Anthracycline derivatives from a marine-derived New Zealand *Streptomycete*

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Dedicated to Professor Rod Rickards on the occasion of his 70th birthday

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Abstract

Four new anthracycline derivatives, *(7S*9R*10R*)*-pyrromycin **1**, *(7R*9R*10R*)*-pyrromycin **2**, 1-hydroxyauramycin T **3**, 1-hydroxysulfurmycin T **4**, and the previously reported 1-hydroxyaclacinomycin B **5** were isolated from a New Zealand marine-derived *Streptomycete*. All five compounds were cytotoxic against the P388 murine leukaemia cell line.

Keywords: *Streptomycete*, anthracyclines, pyrromycins, New Zealand, cytotoxic, P388

Introduction

Actinomycetes are well known as producers of biologically active compounds with members of the genus *Streptomyces,* in particular, being prolific producers of metabolites of a diverse range of biological activities.[1] To date, a large number of anthracyclines have been isolated from a wide variety of *Streptomycetes*.[1] Some of these anthracyclines, such as adriamycin and daunomycin, have found uses in medicine for the treatment of some cancers.[2,3]

Results and Discussion

In continuing studies on bioactive natural products from New Zealand micro-organisms one isolate, a *Streptomyces* sp. (CANU Fox 21-2-6), was of interest. The crude EtOAc extract from the fermentation showed strong cytotoxicity against the murine P388 leukaemia cell line. Furthermore, dereplication of this extract using an in-house approach (HPLC, MS and UV profiles) indicated good potential for novel chemistry. Further investigation led to the isolation of four new anthracycline derivatives, *(7S*9S*10R*)*-pyrromycin **1**, *(7R*9R*10R*)*-pyrromycin

2, 1-hydroxyauramycin T **3**, 1-hydroxysulfurmycin T **4**, as well as the previously reported 1-hydroxyaclacinomycin B **5**.

The *Streptomycete*, (CANU Fox 21-2-6a), was isolated from well-weathered driftwood collected below the low-tide mark at the mouth of the Fox River on the West Coast of New Zealand. After 18 days fermentation in starch-casein broth under static conditions at 26°C, the EtOAc extract prepared from the fermentation broth was fractionated using flash reverse-phase (RP) chromatography. Repeated chromatography on DIOL of selected fractions from the RP column yielded **5**. Further purification of selected DIOL fractions by HPLC yielded **1**, **2**, **3** and **4**.

The molecular formula of **1**, a red solid, was deduced as $C_{30}H_{35}NO_{11}$ (fourteen double bond equivalents) by HRESIMS and from ^{13}C NMR data. The ^{1}H NMR spectrum of **1** in $CDCl_3$ showed three signals above 12 ppm, interpreted as hydrogen-bonded phenolic groups. The ^{13}C NMR experiment confirmed thirty carbon signals, five CH_3, including one OCH_3 and two NCH_3, three CH_2, nine CH, and thirteen quaternary carbon signals.

The UV-visible spectrum of **1,** maxima at 202, 234, 258, 290 and 492 nm, was characteristic of a quinone.[4] That spectral data, coupled with the ^{1}H and ^{13}C NMR chemical shifts, indicated the presence of an anthraquinone moiety containing three phenolic groups. In addition to the fourteen signals that could be assigned to the anthraquinone system, a single anomeric signal at δ_C 101.9 suggested that **1** was also a mono-glycoside.

The partial connectivities from COSY and HSQC NMR experiments established four subunits, **a**, **b**, **c**, and **d** as shown in Figure 1.

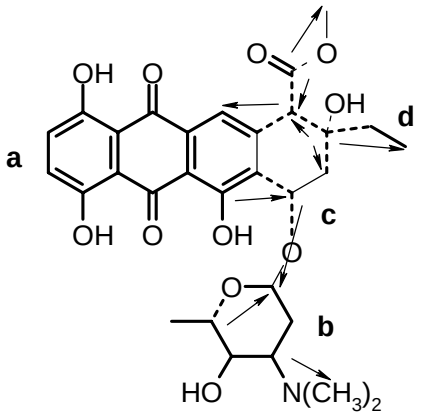


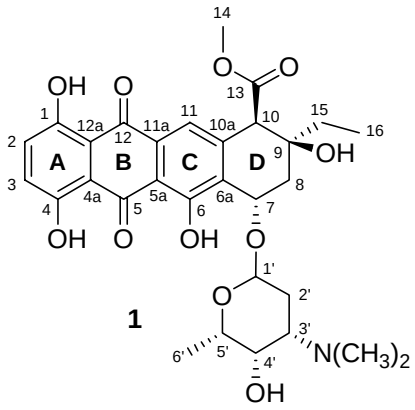
Figure 1. Substructures **a-d** and important CIGAR correlations for **1**.

The assembly of these partial structures followed from a long range ^{1}H-^{13}C correlation NMR (CIGAR) experiment.[5] Correlations were observed from the NCH_3 signals to a carbon in subunit **b**. The anomeric proton (δ_H 5.56) showed strong correlations to two oxygenated CH's (δ_C 72.9 and 68.2) thus linking subunits **b** and **c** and closing the glycosidic ring. A correlation from H7 (δ_H 5.10) to an oxygenated aromatic carbon (δ_C 163.6) connected the subunits **a** and **c**. The H11 aromatic proton (δ_H 7.51) correlated to C10 (δ_C 58.5). H8, H10 and H16 (δ_H 2.28, 2.56, 4.07 and

1.11) all correlated to an oxygenated quaternary carbon (δ_C 72.2) connecting subunit **d**. The *O*CH_3 group and H10 both correlated to a carbonyl (δ_C 172.8) completing the assignment of the planar structure of **1** as a pyrromycin derivative.[6]

Examination of the coupling constants and a 2D NOE experiment enabled assignment of the relative stereochemistry of the anthraquinone moiety and the sugar residue in **1**. The starting point was the H7 proton (δ_H 5.10) which showed a single $^3J_{HH}$ of 5 Hz to H8a (δ_H 2.56) enabling placement of H8a in a pseudo-axial position and H8b in a pseudo-equatorial position. NOE correlations were observed from H15a (δ_H 1.56) to H8b (δ_H 2.28) and from H15b (δ_H 1.76) to H10 (δ_H 4.07) indicating that the ethyl side chain and the carboxyl groups were both pseudo-axial to give the relative stereochemistry of **1** as (*7S*9S*10R**)-pyrromycin. The parent pyrromycin is the (*7S9R10R)*-stereoisomer.[6] This stereoisomer also allows for the formation of the observed hydrogen-bond between the D-ring hydroxyl and carboxyl groups and completed the stereochemical assignment of the anthraquinone.

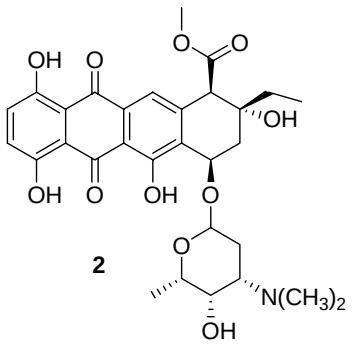
Based on the NOESY data the sugar could be identified as rhodosamine. Particularly important was the NOESY correlation seen from H5' (δ_H 4.26) to H3' (δ_H 3.52) which placed these two protons in axial positions. If one or both of these protons had been in an equatorial position then NOESY correlations would not be observed. H3' also showed NOESY correlations to H4' (δ_H 3.96) and H2' (δ_H 2.08) as expected for *vicinal* axial/equatorial orientations. The final stereocentre in the rhodosamine sugar residue was at the anomeric center. The anomeric proton (H1'; δ_H 5.55) showed no NOESY correlations to either axial proton at H3' and H5' and so could be assigned to an equatorial orientation in keeping with the only observed $^3J_{HH}$ coupling to the H2' protons of 2.5 Hz. This asssigned stereochemistry confirmed the sugar residue as rhodosamine *(1'R*3'S*4'S*5'S*).*



1

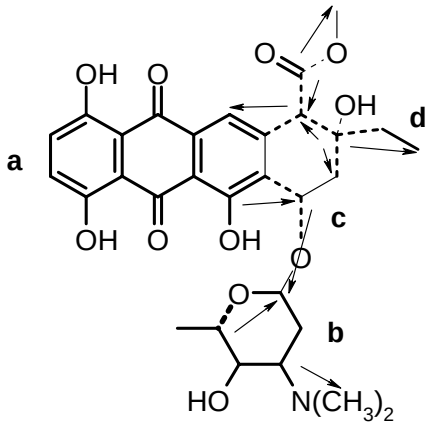
*(7R*9R*10R*)*-pyrromycin **2**, also $C_{30}H_{35}NO_{11}$ from HRESIMS, displayed almost identical spectral properties to **1**, but the observed coupling patterns for H7 and H8 were more complex. H7 (δ_H 4.96) displayed as a triplet ($^3J_{HH}$ 6.5 Hz) and the H8 protons (δ_H 2.62, 2.32) multiplets ($^3J_{HH}$ 7.5, 8 Hz and $^2J_{HH}$ 14 Hz). The H15 ethyl group signals (δ_H 1.49) merged into a multiplet and both H15 and H16 (δ_H 0.98) had been shifted upfield. The variations observed in the ^{1}H

NMR spectrum of **2** were attributed to a change in the stereochemistry of the D-ring. The change in the multiplicity seen for H7 indicated that the D-ring was in a different conformation allowing both couplings to the H8 protons to be observed. The slight increase in polarity and upfield shift of the ethyl protons suggested that the potential for a hydrogen-bond between the D-ring hydroxyl and carboxyl groups was removed thereby placing both groups in pseudo-axial positions and limiting the possible stereoisomers to just two. Energy minimisation using the MM2 parameters in Chem3D® (CambridgeSoft®) showed dihedral angles (H7-C7-C8-H8a and H7-C7-C8-H8b) of 55° and 170° and, 33° and 145° respectively for the possible stereoisomers. Application of the Karplus equation[7] and comparison to experimental values (8 and 7.5 Hz) limited the possibilities to just one stereoisomer, *(7R*9R*10R*)*-pyrromycin **2**.



2

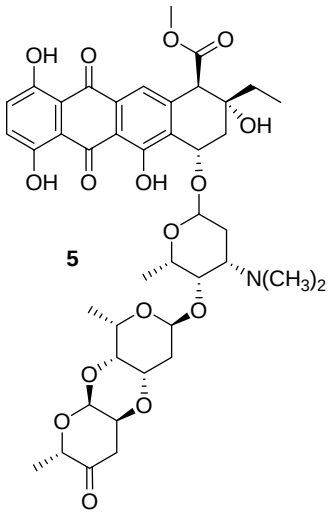
1-Hydroxyauramycin T **3**, $C_{29}H_{33}NO_{11}$ from HRESIMS, also displayed very similar spectral properties to **1**. However, for **3**, the signals arising from the ethyl group in the ^{1}H NMR spectrum of **1** were replaced with a singlet methyl resonating at δ_H 1.43. This, when coupled with the necessary decrease in mass and change in molecular formula allowed **3** to be assigned as the methyl derivative of **1**. The ^{1}H NMR signals for the H7 and H8 protons showed minor variation from those observed for **1** suggesting that the relative stereochemistry is identical to that established for **1**.



In the ^{1}H NMR spectrum of **4**, $C_{31}H_{35}NO_{12}$ from HRESIMS, a methyl ketone (δ_H 2.25) and a pair of isolated doublets (δ_H 2.66, 3.04) replaced the ethyl group signals seen in the ^{1}H NMR

spectrum of **1**. This methyl group showed a long range TOCSY correlation to the isolated doublets and a CIGAR correlation to a carbonyl group (δ_C 210.6) to establish the structure and relative stereochemistry as 1-hydroxysulfurmycin T **4**.

1-Hydroxyaclacinomycin B (**5**) was identified by comparison of NMR data to those in the literature.[8,9]



5

The anthracycline pyrromycin core is well established in a range of mono-, di- and tri-glycosides, but the auramycins and sulfurmycins have previously only been reported as tri-glycosides.[9,10] This is the first report of mono-glycosylated auramycins and sulfurmycins.

The bioactivity evaluation showed that all four compounds displayed very good cytotoxicity against P388 cultured cells with ID_{50} values ranging from 0.4 – 0.06 μg/mL.

Experimental Section

General Procedures. UV spectra were recorded with a Hewlett Packard 8452 diode array spectrometer. Optical rotation values were obtained on a Perkin Elmer 341 polarimeter. ^{1}H, ^{13}C-APT and 2D NMR (^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C CIGAR) spectra were recorded on a Varian INOVA 500 MHz spectrometer. ESI mass spectra were acquired using a Micromass TOF LCT mass spectrometer. Column chromatography used 40 μM Prep LC Bakerbond Octadecyl (C_{18}) and 40 μM Prep LC Bakerbond Diol. Solvents for extraction and chromatography were distilled prior to use. HPLC was carried out using a Shimadzu LC-10ADvp equipped with an SPD-M10Avp photodiode array detector.

Isolation of the microbial strain. The outer layer of driftwood material collected at the mouth of the Fox River on the West Coast of New Zealand was scraped clean to remove superficial organisms. The driftwood was then thinly sliced and sections placed on agar plates containing isolation medium (15 g/L agar and fresh seawater (1 L)) plus the antibiotics chloramphenicol,

ampicillin (100 μg/L) and streptomycin sulphate (50 μg/L)). Microbial colonies growing out of the wood sections were transferred to a medium for sporulation (Gibco PDA 39 g/L and fresh seawater (1 L)) containing the antibiotics chloramphenicol, ampicillin, (100 μg/L) and streptomycin sulphate (50 μg/L)).

Fermentation and isolation. *Streptomyces* sp. (CANU Fox 21-2-6a) was fermented in starch-casein broth (glycerol 10 g/L, peptone 140 0.3 g/L, KNO_3 2 g/L, NaCl 2 g/L, K_2HPO_4 2 g/L, $MgSO_4.7H_2O$ 50 mg/L, $CaCO_3$ 20 mg/L, $FeSO_4.7H_2O$ 10 mg/L, distilled water 1 L, pH 7.0) under static conditions at 26°C for 18 days. The culture broth (10 L) was homogenised and filtered through celite. The cellular material was extracted by stirring with ethyl acetate overnight (3 x 200 mL) as was the culture filtrate (3 x 2 L). The combined ethyl acetate extracts were concentrated under vacuum yielding a deep red viscous oil (7 mL). The residue was fractionated on C_{18} using a steep, stepped solvent gradient (MeOH/H_2O (10%) to MeOH to DCM). The fractions that eluted between MeOH and DCM were combined and repeatedly chromatographed on DIOL with gradient elution by petroleum ether/DCM to EtOAc/DCM to MeOH/EtOAc. Fractions were purified by analytical HPLC on C_{18} and eluted with (MeCN/H_2O (32 %)(0.05 % TFA)) to yield *(7S*9S*10R*)*-pyrromycin (**1**) (2.0 mg), *(7R*9R*10R*)*-pyrromycin (**2**) (1.4 mg), 1-hydroxyauramycin T (**3**) (1.4 mg), 1-hydroxysulfurmycin T (**4**) (1.4 mg).

***(7S*9R*10R*)*-Pyrromycin (1).** Deep red solid; $[\alpha]^{20}_D$ = +128.0° (0.25 mg/mL, MeOH); UV (MeOH) λ_{max} 202, 234, 258, 290, 492; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.51 (s, 1H, H11), 7.08 (s, 2H, H2 H3), 5.56 (d, *J* = 2.5 Hz, 1H, H1'), 5.10 (d, *J* = 5 Hz, 1H, H7), 4.26 (q, *J* = 6.5, 13 Hz, 1H, H5'), 4.07 (s, 1H, H10), 3.96 (s, 1H, H4'), 3.75 (s, 3H, 14*O*Me), 3.52 (m, 1H, H3'), 2.87 (s, 6H, 7'*N*$(Me)_2$), 2.56 (dd, *J* = 5, 15 Hz, 1H, H8ax), 2.28 (d, *J* = 15 Hz, 2H, H8eq H2'ax), 2.08 (dt, *J* = 4, 12.5 Hz, 1H, H2'eq), 1.76 (m, 1H, H15β), 1.56 (m, 1H, H15α), 1.33 (d, *J* = 6 Hz, 3H, 6'Me), 1.11 (t, *J* = 7 Hz, 3H, 16Me); ^{13}C NMR (CD_3OD 125 Mhz) δ 191.6 (C5), 186.7 (C12), 172.8 (C13), 163.6 (C6), 159.4 (C1), 159.0 (C4), 144.1 (C10a), 133.8 (C11a), 133.1 (C6a), 131.5 (C3), 131.1 (C2), 121.2 (C11), 116.0 (C5a), 113.4 (C12a, C4a), 101.9 (C1'), 72.9 (C7), 72.2 (C9), 68.2 (C5'), 66.1 (C4'), 63.9 (C3'), 58.5 (C10), 53.5 (C14 *O*Me), 40.7 (*N*Me), 36.2 (C8), 33.6 (C15), 28.1 (C2'), 17.2 (C6'), 7.5 (C16); HRESIMS *m/z* 585.2218 (calcd for $C_{30}H_{35}NO_{11}$ 585.2210).

***(7R*9R*10R*)*-Pyrromycin (2).** Deep red solid; $[\alpha]^{20}_D$ = +224.0° (0.125 mg/mL, MeOH); UV (MeOH) λ_{max} 202, 234, 258, 290, 492; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.60 (s, 1H, H11), 7.38 (s, 2H, H2 H3), 5.58 (br s, 1H, H1'), 4.96 (t, *J* = 2.5 Hz, 1H, H7), 4.17 (q, *J* = 7, 12.5 Hz, 1H, H5'), 3.99 (s, 1H, H10), 3.95 (br s, 1H, H4'), 3.78 (s, 3H, 14*O*Me), 3.52 (br d, *J* = 11 Hz, 1H, H3'), 2.91 (s, 3H, 7'*N*Me), 2.85 (s, 3H, 7'*N*Me), 2.62 (dd, *J* = 7.5, 14 Hz, 1H, H8ax), 2.32 (dd, *J* = 8, 14 Hz, 1H, H8eq), 2.29 (dd, *J* = 8, 14 Hz, 1H, H2'ax), 2.06 (br s, 1H, H2'eq), 1.49 (m, 2H, H15), 1.33 (d, *J* = 6 Hz, 3H, 6'Me), 0.98 (t, *J* = 7 Hz, 3H, 16Me); HRESIMS *m/z* 585.2211 (calcd for $C_{30}H_{35}NO_{11}$ 585.2210).

1-Hydroxyauramycin T (3). Deep red solid; UV (MeOH) λ_{max} 202, 234, 258, 290, 492; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.67 (s, 1H, H11), 7.37 (s, 2H, H2 H3), 5.56 (br s, 1H, H1'), 5.13 (d, *J* = 5 Hz, 1H, H7), 4.24 (q, *J* = 7.5, 14 Hz, 1H, H5'), 4.09 (s, 1H, H10), 3.91 (br s, 1H, H4'), 3.71 (s, 3H, 14*O*Me), 3.46 (br d, *J* = 11.5 Hz, 1H, H3'), 2.88 (br s, 3H, 7'*N*Me), 2.78 (br s, 3H, 7'*N*Me), 2.58 (dd, *J* = 6, 15 Hz, 1H, H8ax), 2.20 (d, *J* = 15 Hz, 1H, H8eq), 2.15 (m, 1H, H2'ax), 2.02 (dt, *J* = 3.5, 12.5 Hz, 1H, H2'eq), 1.38 (s, 3H, 16Me), 1.31 (d, *J* = 6.5 Hz, 3H, 6'Me); HRESIMS *m/z* 571.2059 (calcd for $C_{29}H_{33}NO_{11}$ 571.2054).

1-Hydroxysulfurmycin T (4). Deep red solid; UV (MeOH) λ_{max} 202, 234, 258, 290, 492; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.71 (s, 1H, H11), 7.36 (s, 2H, H2 H3), 5.49 (br s, 1H, H1'), 5.10 (br s, 1H, H7), 4.26 (q, *J* = 7, 13 Hz, 1H, H5'), 4.24 (s, 1H, H10), 3.93 (br s, 1H, H4'), 3.71 (s, 3H, 14*O*Me), 3.46 (br d, *J* = 11.5, 1H, H3'), 3.04 (d, *J* = 16.5 Hz, 1H, H15α), 2.88 (br s, 3H, 7'*N*Me), 2.77 (br s, 3H, 7'*N*Me), 2.66 (d, *J* = 16.5 Hz, 1H, H15β), 2.53 (br s, 2H, H8), 2.25 (s, 3H, 17Me), 2.15 (m, 1H, H2'ax), 2.03 (m, 1H, H2'eq), 1.32 (d, *J* = 6.5 Hz, 3H, 6'Me); HRESIMS *m/z* 613.2158 (calcd for $C_{31}H_{35}NO_{12}$ 613.2159).

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