Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 12, pp. 1313-1318, 1996

0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00225-9

ISOLATION AND X-RAY CRYSTAL STRUCTURE OF RACEMIC XESTOSPONGIN D FROM THE SINGAPORE MARINE SPONGE *NIPHATES SP*[1]

George R. Pettit,* Brian Orr, Delbert L. Herald,
Dennis L. Doubek, Larry Tackett, and Jean M. Schmidt

Cancer Research Institute and Department of Chemistry
Arizona State University, Tempe, Arizona 85287-1604, USA

Michael R. Boyd

Laboratory of Drug Discovery Research and Development, DTP, DCT, NCI, FCRDC
Frederick, Maryland 21702-1201, USA

Robin K. Pettit

Department of Biology, Western Oregon State College
Monmouth, Oregon 97361, USA

John N. A. Hooper

Sessile Marine Invertebrates, Queensland Museum, South Brisbane
Queensland 4101, Australia

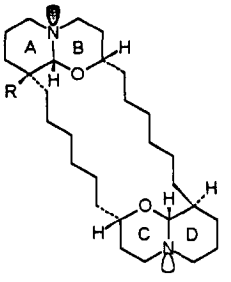
Abstract: (±)-Xestospongin D (2) has been isolated from the Singapore marine sponge *Niphates sp.* The natural product was found to be racemic in contrast to earlier isolations of (+)-xestospongin D from *Xestospongia* and *Haliclona* species. The (±)-xestospongin D was found to inhibit growth of certain human cancer cell lines comprising the NCI panel (*e.g.*, leukemia subpanel, mean GI_{50} 3.62 ± 2.02 x 10^{-6}M; breast subpanel, mean GI_{50} 4.53 ± 1.98 x 10^{-6}M) as well as the murine P388 lymphocytic leukemia (ED_{50} 1.7 μg/mL) and the bacterium *Micrococcus luteus*.

Marine Porifera species in the genera *Niphates* and *Xestospongia* have proven, respectively, to be useful sources of new pyridine[2-6] and quinolizidine derivatives.[7-10] Certain members of the latter group have exhibited significant coronary vasodilative effects, as well as modest murine leukemia cell growth inhibition (P388 and L1210, ED_{50} - 0.1 to 2.5 μg/mL) and antimicrobial activity.[9,11] The first four "1-oxa-quinolizidines" were isolated from the Australian sponge *Xestospongia exigua*, designated xestospongins A-D, with the structure of (−)-xestospongin C (1) determined by X-ray techniques.[7] The structure of (+)-xestospongin D (2) was deduced by its relationship to (−)-xestospongin C (1). Subsequently, xestospongin D was isolated from *Xestospongia*[9] and *Haliclona*[10] species, but optical rotation measurements were not reported.

In 1989 using SCUBA (−3 to −15m) we collected *Niphates sp.* south of the Beting Bemban Besar reef (Republic of Singapore) and found a methanol extract significantly inhibited the P388 lymphocytic leukemia cell line. Bioassay directed separation of the methanol extract from a 1992 recollection (500 kg wet wt) employing a series of solvent partitioning followed by steric exclusion and partition chromatographic procedures (Sephadex LH-20) led to

isolation of an optically inactive 1-oxa-quinolizidine (0.19 g, 3.8 x 10^{-3}% yield) with P388 ED_{50} 1.7 μg/mL: colorless prisms, mp 160-161 °C (ethyl acetate), lit. mp[7] 156-157 °C (diethyl ether); $[\alpha]_D^{24}$ –0.46 (c = 1.08, $CHCl_3$); IR ν_{max} (NaCl) 3389 (OH), 2810 and 2704 cm^{-1} (Bohlmann Bands);[12-14] HRFAB found 463.3895 $[M+1]^+$, $C_{28}H_{50}N_2O_3$ requires 463.3899 $[M+1]^+$; ^1H NMR δ_H (500 MHz, $CDCl_3$) 4.06 (s, H-10), 3.56 (br t, *J* = 10.5 Hz, H-2), 3.36 (br t, *J* = 10.5 Hz, H-2'), 3.13-2.90 (m, H-6'α, H-4, 2XH-4', H-10'), 2.79 (br d, *J* = 9.5 Hz, H-6α), 2.49 (s, OH), 2.34 (br d, *J* = 10 Hz, H-4), 2.21 (br t, *J* = 10.5 Hz, H-6'β), 2.01 (br t, *J* = 10.5 Hz, H-6β) and 1.70-1.00 (m, 18XCH_2 and H-9'); ^{13}C NMR δ_C (125 MHz, $CDCl_3$) 95.62 (C-10'), 90.49 (C-10), 76.36 (C-2), 75.43 (C-2'), 70.80 (C-9), 54.21 and 54.08 (C-4' and C-6'), 52.53 (C-4), 44.35 (C-6), 40.28 (C-9'), 38.32, 36.03, 35.55, 32.20, 32.14, 32.00, 31.54, 31.48, 31.11, 29.43, 28.92, 28.76, 26.04, 25.35, 24.85, 24.76, 22.60, 20.90.

While these results seemed to agree with the proposed (+)-xestospongin D (2) structure, the $[\alpha]_D$ + 18.4 previously reported[7] for that substance was difficult to reconcile unless our specimen was a racemic version of xestospongin D. That interpretation was confirmed by a subsequent X-ray crystal structure determination. Crystal data: wedge-shaped, colorless prismatic crystals of this compound grown with difficulty from ethyl acetate solution; monoclinic space group $P2_1/c$, with a = 9.173(2), b = 30.993(8), c = 10.181(1) Å, β = 106.816(13)° at 25 ± 1°C, V = 2770.7(10) $Å^3$, λ (Cu Kα) = 1.54184 Å, ρ_o = 1.103 g cm^{-3}, ρ_c = 1.109 g cm^{-3} for Z = 4 and F.W. = 462.70, F(000) = 1024. After Lorentz and polarization corrections, merging of equivalent reflections and rejection of systematic absences, 4636 unique reflections (R(int) = 0.0624) remained, of which 3700 were considered observed ($I_o > 2\sigma(I_o)$) and were used in the subsequent absorbtion correction,[15] structure determination[16] and refinement[17]. Final refinement after an additional absorbtion correction[18] resulted in standard R values of 0.0504 and 0.0594 for 2354 ($I > 2\sigma(I_o)$) and 2712 reflections (all data), respectively.



1, R = H (–)-Xestospongin C
2, R = OH (+)-Xestospongin D

The structure of one of the enantiomers present in the asymmetric unit of the crystal cell of (±)-xestospongin D has been displayed in Figure 1[19] and corresponds to the absolute

structure previously proposed[7] for xestospongin D (*i.e.*, the (+)-enantiomer). The absolute stereochemistry at the six chiral centers for this enantiomer were assigned as follows: 2S, 9S, 10R, 2'S, 9'S, 10'R. The crystal structure of this enantiomer closely resembles the X-ray crystal structure of the previously reported (−)-xestospongin C (1).[7] As in the case of xestospongin C, the central macrocyclic ring of (+)-xestospongin D (2) adopts a nearly perfect rectangular [4646] conformation (Figure 1) with approximate dimensions of 7.99 Å (O-1 - O-1') and 4.52 Å (C-13 - C-19). An additional rather striking conformational feature exhibited by both xestospongin C (1) and xestospongin D (2) is the mirror symmetry displayed for the opposing staggered methylene chains which make up the longer sides of the rectangle. The nearly perfect overlap of the corresponding methylene atoms of opposing sides can readily be seen for (+)-xestospongin D (2) when the molecule is viewed from the side (Figure 2). As in the case of (−)-xestospongin C (1), the A,B-rings of (+)-xestospongin D (2) adopt the *cis*-fused conformation and the C,D-rings, a *trans*-decalin fused system.

When (±)-xestospongin D was tested[20] in the U. S. National Cancer Institute's human tumor cell line screen, modest growth-inhibitory activity was observed against a number of the lines. For example, a leukemia cell line subpanel (comprising tested lines CCRF-CEM, HL-60TB, K-562, MOLT-4 and SR) was relatively sensitive, showing a triplicate-test averaged GI_{50} (see ref. 21 for definition) of $3.62 \pm 2.02 \times 10^{-6}$M. A breast tumor subpanel was similarly sensitive; for example, the triplicate-test averaged GI_{50} for the group comprising the MCF7, HS 578T, MDA-MB-435, and MDA-N lines was $4.53 \pm 1.98 \times 10^{-6}$M.

Potential antimicrobial activity of xestospongin D was investigated using disk diffusion assays. Antimicrobial disk susceptibility tests were performed according to the method established by the National Committee for Clinical Laboratory Standards.[22] Immediately prior to the assay, xestospongin D was reconstituted in sterile methanol, and two fold dilutions applied to sterile 6 mm disks. Susceptibility testing was performed with the bacteria *Staphylococcus aureus*, *Enterococcus faecalis*, *Micrococcus luteus*, *Escherichia coli*, and *Neisseria gonorrhoeae*, and the fungi *Candida albicans* and *Cryptococcus neoformans*. Xestospongin D inhibited growth of the Gram-positive opportunist *M. luteus*, with a minimum inhibitory concentration between 12.5-25 μg/disk. *M. luteus* present in normal skin flora of humans has been shown to cause prosthetic valve endocarditis in immunocompromised patients.[23] At up to 100 μg/disk, xestospongin D exhibited no antimicrobial activity against the other six bacteria and fungi tested.

The results of this study emphasize that the occurrence of optical activity in a natural product with chiral atoms cannot be taken for granted.[24] While assignment of absolute stereochemistry to a new compound via correlation to a closely related, parent structure of established absolute stereochemistry is a commonly accepted technique, it is not without its pitfalls. In this respect we have shown that both enantiomers of xestospongin D can occur naturally (albeit, in this case, as a dl-pair). Indeed, the possibility arises that the absolute stereochemistry for (+)-xestospongin D could actually be the mirror image of that proposed in the original publication (as shown in Figure 1). This possibility, although presumably remote, does illustrate that it is best to rely on additional chemical and

physicochemical evidence, such as X-ray methods (anomalous dispersion), optical rotation relationships (*e.g.*, Hudson rule), and absolute structure assignments based on differential enantiomeric reactivity (Horeau and Mosher methods), in order to rule out any ambiguities.

The occurrence of racemic xestospongin D prompts speculation as to its mode of origin. Presumably a C-10 aldehyde biosynthetic precursor of (±)-xestospongin D (2) was hydroxylated and then underwent tautomerisation prior to ring closure. Alternatively the amino-acetal system might be more labile than expected and the loss of asymmetry could occur during a ring opening/ring closure sequence.

Acknowledgment. The research reported herein was made possible by the following financial assistance: Outstanding Investigator Grant CA44344-01A1-06 awarded by the Division of Cancer Treatment, National Cancer Institute, DHHS: the Fannie E. Rippel Foundation, the Arizona Disease Control Research Commission, the Robert B. Dalton Endowment Fund, Virginia Piper, Diane Cummings (The Nathan Cummings Foundation, Inc.), Gary L. Tooker, Polly J. Trautman, and the Eagles Art Ehrmann Cancer Fund. For other very helpful assistance we thank the National University of Singapore (and especially Dr. Chou Loke Ming and Professor T. J. Lam) and Drs. Matthew Suffness, Cherry L. Herald, Zbigniew A. Cichacz, Fiona Hogan, Ms. Denise Nielsen-Tackett, Mr. Lee Williams and Ms. Melanie Filiatrault.

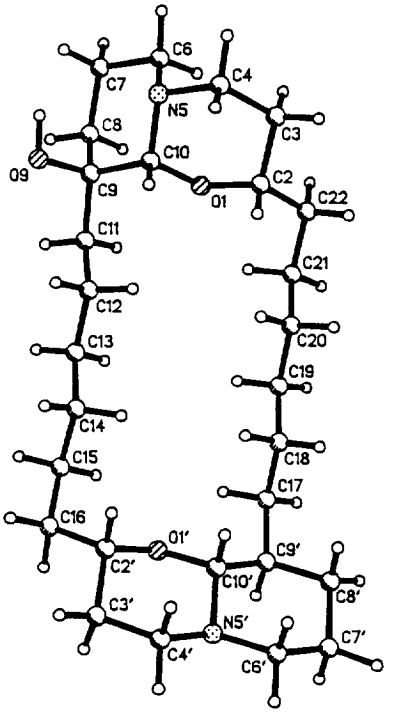


Figure 1. Perspective view of (+)-xestospongin D.

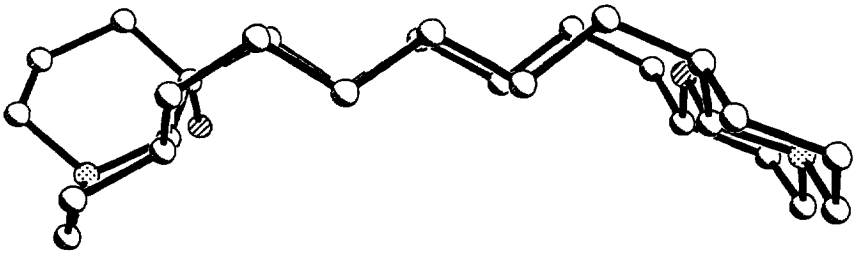


Figure 2. Side view of (+)-xestospongin D (less hydrogens).

Literature Cited

1. Contribution 357 of "Antineoplastic Agents" and for part 356 see: Zhang, X.; Zhang, R.; Zhao, H.; Cai, H.; Gush, K.A.; Kerr, R.G.; Pettit, G.R.; Kraft, A.S. *Cancer Research* **1996**, *56*, 802.
2. Quiñoà, E.; Crews, P. *Tetrahedron Lett.* **1987**, *28*, 2467.
3. Kobayashi, J.; Murayama, T.; Kosuge, S.; Kanda, F.; Ishibashi, M.; Kobayashi, H.; Ohizumi, Y.; Ohta, T.; Nozoe, S.; Sasaki, T. *J. Chem. Soc. Perkin Trans. 1.* **1990**, 3301.
4. Kobayashi, J.; Zeng, C.; Ishibashi, M.; Shigemori, H.; Sasaki, T.; Mikami, Y. *J. Chem. Soc. Perkin Trans. 1.* **1992**, 1291.
5. Talpir, R.; Rudi, A.; Ilan, M.; Kashman, Y. *Tetrahedron Lett.* **1992**, *33*, 3033.
6. Rao, A.V.R.; Reddy, G.R. *Tetrahedron Lett.* **1993**, *34*, 8329.
7. Nakagawa, M.; Endo, M.; Tanaka, N.; Gen-Pei, L. *Tetrahedron Lett.* **1984**, *25*, 3227.
8. Kobayashi, M.; Kawazoe, K.; Kitagawa, I. *Chem. Pharm. Bull.* **1989**, *37*, 1676.
9. Quirion, J-C.; Sevenet, T.; Husson, H-P.; Weniger, B.; Debitus, C. *J. Nat. Prod.* **1992**, *55*, 1505.
10. Venkateswarlu, Y.; Reddy, M.V.R.; Rao, J.V. *J. Nat. Prod.* **1994**, *57*, 1283.
11. Endo, M.; Nakagawa, M.; Hamamoto, Y.; Ishihama, M. *Pure Appl. Chem.* **1986**, *58*, 387.
12. Bohlmann, F. *Angew. Chem.* **1957**, *69*, 641.
13. Uskoković, M.; Bruderer, H.; von Planta, C.; Williams, T.; Brossi, A. *J. Am. Chem. Soc.* **1964**, *86*, 3364.
14. Crabb, T.A.; Newton, R.F.; Jackson, D. *Chem. Rev.* **1971**, *71*, 109.
15. North, A.C.; Phillips, D.C.; Mathews, F.S. *Acta. Cryst.* **1968**, *A24*, 351.
16. Altomare, A.; Cascarano, G.; Giacovazzo, C.; Guagliardi, A.; Burla, M.; Polidori, G.; Camalli, M. *"SIR92 - A Program for Automatic Solution of Crystal Structures by Direct Methods"*, Dipartimento Geomineralogico, Univ. of Bari, Italy.
17. Sheldrick, G.M. "SHELXL93. *Program for the Refinement of Crystals Structures"*, Univ. of Göttingen, Germany, **1993**.
18. Parkin, S.; Moezzi, B.; Hope, H. *J. Appl. Cryst.* **1995**, *28*, 53.

19. Preparation of Figure 1 was done with "SHELXTL-PC Version 5.03, 1994", an integrated software system for the determination of crystal structures from diffraction data, Siemens Industrial Automation, Inc., Analytical Instrumentation, Madison, WI 53719, USA.
20. Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A.; Gray-Goodrich, M.; Campbell, H.; Boyd, M. *J. Natl. Cancer Inst.* 1991, *83*, 757.
21. Boyd, M.R.; Paull, K.A. *Drug Dev. Res.* 1995, *34*, 91.
22. National Committee for Clinical Laboratory Standards. 1990. Approved standard M2-A4. Performance standards for antimicrobic disk susceptibility tests. National Committee for Clinical Laboratory Standards, Villanova, PA.
23. Dürst, U.N.; Bruder, E.; Egloff, L.; Wüst, J.; Schneider, J.; Hirzel, H.O. *Z. Kardiol.* 1991, *80*, 294.
24. Kobayashi, M.; Kawazoe, K.; Kitagawa, I. *Tetrahedron Lett.* 1989, *30*, 4149.

(*Received in USA* 3 *April* 1996; *accepted* 9 *May* 1996)