Synthesis of the Antibiotic (*R*)-Reutericyclin *via Dieckmann* Condensation

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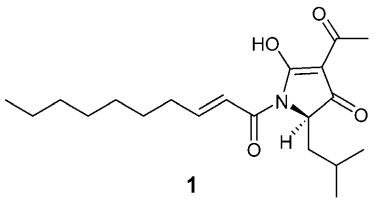
(*R*)-Reutericyclin ((*R*)-**1**), a bactericidal, amphiphilic natural product with a trisubstituted tetramic acid moiety, was prepared in four steps from D-leucine in an overall yield of 24%. The chiral heterocyclic portion of **1** was synthesized by *Dieckmann* cyclization of ethyl *N*-(acetoacetyl)leucinate (**7**), and the resulting pyrrole derivative **8** was *N*-acylated with (*E*)-dec-2-enoyl chloride in the presence of BuLi at $-70°$ (*Scheme 2*). This new procedure is straightforward and allows the synthesis of both antipodes of reutericyclin in an enantiomeric excess (ee) of *ca.* 80%.

Introduction. – Reutericyclin (=(2*R*)-4-acetyl-1,2-dihydro-5-hydroxy-2-(2-methylpropyl)-1-[(2*E*)-1-oxodec-2-enyl]-3*H*-pyrrol-3-one; (*R*)-**1**)[1]) is a tetramic acid that acts against a broad spectrum of *Gram*-positive bacteria. The amphiphilic metabolite was the first low-molecular-weight antibiotic isolated from a lactic acid bacterium, *i.e.*, *Lactobacillus reuteri* LTH2584 [1][2]. Reutericyclin is produced during food industrial sourdough fermentation by *Lactobacillus reuteri*, and contributes to the resistance of its producer strain [3]. The mode of action is based on its proton-ionophoric properties, selectively dissipating the transmembrane proton potential (ΔpH) [4][5].



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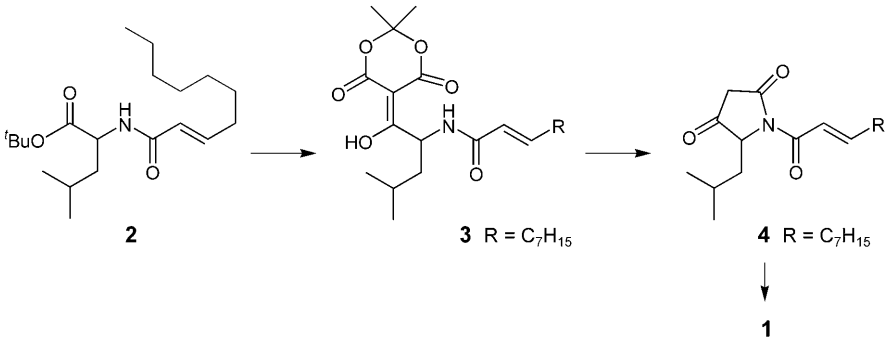
There are two general pathways to synthesize reutericyclin (**1**). In the first published synthesis [6], leucine *tert*-butyl ester is *N*-acylated with (*E*)-dec-2-enoyl chloride to the amide **2** (*Scheme 1*). After ester hydrolysis, the resulting *N*-acylamino acid is reacted with *Meldrum*'s acid to the intermediate condensation product **3**, which thermally cyclizes to the tetramic acid **4**. The Ac group is introduced during the last of the seven steps (starting from leucine) to yield racemic **1**. Racemization is due to the last

[1]) Systematic name of the *enolic* form drawn (tautomerism; see *Figure* in the *Exper. Part*).

step in which $TiCl_4$, a strong *Lewis* acid, is used as a catalyst. The separation of the enantiomers of racemic reutericyclin is expected to be difficult for large-scale preparations. Furthermore, the reaction conditions for the thermal cyclization with *Meldrum*'s acid have to be strictly followed to obtain reproducible results. For these reasons, we developed a second synthetic pathway aiming at the synthesis of the two antipodes of **1**.

Scheme 1. *Published Synthesis of Racemic* **1** [6]



Results and Discussion. – To avoid the vigorous conditions typically required for *C*-acylation, we designed a synthetic route that includes *N*-acylation with (*E*)-dec-2-enoyl chloride in the *last* step. As outlined in *Scheme 2*, the Ac group was introduced first by coupling D-leucine ethyl ester (**5**) with *N*-hydroxysuccinimidyl acetoacetate (**6**) [7]. The resulting leucinate **7** was cyclized to the 1,2-dihydro-3*H*-pyrrol-3-one **8** *via Dieckmann* condensation in the presence of EtONa/EtOH, following a modified procedure to cyclize pyrrole derivatives [8]. Alternative attempts to introduce the acetoacetyl group and to carry out the *Dieckmann* cyclization failed.

The last, crucial step involved *N*-acylation of tautomeric **8** (*cf. Figure*) with (*E*)-dec-2-enoyl chloride. Since **8** is a *cyclic* amide, several bases such as DMAP, Et_3N/*N*,*N*-dimethylaniline (*via* an Me_3SiN group), pyridine (*Schotten–Baumann* conditions), and $LiNH_2$ were tested as acylation catalysts. However, no coupling product with the acid chloride was obtained. Similarly, when (*E*)-dec-2-enoic acid was activated with 1-hydroxy-1*H*-benzotriazole (HOBt) or transformed into a mixed anhydride with isobutyl chlorocarbonate (using $Et(i\text{-}Pr)_2N$ or DBU as base), no reaction to **1** was observed. Attempted condensations by fusion of the neat compounds or in the presence of 18-crown-6 in dimethoxyethane (DME) were also unsuccessful. Finally, BuLi (2.2 equiv.)[2]) was found to remove the amide proton of **8** at $-70°$ under salt formation, and, after 20 min of vigorous stirring, the resulting strong nucleophile was reacted with dec-2-enoyl chloride. After aqueous workup and column chromatographic purification, (*R*)-**1** was obtained as an amorphous powder.

Further purification was achieved by recrystallization. Several solvents were tested to this end, especially EtOH, Me_2CO, AcOEt, Et_2O, MeOH, and petroleum ether.

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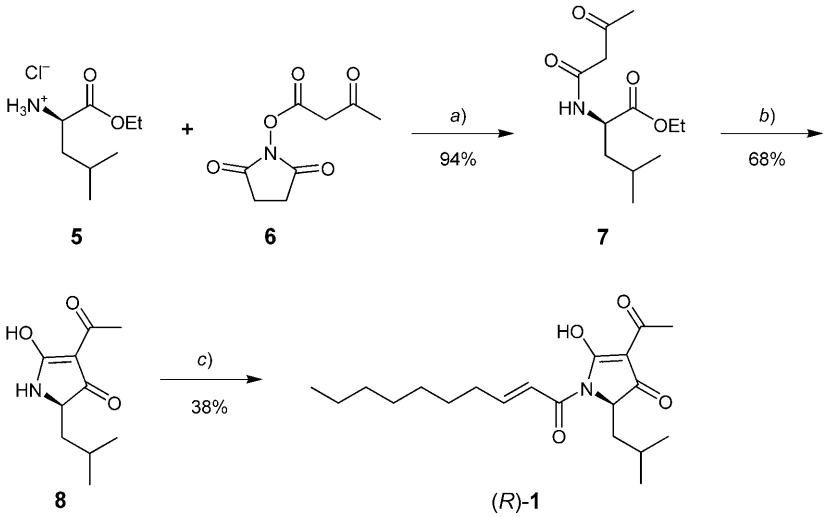
[2]) Only few cases are known in which unbranched BuLi was used in combination with NH compounds [9].

Scheme 2. *Synthesis of (*R*)-***1*** from Ethyl* D-*Valinate*



a) CH_2Cl_2, r.t., 3 h. *b*) EtONa/EtOH, Δ, 3 h. *c*) 1. THF, BuLi (2.2. equiv.), – 70°; 2. (*E*)-dec-2-enoyl chloride; 3. H_3O^+.

Since reutericyclin (**1**) possesses pronounced amphiphilic properties due to its lipophilic alkyl chain and the hydrophilic tetramic acid part, it is soluble in many solvents. In most of the above solvents, it formed a gel upon cooling, rather than crystals; and slow evaporation of the solvent rendered an oily residue. Nevertheless, we finally achieved to obtain a solid material from a cyclohexane/acetone mixture upon slow evaporation.

We observed that the NMR spectra of reutericyclin (**1**) seem to depend on the workup procedure and NMR solvent, which may give rise to different tautomeric forms and H-bonding patterns. Due to staple effects, the ^{1}H NMR spectra of some reutericyclin preparations showed only poorly resolved signals, with almost no fine structures. However, it was possible to record acceptable ^{1}H- and ^{13}C-NMR spectra and to perform a HMQC experiment with a dilute (D_6)DMSO solution. The complete NMR data of **1** have been previously published [1][6].

After total hydrolysis and derivatization of the samples of synthetic **1**, GC/MS analysis on a chiral capillary column showed an enantiomeric purity of *ca*. 90% (80% ee) of the corresponding L- or D-leucine, depending on the configuration of the original substrate **5**.

Experimental Part

General. M.p.: *Büchi SMP-20* apparatus; uncorrected. UV Spectra: *Beckmann DU660* spectrometer; λ_{max} (ε) in nm. Optical rotation: *Perkin-Elmer 341* polarimeter. IR Spectra: *Perkin-Elmer 1600* spectrometer; in cm^{-1}. NMR Spectra: *Bruker DPX-400* and *DRX-500* spectrometers; at 400 or 500 (^{1}H), or at 100 or 125 MHz (^{13}C), resp. HR-EI-MS: *Kratos MS-50* spectrometer (*A.E.I.*, Manchester).

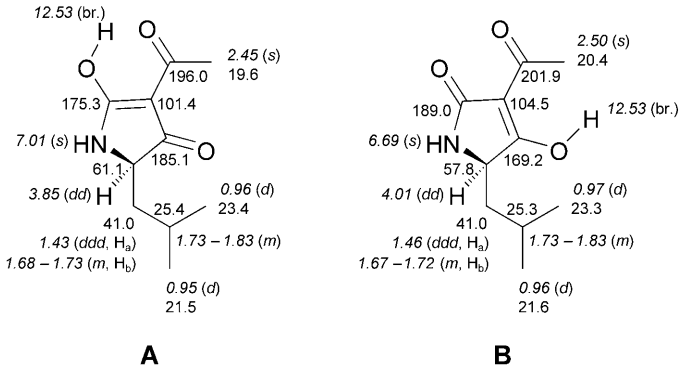


Figure. *^{1}H- and ^{13}C-NMR* (italics) *chemical shifts and multiplicities for compound* **8** *present in two major tautomeric forms.* The observed ratio of **A**/**B** was *ca.* 4:1 in $CDCl_3$ solution at 20°. The two Me groups of the i-Bu side chain were tentatively assigned.

*(*E*)-Dec-2-enoic Acid*. This compound was prepared through *Knoevenagel* condensation of octanal and malonic acid [6][10]. Variations of temp., base, and reaction time were tested, but always a certain amount of *β*-hydroxydecanoic acid was detected, which could not be separated completely from the desired product. A most-useful old variant [11] proved to be stirring at r.t. for 36 h. Yield: 70% (for 0.2 mol).

*(*E*)-Dec-2-enoyl Chloride.* This compound was prepared by heating the acid with $SOCl_2$ followed by distillation [6]. Yield 76% (for 0.12 mol).

Ethyl D-*Leucinate Hydrochloride* (**5**). D-Leucine was heated under stirring in EtOH soln. saturated with HCl gas [12]. Yield: 87% (for 38 mmol). $[\alpha]_D^{20} = -18$ ($c = 5$, EtOH; $[\alpha]_D^{20}$ of commercially available L-enantiomer: +18).

Ethyl N*-(Acetoacetyl)*-D-*leucinate* (**7**) [7]. To a soln. of **5** (4.892 g, 25 mmol) in CH_2Cl_2 (40 ml) was added 2 equiv. of $Et(i\text{-}Pr)_2N$ (6.463 g, 8.56 ml, 50 mmol). Under Ar gas, a previously prepared suspension of commercial *N*-(hydroxysuccinimidyl)acetoacetate (**6**; 4.979 g, 25 mmol) in CH_2Cl_2 (40 ml) was added through a dropping funnel within 15 min, and the resulting mixture was stirred at r.t. for 3 h. The soln. was washed with 5% aq. HCl (5×40 ml), and the aq. layers were re-extracted with CH_2Cl_2 (3×50 ml). The combined org. layers were washed with sat. $NaHCO_3$ soln. (3×) and H_2O (1×). After drying (Na_2SO_4) and evaporation of the solvent, a pale yellow oil was obtained, which was used without further purification. Yield of **7**: 5.71 g (94%). M_r 243.30 g/mol. ^{1}H-NMR ($CDCl_3$): 0.93, 0.94 (2*d*, 2 Me of i-Bu); 1.27 (*t*, *Me*CH_2O); 1.55–1.66 (*m*, CH_2 of i-Bu)); 1.68 (*m*, Me_2CH); 2.27 (*s*, Ac); 3.43 (*s*, AcCH_2); 4.18 (*q*, OCH_2); 4.59 (*td*, NCH); 7.17 (*d*, NH). ^{13}C-NMR ($CDCl_3$): 14.2 (*Me*CH_2O); 22.0, 22.9 (2 Me of i-Bu); 25.0 (Me_2CH); 31.0 (Me of Ac); 41.4 (CH_2 of i-Bu)); 49.7 (AcCH_2); 51.1 (NCH); 61.4 (OCH_2); 165.4 (COO); 172.7 (NCO); 204.1 (C=O of Ac).

*(*2R*)-4-Acetyl-1,2-dihydro-5-hydroxy-2-(2-methylpropyl)-3*H*-pyrrol-3-one* (**8**)[1]. To a soln. of EtONa, prepared by dissolving elemental Na (0.58 g, 1.07 equiv.) in anh. EtOH (18 ml) under Ar, **7** (5.71 g, 23.5 mmol) in EtOH (30 ml) was added through a dropping funnel within 25 min at r.t., and the resulting mixture was refluxed for 3 h. After cooling down, the mixture was neutralized with 10% AcOH in EtOH (4.57 ml), and the solvent was evaporated. CH_2Cl_2 (25 ml) was added to the pale-yellow, gel-like residue, which was washed with H_2O (4×) to remove AcONa. The aq. layers were extracted with CH_2Cl_2 (3×), the combined org. layers were dried (Na_2SO_4), and the solvent was removed on a rotary evaporator. The remaining solid was crystallized from EtOH in several fractions to afford colorless **8**. Yield: 3.152 g (68%). M_r 197.23 g/mol. M.p. 133–134°. UV (EtOH): 215 (60130), 275 (146540). $[\alpha]_D^{20} = +117°$ ($c = 0.1$, EtOH)[3]. IR (KBr): 3226 (NH), 2960 (CH), 1669 (C=O), 1449 (NH), 1367, 1319, 1277, 1229. ^{1}H- and ^{13}C-NMR: see the *Figure*. EI-MS: 197.1 (1.2, M^+), 182.1 (5.5, $[M - CH_3]^+$), 154.1 (25, $[M - Ac]^+$), 141.1 (100, $[M - C_4H_8]^+$), 123.1 (19, $[M - C_4H_8 - H_2O]^+$), 86.1 (30), 70.1 (20).

*(*R*)-Reutericyclin* (= *(*2R*)-4-Acetyl-1,2-dihydro-5-hydroxy-2-(2-methylpropyl)-1-[(*2E*)-1-oxodec-2-enyl]-3*H*-pyrrol-3-one*; (*R*)-**1**). A 250-ml three-neck round-bottom flask equipped with a strong mechanical stirrer was dried and evacuated several times, flooded with Ar gas, and then filled with a soln. of **8** (2.958 g, 15.00

[3]) L-Enantiomer: $[\alpha]_D^{20} = -128°$ (EtOH).

mmol) in anh. THF (100 ml). At $-70°$, 2.2 equiv. of BuLi (1.6M soln. in hexane; 21 ml, 33 mmol) was added through a dropping funnel within 20 min under vigorous stirring. The soln. became turbid as a salt precipitated, first yellowish, then light orange, and stirring was continued for 20 min. Then, 0.95 equiv. of *dec-2-enoyl chloride* (2.83 g, 2.79 ml) in THF (20 ml) was added dropwise within 15 min at $-70°$. The precipitate gradually disappeared, and the soln. was stirred for another 30 min at this temp. After removing the cooling bath, the mixture was stirred for another 2 h, during which time the soln. reached r.t. Ice water (150 ml) with conc. H_2SO_4 (3.234 g, 33 mmol) was slowly added, and the aq. layer was separated and extracted with CH_2Cl_2 (3×50 ml). The org. layers containing THF were diluted with CH_2Cl_2 (70 ml), and then washed neutral with H_2O (4×70 ml). After drying (Na_2SO_4) and evaporation of the solvents, an oily, slightly yellow residue was obtained (4.94 g), which was purified by column chromatography (CC) on silica gel *60 PF254* (*Merck 7747*) with cyclohexane/Me_2CO 3:1 (column: 12 cm × 6 cm (i.d.)). During chromatography, the yellow substance developed a red ring (probably due to decomposition of a byproduct). Of the three major fractions, the first one was resubjected to CC (conditions as above; column: 15 cm × 3.5 cm (i.d.)); the other two fractions contained the pure product (TLC, HPLC). Yield of **1**: 1.985 g (38%). M_r 349.46 g/mol. M.p. 161–164° (dec.). UV (EtOH): 228 (17870), 266 (23875), 278 (25165). Optical rotation (EtOH, $c=0.29$): $[\alpha]_{436}^{20}=+69$, $[\alpha]_{546}^{20}=+19$, $[\alpha]_{578}^{20}=+15$; $[\alpha]_{589}^{20}$ ($[\alpha]_D$) $=+13°$. IR (KBr): 2927 (Me, CH_2), 2856 (CH), 1718 (ketone C=O), 1622 (amide C=O), 1470, 1355, 1319, 1242, 1155, 980 (HC=CH), 761. ^{1}H- and ^{13}C-NMR: see [1][6]. EI-MS: 349.2 (7.5, M^+), 293.1 (42, $[M-C_4H_8]^+$), 250.1 (18, $[M-Ac]^+$), 198.0 (20, $[M+2-C_{10}H_{17}O]^+$), 183.1 (25), 153.2 (71, $C_{10}H_{17}O^+$), 141.1 (100), 69.1 (33) 55.1 (29). HR-EI-MS: 349.2244 (M^+, $C_{20}H_{31}NO_4^+$; calc. 349.2235).

*(*S*)-Reutericyclin*. This enantiomer was prepared as described for (*R*)-**1**, but from (less-expensive) ethyl L-leucinate. The enantiomeric purity of **1** was determined as follows: a sample of synthetic (*S*)-(**1**) from a batch of *ca*. 2 g of product was hydrolyzed with 6N DCl/D_2O at 110° for 24 h. The dried hydrolysate was esterified with DCl/EtOD at 110° for 30 min, and the resulting ester was *N*-protected with trifluoroacetic anhydride to afford *N*-trifluoroacetyl-D-leucine ethyl ester. GC/MS Analysis of this derivative on a capillary coated with *Chirasil-Val* [13] showed two fully separated peaks (Δt_R 2 min) corresponding to a D/L mixture of 9.7:90.3 (80.6% ee).

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