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NOTE

Aranciamycin anhydride, a new anthracycline-type antibiotic isolated from *Streptomyces* sp. Tü 6384*

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Streptomycetes isolated from the rhizosphere of Norway spruce were grown in submerged culture in various complex media, and extracts prepared from culture filtrates and biomass were screened by HPLC-diode array analysis to detect novel secondary metabolites.[2] Strain Tü 6384 was found to produce a new anthracycline-type compound that showed a high similarity with aranciamycin, an antibiotic isolated from *Streptomyces echinatus*, in its UV–visible spectrum.[3] The compound was characterized as aranciamycin anhydride (**1**).

Strain Tü 6384 was isolated from the rhizosphere of Norway spruce collected in Rammert forest near Tübingen, Germany. It was examined for a number of key properties that are known to be of value in streptomycete systematics.[4,5] Whole-cell hydrolysates of strain Tü 6384 contained LL-diaminopimelic acid, and hexa- and octahydrogenated menaquinones with nine isoprene units were the predominant isoprenologs. Partial sequencing of the 16S rRNA gene led to a similarity of 99% with *Streptomyces prunicolor*.

Batch fermentations of strain Tü 6384 were carried out in a 10-l stirred tank fermentor (Biostat S; B Braun, Melsungen, Germany) in a complex medium that consisted of (per liter tap water) oatmeal (Holo Hafergold, Neuform, Germany) 20 g, and 5 ml of a trace element solution that was composed of (per liter deionized water) $CaCl_2 \times 2H_2O$ 3 g, iron(III) citrate 1 g, $MnSO_4 \times 1H_2O$ 200 mg, $ZnCl_2$ 100 mg, $CuSO_4 \times 5H_2O$ 25 mg, $Na_2B_4O_7 \times 10H_2O$ 20 mg, $CoCl_2 \times 6H_2O$ 4 mg and $Na_2MoO_4 \times 2H_2O$ 10 mg; the pH was adjusted to 7.3 (5 M HCl) before sterilization. The fermentor was inoculated with 5% by volume of a shake flask culture grown in a seed medium at 27 °C in 500-ml Erlenmeyer flasks with a single baffle for 72 h on a rotary shaker at 120 r.p.m. The seed medium consisted of glucose 10 g, glycerol 10 g, oatmeal 5 g, soybean meal (Schoenenberger, Magstadt, Germany) 10 g, yeast extract (Ohly Kat, Deutsche Hefewerke, Hamburg, Germany) 5 g, Bacto Casamino acids 5 g and $CaCO_3$ 1 g in 1 l tap water. The fermentation was carried out for 4 days with an aeration rate of 0.5 volume air per volume per min and agitation at 250 r.p.m. The production of **1** reached a maximal yield of 50 mg l^{-1} at 72 h of incubation. The culture filtrate (6 l) was applied to an Amberlite XAD-16 column (60×4 cm i.d.; Rohm and Haas, Frankfurt, Germany), washed with each of 3 l H_2O and H_2O-EtOH (6:4), and **1** was eluted with 3 l EtOH and concentrated *in vacuo*. The crude product was dissolved in CH_2Cl_2 and added to a diol-modified silica gel column (45×2.6 cm i.d., LiChroprep Diol; Merck, Darmstadt, Germany). The separation was accomplished by a step gradient from CH_2Cl_2 to 5% EtOH. The combined fractions containing **1** were concentrated *in vacuo* and yielded 385 mg crude product, which was purified by preparative RP-HPLC (Nucleosil-100 C-18, 10 μm, 25×1.6 cm i.d.; Maisch, Ammerbuch, Germany) with CH_3CN–0.1% HCOOH using a linear gradient elution from 40 to 80% CH_3CN over 20 min at a flow rate of 20 ml min^{-1}. Compound **1**, 45 mg, was obtained as a red-orange powder (Table 1).

The molecular mass of **1** was determined by high-resolution electrospray ionization-FT-ion cyclotron resonance mass spectrometry, which gave the mass of 710.17728, suggesting a molecular formula of $C_{35}H_{34}O_{16}$ (theoretical: 709.17741, Δ=0.13 p.p.m.). The chemical structure of **1** as shown in Figure 1 was determined by ^1H-, ^{13}C- and 2D-NMR experiments (Table 2) and GC–MS. The complete NMR assignments were unambiguously carried out on the basis of COSY and heteronuclear multiple bond correlation (HMBC) experiments. The ^1H-NMR and heteronuclear single quantum coherence (HSQC) data showed a total of 30 carbon-attached protons, among which five methyl, two methylene and eleven methine carbons could be assigned. Inspection of the 2D-NMR data (COSY, HSQC and HMBC) allowed assignment of the structure of **1,** a new derivative of the aranciamycin family, aranciamycin anhydride (Figure 2). The ^1H–^1H–COSY spectra of **1** revealed protons attached to two ethyl, one phenyl and one sugar (hexose) moiety, as shown by the bold lines in Figure 2. All expected

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Table 1 Physico-chemical properties of aranciamycin anhydride[1]

	1
Appearance	Red-orange powder
Optical rotation[a]	$[\alpha]^{20}_{D}$ +82° (c. 0.05, MeOH)
FT-ICR-MS[b]	709.17728 found $(M-H)^-$
	709.17741 calculated $(M-H)^-$
	Δ=0.13 p.p.m.
Molecular formula	$C_{35}H_{34}O_{16}$
UV[c] λ_{max} (MeOH) nm (log ε)	240 (4.48), 260 (4.44), 435 (4.08)
IR[d] V_{max} (cm^{-1})	3503, 2977, 2933, 1766, 1715, 1675, 1625, 1448, 1415, 1380, 1290, 1247, 1191, 1170, 1135, 1109, 1083, 1031, 1001, 957, 840, 758, 735

Abbreviation: ICR, ion cyclotron resonance.
[a]Optical rotation was recorded on a 341 polarimeter (Perkin-Elmer, Überlingen, Germany).
[b]HR-FT-ICR-MS measurement was carried out on an APEX II FT-ICR mass spectrometer (4.7 T, Bruker-Daltonics, Bremen, Germany).
[c]UV–visible spectra were obtained on a HP 1090M diode array detector (Agilent Technologies, Waldbronn, Germany).
[d]Infrared data measurement was carried out on an 881 IR-spectrometer (Perkin-Elmer).

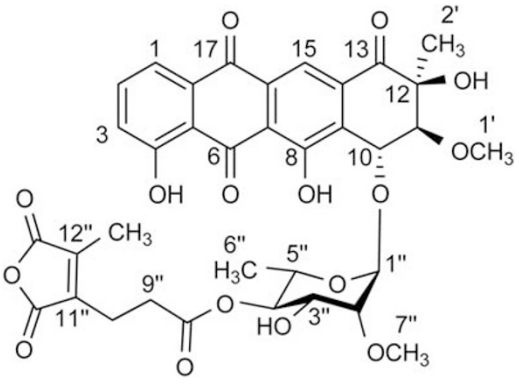

Figure 1 Structure of aranciamycin anhydride (**1**).

Table 2 ^{1}H- and ^{13}C-NMR assignment of 1 in $CDCl_3$-*d* (25 °C)

Position	*δ (^{1}H) (p.p.m.) J in Hz*	*δ (^{13}C) (p.p.m.)*
1	7.88 (d, 7.5)	120.9
2	7.75 (t, 7.9)	138.5
3	7.34 (d, 8.3)	125.3
4	—	163.2
5	—	115.9
6	—	193.2
7	—	119.1
8	—	162.5
9	—	133.3
10	5.19 (d, 2.4)	72.4
11	3.72 (d, 2.5)	85.9
12	—	76.9
13	—	198.9
14	—	136.3
15	8.41 (s)	118.0
16	—	134.0
17	—	180.6
18	—	133.6
1′	3.54 (s)	60.3
2′	1.54 (s)	23.1
1″	5.65 (s, br)	100.5
2″	3.53 (dd, 1.4; 3.6)	80.3
3″	3.59 (dd, 3.6; 9.8)	69.6
4″	4.87 (dd, 9.8; 9.8)	75.1
5″	3.92 (dq, 9.8; 6.2)	67.6
6″	1.23 (d, 6.2)	17.7
7″	3.56 (s)	59.0
8″	—	171.8
9″	2.77 (m)	31.3
10″	2.78 (m)	19.9
11″	—	142.0
12″	—	142.6
13″	—	166.1
14″	—	165.8
15″	2.10 (s)	9.7

HMBC correlations of the aranciamycin moiety could be seen and couplings were in good accordance with the data given in literature.[6] The connection between the sugar moiety and the aranciamycin chromophore was established by the ^{1}H–^{13}C–long-range coupling from C-10 (δ_H 5.19, δ_C 72.4) to H-1″ (δ_H 5.65, δ_C 100.5) and *vice versa* as shown in Figure 2. The constitution of the sugar was determined from COSY and HMBC spectra, which revealed a 6-desoxyhexose moiety. The coupling constants (Table 3) indicated that the sugar was a mannopyranose. A ^{1}H–^{13}C long-range coupling from the methyl group at δ_H 3.56 to C-2″ (δ_C 80.3) of the sugar established the position of the methoxy group. Additional examination of the NMR data and chiral GC–MS analysis of the derivatized hydrolysate allowed us to identify the moiety as β-2-*O*-methyl-L-rhamnose in accordance with the literature.[7] In comparison with the molecular formula of aranciamycin, derivative **1** has an additional $C_8H_6O_4$ moiety. In addition, the NMR spectra showed five quaternary olefinic, two methylene and one methyl moiety compared with the aranciamycin core structure. The ^{1}H–^{13}C long-range couplings from H-9″ (δ_H 2.77) to C-8″ (δ_C 171.8), C-10″ (δ_C 19.9) and C-11″ (δ_C 142.0), from H-10″ (δ_H 2.78) to C-8″, C-9″ (δ_C 31.3) and C-14″ (δ_C 165.8), and from H-15″ (δ_H 2.10) to C-11″, C-12″ (δ_C 142.6) and C-13″ (δ_C 166.1) gave rise to an anhydride structure in excellent accordance with literature values.[8] The connection between the sugar and the anhydride moiety was established by the ^{1}H–^{13}C long-range coupling observed from H-4″ (δ_H 4.87) to the carbonyl of C-8″, resulting in the structure of **1** (Figure 1).

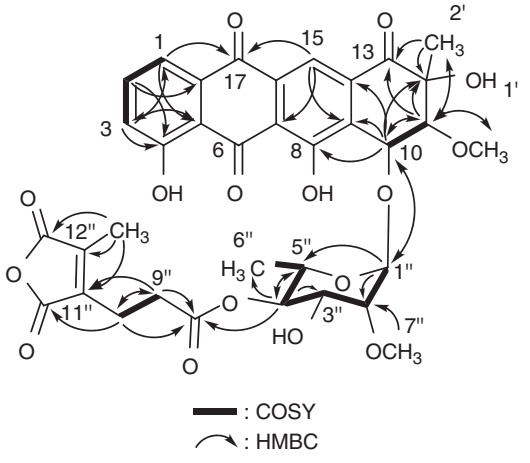

Figure 2 ^{1}H–^{1}H–COSY and HMBC correlations observed in **1**.

Table 3 Growth-inhibitory activity of 1 and aranciamycin (μg ml^{-1}) against selected human tumor cell lines

	1		*Aranciamycin*	
Cell line	*GI_{50}*	*TGI*	*GI_{50}*	*TGI*
HM02	6.5	>10	0.62	1.35
HepG2	7.2	>10	1.15	3.2
MCF 7	>10	>10	1.3	3.3

Abbreviations: GI_{50}, 50% growth inhibition; TGI, 100% growth inhibition.

The antimicrobial activity spectrum of **1** was tested in an agar plate diffusion assay against *Bacillus subtilis* DSM 10, *Escherichia coli* K12, *Saccharomyces cerevisiae* ATCC 9010 and *Botrytis cinerea* Tü 157 in a concentration of 0.1–1 mg ml^{-1}. Similar to aranciamycin, **1** showed weak antibacterial activity only against *Bacillus subtilis*. The inhibitory action of **1** on the growth of tumor cells was compared with aranciamycin and tested according to NCI guidelines[9] with the human tumor cell lines HM02 (gastric adenocarcinoma), MCF 7 (breast carcinoma) and HepG2 (hepatocellular carcinoma). Cells were grown in 96-well microtiter plates in RPMI 1640 with 10% fetal calf serum in a humidified atmosphere of 5% CO_2 in air. Aranciamycin and **1** (0.1–10 μg ml^{-1}) were added to the cells after incubation for 24 h. Stock solutions were prepared in DMSO; the final DMSO concentration of the cultures was 0.1%. The cells were fixed and cell protein analyzed with sulforhodamine B after incubation for 48 h. The cytostatic activity of **1** was somewhat less than that of aranciamycin (Table 3).

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