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Isolation and Structure Elucidation of Erylusamine B, a New Class of Marine Natural Products, Which Blocked an IL-6 Receptor, from the Marine Sponge *Erylus placenta* Thiele[1]

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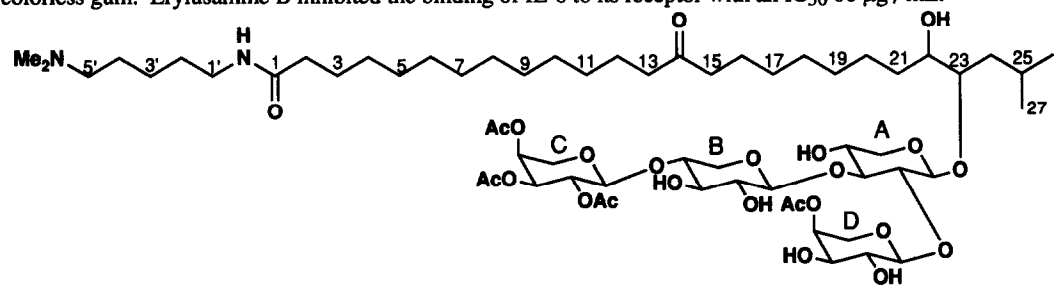
Abstract: Erylusamine B (1) has been isolated as an IL-6 antagonist from the marine sponge *Erylus placenta* Thiele. Its structure was determined by spectral and chemical methods to be *N*-(5-*N*, *N*-dimethylaminopentyl)-14-oxo-22-hydroxy-25-methyl-23-*O*-1β-D-xylopyranosyl-(2→1)-(4-*O*-acetyl-β-L-arabinopyranosyl)-(3→1)-β-D-xylopyranosyl-(4→1)-2,3,4-*O*-triacetyl-β-L-arabinopyranosyl)-hexacosamide.

Interleukin-6 (IL-6) is a multifunctional cytokine which exerts a variety of biological functions in various cells through the ligand-receptor interaction.[2] It is also related to such diseases as inflammation, viral infection, autoimmunity, and cancer. Therefore, antagonists for IL-6 receptors are valuable leads. In the course of our search for such leads from Japanese marine invertebrates, we noticed that the MeOH extract of the marine sponge *Erylus placenta* exhibited antagonistic activity against an IL-6 receptor.[3] Bioassay-guided fractionation yielded an active substance, erylusamine B (1). We describe the isolation and structure elucidation of this metabolite.

The EtOH extracts of the frozen sponge (1.3 kg wet weight), which was collected from a marine cave (-15 m) off Hachijo-jima Island, were concentrated, adjusted to pH 9 with 1N NH_4OH, and extracted with Et_2O and subsequently with *n*-BuOH. The MeOH soluble portion of the *n*-BuOH phase was gel-filtered on Sephadex LH-20 [$CHCl_3$ / MeOH (1:1) containing 0.1 % AcOH]. Active fractions[4] were further fractionated on ODS and silica gel, followed by HPLC on ODS with 78 % MeOH containing 0.1 % TFA to yield erylusamine B (11.2 mg) as a colorless gum. Erylusamine B inhibited the binding of IL-6 to its receptor with an IC_{50} 66 μg / mL.



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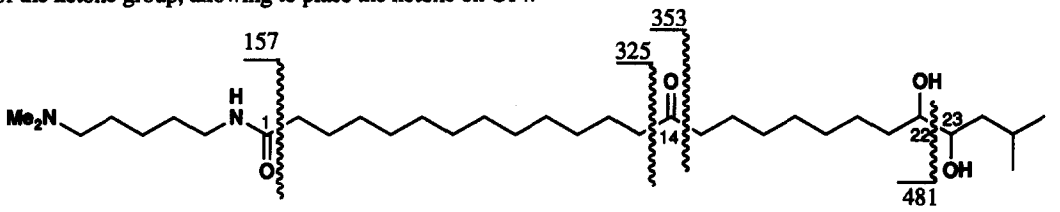
Erylusamine B (1)[5] had a molecular formula of $C_{62}H_{108}N_2O_{24}$ as determined by the HRFAB mass spectrum (MH^+ *m/z* 1265.7295, Δ -7.5 mmu) and ^{13}C NMR data. The IR bands at 3350, 1740, 1715, and 1670 cm^{-1} implied the presence of hydroxyl, ester, ketone, and amide groups. The ^1H NMR spectrum[6] contained two secondary methyls (δ 0.85, 0.87) and a huge methylene envelope at δ 1.25, suggesting the presence of a long aliphatic chain. Six singlet methyls between δ 1.99 and δ 2.25 were assigned as four acetate and two *N*-methyl

groups on the basis of ^{13}C NMR and HMQC[7] data [δ 169.2, 169.9, 170.1, and 170.9 (acetates), 45.0 (*N*-methyl)]. The NMR spectra also revealed numbers of oxy-methines and -methylenes (δ_H 3.10-5.26 ; δ_C 64.0-87.0), together with four anomeric methines between δ 4.4 and δ 4.6 which were attached to carbons resonated at 100-105 ppm, thus suggesting the presence of four sugar units.

The structure of the aglycone was deduced from extensive 2D NMR experiments, together with FABMS data of a hydrolysis product. Starting from an exchangeable amide proton at δ5.68 (NH-1'), five contiguous methylenes (C1'-C5') were inferred from the COSY spectrum. HMBC[8] correlations between the *N*-Me_2 protons and C5' completed an *N,N*-dimethylpentanediamine unit. Incidentally, the amide proton and protons on C1' were correlated with a carbon at δ 173.2 (C1).

Mild acid hydrolysis of Erylusamine B (2N HCl, 80°C, 2h) afforded the diol **2**,[9] whose molecular formula of $C_{34}H_{68}N_2O_4$ was secured by both FABMS and NMR data. The ^1H and ^{13}C NMR spectra contained two oxygenated methines (δ 3.25, 3.41), methylene chains (δ 1.15-1.60), an amide (δ 173.2), and a ketone (δ 213.5), in addition to an *N,N*-dimethylpentanediamine unit. Although the position of a ketone and two hydroxyls was not unambiguously implied by NMR experiments, the problem could be solved by the FAB mass spectrum to obtain the gross structure as shown in Scheme I. The presence of a vicinal diol was inferred from facile cleavage of the C22-C23 bond, which was also confirmed by formation of an acetonide **3**[10] upon treatment with 2,2-dimethoxypropane / *p*-TsOH. Fragment ions at *m/z* 353 and 325 were derived from the fission at both sides of the ketone group, allowing to place the ketone on C14.



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Scheme I

The structure of the tetrasaccharide portion was deduced from NMR data. Starting from the four anomeric protons, interpretation of COSY and HOHAHA spectra revealed the presence of four pentose rings A-D. Rings A and B were assigned as xylopyranose, while rings C and D as arabinopyranose on the basis of vicinal coupling constants. All the anomeric protons were axial as judged from vicinal coupling constants of 7.4-7.7 Hz. Coupling constants of overlapping proton signals were determined by the HMQC spectrum. Hydroxyl groups on C2, C3, and C4 in ring C, and a hydroxyl group on C4 in ring D were acetylated, which was readily substantiated by deshielded proton signals (δ 5.00, 5.06, 5.15, 5.26). The linkage of the sugar units was established by HMBC cross peaks H1C / C4B, H1B / C3A, and H1D / C2A, while the position of the glycoside linkage to the fatty acid moiety was based on an HMBC cross peak H1A / C23.[11]

Erylusamine B is a new class of marine natural products. It is related to glykenines, antimicrobial glycosides known from terrestrial fungus *Basidiomycetes* sp., which are acetylated trisaccharides of tetrahydroxylated C_{26}-fatty acids.[12]

Table 1. NMR Data for Erylusamine B in $CDCl_3$[a]

No.	^{13}C	^{1}H	HMBC correlation
1	173.2 s		
2	36.8 t	2.13 m	C1
3	25.5 t	1.58 m	C1
4-11	29.2 t	1.25 br	
12	24.5 t	1.51 m	C14
13	42.7 t	2.35 m	C14
14	211.8 t		
15	42.7 t	2.35 m	C14
16	24.5 t	1.51 m	C14
17-20	29.2 t	1.25 br	
21	33.0 t	1.38 m	
22	75.0 d	3.25 m	
23	82.0 d	3.41 m	
24	42.0 t	1.15m, 1.38 m	
25	23.0 d	1.79 m	
26	24.0 q	0.85 (d, 6.4)	
27	22.0 q	0.87 (d, 6.2)	
1'NH		5.68 brs	C1
1'	39.2 t	3.22 (q, 6.4)	C1, C2', C3'
2'	23.5 t	1.60 m	
3'	24.1 t	1.32 m	
4'	26.8 t	1.50 m	
5'	59.2 t	2.34 m	
5'NMeX2	45.0 q	2.27 s	5'NMe
1A	103.5 d	4.47 (d, 7.6)	
2A	81.0 d	3.59 m	C1A
3A	86.8 d	3.45 m	C1A, C2A
4A	68.5 d	3.60 m	
5A	65.0 t	3.11 (dd, 11.0, 10.0)	C1A, C3A, C4A
		3.96 (dd, 11.0, 5.6)	C1A, C3A, C4A
1B	103.5 d	4.47 (d, 7.4)	C3A
2B	73.2 d	3.44 m	C1B, C3B
3B	75.0 d	3.52 m	C4B
4B	79.0 d	3.60 m	C3B
5B	63.4 t	3.29 (dd, 11.6, 10.4)	C1B, C3B, C4B
		3.90 (dd, 11.6, 5.4)	C1B, C3B, C4B
1C	101.5 d	4.44 (d, 7.5)	C4B
2C	69.0 d	5.15 (dd, 7.5, 9.8)	C1C, C3C
3C	70.1 d	5.00 (dd, 9.8, 3.3)	C2C
4C	67.5 d	5.26 brs	C2C, C3C
5C	64.2 t	3.69 (dd, 11.3, 1.6)	C1C, C3C, C4C
		4.04 (dd, 11.3, 2.8)	C1C, C4C
2CAc	20.1 q, 169.2 s	2.03 s	
3CAc	20.1 q, 169.9 s	1.99 s	
4CAc	21.0 q, 170.1 s	2.13 s	
1D	104.7 d	4.55 (d, 7.7)	C2A
2D	72.5 d	3.67 m	
3D	71.6 d	3.61 m	C2D
4D	70.2 d	5.06 brs	C2D, C3D
5D	64.8 t	3.53 (dd, 13.3, 1.0)	C4D
		4.02 (dd, 13.3, 2.2)	C1D, C3D, C4D
4DAc	21.0q, 170.9s	2.13 s	

[a] In ppm at 600 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR chemical shifts are referenced to solvent peaks: δ_H 7.25 and δ_C 77.0 for $CDCl_3$.

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3. a) T. Taga, Y. Kawanishi, R. R. Hardy, T. Hirano, T. Kishimoto, *J. Exp. Med.*, **1987**, *166*, 967. b) K. Yamasaki, T. Taga, Y. Hirata, H. Yawata, Y. Kawanishi, B. Seed, T. Taniguchi, T. Hirano, T. Kishimoto, *Science*, **1988**, *241*, 825.
4. Erylusamine B is one of the major components of the IL-6 inhibitory fraction obtained by Sephadex LH-20 chromatography. The structures of other erylusamines will be reported elsewhere.
5. **1**: $[\alpha]_D^{20}$ -5.47° (*c* 0.25, MeOH), IR (film) : ν_{max} 3350, 1740, 1715, 1670, 1450, 1370, 1250, 1200, 1165, 1125, 1090, 1050 cm^{-1}, FABMS : *m/z* 1265 (MH^+), 569, 481, 381, 325, 311, 157, HRFABMS : obsd. $(M+H)^+$ *m/z* 1265.7295 $C_{62}H_{109}N_2O_{24}$ (Δ -7.5 mmu).
6. Erylusamine B gave broad ^1H NMR signals in $CDCl_3$ after purification by ODS HPLC with solvents containing TFA, while the preparation obtained from an alumina column gave sharp signals in $CDCl_3$, thus suggesting that the free amine form of **1** gave sharp ^1H NMR signals in $CDCl_3$, whereas the TFA salt gave broad signals. When the CD_3OD solution was stored in an NMR tube at 4°C for a few months, **1** gave several spots on TLC due to hydrolysis of acetyl groups.
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8. A. Bax, A. Azolos, Z. Dinya, K. Sudo, *J. Am. Chem. Soc*, **1986**, *108*, 8056.
9. **2**: ^1H NMR ($CDCl_3$ / CD_3OD, 3:1) δ 0.88 (3H, d, *J*=6.8 Hz), 0.91 (3H, d, 6.8) 1.15-1.60 (m), 1.70 (1H, m), 2.14 (2H, m), 2.40 (4H, dt, 7.0, 7.0); 2.83 (6H, s, NMex2), 3.04 (2H, m), 3.16 (2H, t, 7.0), 3.42 (2H, m) ; ^{13}C NMR ($CDCl_3$ / CD_3OD, 3:1) 20.7 q, 22.6 t, 23.0 t, 23.8 t, 25.0 t, 25.3 t, 25.7 d, 28.0 t, 28.3-29.2 t, 32.4 t, 35.8 t, 37.8 t, 41.6 t, 42.0 t, 57.2 t, 71.6 d, 74.0 d ; FABMS *m/z* 569 $(M+H)^+$, 481, 353, 325, 157.
10. **3**: ^1H NMR (DMSO-d_6) δ 0.88 (3H, d, *J*=6.8 Hz), 0.90 (3H, d, 6.8), 1.1-1.6 (m), 1.70 (1H, m), 2.01 (2H, t, 7.0), 3.09 (6H, s, NMex2), 2.14 (2H, t, 7.2), 2.37 (4H, t, 7.0), 3.00 (2H, dt, 5.5, 6.5), 3.47 (1H, dd, 8.5, 3.0), 3.58 (1H, dd, 8.5, 3.5) ; ^{13}C NMR (DMSO-d_6) δ 21.8 q, 23.1 t, 23.2 t, 24.2 t, 25.0 t, 25.2 t, 25.5 d, 26.7 t, 27.1 t, 29.4-28.1 t, 32.0 t, 35.3 t, 41.2 t, 41.6 t, 45.2 t, 59.1 t, 78.6 d, 79.1 d, 80.6 d, 107.1 s, 171.9 s, 210.5 s ; FABMS *m/z* 609 $(M+H)^+$.
11. The relevant HMBC cross peak was observed in the spectrum measured in CD_3OD but not in the spectrum in $CDCl_3$. The absolute stereochemistry of arabinose and xylose was determined to be L and D, respectively, by chiral GC analysis, while the absolute stereochemistry at C22 and C23 remains to be elucidated. Determination of stereochemistry of erylusamine B will be reported elsewhere.
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