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Cineromycins, γ-Butyrolactones and Ansamycins by Analysis of the Secondary Metabolite Pattern Created by a Single Strain of *Streptomyces*†

HANS-JÖRG SCHIEWE and AXEL ZEECK*

Institut für Organische Chemie, Universität Göttingen,
Tammannstraße 2, D-37077 Göttingen, Germany

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The antifungal and antibacterial properties of the crude extract from *Streptomyces* sp. (strain Gö 40/10) encouraged us to perform a detailed analysis of its secondary metabolite pattern by chemical screening, which revealed the presence of at least 30 different compounds. Ten of the isolated 18 metabolites proved to be new. Remarkable are hydrogenated (**3**, **4**) and glucosylated (**5**, **6**, **7**) 14-membered macrolides derived from cineromycin B, two γ-butyrolactones (**8**, **9**), the so far unknown naphthomycin K (**14**) and the collinolactones A and B. The constitution and relative stereochemistry of these metabolites were deduced from spectroscopic data. Finally the concept of our one strain/many compounds (OSMAC) method for exploring new microbial secondary metabolites is discussed.

In our chemical screening program[2] we discovered *Streptomyces* sp. Gö 40/10, which exhibited antifungal (*Mucor hiemalis*, *Candida albicans*) and antibacterial (*Bacillus subtilis*) activity together with an unusual extensive secondary metabolite pattern. By HPTLC analysis at least 30 compounds were detected utilizing their color, UV-absorbtion (254 and 366 nm) and/or colorization reactions. Applying the OSMAC (one strain/many compounds) method[3,4] to strain Gö 40/10 we isolated 18 metabolites in yields of 0.1 to 22.0 mg/liter. These metabolites belong to four different chemical classes: 14-membered macrolides, γ-butyrolactones, ansamycins and the collinolactones, new polyketides with a tricyclic cyclodecatriene core[1].

The present paper deals with the purification procedures leading to the various secondary metabolites, their physicochemical properties and structural elucidation studies. The collinolactones are part of a prior publication[1].

Fermentation and Isolation

Fermentation of *Streptomyces* sp. (strain Gö 40/10) was carried out in a soybean meal/mannitol medium at 28°C by using different culture vessels and aeration conditions, which all yields the same product pattern with little variation in the relative amounts of the individual secondary metabolites. On the basis of TLC analysis, time for harvesting was fixed at 72 hours of fermentation. The interesting metabolites were found mainly in the culture filtrate, only the antifungal ansatrienins were isolated from the mycelium extract (acetone). The culture filtrate was separated from the mycelium by filtration and worked up according to Scheme 1. The observed biological activity of the raw extracts could be assigned to the already known components of the mixture[5,11,14].

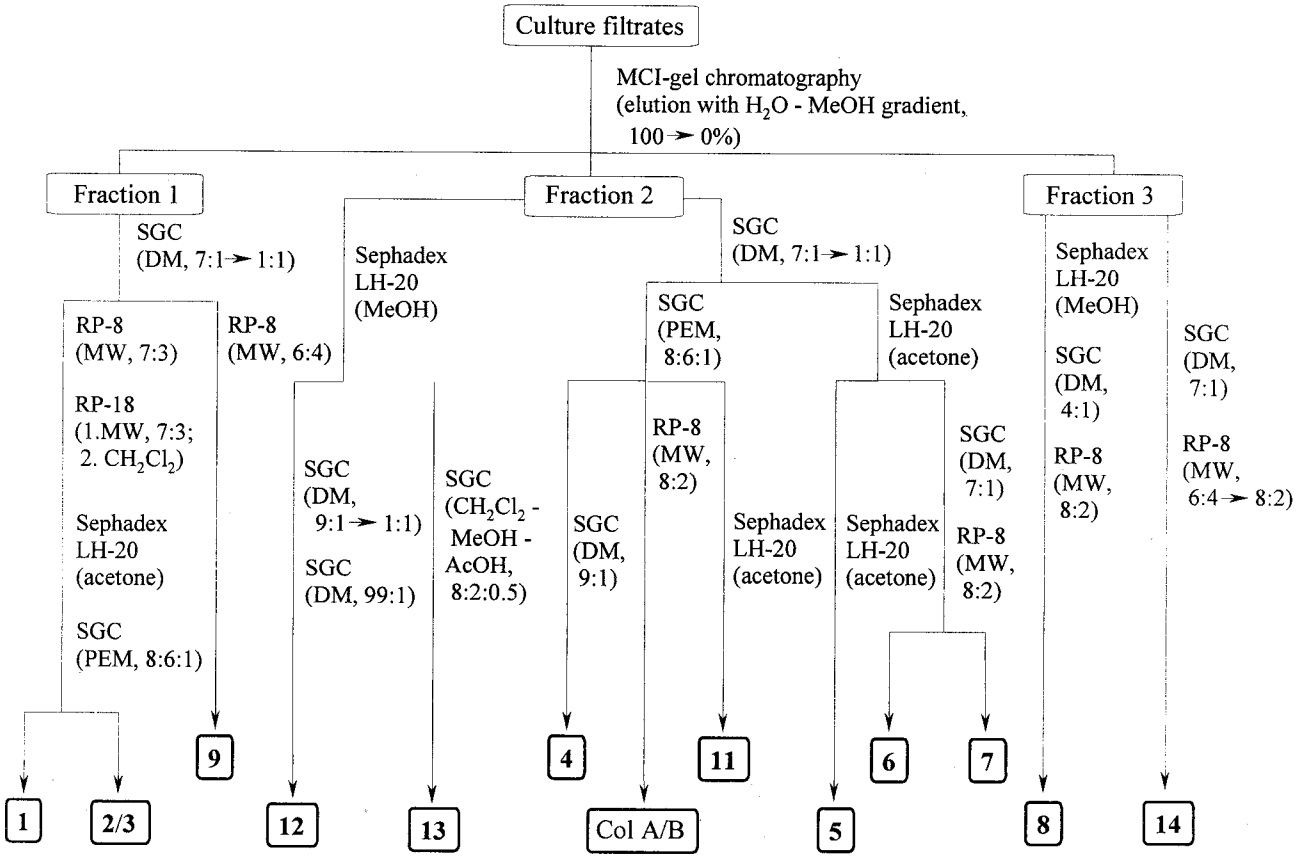
Cineromycins

The 14-membered macrolides were detected by HPTLC as two distinct groups showing similar Rf values and colorization reactions within each of them (Table 1). The more lipophilic metabolites (Rf 0.25~0.57, $CHCl_3$ - MeOH, 9 : 1, violet spots) were identified as cineromycin B (**1**)[5~7] and hydrogenated derivatives of it, the hydrophilic group (Rf 0.08~0.09, $CHCl_3$ - MeOH, 9 : 1, red spots) comprise three glucosylated cineromycins.

The derivatives of **1** with higher Rf values (Table 1) lead to a non-separable 1 : 1 mixture of the regioisomers 2,3-dihydrocineromycin B (**2**)[6,7] and the new 5,6-dihydro-

† Art. No. 38 on secondary metabolites by chemical screening. Art. No. 37: see ref. 1.

Scheme 1. Isolation and purification of the metabolites.



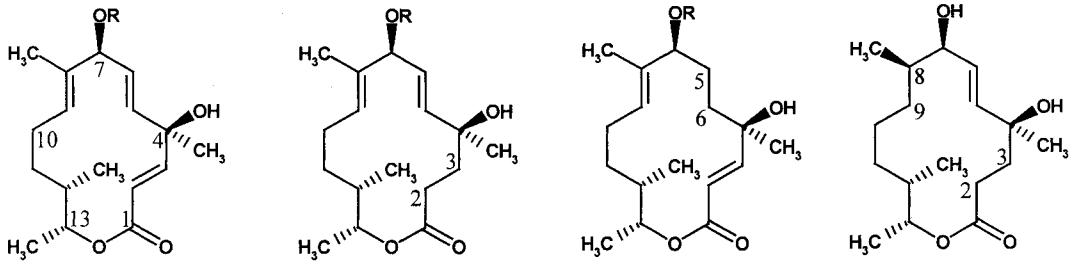
SCG: Silica gel chromatography; DM : CH_2Cl_2 - MeOH; CM : $CHCl_3$ - MeOH; MW : MeOH - H_2O; PEM: Petrolether - EtOAc - MeOH.

Table 1. Rf values and color reactions of the isolated compounds.

Compound	A	B	Anisaldehyde-H_2SO_4	Vanillin-H_2SO_4
1	0.25	0.84	Violet	Red Brown
2/3	0.42	0.76	Violet	Red Brown
4	0.57	0.76	Violet	Red Brown
5	0.08	0.42	Red	Brown
6	0.08	0.45	Red	Brown
7	0.09	0.50	Red	Brown
8	0.38	0.76	Blue Green	Brown
9	0.43	0.62	Light Red	Yellow Green
11	0.69	0.81	Blue Green[a]	
12	0.59	0.86	Orange[a]	
13	0.00	0.66	Dark Green[a]	
14	0.29	0.69	Red[a]	
Col A/B[b)]	0.62	0.77	Petrol	Green

A: $CHCl_3$ - MeOH (9 : 1), B: BuOH - AcOH - H_2O (4 : 1 : 5, upper phase).
[a] Color without staining reagent; [b] collinolactone A/B[1)].

Fig. 1. Structural formulae of cineromycin B derivatives.



1: R = H
5: R = α-glucosyl

2: R = H
6: R = α-glucosyl

3: R = H
7: R = α-glucosyl

4

cineromycin B (**3**). The structure of **3** was verified by both, comparison of its data with those of a semisynthetical derived compound[8] and an independent spectroscopic analysis.

Additionally unknown 2,3,8,9-tetrahydrocineromycin B (**4**) was identified. The HREI-MS (298.2144, $[M^+]$, $C_{17}H_{30}O_4$) indicated four additional hydrogen atoms compared to **1**. The ^1H NMR spectrum is characterized by the loss of three olefinic protons, differences in the multiplicities and chemical shifts of 7-H (**4**: $\delta_{7\text{-H}}=4.09$, dd, $J=2.5$, 9.0 Hz; **1**: $\delta_{7\text{-H}}=4.55$, d, $J=5.0$ Hz) and three additional methylene and one methine group. The position of the C-5/C-6 double bond could be assigned by 2D NMR experiments. Due to the small coupling constant of 2.5 Hz between 7-H and 8-H we assume a *cis* conformation of the two protons, this results in the shown *R* configuration of C-8. Furthermore the known 7-dehydrocineromycin B[6,7] could be isolated in small amounts.

The three members of the more hydrophilic cineromycin B derivatives showed a very similar chromatographic behavior. The application of strong separating steps was limited, because of the instability of the compounds. The structure elucidation of the most hydrophilic compound, 7-*O*-(α-glucosyl)-cineromycin B (**5**) was done with a sample of 80% purity. The FAB-MS (474, $[M+NH_3+H^+]$) suggested a molecular weight of 456 in agreement with an addition of a hexose moiety to **1**. This was supported by the ^1H NMR spectrum, which in comparison to **1** showed additional signals between $\delta=3\sim5$ belonging to an α-glucosyl spin system as was revealed by the $^3J_{1'\text{-H}-2'\text{-H}}$ coupling constant of 4.0 Hz and a COSY experiment. The connection between the glucosyl residue and the aglycon was established by the downfield shift of C-7[9] in comparison to **1** (**5**: $\delta_{C\text{-}7}=78.7$; **1**: $\delta_{C\text{-}7}=75.5$) and the chemical shift of the anomeric carbon atom ($\delta_C=96.6$), leading to the structure depicted for **5**.

The second compound was identified as the new 7-*O*-(α-glucosyl)-2,3-dihydrocineromycin B (**6**). The structure was verified by DCI-MS (481, $[M+Na^+]$) and NMR. The only difference between **5** and **6** was the lack of signals of the C-2/C-3 double bond in the NMR spectra and the presence of two additional methylene groups. The point of attachment of the sugar moiety could be unambiguously determined by the down field shift of C-7 ($\Delta\delta=+6.2$ ppm) and HMBC correlations between both, C-7/1′-H and C-1′/7-H.

The third member of this group, 7-*O*-(α-glucosyl)-5,6-dihydrocineromycin B (**7**), was the most unstable one and already disintegrated after the ^1H NMR experiment. Comparison of this spectrum with that of **5** revealed the absence of the C-5/C-6 double bond and the presence of two additional methylene groups. This result in combination with two fully elucidated glucosylated cineromycin B derivatives leads to the proposal of structure **7** despite the incomplete data.

γ-Butyrolactones

The HREI-MS of lactone I (**8**) ($m/z=228$) indicates the empirical formula $C_{11}H_{16}O_5$. The ^{13}C NMR spectrum includes signals of eleven C-atoms: one methyl and two methylene groups, two aliphatic and four olefinic methine carbon atoms as well as one carbonyl group and a quarternary carbon atom ($\delta=79.1$), which is attached to an oxygen as it is the case for the methylene and one methine group. In the ^1H NMR spectrum in DMSO-d_6 all of the 16 predicted hydrogen atoms are visible. An analysis

Fig. 2. Structural formulae of lactone I (**8**), lactone II (**9**, relative stereochemistry) and butalactin (**10**, relative stereochemistry).

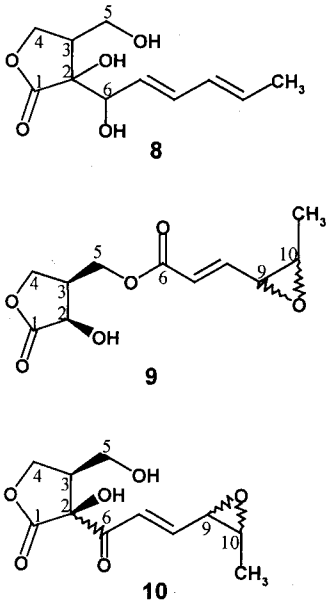
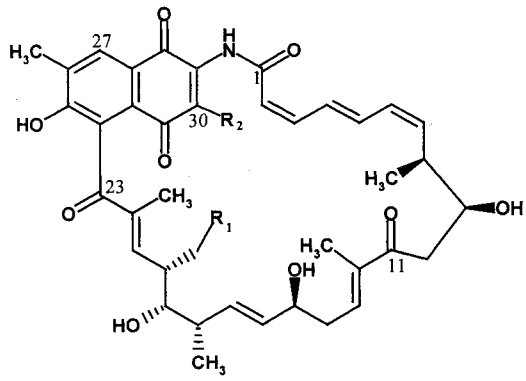


Fig. 3. Structural formulae of the naphthalene ansamycins.



		R_1	R_2
Naphthomycin B	(**11**)	H	Cl
Diastovaricin I	(**12**)	H	OH
Diastovaricin II	(**13**)	H	CysNAc
Naphthomycin K	(**14**)	OH	OH

of the observable $^3J_{H-H}$ coupling constants and a COSY experiment yield two fragments, which could be connected *via* HMBC correlations. Additional 2D NMR correlation spectra lead to the constitution of lactone I as dihydro-3-hydroxy-4-(hydroxymethyl)-3-(1-hydroxy-hexa-2,4-dien-1-yl)-2(3*H*)-furanone (**8**), which fits the characteristic IR absorption at $\tilde{\nu}=1763\,cm^{-1}$. From the $^3J_{H-H}$ coupling constants ($^3J_{7,8}$ and $^3J_{9,10}=15.0$ Hz) a *trans* configuration of the double bonds was deduced.

Lactone II (**9**) shows a molecular ion at $m/z=242$, which led to the molecular formula $C_{11}H_{14}O_6$ (HREI-MS). The ^{13}C NMR spectrum reflects the high degree of oxygenation due to the presence of five signals between $\delta_C=80$ and 55, two of them at $\delta_C=55.5$ and 55.2 could be assigned to an epoxide. The chemical shifts of two olefinic protons at $\delta_C=$ 124.2 (C-7) and 143.4 (C-8) together with the signal at $\delta_C=$ 165.1 (C-6) indicated the presence of an α,β-unsaturated ester. Further ^1H-^1H- and ^1H-^{13}C-shift correlation NMR spectra allows us to establish the constitution of a dihydro-4-[4,5-epoxy-1-oxohex-2-enyloxy-methyl]-3-hydroxy-2(3*H*)-furanone (**9**). Due to the large $^3J_{H-H}$ coupling constant ($^3J_{7,8}=15.5$ Hz) a *trans* configuration was assigned to the double bond, whereas 2-H and 3-H ($^3J_{2,3}=8.0$ Hz) assume a *cis* configuration[10]. To establish the arrangement of 9-H and 10-H a NOESY experiment was conducted, in which correlations between these two protons as well as between the methyl group ($\delta_H=1.31$) and 8-H ($\delta_H=$ 6.80) were observable indicating a *cis* epoxide.

Ansamycins

The most striking feature of *Streptomyces* sp. Gö 40/14 was the production of various colored metabolites with a broad range of polarity. The major constituents of these group could be readily identified by comparison with reference samples from prior work and literature data as ansatrienin A and B[11] (mycotrienine I and II[12]), naphthomycin B (**11**)[13] and diastovaricin I (**12**) and II (**13**)[14]. Additionally naphthomycin K (**14**) was visible as a dark red spot on the unstained TLC. Inspection of the spectroscopic data showed that **14** is closely related to **12**. The molecular mass of **14** is 16 units higher (FAB-MS $m/z=703$ $[M+H]^+$), while comparison of the ^1H NMR data presented the lack of a methyl group as well as additional signals of a hydroxymethylene group ($\delta_H=3.68/3.63$), whose ^{13}C NMR ($\delta_C=64$) fits well. Thus the structure is as depicted for **14** (Fig. 3).

Discussion

Detailed investigation of the secondary metabolite pattern of strain *Streptomyces* sp. Gö 40/10 leads to the isolation and structure elucidation of 18 compounds, ten of them not described in the literature before.

The compounds **5**, **6** and **7** represents the first glycosylated macrolides of the cineromycin B-type. Unfortunately, unlike the structurally related erythromycin A or oleandomycin[15] these metabolites exhibit no antibacterial activity. Nevertheless, together with the new hydrogenated derivatives **3** and **4** they allow us to complete the picture of structural variability of the cineromycin B class: Oxidation/methylation/glucosylation at C-7, oxygenation of 8-Me and saturation of all three double bonds are possible[6,7].

The new γ-butyrolactone **8** is structurally related to A-factor[16], the virginiae butanolides[17] and especially butalactin (**10**)[18], which also carries an hydroxyl group at C-2. Another mutuality of **8**, **9** and **10** is their inability to stimulate aerial mycelium formation of Streptomycetes. It is reasonable to assume a virginiae butanolide-like biosynthesis for **8**, which utilizes a tetraketide moiety[19]. In contrast to **8** the structure of lactone II (**9**) shows similarities to the musacins B_1/B_2[7], the lactonization of which follows a different mode than described for the A-factor analogues.

Naphthomycin K (**14**) is the first representative of the naphthomycin B series (characterized by the *Z/E/Z* configuration of the triene moiety), which is modified in the ansa chain. So far this biosynthetic feature was observed in the rifamycin class only, where the corresponding methyl group (C-28 Me) in proansamycin B-M is eliminated by successively oxidation *via* the alcohol (rifamycin W), the aldehyde and the carboxylic acid, followed by decarboxylation (rifamycin S)[20]. Since chloroansamycins like **11** can be converted to thioansamycins like **13** with thiols *in vitro*[21], it is reasonable to assume that this conversion is non-enzymatic.

An analysis of the polyketide chains of the isolated metabolites revealed the fact, that strain Gö 40/10 must harbour at least six different polyketide synthases of type I, which are used in parallel and lead to six different core structures: Ansatrienins, naphthalene ansamycins, cineromycins, two types of γ-butytolactones and collinolactones. It seems to be a general characteristic of Streptomycetes to contain a complete gene cluster for each different carbon skeleton of the secondary metabolites they synthesize. The combination of cineromycins and musacin-like compounds is reminiscent of *Streptomyces griseoviridis* (strain FH-S 1832)[7], whereas the common occurrence of ansamycin antibiotics and collinolactones is the same as in *Streptomyces collinus* (strain Tü 1892)[22]. A comparative TLC analysis of extracts of the latter and of Gö 40/10 revealed the more lipophilic cineromycins and the γ-butyrolactones as congeners as well, but in very small amounts only. We assume that in some cases gene clusters can occur concurrently in the genome independent of other features of a given strain and that the overall number of such secondary metabolism gene clusters in a given genome is much higher than estimated up to now.

Analyzing the secondary metabolite pattern of Gö 40/10, we illustrated that the application of the OSMAC-method[3,4] is valuable for exploring the potential of different gene clusters and attaining both, new derivatives of known classes of compounds as well as novel structures. This demonstrates that it is worthwhile to investigate carefully selected strains of a family of established producers of secondary metabolites. Based on the finding that more than 50% of the isolated compounds from Gö 40/10 proved to be new, the combination of chemical screening for selecting suitable strains and the OSMAC-method for exploring their biosynthetic potential to the fullest, is a very successful strategy for finding novel secondary metabolites and for getting more information about the occurrence and the regulation of secondary metabolites, biosynthesis genes and enzymes.

Experimental

General

See ref. 1. The ^1H and ^{13}C NMR spectra were measured with Bruker AMX 300 (300 MHz), Varian Unity 300 (300 MHz) and Varian Inova 500 (500 MHz) NMR spectrometer. The FAB-mass spectra were taken by Finnigan MAT 95 A (200 eV, α-nitrobenzylalcohol, glycerol as matrix) mass spectrometer. Staining reagents are described in ref. 3.

5,6-Dihydrocineromycin B (3)

Yield 2.1 mg/liter; Rf (see Table 1); $[\alpha]_D^{20}$ +10 (*c* 0.1, MeOH)†; UV λ_{max}^{MeOH} nm (ε) 204 (7125), 222 (5315), 237 (5967)†; MP 145~155°C†; IR (KBr) cm^{-1} 3426 (br), 2969, 2929, 1709, 1637 (sh), 1458, 1370†; ^1H NMR (500 MHz, $CDCl_3$) δ 0.92 (d, J=7.0 Hz, 3H, 12-CH_3), 1.30 (m, 3H, 13-

† Taken from the 1 : 1 mixture of **2/3**.

CH_3), 1.25~1.30 (m, 2H, 11-CH_2), 1.34 (s, 3H, 4-CH_3), 1.39 (m, 1H, 12-H), 1.51 (d, J=0.5 Hz, 8-CH_3), 1.57 (m, 1H, 6-H_a), 1.80 (m, 1H, 10-H_a), 1.81 (m, 1H, 6-H_b), 1.95 (m, 2H, 5-H_2), 2.09 (m, 1H, 10-H_b), 3.97 (dd, J=15.5, 1.5 Hz, 1H, 7-H), 4.55 (m, 1H, 13-H), 5.49 (m, 1H, 9-H), 5.98 (d, J=15.5 Hz, 1H, 2-H), 6.82 (d, J=15.5 Hz, 1H, 3-H); ^{13}C NMR (see Table 2); DCI-MS m/z 314 (100, [M+NH_3+H^+])$^+$.

2,3,8,9-Tetrahydrocineromycin B (**4**)

Yield 0.1 mg/liter; Rf (see Table 1); $[\alpha]_D^{20}$ −60 (c 0.005, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 201 (1794), 226 (567); MP 88~90°C; IR (KBr) cm^{-1} 3410 (br), 2931, 2874, 1701, 1458, 1211, 1108, 1072, 988; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.87 (d, J=7.0 Hz, 3H, 12-CH_3), 1.01 (m, 1H, 9-H_a), 1.02 (d, J=7.0 Hz, 3H, 8-CH_3), 1.03 (m, 1H, 10-H_a), 1.17 (d, J=6.5 Hz, 3H, 13-CH_3), 1.23 (m, 2H, 11-H_2), 1.27 (s, 3H, 4-CH_3), 1.36 (m, 1H, 10-H_b), 1.41 (m, 1H, 9-H_b), 1.54 (m, 2H, 8-H, 12-H), 1.67 (ddd, J=15.5, 5.5, 4.0 Hz, 1H, 3-H_a), 2.12 (ddd, J=15.5, 8.5, 3.5 Hz, 1H, 3-H_b), 2.38 (ddd, J=18.5, 5.5, 4.0 Hz, 1H, 2-H_a), 2.47 (ddd, J=18.5, 5.0, 3.5 Hz, 1H, 2-H_b), 4.09 (dd, J=9.0, 2.5 Hz, 1H, 7-H), 4.44 (s (br), OH, 1H), 4.64 (dd, J=10.5, 6.0 Hz, 1H, 13-H), 5.56 (d, J=15.0 Hz, 1H, 5-H), 5.92 (dd, J=15.0, 8.5 Hz, 1H, 6-H); ^{13}C NMR (see Table 2); EI-MS m/z (abundance, %) 298 (0.1, [M^+], high resolution found as calcd. for $C_{17}H_{30}O_4$: 298.2144), 280 (20, [M^+-H_2O], high resolution found as calcd. for $C_{17}H_{28}O_3$: 280.2038), 155 (100); DCI-MS m/z 316 (100, [M+NH_3+H^+]).

7-O-(α-Glucosyl)-cineromycin B (**5**)

Yield 0.1 mg/liter; Rf (see Table 1); $[\alpha]_D^{20}$ +38 (c 0.05, MeOH); UV λ_{max}^{MeOH} nm (ε) end adsorption; MP dec; IR (KBr) cm^{-1} 3432, 1634; ^{1}H NMR (500 MHz, acetone-d_6-D_2O) δ 0.88 (d, J=7.0 Hz, 3H, 12-CH_3), 1.15 (m, 1H, 11-H_a), 1.18 (d, J=6.0 Hz, 3H, 13-CH_3), 1.25 (m, 1H, 11-H_b), 1.41 (m, 1H, 12-H), 1.52 (s, 3H, 4-CH_3), 1.70 (s, 3H, 8-CH_3), 1.86 (m, 1H, 10-H_a), 2.10 (m, 1H, 10-H_b), 3.33 (dd, J=9.0, 9.0 Hz, 1H, 4′-H), 3.39 (dd, J=9.5, 4.0 Hz, 1H, 2′-H), 3.48 (m, 1H, 5′-H), 3.66 (m, 2H, 3′-H, 6′-H_a), 3.77 (dd, J=11.0, 2.5 Hz, 6′-H_b), 4.51 (m, 1H, 13-H), 4.60 (d, J=6.0 Hz, 1H, 7-H), 4.87 (d, J=4.0 Hz, 1H, 1′-H), 5.27 (m, 1H, 9-H), 5.66 (dd, J=16.5, 6.0 Hz, 1H, 6-H), 5.83 (d, J=15.5 Hz, 1H, 2-H), 5.98 (dd, J=16.5, 1.0 Hz, 1H, 5-H), 6.95 (d, J=15.5 Hz, 1H, 3-H); ^{13}C NMR (see Table 2); DCI-MS m/z 474 (100, [M+NH_3+H^+]).

7-O-(α-Glucosyl)-2,3-dihydrocineromycin B (**6**)

Yield 0.1 mg/liter; Rf (see Table 1); $[\alpha]_D^{20}$ +81 (c 0.1, MeOH); UV λ_{max}^{MeOH} nm (ε) 204 (11022), 222 (8223), 237 (9231); MP 90°C; IR (KBr) cm^{-1} 3417, 2924, 1730, 1030; ^{1}H NMR (500 MHz, CD_3OD) δ 0.90 (d, J=7.0 Hz, 3H, 12-CH_3), 1.10 (d, J=6.5 Hz, 3H, 13-CH_3), 1.26 (s, 3H, 4-CH_3), 1.39 (m, 2H, 11-CH_2), 1.69 (s, 3H, 8-CH_3), 1.73 (dq, J=7.0, 7.0 Hz, 1H, 12-H), 1.83 (ddd, J=14.0, 8.0, 4.5 Hz, 1H, 3-H_a), 1.90 (ddd, J=14.0, 9.5, 4.0 Hz, 1H, 3-H_b), 1.99 (m, 1H, 10-H_a), 2.21 (m, 2H, 10-H_b, 2-H_a), 2.41 (ddd, J=16.0, 9.5, 4.0 Hz, 1H, 2-H_b), 3.29 (dd, J=9.0, 9.0 Hz, 1H, 4′-H), 3.38 (dd, J=9.5, 4.0 Hz, 1H, 2′-H), 3.58 (ddd, J=9.0, 5.0, 2.5 Hz, 1H, 5′-H), 3.65 (dd, J=9.5, 9.0 Hz, 1H, 3′-H), 3.66

Table 2. ^{13}C NMR data of the new cineromycin B derivatives **3** to **6**.

Compound	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9
3[a]	166.1	118.9	153.8	73.1	39.0	29.7	79.6	135.2	128.8
4[b]	175.5	31.1	35.7	72.2	137.4	130.2	77.5	39.1	30.9
5[c]	166.4	115.3	156.7	73.6	139.7	130.3	78.7	137.4	130.5
6[d]	175.3	30.9	37.9	73.2	139.2	129.3	84.0	137.9	128.1

Compound	C-10	C-11	C-12	C-13	4-CH_3	8-CH_3	12-CH_3	13-CH_3
3	23.8	33.2	38.1	75.5	28.2	11.1	17.2	19.0
4	23.3	33.0	39.3	75.7	30.3	17.5	16.3	18.9
5	25.5	35.1	40.4	75.4	27.2	15.2	16.1	18.4
6	24.1	33.3	36.6	74.5	30.1	12.5	15.6	15.4

Compound	C-1'	C-2'	C-3'	C-4'	C-5'	C-6'
5	96.6	73.4	75.1	72.0	73.4	62.9
6	97.5	73.6	75.1	71.8	73.8	62.6

δ values in ppm, solvent as internal standard.
[a] $CDCl_3$ (125.7 MHz); [b] $CDCl_3$ (75.5 MHz); [c] acetone-d_6 (75.5 MHz); [d] CD_3OD (75.5 MHz).

(dd, J=12.0, 5.0 Hz, 1H, 6′-H_a), 3.74 (dd, J=12.0, 2.5 Hz, 1H, 6′-H_b), 4.52 (m, 1H, 13-H), 4.58 (d, J=6.5 Hz, 1H, 7-H), 4.88 (d, J=4.0 Hz, 1H, 1′-H), 5.38 (m, 1H, 9-H), 5.57 (dd, J=16.0, 1.0 Hz, 1H, 5-H), 5.80 (dd, J=16.0, 6.5 Hz, 1H, 6-H); ^{13}C NMR (see Table 2); DCI-MS (abundance, %) m/z 476 (100, [M+NH_3+H^+]); FAB-MS m/z 457 (100, [M^+−H], neg. mode), 481 (100, [M^++Na], pos. mode).

7-*O*-(α-Glucosyl)-5,6-dihydrocineromycin B (**7**)

Yield 0.1 mg/liter; Rf (see Table 1); $[\alpha]_D^{20}$ +30 (c 0.05, MeOH); UV λ_{max}^{MeOH} nm (ε) end adsorption; MP 78°C; IR (KBr) cm^{-1} 3426, 2924, 2855, 1634, 1053.

Lactone I (Dihydro-3-hydroxy-4-(hydroxymethyl)-3-(1-hydroxy-hexa-2,4-dien-1-yl)-2(3*H*)-furanone, **8**)

Yield 8.1 mg/liter; Rf (see Table 1); $[\alpha]_D^{20}$ +50 (c 0.1, MeOH); UV λ_{max}^{MeOH} nm (ε) 231 (18248); MP >220°C; IR (KBr) cm^{-1} 3411 (br), 2922, 1763, 1383, 1216, 997, 560; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.72 (ddd, J=7.0, 1.5, 0.5 Hz, 3H, 11-H_3), 2.56 (m, 1H, 3-H), 3.37 (dd, J=11.0, 8.0 Hz, 1H, 5-H_a), 3.50 (dd, J=11.0, 5.0 Hz, 1H, 5-H_b), 4.08 (dd, J=9.0, 5.0 Hz, 1H, 4-H_a), 4.24 (dd, J=9.0, 8,0 Hz, 1H, 4-H_b), 4.25 (dd, J=5.5, 0.5 Hz, 1H, 6-H), 4.50 (s (br), 1H, 5-OH), 5.48 (d, J=5.0 Hz, 1H, 6-OH), 5.67 (m, 1H, 7-H), 5.71 (m, 1H, 10-H), 5.80 (s (br), 1H, 2-OH), 6.09 (ddd, J=15.0, 10.0, 1.5 Hz, 1H, 8-H), 6.27 (dd, J=15.0, 10.0 Hz, 1H, 9-H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 18.2 (q, C-11); 41.6 (d, C-3); 61.5 (t, C-5); 70.8 (t, C-4); 74.8 (d, C-6); 79.1 (s, C-2); 128.9 (d, C-7); 130.8 (d, C-10); 132.3 (d, C-9); 134.1 (d, C-8); 180.0 (s, C-1); EI-MS m/z (abundance, %) 228 (8, [M^+] high resolution found as calcd. for $C_{11}H_{16}O_5$: 228.0997), 132 (60), 101 (40), 97 (100); DCI-MS m/z 246 (100, [M+NH_3+H^+]).

Lactone II (Dihydro-4-[4,5-epoxy-1-oxohex-2-enyloxy-methyl]-3-hydroxy-2(3*H*)-furan-one, **9**)

Yield 1.9 mg/liter; Rf (see Table 1); $[\alpha]_D^{20}$ +32 (c 0.1, MeOH); UV λ_{max}^{MeOH} nm (ε) 218 (10799); MP >180°C; IR (KBr) cm^{-1} 3456 (br), 2924, 1779, 1719, 1271, 1186, 984, 818; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.22 (d, J=5.5 Hz, 3H, 11-H_3), 2.86 (m, 1H, 3-H), 3.30 (dq, J=5.5, 4.5 Hz, 1H, 10-H), 3.61 (dd, J=6.5, 4.5 Hz, 1H, 9-H), 4.10~4.36 (m, 4H, 4-H_2, 5-H_2), 4.56 (dd, J=8.0, 6.0 Hz, 1H, 2-H), 6.12 (d, J=15.5 Hz, 1H, 7-H), 6.14 (d, J=6.0 Hz, 2-OH), 6.66 (dd, J=15.5, 6.5 Hz, 1H, 8-H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 13.1 (q, C-11); 38.9 (d, C-3); 55.2 (d, C-9); 55.5 (d, C-10); 61.1 (t, C-4); 67.5 (d, C-2), 67.8 (t, C-5); 124.2 (d, C-7); 143.4 (d, C-8); 165.1 (s, C-6); 176.7 (s, C-1); EI-MS m/z (abundance, %) 242 (8, [M^+] high resolution found as calcd. for $C_{11}H_{14}O_6$: 242.0790), 198 (23), 115 (83); 84 (95); 83 (100); 71 (9); DCI-MS m/z 260 (100, [M+NH_3+H^+]).

Naphthomycin K (**14**)

Yield 0.1 mg/liter; Rf (see Table 1); $[\alpha]_D^{20}$ +88 (c 0.025, MeOH); UV λ_{max}^{MeOH} (ε) 201 (12481), 217 (6709), 233 (7460), 264 (4551), 294 (5958), $\lambda_{max}^{MeOH+HCl}$ 201 (12574), 217 (6654), 233 (7380), 270 (3425), 305 (4692), $\lambda_{max}^{MeOH+NaOH}$ 232 (7226), 264 (3659), 295 (7038), 396 (516); MP >250°C; IR (KBr) cm^{-1} 3429, 2925, 1635, 1030; CD λ_{max}^{MeOH} nm ($[\theta]^{20}$) 252 (−19007), 265 (−24688), 304 (+12894); ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.82 (d, J=7.0 Hz, 3H, 18-CH_3), 0.98 (d, J=6.5 Hz, 3H, 8-CH_3), 1.64 (s, 3H, 12-CH_3), 1.92 (d, J=1.0 Hz, 3H, 22-CH_3), 2.28 (d, J=1.0 Hz, 3H, 14-H_a), 2.40 (m, 1H, 14-H_b), 2.54 (d, J=0.5 Hz, 3H, 8-H), 3.15 (m, 1H, 19-H), 3.63 (m, 1H, 20a-H_a), 3.68 (s (br), 1H, 20b-H_b), 3.82 (m, 1H, 15-H), 4.52 (m, 1H, 19-OH), 4.65 (d, J=6.0 Hz, 1H, 9-OH), 5.18 (dd, J=11.0, 11.0 Hz, 1H, 7-H), 5.22 (dd, J=15.0, 9.0 Hz, 1H, 16-H), 5.38 (dd, J=15.0, 7.0 Hz, 1H, 17-H), 5.58~5.67 (m, 2H, 2-H, 21-H), 5.98 (dd, J=10.5, 10.5 Hz, 1H, 6-H), 6.08 (dd (br), J=11.0, 11.0 Hz, 1H, 3-H), 6.34 (m, 1H, 13-H), 6.45 (ddd, J=15.0, 11.5, 0.5 Hz, 1H, 5-H), 7.12 (t (br), 1H, 4-H), 7.32 (m, 1H, NH), 7.71 (s, 1H, 27-H); ^{13}C NMR (125.7 MHz, DMSO-d_6 - acetone-d_6, 3 : 1 or CD_3OD)† 11 (q, 12-CH_3), 12 (q, 22-CH_3), 18 (q, 18-CH_3), 17 (2×q, 26-CH_3, 8-CH_3), 37 (t, C-14), 39 (d, C-8), 41 (d, C-18), 43 (t, C-10), 46 (d, C-20), 64 (t, 20-CH_2OH), 71 (d, C-9), 72 (d, C-15), 75 (d, C-19), 123 (d, C-2), 129 (d, C-6, C-27), 131†† (s, C-26), 131 (2×d, C-4, C-17), 133 (d, C-5), 134 (d, C-16), 137†† (s, C-12), 138 (2×d, C-3, C-7), 139 (d, C-21), 140 (d, C-13), 153†† (s, C-25), 175†† (2×s, C-28, C-31), 195†† (s, C-23), 199†† (s, C-11); FAB-MS m/z (abundance, %) 703 (100, [M^+], neg. mode).

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† Chemical shifts taken from the HMBC spectra.
†† Detectable only in the HMBC-spectrum in CD_3OD.

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