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Evariquinone, isoemericellin, and stromemycin from a sponge derived strain of the fungus *Emericella variecolor*☆

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Abstract

From a strain of the fungus *Emericella variecolor* derived from the marine sponge *Haliclona valliculata*, two new natural products, evariquinone and isoemericellin, were isolated after HPLC–UV, –MS, and –NMR studies of the extract and their structures were elucidated by mass spectrometry and NMR experiments. Evariquinone showed antiproliferative activity towards KB and NCI-H460 cells at a concentration of 3.16 μg/ml. Furthermore, the fungus was found to produce the known metabolites stromemycin, shamixanthone, and 7-hydroxyemodin. Chemical degradation, NMR decoupling experiments, and spin-system simulation provided evidence for the double bonds in stromemycin to be all *E*-configured. ROESY experiments established the monosaccharide moiety to be glucose.

Keywords: *Emericella variecolor*; *Haliclona valliculata*; Sponge derived fungus; Evariquinone; Isoemericellin; Stromemycin; Shamixanthone; 7-Hydroxyemodin; Structural elucidation; HPLC–NMR coupling

1. Introduction

Strains of the fungus *Emericella variecolor* (anamorph: *Aspergillus variecolor* syn. *A. stellatus*) have been the source of a variety of natural products, mainly sesterterpenes with unusual polycyclic skeletons, for example, astellatol (Sadler and Simpson, 1989) or variecolin (Hensens et al., 1991), and prenylated xanthones (Chexal et al., 1974; Kawahara et al., 1988). Within the scope of a program aiming at the isolation of novel natural products from sponge derived microorganisms (Bringmann and Lang, in press; Bringmann et al., in press), we have isolated and chemically investigated several fungi (Brauers et al., 2000). One strain, isolated from the sponge *Haliclona valliculata* and identified as *E. variecolor*, showed a remarkable diversity of secondary metabolites in the preliminary HPLC screening and was therefore subjected to detailed chemical analysis. In this paper, we describe the isolation and structural elucidation of the new anthraquinone evariquinone (1,2,3-trihydroxy-6-methyl-8-methoxyanthraquinone, **1**; Fig. 1) and the known 1,2,3,8-tetrahydroxy-6-methylanthraquinone (7-hydroxyemodin, **2**), as well as the new prenylxanthone isoemericellin (**3**), accompanied by the biosynthetically related known metabolite shamixanthone (**4**). Furthermore, the *C*-glycosidic depside stromemycin (**5**) was identified, which has recently been patented for its metalloproteinase-inhibiting activity (Hopmann et al., 2001); here first investigations on the double-bond configurations and the identity of the monosaccharide moiety of **5** were conducted.

2. Results and discussion

In the HPLC–UV chromatogram (with photodiode array detection) of the extract of the medium, two peaks

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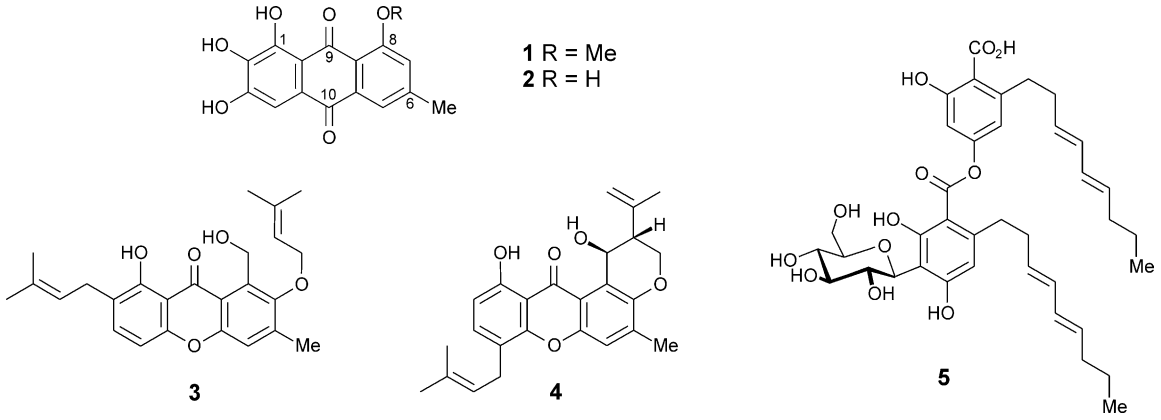


Fig. 1. Natural products from the fungus *Emericella variecolor* isolated from the sponge *Haliclona valliculata*: evariquinone (**1**), 7-hydroxyemodin (**2**), isoemericellin (**3**), shamixanthone (**4**), and stromemycin (**5**).

were observed whose UV spectra gave a first hint at the structural similarity of the compounds. HPLC–NMR investigations further substantiated this assumption. In the ^{1}H NMR spectra of both HPLC peaks, which were acquired in the stop-flow mode using the WET pulse sequence (Ogg et al., 1994) for solvent suppression, resonances were observed for three aromatic protons appearing as singlets in the range from 7.1 to 7.6 ppm and for one methyl group (2.45 ppm). The spectrum of the more polar compound contained one additional *O*-methyl signal (3.95 ppm). For completion of the structural elucidation, the compounds were isolated by preparative HPLC and subjected to NMR and MS measurements.

The high resolution electron impact mass spectrum (HREIMS) of the more polar substance gave a molecular ion peak at *m*/*z* 300.0634 corresponding to a molecular formula $C_{16}H_{12}O_6$. The compound showed NMR spectra typical of a 9,10-anthraquinone, with two ^{13}C NMR carbonyl signals (189 and 183 ppm), 12 signals in the aromatic region of the ^{13}C NMR spectrum, a methyl signal, and an *O*-methyl one. The ^{1}H NMR spectrum indicated the presence of three aromatic protons, an aromatic methyl and an *O*-methyl group (Table 1). The structure was assembled through HMQC, HMBC, and COSY experiments. The position of the aromatic methyl group (2.45 ppm), flanked by two protons (7.60 and 7.25 ppm), was deduced from weak H,H-coupling between the methyl protons and both of these aromatic protons, as well as from HMBC correlations. The *O*-methyl group was found to be attached to the same ring as the aromatic methyl group since both, its protons and one of the aromatic protons, exhibited HMBC correlations to C-8. The third aromatic proton (7.15 ppm) showed no H,H-coupling and no HMBC correlation to any carbon in the first ring and therefore had to be attached to the other half of the anthraquinone. For the protons with resonances at 7.15 and 7.60 ppm, a position next to the same carbonyl group was obvious from the strong HMBC correlations to the ^{13}C signal of C-10 (183.2 ppm). To match the molecular formula, the remaining three substituents at the second ring had to be free hydroxy groups. This was in good agreement with the low-field chemical shifts observed for the corresponding carbons (152.0, 140.5, and 152.7 ppm). Consequently, the compound was established to be 1,2,3-trihydroxy-6-methyl-8-methoxy-anthraquinone (**1**; Fig. 1); since this product was not known from the literature, it was given a new name, evariquinone, after the producing organism, *E. variecolor*.

Based on the structure of **1** the related second compound was easily identified as 1,2,3,8-tetrahydroxy-6-methylanthraquinone (**2**; Fig. 1). All NMR data of **2** corresponded to those of **1** except for the missing signal of an *O*-methyl group. Further evidence of the two structures was gained from an exhaustive *O*-methylation of both, **1** and **2**, with diazomethane. In both cases the product was the same known (Roberge and Brassard, 1981) permethylated derivative, 1,2,3,8-tetramethoxy-6-methylanthraquinone (**6**). The ROESY spectrum of **6**

Table 1
NMR data of evariquinone (**1**)

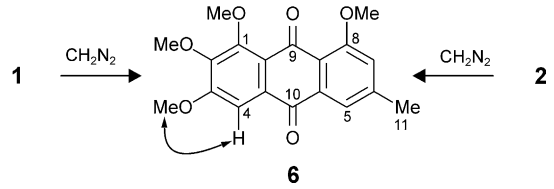
Position	δ_H (ppm)	δ_C (ppm)	HMBC
1		152.0	
2		140.5	
3		152.7	
4	7.15 *s*	108.9	1/3, 2, 4a, 9a, 10
4a		125.9	
5	7.60 *d*	121.5	7, 10, 8a, 11
6		148.7	
7	7.25 *d*	119.8	5, 8, 8a, 11
8		162.3	
8a		119.3	
9		189.2	
9a		112.7	
10		183.2	
10a		136.8	
11	2.45 *s*	22.2	5, 6, 7
8-OMe	3.95 *s*	56.9	8

showed a correlation from H-4 to only one methoxy group (Scheme 1), thus providing additional proof of the proposed substitution pattern, with C-4 unsubstituted.

Compound **2**, commonly known as 7-hydroxyemodin, had previously been isolated from *Aspergillus rugulosus* (Jenkins, 1972; Thomson, 1987) and from a variety of insects belonging to the family of Coccidae (Chan et al., 1966; Banks et al., 1976). The new compound **1** was found to display antiproliferative activity towards KB cells (60% inhibition) and NCI-H460 cells (69% inhibition) at a concentration of 3.16 μg/ml, while the related product **2** was inactive.

Two further peaks in the chromatogram of the extract of the cell-free culture broth showed HPLC-UV spectra typical of xanthones, a structural class known to occur in *A. variecolor* (Chexal et al., 1974, 1975). Isolation by preparative HPLC yielded two yellow, crystalline compounds. The molecular formula $C_{25}H_{28}O_5$ of the first compound was deduced from HREIMS. From its NMR data, a xanthone core and two prenyl groups were identified: one was found to be directly linked to the ring system, the other one via an aromatic oxygen atom. In addition to the OH group (12.9 ppm) and the aromatic methyl substituent (2.47 ppm), which are common to most of the fungal prenylxanthones (Chexal et al., 1974, 1975), a hydroxymethyl group was detected. The substitution pattern of the compound was derived from a series of HMBC and HMQC correlations (Fig. 2), which permitted the assignment of structure **3** to the compound.

A remaining problem was the orientation of the *O*-prenylated ring, viz the question whether the CH_2OH group was on the same side of the molecule as the phenolic hydroxy group (i.e. at C-9) or not (i.e. at C-12). The chemical shift of the hydroxymethylene protons (5.13 ppm) indicated a shielding by the adjacent carbonyl group and thus substantiated the position of this group to be located next to the carbonyl group. Additional evidence was provided by the similarity of the ^1H NMR chemical shifts with those of variecoxanthone B (**7**, Fig. 2) (Chexal et al., 1975), which is also known as emericellin (Ishida et al., 1975). The new compound **3** differed from **7** only by the position of the *C*-prenyl moiety, and was thus named isoemericellin. By the HMBC correlation {H-21↔C-4} (Fig. 2), it was



Scheme 1. Structure of the joint permethylated derivative **6** of both, evariquinone (**1**) and 7-hydroxyemodin (**2**); the arrow denotes the only ROESY interaction of H-4.

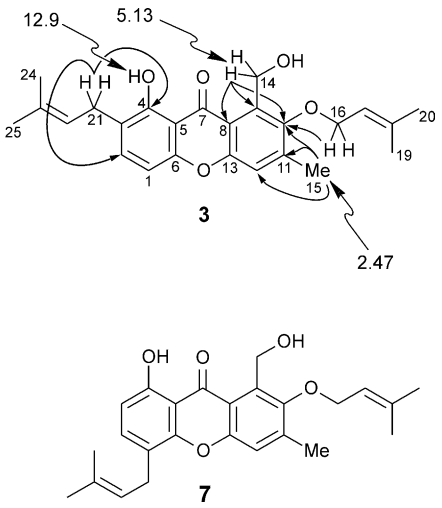


Fig. 2. Selected chemical shifts and HMBC correlations indicative of the constitution of isoemericellin (**3**) and, for comparison, the structure of emericellin (**7**) from *Aspergillus nidulans*.

unequivocally established to have its *C*-prenyl group *ortho* to 4-OH, i.e. at C-3. In the EI mass spectrum major peaks at *m/z* 339 and 340 were observed, indicating a loss of the *O*-prenyl residue (C_5H_9 and C_5H_8, respectively). Subsequent cleavage of a C_4H_8 unit from the *C*-prenyl residue explained the signals at *m/z* 283 and 284 and verified the position of the *C*-prenyl residue *ortho* to the 4-OH group, since this bond rupture requires a six-membered transition state involving an adjacent hydroxy group (Ballantine et al., 1970). Cytotoxicity testing of isoemericellin (**3**) was rendered impossible by the lacking solubility of the compound in DMSO or water. The second yellow compound was identified as shamixanthone (**4**, Fig. 1), a prenylated xanthone previously isolated from *A. variecolor* (Chexal et al., 1974).

HPLC–UV and HPLC–MS (HPLC with mass spectrometric detection) of the mycelium extract showed one prominent UV-absorbing peak with an $[M+H]^+$ of *m/z* 697.6. Using D_2O–MeCN instead of H_2O–MeCN as the eluent (Bringmann et al., 2000) resulted in an $[M+D]^+$ of *m/z* 706.4, the increase being attributable to the presence of eight exchangeable protons in the molecule. Isolation by reversed-phase medium pressure liquid chromatography (RP-MPLC) and column chromatography on silica gel gave 56 mg of an amorphous reddish solid soluble in methanol. The NMR spectra suggested the existence of two nonadienyl residues attached to aromatic systems. Since these residues were not equivalent, the proton spectrum exhibited two sets of—unfortunately overlapping—signals. The non-equivalency was more obvious in the ^{13}C NMR spectrum. The aromatic region of the ^1H NMR spectrum contained three signals, two of which showed a *meta*-coupling (2.3 Hz) to each other, revealing that the structure included at least two aromatic rings. Another

partial structure evident from the NMR data was a sugar moiety, which was identified as glucose in its pyranosidic form using COSY and ROESY correlations, the large coupling constant (9.9 Hz) between H-1″ and H-2″ indicating the β-anomer. Different from related, but *O*-glucosidic natural products (Yasuzawa et al., 1990), the glucose was found to be *C*-glucosidic, as could be deduced from the HMBC correlations between H-1″ and C-1, C-2, and C-3, and from the chemical shift of C-1″ (76 ppm). The ^{13}C chemical shifts of the sugar moiety were in good agreement with those of known aryl *C*-glycosides (cf. e.g. Ferrari et al., 2000). The substitution pattern of the two aromatic rings was assigned using HMQC and HMBC experiments. The partial structures in combination with the molecular mass, as obtained by HPLC-MS and fast atom bombardment mass spectrometry (FABMS), suggested an ether or an ester linkage between the molecular halves. Evidence of an ester function was given in the HPLC–MS and FABMS spectra. Besides a small $[M+H]^+$ peak at *m*/*z* 697.6, a major signal at *m*/*z* 421 containing six exchangeable protons was detected. This was interpreted as the glycosylated part of the molecule after cleavage of the ester bond, proving the attachment of the sugar to the carbonyl side of the ester. Such mass spectrometrically observed ester cleavages have been shown to be typical of depsides (Huneck et al., 1968). In our case the cleavage occurred even under the very mild conditions of electrospray ionization (ESI) used for HPLC–MS. Further information about the ester linkage was obtained from the ROESY correlations of H-8 to both, H-2′ and H-4′ (Fig. 3), proving the attachment of the non-glycosylated ring to the oxygen at C-3′, not to the one at C-1′. The compound was thus identical to stromemycin (**5**), a fungal product recently isolated and structurally largely elucidated, and found to inhibit the human metalloproteinase stromelysin (Hopmann et al., 2001). In our cytotoxicity assays, the compound was inactive against five different cancer cell lines.

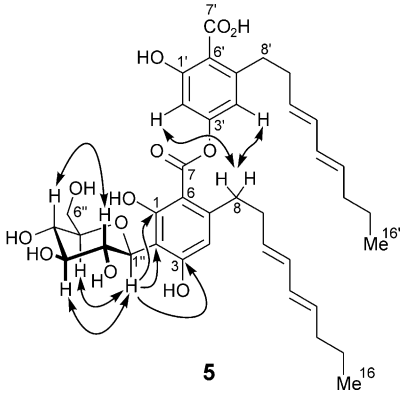


Fig. 3. Selected ROESY and HMBC correlations indicative of the structure of stromemycin (**5**).

For an assignment of the hitherto unknown (Hopmann et al., 2001) configuration of the double bonds by evaluation of the $^3J_{HH}$ coupling constants, the ester bond was cleaved by methanolysis (Scheme 2) to simplify the largely overlapping multiplets present in the ^1H NMR spectrum of **5**; however, even the spectra of the cleavage products **8** and **9** (although again confirming the above attribution of the sugar-bearing molecular position) showed higher-order spectra for the double-bond protons, which allowed no direct access to the coupling constants. Therefore, the spectra of **8** and **9** were decoupled by simultaneous irradiation at the frequencies of the allylic protons H-9 and H-14, and the remaining multiplets were evaluated by analysis of the spin system using the WIN-DAISY software. This resulted in $^3J_{HH}$ coupling constants across the double bonds between 15.2 Hz and 15.3 Hz, thus indicating *E*-configuration at all of the four double bonds.

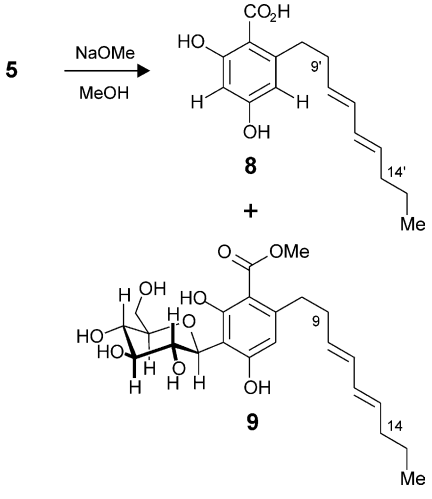
A further compound was gained by extraction of the acidified culture medium. This colorless, crystalline compound was easily identified as 2-furanoic acid. Since the fungus was grown on malt-extract containing medium, and the compound is known to be produced by a variety of fungi by oxidation of the malt-extract constituent furfural (Claydon et al., 1985), this finding was not unexpected.

The results described in this paper provide further insight into the great diversity of natural products from marine fungi, and emphasize the increasing importance of sponge-derived fungi in marine natural products research.

3. Experimental

3.1. General

Mps: uncorr. IR spectra were taken on a Jasco FT/IR-410 spectrometer, CD spectra on a Jasco J-715 spectropolarimeter, and optical rotations on a Perkin-



Scheme 2. Methanolysis of stromemycin (**5**).

Elmer 241MC polarimeter. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) were recorded on a Bruker DMX 600 either in $CDCl_3$ or CD_3OD with the solvent as the int. standard ($CDCl_3$: δ 7.26 and δ 77.01, CD_3OD: δ 3.30 and δ 49.02). Proton-detected, heteronuclear correlations were analyzed using HMQC (optimized for $^1J_{HC} = 145$ Hz) and HMBC (optimized for $^nJ_{HC} = 7$ Hz). ROE effects were measured using a ROESY pulse sequence from the standard Bruker pulse program library. EIMS (70 eV) and HREIMS (70 eV) were determined on Finnigan MAT 8200 and Finnigan MAT 90 instruments. HPLC–ESIMS was performed with a triple-stage quadrupole TSQ 7000 mass spectrometer (temperature of capillary: 210 °C, ESI-voltage: 3.5 kV, N_2 as sheath and auxiliary gas, positive mode). HPLC (analytical): Symmetry C_{18}, 2.1×150 mm (Waters); HPLC (preparative): SymmetryPrep C_{18}, 19×300 mm (Waters); UV-Detection 200–500 nm (photodiode array detector). MPLC: Lobar LichroPrep RP-18, Size B (Merck); iterative calculations of coupling constants in ^{1}H NMR spin systems were performed with WIN-DAISY 4.05 (Bruker). Thin layer chromatography (TLC) was performed on precoated plates (Merck TLC aluminum sheets silica gel 60 F_{254}) with detection by UV light (254 nm) and with toluene–EtOAc–formic acid (5:4:1) as the mobile phase.

3.2. Fungus

The fungal strain, identified as *E. variecolor* Berk. & Broome (Trichocomaceae), was isolated from the interior of a marine sponge, *H. valliculata* Griessinger (Haliclonidae), collected in a depth of 20–30 m at Secca di Capo di Fonza, Elba/Italy, in May 2000. For chemical investigations the fungus was grown on WSA liquid medium (Wickerham, 1951). After a growth period of 14 days, 10 vol.% of EtOAc were added and the mixture was kept at −80 °C until extraction. The strain was deposited in the "Kulturensammlung Marine Pilze Bremerhaven (KMPB)" under the accession no. E-00-6/3.

3.3. Extraction

The fungus (450 g wet wt) was separated from the culture medium and extracted exhaustively with CH_2Cl_2–MeOH (1:1). The medium was extracted three times with 1 l of EtOAc each. Both extracts were dried in vacuo and partitioned between MeOH + 25% H_2O and petrol. The extracted medium was acidified with 100 ml of conc. HCl and extracted twice with 1 l of EtOAc each.

3.4. Evariquinone (**1**) and 7-hydroxyemodin (**2**)

The MeOH fraction of the medium extract was desalted by partitioning between H_2O and EtOAc and the EtOAc fraction subjected to preparative HPLC using H_2O (A) and MeCN + 0.05% TFA (B) as eluents (linear gradient: 0–20 min 25–60% B; flow rate 12 ml/min). Compound **1** was eluted after 16 min; crystallization from MeOH gave 15 mg of orange-colored needles. TLC: $R_f = 0.54$. Mp 238–242 °C (subl.). UV λ_{max}^{EtOH} nm: 213, 248, 285, 424. IR ν_{max}^{KBr} cm^{-1}: 3466, 1620, 1593, 1460, 1330, 1230. EIMS *m*/*z* (rel. int.): 300 (100), 282 (95), 254 (16), 198 (22), 170 (11). HREIMS *m*/*z*: 300.0634 ($C_{16}H_{12}O_6$ requires 300.0634). NMR data (600 MHz, MeOH-d_4), see Table 1.

Compound **2** (16.8 mg, red powder) was eluted at 22 min. TLC: $R_f = 0.67$. Mp 293 °C (dec.); (Chan and Crow, 1966) mp 296–298 °C (dec.). ^{13}C NMR (100 MHz, acetone-d_6): 192.7 (C-9), 181.5 (C-10), 163.1 (C-8), 152.7 (C-3), 152.2 (C-1), 149.8 (C-6), 139.3 (C-2), 134.7 (C-10a), 127.1 (C-4a), 124.4 (C-7), 121.5 (C-5), 114.8 (C-8a), 111.4 (C-9a), 110.1 (C-4), 22.1 (Ar-Me). HREIMS *m*/*z*: 286.0475 ($C_{15}H_{10}O_6$ requires 286.0477). ^{1}H NMR, UV, and IR spectra showed a good correlation with literature data (Banks et al., 1976; Chan and Crow, 1966).

3.5. O-Methylation of **1** and **2**

Anthraquinone **1** or **2** (1 mg) was dissolved in 1 ml of MeOH–H_2O (9:1). A solution of CH_2N_2 in Et_2O (de Boer and Baker, 1963) was added until generation of N_2 ceased and the solution remained yellow. After 72 h the solvent was removed under reduced pressure, and the residue purified by preparative HPLC using H_2O (A) and MeCN (B) as the eluents (linear gradient: 0–25 min 35–100%; flow rate 12 ml/min). Both reactions yielded the same product, 1,2,3,8-tetramethoxy-6-methylanthraquinone (**6**). Mp 160 °C; (Roberge and Brassard, 1981) mp 162–163 °C; the UV and ^{1}H NMR data were in good agreement with results published by Roberge and Brassard (1981).

3.6. Isoemericellin (**3**) and shamixanthone (**4**)

The petrol fraction of the medium extract was subjected to preparative HPLC using H_2O (A) and MeCN + 0.05% TFA (B) as eluents (linear gradient: 0-20 min 70–94% B, 20–60 min 94% B; flow rate 12 ml/min). Isoemericellin (**3**) was eluted from 34 to 36 min, further purification by column cromatography on silica gel with CH_2Cl_2 + 0.5% MeOH as the eluent and recrystallization from MeCN/CH_2Cl_2 yielded 8 mg of thin yellow needles. TLC: $R_f = 0.87$. Mp 112 °C. UV λ_{max}^{EtOH} nm: 384, 295, 270, 236, 203. IR ν_{max}^{KBr} cm^{-1}: 3503, 2972, 2916, 2851, 2360, 1606, 1462, 1438. EIMS *m*/*z* (rel. int.): 408 $[M]^+$ (3), 340 (48), 339 (41), 322 (29), 307 (100), 284 (30), 267 (45), 256 (54), 69 (53), 41 (37). HREIMS *m*/*z*: 408.1940 ($C_{25}H_{28}O_5$ requires 408.1937). NMR data (600 MHz, $CDCl_3$), see Table 2. Shamixanthone (**4**) (16.9 mg, yellow powder) was eluted from 30 to 32 min. TLC: $R_f = 0.91$. Mp 152 °C ; (Chexal et al., 1974) mp 154–156 °C. $[\alpha]_D^{20}$ +25.2° ($CHCl_3$; *c* 0.33); lit. +11.9° ($CHCl_3$; *c* 1.92) (Chexal et al., 1974). ^{1}H NMR,

^{13}C NMR, and EIMS were in good agreement with literature data (Chexal et al., 1974; Holker et al., 1974).

3.7. Stromemycin (5)

The MeOH fraction of the mycelium extract was subjected to MPLC using H_2O (A) and MeCN + 0.05% TFA (B) as eluents (step gradient: 0 min 70% B; 27 min 80% B; 40 min 90% B; 54 min 100% B; flow rate 5 ml/min). Compound **5** was eluted from 44 to 51 min and was further purified by column chromatography on silica gel eluting with CH_2Cl_2–MeOH–HCOOH (100:5:5). To remove the formic acid the fractions containing **5** were washed twice with water. After drying, 56.6 mg of an amorphous reddish powder were obtained. TLC: $R_f = 0.26$. Mp 139 °C. $[\alpha]_D^{20}$ +29° (EtOH; *c* 0.1); (Hopmann et al., 2001) mp and $[\alpha]_D^{20}$ not given. UV λ_{max}^{EtOH} nm: 229, 273, 307. CD: $\Delta\varepsilon_{228}$ −2.2, $\Delta\varepsilon_{271}$ +1.4, $\Delta\varepsilon_{300}$ +0.4, $\Delta\varepsilon_{314}$ +0.9 (EtOH; c 0.05). IR ν_{max}^{KBr} cm^{-1}: 3368 *br*, 3016, 2963, 2924, 1659, 1615, 1575, 1437, 1250, 1157. FABMS *m/z*: 697.2 $[M+H]^+$. NMR data (600 MHz, MeOH-d_4) identical to those previously published by Hopmann et al. (2001).

3.8. Methanolysis of stromemycin (5)

One milligram of **5** was dissolved in a 300 mM solution of NaOMe in MeOH. After 24 h the reaction mixture was acidified with HOAc and subjected to preparative HPLC using H_2O (A) and MeCN + 0.05% TFA (B) as eluents (linear gradient: 0–25 min 40–85% B; flow rate 12 ml/min). The products **8** and **9** were eluted after 8.5 and 13 min, respectively: due to the small quantities, they were characterized solely by ^1H NMR spectroscopy and ESIMS. **8**: ESIMS *m/z*: 277.4 $[M+H]^+$. ^1H NMR (600 MHz, MeOH-d_4): 0.9 (3H, *t*, H-16), 1.4 (2H, *sext*., H-15), 2.03 (2H, *q*, H-14), 2.3 (2H, *q*, H-9), 2.95 (2H, *t*, H-8), 5.8 (2H, *m*, H-10 and H-13), 5.99 (2H, *m*, H-11 and H-12), 6.15 (1H, *d*, H-4), 6.20 (1H, *d*, H-2). **9**: ESIMS *m/z*: 453.7 $[M+H]^+$. ^1H NMR (600 MHz, MeOH-d_4): 0.9 (3H, *t*, H-16), 1.4 (2H, sext., H-15), 2.05 (2H, *q*, H-14), 2.3 (2H, *q*, H-9), 2.86 (2H, *t*, H-8), 3.38 (1H, *m*, H-21), 3.45 (1H, *m*, H-19), 3.46 (1H, *m*, H-20, overlapping with H-19), 3.73 (1H, *dd*, H-22$_\alpha$), 3.84 (1H, *dd*, H-22$_\beta$), 3.91 (3H, *s*, COOMe), 4.02 (1H, *m*, H-18), 4.9 (1H, *d*, H-17), 5.57 (2H, *m*, H-10 and H-13), 6.0 (2H, *m*, H-11 and H-12), 6.3 (1H, *s*, H-4).

Table 2
NMR data of isoemericellin (**3**)

Position	δ_H (ppm)	δ_C (ppm)	HMBC
1	6.87 *d*	106.0	3, 5, 6, 7
2	7.50 *d*	137.3	4, 6, 21
3		123.3	
4		159.1	
4-OH	12.9 *s*		3, 4, 5
5		108.9	
6		154.2	
7		184.9	
8		118.2	
9		134.0	
10		152.7	
11		142.9	
12	7.3 *d*	119.8	7, 8, 9, 10, 15
13		154.3	
14	5.13 *s*	57.4	8, 9, 10
15	2.47 *d*	18.0	10, 11, 12
16	4.45 *d*	72.4	10, 17, 18
17	5.62 *m*	119.8	19, 20
18		139.3	
19	1.72 *d*	18.1	17, 18
20	1.83 *s*	26.1	17, 18
21	3.40 *d*	27.5	2, 4, 22, 23
22	5.35 *m*	122.0	24, 25, 21
23		133.6	
24	1.76 *s*	18.3	22, 23
25	1.78 *d*	26.0	22, 23

3.9. 2-Furanoic acid

The EtOAc extract of the acidified medium was evaporated to 200 ml and extracted twice with 100 ml of aq. NaOH (pH 13). After acidification to pH 1 with conc. HCl, the aqueous phase was re-extracted twice with 100 ml EtOAc. Column chromatography on silica gel with CH_2Cl_2–MeOH (95:5) and subsequent MPLC using H_2O–MeCN–TFA (85:15:0.1) yielded 14.4 mg of a colorless crystalline compound. TLC: $R_f = 0.67$. Mp 129 °C; (Hicks and Feather, 1977) mp 131 °C. It was identified as 2-furanoic acid by comparison with spectroscopical data (NMR, EIMS) of an authentic sample.

3.10. HPLC–NMR

For HPLC–NMR of the MeOH fraction of the medium extract, 10 mg of desalted extract were dissolved in 1 ml MeCN–H_2O (9:1). 75 µl of this solution were subjected to HPLC on an analytical column (Waters Symmetry C_{18}, 3.9×150 mm) with D_2O + 0.5% TFA (A) and MeCN (B) as eluents (linear gradient: 0–20 min 15–100% B; flow rate 1 ml/min). ^1H NMR spectra were acquired in the stop-flow mode with a 60 µl *z*-gradient flow probe (Bruker) in a 14.1 T magnet (600 MHz, Bruker) using the WET sequence for suppression of solvent signals. A MeOH impurity in the eluent served as the internal standard for calibration (δ 3.31 ppm).

3.11. Cytotoxicity assay

Cytotoxicity tests against five tumor cell lines, namely SKOV3 (ATCC HTB-77, human ovarian carcinoma), SF268 (NCI 503138, CNS cancer glioma), KB/HeLa (ATCC CCL17, human cervix carcinoma) NCI-H460 (NCI 503473, non-small cell lung cancer), and RKOp27

(human colon adenocarcinoma) were carried out at Zentaris AG (Frankfurt). After an incubation time of 45 h, cell viability was measured by a colorimetric XTT-assay (Scudiero et al., 1988).

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