REVISED NMR ASSIGNMENTS FOR THE CHOLECYSTOKININ ANTAGONIST ASPERLICIN

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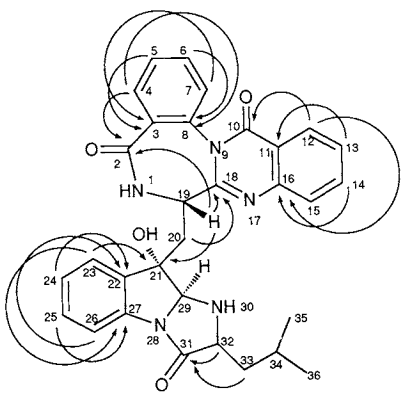
Asperlicin (**1**), a competitive cholecystokinin (CCK) antagonist, produced from *Aspergillus alliaceus*, was originally characterized primarily by means of X-ray crystallography.[1~3] The compound contains a 1,4-benzodiazepine ring with a quinazolone fused to the 1,2-positions, and a 3-substituted moiety condensed from tryptophan and leucine. This was the first reported naturally occurring nonpeptide antagonist of a peptide receptor. Subsequently, several possible therapeutically useful CCK antagonists were synthesized based on the benzodiazepine nucleus of asperlicin.[4] Furthermore, benzodiazepines represent an important class of drugs which are widely used as anti-anxiety agents and hypnotics.[5]

We have recently isolated a series of substance P antagonists, benzomalvins A (**2**), B and C, from a *Penicillium* sp.[6] Structural studies using 2D NMR clearly demonstrated that the benzomalvins possess the same quinazolino-benzodiazepine-dione ring skeleton as in asperlicin (**1**). The structure of **2** was confirmed through total synthesis.[6] Comparison of ^{1}H and ^{13}C NMR spectral data of benzomalvins with literature values for asperlicin,[3] however, showed discrepancies among NMR assignments. In order to resolve this issue and to provide a basis for future structural assignments of new compounds of this general class, we have carried out a detailed NMR analysis leading to unambiguous assignments of both ^{1}H and ^{13}C data for asperlicin, reported herein.

The ^{1}H NMR spectrum of **1** in $CDCl_3$-CD_3OD (4:1) showed the presence of 2 methyls, 2 methylenes, 4 methines and 12 well-resolved aromatic protons. A double quantum filtered phase sensitive COSY experiment[7] revealed ^{1}H-^{1}H couplings and established ^{1}H assignments for a 3-methyl-butyl group (32-H~36-H), a $>CH-CH_2-$ segment (19-H~20-H) and 3 di-substituted benzene moieties. Thus, the remaining methine singlet at δ 5.49 is assigned to 29-H. The ^{13}C NMR studies and a DEPT experiment revealed resonances for all 31 carbons including 12 protonated and 10 non-protonated sp^2 carbons, 2 methyls, 2 methylenes, 4 methines and 1 quaternary carbon. A 2D ^{1}H-detected HMQC experiment[8] yielded ^{13}C-^{1}H direct connectivities and established the corresponding ^{13}C chemical shifts for the protonated moieties described above.

Further ^{13}C δ assignments of 3 di-substituted benzene moieties and 11 non-protonated carbons were achieved by an HMBC experiment,[9] whereby

Fig. 1. Structures of asperlicin (**1**) and benzomalvin A (**2**), and HMBC-derived correlations for non-protonated carbons in **1**.



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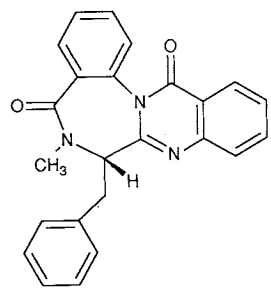

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Table 1. ^{1}H and ^{13}C NMR assignments of asperlicin (**1**).

Atom No.	^{13}C (m)	^{1}H (m, J)	HMBC coupled H No.
2	168.4 (s)	—	4-H, 19-H
3	130.4 (s)	—	5-H, 7-H
4	129.9 (d)	7.96 (dd, 7.5, 1.7)	6-H
5	129.3 (d)	7.66 (br dd, 7.5, 7.5)	7-H
6	127.8 (d)	7.69 (ddd, 7.5, 7.5, 1.7)	7-H
7	128.6 (d)	7.58 (br d, 7.5)	5-H
8	133.7 (s)	—	4-H, 5-H, 6-H, 7-H
10	162.2 (s)	—	12-H
11	121.3 (s)	—	13-H, 15-H
12	127.4 (d)	8.27 (dd, 7.9, 1.0)	13-H, 14-H
13	128.0 (d)	7.55 (ddd, 7.9, 7.9, 1.0)	
14	135.3 (d)	7.82 (ddd, 7.9, 7.9, 1.0)	12-H
15	131.8 (d)	7.67 (br d, 7.9)	
16	146.3 (s)	—	12-H, 14-H
18	155.3 (s)	—	19-H, 20-H
19	50.7 (d)	4.56 (dd, 9.3, 4.8)	20-H
20	34.5 (t)	2.58 (dd, 15.5, 4.8), 2.71 (dd, 15.5, 9.3)	19-H, 29-H
21	80.9 (s)	—	19-H, 20-H, 23-H
22	139.6 (s)	—	20-H, 24-H, 26-H
23	124.0 (d)	7.23 (br d, 7.5)	25-H
24	126.0 (d)	7.09 (br dd, 7.5, 7.5)	26-H
25	129.8 (d)	7.27 (br dd, 7.5, 7.5)	23-H
26	115.9 (d)	7.48 (br dd, 7.5)	24-H
27	136.3 (s)	—	23-H, 25-H
29	86.8 (d)	5.49 (s)	20-H
31	171.4 (s)	—	32-H, 33-H
32	62.0 (d)	4.25 (dd, 10.4, 3.3)	33-H
33	42.9 (t)	1.51 (ddd, 12.5, 10.4, 4.3), 1.73 (ddd, 12.5, 9.5, 3.3)	32-H
34	25.2 (d)	1.84 (m)	32-H, 33-H
35	23.6 (q)	0.90 (d, 6.4)	33-H
36	21.4 (q)	0.95 (d, 6.4)	33-H

the multiple bond ^{1}H-^{13}C couplings necessary to connect non-protonated carbon atoms to protonated ones were observed. Starting from the >CH–CH_2– segment (C-19 ~ C-20), the methine proton (19-H, δ 4.56) was coupled to an amidine carbon (C-18, δ 155.3), an amide carbonyl (C-2, δ 168.4) and a quaternary carbon (C-21, δ 80.9); and its methylene protons (20-H, δ 2.58, 2.71) were coupled to C-18, establishing the δ values of C-2, C-18 and C-21. The ^{3}J coupling of C-2 and 4-H in conjunction with the COSY and HMQC data led us to place δ values on protonated carbons (C-4 ~ C-7) of a benzoic amide moiety. Similarly, ^{3}J couplings of C-10 ~ 12-H, C-21 ~ 23-H and C-31 ~ 33-H resulted in δ assignments of protonated carbons of two other benzoid moieties (C-12 ~ C-15 and C-23 ~ C-26) and a carbonyl carbon (C-31). Furthermore, the chemical shifts of the remaining 6 non-protonated carbons (C-3, C-8, C-11, C-16, C-22 and C-27) of 3 di-substituted benzene rings were also assigned on the basis of the 3-bond couplings as depicted in **1**. The re-assigned ^{1}H and ^{13}C NMR data of **1**, summarized in Table 1, are in excellent agreement with those of benzomalvins.[6]

Acknowledgement

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