Antineoplastic agents. 168. Isolation and structure of axinohydantoin[1]

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Western (Palau) and Eastern (State of Truk) Caroline Islands and Papua New Guinea sponges of the genera *Axinella* and *Hymeniacidon* were found to contain the cytostatic (PS ED_{50} 2.5 and 2.0 μg/mL) and antineoplastic (PS T/C 143 at 3.6 mg/kg and T/C 138 at 3.6 mg/kg) pyrrologuanidines **1***a* and **1***b*. The related hydantoin **2**, designated axinohydantoin, was also isolated from an *Axinella* sp. and its structure was assigned by X-ray crystallographic techniques. Present experience with sponges in the *Axinella* and *Hymeniacidon* genera suggests that the previously known hymenialdisine (**1***b*) and analogous imidazole derivatives may be widely distributed among these and related orange colored Porifera.

Key words: axinohydantoin, hymenialdisine, *Axinella, Hymeniacidon,* cystostatic.

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On a trouvé que les éponges du genera *Axinella* et du *Hymeniacidon* des iles Caroline occidentale (Palau) et orientale (État de Truk) ainsi que de la Nouvelle Guinée contiennent des pyrrologuanidines **1***a* et **1***b* qui sont des cytostatiques (PS ED_{50} 2,5 et 2,0 μg/mL) et des antinéoplasiques (PS T/C 143 à 3,6 mg/kg et T/C 138 à 3,6 mg/kg). À partir d'un *Axinella* sp., on a aussi isolé l'hydantoïne apparentée **2**, appelée axinohydantoïne, et on a déterminé sa structure à l'aide de la diffraction des rayons-X. L'expérience acquise avec les éponges de l'*Axinella* et de l'*Hymeniacidon* genera suggère que l'hyménialdisine (**1***b*), qui était connue antérieurement, ainsi que les dérivés imidazoles analogues sont peut-être très répandus dans ces éponges et dans les Porifera apparentés de couleur orange.

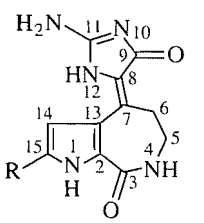
Mots clés : axinohydantoïne, hyménialdisine, *Axinella, Hymeniacidon,* cytostatique.

[Traduit par la revue]

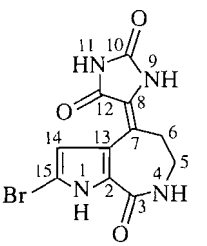
Introduction

Early (2) in our evaluation of marine animals as new sources of potentially useful anticancer drugs, good leads were uncovered among the Porifera, and this initial (1966–1968) promise is now being amply realized (3, 4). In 1979 in Palau we collected a *Hymeniacidon* species (at −40 m) and an *Axinella* sp. that provided extracts with confirmed levels of activity against the U.S. National Cancer Institute's (NCI) murine P388 lymphocytic leukemia (PS system). Other PS active sponge collections were completed in 1981 (Papua New Guinea) and 1985 (Truk, Federated States of Micronesia) that included *Axinella carteri* (Dendy) and a *Hymeniacidon* species.

Initial extracts of each sponge were found to provide a confirmed level of activity against the PS system. By means of PS (*in vitro*) bioassay guided separation procedures, these sponge species led to two cytostatic and antineoplastic alkaloids (**1***a*,*b*) accompanied in the case of *Axinella* sp. by a closely related, but marginally (PS ED_{50} 18 μg/mL) inactive, component (**2**). The PS active marine alkaloids proved to be identical[6] with the known (5–7) hymenialdisine (**1***b*, ref. 6, PS ED_{50} 2.0 μg/mL and T/C 138 at 3.6 mg/kg)[7] and its debromo derivative **1***a* (refs. 5–7, PS ED_{50} 2.5 μg/mL and T/C 143 at 3.6 mg/kg).



1*a*, R = H debromohymenialdisine
1*b*, R = Br hymenialdisine



2
axinohydantoin

The unequivocal X-ray crystal structure of hymenialdisine was nicely established by Cimino *et al.* (5) and reconfirmed in the following year by the Kitagawa group (6). In turn, these advances simplified characterization of the companion substance from *Axinella* sp., herein named axinohydantoin (**2**), as a closely related compound. But establishing the exact geometrical configuration for its hydantoin–lactam sp^2 bond required the following crystal structure determination.

Axinohydantoin (**2**) crystallized from methanol as yellow prisms, which corresponded to $C_{11}H_9BrN_4O_3$ (by hreims) and with one mole of methanol. The structure was solved using

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[1]For contribution 167 refer to ref. 1.

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[5]Revision received March 27, 1990.

[6]By comparison with authentic specimens provided by Dr. I. Kitagawa (see ref. 6).

[7]Interestingly, hymenialdisine was previously active in the KB cell line, but *in*active employing the P388 leukemia: cf. ref. 5. Perhaps the initial negative results were due to the sparingly soluble properties of this pyrrologuanidine.

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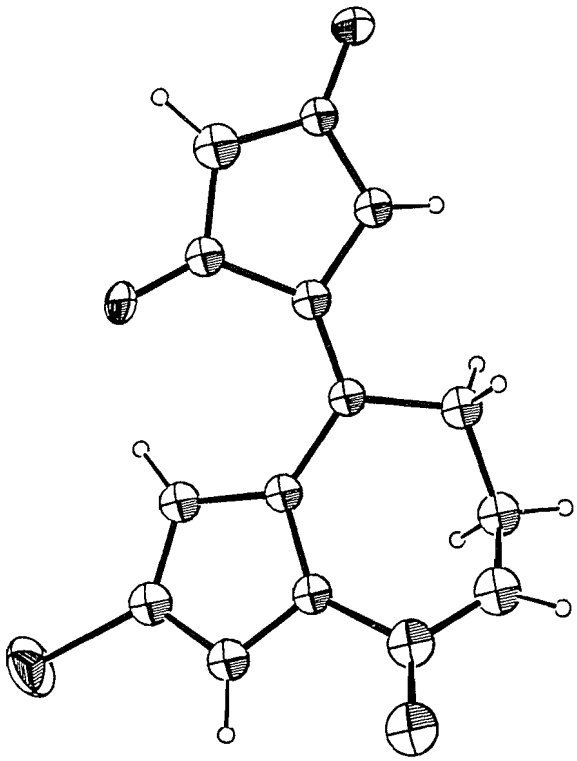

FIG. 1. ORTEP view of a single molecule of **2**, with 50% thermal ellipsoids.

MULTAN (8) and refined to $R = 0.054$ using anisotropic temperature factors for Br and oxygens other than O-3, and isotropic temperature factors for the other non-hydrogens and for hydrogens (unrefined) in calculated positions. HN-1, HN-11, and HN-9 were calculated to be 0.95 Å along a line to the respective oxygen. These positions differed very little from those calculated assuming bonding to trigonal atoms. HN-4 has been shown at the calculated position assuming a trigonal N-4 that is too far (2.4 Å) from the closest O-10 for significant hydrogen bonding; it may actually bend somewhat toward this O-10.

The structure deduced for axinohydantoin (**2**, Fig. 1) was found to be closely related to that of hymenialdisine (**1***b*), with reversal of configuration at the C7—C8 double bond being the most interesting difference. In turn, this suggested that axinohydantoin was not simply a hydrolysis product of guanidine **1***b*. The most prominent bond length difference between the two structures occurs at C10—O10, with 1.23 Å in hydantoin **2** compared to 1.33 Å for C11—N11 in **1***b*. No significant differences in bond angles were observed. An angle of 36° was observed between the least-squares planes of the two nearly planar five-membered rings in hydantoin **2**, compared to 43.8° in guanidine **1***b*. In both cases, the seven-membered ring has adopted a boat conformation with C-5 at the prow, and similar torsion angles except for C2—C3—N4—C5 expanding from −10.5° in **1***b* to −15° in **2**, and C2—C13—C7—C6 contracting from 41.1° in **1***b* to 31° in **2**. The twist angle C13–C7—C8—C12 about the carbon–carbon double bond increases from 0.5° in **1***b* to 10° in **2**, presumably to relieve the steric interaction between O-12 and HC-14.

The arrangement of intermolecular hydrogen bonds governing the packing in hydantoin **2** (see supplementary material)[8] was found to be completely different than that in guanidine **1***b* (6). The hydantoin ring in each molecule was found linked to the hydantoin rings of two other molecules via base-pairing interactions across centers of symmetry. The pyrrole NH proved to be hydrogen bonded to the methanol solvate oxygen and in turn to O-3. Only a few substances (9–12) with a hydantoin system have been isolated from sponges, and one of these, midpacamide, found by Scheuer and co-workers (9) in an unidentified Marshall Island sponge, may be biogenetically related to axinohydantoin. From evidence now in hand, pyrroles **1** and **2** and related substances may prove to be ubiquitous Porifera biosynthetic products.

Experimental

General methods

Marine sponge taxonomic identification was performed in the Smithsonian Institution where voucher specimens are deposited in the collections of the Department of Invertebrate Zoology, National Museum of Natural History. All solvents employed were redistilled. Size exclusion chromatography was accomplished with Sephadex LH-20 (particle size: 25–100 μm) suppled by Pharmacia Fine Chemicals, Uppsala, Sweden. Thin-layer chromatography was carried out with silica gel GHLF Uniplates (Analtech Inc.) and with RP-8 precoated plates (layer thickness: 0.25 mm) from E. Merck, Darmstadt, Germany. High-speed countercurrent chromatography was accomplished with an Ito multilayer coil extractor–separator (P.C. Inc., Potomac, MD) using 2.6 mm i.d. tubing, and an FMI Lab Pump.

Melting points are uncorrected and were determined on a Kofler-type hot-stage apparatus. Ultraviolet spectra were recorded employing a Hewlett–Packard model 8450 uv/vis spectrophotometer and ir spectra with a Nicolet ft-ir model MX-1 instrument. The nmr spectra were measured in DMSO-d_6 using a Bruker AM-400 instrument and are recorded in ppm downfield to TMS (assignments bearing the same superscript may be reversed). The ^{13}C nmr multiplicities were determined with APT experiments based on an average coupling constant of 135 Hz. The eims spectra were recorded with a Kratos AEI 5076 spectrometer at the NSF Regional Facility, University of Nebraska, Lincoln, Nebraska.

Palau Porifera (Axinella *sp. and* Hymeniacidon *sp.) collection and extraction.*

The initial collection of *Axinella* sp. (Demospongiae class, Axinellida order, Axinellidae family) in Palau, Western Caroline Islands, was conducted in May, 1979. The sponge displayed a brownish-yellow exterior of irregular mass. A 2-propanol–$CHCl_3$ extract gave confirmatory in vivo activity with PS T/C 201 at 100 mg/kg, PS ED_{50} = 2.5 μg/mL. A scale-up re-collection (220 kg wet wt.) of this sponge was completed in March, 1985, and preserved in 2-propanol. The preserving solution was separated from the sponge, concentrated to an aqueous slurry, and extracted with CH_2Cl_2 (13). The remaining sponge material was re-extracted with 2-propanol–CH_2Cl_2 (1:1); the extract was separated, solvent removed, and the residue partitioned between CH_2Cl_2 and H_2O. At this early stage a solid precipitate appeared at the CH_2Cl_2–H_2O interface. The precipitate was separated and amounted to

[8]Tables of observed and calculated structure factor amplitudes, calculated hydrogen coordinates, isotropic temperature factors, bond lengths and angles, torsion angles, and a packing diagram may be purchased from the Depository of Unpublished Data, CISTI, National Research Council of Canada, Ottawa, Ont., Canada K1A 0S2.

Tables of positional parameters and bond distances have also been deposited with the Cambridge Crystallographic Data Centre, and can be obtained on request from The Director, Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, U.K.

1.8 kg (PS T/C 227 at 294 mg/kg, ED_{50} 2.8 μg/mL). Analogous solid fractions were also obtained at this initial CH_2Cl_2 step during separations of the sponge extracts summarized below.

The orange sponge *Hymeniacidon* sp. (Demospongiae class, Halichondrida order, Hymeniacidonidae family) was collected (1979), re-collected (in 1985, 218 kg wet wt.), and extracted (2-propanol extract showed PS T/C 130 at 5.5 mg/kg and ED_{50} 27 μg/mL) as summarized above. Removal of solvent from the initial 2-propanol extract led to an aqueous concentrate that contained 1.2 kg of a solid fraction with PS ED_{50} = 3.7 μg/mL.

*Isolation of hymenialdisine (**1**b) and axinohydantoin (**2**)*

A 10 g aliquot from the 1.8 kg of solid precipitate noted above was dissolved in CH_3OH (400 mL) and separated by size exclusion chromatography on a column of Sephadex LH-20 (100 × 10 cm) to yield two major marine alkaloid fractions. When fraction 1 (elution volume: 12.0–12.6 L) was allowed to stand for 24 h at room temperature, axinohydantoin (**2**) slowly crystallized as yellow needles (30 mg): mp >350°C; tlc on silica gel R_f = 0.83, 1-BuOH–AcOH 50% (95:5); tlc on RP-8 R_f = 0.51, CH_3OH–AcOH 5% (1:1); uv (CH_3OH) λ_{max}: 264sh (log ε = 3.88), 345 (log ε = 4.16) nm; ir (KBr) ν_{max}: 1740, 1702, 1638, 1480, 1425, 1407 cm^{-1}; ^1H nmr (DMSO-d_6) δ: 2.67 (2H, m, H-6), 3.22 (2H, m, H-5), 6.66 (1H, s, H-14), 7.89 (1H, t, HN-4), 9.83, 10.91 (2 × 1H, s, HN-9, HN-11), 12.35 (1H, s, HN-1); ^{13}C nmr (DMSO-d_6) δ: 36.2 (t, C-6), 38.5 (t, C-5), 101.6 (s, C-15), 113.9 (d, C-14), 120.0 (s, C-13), 121.2 (s, C-7), 125.5 (s, C-2), 126.5 (s, C-8), 153.8 (s, C-10), 162.7 (s, C-3), 163.3 (s, C-12); hreims *m/z*: 325.9842 and 323.9834 ($C_{11}H_9N_4O_3Br$ requires 325.9836).

Fraction 2 (elution volume: 12.9–13.5 L) yielded a crystalline precipitate (100 mg), which was identified as hymenialdisine (**1***b*) by comparison (uv, ir, ^1H nmr, eims) with an authentic sample (6).

*Truk Porifera (*Axinella carteri*) collection and extraction*

In May 1985, approximately 1 kg of an orange-yellow sponge subsequently identified as *Axinella carteri* (Dendy), was collected in the Truk Lagoon, Federated States of Micronesia, at −13 to −24 m. The preserving solution (2-propanol) was removed and this extract proved toxic down to 50 mg/kg against the PS leukemia. The 2-propanol extract was partitioned between CH_2Cl_2 and H_2O and the resulting CH_2Cl_2 extract was successively partitioned (13) between 9:1–4:1–1:1 MeOH:H_2O with hexane–CCl_4–CH_2Cl_2. The final CH_2Cl_2 extract showed PS T/C 135 at 100 mg/kg and PS cell line ED_{50} = 1.2 μg/mL.

In October 1985, approximately 148 kg (wet wt.) of the sponge was recollected and preserved in MeOH. The MeOH solution was decanted, and the sponge was ground and extracted with MeOH:CH_2Cl_2 (1:1). The original MeOH solution was concentrated to an aqueous phase and extracted with CH_2Cl_2 (3×) followed by 1-BuOH. Study of this 1-BuOH fraction was discontinued when PS results showed minimal activity.

When the ambient temperature extraction of the sponge with MeOH:CH_2Cl_2 was completed, the aqueous MeOH phase was separated and concentrated to an aqueous phase, which was extracted with 1-BuOH (15 L). The 1-BuOH phase was concentrated, redissolved in MeOH (1.5 L), and dried to give a 232 g fraction (PS ED_{50} 1.4 μg/mL). A 97 g aliquot of the MeOH soluble fraction was treated with 1-BuOH (800 mL, 50°C, 12 h) and the relatively insoluble part (50 g, PS ED_{50} 1.5 μg/mL) was collected. The MeOH (600 mL) sparingly soluble portion weighed 4.26 g (PS ED_{50} 0.11 μg/mL).

*Papua New Guinea Porifera (*Hymeniacidon *sp.) collection and extraction*

The collection (May 1981, near Motapure Island, Papua New Guinea) and recollection (October 1983, 44 kg wet wt.) of an orange *Hymeniacidon* sp. as well as the large scale extraction (crude extract PS T/C 136 at 100 mg/kg and ED_{50} 24 μg/mL) and solvent partitioning were performed as described above for *A. carteri*. In this case, when the 934 g initial CH_2Cl_2 fraction was subjected to further separation by MeOH:H_2O with the hexane → CCl_4 → CH_2Cl_2 sequence, a total of 135 g (PS ED_{50} 14 μg/mL) of a solid interfacial fraction was collected and used to isolate hymenialdisines **1***a* and **1***b*.

TABLE 1. Positional and thermal parameters

Atom	*x*	*y*	*z*	*B* (Å^2)
Br	0.56111(6)	0.0097(2)	0.18172(7)	4.40(3)
O3	0.7915(4)	−0.210(1)	0.0865(4)	3.4(2)*
O12	0.7371(3)	−0.194(1)	0.4049(4)	3.0(2)
O10	0.9511(3)	−0.093(1)	0.5586(3)	3.3(2)
OM	0.6407(4)	−0.152(2)	0.0121(5)	7.3(3)
N1	0.6929(4)	−0.069(1)	0.1562(4)	2.3(2)*
N4	0.8760(4)	−0.055(1)	0.1624(5)	2.9(2)*
N11	0.8375(4)	−0.161(1)	0.4951(4)	2.5(2)*
N9	0.9106(4)	−0.056(1)	0.4357(4)	2.0(2)*
C15	0.6581(5)	−0.024(2)	0.2056(5)	2.5(2)*
C14	0.7046(4)	−0.000(2)	0.2706(5)	2.2(2)*
C13	0.7725(4)	−0.036(1)	0.2599(5)	1.8(2)*
C2	0.7630(5)	−0.073(1)	0.1875(5)	2.0(2)*
C3	0.8108(5)	−0.120(2)	0.1407(6)	2.8(2)*
C5	0.8961(5)	0.083(2)	0.2145(5)	2.6(2)*
C6	0.9053(4)	0.024(2)	0.2915(5)	2.4(2)*
C7	0.8393(4)	−0.031(1)	0.3144(5)	1.8(2)*
C8	0.8468(5)	−0.069(1)	0.3839(5)	1.9(2)*
C12	0.7982(5)	−0.147(1)	0.4248(5)	2.0(2)*
C10	0.9045(5)	−0.104(2)	0.5026(5)	1.9(2)*
CM	0.5809(8)	−0.093(2)	−0.0359(8)	6.3(4)*

*Starred atoms were refined isotropically.

Anisotropically refined atoms are given in the form of the isotropic equivalent thermal parameter, defined as $8\pi^2(U_{11} + U_{22} + U_{33})/3$.

*Isolation of hymenialdisines **1**a and **1**b—Procedure A*

An aliquot (250 mL) of the preceding *Axinella carteri* MeOH (600 mL) solution was applied to a column of Sephadex LH-20 (1.9 kg in MeOH). A total of 460 fractions of 20 mL each were collected and a fraction weighing 0.73 g (PS ED_{50} 2.2 μg/mL) was further separated using high speed countercurrent distribution with an Ito coil. A 50 mg aliquot was applied (6 mL) in 1-BuOH:HOAc:H_2O (4:1:5) to the coil with the 1-BuOH phase as stationary (upper) and the aqueous part as mobile (lower) phase. Fractions (120) of 6.5 mL each were collected; fractionation was monitored with ultraviolet detection (254 nm). The fractions were neutralized (pH 7) with aqueous NaOH and refrigerated. Debromohymenialdisine **1***a*, 9 mg, PS ED_{50} = 3.0 μg/mL, crystallized from fractions 28–33 and was identical (tlc, ms, nmr) with an authentic sample (6).

The MeOH less soluble fraction (4.26 g) described above was extracted with MeOH (5 × 25 mL) at 40°C and the solution filtered to give 3.73 g of residue. A 0.90 g portion was triturated with DMSO (10 mL). The soluble portion (0.25 g) was chromatographed on a column of Sephadex LH-20 in MeOH to provide 0.13 g of hymenialdisine (**1***b*) as yellow crystals (PS ED_{50} 0.62 μg/mL), identical (tlc and ms comparisons) with an authentic sample (6).

Procedure B

The 1.2 kg fraction (see above) from the Palau *Hymeniacidon* sp. was further separated by successive Soxhlet extraction (20 g aliquot) with CH_2Cl_2 (6 × 5 L), EtOH (6 × 5 L), and 1-BuOH (6 × 5 L) to give respectively 35 g (PS ED_{50} 26 μg/mL), 500 g (PS ED_{50} 8.6 μg/mL), and 106 g (PS ED_{50} 2.6 μg/mL) fractions. A 10 g sample of the 1-BuOH fraction in MeOH was subjected to chromatography on a column of Sephadex LH-20 (500 g) to give 26 individual (by tlc comparisons) fractions using 4:1 CH_2Cl_2:MeOH. Of these, 56 mg proved to be largely debromohymenialdisine **1***a* (PS ED_{50} 1.4 μg/mL) and hymenialdisine (5.5 mg, **1***b*, PS ED_{50} 7.5 μg/mL) by comparison nmr and tlc.

Procedure C

The 135 g fraction from the Papua New Guinea *Hymeniacidon* sp.

was extracted (Soxhlet procedure with two stainless steel 1-gallon extractors) with EtOH to yield a 22 g alcohol soluble fraction. Treatment of this fraction with CH_2Cl_2:CH_3OH (1:1) yielded a precipitate (4.3 g, PS ED_{50} 8.5 μg/mL), which was extracted with hot 1-BuOH. The 1.5 g 1-BuOH soluble fraction was preabsorbed onto silica gel and separated by chromatography on a column (3 × 62 cm) of silica gel (180 g). Gradient elution with 95:5 CH_2Cl_2:CH_3OH with increments of MeOH provided fractions that yielded (0.17 g and 0.06 g respectively) debromohymenialdisine (**1***a*, PS T/C 143 at 3.6 mg/kg and ED_{50} 2.5μg/mL) and hymenialdisine (**1***b*, PS T/C 138 at 3.6 mg/kg and ED_{50} 2.7 μg/mL). Both **1***a* and **1***b* were identified by direct comparison with authentic samples (6) employing tlc, ^{13}C and ^1H nmr, ms, uv, and ir spectral data.

X-ray structure of axinohydantoin (2)

$C_{11}H_8BrN_4O_3 \cdot CH_3OH$ fw = 357.17

Monoclinic, $C2/c$, a = 19.558(2), b = 7.505(1), c = 19.092(3) Å, β = 103.78(1)°, V = 2754.3 Å^3, Z = 8, ρ_c = 1.72; Nicolet $P2_1$ diffractometer, crystal 0.17 × 0.13 × 0.08 mm, 23°C, MoK_{α_1}, λ = 0.71073 Å, $2\theta/\theta$ scans, $2\theta_{max}$ 50°; 836 of 2446 reflections with $F_o^2 > 3\sigma(F_o^2)$ used; solved by direct methods; R = 0.053, R_w = 0.055 excluding unobserved reflections. Coordinates and isotropic temperature factors of non-hydrogens are given in Table 1.[8]

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