New Cineromycins and Musacins Obtained by Metabolite Pattern Analysis of *Streptomyces griseoviridis* (FH-S 1832)

II. Structure Elucidation†

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A detailed analysis of the secondary metabolite pattern produced by *Streptomyces griseoviridis* (strain FH-S 1832) using a chemical screening method resulted in the detection, isolation and structure elucidation of new 14-membered lactones of the cineromycin B-type [dehydrocineromycin B (**5**), oxycineromycin B (**7**), and 2,3-dihydrocineromycin B (**8**)], as well as new γ-lactones related to nigrosporalactone and 4,5-dihydroxy-octa-2,6-dienoic acid esters named musacins A to F (**10**, **13**~**15**, **17**, **18** and **21**). The constitution of these metabolites were deduced from spectroscopic data as well as chemical transformations. The configuration of musacin D (**10**) was determined by derivatization with chiral acids (Helmchen's method).

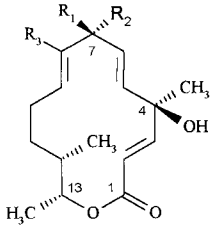
Based on a detailed analysis of the metabolic fingerprint of *Streptomyces griseoviridis* (FH-S 1832) *via* chemical screening[2~4], new secondary metabolites of the cineromycin B-type as well as new γ-lactones and their open ring derivatives called musacins A to F were detected. Investigations in a taxonomical characterization of the producing organism, isolation procedures and studies towards the biological activities of the produced secondary metabolites have been already described in the preceeding paper[1]. In this study we present the structural elucidation of the new metabolites obtained.

Cineromycins

The pure cineromycins were characterized spectroscopically, their molecular formulae were determined by EI- or DCI-mass spectra and their structures were elucidated by detailed analysis of the ^{1}H-, ^{13}C-, ^{1}H-^{1}H and ^{1}H-^{13}C-shift correlation NMR-spectra in comparison to literature data. In agreement to the similarities of the isolated compounds obtained *via* the colorization reactions by staining with different reagents (scheme 1 in ref. 1), the spectroscopical investigations revealed close structural relationships.

With the information obtained from the spectroscopic data (*e.g.* MS: $m/z=312$ $[M+NH_3+H^+]$, 294 $[M^+]$; IR: $\nu=1710\,cm^{-1}$; ^{1}H NMR: four methyl groups, two methylene groups, three aliphatic and five olefinic protons as well as two OH-groups) the compound with Rf 0.59 (ethyl acetate-*n*-hexane, 3:1)[1] was identified as the literature known 14-membered macrolactone cineromycin B (**1**)[5]. In analogy to the closely related albocycline (**2**) oxygenation reaction[6,7] of **1** with *m*-chloroperbenzoic acid (CH_2Cl_2, 0°C) yielded a mixture of the epoxide diastereomers **3** and **4**, whose spectroscopic analysis unambiguously proved the structure of **1**.

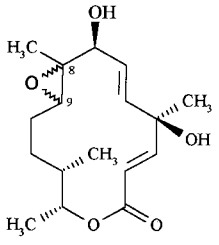
Fig. 1. Structural formulae of cineromycin-type compounds.



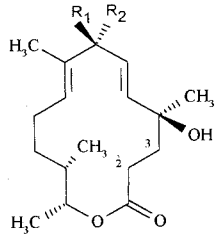
		R_1	R_2	R_3
Cineromycin B	(**1**)	OH	H	CH_3
Albocycline	(**2**)	OCH_3	H	CH_3
Dehydrocineromycin B	(**5**)	$R_1=R_2=O$		CH_3
7-*epi*-cineromycin B	(**6**)	H	OH	CH_3
Oxycineromycin B	(**7**)	OH	H	CH_2OH

† Art. No. 32 on secondary metabolites by chemical screening. Art. No. 31: see ref. 1.

Fig. 2. Structural formulae of cineromycin B derivatives.



8,9-Epoxycineromycin B (**3**, **4**)



		R_1	R_2
2,3-Dihydrocineromycin B	(**8**)	OH	H
2,3-Dihydro-dehydrocineromycin B	(**9**)	$R_1=R_2=O$	

Table 1. ^{13}C NMR data of 8,9-epoxycineromycins B (**3**, **4**; diastereomers), dehydrocineromycin B (**5**), 7-*epi*-cineromycin B (**6**), oxycineromycin B (**7**), 2,3-dihydrocineromycin B (**8**), chemical shifts in $CDCl_3$ (50.3 MHz, δ values in ppm, TMS as internal standard).

Compound	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9
3	166.3	117.2	154.3	72.6	137.6	133.4	71.1	62.3	57.8
4	166.4	116.3	153.6	75.7	137.4	130.0	72.7	63.6	60.4
5	165.7	117.6	152.9	73.9	149.5	128.1	196.8	138.5	145.8
6	166.0	115.0	154.8	73.3	135.4	134.4	76.0	137.3	128.6
7	166.5	114.6	155.6	73.0	136.0	132.9	72.4	139.7	132.5
8[a]	173.9	30.5	36.9	72.3	136.4	129.4	77.9	137.6	126.4

Compound	C-10	C-11	C-12	C-13	4-CH_3	8-CH_3	12-CH_3	13-CH_3
3	24.5	28.6	41.2	76.2	26.3	15.0	15.4	18.9
4	20.4	29.1	39.5	77.8	26.5	10.9	14.9	18.8
5	26.5	32.9	39.9	75.7	24.4	11.4	16.2	18.3
6	25.0	34.0	39.8	75.4	26.8	14.8	16.1	18.4
7	24.4	34.2	39.5	75.5	26.6	60.0[b]	16.0	18.1
8[a]	22.5	31.6	35.9	73.2	29.5	12.5	15.3	15.7

[a] $CDCl_3$, 125.7 MHz; [b] 8-CH_2OH.

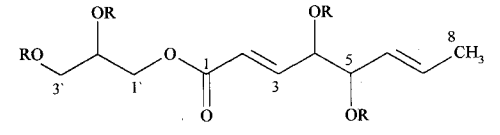
The second compound (Rf 0.95, ethyl acetate-*n*-hexane, 3:1[1]) isolated was found to be the new dehydrocineromycin B (**5**). The structure was verified by both, spectroscopic data (HREI-MS: 292.1674 [M^+, $C_{17}H_{24}O_4$]; presence of a second carbonyl group, $\delta_C=196.8$, IR: $\nu=1640\,cm^{-1}$; lack of the 7-H in the ^1H NMR spectrum), and chemical transformation to cineromycin B (**1**) by reduction with 1.1 eq $NaBH_4$ in MeOH. However, the non-stereo controlled reduction resulted in both, **1** and 7-*epi*-cineromycin B (**6**), which could be separated by column chromatography on silica gel ($CHCl_3$-MeOH, 95:5). In the ^1H NMR spectrum **6** is characterized by differences in the multiplicity of 7-H (**6**: $\delta_{7\text{-H}}=4.45$, d, $J=6$ Hz; **1**: $\delta_{7\text{-H}}=4.50$, m) and a high-field shift of 9-H ($\delta=5.23$, m). On the other hand oxidation of cineromycin B (**1**) with 4-methylmorpholino-4-oxide (NMO) and tetra-*n*-propyl-ammoniumperruthenate (TPAP) at room temperature (12 hours) gave **5** in 36% yield.

The colorless oily oxycineromycin B (**7**) appeared to be more hydrophilic (Rf 0.18, EtOAc-*n*-hexane, 3:1) than cineromycin B (**1**), although a comparison of the NMR data showed a number of similarities. DCI-MS gave $m/z=328$ [$M+NH_3+H^+$], which pointed to a molecular weight of 310 ($C_{17}H_{26}O_5$). An additional hydroxy group was estimated and shown to be located at 8-CH_3 (δ_H 2.22, 8-OH, broad; 4.19 and 4.37, 8-CH_2; δ_C 60.0, 8-CH_2).

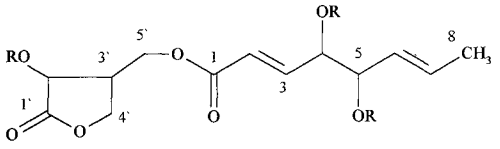
The new colorless crystalline 2,3-dihydrocineromycin B (**8**; Rf 0.40, $CHCl_3$-MeOH, 4:1[1]) showed the molecular ion in the DCI-MS at $m/z=314$ [$M+NH_3+H^+$], which pointed to the molecular formula $C_{17}H_{28}O_4$. A comparison of the NMR spectra with **1** revealed two methylene groups (**8**: δ_H 2.25, 2.39, 2-H_2; δ_C 30.5 (t), C-2 and δ_H 2.02, 2.10, 3-H_2; δ_C 36.9 (t), C-3) instead of the two methine protons of the double bond C-2/C-3 in **1**.

An oxidation with NMO and TPAP of compound **8** in an analogous manner to that performed with cineromycin B (**1**), resulted in 2,3-dihydro-dehydrocineromycin B (**9**).

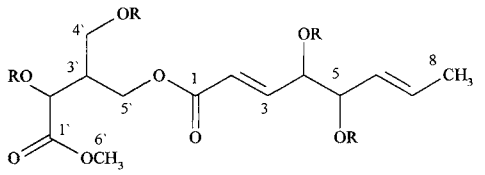
Fig. 3. Structural formulae of the musacins A to C.



Musacin A (**10**) R=H
Musacin A-tetraacetate (**11**) R=Ac



Musacin B_1/B_2 (**12/14**) R=H
Musacin B_1/B_2-triacetate (**13/16**) R=Ac



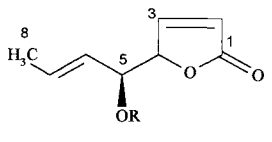
Musacin C (**15**) R=H
Musacin C-tetraacetate (**17**) R=Ac

Musacins A, B_1/B_2 and C

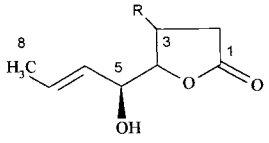
A further group of new secondary metabolites from *Streptomyces griseoviridis* (strain FH-S 1832) are the so-called musacins, which gave blue to violet colorization with blue tetrazolium reagent. While musacin A to C showed brown spots by staining with anisaldehyde, musacin D to F turned blue indicating two subgroups in between the musacins. With the exception of musacin D all compounds appeared to be as colorless oils.

Musacin A (**10**) shows the molecular ion at $m/z=246$ (DCI-MS), while the EI-mass spectrum resulted only in a characteristic fragmentation pattern ($m/z=176$, 155, 84, 71 and 57), analogous to that observed in musacin B and C. The UV-spectrum reveals an absorbance at λ_{max} =209 nm and the IR-spectrum shows two characteristic bands at 3390 and 1710 cm^{-1} indicating hydroxyl groups as well as an α,β-unsaturated ester. The ^{13}C NMR-spectrum includes signals of eleven C-atoms: one methyl and two methylene groups, three aliphatic and four olefinic methine carbon atoms as well as one carbonyl group (Table 2). The downfield shifts of the methylene and the aliphatic methine groups indicate the close vicinity of oxygen atoms. The chemical shifts of one olefinic signal pair [δ_C: 120.6 (C-2) and 148.3 (C-3) together with a signal at δ_C 166.6 (C-1)] indicated the presence of an α,β-unsaturated ester. Additional 2D NMR-correlation spectra lead to the constitution of musacin A as 4,5-dihydroxy-octa-2,6-dienoic acid-2,3-dihydroxy-propylester (**10**). From the $^3J_{H\text{-}H}$ coupling

Fig. 4. Structural formulae of the musacins D to F.



Musacin D (**18**) R=H
19 R=(2′*R*)-$CH_3CH_2CH(Ph)CO$
20 R=(2′*S*)-$CH_3CH_2CH(Ph)CO$



Musacin E=Nigrosporalactone (**21**) R=H
Musacin F (**22**) R=OH

Table 2. ^{13}C NMR data of musacin A (**10**), musacin A-tetraactetate (**11**), musacin B_1 (**12**), musacin B_2-triacetate (**16**), musacin C-tetraacetate (**17**), and musacin D (**18**).

Compound	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′
10[b]	166.6	120.6	148.3	73.8	75.0	130.0	128.1	16.6	65.2	69.8	62.6			
11[a]	165.0	122.9	141.9	72.9	74.3	123.9	132.9	17.9	62.5[e]	69.0	62.3[e]			
12[c]	167.7	121.6	150.3	75.2	76.4	131.5	129.6	18.1	178.6	69.9	45.0	68.2	63.3	
16[d]	165.0	122.4	142.4	72.8	74.2	123.9	133.0	17.8	171.4	67.5	37.3	67.5	61.1	
17[d]	165.1	122.9	141.9	72.8	74.2	123.8	133.0	17.8	170.4	69.5	39.4	60.6[e]	61.5[e]	52.5
18[a]	172.9	122.9	153.2	85.7	72.1	127.5	130.8	17.8						

[a] $CDCl_3$, 50.3 MHz; [b] CD_3OD, 125.7 MHz; [c] CD_3OD, 50.3 MHz; [d] $CDCl_3$, 125.7 MHz; [e] the assignments may be exchangeable.

constants ($^3J_{2,3}$ and $^3J_{6,7}$ = 15.5 Hz) a *trans*-configuration of the double bonds were deduced. Independently, the structure of musacin A (**10**) was confirmed by derivatization with acetic anhydride, which yields musacin A-tetraacetate (**11**).

Compared with **10** musacin B_1 (**12**) differs in its mass (m/z = 286) and an additional carbonyl band (IR: ν 1780), which is caused by a γ-lactone moiety. NMR spectroscopic analysis presented an analogue all-*trans*-4,5-dihydroxy-octa-2,6-dienoic acid moiety, while the alkohol moiety shows both, two methylene groups ($\delta_{5'\text{-H}}$ 4.30 and 4.41; $\delta_{4'\text{-H}}$ 4.06 and 4.44), and two aliphatic methine protons ($\delta_{2'\text{-H}}$ 4.32; $\delta_{3'\text{-H}}$ 2.77) all linked to oxygen atoms, as well as the second carbonyl group (δ_C 178.6). These data were in accordance with the structure **12** (4,5-dihydroxy-octa-2,6-dienoic acid-4-hydroxy-5-oxo-tetrahydrofuran-3-yl-methylester). In support of this structure, treatment of **12** with acetic anhydride yielded the corresponding musacin B_1-triacetate (**13**).

The oily mixture of musacins B_2 and C (**14**, **15**) could not be separated by chromatographic procedures (ratio 1 : 1; Rf see ref. 1). After acetylation, however, separation was successful by column chromatography on silica gel ($CHCl_3$ - MeOH, 98 : 2) and made structure elucidation possible. The first product (**16**) exhibits a molecular mass of 412 (DCI-MS). Based on the obtained NMR data **16** appears to be a triacetate, which is closely related to the corresponding triacetate of musacin B_1 (**13**). Besides an identical elemental composition, both triacetates differ in the stereochemistry at C-2' and/or C-3'. However, detailed stereochemical information is not available from the ^{1}H NMR spectra.

In analogy to **16** the second acetylation product (**17**) shows the spectroscopical characteristics of the 4,5-diacetoxy-octa-2,6-dienoic acid moiety and presented the NMR signals of four acetyl groups. The IR-spectrum exhibits only one strong carbonyl absorbance at 1745 cm^{-1} indicating lack of the lactone group. In place of the alcoholic part of the ester of musacin B, compound **17** possessed a methoxy group (δ_H 3.74, δ_C 52.5), two methylene groups (δ_C 60.6 and 61.5), two methine groups (δ_C 39.4 and 69.5) and one further carbonyl group (δ_C 170.4). Therefore, the tetraacetate **17** is formulated as 4,5-diacetoxy-octa-2,6-dienoic acid-3-acetoxy-2-acetoxymethyl-3-methoxycarbonyl-propylester.

Musacins D, E, and F

Musacin D (**18**) was isolated as a white amorphous compound with a molecular mass of 154 (DCI-MS). An UV-absorption at λ_{max} 206 and IR-bands at ν 1760 cm^{-1} are characteristic hints for an α,β-unsaturated δ-lactone moiety. The ^{13}C NMR-spectrum shows eight signals: one carbonyl group (δ 172.9), four olefinic signals (δ 122.9, 127.5, 130.8, 153.2), two aliphatic methine groups (δ 72.1, 85.7) and a methyl group (δ 17.8). The corresponding proton signals were found in the ^{1}H NMR-spectrum ($CDCl_3$): one methyl group (δ 1.75), six single protons (δ = 4.40, 5.01, 5.52, 5.88, 6.19, 7.52) as well as one OH-group (δ = 2.67, see Table 2). In combination with the ^{1}H,^{1}H-coupling information the constitution of musacin D was determined to be 5-(1-hydroxy-but-2-enyl)-2,5-dihydrofuran-2-one (**18**). The double bond at C-2/C-3 is in *cis*-position ($^3J_{2,3}$ = 6 Hz), while the one at C-6/C-7 is *trans*-configurated ($^3J_{6,7}$ = 15 Hz). Application of Helmchen's-method for secondary alcohols[8] *via* esterification of musacin D (**18**) with 2-(*R*)- and 2-(*S*)-α-phenylbutyric acid and ^{1}H NMR-analysis of the isolated diastereomeric esters **19** and **20** resulted in the *S*-configuration of the center of chirality at C-5.

The oily musacin E (**21**) shows nearly identical spectroscopic data as musacin D (**18**). In comparison the molecular mass (DCI- and EI-MS, m/z = 156) is two mass units higher than **18**. From the ^{13}C and ^{1}H NMR-spectra it was obvious that the double bond at C-2/C-3 is hydrogenated. Thus, musacin E is 5-(1-hydroxy-but-2-enyl)-tetrahydrofuran-2-one (**21**), which appeared to be identical to nigrosporalactone[9], isolated from a *Nigrospora* species.

Inspection of the spectroscopic data showed that musacin F (**22**) is structurally related to musacin E (**21**). The mass difference is 16 additional units (EI-MS m/z 172), while comparison of the ^{13}C and ^{1}H NMR-data in $CHCl_3$ presented the lack of the methyl group as well as additional signals of a methine group linked to oxygen ($\delta_{C\text{-}3}$ 68.0, $\delta_{H\text{-}3}$ 4.45) and an OH-group ($\delta_{3\text{-}OH}$ 1.92). This resulted in the constitution 4-hydroxy-5-(1-hydroxy-but-2-enyl)-tetrahydrofuran-2-one (**22**) for musacin F.

Discussion

The new 14-membered macrolides of the cineromycin B-type **5**, **7**, and **8** exhibit no sugar moiety as found in the clinically used erythromycin A or oleandomycin[10] and are structurally related to albocycline (**2**), isolated from *Streptomyces bruneogriseus*[6,7]. As in the case of albocycline[6], only weak antibacterial activity of **5**, **7**, and **8**, especially against staphylococci, could be observed. From the structural point of view, variability among the cineromycin B-metabolites is observed at C-7 (oxidation/reduction), C-8 (oxygenation of 8-Me), and at the double bond C-2/C-3 (reduction). We assume, that

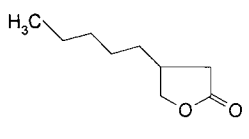
these variations are post-polyketide modifications of an already biosynthesized heptaketide lactone.

The new musacins A to C (**10**, **12**, **14** and **15**) are esters of 4,5-dihydroxyocta-2,6-dienoic acid, which vary in the alcohol part. Obviously, the acid moiety derives from a tetraketide intermediate, while C-1′ to C-3′ (glycerol) of musacin A (**10**) originates from the fermentation medium. The alcohol moiety of musacin C (**15**) appeared to be the methyl ester of the ring opened γ-lactone from musacin B_1/B_2 (**12**/**14**) and might therefore be an artefact from work-up procedures.

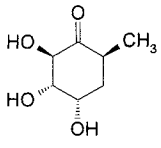
In contrast, musacins D to F (**18**, **21** and **22**) were found to be intramolecular esters of 4,5-dihydroxyocta-2,6-dienoic acid, in which esterification takes place with 4-OH to result in a γ-lactone moiety. Obviously, structural variability is caused by the configuration of the double bond at C-2/C-3. From the biosynthetic point of view, the *trans*-configuration led to intermolecular esterification as found for the musacins A to C, while a *cis*-configuration of the double bond at C-2/C-3 gave musacin D (**18**). Formation of musacin E (**21**) and F (**22**) can be explained by enzymatic reduction or addition of water.

Isolation and structure elucidation of the obtained secondary metabolites from *Streptomyces griseoviridis* (FH-S 1832) require this strain to be classified as a talented organism[1]. Despite the fact that the broad variety of spots with different colorization behaviour to the four staining reagents indicated a number of different structures, the compounds isolated could be divided into two main groups: the 14-membered lactones of the cineromycin-type and the musacin-type metabolites. In addition, γ-nonalactone (**23**) was detected by both, its coconut odor, and by co-chromatography on HPLC with an authentic sample. Cultivation of FH-S 1832 on an oatmeal medium[1] presented a number of differences, which turned out to be variations predominantly in the yields of the already determined metabolites. For example, musacin A (**10**) appeared only as a minor component, which is obviously due to the lack of the biosynthetic precursor glycerol. On the other hand oatmeal fermentation resulted in the isolation of the carba-sugar gabosine F [(2*R*,3*S*,4*S*,6*S*)-2,3,4-trihydroxy-6-methylcyclohexanone] (**24**)[11], which could not be detected on the glycerol medium. Thus, *Streptomyces griseoviridis* (FH-S 1832) produces a variability of structurally diverse secondary metabolites depending on the culture conditions (*e.g.* medium variations).

Fig. 5. Further metabolites of strain FH-S 1832.



γ-Nonalactone (**23**)



Gabosine F (**24**)

Experimental

General

MP's were determined on a Reichert hot-stage microscope and are not corrected. NMR spectra were measured with Varian-VXR-200, Varian-XL-200, Varian-VXR-500 and Bruker-AMX-300 instruments. The multiplicities of the ^{13}C NMR values were assigned by attached proton test (APT) or DEPT techniques. Chemical shifts are expressed in δ values (ppm) with tetramethylsilane (TMS) as internal standard, J in Hz. The mass spectra were taken by Finnigan MAT 311 A and Varian MAT 731 (EI-MS, 70 eV, direct insert, high resolution with perfluorokerosine as standard) and Finnigan MAT 95 A (DCI-MS, 200 eV, NH_3 as reagent gas). IR spectra in pressed KBr discs were recorded on Perkin-Elmer Models 298 and 1600, and the UV spectra on a Kontron Uvikon 860 spectrometer. Optical rotation values were recorded with a Perkin Elmer 241, and CD spectra with a Jasco spectropolarimeter J-500A. TLC was performed on silica gel 60 F_{254} plates, HPTLC-ready-to-use-plates on aluminium foil or glass (Merck) and column chromatography on silica gel 60 (<0.8 mm; Macherey-Nagel) or Sephadex LH-20 (Pharmacia).

Dehydrocineromycin B (5)

Yield 3.4 mg/liter; Rf (see Ref. 1); $[\alpha]_D^{20}$ −68.0 (*c* 0.7 in MeOH); UV λ_{max}^{MeOH} nm (ε) 210 (27,900), $\lambda_{max}^{MeOH+HCl}$ 207 (27,100), $\lambda_{max}^{MeOH+NaOH}$ 206 (19,100); IR (KBr) cm^{-1} 3430, 2960, 2910, 2860, 1710, 1640, 1450, 1380; CD λ_{max}^{MeOH} nm (θ) 249 (−25,100), 242 (−4,000); ^1H NMR: (200 MHz, $CDCl_3$) δ 0.95 (d, J=6.0 Hz, 12-CH_3), 1.28 (m, 11-H_2), 1.28 (d, J=6.0 Hz, 13-Me), 1.45 (m, 12-H), 1.75 (s, 4-CH_3), 1.62 (d, J=1.0 Hz, 8-CH_3), 2.40 (m, 10-H_2), 4.65 (qd, J=16.0 and 6.0 Hz, 13-H), 6.45 (m, 9-H), 5.98 (d, J=16.0 Hz, 5-H), 6.04 (d, J=16.0 Hz, 2-H), 6.27 (d, J=16.0 Hz, 6-H), 6.83 (d, J=16.0 Hz, 3-H); ^{13}C NMR see Table 1; EI-MS (70 eV) *m/z* (abundance, %) 292.1674 (1, M^+, calcd. for $C_{17}H_{24}O_4$ and found), 274 (3, $[M^+-H_2O]$), 124 (34), 95 (59), 69 (33), 55 (49), 43 (100).

Oxycineromycin B (7)

Yield 2.0 mg/liter; Rf (see ref. 1); $[\alpha]_D^{20}$ −56 (*c* 0.7 in MeOH); UV λ_{max}^{MeOH} nm (ε) 211 (33,400), $\lambda_{max}^{MeOH+HCl}$ 204 (23,000), $\lambda_{max}^{MeOH+NaOH}$ 203 (19,000); IR (KBr) cm^{-1} 3430, 2980, 2920, 2160, 1700, 1500, 1360; CD λ_{max}^{MeOH} nm (θ) 240 (−13,600); ^1H NMR (200 MHz, $CDCl_3$) δ 0.88 (d, J=6.0 Hz, 13-Me), 1.24 (m, 11-H_2), 1.24 (d, J=6.0 Hz, 13-CH_3), 1.35 (m, 12-H), 1.54 (s, 4-CH_3), 1.90 (m, 10-H_2), 2.22 (s, 8-OH); 4.19 (d, J=12.0 Hz, 8-H_a), 4.37 (d, J=12.0 Hz, 8-H_b), 4.65 (dq, J=16.0 and 6.0 Hz, 13-H), 4.85 (d, J=6.0 Hz, 7-H), 5.32 (m, 9-H), 5.70 (dd, J=16.0 and 6.0 Hz, 5-H), 5.86 (d, J=16.0 Hz, 2-H), 5.90 (dd, J=16.0 and 1.0 Hz, 6-H), 6.92 (d, J=16.0 Hz, 3-H); ^{13}C NMR (see Table 1); EI-MS (70 eV) *m/z* (abundance, %) 292 (5, $[M^+-H_2O]$), 249 (8), 231 (9), 147 (40), 95

(78), 81 (68), 69 (66), 55 (96), 43 (100); DCI-MS $[M+NH_3+H^+]$, 310 (45, $[M^+]$).

2,3-Dihydrocineromycin B (**8**)

Yield 8.3 mg/liter; Rf (see ref. 1); $[\alpha]_D^{20}$ −15.2 (*c* 0.8 in MeOH); UV λ_{max}^{MeOH} nm end adsorption; MP 114°C; IR (KBr) cm^{-1} 3420, 2980, 2920, 2860, 1720, 1450, 1260; ^1H NMR (500 MHz, $CDCl_3$) δ 0.88 (d, J=7.0 Hz, 12-CH_3), 1.11 (d, J=6.0 Hz, 13-CH_3), 1.28 (s, 4-CH_3), 1.38 and 1.47 (m, 11-H_2), 1.65 (d, J=1.0 Hz, 8-CH_3), 1.72 (m, 12-H), 2.02 and 2.10 (m, 10-H_2), 1.78 and 1.98 (m, 3-H_2), 2.25 and 2.39 (m, 2-H_2), 4.50 (m, 7-H), 4.50 (m, 13-H), 5.24 (m, 9-H), 5.55 (dd, J=16.0 and 6.0 Hz, 5-H), 5.83 (dd, J=16.0 Hz, 2-H); ^{13}C NMR (see Table 1); EI-MS (70 eV) *m/z* (abundance, %) 278 (18, $[M^+-H_2O]$), 222 (9), 193 (9), 154 (30), 95 (46), 69 (26), 55 (51), 43 (100); DCI-MS 314 (100, $[M+NH_3+H^+]$), 296 (46, $[M^+]$).

8,9-Epoxycineromycin B (**3**, **4**)

A solution of 24 mg of **1** and 15 mg of MCPBA dissolved in 10 ml of CH_2Cl_2 was stirred at 0°C for 12 hours. The mixture was evaporated and the crude material was purified by column chromatography (silica gel, AcOEt - *n*-hexane, 3 : 1) to yield 19 mg (76%) of a mixture of the diastereomers **3** and **4**. Repurification by column chromatography (silica gel, $CHCl_3$ - MeOH, 9 : 1) yielded 8 mg (32%) of pure **3**; Rf (AcOEt - *n*-hexane, 3 : 1) 0.58, ($CHCl_3$ - MeOH, 9 : 1) 0.77; $[\alpha]_D^{20}$ −31.0 (*c* 0.3 in $CHCl_3$); UV λ_{max}^{MeOH} 202 (7,100), $\lambda_{max}^{MeOH+HCl}$ 201 (7,200), $\lambda_{max}^{MeOH+NaOH}$ 202 (9,100); IR (KBr) cm^{-1} 3433, 2923, 1716, 1636, 1457, 1260, 1051; ^1H NMR (500 MHz, $CDCl_3$) δ 0.90 (d, J=6.0 Hz, 12-CH_3), 1.02 and 2.02 (m, 10-H_2), 1.02 and 1.36 (m, 11-H_2), 1.27 (s, 8-CH_3), 1.31 (d, J=6.0 Hz, 13-CH_3), 1.42 (m, 12-H), 1.51 (s, 4-CH_3), 2.95 (dd, J=10.0 and 3.0 Hz, 9-H), 4.23 (d, J=6.0 Hz, 7-H), 4.66 (dq, J=16.0 and 6.0 Hz, 13-H), 5.59 (dd, J=16.0 and 6.0 Hz, 6-H), 5.99 (d, J=16.0 Hz, 2-H); 6.03 (dd, J=16.0 and 1.0 Hz, 5-H), 6.78 (d, J=16.0 Hz, 6-H); ^{13}C NMR (see Table 1); EI-MS (70 eV) *m/z* (abundance, %) 310 (1, $[M^+]$), 228 (4), 139 (28), 111 (26), 97 (34), 95 (46), 91 (58), 55 (73), 43 (100); DCI-MS 328 (100, $[M+NH_3+H^+]$), 310 (3%, $[M^+]$). The diastereoisomer **4** was characterized from the mixture of **3** and **4**. ^1H NMR (200 MHz, $CDCl_3$) δ 0.92 (d, J=6.0 Hz, 12-CH_3); 1.02 and 2.02 (m, 10-H_2), 1.02 and 1.36 (m, 11-H_2), 1.22 (s, 8-CH_3), 1.28 (d, J=6.0 Hz, 13-CH_3), 1.42 (m, 12-H), 1.49 (s, 4-CH_3), 2.75 (dd, J=10.0 and 3.0 Hz, 9-H), 3.85 (d, J=6.0 Hz, 7-H), 4.65 (dq, J=16.0 and 6.0 Hz, 13-H), 5.68 (dd, J=16.0 and 6.0 Hz, 6-H), 5.73 (d, J=16.0 Hz, 2-H), 5.87 (dd, J=16.0 and 1.0 Hz, 5-H), 6.90 (d, J=16.0 Hz, 6-H); ^{13}C NMR (see Table 1).

7-*epi*-Cineromycin B (**6**)

A solution of 34 mg of **1** and 5 mg (1.1 eq) of $NaBH_4$ in 10 ml of MeOH was stirred at room temperature for 10 minutes. After addition of 10 ml H_2O and stirring for 30 minutes the mixture was extracted 3 times with $CHCl_3$. The combined organic layers were dried with Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography (silica gel, $CHCl_3$ - MeOH, 95 : 5). Repurification by column chromatography (RP-8 silica gel, MeOH - H_2O, 8 : 2) yielded 9.8 mg (29%) pure cineromycin B (**1**) and 2.2 mg (8%) 7-*epi*-cineromycin B (**6**): Rf (AcOEt - *n*-hexane, 3 : 1) 0.73, ($CHCl_3$ - MeOH, 9 : 1) 0.49; $[\alpha]_D^{20}$ −59.1 (*c* 0.2 in $CHCl_3$). IR (KBr) cm^{-1} 3421, 2958, 2924, 2854, 1700, 1636, 1457, 1379; ^1H NMR (500 MHz, $CDCl_3$) δ 0.87 (d, J=6.0 Hz, 12-CH_3), 1.10 (m, 11-H_2), 1.21 (d, J=6.0 Hz, 13-CH_3), 1.30 (m, 12-H), 1.56 (s, 4-CH_3), 1.69 (d, J=1.0 Hz, 8-CH_3), 1.79 (m, 10-H_2), 4.45 (d, J=6.0 Hz, 7-H), 4.56 (dq, J=6.0 and 6.0 Hz, 13-H), 5.23 (m, 9-H), 5.68 (dd, J=16.0 and 1.0 Hz, 5-H), 5.82 (d, J=16.0 Hz, 2-H), 5.83 (dd, J=16.0 and 1.0 Hz, 6-H), 6.86 (d, J=16.0 Hz, 3-H); ^{13}C NMR (see Table 1); EI-MS (70 eV) *m/z* (abundance, %) 263 (68), 149 (32), 137 (46), 105 (52), 91 (74), 69 (48), 55 (63); DCI-MS 312 (100, $[M+NH_3+H^+]$), 294 (17, $[M^+]$).

2,3-Dihydro-dehydrocineromycin B (**9**)

A solution of 16 mg of 2,3-dihydrocineromycin B (**8**) in 10 ml of CH_2Cl_2 was stirred with 11 mg of 4-methylmorpholine-4-oxide (NMO) and 2 mg of tetra-*n*-propylammoniumperruthenate at room temperature for 12 hours. The reaction mixture was evaporated to dryness and chromatographed on a silica gel column (ethyl acetate - *n*-hexane, 3 : 1) to yield 7.2 mg (63%) of pure **9**. $[\alpha]_D^{20}$ +2.4 (*c* 0.4 in MeOH); UV λ_{max}^{MeOH} nm (ε) 233 (6,000), $\lambda_{max}^{MeOH+HCl}$ 233 (6,500), $\lambda_{max}^{MeOH+NaOH}$ 232 (6,600); IR (KBr) cm^{-1} 3427, 2968, 2927, 1730, 1633, 1377, 1239; ^1H NMR (500 MHz, $CDCl_3$) δ 0.96 (d, J=6.5 Hz, 12-CH_3), 0.88 and 1.76 (m, 12-H_2), 1.22 (d, J=6.5 Hz, 13-CH_3), 1.38 and 1.62 (m, 11-H_2), 1.48 (s, 4-CH_3), 1.80 (d, J=1.0 Hz, 8-CH_3), 1.86 and 1.96 (m, 3-H_2), 1.98 and 2.18 (m, 10-H_2), 2.38 and 2.53 (m, 2-H_2), 4.80 (m, 13-H), 6.24 (d, J=16.0 Hz, 5-H), 6.42 (m, 9-H), 6.58 (d, J=16.0 Hz, 6-H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 11.1 (C-15), 16.4 (C-16), 18.4 (C-17), 23.5 (C-10), 28.9 (C-14), 30.8 (C-12), 31.4 (C-11), 36.4 (C-3), 37.7 (C-12), 72.8 (C-4), 75.0 (C-13), 128.0 (C-6), 137.1 (C-8), 146.7 (C-9), 146.9 (C-5), 174.5 (C-1), 197.1 (C-7); EI-MS (70 eV) *m/z* (abundance, %) 294 (2, $[M^+-H_2O]$), 276 (3 $[M^+-2H_2O]$), 251 (10), 149 (15), 109 (34), 95 (55), 69 (34), 55 (46), 43 (100); DCI-MS 312 (100, $[M+NH_3+H^+]$).

Musacin A (**10**)

Yield 4.0 mg/liter; Rf (see ref. 1); $[\alpha]_D^{20}$ +23.2 (*c* 0.5 in MeOH); UV λ_{max}^{MeOH} nm (ε) 209 (9,500), $\lambda_{max}^{MeOH+HCl}$ 208 (9,600), $\lambda_{max}^{MeOH+NaOH}$ 217 (12,800); CD λ_{max}^{MeOH} nm (θ) 228 (+10,400); IR (KBr) cm^{-1} 3390, 2940, 2920, 2890, 1710, 1660, 1385; ^1H NMR (500 MHz, CD_3OD) δ 1.71 (ddd, J=6.5, 1.5 and 1.0 Hz, 8-H_3), 3.56 (dd, J=5.0 and 2.5 Hz, 3′-H_2), 3.85 (tt, J=5.5 and 5.5 Hz, 2′-H), 3.98 (dd, J=7.0 and 6.0 Hz, 5-H), 4.13 (m, 4-H), 4.13 (ddd, J=11.0, 6.0 and 1.5 Hz, 1′-H_a), 4.22 (ddd, J=11.0, 4.0 and 1.5 Hz,

1'-H_b), 5.53 (ddq, J=15.5, 7.0 and 1.5 Hz, 6-H), 5.73 (dqd, J=15.5, 6.5 and 1.0 Hz, 7-H), 6.10 (dd, J=15.5 and 1.8 Hz, 2-H), 7.07 (dd, J=15.5 and 4.5 Hz, 3-H), ^{13}C NMR (see Table 2); EI-MS (70 eV) *m/z* (abundance, %) 176 (7), 155 (4), 84 (93), 71 (56), 57 (27); DCI-MS 264 (100, [M+NH_3+H^+]), 246 (22, [M^+]).

Musacin A-tetraacetate (4,5-Diacetoxy-octa-2,6-dienoic acid-2,3-diacetoxy-propylester (**11**)

15 mg of **10** and 5 mg of 4-(dimethylamino)pyridine dissolved in 5 ml of acetic anhydride was stirred for 1 hour at room temperature. After addition of 5 ml of ice water, the mixture was extracted three times with 10 ml of $CHCl_3$. The combined organic layers were extracted three times with 10 ml of a saturated solution of $NaHCO_3$ in H_2O and once with H_2O. After drying with Na_2SO_4 the organic layer was concentrated and the residue was purified by column chromatography (silica gel, AcOEt-*n*-hexane 4:1) to yield 12.8 mg (51%) of pure **11**; Rf (AcOEt-*n*-hexane, 2:1) 0.78, ($CHCl_3$-MeOH, 99:1) 0.21; IR (KBr) cm^{-1} 3450, 2930, 1745, 1665, 1370; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.73 (dd, J=6.5 and 1.0 Hz, 8-H_3), 2.06, 2.08, 2.10 and 2.12 (4×s, 4×Ac-H_3), 4.17 (dd, J=12.0 and 5.5 Hz, 1'-H_a), 4.31 (m, 3'-H_a and 3'-H_b), 4.37 (dd, J=12.0 and 4.5 Hz, 1'-H_b), 5.29 (tt, J=5.0 and 4.5 Hz, 2'-H), 5.42 (m, 5-H and 6-H), 5.57 (ddd, J=5.0, 3.5 and 1.5 Hz, 4-H), 5.85 (dqd, J=14.5, 6.5 and 2.5 Hz, 7-H), 6.02 (dd, J=15.5 and 1.5 Hz, 2-H), 6.87 (dd, J=15.5 and 5.0 Hz, 3-H); ^{13}C NMR (50.3 Hz, $CDCl_3$) see Table 2, additional signals 20.7, 20.9, 20.9 and 21.0 (4×q, 4×CH_3CO), 169.6, 169.8, 170.0 and 170.4 (4×s, 4×AcCO); EI-MS (70 eV) *m/z* (abundance, %) 344 (2), 302 (30), 242 (10), 239 (2), 200 (8), 159 (86), 126 (80), 117 (4), 113 (14), 84 (36), 57 (4), 43 (100).

Musacin B_1 (**12**)

Yield 4.5 mg/liter; Rf (see ref. 1); $[\alpha]_D^{17}$ −10.1 (*c* 0.4 in MeOH); UV λ_{max}^{MeOH} nm (ε) 206 (9,000), $\lambda_{max}^{MeOH+HCl}$ 207 (8,800), $\lambda_{max}^{MeOH+NaOH}$ 214 (8,200); CD λ_{max}^{MeOH} nm (θ) 220 (+15,700); IR (KBr) cm^{-1} 3420, 2920, 1780, 1660, 1380; ^{1}H NMR (500 MHz, CD_3OD) δ 1.71 (ddd, J=6.5, 1.5 and 0.5 Hz, 8-H_3), 2.77 (m, 3'-H), 3.98 (dd, J=7.0 and 6.0 Hz, 5-H), 4.06 (dd, J=9.5 and 9.5 Hz, 4'-H_a), 4.14 (ddd, J=5.5, 5.0 and 1.5 Hz, 4-H), 4.30 (dd, J=11.5 and 6.5 Hz, 5'-H_a), 4.32 (d, J=10.0 Hz, 2'-H), 4.41 (dd, J=11.5 and 4.5 Hz, 5'-H_b), 4.44 (dd, J=9.5 and 8 Hz, 4'-H_b), 5.53 (ddq, J=15.0, 7.0 and 1.5 Hz, 6-H), 5.73 (dqd, J=15.0, 6.5 and 1.0 Hz, 7-H), 6.09 (dd, J=15.5 and 2.0 Hz, 2-H), 7.08 (dd, J=15.5 and 4.5 Hz, 3-H), ^{13}C NMR (see Table 2); EI-MS (70 eV) *m/z* (abundance, %) 216 (17), 188 (3), 116 (12), 84 (100), 71 (81); DCI-MS 304 (100, [M+NH_3+H^+]), 246 (95, [M^+]).

Musacin B_1-triactetate (4,5-Diacetoxy-octa-2,6-dienoic acid-4-acetoxy-5-oxo-tetrahydrofuran-3-yl-methylester) (**13**)

Following an analogous acetylation and work-up protocol as described for **11** 15 mg of **12** yielded 2.8 mg (13%) of the triacetate **13**. Purification was performed by column chromatography (silica gel, $CHCl_3$-MeOH, 31:1) followed by preparative scale TLC (silica gel, $CHCl_3$-MeOH, 3:1): Rf (AcOEt-*n*-hexane, 2:1) 0.73, ($CHCl_3$-MeOH, 99:1) 0.18; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.71 (dd, J=6.5 and 1.0 Hz, 8-H_3), 2.02, 2.05 and 2.08 (3×s, 3×Ac-H_3), 2.99 (m, 3'-H), 4.11 (dd, J=9.5 and 9.5 Hz, 4'-H_a), 4.28 (dd, J=11.5 and 6.0 Hz, 5'-H_a), 4.36 (dd, J=11.5 and 4.5 Hz, 5'-H_b), 4.51 (dd, J=9.5 and 8.5 Hz, 4'-H_b), 5.39 (m, 2H, 5-H and 6-H), 5.41 (d, J=9.5 Hz, 2'-H), 5.54 (ddd, J=5.5, 3.5 and 1.5 Hz, 4-H), 5.82 (dqd, J=14.5, 6.5 and 2.5 Hz, 7-H), 5.99 (dd, J=15.5 and 1.5 Hz, 2-H); 6.86 (dd, J=15.5 and 5.5 Hz, 3-H); EI-MS (70 eV) *m/z* (abundance, %) 300 (0.3), 215 (0.4), 84 (4), 71 (4), 43 (100).

Musacin B_2/C (**14**/**15**)

The isolated sample[1] named musacin B_2/C was an oily mixture of two components in a ratio of 1:1, which could not be separated by common chromatographic procedures. Yield 50.7 mg/liter; Rf (see ref. 1); ^{13}C NMR (50.3 MHz, CD_3OD) δ 18.0, 40.5, 45.9, 52.5, 59.7, 62.5, 63.3, 68.3, 69.1. 69.9, 75.2, 75.2, 76.4, 121.7, 121.9, 129.5, 131.3, 131.4, 149.8, 150.0, 167.7, 167.8, 175.8, 178.8.

Musacin B_2-triacetate (4,5-Diacetoxy-octa-2,6-dienoic acid-4-acetoxy-5-oxo-tetrahydrofuran-3-yl-methylester (**16**)

A solution of 66 mg of the mixture **14**/**15** and 10 mg of 4-(dimethylamino)pyridine in 20 ml of acetic anhydride was stirred for 2 hours at room temperature. After treatment as described for **11** the crude material was purified by column chromatography (silica gel, $CHCl_3$-MeOH, 98:2) to yield 12.7 mg (27%) of **16** and 25.2 mg (50%) of **17**. Musacin B_2-triacetate (**16**): Rf (AcOEt-*n*-hexane, 2:1) 0.57, ($CHCl_3$-MeOH, 99:1) 0.18; $[\alpha]_D^{20}$ −45.5 (*c* 0.6 in $CHCl_3$); IR (KBr) cm^{-1} 3440, 2920, 2860, 1800, 1750, 1665, 1380; UV λ_{max}^{MeOH} nm (ε) 204 (14,100), $\lambda_{max}^{MeOH+HCl}$ 205 (15,300), $\lambda_{max}^{MeOH+NaOH}$ 214 (11,800); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.71 (dd, J=6.5 and 1.0 Hz, 8-H_3), 2.03, 2.05 and 2.09 (3×s, 3×Ac-H_3), 3.12 (m, 3'-H), 4.17 (dd, J=11.5 and 5.5 Hz, 5'-H_a), 4.30 (dd, J=11.5 and 4.0 Hz, 5'-H_b), 4.37 (dd, J=10.0 and 1.5 Hz, 4'-H_a), 4.44 (dd, J=10.0 and 6.0 Hz, 4'-H_b), 5.40 (m, 5-H and 6-H), 5.54 (ddd, J=5.0, 3.5 and 1.5 Hz, 4-H), 5.60 (d, J=8.0 Hz, 2'-H), 5.83 (m, 7-H), 5.95 (dd, J=15.5 and 1.5 Hz, 2-H), 6.83 (dd, J=15.5 and 5 Hz, 3-H); ^{13}C NMR (125.7 MHz, $CDCl_3$) see Table 2; additional signals δ 20.4, 20.8 and 21.0 (3×q, 3×CH_3CO), 169.6, 169.7 and 169.9 (3×s, 3×AcCO); EI-MS (70 eV) *m/z* (abundance, %) 342 (10), 300 (84), 258 (15), 216 (8), 157 (16), 126 (90), 113 (22), 84 (44), 71 (56), 57 (38), 43 (100); DCI-MS: 430 (100, [M+NH_3+H^+]).

Musacin C-tetraacetate (4.5-Diacetoxy-octa-2,6-dienoic acid-3-acetoxy-2-acetoxymethyl-3-methoxycarbonyl-propylester (**17**)

Acetylation reaction see **16**. Rf (AcOEt-*n*-hexane,

2:1) 0.74, ($CHCl_3$-MeOH, 99:1) 0.25. $[\alpha]_D^{20}$ +15.4 (*c* 0.5 in $CHCl_3$); IR (KBr) cm^{-1} 3440, 2960, 2920, 2850, 1745, 1660; UV λ_{max}^{MeOH} nm (ε) 205 (14,500), $\lambda_{max}^{MeOH+HCl}$ 204 (15,300), $\lambda_{max}^{MeOH+NaOH}$ 214 (15,800); CD λ_{max}^{MeOH} nm (θ) 209 (−2,400); ^1H NMR (500 MHz, $CDCl_3$) δ 1.70 (dd, *J*=6.5 and 1.0 Hz, 8-H_3), 2.01, 2.03, 2.05 and 2.07 (4×s, 4×Ac-H_3), 2.76 (m, 3'-H), 3.74 (s, 6'-H_3), 4.16 (m, 4'-H_a, 4'-H_b and 5'-H_a), 4.25 (dd, *J*=11.5 and 5.5 Hz, 5-H_b), 5.20 (d, *J*=3.5 Hz, 2'-H), 5.39 (m, 5-H and 6-H), 5.53 (ddd, *J*=5.0, 3.5 and 1.5 Hz, 4-H), 5.82 (m, 7-H), 5.98 (dd, *J*=15.5 and 1.5 Hz, 2-H), 6.85 (dd, *J*=15.5 and 5.0 Hz, 3-H); ^{13}C NMR (125.7 MHz, $CDCl_3$) see Table 2, additional signals 20.5, 20.7, 20.8 and 21.0 (4×q, 4×CH_3-CO), 169.0, 169.7, 169.9 and 169.9 (4×s, 4×Ac-CO); EI-MS (70 eV) *m/z* (abundance, %) 416 (2), 374 (39), 314 (12), 272 (4), 231 (88), 189 (10), 147 (2), 126 (66), 84 (36), 71 (18), 43 (100); DCI-MS 504 (100, $[M+NH_3+H^+]$).

Musacin D (**18**)

Yield 3.0 mg/liter; Rf (see ref. 1), MP 68°C; $[\alpha]_D^{20}$ +182.0 (*c* 0.9 in $CHCl_3$) λ_{max}^{MeOH} nm (ε) 206 (8.300), $\lambda_{max}^{MeOH+HCl}$ 206 (8,700), $\lambda_{max}^{MeOH+NaOH}$ 210 (5,200); CD λ_{max}^{MeOH} nm (θ) 227 (+27,800); IR (KBr) cm^{-1} 3420, 3100, 2950, 2910, 2890, 1740, 1600, 1450, 1335; ^1H NMR (200 MHz, $CDCl_3$) δ 1.75 (ddd, *J*=6.5, 1.5 and 1.0 Hz, 8-H_3), 2.67 (s, 5-OH), 4.40 (dd, *J*=6.5 and 4.5 Hz, 5-H), 5.01 (ddd, *J*=4.5, 2.0 and 1.5 Hz, 4-H), 5.52 (ddq, *J*=15.0, 6.5 and 1.5 Hz, 6-H), 5.88 (dqd, *J*=15.0, 6.5 and 1.0 Hz, 7-H), 6.19 (dd, *J*=6.0 and 2.0 Hz, 2H); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ 17.8 (q, C-8), 72.1 (d, C-5), 85.7 (d, C-4), 122.9 (d, C-2), 127.5 (d, C-6), 130.8 (d, C-7), 153.2 (d, C-3), 172.9 (s, C-1); EI-MS (70 eV) *m/z* (abundance, %) 136 (0.4, $[M^+-H_2O]$), 84 (16), 71 (100), 53 (18); DCI-MS 172 (100, $[M+NH_3+H^+]$).

5-((*S*)-1-(*R*)-2-Phenylbutyroxy)-musacin D (**19**)

A solution of 6 mg of **18**, 8 mg of dicyclohexylcarbodiimide, 5 mg of 4-(dimethylamino)pyridine and 64 mg of (*R*)-2-phenylbutyric acid in 10 ml of CH_2Cl_2 was stirred for 2.5 hours at room temperature. After addition of 10 ml of MeOH the mixture was evaporated and chromatographed on silica gel ($CHCl_3$-MeOH, 98:2) to yield 3.4 mg (29%) of **19**. Rf (AcOEt-*n*-hexane, 2:1) 0.86, ($CHCl_3$-MeOH, 99:1) 0.64; ^1H NMR (200 MHz, $CDCl_3$) δ 0.89 (t, *J*=7.5 Hz, 4'-H), 1.62 (ddd, *J*=6.5, 1.5 and 1.0 Hz, 8-H_3), 1.81 (m, 3'-H_a), 2.09 (m, 3'-H_b), 3.44 (t, *J*=7.5 Hz, 2'-H), 5.06 (ddd, *J*=4.0, 2.0 and 1.5 Hz, 4-H), 5.30 (ddq, *J*=15.0, 7.0 and 1.5 Hz, 6-H), 5.63 (dqd, *J*=15.0, 6.5 and 1.0 Hz, 7-H), 6.18 (dd, *J*=5.5 and 2.0 Hz, 2-H), 7.28 (m, 3-H), 7.28 (m, 5×arom. H). EI-MS (70 eV) *m/z* (abundance, %) 300.1361 (6, [M^+, calcd. for $C_{18}H_{20}O_4$ and found], 217 (4), 147 (10), 119 (100), 91 (94).

5-((*S*)-1-(*S*)-2-Phenylbutyroxy)-musacin D (**20**)

In an analogous procedure as described for **19** 6 mg of **18** were esterified with (*S*)-2-phenylbutyric acid and purified to yield 1.7 mg (15%) of **20**. Rf (AcOEt-*n*-hexane, 2:1) 0.63, ($CHCl_3$-MeOH, 99:1) 0.56; ^1H-NMR (300 MHz, $CDCl_3$) δ 0.89 (t, *J*=7.5 Hz, 4'-H), 1.71 (dd, *J*=6.5 and 1.0 Hz, 8-H_3), 1.81 (m, 3'-H_a), 2.09 (m, 3'-H_b), 3.45 (t, *J*=7.5 Hz, 2'-H), 4.95 (ddd, *J*=4.5, 2.0 and 1.5 Hz, 4-H), 5.39 (m, 5-H), 5.41 (ddq, *J*=14.5, 7.5 and 1.0 Hz, 6-H), 5.82 (dqd, *J*=14.5, 6.5 and 1.5 Hz, 7-H), 6.03 (dd, *J*=6.0 and 2.0 Hz, 2-H), 7.08 (dd, *J*=6.0 and 1.5 Hz, 3-H), 7.30 (m, 5×arom. H); EI-MS (70 eV) *m/z* (abundance, %) 300.1361 (4, [M^+, calcd. for $C_{18}H_{20}O_4$ and found]), 217 (3), 147 (8), 119 (100), 91 (97).

Musacin E (**21**)

Yield 0.4 mg/liter; Rf (see ref. 1); $[\alpha]_D^{20}$ +10.8 (*c* 0.3 in $CHCl_3$) λ_{max}^{MeOH} nm (ε) 201 (1,600), $\lambda_{max}^{MeOH+HCl}$ 201 (1,600), $\lambda_{max}^{MeOH+NaOH}$ 210 (1,700); IR (KBr) cm^{-1} 3440, 2960, 2920, 2850, 1770, 1670, 1460, 1260; ^1H NMR (200 MHz, $CDCl_3$) δ 1.75 (ddd, *J*=6.5, 1.5 and 1.0 Hz, 8-H_3), 1.92 (d, *J*=3.5 Hz, 5-OH), 2.20 (m, 3-H_2), 2.55 (m, 2-H_2), 4.40 (m, 5-H), 4.51 (m, 4-H), 5.43 (ddq, *J*=15.5, 6.5 and 1.5 Hz, 6-H), 5.87 (dqd, *J*=15.5, 6.5 and 1.0 Hz, 7-H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 17.9 (q, C-8), 21.3 (t, C-3), 28.5 (t, C-2), 72.9 (d, C-5), 82.3 (d, C-4), 127.4 (d, C-6), 130.4 (d, C-7), 177.4 (s, C-1); EI-MS (70 eV) *m/z* (abundance, %) 156 (1, [M^+]), 138 (3, $[M^+-H_2O]$, 85 (52), 71 (100); DCI-MS 174 (100, $[M+NH_3+H^+]$).

Musacin F (**22**)

Yield 1.0 mg/liter; Rf (see ref. 1); $[\alpha]_D^{20}$ +10.3 (*c* 0.3 in $CHCl_3$) λ_{max}^{MeOH} nm end absorption; IR (KBr) cm^{-1} 3440, 2930, 1780, 1585, 1200; ^1H NMR (200 MHz, CD_3OD) δ 1.74 (ddd, *J*=6.5, 1.5 and 1.5 Hz, 8-H_3), 2.30 (dd, *J*=18.0 and 1.5 Hz, 2-H_a), 2.89 (dd, *J*=18.0 and 7.0 Hz, 2-H_b), 4.24 (m, 5-H), 4.29 (dd, *J*=3.0 and 1.5 Hz, 4-H), 4.45 (ddd, *J*=7.0, 1.5 and 1.5 Hz, 3-H), 5.53 (ddq, *J*=15.5, 6.0 and 1.5 Hz, 6-H), 5.86 (dqd, *J*=15.5, 6.5 and 1.5 Hz, 7-H); ^{13}C NMR (50.3 MHz, CD_3OD) δ 18.1 (q, C-8), 39.5 (t, C-2), 68.0 (d, C-3), 72.7 (d, C-5), 92.4 (d, C-4), 130.0 (d, C-6), 130.0 (d, C-7), 179.1 (s, C-1); EI-MS (70 eV) *m/z* (abundance, %) 172 (3, [M^+]), 101 (17), 84 (44), 71 (100).

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References

1) Burkhardt, K.; H.-P. Fiedler, S. Grabley, R. Thiericke & A. Zeeck: Secondary metabolites by chemical screening. 31. New cineromycins and musacins obtained by metabolite pattern analysis of *Streptomyces griseoviridis* (FH-S 1832). I. Taxonomy, fermentation, isolation and biological activity. J. Antibiotics 49:

432～437, 1996

2) NAKAGAWA, A.: Chemical screening. *In* The search for bioactive compounds from microorganisms. *Ed.*: S. ŌMURA, pp. 263～280, Springer Verlag, New York, 1992
3) ZÄHNER, H. & H. DRAUTZ: Methods in the search for new secondary metabolites from microorganisms—a comparison. *In* VIII International Symposium on Medicinal Chemistry, Proceedings Vol. 2. *Ed.*, R. DAHLBOHM & J. L. G. NILSSON, pp. 49～68, Swedish Pharmaceutical Press, Stockholm, 1985
4) GRABLEY, S.; J. WINK & A. ZEECK: Chemical screening as applied to the discovery and isolation of microbial secondary metabolites. *In* Biotechnology Focus 3. *Eds.*, R. K. FINN & P. PRÄVE, pp. 359～370, Hanser Publishers, Munich, 1992
5) MIYAIRI, N.; M. TAKASHIMA, K. SHIMIZU & H. SAKAI: Studies on new antibiotics, cineromycins A and B. J. Antibiotics Ser. A 19: 56 ～ 62, 1966
6) NAGAHAMA, N.; M. SUZUKI, S. AWATAGUCHI & T. OKUDA: Studies on a new antibiotic, albocycline. I. Isolation, purification and properties. J. Antibiotics Ser. A 20: 261～266, 1967
7) HARADA, K.; F. NISHIDA, H. TAKAGI, M. SUZUKI & T. IWASHITA: Studies on an antibiotic, albocycline. VII. Minor components of albocycline. J. Antibiotics 37: 1187～1197, 1984
8) HELMCHEN, G.: Über eine Methode zur Bestimmung der absoluten Konfiguration von chiralen sekundären Alkoholen und Aminen: NMR-Spektroskopie von Diastereomeren Estern und Aminen der α-Phenylbutter- und Hydratropasäure. Tetrahedron Lett. 1974: 1527～1530, 1974
9) EVANS, R. H.; G. A. ELLESTAD & M. P. KUNSTMANN: Two new metabolites from an unidentified *Nigrospora* species. Tetrahedron Lett. 22: 1791～1794, 1969
10) ŌMURA, S.: Macrolide antibiotics. Chemistry, biology, and practise. Academic Press, New York, 1984
11) BACH, G.; S. BREIDING-MACK, S. GRABLEY, P. HAMMANN, K. HÜTTER, R. THIERICKE, H. UHR, J. WINK & A. ZEECK: Secondary metabolites by chemical screening. 22. Gabosines, new carba-sugars from streptomyces. Liebigs Ann. Chem. 1993: 241～250, 1993