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Carolactone - a Macrolide Ketocarboxylic Acid Reducing Biofilm Formation by the Caries- and Endocarditis-associated Bacterium *Streptococcus mutans*

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Dedicated to Heinz G. Floss on the occasion of his 75th birthday

Abstract: The macrolide ketocarboxylic acid carolactone (**1**) was isolated from the myxobacterium *Sorangium cellulosum*, strain So ce960, because of its antibiotic activity. Subsequently, carolactone (**1**) was discovered as a highly potent agent against biofilms

containing the caries and endocarditis associated bacterium *Streptococcus mutans*. The 2D structure of **1** was elucidated by HR-MS, IR and 2D NMR spectroscopy. Initially the stereogenic centres were determined by chemical derivatization in combination with

computational methods and finally verified by X-ray analysis.

Keywords: antibiotics • biofilm • *Streptococcus mutans* • structure elucidation • Mosher ester • macrolactone • X-ray analysis

Introduction

Bacterial biofilms often evade host defenses and are inherently resistant to antimicrobial agents. These characteristics impose severe health threats, either because of biofilm growth on natural tissues or medical devices and implants. Since chronic infectious diseases, like endocarditis and periodontitis, or persistent nosocomial infections of implants like stents, bone implants or artificial valves, are associated with microbial biofilms, their eradication has become a major concern in clinical treatment.^[1] Because antibiotic resistance of

bacteria in biofilms is found approximately 1000-fold higher than in planktonic form, there is an urgent need to develop new methods or tools to – ideally – selectively destroy clinically relevant biofilms.^[2]

During our screening efforts to identify antibiotics from myxobacteria^[3], we discovered carolactone (**1**) in the extract of *Sorangium cellulosum*, strain So ce960, because of its activity against the antibiotic-sensitive *E. coli* strain *tolC*.^[4,5] The MIC value for carolactone **1** was determined to be 0.06 µg/mL against *E. coli tolC*, while other bacteria were insensitive, at least up to a concentration of 40 µg/mL. Minor antifungal activity was observed against *Aspergillus niger*, *Pythium debaryanum*, and *Sclerotinia sclerotiorum* in the range of 16 to 20 µg/mL.^[6] Carolactone (**1**) showed no acute toxicity in cell culture assays with L929 mouse fibroblasts.^[7]

In a subsequent screening project based on purified metabolites from myxobacteria, carolactone (**1**) was identified as a highly potent agent severely reducing the number of viable cells in biofilms at nanomolar concentration. Biofilms containing the caries and endocarditis associated bacterium *Streptococcus mutans* were sensitive at 0.005 µg/mL (at which approximately 35% of the cells within the biofilm die; at 0.025 µg/mL 66% die), while planktonic cultures were nearly insensitive to **1**.^[7] Here, we report on the isolation, structure elucidation, stereochemical analysis, and antibiotic activity of carolactone (**1**) produced by *S. cellulosum*, strain So ce960.

Results and Discussion

For production of carolactone (**1**) *S. cellulosum* So ce960, was cultivated in the presence of Amberlite XAD 16 in a medium described recently.^[8] The resin from 100 L of fermentation broth was harvested by sieving and washed with aqueous 30% methanol to remove polar by-products. The crude extract (16.6 g) was

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collected after extraction of the XAD resin with 100% methanol, partitioning between methanol and n-hexane and concentration of the methanol phase. Further enrichment of the biological activity was achieved by gel chromatography on Sephadex LH-20 and by repeated RP MPLC finally yielding carolacton (**1**) (275 mg).

Figure 1. Structure of carolactone (**1**) and NMR assignments

The molecular composition was determined by high-resolution MS which matched well with the NMR data. From $C_{25}H_{40}O_8$ six double bond equivalents were calculated. Two highly abundant carbonyl bands ($\nu = 1731$ and 1715 cm^{-1}) in the IR spectrum of **1** and corresponding carbon signals in the ^{13}C NMR spectrum at δ 172, 175, and 213 ppm (Table 1) indicated the presence of an ester or lactone moiety, respectively, as well as a keto group. The constitution of carolactone (**1**) was finally determined by exploiting several NMR spectroscopic methods. The ^1H , ^{13}C HMQC spectra revealed the correlation of 37 protons and their adjacent carbons, leaving three exchangeable protons.

The correlations in the ^1H , ^1H COSY NMR spectra allowed to establish three structural units A, B, and C (see Figure 1), which were interconnected utilizing the relevant ^1H , ^{13}C HMBC correlations. Since both oxymethine termini (at C9 and C18) of the main unit A were determined to be next neighbors to the carboxy group at C19 ($\delta_{\text{C}} = 175.91$ ppm), the macrolide ring could unequivocally be established. The acylation-dependent downfield shift of 9H ($\delta_{\text{H}} = 4.76$) designated the direction of ring closure. Additional correlations of C9 with the methyl and methine protons at C23 and C7 allowed to connect substructures A and B. Structural element B was initially elucidated from vicinal and long-range correlations in the COSY spectrum and was associated with unit C via the carbonyl group at C5 which was found to be in compliance with the HMBC correlations (Figure 1). The HMBC spectrum also revealed the correlations of the remaining methoxy (C20) and carboxyl group at C1, thus completing the carbon skeleton of carolactone (**1**). Hence, oxymethines C17 and C18 as well as the carboxyl group at C1 remained as those positions bearing exchangeable protons.

The *trans* configuration of the double bonds was assigned from the vicinal coupling constant of 15.5 Hz for the $\Delta^{15,16}$ bond, while ROESY correlations between 6H and 23H₃ as well as 7H and 9H were utilized to determine the *E* configuration at the $\Delta^{7,8}$ bond.

The assignment of configurations of all stereogenic centers initially relied on the chemical derivatization of **1**. To our delight we found that treatment of **1** with *para*-bromobenzoyl chloride led to macrolactonization of the carboxyl group at C1 with the C18 hydroxyl group leading to bislactone **2** in very good yield.

Noteworthy, this transformation resembles a so far unknown Yamaguchi protocol (Scheme 1).^[9] NMR-analysis supported the expected overall rigid conformation which could be employed to further elucidate the structure in combination with computational methods.

Table 1. ^1H and ^{13}C NMR data of carolacton (**1**) in CDCl_3 .^[a]

H	δ_{H}	M	J [Hz]	ROESY ^[b]	C	δ_{C}	M
2H _a	2.67	dd	15.7, 4.2	(3) > 20	1	175.54	s
2H _b	2.45	dd	15.7, 5.9	(3) 21	2	35.81	t
3H	3.75	ddd	8.5, 5.9, 4.2	(2ab, 4) 20, 21	3	80.06	d
4H	3.00	dq	8.5, 6.9	(3), 23, 6 > 7	4	47.27	d
-	-	-	-	-	5	213.29	s
6H	3.50	dq	10.2, 6.8	(22, 7) 23 > 4, 3	6	47.40	d
7H	5.38	dd	10.2, 1.5 (br)	9 > (6), 4 > 10, 22	7	129.36	d
-	-	-	-	-	8	135.80	s
9H	4.76	d	11.3	7 > 23, 12b, 24	9	83.25	d
10H	2.06	tt	11.5, 7.0, 2.6	(24) 23 > 7	10	33.33	d
11H _a	1.77	tt	13.5, 4.0	(11a) 14, 16	11	28.37	t
11H _b	1.00	m	- ^[c]	- ^[c]	-	-	-
12H _a	1.27	m	- ^[d]	- ^[d]	12	18.81	t
12H _b	1.00	m	- ^[c]	- ^[c]	-	-	-
13H _a	1.39	tt	12.8, 4.0	(14) > 25	13	34.39	t
13H _b	1.27	m	- ^[d]	- ^[d]	-	-	-
14H	2.33	dddq	11.1, 9.8, 4.5, 6.6	(25) 16, > (15) 11b, 13a	14	35.66	d
15H	5.47	ddd	15.5, 9.8, 1.9	> 25, 17	15	134.45	d
16H	5.54	dd	15.5, 2.5	18, (17) > 14	16	125.41	d
17H	4.50	dt	3.4, 2.5	18, (16) > 15	17	72.92	d
18H	4.18	d	3.4	(17), 16	18	73.66	d
-	-	-	-	-	19	171.91	s
20H ₃	3.32	s	-	3 > 2a, 4	20	58.01	q
21H ₃	0.94	d	6.8/7	(4) > 3 > 2b	21	12.77	q
22H ₃	1.12	d	6.6	(6) > 7 > 23, 20	22	15.27	q ^[e]
23H ₃	1.72	d	1.1	6 >> 9, 10 > 4, 24	23	12.95	q
24H ₃	0.78	d	7	23, (10), 9 > 12a	24	15.27	q ^[e]
25H ₃	0.99	d	6.6	15 > 13a/b	25	21.64	q

[a] $^1\text{H}/^{13}\text{C}$ at 600/150 MHz; internal reference CDCl_3 signal at $\delta_{\text{H/C}} = 7.27/77.0$ ppm, coupling constants after Gauss multiplication; [b] column shows numbers of correlating protons, > denotes lower signal intensity, coupling vicinal proton numbers given in brackets; [c-e] overlapping signals.

Advantageously, this transformation also protected one hydroxyl group of the diol at C17 and C18 - a prerequisite for the determination of the absolute configuration using the Mosher-ester strategy.^[10] Hence, bicyclic derivative **2** was treated with both enantiomeric MTPA chlorides to form the diastereomeric esters **3a** and **3b** (Scheme 1). Applying the Mosher analysis rules, the absolute configuration at C17 was determined from the calculated $\Delta\delta^{\text{SR}}$ values to be (*R*). The relative configurations of all stereogenic centers of the bislactone **2** were initially achieved by a combination of extended NMR analyses combined with molecular modeling studies (MMFFs; see also supporting information).

Particular focus was first given to the 12-membered ring of carolactone (**1**) and the $^3J_{\text{HH}}$ coupling constants between 9-H/10-H, 14-H/15-H, 16-H/17-H and 17-H/18-H. The small coupling constants (2-4 Hz) between 16-H/17-H and 17-H/18-H correlate well with a dihedral angle of about 60° while the large coupling constants (≈ 10 Hz) between 9-H/10-H and 14-H/15-H refer to dihedral angles of about 160 - 180° . On the basis of these restrictions three possible diastereomers (1st isomer: 9*S*, 10*S*, 14*R*, 17*R*, 18*R*; 2nd isomer: 9*S*, 10*S*, 14*R*, 17*R*, 18*S*; 3rd isomer: 9*S*, 10*S*, 14*S*, 17*S*, 18*S*) were selected for further analysis (Table 2).^[11]

Scheme 1. Macrolactonization of **1**, Mosher-ester formation and relevant $\Delta\delta^{\text{SR}}$ values.

Table 2. Dihedral angle of energy-minimized isomers of lactone ring C9-C19.

$^3J_{HH}$	coupling constant	dihedral angle ϕ of isomers		
		9S, 10S, 14R, 17R, 18R	9S, 10S, 14R, 17R, 18S	9S, 10S, 14S, 17S, 18S
17-H/18-H	~ 4 Hz	54°	69°	57°
16-H/17-H	~ 2 Hz	58°	58°	38°
14-H/15-H	~ 10 Hz	176°	177°	176°
9-H/10-H	~ 10 Hz	174°	178°	177°

The presence of the additional 13-membered ring in bislactone **2** paved the way to include the side chain at C-9 of **1** into the structural analysis. NOESY spectra provided additional distance information that alleviated the choice of potential isomers. In the Monte Carlo searches the dihedral angle between atoms 3-H/4-H that show a large $^3J_{HH}$ coupling constant (≈ 10 Hz) was restricted to 160–180° while the distances between the atom pairs 4-H/7-H and 7-H/10-H were constrained between 2 and 4 Å, accounting for their observed NOE couplings. The calculated structures for all possible diastereomers were then analyzed for discrepancies between their configurations and observed NMR data (i.e. vicinal ¹H coupling constants and NOE couplings). From these structural considerations two diastereomers **2a** and **2b** emerged that only differed in the absolute configuration of the right stereodomain (C3-C10 encircled in orange) with respect to the established domain (C11-C18 encircled in blue) (Figure 2). Importantly, the initial assignment on the stereochemistry of lactone ring C9-C19 could further be confirmed.

In diastereomer **2b** the distance of 4 Å between 12-H_a/24-H₃ is exceptional large; in contrast, relevant NOE-contacts (Figure 2) in the second diastereomer **2a** correspond to distances smaller than 3 Å (Table 3). Assembling all data collected, the complete relative and absolute configuration of **1** could be deduced which is depicted in diastereomer **2a**.

Table 3. Relevant coupling constants and NOE-contacts of diastereomers **2a** and **2b**.

$^3J_{HH}$	Coupling constant	Dihedral angle		Protons	Distances [Å]	
		2a	2b		2a	2b
17-H/18-H	~ 3 Hz	61°	61°	16-H/18-H	2.6	2.7
16-H/17-H	~ 2 Hz	54°	46°	14-H/16-H	2.4	2.4
14-H/15-H	~ 10 Hz	171°	177°	16-H/11-H _a	2.9	2.9
9-H/10-H	~ 12 Hz	176°	159°	15-H/12-H _b	2.7	2.8

6-H/7-H	~ 10 Hz	153°	178°	14-H/11-H _a	2.4	2.3
3-H/4-H	~ 10 Hz	178°	175°	12-H _a /24-H ₃	2.4	4.0
				12-H _b /9-H	2.2	3.1
				10-H/7-H	2.7	3.1
				7-H/4-H	2.3	2.6

Finally, we were able to crystallize carolactone (**1**) from ethyl acetate/petroleum ether. The absolute configuration of **1** is depicted in Figure 2 as the refined result of the X-ray single-crystal structure determination.^[12,13] The relative configuration defined by the spectroscopic and chemical investigations were confirmed by this X-ray analysis. In the solid state the packing of the molecules is characterized by intermolecular hydrogen bridges linking the carboxyl group O1 and O2 with the hydroxyl groups at O5 and O6 of the lactone ring (see Figure 2). Interestingly, our conformational analysis of **1** utilizing NMR data combined with molecular modelling reveal that the conformation **1** is close to identity in solution and in the crystal. Diagnostic data in solution are the expected large vicinal coupling constants between the *trans*-oriented proton pairs 3H/4H, 6H/7H, 9H/10H, and 14H/15H.^[14] In addition, the strongest NOEs in the ROESY spectrum were observed for the proton pairs 9H/7H, 6H/23H₃ and 3H/20H₃ as well as 10H/23H₃, 9/12b.

Figure 2. Resulting isomers **2a** and **2b** from modelling studies on bislactone **2** and selected NOE-contacts (numbering refers to Figure 1). X-ray analysis^[12] and fully assigned structure of carolactone (**1**).

Conclusion

In conclusion, we disclose the isolation and structure elucidation of carolactone (**1**), a metabolite produced by the myxobacterium *Sorangium cellulosum*, strain So ce960, using NMR spectroscopy combined with molecular modeling, chemical derivatization and X-ray analysis. Importantly, carolactone (**1**) shows promise as a lead structure to develop compounds that can reduce the number of viable cells in biofilms of the caries- and endocarditis associated bacterium *Streptococcus mutans*.

Ongoing work in our laboratories aims at the development of carolactone analogues with superior activity and at the elucidation of the mode of action of this fascinating novel class of natural products.

Experimental Section

General: Optical rotation: Perkin Elmer 241 MC polarimeter. – UV: Shimadzu UV/VIS scanning spectrometer UV-2102, solvent methanol [Uvasol, (Merck)]. – IR: Nicolet FT-IR spectrometer 20 DXB. – NMR: Bruker spectrometer DMX 600 (¹H: 600.1 MHz, ¹³C: 150.9 MHz), DRX-500 (¹H: 5400 MHz, ¹³C: 125 MHz) and DPX-400 (¹H: 400 MHz, ¹³C: 100 MHz); internal standard was the solvent signal. Mass spectrometry: EI or DCI: Finnigan spectrometer MAT 95 (EI with 70 eV) (DCI with isobutane), resolution $M/\Delta M = 1000$; high-resolution data from peak matching ($M/\Delta M = 10000$). Biological test: MIC values were determined by serial dilution according to published procedures.^[6]

Analytical thin-layer chromatography was performed using silica gel 60 F₂₅₄ plates (Merck, Darmstadt), and the spots were visualized with UV light at 254 nm or by staining with vanillin/H₂SO₄ in methanol or KMnO₄ in water. RP-HPLC was performed on RP18 with water/methanol gradient elution.

All compounds prepared were isolated after RP-HPLC chromatography (LiChrospher RP18 with water/methanol gradient elution) as pure homogeneous substances according to TLC, and NMR analyses and identified by appropriate spectroscopic methods (¹H and ¹³C NMR, high resolution MS).

Molecular modeling studies were performed using MacroModel (version 7.5) and the MMFF94S force field, together with the generalized Born/Surface area (GB/SA) chloroform solvent model. Structures were subjected to a minimization procedure to the nearest local minimum prior to the generation of new local energy conformers by Monte Carlo searching.

Isolation: Fermentation (100 L) of *S. cellulosum*, So ce960, was performed in presence of 1.5 L Amberlite XAD 16. The resin was harvested by filtration in a process filter (210 µm), washed with water to remove adherent cells, with 30% methanol in water to remove polar constituents and finally eluted with methanol (8 L). After evaporation of the methanol the residual water layer was extracted three times with ethyl acetate. The organic layer was dried with anhydrous sodium sulphate, filtrated, concentrated *in vacuo*, redissolved in methanol and extracted with n-hexane. After removal of the hexane layer the methanol was evaporated to give a crude extract (16.6 g). Further enrichment was achieved by chromatography on Sephadex LH-20 (column 8×79 cm, eluent methanol, flow 28 mL/min). Based on TLC analyses (UV absorption) a fraction (8.0 g) containing carolactone (**1**) was collected and separated further by RP chromatography [Merck Prepar 100 chromatography system; column 10×40 cm, ODS AQ, 120 Å, 15µm (YMC); solvent: methanol/ammonium acetate buffer adjusted to pH 5, 57:43, flow 170 mL/min, UV detection: 210 nm]. The fraction containing carolactone (1.3 g) was further purified by preparative RP MPLC [column 3×48 cm, ODS AQ, 120 Å, 16 µm (Kronlab), solvent: acetonitrile/0.05 M ammonium acetate buffer adjusted to pH 5, 65:35, flow 17 mL/min, UV detection at 206 nm] yielding carolactone (275 mg) after evaporation.

Carolactone (1): C₂₅H₄₀O₈ (468.58).- M.p.: 142 °C (dichloromethane/petroleum ether).- Optical rotation: $[\alpha]_D^{20} = -205$ ($c = 1.07$ in MeOH).- IR (KBr): $\nu = 3419$ (w), 2962 (m), 2931 (m), 1731 (s), 1715 (s), 1456 (w), 1375 (w),

1196 (m), 1127 (w), 1089 (m) cm⁻¹.- UV (MeOH): $\lambda_{\max}$ (lg ϵ) = 204 (4.06), 259 (2.35), 290 (2.58) nm.- TLC (silica gel 254 nm): ethyl acetate/methanol/water 65/30/10, $R_f = 0.62$.- HPLC: solvent A 95/5 water/acetonitrile + 5 mM NH₄Ac, pH 5.5, solvent B 5/95 water/acetonitrile + 5 mM NH₄Ac, pH 5.5; gradient from 10% B to 100% B in 30 min, 10 min 100% B; column: 2×125 mm, Nucleosil 120 5µm C18 (Macherey Nagel), flow 0.3 mL/min; $R_t = 12.8$ min.- ¹H and ¹³C NMR data: see table 1.- ESI-HRMS: [C₂₅H₄₀O₈+H]⁺ calcd.: 469.2796, found: 469.2806.

Bislactone 2: To a solution of carolactone (**1**) (7.5 mg, 16 µmol; 1 eq.) and a catalytical amount of DMAP in CH₂Cl₂ (0.8 mL) was added dropwise a solution of *para*-bromobenzoyl chloride (3.5 mg, 16 µmol; 1 eq.) in CH₂Cl₂ (0.8 mL) at -19 °C. After addition of three drops triethylamine the solution was stirred for 2.5 h at -10 °C. Subsequently, the reaction mixture was terminated by adding saturated ammonium chloride solution at -10 °C. After warming to roomtemperature the solution was washed with brine (1.5 mL). The organic layer was separated and the aqueous layer was extracted twice with CH₂Cl₂ (2 mL). The combined organic layers were concentrated *in vacuo* and purified directly by semi-preparative HPLC (RP-18; H₂O/MeCN = 50:50 → 0:100 in 30 min; 2 t_r = 19 min). Bislactone **2** was collected as a colourless solid (5.1 mg, 11.3 µmol, 71 %). $R_f = 0.72$ (CH₂Cl₂/MeOH = 10:1); $[\alpha]_D^{20} = -32.2^\circ$ ($c = 0.32$, CH₂Cl₂); ¹H NMR (C₆D₆, 500 MHz): $\delta = 5.63$ (brs, 1H, 18-H), 5.59 (ddd, $J = 15.4, 10.0, 2.0$ Hz, 1H, 15-H), 5.54 (dd, $J = 9.5, 1.5$ Hz, 1H, 7-H), 5.09 (dd, $J = 15.4, 2.7$ Hz, 1H, 16-H), 4.91 (d, $J = 11.1$ Hz, 1H, 9-H), 4.52 (ddd, $J = 9.9, 2.7, 2.0$ Hz, 1H, 17-H), 4.11 (d, $J = 9.9$ Hz, 1H, OH), 3.32 (dq, $J = 9.5, 7.2$ Hz, 1H, 6-H), 3.33-3.24 (m, 1H, 3-H), 3.10 (dq, $J = 9.5, 6.8$ Hz, 1H, 4-H), 3.03 (s, 3H, 20-H₃), 2.49 (dd, $J = 12.4, 5.3$ Hz, 1H, 2-H_a), 2.19-2.09 (m, 1H, 14-H), 1.95-1.87 (m, 1H, 10-H), 1.84 (dd, $J = 12.4, 0.8$ Hz, 1H, 2-H_b), 1.59 (d, $J = 1.5$ Hz, 3H, 23-H₃), 1.24-1.12 (m, 2H, 13-H₂), 1.12-1.04 (m, 1H, 12-H_a), 0.98 (d, $J = 7.2, 3$ Hz, 22-H₃), 0.96 (d, $J = 6.8$ Hz, 3H, 21-H₃), 0.95-0.87 (m, 1H, 12-H_b), 0.91 (d, $J = 6.6$ Hz, 3H, 25-H₃), 0.77-0.69 (m, 1H, 11-H_b), 0.70 (d, $J = 7.2$ Hz, 3H, 24-H₃); ¹³C NMR (C₆D₆, 125 MHz): $\delta = 211.3$ (s, C5), 171.8 (s, C1), 167.8 (s, C19), 139.7 (s, C8), 136.6 (d, C15), 127.7 (d, C16), 126.4 (d, C7), 81.8 (d, C3), 78.5 (d, C9), 73.2 (d, C18), 71.2 (d, C17), 57.1 (q, C20), 49.2 (d, C6), 43.0 (d, C4), 38.1 (d, C10), 35.9 (d, C14), 35.4 (t, C13), 33.3 (t, C2), 29.7 (t, C11), 22.6 (q, C25), 18.7 (q, C23), 18.6 (t, C12), 17.7 (q, C22), 15.6 (q, C21), 15.2 (q, C24); HRMS (ESI): m/z for C₂₅H₃₈O₇-Na [M+Na]⁺: calculated 473.2531, found 473.2515.

(S)-Mosher-ester 3a: To a solution of bicyclic derivative **2** (2.1 mg, 4.7 µmol; 1 eq.) and a catalytically amount of DMAP in CH₂Cl₂ (0.4 mL) was added successively (R)-(-)-MTPA-Cl (9 µL, 48.0 µmol; 10 eq.) and triethylamine (10 µL, 70 µmol, 15 eq.) at rt. After stirring for 23 h at 30 °C the reaction mixture was concentrated *in vacuo* and purified directly by semi-preparative HPLC (RP-18; H₂O/MeCN = 50 : 50 → 0 : 100 in 30 min; 3a t_r = 28 min) to afford (S)-Mosher-ester **3a** as a colourless oil (2.8 mg, 4.3 µmol, 89%). $R_f = 0.24$ (PE/EE = 5:1); ¹H NMR (CDCl₃, 400 MHz): $\delta = 7.65$ -7.62 (m, 2H), 7.42-7.38 (m, 3H), 5.97 (ddd, $J = 3.2, 3.2, 1.4$ Hz, 1H), 5.61 (d, $J = 3.2$ Hz, 1H), 5.55 (dd, $J = 15.5, 3.2$ Hz, 1H), 5.46 (ddd, $J = 15.5, 9.4, 1.4$ Hz, 1H), 5.32 (dq, $J = 9.5, 1.2$ Hz, 1H), 4.78 (d, $J = 11.2$ Hz, 1H), 3.59 (s, 3H), 3.55-3.45 (m, 1H), 3.24 (dq, $J = 9.5, 7.2$ Hz, 1H), 2.92 (s, 3H), 2.95-2.86 (m, 1H), 2.79 (dd, $J = 12.6, 5.3$ Hz, 1H), 2.37-2.26 (m, 1H), 2.26 (dd, $J = 12.6, 1.8$ Hz, 1H), 1.98-1.89 (m, 1H), 1.78-1.72 (m, 1H), 1.61 (d, $J = 1.2$ Hz, 3H), 1.39-1.23 (m, 3H), 1.17 (d, $J = 7.2$ Hz, 3H), 1.04 (d, $J = 6.8$ Hz, 3H), 0.95-0.87 (m, 2H), 0.92 (d, $J = 6.7$ Hz, 3H), 0.86 (d, $J = 7.0$ Hz, 3H); HRMS (ESI): m/z for C₃₅H₄₅O₇-Na [M+Na]⁺: calculated 689.7114, found 689.7131.

(R)-Mosher-ester 3b: To a solution of bicyclic derivative **2** (2.0 mg, 4.4 µmol; 1 eq.) in pyridine was added dropwise (S)-(+)-MTPA-Cl (7 µg, 37.4 µmol; 8.5 eq.) at rt and stirred for 19 h. After another dropwise addition of (S)-(+)-MTPA-Cl (7 µL, 37.4 µmol; 8.5 eq.) the solution was stirred for 24 h at 36 °C. The reaction mixture was diluted with a saturated solution of copper sulfate (0.5 mL) and extracted two times with diethyl ether (1 mL). The combined organic layers were washed with water (1 mL) and brine (1 mL), dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The residue was purified by semi-preparative HPLC (RP-18; H₂O/MeCN = 50 : 50 → 0 : 100 in 30 min; 3b t_r = 28 min) to afford (R)-Mosher-ester **3b** as a colourless oil (0.6 mg, 0.9 µmol, 21% or 97% brsm). $R_f = 0.24$ (PE/EE = 5:1); ¹H NMR (CDCl₃, 400 MHz): $\delta = 7.68$ -7.65 (m, 2H), 7.39-7.45 (m, 3H), 5.95 (ddd, $J = 3.2, 3.0, 1.8$ Hz, 1H), 5.62 (d, $J = 3.0$ Hz, 1H), 5.45 (dd, $J = 15.4, 3.2$ Hz, 1H), 5.33 (dq, $J = 9.2, 1.3$ Hz, 1H), 4.99 (ddd, $J = 15.4, 10.2, 1.8$ Hz, 1H), 4.75 (d, $J = 11.0$ Hz, 1H), 3.79 (s, 3H), 3.62-3.53 (m, 1H), 3.27 (dq, $J = 9.5, 7.1$ Hz, 1H), 3.09 (s, 3H), 2.89 (dq, $J = 9.1, 6.8$ Hz, 1H), 2.84 (dd, $J = 12.7, 5.3$ Hz, 1H), 2.32 (dd, $J = 12.7, 2.0$ Hz, 1H), 2.26-2.17 (m, 1H), 1.93-1.89 (m, 1H), 1.76-1.67 (m, 1H), 1.63 (d, $J = 1.3$ Hz, 3H), 1.35-1.20 (m,

3H), 1.18 (d, $J = 7.1$ Hz, 3H), 1.06 (d, $J = 6.8$ Hz, 3H), 0.90-0.75 (m, 2H), 0.85 (d, $J = 7.0$ Hz, 3H), 0.78 (d, $J = 6.7$ Hz, 3H); HRMS (ESI): m/z für $C_{35}H_{45}O_9Na$ $[M+Na]^+$: calculated 689.7114, found 689.7139.

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[13] X-ray structure determination. A well bloc shaped colourless crystal (0.34 x 0.57 x 0.9 mm³) was selected for data collection. The first data set was gathered at 153 K on a X8-Apex Bruker-AXS diffractometer (Mo $K\alpha$ radiation). The 21641 in the range from 1.5 to 32.4° θ collected reflections yielding in 9514 independent reflections ($R_{int} = 0.023$). We determined the acentric orthorhombic space group $P2_12_12_1$ with a unit cell of $a = 6.2270(2)$ Å, $b = 14.5886(6)$ Å, $c = 29.339(1)$ Å. The structure was solved by direct methods^[16], showing the position of all carbon and oxygen atoms. Subsequent refinement revealed the position of all hydrogen atoms in Difference Fourier maps. Full-matrix least-squares refinement against F_o^2 with anisotropic thermal parameters and free refinement of the hydrogen positions (458 parameter) resulting in $R1 = 0.048$ and $wR2 = 0.12$ with $I > 2\sigma_I$. There was one remaining difference peak of around one electron (the other peaks were of around 0.3 e and placed at atom bonds) resulted from a little disorder of O(2). This may be reasonable because of a possible alternative hydrogen bridge to O(7) in the solid state packing.

To determine the absolute structure we collected a second data set with Cu $K\alpha$ radiation. This was done on a 4-circle Stoe Stadi4 diffractometer at 297 K. In the range from 3 to 50° θ 6041 reflection were collected obtaining 2796 ($R_{int} = 0.02$) independent reflections. An empirical absorption correction on psi-scans was applied. To keep the reflex to parameter ratio reliable we reduced the number of parameters to 324 using rigid hydrogen position in the full-matrix least-squares refinement. We applied also a split position to O2 and obtained so for the final refinement residuals of $R1 = 0.04$ and $wR2 = 0.106$. The Flack's absolute structure parameter was determined to 0.0(3). In conjunction with the spectroscopic and chemical investigation results there is no doubt that the determined structure represents the correct enantiomeric form.

Further details of the crystal structure investigation are available from Cambridge Crystallographic Data Center, on quoting the depository number CCDC 735883.

[14] The values of the coupling constant J increase with the conformational stability of the side chain, i.e. 8.5, 10.2, 11.3 Hz, respectively.

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Ketocarboxylic Acid Reducing
Biofilm Formation by the Caries-
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Bacterium *Streptococcus mutans***

Targeting Biofilms: The structure elucidation of carolactone – the first highly potent compound from myxobacteria severely reducing the number of viable cells in biofilms - was achieved by a combination of NMR analyses, chemical derivatization, molecular modeling, and X-ray analysis which allowed confirming the absolute configuration of all stereogenic centers.

Supporting information

Carolactone - a Macrolide Ketocarboxylic Acid Reducing Biofilm Formation by the Caries- and Endocarditis-associated Bacterium *Streptococcus mutans*

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1. Tables of NMR data

Bislactone derivative 2:

Table S1. ^1H and ^{13}C NMR data of bicyclic derivative **2** in C_6D_6 .^a

H	δ_{H}	m	J [Hz]	NOESY ^b	C	δ_{C}	m
-	-	-	-	-	1	171.8	s
2-H _a	2.49	dd	12.4, 5.3	(3), 20	2	33.3	t
2-H _b	1.84	dd	12.4, 0.8	(3), 4, 21			
3-H	3.33-3.24	m	-	(2ab), 20, 21	3	81.8	d
4-H	3.10	dq	9.5, 6.8	(2b), 7, 21	4	43.0	d
-	-	-	-	-	5	211.3	s
6-H	3.32	dq	9.5, 7.2	(22), 23	6	49.2	d
7-H	5.54	dd	9.5, 1.5	22, 10, 4	7	126.4	d
-	-	-	-	-	8	139.7	s
9-H	4.91	d	11.1	12b, 10, 23, 24	9	78.5	d
10-H	1.95-1.87	m	-	(11ab), (9), 7, (24)	10	38.1	d
11-H _a	1.73-1.65	m	-	16, 14, (11b), (10)	11	29.7	t
11-H _b	0.77-0.69	m	-	13ab, (12a), (11a), (10)			
12-H _a	1.12-1.04	m	-	(12b), (11b), 24	12	18.6	t ^e
12-H _b	0.95-0.87	m	- ^c	(12a), 15, 9			
13-H ₂	1.24-1.12	m	- ^d	(14), 15, 11b, 25	13	35.4	t
14-H	2.19-2.09	m	-	16, (15), (13), 11a, (25)	14	35.9	d
15-H	5.59	ddd	15.4, 10.0, 2.0	17, (14), 13, 12b, 25, OH	15	136.6	d

16-H	5.09	dd	15.4, 2.7	18,(17), 14, 12a, 25	16	127.7	d
17-H	4.52	ddd	9.9, 2.7, 2.0	15, (16), (18)	17	71.2	d
18-H	5.63	brs	-	16, (17)	18	73.2	d
-	-	-	-	-	19	167.8	s
20-H ₃	3.03	s	-	2a, 3	20	57.1	q
21-H ₃	0.96	d	6.8	2b, 3, (4)	21	15.6	q
22-H ₃	0.98	d	7.2	(6), 7	22	17.7	q
23-H ₃	1.59	d	1.5	6, 9, 24	23	18.7	q ^c
24-H ₃	0.7	d	7.2	9, (10), 12a, 23	24	15.2	q
25-H ₃	0.91	d	6.6	13, (14), 15, 16	25	22.6	q
OH	4.11	d	9.9	15	-	-	-

^a ¹H/¹³C at 500/125 MHz; internal reference C₆D₆ signal at $\delta_{\text{H/C}} = 7.16/128.06$ ppm, coupling constants after Gauss multiplication; ^b column shows numbers of correlating protons, coupling vicinal or geminal proton numbers given in brackets; ^{c,d,e} overlapping signals.

Mosher-ester **3a/b** $\Delta\delta^{\text{SR}}$ values:

Table S2. Selected ¹H NMR data of Mosher-esters (**3a**) and (**3b**) in CDCl₃^a.

H	(S)-Mosher-ester 3a		(R)-Mosher-ester 3b		$\Delta\delta^{\text{SR}}$
	δ_{H} [ppm] ^b	m, J [Hz]	δ_{H} [ppm] ^b	m, J [Hz]	
2-H _a	2.79	dd, 12.6, 5.3	2.84	dd, 12.7, 5.3	-0.05
2-H _b	2.26	12.6, 1.8	2.32	dd, 12.7, 2.0	-0.06
3	3.50	m	3.58	m	-0.08
6	3.24	dq, 9.5, 7.2	3.27	dq, 9.5, 7.1	-0.03
7	5.32	dq, 9.5, 1.2	5.33	dq, 9.2, 1.3	-0.01
10	1.93	m	1.91	m	+0.02
11-H _a	1.75	m	1.72	m	+0.03
14	2.32	m	2.21	m	+0.11
15	5.46	ddd, 15.5, 9.4, 1.4	4.99	ddd, 15.4, 10.2, 1.8	+0.47
16	5.55	dd, 15.5, 3.2	5.45	dd, 15.4, 3.2	+0.10
18	5.61	d, 3.2	5.62	d, 3.0	-0.01
20-H ₃	2.92	s	3.09	s	-0.17
21-H ₃	1.04	d, 6.8	1.06	d, 6.8	-0.02
22-H ₃	1.17	d, 7.2	1.18	d, 7.1	-0.01
23-H ₃	1.61	d, 1.2	1.63	d, 1.3	-0.02
24-H ₃	0.86	d, 7.0	0.85	d, 7.0	+0.01
25-H ₃	0.92	d, 6.7	0.78	d, 6.7	+0.14

^a ¹H at 400 MHz; internal reference CDCl₃ signal at $\delta_{\text{H}} = 7.26$ ppm, coupling constants after Gauss multiplication; ^b for multiplets the average chemical shift was taken.

2. X-ray Analysis

Crystal data and structure refinement for Carolactone (**1**).

Identification code	sh2814	
Empirical formula	C ₂₅ H ₄₀ O ₈	
Formula weight	468.57	
Temperature	153(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	P2(1)2(1)2(1)	
Unit cell dimensions	a = 6.2270(2) Å	$\alpha = 90^\circ$
	b = 14.5886(6) Å	$\beta = 90^\circ$
	c = 29.3390(11) Å	$\gamma = 90^\circ$
Volume	2665.25(17) Å ³	
Z	4	
Density (calculated)	1.168 Mg/m ³	
Absorption coefficient	0.086 mm ⁻¹	
F(000)	1016	
Crystal size	0.91 x 0.57 x 0.34 mm ³	
Theta range for data collection	1.39 to 32.36°	
Index ranges	-9 ≤ h ≤ 4, -19 ≤ k ≤ 21, -37 ≤ l ≤ 44	
Reflections collected	21641	
Independent reflections	9514 [R(int) = 0.0232]	
Completeness to theta = 32.36°	99.9 %	
Absorption correction	None	
Max. and min. transmission	0.9710 and 0.9262	
Refinement method	Full-matrix least-squares on F ²	
Data / restraints / parameters	9514 / 0 / 458	
Goodness-of-fit on F ²	1.034	
Final R indices [I > 2σ(I)]	R1 = 0.0479, wR2 = 0.1237	
R indices (all data)	R1 = 0.0605, wR2 = 0.1341	
Absolute structure parameter	-0.3(6)	
Largest diff. peak and hole	1.157 and -0.484 e.Å ⁻³	

X-ray structure of Carolactone (1).

Table S3a. Bond lengths (Å).

O(1)-C(1)	1.2072(19)	C(2)-C(3)	1.530(2)	C(10)-C(11)	1.536(2)
O(2)-C(1)	1.332(2)	C(3)-C(4)	1.524(2)	C(10)-C(24)	1.536(2)
O(3)-C(20)	1.422(2)	C(4)-C(21)	1.527(2)	C(11)-C(12)	1.533(2)
O(3)-C(3)	1.4360(19)	C(4)-C(5)	1.528(2)	C(12)-C(13)	1.525(2)
O(4)-C(5)	1.2126(19)	C(5)-C(6)	1.525(2)	C(13)-C(14)	1.540(2)
O(5)-C(17)	1.4231(18)	C(6)-C(7)	1.503(2)	C(14)-C(15)	1.505(2)
O(6)-C(18)	1.4199(17)	C(6)-C(22)	1.527(2)	C(14)-C(25)	1.531(2)
O(7)-C(19)	1.2048(16)	C(7)-C(8)	1.3325(19)	C(15)-C(16)	1.330(2)
O(8)-C(19)	1.3420(16)	C(8)-C(23)	1.5053(19)	C(16)-C(17)	1.502(2)
O(8)-C(9)	1.4721(16)	C(8)-C(9)	1.5055(19)	C(17)-C(18)	1.543(2)
C(1)-C(2)	1.507(2)	C(9)-C(10)	1.5325(19)	C(18)-C(19)	1.5223(18)

Table S3b. Bond angles (°).

C(20)-O(3)-C(3)	112.98(12)	C(7)-C(6)-C(5)	109.03(12)	C(12)-C(13)-C(14)	115.56(12)
C(19)-O(8)-C(9)	116.15(10)	C(7)-C(6)-C(22)	110.36(13)	C(15)-C(14)-C(25)	110.63(15)
O(1)-C(1)-O(2)	122.17(14)	C(5)-C(6)-C(22)	111.76(13)	C(15)-C(14)-C(13)	112.93(12)
O(1)-C(1)-C(2)	124.83(15)	C(8)-C(7)-C(6)	127.81(13)	C(25)-C(14)-C(13)	108.61(13)
O(2)-C(1)-C(2)	112.71(14)	C(7)-C(8)-C(23)	124.79(13)	C(16)-C(15)-C(14)	123.31(14)
C(1)-C(2)-C(3)	115.47(13)	C(7)-C(8)-C(9)	118.34(11)	C(15)-C(16)-C(17)	124.63(13)
O(3)-C(3)-C(4)	104.62(12)	C(23)-C(8)-C(9)	116.85(12)	O(5)-C(17)-C(16)	111.99(12)
O(3)-C(3)-C(2)	109.05(13)	O(8)-C(9)-C(8)	110.34(11)	O(5)-C(17)-C(18)	108.32(10)
C(4)-C(3)-C(2)	114.44(14)	O(8)-C(9)-C(10)	106.12(10)	C(16)-C(17)-C(18)	113.56(12)
C(3)-C(4)-C(21)	113.12(13)	C(8)-C(9)-C(10)	114.06(11)	O(6)-C(18)-C(19)	110.35(11)
C(3)-C(4)-C(5)	106.92(12)	C(9)-C(10)-C(11)	112.69(11)	O(6)-C(18)-C(17)	109.93(11)
C(21)-C(4)-C(5)	110.44(14)	C(9)-C(10)-C(24)	109.02(13)	C(19)-C(18)-C(17)	110.83(11)
O(4)-C(5)-C(6)	121.59(15)	C(11)-C(10)-C(24)	111.82(13)	O(7)-C(19)-O(8)	125.25(12)
O(4)-C(5)-C(4)	120.97(14)	C(12)-C(11)-C(10)	114.23(12)	O(7)-C(19)-C(18)	123.21(12)
C(6)-C(5)-C(4)	117.45(13)	C(13)-C(12)-C(11)	114.26(12)	O(8)-C(19)-C(18)	111.48(11)

3. Molecular Modeling

Molecular modeling studies were performed using MacroModel (version 7.5) and the MMFF94S force field, together with the generalized Born/Surface area (GB/SA) chloroform solvent model. Structures were subjected to a minimization procedure to the nearest local minimum prior to the generation of new local energy conformers by Monte Carlo searching.

Lowest energy conformer of isomer 9*S*, 10*S*, 14*R*, 17*R*, 18*R* (1,000 steps Monte Carlo search):

REMARK	1	PDB output from SCARECROW								
REMARK	1	=====								
ATOM	1	C1	C1	S	0	-8.157	-0.360	-3.157	0.00	0.00
ATOM	2	C2	C2	S	0	-8.915	-0.280	-1.505	0.00	0.00
ATOM	3	C3	C3	S	0	-4.312	1.037	-2.842	0.00	0.00
ATOM	4	C4	C4	S	0	-5.397	1.181	-4.603	0.00	0.00
ATOM	5	C5	C5	S	0	-7.455	1.205	-4.253	0.00	0.00
ATOM	6	C6	C6	S	0	-9.471	-1.931	-0.436	0.00	0.00
ATOM	7	C7	C7	S	0	-8.485	-2.037	1.403	0.00	0.00
ATOM	8	C8	C8	S	0	-6.418	-1.815	1.339	0.00	0.00
ATOM	9	C9	C9	S	0	-5.468	-3.211	0.140	0.00	0.00
ATOM	10	C10	C10	S	0	-3.401	-2.946	-0.065	0.00	0.00
ATOM	11	C11	C11	S	0	-2.874	-1.072	-0.865	0.00	0.00
ATOM	12	C12	C12	S	0	-0.885	-0.956	-1.403	0.00	0.00
ATOM	13	C13	C13	S	0	0.248	0.223	-0.604	0.00	0.00
ATOM	14	O1	O1	S	0	-3.957	-0.736	-2.454	0.00	0.00
ATOM	15	O2	O2	S	0	-3.805	2.338	-1.948	0.00	0.00
ATOM	16	C14	C14	S	0	-0.228	-2.181	-2.893	0.00	0.00
ATOM	17	C15	C15	S	0	-2.424	-3.313	1.727	0.00	0.00
ATOM	18	O3	O3	S	0	-4.917	2.811	-5.552	0.00	0.00
ATOM	19	C16	C16	S	0	-11.521	-1.924	-0.145	0.00	0.00
ATOM	20	O4	O4	S	0	-7.942	2.897	-3.441	0.00	0.00
ATOM	21	H1	H1	S	0	1.177	-1.890	-3.265	0.00	0.00
ATOM	22	H2	H2	S	0	-1.050	-2.009	-4.112	0.00	0.00
ATOM	23	H3	H3	S	0	-0.290	-3.598	-2.475	0.00	0.00
ATOM	24	H4	H4	S	0	1.660	0.324	-0.991	0.00	0.00
ATOM	25	H5	H5	S	0	-0.215	1.135	0.451	0.00	0.00
ATOM	26	H6	H6	S	0	-3.174	-0.044	0.163	0.00	0.00
ATOM	27	H7	H7	S	0	-2.980	-4.002	-1.021	0.00	0.00
ATOM	28	H8	H8	S	0	-6.060	-3.210	-1.217	0.00	0.00
ATOM	29	H9	H9	S	0	-5.729	-4.567	0.687	0.00	0.00
ATOM	30	H10	H10	S	0	-5.904	-1.929	2.725	0.00	0.00
ATOM	31	H11	H11	S	0	-6.100	-0.440	0.893	0.00	0.00

ATOM	32	H12	H12	S	0	-8.805	-3.341	2.042	0.00	0.00
ATOM	33	H13	H13	S	0	-9.022	-0.992	2.311	0.00	0.00
ATOM	34	H14	H14	S	0	-5.021	0.048	-5.480	0.00	0.00
ATOM	35	H15	H15	S	0	-9.146	-3.157	-1.206	0.00	0.00
ATOM	36	H16	H16	S	0	-8.136	1.191	-5.571	0.00	0.00
ATOM	37	H17	H17	S	0	-9.088	1.019	-0.830	0.00	0.00
ATOM	38	H18	H18	S	0	-7.977	-1.680	-3.788	0.00	0.00
ATOM	39	H19	H19	S	0	-0.958	-3.394	1.529	0.00	0.00
ATOM	40	H20	H20	S	0	-2.689	-2.248	2.720	0.00	0.00
ATOM	41	H21	H21	S	0	-2.859	-4.604	2.310	0.00	0.00
ATOM	42	H22	H22	S	0	-11.949	-3.134	0.593	0.00	0.00
ATOM	43	H23	H23	S	0	-11.958	-0.738	0.629	0.00	0.00
ATOM	44	H24	H24	S	0	-12.239	-1.909	-1.442	0.00	0.00
ATOM	45	H25	H25	S	0	-9.257	2.909	-3.374	0.00	0.00
ATOM	46	H26	H26	S	0	-5.351	3.782	-4.759	0.00	0.00

Lowest energy conformer of isomer 9S, 10S, 14R, 17R, 18S (1,000 steps Monte Carlo search):

REMARK	1	PDB output from SCARECROW								
REMARK	1	=====								
ATOM	1	C1	C1	S	0	-9.490	-0.874	0.600	0.00	0.00
ATOM	2	C2	C2	S	0	-10.139	-0.947	2.234	0.00	0.00
ATOM	3	C3	C3	S	0	-6.075	0.872	0.911	0.00	0.00
ATOM	4	C4	C4	S	0	-7.066	0.874	-0.839	0.00	0.00
ATOM	5	C5	C5	S	0	-9.055	0.743	-0.456	0.00	0.00
ATOM	6	C6	C6	S	0	-10.309	-2.642	3.244	0.00	0.00
ATOM	7	C7	C7	S	0	-9.214	-2.619	4.946	0.00	0.00
ATOM	8	C8	C8	S	0	-7.289	-2.052	4.765	0.00	0.00
ATOM	9	C9	C9	S	0	-6.219	-3.167	3.483	0.00	0.00
ATOM	10	C10	C10	S	0	-4.265	-2.641	3.295	0.00	0.00
ATOM	11	C11	C11	S	0	-4.002	-0.734	2.622	0.00	0.00
ATOM	12	C12	C12	S	0	-2.103	-0.261	2.233	0.00	0.00
ATOM	13	C13	C13	S	0	-1.301	1.072	3.058	0.00	0.00
ATOM	14	O1	O1	S	0	-4.957	-0.515	1.005	0.00	0.00
ATOM	15	O2	O2	S	0	-6.307	1.976	2.056	0.00	0.00
ATOM	16	C14	C14	S	0	-1.153	-1.318	0.864	0.00	0.00
ATOM	17	C15	C15	S	0	-3.267	-2.969	5.007	0.00	0.00
ATOM	18	O3	O3	S	0	-6.555	-0.484	-2.027	0.00	0.00
ATOM	19	C16	C16	S	0	-12.237	-2.997	3.663	0.00	0.00
ATOM	20	O4	O4	S	0	-9.699	2.287	0.381	0.00	0.00
ATOM	21	H1	H1	S	0	0.157	-0.794	0.600	0.00	0.00
ATOM	22	H2	H2	S	0	-1.874	-1.307	-0.376	0.00	0.00
ATOM	23	H3	H3	S	0	-0.993	-2.676	1.290	0.00	0.00
ATOM	24	H4	H4	S	0	0.050	1.420	2.778	0.00	0.00

ATOM	25	H5	H5	S	0	-1.971	1.857	4.037	0.00	0.00
ATOM	26	H6	H6	S	0	-4.499	0.154	3.636	0.00	0.00
ATOM	27	H7	H7	S	0	-3.735	-3.542	2.307	0.00	0.00
ATOM	28	H8	H8	S	0	-6.817	-3.129	2.181	0.00	0.00
ATOM	29	H9	H9	S	0	-6.286	-4.541	3.901	0.00	0.00
ATOM	30	H10	H10	S	0	-6.693	-2.125	6.069	0.00	0.00
ATOM	31	H11	H11	S	0	-7.245	-0.670	4.386	0.00	0.00
ATOM	32	H12	H12	S	0	-9.253	-3.930	5.533	0.00	0.00
ATOM	33	H13	H13	S	0	-9.837	-1.737	5.899	0.00	0.00
ATOM	34	H14	H14	S	0	-6.763	2.124	-1.476	0.00	0.00
ATOM	35	H15	H15	S	0	-9.843	-3.735	2.434	0.00	0.00
ATOM	36	H16	H16	S	0	-9.757	0.633	-1.705	0.00	0.00
ATOM	37	H17	H17	S	0	-10.498	0.249	2.922	0.00	0.00
ATOM	38	H18	H18	S	0	-9.147	-2.096	-0.056	0.00	0.00
ATOM	39	H19	H19	S	0	-1.850	-2.875	4.805	0.00	0.00
ATOM	40	H20	H20	S	0	-3.627	-2.019	6.017	0.00	0.00
ATOM	41	H21	H21	S	0	-3.537	-4.283	5.511	0.00	0.00
ATOM	42	H22	H22	S	0	-12.382	-4.241	4.361	0.00	0.00
ATOM	43	H23	H23	S	0	-12.797	-1.962	4.484	0.00	0.00
ATOM	44	H24	H24	S	0	-13.016	-3.076	2.461	0.00	0.00
ATOM	45	H25	H25	S	0	-8.983	2.483	1.425	0.00	0.00
ATOM	46	H26	H26	S	0	-5.296	-0.641	-1.874	0.00	0.00

Lowest energy conformer of isomer 9S, 10S, 14S, 17S, 18S (1,000 steps Monte Carlo search):

REMARK	1	PDB output from SCARECROW								
REMARK	1	=====								
ATOM	1	C1	C1	S	0	11.152	-1.783	-0.236	0.00	0.00
ATOM	2	C2	C2	S	0	11.580	-1.994	1.346	0.00	0.00
ATOM	3	C3	C3	S	0	8.254	-0.174	-1.584	0.00	0.00
ATOM	4	C4	C4	S	0	9.791	0.859	-1.231	0.00	0.00
ATOM	5	C5	C5	S	0	11.343	-0.241	-1.255	0.00	0.00
ATOM	6	C6	C6	S	0	11.169	-3.536	2.303	0.00	0.00
ATOM	7	C7	C7	S	0	9.911	-3.183	3.683	0.00	0.00
ATOM	8	C8	C8	S	0	8.315	-2.318	3.144	0.00	0.00
ATOM	9	C9	C9	S	0	7.265	-3.313	1.927	0.00	0.00
ATOM	10	C10	C10	S	0	5.695	-2.366	1.384	0.00	0.00
ATOM	11	C11	C11	S	0	5.944	-1.440	-0.281	0.00	0.00
ATOM	12	C12	C12	S	0	4.485	-0.365	-0.766	0.00	0.00
ATOM	13	C13	C13	S	0	3.640	-0.636	-2.164	0.00	0.00
ATOM	14	O1	O1	S	0	7.371	-0.390	-0.166	0.00	0.00
ATOM	15	O2	O2	S	0	7.936	-0.782	-2.925	0.00	0.00
ATOM	16	C14	C14	S	0	4.035	1.039	0.357	0.00	0.00
ATOM	17	C15	C15	S	0	4.247	-3.575	1.261	0.00	0.00
ATOM	18	O3	O3	S	0	9.741	1.758	0.294	0.00	0.00

ATOM	19	C16	C16	S	0	12.742	-4.282	3.025	0.00	0.00
ATOM	20	O4	O4	S	0	12.725	0.682	-0.666	0.00	0.00
ATOM	21	H1	H1	S	0	3.051	1.801	-0.161	0.00	0.00
ATOM	22	H2	H2	S	0	5.096	1.843	0.565	0.00	0.00
ATOM	23	H3	H3	S	0	3.603	0.579	1.547	0.00	0.00
ATOM	24	H4	H4	S	0	2.608	0.134	-2.522	0.00	0.00
ATOM	25	H5	H5	S	0	3.966	-1.629	-2.999	0.00	0.00
ATOM	26	H6	H6	S	0	6.159	-2.384	-1.225	0.00	0.00
ATOM	27	H7	H7	S	0	5.400	-1.473	2.352	0.00	0.00
ATOM	28	H8	H8	S	0	6.923	-4.467	2.541	0.00	0.00
ATOM	29	H9	H9	S	0	7.981	-3.662	0.841	0.00	0.00
ATOM	30	H10	H10	S	0	8.603	-1.104	2.643	0.00	0.00
ATOM	31	H11	H11	S	0	7.578	-2.092	4.254	0.00	0.00
ATOM	32	H12	H12	S	0	10.493	-2.415	4.631	0.00	0.00
ATOM	33	H13	H13	S	0	9.590	-4.348	4.290	0.00	0.00
ATOM	34	H14	H14	S	0	12.190	-1.005	2.014	0.00	0.00
ATOM	35	H15	H15	S	0	10.536	-2.794	-0.870	0.00	0.00
ATOM	36	H16	H16	S	0	3.094	-2.939	0.977	0.00	0.00
ATOM	37	H17	H17	S	0	4.464	-4.500	0.303	0.00	0.00
ATOM	38	H18	H18	S	0	4.064	-4.221	2.430	0.00	0.00
ATOM	39	H19	H19	S	0	11.623	-0.616	-2.521	0.00	0.00
ATOM	40	H20	H20	S	0	10.639	-4.460	1.473	0.00	0.00
ATOM	41	H21	H21	S	0	9.885	1.803	-2.191	0.00	0.00
ATOM	42	H22	H22	S	0	9.216	1.021	1.088	0.00	0.00
ATOM	43	H23	H23	S	0	12.467	-5.424	3.688	0.00	0.00
ATOM	44	H24	H24	S	0	13.627	-4.576	2.051	0.00	0.00
ATOM	45	H25	H25	S	0	13.341	-3.435	3.888	0.00	0.00
ATOM	46	H26	H26	S	0	12.267	1.485	0.105	0.00	0.00

Lowest energy conformer of isomer (2a) (10,000 steps Monte Carlo search):

REMARK	1	PDB output from SCARECROW								
REMARK	1	=====								
ATOM	1	C1	C1	S	0	-11.189	-6.474	5.161	0.00	0.00
ATOM	2	C2	C2	S	0	-12.167	-7.133	4.514	0.00	0.00
ATOM	3	C3	C3	S	0	-9.120	-6.288	3.074	0.00	0.00
ATOM	4	C4	C4	S	0	-8.792	-6.100	4.548	0.00	0.00
ATOM	5	C5	C5	S	0	-9.770	-6.947	5.387	0.00	0.00
ATOM	6	C6	C6	S	0	-13.492	-6.497	4.156	0.00	0.00
ATOM	7	C7	C7	S	0	-13.805	-6.601	2.645	0.00	0.00
ATOM	8	C8	C8	S	0	-12.690	-6.167	1.678	0.00	0.00
ATOM	9	C9	C9	S	0	-12.252	-4.706	1.830	0.00	0.00
ATOM	10	C10	C10	S	0	-11.086	-4.268	0.897	0.00	0.00
ATOM	11	C11	C11	S	0	-9.799	-5.113	1.109	0.00	0.00
ATOM	12	C12	C12	S	0	-8.556	-4.585	0.389	0.00	0.00
ATOM	13	C13	C13	S	0	-7.834	-3.559	0.891	0.00	0.00

ATOM	14	O1	O1	S	0	-9.506	-5.107	2.523	0.00	0.00
ATOM	15	O2	O2	S	0	-9.070	-7.370	2.497	0.00	0.00
ATOM	16	C14	C14	S	0	-8.151	-5.389	-0.820	0.00	0.00
ATOM	17	C15	C15	S	0	-6.516	-3.009	0.396	0.00	0.00
ATOM	18	C16	C16	S	0	-11.530	-4.255	-0.569	0.00	0.00
ATOM	19	O3	O3	S	0	-7.429	-6.526	4.711	0.00	0.00
ATOM	20	C17	C17	S	0	-14.615	-7.145	4.972	0.00	0.00
ATOM	21	O4	O4	S	0	-9.656	-8.351	5.141	0.00	0.00
ATOM	22	C18	C18	S	0	-6.414	-1.506	0.648	0.00	0.00
ATOM	23	H1	H1	S	0	-8.914	-5.315	-1.600	0.00	0.00
ATOM	24	H2	H2	S	0	-8.038	-6.445	-0.549	0.00	0.00
ATOM	25	H3	H3	S	0	-7.202	-5.071	-1.256	0.00	0.00
ATOM	26	H4	H4	S	0	-8.217	-3.074	1.791	0.00	0.00
ATOM	27	H5	H5	S	0	-9.998	-6.136	0.766	0.00	0.00
ATOM	28	H6	H6	S	0	-10.862	-3.229	1.179	0.00	0.00
ATOM	29	H7	H7	S	0	-11.953	-4.513	2.866	0.00	0.00
ATOM	30	H8	H8	S	0	-13.117	-4.056	1.644	0.00	0.00
ATOM	31	H9	H9	S	0	-13.057	-6.326	0.656	0.00	0.00
ATOM	32	H10	H10	S	0	-11.838	-6.841	1.802	0.00	0.00
ATOM	33	H11	H11	S	0	-14.705	-6.010	2.431	0.00	0.00
ATOM	34	H12	H12	S	0	-14.063	-7.642	2.403	0.00	0.00
ATOM	35	H13	H13	S	0	-8.929	-5.043	4.811	0.00	0.00
ATOM	36	H14	H14	S	0	-13.471	-5.433	4.422	0.00	0.00
ATOM	37	H15	H15	S	0	-9.527	-6.818	6.448	0.00	0.00
ATOM	38	H16	H16	S	0	-12.001	-8.150	4.163	0.00	0.00
ATOM	39	H17	H17	S	0	-11.386	-5.458	5.501	0.00	0.00
ATOM	40	H18	H18	S	0	-10.793	-3.744	-1.196	0.00	0.00
ATOM	41	H19	H19	S	0	-11.660	-5.269	-0.959	0.00	0.00
ATOM	42	H20	H20	S	0	-12.478	-3.721	-0.684	0.00	0.00
ATOM	43	H21	H21	S	0	-14.434	-7.033	6.046	0.00	0.00
ATOM	44	H22	H22	S	0	-15.578	-6.674	4.746	0.00	0.00
ATOM	45	H23	H23	S	0	-14.704	-8.215	4.754	0.00	0.00
ATOM	46	H24	H24	S	0	-9.502	-8.477	4.183	0.00	0.00
ATOM	47	H25	H25	S	0	-6.440	-3.158	-0.688	0.00	0.00
ATOM	48	H26	H26	S	0	-5.454	-1.117	0.291	0.00	0.00
ATOM	49	H27	H27	S	0	-7.214	-0.966	0.129	0.00	0.00
ATOM	50	H28	H28	S	0	-6.485	-1.268	1.716	0.00	0.00
ATOM	51	C1	UNK	S	1	-5.304	-3.721	1.031	0.00	0.00
ATOM	52	C2	UNK	S	1	-5.298	-3.977	2.541	0.00	0.00
ATOM	53	C3	UNK	S	1	-5.097	-5.487	2.807	0.00	0.00
ATOM	54	C4	UNK	S	1	-5.189	-5.882	4.291	0.00	0.00
ATOM	55	C5	UNK	S	1	-6.537	-5.511	4.854	0.00	0.00
ATOM	56	O1	UNK	S	1	-6.803	-4.401	5.298	0.00	0.00
ATOM	57	O2	UNK	S	1	-4.318	-4.009	0.342	0.00	0.00
ATOM	58	C6	UNK	S	1	-4.211	-3.115	3.182	0.00	0.00
ATOM	59	O3	UNK	S	1	-6.107	-6.196	2.073	0.00	0.00
ATOM	60	C7	UNK	S	1	-5.702	-7.493	1.652	0.00	0.00
ATOM	61	H1	UNK	S	1	-6.265	-3.654	2.935	0.00	0.00
ATOM	62	H2	UNK	S	1	-5.046	-6.960	4.427	0.00	0.00
ATOM	63	H3	UNK	S	1	-4.415	-5.393	4.891	0.00	0.00
ATOM	64	H4	UNK	S	1	-4.270	-3.146	4.274	0.00	0.00
ATOM	65	H5	UNK	S	1	-3.211	-3.447	2.884	0.00	0.00
ATOM	66	H6	UNK	S	1	-4.311	-2.068	2.877	0.00	0.00
ATOM	67	H7	UNK	S	1	-4.108	-5.778	2.427	0.00	0.00
ATOM	68	H8	UNK	S	1	-6.530	-7.948	1.102	0.00	0.00
ATOM	69	H9	UNK	S	1	-4.839	-7.423	0.982	0.00	0.00
ATOM	70	H10	UNK	S	1	-5.466	-8.128	2.509	0.00	0.00

Lowest energy conformer of isomer (2b) (10,000 steps Monte Carlo search):

REMARK 1 PDB output from SCARECROW

REMARK 1 =====

ATOM	1	C1	C1	S	0	2.211	3.420	-2.527	0.00	0.00
ATOM	2	C2	C2	S	0	1.154	2.846	-3.129	0.00	0.00
ATOM	3	C3	C3	S	0	4.185	2.967	-4.737	0.00	0.00
ATOM	4	C4	C4	S	0	4.589	3.380	-3.331	0.00	0.00
ATOM	5	C5	C5	S	0	3.575	2.801	-2.319	0.00	0.00
ATOM	6	C6	C6	S	0	-0.131	3.582	-3.443	0.00	0.00
ATOM	7	C7	C7	S	0	-0.449	3.588	-4.960	0.00	0.00
ATOM	8	C8	C8	S	0	0.696	4.033	-5.886	0.00	0.00
ATOM	9	C9	C9	S	0	1.127	5.491	-5.692	0.00	0.00
ATOM	10	C10	C10	S	0	2.516	5.856	-6.302	0.00	0.00
ATOM	11	C11	C11	S	0	3.670	5.330	-5.392	0.00	0.00
ATOM	12	C12	C12	S	0	5.061	5.928	-5.620	0.00	0.00
ATOM	13	C13	C13	S	0	5.925	5.423	-6.531	0.00	0.00
ATOM	14	O1	O1	S	0	3.763	3.896	-5.620	0.00	0.00
ATOM	15	O2	O2	S	0	4.233	1.784	-5.081	0.00	0.00
ATOM	16	O3	O3	S	0	5.914	2.831	-3.156	0.00	0.00
ATOM	17	C14	C14	S	0	5.415	7.048	-4.669	0.00	0.00
ATOM	18	C15	C15	S	0	7.388	5.777	-6.721	0.00	0.00
ATOM	19	C16	C16	S	0	8.318	4.562	-6.459	0.00	0.00
ATOM	20	O4	O4	S	0	9.344	4.386	-7.128	0.00	0.00
ATOM	21	C17	C17	S	0	7.990	3.618	-5.291	0.00	0.00
ATOM	22	C18	C18	S	0	8.637	4.106	-3.976	0.00	0.00
ATOM	23	C19	C19	S	0	8.186	3.307	-2.735	0.00	0.00
ATOM	24	C20	C20	S	0	6.742	3.583	-2.390	0.00	0.00
ATOM	25	O5	O5	S	0	6.364	4.425	-1.587	0.00	0.00
ATOM	26	C21	C21	S	0	2.559	7.376	-6.502	0.00	0.00
ATOM	27	O6	O6	S	0	8.312	5.494	-3.808	0.00	0.00
ATOM	28	C22	C22	S	0	8.409	2.192	-5.658	0.00	0.00
ATOM	29	C23	C23	S	0	7.593	6.317	-8.138	0.00	0.00
ATOM	30	C24	C24	S	0	-1.291	2.952	-2.666	0.00	0.00
ATOM	31	O7	O7	S	0	3.492	1.374	-2.351	0.00	0.00
ATOM	32	C25	C25	S	0	9.205	6.197	-2.951	0.00	0.00
ATOM	33	H1	H1	S	0	3.489	7.692	-6.984	0.00	0.00
ATOM	34	H2	H2	S	0	1.739	7.703	-7.152	0.00	0.00
ATOM	35	H3	H3	S	0	2.459	7.907	-5.550	0.00	0.00
ATOM	36	H4	H4	S	0	5.647	6.644	-3.679	0.00	0.00
ATOM	37	H5	H5	S	0	6.265	7.646	-5.006	0.00	0.00
ATOM	38	H6	H6	S	0	4.580	7.745	-4.559	0.00	0.00
ATOM	39	H7	H7	S	0	9.726	4.012	-4.081	0.00	0.00
ATOM	40	H8	H8	S	0	6.907	3.607	-5.197	0.00	0.00
ATOM	41	H9	H9	S	0	7.710	6.554	-6.024	0.00	0.00
ATOM	42	H10	H10	S	0	5.574	4.622	-7.185	0.00	0.00

ATOM	43	H11	H11	S	0	3.370	5.506	-4.355	0.00	0.00
ATOM	44	H12	H12	S	0	2.589	5.382	-7.289	0.00	0.00
ATOM	45	H13	H13	S	0	0.351	6.118	-6.149	0.00	0.00
ATOM	46	H14	H14	S	0	1.134	5.759	-4.629	0.00	0.00
ATOM	47	H15	H15	S	0	1.525	3.337	-5.766	0.00	0.00
ATOM	48	H16	H16	S	0	0.365	3.912	-6.925	0.00	0.00
ATOM	49	H17	H17	S	0	-0.748	2.577	-5.266	0.00	0.00
ATOM	50	H18	H18	S	0	-1.321	4.232	-5.137	0.00	0.00
ATOM	51	H19	H19	S	0	8.337	2.230	-2.851	0.00	0.00
ATOM	52	H20	H20	S	0	8.778	3.595	-1.859	0.00	0.00
ATOM	53	H21	H21	S	0	1.217	1.812	-3.466	0.00	0.00
ATOM	54	H22	H22	S	0	2.127	4.455	-2.198	0.00	0.00
ATOM	55	H23	H23	S	0	4.640	4.463	-3.233	0.00	0.00
ATOM	56	H24	H24	S	0	3.908	3.044	-1.304	0.00	0.00
ATOM	57	H25	H25	S	0	-0.047	4.623	-3.107	0.00	0.00
ATOM	58	H26	H26	S	0	-2.223	3.495	-2.857	0.00	0.00
ATOM	59	H27	H27	S	0	-1.103	2.983	-1.587	0.00	0.00
ATOM	60	H28	H28	S	0	-1.446	1.906	-2.952	0.00	0.00
ATOM	61	H29	H29	S	0	3.670	1.071	-3.265	0.00	0.00
ATOM	62	H30	H30	S	0	8.628	6.646	-8.284	0.00	0.00
ATOM	63	H31	H31	S	0	6.942	7.177	-8.332	0.00	0.00
ATOM	64	H32	H32	S	0	7.383	5.552	-8.893	0.00	0.00
ATOM	65	H33	H33	S	0	8.004	1.463	-4.950	0.00	0.00
ATOM	66	H34	H34	S	0	9.499	2.086	-5.671	0.00	0.00
ATOM	67	H35	H35	S	0	8.039	1.921	-6.653	0.00	0.00
ATOM	68	H36	H36	S	0	8.909	7.249	-2.944	0.00	0.00
ATOM	69	H37	H37	S	0	10.231	6.128	-3.325	0.00	0.00
ATOM	70	H38	H38	S	0	9.149	5.818	-1.927	0.00	0.00

Copies of selected 1D and 2D NMR spectra

(S)-Mosher-ester (**3a**)

(R)-Mosher-ester (**3b**)

NOESY NMR spectrum of bislactone 2 in C_6D_6 (500 MHz)

Table S2. MIC Values of Carolacton (**1**).

<i>Organism</i>	MIC [$\mu\text{g/mL}$]
<i>Staphylococcus aureus</i>	>40
<i>Micrococcus luteus</i>	>40
<i>Escherichia coli</i>	>40
<i>Escherichia coli</i> tolC	0.06
<i>Aspergillus niger</i>	20
<i>Pythium debaryanum</i>	16
<i>Sclerotinia sclerotiorum</i>	20
<i>Mucor hiemalis</i>	>40
<i>Saccharomyces cerevisiae</i>	>40
<i>Rhodotorula glutinis</i>	>40