MM 47755, A NEW BENZ[*a*]ANTHRACENE ANTIBIOTIC FROM A STREPTOMYCETE

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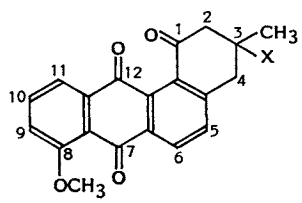
We wish to report the isolation and structure elucidation of 3,4-dihydro-3-hydroxy-8-methoxy-3-methyl-2*H*-benz[*a*]anthracene-1,7,12-trione (**1**) (MM 47755), a new antibiotic from a grey-sporing streptomycete isolated from soil. A minor metabolite from this culture was identified as the previously described[1] 1-hydroxy-8-methoxy-3-methylbenz[*a*]anthracene-7,12-dione (**2**).

The culture was maintained on slants of yeast-malt extract agar (ISP2) (Difco). Growth scraped from agar slants was used to inoculate the fermentation medium (100 ml) contained in 500-ml Erlenmeyer flasks closed with foam bungs. The fermentation medium consisted of soybean flour (Arkasoy 50) 1%, glycerol 2%, maltose 0.2%, $CoCl_2 \cdot 6H_2O$ 0.0005% and trace element solution 1%, pH 7.0. The trace element solution consisted of $CaCl_2 \cdot 2H_2O$ 1%, $MgCl_2 \cdot 6H_2O$ 1%, NaCl 1%, $FeCl_3$ 0.3%, $ZnCl_2$ 0.05%, $CuCl_2 \cdot 2H_2O$ 0.05% and $MnSO_4 \cdot 4H_2O$ 0.05% in distilled water.

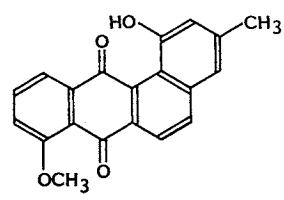
The fermentation flasks were incubated on a gyrotary shaker at 240 rpm and at 26°C. The flasks were harvested at 144 hours and the whole broth clarified by centrifugation. Fermentation and extraction samples were monitored by bioassay on *Staphylococcus aureus* V573. The clarified broth (2 litres) was extracted with EtOAc (3×500 ml), the extract concentrated and applied to a Sephadex LH-20 column (40×410 mm). Elution with MeOH - $CHCl_3$ (1:1) afforded an early peak of antibacterial activity in fractions 2~25. These were not investigated further. Continued elution gave another peak of antibacterial activity (fractions 28~40) and these fractions were bulked (130 ml) and evaporated to dryness.

Chromatography on silica gel, eluting with 20% MeOH in $CHCl_3$, afforded a single peak of antibacterial activity containing a mixture of **1** and **2** (250 mg). These were separated on a second silica gel column eluting with 2% MeOH in $CHCl_3$ to afford the benzanthracene (**2**) (Rf 0.70, 2% MeOH - $CHCl_3$, Merck Silica gel 60 plate, Art. No. 5719) as a yellow solid (2 mg): IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1665, 1620, 1590, 1275; ^1H NMR ($CDCl_3$) δ 2.50 (3H, s, CH_3), 4.08 (3H, s, OCH_3), 7.14 (1H, d, J=1.8 Hz, 2-H), 7.26 (1H, obscured by $CHCl_3$, 4-H), 7.36 (1H, dd, J=8.3 and 0.8 Hz, 9-H), 7.74 (1H, dd, J=8.3 and 7.9 Hz, 10-H), 7.95 (1H, dd, J=7.8 and 0.8 Hz, 11-H), 8.13 (1H, d, J=8.7 Hz, 5-H), 8.30 (1H, d, J=8.7 Hz, 6-H). This compound has been previously reported by MAEHR *et al.*[1] with a structure assigned on the basis of sparse evidence. Agreement with the reported[1] ^1H NMR data is good except for minor discrepancies in the shifts of 4-H and 9-H.

Continued elution with 30% MeOH in $CHCl_3$ furnished **1** (Rf 0.16, 2% MeOH - $CHCl_3$) as a yellow solid (91 mg). Recrystallisation from $CHCl_3$ - cyclohexane afforded yellow prisms: MP 176~177°C; *Anal* Calcd for $C_{20}H_{16}O_5$: C 71.4, H 4.80. Found: C 71.6, H 4.83; $[\alpha]_D^{20}$ −136° (*c* 0.04, MeOH); UV λ_{max}^{MeOH} nm (ε) 263 (33,450), 374 (5,500); $\lambda_{max}^{MeOH-HCl}$ 263 (33,450), 374 (5,500); $\lambda_{max}^{MeOH-NaOH}$ 224 (26,930), 245 (25,200), 263 (25,600), 340 (9,170); IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3500, 1705, 1665, 1590, 1305, 1275, 1110, 1070, 1025, 970, 920; ^{13}C NMR ($CDCl_3$) δ 29.7 (3-CH_3), 43.9 (C-4), 53.6 (C-2), 56.4 (8-OCH_3),



MM 47755 (**1**) X=OH
3 X=H



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72.3 (C-3), 117.2 (C-9), 119.4 (C-11), 120.3 (C-7a), 129.8 (C-6), 133.8 (C-5), 134.1 (C-12b), 134.8 (C-6a), 134.9 (C-12a), 135.4 (C-10), 137.5 (C-11a), 146.8 (C-4a), 159.7 (C-8), 181.2 (C-7), 184.5 (C-12), 197.3 (C-1). Unambiguous carbon assignments were made from a consideration of the 2D ^{1}H-^{13}C correlation spectroscopy (COSY) and correlation *via* long range coupling (COLOC) data.

Treatment of **1** with a mixture of aqueous 1 M potassium hydroxide and MeOH gave a quantitative conversion of **1** to the phenol (**2**) which, after acidification and extraction into $CHCl_3$, exhibited identical spectral properties to the natural product.

The chemical shifts and coupling constants observed for the aromatic protons were consistant with an anthraquinone structure and similar to those reported by MAEHR *et al.*[1] for benzanthracene (**3**). The UV spectrum was also very similar. IR data obtained on **1** indicated the presence of a conjugated carbonyl group at 1705 cm^{-1} (δ_c 197.3) and this, together with the fact that the methyl group had moved upfield to δ 1.50 (see Table 1) compared to its position in **2** of δ 2.50, suggested that ring A was now in a reduced form. This methyl singlet must be connected to the oxygen-bearing quaternary sp^3 carbon found at δ 72.3 and, since there is no coupling between the two methylene groups in ring A, the structure must be as represented in **1**. The corresponding 8-hydroxy compound, known as tetrangomycin, has been reported previously[2].

MM 47755 (**1**) exhibited no antimicrobial activity against Gram-negative bacteria or fungi at 100 μg/ml but weak activity was noted against some Gram-positive organisms, for example an MIC of 32 μg/ml against *Bacillus subtilis*.

Table 1. ^{1}H NMR data of **1** in $CDCl_3$ at 250 MHz (J in Hz).

Assignment	δ	
2-H_2	3.09, 2.99	ABq, J=14.9
3-CH_3	1.50	s
3-OH	2.16	br s
4-H_2	3.16	s
5-H	7.51	d, J=8.0
6-H	8.27	d, J=8.0
8-OCH_3	4.03	s
9-H	7.30	dd, J=7.6, 1.8
10-H	7.70	dd, J=7.6, 7.6
11-H	7.75	dd, J=7.6, 1.8

Addendum in Proof

MM 47755 is identical to the recently reported[3] 6-deoxy-8-*O*-methylrabelomycin.

References

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