SECONDARY METABOLITES BY CHEMICAL SCREENING. PART 19†

SM 196 A AND B, NOVEL BIOLOGICALLY ACTIVE ANGUCYCLINONES FROM *Streptomyces* sp.

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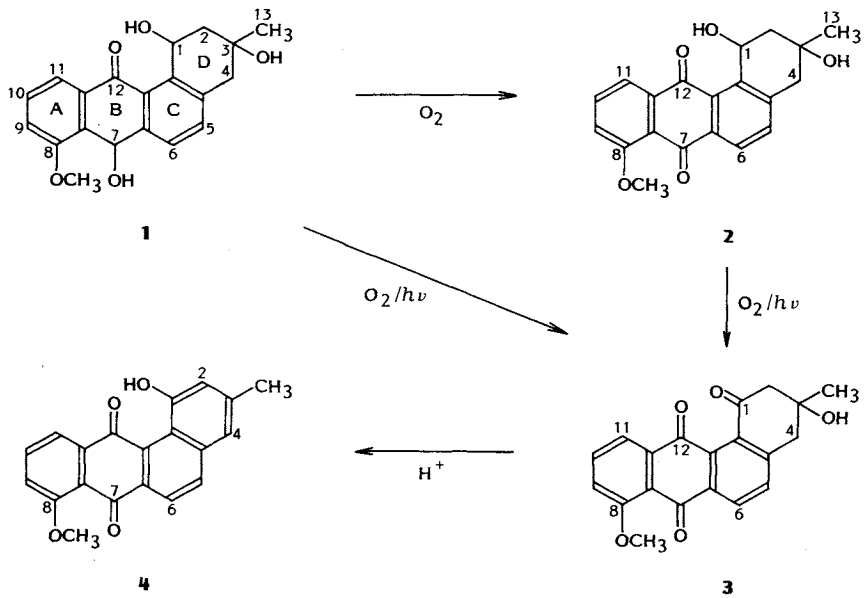
Application of a method of chemical screening[2], which based on the prior work of ZÄHNER *et al.*[3], gave rise to the detection, isolation and structure elucidation of two new angucyclinones[4] exhibiting biological activity. In addition, the known compounds 6-deoxy-8-*O*-methylrabelomycin (**3**)[5,6] and X-14881 E (**4**)[7] were detected in the same culture broth.

The producing organism *Streptomyces* sp. (DSM 4769) was isolated from a soil sample collected near Ajanta (India) and exhibits tightly spiraled, red spores with a smooth surface. Melanin production is positive. Cultivation was carried out in a seed medium (meat flour 2%, malt extract 10%, $CaCO_3$ 1%, pH adjusted to 7.2 prior to sterilization) inoculated from an agar slant culture and incubated for 48 hours at 27°C. The resulted seed medium (5%) was used to inoculate the production medium (glycerol 3%, casein peptone 0.2%, K_2HPO_4 0.1%, NaCl 0.1%, $MgSO_4 \cdot 7H_2O$ 0.05% and 5 ml of a mineral solution containing $CaCl_2 \cdot 2H_2O$ 3 g, $FeC_6H_5O_7$ 1 g, $MnSO_4$ 0.2 g, $ZnCl_2$ 0.1 g, $CuSO_4 \cdot 5H_2O$ 0.025 g, $Na_2B_4O_7 \cdot 10H_2O$ 0.02 g, $Na_2MoO_4 \cdot 2H_2O$ 0.01 g, and $CoCl_2$ 4 mg per liter water). Production was carried out in 300-ml Erlenmeyer flasks containing 100 ml of production medium (200 rpm on a rotary shaker, 30°C) or in 10-liter fermenters (250 rpm, aeration: 4 liters/minute) for 98 hours.

We prepared extracts from the culture filtrate following a procedure described elsewhere[2]. Surprisingly, without the use of any staining reagent a colorless spot on the TLC plate (SM 196 A (**1**): Rf 0.25, $CHCl_3$-MeOH (15:1); Silica gel $60F_{254}$ on glass, Merck, HPTLC-Fertigplatten) immediately turned to brilliant red after exposing the TLC plate

Scheme 1.



1 **2** **3** **4**

† See ref 1.

to UV light (366 nm). In addition, two minor yellow spots (SM 196 B (**2**): Rf 0.40; **3**: Rf 0.33) changed their color to orange. Development of the TLC plate in a second dimension using the same solvent system showed **1** to be converted into **2**, **3**, and **4**, whereas **2** was transformed to **3** and **4**, and **3** gave **4** possibly by H^+-catalysis.

The culture filtrate (8 liters) of a 10-liter fermenter was extracted three times with 5 liters CH_2Cl_2. The organic layer was evaporated to dryness and the remaining oily residue (4 g) was chromatographed on a silica gel column (wrapped with aluminum foil to prevent photo-conversion; 30×3 cm; $CHCl_3$-MeOH (30:1)) to obtain the four detected metabolites: colorless crystalline SM 196 A (**1**, 33 mg), yellow amorphous SM 196 B (**2**, 22 mg), orange amorphous **3** (21 mg), and red **4** (12 mg, Rf 1.0). Due to the chemical instability of the compounds this purification turned out to be difficult, especially in the case of SM 196 B (**2**). The new angucyclinones **1** and **2** are soluble in MeOH, acetone, DMSO, dioxane, and $CHCl_3$ and are insoluble in H_2O or *n*-hexane.

The structures of **1** and **2** were ascertained by ^1H, ^{13}C and 2D NMR techniques. Some of the relevant ^1H and ^{13}C NMR data are summarized in Tables 1 and 2. The compounds of the SM 196 complex exhibit a benz[*a*]anthracene carbon skeleton and therefore belong to the group of angucyclinones[8]. Based on these NMR data, compound **3** was proved to be identical with 6-deoxy-8-*O*-methylrabelomycin[5,6] and **4** with X-14881 E[7]. The colorless metabolite SM 196 A (**1**) possesses a dihydroquinone moiety in ring B and a secondary hydroxy group at C-1. FAB, CI (NH_3), and EI (70 eV) mass spectra of SM 196 A (**1**) showed peaks at *m/z* 338 ($M^+ - H_2$) and 322 ($M^+ - H_2O$).

Table 1. ^{13}C NMR data of SM 196 A (**1**), B (**2**), and X-14881 E (**4**).

C-Atom	SM 196 A (**1**)	SM 196 B (**2**)	X-14881 E (**4**)
1	65.73	65.28	155.10
2	44.14	43.97	119.71
3	69.05	69.18	141.14
4	45.17	45.40	121.11
4a	136.43	142.15	130.67
5	135.70	136.05	137.53
6	128.81	127.27	122.76
6a	143.35	136.05	136.74[a]
7	63.34	182.37	182.12
7a	129.36	120.60	119.15[b]
8	156.81	159.71	159.57
9	114.78	117.69	118.20
10	129.54	135.03	135.21
11	120.21	120.16	120.97
11a	132.74	137.10	137.52
12	187.53	187.84	190.71
12a	128.81	131.06	138.37[a]
12b	140.03	140.08	119.94[b]
13	29.11	29.41	21.19
14	56.04	56.54	56.62

Bruker AM 360, 90 MHz, $CDCl_3$, δ values (ppm) with $CDCl_3$ as an internal standard (δ 77.00).
[a,b] Assignment exchangeable.

Table 2. ^1H NMR data of SM 196 A (**1**) and SM 196 B (**2**).

Proton	SM 196 A (**1**)	SM 196 B (**2**)
1-H	5.59 dddd (J=6.70, 5.87, 4.5, 0.5)	5.48 dddd (J=6.93, 6.07, 4.2, 0.5)
1-OH	5.20 d (J=4.5)	5.05 d (J=4.2)
2-H_a	2.16 ddd (J=−13.63, 5.87, 1.00)	2.15 (J=−13.86, 6.07, 1.00)
2-H_b	2.31 ddd (J=−13.63, 6.70, 1.50)	2.34 ddd (J=−13.86, 6.93, 1.65)
3-OH	1.48 (br)	2.0 (br)
4-H_a	2.90 dddq (J=−16.33, 1.50, 0.4)	2.91 dddq (J=−16.78, 1.65, 0.4)
4-H_b	3.09 ddddq (J=−16.33, 1.00, 0.5, 0.4)	3.10 ddddq (J=−16.78, 1.00, 0.5, 0.4)
5-H	7.48 dt (J=8.07, 0.4)	7.51 dt (J=8.04, 0.4)
6-H	7.85 d (J=8.07)	8.19 (J=8.04)
7-H	5.99 dd (J=2.0, 0.3)	
7-OH	4.13 d (J=2.0)	
9-H	7.20 ddq (J=8.15, 0.74, 0.2)	7.20 ddd (J=8.48, 0.96, 0.2)
10-H	7.50 ddd (J=8.15, 7.92, 0.3)	7.70 dd (J=8.48, 7.79)
11-H	7.92 dd J=7.92, 0.74)	7.87 dd (J=7.79, 0.96)
13-H_3	1.50 s (br)	1.50 s (br)
14-H_3	4.04 d (J=0.2)	4.04 d (J=0.2)

Bruker AM 360, 360 MHz, $CDCl_3$, δ values (ppm) and J (Hz), TMS as internal standard, (^1H) = 0.000 ppm, temperature: 303 K display value.

Table 3. Biological activities of **1** and **2** (MIC in μg/ml).

	Staphylococcus aureus H 503	*Streptococcus pyogenes*		Herpes simplex 1	Herpes simplex 2
		S 308/7	S 77/7		
1	100.0	25.0	12.5	—	—
2	25.0	6.25	6.25	0.55	4.54

The dihydroquinone is rapidly oxidized by oxygen in the air to the corresponding yellow quinone SM 196 B (**2**). Simultaneous radiation (366 nm) led to the orange 6-deoxy-8-*O*-methylrabelomycin (**3**), which exhibits both a quinone moiety in ring B and a ketone moiety in ring D. The brilliant red color observed on the TLC plate after treatment with UV light derives from the dehydration product X-14881 E (**4**), which is to be distinguished from the other compounds by aromatization of ring D. Scheme 1 illustrates the isolation sequence of the SM 196 antibiotic complex. Due to its rapid conversion to **2**, **3**, and **4** on TLC plates it could be assumed that SM 196 A (**1**) is the native natural product. Therefore, no enzymatic steps seem to be involved in the dehydrogenation and dehydration reactions. To study the TLC-plate decomposition in more detail, these reactions were performed on preparative scale using more defined experimental conditions. Stirring of SM 196 A (**1**) in $CHCl_3$ in the presence of oxygen and in the absence of light the quinone **2** was formed within 4 hours. Performing the same reaction by irradiation of **1** with UV light (366 nm) for 6 hours yielded **3** in 60%. A side reaction to X-14881 E (**4**) was observed, which could be explained by hydrochloric acid impurities present in the solvent chloroform. This was proved by the conversion of **3** to **4** in chloroform/1% HCl in the dark.

The described angucyclinones SM 196 A (**1**) and (**2**) exhibit antibacterial[9] and antiviral activity[10] (see Table 3).

Addendum in Proof

Recently, an analog photo-induced oxidation as described for the antibiotics SM 196 A and B was reported for the rubiginones: OKA, M.; M. KONISHI, T. OKI and M. OHASHI: Absolute configuration of the rubiginones and photo-induced oxidation of the C-1 hydroxyl of the antibiotics to a ketone. Tetrahedron Letters 31: 7473~7474, 1990.

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