ASELACINS, NOVEL COMPOUNDS THAT INHIBIT BINDING OF ENDOTHELIN TO ITS RECEPTOR

II. ISOLATION AND ELUCIDATION OF STRUCTURES

JILL E. HOCHLOWSKI, PRESTON HILL, DAVID N. WHITTERN, MICHAEL H. SCHERR, RONALD R. RASMUSSEN, SARAH A. DORWIN and JAMES B. MCALPINE

Pharmaceutical Products Research and Development, Abbott Laboratories, Abbott Park, Illinois, 60064, U.S.A.

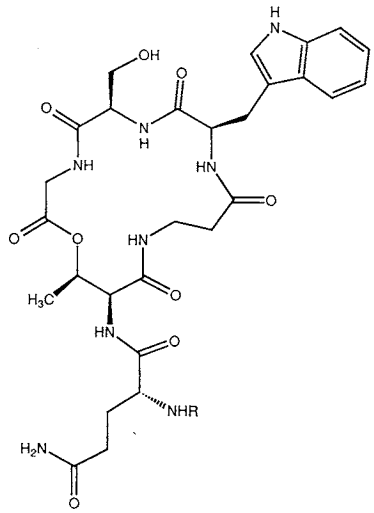
(Received for publication December 3, 1993)

Three novel compounds, named the aselacins, which inhibit the binding of endothelin to its receptor have been isolated from two related *Acremonium* species of fungi grown in stationary culture. These compounds are cyclic pentapeptolides with a ring formed by cyclo[Gly-D-Ser-D-Trp-β-Ala-L-Thr] and an additional exocyclic D-Gln to which is attached a functionalized long chain fatty acid. The aselacins differ in the functionalization of this acid. The structures of the aselacins were determined by amino acid analysis, mass spectrometry and evaluation of 1-D and 2-D homonuclear and heteronuclear ^{1}H, ^{13}C and ^{15}N NMR spectra in protic and aprotic solvents. The stereochemistry of the amino acids present was elucidated by chiral HPLC of hydrolyzed compound.

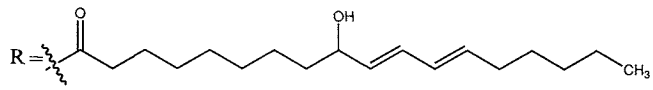
In the course of screening for compounds which inhibit binding of endothelin to its receptor, three novel cyclic pentapeptolides have been isolated from the fungi AB 2093T-194 and AB 2086L-51 grown in stationary culture. A companion paper[1] describes the taxonomy and growth of the producing culture as well as the biological evaluation of the aselacins (**1**~**3**). This paper will discuss the isolation of aselacin A, B and C and the elucidation of their structures.

Isolation of Aselacins A and B

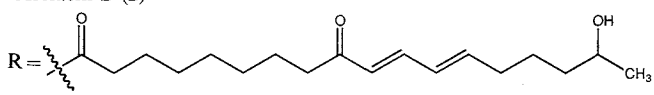
As described in the preceding paper[1], the fungus AB 2093T-194 was grown in stationary culture. After 21 days of growth in four, 20 liters carboys (the four containing a total of 5.6 liters of liquid medium on 2.4 kg of shredded wheat), acetone (1.2 liters) was added to the fungal mass and media and the mixture was allowed to sit at room temperature for six hours after which a mixture of toluene - ethyl acetate (0.75 liters of each) was added and the mixture was left overnight at 4°C. Solvent was then removed from the solid mass and concentrated to a brown oil. This oil was partitioned between heptane - methanol - water - acetic acid (5 liters : 3 liters : 2 liters : 0.02 liter) and the lower layer was concentrated to an oil. This oil was triturated sequentially with heptane, ethyl acetate, ethanol and methanol (2 liters of each). The ethanol soluble material was chromatographed on a Sephadex LH-20 column developed in methanol. The ability of fractions to displace radioactive endothelin from tissue preparations was assayed as described[1]. Active fractions from the LH-20 column were combined, concentrated to dryness and subjected to countercurrent chromatography on an Ito multi-layered coil planet centrifuge in a solvent system of ethyl acetate - ethanol - water (2 : 1 : 2) with the lower phase stationary. Active fractions from this column were combined based upon their behavior on thin layer chromatography (TLC) and dried to yield pure aselacin A (**1**, 241 mg) and a clear oil of mixed components. This oil was subjected to countercurrent chromatography on an Ito multi-layered coil planet centrifuge in a solvent system of chloroform - methanol - water (1 : 1 : 1) with the lower phase stationary. Active fractions from this column were combined based upon their TLC behavior to yield pure aselacin A (9 mg) and pure aselacin B (3 mg). (See Fig. 1 for a schematic of this isolation.)



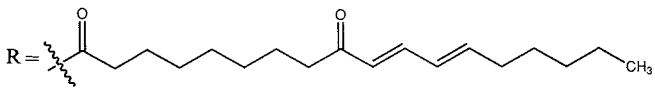
Aselacin A (**1**)



Aselacin B (**2**)



Aselacin C (**3**)



Isolation of Aselacin C

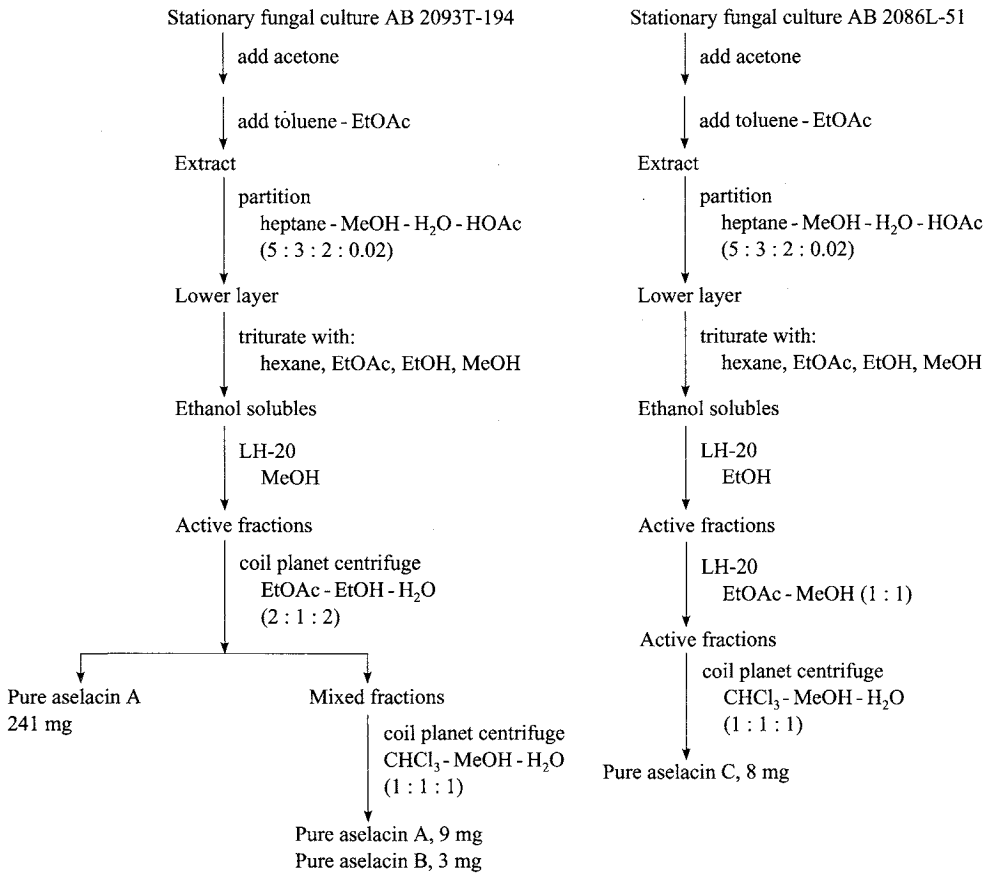
The fungus AB 2086L-51 was grown and extracted in a manner identical to that described above for the fungus AB 2093T-194. Initial work-up procedures, including the partition and extraction were also identical. The ethanol solubles from the partition were chromatographed on a Sephadex LH-20 column developed in ethanol. Active fractions from the LH-20 column were combined, concentrated to dryness and rechromatographed over a Sephadex LH-20 column developed with methanol-ethyl acetate (1 : 1). Active fractions from this column were combined and concentrated to a clear oil. This oil was subjected to countercurrent chromatography on an Ito multi-layered coil planet centrifuge in a solvent system of chloroform-methanol-water (1 : 1 : 1) with the lower phase stationary. Active fractions from this column were combined to yield pure aselacin C (8 mg). (See Fig. 1 for a schematic of this isolation.)

Structure Determination of the Aselacins

Structure Determination of Aselacin A

NMR spectral analysis of aselacin A (**1**) suggested the presence of amino acid residues, and this was confirmed by an amino acid analysis which indicated the presence of tryptophan, threonine, serine, glycine, β-alanine and either glutamic acid or glutamine moieties. A failed Edman degradation suggested that there

Fig. 1. Isolation of the aselacins.



was no *N*-terminal amino acid present in aselacin A. The stereochemistries of the amino acids present in the hydrolysate of aselacin A were determined by chiral HPLC on a CrownPak (Cr^+) column by co-injection with chiral standards (see Table 1). These results indicated that aselacin A contained the amino acids D-Trp, L-Thr, D-Ser, and D-Glu or D-Gln.

Table 1. Comparison of retention times of amino acids in hydrolysate of aselacin A with authentic standards.

Amino acid	D Retention time (minutes)	L Retention time (minutes)	Conditions
Glu	**4.8**	15.6	A
Thr	2.8	**4.9**	A
Ser	**3.1**	3.8	A
Trp	**33.6**	42.0	B

See experimental for description of conditions A and B.

Bold peaks were those enhanced by co-injection.

An NMR spectrum and COSY experiment defined the spin systems for the six amino acids of aselacin A as outlined in Table 2. Carbon signal assignments were made for these same amino acids with an HMBC[2] and an HMQC[3] experiment and are outlined in Table 3. The HMBC experiment allowed the assignment of a pentapeptolide ring comprised of the amino acids cyclo[Gly-D-Ser-D-Trp-β-Ala-L-Thr] with a peptolide linkage through D-Thr by the analysis of long range heteronuclear couplings as follows; the carbonyl carbon signal of serine (δ 173.6) was coupled to the serine α and β proton signals at δ 4.08, 3.83 and 3.50 and to the α protons of glycine at δ 4.05 and 3.59; the carbonyl carbon signal of tryptophan (δ 174.5) was coupled to the tryptophan α

Table 2. ^{1}H NMR assignments of the aselacins (in CD_3OD).

Proton	Aselacin A, shift (multiplicity)	Aselacin B, shift (multiplicity)	Aselacin C, shift (multiplicity)
Gly-α CH_2	4.05 (d, 1H, J=17.6 Hz), 3.59 (d, 1H, J=17.6 Hz)	4.05 (d, 1H, J=17.6 Hz), 3.59 (d, 1H, J=17.6 Hz)	4.05 (d, 1H, J=17.6 Hz), 3.59 (d, 1H, J=17.6 Hz)
Ser-α CH	4.08 (dd, 1H, J=4.9, 3.4 Hz)	4.08 (dd, 1H, J=5.0, 3.3 Hz)	4.08 (dd, 1H, J=4.9, 3.4 Hz)
Ser-β CH_2	3.83 (dd, 1H, J=11.4, 4.9 Hz), 3.50 (dd, 1H, J=11.4, 3.4 Hz)	3.82 (dd, 1H, J=11.5, 5.0 Hz), 3.49 (dd, 1H, J=11.5, 3.4 Hz)	3.82 (dd, 1H, J=11.4, 4.9 Hz), 3.50 (dd, 1H, J=11.4, 3.4 Hz)
Trp-α CH	4.76 (dd, 1H, J=7.1, 5.9 Hz)	4.75 (dd, 1H, J=7.1, 5.9 Hz)	4.75 (dd, 1H, J=7.1, 5.9 Hz)
Trp-β CH_2	3.27 (dd, 1H, J=14.7, 5.9 Hz), 3.18 (dd, 1H, J=14.7, 7.1 Hz)	3.26 (dd, 1H, J=14.7, 5.9 Hz), 3.16 (dd, 1H, J=14.7, 7.1 Hz)	3.26 (dd, 1H, J=14.7, 5.9 Hz), 3.18 (dd, 1H, J=14.7, 7.1 Hz)
Trp-2-H	7.12 (s, 1H)	7.11 (s, 1H)	7.12 (s, 1H)
Trp-4-H	7.48 (br d, 1H, J=8.0 Hz)	7.47 (br d, 1H, J=8.0 Hz)	7.48 (br d, 1H, J=8.0 Hz)
Trp-5-H	7.01 (br dd, 1H, J=8.0, 7.5 Hz)	7.01 (br dd, 1H, J=8.0, 7.5 Hz)	7.00 (br dd, 1H, J=8.0, 7.5 Hz)
Trp-6-H	7.09 (br dd, 1H, J=8.2, 7.5 Hz)	7.08 (br dd, 1H, J=8.2, 7.5 Hz)	7.08 (br dd, 1H, J=8.2, 7.5 Hz)
Trp-7-H	7.34 (br d, 1H, J=8.2 Hz)	7.34 (br d, 1H, J=8.2 Hz)	7.34 (br d, 1H, J=8.2 Hz)
β-Ala-α CH_2	2.89 (ddd, 1H, J=14.0, 11.4, 4.7 Hz), 2.41 (ddd, 1H, J=14.0, 4.7, 4.0 Hz)	2.88 (ddd, 1H, J=14.0, 11.4, 4.6 Hz), 2.42 (ddd, 1H, J=14.0, 4.7, 4.0 Hz)	2.88 (ddd, 1H, J=14.0, 11.4, 4.7 Hz), 2.42 (ddd, 1H, J=14.0, 4.7, 4.0 Hz)
β-Ala-β CH_2	3.71 (ddd, 1H, J=13.4, 11.4, 4.0 Hz), 3.13 (dt, 1H, J=13.4, 4.7 Hz)	3.71 (m, 1H), 3.13 (dt, 1H, J=13.4, 4.7 Hz)	3.72 (ddd, 1H, J=13.4, 11.4, 4.0 Hz), 3.12 (dt, 1H, J=13.4, 4.7 Hz)
Thr-α CH	4.63 (d, 1H, J=2.3 Hz)	4.62 (d, 1H, J=2.2 Hz)	4.62 (d, 1H, J=2.3 Hz)
Thr-β CH	5.59 (qd, 1H, J=6.4, 2.3 Hz)	5.59 (qd, 1H, J=6.4, 2.2 Hz)	5.58 (qd, 1H, J=6.4, 2.3 Hz)
Thr-γ CH_3	1.16 (d, 3H, J=6.4 Hz)	1.15 (d, 3H, J=6.4 Hz)	1.16 (d, 3H, J=6.4 Hz)
Gln-α CH	4.16 (dd, 1H, J=8.8, 6.2 Hz)	4.19 (dd, 1H, J=8.9, 6.2 Hz)	4.18 (dd, 1H, J=8.8, 6.2 Hz)
Gln-β CH_2	1.92 (m, 1H), 1.84 (m, 1H)	1.92 (m, 1H), 1.84 (m, 1H)	1.92 (m, 1H), 1.84 (m, 1H)
Gln-γ CH_2	2.22 (m, 1H), 2.14 (m, 1H)	2.21 (m, 1H), 2.14 (m, 1H)	2.24 (m, 1H), 2.18 (m, 1H)
Fatty acid CH_2-2	2.19 (m, 2H)	2.22 (m, 2H)	2.20 (m, 2H)
Fatty acid CH_2-3	1.51 (m, 2H)	1.55 (m, 2H)	1.54 (m, 2H)
Fatty acid CH_2-4	1.23 (envelope)	1.23 (envelope)	1.25 (envelope)
Fatty acid CH_2-5	1.23 (envelope)	1.23 (envelope)	1.25 (envelope)
Fatty acid CH_2-6	1.23 (envelope)	1.23 (envelope)	1.25 (envelope)
Fatty acid CH_2-7	1.32 (m, 1H), 1.22 (m, 1H)	1.47 (m, 2H)	11.56 (m, 2H)
Fatty acid CH_2-8	1.42 (m, 1H), 1.38 (m, 1H)	2.47 (t, 2H, J=7.2 Hz)	2.49 (t, 2H, J=7.2 Hz)
Fatty acid CH or CO-9	3.96 (q, 1H, J=6.8 Hz)	—	—
Fatty acid CH-10	5.48 (dd, 1H, J=15.2, 7.0 Hz)	6.06 (d, 1H, J=15.5 Hz)	6.06 (d, 1H, 15.5 Hz)
Fatty acid CH-11	6.11 (dd, 1H, J=15.2, 10.4 Hz)	7.18 (dd, 1H, J=15.5, 9.0 Hz)	7.18 (dd, 1H, J=15.5, 9.0 Hz)
Fatty acid CH-12	6.00 (dd, 1H, J=15.2, 10.4 Hz)	6.26 (dd, 1H, J=15.2, 9.0 Hz)	6.20 (dd, 1H, J=15.2, 9.0 Hz)
Fatty acid CH-13	5.65 (dt, 1H, J=15.2, 7.0 Hz)	6.24 (dt, 1H, J=15.2, 6.2 Hz)	6.24 (dt, 1H, J=15.2, 6.2 Hz)
Fatty acid CH_2-14	2.06 (m, 2H)	2.23 (m, 2H)	2.21 (m, 2H)
Fatty acid CH_2-15	1.37 (m, 2H)	1.53 (m, 2H)	1.35 (m, 2H)
Fatty acid CH_2-16	1.27 (m, 2H)	1.49 (m, 2H)	1.32 (m, 2H)
Fatty acid CH_2 or CH-17	1.28 (m, 2H)	3.72 (m, 1H)	1.34 (m, 2H)
Fatty acid CH_3-18	0.89 (t, 3H, J=7.0 Hz)	1.14 (d, 3H, J=7.0 Hz)	0.89 (t, 3H, J=7.0 Hz)

Table 3. ^{13}C NMR assignments of the aselacins (in CD_3OD).

Carbon	Aselacin A shifts	Aselacin B shifts	Aselacin C shifts	Carbon	Aselacin A shifts	Aselacin B shifts	Aselacin C shifts
Gly-CO	169.8	169.8	169.8	Gln-α CH	55.5	55.5	55.5
Gly-α CH_2	42.9	42.9	42.9	Gln-β CH_2	27.2	27.2	27.2
Ser-CO	173.6	173.6	173.6	Gln-γ CH_2	32.3	32.5	32.6
Ser-α CH	59.0	59.0	59.0	Gln-δ CO	177.0	177.0	177.0
Ser-β CH_2	62.0	62.1	62.1	Fatty acid CO	176.8	176.8	176.7
Trp-CO	174.5	175.4	175.4	Fatty acid CH_2-2	36.1	36.1	36.1
Trp-α CH	55.0	55.5	55.5	Fatty acid CH_2-3	26.6	26.6	26.6
Trp-β CH_2	29.2	29.2	29.2	Fatty acid CH_2-4	30.2	30.2	30.2
Trp-C-2	125.1	125.1	125.1	Fatty acid CH_2-5	30.2	30.2	30.2
Trp-C-3	109.8	109.9	109.9	Fatty acid CH_2-6	30.4	30.1	29.2
Trp-C-3a	128.9	128.9	128.9	Fatty acid CH_2-7	26.5	25.5	25.5
Trp-C-4	119.2	119.3	119.3	Fatty acid CH_2-8	38.4	41.0	41.0
Trp-C-5	119.9	119.9	119.9	Fatty acid CH-9	73.4	203.8	203.9
Trp-C-6	122.7	122.7	122.7	or CO-9			
Trp-C-7	112.5	112.5	112.5	Fatty acid CH-10	135.0	128.9	128.9
Trp-C-7a	138.0	138.2	138.0	Fatty acid CH-11	131.7	145.7	145.2
β-Ala-CO	174.5	174.5	174.5	Fatty acid CH-12	131.2	147.1	147.3
β-Ala-α CH_2	35.4	35.4	35.4	Fatty acid CH-13	135.6	130.4	130.2
β-Ala-β CH_2	38.3	38.3	38.2	Fatty acid CH_2-14	33.6	34.1	34.1
Thr-CO	170.8	170.8	170.8	Fatty acid CH_2-15	30.6	26.1	30.1
Thr-α CH	57.3	57.2	57.2	Fatty acid CH_2-16	32.5	39.6	32.4
Thr-β CH	71.5	71.6	71.5	Fatty acid CH_2-17	23.5	68.3	23.5
Thr-γ CH_3	16.7	16.7	16.7	or CH-17			
Gln-CO	176.0	176.0	176.0	Fatty acid CH_3-18	14.4	23.5	14.6

and β proton signals at δ 4.76, 3.27 and 3.18 and to the α proton of serine at δ 4.08; the carbonyl carbon signal of β-alanine (δ 174.5) was coupled to the β-alanine α and β proton signals at δ 2.89, 2.41, 3.71 and 3.13 and to the α proton of tryptophan at δ 4.76; the carbonyl carbon signal of threonine (δ 170.8) was coupled to the threonine α and β proton signals at δ 4.63 and 5.59 (the chemical shift of which clearly indicates an ester linkage) and to the β protons of β-alanine at δ 3.71 and 3.13; the carbonyl carbon signal of glycine (δ 169.8) was coupled to the glycine α-proton signals at δ 4.05 and 3.59 and to the β proton of threonine at δ 5.59. Threonine must therefore be attached through its β carbon to glycine thus forming a peptolide ring.

Table 4. ^{15}N NMR spectrum of aselacin A (DMSO-d_6).

Nitrogen	^{15}N chemical shift[a]	^{1}H chemical shift
Gly-α-NHCO	103.9	7.80
Ser-α-NHCO	120.7	8.99
Trp-α-NHCO	127.2	7.60
Trp-NH-1	131.2	10.88
β-Ala-β-NHCO	111.1	7.35
Thr-α-NHCO	110.5	8.38
Gln-α-NHCO	126.1	8.41
Gln-δ-NH_2CO	108.0	7.30, 6.80

[a] Nitrogen shifts referenced to an external standard of 40% $HCONH_2$ in DMSO-d_6 assigned a value of δ 112.4 ppm.

Further long range couplings were observed between the carbonyl carbon signal of glutamine (δ 176.0) and its α and β proton signals at δ 4.16, 1.92 and 1.84 and to the β proton signal of threonine thus indicating that glutamine was exocyclic to the pentapeptolide previously described. This residue was determined to be a glutamine (rather than glutamic acid) with substitution on the α amino group and unsubstituted at the δ-carboxyamide by acquiring in DMSO-d_6; a ^{15}N NMR spectrum and DEPT, a ^{1}H-^{15}N HMQC and a ^{1}H COSY spectrum which allowed the assignment of all proton and nitrogen signals (see Table 4) of **1** in this aprotic solvent. The ^{15}N DEPT contained eight nitrogen signals for the six

amino acids, one of which was an NH_2. The D-Gln-δ-$CONH_2$ protons with signals at δ 7.30 and 6.80 were attached to a nitrogen at δ 108.0 and a single D-Gln-α-NH proton with a signal at δ 8.41 was attached to a nitrogen at δ 126.1.

A fast atom bombardment positive ion mass spectrum of aselacin A gave a molecular ion of m/z 909 (M+H) for a molecular formula encompassing the six previously discussed amino acids plus an additional $C_{18}H_{31}O$. Remaining carbon signals in the ^{13}C NMR spectrum (see Table 3) included those for one amide, two disubstituted olefins, one oxygen substituted methine, one methyl and 11 methylenes. A COSY experiment allowed assignment to these signals of an 18-carbon fatty acid with a diene at C-10~C-13 and an hydroxyl at C-9. Electrospray MS-MS indicated that this fatty acid is attached to the exocyclic glutamine as evidenced by a major fragmentation peak at m/z 503 for the cyclopentapeptolide with concomitant loss of glutamine and the fatty acid. This attachment is further supported by a ROESY[4] experiment in DMSO-d_6 in which a nuclear Overhauser effect (NOE) is observed between the α amine proton (δ 8.41) of glutamine and the C-2 methylene protons (δ 2.19) of the fatty acid.

Structure Determination of Aselacin B

A fast atom bombardment positive ion mass spectrum of aselacin B gave a molecular ion of m/z 923 (M+H) which was 14 mass units higher than that for aselacin A. Homonuclear and heteronuclear 1-D and 2-D experiments indicated that the amino acid portion of aselacin B was identical to that for aselacin A (see Tables 2 and 3). Thus the aselacins differed from one another solely in the fatty acid side chain attached to each of their glutamine residues.

The ^{13}C NMR spectrum (see Table 3) of aselacin B contained a signal at δ 203.8 indicating the presence of a ketone functionality. An HMBC experiment showed long range coupling between this ketone and a diene olefinic system with proton signals at δ 6.06 (C-10), 7.18 (C-11), 6.26 (C-12) and 6.24 (C-13) for an $\alpha\beta$, $\gamma\delta$-unsaturated ketone.

Aselacin B contained an hydroxymethine with a carbon signal at δ 68.3 (C-17) and a proton at δ 3.72 which were not present in aselacin A. The δ 3.72 (C-17) proton signal was coupled to a methyl doublet signal at δ 1.14 indicating that oxidation has occured at the C-17 position of the fatty acid chain in aselacin B relative to that of aselacin A. A COSY experiment showed further coupling from the δ 3.72 proton signal to methylene proton signal at δ 1.49 (C-16) which was also coupled to a methylene proton signal at δ 1.53 (C-15) which was also coupled to a methylene proton signal at δ 2.23 (C-14) which coupled into the δ proton of the $\alpha\beta$, $\gamma\delta$-unsaturated ketone group thus placing that functionality at carbons 9~13 of the octadecanoic acid side chain.

Structure Determination of Aselacin C

A fast atom bombardment positive ion mass spectrum of aselacin C gave a molecular ion of m/z 907 (M+H) which was 2 mass units lower than that for aselacin A. Homonuclear and heteronuclear 1-D and 2-D experiments indicated that the amino acid portion of aselacin C was identical to that for aselacin A (see Tables 2 and 3). Aselacins A and C therefore differed in their fatty acid side chains. The ^{13}C NMR spectrum (see Table 3) of aselacin C contained a signal at δ 203.9 indicating the presence of a ketone functionality. An HMBC experiment showed long range coupling between this ketone and a diene olefinic system with proton signals at δ 6.06 (C-10), 7.18 (C-11), 6.20 (C-12) and 6.24 (C-13) for an $\alpha\beta$, $\gamma\delta$-unsaturated ketone comparable to that present in the structure of aselacin B. The remainder of the fatty

acid side chain of aselacin C could be analyzed by a COSY experiment, and is identical to that of aselacin A.

Experimental

General Procedures

Melting points were determined on a Hoover Unimelt and are reported uncorrected. Optical rotations were measured on a Perkin-Elmer Model 241 polarimeter in a 10 cm cell. Rf values reported were determined on Merck Kieselgel 60 F_{254} TLC plates and were visualized using ceric sulfate spray reagent[5]. Fast atom bombardment mass spectra were measured on Kratos MS-50 mass spectrometer and electrospray mass spectra on a Finnigan TSQ 700 triple quadrupole mass spectrometer. Ultraviolet spectra were recorded on a Perkin-Elmer Lambda 3B UV-visible spectrophotometer and infrared spectra on a Nicolet model 60SX FT-IR. Nuclear magnetic resonance spectra were acquired on a General Electric GN500 spectrometer. Chiral HPLC analyses were performed on a Perkin-Elmer HPLC with an LCD SM5000 diode array detector set at 200 nm and a Diacel CrownPak (Cr^+) column.

Aselacin A

Aselacin A is a white powder, mp 177~179°C, $C_{46}H_{68}N_8O_{11}$, $[\alpha]_D$ −110° (*c* 0.83, MeOH), with an Rf 0.90 in methanol, Rf 0.25 in ethyl acetate-methanol (4:1), Rf 0.0 in methylene chloride. An ultraviolet spectrum acquired in methanol contains bands at: λ_{max} 224 (ε 10,000), 274 (1,800), 281 (1,900), 291 (1,600). These bands are unchanged upon addition of acid or base. An infrared spectrum acquired as a KBr pellet contains bands at: 3290, 3058, 2928, 2855, 1737, 1664, 1638, 1544, 1458, 1440, 1379, 1341, 1273, 1236, 1062, 989, 942 and 742 cm^{-1}.

Aselacin B

Aselacin B is a clear oil, $C_{46}H_{66}N_8O_{12}$, $[\alpha]_D$ −14° (*c* 0.1, MeOH), with an Rf 0.84 in methanol, Rf 0.15 in ethyl acetate-methanol (4:1), Rf 0.0 in methylene chloride. An ultraviolet spectrum acquired in methanol contains bands at: λ_{max} 223 (ε 10,500) and 274 (8,600). These bands are unchanged upon addition of acid or base. An infrared spectrum acquired as a microscope sample contains bands at: 3300, 3116, 3057, 2930, 1737, 1663, 1593, 1546, 1455, 1441, 1237, 1062 and 1004 cm^{-1}.

Aselacin C

Aselacin C is a white powder, mp 161~167°C, $C_{46}H_{68}N_8O_{11}$, $[\alpha]_D$ −20° (*c* 0.3, MeOH), with an Rf 0.90 in methanol, Rf 0.35 in ethyl acetate-methanol (4:1), Rf 0.0 in methylene chloride. An ultraviolet spectrum acquired in methanol contains bands at: λ_{max} 219 (ε 9,400) and 275 (6,600). These bands are unchanged upon addition of acid or base. An infrared spectrum acquired as a microscope sample contains bands at: 3300, 3056, 2929, 2856, 1740, 1666, 1636, 1545, 1459, 1379, 1356, 1272, 1239, 1084, 1005 and 943 cm^{-1}.

Amino Acid Analysis of Aselacin A

Aselacin A was heated at 155°C in 6 N HCl with 5% thioglycolic acid for 2 hours. Hydrolysate was analyzed on a Beckman 6300 amino acid analyzer with a 120 mm sodium cation exchange column by standard elution methods. Amino acids present in the hydrolysate were determined by comparison of retention times to those of standard samples.

Chiral HPLC of Aselacin A

Hydrolysis for chiral HPLC was carried out in a similar manner to that for amino acid analysis. Two sets of conditions were required for analysis of all amino acids present in aselacin A. In condition A (as listed in Table 1), the crude hydrolysate was passed over a Varian SCX (benzenesulfonic acid) ion exchange column washed with water and eluted with 0.01 M HCl. Lyophilized eluate was chromatographed on a CrownPak (Cr^+) column eluted with aqueous 0.02 M $HClO_4$, pH 1.6 with the column temperature maintained at 0°C and a flow rate of 0.5 ml/minute. In condition B, crude hydrolysate was applied directly to a CrownPak (Cr^+) coulmn eluted with aqueous 0.005 M $HClO_4$, pH 2.2 with the column temperature maintained at 25°C and a flow rate of 1 ml/minute.

References

1) JACKSON, M.; N. S. BURRES, J. P. KARWOWSKI, L. A. ALDER, P. E. HUMPHREY, W. L. KOHL & J. B. MCALPINE: Aselacins, novel compounds that inhibit binding of endothelin to its receptor. I. The producing organism, fermentation and biological activity J. Antibiotics 47: 523~527, 1994
2) MULLER, L.: Sensitivity enhanced detection of weak nuclei using heteronuclear multiple quantum coherence. J. Am. Chem. Soc. 101: 4481~4484, 1979
3) BAX, A. & M. F. SUMMERS: ^{1}H and ^{13}C assignments from sensitivity-enhanced detection of heteronuclear multiple-bond connectivity by 2D multiple quantum NMR. J. Am. Chem. Soc. 108: 2093~2094, 1986
4) BOTHNER-BY, A. A.; R. L. STEPHENS, J. M. LEE, C. D. WARREN & R. W. JEANLOZ: Structure determination of a tetrasaccharide: Transient nuclear Overhauser effects in the rotating frame. J. Am. Chem. Soc. 106: 811~813, 1984
5) KREBS, K. G.; D. HEUSSER & H. WIMMER: Spray reagents in thin layer chromatography. *In* Thin-layer Chromatography. A Laboratory Handbook. *Ed.*, E. STAHL, p. 861, Springer-Verlag, New York, 1969