. Wu, and K. Zou, *Z. Naturforsch.*, **616**, 626 (2006).