Blanchaquinone: A New Anthraquinone from an Australian *Streptomyces* sp.

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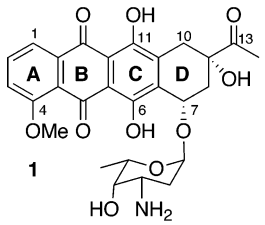
Chemical analysis of an Australian *Streptomyces* species yielded a range of known anthracyclines and biosynthetically related metabolites, including daunomycin (**1**), ϵ-rhodomycinone (**2**), 11-hydroxyauramycinone (**3**), 11-hydroxysulfurmycinone (**4**), aklavinone (**5**), bisanhydro-γ-rhodomycinone (**6**), and the anthraquinone **7**, as well as the hitherto unreported blanchaquinone (**8**). The structure assigned to **8** was secured by detailed spectroscopic analysis and correlation to known analogues, such as the anthraquinone **7**. This account also represents the first natural occurrence of **3**, **4**, and **7** and the first spectroscopic characterization of 11-hydroxysulfurmycinone (**4**).

The anthracyclines are a group of antibiotic glycosides produced by various *Streptomyces* species that include the early cancer chemotherapy agent daunomycin (**1**).[1] *Streptomyces* species are also known as a source of anthraquinones with varying levels of oxidation and aromatic substitution, many of which display biological activities. In the course of our investigations into Australian microbes as a potential source of new antibiotics a *Streptomyces* strain (MST-77755) was found to yield a range of anthracyclines and biosynthetically related metabolites, including the previously unreported anthraquinone blanchaquinone (**8**).

The MeOH extract of the mycelium of a *Streptomyces* strain (MST-77755) was noted to exhibit significant antibacterial and cytotoxic properties. Repeated chromatographic fractionation of this extract yielded eight pure compounds, six of which were identified on the basis of spectroscopic comparisons to literature data as the known compounds daunomycin (**1**),[2] ϵ-rhodomycinone (**2**),[3] 11-hydroxyauramycinone (**3**),[4] aklavinone (**5**),[5] bisanhydro-γ-rhodomycinone (**6**),[6] and the anthraquinone **7**.[7] Other compounds isolated and identified during this study included the known semisynthetic product 11-hydroxysulfurmycinone (**4**) and a new anthraquinone, blanchaquinone (**8**).

Although known as a semisynthetic product derived from enzymatic hydroxylation of sulfurmycinone[8] (**9**), the identification of 11-hydroxysulfurmycinone (**4**) was problematic due to the paucity of published spectroscopic data; indeed, the existing literature proof for **4** seems to rest entirely on a single TLC analysis! Our reisolation of 11-hydroxysulfurmycinone (**4**) represents only the second published record of this compound and its first reported occurrence as a natural product. Reisolation provided an opportunity to revisit the structure assignment with a more complete spectroscopic data set.

HRESI(+)MS of 11-hydroxysulfurmycinone (**4**) yielded a pseudo-molecular ion $[M + Na]^+$ corresponding to a molecular formula ($C_{23}H_{20}O_{10}$, Δmmu = 0.7) requiring 14 DBE. The ^{1}H NMR ($CDCl_3$) data for **4** indicated an excellent correlation to 11-hydroxyauramycinone (**3**). Minor ^{1}H NMR chemical shift differences were observed between **4** and **3** about the D ring, with major differences suggesting replacement of the tertiary methyl (δ 1.44, s) in **3** with a



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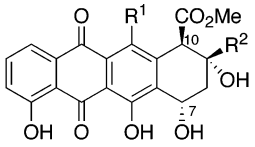
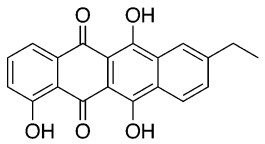


2 R^1= OH, R^2 = Et
3 R^1= OH, R^2 = Me
4 R^1= OH, R^2 = CH_2COCH_3
5 R^1= H, R^2 = Et
10 R^1= H, R^2 = Me
9 R^1= H, R^2 = CH_2COCH_3



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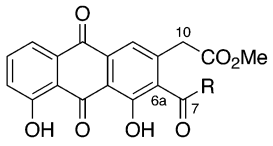
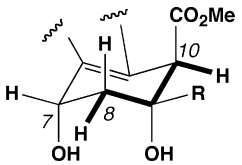


7 R = Et
8 R = *n*-butyl

2-oxopropan-1-yl (CH_2COCH_3: δ 2.86 and 2.65, AB_q, J = 17.4 Hz; CH_2COCH_3: δ 2.28, s) substituent in **4**. Such a side-chain was identical to that of sulfurmycinone[9] (**9**), consistent with **4** being the reported enzymatic hydroxylation product of **9**, namely, 11-hydroxysulfurmycinone. Careful analysis of the ^{1}H NMR coupling constants for **4** (see Figure 1), including a noteworthy $J_{8eq,10}$ W coupling, confirmed a common relative stereochemistry between **4** and **9**. Although the D ring in such systems does not strictly adopt a chair conformation as displayed in Figure 1, the observation of a long-range coupling is nonetheless consistent with the W "type" configuration as indicated. Since the absolute configuration for sulfurmycinone (**9**) has been



J (Hz)	**3**	**4**
$J_{7eq,8ax}$	5.0	5.0
$J_{7eq,8eq}$	1.5	1.7
$J_{8ax,8eq}$	15	14.5
$J_{8eq,10eq}$	1.5	1.5

Figure 1. ^{1}H NMR ($CDCl_3$, 400 MHz) J values about ring D in 11-hydroxyauramycinone (**3**) and 11-hydroxysulfurmycinone (**4**).

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described[9] and 11-hydroxysulfurmycinone (**4**) has been derived directly from **9**, the absolute configuration for **4** is as indicated.

HRESI(+)MS analysis of blanchaquinone (**8**) yielded a pseudomolecular ion $[M + Na]^+$ corresponding to a molecular formula ($C_{22}H_{20}O_7$, Δmmu = −0.7) requiring 13 DBE. The UV and IR spectra for **8** correlated exceptionally well with those for the anthraquinone **7**. Further spectroscopic comparisons between **7** and **8** revealed common ^{1}H NMR features spanning all aromatic protons, the hydrogen-bonded phenolics, and the methyl acetate side-chain. From the evidence presented above it was concluded that **8** was a closely related $(CH_2)_2$ homologue of **7**. The only significant difference in the ^{1}H NMR data between **7** and **8** focused around the C-6a side-chain. The propanoyl side-chain so evident in **7** had been replaced by a pentanoyl moiety [δ 2.98 (t, 7.5 Hz), 1.69 (m), 1.40 (m), and 0.94 (t, 7.3 Hz)] in **8**. Hence the structure for blanchaquinone (**8**) was proposed as shown.

Of the eight compounds isolated, daunomycin (**1**), ϵ-rhodomycinone (**2**), and aklavinone (**5**) are all commonly found in *Streptomyces* species, with the latter two being intermediates in the biosynthesis of the former.[10] To date, 11-hydroxyauramycinone (**3**) and 11-hydroxysulfurmycinone (**4**) have only been reported as products generated by microbial conversion of authentic auramycinone (**10**) and sulfurmycinone (**9**) by an *S. coeleorubidus* strain.[8] Similarly, while the anthraquinone **7** has been isolated several times, these have all arisen from mutant *S. galileus* species blocked in anthracycline production.[7,11,12] Our study represents (a) the first isolation of the anthraquinone **7** from a natural, nonmutant *Streptomyces* strain, (b) the first natural occurrence of 11-hydroxyauramycinone (**3**) and 11-hydroxysulfurmycinone (**4**), (c) the first characterization of 11-hydroxysulfurmycinone (**4**), and (d) the first report of the new anthraquinone, blanchaquinone (**8**).

The pure compounds **1**−**8** were assessed for biological activity against a number of assays. Daunomycin (**1**) was the only metabolite isolated from this *Streptomyces* strain (MST-77755) that displayed significant antibacterial properties, returning an MIC against *Bacillus subtilis* of 0.6 μg/mL. In contrast, several compounds displayed significant cytotoxic properties (NS-1), these being daunomycin (**1**), ϵ-rhodomycinone (**2**), 11-hydroxyauramycinone (**3**), 11-hydroxysulfurmycinone (**4**), bisanhydro-γ-rhodomycinone (**6**), and the two anthraquinones **7** and **8** (LD_{99} values were 0.1, 0.8, 6, 13, 13, 3, and 6 μg/mL respectively).

Experimental Section

General Experimental Procedures. General experimental procedures are as for previous work,[13] except for the following: Initial HPLC work was carried out on a system consisting of two Shimadzu LC-8A preparative liquid chromatographs with static mixer, a Shimadzu SPD-M10AVP diode array detector, and a Shimadzu SCL-10AVP system controller. UV−vis absorption spectra were obtained using a Shimadzu UV-1650PC spectrophotometer, while infrared (IR) spectra were acquired using a Shimadzu FTIR-8400 spectrometer.

Biological Material. The *Streptomyces* strain (MST-77755) was isolated from a soil sample collected near Blanchetown, South Australia.

Assay Details. Cytotoxic activity was determined in a microtiter plate, cell proliferation assay. Briefly, murine NS-1 cells in RPMI 1640 medium (200 μL, 5 × 10^4 cells/mL), supplemented with 1 mM sodium pyruvate and 5% (v/v) newborn calf serum, were added to the wells of a microtiter plate containing serial 2-fold dilutions of the test compound. The plates were incubated at 37 °C in the presence of 5% CO_2. A qualitative assessment of cell proliferation was made at 72 h, with the LD_{99} determined as the lowest concentration of the test compound at which no cell proliferation was observed.

Antibacterial activity was determined in an agar-based, microtiter plate assay. Briefly, an aliquot of an overnight fermentation of *Bacillus subtilis* (ATCC 6633) was applied to the surface of an agar matrix that contained the test compound, then incubated at 28 °C. A qualitative assessment of bacterial growth was made at 24 h, with the MIC determined as the lowest concentration of the test compound at which no growth of bacteria was observed.

Extraction and Isolation. A liquid fermentation (50 mL in each of 20 × 250 mL Erlenmeyer flasks, shaken at 300 rpm at 28 °C for 7 days in ISP2 media) was filtered to collect the mycelium, which was extracted with MeOH (∼500 mL). This extract was concentrated in vacuo to an aqueous residue that was diluted with H_2O to a final volume of 1000 mL. This aqueous material was sequentially eluted through two parallel C_{18} SPE cartridges (2 × 10 g, Varian HF C_{18}), first with 50% MeOH/H_2O (2 × 40 mL), then MeOH (2 × 40 mL). Evaporation of the combined MeOH eluants gave a residue that was subjected to preparative HPLC (60 mL/min with gradient elution of 40% to 100% MeCN/H_2O over 20 min followed by MeCN for 10 min, through a 5 μm Platinum EPS C_{18} 50 × 100 mm column). One hundred 20 mL fractions were collected, concentrated in vacuo, and combined on the basis of analytical HPLC and bioassay analysis.

An early eluting bioactive and highly colored (red) fraction was further partitioned by C_{18} HPLC (2.5 mL/min eluting with 45−65% MeCN/H_2O, 5 μm Phenomenex LUNA C_{18}(2) 21.2 × 150 mm column) to give daunomycin (**1**) (38 mg, 4.1%) and aklavinone (**5**) (2.0 mg, 0.2%), plus an unresolved two-component mixture. The latter mixture was resolved by preparative TLC (eluting with 50:50:1 EtOAc/hexane/HCO_2H, Merck Kieselgel 60 GF_{254} 20 × 20 cm glass plates, 0.1 mm thickness) to yield 11-hydroxyauramycinone (**3**) (0.6 mg, 0.06%) and 11-hydroxysulfurmycinone (**4**) (0.6 mg, 0.06%). Later eluting material from the crude extract fractionation was partitioned by C_{18} HPLC (2.5 mL/min eluting with 70−100% MeCN/H_2O, 5 μm Phenomenex LUNA C_{18}(2) 21.2 × 150 mm column) to yield ϵ-rhodomycinone **2** (73 mg, 7.8%), the anthraquinone **7** (1.2 mg, 0.12%), blanchaquinone (**8**) (0.7 mg, 0.08%), and bisanhydro-γ-rhodomycinone (**6**) (0.5 mg, 0.05%). All percent yields reflect the estimated percent content of each metabolite in the total crude extractables. The relative order of elution of isolated compounds on analytical C_8 HPLC is as follows: daunomycin, 11-hydroxysulfurmycinone, 11-hydroxyauramycinone, ϵ-rhodomycinone, the anthraquinone **7**, blanchaquinone, and bisanhydro-γ-rhodomycinone.

Daunomycin (1): red solid; HRESI(+)MS *m/z* 528.1866 ($[M + Na]^+$, Δmmu = 0.3); $[\alpha]_D$, UV, ^{1}H and ^{13}C NMR were in good agreement with the literature values.[1,2,14]

ϵ-Rhodomycinone (2): red solid; HRESI(+)MS *m/z* 451.1002 ($[M + Na]^+$, Δmmu = −0.3); UV, ^{1}H and ^{13}C NMR were in good agreement with the literature values.[3,15]

11-Hydroxyauramycinone (3): red solid; HRESI(+)MS *m/z* 437.0850 ($[M + Na]^+$, Δmmu = 0.2); $[\alpha]_D$, UV, and ^{1}H NMR were in good agreement with the literature values.[4]

11-Hydroxysulfurmycinone (4): red solid; $[\alpha]^{20}_D$ +545°[16] (*c* 0.008, $CHCl_3$); UV (MeOH) λ_{max} (ϵ) 234 (37 000), 253 (22 000), 291 (7600), 492 (11 400), 526 (7700), 579 (1000) nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.42, 12.93, 12.19 (all 1H, s, OH), 7.87 (1H, dd, J = 1.2, 7.3 Hz, H-1), 7.68 (1H, dd, J = 7.3, 8.5 Hz, H-2), 7.30 (1H, dd, J = 1.2, 8.5 Hz, H-3), 5.15 (1H, dd, J = 4.6, 8.8 Hz, H-7), 4.50 (1H, d, J = 1.5 Hz, H-10), 4.10 (1H, d, J = 9.3 Hz, 7-OH), 3.67 (3H, s, CO_2CH_3), 2.86, 2.65 (both 1H, d, J = 17.4 Hz, CH_2COCH_3), 2.28 (3H, s, CH_2COCH_3), 2.25 (1H, ddd, J = 1.5, 1.7, 14.5 Hz, H-8_{eq}), 2.07 (1H, dd, J = 5.0, 14.5 Hz, H-8_{ax}); ESI(+)MS *m/z* 935 $[2M + Na]^+$, 479 $[M + Na]^+$; HRESI(+)MS *m/z* 479.0947 ($[M + Na]^+$, Δmmu = 0.7).

Aklavinone (5): yellow solid; HRESI(−)MS *m/z* 411.1071 ($[M - H]^-$, Δmmu = 0.9); $[\alpha]_D$, UV, and ^{1}H NMR were in good agreement with the literature values.[5,17,18]

Bisanhydro-γ-rhodomycinone (6): red solid; HRESI(−)-MS *m*/*z* 333.0767 ($[M - H]^-$, Δmmu = 0.4); UV and ^{1}H NMR were in good agreement with the literature values.[6,19]

Anthraquinone 7: yellow solid; HRESI(+)MS *m*/*z* 391.0792 ($[M + Na]^+$, Δmmu = −0.2); UV, IR, and ^{1}H NMR were in good agreement with the literature values.[7]

Blanchaquinone (8): yellow solid; UV (cyclohexane) λ_{max} (ϵ) 232 (74 000), 255 (45 000), 290 (24 000), 435 (26 000) nm; IR ($CHCl_3$) 2928, 2856, 1736, 1694, 1682, 1624, 1450, 1375, 1286, 1265 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.45, 11.93 (both 1H, s, OH), 7.83 (1H, dd, J = 1.1, 7.4 Hz, H-1), 7.71 (1H, s, H-11), 7.70, (1H, dd, J = 7.4, 8.5 Hz, H-2), 7.32 (1H, dd, J = 1.2, 7.8 Hz, H-3), 3.77 (2H, s, H-10), 3.69 (3H, s, CO_2CH_3), 2.98 (2H, t, J = 7.5 Hz, $COCH_2CH_2CH_2CH_3$), 1.69 (2H, m, J = 7.5 Hz, $COCH_2CH_2CH_2CH_3$), 1.40 (2H, m, J = 7.5 Hz, $COCH_2CH_2CH_2CH_3$), 0.94 (3H, t, J = 7.3 Hz, $COCH_2CH_2CH_2CH_3$); ESI(+)MS *m*/*z* 815 $[2M + Na]^+$, 419 $[M + Na]^+$; HRESI(+)MS *m*/*z* 419.1100 ($[M + Na]^+$, Δmmu = −0.7).

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