CEPACIN A AND CEPACIN B, TWO NEW ANTIBIOTICS PRODUCED BY *PSEUDOMONAS CEPACIA*

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Two new acetylenic antibiotics, cepacins A and B, have been isolated from the fermentation broth of *Pseudomonas cepacia* SC 11,783 and assigned structures **1** and **2**. Cepacin A has good activity against staphylococci (MIC 0.2 μg/ml) but weak activity against streptococci (MIC 50 μg/ml) and the majority of Gram-negative organisms (MIC values 6.3 $\sim >$ 50 μg/ml). Cepacin B has excellent activity against staphylococci (MIC $<$0.05 μg/ml) and some Gram-negative organisms (MIC values 0.1 $\sim >$ 50 μg/ml).

A screen designed to detect new macrolide-like antibiotics yielded two novel acetylenic antibiotics, cepacins A and B (**1** and **2**) from fermentations of *Pseudomonas cepacia* SC 11,783. Although this is the first report of compounds of this type being produced by strains of *P. cepacia*, related acetylenes are produced as fermentation products by other classes of organisms. For example, biformin[1], a triacetylene-epoxide is produced by the basidiomycete *Polyporus biformis* as first described in 1947[2]. Nemotin (**3**), an allene-diyne-γ-lactone[3], is produced by *Poria tenuis, Poria corticola* and an unidentified basidiomycete[4,5]. Mycomycin[6], an allene-diyne also containing a diene, is produced by a *Nocardia acidophilis*[7,8]. Marasin[9,10] and a host of related allene-diynes[11] have been isolated from fungal cultures.

Strain Description

The organism that produces the cepacins was isolated from a soil sample obtained in West Windsor, New Jersey. The soil sample was plated onto medium containing: synthetic sea salts* 1 g, aspartic acid 0.05 g, asparagine 0.05 g, yeast extract 1.0 g, KH_2PO_4 0.02 g, K_2HPO_4 0.02 g, compost extract** 300 ml, glycerol 5 ml and distilled water 700 ml. The medium was adjusted to pH 6.5 and sterilized in an autoclave at 121°C for 30 minutes. After cooling to room temperature, the medium was supplemented with filter-sterilized solutions of 0.1% biotin 1 ml, 0.2% thiamine 1 ml and 1% actidione 10 ml.

After 24 $\sim$ 72 hours incubation at 28°C, colonies of *P. cepacia* were isolated from the plated soil. The isolated colonies were picked off and maintained on an agar medium composed of: yeast extract 1 g, beef extract 1 g, NZ Amine A (Sheffield Chemicals) 2 g, glucose 10 g, agar 15 g and distilled water to 1 liter. The medium was adjusted to pH 7.3 and sterilized in an autoclave at 121°C for 30 minutes.

P. cepacia SC 11,783 is a Gram-negative rod, motile by means of multitrichous polar flagella. It is non-fluorescent, oxidase positive, and accumulates poly-β-hydroxybutyrate as intracellular reserve material. Arginine dihydrolase is lacking, nitrate is reduced to nitrite, and growth occurs at 41°C.

* Synthetic sea salts are commercially available as Instant Ocean from Sea Aquarium Systems, 33208 Lakeland Blvd., Eastlake, Ohio 44094, U.S.A.

** Compost extract is prepared by bringing to a boil a suspension of leaf litter in tap water (2: 1) and then allowing the mixture to simmer for 30 minutes. After cooling, the extract was filtered, initially through cheese-cloth and finally through Whatman No. 4 filter paper. The resulting liquid was sterilized in an autoclave at 121°C for 20 minutes.

The following compounds are utilized as the sole carbon source on the basal medium described in STANIER *et al.*[12]: glucose, xylose, arabinose, fructose, sucrose, ribose, mannitol, sorbitol, salicin, acetate, citrate, *d*-tartrate and putrescine. Rhamnose, maltose, lactose and erythritol are not utilized.

P. cepacia SC 11,783 is identical to *P. cepacia* ATCC 17759 and matches the published description of this species[18] except for the failure to produce a yellow intracellular pigment. This organism has been deposited in the American Type Culture Collection under the accession number ATCC 39356.

Fermentation

Seed culture was prepared by transferring a loopful of surface growth from an agar slant of *P. cepacia* SC 11,783 into each of four 500-ml Erlenmeyer flasks, each containing 100 ml of the following sterilized medium: yeast extract 0.4%, malt extract 1.0% and dextrose 0.4% in distilled water. The pH was adjusted to 7.3 with NaOH before sterilization. The flasks were incubated at 25°C on a rotary shaker (300 rpm, 5 cm stroke) for approximately 24 hours. A 1% (v/v) transfer of this culture growth was made to forty 500-ml Erlenmeyer flasks each containing 100 ml of the sterilized medium described above. The flasks were incubated for approximately 24 hours under the same conditions previously described. A 1.5% (v/v) transfer of the resulting culture growth was used to inoculate a 380-liter stainless steel fermentation tank containing 250 liters of the same sterilized medium described above. The fermentation was continued for approximately 20 hours at 25°C using an agitation rate of 130 rpm and an air flow of 283 liters per minute. After the appropriate fermentation time, a 3.5% (v/v) transfer of this culture growth was used to inoculate a 5,000-liter stainless steel fermentation tank containing 3,500 liters of the sterilized medium described above. The fermentation was continued for approximately 14 hours an agitation rate of 100 rpm and an air flow of 2,830 liters per minute. A polyalkylene glycol, Ucon LB-625, was at 25°C using added as needed to control foaming. Antibiotic production was determined by a paper disc agar diffusion assay using *Staphylococcus aureus* SC 11,348 as a test organism. At harvest, the fermentation broth was adjusted to pH 5 with HCl and centrifuged.

Isolation

Isolation of cepacins A and B was monitored by assay with *S. aureus* SC 11,348. The antibiotics in the broth supernate are very labile to acid and base and are most stable at about pH 5.

Purification by adsorption chromatography on silica gel and partition chromatography on Sephadex LH-20 is outlined in Fig. 1. Cepacin A was obtained as a glassy residue and cepacin B as a crystalline solid. The pure antibiotics are very unstable as solvent-free residues, rapidly converting to dark-brown insoluble material. They can, however, be stored in dilute solution (ethyl acetate, methylene chloride, or chloroform) in the dark at −20°C for extended periods without substantial loss of purity.

Characterization and Structure

Characterization data for cepacins A and B are listed in Table 1. The UV spectra of these antibiotics are nearly identical to that of nemotin (**3**)[8], and the IR spectra indicate a close relationship also. The latter show absorption characteristic of hydroxyl, acetylene, allene and γ-lactone functions. The structural relationship of the cepacins to nemotin is further supported by a base catalyzed transformation of the UV chromophore that is essentially identical to that observed in the transformation of nemotin (**3**) to nemotin A (**4**)[14]. Treatment of either cepacin A or B with base results in a rapid conversion ($t_{1/2}$ *ca.* 5 minutes at 20°C and pH 8.9 in EtOH - H_2O, 1: 9) of the diyne-allene to a triyne-ene chromophore (*e.g.* **2**→**5**) with concomitant loss of antimicrobial activity.

Fig. 1. Isolation of cepacins A and B.

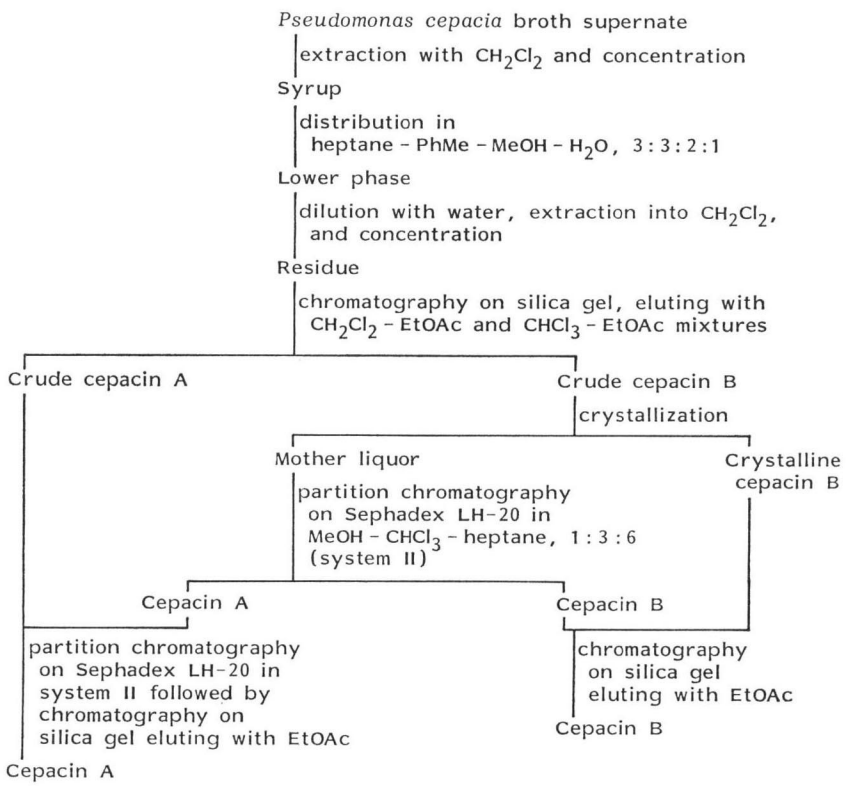


Table 1. Properties of cepacins A and B.

	Cepacin A (**1**)	Cepacin B (**2**)
Appearance	Amorphous	Crystalline solid
Empirical formula (MW)	$C_{16}H_{14}O_4$ (270.29)	$C_{16}H_{14}O_5$ (286.29)
UV λ_{max}^{EtOAc} nm (ε)	248 (10,300), 261.5 (15,200), 277 (12,400)	248 (9,700), 262 (15,100), 277 (12,000)
IR $\nu_{max}^{CHCl_3}$ cm^{-1}	3583, 3306, 2215, 1952, 1773, 1179, 971, 897	3582, 3530, 3306, 2215, 1952, 1777, 1180, 887
$[\alpha]_D^{22}$ (*c* 0.25, $CHCl_3$)	−129°	−214°
Rf on silica gel $CHCl_3$ - EtOAc, 1 : 1	0.20	0.15

Structures **1** and **2** for cepacin A and cepacin B, respectively, were derived from their ^1H NMR spectra (Table 2) in conjunction with the hydrogenation experiments described below. Decoupling experiments summarized in Tables 3 and 4 were used to make the assignments for **1** and **2** given in Table 2. The ethereal protons are found at relatively high field (2.74~3.47 ppm) indicating that they are epoxide protons[15], and the homoannular coupling constants (1.9~2.3 Hz) further reveal that the epoxides are *trans* substituted[16].

The multiplicity of peaks in the ^1H NMR spectrum of cepacin A in $CDCl_3$ suggests a 1 : 1 mixture of diastereomers. This proposition is supported by a spectrum measured in $CDCl_3$ - C_6D_6 (1 : 1) (Table 2) in which the true coupling constants remain essentially unchanged while the $\Delta\delta$s exhibit substantial solvent-induced variations. It is also possible in this solvent system to observe the coupling constant,

Table 2. ^{1}H NMR data for cepacins A and B and derivatives[a].

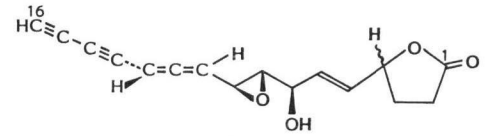
Position	Cepacin A (1)						Cepacin B (2)		Hydrogenolysis product (6)		Hydrogenation product (8)	
	$CDCl_3$[b]			$CDCl_3$ & C_6D_6[b]			$CDCl_3$[b]		$CDCl_3$[b]		$CDCl_3$[b]	
	δ	J	$\Delta\delta$	δ	J	$\Delta\delta$	δ	J	δ	J	δ	J
2	2.47 2.58	m m		2.06 2.14	m 17.6, 9.5, 5.0		2.55 2.66	17.9, 9.6, 8.2 17.9, 9.8, 5.7	2.53 2.67	17.9, 9.5, 8.4 17.9, 9.5, 6.3	2.54 2.65	17.9, 9.8, 8.1 17.9, 10.0, 5.9
3	2.05	m		1.5 1.8	m m		2.31	m	2.31	m	2.2~2.4	m
4	5.03	7.6, 7.6, ?	2.8	4.50	7.2, 7.2, ?	7.2	4.61	7.0, 7.0, 3.6	4.60	7.5, 6.7, 3.2	4.60	7.5, 6.9, 3.7
5	5.91	m		5.64[c]	15.7, 6.7		3.69	4.0, 4.0	3.60	4.4, 3.2	3.64	4.4, 4.1
6				5.65[c]	15.8, 8.1		3.21	4.0, 2.1	3.19	4.4, 2.3	3.16	4.4, 2.3
7	4.17	4.6, ?	1.5	3.90	4.6, ?		3.15	4.3, 2.1	3.03	4.9, 2.1	3.05[c]	4.6, 2.1
8	3.02	4.6, 2.1	3.7	2.74	4.6, 2.1	5.5	3.01	4.3, 1.8	3.51	7.3, 5.3, 5.3	2.76[c]	4.6, 2.1
9	3.47	7.8, 2.0, 0.6		3.26	7.6, 2.1	3.1	3.46	7.9, 1.9, 0.6			2.95	6.1, 4.7, 2.1
10	5.33	7.9, 6.8, 0.6		5.02	*ca.* 6.4, 6.4		5.31	7.9, 6.7, 0.6				
12	5.68	6.7		5.34	6.4		5.70	6.7, 0.9, 0.9				
16	2.50	m (J<1)		2.11	m (J<1)		2.52	m (J<1)				

[a] Spectra were obtained at 400 MHz. Chemical shifts (δ) are in parts per million downfield from internal Me_4Si. Coupling constants (J) and multiplicity due to isomer mixtures ($\Delta\delta$) are in Hz ($\pm$0.3).

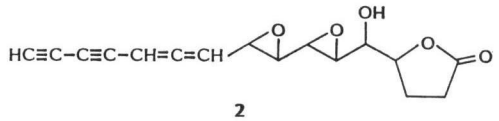
[b] A small quantity of CD_3OD was added to the samples. In its absence, the protons on carbons bearing hydroxyl groups gave broad peaks.

[c] These assignments may be reversed.

15.8 Hz, for the olefinic protons, establishing the *trans* stereochemistry of this linkage. The peak in the infrared spectrum at 971 cm^{-1} is also indicative of a *trans* olefin.



1



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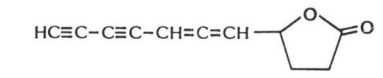

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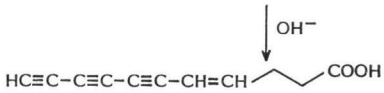


4

The major product from the catalytic hydrogenation of **2** in the presence of Pd/C or rhodium (Rh)/Al_2O_3 is **6** which is also produced by catalytic hydrogenation of base-rearrangement product **5** (Scheme 1). The structure of **6** is supported by the IR spectrum (γ-lactone: 1772 cm^{-1}), the NMR data (see Table 2 and experimental section), and by the high resolution mass spectral data obtained for the trimethylsilyl derivative **7** (summarized in Scheme 1). When rhodium was used as the hydrogenation catalyst in order to minimize hydrogenolysis[17], **6** was still by far the most abundant product. It is evident that the epoxide adjacent to the allene function is very susceptible to hydrogenolysis. However, a minor product is also produced (13% yield) that can be obtained in slightly better yield (25%) by partial reduction with

Table 3. NMR decoupling observations for cepacin A (**1**) in $CDCl_3$.

Irradiated group (δ)	Observed group (δ)							
	2.05	3.02	3.47	4.17	5.03	5.33	5.68	5.91
3.02	—	*	J=2→0	Sharpens	—	—	—	—
3.47	—	J=2.1→0	*	Sharpens slightly	—	J=*ca.* 7→0	—	—
4.17	—	J=4.6→0	—	*	—	—	—	Simplifies
5.03	Simplifies	—	—	Sharpens slightly	*	—	—	Simplifies
5.33	—	—	J=7.8→0	—	—	*	J=6.7→0	—
5.91	—	—	—	→dd, J=4.6; $\Delta\delta$=1.5	→dt, J=7.6, 7.6; $\Delta\delta$=2.8	—	—	*

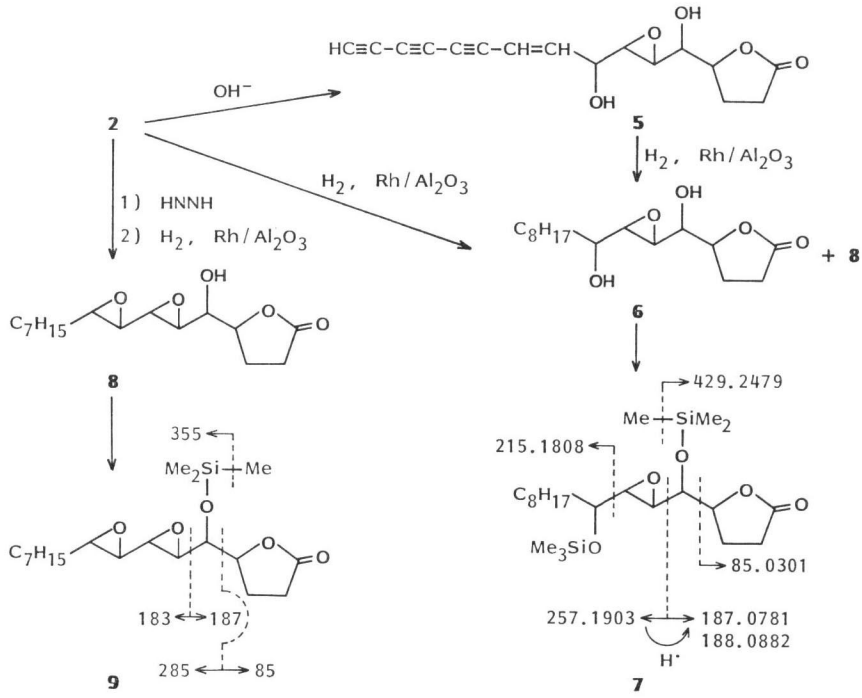
Table 4. NMR decoupling observations for cepacin B (**2**) in $CDCl_3$.

Irradiated group (δ)	Observed group (δ)							
	2.31	3.01	3.15	3.21	3.46	3.69	4.61	5.31
3.01	—	*	Partially decoupled[a]	—	J=1.9→0	—	—	—
3.15	—	Partially decoupled[a]	*	[a]	Partially decoupled[b]	—	—	—
3.21	—	—	[a]	*	—	J=4.0→0	—	—
3.46	—	dd→d	—	—	*	—	—	t→d
3.69	—	—	—	J=4.0→0	—	*	J=3.6→0	—
4.61	Simplifies	—	—	—	—	J=3.6→0	*	—

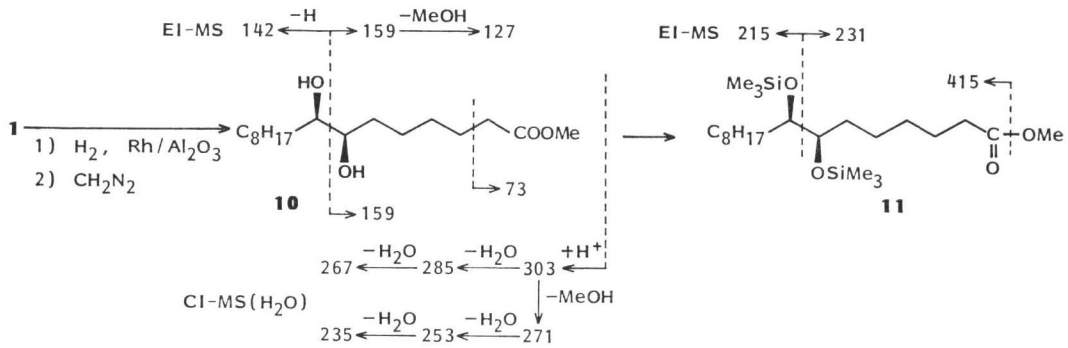
[a] The chemical shift difference between the irradiated and observed groups is too small.

[b] The group at 3.01 ppm presumably received enough energy from the 3.15 ppm irradiation to give partial decoupling of the group at 3.46 ppm.

Scheme 1.



Scheme 2.



diimide followed by catalytic reduction. This was identified as the simple reduction product, **8**, from its ^{1}H NMR spectrum (Table 2) and the mass spectrum of the trimethylsilyl derivative, **9**, (summarized in Scheme 1).

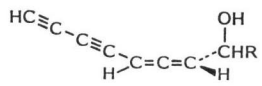
Catalytic hydrogenation of cepacin A (**1**), Scheme 2, results in hydrogenolysis of both the epoxide and the γ-lactone to give (+)-*threo*-7,8-dihydroxypalmitic acid, isolated as its methyl ester, **10**. The gross structure is supported by its NMR spectra (experimental section) and by the mass spectra of **10** and its bis-trimethylsilyl derivative, **11** (Scheme 2). The chemical shift of the C-7, 8 protons in benzene-d_6 (δ 3.34 in a 5% solution) allows assignment of the *threo* stereochemistry[18]. (It should be noted that the chemical shift of these protons is substantially concentration dependent, occurring at δ 3.20 in a 0.5% solution.) The optical rotation of **10** in MeOH ($[\alpha]_{546}^{25}$ +24° (*c* 0.3)) is nearly the same as that reported for methyl *threo*-9,10-dihydroxystearate (+22.5°) and for methyl *threo*-12,13-dihydroxystearate (+23.7°),

Table 5. Antibacterial activity (MIC, μg/ml) of cepacins A and B.

Organism	SC#	A	B	Organism	SC#	A	B
Staphylococcus aureus	1276	0.2	<0.05	*Proteus mirabilis*	3855	>50	6.3
〃 〃	2399	0.1	<0.05	*P. rettgeri*	8479	>25	6.3
〃 〃	2400	0.2	<0.05	*P. vulgaris*	9416	1.6	<0.05
〃 〃	10165	0.2	<0.05	*Salmonella typhosa*	1195	25	0.4
Streptococcus faecalis	9011	50	>50	*Shigella sonnei*	8449	50	0.8
S. agalactiae	9287	50	>50	*Enterobacter cloacae*	8236	>50	25
Micrococcus luteus	2495	0.2	3.13	*E. aerogenes*	10078	>50	3.1
Escherichia coli	8294	50	0.78	*Citrobacter freundii*	9518	50	0.8
〃 〃	10857	1.6	0.1	*Serratia marcescens*	9783	>50	>50
〃 〃	10896	12.5	0.4	*Pseudomonas aeruginosa*	9545	>50	>50
〃 〃	10909	6.3	0.2	〃 〃	8329	>50	>50
Klebsiella aerogenes	10440	>50	0.78	*Acinetobacter calcoaceticus*	8333	>50	>50
K. pneumoniae	9527	>50	>50				

Minimum inhibitory concentrations were determined by a two-fold agar dilution method on Yeast Beef Agar (BBL). Final inoculum level was 10^4 colony-forming units.

both of which have been shown to have the *R,R* absolute configuration[19]. Since the molecular rotation for a compound containing a *threo*-vicinal diol positioned remotely from either end of a linear aliphatic system (and containing no other asymmetric function) should be nearly constant, the *R,R* configuration can also be assigned to **10** and to be corresponding positions in **1**.



12

The absolute configuration of allenes has been correlated with optical rotation in studies by LOWE[20] and BREWSTER[21]. Molecular rotations for cepacin A and cepacin B, $-348°$ and $-612°$ respectively (calculated from the $[\alpha]_D$ values in Table 1), are in reasonable accord with the calculated[21] value, $-445°$, for **12** (*R* enantiomer). Rotations for cepacins A and B are approximate because of instability of the compounds, but the magnitudes are large. Since diyne-allenes typically have large rotations, the rotations of cepacins A and B would be dominated by this array, allowing assignment of the *R* configuration for the allene in both antibiotics[11]. As shown above, cepacin A is a 1 : 1 mixture of diastereomers, and the only asymmetric center where a mixture remains possible is at C-4. Thus the absolute stereochemistry of the cepacin A mixture can be depicted as in structure **1**.

Biological Properties

As seen from the data in Table 5, cepacins A and B both exhibit antibacterial activity with a high degree of activity against staphylococci. Interestingly, the compounds are poorly active against streptococci. Cepacin B is significantly more active than cepacin A against both Gram-positive and Gram-negative organisms.

Both compounds are relatively toxic to mice. On ip administration, cepacin A had an LD_{50} of 30 mg/kg and cepacin B had an LD_{50} of 25 mg/kg.

Experimental

^1H NMR spectra were recorded at 400 MHz on a Jeol Model GX400 spectrometer. ^{13}C NMR spectra were determined at 15 and 67.5 MHz on Jeol Model FX60Q and FX270 spectrometers.

IR and UV spectra and optical rotations were measured on Perkin-Elmer Model 983, Model 202 and Model 141 instruments, respectively. Mass spectra were determined on an AEI MS-902 double-focusing mass spectrometer and on an Extranuclear Laboratories modified Simulscan instrument. Thin-layer chromatography was done on Merck silica gel 60-F_{254} plates and silica gel column chromatography was done on Whatman LPS-1 silica gel. Cepacins A and B were stored as solutions in CH_2Cl_2 or EtOAc at −20°C. Removal of solvent *in vacuo* and immediate redissolution could be done without extensive decomposition.

Isolation of Cepacins A (**1**) and B (**2**)

The broth supernate from a 3,000-liter fermentation of *P. cepacia* SC 11,783 was extracted with 1,000 liters of CH_2Cl_2. Concentration of the extract gave 2 kg of residue that was distributed in 7 liters of each phase of heptane - PhMe - MeOH - H_2O, 3: 3: 2: 1 (System I). After separation of the phases by centrifugation, the upper phase was extracted with another 7-liter portion of System I lower phase, and the two resulting portions of lower phase were washed successively with a single 7-liter portion of System I upper phase. The combined lower phase was diluted with an equal volume of water, extracted with 6 liters of CH_2Cl_2, and the extract concentrated *in vacuo*, giving 75 g of residue. Chromatography of the residue on a 1.8-liter column of silica gel, eluting with CH_2Cl_2 followed by CH_2Cl_2 - EtOAc, 4: 1, and monitoring by TLC, gave 11 g of material rich in cepacins A and B. Chromatography of this material on a 2.75-liter column of silica gel, eluting with $CHCl_3$ followed by $CHCl_3$ - EtOAc, 3: 1, gave 0.72 g of crude cepacin A (47% pure by UV analysis) and 1.34 g of crude cepacin B (44% pure by UV analysis) containing some cepacin A.

Crude cepacin B was dissolved in 10 ml of MeOH - $CHCl_3$ - heptane, 1: 3: 6, (System II) and the resulting crystals removed and immediately redissolved in CH_2Cl_2. Concentration of the mother liquor gave a second crop of crystalline cepacin B which was combined with the solution of the first crop. The mother liquor was chromatographed at 5°C on a 500-ml column of Sephadex LH-20 eluting with System II. Fractions containing cepacin B were concentrated and the residue (*ca.* 26 mg) combined with the CH_2Cl_2 solution of the crystalline material. The resulting solution was passed through a 50-ml column of silica gel and the antibiotic eluted with EtOAc to give 200 ml of a solution containing 461 mg of cepacin B. The antibiotic was stored in this form at −20°C. ^{13}C NMR (CD_3CN) δ 24.3 (t, C_3), 28.4 (t, C_2), 52.7 (d), 54.4 (d), 57.0 (d), 58.6 (d), 68.0 (C_{15}), 68.4 (s, C_{13}), 72.9 (d), 73.7 (d, C_{16}), 78.4 (d), 81.7 (d), 94.2 (d), 178.2 (s, C_1), 217.9 (s, C_{11}).

Fractions from the Sephadex LH-20 chromatography that contained cepacin A were combined with the crude cepacin A from the silica gel chromatography and rechromatographed on Sephadex LH-20 as described above. Fractions containing cepacin A were combined, partially concentrated to remove MeOH, and the resulting solution passed through a 25-ml column of silica gel. Elution with EtOAc gave 140 ml of a solution containing 246 mg of cepacin A. The antibiotic was stored in this form at −20°C. ^{13}C NMR (CD_3CN) δ 29.0 (t, C_2 or C_3), 29.2 (t, C_2 or C_3), 52.5 (d), 62.7 (d), 68.0 (s C_{15}), 68.3 (s, C_{13}), 71.3 (d), 73.6 (d, C_{16}), 75.1 (s, C_{14}), 78.1 (d), 80.9 (d), 94.5 (d, C_{10} or C_{12}), 130.9 (d, C_5 or C_6), 132.5 (d, C_5 or C_6), 178.0 (s, C_1), 217.7 (s, C_{11}).

Catalytic Reduction of Cepacin B (**2**)

A solution of 15 mg of cepacin B in 15 ml of EtOAc - 95% EtOH, 1: 1, was stirred with 150 mg of 5% Rh/Al_2O_3 under H_2 (1 atm, 20°C) for 1 hour. Isolation of the major product by preparative TLC (silica gel; $CHCl_3$ - MeOH, 19: 1; Rf 0.31 ~ 0.44; detection with rhodamine B) gave 11 mg of **6**: IR ($CDCl_3$) 1772 cm^{-1} (γ-lactone); ^1H NMR, see Table 2; MS of trimethylsilyl derivative (**7**), see Scheme 1; ^{13}C NMR (CD_3OD) δ 14.4 (q), 23.6 (t), 24.5 (t), 24.6 (t), 29.1 (t), 30.3 (t), 30.6, 30.7 (t), 32.9 (t), 35.0 (t), 57.7 (d), 60.2 (d), 72.3 (d), 74.0 (d), 82.7 (d), 179.9 (s).

A solution of 1 mg of cepacin B in 1 ml of 95% EtOH - HOAc, 9: 1, was stirred with 15 mg of 5% Rh/Al_2O_3 under H_2 (1 atm, 20°C) for 1 hour. Preparative TLC (silica gel; $CHCl_3$ - MeOH, 9: 1; detection with dichlorofluorescein) showed the major product (**6**) at Rf 0.38 ~ 0.45 and a minor product, **8**, 130 μg, at Rf 0.61 ~ 0.64: MS, see Scheme 1.

Diimide Reduction of Cepacin B (**2**)

A solution of 1 mg of cepacin B and 25 mg of triisopropylbenzenesulfonyl hydrazide in THF was

kept at 60°C for 15 hours[22]. The solvent was removed and a solution of the residue in methyl *tert*-butyl ether (MTBE) was washed with aqueous $NaHCO_3$ and brine, dried (Na_2SO_4) and concentrated. Column chromatography of the residue on silica gel eluting with MTBE followed by EtOAc gave 0.66 mg of partially reduced product. This was treated with H_2 and Rh/Al_2O_3 as above, giving (after purification by TLC) 0.25 mg of **8**: $[\alpha]_D^{24}$ $-12\pm5°$ (*c* 0.1, $CHCl_3$); ^1H NMR ($CDCl_3+CD_3OD$) δ 4.60 (1H, ddd, J=7.5, 6.9, 3.7 Hz, C_4H), 3.64 (1H, dd, J=4.4, 4.1 Hz, C_5H), 3.16 (1H, dd, J=4.4, 2.3 Hz, C_6H), 3.05 (1H, dd, J=4.6, 2.1 Hz, C_7 or C_8H), 2.95 (1H, ddd, J=6.1, 4.7, 2.1 Hz, C_9H), 2.76 (1H, dd, J=4.6, 2.1 Hz, C_7 or C_8H).

Catalytic Reduction of Cepacin A (**1**)

A solution of 21 mg of cepacin A in 20 ml of EtOAc - 95% EtOH, 1: 1, was stirred with 56 mg of 5% Rh/Al_2O_3 under H_2 (1 atm, 20°C) for 1 hour. Removal of catalyst and solvent gave 19.6 mg of crude reduction product. The IR spectrum in $CHCl_3$ shows both γ-lactone (1765 cm^{-1}) and carboxylic acid absorption (1708 cm^{-1}), the latter being about twice as strong as the former. The product was treated with CH_2N_2 in Et_2O and then chromatographed on a silica gel column, eluting with PhMe - Et_2O, 3: 1. A center cut of the main component gave 4.0 mg of **10** as a crystalline solid: mp 46~48°C; IR ($CHCl_3$) 1728 cm^{-1} (ester C=O); ^1H NMR (5% in C_6D_6) δ 0.93 (3H, t, J=6.8 Hz, $C_{16}H$), 2.15 (2H, t, J=7.3 Hz, C_2H), 3.34 (2H, m, $C_{7,8}H$) and 3.38 (3H, s, OCH_3); ^{13}C NMR (CD_3CN) δ 14.7, 23.6, 25.9, 26.4, 26.8, 30.1, 30.3, 30.6, 30.7, 32.9, 34.2, 34.4, 34.8, 51.2, 74.9 (2C), 175.0; $[\alpha]_D^{25}$ $+20°$ (*c* 0.3, MeOH).

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References

1) JONES, E. R. H.; J. S. STEPHENSON, W. B. TURNER & M. C. WHITING: Chemistry of the higher fungi. XIII. Synthesis of (a) a C_9 triacetylenic epoxy-alcohol, a *Coprinus quadrifidus* metabolite and (b) a C_9 triacetylenic 1,2-diol. The structure of biformyne 1. J. Chem. Soc. 1963: 2048~2055, 1963
2) ROBBINS, W. J.; F. KAVANAGH & A. HERVEY: Antibiotics from basidiomycetes. II. *Polyporus biformis*. Proc. Nat. Acad. Sci. U.S.A. 33: 176~182, 1947
3) BU'LOCK, J. D.; E. R. H. JONES & P. R. LEEMING: Chemistry of the higher fungi. V. The structures of nemotinic acid and nemotin. J. Chem. Soc. 1955: 4270~4276, 1955
4) ANCHEL, M.; J. POLATNICK & F. KAVANAGH: Isolation of a pair of closely related antibiotic substances produced by three species of basidiomycetes. Arch. Biochem. 25: 208~220, 1950
5) KAVANAGH, F.; A. HERVEY & W. J. ROBBINS: Antibiotic substances from basidiomycetes. V. *Poria corticola*, *Poria tenuis* and an unidentified basidiomycete. Proc. Nat. Acad. Sci. U.S.A. 36: 1~7, 1950
6) CELMER, W. D. & I. A. SOLOMONS: Mycomycin. III. The structure of mycomycin, an antibiotic containing allene, diacetylene and *cis*, *trans*-diene groupings. J. Am. Chem. Soc. 75: 1372~1376, 1953
7) JOHNSON, E. A. & K. L. BURDON: Mycomycin — A new antibiotic produced by a moldlike actinomycete active against the bacilli of human tuberculosis. J. Bacteriol. 54: 281, 1947
8) CELMER, W. D. & I. A. SOLOMONS: Mycomycin. I. Isolation, crystallization and chemical characterization. J. Am. Chem. Soc. 74: 2245~2248, 1952
9) BENDZ, G.; G. WALLMARK & K. ÖBLOM: The antibiotic agent from *Marasmius ramealis*. Nature 162: 61~62, 1948
10) BENDZ, G.: Marasin, an antibiotic polyacetylene, isolated from the culture medium of *Marasmius ramealis*. Arkiv Kemi 14: 305~321, 1959
11) BEW, R. E.; J. R. CHAPMAN, E. R. H. JONES, B. E. LOWE & G. LOWE: Natural acetylenes. XVIII. Some allenic polyacetylenes from basidiomycetes. J. Chem. Soc. (C) 1966: 129~135, 1966
12) STANIER, R. Y.; N. J. PALLERONI & M. DOUDOROFF: The aerobic pseudomonads: A taxonomic study. J. Gen. Microbiol. 43: 159~271, 1966
13) BALLARD, R. W.; N. J. PALLERONI, M. DOUDOROFF, R. Y. STANIER & M. MANDEL: Taxonomy of the aerobic pseudomonads: *Pseudomonas cepacia*, *P. marginata*, *P. alliicola* and *P. caryophylli*. J. Gen. Microbiol. 60: 199~214, 1970

14) BU'LOCK, J. D.; E. R. H. JONES, P. R. LEEMING & J. M. THOMPSON: Chemistry of the higher fungi. VI. Isomerization reactions of naturally occurring allenes. J. Chem. Soc. 1956: 3767~3771, 1956
15) JACKMAN, L. M. & S. STERNHELL: Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry. 2nd ed., pp. 224 & 229, Pergamon Press, New York, 1969
16) JACKMAN, L. M. & S. STERNHELL: Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry. 2nd ed., p. 287, Pergamon Press, New York, 1969
17) HOUSE, H. O.: Modern Synthetic Reactions. 2nd ed., p. 13, W. A. Benjamin, Inc., Menlo Park, 1972
18) KURANOVA, I. L. & L. V. BALYKINA: Determination of *threo*- and *erythro*-configuration of vicinal diastereomeric derivatives of higher fatty acids using proton NMR spectra. Khim. Prir. Soedin. 1978: 299~305, 1978
19) MORRIS, L. J. & M. L. CROUCHMAN: The absolute optical configurations of the isomeric 9,10-epoxystearic, 9,10-dihydroxystearic and 9,10,12-trihydroxystearic acids. Lipids 7: 372~379, 1972
20) LOWE, G.: The absolute configuration of allenes. J. Chem. Soc., Chem. Comm. 1965: 411~413, 1965
21) BREWSTER, J. H.: Helix models of optical activity. *In* Topics in Stereochemistry. Vol. 2, *Ed.*, N. L. ALLINGER & E. L. ELIEL, pp. 1~72, John Wiley and Sons, Inc., New York, 1967
22) CUSACK, N. J.; C. B. REESE, A. C. RISIUS & B. ROOZPEIKAR: 2,4,6-Tri-isopropylbenzenesulfonyl hydrazide: A convenient source of di-imide. Tetrahedron 32: 2157~2162, 1976