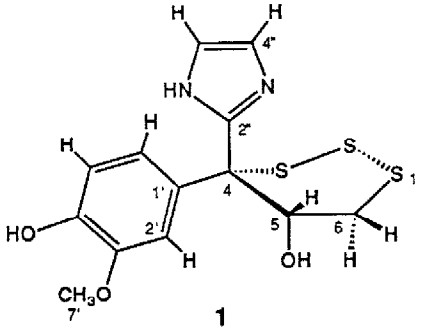
Tetrahedron Letters,Vol.30,No.28,pp 3703-3706,1989 0040-4039/89 $3.00 + .00
Printed in Great Britain Maxwell Pergamon Macmillan plc

A BIOLOGICALLY ACTIVE 1,2,3-TRITHIANE DERIVATIVE FROM THE NEW ZEALAND ASCIDIAN *APLIDIUM* SP. D.

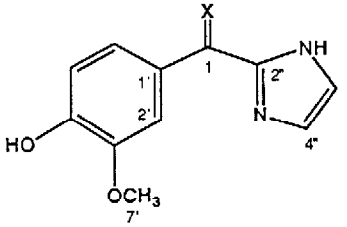
Brent R. Copp, John W. Blunt* and Murray H. G. Munro*
Department of Chemistry, University of Canterbury, Christchurch, New Zealand
Lewis K. Pannell
National Institute of Diabetes, Digestive and Kidney Diseases, NIH, Bethesda, MD 20892, USA

SUMMARY: *cis*-5-Hydroxy-4-(4'-hydroxy-3'-methoxyphenyl)-4-(2"-imidazolyl)-1,2,3-trithiane **1** was isolated from the New Zealand ascidian *Aplidium* sp. D. In neutral or slightly basic solution **1** interconverts to the *trans* isomer **3**. These isomers are the precursors to 2-vanilloyl imidazole **2**, previously reported from an extract of an Australian species of *Aplidium*. Both trithiane isomers are active against P388 leukemia cells *in vitro*.

As part of the search for biologically active compounds from New Zealand marine organisms, studies have been conducted on a previously undescribed compound ascidian, *Aplidium* sp. D.[1] The methanol-toluene extract of this species exhibited *in vitro* antimicrobial, antileukemic and cytotoxic properties. We report here the isolation of an unusual 1,2,3-trithiane derivative **1**, a major component present in the extract. This compound is shown to be the precursor for 2-vanilloyl imidazole **2**, previously reported as a natural product from an Australian ascidian, *Aplidium pliciferum*.[4] Only two other naturally occurring 1,2,3-trithiane derivatives, both unrelated to **1**, have been reported.[5,6]



1



2 X = O
4 X = S

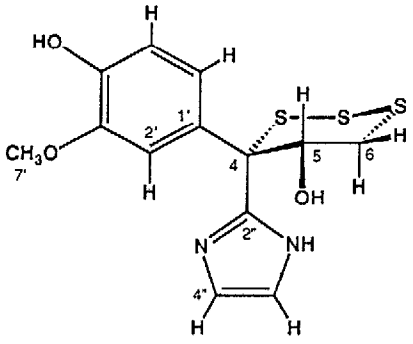
Bioassay directed fractionation of a freshly prepared methanol-water extract of the ascidian using reverse phase chromatography[7] yielded **1** as a yellow gum (48 mg, 0.05% wet weight). In contrast, similar separations on an equivalent extract that had been allowed to stand in neutral solution at room temperature for one month failed to yield **1**. Rather, the major component was shown to be **2** by a single crystal X-ray diffraction study,[8] and subsequently by comparison with the data recently reported for this compound.[4] Re-examination of the freshly prepared ascidian extract failed to indicate the presence of **2**, suggesting it is not a naturally occurring metabolite of *Aplidium* sp. D. No evidence for the occurrence of thiazole-containing compounds was found in either extract, in contrast to the previous report on the co-occurrence of these compounds with **2** in *Aplidium pliciferum*.[4]

High resolution EI and negative ion DCI mass spectroscopy[9] of **1** indicated a molecular formula of $C_{13}H_{14}N_2O_3S_3$, supported by the appropriate isotope pattern, while the ^1H and ^{13}C NMR spectra[9,10] of **1** were consistent with the presence of 3-methoxy-4-hydroxyphenyl and 2-substituted imidazole rings as in

2. In addition, proton resonances at 2.94, 3.69 and 5.25 ppm indicated the presence of a -CH_2-CH(X)- system, confirmed by a COSY NMR experiment. ^{13}C NMR resonances associated with this system were observed at 40.13 and 84.35 ppm, together with a quaternary carbon resonance at 68.09 ppm. All protonated carbons were assigned by a HETCOR NMR experiment, while the long range ^{1}H-^{13}C correlations shown in Figure 1 were established by an HMBC NMR experiment (optimised for 8.3 Hz couplings).[11]

The presence of a 1,2,3-trithiane ring in **1** was suggested by the observation of three sequential losses of ^{32}S in the low and high resolution EI mass spectrum i.e. 342 (M^+), 310 (M^+-S), 278 (M^+-S_2), 246 (M^+-S_3) while chemical shift considerations required an hydroxyl group on the methine carbon (C-5, 84.35; H-5, 5.25 ppm) and sulfur substitution on the methylene carbon (C-6, 40.13; 2H-6, 3.69, 2.94 ppm). The chemical shift of C-4 (68.09 ppm) was consistent with sulfur substitution, leading to the structure of **1** as shown.

An acidic (4% TFA) CD_3OD solution of **1** was stable with no decompositon detectable by ^{1}H NMR after one month. However, slightly alkaline (0.02% w/v NaOH) or even neutral CD_3OD solutions of **1** were observed to change over several weeks to give a mixture of **1** and two new, major components. One of these components, **3**, was closely related to **1**. This new compound possessed the same alkyl and aromatic functionalities, long range ^{1}H-^{13}C correlations (as determined by an HMBC experiment) and low and high resolution mass spectroscopic data as observed for **1**, indicating that they were isomers. Comparison of the ^{1}H and ^{13}C NMR data of **1** with those of **3**[12] indicated that they were related by stereochemical inversion at C-4.



3

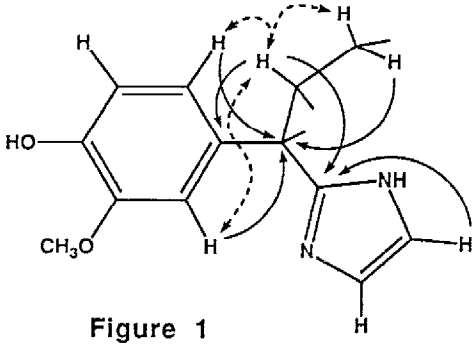


Figure 1

Connectivities found for **1** and **3** from HMBC (⟶) and difference NOE (⇢) NMR experiments.

The preferred solution state conformations of **1** and **3** were investigated by use of ^{1}H NMR difference NOE experiments[13] and application of the modified Karplus equation.[14] The vicinal coupling constants observed for **3** ($^{3}J_{H5,H6}$ 3.8 and 10.2 Hz) were satisfied by the -CH_2-CH(OH)- dihedral angles of about 67° and 169°. These inferred dihedral angles and the observed NOE's (all >4%, see Figure 1) are best satisfied by the structure as shown in **3** (arbitrarily assigned as (4S, 5S)).

In the natural trithiane **1**, the vicinal coupling constants for the -CH_2-CH(OH)- system were equivalent ($^{3}J_{H5,H6}$ 7.2 Hz), which corresponds only with dihedral angles of about 40° and 144°. Alternative arrangements with equivalent $^{3}J_{HH}$ values require H-5 to bisect the C-6 hydrogens, but such arrangements would have *J* values much smaller than the observed values.[15] In addition to these angular requirements, the observation of a significant (5%) NOE between H-5 and only one of the C-6 hydrogens (3.69 ppm) and to each of the two *ortho* protons (H-2' (5%), H-6' (4%)) required the solution conformation of **1** to be a skew boat, shown arbitrarily as (4R, 5S) in **1**. The skew boat conformation of the 1,2,3 trithiane ring has previously been shown to be an accessible local energy minimum.[16] To determine if the -CH_2-CH(OH)- coupling constants observed for **1** were due to an averaging caused by the rapid interconversion of two or more conformations, variable temperature ^{1}H and ^{13}C NMR experiments were run. As no signal splitting or broadening was observed over the temperature range of 213 to 353 K, one conformation, **1**, must have been predominant.[17]

The other main component present in the mixture formed on the treatment of **1** under basic conditions possessed very similar ^{1}H and ^{13}C NMR data[18] to those observed for 2-vanilloyl imidazole **2**, implying the presence of a conjugative electron withdrawing group at C-1. Upon acidification of the CD_3OD solution to stop further decompositon of **1**, the ^{1}H and ^{13}C NMR signals due to this new compound diminished and signals assignable to the ketone **2** appeared. The transformation to **2** was complete after 2 days at room temperature. The structure of this transient intermediate is proposed as being the thione **4**. All attempts to isolate the thione **4** by HPLC resulted in the isolation of the ketone **2**, consistent with the low stability of **4** to aqueous acidic solutions,[19] as employed in the HPLC separations.

The natural trithiane **1** showed modest activity against the P388 murine leukemia *in vitro* (IC_{50} 13±1 μg/ml), was cytotoxic against the slow growing BSC cells used in the antiviral screen, but showed no antiviral efficacy. The trithiane was also inhibitory against the gram positive bacterium *Bacillus subtilis* and the fungus *Candida albicans* (MIC values ~20μg/disc), but inactive against the gram negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*. The trithiane **3** was active against P388 *in vitro* with IC_{50} 12 μg/ml, was cytotoxic to BSC cells with no antiviral efficacy, and inhibited *Bacillus subtilis* and *Candida albicans*. 2-Vanilloyl imidazole had no detectable biological activities in our assay system.

ACKNOWLEDGEMENTS We thank Dr Chris Battershill for collection and identification of the ascidian, Mrs Gillian Barns and Mrs Fiona Dale for biological assay results, and Nicola Kinzett and Dr W.T. Robinson for assistance with the X-ray crystallographic study. Financial assistance from the New Zealand Cancer Research Society, NZ University Grants Committee and SeaPharm Inc./Harbor Branch Oceanographic Institution is also gratefully acknowledged.

REFERENCES AND NOTES

1. *Aplidium* sp. D (Chordata, Aplousobranchia, Polyclinidae) remains undescribed in the taxonomic literature. It is distinct from all other *Aplidium* species described for New Zealand.[2,3] Colonies are cherry in colour and grow as small globes up to 4 cm in diameter. The top layer of the test is tough and cartilagenous with many white test cells. Zooids are comparatively long (up to 8 mm) and the branchial siphon has six lobes. The arterial opening has a single simple lappet. The specimens used in this study were collected by SCUBA at Kaikoura, New Zealand in December 1982. The type specimen for this species is held at the University of Canterbury as collection number 821210-1-04.
2. Brewin, B. *Trans. Proc. Roy. Soc. N.Z.* **1946**, *76*, 87-131.
3. Millar, R.H. *NZOI Memoirs* **1982**, *85*, 22-41.
4. Arabshahi, L.; Schmitz, F.J. *Tetrahedron Lett.* **1988**, *29*, 1099-102.
5. Anthoni, U.; Christophersen, C.; Ogard Madsen, J.; Wium-Andersen, S.; Jacobsen, N. *Phytochemistry* **1980**, *19*, 1228-9.
6. Tressel, R.; Holzer, M.; Apetz, M. *J. Agric. Food Chem.* **1977**, *25*, 455-9. Kasai, T.; Sakamura, S. *Agric. Biol. Chem.* **1982**, *46*, 821-2.
7. Blunt, J.W.; Calder, V.L.; Fenwick, G.D.; Lake, R.J.; McCombs, J.D.; Munro, M.H.G.; Perry, N.B. *J. Nat. Prod.* **1987**, *50*, 290-2.
8. Compound **2**: ^{1}H NMR, HRMS and melting point were identical to those previously reported.[4] ^{13}C NMR (75 MHz, CD_3OD) 179.80 (C-1), 155.16 (C-4'), 149.74 (C-3'), 128.23 (C-1'), 127.57 (C-6'), 124.68 (C-4", C-5"), 116.55 (C-5'), 114.04 (C-2'), 56.81 (C-7'), C-2" was not observed; ν_{max} (KBr) 3450, 1680, 1600, 1510, 1390, 1360, 1120, 780 cm^{-1}; λ_{max} (CH_3OH) 240 nm (ε 2300), 291 (2400), 324 (2600); λ_{max} (CH_3OH-KOH) 369 nm (ε 4300).
   The single crystal X-ray data were collected at 173K on a Nicolet XRD R3 four circle diffractometer, using Mo Kα (λ 0.71069 Å) monochromated radiation. The cell parameters were determined by least squares refinement of 22 accurately centered reflections in the range $12^{o}<2\theta<32^{o}$. The data was corrected for Lorentz polarisation effects. The space group was determined unambiguously as a result of the structural analysis. Crystal data: $C_{11}H_{10}N_2O_3$, crystal dimensions 0.76x0.23x0.30 mm, a = 10.407(1), b = 9.661(1), c = 10.461(1) Å, β=102.51(9)°, U=1026.8(8) $Å^3$, D_{cal}=1.406 g cm^{-3} , space group monoclinic, $P2_{1/n}$ (Z=4), F(000)=455.91. 2θ ω-scans at a scan rate of 14.65° min^{-1} over a scan range of $4 < 2\theta < 50^{o}$, and a background scan ratio of 0.1, yielded 1608 reflections of which 1200 were considered unique ($I>3\sigma(I)$) and used in the structure refinement. Crystal stability was monitored by recording three check reflections every 100 reflections and no significant variations were observed. Direct methods using the program SOLV

revealed the positions of the C, N and O atoms and these were refined by blocked cascade least-squares techniques. Hydrogen atoms were inserted at calculated positions using a riding model with thermal parameters equal to 1.2U of their carrier atoms. Anisotropic thermal parameters were assigned to non-hydrogen atoms and the refinement on 152 least-squares parameters converged with R=0.0387, wR=0.0416 and a maximum least-squares shift error of 0.006. The function minimised in the refinement was $\Sigma\ \omega\ (|F_o|-|F_c|)^2$ where $\omega = [\sigma^2\ (F_o) + 0.00121\ F_o^2]^{-1}$. All programs used for the data collection and structure solution are contained in the SHELXTL (Version 4.0) package (Sheldrick, G.M. SHELXTL User Manual, Revision 4, Nicolet XRD Corporation, Madison, Wisconsin, 1984).

Atomic coordinates ($x10^4$) and isotropic thermal parameters ($Å^2x10^3$):

Atom	x	y	z	U	Atom	x	y	z	U
C-1'	2193(2)	5850(2)	4738(2)	22(1)	C-2"	2415(2)	4991(2)	7080(2)	21(1)
C-2'	2870(2)	6080(2)	3736(2)	23(1)	C-4"	1711(2)	5654(2)	8753(2)	30(1)
C-3'	2291(2)	6856(2)	2659(2)	24(1)	C-5"	2110(2)	4321(2)	8985(2)	30(1)
C-4'	1036(2)	7429(2)	2570(2)	23(1)	O-1	3571(1)	3988(1)	5650(1)	25(1)
C-5'	390(2)	7230(2)	3575(2)	25(1)	O-3'	2828(2)	7132(2)	1610(1)	31(1)
C-6'	956(2)	6436(2)	4654(2)	24(1)	O-4'	440(2)	8179(2)	1513(2)	30(1)
C-7'	4069(2)	6504(3)	1608(2)	37(1)	N-1"	2577(2)	3917(2)	7930(2)	23(1)
C-1	2790(2)	4893(2)	5808(2)	21(1)	N-3"	1899(2)	6075(2)	7570(2)	26(1)

Structure factor and molecular dimension tables have been deposited with the Cambridge Crystallographic Data Centre.

9. Compound **1**: ^1H NMR (300 MHz, CD_3OD + 4% TFA) 7.53 (2H, bs, H-4", H-5"), 6.96 (1H, d, *J*=2.3 Hz, H-2'), 6.79 (1H, d, *J*=8.5 Hz, H-5'), 6.61 (1H, dd, *J*=2.3, 8.5 Hz, H-6'), 5.25 (1H, t, *J*=7.2 Hz, H-5), 3.82 (3H, s, 3H-7'), 3.69 (1H, dd, *J*=7.2, 11.0 Hz, H-6β), 2.94 (1H, 7.2, *J*=11.0 Hz, H-6α); ^{13}C NMR (75 MHz, CD_3OD + 4% TFA) 149.89 (C-3'), 149.34 (C-4'), 147.96 (C-2"), 127.65 (C-1'), 121.95 (C-6'), 121.09 (C-4", C-5"), 117.07 (C-5'), 111.96 (C-2'), 84.35 (C-5), 68.09 (C-4), 56.82 (C-7'), 40.13 (C-6); ν_{max} (smear) 3400, 1680, 1600, 1525, 1390, 1180, 1130, 800, 730 cm^{-1}; λ_{max} (CH_3OH) 283 nm (ε 3900); λ_{max} (CH_3OH-KOH) 216 nm (ε 13750), 255 (8100), 290 (5800); $[\alpha]^{23}$ (λ) 0° (280 nm), +5400° (296), 0° (319), -4400° (360) (c 0.1 in CH_3OH); CD (CH_3OH) λ_{ext} 300 nm (Δε 0), 325 (-16.1); HRDCIMS m/z 342.0161, $C_{13}H_{14}N_2O_3S_3$ requires 342.0166.
10. ^1H and ^{13}C NMR spectra were recorded on a Varian XL300 spectrometer. Mass spectra were recorded on either a VG7070E or a Kratos MS80RFA mass spectrometer.
11. Bax, A.; Summers, M.F. *J. Amer. Chem. Soc.* **1986**, *108*, 2093-4.
12. Compound **3**: ^1H NMR (300 MHz, D_2O) 7.60 (2H, bs, H-4", H-5"), 6.79 (1H, d, *J*=8.5 Hz, H-5'), 6.72 (1H, d, *J*=2.4 Hz, H-2'), 6.30 (1H, dd, *J*=2.4, 8.5 Hz, H-6'), 4.99 (1H, dd, *J*=3.8, 10.2 Hz, H-5), 3.75 (3H, s, 3H-7'), 3.24 (1H, dd, *J*=10.2, 14.5 Hz, H-6α), 3.16 (1H, dd, *J*=3.8, 14.5 Hz, H-6β); ^{13}C NMR (75 MHz, CD_3OD + 4% TFA) 149.71 (C-3'), 149.52 (C-4'), 146.85 (C-2"), 130.62 (C-1'), 121.63 (C-4", C-5", C-6'), 117.03 (C-5'), 111.74 (C-2'), 74.47 (C-5), 60.78 (C-4), 56.82 (C-7'), 40.13 (C-6); ν_{max} (smear) 3180, 1680, 1600, 1520, 1440, 1200, 1140, 840, 800, 720 cm^{-1}; λ_{max} (CH_3OH) 220 nm (ε 10300), 283 (3400); λ_{max} (CH_3OH-KOH) 213 nm (ε 10900), 289 (5200); $[\alpha]^{23}$ (λ) 0° (227 nm), +4150° (245) (c 0.1 in CH_3OH); CD (CH_3OH) λ_{ext} 280 nm (Δε 0), 290 (-1.6); HREIMS m/z 342.0133, $C_{13}H_{14}N_2O_3S_3$ requires 342.0166.
13. ^1H NOE difference experiments were performed on undegassed solutions using a low power cycling method, as described in Kinns, M.; Sanders, J.K.M., *J. Mag. Reson.* **1984**, *56*, 518-20.
14. Haasnoot, C.A.G.; de Leeuw, F.A.A.M.; Altona, C. *Tetrahedron* **1980**, *36*, 2783-92.
15. Jackman, L.M.; Sternhell, S. "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry" **1969** 2nd Edition; Pergamon Press, Oxford.
16. Allinger, N.L.; Hickey, M.J.; Kao, J. *J. Am. Chem. Soc.* **1976**, *98*, 2741-5.
17. Anet, F.A.L.; Basus, V.J. *J. Mag. Reson.* **1978**, *32*, 339-43.
18. Compound **4**: ^1H NMR (300 MHz, CD_3OD + 4% TFA) 7.65 (1H, dd, *J*=2.0, 8.5 Hz, H-6'), 7.63 (1H, d, *J*=2.0 Hz, H-2'), 7.55 (2H, bs, H-4", H-5"), 6.97 (1H, d, *J*=8.5 Hz, H-5'), 3.91 (3H, s, 3H-7'); ^{13}C NMR (75 MHz, CD_3OD + 4% TFA) 155.55 (C-4'), 149.49 (C-3'), 127.52 (C-6'), 123.96 (C-4", C-5"), 116.67 (C-5'), 113.76 (C-2'), 56.82 (3H, C-7'), no signals were observed for C-1, C-1' and C-2".
19. Campaigne, E. In "The Chemistry of the Carbonyl Group", Vol 1; Editor Patai, S; Interscience Publishers, London, 1966; p938.

(Received in UK 13 April 1989)