ASPERLICIN, A NOVEL NON-PEPTIDAL CHOLECYSTOKININ ANTAGONIST FROM *ASPERGILLUS ALLIACEUS*

STRUCTURE ELUCIDATION

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Asperlicin (**1**, $C_{31}H_{29}N_5O_4$) is a novel cholecystokinin antagonist produced by *Aspergillus alliaceus*. The structure of asperlicin has been determined by NMR and mass spectral analysis, and X-ray crystallography.

In the preceding paper,[1] the fermentation, isolation and characterization of the cholecystokinin antagonist asperlicin[2] has been reported. In the present paper, the structure determination of asperlicin is described.

Structure of Asperlicin

Asperlicin (**1**) exhibits a UV absorption maximum in methanol at 310.5 nm (ε 4,075) with shoulders at 322 nm (3,135), 278 nm (10,350), 266 nm (14,420), 258 nm (17,760), and 230 nm (48,490). The compound affords a useful electron impact mass spectrum with a molecular ion observed at *m/z* 535 corresponding to the molecular formula $C_{31}H_{29}N_5O_4$ by high resolution exact mass measurement (*cf.* Table 1 for the results of scanning HR-MS measurements). The molecular weight was corroborated by fast atom bombardment (FAB) analysis which disclosed $(M+H)^+$ at *m/z* 536. Treatment of **1** with bistrimethylsilyltrifluoroacetamide (BSTFA) in pyridine at room temperature for 10 minutes afforded a di-trimethylsilyl derivative (M^+ *m/z* 679; perdeutero-BSTFA analog, M^+ *m/z* 697).

Linked-scan metastable MS analyses utilizing both the B^2/E and B/E methods[3,4] coupled with the high resolution MS assignments (Table 1) allowed determination of the fragmentation pathways shown in Fig. 1. Leucine and anthranilic acid in the approximate ratio of 1: 2 were identified in the acid hydrolysate of **1** as their trimethylsilyl derivatives by GC-MS.

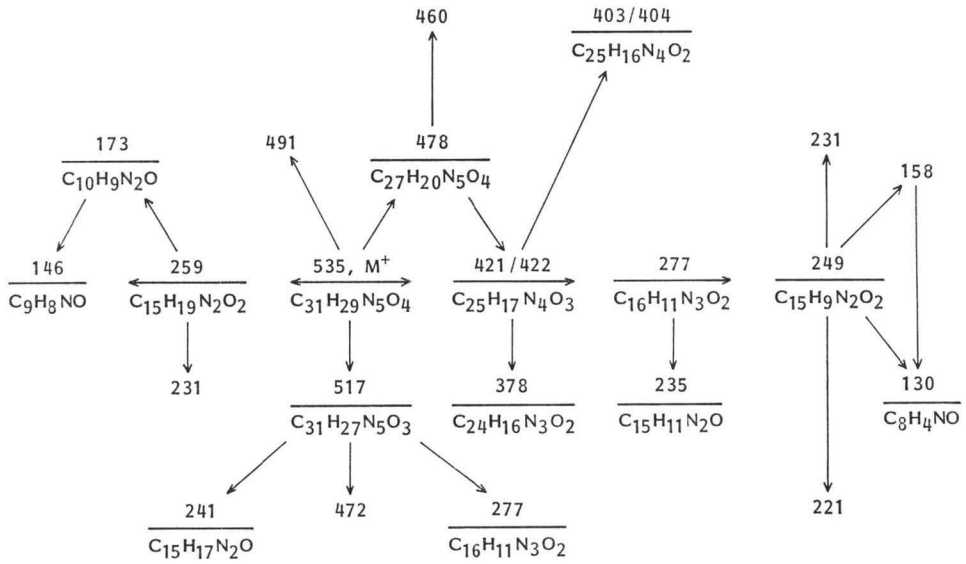
^1H NMR spectra in a variety of solvents indicated 29 protons, three of which are exchangeable. The relationship between the twelve aromatic protons was deduced on the basis of extensive decoupling studies in various solvent mixtures and was shown to consist of three sets of four contiguous proton resonances, consistent with two anthranilate and one modified tryptophan structural subunits. A leucine moiety as well as a $-CH_2CHNH-$ residue were also indicated. ^{13}C NMR spectra corroborated the ^1H NMR findings and further indicated the presence of a tertiary hydroxyl group. These findings are consistent with a modified tryptophan residue, (a), and two anthranilate residues, (b), which together account for all 31 carbons and 29 protons in the molecular formula for **1**.

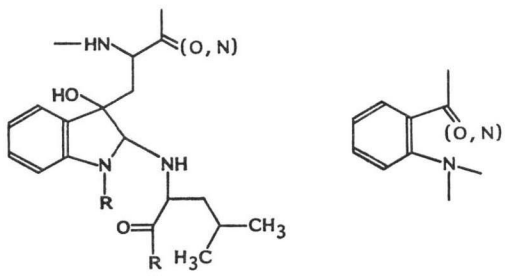
Structure **1** is assigned for asperlicin based upon the structural subunits (a) and (b), which were determined by examination of ^1H and ^{13}C NMR spectra, and the MS fragmentation pathways exhibited

Table 1. Empirical formula assignments for the significant ions in the high resolution mass spectrum of **1**.

Found (m/z)	Formula	Δ (mmu) (Found−Calcd)	Found (m/z)	Formula	Δ (mmu) (Found−Calcd)
535.2205	$C_{31}H_{29}N_5O_4$	−1.5	249.0671	$C_{15}H_9N_2O_2$	0.7
517.2101	$C_{31}H_{27}N_5O_3$	−1.3	241.1355	$C_{15}H_{17}N_2O$	1.4
478.1504	$C_{27}H_{20}N_5O_4$	−1.1	235.0846	$C_{15}H_{11}N_2O$	−2.5
422.1397	$C_{25}H_{18}N_4O_3$	1.8	213.1419	$C_{14}H_{17}N_2$	2.7
404.1248	$C_{25}H_{16}N_4O_2$	−2.5	173.0729	$C_{10}H_9N_2O$	1.4
394.1418	$C_{24}H_{18}N_4O_2$	−1.2	146.0606	C_9H_8NO	0
378.1298	$C_{24}H_{16}N_3O_2$	5.5	132.0463	C_8H_6NO	1.4
360.1178	$C_{24}H_{14}N_3O$	4.1	130.0299	C_8H_4NO	0.6
292.1117	$C_{17}H_{14}N_3O_2$	3.1	120.0451	C_7H_6NO	0.2
290.0918	$C_{17}H_{12}N_3O_2$	−1.2	104.0526	C_7H_6N	2.6
277.0869	$C_{16}H_{11}N_3O_2$	1.8	102.0385	C_7H_4N	4.1
275.0836	$C_{17}H_{11}N_2O_2$	1.5	90.0374	C_6H_4N	3.0
259.1486	$C_{15}H_{19}N_2O_2$	3.9	86.0972	$C_5H_{12}N$	0.2

Fig. 1. Mass spectral fragmentation pathways of **1** (determined by U_a/B and U_a/B^2 linked-scan analyses).





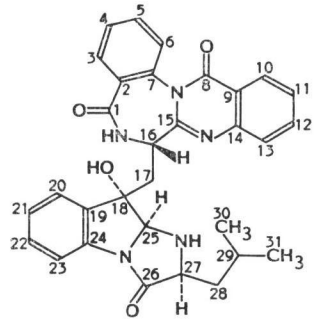
a b

in the electron impact mass spectrum of **1** as disclosed by the linked-scan metastable studies (Fig. 1). The structure of asperlicin (**1**) bears some structural similarity to the previously reported fungal tremorgin tryptoquivaline.[5] The ^{13}C NMR spectrum of **1** is assigned in Fig. 2. The aromatic methines were assigned based upon a 2D-chemical shift correlation map.

X-Ray Crystallographic Analysis

The relative and absolute stereochemistry of **1** was assigned by single crystal X-ray diffraction experiments. Suitable crystals of the hydrochloride of **1** formed from acetonitrile with space group symmetry of $P4_12_12$ and cell constants of $a=20.644$ (3)Å

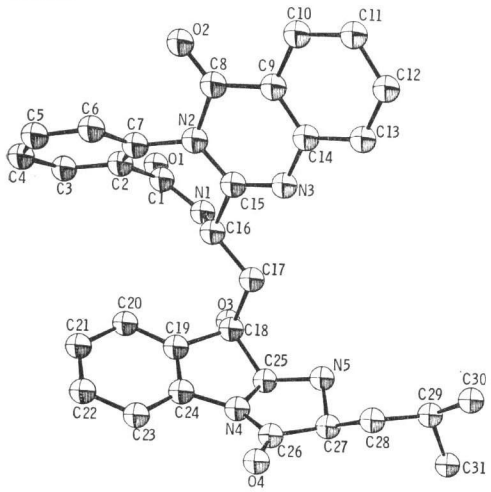
Fig. 2. ^{13}C NMR assignments of asperlicin (**1**) in $CDCl_3$.



Chemical shift (ppm)	Multiplicity	Assignment (carbon No.)	Chemical shift (ppm)	Multiplicity	Assignment (carbon No.)
21.5	q	C31	128.2*	d	C6
23.6	q	C30	129.3	d	C11
25.2	d	C29	129.9	d	C10
34.2	t	C17	130.0	d	C22
42.8	t	C28	130.4	s	C9
50.9	d	C16	131.9	d	C12
62.0	d	C27	133.8	s	C19
81.4	s	C18	135.3	d	C5
87.0	d	C25	136.7	s	C14
116.3	d	C23	139.2	s	C24
121.6	s	C2	146.3	s	C7
124.2	d	C20	155.5	s	C15
125.8	d	C21	162.2	s	C26
127.2*	d	C13	168.3	s	C8
127.7	d	C4	171.2	s	C1
128.0	d	C3			

* Assignments may be interchanged.

Fig. 3. A computer-generated drawing showing the absolute stereochemistry of **1** derived from the X-ray coordinates with hydrogens omitted for clarity.



and $c=14.089$ (1)Å for $Z=8$. Of the 2,348 reflections measured with an automatic four circle diffractometer equipped with Cu radiation, 2,055 were observed ($I \geq 3\sigma I$). The structure was solved with a multi-solution tangent formula approach and difference Fourier analysis and refined using full-matrix least-squares techniques.[6] One enantiomer refined to an R factor of 0.0997 while the other minimized to 0.0957, a statistically significant difference.[7] Careful remeasurement of fifteen enantiomer sensitive reflections confirmed this assignment of the absolute configuration. A molecule of acetonitrile was found in the crystal lattice. Hydrogens were assigned isotropic temperature factors corresponding to their attached atoms. The function $\sum w(|F_o|-|F_c|)^2$ with

$w=1/(\sigma F_o)^2$ was minimized to give an unweighted residual of 0.058. No abnormally short intermolecular contacts were noted. Tables containing the crystallographic coordinates, bond distances and bond angles have been deposited with the Cambridge Crystallographic Data Centre, Cambridge, England. Fig. 3 is a computer-generated perspective drawing of **1** from the final X-ray coordinates showing the determined relative and absolute stereochemistry.

Materials and Methods

General Methods

^1H NMR and ^{13}C NMR spectra were recorded on a Varian SC-300 instrument. The ^{13}C NMR 2D-chemical shift correlation was obtained on a Nicolet NT300 instrument. Low and high resolution electron impact mass spectra were recorded on a Varian MAT212 instrument, and FAB spectra were obtained on a Varian MAT731 instrument. All X-ray diffraction data were obtained with an Enraf-Nonius CAD-4 diffractometer.

Asperlicin (1)

Asperlicin (**1**): ^1H NMR (CD_3OD - $CDCl_3$, 1:4; 25°C) δ in ppm 0.88 (3H, d, J=6.2 Hz), 0.94 (3H, d, J=6.2 Hz), 1.52 (1H, ddd, J=4, 10.5 & 12.5 Hz), 1.77 (1H, ddd, J=3, 9.5 & 12.5 Hz), 1.84 (1H, m), 2.53 (1H, dd, J=4.5 & 15.5 Hz), 2.72 (1H, dd, J=9.5 & 15.5 Hz), 4.25 (1H, dd, J=3 & 10.5 Hz), 4.37 (1H, dd, J=4.5 & 9.5 Hz), 5.50 (1H, s), 7.10 (1H, br t, J=7.5 Hz), 7.24 (1H, br d, J=8 Hz), 7.28 (1H, br t, J=7.5 Hz), 7.49 (1H, br d, J=8 Hz), 7.56 (1H, br t, J=8 Hz), 7.60 (1H, d, J=7.5 Hz), 7.64 (1H, d, J=7.5 Hz), 7.67 (1H, t, J=8 Hz), 7.69 (1H, t, J=8 Hz), 7.83 (1H, dt, J=1 & 7.5 Hz), 7.98 (1H, dd, J=1.5 & 7.5 Hz), and 8.28 (1H, dd, J=1 & 8 Hz); $[\alpha]_D$ −185.3° (c 0.2, MeOH).

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