A New Quinolizidine Alkaloid from the Papua New Guinean Sponge *Xestospongia exigua*

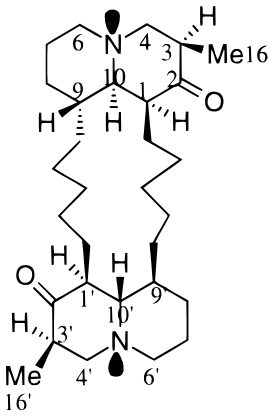
Tetsuo Iwagawa,*,† Masafumi Kaneko,† Hiroaki Okamura,† Munehiro Nakatani,† Rob W. M. van Soest,‡ and Motoo Shiro§

Faculty of Science, Kagoshima University, Korimoto Kagoshima 890-0065, Japan, Institute of Taxonomic Zoology, University of Amsterdam, P.O. Box 4766, 1009 AT, Amsterdam, The Netherlands, and Rigaku Corporation, 3-9-12 Matsubara-cho Akishmima-shi, Tokyo 196-0003, Japan

Received March 3, 2000

A new bis-quinolizidine alkaloid, xestosin A (**1**), possessing *cis*- and *trans*-quinolizidine nuclei, has been isolated from the Papua New Guinean sponge *Xestospongia exigua*. The structure was determined by spectrometric and single-crystal X-ray analyses.

Thirteen new bis-oxaquinolidizidine alkaloids and two known bis-quinolizidine alkaloids, petrosin and petrosine A, have been isolated from marine sponges of the genus *Xestospongia*.[1,2] They possess either vasodilatory or ichthyotoxic activity. In the course of our investigation of bioactive compounds from marine sponges,[3] we have examined the methanolic extract of the Papua New Guinean sponge *Xestospongia exigua* Knight (Renieridae), affording a new bis-quinolizidine alkaloid named xestosin A (**1**). In this report, we describe the isolation and characterization of this compound.



1

Xestosin A (**1**), was isolated as prisms, mp 175−177 °C, and did not exhibit optical rotation. The molecular formula $C_{30}H_{50}N_2O_2$ was determined on the basis of the M^+ peak in the HREIMS spectrum (*m/z* 472.3825, Δ −4.7 mmu) and the NMR data. The IR spectrum indicated absorption bands indicative of a trans-fused quinolizidine system (2805 and 2760 cm^{-1})(Bohlmann bands)[4] and a carbonyl (1709 cm^{-1}). The ^{13}C NMR spectrum showed the presence of resonances due to two methyls, 18 sp^3 methylenes, eight sp^3 methines, and two carbonyls, suggesting a pentacyclic ring. Nine resonances [δ 25.1 (t, C-7) or 25.3 (t, C-7), 28.7 (t, C-8), 37.6 (d, C-9), 40.8 (d, C-3), 49.8 (d, C-1), 56.0 (t, C-6), 64.9 (t, C-4), 71.3 (d, C-10), 214.5 (s, C-2)] in the ^{13}C NMR spectrum seemed to be due to a trans-fused quinolizidine ring, as observed in petrosin.[5] In the $^1H-^1H$ COSY NMR spectrum, resonances due to methyl protons at C-16 (δ 0.94, 3H, d, J = 6.6 Hz) were coupled to H-3 (δ ca. 2.87, m, 1H), which in turn was coupled to H-4 (δ ca. 1.84, 1H, overlapped, H-4α, δ ca. 3.00, 1H, overlapped, H-4β). Methylene protons at C-6, which were correlated with C-10 (δ 71.3, d) in the HMBC experiments (Figure 1), were observed at δ ca. 1.89 (1H, overlapped, H-6α) and δ ca. 2.92 (1H, overlapped, H-6β). Furthermore, the presence of one more quinolizidine moiety was inferred from characteristic resonances due to three carbons adjacent to a nitrogen atom [δ 62.2 (C-4′), 48.5 (C-6′), and 63.8 (C-10′)] in the ^{13}C NMR data. In the ^1H NMR spectrum, the downfield chemical shift of H-10′ at δ 2.75 (1H, br s) suggested a cis-fused quinolizidine ring, inasmuch as the trans one appeared upfield (δ 1.77, H-10). Methyl protons at C-16′ (δ 0.94, 3H, d, J = 6.0 Hz) were correlated to C-4′ (δ 62.2, t) and H-4′β (δ ca. 3.06, 1H, m) to C-6′ (δ 48.5, t) in the HMBC spectrum. H-6′ methylene protons (δ ca. 2.54, 1H, m, H-6′α, δ 2.98, 1H, overlapped, H-6′β) were also shifted downfield due to the anisotropic effect of nitrogen. Unfortunately, it seemed impossible to determine the stereochemistry of the linkage between the two quinolizidines and a pair of five-methylene groups of **1** on the basis of the ^1H and ^{13}C NMR spectra, inasmuch as resonances in the ^1H NMR spectrum were overlapped with each other. Thus, single crystals of

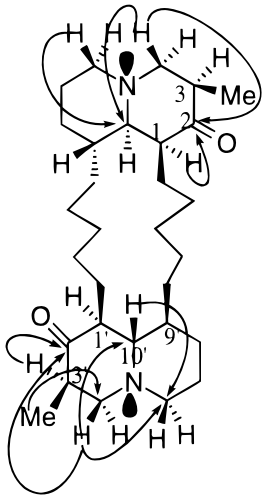


Figure 1. Selected HMBC correlations of **1**.

* To whom correspondence should be addressed. Tel.: (81)-99-285-8115. Fax: (81)-99-285-8117. E-mail: iwagawa@sci.kagoshima-u.ac.jp.
† Kagoshima University.
‡ University of Amsterdam.
§ Rigaku Corporation.

10.1021/np000111b CCC: $19.00
Published on Web 07/19/2000

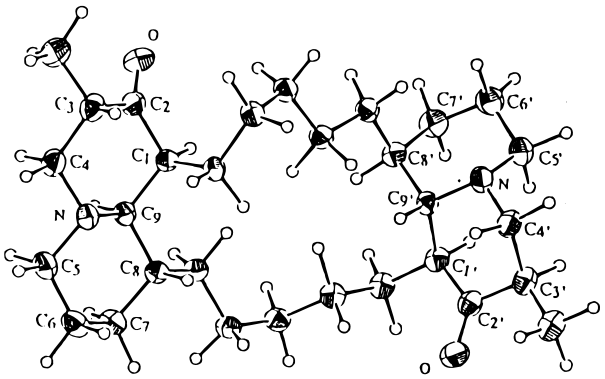


Figure 2. Perspective ORTEP drawing of **1**.

1 were submitted for X-ray analysis.[6] A computer-generated perspective drawing of the final X-ray model is given in Figure 2. Therefore, xestosin A (**1**) was composed of cis- and trans-fused quinolizidine rings linked together through a pair of five-methylene groups. This is the first isolation of a bis-quinolizidine alkaloid possessing cis- and trans-fused quinolizidine rings; bis-quinolizidines isolated previously possess trans-fused quinolizidine moieties.

Experimental Section

General Experimental Procedures. UV and IR spectra were recorded on UV-210 and JASCO FT/IR 5300 spectrometers, respectively. NMR spectra were recorded with either a JEOL JNM-GX 400 or a JNM-A 600 spectrometer using TMS as internal standard and $CDCl_3$ as solvent. MS spectra were obtained with a JEOL JMS DX-300 instrument. A Rigaku AFC7R diffractometer was used in the X-ray work.

Animal Material. The sponge *X. exigua* (collection no. 167) was collected using scuba at −15 m at Fly Islands, Papua New Guinea, and was frozen immediately after collection. The sponge was compared to the type material of *Xestospongia exigua* (Kirkpatrick, 1900), and the characteristics were found to match. Voucher material is kept in the collections of the Zoological Museum, Amsterdam, under registration number ZMA POR. 11476.

Extraction and Isolation. The fresh organism (dry wt, 400 g) was chopped into small pieces and extracted with MeOH (40 L). The MeOH extract was suspended in H_2O and extracted with CH_2Cl_2. A portion (3 g) of the CH_2Cl_2 extract (8.2 g) was absorbed on Si gel and subjected to chromatography on Si gel (40 g) packed in hexane, and fractions (100 mL) were collected as follows: 1−2 (CH_2Cl_2−hexane, 4:1), 3−4 (CH_2Cl_2), 5−6 (MeOH−CH_2Cl_2, 1:49), 7−8 (MeOH−CH_2Cl_2, 1:19), 9−10 (MeOH−CH_2Cl_2, 1:9), 11−12 (MeOH−CH_2Cl_2, 1:4), 13−14 (MeOH−CH_2Cl_2, 1:1), and 15−16 (MeOH). Fractions 6−8 (894 mg) were chromatographed on Si gel using EtOAc and hexane, increasing the proportion of MeOH to elute the fractions. The fraction eluted with EtOAc−hexane (1:1) gave xestosin A (**1**) (7 mg) after recrystallization from MeOH−CH_2Cl_2.

Xestosin A (1): prisms from CH_2Cl_2−hexane, mp 175−177 °C; IR (film) ν_{max} 2805, 2760, 1709 cm^{-1}; ^1H NMR (600 MHz) δ ca. 0.72 (1H, overlapped, H-8), 0.94 (3H, d, J = 6.6 Hz, H-16), 0.94 (3H, d, J = 6.0 Hz, H-16′), ca. 1.43 (1H, overlapped, H-9), 1.62 (1H, overlapped, H-9′), ca. 1.77 (1H, br d, J = 9.4 Hz, H-10), ca. 1.84 (1H, overlapped, H-4α), ca. 1.86 (1H, overlapped, H-6α), ca. 1.88 (1H, overlapped, H-8), 2.54 (1H, m, H-6′α), 2.58 (1H, overlapped, H-1), ca. 2.64 (2H, overlapped, H-3′, H-4′α), 2.75 (2H, br s, H-1′, H-10′), ca. 2.87 (1H, m, H-3), ca. 2.91 (1H, overlapped, H-6β), ca. 2.98 (1H, overlapped, H-6′β), ca. 3.00 (1H, overlapped, H-4β), ca. 3.06 (1H, m, H-4′β), ^{13}C NMR (100 MHz) δ 11.2 (q, C-16), 12.3 (q, C-16′), 22.0 (C-7′), [25.1 or 25.3 (t, C-7), 25,9 (t), 26.1 (t), 26.5 (t), 28.7 (t, C-8), 28.9 (t), 30.0 (t), 31.7 (t), CH_2 × 13], 36.1 (d, C-9′), 37.6 (d, C-9), 40.3 (d, C-3′), 40.8 (d, C-3), 46.4 (d, C-1′), 48.5 (t, C-6′), 49.8 (d, C-1), 56.0 (t, C-6), 62.2 (t, C-4′), 63.8 (d, C-10′), 64.9 (t, C-4), 71.3 (C-10), 212.7 (s, C-2′), 214.5 (C-2); HREIMS m/z 470.3825 (M^+, calcd for $C_{30}H_{50}N_2O_2$, 470.3871).

X-ray Analysis of 1. Crystal data: $C_{30}H_{50}O_2$, colorless prisms, monoclinic space group $P2_1/c$ (#14), a = 11.012(3) Å, b = 14.707(3) Å, c = 17.817(3) Å, β = 107.80(2)°, V = 2747.5(10) Å^3, Z = 4, D_x 1.138 g/cm^3, $F(000)$ = 1040.00, μ(Cu Kα) = 5.37 cm^{-1}. Intensity data were collected on a Rigaku AFC7R diffractometer using graphite monochromated Cu Kα radiation and a 12-kW radiation anode generator; 4440 reflections, of which 4291 had $I > 3\sigma(I)$, were collected in the range 49.1° < 2θ < 50.0°. The structure was solved by direct methods (SHELXS86)[6] and expanded using Fourier techniques (DIRDIF 92).[7] The non-hydrogen atoms were refined anisotropically. Hydrogen atoms, excluding those of water, were included but not refined. It was refined by full-matrix least-squares and converged with R = 0.048 and R_w = 0.072. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at Rigaku Corporation.

Acknowledgment. We are grateful to Dr. Y. Kumaki for measuring NMR spectra. We are also indebted to the staff of Keiten-maru (Kagoshima University) and Drs. N. Balat, A. Murphy, and J. Willet (The Papua New Guinea University of Technology) for collecting samples.

References and Notes

(1) Nakagawa, M.; Endo, M.; Tanaka, N.; Lee G.-P. *Tetrahedron Lett.* **1984**, *25*, 3227−3230.
(2) Kobayashi, M.; Miyamoto, Y.; Aoki, S.; Murakami, N.; Kitagawa, I.; In, Y.; Ishida, T. *Heterocycles* **1998**, *47*, 195−203, and the references cited therein.
(3) Iwagawa, T.; Kaneko, M.; Okamura, H.; Nakatani, M.; van Soest, R. W. M. *J. Nat. Prod.* **1998**, *61*, 1310−1312.
(4) Bohlmann, F. *Angew. Chem.* **1957**, *69*, 641−642.
(5) Braekman, J. C.; Daloze, D.; Defay, N.; Zimmermann, D. *Bull. Soc. Chem. Belg.* **1984**, *93*, 941−944.
(6) Sheldrick, G. M. *Crystallographic Computing 3*; Sheldrick, G. M., Kruger, C., Gould, R., Eds.; Oxford University Press: Oxford, 1985; pp 175−189.
(7) Beurskens, P. T.; Admiraal, G.; Beurskens, G.; Bosman, W. P.; Garcia-Granda, S.; Gould, R. O.; Smits, J. M. M.; Smykalla, C. *The DIRDIF program system*, Technical Report of the Crystallography Laboratory; University of Nijmegen: Nijmegen, The Netherlands, 1992.

NP000111B