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ISOLATION AND STRUCTURE OF THE EXCEPTIONAL PTEROBRANCHIA HUMAN CANCER INHIBITORS CEPHALOSTATINS 16 and 17[1]

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Abstract: The Southeast African marine worm Cephalodiscus gilchristi has been found to contain two new and exceedingly potent human cancer cell growth inhibitors Cephalostatins 16 (3) and 17 (4).

Over the past 21 years we have vigorously pursued the antineoplastic constituents of Cephalodiscus gilchristi (Phylum Hemichordata, Class Pterobranchia, Order Cephalodiscida), a tiny (a few mm) marine worm found in the Indian Ocean off Southeast Africa. In the period 1987-91 we finally succeeded in isolating and elucidating the structures of cephalostatins 1-9[2a,b] (10^{-4} to 10^{-6}% yields) from a very difficultly separable series of murine P388 lymphocytic leukemia (PS system) inhibitory fractions obtained from 166 kg (wet wt. including coenecium) from a 1981 scaleup recolletion of the worm.

By 1990 the supply of cephalostatin 1 for preclinical development became an urgent problem. That necessity was further accentuated by evidence for yet unknown and very potent cephalostatins as trace constituents in the 166 kg recollection. Those requirements led to a 450 kg (wet wt as above) recollection (1990) that has provided new cephalostatins 10-15.[2c-e] While potent and very useful for structure/activity relationships, these new cephalostatins were unable to favorably compete with or exceed cephalostatins 1 (1) and 7 (2) as human cancer cell growth inhibitors. As reported in the sequel, we have now succeeded in discovery of two trace (~10^{-7}% yields) components of C. gilchristi, designated cephalostatins 16 (3) and 17 (4), that approximate the potency of cephalostatins 1 and 7. Interestingly, these exceptionally potent cephalostatins continue to exhibit the perhydropyran/spiroketal ring systems so charateristic of remarkable marine sponge antineoplastic constituents we have recently discovered such as spongistatin 1[3] and the halistatins.[4] Other perhydropyran systems can lead to extraordinary toxicity where maitotoxin[5] and related dinoflagellate biosynthetic products are classic illustrations.

A P388 cell line active fraction prepared[2d] from 450 kg (wet wt.) of C. gilchristi was subjected to Sephadex LH-20 column partition chromatography and reversed-phase semi-preparative HPLC (C8, acetonitrile-methanol-water, 10:10:12, as mobile phase) to afford two optically active, colorless, amorphous solids with mp >300°C: cephalostatins 16 (3, 4.1 mg,

PS ED_{50} <0.001 μg/mL) and 17 (4, 3.8 mg, PS ED_{50} 0.0041 μg/mL).

Cephalostatin 16 (3) was obtained in $9x10^{-7}$% yield: Rf 0.84 (SiO_2 plate, 90:10:0.8 dichloromethane-methanol-water); $[\alpha]_D$ +55° (c, 1.73, CH_3OH); HRFABMS, m/z 911.54220 $[M+H]^+$ for $C_{54}H_{75}N_2O_{10}$ (Δ + 0.03 mmu); UV (CH_3OH), λ_{max} 288 (ε 11600) and 305 (shoulder) nm and IR (KBr), ν_{max} 3453, 2928, 1711, 1632, 1449, 1400, 1045 and 889 cm^{-1}. Analysis (Table 1) of the APT, ^1H-^1H COSY and X-H-COSY experiments with steroidal alkaloid 3 confirmed the proton and carbon resonances as corresponding to six singlet methyl, two doublet methyl, sixteen methylene (including two linked to oxygen), thirteen methine (2 sp^2 and 11 sp^3, with three linked to oxygen) and seventeen quaternary (7 sp^2 and 10 sp^3, with 7 bonded to oxygen) carbons. Interpretation of the ^1H-^1H COSY, X-H COSY, TOCSY and HMBC 2D-NMR experimental results indicated that steroid 3 possessed a structure analogous to that of cephalostatin 1. However, the right side steroid F ring unit was found to be a tetrahydropyran (with C-27 methyl and tertiary hydroxyl groups) connected to the E ring at C-22 in a spiroketal system as in cephalostatins 1-15. The NOE correlations of the right-side unit of steroid 3 begin with the equatorial proton at C-23 (δ 2.55, m) and proceed to a methyl at C-21 (δ 1.27, d), as well as from the C-21 methyl to a hydroxyl proton at C-17 (δ 5.09, s). Computer-assisted energy minimization (molecular-modeling software, Chem. 3D) in conjunction with molecular modeling for rings C-F (Figure 1) revealed that the configuration at C-22 should be (S). The stereochemical assignment at C-22 was also supported by considerations relating to the X-ray crystal structure of cephalostatin 1 (1). Based on the NOE correlations between the methyl (δ 1.23, s) at C-27 and the hydrogen atoms at C-24 (δ 1.87, m and 2.16, m) and at C-26 (δ 3.61, d and 4.01, d, J=14.5 Hz), it became apparent that the 27-methyl group was equatorial. In this configuration the 27-methyl group would be closest to both the axial and equatorial protons at C-24 and C-26 (Figure 2).

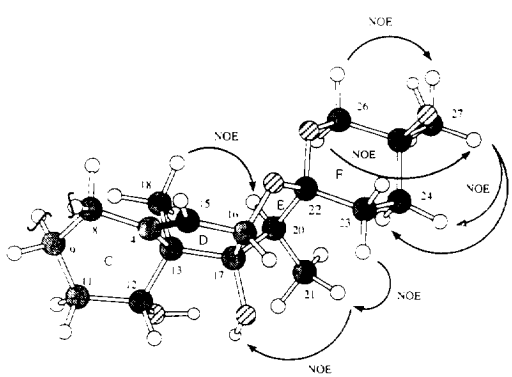


Figure 1: Selected NOE enhancements of cephalostatin 16 (1) displayed on computer-assisted energy minimization modeling for rings C-F.

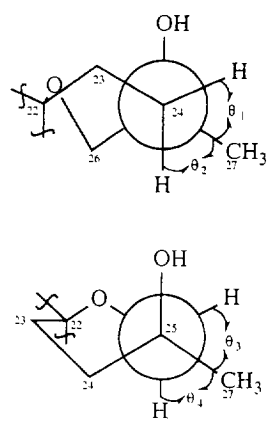
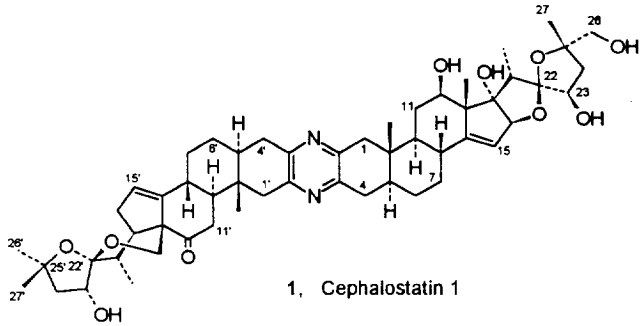
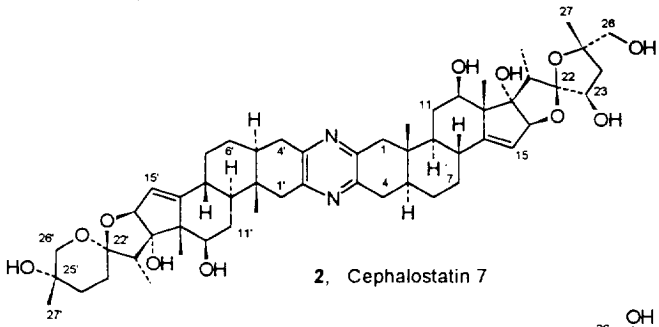


Figure 2:

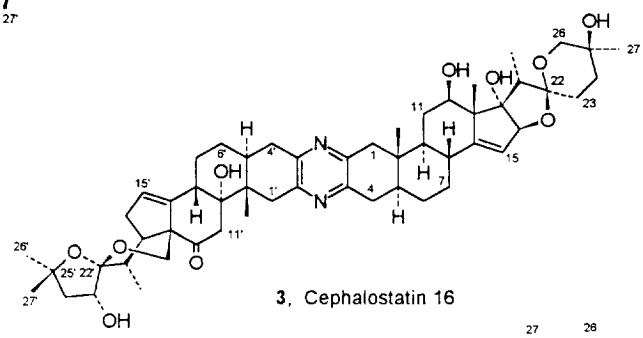
$\theta_1 = \theta_2 = \theta_3 = \theta_4 = 60^0$



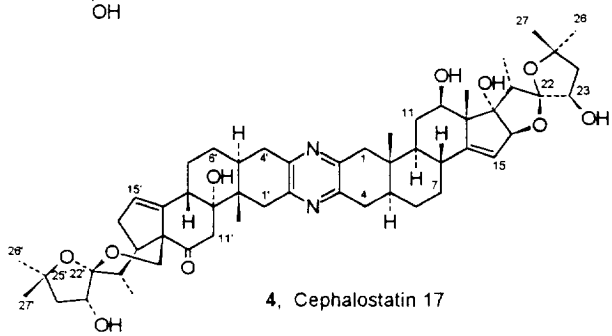
1, Cephalostatin 1



2, Cephalostatin 7



3, Cephalostatin 16



4, Cephalostatin 17

Table : 1

The high field (500MHz) ^{1}H, ^{13}C-NMR spectral data in cephalostatin 16 (**1**) and assignments (in C_5D_5N)

	^{13}C ppm	^{1}H ppm	J (Hz)	HMBC (H to C)		^{13}C ppm	^{1}H ppm	J (Hz)	HMBC (H to C)
Right side					Left side				
1	46.03 d	2.60 m		10.	1'	39.50 t	2.95 m		5',10',19'.
		3.12 m		10.			3.77 m		9'.
2	149.67 s				2'	150.17 s			
3	148.28 s				3'	148.55 s			
4	35.77 t	2.68 m			4'	36.19 t	2.72 m		5',10'.
		2.90 m		10.			3.08 m		5',10'.
5	41.76 d	1.62 m			5'	34.21 d	2.05 m		
6	28.27 t				6'	28.14 t			
7	28.96 t	1.36 m			7'	24.56 t	1.92 m		
		1.74 m					2.01 m		
8	34.01 d	2.08 m			8'	38.96 d	2.77 m		9'.
9	52.88 d	0.85 m			9'	78.72 s			
10	36.30 s				10'	42.22 s			
11	28.96 t	1.36 m			11'	123.60 d	6.47 s		8',9',10',13'.
		1.74 m							
12	75.66 d	4.20 m			12'	211.06 s			
13	55.81 s				13'	61.54 s			
14	154.83 s				14'	148.55 s			
15	119.89 d	5.59 brs		8,13,14,17.	15'	124.42 d	5.57 brs		13',14',16',17'
16	93.74 d	5.18 brs		13,14,15,17.	16'	32.54 t	2.12 m		
							2.90 m		13',15'.
17	91.31 s				17'	36.02 d	2.81 m		16'.
18	13.01 q	1.35 s		12,13,14,17.	18'	64.04 t	4.16 dd	13.0.	12',13',17',22'
19	11.77 q	0.78 s		1,5,9,10.	19'	15.03 q	0.98 s		1',5',9',10'.
20	48.46 d	2.22 m		13,21,22.	20'	32.88 d	3.20 m		16',17',21',22'
21	8.16 q	1.27 d	7.0	17,20,22.	21'	15.47 q	1.40 d	6.8	17',20',22'.
22	107.90 s				22'	110.93 s			
23	27.72 t	2.55 m		23,24.	23'	81.57 d	4.82 t	7.0	
		1.57 m							
24	33.28 t	1.87 m			24'	47.34 t	1.93 dd	12.2	23',27'.
		2.16 m					2.36 dd	12.2,7.0	22'.
25	65.76 s				25'	81.10 s			
26	70.18 t	3.61 d	14.5	25.	26'	29.77 q	1.46 s		24',25',27'.
		4.01 d	14.5	24,25.					
27	27.00 q	1.23 s		24.	27'	28.93 q	1.40 s		24',25',26'.
17-OH		5.09 s		17.	9'-OH		6.02 s		9'.
12-OH		4.71 s		11,12.					

Cephalostatin 17 (4) was obtained in 8.4×10^{-7}% yield: Rf 0.82 (SiO_2, plate 90:10:0.8 dichloromethane-methanol-water); $[\alpha]_D$ +70° (c, 0.70, CH_3OH); UV (CH_3OH), λ_{max} 288 (ϵ 15000) and 305 (shoulder) nm; IR (KBr), ν_{max} 3439, 2928, 1713, 1464, 1400, 1090, 1051, 952 and 891 nm^{-1}. The molecular formula of cephalostatin 17 (4) was found to be $C_{54}H_{75}N_2O_{10}$ by HRFABMS, m/z 911.5402 $[M+H]^+$, Δ -2.16 mmu. Analyses of the high field 2D-NMR data from ^1H-^1H COSY, HMQC, ROESY and HMBC experiments with cephalostatin 17 compared to those obtained with cephalostatin 1 (1, an x-ray crystal structure) resulted in assignment of structure 4.

Cephalostatins 16 (3) and 17 (4) were comparatively evaluated, alongside cephalostatin 1 (1), in the U.S. National Cancer Institute's human tumor, disease-oriented *in vitro* primary screen.[6-8] Each compound was tested in quadruplicate at three different concentration ranges (10^{-6}, 10^{-7} and 10^{-8}M upper limits; five, $\log_{10}$-spaced concentrations in each range) against the entire group of 60 cell lines which the screening panel comprises. The standard cephalostatin 1 (1) yielded the expected overall panel GI_{50} value of approximately 1 nmolar, and produced the distinctive mean-graph "fingerprint" of differential cellular response which is typical of all members of the cephalostatin series heretofore studied (e.g. see ref 2b). In the present investigation, cephalostatins 16 (3) and 17 (4) likewise produced the characteristic cytotoxicity profile, as confirmed by Compare[7] pattern-recognition analyses; the corresponding correlation coefficients were 0.96 and 0.92 for steroidal alkaloids 3 and 4, respectively, against the standard (1). The latter analyses implied that the cytotoxic mechanism(s) of these newest cephalostatins did not diverge substantially from the rest of the series. The overall panel-averaged cytotoxic potencies were comparable (e.g. 1 nmolar and 4 nmolar for alkaloids 3 and 4 respectively) to the benchmark compound (1).

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