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

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Abstract: The structure of cepaciamide A was revised to be (3R,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<sub>3</sub>- and C<sub>2</sub>--positions and the position of the cyclopropane ring by using synthetic methods. The structure of cepaciamide B was also determined to be <math>(3R,3'S,2"S,11"Z)-3-[3'-(2"-M)]

Cepaciamide A (1a) was isolated from *Pseudomonas cepacia* D-202 as a novel fungitoxic 3-amino-2piperidinone-containing lipid against *Botrytis cinerea* and *Penicillium expansum*, which cause the storage rot of beet roots.<sup>1</sup> 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 (3R)-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.<sup>2</sup> Therefore, both parts 3b and 3c are synthetic targets based on the previously proposed structure (3a).

(*R*)-Ornithine hydrochloride was cyclized by the known method to (3R)-amino-2-piperidinone,<sup>3</sup> which was purified as stable Boc derivative 4<sup>4</sup> (Scheme 1). According to the method reported by Oikawa and Kusumoto,<sup>5</sup> chiral ester 5 was synthesized from *t*-butyl (2*E*)-hexadecenoate *via* asymmetric dihydroxylation using AD-mix- $\beta^6$  in 3 steps. After removal of the Boc group of 4 and *t*-butyl ester of 5 with TFA, condensation with diethylphosphoryl cyanide (DEPC)<sup>7</sup> and subsequent deacetylation gave the desired amide 3b possessing  $(3R, 3^{2}R)$ -configuration. However, the <sup>1</sup>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<sub>3</sub>-position is opposite because the (3R)-configuration is undoubtedly determined from the CD spectra of model amides.<sup>2</sup> Next, we synthesized *ent*-5 possessing (*S*)-configuration by using AD-mix- $\alpha$ ,<sup>6</sup> which was converted to amide 1b possessing  $(3R, 3^{2}S)$ -configuration. The spectral data (<sup>1</sup>H-NMR, IR, MS and CD) of synthetic 1b were completely identical with those of 1b derived from 1a. In the <sup>1</sup>H-NMR spectra of 1b and 3b, chemical shifts of C<sub>3</sub>-H ( $\delta$  4.25 ppm for 1b;  $\delta$  4.36 ppm for 3b ) and C<sub>2</sub>-Ha ( $\delta$  2.27 ppm for 1b;  $\delta$  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)<sub>2</sub>NH / CH<sub>3</sub>CN, (2) (Boc)<sub>2</sub>O / CHCl<sub>3</sub>, 82%, 2 steps [B] (1) AD-mix- $\beta$ , MeSO<sub>2</sub>NH<sub>2</sub> / *t*-BuOH-H<sub>2</sub>O, 99%, >99% ee, (2) MeC(OMe)<sub>3</sub>, TMSCl / CH<sub>2</sub>Cl<sub>2</sub>, 56%, (3) *n*-Bu<sub>3</sub>SnH, AIBN / PhH, 91% [B'] same as [B], except for (1) AD-mix- $\alpha$ , MeSO<sub>2</sub>NH<sub>2</sub> / *t*-BuOH-H<sub>2</sub>O, 83%, >99% ee, (2) 64%, (3) 91% [C] (1) TFA / CH<sub>2</sub>Cl<sub>2</sub>, (2) DEPC, Et<sub>3</sub>N / CH<sub>2</sub>Cl<sub>2</sub>, 75%, 2 steps (3) K<sub>2</sub>CO<sub>3</sub> / 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 <sup>1</sup>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. (2S)-Epimer 3d was also synthesized *via* Mitsunobu inversion;<sup>9</sup> however, the <sup>1</sup>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, <sup>10,11</sup> ester 9b was synthesized as a new promising candidate from commercially available methyl (Z)-vaccenate 11 (Scheme 2).  $\alpha$ -Hydroxylation using (+)-(camphorylsulfonyl)oxaziridine<sup>12</sup> 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<sup>13</sup> 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 <sup>1</sup>H-NMR spectrum of synthetic **9b**, <sup>14</sup> 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**.<sup>11</sup> In this way, the  $C_{11,12}$ -position of the cyclopropane ring was revealed.

The stereochemistry at the C<sub>2</sub>- and C<sub>11,12</sub>-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<sub>7</sub>-unit as the Grignard reagent. Further elongation of 12 with the C<sub>8</sub>-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.<sup>9</sup> 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).<sup>15</sup> Both 14a and 15a also looked just like the same single diastereomer and also like 1c itself in their <sup>1</sup>H-NMR spectra. This fact means that the influence of the C<sub>11,12</sub>-stereogenic centers is negligible in determining the stereochemistry at the C<sub>2</sub>-position. The  $\Delta\delta$  (0.05 ppm) of the C<sub>2</sub>-methine protons between 14b ( $\delta$  4.79 ppm) and 15b ( $\delta$  4.74 ppm) is larger than that of the corresponding MTPA esters. The <sup>1</sup>H-NMR spectrum of the (*aR*)-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]  $Et_2Zn$ ,  $CH_2I_2 / CH_2CI_2$ ; [C] (1) BrMg(CH<sub>2</sub>)<sub>7</sub>OTHP, CuI / THF, (2) TBDPSCI, imidazole / DMF, (3) PPTS / EtOH, (4) CBr<sub>4</sub>, Ph<sub>3</sub>P / CH<sub>2</sub>Cl<sub>2</sub>, 73%, 4 steps; [D] (1) *n*-BuLi, 1-Octyne / THF-HMPA, (2) DDQ / CH<sub>2</sub>Cl<sub>2</sub>-H<sub>2</sub>O, (3) H<sub>2</sub>, Lindlar cat. / EtOAc, (4) Swern oxid.; (5) NaClO<sub>2</sub> NaH<sub>2</sub>PO<sub>4</sub>, 2-methyl-2-butene / *t*-BuOH-H<sub>2</sub>O, (6) CH<sub>2</sub>N<sub>2</sub> / Et<sub>2</sub>O, (7) TBAF / THF, 31%, 7 steps; [E] (1) DEAD, Ph<sub>3</sub>P, PhCO<sub>2</sub>H / Et<sub>2</sub>O, (2) K<sub>2</sub>CO<sub>3</sub> / MeOH, 84%, 2 steps; [F] (*aR*)-MBNC, 4-pyrrolidinopyridine / toluene; [G] (1) Swern oxid., (2) Ph<sub>3</sub>P<sup>+</sup>-(CH<sub>2</sub>)<sub>9</sub>OH·Br<sup>-</sup>, *n*-BuLi / THF, (3) MPMCI, NaH / DMF, (4) PPTS / EtOH, 35%, 4 steps; [H] (1) NaIO<sub>4</sub> / THF-H<sub>2</sub>O, (2) Ph<sub>3</sub>P<sup>+</sup>-(CH<sub>2</sub>)<sub>9</sub>CH·Br<sup>-</sup>, *n*-BuLi / THF, 94%, 2 steps; [I] (1) KO<sub>2</sub>CN=NCO<sub>2</sub>K, AcOH / pyridine, (2) DDQ / CH<sub>2</sub>Cl<sub>2</sub>-H<sub>2</sub>O, 98%, 2 steps; [J] (1) TsCl, pyridine / CH<sub>2</sub>Cl<sub>2</sub>, (2) LiAlH<sub>4</sub> / Et<sub>2</sub>O, 75%, 2 steps; [K] I<sub>2</sub> / MeSSMe; [L] (*aR*)-MBNC, DCC, 4pyrrolidinopyridine / toluene

The stereochemistry at the  $C_{11,12}$ -positions was next determined. Chiral cyclopropane 7<sup>8</sup> was converted to diol 16 via elongation of the C<sub>9</sub>-unit by Wittig reaction.<sup>16</sup> Oxidative cleavage of 16 and subsequent elongation of the C<sub>5</sub>-unit by Wittig reaction gave diene 17. Diimide reduction of 17<sup>17</sup> and subsequent deprotection of MPM ether gave saturated alcohol 18;  $[\alpha]_D^{24}$  -2.05° (c 0.92, CHCl<sub>3</sub>), which has been previously obtained from 1c;  $[\alpha]_D^{23}$  -3.30° (c 0.40, CHCl<sub>3</sub>).<sup>2</sup> Since both <sup>1</sup>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 (3R, 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/z663 (MH<sup>+</sup>) for 1a and at m/z 649 (MH<sup>+</sup>) for 2a. Their difference (m/z 14) corresponds to one methylene group. In the <sup>1</sup>H-NMR spectrum of the mixture, one olefinic signal ( $\delta$  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.<sup>18</sup> While 1b and 1c were identified as those previously derived from 1a in all respects, 2c gave an olefinic signal ( $\delta$  5.34 ppm, 2H, m) without cyclopropane signals in the <sup>1</sup>H-NMR spectrum. These results and the occurrence of ornithine-containing lipids such as 9a and 10a<sup>11</sup> suggested that 2c would be methyl (2S, 11Z)-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<sub>11</sub>- and C<sub>12</sub>-positions. To confirm the absolute stereochemistry, the specific rotation of 2c,  $[\alpha]_D^{23} + 2.20^{\circ}(c \ 0.90, \text{ CHCl}_3)$ , was compared with that of ent-13,  $[\alpha]_{D}^{22}$  +6.38° (c 1.39, CHCl<sub>3</sub>). Although 2c was still contaminated by a small amount of impurities, both specific rotations had the same sign. Furthermore, 2c was converted to (aR)-MBNC ester 20, whose <sup>1</sup>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 (3R,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.<sup>20</sup>

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