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STRUCTURE OF COMPLESTATIN, A VERY STRONG INHIBITOR OF PROTEASE ACTIVITY OF COMPLEMENT IN THE HUMAN COMPLEMENT SYSTEM

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Summary: The structure of complestatin, which strongly inhibits the protease activity of complements in the human complement system, has been determined as shown in Fig. 2 mainly based on HMBC. Its structure is closely related to glycopeptide antibiotics.

Complestatin (I) is a peptide isolated from the mycelium of Streptomyces lavendulae[1], and strongly inhibits the hemolysis of sensitized erythrocytes by the complement system[2]. To the best of our knowledge, I is the most potent compound among the known inhibitors with anti-complement activity such as flufenamic acid[3], leupeptin[4] and K-76[5]. In this paper we wish to report the structural determination of I facilitated by extensive use of Heteronuclear Multiple Bond Correlation (HMBC)[6].

The physicochemical properties of I were as follows: mp. >300°C (dec.), $[\alpha]_D^{24}$=+24.5° (c=0.13, MeOH-0.01N NaOH 2:1); UV λ_{max}^{MeOH} (ε) 282 (13800) and 292 (13200) nm; IR ν_{max}^{KBr} 3400 (OH), 1650 and 1510 (amide) cm^{-1}; color reaction, positive to Liebermann and Ehrlich, negative to Molisch and ninhydrin; elemental analysis, found C 54.13, H 3.81, N 7.27, Cl 15.45%, calcd. for $C_{61}H_{45}O_{15}N_7Cl_6$, C 55.14, H 3.41, N 7.38, Cl 16.01; HR-FABMS; M^+ (m/z), found 1325.1060, calcd. 1325.1110.

Due to severe overlapping of the ^{13}C signals in the aromatic region, the number of sp^2 quaternary carbons was determined to be 24 by spin echo experiments[7]. The ^1H- and ^{13}C-NMR spectral data summarized in Table 1 showed the presence of 1 X $-NCH_3$, 2 X CH_2, 6 X CH, 20 X -CH=, 24 X -C=, 1 X -COOH, 6 X -CO-NH- and 1 X -C=O.

Acid hydrolysis of I (1N-HCl/CH_3COOH, 105°C, 22 hr) gave three main products (II, III and IV). Two of them obtained in the ratio of 1:2 were identified as 4-hydroxyphenylglycine (II) and 3,5-dichloro-4-hydroxyphenylglycine (III) by spectral analysis. Their absolute configurations were determined to be both D by comparison with a standard compound ($[\alpha]_D^{25}$=-128.7°; II, $[\alpha]_D^{25}$=-125.5°), and an authentic sample with L-(+)-configuration

Table 1 ^{13}C and ^{1}H NMR spectral data of complestatin (in DMSO-d_6)

group	C-13	H-1 (J_{Hz})
	1 127.8	
	2 128.2	7.109(8.0)
	3 115.3	6.765(9.2)
	4 157.1	
A	5 115.3	6.765(9.2)
	6 128.2	7.109(8.0)
	C=O 171.3	
	CH 55.8	5.065(7.2)
	NH	8.507(6.3)
	1 131.0	
	2 127.0	7.341
	3 122.0	
	4 148.7	
B	5 122.0	
	6 127.0	7.341
	C=O 169.2	
	CH 51.5	5.196(7.0)
	NH	8.741(6.2)
	1 131.9	
	2 126.7	7.305
	3 121.7	
	4 148.1	
C	5 121.7	
	6 126.7	7.305
	C=O 169.8	
	CH 55.2	5.563(8.4)
	NH	7.877(7.8)

group	C-13	H-1 (J_{Hz})
	1 134.5	
	2 131.5	7.825(2.0,8.8)
	3 123.1	6.867(2.6,8.2)
	4 155.2	
D	5 121.7	7.079(2.5,7.0)
	6 130.6	7.192(2.0,8.6)
	C=O 168.4	
	CH 61.3	5.065(7.2)
	CH_2 35.0	3.050
	NCH_3 31.2	2.985
	1 126.4	
	2 129.5	5.108(3.5)
	3 131.1	
E	4 139.4	
	5 149.6	
	6 110.5	5.475(4.0)
	C=O 167.6	
	CH 55.0	5.563(8.4)
	NH	8.285(9.2)
	1 10.9	
	2 123.6	7.272(2.8)
	3 111.5	
	4 126.3	
	5 118.4	7.435(9.0)
F	6 123.7	6.830(1.5,8.2)
	7 134.4	
	8 114.4	7.249

group	C-13	H-1 (J_{Hz})
	9 136.3	
	C=O 170.3	
	CH 57.1	4.182
	CH_2 28.2	2.886, 3.5
	NH	8.863(6.8)
	1 124.1	
	2 130.1	7.776
	3 122.5	
G	4 155.8	
	5 122.5	
	6 130.1	7.776
	αC=O 185.7	
	βC=O 163.7	

obtained by acid hydrolysis of enduracidin[8] ($[\alpha]_D^{25}$= +87.6°; III, $[\alpha]_D^{25}$=-81.6°). IV[9] was determined to be 2-(3,5-dichloro-4-hydroxyphenyl)-2,2-dihydroxyacetic acid based on NMR spectral comparison with III. Since there existed no ketal carbon in I, the ketal carbon (C-2) of IV was present as a ketone in the parent compound[10].

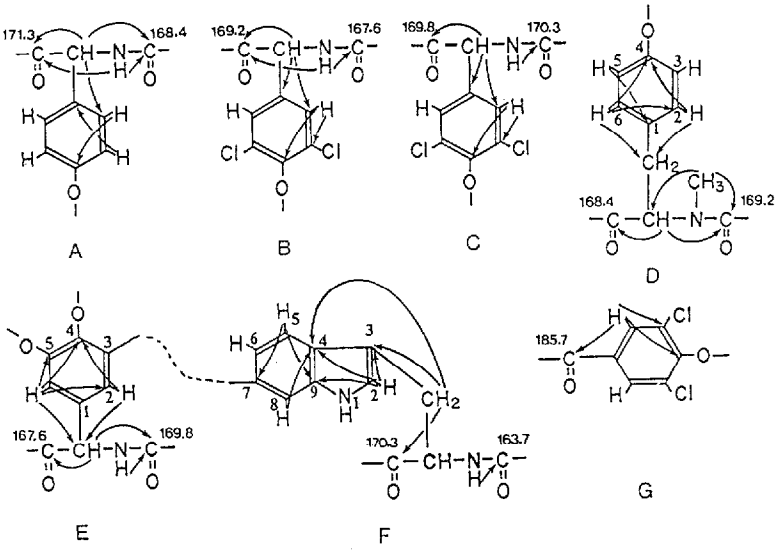
Detailed analysis of the COSY spectrum of I revealed the presence of the following units; 4 X -CO-NH-CH-, 1 X -CO-NH-CH-CH_2-, 1 x -CH_2-CH-N-, 2 X 1,4-disubstituted aromatic systems and two ortho-coupled aromatic protons.

Analysis of the HMBC spectrum of I established seven partial structures, i.e., six amino acid units and one fragment with a ketone function (A to F and G, respectively, in Fig. 1). The arrows in Fig. 1 indicate long range couplings between protons and carbons ($^{2}J_{C-H}$ or $^{3}J_{C-H}$). It is clear that II, III, and IV obtained by acid hydrolysis were originated from unit A, units B and C, and unit G, respectively. Taking account of the overlapping carbonyl carbons, Fig. 1 accommodates all the carbons present in I.

Non equivalent proton chemical shifts of D-2 and D-6, and D-3 and D-5 of the symmetric aromatic side chain in partial structure D suggested that the oxygen atom at D-4 is protected by a bulky group which restricts free rotation of the aromatic ring D.

The large $^{1}J_{C-H}$ value[11] observed with F-2 proton together with positive Ehrlich and Liebermann reactions of I indicated the presence of an indole

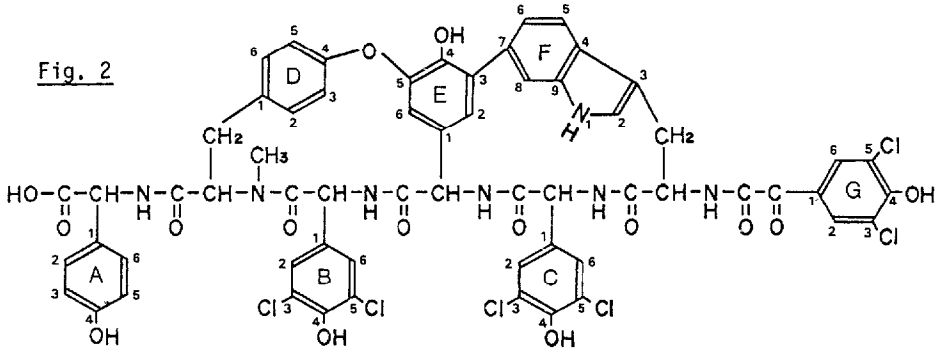
Fig. 1



ring in unit F. This structure is supported by the $^{2}J_{C-H}$ and $^{3}J_{C-H}$ long range couplings between F-2, F-5 and F-8 protons and relevant carbons shown in Fig. 1. In addition, the direct linkage between F-7 and E-3 carbons was revealed by long range coupling between E-3 (δ_C 131.0) and the protons at F-6 (δ_H 6.830) and F-8 (δ_H 7.249), and F-7 (δ_C 134.5) and the protons at F-5 (δ_H 7.435) and E-2 (δ_H 5.108).

The sequence of six amino acid units could be obtained by analysis of long range couplings between α-methine and/or amide protons and the carbonyl carbon of the adjacent amino acid fragment (see Fig. 1). The analysis was started from unit D, in which the long range couplings between the N-methyl protons and a carbonyl carbon (δ_C 169.2) enabled to distinguish the carbonyl carbon (δ_C 168.4) in the unit D from one belonging to the next amino acid residue (δ_C 169.2). The latter carbonyl carbon is present in unit B connecting units B and D. By repeating this analytical procedure, the sequence A-D-B-E-C-F- could be established. Since unit G gave IV (vide supra), G must be located at the next position of unit F.

Fig. 2



As explained above, the phenolic hydroxy group of unit D must be protected to restrict free rotation of the ring D. Since acid hydrolysis gave phenolic compounds II, III and IV (vide supra) which apparently derived from

units A, B, C and G, unit D must be combined to one of the hydroxy groups of unit E. This position was determined by observing the deuterium-induced upfield shift in the ^{13}C-NMR spectrum of I taken in d_6-DMSO added with one drop of 1:1 mixture of H_2O and D_2O. The carbon signal of E-5 (δ_C 149.6) showed no shift, while line broadening was observed with E-4 resonance (δ_C 139.4) suggesting the linkage of unit D to E-5 carbon. Thus, the structure of complestatin is established as shown in Fig. 2.

I is structurally related to glycopeptide antibiotics such as vancomycin[12], ristocetin[13], teicoplanin[14] and chloropolysporin[15]. The main differences are that I has no sugar units, and possesses the indole nucleus instead of the modified tyrosine or β-hydroxytyrosine unit.

It is interesting that the biological activities of I and glycopeptide antibiotics are markedly different; the latter show very strong antibacterial activity to Gram positive bacteria, while I inhibited the growth of a few Gram positive bacteria at a very high concentration (ca. 2000 μg/ml). Tested so far, glycopeptide antibiotics showed no anti-complementary activity. It is also very important that a deglycosylated derivative of chloropolysporin, which had almost same activity as the parent compound *in vitro*,[16] was inactive against the complementary system[17]. These findings suggest that difference of the biological activities between glycopeptide antibiotics and complestatin is not due to the presence of sugar units in the former group.

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10) Similar phenomenon was observed with phenylglyoxylic acid, which showed the molecular ion peak corresponding to the ketone form. On the other hand, the ^1H-NMR spectrum of this compound indicated the presence of a 1:1 mixture of the ketone and hydrated forms 12 hours after dissolving in a solvent.
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