Pergamon

Tetrahedron Letters, Vol. 37, No. 7, pp. 1039-1042, 1996
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0040-4039/96 $15.00 + 0.00

0040-4039(95)02342-9

Structural Identification of Cepaciamide A, a Novel Fungitoxic Compound from *Pseudomonas cepacia* D-202

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Abstract: A novel fungitoxic compound, cepaciamide A, was isolated from *Pseudomonas cepacia* D-202. Its structure and stereochemistry were elucidated by the spectroscopic and synthetic methods.

Pseudomonas cepacia D-202 has been recognized as a biological control agent against *Botrytis cinerea* and/or *Penicillium expansum,* which causes beet roots rot in Japan.[1] In the investigation of the fungitoxic compounds from *P. cepacia* D-202, we have isolated a series of biologically active phospholipids and piperidinone -containing lipids. We report here the isolation, structural identification and fungitoxic activity of a novel compound, cepaciamide **A** (**1**), from *P. cepacia* D-202.

Isolation: The organism, *P. cepacia* D-202, was grown on King's B medium at 27°C for two days. The fermented broth was centrifuged and the bacterial cells were freeze-dried and extracted by $CHCl_3$:MeOH (1:1). After filtration, the filtrate was evaporated to dryness, and the residue was extracted with acetone. The acetone extract showed the fungitoxic activity against *B. cinerea* and was separated by flash column chromatography on silica gel with eluents $CHCl_3$:MeOH (3:1) to give the active fraction I containing compound **1** (Fig.1), which was purified by HPLC on an ODS column with eluents MeOH:CH_3CN:$(CH_3)_2CHOH$ (50:25:25).

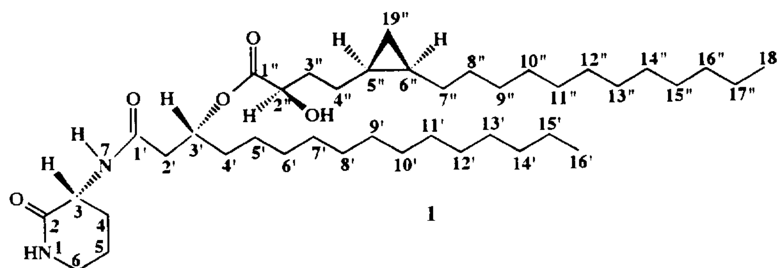


Fig.1: Structure of cepaciamide A.

Structural identification: Compound **1**, mp 98-102°, gave a $[MH]^+$ at *m/z* 663 in FDMS. Its IR absorption bands at 1724 and 1620 cm^{-1} indicated the presence of the ester and amide groups, which were supported by its chemical shifts of δ 170.78, 175.41 and 176.71 for the three carbonyls in the ^{13}C NMR spectrum. In addition, the chemical shifts of δ 1.15-1.48 for 46 protons suggested the presence of fatty acids. Its another IR absorption band at 3320 cm^{-1} together with its proton chemical shift of δ 4.12 for the methine bearing oxygen revealed the presence of the hydroxyl group.

Hydrolysis of **1** with 0.2 *M* NaOH gave a fatty acid (**1a**) and the nitrogen-containing residue (**1b**) (Fig.2). The methyl ester (**1c**) of **1a** gave a $[MH]^+$ ion in the HREIMS at *m/z* 326.2841 corresponding to a molecular formula of $C_{20}H_{38}O_3$ (Δ0.3 mmu) requiring two sites of unsaturation. The proton signal of δ 4.13 together with carbon signal of δ 70.46 of **1c** were assigned to α-hydroxyl group from change of the signal of 2"-H (δ 4.13) into a singlet by irradiation of 3"-H (δ 1.62) in the ^1H-NMR decoupling experiment. The proton signals δ -0.33, 0.58, 0.66 of **1c** revealed the presence of *cis*-1,2-disubstituted cyclopropane ring, which was consistent with those of synthetic product of *cis*-5, 6-methylenoctacanoic acid.[2] It was established for the cyclopropane of **1c** to be located on 5"-C and 6"-C by hydrogenation of **1c** with 10% PtO_2 and subsequent oxidation with CrO_3 to give four degradation products, which were identified by GCMS to be dimethyl succinate (M^+ m/z 146), methyl tridecanoate (M^+ m/z 228), methyl dodecanoate (M^+ m/z 214) and dimethyl glutarate (M^+ m/z 160).[3]

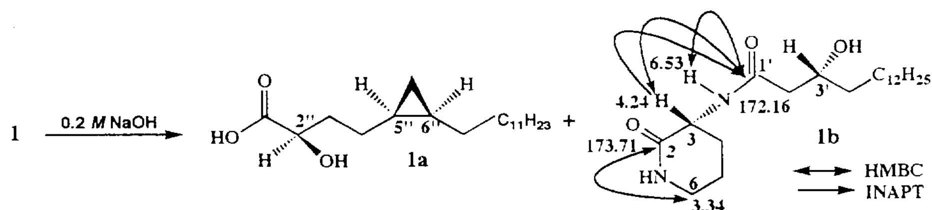
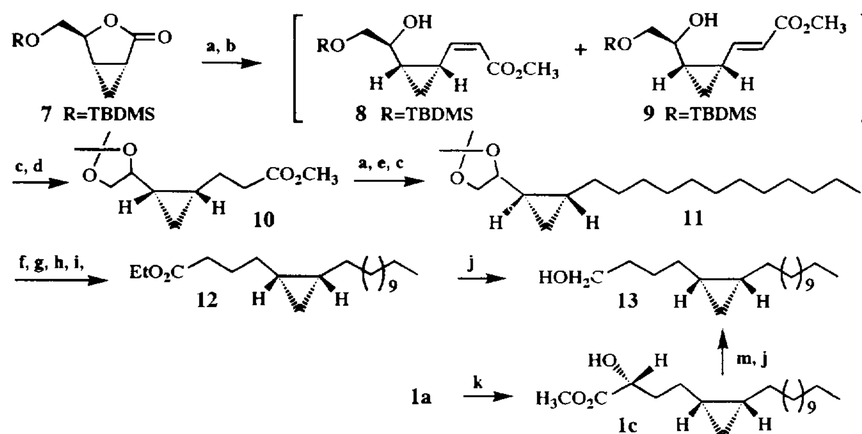


Fig.2: Hydrolysis of 1 with alkaline conditions and NMR data of 1b.

The nitrogen-containing residue **1b** gave a $[MH]^+$ ion at *m/z* 368.3049 corresponding to a molecular formula of $C_{21}H_{40}O_3N_2$ (Δ0.1 mmu) requiring three sites of unsaturation in the HREIMS. Its NMR spectral studies suggested that **1b** was composed of β-hydroxyhexadecanoic acid and 3-amino-2-piperidinone. The presence of a β-hydroxyl group in **1b** was revealed from the change of two doublet doublet signals of 2'-Ha (δ 2.22) and 2'-Hb (δ 2.40) into doublets by the irradiation of 3'-H (δ 3.96) in the ^1H-NMR decoupling experiment. Further the HMBC correlation between 3'-H (δ 3.96) and 1'-C (δ 172.16) confirmed the presence of β-hydroxyl group in **1b**. The presence of piperidinone was revealed with its ^1H-^1H COSY and HMBC spectra; the correlations between 7-NH (δ 6.53) and 3-H (δ 4.24), 3-H and 4-H (δ 1.59 and 2.50), 4-H and 5-H (δ 1.93), 5-H and 6-H (δ 3.34), 6-H and 1-NH (δ 5.76) and the relationship between 6-H (δ 3.34) and 2-C (δ 173.71) (Fig.2). This was confirmed from the identical ^1H-NMR data with those of synthetic 3-amino-2-piperidinone.[10] The HMBC correlations between 1'-C (δ 172.16) and 3-H (δ 4.24) and between 1'-C and 7-NH (δ 6.53) suggested that β-hydroxyhexadecanoic acid was connected to 7-N in 3-amino-2-piperidinone moiety of **1b** as an amide bond. This connection was proved from the significant enhancement of signal due to 1'-C (δ 172.16) by selective irradiation of 3-H (δ 4.24) in the INAPT experiment (Fig 2).

Absolute configurations: **1a** $[\alpha]_D^{23}$= -3.5° (c=0.6, $CHCl_3$) has three asymmetric centers. One on 2"-C was determined as *R* by the advanced Mosher method.[4] The other two on 5"-C and 6"-C were determined by comparing the optical rotation values of its reduction product with that of the synthetic product **13** (Scheme 1). In the synthetic procedure, the stereochemically-defined lactone (**7**) was prepared from D-mannitol by the known procedure.[5] The reduction of **7** with DIBAL followed the Wittig reactions produced **8** and **9**. Hydrogenation of the alkenes, **8** and **9**, followed by reaction with acetone dimethylacetal gave the segment **10**. Finally the synthesis of **13** was completed from **10** by the sequence of reactions involving the Wittig reaction as key steps (Scheme 1). On the other hand, the methyl ester **1c** was treated with *p*-toluenesulfonyl chloride and subsequently reduced with $LiAlH_4$ to give **13**, which showed $[\alpha]_D^{23}$= -3.3° (c=0.4, $CHCl_3$) consistent with that ($[\alpha]_D^{23}$= -3.6° (c=0.28, $CHCl_3$)) of synthetic (5*S*, 6*R*)-methylenoctadecanol (**13**).

Therefore, the absolute configurations of the cyclopropane ring in **1a** was determined to be as *S* for 5"-C and *R* for 6"-C, and the structure of **1a** was determined as (2*R*, 5*S*, 6*R*)-2-hydroxy-5, 6-methylenoctadecanoic acid.



a: DIBAL, ether, -78°C, 90%; b: Ph_3P=$CHCO_2CH_3$, THF, 50°C, 8h, 89%; c: Pd/C, H_2, MeOH, rt, 6h; d: $Me_2C(OMe)_2$, $TsOH.H_2O$, acetone, rt, 92%; e: Ph_3P=$CH(CH_2)_7CH_3$, THF, -78°C, 2h; -78 - 0°C, 1h; 0°C, 1h, 38%; f: 33% H_2SO_4, MeOH, rt, 20min, 67%; g: $HIO_4.H_2O$, MeOH, 20min, 81%; h: Ph_3P=$CHCH_2CO_2Et$, THF, -78°C, 1h; -78° - 0°C, 1h; 0°C, 12h, 60%; i: Pd/C, H_2, EtOH, 0°C, 4h, 15%; j: $LiAlH_4$, ether, rt, 8h; k: CH_2N_2, ether, 0°C, 98%; m: TsCl, CH_2Cl_2, pyridine, 0°C, 8h, 95%.

Scheme 1: Synthesis of (5*S*, 6*R*)-5, 6-methylenoctadecanol and reduction of 1a.

There are two asymmetric centers in **1b**. One on 3'-C was determined as *R* by the advanced Mosher method,[4] and another one on 3-C in the piperidinone ring could not be determined by studying its NMR spectra. Since **1b** has a negative absorption at 230 nm in its CD spectrum, elucidation of the absolute configuration by the CD spectra was attempted. Thus the three model compounds **3**, **4** and **5** (Fig.3) were synthesized from L-ornithine[6] with (*R*)-3-hydroxybutyric acid, (±)-3-hydroxybutyric acid and (*S*)-3-hydroxybutyric acid respectively. All CD spectra of three model compounds showed a positive absorption at 230 nm. This fact suggested that the absorption of the CD spectra depended on the asymmetric center at 3-C in 3-amino-2-piperidinone moiety, and was not affected by the stereochemistry at 3'-C in the side-chain. The negative absorption of **1b**, opposite to that of model compounds (**3**-**5**) in CD spectra, established that the piperidinone moeity of **1b** was formed from D-ornithine, indicating the absolute configuration of 3-C in **1b** to be *R*. Therefore, **1b** was determined as (3*R*, 3'*R*)-3-(*N*-3'-hydroxyhexadecanamido)-2-piperidinone. Finally the structure of **1** was revealed as (3*R*, 3'*R*, 2"*R*, 5"*S*, 6"*R*)-3-N-[3'-(2"-hydroxy-5", 6"-methylenoctadecanoyl)- hexadecamido]-2-piperidinone and was named as **cepaciamide A**.

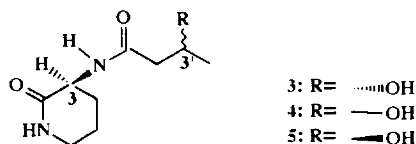


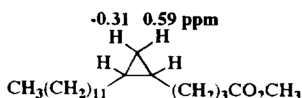
Fig.3: Structures of 3-5.

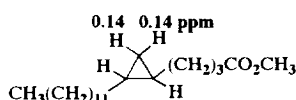
Fungitoxic activity: Bioassay for the fungitoxic activity against *Botrytis cinerea* was used as the monitoring method for the isolation. Compound **1** showed 52% fungitoxic activity against *B. cinerea* at the concentration of 100 ppm comparing with the control. It is interesting to note that the hydrolysis product **1b** showed much stronger fungitoxic activity (84%) than **1**.

Acknowledgement: We thank Mr. K. Watanabe and Ms. E. Fukushi in our department for FD-MS, EI-MS, and HR-EI-MS measurements.

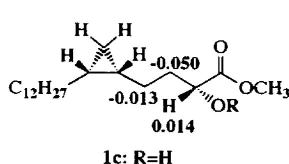
References and notes:

1. a) Ishikuri, S.; Uchino, H. and Kanzawa, K. *Ann. Phytopath. Soc. Jpn.* 1992, **58**, 456-460.; b) Tsuchiya K.; Homma Y., Komoto Y. and Suzui T.; *Ann. Phytopath. Soc. Jpn.* 1995, **61**, 318-324.; c) *P. cepacia* D-202 was deposited in the Fermentation Research Institute, Agency of Industrial Science and Technology Japan, under the accession number FERMP-11559.
2. a) Denmark, S. E. and Edwards, J. P. *J. Org. Chem.* 1991, **56**, 6974-6981.; b)Yamanoi, K. and Ohfune, Y. *Tetrahedron Lett.*, 1988, **29**, 1181-1184.; c)

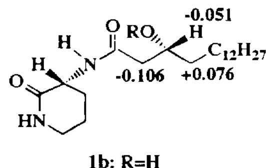




3. Kaneshiro, T. and Marr, A. G. *J. Biol. Chem.*, 1961, **236**, 2615-2614.
4. a) Ohtani, I.; Kusumi, T.; Ishitsuka, M. O. and Kakisawa, H. *Tetrahedron Lett.* 1989, **30**, 3147.; b) Difference of chemical shifts between (*S*)-MTPA and (*R*)-MTPA esters of **1c** and **1b**:



1c: R=H



1b: R=H

5. Pail, I. B.; Trafford, C.; Colin, D. and John, M. *J. Chem. Soc. Perkin. 1*, 1994, 1503-1508.
6. Pellegata, R.; Pinza, M. and Pifferi, G. *Synthesis*, 1978, 614-616.
7. **1**: FDMS: *m/z* [MH]$^+$ 663; IR (film): 3320, 1724, 1620 cm^{-1}; ^{1}H-NMR (500MHz, $CDCl_3$+CD_3OD): δ -0.33 (1H, m), 0.58 (1H, m), 0.66 (2H, m), 0.86 (6H, t), 1.15-1.48 (46H, m), 1.53-1.76 (3H, m), 1.78-1.98 (2H, m), 1.94 (2H, m), 2.36 (1H, m), 2.42 (2H, m), 3.17 (2H, m), 4.12 (1H, m), 4.22 (1H, m), 5.23 (1H, m); ^{13}C NMR (67.5 Mz, $CDCl_3$+CD_3OD, multiplicities by DEPT) δ 11.23 (t), 14.28 (q), 16.15 (d), 23.05 (t), 23.38 (t), 25.30 (t), 25.62 (t), 27.58 (t), 29.14 (t), 29.61 (t), 29.74 (t), 29.97 (t), 30.10 (t), 30.56 (t), 30.65 (t), 32.34 (t), 35.02 (t), 35.36 (t), 39.69 (t), 42.68 (t), 54.37 (d), 70.69 (d), 72.95 (d), 170.78 (s), 175.41 (s), 176.71 (s).
8. **1c**: $[\alpha]_D^{23}$= -3.4 (c=0.3, $CHCl_3$); EIHRMS: *m/z* 326.2841 found, 326.2839 calcd. for $C_{20}H_{38}O_3$; IR (film): 3320 (OH), 1724 (CO) cm^{-1}; ^{1}H-NMR (500MHz, $CDCl_3$): δ -0.33 (19-Ha, m), 0.58 (19-Hb, m), 0.66 (5-H, 6-H, m), 0.87 (3H, t, *J*=5.94Hz), 1.17 (4H, m), 1.27-1.31 (24H, m), 1.37 (4H, m), 1.62 (3-Ha m), 1.72 (3-Hb, m), 2.67 (OH, br.), 3.78 (3H, s), 4.13 (2-H, m); ^{13}C NMR (125 Mz, $CDCl_3$): δ 14.12, 15.74, 22.70, 24.73, 28.70, 28.72, 29.30, 29.35, 29.46, 29.58, 29.62, 30.17, 30.19, 34.42, 52.48, 70.46, 173.26.
9. **1b**: HREIMS: *m/z* 368.3049 found, 368.3050 calcd. for $C_{21}H_{40}O_3N_2$; ^{1}H-NMR (500MHz, $CDCl_3$): δ 0.86 (3H, t, *J*=6.8Hz), 1.23 (20H, m), 1.40 (2H, m), 1.52 (2H, m), 1.59 (2H, m), 1.93 (2H, m), 2.22 (2-Ha, dd, *J*=9.2, 15.1Hz), 2.40 (2-Hb, dd, J=2.5, 15.1Hz), 2.50 (1H, m), 3.34 (2H, m), 3.96 (1H, m), 4.24 (1H, m), 5.76 (1-NH, br.s), 6.53 (7-NH, br.s); ^{13}C NMR (125 MHz, $CDCl_3$, mutiplicities by DEPT), δ 14.82 (q), 21.84 (t), 23.39 (t), 26.23 (t), 27.80 (t), 30.05 (t), 30.26 (t), 30.29 (t), 30.36 (t), 32.62 (t), 37.44 (t), 42.55 (t), 43.37 (t), 51.48 (d), 69.34 (d), 172.16 (s), 173.71(s).
10. (*S*)-3-[*N*-(*R*)-3'-hydroxybutanamido]-2-piperidinone (**3**). $[\alpha]_D^{23}$= -12.40° (c=0.5, MeOH); FDMS: *m/z* [MH]$^+$ 201; HREIMS: *m/z*: [M-C_2H_3]$^+$ 173.0933 found, 173.0932 calcd. for $C_7H_{13}O_3N_2$; ^{1}H-NMR (270 MHz, CD_3OD): δ 1.22 (3H, d, *J*=5.9Hz), 1.67 (1H, m), 1.93 (2H, m), 2.25 (1H, m), 2.37 (2H, m), 3.47 (2H, m), 4.20 (1H, m), 4.39 (1H, m) ppm.

(Received in Japan 2 *October* 1995; *revised* 6 *December* 1995; *accepted* 7 *December* 1995)