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Tetrahedron Letters 44 (2003) 3897–3899

TETRAHEDRON LETTERS

# Distomadines A and B, novel 6-hydroxyquinoline alkaloids from the New Zealand ascidian, *Pseudodistoma aureum*

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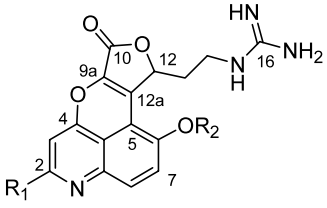
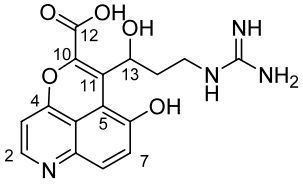
Received 11 March 2003; accepted 28 March 2003

**Abstract**—Distomadines A and B, novel tetracyclic guanidine-containing 6-hydroxyquinoline alkaloids were isolated from the New Zealand ascidian *Pseudodistoma aureum* and characterised by interpretation of spectroscopic data and chemical derivatisation. Distomadine A exhibited mild antifungal activity but failed to exhibit any biological activity in a range of antitumour, cytotoxicity, anti-inflammatory, and antimycobacterial tests. The known methyl esters of fatty acids eicosapentaenoic acid (EPA), docosahexaenoic acid and eicosatetraenoic acid were also identified in the extract with EPA methyl ester exhibiting mild cytotoxicity to a non-malignant cell line. 

As part of our ongoing search for new chemical entities from New Zealand ascidians[1] we have studied biologically active extracts derived from the organism *Pseudodistoma aureum* (Brewin, 1957) (Polyclinidae) collected in the far North of New Zealand. Previous studies of ascidians of the genus *Pseudodistoma* have led to the discovery of purines,[2] cytotoxic amines,[3] aminols[4] and alkaloids.[5] Bioassay (cytotoxicity) directed fractionation of a $CH_2Cl_2$–MeOH extract of the organism (85 g dry wt) using repeated reversed-phase $C_{18}$ flash column chromatography (MeOH:$H_2O$) followed by HPLC ($C_{18}$; MeCN:$H_2O$ (90:10); 5 mL/min) afforded the known compounds all-(*Z*)-5,8,11,14,17-eicosapentaenoic acid (EPA) methyl ester (11.0 mg, 0.013% dry wt), all-(*Z*)-4,7,10,13,16,19-docosahexaenoic acid methyl ester (2.0 mg, 0.0024% dry wt) and all-(*Z*)-5,8,11,14-eicosatetraenoic acid methyl ester (2.5 mg, 0.0029% dry wt) which were identified by comparison of their spectroscopic data with published data.[6] Investigation of a portion of the inactive fractions using reversed-phase $C_{18}$ flash column chromatography (aq. $NH_3$ 0.1% through to MeOH) followed by size exclusion chromatography on Sephadex LH-20 (aq $NH_3$ 0.1%/MeOH) yielded distomadine A (**1**) as an optically active ($[\alpha]_D^{20} = +47$ (*c* 0.3, MeOH)) fluorescent yellow powder (4.5 mg, 0.03% dry wt, mp 232° (dec)) and a 1:2 mixture of distomadine B (**2**) and 2′-deoxyadenosine (4.2 mg, 0.01% dry wt).




**1** $R_1$=$R_2$=H
**2** $R_1$=$CO_2H$, $R_2$=H
**3** $R_1$=H, $R_2$=$CH_3$




**4**

A molecular formula of $C_{16}H_{14}N_4O_4$ for **1** was established by HRFAB mass spectrometry [*m*/*z* 327.1084 (M+H), Δ +0.9 mmu]. UV absorptions at 242 nm (log ε 4.31), 293 (3.61), 313 (3.65), 379 (3.58), 399 (3.59) and 438 (3.45), a bathochromic shift of 47 nm upon addition of base, and fluorescence (250 nm excitation, 450 nm emission) suggested the presence of an extended aromatic chromophore. In the $^1$H NMR spectrum, four aromatic protons were observed as two pairs of mutually coupled doublets, with one of the pairs (δ 8.08 and 6.56) exhibiting a coupling constant (*J* 5.2 Hz) indicative of the presence of a pyridine ring (Table 1). Also observed in the $^1$H NMR spectrum was a 6-proton spin system comprising an exchangeable proton (δ 7.28), a methylene multiplet (δ 3.41), a diastereotopic methylene pair (δ 2.83 and δ 1.98) and a methine (δ 5.64). The $^{13}$C

*Keywords*: ascidian; *Pseudodistoma aureum*; distomadines; alkaloid; quinoline; guanidine.

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0040-4039/03/$ - see front matter 
doi:10.1016/S0040-4039(03)00831-1

**Table 1.** $^{1}H$ and $^{13}C$ NMR data for distomadine A (**1**) and hydroxy acid **4**

| C no. | Distomadine A (**1**) ($CD_3OD$) | | | C no. | **4** (DMSO-$d_6$)[b] | | |
|---|---|---|---|---|---|---|---|
| | $^{13}C$ (ppm) (mult, $J_{CH}$ in Hz) | $^{1}H$ (ppm) (mult, $J$, Hz) | HMBC ($^{1}H\rightarrow^{13}C$) | | $^{13}C$ (ppm) | $^{1}H$ (ppm) (mult, $J$, Hz) | HMBC $^{1}H\rightarrow^{13}C$ |
| 2 | 148.9 (d, 179) | 8.08 (d, 5.2) | 3, 4, 4a, 8a | 2 | 145.4 | 7.86 (d, 4.9) | 3, 4, 8a |
| 3 | 105.4 (dd, 167, 9) | 6.56 (d, 5.2) | 2, 4, 4a, 5 | 3 | 100.5 | 6.15 (d, 4.9) | 2, 4, 4a |
| 4 | 161.5 (d, 7) | | | 4 | 157.9 | | |
| 4a | 123.5 (d, 6) | | | 4a | 122.4 | | |
| 5 | 106.0 (d, 7) | | | 5 | 107.8 | | |
| 6 | 161.0 (d, 9) | | | 6 | 158.6 | | |
| 7 | 130.7 (d, 159) | 7.06 (d, 9.4) | 5, 6, 8a, 12a | 7 | 130.3 | 6.78 (d, 9.1) | 5, 8a |
| 8 | 131.2 (d, 161) | 7.50 (d, 9.4) | 4, 4a, 5, 6 | 8 | 125.3 | 7.16 (d, 9.1) | 4a, 6 |
| 8a | 143.7 (m) | | | 8a | 141.8 | | |
| 9a | 134.9 (s) | | | 10 | 143.4 | | |
| 10 | 166.4 (s) | | | 11 | 118.6 | | |
| 12 | 79.7 (dd, 158, 3) | 5.64 (dd, 8.2, 1.8) | 9a, 10, 12a, 13, 14 | 12 | 167.0 | | |
| 12a | 143.4 (d, 3) | | | 13 | 64.3 | 4.96 (dd, 8.8, 5.4) | 5, 10, 11, 14, 15 |
| 13a | 34.1 (t, 132) | 2.83 (m) | 12a, 14 | 14a | 36.3 | 1.90 (m) | 11, 13, |
| 13b | | 1.98 (m) | 12, 14 | 14b | | 1.70 (m) | 11, 13, 15 |
| 14 | 39.8 (t, 140) | 3.41 (m) | 12, 13, 16 | 15a | 38.1 | 3.28 (m) | 13 |
| 15 | | 7.28 (t, 5.7)[a] | | 15b | | 3.06 (m) | 13, 14, 17 |
| 16 | 158.7 (s) | | | 17 | 157.8 | | |

[a] $H_2O/D_2O$ 9:1. A broad singlet was also observed at $\delta$ 6.64 (3H). Due to the line width, the signal could not be assigned to specific protons in the molecule.

[b] Broad singlets were also observed at $\delta$ 8.82 (1H) and 7.79 (3H). Due to the line width, the signals could not be assigned to specific protons in the molecule.

NMR spectrum indicated the presence of two $sp^3$ methylene, one $sp^3$ methine, four aromatic methine, one guanidine, and one ester carbonyl as well as seven quaternary olefinic resonances. Extensive interpretation of COSY, $^{1}H$–$^{13}C$ gHSQC and $^{1}H$–$^{13}C$ and $^{1}H$–$^{15}N$ gHMBC NMR data allowed construction of two fragments comprised of a 4,5,6-trisubstituted quinoline ring system (N1–C8a) bearing oxygen substitution at C-4 and C-6, and a second fragment comprising an alkyl amine side chain (H-12 to NH-15). The observation of a long range $^{1}H$–$^{13}C$ HMBC correlation from 2H-14 to a $^{13}C$ resonance at $\delta$ 158.7 (C-16) suggested the presence of a guanidine group, which was confirmed by a positive Sakaguchi spot test[7] and by preparation of a 2,4-dimethylpyrimidine derivative by reaction of **1** with acetylacetone.[8] HMBC correlations from H-12 ($\delta$ 5.64) in **1** established connectivity to an ester carbonyl ($\nu$ 1745 $cm^{-1}$, $\delta$ 166.4, C-10) and both carbons of a tetrasubstituted olefinic bond ($\delta$ 134.9, C-9a; $\delta$ 143.4, C-12a). A lack of inter-fragment HMBC correlations observed in a range of experiments ($^{x}J_{CH}$ 4, 8.3 and 10 Hz) necessitated the preparation of derivatives to aid the structure solution. Treatment of **1** with diazomethane afforded 6-methoxy distomadine A (**3**), the location of the methoxy group being established by observation of an HMBC correlation between the new methyl signal ($\delta$ 3.96) and C-6 ($\delta$ 148.8), and by ROESY through-space interactions between the methyl group and H-7 ($\delta$ 7.33).[9] Reaction of distomadine A (**1**) with NaOH–MeOH afforded a single more polar product (**4**),[10] the spectroscopic data for which, including the lack of observation of an HMBC correlation from H-13 to C-12 (note numbering of **4**) and the prominent upfield shifts of H-13 ($\Delta\ \delta$ 0.68) and C-13 ($\Delta\ \delta$ 15.4), suggested opening of the lactone ring present in **1** (Table 1). Crucial however was the detection of a new $^{3}J_{CH}$ HMBC correlation from H-13 to C-5 ($\delta$ 107.8) thereby securing inter-fragment connectivity and establishing the planar structure of **4**, and hence by relationship, the natural product distomadine A (**1**). All attempts to derivatise **4** in order to secure absolute stereochemistry were unsuccessful.

Distomadine B (**2**) was characterised as the 2-carboxylic acid derivative of **1** by HRFABMS and NMR analysis.[11]

Ascidians are noted for their ability to biosynthesise amino-acid derived metabolites[12]—the distomadines are probably derived from tryptophan (hydroxyquinoline precursor) and homoarginine.

EPA methyl ester exhibited moderate BSC-1 cytotoxicity (zone size 3.5–4.5 mm, 120 μg loading), but no cytotoxicity against P388 murine leukaemia cells or antimicrobial activity. Distomadine A (**1**) exhibited mild antifungal activity towards *Candida albicans* (6 mm excess radius at 600 μg) but was inactive in a range of assays including antitumour, antiviral, anti-inflammatory and antimycobacterial assays.

## Acknowledgements

We gratefully acknowledge funding from the Auckland Medical Research Foundation and The University of Auckland Research Committee. We thank Dr. Chris Battershill (NIWA, Wellington, New Zealand; current address: AIMS, Townsville, Australia) for initial collections and identification of the organism (voucher coded 1999LH3-3 held at the University of Auckland). D.R.A. wishes to thank the University of Auckland for a doctoral scholarship. We also thank Mrs. Gill Ellis for cytotoxicity and antimicrobial assays, Dr. M. Berridge and A. Tan (Malaghan Medical Research Institute, Wellington, NZ) for anti-inflammatory assays, Dr. V. Narayanan (NCI, Bethesda) for in vitro human antitumour assays, and Dr. J. Maddry (TAACF) for antimycobacterial testing.

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8. Prepared using a literature procedure (Yorke, S. C.; Blunt, J. W.; Munro, M. H. G.; Cook, J. C.; Rinehart, K. L. *Aust. J. Chem.* **1986**, *39*, 447–455) in 88% yield. $[\alpha]_D^{20}=+1.4$ (*c* 0.14, MeOH); HRFABMS *m*/*z* 391.1404 (M+H), Δ +0.2 mmu. NMR ($D_2O$) resonances assigned to 2,4-dimethylpyrimidine ring: $^1H$ $\delta$ 5.94 (1H, s), 1.88 (3H, br s), 1.53 (3H, br s); $^{13}C$ $\delta$ 170.0 (2C, s), 162.4 (1C, s), 112.4 (1C, d), 24.7 (2C, q).
9. $[\alpha]_D^{20}=+18$ (*c* 0.16, MeOH); HRFABMS *m*/*z* 341.1253 (M+H), Δ −0.3 mmu.
10. $[\alpha]_D^{20}=-20$ (*c* 0.3, MeOH); HRFABMS *m*/*z* 345.1197 (M+H), Δ +0.2 mmu. IR $\nu_{max}$ (dry film) 3306 (br), 1667 $cm^{-1}$; UV (MeOH) $\lambda_{max}$ 248 nm (log $\varepsilon$ 4.12), 306 (3.39), 364 (3.47), 380 (3.44).
11. Distomadine B (**2**): $C_{17}H_{14}N_4O_6$ HRFABMS 371.0962 (M+H) Δ +3.0 mmu. $^1H$ NMR (400 MHz, DMSO-$d_6$/$D_2O$), $\delta$ 7.57 (1H, d, $J$=9.4 Hz, H-8), 6.98 (1H, s, H-3), 6.74 (1H, d, $J$=9.4 Hz, H-7), 5.47 (1H, dd, $J$= 7.9, 2.0 Hz, H-12), 3.28 (2H, m, $H_2$-14), 2.62 (1H, m, H-13a), 1.91 (1H, m, H-13b); $^{13}C$ NMR (100 MHz, DMSO-$d_6$/$D_2O$), $\delta$ 168.1 (C-4), 164.1 (C-6), 158.5 (C-2), 157.1 (C-16), 143.8 (C-9a), 139.9 (C-8a), 131.7 (C-8), 131.1 (C-7), 130.9 (C-12a), 122.8 (C-4a), 103.7 (C-3), 102.2 (C-5), 77.4 (C-12), 38.5 (C-14), 32.6 (C-13), COOH and C-10 not observed.
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