Juglomycins G-J: Isolation from Streptomycetes and Structure Elucidation

R. P. Maskey, S. Fotso, H. Lessmann, I. Grün-Wollny[a], H. Lackner, and H. Laatsch[*]

Department of Organic and Biomolecular Chemistry, University of Göttingen,
Tammanstrasse 2, D-37077 Göttingen, Germany
[a] Labor Grün-Wollny, Versaillerstr. 1, D-35394 Giessen

Reprint requests to Prof. Dr. H. Laatsch. Fax: +49(0)551-399660. E-mail: hlaatsc@gwdg.de

Z. Naturforsch. **60b**, 183-188 (2005): received June 22, 2004

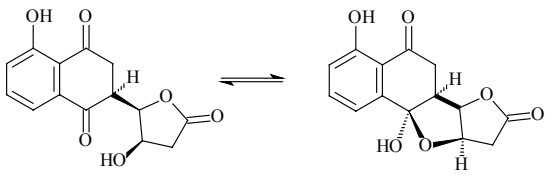
Dedicated to Prof. Dr. K. Krohn on the occasion of his 60th birthday

From the *Streptomyces* spp. 815 and GW4184, four new 4-juglon-2-ylbutyric acid derivatives, the juglomycins G – J (**3a**, **4a**, **4c**, **5c**), have been isolated and characterised. The structures were derived from the 1D and 2D NMR data and mass spectra and confirmed by comparison with structurally related compounds. The absolute configuration was deduced from the CD spectra. The new natural products exhibited weak antibacterial activity similar to juglomycin A (**5a**).

Key words: Juglomycin, Juglon, Streptomycetes

Introduction

Juglomycins [1], broad-spectrum antibiotics with cytotoxic activity, are biogenetically (folded polyketides [2]) related to the benzoisochromanquinones of the nanaomycin/kalafungin type [3] and even to α-methylanthraquinone derivatives [2], usually found in plants or insects (Aloe, Kermes *etc*). An important key-intermediate on the way to different juglomycins is apparently the (2S,3´R,4´R)-2,3-dihydrojuglomycin A (**1**) in equilibrium with its tetracyclic (1R,2S,3´R,4´R)-hemiketal **2** [1a,c]. From here, the pathway continues to juglomycin C (**3e**), then splitting into two directions: (A) to the monomeric juglomycin variants A - F and the α-methylanthraquinones [1c,2,4,5], and (B) to dimerisation products of hitherto unprecedented structures [1c,6], and finally to the deeply red pentacyclic juglorubin [7].



1 **2**

While searching for further intermediates, the new juglomycins G, H and I (**3a**, **4a**, **4c**) [1c] have been found in the same terrestrial *Streptomyces* sp. 815, that generated the above mentioned monomeric and dimeric metabolites including α-methylanthraquinones. Surprisingly, a *Streptomyces* strain GW4184 recently isolated by one of the authors (I. G.-W.) also delivered the juglomycins A and B (**5a**, **5b**) [1], juglorubin [7], oviedomycin [8], GTRI-02 [9], and a new member of the juglomycin family, which was named juglomycin J (**5c**). Here, we describe the new juglomycins G – J and the isolation procedures; a subsequent paper [6] will deal with the novel dimerisation products of pathway B.

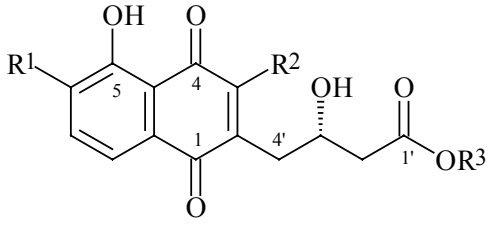
Results and Discussion

Juglomycin G (**3a**)

In M_2 medium, the *Streptomyces* strain 815 produced a series of juglomycin variants and derivatives, which were responsible for the high antibacterial activity of the crude extract. After complex separation procedures, a further quinone **3a** was isolated, which eluted from silica gel only slowly with a strong tailing. On spraying with dilute sodium hydroxide, the yellow spot gave an intensive blue colouration characteristic of *peri*-hydroxy quinones (λ_{max} 451 → 608 nm). The molecular formula $C_{14}H_{12}O_7$ was obtained by EI HRMS of the molecular peak at *m/z* 292. The IR spectrum delivered characteristic bands at 3432–3222 (OH), 1706 (carboxylic acid) and 1636/1609 cm^{-1} (chelated and non-chelated carbonyls of the quinone). The presence of a free carboxylic acid was confirmed by the smooth formation of the methyl ester **3b** on treatment with MeOH/HCl. A non-chelated phenolic OH group was identified by

the formation of **3c** from **3b** on careful treatment with diazomethane.

The aliphatic region of the ^{1}H NMR spectrum with signals of a –$CH_2CH(OH)CH_2CO$–fragment was very similar to that of juglomycin C (**3e**) [1]. A quinonoid proton at δ = 6.93 as in the juglomycins A – C [1] showed allylic couplings of 1.2 and 1.0 Hz to the 4'-CH_2 group. Instead of the signals for three consecutive aromatic protons in **3e**, only the AB system of two *ortho*-protons at δ = 7.60 and 7.24 (J = 8.3 Hz) was observed. Formula and spectroscopic data led to a 5,6-dihydroxynaphthoquinone chromophore, as 5,8-dihydroxynaphthoquinones (naphthazarines) would show *two* chelated OH signals, different low-field proton shifts and a red instead of a yellow colour [10,11]. The resulting structure **3a** was supported by a proton-coupled ^{13}C NMR spectrum, in which only C-7 (δ = 121.8, J = 166/1.5 Hz) showed a ^3J-coupling with a phenolic proton, and by comparison of the proton peaks with those of diomelquinone A [12] and arizonin B1 [13]. The position of the side chain at C-2 was given by the ^3J-couplings from both 4'-H and 3-H to the non-chelated carbonyl carbon (δ = 183.9). The configuration at C-3' was supposed to be (S) as in **3e** [1], which completes the structure of the new juglomycin G (**3a**).



3	**a**	**b**	**c**	**d**	**e**	**f**	**g**
R^1	OH	OH	OCH_3	OCH_3	H	H	H
R^2	H	H	H	H	H	OH	CH_3
R^3	H	CH_3	CH_3	H	H	H	H

Juglomycin H (**4a**)

After methylation of a crude quinone mixture (fraction C6.2; see exp.), juglomycin H (**4a**) was isolated as its crystalline methyl ester **4b**, colourless needles with m.p. 124 °C. **4b** gave a yellow fluorescence under 366 nm and no bathochromic shift of the UV maximum (333 nm) after addition of alkali. The IR spectrum clearly indicated hydroxy groups (3241 cm^{-1}), ester carbonyls (1742 cm^{-1}) and a chelated ketone carbonyl (1633 cm^{-1}). EI HRMS of the molecular peak delivered the formula $C_{15}H_{18}O_6$ (*m/z* 294.1103), which finally gave $C_{14}H_{16}O_6$ for the natural juglomycin H (**4a**).

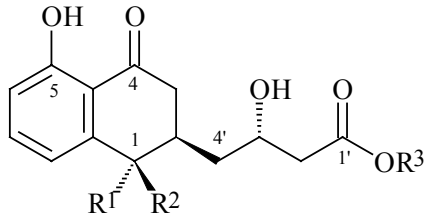
The proton NMR spectrum of **4b** depicted low field signals at δ = 12.39 for a chelated OH group and for three consecutive aromatic protons, the *peri*-proton of which (δ = 7.19) showed a long range coupling (J = 1.0 Hz) with 1-H* at δ = 4.59, thus indicating an aliphatic CH group bearing oxygen. The high-field region showed signals of three methylene and three methine groups, two of the latter being connected to oxygen. The proton at δ = 4.59 coupled with an OH proton (δ = 5.05) and 2-H. 2-H also coupled with two methylene groups, one of which (δ = 1.97/1.48) represents the link to the β-hydroxybutyric acid side chain as in juglomycin C (**3e**). Characteristic ^{13}C signals at δ = 72.5 (C-1) and δ = 204.6 (C-4) supported the structure. From the *vicinal* coupling (J = 8.7 Hz, Θ = 170°) between 1-H and 2-H, a *bis*-axial *trans* position of the protons can be derived. In isoshinanolone [14,15] (~ 60°), the corresponding protons show an eq./ax. coupling of 2.5 Hz.

Due to the weakly positive CD effect at 333 nm ($n\rightarrow\pi^*$, R-Band) of **4b**, the configuration at C-2 should be (R) [16,17] and ultimately (S) at C-1. In the CD spectrum of isoshinanolone (1R, 2R) also a weakly positive effect at 330 nm has been observed [14], while its CD curve in the short wave length region is mirror imaged. Due to the identical skeleton and the same source, the configuration at C-3' is believed to be (S) as in juglomycin C (**3e**) [1]. This leads to a 1S,2R,3'S-configuration of **4b** and juglomycin H (**4a**).

Juglomycin I (**4c**)

Juglomycin I (**4c**) was also isolated as its methyl ester **4d** from fraction C6.2, however, in contrast to **4b** it afforded a yellowish oil which did not crystallise. The same molecular weight and very similar UV/vis, IR and NMR spectra indicated a close similarity between **4b** and **4d**; from the ^{1}H and ^{13}C NMR spectra, an identical C,C-connectivity was determined. The most striking difference was a downfield shift ($\Delta\delta \approx 0.4$) of the 1-H signal of **4d**, which shows a coupling constant of 2.9 Hz and indicates that here 1-H is in equatorial position and that **4c** is a diastereomer of **4a**. Below 300 nm, the CD spectrum of **4d** is mirror imaged to that of **4b**, with a weakly positive CD effect at 334 nm. Thus, the CD spectrum of **4d** has the same shape as that of isoshinanolon, and therefore the absolute configuration in **4c**/**4d** is fixed as 1R,2R,3'S. Only very few 4-hydroxy-3-alkyltetralones of this type have been isolated from microorganisms so far, scytalol D [18] being one of them.

* The numbering of the carbon atoms in juglomycin H (**4a**) and I (**4c**) is done in analogy to juglomycin C (**3e**).

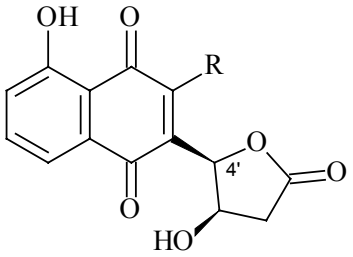


4	**a**	**b**	**c**	**d**
R^1	OH	OH	H	H
R^2	H	H	OH	OH
R^3	H	CH_3	H	CH_3

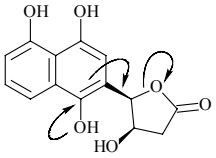
Juglomycin J (**5c**)

Juglomycin J (**5c**) was obtained from extracts of the *Streptomyces* sp. GW4184 as a yellow solid with the molecular formula $C_{15}H_{12}O_7$ (HRMS *m/z* 304.0633). The IR spectrum showed characteristic bands of OH groups (3420 cm^{-1}), of a lactone carbonyl (1780 cm^{-1}) and of chelated and non-chelated quinone carbonyls (1642 and 1606 cm^{-1}). The ^1H and ^{13}C NMR spectra resembled those of juglomycin A (**5a**) and B (**5b**) [1,19], also isolated from this strain. The proton spectrum indicated a chelated OH group (δ = 11.60), three consecutive aromatic protons, two methine groups bearing oxygen and a methylene group of a lactone ring. The only difference was the substitution of the quinonoid proton [3-H in juglomycins A (**5a**) and B (**5b**)] by a methoxy group (δ = 4.19). According to the molecular formula, the ^{13}C spectrum showed 15 signals including the typical carbonyl peaks of the quinone system (δ = 187.1 and 184.1) and the lactone ring (δ = 176.5) and three aromatic methine peaks. Additionally, five quaternary aromatic carbons, two of them being phenolic (δ = 162.2, 159.5), two aliphatic methine carbons bearing oxygen and a methylene group were identified. A methoxy signal at δ = 62.9 supported the structure of a 3-methoxy variant of juglomycin A or B, which was confirmed by comparable NMR data of the juglomycins A-D [1, 19]. The CD spectrum of **5c** showed the same shape as that of juglomycin B (**5b**), indicating that both metabolites have the same absolute configuration. Juglomycin J (**5c**) is therefore 3-methoxyjuglomycin B with a (3'R,4'S) configuration.

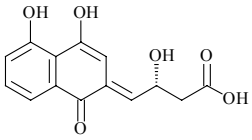
In agar diffusion assays, the juglomycins G (**3a**) and J (**5c**) were weakly active against *Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Candida albicans*, and *Mucor miehei*, slightly less than juglomycin A (**5a**). The only difference is a strong activity of **5a** against *Streptomyces viridochromogenes*, where **5c** was inactive. Both **5a** and **5c** showed similar activities against the microalgae *Chlorella vulgaris* and *Chlorella sorokiniana.*



5a: R = H
5b: R = H, 4'S instead of 4'R
5c: R = OCH_3, 4'S instead of 4'R



6



7

In a hypothetic biosynthetic scheme, the monomeric and also the dimeric [6] juglomycins can be derived easily from the heptaketide 2,3-dihydrojuglomycin A (**1**) [1c]. Enolisation of the latter and rearrangement of the resulting **5a**-hydroquinone (**6**) should deliver the quinonemethide **7**, which can re-cyclise and yield (after oxidation) juglomycin A (**5a**) or B (**5b**). The methide **7** is a tautomer of juglomycin C (**3e**), whose hydroxylation would afford the juglomycins D (**3f**) and G (**3a**). Juglomycin J (**5c**) can be obtained from juglomycin B (**5b**) by addition of methanol and oxidation, the juglomycins H (**4a**) and I (**4c**) are reduction products of **1**. The origin of the methyl group in juglomycin Z (**3g**) has not been elucidated so far [20].

Experimental Section

Spectroscopy: ^1H and ^{13}C NMR: Varian VXR 500, Unity 300 and Bruker AMX 300; MS: Finnigan-MAT-95 (DCI: 200 eV/NH_3); ESI-MS: Finnigan LCQ with quaternary pump Rheos 4000 (Flux Instrument); IR and UV/vis: 1600 Series FT-IR (KBr pellets) and Lambda 15 UV/vis spectrometers, both Perkin-Elmer; CD: Jasco J500A, opt. rotation: Perkin-Elmer 241. – *Chromatography*: R_f values from TLC on Polygram SIL G/UV_{254}; impregnated (= impr.) silica gel for PTLC: 40 g of silica gel P/UV_{254} for PTLC was suspended in 100 ml of 1% aqueous KH_2PO_4 solution, dispensed on a 20×40 cm glass plate, air-dried at 20 °C and activated for 3 h at 130 °C; for CC: 200 g of neutral silica gel 60, 0.05-0.2 mm, suspended in 500 ml of 1% KH_2PO_4 , was filtered, air-dried for 3 days and activated for 4 h at 130 °C. All silica gels: Macherey, Nagel & Co., Düren. Size exclusion chromatography: Sephadex LH-20 (Pharmacia). See

also [1c]. – *Solvent systems*: SS-1: C_6H_{12}/EtOAc/AcOH (10:10:1); SS-2: C_6H_{12}/EtOAc/AcOH (10:5:1); SS-3: $CHCl_3$/C_6H_{12}/MeOH (9:6:1); SS-4: $CHCl_3$/acetone/EtOAc (9:1:1); SS-5: $CHCl_3$/acetone/AcOH (9:1:1); SS-6: 1% aqu. KH_2PO_4 solution/EtOH (5:1); SS-7: $CHCl_3$/C_6H_{12}/MeOH (50:50:3); SS-8: $CHCl_3$/MeOH (98:2).

Streptomyces sp. 815 - extraction and isolation procedures

The cultivation of strain 815 has been described previously [1]. After 36 - 60 hrs of growth, the culture broth (40 l) was mixed with 2 kg of Hyflo-Celite and filtered. – The *mycelial cake* was stirred with 5 l of acetone in a dark room for 1 hour and filtered. After evaporation of the acetone under vacuum, the residue was mixed with 200 g of Celite, washed twice with each 1 l of dilute $NaHCO_3$ solution and the filtration residue extracted with 2 l of acetone. The acetone was again removed *i. vac*. and the remaining water phase extracted three times with each 300 ml of $CHCl_3$. The concentrated $CHCl_3$ solution was defatted with petrol ether to give 2.0 g of crude product **D**.

The *culture filtrate* was set to pH 3.0 (1 N HCl) and the precipitate filtered off after addition of 1 kg of Celite. This filter cake was extracted with 5 l of ethyl acetate, the solvent evaporated and the residue suspended in 500 ml of phosphate buffer (pH 7.4). The fats and the remaining juglomycins A (**5a**) and B (**5b**) were extracted twice with each 300 ml of $CHCl_3$. Finally, the aqueous solution was brought to pH 3.0 (1 N HCl) and again extracted with 300 ml of $CHCl_3$ to obtain 1.6 g of crude product **E**. – After removing the above precipitate, the culture filtrate was extracted with 4 × 5 l of ethyl acetate, the extract shaken with 5 l of phosphate buffer (pH 7.4) and the organic phase evaporated to dryness. The defatted residue (petrol ether) yielded 2.5 g of crude product **A**. The aqueous phase was acidified (pH 3.0, N HCl) and extracted three times with each 3 l of $CHCl_3$ (yield: 15.5 g crude product **B**) and then with 3 × 3 l ethyl acetate to deliver 16 g of crude product **C**.

From the crude product **A**, juglomycin A (**5a**) and B (**5b**) and 2,3-dihydrojuglomycin A (**1**) were isolated as described previously [1]. – The crude product **B** was separated on impr. silica gel (SS-1, 4 columns, 35 × 5 cm) to give the fractions B1 [residues of juglomycin A (**5a**) and B (**5b**)], B2 [14 g of chromatographically pure juglomycin C (**3e**)], 65 mg B3 and 370 mg B4. Purification of fraction B3 by PTLC (impr. silica gel, SS-2, developed twice) afforded 25 mg juglomycin C (**3e**) and 16 mg *O*-demethyl-aloesaponarin I [2,21]. As the fraction B4 seemed to be instable for further purification, 320 mg were treated with CH_2N_2 in methanol. The product could then be separated by PTLC (silica gel, SS-3) to yield 3-O-methyl juglomycin D methyl ester (185 mg) and juglocombin A-dimethyl ester [6] (62 mg). Separation of the unmethylated fraction B4 (50 mg) by PTLC on impr. silica gel (SS-2) afforded 24 mg of juglomycin D (**3f**) and 17 mg of impure and unstable juglocombin A [6], which was precipitated from chloroform with cyclohexane (13 mg).

The crude product **C** was pre-separated on neutral silica gel 60 (SS-5, each 4 g on a 40 × 5 cm column) to afford fractions C1 – C6. Fraction C1 contained mainly fats and C3 did not show any interesting bands. Fraction C2 yielded 280 mg of pure juglomycin C (**3e**). Chromatography of C4 (1.4 g) on silylated RP silica gel 60 (SS-6, 30 × 4 cm columns, 0.7 bar) delivered 510 mg of juglomycin D (**3f**) and 184 mg of juglorescein [6]. – Fraction C5 (560 mg) was treated with 10 % methanolic HCl for 1 hour at 20 °C. Repeated separations of the product (PTLC on impr. silica gel, SS-7 and analogous systems of different polarity) delivered juglomycin C methyl ester (12 mg), juglomycin D methyl ester (78 mg), juglomycin G methyl ester (**3b**, 23 mg), juglorescein dimethyl ester [6] (16 mg), juglocombin A-dimethyl ester [6] (32 mg), juglocombin B trimethyl derivative [6] (105 mg), and the juglochromans A-D [6] as dimethyl esters (4 – 22 mg). – After PTLC on impr. silica gel (SS-1) fraction C6 yielded 52 mg of juglomycin G (**3a**) and a broad zone of a mixture of several components (C6.2). These were methylated with diazomethane/methanol and then purified by PTLC (impr. silica gel, SS-8) to afford juglomycin H-methyl ester (**4b**, 14 mg) and juglomycin I methyl ester (**4d**, 8 mg).

The crude products **D** and **E** contained remaining fats, colourless by-products, some juglomycin C (**3e**), a mixture of other juglomycin derivatives (similar to fraction C5), 3-O-demethyl-aloesaponarin I and sabaramycine, a known polyene [22].

Juglomycin G (**3a**)

Fine red needles from $CHC1_3$/MeOH/C_6H_{12}, m.p. 120 °C (dec.). – UV/vis (MeOH): λ_{max} (lg ε) = 451 (3.53), 270 (4.06), 220 (4.42) nm; (MeOH/NaOH): λ_{max} (lg ε) = 608 (3.90), 407 (3.16), 290 (sh, 3.91), 265 (sh, 4.04), 235 (4.38) nm. – IR (KBr): ν = 3424, 3222, 2945, 2678, 1706, 1636, 1609, 1464, 1389, 1293, 1271, 1202, 1131, 1055, 1032, 839, 760, 738 cm^{-1}. – $[\alpha]_D^{20}$: +19.7° (c = 0.16, MeOH). – ^{1}H NMR (acetone-d_6, 200 MHz): δ = 11.20 (s br, 1 H, 5-OH), 7.60 (d, ^3J = 8.3 Hz, 1 H, 8-H), 7.24 (d, ^3J = 8.3 Hz, 1 H, 7-H), 6.93 (dd, J = 1.2, 1.0 Hz, 1 H, 3-H), 4.34 (dddd, J = 8.4, 8.1, 4.7, 4.4 Hz, 1 H, 3'-H), 2.85 (ddd, J = 13.5, 4.4, 1.2 Hz, 1 H, 4'-H_a), 2.65 (ddd, J = 13.5, 8.4, 1.0 Hz, 1 H, 4'-H_b), 2.63 (dd, J = 15.3, 4.7 Hz, 1 H, 2'-H_a), 2.50 (dd, J = 15.3, 8.1 Hz, 1 H, 2'-H_b). – ^{13}C-NMR (acetone-d_6, 50.3 MHz): δ = 191.9 (C-4), 183.9 (C-

1), 173.0 (C-1'), 152.9 (C-5), 151.4 (C-6), 150.2 (C-2), 137.0 (C-3), 124.6 (C-8a), 121.8 (C-7), 120.8 (C-8), 116.4 (C-4a), 67.4 (C-3'), 42.5 (C-2'), 38.2 (C-4'). – MS (EI, 70 eV): *m/z* (%) = 292 ($M^{+\cdot}$, 8), 274 ($[M - H_2O]^{+\cdot}$, 42), 232 (13), 204 (100), 189 (12), 176 (33), 147 (17); EI HRMS = 292.0583 (calcd. 292.0583 for $C_{14}H_{12}O_{7,}$).

Juglomycin G methyl ester (**3b**): From the methylated (MeOH/HCl) fraction C5, 23 mg **3b** were obtained as red needles from $CHCl_3/C_6H_{12}$ with m.p. 112 °C (dec.). – ^{1}H NMR (acetone-d_6, 200 MHz): δ = 3.62 (s, 3 H, $COOCH_3$) – ^{13}C NMR (acetone-d_6, 50.3 MHz): δ = 51.6 ($COOCH_3$); for the remaining peaks see **3a**.

6-O-Methyljuglomycin G methyl ester (**3c**): By methylation of 20 mg **3b** in methanol with diazomethane/ether at 0 °C, **3c** (14 mg, 64 %) was obtained as reddish yellow needles from $CHC1_3/C_6H_{12}$ with m.p. 131 °C (dec.). – ^{1}H NMR (acetone-d_6,, 200 MHz): δ = 7.66 (d, *J* = 8.3 Hz, 1 H, 8-H), 7.38 (d, *J* = 8.3 Hz, 1 H, 7-H), 4.02 (s, 3 H, 6-OMe), 3.65 (s, 3 H, $COOCH_3$); for the remaining signals see **3a**.

6-O-Methyl-juglomycin G (**3d**): **3c** (10 mg) was treated with 5 ml of 10% aq. Na_2CO_3 (1 h, 20 °C) and the acidified solution extracted with ethyl acetate. PTLC (imp. silica gel, SS-5) gave 7.5 mg of **3d**, which crystallised from $CHCl_3$/MeOH/C_6H_{12} as compact red needles; m.p. 150-152 °C (dec.). – UV/vis (MeOH): λ_{max} (lg ε) = 446 (3.59), 269 (4.06), 221 (4.46); (MeOH/NaOH): λ_{max} (lg ε) = 538 (3.76), 305 (sh, 3.86), 272 (4.02), 230 (4.53), 208 (4.38) nm. – IR (KBr): ν = 3482, 2940, 2927, 1707, 1641, 1625, 1598, 1483, 1458, 1370, 1269; 1211, 1138, 1052, 778 cm^{-1}. – ^{1}H NMR (acetone-d_6, 200 MHz): δ = 12.36 (s br, 1 H, 5-OH), 7.60 (d, *J* = 8.3 Hz, 1 H, 8-H), 7.34 (d, *J* = 8.3 Hz, 1 H, 7-H), 3.99 (s, 3 H, 6-OMe); the remaining shifts were similar to those in **3a**.

Juglomycin H methyl ester (**4b**)

Colourless needles from $CHCl_3$/MeOH/C_6H_{12} with m.p. 124 °C. – UV/vis (MeOH): λ_{max} (lg ε) = 333 (3.50), 259 (3.85), 214 (4.14) nm; IR (KBr): ν = 3241, 2969, 2947, 2917, 1743, 1633, 1456, 1436, 1340, 1268, 1213, 1154, 1078, 1043, 987, 962, 816, 792, 751 cm^{-1}. – $[\alpha]_D^{20}$ = –22.8 (c = 0.16, MeOH); CD (MeOH): λ_{ext} ($[\theta]^{22}$) = 333 (+2770), 258 (-17440), 232 (-4760), 212 (+36300) nm. – ^{1}H NMR (acetone-d_6, 200 MHz): δ = 12.39 (s, H/D exch., 1 H, 5-OH), 7.53 (dd, *J* = 8.0, 7.3 Hz, 1 H, 7-H), 7.19 (ddd, *J* = 7.3, 1.1, 1.0 Hz, 1 H, 8-H), 6.81 (ddd, *J* = 8.0, 1.1, 0.5 Hz, 1 H, 6-H), 5.05 (d, H/D exch., *J* = 6.3 Hz, 1 H, 1-OH), 4.59 (ddd br, *J* = 8.7, 6.3, 1.0 Hz, 1 H, 1-H), 4.28 (d, H/D exch., *J* = 5.0 Hz, 1 H, 3'-OH), 4.21 (m, 1 H, 3'-H), 3.62 (s, 3 H, OCH_3), 2.96 (dd, *J* = 17.0, 3.9 Hz, 1 H, 3-H_a), 2.58 (dd, *J* = 17.0, 10.4 Hz, 1 H, 3-H_b), 2.50 (dd, *J* = 14.6, 5.3 Hz, 1 H, 2'-H_a), 2.47 (dd, *J* = 14.6, 7.0 Hz, 1 H, 2-H_b), 2.43 (m, 1 H, 2-H), 1.97 (ddd, *J* = 13.6, 9.8, 3.2 Hz, 1 H, 4'-H_a), 1.48 (ddd, *J* = 13.6, 8.4, 2.6 Hz, 1 H, 4-H_b). – ^{13}C NMR (acetone-d_6, 50.3 MHz): δ = 204.6 (C-4), 172.6 (C-1'), 163.0 (C-5), 149.1 (C-8a), 137.6 (C-7), 118.5 (C-8), 116.8 (C-6), 116.2 (C-4a), 72.5 (C-1), 66.1 (C-3'), 43.5 (C-2'), 42.0 (C-4'), 40.3 (C-2), 40.1 (C-3). – MS (EI, 70 eV): *m/z* (%) = 294 ($M^{+\cdot}$, 48), 276 ($[M - H_2O]^{+\cdot}$, 25), 244 (22), 216 (18), 176 (100), 160 (8), 121 (25); EI HRMS = 294.1103 (calcd. 294.1103 for $C_{15}H_{18}O_{6,}$).

Juglomycin I methyl ester (**4d**)

Yellowish oil. UV/vis (MeOH): λ_{max} (lg ε) = 333 (3.68), 258 (4.01), 217 (4,21) nm. – IR (film, $CHCl_3$): ν = 3475, 2955, 2918, 1721, 1640, 1456, 1440, 1344, 1244, 1210, 1046, 996, 799, 668 cm^{-1}. – $[\alpha]_D^{25}$ = +14.9 (c = 0.28, MeOH); CD (MeOH): λ_{ext} ($[\theta]^{22}$) = 334 (+1780), 258 (+8320), 214 (-22120) nm. – ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 12.30 (s, 1 H, 5-OH), 7.50 (dd, *J* = 8.4, 7.7 Hz, 1 H, 7-H), 7.01 (ddd, *J* = 7.7, 1.1, <1 Hz, 1 H, 8-H), 6.92 (dd, *J* = 8.4, 1.1 Hz, 1 H, 6-H), 4.97 (d, *J* = 2.9 Hz, 1 H, 1-H), 4.19 (m, 1 H, 3'-H), 3.73 (s, 3 H, OCH_3), 2.85 (dd, *J* = 17.3, 9.8 Hz, 1 H, 3-H_a), 2.66 (dd, *J* = 17.3, 4.2 Hz, 1 H, 3-H_b), 2.57 (m, 1 H, 2-H), 2.52 (dd, *J* = 17.0, 3.5 Hz, 1 H, 2'-H_a), 2.47 (dd, *J* = 17.0, 8.9 Hz, 1 H, 2'-H_b), 1.76 (ddd, *J* = 14.6, 10.4, 8.2 Hz, 1 H, 4'-H_a), 1.54 (ddd, *J* = 14.6, 6.2, 1.9 Hz, 1 H, 4'-H_b). – ^{13}C NMR ($CDCl_3$, 125.7 MHz): δ = 203.9 (C-4), 173.4 (C-1'), 162.4 (C-5), 145.0 (C-8a), 137.1 (C-7), 119.0 (C-8), 117.7 (C-6), 115.2 (C-4a), 69.7 (C-1), 66.7 (C-3'), 52.0 (OCH_3), 41.4 (C-2'), 40.5 (C-4'), 38.1 (C-2), 37.4 (C-3). – MS (EI, 70 eV): *m/z* (%) = 294 ($[M]^{+\cdot}$, 23), 276 (100, $[M - H_2O]^{+\cdot}$, 100), 258 ($[M - 2\ H_2O]^{+\cdot}$, 40), 226 (35), 203 (42), 176 (66), 174 (77), 121 (85); $C_{15}H_{18}O_6$ (294.30).

Streptomyces sp. GW4184: Fermentation and isolation of the metabolites

A well grown agar culture of the terrestrial *Streptomyces* isolate GW4184 was used to inoculate 100 Erlenmeyer flasks each filled with ca. 250 ml of M_2 medium. The culture was shaken for 3 days at 28 °C and turned first yellow and then brown-red. The culture broth was mixed with 2 kg Celite, filtered over a press filter and the mycelial cake extracted with ethyl acetate to deliver 4.6 g extract. The filtrate was extracted with XAD 16 resin and the latter eluted with methanol (3.5 g residue after evaporation). Both extracts were combined and separated by column chromatography on silica gel with CH_2Cl_2/MeOH to afford four fractions. Re-chromatography of fraction I on Sephadex LH-20 (CH_2Cl_2/MeOH 1:1) and finally by preparative HPLC afforded Juglomycin A (**5a**), B (**5b**) and J (**5c**) (8.5, 4 and 5 mg). The purification of fraction II on RP18 silica gel (41-65 µm,

MeOH) gave 10 mg of oviedomycin [8]. Preparative HPLC of fraction III yielded 4 mg GTRI-02 [10], 8 mg of juglorescein [6] and 3 mg of oviedomycin. Fraction IV was triturated with CH_2Cl_2/MeOH (1:1) and the solid discarded. The soluble part was chromatographed on Sephadex LH-20 (CH_2Cl_2/MeOH, 1:1) and the polar red main fraction further purified by preparative HPLC to afford 18 mg of juglorubin [7].

Juglomycin J (**5c**)

Yellow solid with UV/vis (MeOH): λ_{max} (lg ε) = 421 (3.51), 283 (3.94), 241 (4.04) nm; IR (KBr): ν = 3420, 2953, 2926, 2854, 1780, 1642, 1606, 1516, 1457, 1367, 1273, 1193, 1158, 1083, 1049, 1002, 944, 907, 882, 837, 757, 710 cm^{-1}. – CD (MeOH): λ_{ext} ($[\theta]^{22}$) = 322 (678), 284 (-4342), 258 (+1894) nm. – ^{1}H NMR (acetone-d_6, 300 MHz): δ = 11.60 (s br, 1 H, 5-OH), 7.76 (dd, J = 8.4, 7.5 Hz, 1 H, 7-H), 7.57 (dd, J = 7.5, 1.1 Hz, 1 H, 8-H), 7.30 (dd, J = 8.4, 1.1 Hz, 1 H, 6-H), 5.62 (d, J = 2.6 Hz, 1 H, 4'-H), 4.64 (dt, J = 7.5, 2.6 Hz, 1 H, 3'-H), 4.21 (s, 3 H, 3-OCH_3), 3.15 (dd, J = 18.0, 7.5 Hz, 1 H, 2'-H_a), 2.47 (dd, J = 18.0, 2.6 Hz, 1 H, 2'-H_b). – ^{13}C NMR (acetone-d_6, 75.5 MHz): δ = 187.1 (C-4), 184.1 (C-1), 176.5 (C-1'), 162.2 (C-5), 159.5 (C-3), 138.0 (C-7), 132.8 (C-2), 130.5 (C-8a), 124.6 (C-6), 119.4 (C-8), 115.6 (C-4a), 82.3 (C-4'), 72.1 (C-3'), 62.9 (OCH_3), 39.1 (C-2'). – (+)-ESI MS: *m/z* (%) = 631 ($[2M+Na]^+$, 100), 327 ($[M+Na]^+$, 20); (-)-ESI MS: *m/z* (%) = 629 ($[2M+Na-2H]^+$, 37), 303 ($[M-H]^+$, 100); ESI HRMS: 305.0656 (M+H, calcd. 305.0655 for $C_{15}H_{13}O_7$), 327.0475 (M+Na, calcd. 327.0475 for $C_{15}H_{12}O_7Na$).

Acknowledgements

We thank R. Machinek and his team for the NMR spectra, Dr. G. Remberg and Dr. H. Frauendorf for the mass spectra and N. Sieck, M. Lackner and H. Müller for technical and microbiological assistence. This work was supported by grants from Deutsche Forschungsgemeinschaft and the Bundesministerium für Bildung und Forschung (BMBF, grant 03F0346A). S. F. is thankful for a DAAD research grant.

---

[1] a) H. Lessmann, J. Krupa, H. Lackner, P. G. Jones, Z. Naturforsch. **44b**, 353 (1989); b) J. Krupa, H. Lackner, P. G. Jones, K. Schmidt-Bäse, G. M. Sheldrick, Z. Naturforsch. **44b**, 345 (1989); c) H. Lessmann, Ph.D. Thesis, University of Goettingen (1993); Cuvillier-Verlag, Göttingen (1994), ISBN 3-89588-035-3.

[2] J. Krupa, H. Lessmann, H. Lackner, Liebigs Ann. Chem. 699 (1989).

[3] a) Y. Iwai, K. Kimura, Y. Takahashi, K. Hinotozawa, H. Shimizu, H. Tanaka, S. Ōmura, J. Antibiot. **36**, 1268 (1983); b) S. P. Cole, B. A. M. Rudd, D. A. Hopwood, C. Chang, H. G. Floss, J. Antibiot. **40**, 340 (1987).

[4] P. L. Bartel, C. Zhu, J. S. Lampel, D.C. Dosch, N. C. Connors, W. R. Strohl, J. M. Beale (Jr.), H. G. Floss, J. Bacteriol. **172**, 4816 (1990).

[5] W. R. Strohl, N. C. Connors, Mol. Microbiol. **6**, 147 (1992).

[6] Part II of this investigation: H. Lessmann, R.P. Maskey, S. Fotso, H. Lackner, H. Laatsch, Z. Naturforsch. **60b**, 189 (2005).

[7] H. Lessmann, J. Krupa, H. Lackner, Z. Naturforsch. **48b**, 672 (1993).

[8] C. Mendez, E. Kuenzel, F. Lipata, F. Lombo, W. Cotham, M. Walla, D. W. Bearden, A. F. Brana, J. A. Salas, J. Rohr, J. Nat. Prod. **65**, 779 (2002).

[9] W.-H. Yeo, B.-S. Yun, S.-S. Kim, E.-K. Park, Y.-H. Kim, I.-D. Yoo, S.-H. Yu, J. Antibiot. **51**, 952 (1998).

[10] H. A. Khan, I. Chandresekharan, A. Ghanim, Phytochemistry **22**, 614 (1983).

[11] H. Laatsch, Liebigs Ann. Chem. 297 (1987).

[12] H. Laatsch, Liebigs Ann. Chem. 1847 (1985).

[13] J. E. Hochlowski, G. M. Brill, W. W. Andres, S. G. Spanton, J. B. McAlpine, J. Antibiot. **40**, 401 (1987).

[14] G. M. K. B. Gunaherath, A. A. L. Gunatilaka, M. U. S. Sultanbawa, S. Balasubramaniam, Phytochemistry **22**, 1245 (1983).

[15] a) M. Tezuka, C. Takahashi, M. Kuroyanagi, M. Satake, K. Yoshihira, S. Natori, Phytochemistry **12**, 175 (1973); b) M. B. Zakari, J. A. D. Jeffreys, P. G. Waterman, S.-M. Zhong, Phytochemistry **23**, 1481 (1984).

[16] G. Snatzke, Tetrahedron **21**, 413, 421, 439 (1965).

[17] M. Legrand, M. J. Rougierin, in (H. B. Kagan (ed.): Stereochemistry – Fundamentals and Methods, Thieme, Stuttgart, Vol. 2, p. 34 (1977).

[18] E. Thines, H. Anke, O. Sterner, J. Antibiot. **51**, 387 (1998).

[19] N. Tanaka, H. Ogata, K. Ushiyama, H. Ono, J. Antibiot. **24**, 222 (1971).

[20] H.-P. Fiedler, A. Kulik, T. C. Schüz, C. Volkmann, A. Zeeck, J. Antibiot. **47**, 1116 (1994).

[21] A. Yagi, K. Makino, I. Nishioka, Chem. Pharm. Bull. **22**, 1159 (1974).

[22] K. Baradaran-Heravi, Ph.D. Thesis, University of Goettingen (1989).