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Full Papers

Julichrome Q_6 Glucuronide, a Monomeric Subunit of the Julimycin B-I Complex from a Terrestrial *Streptomyces* sp.[⊥]

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The terrestrial *Streptomyces* sp. isolate GW6225 afforded julichrome Q_6 glucuronide (**9**), the first monomeric member of the julimycin-B complex, and additionally the julichromes $Q_{1\cdot2}$ (**7a**), $Q_{1\cdot5}$ (**7b**), and $Q_{3\cdot5}$ (**8**), which were fully characterized by 2D NMR spectra. Additional new microbial compounds were 4-acetylchrysophanol (**6a**) and *N*-phenyl-β-naphthylamine (**10**). The isomeric *N*-phenyl-α-naphthylamine (**11**) was found in the marine *Streptomyces* sp. B8335 and was also characterized. The high antibiotic activity of *Streptomyces* sp. GW6225 extracts was probably due to the thiazolyl cyclopeptide nosiheptide.

Strains of *Streptomyces shiodaensis* produce a group of 20 unique dimeric naphthalene derivatives, the julichromes or julimycins.[1–4] The parent compound is julichrome $Q_{6\cdot6}$ (**5b**), a member of the julimycin B-I complex. The other julichromes are formally derived from **1** by a stepwise hydroxylation, epoxidation, and/or oxidation: As the end product, the dimeric anthraquinone julichrome $Q_{5\cdot5}$ (**6b**) is formed via symmetrical and unsymmetrical intermediates.

Julichrome biosynthesis has not been investigated. It seems plausible, however, that first the nonaketide precursor **1** cyclizes to the ketoacid **2**, which would yield **3a** after decarboxylation and **5a** after further reduction and acetylation, or could be stabilized as the enol ether spectomycin A2 (**4a**).[5] Oxidation of phenols **3a**–**5a** should result in the respective dimers spectomycin B1 (**4b**)[5] and julichrome $Q_{6\cdot6}$ (**5b**). In a similar way, the 5,5′-dimeric naphthalenes A-39183-A (**3b**) and A-39183-B (setomimycin)[6] could be formed. While the monomer **4a** is known as a natural product, **3a**, **5a**, and **6a** have not yet been described.

The terrestrial *Streptomyces* sp. isolate GW6225 afforded three known members of the julimycin B family, namely, the julichromes $Q_{1\cdot2}$ (**7a**), $Q_{1\cdot5}$ (**7b**), and $Q_{3\cdot5}$ (**8**). We report here for the first time the full data assignments for **7b** and **8** using 2D NMR spectra. Additionally, we isolated the missing julichrome precursor **5a** as its glucuronide **9**, which is the first monomeric julichrome derivative, and the first glycoside in the julichrome series as well.

Derivatives of the julichrome $Q_{x\cdot5}$ series contain a 4-acetylchrysophanol unit **6a**, which we have isolated now for the first time. The co-occurrence of dimeric julichromes and potential monomeric precursors strongly supports the supposed biosynthetic origin of julichromes by phenol oxidation.

Besides the julichromes, *N*-phenyl-β-naphthylamine (**10**) was isolated as a new microbial product. The isomeric *N*-phenyl-α-naphthylamine (**11**) had been isolated previously in our group from the marine *Streptomyces* sp. isolate B8335,[7] but has not yet been described in detail.

Results and Discussion

Fermentation and Isolation. A 20 L shaker culture of *Streptomyces* sp. GW6225 was grown at 28 °C for 5 days, giving a yellowish-brown culture broth. In a series of chromatographic steps, the crude extract obtained after usual workup[8] gave seven compounds in amounts between 1 and 16 mg. The yellow zones containing the julichromes turned reddish-brown with anisaldehyde/H_2SO_4 spraying reagent on TLC, and the colorless zone of **10** gave a violet band (for R_f values, see Experimental Section). Three further yellow bands containing *peri*-hydroxyquinones turned red with

[⊥] Dedicated to Prof. Dr. L.-F. Tietze on the occasion of his 65th birthday.

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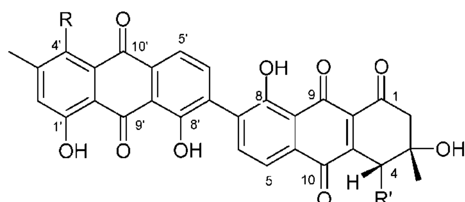
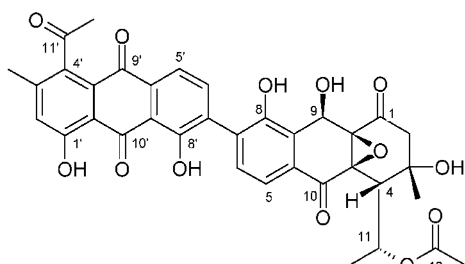


7a: R =R' = $CH(CH_3)OCOCH_3$
7b: R = $COCH_3$, R' = $CH(CH_3)OCOCH_3$



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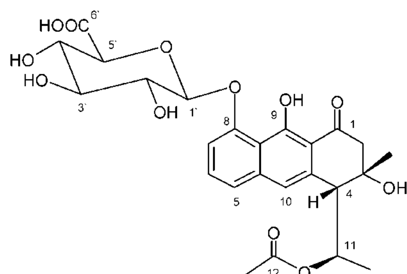
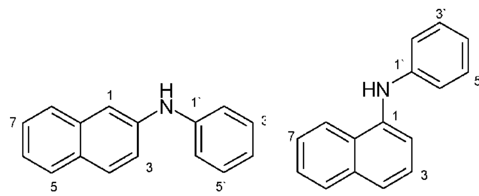


9



10 **11**

dilute NaOH. Nosiheptide[9] was obtained from a highly polar, intensively yellowish-green fluorescent band.

Julichromes. The julichromes $Q_{1\cdot5}$ (**7b**) and $Q_{3\cdot5}$ (**8**) were obtained as red and orange solids, respectively. Both compounds have been described previously;[1,2] however, NMR data were not published. Interpretation of the H,H COSY, HMQC, and HMBC correlations, the molecular weights, and corresponding formulas (by HRMS) unambiguously confirmed their structures and led to full assignment of their shift values (Table 1). Nosiheptide and julichrome $Q_{1\cdot2}$ (**7a**) were identified by comparison of their spectroscopic data with authentic samples and literature data.[10–12]

Julichrome Q_6 glucuronide (**9**) was obtained as a polar yellowish-green solid. It showed a green UV fluorescence at 366 nm and exhibited no color reaction with concentrated H_2SO_4 or NaOH spraying reagents. The UV/vis spectra were identical in neutral and acidic solution; in basic solution, the absorption of **9** displayed a bathochromic shift. A molecular weight of 520 was found by (+)- and (−)-ESIMS, and (+)-HRESIMS confirmed the molecular formula as $C_{25}H_{28}O_{12}$.

A combination of ^{13}C and HMQC NMR spectra revealed all 25 carbon resonances of **9**, three carbonyls, 10 in the aromatic/olefinic region, and two at chemical shifts indicative of oxygenated carbons. An oxymethine carbon at δ 100.5 and five oxymethines between δ 75.8 and 67.6 pointed to a sugar unit. Of the remaining resonances, three could be attributed to methyl carbons.

The ^1H NMR spectrum of **9** displayed a singlet from an acidic proton at δ 14.79, along with resonances from a 1,2,3-trisubstituted aromatic moiety; a further resonance appeared as a 1H singlet at δ 7.22. In the aliphatic region, two methyl singlets, the AB pattern of an isolated methylene group, and resonances of a CH_3–CH(O)–CH fragment were visible.

An anomeric proton at δ 5.21 (d, J =7.6 Hz), COSY correlations connecting the other four oxymethine protons, and an HMBC correlation between H-5′ and C-1′ confirmed the expected sugar moiety; however, no terminal methyl or oxymethylene group was visible. Instead, HMBC correlations from H-5′ (δ 3.91) to the carbonyl carbon of an acid (δ 170.2) and to the anomeric carbon pointed to a uronic acid (Figure 1). (The same numbering system was used as for the julichromes, to facilitate comparison of their NMR shifts.)

In the (−)-ESIMS/MS spectrum, a fragment peak at *m/z* 343 (M – [hexuronyl] – H) was consistent with a glucuronic acid moiety, which was further confirmed on the basis of H,H COSY and NOE data. The relative configuration was derived from the coupling constants (7.6–9.7 Hz), which indicated that all ring protons were axially oriented. This was further confirmed by an NOE of H-1′ with H-5′ and by the high similarity with compounds containing the same sugar moiety.[13,14] Glucuronic acid occurs usually in the D-form, and according to the Klyne rule,[15] D-sugars usually form β-glycosides, and *vice versa.* Thus, a β-D-configuration of the glucuronopyranosyl moiety in **9** is most likely.

The anomeric proton H-1′ (δ 5.21) exhibited a ^3J correlation with C-8 (δ 156.6) in the trisubstituted aromatic ring (Figure 1). H-5 (δ 7.46) displayed a ^3J coupling with the 1H singlet from the methine carbon C-10 (δ 119.4) and *vice versa*, confirming a *peri*-position for both substituents, which was further established by the NOESY spectrum. The methine doublet of H-7 exhibited a ^3J cross-signal with the quaternary carbon atom of C-8a, while the triplet of H-6 displayed a ^3J coupling with C-10a. As the aromatic methine singlet from H-10 exhibited two ^3J correlations with C-8a and C-9a, the residual sp^2 oxycarbon resonance at δ 164.0 must be that of C-9, establishing an 8-glucuronopyranosyl-α-naphthol partial structure.

The sp^3 methine singlet of H-4 (δ 2.88) exhibited ^3J correlations with the methylene carbon C-2 (δ 48.7), and the methyl singlet from 3-CH_3 (δ 29.9) and, concomitantly, 2-CH_2 (δ 2.60, 2.85) and 3-CH_3 (δ 1.16) coupled with C-4. The latter two resonances of C-2 and 3-CH_3 were combined in the partial structure CH_2–C(CH)(CH_3)–OH. The methylene protons in the latter fragment (H_2-2, δ 2.85 and 2.60) exhibited cross-signals with the ketone carbonyl of C-1 (δ 204.0), which was confirmed to be linked at C-9a via the ^4J correlation from the aromatic proton, H-10 (δ 7.22). This confirmed the connection of the 8-glucuronopyranosyl-α-naphthol partial structure with the –CH_2–C(CH)(CH_3)–OH fragment via a carbonyl group and CH-4. COSY data indicating a vicinal relationship between the H-4 methine (δ 2.88) and the H-11 (δ 5.62) oxymethine protons confirmed their positions on adjacent carbons. The CH_3-11 methyl was, in turn, identified and assigned by a COSY correlation to H-11. Finally, the attachment of the acetate group to C-11 was confirmed by a ^3J HMBC correlation between H-11 (δ5.62) and the C-12 carbonyl (δ 169.1). As a result, the final structure of **9** was deduced.

In the aglycone, the singlet from 3-CH_3 showed an NOE with H-4, so that 3-CH_3 and 4-H must be cofacial as in all other julichromes. The CD spectra of **9** were very similar to those of julichrome $Q_{5\cdot6}$ and indicated an identical absolute configuration in ring C of both compounds.[1]

4-Acetylchrysophanol (**6a**) was obtained from the first UV-absorbing, yellow fluorescent TLC zone, which turned brown with concentrated H_2SO_4 and red with NaOH, indicating a *peri*-hydroxyquinone.

Table 1. ^{13}C and ^{1}H NMR Spectroscopic Data for Julichromes $Q_{1\cdot5}$ (**7b**) and $Q_{3\cdot5}$ (**8**) and 4-Acetylchrysophanol (**6a**) in $CDCl_3$

	julichrome $Q_{1\cdot5}$ (**7b**)		julichrome $Q_{3\cdot5}$ (**8**)		4-acetylchrysophanol (**6a**)	
position	δ_C[a]	δ_H[b] (J in Hz)	δ_C[a]	δ_H[c] (J in Hz)	δ_C[a,d]	δ_H[c] (J in Hz)
1	194.7	-	204.1	-		
2	50.7	2.78(s)	50.4	2.78(d,18.5,Ha), 2.74(d,18.9,Hb)		
3	70.4	-	69.5	-		
3-CH_3	31.4	1.44(s)	33.8	1.65(s)		
4	48.1	3.58(d,1.5)	45.2	3.30(d,3.8)		
4a	153.1	-	62.7	-		
5	119.0	7.81(brs)	120.5	7.83(d,8.1)		
6	138.1	7.81(brs)	132.0	7.46(d,8.1)		
7	131.3	-	130.7	-		
8	159.6	-	153.0	-		
8a	114.9	-	126.0	-		
9	186.8	-	60.3	6.01(s)		
9a	133.5	-	63.7	-		
10	184.4	-	187.4	-		
10a	132.8	-	128.5	-		
11	68.1	5.82(m)	68.3	5.60(m)		
11-CH_3	20.6	1.22(d,6.6)	21.9	1.38(d,6.5)		
12	170.5	-	170.6	-		
13	21.3	1.91(s)	21.4	1.80(s)		
1′	162.5	-	162.6	-	162.4	-
2′	126.2	7.20(s)	126.3	7.17(s)	126.0	7.15(d,0.7)
3′	144.9	-	145.2	-	144.6	-
3′-CH_3	19.6	2.36(s)	19.6	2.33(s)	19.6	2.32(d,0.7)
4′	137.3	-	137.4	-	137.2	-
4a′	129.6	-	129.5	-	129.7	-
5′	119.8	7.88(d,7.8)	120.5	7.86(d,7.8)	120.4	7.75(dd,7.5,1.1)
6′	138.7	7.78(d,7.8)	139.3	7.75(d,7.8)	137.4	7.59(dd,8.5,7.5)
7′	131.6	-	133.3	-	125.0	7.30(dd,8.4,1.1)
8′	160.0	-	158.7	-	162.4	-
8a′	115.7	-	115.7	-	115.4	-
9′	192.5	-	192.7	-	192.5	-
9a′	113.5	-	113.3	-	113.5	-
10′	181.9	-	181.7	-	182.3	-
10a′	133.0		132.7	-	132.9	-
11′	204.7	-	204.8	-	204.8	-
11′-CH_3	31.1	2.55(s)	31.1	2.52(s)	31.1	2.51(s)
8-OH	-	12.54	-	-	-	-
1′-OH	-	12.14	-	12.08(s)	-	12.19(s)
8′-OH	-	12.53	-	12.88(brs)	-	11.98(s)
				4.15(brs,OH)		

[a] 125 MHz; [b] 300 MHz; [c] 600 MHz [d] The atom numbers correspond to the dashed atom numbers in **7b** and **8**.

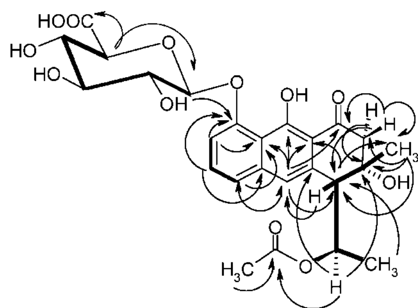


Figure 1. ^{1}H,^{1}H COSY (–) and selected HMBC (→) correlations of julichrome Q_6 glucuronide (**9**).

The ^{1}H and ^{13}C NMR data of **6a** showed close similarity with those of the anthraquinone part in **7b** and **8** (Table 1). Whereas the latter compounds showed an AB spin pattern for the 5′,6′ protons, in **6a** a set of aromatic protons (dd at δ 7.75 and 7.30, td at 7.59) indicated a 1,2,3-trisubstituted benzene ring. According to the molecular formula ($C_{17}H_{12}O_5$ by (−)-HRESIMS), structure **6a** was assumed, which was fully confirmed by the HMBC NMR data (Figure 2). 4-Acetylchrysophanol (**6a**) is a logical byproduct of julichromes, but has not been described before.

***N*-Phenyl-*β*-naphthylamine.** In fraction **I**, besides fats, a UV-absorbing and blue fluorescent band was visible, which turned violet with anisaldehyde/H_2SO_4 and pink with Ehrlich's reagent. The molecular weight of the colorless solid obtained by chromatography

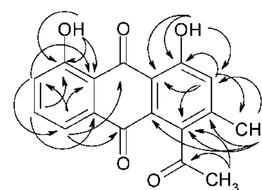


Figure 2. HMBC correlations of 4-acetylchrysophanol (**6a**).

on Sephadex LH-20 was established by EIMS as 219, and HREIMS confirmed a molecular formula of $C_{16}H_{13}N$.

The ^{1}H NMR spectrum displayed 12 aromatic protons along with one broad 1H singlet at δ 5.93, most likely pointing to a nitrogenous aromatic system bearing at least three aromatic nuclei. As the aromatic proton resonances appeared in the region δ 7.73–6.98, the nitrogen atom was not incorporated in the aromatic nuclei. This pointed to an α- or *β*-naphthylanilino system (**10**, **11**).

The ^{1}H NMR spectra displayed two *ortho*-coupled 1H doublets at δ 7.73 and 7.22, along with a *m*-coupled doublet at δ 7.45 ($J \approx$ 2.1). Additionally, it showed a resonance at δ 7.22 (dd), whose coupling constants confirmed a *m*-position to the proton at δ 7.45 and an *o*-position with respect to that at δ 7.73 ($J \approx 8.7$), resulting in an aromatic 1,3,4-trisubstituted nucleus. This established the structure of *N*-phenyl-*β*-naphthylamine (**10**) and not of *N*-phenyl-α-naphthylamine (**11**). The ^{13}C NMR spectrum of **10** displayed 14 carbon resonances instead of 16 due to the monosubstituted benzene

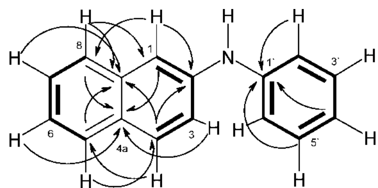


Figure 3. ^{1}H,^{1}H COSY (–) and HMBC NMR correlations (→) of *N*-phenyl-*β*-naphthylamine (**10**).

Table 2. Cytotoxic Activities of Julichromes $Q_{1\cdot5}$ (**7b**), $Q_{3\cdot5}$ (**8**), and Q_6 Glucuronide (**9**)

	antitumor potency[a]		tumor selectivity[b]	
compound	mean IC_{50} [μM]	mean IC_{70} [μM]	*n*/total	%
julichrome $Q_{1\cdot5}$ (**7b**)	5.5	10.0	2/36	6%
julichrome $Q_{3\cdot5}$ (**8**)	6.3	10.9	0/36	0%
julichrome Q_6 glucuronide (**9**)	12.3	20.4	2/36	6%

[a] Mean IC values, determined as average of 36 human tumor cell lines tested. [b] Individual IC_{50} < ½ mean IC_{50}; e.g., if mean IC_{50} = 2.0 μM, the threshold for above average sensitivity was IC_{50} < 1.0 μM.

ring. Structure **10** was further confirmed by HREIMS, H,H COSY, and HMBC correlations (Figure 3).

***N*-Phenyl-α-naphthylamine.** Separation of extracts of the marine *Streptomyces* sp. isolate B8335 gave a UV-absorbing zone with a similar behavior to that for **10** (violet with anisaldehyde/H_2SO_4, pink with Ehrlich's reagent), and HRMS afforded the same molecular formula, $C_{16}H_{13}N$.

The ^{1}H NMR spectrum showed several multiplets in the range δ 8.02–6.88 with an intensity of 12 protons, corresponding to at least three aromatic nuclei. The ^{13}C/APT NMR spectra showed resonances of 12 methine groups and 4 quaternary carbon atoms. These data supported the assumption that again two fused aromatic rings (naphthalene) and a phenyl ring were present and that both were connected via a heteroatom. The similarity of both ^{1}H and ^{13}C NMR spectra and also of the EI mass fragmentation to those of **10** confirmed the structure of a positional isomer, α-naphthylaniline (**11**). This conclusion was further confirmed by comparison with an authentic sample of **11** as well as reference data.[16]

Both isomers, α- and *β*-naphthylaniline, have been isolated previously from plants: **10** is known from *Aconitum karakolicum*, *Acroptilon repens*, *Daucus carota*, and *Eichhornia crassipes*,[17–19] while **11** was obtained as a constituent of *Narcissus tazetta* and *Eichhornia crassipes.*[20,21] Both isomers are used as indicators in the analysis of organometallic reagents. As they are also efficient antioxidants with technical applications (e.g., stabilization of rubber), it may be assumed that they were extracted as artifacts during workup. We were able, reproducibly, to isolate both isomers from their respective strains avoiding all contacts with plastic materials. As both compounds were not formed by dozens of other strains using identical conditions, an artificial origin of **10** or **11** was definitely ruled out.

Biological Properties. Antibacterial and antifungal activities were qualitatively determined using the agar diffusion method. Although a pronounced antibacterial activity of most julimycines against Gram-positive bacteria was reported,[22] julichrome Q_6 glucuronide (**9**) displayed no activity against a number of bacteria, fungi, and microalgae (see Experimental Section). It showed, however, a moderate and rather unselective cytotoxic activity against a range of human tumor cell lines with a mean IC_{50} of 12.3 μM (mean IC_{70} = 20.4 μM). A similar biological profile was observed for the julichromes $Q_{1\cdot5}$ and $Q_{3\cdot5}$ (**7b**, **8**), whose cytotoxic activity was approximately 2-fold more pronounced (Table 2). The antibacterial activity of the crude extract was likely due to nosiheptide.[9]

Experimental Section

General Experimental Procedures. Materials and methods and antimicrobial tests were used as described earlier.[8] Optical rotation was measured on a Perkin-Elmer polarimeter, model 241. UV/vis spectra were recorded on a Perkin-Elmer Lambda 15 UV/vis spectrometer. NMR spectra were measured on Bruker AMX 300 (300.135 MHz), Varian Unity 300 (300.145 MHz), and Varian Inova 600 (600.7 MHz) spectrometers. ESIMS was recorded on a Finnigan LCQ with a Rheos 4000 (Flux Instrument) quaternary pump. ESI-HRMS were measured on a Micromass LCT mass spectrometer coupled with a HP1100 HPLC with a diode array detector. Reserpine (MW = 608) and leucine-enkephalin (MW = 555) were used as standards in positive and negative mode. High-resolution mass spectra (HRMS) were recorded by ESIMS on an Apex IV 7 Tesla Fourier-transform ion cyclotron resonance mass spectrometer (Bruker Daltonics, Billerica, MA). ESIMS/MS was performed with a normalized collision energy of 35%. EIMS spectra were recorded on a Finnigan MAT 95 spectrometer (70 eV) with perfluorkerosine as reference substance for EI-HRMS. Flash chromatography was carried out on silica gel (230–400 mesh). R_f values were measured on Polygram SIL G/UV_{254} (Macherey-Nagel & Co.). Size exclusion chromatography was done on Sephadex LH-20 (Pharmacia).

Taxonomy of Strain GW8227. Strain GW6225 was isolated from sand of a desert in Arizona. The aerial spore mass color was gray with shades of blue, and the reverse was brown on yeast extract-malt agar. A brown, diffusible pigment was formed. The strain showed aerial hyphae with spiral chains of spores. It most probably belongs to the genus *Streptomyces*. The isolate GW6225 is maintained on YS slant agar containing 0.2% yeast extract, 1% soluble starch, and 1.5% agar, adjusted to pH 7.2, at the Labor Grün-Wollny, Versaillerstr. 1, D-35394 Giessen, Germany.

M_2 Medium. Glucose (4 g), yeast extract (4 g), and malt extract (10 g) were dissolved in 1 L of tap water. The pH of the solution was adjusted to 7.8 and sterilized by autoclaving for 33 min at 121 °C and 1.2 bar.

Fermentation of Isolate GW8227. The terrestrial isolate *Streptomyces* sp. GW6225 was inoculated from its soil culture on three agar plates of M_2 medium and incubated for 96 h at 28 °C. The well-developed colonies were used to inoculate 100 of 1 L Erlenmeyer flasks each containing 200 mL of M_2 medium, which were grown in shake culture (95 rpm) at 28 °C for 5 days. The resulting gray culture broth was mixed with ca. 1 kg diatomaceous earth (Celite) and pressed through a pressure filter, affording the aqueous filtrate and a mycelial fraction. The aqueous fraction was extracted with EtOAc, and the mycelium was extracted (3×) with EtOAc followed by acetone. The acetone was evaporated, and the aqueous residue extracted by EtOAc. Both organic phases were combined and evaporated to dryness, yielding 6.3 g of a greenish-brown crude extract.

Isolation. The crude extract was subjected to flash chromatography on silica gel with a MeOH/CH_2Cl_2 gradient (1.5 L 0% MeOH, 1 L 3% MeOH, 0.7 L 7% MeOH, 0.8 L 10% MeOH, 0.6 L 20%, 0.7 L 50% MeOH) to give six fractions, monitored by TLC. Refractionation and purification of fractions I (0.3 g), IV (1.3 g), and V (1.2 g) and the polar fraction VI (0.2 g) was accomplished using a series of chromatographic processes including preparative TLC, Sephadex LH-20, and silica gel column chromatography (see separation scheme in Supporting Information). This gave *N*-phenyl-*β*-naphthylamine (**10**, 16.3 mg, colorless solid) from I and 4-acetylchrysophanol (**6a**, 3.6 mg, yellow solid) and julichrome $Q_{1\cdot2}$ (**7a**, 1.3 mg, red solid) from II. Julichromes $Q_{1\cdot5}$ (**7b**, 3.6 mg, red solid) and $Q_{3\cdot5}$ (**8**, 6.3 mg, orange solid) and nosiheptide (6.6 mg) were obtained from fraction V. Finally, the polar, new julichrome Q_6 glucuronide (**9**, 6.3 mg, yellowish-green solid) was obtained from fraction VI by the same technique of purification (separation diagram, see Supporting Information).

A 25 L culture of the marine *Streptomyces* isolate B8335 was fractionated in a similar fashion. Column chromatography on silica gel, PTLC, and separation on Sephadex LH-20 delivered actinomycin D and *N*-phenyl-α-naphthylamine (**11**, 8 mg) as a colorless UV-absorbing solid, which turned violet with anisaldehyde/sulfuric acid.

Julichrome $Q_{1\cdot5}$ (7b): red solid, R_f = 0.37 (CH_2Cl_2/4% MeOH); ^{1}H NMR and ^{13}C NMR, see Table 1; (+)-ESIMS *m/z* (%) 1328 [2M + Na + H]$^+$ (35), 675 [M + Na]$^+$ (100); (−)-ESIMS *m/z* (%) 1303 [2M – H]$^-$ (20), 651 [M − H]$^-$ (100); (+)-HRESIMS *m/z* 653.1654820 (calcd for [M + H]$^+$, $C_{36}H_{29}O_{12}$, 653.16535).

Scheme 1

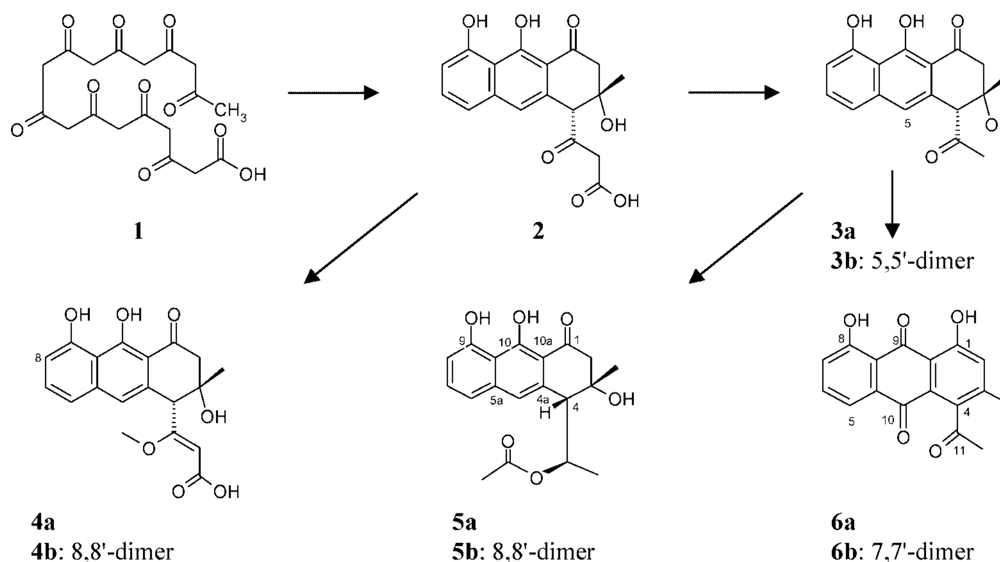


Table 3. ^{13}C (125 MHz) and ^{1}H NMR (600 MHz) Spectroscopic Data for Julichrome Q_6 Glucuronide (**9**) in DMSO-d_6

position (C/H no.)	δ_C	δ_H (J in Hz)
1	204.0	
2	48.7	2.85 (d, 18.6 Hz, H_a), 2.60 (dd, 18.5, 1.0 Hz, H_b)
3	68.8	
3-CH_3	29.9	1.16 (s)
4	54.8	2.88 (s)
4a	135.8	
5	121.1	7.46 (d, 8.1 Hz)
6	131.2	7.59 (t, 8.0 Hz)
7	110.2	7.17 (d, 7.9 Hz)
8	156.6	
8a	114.9	
9	164.0	
9a	111.2	
10	119.4	7.22 (s)
10a	138.7	
11	67.6	5.62 (dq, 6.2, 1.8 Hz)
11-CH_3	19.3	1.22 (d, 6.5 Hz)
12	169.1	
13	20.7	1.68 (s)
1′	100.5	5.21 (d, 7.6 Hz)
2′	73.2	3.48 (t, 8.6 Hz)
3′	75.8	3.37 (t, 9.1 Hz)
4′	71.4	3.44 (t, 9.3 Hz)
5′	75.2	3.91 (d, 9.7 Hz)
6′	170.2	
OH		14.79 (brs)

Julichrome $Q_{3\cdot5}$ (8): orange solid, $R_f = 0.25$ (CH_2Cl_2/4% MeOH); ^{1}H NMR and ^{13}C NMR, see Table 1; (+)-ESIMS m/z (%) 1363 [2M + Na]$^+$ (22), 715 [M − H + 2Na]$^+$ (100), 693 [M + Na]$^+$ (33); (−)-ESI MS m/z 669 [M − H]$^-$; (+)-HRESIMS m/z 671.175968 (calcd for [M + H]$^+$, $C_{36}H_{31}O_{13}$, 671.17592).

Julichrome Q_6 glucuronide (9): yellowish-green solid, $R_f = 0.41$ (CH_2Cl_2/20% MeOH); $[\alpha]_D^{20} = -70$ (c 1.0, MeOH); UV/vis (23.9 μg/mL MeOH) λ_{max} (neutral) 221, 265, 291, 301, 315, 391; (acidic) 221, 265, 291, 301, 315, 391; (basic) 208, 223, 265, 291, 333 (br), 406; CD (c 39 μg/mL, MeOH) $[\theta]_{350}$ 0, $[\theta]_{311}$ −7990, $[\theta]_{265}$ +9900, $[\theta]_{240}$ 0, $[\theta]_{226}$ −27600; ^{1}H NMR and ^{13}C NMR, see Table 3; (+)-ESIMS; (+)-ESIMS m/z (%) 1063 [2M + Na]$^+$ (22), 543 [M + Na]$^+$ (100); (–)-ESIMS m/z (%) 1039 [2M − H]$^-$ (30), 519 [M − H]$^-$ (100). (+)-HRESIMS m/z 521.16550 [M + H]$^+$, 543.14729, 559.12133 (calcd for [M + Na]$^+$, $C_{25}H_{29}O_{12}$, 521.16535, for $C_{25}H_{28}O_{12}Na$, 543.14730, and for [M + K]$^+$, $C_{25}H_{28}O_{12}K$, 559.12124).

4-Acetylchrysophanol (6a): yellow solid, $R_f = 0.28$ (CH_2Cl_2). ^{1}H NMR and ^{13}C NMR, see Table 1; (+)-ESIMS m/z 615 ([2M + Na]$^+$); (−)-ESIMS m/z (%) 295 ([M − H]$^-$, 100%), 613 ([2M − 2H + Na]$^-$, 31%); EIMS m/z (%) 296 ([M]$^{+\cdot}$, 16%), 281 ([M − CH_3]$^{+\cdot}$, 100%), 225 (6%), 159 (16), 83 (10), 69 (14), 57 (32), 43 (21); (−)-HRESIMS m/z 295.06148 [M − H] (calcd for $C_{17}H_{11}O_5$, 295.06011).

***N*-Phenyl-*β*-naphthylamine (10):** colorless solid, $R_f = 0.84$ (CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.73 (1 H, d, $J = 8.7$ Hz, 4-H), 7.72 (1H, d, $J = 8.0$ Hz, 5-H), 7.63 (1H, d, $J = 8.2$ Hz, H-8), 7.45 (1H, d, $J = 2.1$ Hz, 1-H), 7.31 (1H, dt, $J = 8.1$ Hz, 1.2 Hz, 7-H), 7.29 (3 H, m, 3′/5′-H, 6-H), 7.22 (1H, dd, $J = 8.7$, 2.2 Hz, 3-H), 7.17 (2H, dd, $J = 8.5$, 1.0 Hz, 2′/6′-H), 6.98 (1H, t, $J = 7.3$ Hz, 4′-H), 5.93 (brs, NH). ^{13}C/APT NMR ($CDCl_3$, 50 MHz) δ 142.9 (C_q-1′), 140.8 (C_q-2), 134.6 (C_q-8a), 129.4 (CH-3′/5′), 129.2 (CH-4; C_q-4a), 127.6 (CH-5), 126.5 (CH-7/8), 123.5 (CH-6), 121.5 (CH-4′), 120.0 (CH-3), 118.3 (CH-2′/6′), 111.6 (CH-1). EIMS (70 eV) m/z (%) 219 ([M]$^{+\cdot}$, 100), 217 (63), 115 (26), 108 (44), 102 (14), 96 (13), 77 (12).

N-Phenyl-α-naphthylamine (11): colorless solid, $R_f = 0.90$ ($CHCl_3$/MeOH 5%); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.02 (1H, m, Ar-H), 7.86 (1H, m, Ar-H), 7.57 (1H, m, Ar-H), 7.52–7.46 (2H, m, 2 Ar-H), 7.38 (1H, m, 2-H), 7.22 (1 H, dt, $^3J = 8.5$ Hz, $^4J = 1.3$ Hz, Ar-H), 7.01, 6.99 (2H, dd, $J = 8.1$, 1.2 Hz, 2 Ar-H), 6.88 (1H, t, $^3J = 7.9$ Hz, Ar-H); ^{13}C/APT NMR ($CDCl_3$, 50 MHz) δ 144.7 (C_q-1), 138.7 (C_q-1′), 134.6 (C_q-4a), 129.3 (CH-3′/5′), 128.5 (CH-5), 127.7 (C_q-8a), 126.1 (CH-3), 126.0 (CH-6), 125.6 (CH-7), 122.9 (CH-8), 121.8 (CH-4), 120.4 (CH-4′), 117.4 (CH-2′/6′), 115.8 (CH-2); EIMS (70 eV) m/z (%) 219 ([M]$^{+\cdot}$, 100), 218 (42), 165 (9), 109 (17), 85 (6), 71 (7), 57 (12), 43 (8).

Biological Activity. For the agar diffusion tests, the compounds were dissolved in CH_2Cl_2/10% MeOH at a concentration of 1 mg/mL. Aliquots were soaked on filter paper disks (9 mm ∅, no. 2668, Schleicher & Schüll, Germany) and dried for 1 h at room temperature under sterile conditions. The paper disks were placed on inoculated agar disks and incubated for 24 h at 38 °C in the dark (bacteria and fungi) or room temperature in sunlight (algae). Julichrome Q_6 glucuronide (**9**) was inactive at 40 μg/platelet against *Escherichia coli*, *Staphylococcus aureus*, *Streptomyces viridochromogenes*, *Bacillus subtilis*, *Candida albicans*, and the microalgae *Chlorella vulgaris*, *Chlorella sorokiniana*, and *Scenedesmus subspicatus*.

Antitumor Test. A modified propidium iodide assay was used to examine the antiproliferative activity of the compounds against human tumor cell lines. The test procedure has been described elsewhere.[23] Cell lines tested were derived from patient tumors engrafted as a subcutaneously growing tumor in NMRI nu/nu mice or obtained from American Type Culture Collection, Rockville, MD, National Cancer Institute, Bethesda, MD, or Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany.

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Supporting Information Available: Workup procedure of the terrestrial *Streptomyces* sp. GW6225, $^1H,^1H$ COSY and HMBC NMR correlations of julichromes Q_{1-5} (**7b**) and Q_{3-5} (**8**), julichrome Q_6 glucuronide (**9**), and *N*-phenyl-β-naphthylamine (**10**). This material is available free of charge via the Internet at http://pubs.acs.org.

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