NOTES

New Inhibitors of 3α-Hydroxysteroid Dehydrogenase, 0231A and 0231B from *Streptomyces* sp. HKI 0231

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3α-Hydroxysteroid dehydrogenase is one of the key enzymes involved in inflammatory processes[1]. Hence, inhibitors of this enzyme are interesting as lead structures for the development of anti-inflammatory agents. In a screening for new microbial metabolites inhibiting 3α-hydroxysteroid dehydrogenase we disclosed *Streptomyces* sp. HKI 0231 as the producer of new inhibitors, 0231A (**1**) and 0231B (**2**). Both compounds were formed as minor components in addition to angucyclin-type metabolites such as tetrangulol methylether[2~4].

Here we report isolation and structure of these unusual polycyclic metabolites. 10 liters of fermentation broth of *Streptomyces* sp. HKI 0231 were extracted three-times by 10 liters of ethyl acetate. The residue of extract (1.6 g) was subjected to chromatography on Sephadex LH-20 (MeOH) whereby **1** and **2** were eluted together with tetrangulol methylether and other angucycline metabolites. Thereafter this fraction was chromatographed on silica gel 60 (0.063~0.1 mm, column 5×40 cm, elution by $CHCl_3$). The tetrangulol methylether was first eluted followed by **1** and **2** as a mixture which appeared as fairly yellowish fractions showing bright fluorescence under UV light (λ_{max} 485~495 nm). Final purification was accomplished by preparative HPLC (Nucleosil RP_{18}, 7 μm, gradient 95% H_2O to 95% acetonitrile, 30 minutes) to yield 18 mg of **1** and 15 mg of **2** as yellow solids. The physico-chemical properties of **1** and **2** are shown in Table 1. The UV-VIS

Table 1. Physico-chemical properties of 0231 A (**1**) and 0231 B (**2**).

	1	**2**
Appearance	yellow solid	yellow solid
Melting point (uncorr.)	125 °C	135 °C
Molecular weight	361	331
HREI-MS	361.1296 (M^+)	300.0997 $(M\text{-}OCH_3)^+$
Formula	$C_{22}H_{19}NO_4$ (calcd. 361.1278)	$C_{21}H_{17}NO_3$ (calcd. 300.1025 for $C_{20}H_{14}NO_2$)
UV-VIS (λ_{max} (nm), MeOH)	240, 285, 365, 380, 425, 440, 462	240, 285, 365, 380, 420, 440, 460
$[\alpha]_D$ (MeOH; 3.5 mg/ml, 0.5 cm)	0 °	0 °
IR (λ_{max}, cm^{-1}, KBr)	744, 795, 852, 885, 978, 1026, 1070, 1110, 1134, 1184, 1265, 1307, 1334, 1402, 1437, 1461, 1484, 1555, 1586, 1590, 1627, 1720, 2950, 3420	737, 790, 837, 867, 897, 954, 1025, 1030, 1086, 1137, 1190, 1270, 1331, 1339, 1399, 1433, 1487, 1579, 1585, 1625, 1629, 1716, 2986, 3420
Fluorescence (λ_{max}, in MeOH)	493 nm	487 nm
(λ_{max} excitation):	(380 nm)	(440 nm)

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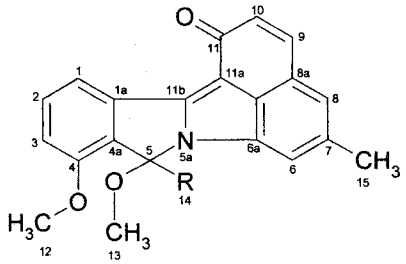
and fluorescence spectra attested to the presence of an extended conjugated π-electron system. The absorptions at λ_{max} 1720 and 1716 cm^{-1} in the IR spectra of **1** and **2** indicated two carbonyl groups in both molecules. Measurement of optical rotation (in MeOH) indicated that both compounds are inactive. Thus compound **2** could be a mixture of enantiomers. The ESI mass spectrum of **1** displayed ion peaks of *m/z* 362.2 $(M+H)^+$, 745.6 $(2M+Na)^+$ and 1105.9 $(3M+Na)^+$. In the ESI-MS of **2** pseudomolecular ions with *m/z* 332.0 ($[M+H]^+$), *m/z* 353.7 ($[M+Na]^+$), *m/z* 685.4 ($[2M+Na]^+$) and *m/z* 1016.0 ($[3M+Na]^+$) were visible. The molecular weight and the chemical formula of **1** and **2** as shown in Table 1 were furnished by HREI-MS (**1**: M^+: *m/z* 361.1296 (80%), calcd. 361.1314 for $C_{22}H_{19}NO_4$; **2**: $[M-OCH_3]^+$: *m/z* 300.09970 (80%), calcd. 300.10248 for $C_{20}H_{14}NO_2$). In addition the HREI-MS of **1** displayed *m/z* 330.1161 ($[M-OCH_3]^+$, 100%, calcd. 330.1130 for $C_{21}H_{16}NO_3$) and *m/z* 315.0874 ($[M-OCH_3-CH_3]^+$, 80%, calcd. 315.0859 for $C_{20}H_{13}NO_3$).

The number, bonding type and multiplicity of carbon atoms in **1** and **2** (Fig. 1) were determined by the ^{13}C and DEPT NMR spectra. The presence of quaternary carbon signals at 181.6 ppm in **1** and 181.3 ppm in **2** showed the presence of carbonyl groups in conjugation to double bonds. The quaternary carbon signal at 114.2 ppm in the ^{13}C spectrum of **1** was assigned to an *ortho* carbonic acid amide (C-5). In the ^{13}C spectrum of **2** a signal at 86.7 ppm attested to a semi aminal-type structure. Moreover, the number of methoxyl and methyl groups in both molecules was determined doubtlessly from the ^1H and ^{13}C NMR spectra. The positions of protons in **1** and **2** were suggested from the 2D NMR spectra ($^1H,^1H$-COSY, HSQC). In addition to the singlet proton signals of the methyl and methoxyl groups the ^1H NMR spectrum of **2** displayed 6.77 ppm for H-5. Its unusual deep-field shift can be explained by the electronic effects of the neighboured

Fig. 1. Structures of 0231A (**1**) and 0231B (**2**).



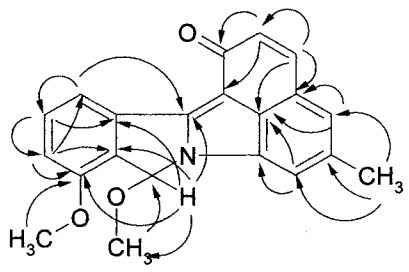
1 R = OCH_3

2 R = H

Table 2. Assignment of ^1H and ^{13}C NMR spectra of **1** and **2** (500 MHz, $CDCl_3$, TMS as internal standard, δ in ppm, *J* in Hz, s: singlet, d: doublet).

position	1		2	
	δ_C	δ_H	δ_C	δ_H
C-1	118.2	8.22 d, 78.3	118.0	8.22 d, 7.1
C-2	133.8	7.61 dd, 8.8, 7.9	132.9	7.57 dd, 7.1, 8.5
C-3	113.6	7.06 d, 7.9	113.1	7.03 d, 8.5
C-4	155.7	-	156.1	-
C-4a	125.2	-	127.7	-
C-5	114.2	-	86.7	6.77 s
C-6a	130.3	-	131.3	-
C-6	114.2	7.54 d, 0.5	113.3	7.45 d, 0.5
C-7	135.3	-	134.7	-
C-8	124.0	7.35 d, 0.5	123.7	7.33 d, 0.5
C-8a	109.4	-	109.1	-
C-9	137.8	7.66 d, 9.6	137.4	7.64 d, 9.6
C-10	132.4	6.71 d, 9.6	132.6	6.71 d, 9.6
C-11	181.6	-	181.3	-
C-11a	124.5	-	124.5	-
C-11b	146.1	-	148.6	-
C-12	55.9	4.00 s	55.8	3.99 s
C-13	52.6	3.10 s	51.0	2.97 s
C-14	52.6	3.10 s	-	-
C-15	22.1	2.58 s	22.1	2.58 s

Fig. 2. Instructive C,H long-range couplings in the HMBC spectrum of **2**.



aromatic ring and the two heteroatoms.

For the assignment of ^1H and ^{13}C signals as shown in Table 2 the observable C,H long-range couplings in the HMBC spectra (Fig. 2) were of pivotal importance. The positions of the methoxyl and methyl groups were thus assignable. In the HMBC spectrum of **1** instructive C,H long-range correlations were observed between both H-13 and H-14 with C-5 confirming that this carbon atom belongs to the diester of an *ortho*-carbonic acid amide. The HMBC spectrum of **2** showed C,H long-range couplings of H-5 with C-13, C-4a, C-1a, C-11b and C-4 which proved the position of the semiaminal-type C-5. Correlations between H-5 and C-4 in **2** confirmed the methoxyl substituent at C-12. It can be suggested that its position in **1** is the same. The observable NOE between H-5 (6.77 ppm) and H-6 (7.45 ppm) in the NOESY spectrum supported the structure of **2** as shown in Fig. 1.

Thus the structures of **1** and **2** as new polycyclic heteroaromatic systems were assigned on the basis of mass spectrometry, 1D and 2D NMR measurements (Table 2). The observed bright fluorescence is explainable by the presence of a new conjugated oxonaphthopyrrolo chromophore.

Coproduction of tetrangulol methylether[2~4] and other angucycline type metabolites suggested that **1** and **2** are shunt metabolites of the angucycline biosynthetic pathway[4]. Their formation could be explained by the oxidation of a quinone carbonyl, introduction of nitrogen, dehydratation and rearrangement of the cyclic system.

Both **1** and **2** displayed no activity against a series of Gram-positive and Gram-negative bacteria and fungi in concentration <200 μg/ml. However, 0231A (**1**) and 0231B (**2**) inhibited 3α-hydroxysteroid dehydrogenase with IC_{50}=10.5 μg/ml and 2.5 μg/ml, respectively[1].

Experimental

Mass spectra were recorded on a high-resolution sector-field mass spectrometer AMD-402 (AMD Intectra, Harpstedt, Germany), NMR spectra on a Bruker Avance DRX 500 spectrometer, UV-VIS and IR fluorescence spectra on Shimadzu spectrometers. Optical rotation was measured with a Kernchen polarimeter (Dr. KERNCHEN OPTICS, Seelze, Germany).

The strain *Streptomyces* sp. HKI 0231 was isolated from a soil sample collected near the Grotta dei Cervi (Porto Badisco, Italy). It was identified as *Streptomyces* sp. due to its morphological and physiological characteristics and was deposited in the strain collection of the Hans-Knöll-Institute for Natural Products Research Jena (Germany). A seed culture was prepared by inoculating 1~2 cm^2 agar pieces of a surface culture into Erlenmeyer flasks containing 100 ml of a medium composed as follows (g/liter): glucose 15, soybean meal 15, NaCl 5, $CaCO_3$ 1, KH_2PO_4 3, pH 6.5 (28°C, rotary shakers, 180 r.p.m., 48 hours). 5 ml of the seed culture were used to inoculate 100 ml medium in 500 ml Erlenmeyer flasks composed of (g/liter): mannitol 20, soybean meal 20, pH 6.5 (sterilization for 25 minutes at 110°C). Cultivation was carried out for 120 hours on rotary shakers (180 r.p.m., 28°C).

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