8. Metabolic Products of Microorganisms

Part 265[1])

Prelactones C and B, Oligoketides from *Streptomyces* Producing Concanamycins and Bafilomycins

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Prelactones C (**3**) and B (**8**) were isolated from the concanamycin-producing *Streptomyces sp.* (strain Gö 22/15) and bafilomycin-producing *Streptomyces griseus* (strain Tü 2599), respectively, by chemical screening methods. The constitution and relative configuration of **3** and **8** were established by one- and two-dimensional NMR methods. The absolute configuration of **3** was determined using the *Helmchen* method and that of **8** by CD spectra. The structural properties and absolute configuration of these new δ-lactones reveal strong similarities to the hemiacetal portion of the corresponding macrolide antibiotics. Their possible role in the early polyketide formation of unusual macrolactones is discussed.

Introduction. – Concanamycin A (**1**) [2] and bafilomycin A_1 (**2**) [3] belong to a new class of so-called 'unusual macrolides' with broad biological activities [4]. These polyketide-derived secondary metabolites [5] [6] are unique in the formation of an intramolecular hemiacetal within a long side chain, which is attached to the macrocyclic lactone. The concanamycins [2] [7] represent a family of 18-membered macrolides and are closely related to virustomycin [8] and the viranamycins [9]. Bafilomycins, hygrolidins [10], and the recently discovered PD 118,576 complex [11] belong to the 16-membered family. The members of both families differ in their polyketide starter unit, the number of the ketide building blocks in the macrocyclic lactone, and the substituents, which are attached to the hemiacetal portion (carbohydrates, fumaric acid, or fumaric-acid derivatives). Furthermore, they contain one (hygrolidines) or two MeO groups in unusual positions, pointing out that there could be another precursor for the polyketide chain than acetate, propionate, and butyrate, respectively. The comparable centers of chirality are identical within both families. Because of their unique structures, we decided to start investigations regarding biogenesis and biosynthesis of this unusual macrolides [6].

The biogenesis of 'classical *Woodward*' macrolide antibiotics by a head-to-tail condensation from short fatty acids, and some late transformations in the biosynthetic sequence (*e.g.* glycosylation, oxidation, and alkylation) were well studied, while knowledge about the assembly process from simple building blocks is still poorly understood [12] [13]. Obviously, the polyketide formation has certain analogies with the biosynthesis of long-chain fatty acids although the stereochemical integrity and structural diversity of

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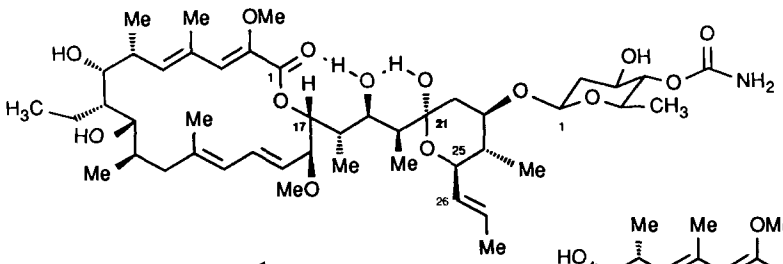

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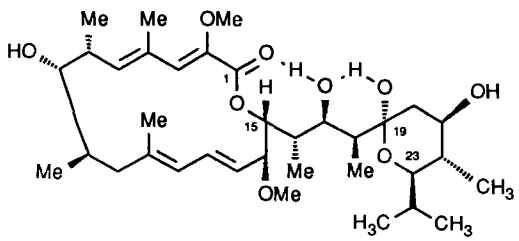

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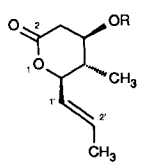
secondary metabolites finds limited precedent in the fatty-acid system. For this fact, macrolide-producing organisms are believed to possess a complex polyketide synthase, which selects the correct substrate and functionalizes the C(β) atom at each step [14]. This thesis gets strong support by the *in vivo* incorporation experiments with thioester-activated di- and triketides by the groups of *Cane* [15], *Hutchinson* [16], and others. The low level of intact incorporation of their potential intermediates is probably caused by microbial degradation of the precursors. *Vederas* solved the problem in his investigations regarding dehydrocurvularin by adding β-oxidation inhibitors to the fermentation broth [17]. The competition between polyketide formation and β-oxidation might be the explanation for the fact that there was no example for the presence of early intermediates from a macrolide-producing strain, until branched-chain fatty acids were isolated from mutants of tylosin- and mycinamycin-producing organisms (see [18] and [19] [20], resp.).

In the course of our studies on concanamycins and bafilomycins, we isolated two δ-lactones called prelactones C and B applying the chemical-screening method [21] [22] to the extracts of the producing strains. The present work describes the isolation and structure elucidation of these new metabolites and discusses their possible role in the formation of unusual macrolides.

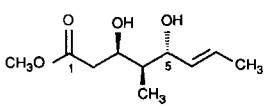
Isolation. – The prelactones C (**3**) and B (**8**) were isolated from the mycelium extract (1 mg/l) of *Streptomyces sp.* (strain Gö 22/15) and the culture-filtrate extract (0.2 mg/l) of *Streptomyces griseus* (strain Tü 2599 ana 18; see *Exper. Part*). Compound **3** was detected by an intensive green-blue color reaction after spraying silica gel TLC plates with anisaldehyde/H_2SO_4 reagent and could be also detected in the culture-filtrate extract of strain Gö 22/15 in a yield of *ca.* 2 mg/l. Compound **8** showed no specific color reaction and was isolated together with a still unidentified sesquiterpene ($C_{15}H_{26}O_2$), which gave a dark blue color reaction upon spraying with vanillin/H_2SO_4 reagent.

Structure Determination. – The combination of DCI-MS and HR-EI-MS data of prelactone C (**3**) gave an empirical formula $C_9H_{14}O_3$, which is supported by NMR data. The IR spectrum showed a strong absorbance at 1740 cm^{-1}, suggesting the presence of an ester or lactone functionality. Complete analysis of one- and two-dimensional ^1H- and

^{13}C-NMR spectra of prelactone C (**3**) and its 2-bromobenzoate **4** led to the connectivity within a C_7-skeleton. Furthermore, the not crystallizing **4** showed a diagnostic downfield shift of 1.39 ppm for the δ 3.79 resonance of **3**. Thus, the only free secondary OH group is located at C(4) (pyran numbering). The presence of only three O-atoms, which were assigned to an ester and one free secondary alcohol group, suggested the presence of a δ-lactone, as shown in formula **3** of prelactone C. The detailed analysis of the vicinal ^{1}H,^{1}H-coupling constants of **3** (*Table*) allowed the assignment of the relative configuration. The magnitudes of $^3J(5,6) = 10$ and $^3J(4,5) = 7$ Hz indicated that all residues of the six-membered ring are oriented equatorially, within a twisted half-chair conformation [23]. To establish the absolute configuration of **3**, we applied the *Helmchen* method for secondary alcohols [24]. This method is based on typical ^{1}H-NMR-signal shifts caused by an acylation with 2-phenylbutyric acid. Separate esterification of **3** with both enantiomers of 2-phenylbutyric acid gave the (2″*S*)-2″-phenylbutyrate **5** and the (2″*R*)-2″-phenylbutyrate **6**. The ^{1}H-NMR spectra of **5** showed a characteristic high-field shift on 2 H–C(3), while **6** showed a strong effect on Me–C(5) and a moderate on H–C(6), H–C(1′) and H–C(2′) (*Table*). According to the rules of *Helmchen*, the configuration at C(4) of **3** can be deduced to be (*R*). Thus, **3** was identified as (4*R*,5*S*,6*R*)-tetrahydro-4-hydroxy-5-methyl-6-[(*E*)-prop-1-enyl]-2*H*-pyran-2-one.



3 R = H
4 R = CO(2-BrC$_6$H$_4$)
5 R = (2″*S*)-CH$_3$CH$_2$CH(Ph)CO
6 R = (2″*R*)-CH$_3$CH$_2$CH(Ph)CO



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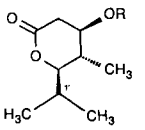
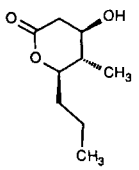


8 R = H
9 R = Ac



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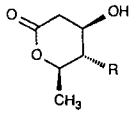


11 R = Me
12 R = Et

During the isolation and purification of **3**, especially in the presence of MeOH, a new, more lipophilic compound with similar color reactions upon spraying with anisaldehyde/H_2SO_4 reagent was formed. For this compound, which proved to be rather unstable, only few data were available. The FAB-MS, in addition with the ^{1}H-NMR data, led to the proposal that this compound is derived from **3** by methanolysis yielding the methyl ester **7**.

The analogous examination of the spectroscopical data of prelactone B led to the result that the propenyl group in **3** is replaced by an isopropyl group as shown in formula **8**. This fact is supported by the empirical formula $C_9H_{16}O_3$ which was determined by the combination of DCI-MS and HR-EI-MS methods. The assignment of the relative configuration of **8** by the ^{1}H-NMR coupling constants was not possible because of the identical chemical shifts for H–C(4) and H–C(6). Fortunately, monoacetate **9** proved to be suitable for the analysis of the coupling constants (*Table*). We found the same all-equatorial orientation of the substituents of the lactone ring as in **3**. The absolute configuration of **8** could be ascertained by comparison of the CD spectrum of **8** with that of 1′,2′-dihydroprelactone C (**10**). The latter was prepared from **3** by catalytic hydrogenation to exclude effects of the double bond. The CD spectra of **8** and **10** showed similar *Cotton* effects with a negative first CD band centered at 236 nm and a positive second CD band at

Table. *^{1}H-NMR Data of Prelactone C* (**3**), *Prelactone B* (**8**), *Their Derivatives* **5**, **6**, *and* **9**, *and the C(22) to C(28) Segment of Concanamycin A* (**1**). δ in ppm rel. to TMS (= 0 ppm), J in Hz.

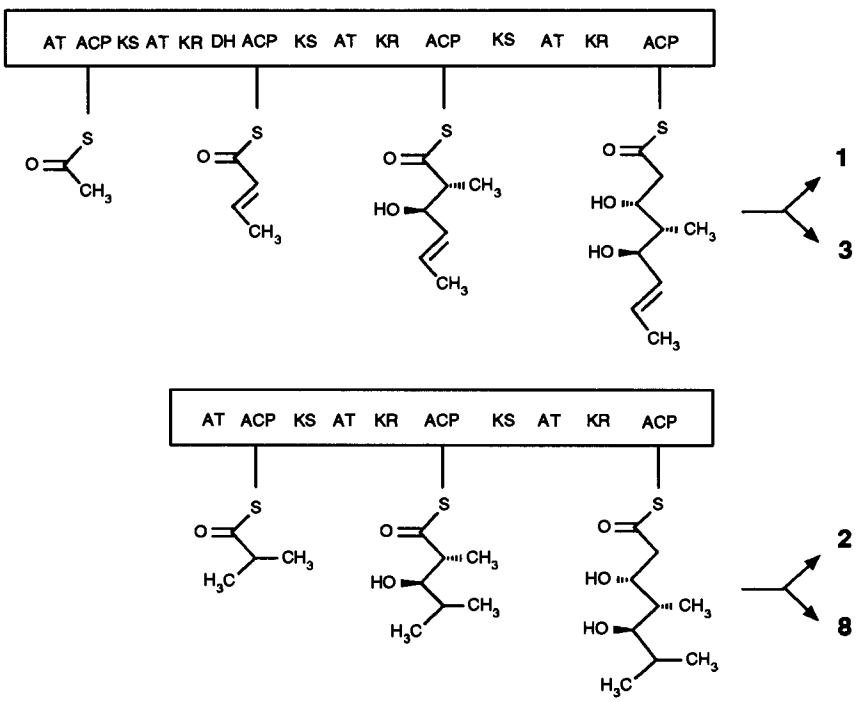
	3[a])	**3**[b])	**5**[b])	**6**[b])	**8**[b])	**9**[b])		**1**[c])
H_a–C(3)	2.40	2.50	2.35	2.52	2.48	2.54	H_a–C(22)	1.11
	(*dd*, *J* = 17,7)	(*dd*, *J* = 17,8)	(*dd*, *J* = 17,7)	(*dd*, *J* = 17,7)	(*dd*, *J* = 17,7)	(*dd*, *J* = 17,6)		(*m*)
H_b–C(3)	2.91	2.94	2.89	2.97	2.94	2.94	H_b–C(22)	2.28
	(*dd*, *J* = 17,6)	(*dd*, *J* = 17,6)	(*dd*, *J* = 17,6)	(*dd*, *J* = 17,6)	(*dd*, *J* = 17,6)	(*dd*, *J* = 17,6)		(*dd*, *J* = 12,5)
H–C(4)	3.72	3.79	4.87	4.85	3.75	4.89	H–C(23)	3.76
	(*ddd*, *J* = 7,7,6)	(*m*)	(*ddd*, *J* = 8,7,6)	(*ddd*, *J* = 8,7,6)	(*m*)	(*ddd*, *J* = 7,6,6)		(*m*)
H–C(5)	1.60	1.68	obs.	obs.	1.75	1.90–2.05	H–C(24)	1.24
	(*ddq*, *J* = 10,7,6)	(*m*)			(*ddq*, *J* = 10,7,6)	(*m*)		(*m*)
Me–C(5)	1.04	1.04	0.95	0.75	1.02	1.04	H–C(25)	0.89
	(*d*, *J* = 6.5)	(*d*, *J* = 7)	(*d*, *J* = 6.5)	(*d*, *J* = 6.5)	(*d*, *J* = 6.5)	(*d*, *J* = 6.5)		(*d*, *J* = 6)
H–C(6)	4.30	4.21	4.28	4.19	3.75	3.80	H–C(26)	3.97
	(*dd*, *J* = 10,8)	(*m*)	(*dd*, *J* = 10,8)	(*dd*, *J* = 11,8)	(*m*)	(*dd*, *J* = 10,2)		(*dd*, *J* = 10,8)
H–C(1′)	5.46	5.45	5.41	5.34	1.99	1.90–2.05	H–C(26)	5.29
	(*ddq*, *J* = 15,8,2)	(*ddq*, *J* = 15,8,2)	(*ddq*, *J* = 15,8,2)	(*ddq*, *J* = 15,8,2)	(*dsept.*, *J* = 6.5,2)	(*m*)		(*ddq*, *J* = 15,8,1)
H–C(2′)	5.83	5.81	5.82	5.78	–	–	H–C(27)	5.54
	(*ddq*, *J* = 15,6,1)	(*ddq*, *J* = 15,6,1)	(*dq*, *J* = 15,6.5)	(*dq*, *J* = 15,6.5)				(*ddq*, *J* = 15,6,1)
Me(3′)	1.75	1.76	1.75	1.76	0.92 (*d*, *J* = 6.5)	0.90 (*d*, *J* = 6.5)	H–C(28)	1.59
or Me_2C(1′)	(*dd*, *J* = 6.5,2)	(*dd*, *J* = 6.5,2)	(*dd*, *J* = 6.5,2)	(*dd*, *J* = 6.5,2)	1.10 (*d*, *J* = 6.5)	1.08 (*d*, *J* = 6.5)		(*dd*, *J* = 6,1)

[a]) CD_3OD; 500 MHz, [b]) $CDCl_3$, 200 MHz. [c]) $CDCl_3$, 500 MHz [6].

212 nm. For this reason, **8** was identified as (4*R*,5S,6*R*)-tetrahydro-4-hydroxy-6-isopropyl-5-methyl-2*H*-pyran-2-one.

Discussion. – Prelactone C (**3**) and B (**8**) are identical in ring size, substituent pattern, and configuration, having the 6-membered hemiacetal portion of the concanamycins and bafilomycins, respectively. These new oligoketides might represent the early chain-elongation steps of the polyketide biosynthesis of the macrolides themselves, as depicted in the *Scheme*. Further chain elongation of the last ACP thioester leads to the macrolides **1** and

Scheme. *Model for the Early Biosynthesis of* **1** *and* **2**. The starter unit and the hypothetic intermediates at the end of each elongation step are shown as the ACP thioesters.



Abbreviations represent the polyketide synthase functions in analogy to fatty acid synthase functions: AT (acyltransferase); ACP (acyl-carrier protein); KS (β-ketoacyl-ACP synthase); KR (β-ketoacyl-ACP reductase); DH (deyhdratase).

2, respectively, while an intramolecular lactonisation leads to a detaching of the oligoketide from ACP as prelactone C and B, respectively. In cases where a δ-lactonisation is energetically favored, the detaching competes with the elongation, and there is a chance to isolate early intermediates. The advantage of the δ-lactones seems to be that a reentry in the macrolide pathway or a rapid microbial degradation, especially by β-oxidation is prevented. Thus, the lactones accumulate, and they can be seen as shunt products of the macrolide formation. The fact that **3** is earlier detectable from fermentation broth than **1**, ruled out that the prelactones might be degradation products of the macrolides themselves.

The isolated prelactones seem to be the first wild-strain-derived compounds representing early steps of the polyketide pathway. Additionally, **3** and **8** reveal the similarity of the biosynthesis of two different families of macrolides. Similar polyketide synthase systems can be postulated for other macrolides, so prelactones are expected to be widespread, if the structural facts favor them, *e.g.* prelactone V (**11**) from the viranamycin-producing organism or prelactone E (**12**) from the strain producing the C_2-symmetric polyketide-derived macrodiolide elaiophylin [25]. The discovery of **3** and **8** strongly supports the current and widely accepted hypothesis of step by step functionalization of growing polyketide chains, proposed by *Cane, Hutchinson* and others. For a more detailed understanding of the role of these prelactones in the macrolide biosynthesis, we started with incorporation experiments.

The discovery of prelactone C (**3**) emphasizes the value of the chemical-screening method for revealing biosynthetic sequences in microorganisms. Prelactone C (**3**) has no common biological activity, no color, and only UV end absorption, so that it could only be detected by a chemical color reaction on TLC plates. The lack of the double bond might be the reason for the fact that prelactone B gave no striking color reaction, but with the knowledge of **3** and the hypothesis of its existence, we were able to isolate **8** as prelactone of the bafilomycins.

We are greatful to Prof. Dr. *H. Zähner*, Institut für Mikrobiolgie der Universität Tübingen, for providing us with crude extracts of the strain Tü 2599 ana 18, and to Mrs. *J. Gerber-Nolte* for skilful technical assistence.

Experimental Part

General. TLC: Silica gel *60* F_{254} precoated plates (*Merck*); detection after staining with anisaldehyde reagent (79 ml of AcOEt, 15 ml of MeOH, 5 ml of H_2SO_4, 1 ml of anisaldehyde) and heating up; R_f values were determined on 25 × 25-cm plates, evaluation length was 20 cm. Column chromatography (CC): Silica gel *60* (< 0.8 mm; *Macherey-Nagel*) and *Sephadex LH-20* (*Pharmacia*). Optical rotation: *Perkin-Elmer*, model *241*, with thermostat; temp. 20°. IR Spectra: *Perkin-Elmer*, model *298*. CD Spectra: *Jasco* spectropolarimeter *J-500A* with *Biotronic IF 500-II* interface, coupled with a *Dawicontrol DC I6 AT/120* personal computer; $[\Theta]^{20}$ (nm). NMR Experiments: *Varian-VXR-200* and *-VXR-500* instruments; TMS (^{1}H) or solvent signal (^{13}C) as internal standards; ^{13}C multiplicities from APT experiments; assignments with the help of ^1J(C,H)-COSY and HETCOR experiments; * or ** means that assignments may be interchanged. MS: *Finnigan MAT 311 A* for EI-MS (70 eV) and *Finnigan 95 A* for DCI-MS (200 eV, NH_3 as reactand gas) and FAB-MS (3-nitrobenzyl alcohol as matrix).

Fermentation and Isolation. a) *Streptomyces sp.* (strain Gö 22/15) was cultivated in a medium (150 ml in a 100-ml *Erlenmeyer* flask) consisting of 1% of malt extract, 0.4% of yeast extract, and 0.4% of D-glucose and was shaken at 250 rpm and 28° for 48 h. An aliquote of this seed culture (10%) was used to inoculate the same medium for production in a 50-l fermentor (*Braun*, model *Biostat U*); starting pH was 6.6. Maximum production of **3** was reached after 60 and for **1** after 76 h at pH 7.5. The culture filtrate and the mycelium were separated by filtration after adding 3 kg of *Hyflocelite*. The mycelium cake was suspended in 20 l of acetone and treated with an *ultraturrax* to destroy the cells. The cells were filtered, the soln. was concentrated and extracted twice with AcOEt. The combined org. layers were dried (Na_2SO_4). Concanamycins A–C, and prelactone C were separated as follows. The mycelium extract (8.06 g) was submitted to CC (silica gel; $CHCl_3$/MeOH 100:0 to 90:10) to give crude **3** (214 mg), concanamycin C (1.06 g), and concanamycin A (**1**; 1.67 g; containing 30% of concanamycin B, the 8-deethyl-8-methyl homologue of **1**). CC (1. *Sephadex LH-20*, MeOH; 2. silica gel, $CHCl_3$/MeOH 93:7) of the crude **3** yielded pure **3** (53 mg) and **7** (4 mg).

b) *Streptomyces griseus* (strain 2599 ana 18) was cultivated and fermented as described [3]. From 200 l of culture broth, 14.64 g of culture-filtrate extract were obtained. CC (silica gel, AcOEt/hexane 1:4 to 4:1, then $CHCl_3$/MeOH 80:20) gave bafilomycin Z (160 mg), D (187 mg), and A_1 (412 mg), crude **8** (208 mg), and bafilomycin B_1 (760 mg) and C_1 (314 mg). The crude **8** was resubmitted to CC (*Sephadex LH-20*, $CHCl_3$/MeOH 6:4) yielding pure **8** (34 mg) and an unidentified sesquiterpene (69 mg).

Prelactone C (= *(4R,5S,6R)-Tetrahydro-4-hydroxy-5-methyl-6-[(E)-prop-1-enyl]-2H-pyran-2-one*; **3**). Oil. R_f (AcOEt/hexane 2:1) 0.41. $[\alpha]_D^{20} = +57.6$ ($c = 0.5$, MeOH). IR (KBr): 3480, 2980, 2930, 1740, 1270, 1010. CD (MeOH): −800 (268), +1100 (245), −900 (220). ^{1}H-NMR: *Table*. ^{13}C-NMR (50.3 MHz, $CDCl_3$): 13.7 (*q*, Me−C(5)); 17.7 (*q*, C(3′)); 39.1 (*t*, C(3)); 41.5 (*d*, C(5)); 69.6 (*d*, C(4)); 84.1 (*d*, C(6)); 127.6 (*d*, C(2′)); 132.6 (*d*, C(1′)); 170.2 (*s*, C(2)). EI-MS: 152 (12, $[M - H_2O]^+$), 109 (10), 82 (46), 71 (100). HR-MS: 152.0843 ($C_9H_{12}O_2$, calc. 152.0840).

Tetrahydro-5-methyl-2-oxo-6-[(E)-prop-1-enyl]-2H-pyran-4-yl 2-Bromobenzoate (**4**). A soln. of 10 mg of **3**, 10 mg of 4-(dimethylamino)pyridine, and 50 µl of 2-bromobenzoyl chloride in 1 ml of pyridine was stirred overnight at r.t. After addition of 7 ml of H_2O and stirring for 1 h, the mixture was extracted 3 times with $CHCl_3$. The combined org. layers were dried (Na_2SO_4), filtered, and concentrated. The residue was purified by CC (silica gel, AcOEt/hexane 1:2): **4** (10 mg, 48%). R_f (AcOEt/hexane 1:2) 0.60. ^{1}H-NMR (500 MHz, $CDCl_3$): 1.22 (*d*, $J = 7$, Me−C(5)); 1.78 (*dd*, $J = 6$, 1.5, Me(3′)); 2.06 (*ddq*, $J = 10$, 7, 6, H−C(5)); 2.75 (*dd*, $J = 17$, 7, H_b−C(3)); 3.12 (*dd*, $J = 17$, 6, H_a−C(3)); 4.33 (*dd*, $J = 10$, 8, H−C(6)); 5.18 (*ddd*, $J = 7$, 7, 6, H−C(4)); 5.48 (*ddq*, $J = 15$, 8, 1.5, H−C(1′)); 5.88 (*ddq*, $J = 15$, 6, 0.5, H−C(2′)); 7.32 (*m*, 2 arom. H); 7.66 (*m*, 1 arom. H); 7.77 (*m*, 1 arom. H). EI-MS: 202 (8), 200 (8), 185 (10), 183 (10), 101 (20), 82 (25), 59 (66), 43 (100).

Tetrahydro-5-methyl-2-oxo-6-[(E)-prop-1-enyl]-2H-pyran-4-yl (S)-2-Phenylbutyrate (**5**). A soln. of 10.1 mg of **3**, 170 mg of DCC, 30 of 4-(dimethylamino)pyridine and 170 µl of (*S*)-2-phenylbutyric acid was stirred for 1.5 h at r.t. After addition of 5 ml of MeOH, the mixture was evaporated. The crude material was purified by CC (silica gel, AcOEt/hexane 1:2): 7.9 mg (43%) of **7**. R_f (AcOEt/hexane 1:2) 0.69. $[\alpha]_D^{20} = +20$ ($c = 0.6$, $CDCl_3$). ^{1}H-NMR: *Table*; 0.92 (*t*, $J = 8$, Me(4″)); 1.8–1.9 (*m*, H_a−C(3″)); 2.0–2.2 (*m*, H_b−C(3″)); 3.46 (*t*, $J = 8$, H−C(2″)); 7.2–7.35 (*m*, 5 arom. H). EI-MS: 316 (3, M^+), 267 (3), 152 (19), 119 (71), 91 (100).

Tetrahydro-5-methyl-2-oxo-6-[(E)-prop-1-enyl]-2H-pyran-4-yl (R)-2-Phenylbutyrate (**6**). As described for **5**, 5.1 mg of **3** were esterified with (*R*)-2-phenylbutyric acid: 4.0 mg (43%) of **6**. R_f (AcOEt/hexane 1:2) 0.76. $[\alpha]_D^{20} = -23$ ($c = 0.4$, $CDCl_3$). ^{1}H-NMR: *Table*. 0.90 (*t*, $J = 8$, Me(4″)); 1.8–1.9 (*m*, H_a−C(3″)); 2.0–2.2 (*m*, H_b−C(3″)); 3.44 (*t*, $J = 8$, H−C(2″)); 7.2–7.35 (*m*, 5 arom. H). EI-MS: 316 (5, M^+), 152 (40), 119 (100), 91 (95).

Methyl (E)-3,5-Dihydroxy-4-methyloct-6-enoate (**7**). R_f (AcOEt/hexane 2:1) 0.45. IR (KBr): 3440, 2980, 1720, 1010. ^{1}H-NMR (200 MHz, $CDCl_3$): 0.92 (*d*, $J = 7$, Me−C(4)); 1.73 (br. *d*, $J = 6$, Me(8)); *ca.* 1.85 (*m*, H−C(4)); 2.40 (*dd*, $J = 16$, 3, H_3−C(2)); 2.62 (*dd*, $J = 16$, 10, H_b−C(2)); 3.63 (*s*, MeO); 4.04 (*m*, H−C(5)); 4.39 (*m*, H−C(3)); 5.52 (*ddq*, $J = 15$, 6, 1.5, H−C(6)); 5.72 (*ddq*, $J = 15$, 6, 0.5, H−C(7)). FAB-MS: 225 ($[M + Na]^+$).

Prelactone B (= *(4R,5S,6R)-Tetrahydro-4-hydroxy-6-isopropyl-5-methyl-2H-pyran-2-one*; **8**). R_f (AcOEt/hexane 2:1) 0.38. $[\alpha]_D^{20} = +38.3$ ($c = 0.6$, MeOH). IR (KBr): 3400, 2980, 2920, 1740, 1250, 1210. CD (MeOH): −700 (235), +1000 (212). ^{1}H-NMR: *Table*. ^{13}C-NMR (50.3 MHz, $CDCl_3$): 13.6 (*q*, 1 *Me*−C(1′))*; 14.0 (*q*, *Me*−C(5))*; 20.0 (*q*, 1 *Me*−C(1′))*; 39.0 (*d*, C(5)); 39.1 (*t*, C(3)); 69.8 (*d*, C(4)); 86.2 (*d*, C(6)); 170.9 (*s*, C(2)). EI-MS: 129 (100, $[M - C_3H_7]^+$), 111 (80), 87 (77), 58 (72). HR-MS: 129.0552 ($C_6H_9O_3$, calc. 129.0551).

Tetrahydro-6-isopropyl-5-methyl-2-oxo-2H-pyran-4-yl Acetate (**9**). A soln. of 15.4 mg of **8** and 10 mg of 4-(dimethylamino)pyridine in 1 ml of Ac_2O was stirred for 2 h at r.t. After addition of 10 ml of phosphate buffer pH 7 (*Merck*), the mixture was extracted twice with $CHCl_3$. The combined org. layers were dried (Na_2SO_4), filtered, and concentrated. The residue was purified by CC (silica gel, AcOEt/hexane 1:2): **9** (8.9 mg, 47%). R_f (AcOEt/hexane 1:2) 0.75. $[\alpha]_D^{20} = +20$ ($c = 0.8$, $CDCl_3$). ^{1}H-NMR: *Table*. ^{13}C-NMR (50.3 MHz, $CDCl_3$): 14.1 (*q*, 1 *Me*−C(1′))*; 14.1 (*q*, *Me*−C(5))*; 19.9 (*q*, 1 *Me*−C(1′))*; 21.0 (*q*, *Me*CO); 35.5 (*t*, C(3)); 36.3 (*d*, C(5)); 71.8 (*d*, C(4)); 85.6 (*d*, C(6)); 170.3 (*s*, C(2))**; 171.0 (*s*, MeCO)**. EI-MS: 171 (17, $[M - C_3H_7]^+$), 111 (100), 43 (82).

(4R,5S,6R)-Tetrahydro-4-hydroxy-5-methyl-6-propyl-2H-pyran-2-one (**10**). At r.t., 10.1 mg of **3** were hydrogenated for 1 h in 5 ml of AcOEt containing 5 mg of 10% Pd/C at 1 atm. After filtration, the catalyst was washed with 10 ml of AcOEt, and the resulting soln. was concentrated. CC (silica gel, AcOEt) furnished 5.3 mg (52%) of **10**. Colorless oil. R_f (AcOEt/hexane 2:1) 0.41. $[\alpha]_D^{20} = +78.3$ ($c = 0.4$, MeOH). IR (KBr): 3480, 2980, 2930, 1740, 1270, 1010. CD (MeOH): −500 (236), +2400 (213). ^{1}H-NMR (200 MHz, $CDCl_3$): 0.94 (*t*, $J = 6$, Me(3′)); 1.10 (*d*, $J = 7$, Me−C(5)); 1.3–1.8 (*m*, 2 H−C(1′), 2 H−C(2′)); 2.49 (*dd*, $J = 17$, 8, H_a−C(3)); 2.92 (*dd*, $J = 17$, 5, H_b−C(3)); 3.7–3.9 (*m*, H−C(4), H−C(6)). EI-MS: 129 (22, $[M - C_3H_7]^+$), 111 (20), 87 (20), 58 (100), 43 (58).

REFERENCES

[1] O. Potterat, H. Zähner, C. Volkmann, A. Zeeck, *J. Antibiot.*, in press.
[2] J.W. Westley, C.-M. Liu, L.H. Sello, R.H. Evans, N. Trope, J.F. Blount, A.M. Chiu, L.J. Todaro, P.A. Miller, *J. Antibiot.* **1984,** *37,* 1738.
[3] G. Werner, H. Hagenmaier, H. Drautz, A. Baumgartner, H. Zähner, *J. Antibiot.* **1984,** *37,* 110.
[4] S. Omura, in 'Macrolide Antibiotics: Chemistry, Biology, and Practice', Ed. S. Omura, Academic Press, Inc., Orlando, 1984, p. 510–550.
[5] O.D. Hensens, R.L. Monaghan, L. Huang, G. Albers-Schönberg, *J. Am. Chem. Soc.* **1983,** *105,* 3672.
[6] K.U. Bindseil, A. Zeeck, in preparation.
[7] J.-T. Woo, C. Shinohara, K. Sakai, K. Hasumi, A. Endo, *J. Antibiot.* **1992,** *45,* 1108.
[8] S. Omura, N. Imamura, K. Hinotozawa, K. Otoguru, G. Lukacs, R. Faghih, R. Tollmann, B.H. Arison, J.L. Smith, *J. Antibiot.* **1983,** *36,* 1783.
[9] Y. Hayakawa, K. Takaku, K. Furihata, K. Nagai, H. Seto, *J. Antibiot.* **1991,** *44,* 1294.
[10] H. Seto, H. Akao, F. Furihata, N. Otake, *J. Antibiot.* **1984,** *37,* 610.
[11] G.M. Hatfield, R.W. Woodard, J.-K. Son, *J. Nat. Prod.* **1992,** *55,* 753.
[12] D. O'Hagen, *Nat. Prod. Rep.* **1989,** *6,* 205.
[13] D. O'Hagen, in 'The Polyketide Metabolites', Ed. D. O'Hagen, Ellis Horwood Ltd., New York, 1991, p. 116–137.
[14] S. Donadio, M.J. Staver, J. Mc Alpine, S.J. Swanson, L. Katz, *Science* **1991,** *252,* 675.
[15] D.E. Cane, C. Yang, *J. Am. Chem. Soc.* **1987,** *109,* 1255.
[16] S. Yue, J.S. Duncan, Y. Yamamoto, C.H. Hutchinson, *J. Chem. Soc.* **1987,** *109,* 1253.
[17] Z. Li, F.M. Martin, J.C. Vederas, *J. Am. Chem. Soc.* **1992,** *114,* 1531.
[18] M.L.B. Huber, J.W. Pascal, J.P. Leeds, H.A. Kirst, J.A. Wind, F.D. Miller, J.R. Turner, *Antimicrob. Agents Chemother.* **1990,** *34,* 1535.
[19] K. Kinoshita, S. Takenaka, M. Hayashi, *J. Chem. Soc., Chem. Commun.* **1988,** 943.
[20] K. Kinoshita, S. Takenaka, M. Hayashi, *J. Chem. Soc., Perkin Trans. 1* **1991,** 2547.
[21] K.U. Bindseil, T. Henkel, A. Zeeck, D. Bur, D. Niederer, U. Séquin, *Helv. Chim. Acta* **1991,** *74,* 1281.
[22] H. Zähner, H. Drautz, W. Weber, in 'Bioactive Microbial Products Search and Discovery', Eds. J.D. Bu'Lock, J.L. Nisbert, and D.J. Winstanley, Academic Press, London–New York, 1982, p. 51–70.
[23] Y. Romeyke, M. Keller, H. Kluge, S. Grabley, P. Hammann, *Tetrahedron* **1991,** *47,* 3335.
[24] G. Helmchen, *Tetrahedron Lett.* **1974,** *16,* 1527.
[25] M. Gerlitz, P. Hammann, R. Thiericke, J. Rohr, *J. Org. Chem.* **1992,** *57,* 4030.