Isolation, Structure Elucidation and Activity of Anthracycline Acetates from a Terrestrial *Streptomyces* sp.

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The red coloured ethyl acetate extract of the *Streptomyces* sp. isolate GW37/3236 delivered the two new antibiotics 13-O-acetyl-bisanhydro-13-dihydrodaunomycinone (**3c**) and 4,13-O-diacetyl-bisanhydro-4-O-demethyl-13-dihydrodaunomycinone (**3d**) and additionally several known compounds. The quinones **3c** and **3d** are the first naturally occurring quinone acetates. Their structures were derived by comparison of the NMR data with those of bisanhydro-13-dihydrodaunomycinone (**3b**) and by interpretation of the 2D NMR data accompanied by the molecular weight and formula. 2-Acetamido-3-hydroxybenzamide (**5**) was also isolated from the extract and was identified by comparison of the NMR data with those of 2-acetamidobenzamide and by 2D NMR correlations. 6,9,11-Trihydroxy-4-methoxy-5,12-naphthacenedione (**4**) is isolated for the first time as a natural product.

Key words: Streptomyces sp., Daunomycinones

Introduction

The anthracycline antibiotics are forming a unique group of red pigments [1] which have attracted the interest of chemists for the past twenty years and led to many synthetic approaches, due to their outstanding antitumor and antibacterial activities [2]. Many members of the anthracycline family, especially the antibiotics like daunomycin [3, 4] and doxorubicin [5] are clinically very useful antineoplastic agents with a broad spectrum of activity extending to certain solid tumours that are normally resistant to most other modes of chemotherapy [6, 7]. The clinical use of these drugs, however, is hampered by a number of undesired side effects, the most serious being the dose-related cardio toxicity. There is therefore a great interest in related natural or synthetic compounds having improved therapeutic indices [6 – 8], and many efforts have been made to modify the anthracycline molecule with the objective of developing analogues with a wider spectrum of activity and reduced toxicity [7].

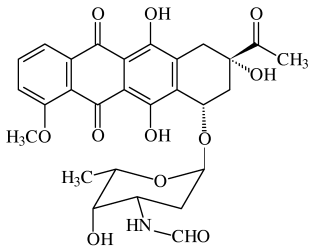
In connection with our search for new antibiotics with potential medical application, the terrestrial *Streptomyces* sp. isolate GW 37/3236 was investigated. The red coloured extract showed a high anti-microbial activity, and TLC delivered several red zones, which turned blue with dilute sodium hydroxide, pointing to *peri*-hydroxyquinones. The separation of the extract led to the isolation of the known anthracycline baumycin C1 (**1**) and anthracyclinones 7-deoxy-13-dihydrodaunomycinone (**2a**) [10], daunomycinone (**2b**) [10], 13-dihydrodaunomycinone (**2c**) [10], 11-deoxybisanhydro-13-dihydrodaunomycinone (**3a**) [11], and bisanhydro-13-dihydrodaunomycinone (**3b**) [12]. The extract also delivered 6,9,11-trihydroxy-4-methoxy-5,12-naphthacenedione (**4**) which was reported so far only as a degradation product of daunorubicin [13], and two new anthracyclinone antibiotics 13-O-acetyl-bisanhydro-13-dihydrodaunomycinone (**3c**) and 4,13-O-diacetyl-bisanhydro-4-O-demethyl-13-dihydrodaunomycinone (**3d**). In addition we found a simple new natural product, 2-acetamido-3-hydroxybenzamide (**5**). We report here the structure elucidation of these products and their biological properties.

Results and Discussion

The terrestrial *Streptomyces sp*. GW 37/3236 was cultivated in M_2 medium and worked up at our standard conditions [14]. The resulting crude extract was subjected to flash column chromatography using a

C No.	**3c**		**3d**	C No.	**3c**		**3d**
	^{13}C	^{1}H	^{1}H		^{13}C	^{1}H	^{1}H
1	118.8	8.12 (dd, 7.9, 0.7)	8.47 (dd, 7.9, 1.2)	11	168.9	–	–
2	134.2	7.75 (t, 7.9)	7.83 (t, 7.9)	11a	106.8	–	–
3	115.8	7.30 (d, 7.9)	7.43 (dd, 7.9, 1.2)	12	175.5	–	–
4	160.8	–	–	12a	134.9	–	–
4a	121.1	–	–	13	71.7	6.05 (q, 6.7)	6.05 (q, 6.7)
5	177.8	–	–	14	22.3	1.64 (d, 6.7)	1.64 (d, 6.7)
5a	106.8	–	–	4-OCH$_3$	56.6	4.10 (s)	–
6	168.1	–	–	4-OCOCH$_3$	–	–	–
6a	131.3	–	–	4-OCOCH$_3$	–	–	2.48 (s)
7	126.3	8.48 (d, 8.1)	8.48 (d, 8.3)	13-OCOCH$_3$	170.2	–	–
8	130.0	7.77 (dd, 8.1, 1.5)	7.79 (dd, 8.3, 1.9)	13-OCOCH$_3$	21.3	2.16 (s)	2.15 (s)
9	146.0	–		6-OH	–	15.94 (s)	15.61 (s)
10	122.7	8.42 (d, 1.5)	8.44 (d, 1.9)	11-OH	–	15.24 (s)	15.20 (s)
10a	131.5	–	–	–	–	–	–

Table 1. ^{1}H (300 MHz, $CDCl_3$) and ^{13}C (75.5 MHz, $CDCl_3$) NMR data of **3c** and **3d** (δ values, *J* in Hz).



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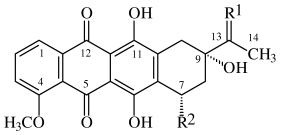
CH_2Cl_2/MeOH gradient and separated into 5 fractions. Further purification of these fractions on PTLC and Sephadex LH-20 yielded six known and four new natural products.

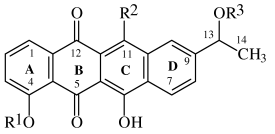
Compound **1**, **2a** ∼ **c** and **3a** ∼ **b** were all obtained as red solids with very similar ^{1}H and ^{13}C NMR spectra. The search in AntiBase [15] with the information from NMR and mass data led to the identification of these compounds as baumycin C1 (**1**) [9], 7-deoxy-13-dihydrodaunomycinone (**2a**) [10], daunomycinone (**2b**) [10], 13-dihydrodaunomycinone (**2c**) [10], 11-deoxybisanhydro-13-dihydrodaunomycinone (**3a**) [11], and bisanhydro-13-dihydrodaunomycinone (**3b**) [12] by comparison of the NMR data with the literature values.

Compound **3c** was obtained as a red solid with the mass of *m/z* 406 (EI HRMS $C_{23}H_{18}O_7$). The ^{1}H NMR spectrum of this compound was very similar to that of bisanhydro-13-dihydrodaunomycinone (**3b**). The spectrum depicted two sets of aromatic protons for 1,2,3- (δ 8.00, 7.65, 7.20, ring A) and for 1,2,4-substituted (δ 8.35, 8.30, 7.70, ring D) benzene rings. Two chelated hydroxy protons gave signals at δ 15.80 and 15.10, and a methoxy group a singlet at δ 4.00. Additionally, a quartet of a methine group bearing an oxygen atom at δ 5.95 and a methyl doublet at δ 1.60 representing a $CH(OR)CH_3$ fragment were visible. A methyl signal at δ 2.10 was due to an acetate group, which is lacking in the spectra of **3b**. The difference between **3b** and **3c** in the mass and molecular formula of Δm = 42 which corresponds to C_2H_2O suggest that **3c** is an acetate derivative of **3b**. The ^{13}C and APT NMR spectra indicated 23 signals as demanded by the molecular formula. It depicted three carbonyl carbons, two of a quinone system and one for an ester group. The spectrum also contained six aromatic methine carbons and an aliphatic methine group bearing oxygen. Of the ten aromatic quaternary carbon atoms, three were oxygenated. Additionally, a methoxy, an acetate methyl and a methyl group connected to sp^3 carbon atoms were observed. Careful interpretation of the 1D and 2D NMR data in combination with the molecular formula led to 13-O-acetyl-bisanhydro-13-dihydrodaunomycinone (**3c**), which is a new natural product.

Compound **3d** was obtained as a red solid as well with the molecular weight of *m/z* 434 ($C_{24}H_{18}O_8$). The ^{1}H NMR spectra of **3c** and **3d** showed a close similarity, the only difference being the replacement of the methoxy signal in **3c** by an acetate signal in **3d**. This was confirmed by differences in mass and molecular formula of Δm = 28 and CO, respectively. The protons of the aromatic ring D and of the side chain in **3c** and **3d** showed nearly identical chemical shifts. On the other hand, all the proton signals of ring A in **3d** were shifted to deeper field with respect to **3c**, indicating that the substitution had taken place here. While H-1 and H-3 in **3c** absorbed at δ 8.12 and 7.30, respectively, these signals were shifted to 8.47 and 7.43 in the acetate **3d**. These shift differences are very similar to



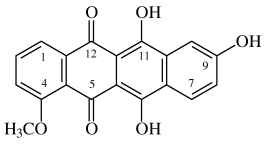
2a: R^1 = OH, H; R^2 = H
2b: R^1 = O; R^2 = OH
2c: R^1 = OH, H; R^2 = OH



3a: R^1 = CH_3; R^2 = H; R^3 = H
3b: R^1 = CH_3; R^2 = OH; R^3 = H
3c: R^1 = CH_3; R^2 = OH; R^3 = $COCH_3$
3d: R^1 = R^3 = $COCH_3$; R^2 = OH

those between pairs of related quinones, *e.g.* the acetate and the methyl ether of 5-hydroxynaphthoquinone [16] (juglon). On the basis of these facts, we assign the structure 4,13-O-diacetyl-bisanhydro-4-O-demethyl-13-dihydrodaunomycinone (**3d**) for this compound, which also represents a new quinone. Although several quinones with acetate groups in benzyl position are known [17] **3d** is the first natural *peri*-acetoxyquinone isolated so far. Both **3c** and **3d** were racemic due to their missing optical rotation and CD effects (in $CHCl_3$).

The molecular weight of compound **4** was found to be *m/z* 336. ^{1}H and ^{13}C NMR spectra of **4** were similar to those of **3b** and **3c** in the aromatic region, however, in the aliphatic region the signals of the side chain in **3b** and **3c** were missing. The latter was identified as 6,9,11-trihydroxy-4-methoxy-5,12-naphthacenedione (**4**) by a search in AntiBase with the NMR data and the molecular weight, and confirmed by comparison of the NMR data with the literature values [13]. So far, 6,9,11-trihydroxy-4-methoxy-5,12-naphthacenedione (**4**) has been reported only as degradation product of daunorubicin [13]. It was isolated here for the first time from natural sources.



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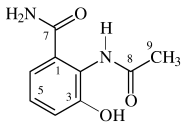

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In addition to the red quinones mentioned above, the crude extract delivered a colourless solid with the molecular weight *m/z* 194 ($C_9H_{10}N_2O_3$). Compound **5** gave a strong blue fluorescence under 366 nm similar to anthranilic acid, anthranilamide and 2-acetamidobenzamide [18], which have been frequently isolated in our group. By careful interpretation of the 1D and 2D NMR data and by comparison with the NMR data of the derivatives mentioned above, it was identified as a new natural product, 2-acetamido-3-hydroxybenzamide (**5**). The free hydroxyanthranilic acid and several of their derivatives are common metabolites, whereas derivatives of the isomeric 3-aminosalicylic acid are unknown from microorganisms making this substituent pattern unlikely. The related 2-acetamidobenzamide is reported to have antifungal properties. In our test, however, **5** and 2-acetamidobenzamide both were inactive in the agar diffusion test against *Candida albicans* and *Mucor miehei* at concentrations of 40 μg/test plate.

Biological Activity

Antibacterial and antifungal activities were semi-quantitatively determined using the agar diffusion method with 9 mm paper disc with 5, 10 and 20 μg of bisanhydro-13-dihydrodaunomycinone (**3b**), 13-O-acetyl-bisanhydro-13-dihydrodaunomycinone (**3c**) and 4,13-O-diacetyl-bisanhydro-4-O-demethyl-13-dihydrodaunomycinone (**3d**)/disk. **3b** $\sim$ **3d** showed a comparable weak activity against *Bacillus subtilis*, *Streptomyces viridochromogenes* (Tü 57), *Staphylococcus aureus*, *Escherichia coli* and no activity against *Candida albicans*, *Mucor miehei*, *Chlorella vulgaris*, *Chlorella sorokiniana* and *Scenedesmus subspicatus*. The strong activity of the crude extract must be accounted mainly to the presence of the known antibiotics like baumycin C1 (**1**). The new natural products were active against human cancer cell lines LXFA 629L and LXFL 529L (lung), MAXF 401NL (breast), MEXF 462NL (melanoma), RXF 944L (kidney) and UXF 1138L (uterus) with IC_{50} value of > 6 μg/ml.

Experimental Section

NMR spectra were measured on Bruker AMX 300 (300.135 MHz), Varian Unity 300 (300.145 MHz) and Varian Inova 500 (499.876 MHz) spectrometers. HPLC/ESI-MS was recorded on a Finnigan LCQ with quarternary pump Rheos 4000 (Flux Instrument) and nucleosil column EC 125/2, 100-5, C18 (Macherey-Nagel & Co., Düren, Germany). EIMS was recorded on a Finnigan MAT95 (70 eV) and perfluorokerosene was used as reference substance in HREIMS. IR spectra were recorded on a Perkin-Elmer 1600 Series FT-IR spectrometer (KBr pellets). Preparative HPLC was performed using an RP18 column (Eurochrom Eurospher RP 100-C18, 5 μm) using a Knauer variable wavelength monitor at 202 nm. Flash chromatography was carried out on silica gel (30 – 60 µm, J. T. Baker). Thin layer chromatography (TLC) was performed on Polygram SIL G/UV_{254} (Macherey-Nagel & Co.). R_f values were measured

on Polygram SIL G/UV_{254} (Macherey-Nagel & Co.) with CH_2Cl_2/10% MeOH. Size exclusion chromatography was done on Sephadex LH-20 (Pharmacia).

M_2 medium

Malt extract (10 g), yeast extract (4 g) and glucose (4 g) were dissolved in 1 l of tap water and the medium was adjusted to pH 7.8 with 2 N NaOH and sterilized for 33 min at 121 °C. After sterilization an end pH 7.0 of the medium is attained.

Description of the producer

The actinomycete strain GW37/3236 was obtained from the strain collection of bioLeads in Heidelberg, Germany. This strain was Gram-positive, aerobic, non-acid fast, and produced a highly branched aerial mycelium with *flexuous* spore chains. Neither sporangia, or motile spores nor any other special morphological structures were observed. The colour of aerial hyphae and spore mass was light pink on yeast extract-malt agar, oatmeal and soil extract agar. The substrate mycelium was dark red on most media. The strain formed no melanin pigments on tyrosine agar slants. The strain is deposited in the culture collection of actinomycetes at bioLeads GmbH, Waldhofer Straße 104, 69123 Heidelberg, Germany.

Fermentation and work-up procedure

With a well grown agrar culture of the terrestrial *Streptomyces* sp. isolate GW37/3236, 100 of 1 l-Erlenmeyer flask each containing 250 ml of M_2 medium were inoculated and incubated for 3 days at 28 °C on a linear shaker (110 rpm). The 25-liter culture broth was mixed with ca. 1 kg diatom earth and filtered through a press filter to separate mycelia and water phase. The mycelial cake and the filtrate were separately extracted each three times with ethyl acetate (*ca.* 2 l each). Since the chemical compositions of both organic phases were similar, they were combined and concentrated under reduced pressure to yield 5 g of a dark red oily crude extract.

Chromatographic separation of the crude extract (Fig. 1) yielded baumycin C1 (**1**, 3.5 mg, $R_f = 0.42$), 7-deoxy-13-dihydrodaunomycinone (**2a**, 3.4 mg, $R_f = 0.49$), daunomycinone (**2b**, 1.9 mg, $R_f = 0.58$), 13-dihydrodaunomycinone (**2c**, 4.3 mg, $R_f = 0.45$), 11-deoxybisanhydro-13-dihydrodaunomycinone (**3a**, 2.5 mg, $R_f = 0.60$), bisanhydro-13-dihydrodaunomycinone (**3b**, 8.5 mg, $R_f = 0.63$), 13-O-acetylbisanhydro-13-dihydrodaunomycinone (**3c**, 6.4 mg), 4,13-O-diacetyl-bisanhydro-4-O-demethyl-13-dihydrodaunom ycinone (**3d**, 0.9 mg), 6,9,11-trihydroxy-4-methoxy-5,12-naphthacenedione (**4**, 0.9 mg, $R_f = 0.62$) and 2-acetamido-3-hydroxybenzamide (**5**, 4.4 mg).

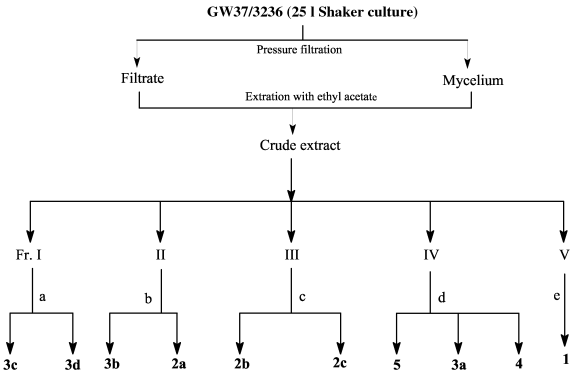

Fig. 1. Work up scheme of the strain *Streptomyces* sp. isolate GW37/3236; a) PTLC with CH_2Cl_2; b) precipitation from CH_2Cl_2 solution with MeOH and then PTLC with CH_2Cl_2/5% MeOH/0.1% TFA; c) Sephadex LH-20 with CH_2Cl_2/40% MeOH followed by PTLC with CH_2Cl_2/5% MeOH; d) Sephadex LH-20 with CH_2Cl_2/60% MeOH followed by PTLC CH_2Cl_2/5% MeOH; e) PTLC with cyclohexane/85% ethyl acetate/0.1% TFA.

*13-O-Acetyl-bisanhydro-13-dihydrodaunomycinone (***3c***)*

Red solid, $R_f = 0.87$ (CH_2Cl_2/5 % MeOH). UV/vis (MeOH): λ_{max} (lg ε) = 263 (4.67), 469sh (4.03), 496 (4.17), 532 (4.11) nm; IR (KBr): ν = 3430, 2925, 2856, 1737, 1616, 1578, 1509, 1461, 1440, 1413, 1372, 1269, 1234, 1066, 1045, 1018, 938, 911, 825, 752, 703, 607, 452 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ = see Table 1; EI-MS: *m/z* (%) = 406 (M^+, 100), 363 (4), 347 (16), 328 (20), 121 (6), 91 (8), 43 (12); (+)-ESI-MS: *m/z* = 429 ($[M+Na]^+$); (-)-ESI-MS: *m/z* (%) = 833 ($[2M+Na-2H]^-$, 100), 406 (M^-, 20); EI-HRMS = 406.1051 (calcd. 406.10016 for $C_{24}H_{18}O_8$).

*4,13-O-Diacetyl-bisanhydro-4-O-demethyl-13-dihydrodaunomycinone (***3d***)*

Red solid, R_f = 0.90 (CH_2Cl_2/5% MeOH). UV/vis (MeOH): λ_{max} (lg ε) = 266, 460 (sh), 489, 523, 554 nm, IR (KBr): ν = 2924, 2853, 1736, 1661, 1636, 1578, 1464, 1414, 1374, 1243, 1212, 1071, 1036, 962, 887, 861, 757, 722, 702, ^1H NMR ($CDCl_3$, 300 MHz) see Table 1; EI-MS: *m/z* (%) = 434 (M^+, 24), 392 (100), 350 (8), 332 (38), 199 (36), 90 (8), 57 (8), 43 (14); (+)-ESI-MS: *m/z* (%) = 891 ($[2M+Na]^+$, 100), 457 ($[M+Na]^+$, 30); (-)-ESI-MS: *m/z* (%) = 889 ($[2M+Na-2H]^-$, 100), 433 ($[M-H]^-$, 5); EI-HRMS = 434.1003 (M, calcd. 434.10016 for $C_{23}H_{18}O_7$).

*2-Acetamido-3-hydroxybenzamide (***5***)*

Colourless solid, R_f = 0.16 (CH_2Cl_2/5% MeOH). UV/vis (MeOH): λ_{max} (lg ε) = 240 (sh), 295 (3.68) nm; IR (KBr):

ν = 3371, 3187, 3065, 1681, 1657, 1603, 1574, 1467, 1433, 1386, 1367, 1320, 1289, 1259, 1189, 1132, 1030, 990, 939, 793, 742, 718, 608, 579, 524, 440; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 6.97 (dd, *J* = 8.3, 1.5 Hz; 1 H, H-4), 7.12 (t, *J* = 7.9 Hz; 1 H, H-5), 7.05 (dd, *J* = 7.9, 1.5 Hz; 1 H, H-6), 2.03 (s; 3 H, CH3), 9.60 (s br; 1 H, NH)[a], 7.64, 7.41 (2 s br; 2 H, NH_2)[a], 10.08 (s br; 1 H, OH)[a]; [a] assignment may be exchanged. ^{13}C NMR (75.5 MHz, DMSO-d_6): δ = 131.5 (C_q-1), 123.1 (C_q-2), 151.2 (C_q-3), 119.1 (CH-4), 126.3 (CH-5), 119.3 (CH-6), 170.1 (C_q-7), 169.6 (Cq-8), 23.1 (CH3-9). EI-MS: *m/z* (%) = 194 (M^+, 36), 177 (43), 152 (48), 135 (100), 107 (72), 79 (12), 52 (16), 43 (36); (+)-ESI-MS: *m/z* = 195 ($[M+H]^+$, 30); (-)-ESI-MS: *m/z* = 193 ($[M-H]^-$); EI-HRMS = 194.0689 (M, calcd. 194.06914 for $C_9H_{10}N_2O_3$).

Acknowledgements

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[1] T. W. Goodwin, Chemistry and Biochemistry of Plants Pigments, p. 324, Acad. Press. London and New York (1965).
[2] B. A. Keay, R. Rodrigo, Tetrahedron **40**, 4597 (1984).
[3] a) E. Shanahan-Pendergast, WO 0253138 (2002); Chem. Abstr. **137**:88442 (2002); b) T. Sasaki, Y. Tsukada, H. F. Deutsch, H. Hirai, Cancer Chemother. Pharmacol. **13**, 75 (1984).
[4] J. Y. Yang, H. Y. Luo, Q. Y. Lin, Z. M. Liu, L. N. Yan, P. Lin, J. Zhang, S. Lei, World J. Gastroenterol. **8**, 644 (2002).
[5] D. A. Gewirtz, Biochem. Pharmacol. **57**, 727 (1999).
[6] A. Di Marco, M. Gaetani, P. Orezzi, B. M. Scarpinato, R. Silvestrini, M. Soldati, T. Dasdia, L. Valentini, Nature **201**, 706 (1964).
[7] D. J. Taatjes, G. Gaudiano, K. Resing, T. H. Koch, J. Med. Chem. **39**, 4135 (1996).
[8] T. H. Smith, A. N. Fujiwara, W. W. Lee, H. Y. Wu, D. W. Henry, J. Org. Chem. **42**, 3653 (1977).
[9] T. Takahashi, H. Naganawa, T. Takeuchi, H. Umezawa, T. Komiyawa, T. Oki, T. Inui, J. Antibiot. **30**, 622 (1977).
[10] J. D. Brand, J. F. Fisher, J. Org. Chem. **55**, 2518 (1990).
[11] J. V. Jizba, P. Sedmera, J. Vokoun, M. Blumauerova, Z. Vanek, Coll. Czech. Chem. Commun. **45**, 764 (1980).
[12] A. Mohamed, E. Sallam, R. W. Whistler, J. M. Cassady, Can. J. Chem. **63**, 2697 (1985).
[13] J. H. Beijnen, R. P. Potman, R. D. van Ooijen, R. J. Driebergen, M. C. H. Voskuilen, J. Renema, W. J. M. Underberg, Int. J. Pharmaceutics **34**, 247 (1987).
[14] R. P. Maskey, R. N. Asolkar, E. Kapaun, I. Wagner-Döbler, H. Laatsch, J. Antibiot. **55**, 643 (2002).
[15] H. Laatsch, AntiBase 2002, A Natural Products Database for Rapid Structure Determination. Chemical Concepts, Weinheim 2002; see Internet http://www.gwdg.de/∼ucoc/Laatsch/.
[16] H. Laatsch, Liebigs Ann. Chem. 1321 (1980).
[17] R. H. Thomson, Naturally Occurring Quinones III, Chapman and Hall, London (1987); b) G. K. Poch, J. B. Gloer, C. A. Shearer, J. Nat. Prod. **55**, 1093 (1992).
[18] a) N. Phay, H. Yada, T. Higashiyama, A. Yokota, A. Ichihara, F. Tomita, J. Antibiot. **49**, 703 (1996); b) M. Shaaban, R. P. Maskey, I. Wagner-Doebler, H. Laatsch, J. Nat. Prod. **65**, 1660 (2002).