Notes

FUJIANMYCINS A AND B, NEW BENZ[a]ANTHRAQUINONE ANTIBIOTICS FROM A *STREPTOMYCES* SPECIES

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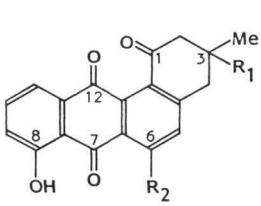
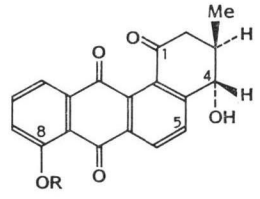
The small group of antibiotics from *Streptomyces* species which are derivatives of the angularly-fused benz[a]anthraquinone structure includes tetrangomycin (**1**)[1,2], its aromatization product tetrangulol[1,2], and its 6-hydroxy-analogue rabelomycin (**2**)[3]. From an unidentified *Streptomyces* species, obtained from a soil sample collected in Fujian Province during the course of a screening program conducted by the Institute of Antibiotics of the Chinese Academy of Medical Sciences, we have obtained two new members of this benz[a]anthraquinone group. We describe here the production, isolation and structural analysis of these compounds, named fujianmycins A (**4**) and B (**5**).

The inoculum of the *Streptomyces* sp. (IA-CAS isolate No. 114) was prepared in a medium containing beef extract 0.5%, mycological peptone 0.5%, yeast extract 0.5%, enzymatic digest of casein 0.3%, sodium chloride 0.15%, glucose 2.0% in distilled water adjusted to pH 7.5. Antibiotics were produced by growth at 28°C in baffled conical flasks (250 ml) containing a medium (100 ml) consisting of mycological peptone 0.4%, yeast extract 0.1%, Bacto-soytone 0.5%, glucose 2.5%, calcium carbonate 0.2% in distilled water. After fermentation for 72 hours, the broth (pH 7.8) was adjusted to pH 6.5 with hydrochloric acid (8 N) and filtered. The filtrate (7.8 liters) was extracted (×3) with equal volumes of EtOAc, and the extracts were dried and evaporated. The resulting tar (929 mg) in CH_2Cl_2 (100 ml) was washed successively with cold $NaHCO_3$ (2%, 2×100 ml) and water (2×50 ml), dried, and re-evaporated. The residue (116 mg) was chromatographed on Sephadex LH-20 in MeOH - $CHCl_3$ (1 : 99) to give a yellow fraction (32 mg) containing fujianmycins A and B. Preparative TLC on silica gel in toluene - EtOAc (3 : 7) gave fujianmycin A (10 mg) and fujianmycin B (9.8 mg).

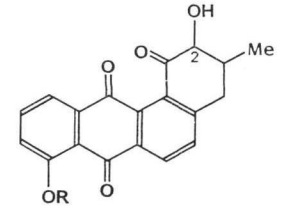
Fujianmycin A formed yellow needles from CH_2Cl_2 - hexane (1 : 1), mp 182~185°C. MS *m/z* 322.0817 (M, $C_{19}H_{14}O_5$ requires 322.0841), 304(M−H_2O), 294(M−CO), 280.0349 (M−CH_2-CHMe, $C_{16}H_8O_5$ requires 280.0372), 264.0410 (M−CHOHCHMe, $C_{16}H_8O_4$ requires 264.0423), 251.0331(M−C_4H_7O, $C_{15}H_7O_4$ requires 251.0344); UV λ_{max}^{MeOH} nm 273 and 402, altered to 267, 326 and 490 on addition of NaOH; IR λ_{max} (CH_2Cl_2 - CCl_4, 3 : 7) cm^{-1} 1640 (H-bonded quinone C=O), 1678 (quinone C=O), 1710 (ketonic C=O), 2500~3300 (H-bonded OH); $[\alpha]_D^{20}$ +17° (*c* ~0.08, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 1.26 (3H, d, $CHCH_3$), 2.35 (1H, m, 3-H), 2.58 (1H, dd, $J_{2ax,2eq}$=16.4 Hz, $J_{2ax,3}$=10.3 Hz, 2-H_{ax}), 3.14 (1H, dd, $J_{2eq,2ax}$=16.4 Hz, $J_{2eq,3}$=



1 R_1 = OH, R_2 = H
2 R_1 = R_2 = OH
3 R_1 = R_2 = H



4 R = H
5 R = Me



6 R = H or Me

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6.1 Hz, 2-H_{eq}), 4.51 (1H, d, $J_{4ax,3}$=9.3 Hz, 4-H_{ax}), 7.29 (1H, dd, *J*s not readable due to $CHCl_3$, 9-H), 7.68 (2H, m, 10-H and 11-H), 8.09 (1H, d with slight broadening, $J_{5,6}$=8.3 Hz, 5-H), 8.41 (1H, d, $J_{6,5}$=8.3 Hz, 6-H), 12.30 (1H, s, exchanged with D_2O, H-bonded OH).

Fujianmycin B crystallized as yellow needles from CH_2Cl_2 - hexane (1 : 1), mp 233~235°C.

Anal Calcd for $C_{20}H_{16}O_5$: C 71.4, H 4.8.
Found: C 71.7, H 5.6.

MS *m/z* 336.1005 (M, $C_{20}H_{16}O_5$ requires 336.0998), 318 (M−H_2O), 308 (M−CO), 294.0528 (M−CH_2-CHMe, $C_{17}H_{10}O_5$ requires 294.0528), 278.0562 (M−CHOHCHMe, $C_{17}H_{10}O_4$ requires 278.0579), 265.0505(M−C_4H_7O, $C_{16}H_9O_4$ requires 265.0501); UV λ_{max}^{MeOH} nm (ε) 273 (22,850) and 382 (3,360), unaltered on addition of NaOH; IR λ_{max} (CH_2Cl_2 - CCl_4, 1 : 1) cm^{-1} 1675 (quinone C=O), 1705 (ketonic C=O), 3600 (OH); $[\alpha]_D^{20}$ +50.0° (*c* 0.176, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 1.26 (3H, d, *J*=6.6 Hz, CHCH_3), 2.35 (1H, m, 3-H), 2.56 (1H, dd, $J_{2ax,2eq}$=16.4 Hz, $J_{2ax,3}$=10.7 Hz, 2-H_{ax}), 3.09 (1H, dd, $J_{2eq,2ax}$=16.4 Hz, $J_{2eq,3}$= 5.6 Hz, 2-H_{eq}), 4.05 (3H, s, OCH_3), 4.50 (1H, d, $J_{4ax,3}$=9.2 Hz, 4-H_{ax}), 7.31 (1H, dd, $J_{9,10}$= 7.9 Hz, $J_{9,11}$=1.5 Hz, 9-H), 7.71 (1H, t, $J_{10,9}\approx J_{10,11}\approx$7.8 Hz, 10-H), 7.77 (1H, dd, $J_{11,10}$= 7.7 Hz, $J_{11,9}$=1.6 Hz, 11-H), 8.00 (1H, d with slight broadening, $J_{5,6}$=8.2 Hz, 5-H), 8.34 (1H, d, $J_{6,5}$=8.2 Hz, 6-H).

These spectral data indicate that the neutral fujianmycin B is the methyl ether of the phenolic fujianmycin A. In confirmation, methylation of fujianmycin A (MeI-Ag_2O, $CHCl_3$, room temp, 12 hours) gave an *O*-methyl derivative identical in Rf value on TLC, mass and ^1H NMR spectra with fujianmycin B, together with a minor *O*,*O*-dimethyl derivative (MS *m/z* 350 (M, $C_{21}H_{18}O_5$)) in which an alcoholic hydroxyl function has also been methylated.

The chromophore of both fujianmycins in neutral solution closely resembles that of the 3,4-dihydro-8-hydroxy-benz[a]anthracene-1,7,12-(2*H*)-trione tetrangomycin (**1**)[1,2], which is isomeric with fujianmycin A. (The chromophores of the phenols fujianmycin A and tetrangomycin in alkaline solution do not correspond, since tetrangomycin undergoes rapid base-catalysed dehydration and aromatization to tetrangulol under these conditions[1,2], in contrast to the present compounds which are stable). As expected, fujianmycin A shows IR absorption maxima corresponding to the ketonic carbonyl and the free and H-bonded quinone carbonyl groups of tetrangomycin (**1**). Furthermore, ^1H NMR spectra of both fujianmycins show the two- and three-proton vicinal aromatic spin systems of tetrangomycin (**1**), while fujianmycin A shows a strongly hydrogen-bonded phenolic group at δ 12.30 which is methylated in fujianmycin B. The remaining eight protons of the fujianmycins are contained in the system -CH_2CHMeCHOH-, for which assignments and spin coupling constants (fully confirmed by double resonance experiments) are presented in the data above. This segment, linked to the carbonyl group located at C-1 from electronic spectra, must form the angularly-fused fourth ring of the fujianmycins. Two orientations of the segment are possible, leading to the 4-hydroxyl structures **4** and **5** or to structures of the 2-hydroxyl type **6**. The observation in ^1H NMR spectra of fujianmycin B of an 0.8 Hz coupling between the methine proton of the secondary alcohol and the aromatic proton at C-5 confirms the position of the carbonyl group and established the structures **4** and **5** for the fujianmycins. This coupling, which is clearly defined in resolution-enhanced spectra, is removed only upon irradiation of the benzylic methine proton.

The *trans* relative configuration of the methyl and hydroxyl substituents of the fujianmycins (**4**) and (**5**) follows from the magnitude of the proton coupling constants[4] in their hydroaromatic rings, particularly the two large (9~11 Hz) couplings between the three *quasi*-axial protons at C-2, C-3 and C-4. The two antibiotics must have the same absolute configuration, since they have positive D-line rotations and effectively the same chromophore.

Fujianmycin A (**4**) showed antibacterial activity in disc assays against *Bacillus subtilis* on seeded agar plates at 50 μg/ml. In contrast, its methyl derivative, fujianmycin B (**5**) was active only at much higher concentrations, indicating the importance of the phenolic function for antimicrobial activity.

Another benz[a]anthraquinone from *Streptomyces* species, ochromycinone, was suggested to have either the 8-hydroxyl structure **3** or the corresponding 11-hydroxyl structure, the former being preferred on biogenetic grounds[5]. Disregarding questions of absolute configuration at C-3, structure **3** represents a deoxy-derivative of both tetrangomycin (**1**) and fujianmycin A (**4**),

and this 8-hydroxyl formulation for ochromycinone is confirmed by the close correspondence of the electronic spectra of these three phenols: λ_{max}^{MeOH} nm **1** 267, 400; **3** 265, 405; **4** 273, 402.

The anthraquinones fujianmycins A (**4**) and B (**5**), ochromycinone (**3**), tetrangomycin (**1**), rabelomycin (**2**) and tetrangulol are clearly related biosynthetically to the naphthoquinones of the aquayamycin[6], yoronomycin[7], P-1894 B[8,9], vineomycin[9,10], and sakyomicin[11,12] group, in which the [a]-ring junction bond of the 3,4-dihydrobenz[a]anthracene-1,7,12(2*H*)-trione system has been oxidised to a 4a,12b-diol.

Acknowledgments

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