NOVEL CHOLECYSTOKININ ANTAGONISTS FROM *ASPERGILLUS ALLIACEUS*

II. STRUCTURE DETERMINATION OF ASPERLICINS B, C, D, AND E

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Asperlicins B (**1**, $C_{31}H_{29}N_5O_5$), C (**2**, $C_{25}H_{18}N_4O_2$), D (**3**, $C_{25}H_{18}N_4O_2$), and E (**4**, $C_{25}H_{18}N_4O_3$) are novel cholecystokinin antagonists produced by *Aspergillus alliaceus*. The structures of these compounds have been determined by ^1H NMR and MS analysis.

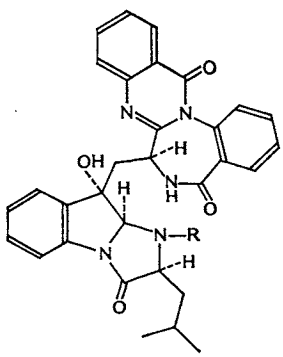
The fermentation, isolation, and characterization of four novel analogs (**1**~**4**) of asperlicin[1] (**5**), a potent non-peptidal cholecystokinin antagonist produced by *Aspergillus alliaceus*, have been reported in the preceding paper[2]. In this paper, the structure determination of these compounds is described.

Results and Discussion

The MS fragmentation scheme, ^{13}C NMR carbon assignments, and ^1H NMR spectrum of **5** have been previously reported[1]. The structures of the present compounds were determined based upon interpretation of their ^1H NMR and MS and comparison with **5**. Compound **1** most closely resembles **5** in that the leucyl moiety amino group has been oxidized to the corresponding hydroxylamine. Compounds **2**~**4** are deleucyl analogs of **5**.

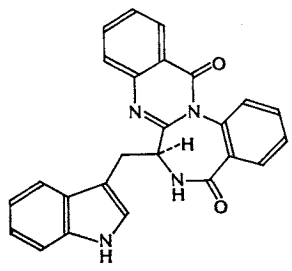
Compound **1** has the molecular formula $C_{31}H_{29}N_5O_5$ by high resolution mass spectra (HR-MS). This formula differs from that of asperlicin (**5**, $C_{31}H_{29}N_5O_4$) in that it contains one equivalent more of oxygen. The ^1H NMR spectrum and MS fragmentation of **1** are quite similar to those of **5** and consistent with the conversion of the leucyl amino group in **5** to the *N*-hydroxy derivative in **1**. This assignment was confirmed by conversion of a sample of **5** to its *N*-hydroxy derivative by treatment with *m*-chloroperbenzoic acid. The spectral data for the naturally occurring asperlicin

Fig. 1. Structures of asperlicin B (**1**) and asperlicin (**5**).



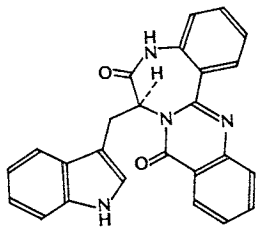
1 R=OH
5 R=H

Fig. 2. Structure of asperlicin C (**2**).



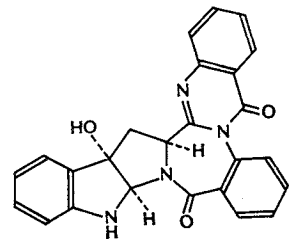
2

Fig. 3. Structure of asperlicin D (3).



3

Fig. 4. Structure of asperlicin E (4).



4

B (1) and the *N*-hydroxy derivative synthesized from 5 were identical.

The molecular formula $C_{25}H_{18}N_4O_2$ was determined for asperlicin C (2) by HR-MS and this formula is $C_6H_{11}NO_2$ (or $C_6H_{11}NO$ (leucyl) plus O) less than that of 5. The MS of 2 exhibits intense ions at *m/z* 277 ($C_{16}H_{11}N_3O_2$) and 130 (C_9H_8N) which define the condensed bis-anthranilate and tryptophan-derived indole moieties, respectively. These ions are analogous to those characteristic of 5. The compound forms a di-trimethylsilyl derivative and the neat *m/z* 277 and 130 species each have one silylatable function associated with them. The lack of the leucyl side chain is evident from the ^1H NMR spectrum in $CDCl_3$. In addition, the twelve aromatic protons and $CH_2CHXNHCO$ moiety are present as in 5. However, the tertiary hydroxy group seen in 5 appears to be missing and instead a broad singlet appears at 7.95 ppm which is coupled to an exchangeable proton at 8.11 ppm (NH). These data are consistent with the deleucyl-deoxyasperlicin structure assigned as asperlicin C (2).

The molecular formula $C_{25}H_{18}N_4O_2$ was determined for asperlicin D (3) and this formula is identical to that of 2. Thus, 3 also appears to be a deleucyl-deoxyasperlicin. The characteristic MS fragment ions at *m/z* 277 and 130 are present however the general fragmentation of 3 is quite different from that of 2. Most critically, unusual ions at *m/z* 184 and 156 are apparent. The ^1H NMR spectrum of 3, although most similar to that of 2, also displays some characteristic differences. As in 2, it lacks the leucyl side chain but retains the twelve aromatic protons. The CH_2CHX pattern (AMX) is still intact, however the methine proton at 6.92 ppm is considerably downfield of its position in 2 (at 4.58 ppm) and it is no longer coupled to an NH proton. Instead, an NH proton occurs as a broad singlet at 7.94 ppm. The ion series [406 (M^+)]→[184 ($C_{11}H_8N_2O$)]→[156 ($C_{10}H_8N_2$)]→[130 (C_9H_8N)] was disclosed by linked-scan analysis. The most structurally significant interpretation of this prominent fragmentation is simply that a carbonyl group is now associated with the tryptophan residue fragmentation. This is not seen in either 5 or 2 wherein such an ion could only occur *via* cleavage of the anthranilate carboxyl-aryl bond, which is not generally expected to be a facile fragmentation. The *m/z* 277 fragment ion is observed in 3 and is very strong, which suggests that the condensed bis-anthranilate moiety is substantially intact.

Thus, since the tryptophan residue's C-1 carbon appears to be a carbonyl group it is reasonable to assign alternate structure 3 to asperlicin D. This structure readily accounts for the downfield shift of the CH_2CHX methine proton in the ^1H NMR spectrum since the proton now sits in the deshielding cone of the benzene ring fused to the 7-membered ring.

Biogenetically, compounds 2 and 3 can both result from a postulated tryptophan-anthranilate diketopiperazine precursor. Condensation of a second anthranilate residue at the diketopiperazine

tryptophan amino group/anthranilate carboxyl position with elimination of two equivalents of water affords **3**, whereas condensation at the diketopiperazine anthranilate amino/tryptophan carboxyl position yields **2**.

Asperlicin E (**4**) has the empirical formula $C_{25}H_{18}N_4O_3$ by HR-MS. This formula is $C_6H_{11}NO$ less than that of **5** suggesting that **4** is a deleucylasperlicin. However, **4** does not exhibit the characteristic *m/z* 277 ion in its MS which is observed in the other asperlicins (**1**~**3** and **5**). Instead, an intense *m/z* 275 ion is observed which has the empirical formula $C_{17}H_{11}N_2O_2$ (*cf.* *m/z* 277 $C_{16}H_{11}N_3O_2$). The *m/z* 275 ion does not contain a silylatable functionality as does the *m/z* 277 ion.

The lack of the leucyl side chain in **4** is confirmed by ^{1}H NMR in $CDCl_3$. The standard twelve aromatic protons are observed as in the other components, as well as two exchangeable protons at 5.18 ppm (NH) and 2.59 ppm (tertiary hydroxyl) as seen in **5**. Additionally, the tryptophan residue's CH_2CHX methine proton is not coupled to an adjacent amide proton and the NH proton is coupled to a doublet proton at 5.57 ppm (J=1.5 Hz). These data suggest that the tryptophan residue's α-nitrogen has condensed with the indole moiety as in structure **4**. The *m/z* 275 ion can be explained as resulting from concerted loss from the molecular ion of the indole ring system (C_8H_7NO) together with the condensed α-nitrogen (N) of the tryptophan moiety.

The structures of asperlicins C and E[3], and D (M. G. Bock *et al.*; unpublished results) have been confirmed subsequently by total synthesis[3].

Experimental

^{1}H NMR spectra were recorded on a Varian SC-300 instrument. MS were recorded on a Finnigan-MAT MAT212 instrument at 90 eV in the electron impact (EI) mode. Exact mass measurements using perfluorokerosene as the internal standard and either the peak matching method or scanning high resolution and linked-scan metastable MS analyses utilizing both B^2/E and B/E methods were recorded on the same instrument. Trimethylsilyl derivatives were prepared with a 1:1 mixture of bistrimethylsilyltrifluoroacetamide and pyridine at 50°C for 1 hour.

Asperlicin B (**1**)

UV λ_{max}^{MeOH} nm (ε) 228 (sh, 47,000), 256 (sh, 16,200), 265 (sh, 13,200), 280 (sh, 9,800), 310 (4,000); ^{1}H NMR ($CDCl_3$ - DMSO-d_6, 10:1) δ 0.94 (3H, d, J=6.5 Hz), 1.02 (3H, d, J=6.5 Hz), 1.61 (1H, dt, J=6.5, 6.5 and 14 Hz), 1.78 (1H, ddd, J=5.5, 8 and 14 Hz), 2.07 (1H, m), 2.73 (2H, d, J=6.5 Hz), 4.05 (1H, dd, J=5.5 and 6.5 Hz), 4.55 (1H, t, J=6.5 Hz), 5.24 (1H, s), 7.01 (1H, br t, J=7.5 Hz), 7.21 (1H, br d, J=7 Hz), 7.24 (1H, br t, J=8 Hz), 7.48 (1H, d, J=8 Hz), 7.53 (1H, t, J=8 Hz), 7.56~7.70 (5H, m), 7.80 (1H, br t, J=7.5 Hz), 8.04 (1H, dd, J=1.5 and 7.5 Hz), 8.30 (1H, br d, J=8 Hz); MS *m/z* (relative intensity %, formula) 551.2145 (12, M, $C_{31}H_{29}N_5O_5$), 534 (28), 515 (73), 472 (93), 431 (22), 429 (20), 404 (53), 299 (28), 277 (27), 249 (100), 197 (51), 146 (61), 130 (59), 102 (34), 93 (74).

Conversion of Asperlicin to Asperlicin B

1.0 mmol asperlicin was treated with 2.4 mmol *m*-chloroperbenzoic acid in 80 ml CH_2Cl_2 plus 9 ml glacial acetic acid for 2.5 hours at room temperature. Approximately 70% of the starting material had disappeared by that time. The reaction mixture was diluted out to 160 ml with CH_2Cl_2 and washed twice each with dilute $NaHCO_3$ and dilute Na_2CO_3. After evaporation of the solvent, the residue was fractionated by two successive silica gel column chromatographic steps (CH_2Cl_2 - EtOAc (1:4), then CH_2Cl_2 - MeOH (97:3)) to afford pure asperlicin B in 40% yield.

Synthetic **1** afforded the same critical data as the natural product.

Asperlicin C (**2**)

UV λ_{max}^{MeOH} nm (ε) 222 (56,300), 268 (15,100), 278 (14,000), 289 (sh, 8,480), 310 (4,650), 321 (sh, 3,590); ^{1}H NMR ($CDCl_3$) δ 3.54 (1H, dd, J=8 and 15 Hz), 3.86 (1H, dd, J=6 and 15 Hz), 4.58 (1H,

dt, J=6, 6 and 8 Hz), 6.10 (1H, d, J=6 Hz), 7.15 (1H, br s), 8.11 (1H, br s, NH); ^{1}H NMR ($CDCl_3$-DMSO-d_6, 10:1) δ 3.47 (1H, dd, J=8 and 15 Hz), 3.81 (1H, dd, J=6 and 15 Hz), 4.42 (1H, dt, J=8, 6 and 6 Hz), 6.99 (1H, dd, J=7.5 Hz), 7.08 (1H, br t, J=7.5 Hz), 7.26 (1H, br s), 7.36 (1H, d, J=8 Hz), 7.58 (5H, m), 7.84 (3H, m), 8.28 (1H, d, J=8 Hz), 8.54 (1H, d, J=6 Hz, NH), 10.35 (1H, br s, NH); MS m/z (relative intensity %, formula) 406.1438 (20, M, $C_{25}H_{18}N_4O_2$), 286.0970 (9, $C_{18}H_{12}N_3O$), 277.0845 (60, $C_{16}H_{11}N_3O_2$), 260.0939 (64, $C_{17}H_{12}N_2O$), 249.0676 (33, $C_{15}H_9N_2O_2$), 234 (15), 158.0852 (8, $C_{10}H_{10}N_2$), 130.0671 (100, C_9H_8N), 103 (15), 102 (15).

Asperlicin D (3)

UV λ_{max}^{MeOH} nm (ε) 222 (61,060), 283 (sh, 13,790), 290 (14,600), 310 (5,920); ^{1}H NMR ($CDCl_3$) δ 3.02 (1H, dd, J=8.5 and 15 Hz), 3.18 (1H, dd, J=10 and 15 Hz), 6.87 (1H, d, J=8 Hz), 6.89 (1H, br s), 6.92 (1H, br t, J=9 Hz), 6.98 (1H, t, J=7 Hz), 7.04 (1H, dt, J=1 and 7.5 Hz), 7.25 (1H, br d, J=8 Hz), 7.35 (1H, br d, J=8 Hz), 7.42 (1H, t, J=7.5 Hz), 7.45 (1H, dt, J=2 and 8 Hz), 7.56 (1H, dt, J=1.5 and 8 Hz), 7.76 (2H, m), 7.94 (1H, br s), 8.25 (1H, dd, J=1.5 and 8 Hz), 8.30 (1H, br d, J=8 Hz), 9.30 (1H, br s); MS m/z (relative intensity %, formula) 406.1407 (6, M, $C_{25}H_{18}N_4O_2$), 388.1318 (2, $C_{25}H_{16}N_4O$), 362.1271 (2, $C_{24}H_{16}N_3O$), 277.0833 (2, $C_{16}H_{11}N_3O_2$), 237.0899 (34, $C_{14}H_{11}N_3O$), 223.0883 (32, $C_{14}H_{11}N_2O$), 184.0655 (40, $C_{11}H_8N_2O$), 170.0601 (44, $C_{11}H_8NO$), 156 (2), 130.0671 (100, C_9H_8N); MS (linked-scan) m/z parent→daughter, (s) 406→388, 377, 362, 288, 277, 223, 184; 388→360, 344, 328; 288→260→170→142; 184→156, 130; 156→130→103.

Asperlicin E (4)

UV λ_{max}^{MeOH} nm (ε) 213 (sh, 37,150), 227 (38,450), 268 (8,680), 277 (sh, 7,830), 310 (sh, 4,850), 324 (3,200); ^{1}H NMR ($CDCl_3$) δ 2.59 (1H, s), 2.78 (1H, dd, J=9.5 and 14 Hz), 3.89 (1H, dd, J=1.5 and 14 Hz), 4.82 (1H, dd, J=1.5 and 9.5 Hz), 5.18 (1H, br s, NH), 5.57 (1H, d, J=1.5 Hz), 6.32 (1H, d, J=8 Hz), 6.82 (1H, br t, J=7.5 Hz), 6.91 (1H, dt, J=1.5 and 8 Hz), 7.29 (1H, br d, J=7.5 Hz), 7.40 (1H, br t, J=7.5 Hz), 7.52 (1H, br t, J=7 Hz), 7.61 (3H, m), 7.63 (1H, dt, J=1.5 and 7.5 Hz), 8.02 (1H, dd, J=2 and 7.5 Hz), 8.15 (1H, dd, J=1.5 and 8 Hz); MS m/z (relative intensity %, formula) 422.1376 (56, M, $C_{25}H_{18}N_4O_3$), 404.1224 (3, $C_{25}H_{16}N_4O_2$), 285.0894 (24, $C_{18}H_{11}N_3O$), 275.0823 (100, $C_{17}H_{11}N_2O_2$), 247.0883 (54, $C_{16}H_{11}N_2O$), 234.0797 (8, $C_{15}H_{10}N_2O$), 203 (6), 146.0590/146.0221 (24, $C_9H_8NO/C_8H_4NO_2$), 130.0654/130.0298 (28, C_9H_8N/C_8H_4NO), 120.0432 (9, C_7H_6NO), 102.0376 (16, C_7H_4N).

References

1) Liesch, J. M.; O. D. Hensens, J. P. Springer, R. S. L. Chang & V. J. Lotti: Asperlicin, a novel nonpeptidal cholecystokinin antagonist from *Aspergillus alliaceus*. Structure elucidation. J. Antibiotics 38: 1638~1641, 1985
2) Goetz, M. A.; R. L. Monaghan, R. S. L. Chang, J. Ondeyka, T. B. Chen & V. J. Lotti: Novel cholecystokinin antagonists from *Aspergillus alliaceus*. I. Fermentation, isolation, and biological properties. J. Antibiotics 41: 875~877, 1988
3) Bock, M. G.; R. M. DiPardo, S. M. Pitzenberger, C. F. Homnick, J. P. Springer & R. M. Freidinger: Total synthesis of nonpeptidal cholecystokinin antagonists from *Aspergillus alliaceus*. J. Org. Chem. 52: 1644~1646, 1987