# 124. Metabolic Products of Microorganisms

Part 262[1])

## The Absolute Configuration of Sphydrofuran, a Widespread Metabolite from Streptomycetes

by **Kai U. Bindseil, Thomas Henkel,** and **Axel Zeeck***

Institut für Organische Chemie der Universität Göttingen, Tammannstr. 2, D-3400 Göttingen

and **Daniel Bur**[a]), **Daniel Niederer**[b]), and **Urs Séquin**[b])*

[a]) Institut für Anorganische Chemie der Universität Basel, Spitalstrasse 51, CH-4056 Basel
[b]) Institut für Organische Chemie der Universität Basel, St. Johanns-Ring 19, CH-4056 Basel

(22.VII.91)

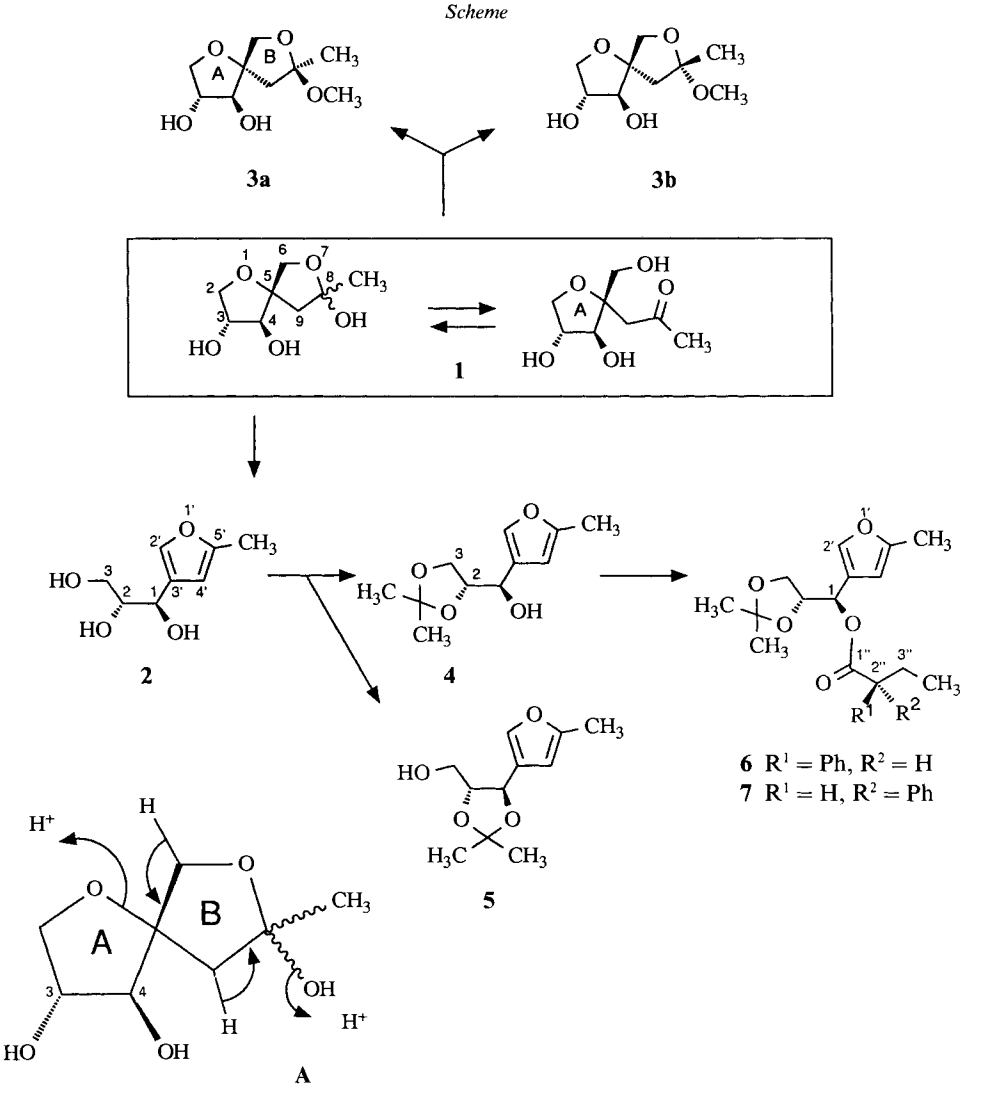
Sphydrofuran (**1**) was isolated from the culture filtrate of *Streptomyces sp.* (strain Gö 28 and Tü 3616) by chemical screening methods. Metabolite **1** is an anomeric and ring-chain tautomeric mixture and could easily be transformed into the stable furan derivative **2** under acidic conditions. The constitution and relative configuration of **1** was established by X-ray crystallography of its 8-*O*-methyl derivative **3a**. The absolute configuration at C(4) of **1** and thus of the whole molecule (3*R*,4*S*,5*S*) could be determined starting from **2** using the *Helmchen* method.

**Introduction.** – In the course of our chemical screening studies, different staining reagents proved to be useful tools to detect colorless or pale secondary metabolites and to reveal biosynthetic sequences in microorganisms [2–4]. The discoloration of sphydrofuran (**1**) and of its anhydro derivative 1-(5-methylfur-3-yl)propane-1,2,3-triol (**2**) from colorless to intensive pink on silica-gel TLC plates after staining with *Ehrlich*'s reagent is one of the first examples for the chemical screening method [5] and probably the reason that these metabolites were detected in different strains and seem to be widespread. The unknown configuration of **1** affected our biosynthetic studies adversely [6]. This paper deals with the elucidation of the relative and absolute configuration of **1** and some of its derivatives.

**Isolation and Transformation Reactions.** – The AcOEt extracts of the culture filtrate of the strains Tü 3616 as well as Gö 28, grown on a oatmeal or soybean meal/mannitol medium, respectively, led to raw extracts which, on silica-gel TLC plates in $CHCl_3$/MeOH systems, showed numerous colorless compounds with similar color reactions on spraying with *Ehrlich*'s reagent. The major component was identified as sphydrofuran (**1**; see *Scheme*) [5] [7]. During the isolation procedure, especially under acid conditions, the amounts of **1** decreased to the benefit of **2**. In the presence of MeOH, the amounts of two other compounds increased; they could be identified as the known anomeric 8-*O*-methyl derivatives **3a** and **3b** of **1**. Compounds **2** as well as **3a** and **3b** are artifacts derived from **1**.

[1]) Part 261: [1].

*Scheme*




Their structures have been described in detail [5] [7] and led to a proposal for the relative configuration of the chiral centers within **3a** and **3b**. The analysis of the complex $^1$H- and $^{13}$C-NMR spectra of **1** suggested an equilibrium mixture consisting of two anomeric, bicyclic hemiacetals and a monocyclic ketone.

The furan derivative **2** could be obtained from **1** under mild acidic conditions, probably starting by an elimination of $H_2O$ followed by a ring-A-opening elimination (see **A**), facilitated by the fact that the energetically favored furan was formed (*Scheme*). This reaction took place under retention of configuration at C(3) and C(4). The acetals **3a** and **3b**, containing the unchanged spiro center of **1**, were formed by treatment of **1** with $10^{-3}$ M

HCl in MeOH in the ratio 1 : 0.7. They are stable under neutral conditions and could easily be hydrolyzed to **1** and its elimination product **2**. Thus, **2**, **3a**, and **3b** were the better starting materials to prove the configuration of **1**.

**Structural Elucidation.** – The constitutions of **2**, **3a**, and **3b** were examined by one- and two-dimensional $^1$H- and $^{13}$C-NMR methods. Comparison of the $^1$H-NMR data and optical rotation values showed that the isolated compounds were identical with those described by *Umezawa* and coworkers [5] [7]. First we tried to prove the predicted relative configuration by NMR methods. The small coupling constant ($J(3,4) < 2$ Hz) in **3a** and **3b** revealed the *trans*-configuration of the vicinal diol in the five-membered ring [8]. But all further attempts to determine the relative configuration by NOE or 2D-ROESY experiments failed. Finally, an X-ray structure analysis of **3a** provided the relative configurations unambiguously (see the *Fig.* and *Table 1*).

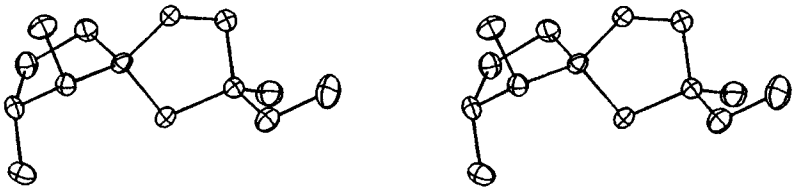


Figure. *Stereo ORTEP plot of* **3a**

Table 1. *Crystallographic Data of* **3a**

| | | | |
|---|---|---|---|
| Formula | $C_9H_{16}O_5$ | $\theta_{max}$ [°] | 27 |
| Space group | Orthorhombic, $P2_12_12_1$ | Radiation [Å] | Mo$K_\alpha$ ($\lambda = 0.71069$) |
| $a$ [Å] | 5.639(1) | Scan mode | $\omega/2\theta$ |
| $b$ [Å] | 9.577(2) | Collected intensities | $+h, +k, +l$ |
| $c$ [Å] | 19.120(6) | Absorption [cm$^{-1}$] | 0.68 |
| $\alpha$ [°] | 90.0 | No. of ind. reflections | 1029 |
| $\beta$ [°] | 90.0 | No. of refl. used in ref. | 765 ($\|F_o\| > 2\sigma(F_o)$) |
| $\gamma$ [°] | 90.0 | No. of variables | 136 |
| $V$ [Å$^3$] | 1033(1) | Observations/parameter | 5.6 |
| $Z$ | 4 | Max. and min. $\Delta\rho$ [e·Å$^{-3}$] | 0.28, −0.20 |
| $F$(000) | 440 | Final $R$ | 0.062 |
| Temperature [K] | 293 | | |

To establish the absolute configuration of **1**, the absolute configuration of one chiral center of **1** or of its derivatives has to be determined. Because of its stability, we chose furan derivative **2** to which we applied the *Helmchen* method for secondary alcohols [9]. This method is based on typical $^1$H-NMR-signal shifts caused by an acylation with 2-phenylbutyric acid. For an unambiguous shift analysis and to guarantee the essential minimum-energy conformations, it was necessary to esterify only one of the secondary OH groups of **2**. Thus, **2** was first treated with 2,2-dimethoxypropane and malonic acid to give the (isopropylidenedioxy)propanols **4**/**5** which were separated by chromatography (silica gel, $CHCl_3$/MeOH; see *Scheme*). Compound **4** proved to be suitable for the elucidation of the configuration at C(1) (=C(4) in **1**). Esterification of 4 with both enantiomers of 2-phenylbutyric acid resulted in the diastereoisomeric 2-phenylbutyrates

**6** and **7**. The $^1$H-NMR spectra of the (2″*S*)-2″-phenylbutyrate **6** showed a remarkable high-field shift of $H_a$–C(3), $H_b$–C(3), and the $Me_2C$ group, while (2″*R*)-2″-phenylbutyrate **7** showed such effects for H–C(2′), H–C(4′), and Me–C(5′) (*Table 2*). According to the rules of *Helmchen*, the configuration at C(1) of **6** and **7** can be deduced unequivocally to be *R*. Thus, for C(4) of **1**, **3a**, and **3b** follows the (*S*)-configuration. Taking into account the X-ray data of **3a**, its structure can now be described as (3*R*,4*S*,5*S*,8*R*)-8-methoxy-8-methyl-1,7-dioxaspiro[4.4]nonane-3,4-diol, which represents also the stable chiral centers of sphydrofuran (**1**) itself.

Table 2. *$^1$H-NMR Data* ($CDCl_3$, 200 MHz) *of (1*R*,2*R*)-1-(5-Methylfur-3-yl)propane-1,2,3-triol* (**2**) *and of Its Derivatives* **4**–**7**. $\delta$ in ppm rel. to TMS ( = 0 ppm), *J* in Hz.

| | **2** | **4** | **5**[a]) | **6** | **7** |
|---|---|---|---|---|---|
| H–C(1) | 4.59 | 4.47 | 4.82 | 5.68 | 5.72 |
| | (*d*, *J* = 5) | (*d*, *J* = 7) | (*d*, *J* = 9) | (*d*, *J* = 7) | (*d*, *J* = 7) |
| H–C(2) | 3.5 3.7 (*m*) | 4.22 | 3.95 | 4.32 | 4.29 |
| | | (*ddd*, *J* = 7, 6, 6) | (*ddd*, *J* = 9, 4, 3) | (*ddd*, *J* = 7, 7, 5.5) | (*ddd*, *J* = 7, 7, 6) |
| $H_a$–C(3) | 3.5–3.7 (*m*) | 3.93 | 3.85 | 3.89 | 3.82 |
| | | (*dd*, *J* = 9, 6) | (*dd*, *J* = 12, 3) | (*dd*, *J* = 8.5, 7) | (*dd*, *J* = 9, 7) |
| $H_b$–C(3) | 3.48 | 3.71 | 3.60 | 3.66 | 3.53 |
| | (*dd*, *J* = 11, 6) | (*dd*, *J* = 9, 6) | (*dd*, *J* = 12, 4) | (*dd*, *J* = 8.5, 5.5) | (*dd*, *J* = 9, 6) |
| H–C(2′) | 7.34 | 7.30 | 7.32 | 7.02 | 7.30 |
| | (*d*, *J* = 1) | (*d*, *J* = 1) | (*d*, *J* = 1) | (*d*, *J* = 1) | (*d*, *J* = 1) |
| H–C(4′) | 6.10 | 6.01 | 6.03 | 5.69 | 5.95 |
| | (*dd*, *J* = 1, 1) | (*dd*, *J* = 1, 1) | (*dd*, *J* = 1, 1) | (*dd*, *J* = 1, 1) | (*dd*, *J* = 1, 1) |
| Me–C(5′) | 2.25 | 2.26 | 2.28 | 2.16 | 2.24 |
| | (*d*, *J* = 1) | (*d*, *J* = 1) | (*d*, *J* = 1) | (*d*, *J* = 1) | (*d*, *J* = 1) |
| $Me_2C$ | – | 1.38, 1.48 | 1.48, 1.51 | 1.36, 1.42 | 1.28, 1.28 |

[a]) For convenience, **5** is numbered like **4**; systematic name in the *Exper. Part.*

**Discussion.** – Sphydrofuran (**1**) is a widespread but structurally unique secondary metabolite produced by Actinomycetes [10]. We found no inhibition activity for **1** against various organisms, but a growth promotion for some bacteria and viruses under the influence of **2**. Biosynthetical studies of this spiro-linked polyol, which shows some similarities with monosaccharides, will be subject of a further investigation [6]. At first sight, we propose a biogenesis starting from the carbohydrate metabolism (A type) [11]. Minor components detectable in the raw extracts derived from strain Gö 28 may reveal parts of the biosynthetic sequence.

We are grateful to Prof. Dr. *H. Zähner* and Dr. *H. Drautz*, Institut für Mikrobiologie I der Universität Tübingen, for providing us with crude extracts of the strain Tü 3616. We wish to thank the *Fonds der Chemischen Industrie* and the *Swiss National Science Foundation* for financial support.

## Experimental Part

*General.* TLC: Silica gel *Sil G-25 $UV_{254+366}$* precoated plates (*Macherey-Nagel*); detection after staining with *Ehrlich*'s reagent (74 ml of MeOH, 25 ml of 12M HCl, 1 g of 4-(dimethylamino)benzaldehyde) 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*). M.p.: *Reichert* hot-stage microscope; not corrected. Optical rotation: *Perkin-Elmer*, model *241*, with thermostat; temp. 20°. IR spectra: *Perkin-Elmer*, model *298*. NMR experiments: *Varian-VXR-200*, *-VXR-400*, and *-VXR-500* instruments; TMS ($^1$H) or solvent signal ($^{13}$C) as internal standards; $^{13}$C multiplicities from APT experiments. MS: *Finnigan MAT 311 A* (70 eV) and *Varian MAT 731* (70 eV) for high resolution (HR).

*Fermentation. Streptomyces sp.* (strain Gö 28) was cultivated in *Erlenmeyer* flasks (1000 ml) containing 150 ml of culture medium (2% soybean meal, 2% mannitol, pH 7.2). After shaking for 72 h at 28°, the mycelium was separated by filtration after addition of *Hyflocelite*. The culture filtrate was extracted twice by AcOEt. The solvent was removed and the crude residue separated by repeated chromatography on precoated (pH 10) silica gel ($CHCl_3$/MeOH 8:2) and *Sephadex LH-20* (MeOH). The fractions containing **1** and **2** were detected by TLC control (*Ehrlich*'s reagent). For further details, see [6].

*(3R,4S,5S)-8-Methyl-1,7-dioxaspiro[4.4]nonane-3,4,8-triol* (**1**). $R_f$ ($CH_2Cl_2$/acetone 4:1) 0.09. $[\alpha]_D^{20} = +16$ ($c = 0.5$, $H_2O$). IR (KBr): 3380, 2930, 1630, 1570.

*(1R,2R)-1-(5-Methylfur-3-yl)propane-1,2,3-triol* (**2**). $R_f$ ($CH_2Cl_2$/acetone 4:1) 0.13. $[\alpha]_D^{20} = +137$ ($c = 1.0$, MeOH). IR (KBr) 3380, 2930, 1630*s*, 1570*s*. $^1$H-NMR: *Table 2*. $^{13}$C-NMR (50.3 MHz, ($D_6$)acetone): 13.4 (*q*, *Me*–C(5′)); 64.0 (*t*, C(3)); 68.0 (*d*, C(2)); 76.0 (*d*, C(1)); 106.3 (*d*, C(4′)); 128.6 (*s*, C(3′)); 138.9 (*d*, C(2′)); 153.8 (*s*, C(5′)). EI-MS (70 eV): 172 (6, $M^+$), 154 (9, $[M - H_2O]^+$), 111 (100), 83 (40).

*(3R,4S,5S,8R)-8-Methoxy-8-methyl-1,7-dioxaspiro[4.4]nonane-3,4-diol* (**3a**). Needles. $R_f$ ($CH_2Cl_2$/acetone 4:1) 0.19. M.p. 95°. $[\alpha]_D^{20} = -52$ ($c = 0.3$, MeOH). IR (KBr): 3400*s* (br.), 2990, 2940, 1470*w*, 1430*w*, 1385*m*, 1315*m*, 1185*m*, 1050*s*. $^1$H-NMR (400 MHz, $CDCl_3$): 1.48 (*s*, Me–C(8)); 2.18 (*d*, $J = 14$, $H_a$–C(9)); 2.32 (*d*, $J = 14$, $H_b$–C(9)); 3.07 (*d*, $J = 4$, OH–C(4) or OH–C(3)); 3.23 (*s*, MeO–C(8)); 3.61 (*d*, $J = 3$, OH–C(3) or OH–C(4)); 3.66 (*dd*, $J = 5$, 10, $H_a$–C(2)); 3.81 (*d*, $J = 10$, $H_a$–C(6)); 4.10 (*m*, $H_b$–C(2), H–C(4)); 4.14 (*d*, $J = 10$, $H_b$–C(6)); 4.22 (*m*, H–C(3)). $^1$H-NMR (500 MHz, ($D_6$)acetone): 1.37 (*s*, Me–C(8)); 1.96 (*dd*, $J = 14$, 1, $H_a$–C(9)); 2.33 (*d*, $J = 14$, $H_b$–C(9)); 3.13 (*s*, MeO–C(8)); 3.58 (*dd*, $J = 10$, 3, $H_a$–C(2)); 3.72 (*dd*, $J = 10$, 1, $H_a$–C(6)); 4.03 (*dd*, $J = 10$, 5, $H_b$–C(2)); 4.08 (*dd*, $J = 5$, 2, H–C(4)); 4.10 (*d*, $J = 10$, $H_b$–C(6)); 4.17 (*m*, H–C(3)); 4.24 (*d*, $J = 4$, OH–C(3)); 4.34 (*d*, $J = 5$, OH–C(4)). $^{13}$C-NMR (50.3 MHz, ($D_6$)acetone): 22.2 (*q*, *Me*–C(8)); 48.5 (*q*, *Me*O–C(8)); 50.6 (*t*, C(9)); 72.9 (*t*, C(6)); 73.5 (*t*, C(2)); 78.9 (*d*, C(3)); 82.5 (*d*, C(4)); 93.4 (*s*, C(5)); 109.0 (*s*, C(8)). $^{13}$C-NMR (101 MHz, $CDCl_3$): 20.9 (*q*, *Me*–C(8)); 48.6 (*q*, *Me*O–C(8)); 49.7 (*t*, C(9)); 71.4 (*t*, C(2)); 71.5 (*t*, C(6)); 77.0 (*d*, C(3)); 82.2 (*d*, C(4)); 91.2 (*s*, C(5)); 108.1 (*s*, C(8)). EI-MS: 189 (10, $[M - Me]^+$), 173 (20, $[M - MeO]^+$), 159 (14), 82 (40).

*X-Ray Structure Determination for* **3a**. Crystals (colorless prisms) were obtained from $Et_2O$. Reflection intensities were collected at r.t. on a four-circle diffractometer *Enraf-Nonius CAD4* equipped with a graphite monochromator and using Mo$K_\alpha$ radiation. Unit-cell parameters were determined from 25 accurately centered, independent, and strong reflexions by least-squares method. Four standard reflexions monitored every 3600 s during data collection showed no intensity loss. The usual conrrections except for absorption were applied. The structure was solved by direct methods with SHELXS-86 [12] and refined with SHELXS-76 [13]. Non-H-atoms were refined anisotropically. The positions for H-atoms were calculated. Details of crystal data and parameters of data collection are given in *Table 1*. Crystallographic data are deposited with the *Cambridge Crystallographic Data Centre*, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, England.

*(3R,4S,5S,8S)-8-Methoxy-8-methyl-1,7-dioxaspiro[4.4]nonane-3,4-diol* (**3b**). $R_f$ ($CH_2Cl_2$/acetone 4:1) 0.17. $[\alpha]_D^{20} = +111$ ($c = 0.3$, MeOH). IR (KBr): 3400*s* (br.), 2990, 2940, 1470*w*, 1430*w*, 1385*m*, 1315*m*, 1185*m*, 1050*s*. $^1$H-NMR (200 MHz, ($D_6$)acetone): 1.31 (*s*, Me–C(8)); 1.96 (*dd*, $J = 14$, 1, $H_a$–C(9)); 2.26 (*d*, $J = 14$, $H_b$–C(9)); 3.17 (*s*, MeO–C(8)); 3.58 (*dd*, $J = 10$, 3, $H_a$–C(2)); 3.68 (*dd*, $J = 10$, 1, $H_a$–C(6)); 4.03 (*dd*, $J = 10$, 5, $H_b$–C(2)); 4.0–4.1 (*m*, overlapped, H–C(4)); 4.22 (*d*, $J = 10$, $H_b$–C(6)); 4.17 (*m*, H–C(3)).

*2,3-(Isopropylidenedioxy)-1-(5-methylfur-3-yl)propan-1-ol* (**4**) *and 2,3-(Isopropylidenedioxy)-3-(5-methylfur-3-yl)propan-1-ol* (**5**). A soln. of 25 mg of **1** and 30 mg of malonic acid in 2,2-dimethoxypropane was stirred for 12 h at r.t. After evaporation, the residue was purified by CC ($SiO_2$, $CHCl_3$/MeOH 95:5, and *Sephadex LH-20*, MeOH): 7 mg (23%) of **4** and 6 mg (20%) of **5**.

**4**: $R_f$ ($CHCl_3$/MeOH 9:1) 0.62. $[\alpha]_D^{20} = -18$ ($c = 0,4$, MeOH). IR (KBr): 3420, 2990, 1765, 1380, 1070. $^1$H-NMR: *Table 2*. $^{13}$C-NMR (50.3 MHz, $CDCl_3$): 13.6 (*q*, *Me*–C(5′)); 25.3 (*q*, $Me_2$C); 26.9 (*q*, $Me_2$C); 66.2 (*t*,

C(3)); 68.6 (*d*, C(2)); 79.3 (*d*, C(1)); 104.7 (*d*, C(4')); 110.1 (*s*, $Me_2C$); 125.2 (*s*, C(3')); 138.3 (*s*, C(2')); 153.3 (*s*, C(5')). EI-MS: 212 (3, $M^+$), 197 (3), 154 (10), 137 (14), 111 (35). HR-MS: 212.10486 ($C_{11}H_{16}O_4$, calc. 212.10486).

**5**: $R_f$ ($CHCl_3$/MeOH 9:1) 0.81. $[\alpha]_D^{20} = +5$ ($c = 0.6$, MeOH). $^1$H-NMR: *Table 2*. $^{13}$C-NMR (50.3 MHz, $CDCl_3$; numbering as for **4**): 14.2 (*q*, *Me*–C(5')); 27.6 (*q*, *Me*$_2$C); 27.8 (*q*, *Me*$_2$C); 61.2 (*t*, C(3)); 72.2 (*d*, C(2)); 82.3 (*d*, C(1)); 105.1 (*d*, C(4')); 109.7 (*s*, $Me_2C$); 123.7 (*s*, C(3')); 139.5 (*d*, C(2')); 154.2 (*s*, C(5')). EI-MS: 212 (3, $M^+$), 197 (2), 154 (6), 137 (18), 111 (18).

*2,3-(Isopropylidenedioxy)-1-(5-methylfur-3-yl)propyl (*S*)-2-Phenylbutyrate* (**6**). A soln. of 6 mg of **4**, 100 mg of DCC, 20 mg of 4-(dimethylamino)pyridine, and 0.1 ml of (*S*)-2-phenylbutyric acid was stirred for 1 h at r.t. After addition of 5 ml of MeOH, the mixture was evaporated. The crude material was purified by CC (silica gel, $CHCl_3$) and gel chromatography (*Sephadex LH-20*, MeOH): 3 mg (30%) of **6**. $R_f$ ($CHCl_3$/MeOH 9:1) 0.85. $^1$H-NMR: *Table 2*; 0.88 (*t*, $J = 8$, Me(4'')); 1.80 (*m*, $H_a$–C(3'')); 2.0–2.3 (*m*, $H_b$–C(3'')); 3.52 (*t*, $J = 8$, H–C(2'')); 7.2–7.4 (*m*, 5 arom. H). EI-MS: 358 (8, $M^+$), 258 (18), 212 (22), 119 (84) 101 (100).

*2,3-(Isopropylidenedioxy)-1-(5-methylfur-3-yl)propyl (*R*)-2-Phenylbutyrate* (**7**). As described for **6**, 6 mg of **4** were esterified with (*R*)-2-phenylbutyric acid: 3.6 mg (35.5%) of **7**. $R_f$ ($CHCl_3$/MeOH 9:1) 0.86. $^1$H-NMR: *Table 2*; 0.86 (*t*, $J = 8$, Me(4'')); 1.80 (*m*, $H_a$–C(3'')); 2.10 (*m*, $H_b$–C(3'')); 3.50 (*t*, $J = 8$, H–C(2'')); 7.2–7.4 (*m*, 5 arom. H). EI-MS: 358 (20, $M^+$), 258 (22), 212 (28), 119 (72), 101 (100).

## REFERENCES


[1] H. Hoff, H. Drautz, H. Zähner, W. Keller-Schierlein, S. Philipps, M. Ritzau, A. Zeeck, submitted to *J. Antibiot.*

[2] H. Zähner, H. Drautz, P. Fiedler, R. Grote, W. Keller-Schierlein, W. A. König, A. Zeeck, in 'Biology of Actinomycetes '88', Proceedings of the 7th International Symposium on Biology of Actinomycetes, Eds. Y. Okahami, T. Beppu, and H. Ogarawa, Japan Scientific Press, Tokyo, 1988.

[3] S. Grabley, J. Wink, A. Zeeck, in 'Jahrbuch der Biotechnologie', Eds. P. Praeve, M. Schlingmann, W. Crueger, K. Esser, R. Thauer, and F. Wagner, Carl Hanser Verlag, München–Wien, 1990, Vol. 3, p. 379–390.

[4] D. Niederer, U. Séquin, H. Drautz, H. Zähner, *Helv. Chim. Acta* **1990,** *73,* 2129.

[5] S. Umezawa, T. Usui, H. Umezawa, T. Tsuchiya, T. Takeuchi, M. Hamada, *J. Antibiot.* **1971,** *24,* 85.

[6] K. U. Bindseil, T. Henkel, A. Zeeck, in preparation.

[7] T.Usui, S. Umezawa, T. Tsuchiya, H. Naganawa, T. Takeuchi, H. Umezawa, *J. Antibiot.* **1971,** *24,* 93.

[8] J. D. Stevens, H. G. Fletcher, *J. Org. Chem.* **1968,** *33,* 1799.

[9] G. Helmchen, *Tetrahedron Lett.* **1974,** *16,* 1527.

[10] S. Breiding-Mack, Ph. D. thesis, University of Göttingen, 1984; I. Steinhoff, Ph. D. thesis, University of Göttingen, 1987.

[11] J. Rohr, A. Zeeck, in 'Jahrbuch der Biotechnologie', Eds. P. Praeve, M. Schlingmann, W. Crueger, K. Esser, R. Thauer, and F. Wagner, Carl Hanser Verlag, München–Wien, 1988, Vol. 2, p. 263–295.

[12] G. M. Sheldrick, Universität Göttingen, 1986.

[13] G. M. Sheldrick, University of Cambridge, 1976.