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Synthesis and Antibacterial Activity of Four Stereoisomers of the Spider-Pathogenic Fungus Metabolite Torrubiellone D

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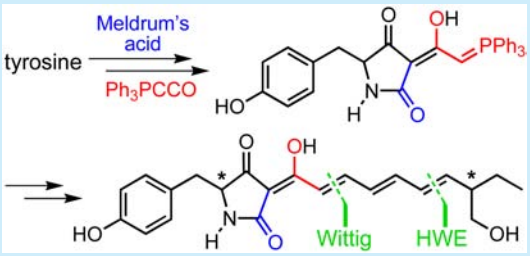
Supporting Information

ABSTRACT: Four stereoisomers of the spider-pathogenic fungus metabolite torrubiellone D were synthesized for the first time in 10% overall yield starting from L-tyrosine or D-tyrosine. The 3-decatrienoyl side chain was assembled and attached via (*E*)-selective HWE and Wittig olefinations. Their antibiotic activities against drug-susceptible *Escherichia coli* strains differed considerably.



Many fungi of the order Hypocreales are pathogenic to insects and feed on them.[1] They are also a rich source of structurally diverse metabolites that may contribute to the infestation of the host and to the defense of its resources against competitors.[1,2] These metabolites are therefore of particular interest as potential leads for new drugs and insecticides. As part of a screening program in Thailand,[3] Isaka et al.[4] assessed the metabolite profiles of 16 *Torrubiella* species,[5] the most prolific of which, *Torrubiella* sp. BCC 2165, was found to produce four hitherto unknown alkaloids, three 2-pyridones and a tetramic acid, torrubiellone D (**1**).

Their structures were elucidated except for the configuration of the stereocenters. A total synthesis of the pyridone (+)-torrubiellone C (**2**) by Gademann et al. proved that the natural (−)-enantiomer, the presumed metabolic product of the tetramic acid torrubiellone D (**1**), has an (*R*)-configured stereocenter in the side chain.[6] Cursory tests of compounds **1** and **2** on *Plasmodium falciparum*, *Mycobacterium tuberculosis*, and three cancer cell lines were negative.[4] We have now synthesized the four diastereomers **1a**−**d** in order to assign the stereochemistry of the natural product and also to evaluate their antibacterial activities (Figure 1).

First, the *N*,*O*-bisprotected tetramic acids (*S*)-**5** and (*R*)-**5** were prepared via a previously published general route[7] starting from enantiopure tyrosine as shown exemplarily for (*S*)-**5** in Scheme 1. L-Tyrosine was Boc-protected to give carbamate (*S*)-**3** which, in turn, was silylated to afford amino acid derivative (*S*)-**4**. This was cyclized to (*S*)-**5** with Meldrum's acid using a modification of Hosseini's protocol.[8]

The 3-decatrienoyl side chain of **1** was then attached to the tetramic acid **5** by first acylating the latter with the cumulated phosphorus ylide $Ph_3P{=}C{=}C{=}O$ to give a 3-acyl ylide which would be used to olefinate a suitably protected octadienal. By a similar approach, we previously synthesized ravenic acid.[9] The required octadienal **16** was prepared in both enantiomeric forms from purchasable enantiopure 2-phenylbutyric acids **6** as

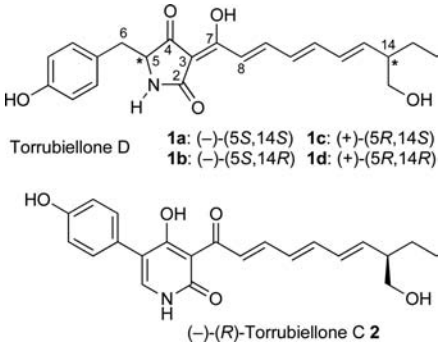


Figure 1. Structures of diastereoisomers of torrubiellone D (**1**) and of natural (−)-torrubiellone C (**2**).

outlined exemplarily for (*R*)-**16** in Scheme 2. Acid (*R*)-**6** was reduced with $LiAlH_4$ to give alcohol (*R*)-**7**, which was converted to the acetate (*R*)-**8** with acetic anhydride in the presence of catalytic copper(II) triflate according to a method by Firouzabadi.[10] Oxidative cleavage of the phenyl ring with $NaIO_4/RuCl_3$ gave the carboxylic acid (*S*)-**9** in 56% yield. The latter was treated with (trimethylsilyl)diazomethane and the resulting diester was selectively saponificated without prior purification to afford 2-(hydroxymethyl)butyrate (*S*)-**10** in 98% over the last two steps.[11] Silylation of the hydroxy group furnished TBS-ether (*S*)-**11**, the methoxycarbonyl residue of which was reduced with DIBAL-H in THF at −78 °C to give the aldehyde (*S*)-**12** in 59% yield. This aldehyde was then olefinated with the anion of phosphonate **13**, generated with LiHMDS in THF. The product ethyl dienoate (*R*)-**14**, obtained in 64% yield, was reduced in 95% yield to the alcohol (*R*)-**15**

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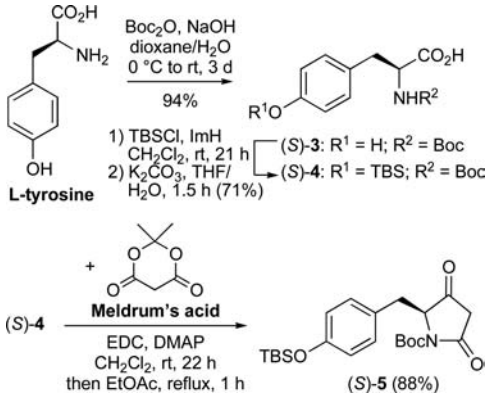
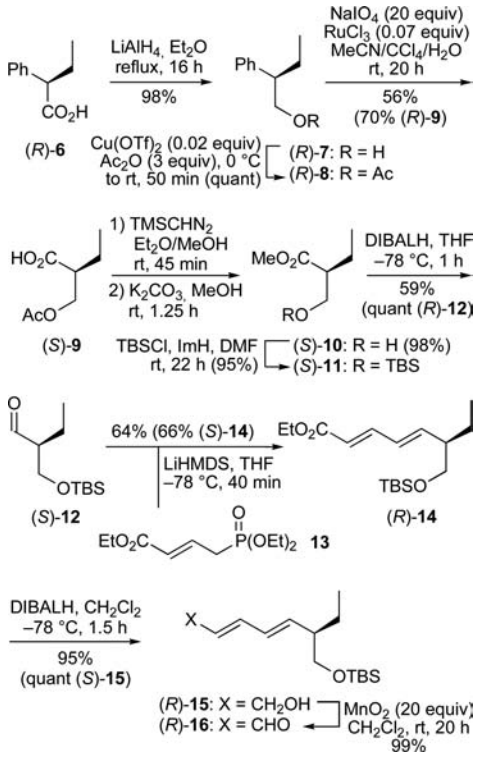
Scheme 1. Synthesis of *N,O*-Bisprotected Tetramic Acid (*S*)-5



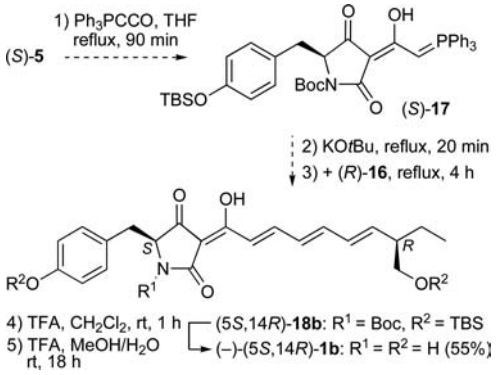
Scheme 2. Synthesis of the Side-Chain Precursor (*R*)-16



with DIBAL-H in dichloromethane. (*R*)-**15** was oxidized with MnO_2 to the dienal (*R*)-**16** almost quantitatively.

Finally, in a sequence of three consecutive reactions in one pot, this aldehyde was converted to the bisprotected tetramic acid (5*S*,14*R*)-**18b** which gave the torrubiellone D isomer (−)-(5*S*,14*R*)-**1b** in 55% overall yield after deprotection (Scheme 3). First, tetramic acid (*S*)-**5** was 3-acylated with Ph_3PCCO to afford the acyl ylide (*S*)-**17** which was deprotonated right away with potassium *tert*-butoxide to give a Wittig-active species of hitherto unknown structure. This, in turn, was treated with aldehyde (*R*)-**16**.[9] The resulting mixture was heated at reflux to afford compound (5*S*,14*R*)-**18b** as the product of an (*E*)-selective Wittig alkenation. It was deprotected stepwise, first with trifluoroacetic acid in dichloromethane and then with the same reagent in a methanol/water mixture to afford the target compound (−)-(5*S*,14*R*)-**1b**. The other three stereoisomers **1a**, **1c**, and **1d** were prepared analogously (cf. the Supporting Information).

Scheme 3. Attachment of the Side Chain via a 3-Acylylidation−Wittig Olefination Sequence



Since all four synthetic stereoisomers of torrubiellone D showed specific optical rotations which deviated from that reported by Isaka et al.[4] for their natural isolate (Table 1), we

Table 1. (Specific) Optical Rotations (*c* = 0.12, MeOH)

	Isaka[4]	1a	1b	1c	1d
α		−0.62	−0.63	+0.64	+0.65
$[\alpha]_D^{23}$	−182	−517	−525	+533	+542

confirmed their stereochemical identity and purity by analytical HPLC on a chiral Phenomenex Lux Amylose-1 column, in comparison to authentic diastereomeric mixtures. Figure 2 shows this for the (5*S*)-torrubiellones D **1a** and **1b** and a mixture of these synthesized from racemic aldehyde **16**.

As the topmost chromatogram, recorded of the diastereomeric mixture of (5*S*)-torrubiellones D, turned out to be an overlay of the chromatograms recorded of the pure synthetic (5*S*)-diastereomers **1a** and **1b**, we can rule out a side-chain racemization during the synthesis of the four stereoisomers. The additional peaks at earlier retention times in the chromatograms of **1a** and **1b** are additive in the chromatogram of the diastereomeric mixture and thus are very likely not impurities but tautomers or rotamers with respect to the C3−C7 bond of the 3-acyltetramic acid moiety. This assumption is also supported by the fact that all peaks showed the same characteristic UV absorption.

The optical rotation of Isaka's natural isolate deviates significantly from those of our pure synthetic stereoisomers. Optical rotations of 3-acyltetramic acids depend decisively on the solvent[12−14] and on the age of the sample solutions since these parameters govern the ratio of tautomers and rotamers whose individual specific optical rotations may vary considerably. Hence, it is hard to tell whether Isaka's natural isolate contained impurities, artifacts, several stereoisomers, or merely a different combination of tautomers or rotamers of one particular of the four possible stereoisomers. It is also worth noting that the configuration of the stereogenic center in the side chain has virtually no influence on the magnitude of the specific optical rotations of the four stereoisomers. Moreover, they all gave rise to virtually identical NMR spectra which are also congruent to the NMR data published by Isaka. The optical rotation of −182 quoted for his natural product isolate would best agree with a mixture of (5*S*)- and (5*R*)-stereoisomers since racemization at C5 is a well-known aspect of tetramic acid chemistry.

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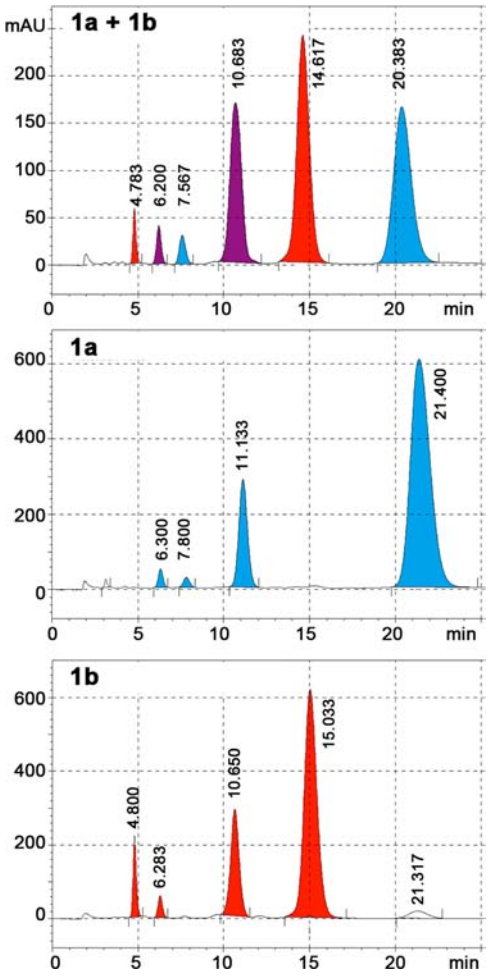

Figure 2. HPLC chromatograms for (5*S*)-torrubiellones D. (Top) Diastereomeric mixture of **1a** and **1b**; (middle) pure **1a**; (bottom) pure **1b** (Phenomenex Lux Amylose-1 100 × 4.6 mm chiral column, mobile phase 40% *n*-hexane, 60% ethanol with 0.1% TFA, flow rate 1 mL/min).

The four synthetic stereoisomers **1a**–**d** of torrubiellone D were finally tested for antibacterial activity against five different bacteria: the Gram-positive strains *Staphylococcus aureus* (DSM346) and *Enterococcus faecium* (DSM20477) and the Gram-negative strains *Escherichia coli* K12 wild-type, *Escherichia coli* Δ*To*lC mutant (JW5503), which lacks the ArcAB–TolC efflux system, and *Escherichia coli* D21f2 with truncated lipopolysaccharide (LPS) core (cf. the Supporting Information for experimental details). The four isomers displayed only weak activity against the Gram-positive bacteria with little variance between the compounds and the two strains. The *S. aureus* was slightly more susceptible to the (14*S*)-isomers **1a** and **1c** (Table 2). A more nuanced picture emerged from the tests with the Gram-negative *E. coli* strains. Wild-type *E. coli* K12 was not susceptible to any of the compounds, which was obviously due to insufficient penetration through the outer LPS layer and to efficient drug efflux pumps of the ArcAB–TolC type. The *E. coli* mutants which had a truncated LPS layer (D21f2) or lacked the TolC efflux pump (Δ*To*lC) were more susceptible than the K12 wild-type. The (5*R*)-isomers **1c** and **1d** gained most strongly from the absence of efflux pumps and reached IC_{50} values of ca. 13 μg/mL (i.e., ca. 35 μM) against *E. coli* Δ*To*lC.

Table 2. IC_{50} Values (μg/mL) of 1a–d for Various Bacteria[a]

	1a	1b	1c	1d
S. aureus	37	53	44	55
E. faecium	40	38	49	39
E. coli K12	>100	>100	>100	>100
E. coli Δ*To*lC	83	30	13	14
E. coli D21f2	62	41	37	39

[a]*S. aureus*: Gram-positive. *E. faecium*: Gram-positive. *E. coli* K12: wild-type, Gram-negative. *E. coli* Δ*To*lC: mutant lacking the ArcAB–TolC efflux system. *E. coli* D21f2: supersusceptible mutant with truncated lipopolysaccharide core.[15]

The (*S*,*S*)-isomer **1a** was least efficacious against both *E. coli* mutants.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b00245.

Experimental details of chemical syntheses and biological tests, characterizations, and NMR spectra of new compounds (PDF)

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Notes

The authors declare no competing financial interest.

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■ DEDICATION

This paper is dedicated to Professor Steven Victor Ley (University of Cambridge) on the occasion of his 70th birthday.

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