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Fasicularin, a Novel Tricyclic Alkaloid from the Ascidian *Nephteis fasicularis* with Selective Activity Against a DNA Repair-Deficient Organism

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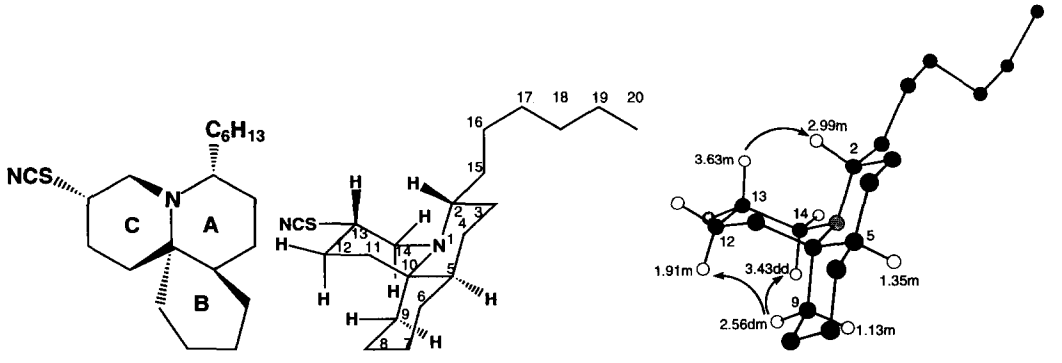
Abstract: A novel tricyclic alkaloid, fasicularin (**1**), was isolated from the Micronesian ascidian *Nephteis fasicularis*. The structure of **1** was elucidated primarily by interpretation of spectral data. Fasicularin was found to be active in a DNA damaging assay.

Ascidians are marine invertebrates which have increasingly become targets for marine natural product research. The majority of compounds produced by these organisms contain nitrogen derived from amino acids and are known to possess interesting biological activities.[1,2] As part of our continuing search for biologically active antitumor agents, we initiated a yeast-based high throughput screen to evaluate natural product extracts in a DNA damaging assay.[3] An extract of the ascidian *Nephteis fasicularis*,[4] collected in Pohnpei, showed activity consistent with induction of DNA damage and was therefore selected for fractionation.

The methanol-methylene chloride (1:1) soluble extract (11.5 g) of the freeze-dried ascidian (75 g), was chromatographed over a column of Sephadex LH-20 to yield fractions that were tested a against wild type yeast strain, a strain deficient in double-stranded DNA repair (RAD-52) and a strain deficient in both double-stranded DNA repair and DNA topoisomerase I. Further purification of the active fractions by column chromatography (RP-18) followed by HPLC (RP-18) yielded fasicularin (**1**)[5] as a colorless gum. Compound **1** was structurally similar to the cylindricines reported from the Australian ascidian *Clavilina cylindrica*.[6-8]

The molecular formula for **1** was established by HRDCIMS which provided a molecular ion at *m/z* 334.2521 for $C_{20}H_{34}N_2S$ (calcd 334.2521, 3 measurements). The IR spectrum exhibited a strong absorbance at 2152 cm^{-1} suggesting that the sulfur was present as a thiocyanate group. The presence of ions at *m/z* 308 and *m/z* 276 in the low resolution DCIMS were indicative of the loss of HCN and NCSH, respectively, from the protonated molecular ion. In addition, the peak at *m/z* 249 was interpreted as the loss of the C_6H_{13} side chain from the protonated molecular ion. The ^1H NMR spectrum of fasicularin (**1**) contained a number of overlapping multiplets between δ 3.63 and 1.05 and a methyl triplet at δ 0.86 (J = 7.0 Hz). In the COSY spectrum of **1**, the following connectivities were observed: the H-13 methine multiplet (δ 3.63) correlated to the H-14 methylene (δ 3.43 and 3.34) and to the H-12 methylene (δ 1.98, 1.91); the H-2 methine multiplet (δ 2.99) showed crosspeaks to the H-3 methylene (δ 1.60, 1.42) and to H-15 (δ 1.53) of the n-hexyl side chain; the H-5 methine (δ 1.35) correlated to the H-4 and H-6 methylenes (δ 1.24, 1.17 and 1.21, 1.05), and finally, the H-20 methyl triplet coupled to the H-19 methylene (δ 1.23).



1

Figure 1

The ^{13}C GASPE spectrum of **1** indicated the presence of 20 carbons including one methyl, fourteen methylenes, three methine and two quaternary carbons, among which the quaternary resonance at δ 111.5 was assigned to the thiocyanate carbon (previously suggested by ms data). Typically thiocyanate carbons resonate near δ 112, while isothiocyanate carbons resonate near δ 129.[9] The C-10 (δ 57.0) signal in the HMBC spectrum of **1** correlated with the H-12 and H-14 methylene protons in ring C; with H-6 (δ 1.21), H-8 (δ 1.49), and H-9 (δ 2.56) in ring B and with H-4 in ring A, thus establishing itself as the bridgehead between the three rings. The H-15 methylene protons correlated to C-2 (δ 52.7) and C-16 (δ 24.2) indicating that the n-hexyl chain was attached at C-2, and H-13 showed a cross peak to the thiocyanate carbon establishing that the thiocyanate was attached at C-13.

The relative stereochemistry of fasicularin (**1**), shown in Figure 1, was determined by nOe difference experiments and coupling constant analyses. The H-13 methine shared a 12.0 Hz axial/axial coupling with H-14 at δ 3.43 and H-12 at δ 1.91. H-9 at δ 2.56 was found to be equatorial by the small coupling it shared with the H-8 protons. The above coupling constants and the large mutual nOe between H-13ax and H-2ax suggested a cis fusion between rings A and C. Enhancement of H-14ax and H-12ax upon saturation of H-9eq suggested a trans fusion between rings A and B. The chemical shift of methine H-5 was δ1.35 which is noticeably upfield, as was methylene H-9ax at δ1.13 (compared to H-9eq at δ2.56). Both H-5 and H-9ax are in the shielding cone of the nitrogen lone pair which accounts for their upfield shifts.

Fasicularin (**1**) demonstrated selective activity in the yeast strain in which the RAD 52 gene had been deleted. Deletion of this gene renders the organism incapable of recombination and repair of DNA double strand breaks and selectively increases sensitivity to agents that produce DNA damage by diverse mechanisms.[3] Deletion of the RAD 52 gene made the yeast >20-fold hypersensitive to fasicularin. The compound was subsequently shown to be cytotoxic to Vero cells with an IC_{50} of 14 ug/ml.

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4. The stalked green ascidian (POH93-073) was collected by hand using SCUBA at a depth of 5 meters in Pohnpei, Federated States of Micronesia, in February of 1993. The ascidian was immediately frozen and stored until extraction. A preserved voucher sample of the ascidian has been identified by Dr. Francois Monniot, Paris Museum of Natural History, as *Nephteis fasicularis* (Family Polycitoridae). The voucher sample of this ascidian has been deposited in the Paris Museum of Natural History, sample number MNHN: A3 Nep 2.
5. Fasicularin (**1**): Isolated as a colorless gum; IR (neat) ν_{max} 3020, 2800, 2152, 1464, 1447, 1110 cm^{-1}; CIMS (CH_4) *m/z* 335 (M+H, 24%), 308 (M+H-HCN, 18%), 276 (M+H-NCSH, 100%), 249 (M+H-C_6H_{14}, 31%), 192 (*m/z* 249-NCS+H, 20%); ^1H NMR (400 MHz, pyridine-d_5) δ 3.63 (m, 1H, H-13), 3.43 (dd, *J* = 12.0, 14.5, 1H, H-14ax), 3.34 (dd, *J* = 2.9, 14.5, 1H, H-14eq), 2.99 (m, 1H, H-2), 2.56 (dm, *J* = 11.8, 1H, H-9eq), 1.98 (m, 1H, H-12eq), 1.91 (m, 1H, H-12ax), 1.84 (m, 1H, H-11ax), 1.60-1.42 (m, 7H, H-3, 3, 7, 8, 11eq, 15, 15), 1.37-1.21 (m, 11H, H-4, 5, 6, 16-19), 1.18-1.05 (m, 5H, H-4, 6, 7, 8, 9ax), 0.86 (t, *J* = 7.0, 3H, CH_3-20); ^{13}C GASPE NMR δ (100 MHz, pyridine-d_5) 111.5 (s, SCN), 57.0 (s, C-10), 52.7 (d, C-2), 46.4 (t, C-14), 45.9 (d, C-5), 40.1 (d, C-13), 34.1 (t, C-15), 33.9 (t, C-9), 32.2 (t, C-3), 32.1 (t, C-18), 30.2 (t, C-17), 29.5 (t, C-6), 27.6 (t, C-12), 27.1 (t, C-4), 26.3 (t, C-7), 24.2 (t, C-16), 22.9 (t, C-19), 22.7 (t, C-8), 19.3 (t, C-11), 14.3 (q, C-20).
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