STEREOCHEMISTRY OF ENACYLOXINS 1. ABSOLUTE CONFIGURATION OF THE CYCLOHEXANE RING PART OF ENACYLOXINS, A SERIES OF ANTIBIOTICS FROM *Frateuria* sp. W-315†

Toshitaka Fujimori,[a] Osamu Nakayama,[a] Hiromasa Kiyota,[a]*
Yu-ichi Kamijima,[a] Toshihiko Watanabe[b] and Takayuki Oritani[a]

[a]Lab. of Applied Bioorganic Chemistry, Division of Life Science, Graduate School of Agricultural Science, Tohoku University, 1-1 Tsutsumidori-Amamiya, Aoba-ku, Sendai 981-8555, and [b]Department of Civil Engineering, Faculty of Engineering, Tohoku Institute of Technology, 35–1, Yagiyama–kasumicho, Taihaku–ku, Sendai 982–8577, Japan

Abstract

The stereochemical study of the cyclohexane ring part of enacyloxins (ENXs), a series of antibiotics isolated from *Frateuria* sp. W-315, was examined. The relative configuration was elucidated by the coupling constant values of the ^{1}H NMR spectrum. The absolute structure was determined to be 1*S*, 3*R*, 4*S*, because the CD spectrum of the corresponding 3,4-dibenzoate derivative showed negative chirality. This conclusion was confirmed by the synthesis of the dibenzoate from tri-*O*-acetyl-D-glucal.

Introduction

Enacyloxins (ENXs) are polyhydroxy–polyenic and yellow–colored antibiotics produced by *Frateuria* sp. W–315 in a Czapek–Dox medium spent by *Neurospora crassa* (1). ENX IIa **1** is a main product and has antibiotic activity against Gram–positive and Gram–negative bacteria (2). ENX IIa was produced from ENX IVa **3** by enacyloxin oxidase (3). Their mode of action was considered to be an inhibition of peptide biosynthesis as clarified by our French congener who are working on biosynthesis of peptide (4). In this paper, we describe the structure elucidation of the cyclohexane part of enacyloxins.

† This work was carried out under a proposal and supervision of the late Dr. Takeyoshi Sugiyama, who passed away on September 5, 1999 at 53 years of age. This paper is dedicated to his memory.

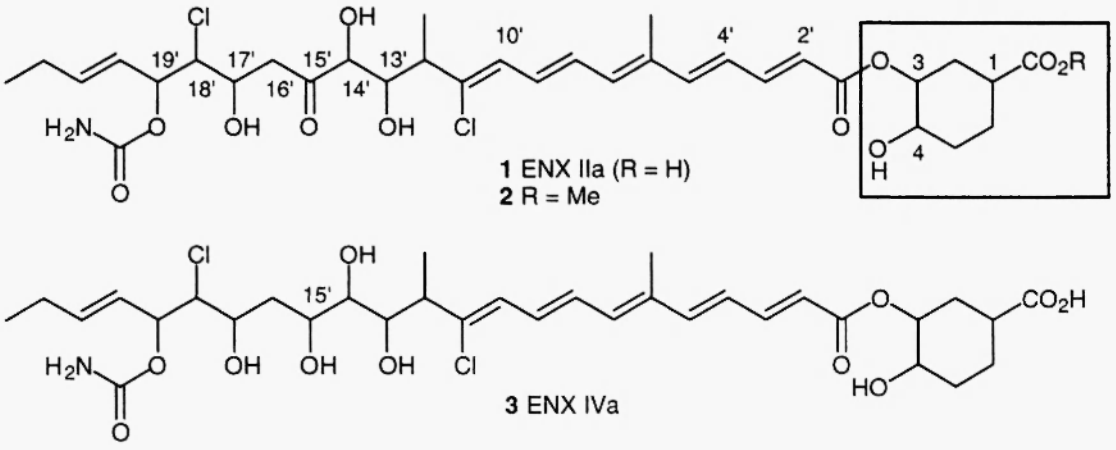
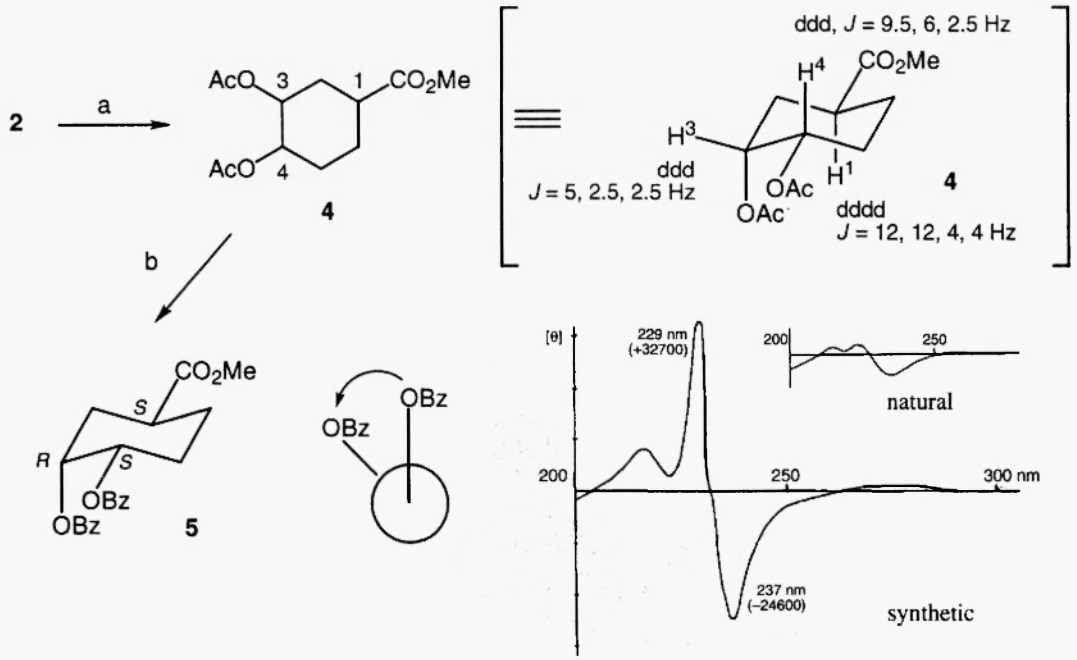


Fig. 1. Enacyloxin IIa **1** and its derivatives.

Results and Discussion

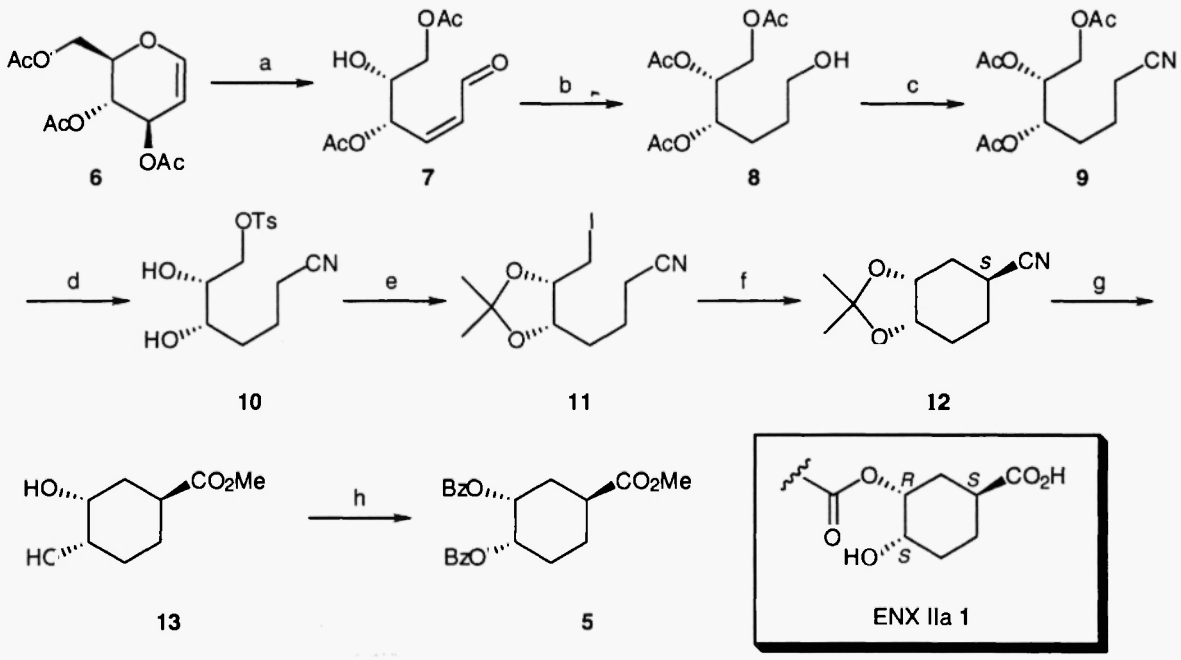
Acetylation of poly-hydroxy groups of **2** and ozonolysis of the resulting acetate followed by re-acetylation gave **4**. The relative configuration of **4** was elucidated by its ^{1}H NMR coupling constant values (Scheme 1). Methanolysis of **4** followed by benzoylation afforded dibenzoate **5**. Since CD spectrum of **5** showed negative chirality, the absolute configuration was determined by applying the dibenzoate chirality rule (5) to be 1*S*, 3*R*, 4*S*.



Scheme 1. Derivation of **2** and CD spectra of natural and synthetic **5**.

a) i. Ac_2O, Py. ii. O_3, MeOH, then $NaBH_4$. iii. Ac_2O, Py. b) i. DBU, MeOH. ii. BzCl, Py.

The absolute configuration was also confirmed by the chemical synthesis of **5**. As shown in Scheme 2, tri-*O*-acetyl D-glucal **6** was converted to enal **7** (6), which was acetylated, hydrogenated and reduced to give **8**. The alcohol **8** was tosylated followed by treatment with NaCN to give nitrile **9**. The triacetyl group was removed and the resulting primary hydroxy group was selectively tosylated to give **10**. The other two vicinal hydroxy groups were protected as acetonide and the tosyloxy group was substituted by iodo group to give **11**. Formation of the cyclohexane ring was achieved by NaHMDS in THF (7) to give **12** as a separable diastereomeric mixture (1*S*/1*R* = 3:2) (8). Acidic treatment of **12** in MeOH afforded the desired methyl (1*S*, 3*R*, 4*S*)-3,4-dihydroxycyclohexanecarboxylate **13**. The corresponding acid with the same configuration was formerly isolated from *Lactobacillus* (9). Benzoylation of **13** gave **5** (10), which showed the same negative chirality with the natural derivative. Consequently, the absolute structure of the cyclohexane part of ENX IIa **1** was unambiguously determined to be (1*S*, 3*R*, 4*S*)-3,4-dihydroxycyclohexanecarboxylic acid.



Scheme 2. Synthesis of **5** from tri-*O*-acetyl-D-glucal **6**.

a) $Hg(OAc)_2$, 1,4-dioxane, dil H_2SO_4 (97%). b) i. Ac_2O, Py. ii. H_2, Pd-C, EtOAc. iii. $NaBH_4$, EtOH (52%). c) i. TsCl, Py. ii. NaCN, DMSO (91%). d) i. sat. NH_3 in MeOH. ii. TsCl, Py. e) i. acetone, TsOH. ii. NaI, acetone, reflux (46% from **9**). f) NaHMDS, THF [36% of **12** and 24% of (1*R*)-**12**]. g) sat. HCl-MeOH, reflux (57%). h) BzCl, Py.

Conclusion

In conclusion, the absolute structure of the cyclohexane part of ENX IIa **1** was determined to be (1*S*, 3*R*, 4*S*)-3,4-dihydroxycyclohexanecarboxylic acid by the CD chirality rule of the corresponding dibenzoate and the chemical synthesis from tri-*O*-acetyl-D-glucal.

Acknowledgement

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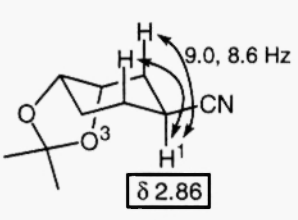
8 These isomers were separated by PTLC [hexane/EtOAc = 2:1; R_f, **12**>(1*R*)-**12**]. The configuration of 1-position was determined from comparison of the ^{1}H-NMR chemical shifts of both isomers: the H-1 peak of **12** was shifted lower field than that of (1*R*)-**12** due to the deshielding effect of the O-3. This was also confirmed by converting **12** to **5**.



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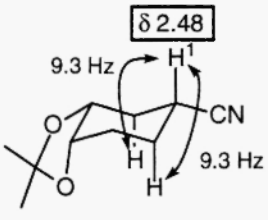

(1*R*)-**12**

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10 **5**: a colorless oil, $[\alpha]_D^{22} = -36.8°$ (*c* 0.540, $CHCl_3$). ^{1}H-NMR δ (400 MHz, $CDCl_3$): 8.07 (2H, m), 7.91 (2H, m), 7.59 (1H, m), 7.48 (3H, m), 7.35 (2H, m), 5.70 (1H, ddd, *J* =5.6, 2.6, 2.6 Hz), 5.22 (1H, ddd, *J* = 9.0, 6.4, 2.6 Hz), 2.85 (1H, dddd, *J* = 11.7, 11.4, 4.0, 3.7 Hz), 2.39 (1H, dddd, *J* = 14.3, 4.8, 3.7, 2.6 Hz), 2.20 (1H, m), 2.10 (2H, m), 1.77 (1H, m). CD ($CHCl_3$): λ_{ext} 237 nm (θ_2 −24600), λ_{ext} 229 nm (θ_2 +32700).

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