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ORIGINAL ARTICLE

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Bendigoles A~C, New Steroids from *Gordonia australis* Acta 2299†

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Abstract Bendigoles A~C are the first secondary metabolites to be isolated from a member of the actinomycete genus *Gordonia*. They were detected in a culture filtrate extract of *Gordonia australis* Acta 2299 by HPLC-diode array analysis and characterized as new steroids by mass spectrometry and NMR experiments. Bendigole C show binding affinity to the human progesterone and A~C to androgen receptor but are inactive at mineralocorticoid and estrogen receptors. In *in vitro* transactivation studies bendigoles A and C showed moderate and weak androgenic activities.

Keywords bendigole A, bendigole B, bendigole C, *Gordonia*, steroids, fermentation, isolation, structure elucidation

Introduction

Freshly isolated actinomycetes from selected terrestrial, limnetic and marine ecosystems were screened for the production of secondary metabolites to detect novel compounds for pharmaceutical applications (http://www.actapharm.org). The strains were cultivated as shake flask cultures in different complex media. Extracts were generated from culture filtrates and mycelia at various fermentation times and their secondary metabolite profiles evaluated by HPLC-diode array analysis and an in-house developed HPLC-UV-Vis database [2]. Strain Acta 2299, which was isolated from activated sludge foam [3] near Bendigo (Victoria, Australia), was of particular interest because of the appearance of two characteristic metabolites in HPLC runs of the culture filtrate extract at retention times of 8.1 and 9.3 minutes, respectively, in our standardized gradient elution profile. Their UV-visible spectra were not congruent with any of the 867 reference compounds stored in the database. HPLC-ESI-MS analysis of the culture filtrate extract revealed a molecular mass of 358.4 Da for the compound with a retention time of 8.1 minutes and 356.4 Da for the compound with a retention time of 9.3 minutes, which were named bendigole A (**1**) and bendigole B (**2**), respectively. A further minor congener of this family, bendigole C (**3**), was also detected. Their structures are shown in Fig. 1. The present work describes

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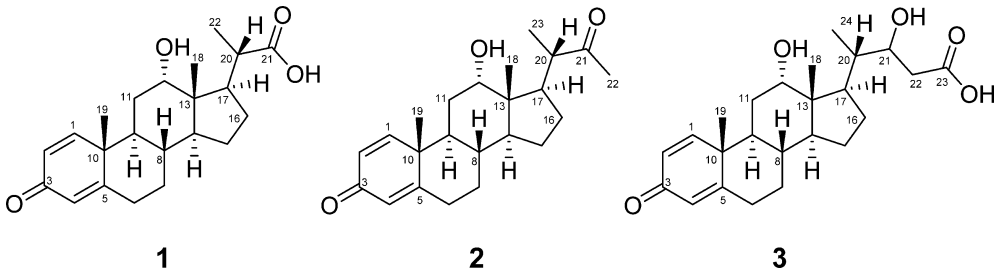

Fig. 1 Structures of bendigoles A (**1**), B (**2**) and C (**3**).

the fermentation, isolation, structure elucidation and the biological activity of the bendigoles.

Materials and Methods

Producing Organism and Taxonomy

Strain Acta 2299 was isolated from activated sludge foam collected from the Bendigo Biological Nutrient Removal Plant, Victoria, Australia. The microorganism was examined for a range of genotypic and phenotypic properties known to be useful in the classification of actinomycetes isolated from activated sludge foam [3, 4].

Fermentation and Isolation

Batch fermentations of strain Acta 2299 were carried out in a 10-liter stirred-tank fermentor (Biostat S, B. Braun Melsungen, Germany) in a medium which consisted of soluble starch 10 g, glucose 10 g, glycerol 10 g, cornsteep powder (Marcor) 2.5 g, Bacto peptone 5.0 g, yeast extract (Ohly Kat, Deutsche Hefewerke) 2.0 g, NaCl 1.0 g and $CaCO_3$ 3.0 g in 1.0 liter tap water; the pH was adjusted to 7.3 with 1.0 M NaOH prior to sterilization. The fermentor was inoculated with 5.0% by volume of a shake flask culture grown in a seed medium at 27°C in 500 ml-Erlenmeyer flasks with one baffle for 48 hours on a rotary shaker at 120 rpm. The seed medium consisted of glucose 10 g, glycerol 10 g, oatmeal 5.0 g, soybean meal 10 g, yeast extract (Ohly Kat) 5.0 g, Bacto casamino acids 5.0 g and $CaCO_3$ 1.0 g in 1.0 liter tap water. The fermentation was carried out for 6 days with an aeration rate of 0.5 vvm and agitation at 250 rpm.

Hyphlo Super-cel (2.0%) was added to the fermentation broth which was separated by multiple sheet filtration into culture filtrate and mycelium. The mycelium was discarded and the culture filtrate (7.0 liters) applied to an Amberlite XAD-16 column (resin volume 1.0 liter); the resin was washed with H_2O and H_2O - MeOH (4 : 6). Bendigoles were eluted with MeOH, concentrated *in vacuo* to an aqueous residue; the latter was adjusted to pH 4.0 with 1.0 M HCl and extracted four times with EtOAc. The organic extracts were combined and concentrated *in vacuo* to dryness. The crude product was dissolved in CH_2Cl_2 and added to a silica gel column (45×2.6 cm, silica gel SI 60, Merck), the separation was accomplished by a linear gradient from CH_2Cl_2 to CH_2Cl_2 - MeOH (9 : 1) within 4 hours at a flow rate of 5.0 ml/minute. Fractions containing bendigoles were purified by Sephadex LH-20 (90×2.5 cm) using MeOH as the eluent. To obtain pure **1**, **2**, and **3**, each fraction was subjected to preparative HPLC using a C18 column (Grom-Sil 300 ODS-5 St, 10 μm, 25×2.0 cm) with CH_3CN - 0.1% HCOOH (**1**: a linear gradient from 20 to 40% CH_3CN over 40 minutes, **2**, **3**: a linear gradient from 35 to 50% CH_3CN over 40 minutes) at a flow rate of 15 ml/minute.

HPLC-DAD Analyses

The chromatographic system consisted of a HP 1090M liquid chromatograph equipped with a diode-array detector and a HP Kayak XM 600 ChemStation (Agilent, Germany). Multiple wavelength monitoring was performed at 210, 230, 260, 280, 310, 360, 435 and 500 nm, and UV-visible spectra measured from λ=200 to 600 nm. A 10-ml aliquot of the fermentation broth was centrifuged, and the supernatant adjusted to pH 4.0 and extracted with the same volume of EtOAc. After centrifugation, the organic layer was concentrated to dryness *in vacuo* and resuspended in 1.0 ml MeOH. 10-μl aliquots of the samples were injected onto an HPLC column (125×4.6 mm) fitted with a guard-column (20×4.6 mm) filled with 5.0-μm Nucleosil-100 C-18 (Maisch, Germany). The samples were analysed by linear gradient elution using 0.1% *ortho*-phosphoric acid as solvent A and CH_3CN as solvent B at a flow rate of 2.0 ml/minute. The gradient was from 0 to 100% for solvent B in 15 minutes with a 2-minute hold at 100% for solvent B.

Structural Elucidation

LC-MS experiments were performed on an Applied Biosystems QTrap 2000 (Applied Biosystems, Germany) coupled to an Agilent 1100 HPLC system (Agilent,

Germany). ESI-FT-ICR mass spectra were recorded on an APEX II FTICR mass spectrometer (4.7 T, Bruker-Daltonics, Germany), and NMR experiments on a DRX 500 NMR spectrometer (Bruker, Germany) equipped with a BBI probehead with z gradients. X-Ray diffraction data were collected on an Oxford Diffraction Xcalibur S diffractometer. The diffractometer is equipped with a Sapphire CCD detector and an enhanced monochromated MoKα source on a four-circle kappa platform. The diffraction frames were integrated by using the CrysAlisRed program, the set of data was corrected for empirical absorption with SCALE3 ABSPACK [5].The structure was solved by direct methods and refined using the program SHELX97 [6].

Crystallographic data for the structure in this paper have been deposited with the Cambridge Crystallographic Data Centre, CCDC, No. 674696. Copies of the data may be obtained free of charge at http://www.ccdc.cam.ac.uk/data_request/cif.

Biological Activity

Receptor binding studies were essentially performed as described before [7]. In brief, increasing amounts of test compound were incubated with human progesterone receptor (hPR) and human androgen receptor (hAR) preincubated with ^{3}H-labelled progesterone and ^{3}H-labelled methyltrienolone (R1881) in competition experiments. Displacement of the labelled reference compounds was determined, and a displacement curve was determined. Receptor binding affinities are expressed as IC_{50} values of the displacement curves. Experiments for hMR and hER were carried out accordingly using aldosterone and estrogen as reference compounds (data not shown).

Transactivation experiments were performed as described before [7]. In brief, PC3 cells stably expressing hAR and stably transfected with a MMTV-Luc reporter plasmid containing the reporter gene luciferase (*luc*) under the control of the steroid hormone responsive murine mammary tumor virus promoter (MMTV) were incubated with increasing amounts of test compound, and luciferase activity was determined after 24 hours. A dose response curve was determined expressing androgenic activity relative to the effect of 1.0 nM methyltrienolone which was arbitrarily set as 100%. Androgenic activity is expresses as EC_{50} value for each dose response curve.

Results

Taxonomy of the Producing Strain

Strain Acta 2299 was Gram-positive, acid-alcohol fast and formed moderately branched hyphae which fragmented into rods and cocci. The pale beige to peach colonies contained *meso*-diaminopimelic acid and *N*-glycolated muramic acid in the peptidoglycan, arabinose and galactose in the whole cell hydrolysate, and dehydrogenated menaquinones with nine isoprene units as the predominant isoprenologue, properties consistent with the classification of the organism in the genus *Gordonia* [3]. The organism also contained mycolic acids, the methyl esters of which had the same Rf value as *Gordonia* marker strains. In addition, the strain formed a distinct phyletic line in the *Gordonia* 16S rRNA gene tree together with isolates provisionally assigned the name "*Gordonia australis*".

Screening, Fermentation and Isolation

A crude extract of the culture filtrate of strain Acta 2299 was screened using an HPLC-DAD method [2], the resultant elution profile showed two characteristic peaks with nearly congruent UV-visible spectra (Fig. 2). The absence of spectral matches with 867 reference compounds stored in the HPLC-UV-Vis database prompted us to undertake the isolation and structure elucidation of the unknown metabolites.

Strain Acta 2299 was grown in a 10-liter fermentor using a complex production medium. The culture reached a maximal biomass of 3 vol-% after 5 days. Production of bendigoles started at 48 hours yielding 20 mg/liter of bendigole A (**1**) and 12.5 mg/liter of bendigole B (**2**) after 6 days of fermentation. When the strain was grown in the seed medium for 4 days resulting in a high biomass formation, no bendigoles were detected by HPLC-MS in dichloromethane extracts neither in the biomass nor in the supernatant.

1 and **2** were isolated from the culture filtrate by Amberlite XAD-16 chromatography and subsequently purified by a succession of selective chromatographic steps. Bendigole C (**3**) was isolated as a minor congener during the purification procedure.

Structural Elucidation

The mass spectra derived from HPLC-ESI-MS chromatograms revealed molecular masses for **1** [$(M+H)^+$=359.5], **2** [$(M+H)^+$=357.5] and **3** [$(M+H)^+$=403.6]. The exact molecular masses were determined by high-resolution ESI-FT-ICR-MS, as 717.43644 Da [$(2M+H)^+$] (**1**), 713.47809 Da [$(2M+H)^+$] (**2**) and 805.48894 Da [$(2M+H)^+$], corresponding to the molecular formulae $C_{22}H_{30}O_4$ (**1**) [$(2M+H)^+_{theor}$=717.43610; Δm=0.475 ppm], $C_{23}H_{32}O_3$ (**2**) [$(2M+H)^+_{theor}$=713.47757; Δm=0.733 ppm] and $C_{24}H_{34}O_5$ (**3**) [$(2M+H)^+_{theor}$=805.48853; Δm=0.511 ppm].

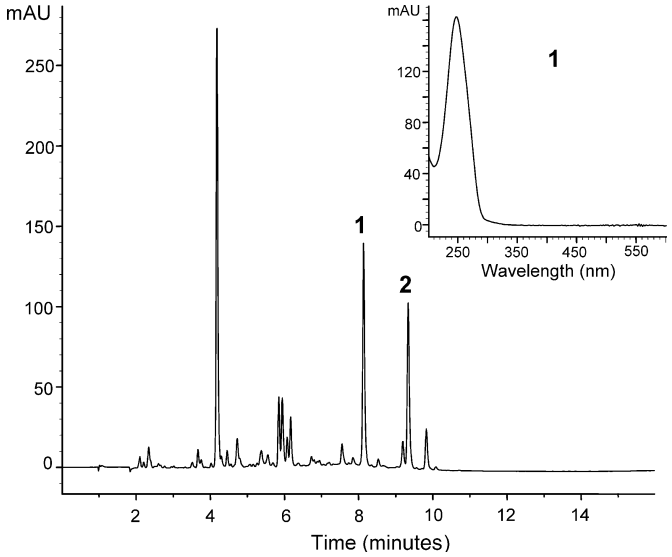

Fig. 2 HPLC analysis of a culture filtrate extract of *Gordonia australis* Acta 2299 at a fermentation time of 96 hours, monitored at λ=260 nm, and exemplary chosen UV-visible spectrum of bendigole A (**1**).

Table 1 Physico-chemical properties of bendigoles A~C

	A (**1**)	B (**2**)	C (**3**)
Appearance	White powder	White powder	White powder
Molecular weight	358	356	402
Molecular formula	$C_{22}H_{30}O_4$	$C_{23}H_{32}O_3$	$C_{24}H_{34}O_5$
ESI-FT-ICR MS (*m*/*z*)			
Found	717.43644	713.47809	805.48894
	$(2M+H)^+$	$(2M+H)^+$	$(2M+H)^+$
Calc.	717.43610	713.47757	805.48853
UV λ_{max}^{MeOH} [nm] (ε [cm^2 μmol^{-1}])	247 (3.30)	246 (2.26)	244 (3.96)
IR ν_{max} (cm^{-1})	3432, 2968, 2936, 2872, 2853, 1724, 1706, 1657, 1614, 1599	3441, 2933, 2916, 2872, 2849, 1703, 1658, 1617, 1600	3421, 2961, 2935, 2872, 1726, 1656, 1614, 1599

The ^{1}H-NMR-spectrum of **2** showed three signals in the aromatic region, two signals around 4 ppm and a lot of multipletts in the aliphatic region between 2.5 and 1 ppm. ^{13}C-NMR and DEPT spectra revealed the presence of four methyl, five methylene, three aromatic, six methine and five quaternary carbons, including one ketone (δ=211.9 ppm). The correlation of ^{1}H-NMR-signals to the corresponding C-atoms was carried out in a Heteronuclear Multiple Quantum Coherence (HMQC) NMR experiment. One signal in the ^{1}H-NMR-spectrum could not be assigned to any C-atom suggesting the presence of a hydroxy group. The structure was fully elucidated using COSY and HMBC spectra. The ^{1}H-^{1}H-COSY experiment revealed correlations from H-1 to H-2, H_2-6 to H-8, H-8 to H-9 and to H-14, from H-9 to H_2-11, from H_2-11 to H-12, from H_2-16 to H-17, from H-17 to H-20 and from H-20 to H_3-23 (Fig. 3). The spin system H_2-14 to H_2-17 could not be established unambiguously since the chemical shifts of the protons H-14 (1.49 ppm), H_2-15 (1.50/1.09 ppm) and H_2-16 (1.50/1.08 ppm) are very similar. The structure was fully elucidated using the HMBC spectrum. Selected correlations from the HMBC spectrum shown in Fig. 3 provide evidence for the positions of the methyl groups and hydroxy group, and for the presence of a methyl-ketone as well as its position. In parallel to NMR studies, crystallisation experiments were performed with a single crystal being obtained for compound **2**. Subsequent X-ray structural determination fully confirmed the structure and revealed the relative stereochemistry for compound **2** (Fig. 4). The relative stereochemistry was also examined in

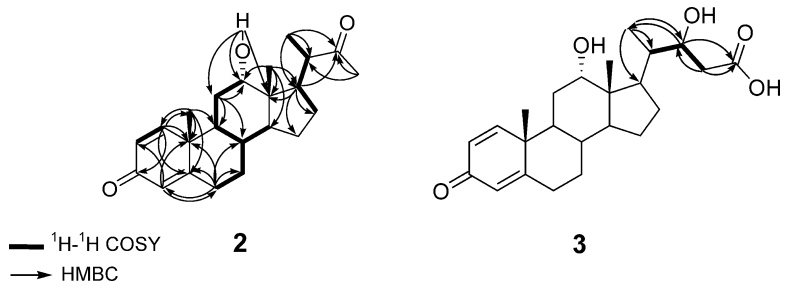

Fig. 3 Selected 2D NMR correlations for bendigoles B (**2**) and C (**3**).

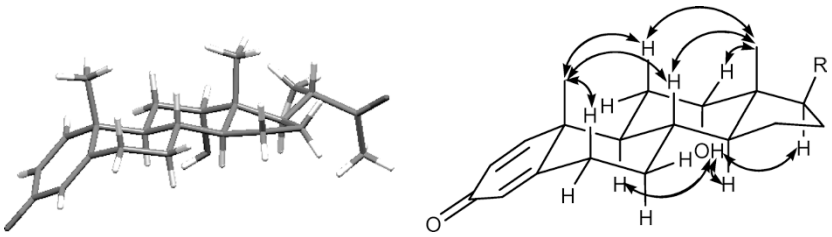

Fig. 4 X-Ray structure of bendigole B (**2**) and selected NOE correlations of **2**.

NOESY experiments (Fig. 4). In the NOESY spectrum of compound **2**, H-8 showed NOE correlations to H_3-18 and H_3-19, suggesting a β-orientation of H-8. H-14 did not exhibit a NOE correlation to H_3-18, but showed a correlation to the 12-OH proton, which also showed NOE correlations to H-9 and H-17, thereby suggesting a-orientation of these protons. These observations were all in accordance with the crystal structure of compound **2**.

Comparison of the molecular formulae of **1**, $C_{22}H_{30}O_4$, and **2**, $C_{23}H_{32}O_3$, shows that a methyl group is replaced by a hydroxy group in compound **1**; this assumption was confirmed in NMR experiments. The ^{13}C-NMR and DEPT spectra of **1** revealed the presence of three methyl, five methylene, three aromatic, six methine and five quaternary carbons. Compared to compound **2**, a methyl group (C-22) was missing as well as the ketone signal (C-21). Instead, there was an additional signal at 177.7 ppm, characteristic for a carboxylic acid. In the ^{1}H-NMR-spectrum the signal of the methyl group CH_3-22 was missing compared to the ^{1}H-NMR-spectrum of compound **2**, while a broad signal appeared at 11.8 ppm. Further NMR-experiments confirmed that the methyl-ketone in compound **2** is replaced by a carboxylic acid in compound **1** (Fig. 1). The stereochemistry was verified in NOESY experiments. In the case of compound **1** similar NOE correlations were observed, from H-8 to H_3-18 and H_3-19, from H-14 to the 12-OH proton and to H-9. Hence the stereochemistry for compound **1** is, as expected, similar to that of compound **2**.

The formal difference in the molecular formulae of compounds **1** and **3** is C_2H_4O. The ^{13}C-NMR and DEPT spectra of **3** revealed the presence of three methyl, six methylene, three aromatic, seven methine and five quaternary carbons; therefore, the difference between the two compounds was $CHCH_2OH$. The structure was fully elucidated with the help of 2D NMR experiments. The chemical shift of the additional CH group together with the observed correlations in the COSY and the HMBC experiments revealed that the side chain was extended by a $CHOHCH_2$ group. In the COSY spectrum additional correlations were observed from H-20 to H-21 and from H-21 to H_2-22 (Fig. 3). In the HMBC spectrum H-21 showed correlations to C-23 and C-24, H_2-22 to C-24 and C-21 and H_3-24 to C-17, C-20 and C-21. These observed correlations gave proof for the structure of compound **3**.

Biological Activity

Bendigoles A (**1**), B (**2**) and C (**3**) have a 3-oxo group (ring A) and polar groups at the D ring which is common to steroid hormone receptor ligands, such as cortisol, aldosterone, progesterone and testosterone. Table 3 summarizes the binding affinities of **1**∼**3** to hPR and hAR. Binding affinities are expressed as IC_{50} values for the displacement curve and relative binding affinity (RBA), where the RBA of the reference compounds was arbitrarily set to 100%. This comparative study was performed using cytosol from insect cells expressing the respective steroid receptors. The ability of **1**∼**3** to displace the radioactively labelled reference ligands was determined in competition

Table 2 ^{1}H- and ^{13}C-NMR spectral data of bendigoles A (**1**), B (**2**) and C (**3**) in DMSO-d_6

	1 in DMSO-d_6		**2** in DMSO-d_6		**3** in DMSO-d_6	
No.	δ (^{1}H) [ppm] J in Hz	δ (^{13}C) [ppm]	δ (^{1}H) [ppm] J in Hz	δ (^{13}C) [ppm]	δ (^{1}H) [ppm] J in Hz	δ (^{13}C) [ppm]
1	7.13 d (10.2)	156.2 CH	7.12 d (10.1)	156.1 CH	7.13 d (10.0)	156.3 CH
2	6.10 dd (10.2, 1.75)	126.4 CH	6.11 dd (10.1, 1.6)	126.4 CH	6.10 dd (10.0, 1.68)	126.4 CH
3	—	185.1 C	—	185.0 C	—	185.1 C
4	5.97 br s	122.7 CH	5.97 br	122.7 CH	5.96 br s	122.7 CH
5	—	169.8 C	—	169.8 C	—	169.9 C
6	2.45 m/2.31 m	31.8 CH_2	2.46/2.31 m	31.8 CH_2	2.45/2.30 m	31.8 CH_2
7	1.85 m/0.94 m	33.1 CH_2	1.85/0.94 m	33.1 CH_2	1.86/0.92 m	33.1 CH_2
8	1.63 m	34.8 CH	1.64 m	34.8 CH	1.63 m	34.8 CH
9	1.35 m	45.6 CH	1.36 m	45.6 CH	1.34 m	45.6 CH
10	—	42.9 C	—	42.9 C	—	42.9 C
11	1.81 m/1.70 m	30.1 CH_2	1.80/1.71 m	30.0 CH_2	1.80/1.68 m	29.9 CH_2
12	3.78 br	70.4 CH	3.80 br	70.4 CH	3.81 br	70.5 CH
13	—	46.0 C	—	46.3 C	—	46.3 CH
14	1.48 m	46.1 CH	1.49 m	45.9 CH	1.44 m	45.8 CH
15	1.50 m/1.11 not detectable	23.3 CH_2	1.50/1.09 m	23.5 CH_2	1.52/1.11 m	23.4 CH_2
16	1.60 m/1.23 m	26.0 CH_2	1.50/1.08 m	25.9 CH_2	1.65/1.24 m	26.1 CH_2
17	2.14 m	43.2 CH	2.14 m	42.4 CH	1.64 m	43.2 CH
18	0.68 s	12.3 CH_3	0.70 s	12.3 CH_3	0.69 s	12.2 CH_3
19	1.16 s	18.1 CH_3	1.17 s	18.0 CH_3	1.16 s	18.0 CH_3
20	2.14 m	41.9 CH	2.34 m	49.4 CH	1.49 m	41.5 CH
21	1.11 d (5.67)	15.8 CH_3	1.07 d (6.87)	14.6 CH_3	3.91 m	68.7 CH
22	—	177.7 C	—	211.9 C	2.17/1.94 m	35.7 CH_2
23	—	—	2.03 s	27.4 CH_3	—	174.1 C
24	—	—	—	—	0.86 m	10.6 CH_3

Table 3 Receptor binding affinities of bendigoles A (**1**), B (**2**) and C (**3**)

	Human progesterone receptor binding	Human androgen receptor binding	Mineralocorticoid receptor binding	Estrogen receptor alpha binding	Estrogen receptor beta binding
Reference compound	Progesterone	Methyltrienolone (R1881)	Aldosterone	Estrogen	Estrogen
Unit	IC_{50} [μM]/RBA [%]	IC_{50} [μM]/RBA [%]	IC_{50} [μM]/RBA [%]	IC_{50} [μM]/RBA [%]	IC_{50} [μM]/RBA [%]
Reference	0.029/100	0.032/100	12/100	32/100	5.9/100
Bendigole A (**1**)	no affinity	8.9/0.4	no affinity	no affinity	no affinity
Bendigole B (**2**)	no affinity	1.9/1.7	no affinity	no affinity	no affinity
Bendigole C (**3**)	1.01/2.8	1.4/2.3	no affinity	no affinity	no affinity

experiments. Only bendigole C (**3**) showed some affinity to the hPR with an RBA of 2.8% compared to progesterone. Fig. 6a shows the displacement curves for progesterone as reference compound (open circles) and **3** at the hPR. No displacement was seen for **1** and **2** (data not shown). Fig. 6b shows the displacement curves for **1**～**3** at the hAR and methyltrienolone as reference compound (open circles). All three bendigoles displayed affinity to the hAR with RBA values of 0.4, 1.7 and 2.3%, respectively, compared to the high affinity AR ligand methyltrienolone (R1881). Apparently **3** accommodates best in the hAR and hPR ligand binding pockets which indicates that the length of

the side chain in position 17 does not hinder binding. The moderate binding affinity may be due to the uncommon α-hydroxy group at position 12, which is not present in any of the physiological steroid hormone receptor ligands in man.

No binding affinity of bendigoles was observed to the human mineralocorticoid receptor (hMR). The physiological ligands of the hMR, aldosterone and cortisol, bear hydroxy groups in position 11 and 21, which are not present in bendigoles. This may explain their inability to displace physiological ligands in a competition assay. Furthermore, bendigoles showed no affinity to the human estrogen receptors, which is expected since estrogen receptors require ligands with an aromatic A ring and a 3-hydroxyl function.

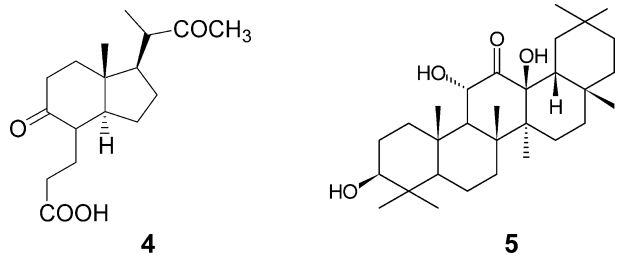

Fig. 5 Structures of 3-[7aβ-methyl-5-oxo-1β-(1α-methyl-2-oxopropyl)-3aαh-hexa-hydroindan-4α-yl]-propionic acid (**4**) and 3,11,13-trihydroxy-12-oleanone (**5**) from *Gordonia* species.

To determine the androgenic or antiandrogenic activities of bendigoles *in vitro* in more detail, transactivation studies were performed using cells stably expressing hAR and the reporter gene luciferase under the control of the steroid-responsive murine mammary tumour virus (MMTV) promoter. As expected from their affinities to the hAR, **1** and **3** exhibited a dose dependent androgenic effect *in vitro* (Fig. 7). Table 4 shows the determined EC_{50} values for all bendigoles. **1** and **3** showed an EC_{50} of 195 and 40 nM, respectively, indicating that the bendigoles are moderate androgenic compounds. Again, bendigole C (**3**) turned out

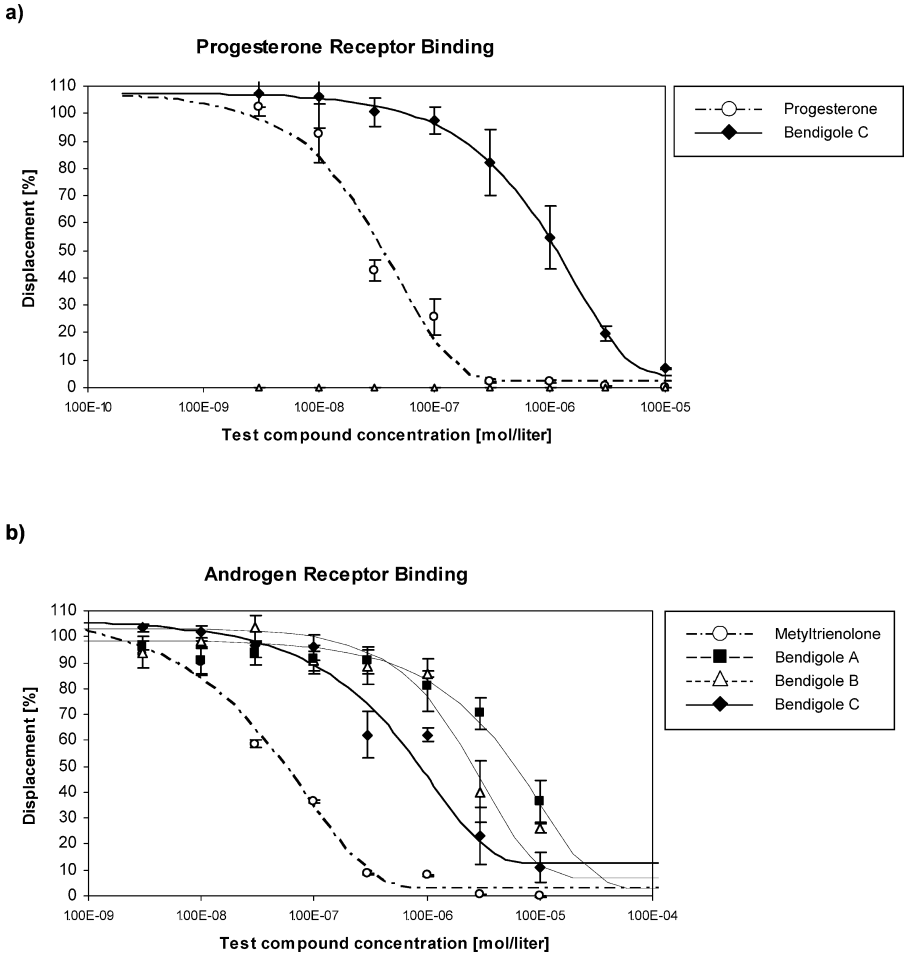

Fig. 6 Receptor binding analyses of bendigoles A (**1**), B (**2**) and C (**3**) at human progesterone receptor (**a**) and human androgen receptor (**b**).

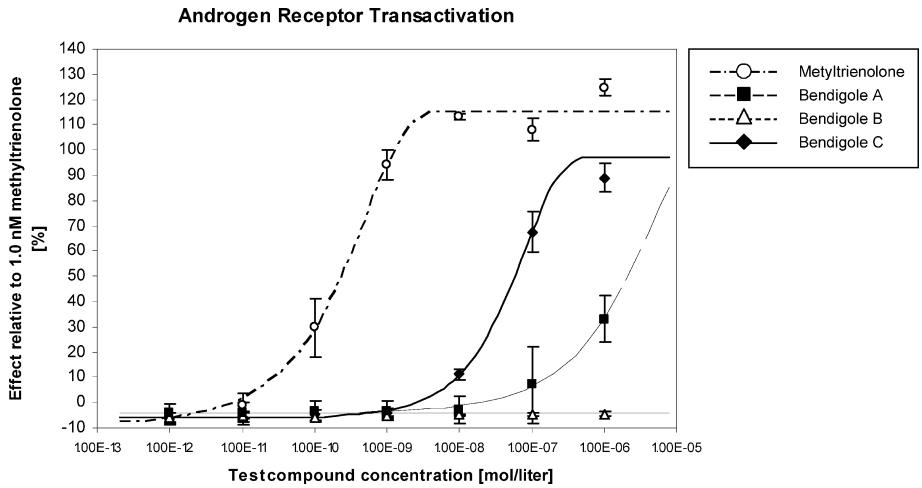

Fig. 7 Androgen receptor transactivation studies of bendigoles A (**1**), B (**2**) and C (**3**).

to be the most potent of the three bendigoles. However, in spite of the hAR binding capacity determined, **2** showed no transactivating capacity *in vitro* which indicates that the compound is not able to fully activate the receptor protein. This may be due to the missing hydroxyl function at the D ring or at the side chain in position 17. No antiandrogenic properties were observed (data not shown). Likewise, none of the bendigoles showed progestagenic or antigestagenic activites at hPR *in vitro* (data not shown).

Table 4 *In vitro* androgenic activity of bendigoles A (**1**), B (**2**) and C (**3**)

	Androgen receptor transactivation agonism
Reference compound	Methyltrienolone (R1881)
Unit	EC_{50} [nM]
Reference	0.26
Bendigole A (**1**)	195.00
Bendigole B (**2**)	no activity
Bendigole C (**3**)	40.00

Discussion

Steroids are ubiquitous cell constituents in eukaryotes which control the fluidity and flexibility of the cell membranes [8], whereas prokaryotes contain hopanoids, pentacyclic terpenes, which have the same function as steroids [9]. However, some reports demonstrate the presence of steroids in bacterial cells [10]. The steroids 4α-methyl-5α-cholest-8(14)-en-3β-ol, 4,4-dimethyl-5α-cholest-8(14)-en-3β-ol, 4α-methyl-5α-cholest-8(14),24-dien-3β-ol and 4,4-dimethyl-5α-cholest-8(14),24-dien-3β-ol were isolated from a *Methylococcus capsulatus* strain grown on methane as the sole carbon source [11, 12]. Two further methylotrophic bacteria, *Methylobacterium organophilum* and *Methylosphaera hansonii*, were found to contain 4,4-dimethyl sterol and lanosterol, respectively [13, 14]. Kohl *et al.* [15] have convincingly shown that myxobacterium *Nannocystis excedens* produces 4-demethylsterols; they identified cholest-8(9)-en-3-β-ol as the main sterol compound according to MS, NMR and X-ray analyses. They also showed that the sterols were absent from the cultivation media and corroborated their findings with radiolabelling experiments. These findings were extended by Bode *et al.* [16] in a screening programme. The experiment was designed to detect steroids, squalene and its dehydroderivates involving 88 strains representing genera of the order *Myxococcales*, and it was found that the biosynthetic pathway of steroids is widespreaded in this group of microorganisms. At the genetic level, Pearson *et al.* [17] compared nine sequences for eukaryotic squalene monooxygenase and 12 sequences for eukaryotic oxidosqualene cyclases with available prokaryotic genomes. In this study it was unambiguously shown that the sterol biosynthetic pathway is present in *Methylococcus capsulatus* and in the planctomycete *Gemmata obscuriglobus*.

Bendigoles B (**2**) and C (**3**) are new steroids isolated from *Gordonia australis* Acta 2299. Bendigole A (**1**) has already been reported as a degradation product from bile acids [18], but this is the first time that the compound has been found as a metabolic product of an organism. To date, only three compounds have been described from members

of the genus *Gordonia*, (i) the acidic polysaccharide gordonan, which shows a cell aggregation-inducing activity in insect BM-N4 cells [19], (ii) the biotransformation product 3-[7aβ-methyl-5-oxo-1β-(1α-methyl-2-oxopropyl)-3aαh-hexa-hydroindan-4α-yl]-propionic acid (**4**; Fig. 5) from a mutant of *G. rubropertinctus* cultured in a medium that contained sterols as substrate [20], and (iii) the sterol 3,11,13-trihydroxy-12-oleanone (**5**; Fig. 5) which was isolated as a cell constituent of *G. dassanayekei* and *G. zeylanica* [21]. The bendigoles are the first secondary metabolites and the first sterols detected in a member of the genus *Gordonia* as it was shown that they were not part of medium constituents, were not produced in the seed medium that permited optimal growth, but were produced and excreted from the cells only under specific growth conditions.

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