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Volatile Lactones − (5*S*,*S*)-5-Methyl-3-(methylalkyl)furan-2(5*H*)-ones − Identified in the Submerged Cultivation of *Streptomyces Avermitilis*

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Six new compounds have been identified in the volatile fractions produced during the submerged cultivation of *Streptomyces avermitilis*. By recording the GC/MS, GC/FTIR, CD, ^{1}H and ^{13}C NMR data and by performing chemical degradation experiments, these compounds were determined to be (5*S*,*S*)-5-methyl-3-(methylalkyl)furan-2(5*H*)-ones. Herein, the existence of volatile lactones with an anteiso structure of the side-chain is thus documented for the first time.

Introduction

The production of important biologically active compounds by actinomycetes[1,2] is usually followed by the formation of volatile substances with strong odor. The odors of soil were subjected to scientific scrutiny for the first time in the 19th century.[3] Among the soil microorganisms, actinomycetes growing in a pure surface or submerged culture are characterized by the production of a strong and earthy odor. Gerber and Lechevalier[4] and Gerber[5] obtained diethyl ether soluble extracts from actinomycetes that had a highly concentrated odor, which, after dilution, became earthy-smelling. Rosen et al.[6] described odor production by the odoriferous strain *Streptomyces griseoluteus*, whilst Dougherty et al.[7] studied the volatile metabolites of actinomycetes by NMR spectroscopy and mass spectrometry. They obtained some partial chemical structures of the odor components (five- or six-membered lactones), but later the chemical structures were revised.[8] Similar compounds, that is, five- to seven-membered lactones with a branched side-chain, but with slightly different structures, have recently been identified in the North Sea *Streptomyces*,[9] for example, 10-methylundec-2-en-4-olide, 10-methyldodec-2-en-4-olide, 10-methylundec-3-en-4-olide, and 10-methyldodec-3-en-4-olide, and in a marine streptomycetes, (*R*)-10-methylundecan-6-olide and (6*R*,10*S*)-10-methyldodecan-6-olide.[10]

As part of an extensive research of the physiology of different *Streptomyces* strains,[11–14] producers of important biologically active compounds, we studied the effect of the type of cultivation on the total odor composition of selected streptomycetes. The components of the chemical odor excreted by cultivated *Streptomyces avermitilis* are commonly present in the air above the surface of the culture liquor. At present, a total of 67 compounds comprising monoterpenes, hydrocarbons, oxygenated compounds, and aromatics have been identified as odor components in different species of *Streptomyces*.[11–14]

In this study, six butyrolactones **1**–**6**, which have previously not been described, were identified in the submerged culture of *Streptomyces avermitilis*.[7,8,11–14] Their structures were inferred from the mass fragmentation pattern obtained by GC/MS and GC/FTIR analyses without their isolation as individual components. Compounds **1**–**6** were identified as (5*S*,*S*)-5-methyl-3-(methylalkyl)furan-2(5*H*)-ones and their structures were confirmed by their synthesis in five reaction steps.

Results and Discussion

Compounds from the CH_2Cl_2 extract of *S. avermitilis* were separated by preparative TLC and the appropriate fraction (fraction 3, given in Table 1), containing compounds with a conjugated system (unsaturated ketone) that exhibit an absorption at 240 nm in the UV spectrum and give a positive Kedde reaction, was further investigated. In general, unsaturated five-membered lactones, for example, cardenolides, react with aromatic nitro compounds in alkaline solution (e.g., the Kedde reagent) to form typical and very specific violet-red or blue-violet products (Meisenheimer adducts).[15]

Fraction 3 (8.56 mg) was investigated by GC/MS and GC/FTIR analyses using a capillary column. Its composition is given in Table 1 and is illustrated in Figure 1. A total of 15 compounds, six of which have not previously been described, were identified by capillary chromatography. Compound **2** (peak 8) was present in the highest amount, among the compounds so far described, and was therefore the first to be identified. It gave a molecular ion at *m*/*z* (%)

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Table 1. Compounds identified in fraction 3.

Peak no.	Compound	%
1	3,5-Dimethylfuran-2(5*H*)-one	4.41
2	3-Ethyl-5-methylfuran-2(5*H*)-one	10.86
3	3-Methyl-5-propylfuran-2(5*H*)-one	9.06
4	3-Butyl-5-methylfuran-2(5*H*)-one	4.48
5	5-Methyl-3-(2-methylbutyl)furan-2(5*H*)-one (**1**)	3.31
6	5-Methyl-3-pentylfuran-2(5*H*)-one	7.44
7	5-Isohexyl-3-methylfuran-2(5*H*)-one	11.11
8	5-Methyl-3-(2-methylpentyl)furan-2(5*H*)-one (**2**)	9.91
9	5-Hexyl-3-methylfuran-2(5*H*)-one	19.36
10	5-Isoheptyl-3-methylfuran-2(5*H*)-one	4.83
11	5-Methyl-3-(2-methylhexyl)furan-2(5*H*)-one (**3**)	3.35
12	5-Heptyl-3-methylfuran-2(5*H*)-one	7.44
13	5-Methyl-3-(2-methylheptyl)furan-2(5*H*)-one (**4**)	1.97
14	5-Methyl-3-(2-methyloctyl)furan-2(5*H*)-one (**5**)	1.66
15	5-Methyl-3-(2-methylnonyl)furan-2(5*H*)-one (**6**)	0.81

= 182 (18) and a base ion at *m*/*z* (%) = 71 (100), together with a characteristic diagnostic ion at *m*/*z* (%) = 112 (54) (Figure 2). The base ion at *m*/*z* = 71 suggested the presence of C_5H_{11} in the molecule. The rearranged fragment ion at *m*/*z* = 112 belongs to another part of the molecule because the sum of *m*/*z* = 71 and *m*/*z* = 112 is *m*/*z* = 182 + H. The GC/FTIR spectrum of **2** exhibited an unusually high wavelength, at 1785 cm^{-1}, for the C=O band, which is evidence

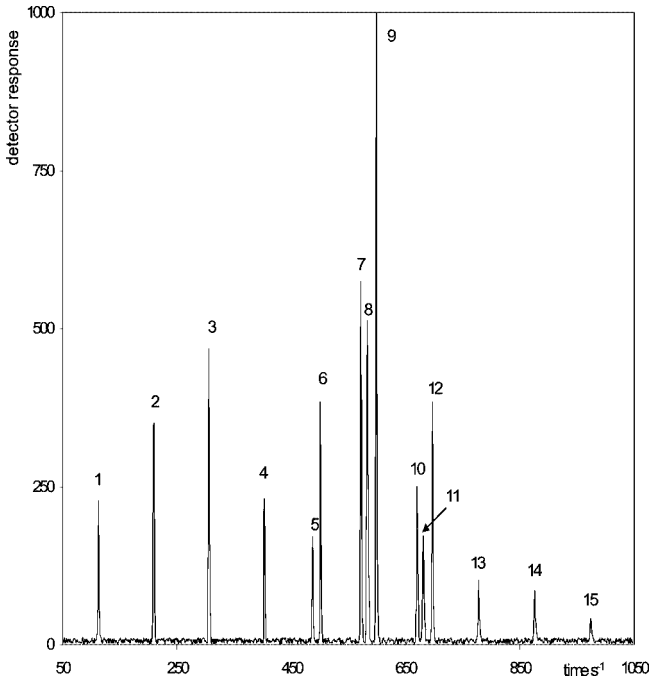


Figure 1. Total ion current of total lactones from fraction 3 obtained after preparative TLC from the submerged cultivation of *Streptomyces avermitilis*.

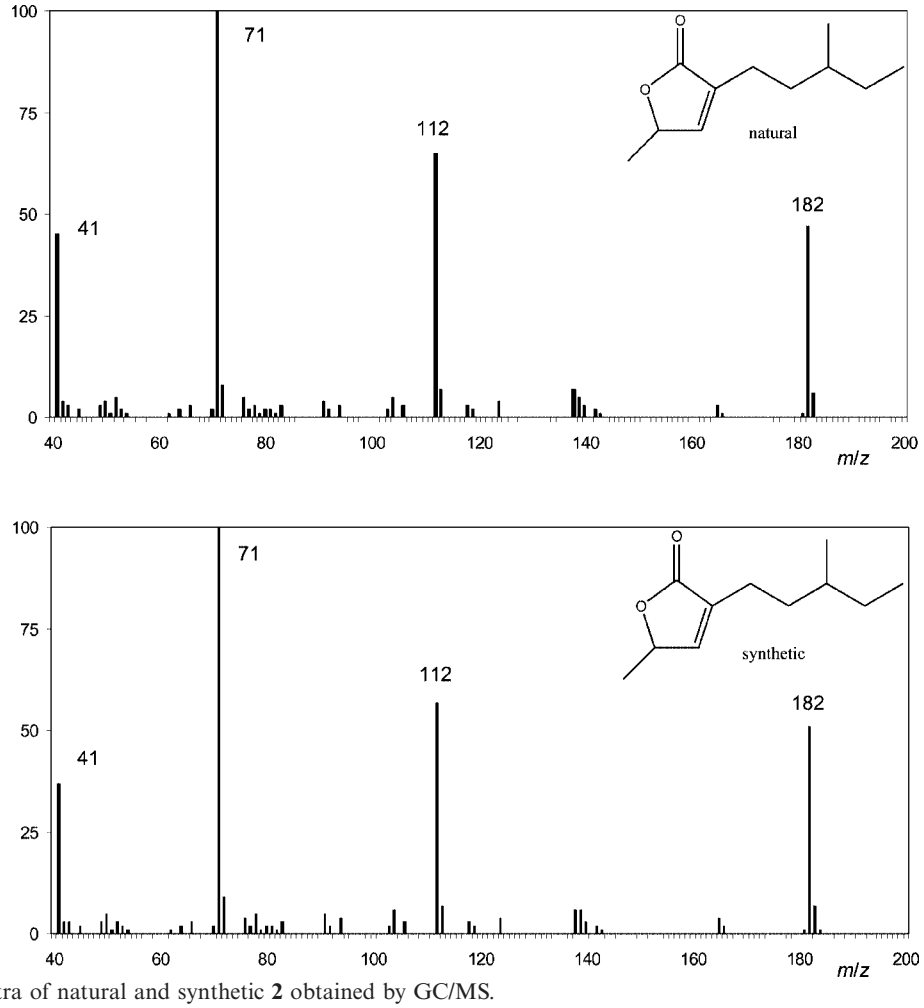


Figure 2. Mass spectra of natural and synthetic **2** obtained by GC/MS.

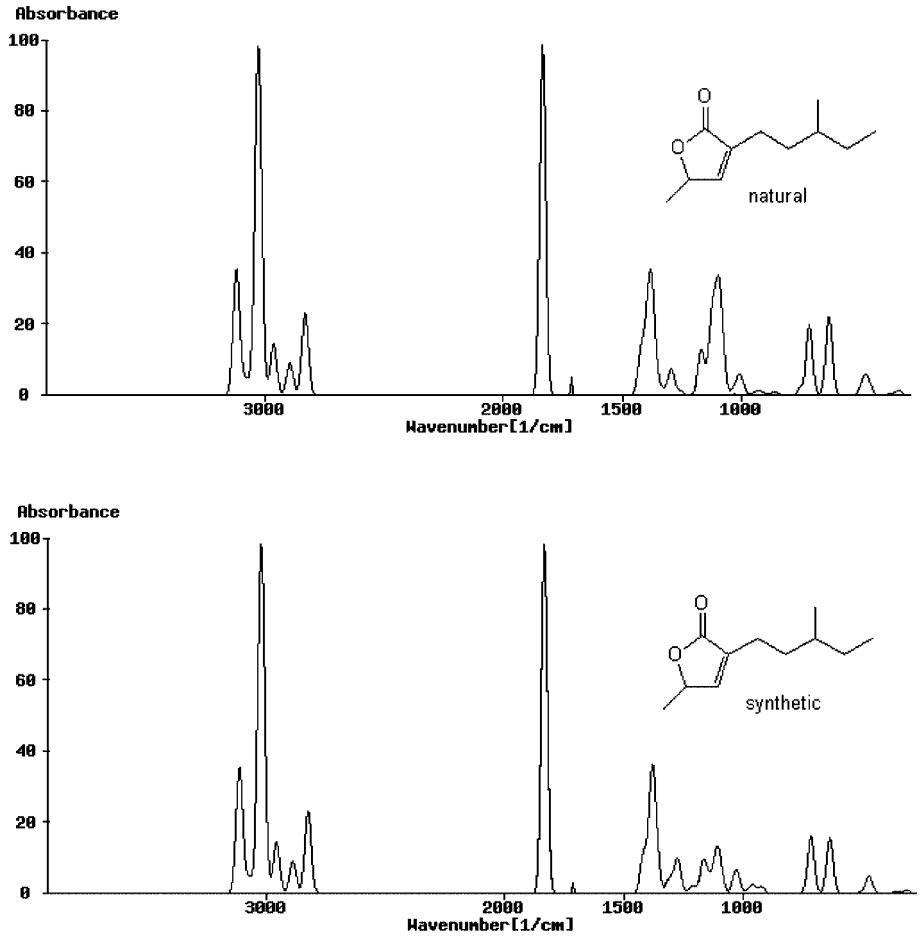


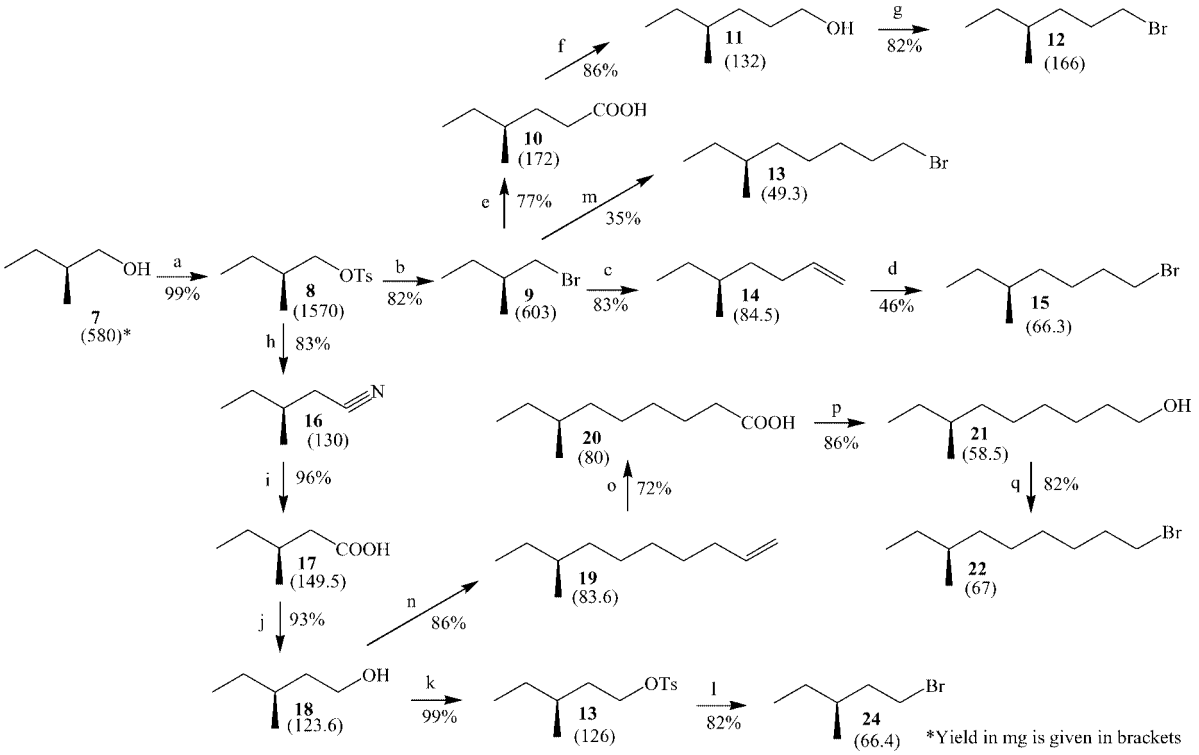
Figure 3. GC/FTIR spectra of natural and synthetic lactone **2**.

for the presence of a five-membered conjugated lactone moiety (Figure 3). The putative fragment ion of a lactone with a methylene moiety corresponds to *m*/*z* = 111, which actually appeared as the rearranged ion at *m*/*z* = 112. If compound **2** is assumed to be a product of butyrolactone present in the species, the structure of **2** is 5-methyl-3-(3′-methylpentyl)furan-2(5*H*)-one. Other compounds, that is, peaks 5, 11, and 13–15, were identified as lower and higher homologs of compound **2** (peak 8). The remaining peaks in Figure 1 (Table 1) were also identified; they were found to derive from known compounds.[7,8,11–14]

To confirm the presumed structure of the lactones, including the stereospecificity of the side-chain, a homologous series of anteisoalkyl bromides (**9**, **12**, **13**, **15**, **22**, and **24**) known from the literature were prepared by a sequence of reactions (see Scheme 1). The starting material was the commercially available, optically active amyl alcohol [(*S*)-2-methylbutan-1-ol] (**7**). A series of well-known reactions were used to prepare all the required bromides in satisfactory yields (given in both mg and %). If the products were not produced in satisfactory yields, or were contaminated by side-products, another sequence of reactions was used. To prepare the bromide **24** we wanted to repeat the sequence **9** → **14** → **15** but the yield was very low. This is why we used the somewhat convoluted synthesis to obtain bromide **22**. In this case, reaction of the alcohol derivative **8** with pent-1-en-5-ylmagnesium bromide gave yields below 10% of the required compound **22**. We therefore chose other reactions that gave several-fold higher yields. Also, the addition of hydrogen bromide to compound **19**, analogous to the addition of HBr to compound **14**, provided negligible yields and a detour via intermediates **20** and **21** was therefore used.

We alkylated both diastereoisomeric adducts produced by Diels–Alder synthesis and subsequent pyrolysis to provide an easy entry to optically active (5*S*,*S*)-5-methyl-3-(methylalkyl)furan-2(5*H*)-ones **1**–**6** in good yields. A related route has been published[16,17] based on the alkylation of adducts from maleic anhydride and cyclopentadiene that yields racemic furan-2(5*H*)-ones; this was, however, unsuitable for our purposes. The optically active starting material 5-methylfuran-2(5*H*)-one (**27**) is readily available.[18]

The *endo* and *exo* adducts **28a** and **28b**, respectively, were alkylated by the appropriate anteisoalkyl bromides (**9**, **12**, **13**, **15**, **22**, and **24**) and the mixture of tricyclic adducts **29a** and **29b** underwent pyrolysis at 240 °C for 5 d. Optical rotations of the (5*S*,*S*)-5-methyl-3-(methylalkyl)furan-2(5*H*)-ones **1**–**6** obtained from the *endo* and *exo* precursors were in good accord with those of model compounds obtained by synthesis. For instance, the literature gives a value of



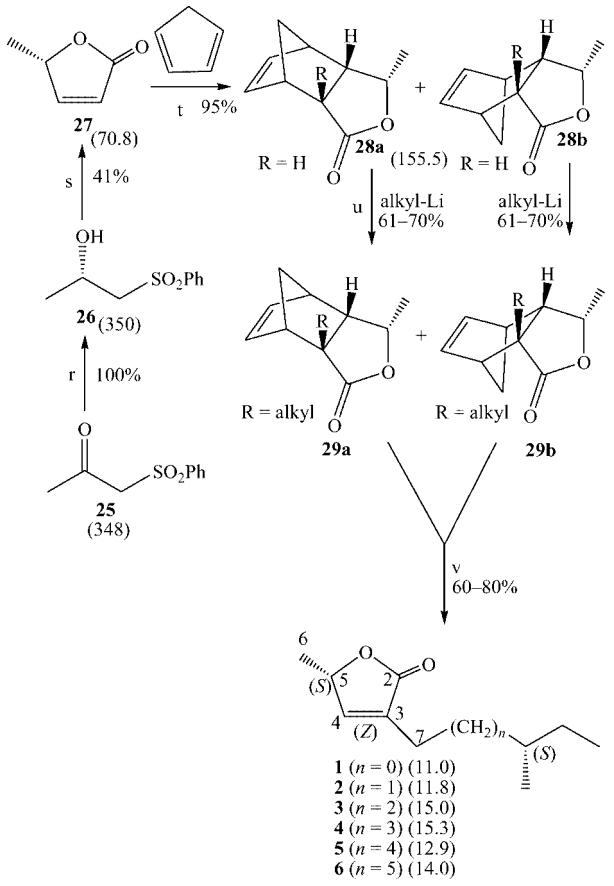
Scheme 1. Synthesis of bromides. Reagents and conditions: a) TsCl/Py/0 °C/18 h;[30–31] b) LiBr/DMF/20 °C/5 h;[30] c) allylmagnesium bromide/Et_2O/20 °C/2 weeks;[32–33] d) 1. B_2H_6/THF/0 °C/30 min/20 °C/1 h; 2. MeOH; 3. Br_2/–5 to 0 °C; 4. MeONa/MeOH/<5 °C/45 min;[34] e) 1. diethyl malonate/NaOMe/20 °C/0.08 h; 2. KOH/MeOH/reflux/overnight.[35] f) $LiAlH_4$/Et_2O/20 °C/overnight;[35] g) 1. Ph_3P, imidazole/CH_2Cl_2/0 °C; 2. Br_2/5 h;[35–36] h) NaCN/DMSO/20 °C/14 h;[37] i) H_2O/H_2SO_4 (1:1)/reflux/6 h;[37] j) $LiAlH_4$/Et_2O/20 °C/2 h;[37] k) TsCl/Py/0 °C/12 h;[37] l) LiBr/DMF/20 °C/5 h;[37] m) 1. Mg/Et_2O; 2. 1,4-dibrombutane/THF/20 °C/overnight;[38] n) 1. TsCl/THF; 2. pent-4-en-1-ylmagnesium bromide/Et_2O/20 °C/14 h;[39] o) $KMnO_4$, $CuSO_4 \cdot 5H_2O$, *t*BuOH/CH_2Cl_2/25 °C/4 h;[40–41] p) $LiAlH_4$/Et_2O/20 °C/overnight;[35–37] q) 1. Ph_3P, imidazole/CH_2Cl_2/0 °C; 2. Br_2/5 h.[35–42]

$[a]_D$ = +79.2 for (5*S*)-3-butyl-5-methylfuran-2(5*H*)-one,[19] (5*S*)-3-hexadecyl-5-methylfuran-2(5*H*)-one has been reported to give $[a]_D$ = +28.8, and $[a]_D$ = –22.8 was found for (5*R*)-3-hexadecyl-5-methylfuran-2(5*H*)-one,[20] verifying that the configurational integrity of the starting (*S*)-5-methylfuran-2(5*H*)-one (**27**) was preserved throughout the overall sequence.

Compound **2** can again serve as a representative of these lactones. This compound was obtained after synthesis as an optically active colorless oil having $[a]_D^{23}$ = +89.2 and with elemental composition $C_{11}H_{18}O_2$, determined by HREIMS measurements on the molecular ion at *m*/*z* = 182.1310. Its ^{1}H NMR spectrum displayed signals arising from a vinylic proton at δ = 7.00 (d, *J* = 1.6 Hz, 1 H, 4-H) ppm, a methyl signal at δ = 1.42 (d, *J* = 6.86 Hz, 3 H, 6-H) ppm, and a methine signal at δ = 5.19 (dd, *J* = 1.6, 6.86 Hz, 1 H, 5-H) ppm, suggesting an α,β-unsaturated γ-lactone system, which was confirmed by the presence in the ^{13}C NMR spectrum of signals at δ = 170.1 (s, C-2), 132.0 (s, C-3), 144.7 (d, C-4), and 78.1 (d, C-5) ppm and in the IR spectrum of a strong absorption at 1785 cm^{-1}. In addition, the ^{1}H NMR spectrum of **2** exhibited a triplet methyl signal at δ = 0.86 (t, *J* = 7.06 Hz, 3 H, 11-H) ppm attributable to a terminal methyl group and strong broad multiplets at δ = 1.15–1.35, 1.75 and 2.35 ppm due to protons in a side-chain. Also a doublet at δ = 0.93 (d, *J* = 6.16 Hz, 3 H) ppm, which is appropriate to a branched side-chain, was observed. These data led us to assign a structure containing a 5-methylfuran-2(5*H*)-one moiety linked to a branched alkyl chain, as formulated in **2**. The UV and CD spectra are fully in agreement with the above data and with literature data.[20–22] The ^{1}H and ^{13}C NMR spectra of other alkyl-5-methylfuran-2(5*H*)-ones (**1**, **3**–**6**) confirmed that the standards synthesized in this study have the appropriate structures to those illustrated in Scheme 2.

Further experiments were carried out to confirm the suggested structures including the configuration of the two stereogenic centers. The first step included the measurement of ^{1}H and ^{13}C NMR spectra of fraction 3; the results are given below.

The strong absorption at 1785 cm^{-1} in the IR spectrum and the positive Kedde reaction[15] suggested the presence of an α,β-unsaturated γ-lactone structure in fraction 3. This finding was confirmed by comparison of the ^{1}H NMR spectroscopic data of fraction 3 with previously isolated compounds[16] and also by a 2D NMR experiment. The 500 MHz COSY spectra of fraction 3 (from the band giving a positive reaction with the Kedde reagent) revealed an α,β-unsaturated γ-lactone spin system (4-H, 5-H, and Me-5). The ^{1}H-^{13}C direct heteronuclear correlation spectrum of the above mixture showed that these protons are attached to five carbon atoms [δ = 144.3/7.00 (4-H), 78.1/5.19 (5-H),



Scheme 2. Synthesis of lactones. Reagents and conditions: r) 1. (*R*)-MeO-BIPHEP, codRu(2-methylallyl)$_2$/acetone; 2. MeOH/acetone/30 min;[43] s) 1. *n*-butyllithium/THF/–78 °C/30 min/–10 °C/2 h; 2. sodium iodoacetate/–78 °C/0 °C/15 h;[43] t) 1. $ZnCl_2$, $EtAlCl_2$/20 °C/30 min; 2. cyclopentadiene/100 °C/8 h;[44] u) 1. LDA/THF/–78 °C/1 h; 2. RBr/–78 °C/2 h;[45] v) toluene/240 °C/5 d.[45]

and 19.2/1.42 (6-H) ppm]. In addition to the signals corresponding to the methyl unsaturated lactone, the ^{1}H NMR spectra showed chemical shifts characteristic of an end methyl group (δ = 0.85 ppm) and an anteisomethyl group (δ = 0.83 ppm), but no carbinol methine protons or carbon atoms were present, indicating the absence of hydroxy groups, and no signal related to the expected tetrahydrofuran moiety.

Two experiments were performed to elucidate the stereochemistry of both the methyl group on the five-membered ring and that in the side-chain. The first experiment involved the measurement of the CD spectrum to clarify the stereochemistry of the ring-located methyl group.

The absolute configuration of the identified compounds was obtained by comparing the CD spectra of the mixture with those of compounds identified previously[20] and of reference butenolides (compounds **A**, **B**,[20] **C**,[21] **D**, and **E**[22]) of known absolute configuration. Despite the fact that fraction 3 contained only one chromophore and that the next stereogenic center bearing a methyl function in some compounds is distant, it should be possible to compare the CD spectra of fraction 3 and reference butenolides[20–22] because the Cotton effects of the unsaturated conjugated lactone should not be affected by other functionalities in the molecule. First, we recorded the UV spectra of fraction 3. Two absorption bands are expected in the spectra of unsaturated lactones, the first appearing in the short-wavelength π-π range around 210 nm and the second n-π band at approximately 235 nm. Fraction 3 shows an absorption maximum at 208 nm and the n-π absorption is located at around λ = 240 nm. Apparently, there are two Cotton effects in all the compounds which are comparable in prefix and size. Compounds **A** and **B**[20] had λ_{max} = 210 ($\log\varepsilon$ = +0.95) and 208 nm ($\log\varepsilon$ = +0.76), respectively; compound **C**[21] had λ_{max} = 234 nm ($\log\varepsilon$ = –1.06), compound **D**[22] had λ_{max} = 211 ($\Delta\varepsilon$ = +8) and 240 nm ($\Delta\varepsilon$ = –0.5), compound **E**[22] had λ_{max} = 212 ($\Delta\varepsilon$ = +6) and 230 nm ($\Delta\varepsilon$ = –0.4), and our mixture of fraction 3 had λ_{max} = 209 ($\log\varepsilon$ = +2.17) and 237 nm ($\log\varepsilon$ = –0.63), enabling us to conclude that the absolute configuration of compounds from fraction 3 at C-5 is (*S*).

The other experiment concerned the configuration of the methyl group in the anteiso position of the side-chain. A mixture of acids obtained by oxidative ozonolysis of fraction 3 was purified by chromatography on a chiral stationary phase. Comparison of the retention characteristics of the (*S*) enantiomers of synthetically prepared standards (Scheme 1) with those of the anteiso acids derived from fraction 3 showed identical retention times (see Table 2). These results indicate that the absolute configuration of both chiral centers is (*S*) and, hence, compounds **1**–**6** are (5*S*,*S*)-5-methyl-3-(methylalkyl)furan-2(5*H*)-ones (Table 3).

Table 2. The presence of chiral degradation products (determined by chiral capillary GC) after oxidation of fraction 3.

Methyl ester	R_t of products [min^{-1}]	
	Standards	After degradation of fraction 3
(*S*)-3-Methylpentanoic acid	7.13	7.19
(*S*)-4-Methylhexanoic acid	9.27	9.32
(*S*)-5-Methylheptanoic acid	11.42	11.45
(*S*)-6-Methyloctanoic acid	13.57	13.60
(*S*)-7-Methylnonanoic acid	15.72	15.74
(*S*)-8-Methyldecanoic acid	17.85	17.87

Table 3. ^{13}C NMR spectroscopic data of furan-2(5*H*)-ones **1**–**6**.

C no.	**1**	**2**	**3**	**4**	**5**	**6**
2	169.1	170.1	170.1	170.1	170.1	170.1
3	132.0	132.0	132.7	132.7	132.7	132.7
4	144.7	144.7	146.3	146.3	146.3	146.3
5	78.1	78.1	78.1	78.1	78.1	78.1
6	19.2	19.2	19.2	19.2	19.2	19.2
7	34.8	22.6	27.8	27.4	27.4	27.4
8	31.1	30.4	25.7	25.7	25.7	25.7
9	31.9	36.5	36.2	29.3	28.4	28.6
10	11.5	29.7	34.2	36.0	24.2	27.9
11	18.4	11.4	29.3	33.9	36.2	24.4
12	–	19.2	11.3	29.3	33.9	36.2
13	–	–	19.0	11.3	29.3	33.9
14	–	–	–	19.0	11.3	29.3
15	–	–	–	–	19.0	11.3
16	–	–	–	–	–	19.0

Conclusions

This study describes the structures of six heretofore unknown volatile odor components, lactones **1**–**6** (Table 4). These compounds were found to be produced during the submerged cultivation of *Streptomyces avermitilis*; to confirm their structures, they were prepared by a series of synthetic reactions. The production of odor components by actinomycetes is associated with the production of antibiotics and their structures are similar to those of butyrolactone autoregulators which control cytodifferentiation and secondary metabolite production, such as the A-factor, factor 1, Grafe's factors, virginiae butanolides A–E and IM-2.[23,24]

Table 4. List of major fragments of the individual compounds (all six lactones, **1**–**6**).

Compd.	M^+	M – Me	M – Et	M – iBu	M – sc[a]	Base peak
1	168	153	139	–	112	71
2	182	167	153	125	112	71
3	196	181	167	139	112	71
4	210	195	181	153	112	71
5	224	209	195	167	112	71
6	238	223	209	181	112	71

[a] M – side-chain.

Experimental Section

General: UV spectra were measured in MeOH within the range of 205–300 nm with a Cary 118 (Varian) apparatus. A Perkin-Elmer (Perkin-Elmer, Norwalk, CT, USA) 1310 IR spectrometer was used to record IR spectra with samples prepared as thin films. Optical rotations were measured with a Perkin-Elmer 243 B polarimeter. Circular dichroism (CD) measurements were carried out with a Jasco-500A spectropolarimeter at 24 °C under dry N_2. NMR spectra were recorded with a Bruker AMX 500 spectrometer (Bruker Analytik, Karlsruhe, Germany) at 500.1 (^{1}H) and 125.7 (^{13}C) MHz and a 400 MHz Varian Inova NMR spectrometer was used to record the NMR spectra of the bromides. High- and also low-resolution mass spectra were recorded using a VG 7070E-HF spectrometer (70 eV). GC/MS data were obtained using a Finnigan 1020 B (Finnigan MAT, San Jose, CA, USA) single-state quadrupole GC/MS instrument in the EI mode. Gas chromatography was performed with a Hewlett Packard HP 5980 gas chromatograph (Hewlett Packard, Czech Republic). GC/FTIR analyses were performed with a Model 8700 gas chromatograph coupled through a GC/IR interface to a 1710 FTIR spectrometer (all components from Perkin-Elmer). A Perkin-Elmer bonded 5% methylphenylsilicone 10 m × 0.53 mm fused silica capillary column with 5 μm film thickness was used. Liquid samples of 1–2 μL were injected into a packed column injector heated to 300 °C. The column was kept at 40 °C for 5 min after injection, then programmed to 220 °C at 10 °C min^{-1} and kept at 220 °C for 8 min. Helium was used as the carrier gas. Throughout the analyses the transfer line to the FTIR spectrometer and the gold-coated light-pipe were heated to 290 °C. Spectra were recorded at 5 cm^{-1} resolution. An FS capillary column HYDRODEX β-3P ID 0.25 mm, length 25 m with heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin as the stationary phase from Macherey-Nagel GmbH & Co. KG, Düren, Germany was used. Oven temperature: 50–150 °C at 2 °C min^{-1}, then to 240 °C at 5 °C min^{-1}, carrier gas: helium, 20 cm s^{-1}, detector: FID, 300 °C, injection of 1 μL mixture in dichloromethane (for standards: containing 0.5 mg mL^{-1} of each sample), split (100:1), 300 °C. All the following compounds were purchased from Sigma-Aldrich (Prague, Czech Republic). The mutant strain *Streptomyces avermitilis* C-18 obtained by genetic improvement of ATCC 1267 was used.[11–14] The inoculum was cultivated for 24 h in a complex medium containing 0.5% glucose, 1.5% soybean meal, and 0.5% yeast extract. The cultivation was carried out in the following synthetic production medium: 3% glucose, 0.2% $(NH_4)_2SO_4$, 0.5% $CaCO_3$, 0.2% NaCl, 0.05% K_2HPO_4, 0.005% $FeSO_4{\cdot}7H_2O$, 0.005% $MnSO_4{\cdot}7H_2O$, 0.005% $ZnSO_4{\cdot}7H_2O$, 0.01% $MgSO_4{\cdot}7H_2O$. The cultivation was performed in a mechanically stirred 50 L fermentor (Bioengineering, Switzerland) at 28 °C, aeration rate 0.6 VVM, and impeller tip speed 0.5–0.6 m s^{-1}. The effluent air from the fermentor was washed in a gas washing bottle containing CH_2Cl_2. The resulting solution was concentrated and used to isolate the compounds. The residue was subjected to preparative TLC on silica gel with dichloromethane/ethyl acetate (5:2, v/v) as eluent. Fraction 3 was obtained after detection in UV and also after spraying with the Kedde reagent (10 mg of 3,5-dinitrobenzoic acid dissolved in 1 mL of a 0.5 N solution of KOH in 50% aqueous methanol directly before spraying) as a colorless oil. It was found to contain an α,β-unsaturated γ-lactone (8.56 mg). This fraction was then used for the following analyses: a) GC/MS, b) GC/FTIR, c) CD measurement, ^{1}H and ^{13}C NMR spectra, and d) oxidative ozonolysis. A stream of 4% ozone was passed through a solution of fraction 3 in dichloromethane (1 mL) at –78 °C for 5 min. The solution was flushed with nitrogen and concentrated. The residue was dissolved in 90% HCOOH (0.5 mL) and 30% hydrogen peroxide (0.1 mL) was added. After gentle heating, the mixture was refluxed for 70 min. The mixture was concentrated and the residue dissolved in methanol (0.5 mL) and treated with excess diazomethane in diethyl ether. The resulting solution of methyl esters was further separated by chiral GC. Fraction 3: UV: λ_{max} (log ε) = 209 (+2.17), 237 (–0.63) nm. FTIR: $\tilde{\nu}_{max}$ = 3075, 2955, 2915, 2850, 1785, 1655, 1470, 1375, 1080, 860, 718 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.15–5.20 (5-H), 7.00–7.06 (4-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 169.1–170.1 (C-2), 132.0–133.0 (C-3), 144.7–146.7 (C-4), 78.1–78.2 (C-5), 19.2–19.4 (C-6) ppm. The appropriate alkyl bromides were prepared according to Scheme 1, which also gives the yields in both % and mg; their physical and chemical characteristics are given below.

(*S*)-1-Bromo-2-methylbutane (9): The yield of **9** was 603 mg, as a colorless oil with b.p. 102–106 °C. $[\alpha]_D^{23}$ = +4.2 (*c* = 1.48, $CHCl_3$); ref.[25] $[\alpha]_D$ = +4.16 (*c* = 4.8, $CHCl_3$). IR (film): $\tilde{\nu}_{max}$ = 2985 (s), 2945 (s), 2895 (s), 1463 (m), 1382 (m), 1252 (m), 1207 (m) cm^{-1}. ^{1}H NMR (400.1 MHz, $CDCl_3$): δ = 3.40 (dd, *J* = 6.2, 10.0 Hz, 1 H, 1a-H), 3.42 (dd, *J* = 5.1, 10.0 Hz, 1 H, 1b-H), 1.79–1.85 (m, 1 H, 2-H), 1.45, 1.26 (m, 2 H, 3-H), 0.92 (t, *J* = 6, 3.9 Hz, 3 H, 4-H), 0.98 (d, *J* = 6.1 Hz, 3 H, 5-H) ppm. HRMS: calcd. for $C_5H_{11}{}^{79}Br$ 150.0044 $[M]^+$; found 150.0041 (Δ = 0.3 ppm).

(*S*)-1-Bromo-3-methylpentane (24): The yield of **24** was 66.4 mg, as a colorless oil with b.p. 146–148 °C. $[\alpha]_D^{23}$ = +5.3 (*c* = 0.59, $CHCl_3$); ref.[26] $[\alpha]_D^{25}$ = +19.6 (neat). IR (film): $\tilde{\nu}_{max}$ = 2989 (s), 2947 (s), 2886 (s), 1462 (m), 1377 (m), 1251 (m), 1212 (m) cm^{-1}. ^{1}H NMR (400.1 MHz, $CDCl_3$): δ = 3.41 (t, *J* = 2 Hz, 1-H), 1.90–2.05 (m, 4 H, 2,3-H), 1.23–1.44 (m, 2 H, 4-H), 0.89 (t, *J* = 6.9 Hz, 3 H, 5-H), 0.96 (d, *J* = 6.1 Hz, 3 H, 6-H) ppm. HRMS: calcd. for $C_6H_{13}{}^{79}Br$ $[M]^+$; found 164.0201164.0196 (Δ = 0.5 ppm).

(*S*)-1-Bromo-4-methylhexane (12): The yield of **12** was 166 mg with b.p. 166–169 °C. $[\alpha]_D^{23}$ = +5.16 (*c* = 0.31, $CHCl_3$); ref.[27] $[\alpha]_D^{23}$ = +5.51 (c, 0.47 in chloroform), ref.[17] $[\alpha]_D^{21}$ = +11.0 (neat). IR (film):

$\tilde{\nu}_{max}$ = 2986 (s), 2951 (s), 2892 (s), 1466 (m), 1375 (m), 1253 (m), 1214 (m) cm^{-1}. ^1H NMR (400.1 MHz, $CDCl_3$): δ = 3.37 (t, J = 7.0 Hz, 2 H, 1-H), 1.50–1.80 (m, 4 H, 2,4-H), 1.21–1.43 (m, 4 H, 3,5-H), 0.90 (t, J = 6.7 Hz, 3 H, 6-H), 0.96 (d, J = 6.1 Hz, 3 H, 7-H) ppm. HRMS: calcd. for $C_7H_{15}{}^{79}Br$ 178.0357[M]$^+$; found 178.0355 (Δ = 0.2 ppm).

(*S*)-1-Bromo-6-methyloctane (13): The yield of **15** was 49.3 mg with b.p. 101–104 °C (18 Torr). $[\alpha]_D^{24}$ = +5.47 (c = 0.13, $CHCl_3$); ref.[29] $[\alpha]_D^{20}$ = +2.16 (neat). IR (film): $\tilde{\nu}_{max}$ = 2933 (s), 2857 (s), 1464 (s), 1378 (m), 1242 (m), 645 (m) cm^{-1}. ^1H NMR (400.1 MHz, $CDCl_3$): δ = 3.34 (t, J = 7.0 Hz, 2 H, 1-H), 1.50–1.75 (m, 4 H, 2,6-H), 1.10–1.45 (m, 8 H, 3,4,5,7-H), 0.89 (t, J = 6.7 Hz, 3 H, 8-H), 0.93 (d, J = 6.1 Hz, 3 H, 9-H) ppm. HRMS: calcd. for $C_9H_{17}{}^{79}Br$ 206.0670 [M]$^+$; found 206.0673 (Δ = –0.3 ppm).

(*S*)-1-Bromo-5-methylheptane (15): The yield of **13** was 66.3 mg with b.p. 185–189 °C. $[\alpha]_D^{23}$ = +5.67 (c = 0.17; $CHCl_3$); ref.[28] $[\alpha]_D^{24}$ = +7.25. IR (film): $\tilde{\nu}_{max}$ = 2973 (vs), 2924 (vs), 2875 (s), 1463 (m), 1382 (m) 1250 (m), 1197 (w), 760 (w), 725 (w) cm^{-1}. ^1H NMR (400.1 MHz, $CDCl_3$): δ = 3.35 (t, J = 7.0 Hz, 2 H, 1-H), 1.50–1.75 (m, 4 H, 2,5-H), 1.10–1.45 (m, 6 H, 3,4,6-H), 0.89 (t, J = 6.7 Hz, 3 H, 7-H), 0.94 (d, J = 6.1 Hz, 3 H, 8-H) ppm. HRMS: calcd. for $C_8H_{17}{}^{79}Br$ 192.0514 [M]$^+$; found 192.0511 (Δ = 0.3 ppm).

(*S*)-1-Bromo-7-methylnonane (22): The yield of **22** was 67 mg with b.p. 101–104 °C (18 Torr). $[\alpha]_D^{24}$ = +4.31 (c = 0.09, $CHCl_3$). IR (film): $\tilde{\nu}_{max}$ = 2930 (s), 2855 (s), 1465 (s), 1380 (m), 1240 (m), 645 (m) cm^{-1}. ^1H NMR: δ = 3.34 (t, J = 7.0 Hz, 2 H, 1-H), 1.50–1.75 (m, 4 H, 2,7-H), 1.10–1.45 (m, 8 H, 3,4,5,8-H), 0.89 (t, J = 6.7 Hz, 3 H, 9-H), 0.93 (d, J = 6.1 Hz, 3 H, 10-H) ppm. HRMS: calcd. for $C_{10}H_{21}{}^{79}Br$ 220.0827 [M]$^+$; found 220.0822 (Δ = 0.5 ppm).

(5*S*)-5-Methylfuran-2(5*H*)-one (27):[18] The yield of enantiomerically pure **27** as a colorless oil was 70.8 mg with b.p. 40–42 °C (1.3 Torr). $[\alpha]_D^{23}$ = +114 (c = 0.14, $CHCl_3$); ref.[17] $[\alpha]_D^{23}$ = +95 (c 0.5, $CHCl_3$). IR (film): $\tilde{\nu}_{max}$ = 3090 (s), 2980 (s), 2940 (s), 1840 (s), 1760 (s), 1635 (s), 1450 (m) cm^{-1}. ^1H NMR (400.1 MHz, $CDCl_3$): δ = 1.44 (d, J = 6.8 Hz, 3 H, 5-H), 5.13 (ddq, J = 1.1, 1.7, 6.8 Hz, 4-H), 6.08 (dd, J = 1.7, 5.6 Hz, 1 H, 2-H), 7.56 (dd, J = 1.1, 5.6 Hz, 1 H, 3-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 18.6 (q), 77.5 (d), 121.7 (d), 157.6 (d), 174.4 (s) ppm. HRMS: calcd. for $C_5H_6O_2$ 98.0368 [M]$^+$; found 98.0365 (Δ = 0.3 ppm).

Preparation of Furan-2(5*H*)-ones: Reactions were carried out under argon and were performed in glass reactors fitted with a septum equipped with Teflon stoppers. $ZnCl_2$ (0.1 equiv.) and $EtAlCl_2$ (0.3 equiv.) were added to a solution of lactone **27** (0.724 mmol) in dichloromethane (1.1 mL). The mixture was stirred at 20 °C for 30 min. Then cyclopentadiene, the monomer obtained by cracking the commercially obtained dimer (5.43 mmol, 0.9 mL), was added and the resulting solution was stirred at 20 °C for 8 h. An aliquot of the reaction mixture was then poured into ice/10% aqueous sodium hydrogen carbonate. The layers were separated and the aqueous phase was extracted with dichloromethane. The combined extracts were dried and the solvent was evaporated. The residue was purified by flash chromatography on a 20×300 mm column to afford the pure isolated products (95% yield, ratio *endo*/*exo* = 6). A solution of adducts **28a**,**b** (0.4 mmol mL^{-1}) in THF was added to a solution of LDA (0.2 mmol) in anhydrous THF (1.5 mL) at –78 °C under argon and the mixture was stirred at –78 °C for 1 h. Then, alkyl halide (0.6 mmol) was added. After 2 h at –78 °C, the solution was warmed to room temperature, diluted with CH_2C1_2, washed with a 1% aqueous HCl solution and with aqueous sodium thiosulfate, and dried with sodium sulfate. The solvent was removed and the residues **29a**,**b** were pyrolyzed, see below. A solution of compound **29a**,**b** (0.7 mmol) in a solvent (30 mL) was heated at 240 °C in toluene in a sealed tube for 5 d. The solvent was distilled off and the residue purified by chromatography on silica gel (hexane/diethyl ether) to afford the appropriate butenolide. The yields varied from 60 to 80% (see Scheme 2 for yields in both % and mg).

(5*S*,*S*)-5-Methyl-3-(2-methylbutyl)furan-2(5*H*)-one (1): Yield: 36.6% (11.0 mg) as a colorless oil. $[\alpha]_D^{23}$ = +91.7 (c = 0.09, CH_2Cl_2). ^1H NMR: δ = 0.85 (t, J = 7.0 Hz, 3 H, 10-H), 0.83 (d, J = 6.6 Hz, 3 H, 11-H), 1.13 (m, 1 H, 9a-H), 1.33 (m, 1 H, 9b-H), 1.42 (d, J = 6.8 Hz, 3 H, 6-H), 1.76 (m, 1 H, 8-H), 1.90 (dd, J = 14.5, 9.2 Hz, 1 H, 7a-H), 2.10 (dd, J = 14.5, 4.9 Hz, 1 H, 7b-H), 5.19 (dd, J = 1.6, 6.8 Hz, 1 H,5-H), 7.06 (d, J = 1.6 Hz, 1 H, 4-H) ppm. HRMS: calcd. for $C_{10}H_{16}O_2$ 168.1150 [M]$^+$; found 168.1154 (Δ = 0.4 ppm);

(5*S*,*S*)-5-Methyl-3-(3-methylpentyl)furan-2(5*H*)-one (2): Yield: 41.0% (11.8 mg) as a colorless oil. $[\alpha]_D^{23}$ = +89.2 (c = 0.08, CH_2Cl_2). ^1H NMR: δ = 0.85 (t, J = 7.0 Hz, 3 H, 11-H), 0.83 (d, J = 6.1 Hz, 3 H, 12-H), 1.15–1.35 (m, 4 H, 8,10-H), 1.42 (d, J = 6.8 Hz, 3 H, 6-H), 1.75 (m, 1 H, 9-H), 2.35 (m, 2 H, 7-H), 5.19 (dd, J = 1.6, 6.8 Hz, 1 H, 5-H), 7.00 (d, J = 1.6 Hz, 1 H, 4-H) ppm. HRMS: calcd. for $C_{11}H_{18}O_2$ 182.1307 [M]$^+$; found 182.1310 (Δ = 0.3 ppm).

(5*S*,*S*)-5-Methyl-3-(4-methylhexyl)furan-2(5*H*)-one (3): Yield: 44.9% (15.0 mg) as a colorless oil. $[\alpha]_D^{23}$ = +86.1 (c = 0.09, CH_2Cl_2). ^1H NMR: δ = 0.85 (t, J = 7.0 Hz, 3 H, 12-H), 0.83 (d, J = 6.6 Hz, 3 H, 13-H), 1.16–1.45 (m, 6 H, 8,9,11-H), 1.42 (d, J = 6.8 Hz, 3 H, 6-H), 1.75 (m, 1 H, 10-H), 2.28 (m, 2 H, 7-H), 5.19 (dd, J = 1.6, 6.8 Hz, 1 H, 5-H), 7.00 (d, J = 1.6 Hz, 1 H, 4-H) ppm. HRMS: calcd. for $C_{12}H_{20}O_2$ 196.1463 [M]$^+$; found 196.1468 (Δ = 0.5 ppm).

(5*S*,*S*)-5-Methyl-3-(5-methylheptyl)furan-2(5*H*)-one (4): Yield: 51.1% (15.3 mg) as a colorless oil. $[\alpha]_D^{23}$ = +83.5 (c = 0.07, CH_2Cl_2). ^1H NMR: δ = 0.85 (t, J = 7.0 Hz, 3 H, 13-H), 0.83 (d, J = 6.6 Hz, 3 H, 14-H), 1.16–1.45 (m, 8 H, 8–10,12-H), 1.42 (d, J = 6.8 Hz, 3 H, 6-H), 1.75 (m, 1 H, 11-H), 2.29 (m, 2 H, 7-H), 5.19 (dd, J = 1.6, 6.8 Hz, 1 H, 5-H), 7.00 (d, J = 1.6 Hz, 1 H, 4-H) ppm. HRMS: calcd. for $C_{13}H_{22}O_2$ 210.1620 [M]$^+$; found 210.1624 (Δ = 0.4 ppm).

(5*S*,*S*)-5-Methyl-3-(6-methyloctyl)furan-2(5*H*)-one (5): Yield: 50.3% (12.9 mg) as a colorless oil. $[\alpha]_D^{23}$ = +84.6 (c = 0.11, CH_2Cl_2). ^1H NMR: δ = 0.85 (t, J = 7.0 Hz, 3 H, 14-H), 0.83 (d, J = 6.6 Hz, 3 H, 15-H), 1.16–1.45 (m, 10 H, 8–11,13-H), 1.42 (d, J = 6.8 Hz, 3 H, 6-H), 1.75 (m, 1 H, 12-H), 2.29 (m, 2 H, 7-H), 5.19 (dd, J = 1.6, 6.8 Hz, 1 H, 5-H), 7.00 (d, J = 1.6 Hz, 1 H, 4-H) ppm. HRMS: calcd. for $C_{14}H_{24}O_2$ 224.1776 [M]$^+$; found 224.1779 (Δ = 0.3 ppm).

(5*S*,*S*)-5-Methyl-3-(7-methylnonyl)furan-2(5*H*)-one (6): Yield: 51.2% (14.0 mg) as a colorless oil. $[\alpha]_D^{23}$ = +80.2 (c = 0.07, CH_2Cl_2). ^1H NMR: δ = 0.85 (t, J = 7.0 Hz, 3 H, 15-H), 0.83 (d, J = 6.6 Hz, 3 H, 16-H), 1.16–1.45 (m, 12 H, 8–12,14-H), 1.42 (d, J = 6.8 Hz, 3 H, 6-H), 1.75 (m, 1 H, 13-H), 2.29 (m, 2 H, 7-H), 5.19 (dd, J = 1.6, 6.8 Hz, 1 H, 5-H), 7.00 (d, J = 1.6 Hz, 1 H, 4-H) ppm. HRMS: calcd. for $C_{15}H_{26}O_2$ 238.1933 [M]$^+$; found 238.1938 (Δ = 0.5 ppm).

Preparation of Methyl Esters for Chiral Gas Chromatography: A mixture of magnesium metal (0.15 mmol) and THF (10 mL) was cooled to room temperature, after which the 1-alkyl bromide (0.1 mmol) was injected with a syringe through the septum into the flask. The reaction mixture was refluxed for 6 h and then cooled to room temperature. A large excess of chunks of dry ice was added quickly to the Grignard reagent through the extra neck of the flask. The mixture was stirred vigorously, warmed to room temperature, acidified with 20% hydrochloric acid (5 mL), and extracted with three 10-mL portions of diethyl ether. The ether extracts were combined, washed with two 50-mL portions of water, dried with anhydrous sodium sulfate, and concentrated to give 60–70% of the carboxylic acid as a slightly yellow oil. The appropriate methyl esters were prepared as described above.

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Note Added in Proof (July 10, 2006): A recent paper[46] reported on three new 5-alkenyl-3,3(2*H*)-furanones from two *Streptomyces* species using a genomic screening approach.

[1] S. Shapiro (Ed.), "Regulation of Secondary Metabolism" in *Actinomycetes*, CRC Press, Boca Raton, **1989**.
[2] V. Behal, *Adv. Appl. Microbiol.* **2000**, *47*, 113–156.
[3] M. Berthelot, G. Andre, *C. R. Hebd. Seances Acad. Sci.* **1891**, *112*, 598–599.
[4] N. N. Gerber, H. A. Lechevalier, *Appl. Microbiol.* **1965**, *13*, 935–938.
[5] N. N. Gerber, *Tetrahedron Lett.* **1968**, *9*, 2971–2974.
[6] A. A. Rosen, R. S. Safferman, C. I. Mashni, A. H. Romano, *Appl. Microbiol.* **1968**, *16*, 178–179.
[7] J. D. Dougherty, R. D. Campbell, R. L. Morris, *Science* **1966**, *152*, 1372–1373.
[8] N. N. Gerber, *Tetrahedron Lett.* **1973**, *14*, 771–774.
[9] J. S. Dickschat, T. Martens, T. Brinkhoff, M. Simon, S. Schulz, *Chem. Biodiv.* **2005**, *2*, 837–865.
[10] K. Stritzke, S. Schulz, H. Laatsch, E. Helmke, W. Beil, *J. Nat. Prod.* **2004**, *67*, 395–401.
[11] T. Rezanka, D. Libalova, J. Votruba, I. Viden, *Biotechnol. Lett.* **1994**, *16*, 75–78.
[12] T. Rezanka, J. Votruba, *Folia Microbiol.* **1998**, *43*, 47–50.
[13] T. Rezanka, J. Votruba, *Bioprocess. Eng.* **1998**, *19*, 159–160.
[14] J. Jachymova, J. Votruba, I. Viden, T. Rezanka, *Folia Microbiol.* **2002**, *47*, 37–41.
[15] K. A. Kovar, G. Francas, R. Seidel, *Arch. Pharm.* **1977**, *310*, 40–47.
[16] P. Canonne, M. Akssira, G. Lemay, *Tetrahedron Lett.* **1983**, *24*, 1929–1932.
[17] P. Canonne, M. Akssira, G. Fytas, *Tetrahedron* **1984**, *40*, 1809–1815.
[18] Z. Chen, R. M. Ortuno, *Tetrahedron: Asymmetry* **1999**, *10*, 1369–1380.
[19] K. Nishide, A. Aramata, T. Kamanaka, T. Inoue, M. Node, *Tetrahedron* **1994**, *50*, 8337–8346.
[20] T. Rezanka, V. M. Dembitsky, *Tetrahedron* **2001**, *57*, 8743–8749.
[21] B. B. Zeng, T. S. Hu, W. Yun, Y. K. Wu, Y. L. Wu, *Enantiomer* **2002**, *7*, 133–137.
[22] A. Gypser, C. Bulow, H. D. Scharf, *Tetrahedron* **1995**, *51*, 1921–1930.
[23] B. J. Rawlings, *Nat. Prod. Rep.* **1997**, *14*, 335–358.
[24] S. Horinouchi, T. Beppu, *Annu. Rev. Microbiol.* **1992**, *46*, 377–398.
[25] V. N. Odinokov, V. R. Akhmetova, Kh. D. Khasanov, A. A. Abduvakhabov, V. R. Sultanmuratova, G. A. Tolstikov, *Russ. J. Org. Chem. (Engl. Transl.)* **1992**, *28*, 915–918.
[26] L. Lardicci, G. P. Giacomelli, R. Menicagli, *Tetrahedron Lett.* **1972**, *13*, 687–690.
[27] C. Djerassi, L. E. Geller, *J. Am. Chem. Soc.* **1959**, *81*, 2789–2794.
[28] S. F. Velick, J. English, *J. Biol. Chem.* **1945**, *160*, 473–480.
[29] P. A. Levene, R. E. Marker, *J. Biol. Chem.* **1933**, *103*, 299–309.
[30] W. Dienet, J. Frelek, G. Snatzke, *Collect. Czech. Chem. Commun.* **1991**, *56*, 954–965.
[31] A. Tai, E. Syouno, K. Tanaka, M. Fujita, T. Sugimura, Y. Higashiura, M. Kakizaki, H. Hara, T. Naito, *Bull. Chem. Soc. Jpn.* **2002**, *75*, 111–122.
[32] H. Schechter, D. K. Brain, *J. Am. Chem. Soc.* **1963**, *85*, 1806–1812.
[33] K. Mori, *Tetrahedron* **1974**, *30*, 3817–3820.
[34] L. A. Aronica, S. Terreni, A. M. Caporusso, P. Salvadori, *Eur. J. Org. Chem.* **2001**, 4321–4329.
[35] M. Larsson, B. V. Nguyen, H. E. Högberg, E. Hedenström, *Eur. J. Org. Chem.* **2001**, 353–363.
[36] V. N. Odinokov, G. Y. Ishmuratov, R. Y. Kharisov, E. P. Serebryakov, G. A. Tolstikov, *Zh. Org. Khim.* **1992**, *28*, 1615–1618.
[37] Y. Nakamura, K. Mori, *Eur. J. Org. Chem.* **1999**, 2175–2182.
[38] K. M. Stephen, R. Buchecker, *Helv. Chim. Acta* **1988**, *71*, 451–460.
[39] Y. Naoshima, Y. Munakata, S. Yoshida, A. Funai, *J. Chem. Soc., Perkin Trans. 1* **1991**, 549–553.
[40] S. Baskaran, J. Das, S. Chandrasekaran, *J. Org. Chem.* **1989**, *54*, 5182–5184.
[41] V. N. Odinokov, O. S. Kukovinets, V. G. Kasradze, A. V. Dolidze, E. P. Serebryakov, L. V. Spirikhin, G. A. Tolstikov, *Zh. Org. Khim.* **1993**, *29*, 1936–1941.
[42] S. Génard, H. Patin, *Bull. Soc. Chim. Fr.* **1991**, *128*, 397–406.
[43] Z. Chen, R. M. Ortuno, *Tetrahedron: Asymmetry* **1999**, *10*, 1369–1380.
[44] R. M. Ortufio, M. Ballesteros, R. Batllori, M. Monsalvatje, J. Corbera, F. Sanchez-Ferrando, J. Font, *Tetrahedron Lett.* **1987**, *28*, 3405–3408.
[45] J. Corbera, J. Font, M. Monsalvatje, R. M. Ortuno, F. Sanchez-Ferrando, *J. Org. Chem.* **1988**, *53*, 4393–4395.
[46] A. H. Banskota, J. B. McAlpine, D. Sorensen, M. Aouidate, M. Piraee, A. M. Alarco, S. Omura, K. Shiomi, C. M. Farnet, E. Zazopoulos, *J. Antibiot.* **2006**, *59*, 168–176.

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