# Bacillomycin $L_c$, a New Antibiotic of the Iturin Group: Isolations, Structures, and Antifungal Activities of the Congeners

STEVEN M. ESHITA and NICK H. ROBERTO

U.S.D.A. Forest Service, Forestry Sciences Laboratory,
359 Main Road, Delaware, OH 43015, U.S.A.

JOHN M. BEALE* and BLAIN M. MAMIYA

Division of Medicinal Chemistry, College of Pharmacy, The University of Texas at Austin,
Austin, TX 78712-1074, U.S.A.

RYAN F. WORKMAN

Department of Chemistry, Purdue University,
West Lafayette, IN 47906, U.S.A.

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Bacillomycin $L_c$, a new antifungal antibiotic of the iturin class, was isolated from a strain of *Bacillus subtilis* as a set of five congeners. The structure as determined by chemical and spectrometric analyses has been shown to differ from that of bacillomycin L by sequence changes from aspartate-1 to asparagine-1 and from glutamine-5 to glutamate-5. The five congeners differ from each other only in the structure of the aliphatic side chain of the constituent $\beta$-amino acid. The hydrophobicity of the $\beta$-amino acid affects the antifungal activity of the congener, as activity increased in the order of increased congener retention on a reversed-phase HPLC column.

Iturins are antifungal antibiotics produced by *Bacillus subtilis* that are characterized by a cyclic peptidolipid structure consisting of eight amino acids. Their common structure consists of a macrocycle of seven $\alpha$-amino acids in a LDDLLDL configuration sequence, closed by a $\beta$-amino acid linkage.[1]

During a screening of tree xylem for microorganisms with biological control potential against tree phytopathogens, several *Bacillus* species were isolated displaying *in vitro* activity against *Ophiostoma ulmi* (Buisman) Nannf., the Dutch elm disease fungus.[2] Among these, *Bacillus subtilis* isolate FS94-14 showed the greatest activity, prompting this study to characterize the antifungal compounds produced by this organism.

We report the isolations, structures, and antifungal activities of individual congeners of a new iturin antibiotic. Previous studies of structure-function relationships for iturins have reported the effects of the component $\alpha$-amino acids and their modification on activity.[3~5] Here the effect of different $\beta$-amino acid substituents on the antifungal activity of an iturinic compound is demonstrated.

## Materials and Methods†

### Organisms

*Bacillus subtilis* FS94-14 was isolated from American elm tree xylem.[2] *Ophiostoma ulmi* isolate Cu71-7857 was obtained from FRANCIS W. HOLMES, Shade Tree Laboratories, University of Massachusetts at Amherst. *Verticillium dahliae* and *Ceratocystis fagacearum* were from LAWRENCE R. SCHREIBER, U.S. Department of Agriculture, Agricultural Research Service-National Arboretum, while *Cryphonectria parasitica* was from FREDERICK BERRY, U.S.D.A. Forest Service, both at Delaware, Ohio.

### Reagents

Bacillomycin L was a generous gift from JANN ICHIDA, U.S. Department of Agriculture, Agricultural Research Service, Delaware, Ohio, who obtained the sample from FRANCOISE PEYPOUX, Université Claude Bernard, Lyon 1, Villeurbanne, France. Pico-Tag amino acid analysis reagents were from Waters. Marfey's reagent, 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (FDAA), was purchased from Pierce. Immobilon-P polyvinylidene difluoride (PVDF) membranes were from Millipore. Amphotericin B and nystatin were from Sigma. The NMR solvents

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and N-bromosuccinimide were obtained from Aldrich.

### Fermentation/Production

Potato dextrose broth was inoculated with *Bacillus subtilis* FS94-14 to a concentration of $5.5 \times 10^5$ cells/ml. Twenty four cultures of 360 ml each in 2-liter Erlenmeyer flasks were incubated without agitation at 28°C for three days before harvest.

### Isolation, Purification

Culture filtrate was concd to 0.6~0.7 g total solids/ml. Impurities were precipitated by mixing $Me_2CO$ - MeOH - concd sup, 4:2:1 (in volume), and removed by centrifugation. The resulting sup was concd to between 1/20 and 1/30 vol, mixed with 1 vol of MeOH, and stored at 4°C for several days, resulting in a white precipitate. The brown sup and cold MeOH washes of the precipitate were pooled and concd to 40 ml. Twenty ml aliquots were loaded on a 2.5×62 cm Sephadex LH-20 (Pharmacia) column and eluted at 0.8 ml/minute with propanol - MeOH - $H_2O$, 18:6:1 (in volume). Antifungal activity was monitored by paper disk diffusion on agar seeded with *O. ulmi* conidia.[2] Active fractions were dried and resuspended in MeOH to approximately 90 mg solids/ml, and passed through a 0.2 micron filter.

Reversed-phase HPLC was carried out in two steps. In the first, one ml aliquots of the active filtrate were injected on a 1.9×15 cm μBondapak $C_{18}$ column (Waters) and eluted at 25 ml/minute with a linear gradient of 46~68% (v/v) MeOH - $H_2O$ in 20 minutes, followed by 68~95% (v/v) MeOH - $H_2O$ in 5 minutes. Fractions were collected at Rt intervals of 11~18.5 minutes (Fraction I) and 18.5~22 minutes (Fraction II), and concd by rotary evaporation to 12 mg/ml and 10 mg/ml in total iturins, respectively. Both fractions were processed in a second HPLC step by repetitive 50 μl injections on a 0.8×10 cm Nova-Pak $C_{18}$ Radial-Pak cartridge (Waters) with a 2.5 ml/minutes flow rate, monitored at 214 nm. Fraction I yielded congeners #0, #1, and #2, collected at average peak Rt values of 10.5, 16.5, and 18 minutes, respectively, eluted with 35% (v/v) $CH_3CN$, 0.01% (v/v) trifluoroacetic acid (TFA), in $H_2O$. Fraction II yielded congener #3 at an average Rt of 12.8 minutes and congener #4 at an average Rt of 14.6 minutes, both eluted with 38% (v/v) $CH_3CN$, 0.01% (v/v) TFA, in $H_2O$.

### α-Amino Acid Composition

The amino acid compositions were determined by the Waters Pico-Tag method.[6] Hydrolysis was carried out at 105°C for 24 hours and at 150°C for 4, 8, and 16 hours using 6 N HCl with 1% (v/v) phenol.

### Edman Sequencing

Congeners were cleaved using N-bromosuccinimide (NBS) treatment[7] to generate a free N-terminus on the carboxy side of the tyrosine. Cleavage products were isolated using reversed-phase HPLC and sequenced on an Applied Biosystems 477A instrument with a 120A on-line analyzer.

### Stereochemistries of the α-Amino Acids

Amino acid chirality was determined by reacting acid hydrolysates of the congeners with FDAA,[8] producing diastereomeric derivatives resolved by reversed-phase HPLC. The chiralities of specific amino acid positions in the sequence for congener #3 were determined by comparing the chiral composition of the Edman-degraded peptide with the composition of the intact peptide. NBS-cleaved #3 was applied to Immobilon-P PVDF and degraded through three Edman chemistry cycles on the protein sequencer. The PVDF-bound residual peptide was vapor-hydrolyzed using 2:1 (v/v) 12 N HCl - TFA at 114°C for 24 hours. Amino acids were extracted using 0.1 N HCl, 30% (v/v) MeOH, dried, then derivatized with FDAA.

### NMR

The NMR experiments were carried out on IBM AF-300 or Bruker AM-500 superconducting magnets at 7.1 and 11.75 tesla, respectively, or on General Electric GN-500 or Bruker AMX-500 instruments. The samples contained 3~6 mg of compound in 0.5 ml of DMSO-$d_6$, and the experiments were carried out at 40°C and referenced internally to the solvent at $\delta$ 2.49. The double-quantum filtered COSY (DQF-COSY)[9] was acquired in the phase-sensitive mode using the States hypercomplex method,[10] while the total correlated spectroscopy (TOCSY)[11] and the rotating-frame Overhauser effect spectroscopy (ROESY)[12] experiments were acquired in the phase-sensitive mode by the time-proportional phase incrementation method.[13] DQF-COSY, TOCSY, and ROESY were recorded with 512 $t_1$ increments, using 64 scans per $t_1$ increment, each with 2048 data points. The spin-lock time for the TOCSY experiment was 60 msec while the ROESY experiment used 100 msec. The data were zero-filled in $F_1$ to give a matrix of either 2048×2048 data points or 2048×1024 data points. The data were processed with a sine-bell squared window function shifted by $\pi/3$ radians in both dimensions. The $^{13}C$-$^1H$ inverse heteronuclear multiple-quantum correlated spectroscopy experiment (HQMC) was recorded and the data analyzed as above. The two-dimensional double quantum experiment and the inverse heteronuclear multiple-bond correlated spectroscopy experiment (HMBC) were acquired in the absolute value mode. These two experiments were recorded with 256 $t_1$ increments, with 32 to 64 scans per $t_1$ increment, each with 1024 data points. The data were zero-filled to give a matrix of 1024×1024 data points. Solvent suppression of residual HDO, when necessary, was achieved by solvent irradiation using a low-power presaturation pulse.

### FAB-MS

Mass spectra were obtained for each of the five con-

geners with a VG-70-SEQ fast-ion-bombardment mass spectrometer operated in the positive ion mode with 8 kV xenon bombardment. Bacillomycin L was used as a control sample. Samples were embedded in glycerol matrices on a direct insertion probe. Aqueous NaCl and KCl were added to the matrix to generate $Na^+$ and $K^+$-doped spectra to aid in the identification of the molecular ion peaks.

### Antifungal Activity

The antifungal activity of the purified antibiotics was measured by the inhibition of radial growth from a mycelial agar cylinder placed on antibiotic agar. The fungi used for the test were *Ophiostoma ulmi* (Buisman) Nannf., *Verticillium dahliae* Kleb., *Ceratocystis fagacearum* (Bretz) Hunt, and *Cryphonectria parasitica* (Murrill) Barr—the causal agents of Dutch elm disease, Verticillium wilt of maples, oak wilt, and chestnut blight, respectively. Amphotericin B and nystatin were dissolved in MeOH and incorporated into potato dextrose agar at final concentrations of 10, 3, 1, 2/3, and 1/3 $\mu$g/ml. Bacillomycin $L_c$ congeners were made to stock concentrations of 1 mg/ml in MeOH (quantitated by HPLC) and incorporated into potato dextrose agar at final concentrations of 50, 30, 20, 15, 10, and 7 $\mu$g/ml for congeners #0~#2; and final concentrations of 10, 7, 6, 5, 3, and 1 $\mu$g/ml for congeners #3 and #4. Concentrations were tested in triplicate. Fungal mats were grown in 100 mm Petri dishes on 25 ml of potato dextrose agar. Mycelial plugs of 5 mm diameter were cut with a cork borer, and placed inverted and centered on 2 ml of antibiotic agar in 9.6 $cm^2$ wells of 6-well tissue culture plates. Plates were incubated at 30°C. Growth diameters were compared with antibiotic-minus controls after 3 days for *O. ulmi* and *C. parasitica*, while *V. dahliae* and *C. fagacearum* required 7 days for sufficient growth.

## Results

### Isolation, Purification

*Bacillus subtilis* isolate FS94-14 was grown in still cultures of potato dextrose broth, thus producing antibiotics active against *Ophiostoma ulmi*, the Dutch elm disease fungus, in the culture filtrate. The purification method consisted of organic solvent extraction, LH-20 chromatography, and two stages of HPLC purification, with antifungal activity monitored by agar diffusion bioassay. The resulting pure active compounds were numbered #1 to #4 based on their order of elution (early to late) by reversed-phase HPLC. Subsequently, a fifth related compound was detected eluting earlier in Fraction I by UV photodiode array spectroscopy. This compound, less active than the other four, was designated #0.

### Structures of the Congeners

Amino acid compositions of the acid-hydrolyzed congeners were consistent with the composition of bacillomycin L.[14]

$$Asx_2, Glx_1, Ser_2, Thr_1, Tyr_1 .$$

The chiral composition of the amino acids was determined by derivatization of acid hydrolysates with FDAA and identification of the resulting diastereomers by HPLC. All five congeners yielded the following equimolar composition:

D-Asx, L-Asx, L-Glx, D-Ser, L-Ser, L-Thr, L-Tyr .

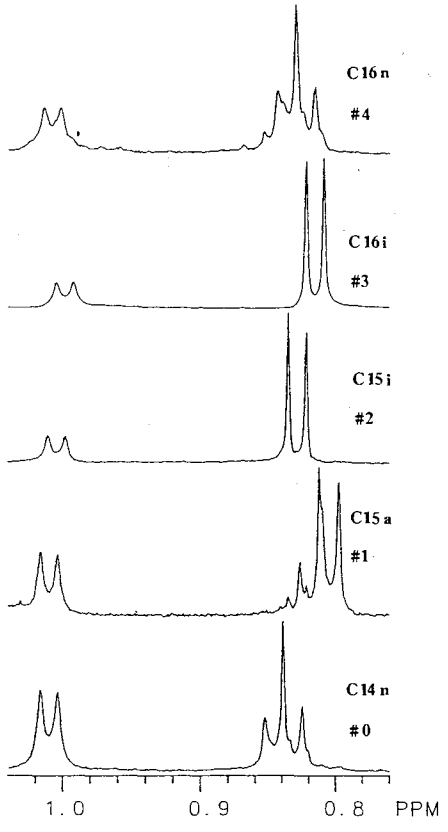
Edman sequencing was attempted on both uncleaved and cleaved congeners. All intact congeners failed to sequence. Congeners cleaved on the carboxy side of the tyrosine residue by treatment with NBS yielded the sequence:

Asn-Ser-Glu-Ser-Thr .

The sequencing reactions did not proceed past threonine. For bacillomycins D and L this stoppage is due to the $\beta$-amino acid at the next residue.[15]

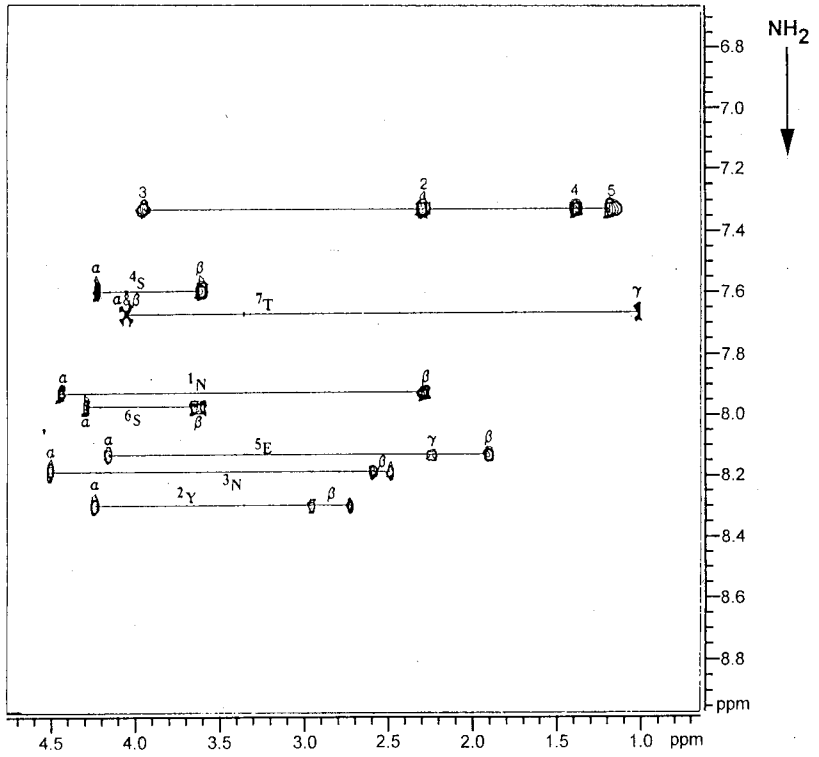
The chiral ambiguities for the Asn and Ser positions in the Edman sequence were resolved for congener #3 by chiral composition analysis on the NBS-cleaved,

Fig. 1. Upfield (0.76 to 1.04 ppm) $^1H$ NMR spectra for congeners #0 through #4.




n=normal, i = iso, a = anteiso.

Fig. 2. TOCSY spectrum of congener #3, expansion of NH to αCH, βCH, and γCH.




E = glutamate, N = asparagine, S = serine, T = threonine, Y = tyrosine; 60 msec spin-lock.

Edman-degraded peptide. After three cycles of Edman chemistry, the residual peptide showed losses in D-Asx, L-Ser, and L-Glx, corresponding to the Asn-Ser-Glu region of the sequence. The chiral sequence for #3 is therefore:

D-Asn—L-Ser—L-Glu—D-Ser—L-Thr .

The remaining α-amino acid positions are L-Asx and D-Tyr.

The one-dimensional $^1$H NMR spectra of the five congeners (Fig. 1) differed in the 0.76 to 1.04 ppm region, indicating different terminal branch structures of aliphatic chains. Congeners #0 and #4 are *normal*-type compounds, congeners #2 and #3 are *iso*-type compounds, and congener #1 is an *anteiso*-type compound.

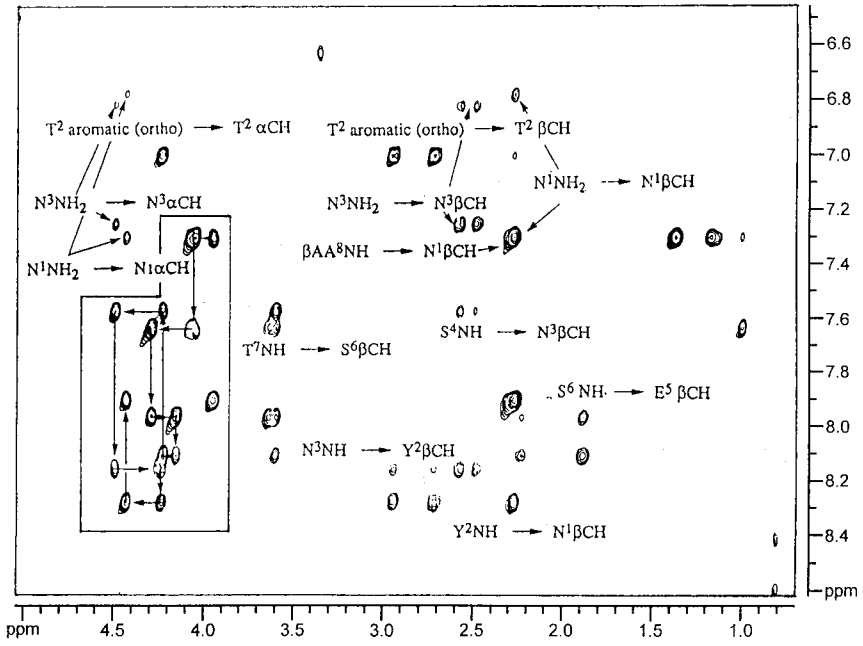
The TOCSY spectrum for congener #3 (Fig. 2) confirmed the seven amino acids detected by chemical methods and revealed an eighth amino acid of aliphatic character with four correlations to an amide proton resonance at $\delta$ 7.33 ppm. DQF-COSY experiments (spectra not shown) revealed connectivities from the amide proton to the β-proton at $\delta$ 3.94 ppm, from the β-proton to both the α-protons at $\delta$ 2.27 ppm and the γ-protons from the aliphatic side chain at $\delta$ 1.39 ppm, and from the γ-protons to other protons further along the aliphatic chain which show resonance overlap at $\delta$ 1.20 ppm. The eighth amino acid is therefore a β-amino acid with an aliphatic chain branched from the β-carbon. The proton resonance assignments for congener #3 are listed in Table 1.

Table 1. $^1$H resonance assignments for congener #3 in DMSO-$d_6$.

| Amino acid | NH | αCH | βCH | γCH | Other |
|---|---|---|---|---|---|
| 1-L-Asn | 7.93 | 4.43 | 2.28 | | $NH_2$ 7.30, 6.78 |
| 2-D-Tyr | 8.30 | 4.22 | 2.95, 2.72 | | Aromatic H-2 & H-6 7.00 H-3 & H-5 6.64 |
| 3-D-Asn | 8.22 | 4.49 | 2.59, 2.49 | | $NH_2$ 7.26, 6.83 |
| 4-L-Ser | 7.60 | 4.21 | 3.61 | | |
| 5-L-Glu | 8.14 | 4.14 | 1.91 | 2.24 | |
| 6-D-Ser | 7.95 | 4.28 | 3.63 | | |
| 7-L-Thr | 7.66 | 4.04 | 4.09 | 1.02 | |
| β-Amino acid | 7.33 | H-2 2.27 | H-3 3.94 | H-4 1.39 | Other H's 1.20 |

The ROESY experiment for congener #3 (Fig. 3) yielded the complete sequence following the correlations from the backbone amide from one residue to the α-proton of the preceding residue. The peptide is cyclic, as backbone correlations can be traced to start and end at the same residue. Starting with the D-asparagine from

Fig. 3. ROESY spectrum of congener #3, expansion of NH to αCH, βCH, and γCH.




E=glutamate, N=asparagine, S=serine, T=threonine, Y=tyrosine; 100 msec spin-lock.

Table 2. $^{13}C$ resonance assignments for congener #3 in DMSO-$d_6$.

| Amino acid | Carbonyl | Cα | Cβ | Cγ | Other |
|---|---|---|---|---|---|
| 1-L-Asn | 171.84<br>amide 171.44 | 50.19 | 40.52 | | |
| 2-D-Tyr | 171.31 | 55.40 | 35.47 | | Aromatic<br>C-1, 127.98<br>C-2 & C-6, 129.86<br>C-3 & C-5, 114.94<br>C-4, 155.63 |
| 3-D-Asn | 171.21<br>amide 171.52 | 50.56 | 36.76 | | |
| 4-L-Ser | 170.13 | 55.18 | 61.17 | | |
| 5-L-Glu | 171.74<br>acid 171.86 | 53.27 | 26.85 | 31.19 | |
| 6-D-Ser | 170.41 | 55.25 | 61.11 | | |
| 7-L-Thr | 169.35 | 58.67 | 65.74 | 19.91 | |

| β-Amino acid | | | | | | | |
|---|---|---|---|---|---|---|---|
| C-1 | 170.91 | | | | | | |
| C-2 | 36.84 | | | | | | |
| C-3 | 46.15 | | | | | | |
| C-4 | 33.80 | | | | | | |
| C-5 | 25.22 | | | | | | |
| C-6 to C-12 | 26.67 | 28.60 | 28.90 | 28.94 | 28.96 | 28.99 | 29.22 |
| C-13 | 38.99 | | | | | | |
| C-14 | 27.27 | | | | | | |
| C-15 & C-16 | 22.42 | | | | | | |

the chiral sequence, the backbone amide at δ 8.22 ppm shows correlation to the α-proton of L-tyrosine at δ 4.22 ppm, whose backbone amide at δ 8.30 ppm shows correlation to the α-proton of L-Asx at δ 4.43 ppm. L-Asx is an asparagine which has amide proton resonances at δ 7.30 and δ 6.78 ppm correlated to its β-carbon protons at δ 2.28 ppm. The backbone amide of L-asparagine at δ 4.43 ppm correlates to the β-amino acid α-protons at δ 2.27 ppm and to the β-proton at δ 3.94 ppm. The β-amino acid amide at δ 7.33 ppm shows correlation to the L-threonine α-carbon at δ 4.04 ppm. The remainder of the sequence matches the Edman chemistry sequence

Table 3. FAB-MS data for bacillomycin L and bacillomycin $L_c$ congeners #0 through #4.

| Sample | $M+H^+$ | M | $M+Na^+$ | $M+K^+$ | β-Amino acid | | Molecular formula |
|---|---|---|---|---|---|---|---|
| | | | | | # of carbons | branch | |
| Bacillomycin L | 1021 | 1020 | 1043 | 1059 | 14 | Mixture | $C_{46}H_{72}N_{10}O_{16}$ |
| | 1035 | 1034 | 1057 | 1073 | 15 | | $C_{47}H_{74}N_{10}O_{16}$ |
| | 1049 | 1048 | 1071 | 1087 | 16 | | $C_{48}H_{76}N_{10}O_{16}$ |
| #0 | 1021 | 1020 | 1043 | 1059 | 14 | Normal | $C_{46}H_{72}N_{10}O_{16}$ |
| #1 | 1035 | 1034 | 1057 | 1073 | 15 | Anteiso | $C_{47}H_{74}N_{10}O_{16}$ |
| #2 | 1035 | 1034 | 1057 | 1073 | 15 | iso | $C_{47}H_{74}N_{10}O_{16}$ |
| #3 | 1049 | 1048 | 1071 | 1087 | 16 | iso | $C_{48}H_{76}N_{10}O_{16}$ |
| #4 | 1049 | 1048 | 1071 | 1087 | 16 | Normal | $C_{48}H_{76}N_{10}O_{16}$ |

above.

The $^{13}C$ DEPT, HMQC, and HMBC experiments (spectra not shown) were used to assign all the carbon resonances and their multiplicities for congener #3 (Table 2). Fifteen carbon resonances were resolved for the β-amino acid, with resonance overlap at δ 22.42 ppm for the two terminal carbons of this *iso*-type compound.

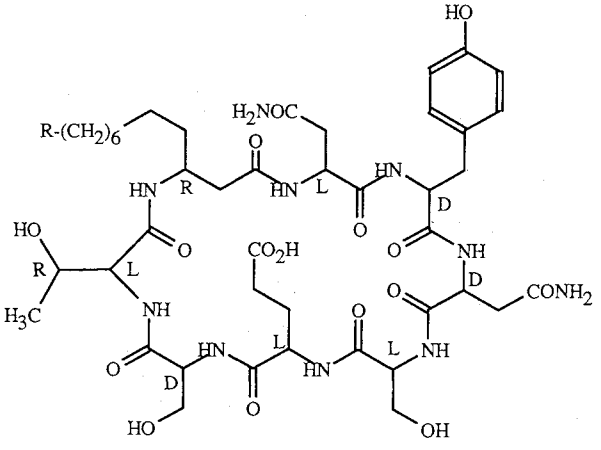
FAB-MS (Table 3) revealed mass matches for congener #0 with $C_{14}$-bacillomycin L, congeners #1 and #2 with $C_{15}$-bacillomycin L, and congeners #3 and #4 with $C_{16}$-bacillomycin L.[15] The 14 and 28 mass unit differences between the congeners are consistent with differences of one and two methylene groups, respectively, in the aliphatic side chain of the β-amino acid.

The resulting structure model for the five congeners of this antibiotic is shown in Fig. 4. Double quantum 2-D spectra for the five congeners (not shown) revealed no differences among the structures other than the different termini of the aliphatic chain of the β-amino acid. The chiral sequence positions determined for D-serine, L-serine, D-asparagine, and L-asparagine of congener #3 were assumed to apply to the other congeners.

## Antifungal Activity

Table 4 shows the concentrations of antibiotics in potato dextrose agar inhibiting the diameter of fungal growth by half. The congeners of bacillomycin $L_c$ showed increased activity with increased retention. The range in congener activity was greatest for *O. ulmi*, spanning an order of magnitude between *n*-$C_{14}$ and *n*-$C_{16}$ β-amino acid structures. *C. parasitica* and *O. ulmi* were more sensitive to antibiotic inhibition than the slower growing fungi, *C. fagacearum* and *V. dahliae*. No congener was as active as the polyene antifungal antibiotics, amphotericin B and nystatin.

Fig. 4. Chemical structures of bacillomycin $L_c$.



Bacillomycin $L_c$

R=$CH_3(CH_2)_2$- (#0), $CH_3CH_2CH_3CH$- (#1), $(CH_3)_2CHCH_2$- (#2), $(CH_3)_2CHCH_2CH_2$- (#3), $CH_3(CH_2)_4$- (#4).

## Discussion

We have designated our new antibiotic bacillomycin $L_c$. The primary structure is identical to an unknown peptide antibiotic sequenced by FAB-MS.[16] Here we have additionally determined the α-amino acid chiralities and the structures of the β-amino acids for all congeners, including the $C_{16}$ species. Bacillomycin $L_c$ differs from bacillomycin L by the sequence positions of a side-chain amide and a carboxylic acid. In the case of bacillomycin L, the carboxylic acid is at position 1 (L-aspartic acid) and an amide is at position 5 (L-glutamine).[15] Alternatively, the structure can be interpreted as a substitution of L-serine for L-proline in bacillomycin D.[15] The β-amino acids of the congeners are the same for bacillomycins $L_c$, L, and D.

We have not determined whether the differences in bacillomycin structures are the result of inherent differences in the biosynthetic pathways, or are artifacts

Table 4. Antibiotic concentrations inhibiting fungal growth by 50% on potato dextrose agar.

| Fungus | Antibiotic [in μg/ml] | | | | | | |
|---|---|---|---|---|---|---|---|
| | Bacillomycin $L_c$ congeners | | | | | Amphotericin B | Nystatin |
| | *n*-$C_{14}$ | *anteiso*-$C_{15}$ | *iso*-$C_{15}$ | *iso*-$C_{16}$ | *n*-$C_{16}$ | | |
| *Ophiostoma ulmi* | 50 | 20~30 | 20 | 7 | 6 | 1 | 3 |
| *Ceratocystis fagacearum* | >50 | 30 | 20 | >10 | 10 | 1~10 | 3 |
| *Verticillium dahliae* | >50 | >30 | >20 | >10 | >10 | >10 | >10 |
| *Cryphonectria parasitica* | 10~15 | 10~20 | 10 | 7 | 5 | 1/3~2/3 | 1/3~1 |

due to differences in the fermentation and isolation conditions. Concerning the latter, we avoided the strongly acidic conditions previously used to isolate bacillomycins and other iturins.[17,18]

Two asymmetric centers with undetermined stereochemistries remain. We have not determined the absolute configuration at C-3 of the β-amino acids. The iturin antibiotics reported thus far have been shown to contain the LDDLLDL pattern for the α-carbons of the α-amino acids. For iturin A, the C-3 position of the β-amino acid has been determined to be (*R*);[19] we assumed this to be the case in the structure shown in Fig. 4. In addition, the absolute configuration at C-12 for the *anteiso*-$C_{15}$ β-amino acid has not been identified directly for any of the iturin antibiotics. However, *anteiso* fatty acids isolated from *B. subtilis* have been reported as exclusively L(+).[20] If these are the precursors of the *anteiso*-β-amino acid, then the absolute configuration at C-12 is (*S*).

The purification and structure identification of the individual congeners permitted us to test the effect of different β-amino acid structures on activity. The assay showed positive correlation between the hydrophobicity of the β-amino acid residue and the antifungal activity. Activity increased with more carbons in the side chain (*n*-$C_{16}$ > *n*-$C_{14}$; *iso*-$C_{16}$ > *iso*-$C_{15}$); activity decreased with chain branching (*iso*-$C_{16} \leq$ *n*-$C_{16}$) and with branching further from the end of the chain (*anteiso*-$C_{15} \leq$ *iso*-$C_{15}$). The difference in congener hydrophobicity or amphiphilicity may affect the molecule's interaction with membranes, a putative target site for the action of iturins.[4,21]

The antifungal activity of a mixture of congeners might be affected similarly by the β-amino acid composition in the mixture. Iturin $A_L$, a mixture of iturins A with a high percentage of $C_{16}$-β-amino acids, was reported to have a higher antifungal activity compared to an iturins A mixture with a lower percentage of long chain congeners.[22] If this relationship applies to all iturins, reports of antifungal activity for both native and derivatized iturins should be qualified with the congener composition used in the measurement.

This structure-activity correlation suggests two strategies by which iturin antifungal activity might be increased: a) synthesis of new, more hydrophobic β-amino acid iturins, or b) skew in the distribution of congeners produced toward existing long chain species. Concerning the latter, congener compositions for several iturins were modified by specific β-amino acid additions to the culture medium.[18,23,24,25] Significantly, bacillomycin $F_a$,[23] bacillomycin $F_b$,[18] and mycosubtilin[25] were produced as 90% *anteiso*-$C_{17}$ congener mixtures in media supplemented with DL-isoleucine. For bacillomycin $L_c$, we measured an activity increase in the culture medium when supplemented with DL-valine, associated with an increase in the production of the *iso*-$C_{16}$ congener (unpublished data). There are no reports of the biosynthesis of novel, longer chain iturins resulting from modifications to the culture medium. Studies of iturin biosynthesis could provide insight into the composition limits in β-amino acid chain-length incorporation, toward the goal of activity enhancement.

Acknowledgments

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