VOL. 56 NO. 11, NOV. 2003 THE JOURNAL OF ANTIBIOTICS pp. 942 — 949

Himalomycin A and B: Isolation and Structure Elucidation of New Fridamycin Type Antibiotics from a Marine *Streptomyces* Isolate†

RAJENDRA P. MASKEY[a], ELISABETH HELMKE[b] and HARTMUT LAATSCH[a,*]

[a] Department of Organic Chemistry, University of Göttingen, Tammannstrasse 2, Göttingen D-37077, Germany
[b] Alfred-Wegner-Institute for Polar and Marine Research, Am Handelshafen 12, Bremerhaven D-27570, Germany

(Received for publication July 7, 2003)

In our screening of marine *Streptomycetes* for bioactive compounds, in addition to the known metabolites rabelomycin (**1**), fridamycin D (**2b**), *N*-benzylacetamide and *N*-(2′-phenylethyl)acetamide, two new anthracycline antibiotics designated as himalomycin A (**2c**) and B (**2d**) were isolated from the culture broth of the marine *Streptomyces* sp. isolate B6921. The structure of the new antibiotics was determined by comparison of the NMR data with those of fridamycin D (**2b**) and by detailed interpretation of mass, 1D and 2D NMR spectra.

Among the more than 45 angucycline antibiotics, less than 10 are C glycosides. Biogenetically related with these tetracyclic quinones is a small group of antibiotics consisting of fridamycins A, B, D (**2b**) and E[1,2], amicenomycin B[3], vineomycin B_2[4,5] and vineomycin C[6], where according to the investigations of IMAMURA *et al.*[7], the angular ring was opened and anthraquinones with substituted side chains at C-2 were resulting. These antibiotics possess antibacterial and antitumor activities as well. In the course of our screening program for novel bioactive principles from marine actinomycetes, we have isolated two new quinone antibiotics of this group, himalomycin A (**2c**) and B (**2d**). In this paper we report the taxonomy of the producing strain, the structure elucidation as well as the biological activity of **2c** and **2d**.

The ethyl acetate extract of *Streptomyces* isolate B6921 drew our attention due to its high biological activity against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, and *Streptomyces viridochromogenes* (Tü 57). A striking nonpolar yellow band on TLC gave an orange to violet colouration with anisaldehyde/sulphuric acid which turned to pink after a few minutes. Some middle polar yellowish orange bands giving an orange fluorescence on TLC turned brown to grey with anisaldehyde/sulphuric acid and deserved further interest. The colour change from orange to red on treatment with diluted sodium hydroxide indicated them to contain the *peri*-hydroxy quinone moiety. Fermentation on a 20-liter scale delivered sufficient material for the structure elucidation.

Taxonomy of the Producing Strain

The *Streptomyces* sp. isolate B6921 has been derived from sandy sediment of a coastal site of Mauritius (Indian Ocean) and was isolated on Olson medium containing 22 g actinomycete isolation agar (Difco) and 5 g glycerol in 1 liter of 50% natural seawater. The reference culture of the strain is held in the Collection of Marine Actinomycetes at the Alfred-Wegener-Institute for Polar and Marine Research in Bremerhaven.

The almost complete 16S rRNA gene sequence of the strain B6921 shows a 99.3% similarity to its closest relative *Streptomyces cyaneus* (Krasilnikov 1941, GenBank accession no. AJ399470). Dependent on the medium, the substrate mycelium varies from dark red to beige. Aerial mycelium is white to reddish grey and spores are borne in

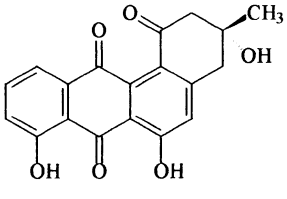
† Art. No. XXV on Marine Bacteria. Art. XXIV: F. C. LI, R. P. MASKEY, S. QIN & H. LAATSCH: Chinikomycin A and B: Isolation, structure elucidation and biological activity of antibiotics with a novel carbon skeleton from a marine *Streptomyces* sp. isolate M045. J. Antibiotics, submitted June 2003

* Corresponding author: hlaatsc@gwdg.de

mature spiral chains. Melanin pigment is neither produced on peptone - yeast extract - iron agar[8] nor on tyrosine agar[8]. Optimum growth temperature is at 30°C. The strain grows very well at 45°C but only weakly at 10°C. Growth occurs in media from 0% up to 7% seawater salinity. Starch, casein, and gelatine are degraded. Chitin and esculin are weakly cleaved, cellulose is not hydrolized. The strain is catalase positive. Nitrate reductase is not formed, H_2S is produced. The utilization of carbon sources was tested with SFN2-Biolog plates (Hayward, CA, USA) using BMS-N without agar as basal medium[9]. A wide range of organic compounds can be utilized for growth: cyclodextrin, dextrin, tween 40, tween 80, L-arabinose, D-arabitol, cellobiose, D-fructose, D-galactose, gentiobiose, D-glucose, *m*-inositol, lactulose, maltose, D-mannose, β-methyl-D-glucoside, L-rhamnose, sucrose, D-trehalose, turanose, D-galacturonic acid, quinic acid, succinamic acid, alaninamide, L-alanine, L-Alanyl-glycine, L-asparagine, L-aspartic acid, L-leucine, L-phenylalanine, L-proline, L-serine, urocanic acid, inosine, thymidine, putrescine, 2-aminoethanol, glycerol. Weak growth was obtained with: glycogen, *N*-acetyl-D-glucosamine, D-lactose, D-mannitol, methylpyruvate, mono-methylsuccinate, citric acid, D-gluconic acid, D-glucosaminic acid, D-glucoronic acid, α-hydroxy butyric acid, β-hydroxy butyric acid, malonic acid, L-glutamic acid, L-histidine, and threonine.

Fermentation and Isolation

Well-grown agar subcultures of *Streptomyces* sp. isolate B6921 served to inoculate twelve of 1-liter-Erlenmeyer flasks each containing 200 ml of M_2^+ medium. The flasks were incubated with 95 rpm at 28°C for 3 days and were used to inoculate a 20-liter jar fermentor which was held at 28°C for 72 hours.



1

The crude extract, obtained after usual work-up[10] of the culture broth, was subjected to flash chromatography on silica gel with a MeOH/$CHCl_3$ gradient to give eight fractions. Further separation of the less polar fractions yielded *N*-benzylacetamide, *N*-(2′-phenylethyl)acetamide, rabelomycin (**1**), and fridamycin D (**2b**). The more polar fractions delivered the new quinones himalomycin A (**2c**), and himalomycin B (**2d**). *N*-Benzylacetamide[11], *N*-(2′-phenylethyl)acetamide[11] and rabelomycin (**1**)[12] were identified by direct comparison of their NMR spectra with reference data from our spectra collection.

Results and Discussion

Separation of fractions I, V and VII delivered three hydroxyquinones with similar UV spectra indicating similar or identical chromophores. The molecular weights of these compounds were determined to be 596, 824 and 828, and ESI-HR mass spectrometry of the molecular signals led to the molecular formulae $C_{31}H_{32}O_{12}$, $C_{43}H_{52}O_{16}$ and $C_{43}H_{56}O_{16}$ for **2b**~**2d**, respectively.

The proton NMR spectrum of compound **2b** showed two signals at δ 13.32 and 13.04 which could be assigned to chelated *peri*-hydroxy groups. Additionally it showed two sets of *ortho*-coupled protons each of intensity 2 representing two 1,2,3,4-tetrasubstituted aromatic systems. Between δ 5.2~2.5, several signals typical for sugars were observed. In addition, two methyl doublets at δ 1.41 and 1.36 and a singlet for a methyl group at δ 1.32 were visible. ^{13}C NMR and APT spectra showed carbonyl signals of a ketone at δ 207.7, of quinone carbonyls at δ 188.0 and 187.9 and an acid or ester at δ 177.0. In the sp^2-region, twelve signals corresponding to two isolated aromatic system were visible. A signal at δ 91.4 was assigned to the acetal carbon atom of a sugar, and correspondingly in the region of the carbon atoms connected with oxygen, several signals were visible. Furthermore, methylene carbons at δ 44.7, 41.0, 39.9 and 36.6 and three methyl signals at δ 27.2, 17.2 and 16.2 were observed.

Compound **2c** delivered ^1H NMR data showing a close similarity with those of compound **2b**. Signals for two chelated hydroxy groups at δ 13.13 and 13.06 and two sets of aromatic proton signals each with *ortho*-couplings were found as well. In the region of sugars, more signals were observed than in the case of compound **2b**, and the spectrum showed three acetal carbon signals at δ 98.4, 91.6 and 91.4.

The ^1H NMR spectrum of compound **2d** showed the same pattern in the aromatic region, four anomeric signals and many methylene and methine signals of sugars at δ 4.02~1.42. Furthermore, four methyl doublets and a methyl singlet were detected. As in the case of compound **2c**, the ^{13}C and APT NMR spectra showed 43 C signals. There were again quinone carbonyl signals visible, however, the signal of the aliphatic ketone was substituted by a CH–O signal in the aliphatic region. Twelve sp^2 carbon

Fig. 1. Working up scheme of the *Streptomyces* sp. isolate B6921.

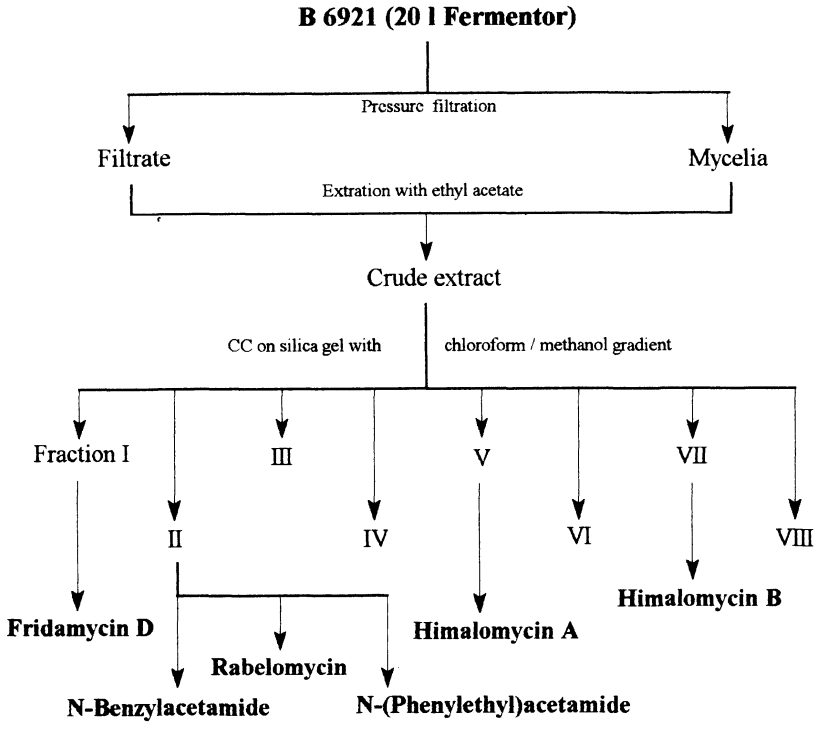
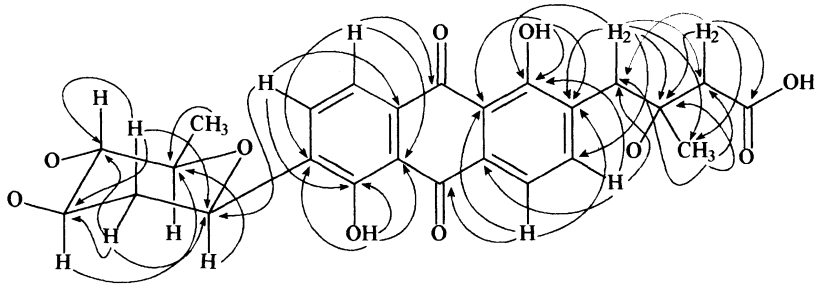


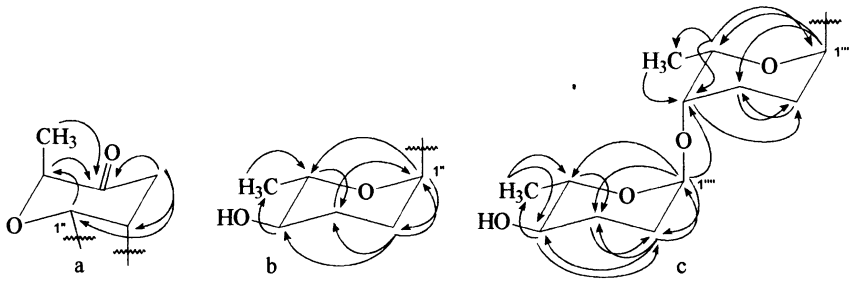
Fig. 2. HMBC correlations in the aglyca of fridamycin D (**2b**), himalomycin A (**2c**) and B (**2d**).



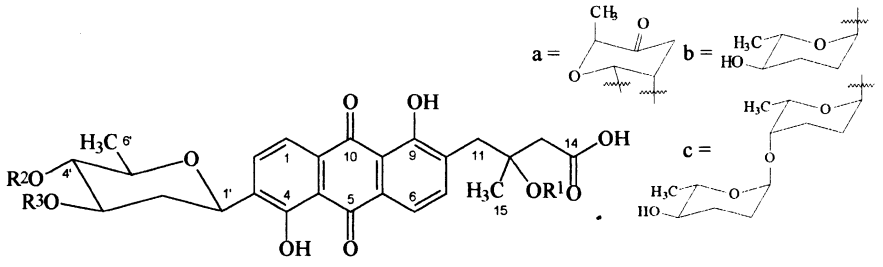
signals between δ 161.8~115.6 indicated two isolated aromatic rings. According to the acetal carbon signals, to the number of carbon atoms connected to oxygen, and the four methyl doublets, four sugar units were present.

According to their H,H COSY, HMQC and HMBC couplings, all three compounds were C-glycosides of the same aglycon (Fig. 1), having the skeleton of fridamycin E (**2a**). The sugars were identified as cinerulose, amicetose and rhodinose by further 2D NMR correlations (Fig. 2). Their relative stereochemistry was determined by aid of the coupling constants of the proton signals. Connection of the aglycon with the sugar residue assisted by the HMBC couplings and the optical rotation led to the known quinone fridamycin D[1] for compound **2b** and the new quinone antibiotics himalomycin A (**2c**) and himalomycin B (**2d**). In the HMBC spectrum of **2b**, the proton signals at δ 3.81 (3′-H) and 3.49 (4′-H) were coupling to the carbon signals at δ 71.1 (C-2″) and 91.4 (C-1″), respectively, which shows the connection of the cinerulose (Fig. 3) to the aglycon giving the final structure of fridamycin D (**2b**). Similarly the signal at δ 3.81 (3′-H) showed coupling to δ 71.4 (C-2″), the anomeric proton signal of sugar a at δ 5.17 (H-1″) to δ 74.5

Fig. 3. HMBC correlations in the sugars of fridamycin D (**2b**, fragment a), and himalomycins A (**2c**, a and c) and B (**2d**, b and c).



a=L-cinerulosyl, b=L-amicetosyl, c=L-amicetosyl-L-rhodinosyl residues.



2a: $R^1 = R^2 = R^3 = H$
2b: $R^1 = H$, R^2 = C-1", R^3 = C-2" of a
2c: R^1 = c, R^2 = C-1", R^3 = C-2" of a
2d: R^1 = c, R^2 = b, $R^3 = H$

(4′-C) and another anomeric proton at δ 5.25 (1‴-H) to δ 77.7 (C-12) in the HMBC spectrum of **2c** to give the connection of the sugar moieties a and c to the aglycon of himalomycin A (**2c**). In the same way the connectivity of the sugar moiety b to C-4′ and c to C-12 was derived by the HMBC couplings of the proton signal at δ 3.11 (4′-H) and 5.24 to δ 98.8 (C-2″) and 77.3 (C-12) respectively. The structure of the latter compounds is further supported by the similarity of their NMR data with those of **2b** (see Tables 1 and 2), and the isolation of a potential biosynthetic precursor, rabelomycin[7] (**1**), from the same strain. The absolute configuration of the *O*-glycosidic sugar residues in **2c** and **2d** could not be determined due to the lack of material. It should be stated, however, that with one exception[13], the cinerulose, amicetose, and rhodinose residues in other antibiotics[14] are L-configured. Additionally, our fridamycin D (**2b**) sample gave an optical rotation corresponding to the literature. We assume therefore that the sugar moieties in **2b**/**2c** are also L-configured.

Biological Properties

Antibacterial, antifungal and antialgal activities were qualitatively determined using the agar diffusion method. At concentrations of ~50 μg/disk, compounds **2b**~**2d** exhibited strong antibacterial activity against *Bacillus subtilis*, *Streptomyces viridochromogenes* (Tü 57), *Staphylococcus aureus* and *Escherichia coli*, which were comparable to each other (Table 4). They exhibited no antialgal activity against the tested micro-algae *Chlorella vulgaris*, *Chlorella sorokiniana* and *Scenedesmus suspicatus* and also no antifungal activity against *Candida albicans* and *Mucor miehei*.

Experimental

Material and methods and antimicrobial tests were used as described earlier[10]. ESI-HRMS were measured on Micromass LCT mass spectrometer coupled with a HP

Table 1. ^{13}C NMR shifts (δ, $CDCl_3$, 75.5 MHz) of fridamycin D (**2b**), himalomycin A (**2c**) and B (**2d**).

C No.	2b	2c	2d	C No.	2b	2c	2d
1	119.1	119.4	119.1	4'	74.5	74.5	88.6
2	133.4	133.3	133.4	5'	74.6	74.6	74.6
3	137.9	137.7	138.8	6'	17.5	17.5	18.5
4	158.9	158.9	159.0	1"	91.4	91.4	98.8
4a	115.5	115.5	115.7	2"	71.1	71.1	30.1
5	187.9	188.1	188.5	3"	39.9	39.9	27.5
5a	131.9	131.8	131.8	4"	207.7	207.7	71.2
6	119.5	118.5	118.5	5"	77.5	77.8	71.4
7	139.6	139.6	140.2	6"	16.2	16.1	17.7
8	134.2	134.4	135.7	1"'	-	91.6	91.4
9	161.1	161.5	161.8	2"'	-	25.9	25.8
9a	115.7	115.6	115.6	3"'	-	24.5	24.6
10	188.0	188.2	188.4	4"'	-	74.2	75.1
10a	132.0	132.0	132.1	5"'	-	67.7	66.7
11	41.0	38.2	38.3	6"'	-	16.7	16.9
12	72.0	77.7	77.3	1""	-	98.4	98.6
13	44.7	44.9	44.1	2""	-	29.8	30.1
14	177.0	172.3	171.8	3""	-	27.7	28.0
15	27.2	23.2	22.8	4""	-	72.1	71.7
1'	71.5	71.5	71.2	5""	-	70.2	70.5
2'	36.6	36.6	39.3	6""	-	17.8	17.8
3'	77.0	77.0	71.5	-	-	-	-

1100 HPLC with a Diode Array Detector. Reserpin (MW=608) and leucin-enkephalin (MW=555) were used as standards in positive and negative mode.

M_2^+ Medium

Malt extract (10 g), yeast extract (4 g) and glucose (4 g) were dissolved in artificial sea water (0.5 liters) and tap water (0.5 liters). Before sterilisation, the pH was adjusted to 7.8 by addition of 2 N NaOH.

Fermentation of *Streptomyces* sp. Isolate B6921

The marine isolate *Streptomyces* sp. B6921 was inoculated from its soil culture on three M_2^+ agar plates prepared with 50% seawater. After incubation for 72 hours at 28°C the well-developed colonies were used to inoculate twelve 1 liter-Erlenmeyer flasks each containing 200 ml of M_2^+ medium. The precultures were shaken at 95 rpm for 3 days at 28°C and afterwards transferred under sterile conditions into a 20-liter jar fermentor, containing 18 liters of M_2^+ medium. Incubation was carried out at 28°C for 3 days under a continuous stream of sterile air (6 liters/minute) and agitation of 250 rpm. The pH was maintained at 6.50±1.25 by adding automatically 2 N NaOH and 2 N HCl. Foaming was controlled by addition of 10% Niax PPG 2025 (Union Carbide Belgium N.V. Zwijndrecht) in ethanol.

The entire culture broth was mixed with *ca.* 1 kg diatom earth, pressed through a pressure filter and both filtrate and residue were extracted with ethyl acetate. As the TLC of both extracts showed similar compositions, they were combined and evaporated at 30°C under vacuum to yield 1.93 g of crude extract which was subjected to column chromatography (3×60 cm, 270 g silica gel) with a chloroform/methanol gradient (0 to 10% MeOH) and

Table 2. ^{1}H NMR shifts (δ, J [Hz]; $CDCl_3$, 500 Hz) of fridamycin D (**2b**), himalomycin A (**2c**) and B (**2d**).

C-No.	**2b**	**2c**	**2d**[a]
1	7.79 (d, 7.6)	7.83 (d, 7.9)	7.87 (d, 7.8 Hz)
2	7.90 (d, 7.8)	7.89 (d, 7.9)	7.96 (d, 7.8)
6	7.84 (d, 7.8)	7.75 (d, 7.9)	7.82 (d, 7.8)
7	7.64 (d, 7.8)	7.66 (d, 7.9)	7.89 (d, 7.8)
4-OH	13.04 (s)	13.06 (s)	-
9-OH	13.32 (s)	13.13 (s)	-
11	3.21 (d, 13.7) 3.08 (d, 13.7)	3.19 (d, 13.4) 3.15 (d, 13.6)	3.35 (d, 13.3) 3.21 (d, 14.2)
13	2.60 (s)	2.71 (s)	2,79 (d, 14.9) 2.63 (d, 15.1)
15	1.32 (s)	1.32 (s)	1.45 (s)
1'	4.99 (dd, 11.1, 1.9)	4.98 (dd, 9.9, 1.5)	4.91 (d br, 11.4)
2'	2.47 (ddd, 12.7, 4.6, 2.2) 1.51 (ddd, 11.5, 11.5, 11.5)	2.47 (ddd, 12.8, 4.5, 1.9) 1.51 (ddd, 11.5, 11.5, 11.5)	2.48 (ddd, 13.0, 5.0, 1.9) 1.42 (m, overlapped)
3'	3.81 (ddd, 11.6, 9.2, 4.6)	3.81 (ddd, 13.6, 9.1, 4.7)	3.60 (ddd, 11.3, 8.4, 5.2)
4'	3.49 (dd, 9.1, 9.1)	3.49 (dd, 9.2, 9.2)	3.11 (dd, 8.9, 8.9)
5'	3.57 (dq, 9.0, 6.1)	3.56 (dq, 9.1, 6.2)	3.59 (dq, 9.4, 6.2)
6'	1.41 (d, 5.8)	1.40 (d, 6.2)	1.36 (d, 5.9)
1"	5.27 (d, 3.0)	5.17 (d, 2.9)	4.96 (s br)
2"	4.32 (ddd, 3.0, 3.0, 3.0)	4.32 (ddd, 3.0, 3.0, 3.0)	1.92 (m) 1.85 (m)
3"	2.68 (dd, 17.3, 3.5) 2.62 (dd, 17.3, 3.4)	2.66 (dd, 17.1, 3.2) 2.61 (dd, 17.3, 2.8)	1.82 (m) 1.75 (m)
4"	-	-	3.25 (ddd, 10.3, 9.1, 4.1)
5"	4.72 (q, 6.8)	4.72 (q, 6.9)	3.88 (dq, 9.1, 6.9)
6"	1.36 (d, 6.8)	1.37 (d, 6.7)	1.23 (d, 6.2)
1'''	-	5.25 (s br)	5.24 (s br)
2'''	-	2.06 (m) 1.43 (m)	2.02 (m) 1.38 (m)
3'''	-	1.95 (m) 1.79 (m)	2.01 (m) 1.77 (m)
4'''	-	3.42 (s, br)	3.42 (s br)
5'''	-	4.02 (q br, 6.7)	4.02 (q br, 6.5)
6'''	-	1.11 (d, 6.7)	1.03 (d, 6.4)
1''''	-	4.75 (s br)	4.71 (s, br)
2''''	-	1.93 (m) 1.72 (m)	1.79 (m) 1.66 (m)
3''''	-	1.82 (m) 1.76 (m)	1.82 (m) 1.75 (m)
4''''	-	3.26 (ddd, 10.2, 9.2, 6.2)	3.13 (m)
5''''	-	3.62 (dd, 9.2, 6.2)	3.63 (dd, 9.2, 6.2)
6''''	-	1.22 (d, 6.2)	1.14 (d, 6.2)

[a] acetone-d_6

Table 3. Physico-chemical properties of fridamycin D (**2b**), himalomycins A (**2c**) and B (**2d**).

	2b	**2c**	**2d**
Properties		Yellow to orange solid	
R_f (CH_2Cl_2/10 % MeOH)	0.58	0.35	0.33
Molecular formula	$C_{31}H_{32}O_{12}$	$C_{43}H_{52}O_{16}$	$C_{43}H_{56}O_{16}$
(+)-ESI-MS	-	1671 ($[2M+Na]^{+1}$), 847 ($[M+Na]^{+1}$)	1679 ($[2M+Na]^{+1}$), 851 ($[M+Na]^{+1}$)
(-)-ESI-MS	1191 ($[2M-1]^{-1}$), 595 ($[M-H]^{-1}$)	1648 ($[2M]^{-1}$), 823 ($[M-H]^{-1}$)	1656 ($[2M]^{-1}$), 828 ($[M]^{-1}$)
ESI-HRMS	found 596.1899	found 824.3292	found 828.3568
	calctd. 596.1893	calctd. 824.3255	calctd. 828.3568
IR (KBr) ν cm^{-1}	3430, 2926, 1732, 1629, 1429, 1373, 1258, 1103, 1075, 805	3442, 2927, 1733, 1630, 1431, 1376, 1259, 1099, 1076, 1000, 988, 799, 467	3439, 2925, 2854, 1628, 1432, 1376, 1260, 1119, 1051, 994, 799, 440
UV/VIS (MeOH): λ_{max} (lg ε)	254 (4.14), 293 (3.85), 443 (3.72)	254 (4.32), 290 (3.95), 441 (3.88)	256 (4.36), 292 (3.96), 434 (3.97)
$[\alpha]^{20}_D$ (mg/100 ml, MeOH)	+40.0 (61)	+30.0 (74)	+30 (71)

Table 4. Antibacterial activities of **2b**~**2d** (diameter of inhibition zones in mm).

	EC	BS	SV	SA
Fridamycin D (**2b**)	24	32	25	26
Himalomycin A (**2c**)	25	32	26	23
Himalomycin B (**2d**)	24	33	28	25

EC=*Escherichia coli*, SA=*Staphylococcus aureus*, SV=*Streptomyces viridochromogenes*, BS=*Bacillus subtilis*.

separated under TLC control into fraction I (448 mg), II (138 mg), III (40 mg), IV (70 mg), V (222 mg), VI (36 mg), VII (86 mg), and VIII (80 mg). Fractions I, II, V and VII contained the yellow quinone zones responsible for the antibacterial activities.

From fraction I, 4 mg of fridamycin D (**2b**) was obtained as a yellow solid after purification on Sephadex LH-20 (4×100 cm, CH_2Cl_2/40% MeOH) followed by PTLC ($CHCl_3$/5% MeOH and then $CHCl_3$/5% MeOH/0.1% AcOH). Fraction II showing a yellow zone at Rf=0.50 ($CHCl_3$/10% MeOH) on TLC was fractionated on Sephadex LH-20 (4×100 cm, $CHCl_3$/40% MeOH) and further purified by preparative HPLC (MeCN/40% H_2O). *N*-Benzylacetamide (7 mg), *N*-(2′-phenylethyl)acetamide (4 mg), and rabelomycin (**1**, 25 mg) were obtained in the order of increasing retention time.

Similarly, fraction V was separated first on Sephadex LH-20 (4×100 cm, CH_2Cl_2/40% MeOH) and then by preparative HPLC (MeCN, 10 ml/minute). Further purification on Sephadex (1×60 cm, $CHCl_3$/40% MeOH) resulted in 2.5 mg of yellow himalomycin A (**2c**). Fraction VII was also first separated on Sephadex LH-20 (4×100 cm, $CHCl_3$/40% MeOH) to enrich the yellow compound, which was then finally purified by preparative HPLC (MeCN/H_2O gradient starting with 40% H_2O, 10 ml/minute) and PTLC (20×20 cm, $CHCl_3$/5% MeOH/0.1% AcOH) to yield 2.8 mg of himalomycin B (**2d**) as a yellow solid.

Acknowledgements

We thank F. HUTH, H. FRAUENDORF and R. MACHINEK for the spectral measurements and F. LISSY and A. MAEDLER for microbiological work. This work was supported by a grant from the Bundesministerium für Bildung and Forschung (BMBF, grant 03F0348A).

References

1) KRICKE, P.: Fridamycin, Anthracyclin-Analoge Antibiotica aus Tü 1989: Strukturaufklärung und Syntheseversuche. PhD thesis, University of Göttingen, 1984
2) KROHN, K. & W. BALTUS: Synthesis of *rac*- and *ent*-fridamycin E. Tetrahedron 44: 49~54, 1988
3) KAWAMURA, N.; R. SAWA, Y. TAKAHASHI, T. SAWA, N. KINOSHITA, H. NAGANAWA, M. HAMADA & T. TAKEUCHI: Amicenomycins A and B, new antibiotics from *Streptomyces* sp. MJ384-46F6. J. Antibiotics 48: 1521~1524, 1995
4) ŌMURA, S.; H. TANAKA, R. ŌIWA, J. AWAYA, R. MASUMA & K. TANAKA: New antitumor antibiotics, OS-4742 A1, A2, B1 and B2 produced by a strain of *Streptomyces*. J. Antibiotics 30: 908~916, 1977
5) IMAMURA, N.; K. KAKINUMA, N. IKEKAWA, H. TANAKA & S. ŌMURA: The structure of vineomycin B2. J. Antibiotics 34: 1517~1518, 1981
6) ALVI, K. A.; D. D. BAKER, V. STIENECKER, M. HOSKEN & B. G. NAIR: Identification of inhibitors of inducible nitric oxide synthase from microbial extracts. J. Antibiotics 53: 496~501, 2000
7) IMAMURA, N.; K. KAKINUMA, N. IKEKAWA, H. TANAKA & S. ŌMURA: Biosynthesis of vineomycins A1 and B2. J. Antibiotics 35: 602~607, 1982
8) SHIRLING, E. B. & D. GOTTLIEB: Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol. 16: 313~340, 1966
9) HELMKE, E. & H. WEYLAND: *Rhodococcus marinonascens* sp. nov., an actinomycete from the sea. Int. J. Syst. Bacteriol. 34: 127~138, 1984
10) BIABANI, M. A. F.; M. BAAKE, B. LOVISETTO, H. LAATSCH, E. HELMKE & H. WEYLAND: Anthranilamides: new antimicroalgal active substance from a marine *Streptomyces* sp. J. Antibiotics 51: 333~340, 1998
11) MASKEY, R. P.; R. N. ASOLKAR, E. KAPAUN, I. WAGNER-DÖBLER & H. LAATSCH: Phytotoxic arylethylamides from limnic bacteria using a screening with microalgae. J. Antibiotics 55: 643~649, 2002
12) LIU, W.-C.; W. L. PARKER, D. S. SLUSARCHYK, G. L. GREENWOOD, S. F. GRAHAM & E. MEYERS: Isolation, characterization, and structure of rabelomycin, a new antibiotic. J. Antibiotics 23: 437~441, 1970
13) MATSUZAWA, Y.; O. TOSHIKAZU, T. TAKEUCHI & H. UMEZAWA: Structure-activity relationships of anthracyclines relative to cytotoxicity and effects on macromolecular synthesis in L1210 leukemia cells. J. Antibiotics 34: 1596~1607, 1981
14) a) HOSHINO, T.; M. TAZOE, S. NOMURA & A. FUJIWARA: New anthracycline antibiotics, auramycins and sulfurmycins II. Isolation and characterization of 10 minor components (C~G). J. Antibiotics 35: 1271~1279, 1982
b) UCHIDA, T.; M. IMOTO, Y. WATANABE, K. MIURA, T. DOBASHI, N. MATSUDA, T. SAWA, H. NAGANAWA, M. HAMADA, T. TAKEUCHI & H. UMEZAWA: Saquayamycins, new aquayamycin-group antibiotics. J. Antibiotics 38: 1171~1181, 1985
c) SAWADA, Y.; T. TSUNO, T. MIYAKI, T. NAITO & T. OKI: New cirramycin-family antibiotics F-1 and F-2 selection of producer mutants, fermentation, isolation, structure elucidation and antibacterial activity. J. Antibiotics 42: 242~253, 1989