Pergamon

Tetrahedron Letters 40 (1999) 935–938

TETRAHEDRON LETTERS

Study on Fungitoxic 3-Amino-2-piperidinone-containing Lipids: Revised Structure of Cepaciamide A and Structural Determination of its Closely Related Lipid, Cepaciamide B

Hiroaki Toshima,* Kazuko Maru, Ying Jiao, Teruhiko Yoshihara, and Akitami Ichihara

Department of Bioscience and Chemistry, Faculty of Agriculture, Hokkaido University, Sapporo 060-8589, Japan

Received 28 October 1998; revised 19 November 1998; accepted 20 November 1998

Abstract: The structure of cepaciamide A was revised to be (3*R*,3'*S*,2"*S*,11"*S*,12"*R*)-3-[3'-(2"-hydroxy-11",12"-methyleneoctadecanoyloxy)hexadecamido]-2-piperidinone with respect to the absolute configuration of the $C_{3'}$- and $C_{2"}$-positions and the position of the cyclopropane ring by using synthetic methods. The structure of cepaciamide B was also determined to be (3*R*,3'*S*,2"*S*,11"*Z*)-3-[3'-(2"-hydroxy-11"-octadecenoyloxy)hexadecamido]-2-piperidinone.

Cepaciamide A (**1a**) was isolated from *Pseudomonas cepacia* D-202 as a novel fungitoxic 3-amino-2-piperidinone-containing lipid against *Botrytis cinerea* and *Penicillium expansum*, which cause the storage rot of beet roots.[1] In the further search for other fungitoxic metabolites, a mixture containing **1a** and its closely related lipid, cepaciamide B (**2a**), was obtained (Fig. 1). In order to examine the structure-activity relationship of cepaciamides as a biocontrol agent, we began a synthetic approach. In this process, we found some errors in the previously proposed structure of cepaciamide A as **3a**. We describe here the revised structure of cepaciamide A (**1a**) and the structural determination of cepaciamide B (**2a**).

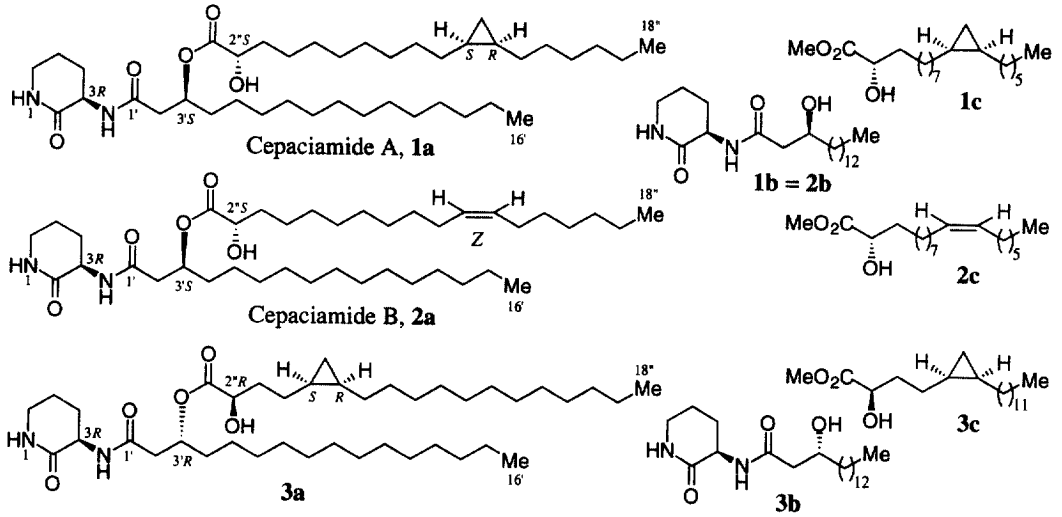


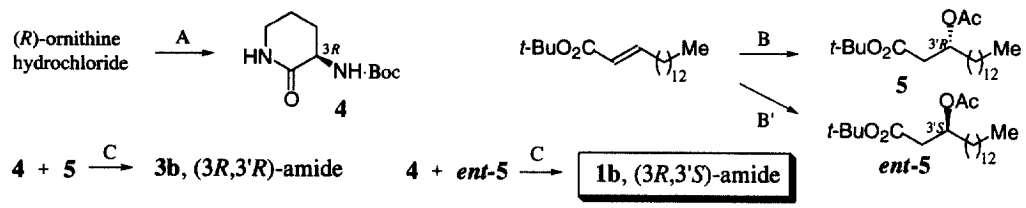
Fig. 1 Structures of cepaciamides A and B

Revised Structure of Cepaciamide A

Cepaciamide A is constituted of (3*R*)-amino-2-piperidinone and two hydroxy-fatty acids (amide-linked $C_{16:0}$ and ester-linked $C_{19:0}$). Weak alkaline hydrolysis of cepaciamide A gave an amide-part and an acid-part. The latter part was isolated as a methyl ester after treatment with diazomethane.[2] Therefore, both parts **3b** and **3c** are synthetic targets based on the previously proposed structure (**3a**).

0040-4039/99/$ - see front matter
PII: S0040-4039(98)02493-9

(*R*)-Ornithine hydrochloride was cyclized by the known method to (3*R*)-amino-2-piperidinone,[3] which was purified as stable Boc derivative **4**[4] (Scheme 1). According to the method reported by Oikawa and Kusumoto,[5] chiral ester **5** was synthesized from *t*-butyl (2*E*)-hexadecenoate *via* asymmetric dihydroxylation using AD-mix-β[6] in 3 steps. After removal of the Boc group of **4** and *t*-butyl ester of **5** with TFA, condensation with diethylphosphoryl cyanide (DEPC)[7] and subsequent deacetylation gave the desired amide **3b** possessing (3*R*,3'*R*)-configuration. However, the ^{1}H-NMR spectrum of **3b** was not identical with that of amide **1b** derived from **1a**. This result means that the absolute configuration of the C_3-position is opposite because the (3*R*)-configuration is undoubtedly determined from the CD spectra of model amides.[2] Next, we synthesized *ent*-**5** possessing (*S*)-configuration by using AD-mix-α,[6] which was converted to amide **1b** possessing (3*R*,3'*S*)-configuration. The spectral data (^{1}H-NMR, IR, MS and CD) of synthetic **1b** were completely identical with those of **1b** derived from **1a**. In the ^{1}H-NMR spectra of **1b** and **3b**, chemical shifts of C_3-H (δ 4.25 ppm for **1b**; δ 4.36 ppm for **3b**) and C_2-Ha (δ 2.27 ppm for **1b**; δ 2.39 ppm for **3b**) are characteristically different. Therefore, the amide-part structure of cepaciamide A was revised from **3b** to **1b**.



Scheme 1: [A] (1) $(TMS)_2NH$ / CH_3CN, (2) $(Boc)_2O$ / $CHCl_3$, 82%, 2 steps [B] (1) AD-mix-β, $MeSO_2NH_2$ / *t*-BuOH-H_2O, 99%, >99% ee, (2) $MeC(OMe)_3$, TMSCl / CH_2Cl_2, 56%, (3) *n*-Bu_3SnH, AIBN / PhH, 91% [B'] same as [B], except for (1) AD-mix-α, $MeSO_2NH_2$ / *t*-BuOH-H_2O, 83%, >99% ee, (2) 64%, (3) 91% [C] (1) TFA / CH_2Cl_2, (2) DEPC, Et_3N / CH_2Cl_2, 75%, 2 steps (3) K_2CO_3 / MeOH, 99%

The synthesis of **3c** was started from epoxide **6b** obtained from (*S*)-glycidol **6a** and known chiral cyclopropane derivative **7**[8] (Fig. 2). The full carbon skeleton of **3c** was constructed *via* coupling between **6b** and the sulfone anion of **8** and carbon-chain elongation with Wittig reagent. However, the ^{1}H-NMR spectrum of **3c** was not identical with that of the **1c** derived from **1a**. The behavior of **3c** (*Rf* = 0.38) and **1c** (*Rf* = 0.33) on silica gel TLC (hexane:EtOAc = 10:1) was also different. (2*S*)-Epimer **3d** was also synthesized *via* Mitsunobu inversion;[9] however, the ^{1}H-NMR spectrum and *Rf* = 0.38 were also not identical with those of **1c**. Therefore, the position of the cyclopropane ring is not correct in the proposed structure as **3c**.

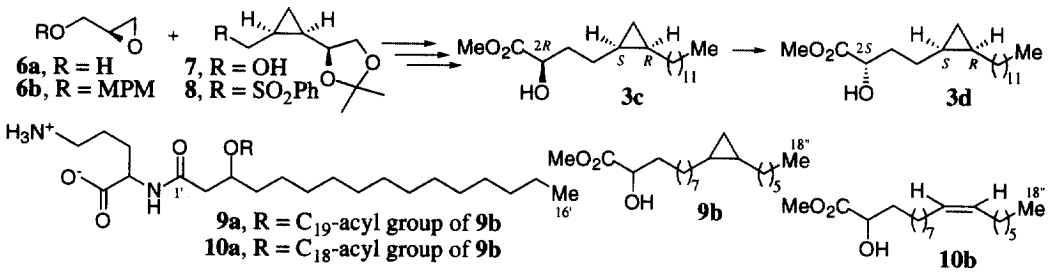
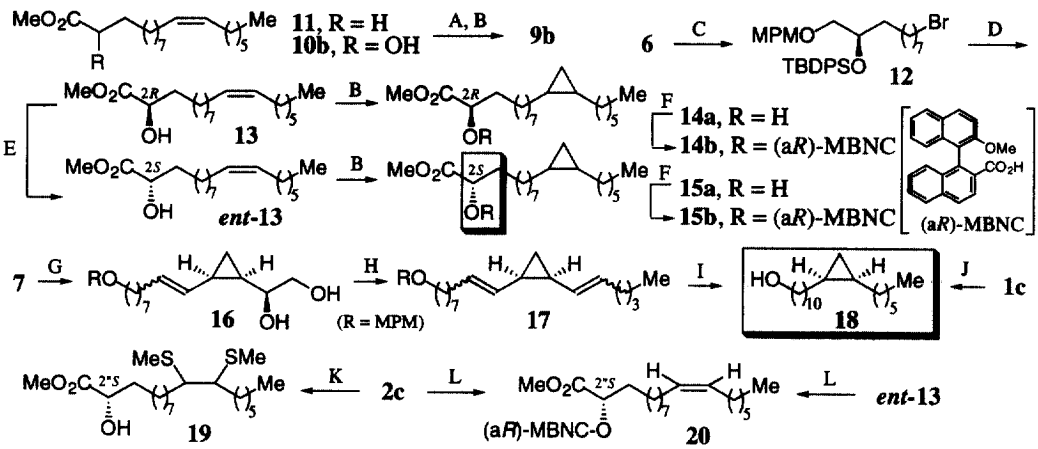


Fig. 2 The brief synthetic route of the proposed fatty acid methyl ester (**3c**) and the structures of ornithine-containing lipids (**9a** and **10a**) closely related to cepaciamides

Since the plane-structures of ornithine-containing lipids (**9a** and **10a**) have been reported,[10,11] ester **9b** was synthesized as a new promising candidate from commercially available methyl (*Z*)-vaccenate **11** (Scheme 2). α-Hydroxylation using (+)-(camphorylsulfonyl)oxaziridine[12] for the enolate of **11** resulted in poor asymmetric induction (*ca.* 10% *e.e.*, determined from the corresponding MTPA esters) to give **10b**. Non-diastereoselective cyclopropanation of **10b** with diethylzinc and diiodomethane[13] would proceed to give **9b** as a

mixture of diastereomers because the C_2-stereogenic center is too remote to affect the $C_{11,12}$-olefinic position. The ^{1}H-NMR spectrum of synthetic **9b**,[14] which looked just like a single diastereomer, was similar to that of **1c** derived from cepaciamide A. In the EIMS spectra of both **1c** and synthetic **9b**, the two characteristic fragment ion peaks (*m/z* 169, 155) due to the cleavage of the cyclopropane ring were observed and found to be identical with those of **9b** derived from ornithine-containing lipid **9a**.[11] In this way, the $C_{11,12}$-position of the cyclopropane ring was revealed.

The stereochemistry at the C_2- and $C_{11,12}$-positions must be determined independently because the stereogenic centers being far apart would provide no information on their relative configuration. Bromide **12** was synthesized from **6**[8] in 4 steps *via* elongation of the C_7-unit as the Grignard reagent. Further elongation of **12** with the C_8-unit as an acetylide of 1-octyne and subsequent stepwise conversion of the functional groups gave chiral ester **13**. ***Ent*-13** was also synthesized *via* Mitsunobu inversion.[9] Non-diastereoselective cyclopropanation of respective enantiomers (**13** and ***ent*-13**) gave **14a** and **15a** as a mixture of diastereomers, which were converted to (a*R*)-MBNC esters (**14b** and **15b**).[15] Both **14a** and **15a** also looked just like the same single diastereomer and also like **1c** itself in their ^{1}H-NMR spectra. This fact means that the influence of the $C_{11,12}$-stereogenic centers is negligible in determining the stereochemistry at the C_2-position. The $\Delta\delta$ (0.05 ppm) of the C_2-methine protons between **14b** (δ 4.79 ppm) and **15b** (δ 4.74 ppm) is larger than that of the corresponding MTPA esters. The ^{1}H-NMR spectrum of the (a*R*)-MBNC ester of **1c** was similar to that of **15b**. In this way, the (2*S*)-configuration of **1c** was revealed.



Scheme 2: [A] LDA, (+)-(camphorylsulfonyl)oxaziridine / THF-HMPA; [B] Et_2Zn, CH_2I_2 / CH_2Cl_2; [C] (1) $BrMg(CH_2)_7OTHP$, CuI / THF, (2) TBDPSCl, imidazole / DMF, (3) PPTS / EtOH, (4) CBr_4, Ph_3P / CH_2Cl_2, 73%, 4 steps; [D] (1) *n*-BuLi, 1-Octyne / THF-HMPA, (2) DDQ / CH_2Cl_2-H_2O, (3) H_2, Lindlar cat. / EtOAc, (4) Swern oxid.; (5) $NaClO_2$ NaH_2PO_4, 2-methyl-2-butene / *t*-BuOH-H_2O, (6) CH_2N_2 / Et_2O, (7) TBAF / THF, 31%, 7 steps; [E] (1) DEAD, Ph_3P, $PhCO_2H$ / Et_2O, (2) K_2CO_3 / MeOH, 84%, 2 steps; [F] (a*R*)-MBNC, 4-pyrrolidinopyridine / toluene; [G] (1) Swern oxid., (2) Ph_3P^+-$(CH_2)_9OH$-Br^-, *n*-BuLi / THF, (3) MPMCl, NaH / DMF, (4) PPTS / EtOH, 35%, 4 steps; [H] (1) $NaIO_4$ / THF-H_2O, (2) Ph_3P^+-$(CH_2)_4CH_3$-Br^-, *n*-BuLi / THF, 94%, 2 steps; [I] (1) $KO_2CN{=}NCO_2K$, AcOH / pyridine, (2) DDQ / CH_2Cl_2-H_2O, 98%, 2 steps; [J] (1) TsCl, pyridine / CH_2Cl_2, (2) $LiAlH_4$ / Et_2O, 75%, 2 steps; [K] I_2 / MeSSMe; [L] (a*R*)-MBNC, DCC, 4-pyrrolidinopyridine / toluene

The stereochemistry at the $C_{11,12}$-positions was next determined. Chiral cyclopropane **7**[8] was converted to diol **16** *via* elongation of the C_9-unit by Wittig reaction.[16] Oxidative cleavage of **16** and subsequent elongation of the C_5-unit by Wittig reaction gave diene **17**. Diimide reduction of **17**[17] and subsequent deprotection of MPM ether gave saturated alcohol **18**; $[\alpha]_D^{24}$ -2.05° (*c* 0.92, $CHCl_3$), which has been previously obtained from **1c**; $[\alpha]_D^{23}$ -3.30° (*c* 0.40, $CHCl_3$).[2] Since both ^{1}H-NMR spectra were completely identical and both specific rotations had the same sign, the (11*S*,12*R*)-configuration of **1c** was revealed. Therefore, the absolute structure of cepaciamide A was revised from **3a** to **1a** as (3*R*,3'*S*,2"*S*,11"*S*,12"*R*)-3-[3'-(2"-hydroxy-11",12"-methyleneoctadecanoyloxy)hexadecamido]-2-piperidinone.

Structural Determination of Cepaciamide B

A mixture of major **1a** and minor **2a** could not be separated by HPLC in spite of considerable effort because of their structural similarity. In the FDMS spectrum of the mixture, two ion peaks were detected at *m/z* 663 (MH^+) for **1a** and at *m/z* 649 (MH^+) for **2a**. Their difference (*m/z* 14) corresponds to one methylene group. In the ^{1}H-NMR spectrum of the mixture, one olefinic signal (δ 5.34 ppm) was observed in addition to the signals of **1a**. Alkaline hydrolysis gave the common amide (**1b** = **2b**) and two fatty acids, which were separated as the corresponding methyl esters (**1c** and **2c**) by HPLC.[18] While **1b** and **1c** were identified as those previously derived from **1a** in all respects, **2c** gave an olefinic signal (δ 5.34 ppm, 2H, m) without cyclopropane signals in the ^{1}H-NMR spectrum. These results and the occurrence of ornithine-containing lipids such as **9a** and **10a**[11] suggested that **2c** would be methyl (2*S*, 11*Z*)-2-hydroxy-11-octadecenoate. To determine the position of olefin, **2c** was converted to bis-sulfide **19**,[19] whose EIMS spectrum gave two characteristic fragment ions (*m/z* 261, 145) cleaved between the C_{11}- and C_{12}-positions. To confirm the absolute stereochemistry, the specific rotation of **2c**, $[\alpha]_D^{23}$ +2.20° (*c* 0.90, $CHCl_3$), was compared with that of ***ent*-13**, $[\alpha]_D^{22}$ +6.38° (*c* 1.39, $CHCl_3$). Although **2c** was still contaminated by a small amount of impurities, both specific rotations had the same sign. Furthermore, **2c** was converted to (a*R*)-MBNC ester **20**, whose ^{1}H-NMR spectrum was completely identical with that of synthetic **20** from ***ent*-13**. In this way, the structure of **2c** was obviously determined as we had assumed. Therefore, the absolute structure of cepaciamide B (**2a**) is (3*R*,3'*S*,2"*S*,11"Z)-3-[3'-(2"-hydroxy-11"-octadecenoyloxy)hexadecamido]-2-piperidinone.

The total syntheses of cepaciamides (**1a** and **2a**) and the experiments supporting our proposal that cepaciamides are not artifacts, will be described in the following paper.[20]

Acknowledgment: We are grateful to Dr Y. Fukushi (Hokkaido University) for the gift of (a*R*)-MBNC, and also to Mr. K. Watanabe and Dr. E. Fukushi in our faculty for measuring the MS spectra.

References and Notes

1. Ishikuri, S.; Uchino, H.; Kanazawa, K. Ann. *Phytopath. Soc. Japan* **1992**, *58*, 456-460.
2. Jiao, Y.; Yoshihara, T.; Ishikuri, S.; Uchino, H.; Ichihara, A. *Tetrahedron Lett.* **1996**, *37*, 1069-1042.
3. Pellegata, R.; Pinza, M.; Pifferi, G. *Synthesis* **1978**, 614-616.
4. Satisfactory spectral data (^{1}H-NMR, ^{13}C-NMR, IR, HRMS, $[\alpha]_D$) were obtained for all new compounds.
5. Oikawa, M.; Kusumoto, S. *Tetrahedron: Asymmetry* **1995**, *6*, 961-966.
6. Kolb, H. C.; VanNieuwenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, *94*, 2483-2547.
7. Yamada, S.; Kasai, Y.; Shioiri, T. *Tetrahedron Lett.* **1973**, 1595-1598.
8. Morikawa, T.; Sakai, H.; Hanai, R.; Shibuya, A.; Taguchi, T. *J. Org. Chem.* **1994**, *59*, 97-103.
9. Mitsunobu, O. *Synthesis* **1981**, 1-28.
10. Knoche, H. W.; Shively, J. M. *J. Biol. Chem.* **1972**, *247*, 170-178.
11. Kawai, Y.; Yano, I.; Kaneda, K.; Yabuuchi, E. Eur. *J. Biochem.* **1988**, *175*, 633-641.
12. Davis, F. A.; Chen, B.-C. *Chem. Rev.* **1992**, *92*, 919-934.
13. Furukawa, J.; Kawabata, N.; Nishimura, J. *Tetrahedron Lett.* **1966**, 3353-3354.
14. Although *trans*-cyclopropane derivative **9c** corresponding to **9b** was also synthesized from methyl (*E*)-vaccenate, the ^{1}H-NMR spectrum of **9c** was not identical with that of **9b**.
15. Fukushi, Y.; Yajima, C.; Mizutani, J. *Tetrahedron Lett.* **1994**, *35*, 599-602.
16. Hands, A. R.; Mercer, A. J. H. *J. Chem. Soc. (C)* **1968**, 1331-1337.
17. Hamersma, J. W.; Snyder, E. I. *J. Org. Chem.* **1965**, *30*, 3985-3988.
18. Inertsil ODS™ (4.6φ x 250 mm) was used for separation with MeOH-H_2O (9:1) eluent.
19. Shibahara, A.; Yamamoto, K.; Nakayama, T.; Kajimoto, G. *Yukagaku* **1985**, *34*, 619-625 (in Japanese).
20. Toshima, H.; Maru, K.; Saito, M.; Ichihara, A. *Tetrahedron Lett.* **1999**, *40*, 939-942.