NOTE

Antibiotic R2, a new angucyclinone compound from *Streptosporangium* sp. Sg3

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Angucyclinones are a subclass of angucyclines, which are defined as microbial quinone natural products related to tetracyclines and anthracyclines and bearing, as characteristic structural feature, a tetracyclic benz[*a*]anthracene ring system assembled in an angular manner.[1] This structural moiety is biosynthetically derived from a decaketide chain formed via the polyketide biosynthetic pathway. The term 'angucycline' includes molecules with hydrolysable sugar moieties, whereas 'angucyclinone' refers to a sugarless compound or a compound with a *C*-glycosidic linked sugar moiety.[1,2] Angucyclines are isolated from the fermentation broth of actinomycetes, mostly from the genus *Streptomyces*, but also from *Actinomadura*, *Nocardia* and *Streptosporangium* genera.[1] Angucyclines show a broad spectrum of biological activities including antitumor,[1,2] antibacterial, antifungal, antiviral,[3,4] enzyme inhibitory[5,6] and platelet aggregation inhibitory properties.[7,8] In a continuous search for new bioactive compounds from actinomycetes other than the genus *Streptomyces*, several Saharan soil samples collected in arid ecosystems were explored.[9–11] Among the isolates, we were interested by a new actinomycete strain belonging to the genus *Streptosporangium* and producing a new compound (**R2**) identified as a new angucyclinone. This paper describes the isolation, structure elucidation and antimicrobial activities of compound **R2**.

Details on the isolation and the taxonomy of the producing organism *Streptosporangium* sp. Sg3 were described in a previous paper.[11] To isolate compound **R2**, *Streptosporangium* sp. Sg3 was cultivated at 30 °C for 9 days in Erlenmeyer flasks (500 ml) containing 100 ml of ISP2 broth (yeast extract 4 g, malt extract 10 g, glucose 4 g, in 1 l distilled water, pH 7.2) on a rotary shaker (250 r.p.m.). The cultures (8 l) were centrifuged and filtered to remove mycelium. The culture filtrate was extracted with an equal volume of *n*-butanol to generate a crude extract (1.9 g). The latter was fractionated by size exclusion chromatography on Sephadex LH-20 (75% MeOH in H_2O), resulting in five fractions, I–V. Fraction IV including **R2**, was of red color and exhibited an antibacterial activity. It was subjected to semipreparative reversed-phase HPLC using an Interchim UP5ODB column (250×7.8 mm) (Interchim, Montluçon, France) and developed using a continuous grade from 20 to 75% MeOH in H_2O (UV detection at 220 nm), yielding three active fractions (**1**, **2**, **3**). The major peak **2** with the main antibacterial activity was subjected to repeated HPLC to yield 3.5 mg of compound **R2**.

Compound **R2** was obtained as a red powder (optical rotation $[\alpha]_D^{21}$ +56 (*c* 0.11, MeOH). The ESI-MS spectrum contained an ion peak at *m/z* 475.1 $[M–H]^-$, and its molecular formula was determined by HRESI-MS analysis as $C_{26}H_{19}O_9$ (calcd 475.43 for $(M–H)^-$, found 475.43). The UV absorption maximum at 535 nm was because of the red color and suggested a quinone chromophore. Absorptions at 3259, 2949 and 1721 cm^{-1} in the IR spectrum of **R2** were characteristic of hydroxy, methyl and carbonyl groups, respectively. **R2** was soluble in MeOH and DMSO, and insoluble in chloroform, *n*-hexane and H_2O.

The structure of **R2** (Figure 1) was determined by ^1H and ^{13}C NMR spectroscopy and by using ^1H–^1H COSY45, ^1H–^{13}C HMQC and ^1H–^{13}C HMBC experiments. The ^1H and ^{13}C chemical shifts of compound **R2** are given in Table 1. The ^{13}C and Heteronuclear Single-Quantum Correlation spectra showed 26 carbon signals with a large number of quaternary carbons (17 out of 26). From the ^{13}C data, it was possible to discern two keto-carbonyl groups (δc 188.4 and 186.5), one carboxylic acid group (δc 174.1), 19 sp^2-hybridized carbons (δc from 160.2 to 114.7), two sp^3-hybridized carbons bearing an electronegative heteroatom (δc 73.0 and 72.5), one sp^3-hybridized carbon (δc 22.6) and two methyl groups (δc 22.4 and 12.4). In CD_3OD, the ^1H NMR spectrum revealed two ortho-coupled aromatic protons (δ_H 7.82 and 7.66, 2H, J=7.5 Hz), two aromatic protons (δ_H 8.24 and 7.02, 2H, s), an AB system (δ_H 4.45 and 4.36, J_{AB}=10.7 Hz), an ethyl group (δ_H 2.83 and 1.32, 5H, J=7.4 Hz) and a methyl group (δ_H 2.70, 3H, s). In DMSO, NMR signals were significantly broader but the

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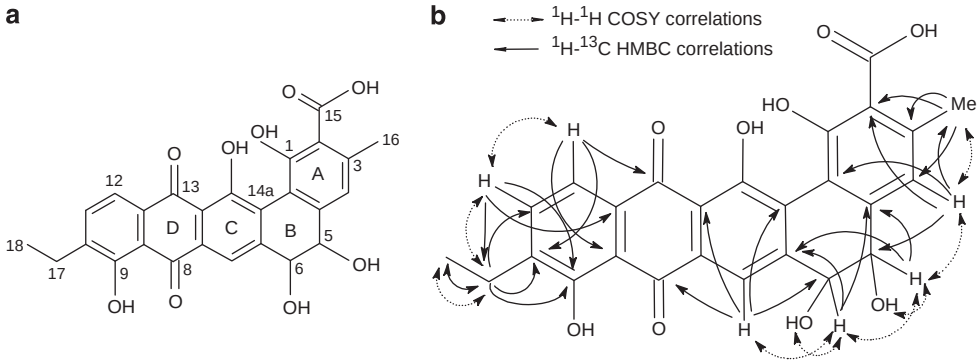

Figure 1 Structure of angucyclinone **R2** (**a**), and HMBC and COSY correlations (**b**).

Table 1 ^{1}H and ^{13}C NMR data assignments of CD_3OD at 298 K

Position	δ_C, *mult.*	δ_H (*J in Hz*)
1	160.2, qC[a]	—
2	118.5, qC	—
3	143.1, qC	—
4	116.9, CH	7.02,s
4a	147.4, qC	—
5	72.5, CH	4.36, d (10.7)
6	73.0, CH	4.45, d (10.7)
6a	141.9, qC	—
7	114.7, CH	8.24, s
7a	131.6, qC	—
8	188.4, qC	—
8a	115.2 qC	—
9	160.1, qC	—
10	139.9, qC	—
11	135.3, CH	7.66, d (7.5)
12	118.7, CH	7.82, d (7.5)
12a	132.0, qC	—
13	186.5, qC	—
13a	117.4, qC	—
14	158.6, qC[a]	—
14a	128.6, qC	—
14b	114.9, qC	—
15	174.1, qC	—
16	22.4, CH_3	2.70, s
17	22.6, CH_2	2.83, q (7.4)
18	12.4, CH_3	1.32, t (7.4)
OH-1[b,c]	—	17.64, s
OH-5[c]	—	5.69, s
OH-6[c]	—	5.92, s
OH-9[b,c]	—	13.98, s
OH-14[b,c]	—	13.08, s
COOH[b,c]	—	13.20, s

[a,b]Resonance assignment may be interchangeable.
[c]In DMSO-d_6.
Mult. stands for multiplicity. qC, CH, CH_2 and CH_3 indicate respectively quaternary, tertiary, secondary and primary carbons.

^{1}H NMR spectrum showed additional resonances: four enol or carboxylic acid protons (δ_H 17.64, 13.98, 13.20 and 13.08, 4H, s) and two hydroxyl signals (δ_H 5.92 and 5.69, 2H, s). The 2D ^{1}H–^{1}H and ^{1}H–^{13}C experiments and especially the long range ^{1}H–^{13}C couplings observed in the HMBC spectrum permitted to establish the presence of a benzo[*a*]naphtacenequinone skeleton. Only four carbons did not show any correlation in the HMBC spectrum (C1, C7a, C14 and C15). Their assignment has been confirmed by comparison with NMR data of the pradimicin antibiotic family.[12] The peak of the four enol or carboxylic acid protons was too broad to give HMBC correlations and so could not be exactly assigned. The observed coupling constant of 10.7 Hz between H5 and H6 suggested that the dihedral angles between H5 and H6 are near 180° and is consistent with a *trans*-diaxal orientation. This value is similar with ones found for related compounds[3,12] where a 5S,6S absolute configuration has been deduced.

The structure of **R2** was determined to be a benzo[*a*]naphtacene-2-carboxylic acid,4,7,11,12-tetrahydro-1,5,6,9,14-pentahydroxy-8,13-dioxo-3-methyl-10-ethyl.

The minimum inhibitory concentrations (MICs) of **R2** were determined by a conventional agar dilution method using nutrient agar medium. Compound **R2** showed strong activity against the Gram-positive bacteria *Micrococcus luteus* ATCC 9314 and *Bacillus subtilis* ATCC 6633 (MICs=0.5 and 1 μg ml^{-1}, respectively), and moderate activity against *Staphylococcus aureus* CIP 7625 (MIC=10 μg ml^{-1}). A weak activity was observed against *Listeria monocytogenes* CIP 82110 and *Mycobacterium smegmatis* ATCC 607 (MICs=40 and 50 μg ml^{-1}, respectively). Compound **R2** showed no activity (MICs > 100 μg ml^{-1}) against the Gram-negative bacteria (*Escherichia coli* ATCC 10536 and *Pseudomonas fluorescens* number 412), the fungi (*Mucor ramannianus* NRRL 1829 and *Aspergillus carbonarius* number M333) and the yeasts (*Saccharomyces cerevisiae* ATCC 4226 and *Candida albicans* number 224).

In conclusion, from the results presented in this paper, we found that strain *Streptosporangium* sp. Sg3 produces a new angucyclinone that displayed antibacterial activities.

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