Three new glycolipids from a Red Sea sponge of the genus Erylus

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Three New Glycolipids from a Red Sea Sponge of the Genus Erylus

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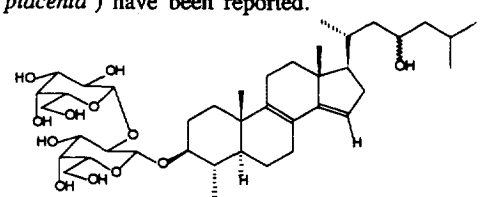
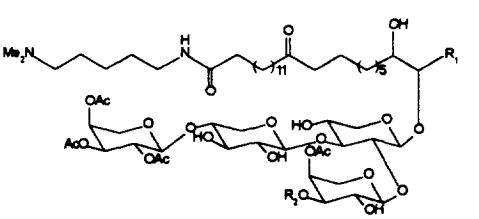
Abstract: Three new cytotoxic glycolipids, erylusamine TA (**8**), erylusine (**9**) and erylusidine (**10**) have been isolated from a Red Sea sponge tentatively identified as *Erylus cf. lendenfeldi*. The structure of the three compounds was determined by interpretation of 2D NMR and FABMS data as trisaccharides of long-chain oxodihydroxyfatty acid amides formed with N,N-dimethyl-1,5-pentanediamine to yield **8** with ω-N,N-dimethylaminobutyl-N-methyl-1,3 -propanediamine to yield **9** and with 4-(aminobutyl) guanidine to yield **10** (R_3=$COCH_2CH(CH_3)_2$).

Our search for biologically active marine natural products has led to the isolation of two oligo steroidal glycosides: eryloside A (**1**) and B (**2**), from the Red Sea sponge *Erylus lendenfeldi* (family Geodiidae), collected in the Gulf of Eilat[1]. An *Erylus* specimen from the Southern part of the Red Sea was examined during an expedition to the Dahlak archipelago (near Eritrea) and tentatively identified as *Erylus cf. lendenfeldi*. Several Indo-Pacific and Caribbean members of the genus *Erylus* have been examined during the last few years. Interestingly, complex carbohydrates such as the erylosides (from *E. lendenfeldi*[1], *E.sp.*[2], *E. goffrilleri*[3] and *E. formosus*[4]) (e.g. **1**) and the erylusamines (**3-7**) (from *E. placenta*[5]) have been reported.



Eryloside A (**1**)

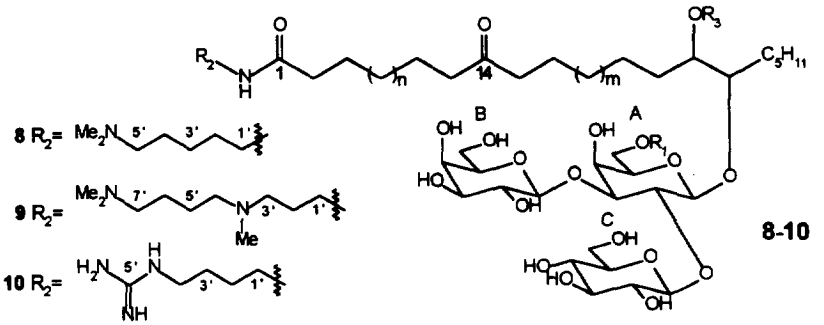


Erylusamine A-E (**3-7**) R_1=C_3H_7-C_5H_{11}, R_2=H, Ac.

Investigation of the cytotoxic extract of *Erylus* cf. *lendenfeldi* led to the isolation of three new glycolipids: erylusamine TA (**8**), erylusine (**9**), and erylusidine (**10**). This paper deals with the isolation and structure determination of these three novel metabolites.

The sponge was frozen shortly after collection and kept frozen until required. The methanol-ethyl acetate (1:1) extract (2.5 gr) of the wet specimen was divided between aq. MeOH and petroleum ether, CCl_4 and $CHCl_3$. The $CHCl_3$ soluble was subjected to counter current chromatography[6]. Several solvent systems were used for the purification of the compounds. Thus, erylusamine TA (**8**, 6 mg) was obtained with $CHCl_3$-MeOH-H_2O, 5:5.7:3, erylusine (**9**, 10 mg) with $CHCl_3$-MeOH-nPrOH-H_2O, 5:6:1:4, and erylusidine (**10**, 7 mg) with the latter system containing 0.1% NH_4OH; all three glycosides

were obtained as colorless oils.



Erylusamine TA (**8**): R_1=Ac, R_2=$CH_2CH_2CH_2CH_2CH_2NMe_2$, R_3=H, n=8, m=2.
Erylusine (**9**): R_1=Ac, R_2=$CH_2CH_2CH_2NMeCH_2CH_2CH_2CH_2NMe_2$, R_3=H, n=8, m=2.
Erylusidine (**10**): R_1=H, R_2=$CH_2CH_2CH_2CH_2NHC{=}NH(NH_2)$, R_3=$COCH_2CH(CH_3)_2$, n=8, m=3.

Erylusamine TA (**8**) had a molecular formula of $C_{54}H_{100}N_2O_{20}$, as supported by both FABMS and NMR data (Table 1). The IR bands at 3359, 1736, 1704 and 1642 cm^{-1} implied the presence of hydroxyl, ester, ketone and amide groups which were further confirmed by the NMR data. The ^1H NMR spectrum of **8** (500 MHz, CD_3OD:$CDCl_3$ 1:1) contained one primary methyl (δ 0.88t) and a huge methylene envelope at δ 1.30, suggesting the presence of a long aliphatic chain(s). Three singlet methyls at δ 2.06 (3H) and δ 2.67 (6H) were assigned as one acetate and two equivalent N-methyl groups, respectively, on the basis of ^{13}C NMR and the HMQC[7] data [δ 171.0, 20.0 (acetate) and 42.4 (N-methyl)]. The NMR spectra also revealed 14 oxygenated methines and 3 oxygenated methylene groups (δ_H 3.16-4.27 and δ_C 60.6-86.5), together with three anomeric methines at δ 4.42, 4.53 and δ 4.73 which were attached to carbons resonated at δ 102.5, 103.8 and δ 103.0, respectively, thus suggesting the presence of three sugar units.

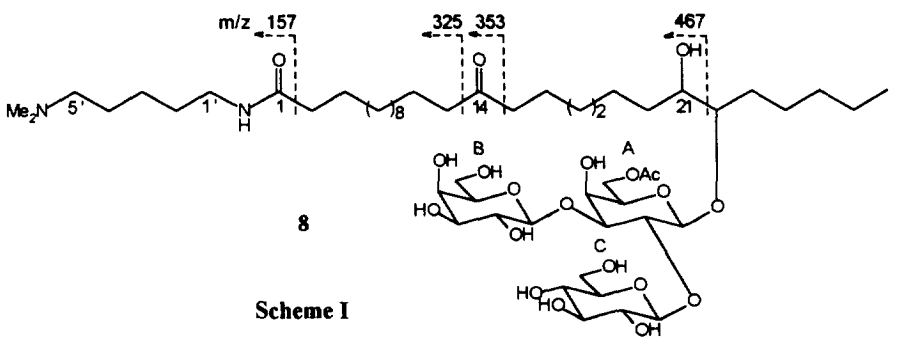
The structure of the trisaccharide portion was deduced from NMR data. Starting from the three anomeric protons, analysis of the COSY spectrum revealed the presence of three pyranose rings designated A-C. Rings A and B were assigned as galactopyranose and ring C as glucopyranose, on the basis of vicinal coupling constants (Table 1) and ROESY[8] correlations; correlations between the following protons: H1Aax/H3Aax, H1Aax/H5Aax, H4Aeq/H3Aax, H4Aeq/H5Aax, H1Bax/H3Bax, H1Bax/H5Bax, H4Beq/H3Bax, H4Beq/H5Bax, H1Cax/H3Cax and H1Cax/H5Cax. All the anomeric protons were axial as judged from vicinal coupling constants of 7.5Hz and 8.0Hz[9]. Coupling constants of overlapping proton signals were determined by 1D TOCSY experiments[10]. The hydroxyl group on C6 in ring A was acetylated, which was readily substantiated by the deshielded H_2-6 proton signal (δ 4.27). The latter protons showed, as expected, an HMBC[11] correlation to the carbon of δ 171.0. The linkage of the sugar units among themselves was established by HMBC cross peaks H1B/C3A and H1C/C2A.

The structure of the aglycone was deduced from extensive 2D NMR experiments together with FABMS data. Starting from an exchangeable amide proton at δ 7.8 (brs, NH-1), five continuous methylenes (C1′-C5′) were inferred from the COSY spectrum. An HMBC correlation between the NMe_2 protons and C5′ completed the suggested N,N-dimethylpentanediamine unit. The latter unit was fully confirmed by the *m/z* 157 fragment (Scheme I). An HMBC correlation of the protons on C1′ with the carbon resonating at δ 174.8, established the linkage to the long chain fatty acid.

The ^{1}H and ^{13}C NMR data of the aglycone contained, in addition to the sugar unit resonances, two methineoxy groups (δ_H 3.32, 3.52, and δ_C 86.5, 73.0, respectively) a methylene chain(s) (δ 1.30-1.64), a primary methyl (δ 0.88t), an amide (δ 174.8), and a ketone (δ 213.0), in addition to the N,N-dimethylpentanediamine unit. Although the position of the ketone and the two oxygenated methines was not clearly defined by NMR experiments, this problem was solved by the FAB mass spectrum which revealed the gross structure as shown in Scheme I. The presence of two vicinal methineoxy groups was inferred from the COSY experiment and the cleavage of the C21-C22 bond (*m/z* 467). Furthermore, fragment ions at *m/z* 353 and 325 were derived from the fission on both sides of the ketone group, allowing placement of the ketone on C14. Because of the presence of a dimethylamine group at the end of a long aliphatic chain, charge-remote fragmentation was observed in the FAB mass spectrum[12]. Of special importance was a large fragment ion at *m/z* 569 corresponding to the entire aglycone.

The position of the glycoside linkage to the fatty acid moiety was based on an HMBC cross peak H1A/C22 and the *m/z* 467 fragment.

Erylusamine TA (**8**) has a similar structure to erylusamines A-E(**3-7**) reported earlier by Fusetanis's group[5] *vide supra*.



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Scheme I

Erylusine (**9**) had a molecular formula of $C_{57}H_{107}N_3O_{20}$ as revealed by both FABMS and NMR data. The ^{1}H NMR spectrum of **9** (Table 2) was very similar to that of **8** except for the presence of an additional singlet methyl at δ 2.60 which was assigned as N-methyl group on the basis of the ^{13}C NMR and HMQC data (δ 38.8).

Interpretation of the COSY, HMQC and HMBC spectra of **9** allowed assignments of three pyranose rings. The carbon chemical shifts and coupling constants of the three pyranose protons (Table 2) suggested that the structure of the trisaccharide moiety in **9** was identical to that in **8**.

An ω-N,N-dimethylaminobutyl-N-methyl-1,3-propanediamine unit was assigned from the COSY spectrum, integration of the ^{1}H NMR spectrum and HMBC cross peaks 7'NMe/C7', 3'NMe/C3' and C4'. In addition, the protons on C1' correlated with a carbon at δ 172.6. Unlike **8**, whose aglycone gross structure was obtained from the FABMS, the FAB mass spectrum of **9** was not informative. Tentatively, on the basis of similar NMR data of **8** and **9**, we suggest that the rest of the aglycone structure of **9** is the same as that of **8**. Furthermore, the HMBC cross peak H1A/C22 disclosed the linkage of the trisaccharide unit to the oxygenated methine C22.

Erylusidine (**10**) had a molecular formula of $C_{56}H_{104}N_4O_{20}$, as was indicated by both the FABMS and NMR data. The NMR spectra of **10** (Table 3) were very similar to that of **8** except for the absence of an acetate and the N,N-dimethyl group, and the presence of a guanidine moiety suggested by a positive

Sakaguchi test, and confirmed by a quaternary carbon signal at δ 156.7 ppm in the ^{13}C NMR spectrum. Furthermore, the NMR data also suggested the presence of an isobutyl ester which was elucidated on the basis of COSY, HMQC and HMBC data (δ_H 0.99, 2.10, 2.25 and δ_C 21.5, 25.3, 43.0, 173.0).

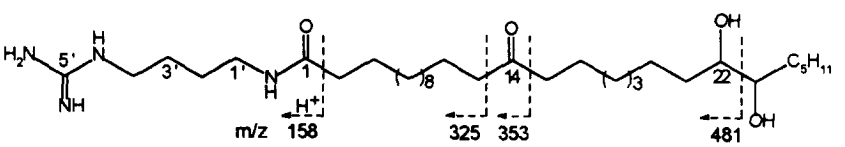
Interpretation of the NMR data suggested that the trisaccharide moiety in **10** differs from the trisaccharide moiety in **8** and **9**, in the absence of the acetate group on C6 in ring A.

The presence of a 4-(aminobutyl)guanidine (agmatine) moiety was revealed by COSY cross-peaks, HMBC correlations and FABMS fragmentations of diol **11** which was obtained together with **12** upon mild acid hydrolysis of **10** (Scheme II). The HMBC cross peak H_2-1′/C1 indicated that the agmatine moiety was attached at C1 through an amide bond. The position of the ketone and the two oxygenated methines were deduced by FABMS data of **11**. Due to the presence of a guanidine group at the end of a long aliphatic chain, charge-remote fragmentation was observed in the FAB mass spectrum of **3** and **4**[12].

HMBC cross peaks H1A/C23 and H_2-22/C1″ disclosed the linkage of the trisaccharide unit to the oxygenated methine C23 and of the isobutyl ester to the oxygenated methine C22.

It is important to mention that all three new compounds (**8-10**) were accompanied by small amounts of other homologues which could not be completely removed from the major three compounds by CCC and HPLC chromatographies. Thus, the molecule ion of compound **8** (MH^+ 1097) was accompanied by small peaks at *m/z* 1083 and 1111 and the aglycone *m/z* 569 by 555 and 583. Similarly, erylusine (**9**) (MH^+ 1154) was accompanied by *m/z* 1126, 1140 and 1168 and erylusidine (**10**) (MH^+ 1153) by *m/z* 1167 (and its hydrolysis, aglycone, product *m/z* 667 by 681).

As we could not obtain single homologues, the three new compounds have not yet been bio-tested again for their cytotoxicity. Such a study is now underway.



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Experimental

General experimental procedures - IR spectra was recorded on a Nicolet 205 FT-IR spectrometer. Optical rotations were measured for solutions in MeOH with a Perkin-Elmer 141 polarimeter with a 10 cm microcell. NMR spectra were taken with a Bruker ARX-500 spectrometer operating at 500 and 125 MHz for ^1H NMR and ^{13}C NMR, respectively. Chemical shifts were referenced to solvent peaks: δ_H 3.30 and δ_C 49.0 for CD_3OD, δ_H 2.49 and δ_C 39.5 for DMSO-d_6. Mass spectra were recorded on a VG Autospec M250Q mass spectrometer DTT/DTE (Magic bullet), was used as a matrix in the FAB mass spectra counter current chromatography (CCC) was performed on a P.C. INC apparatus Model: HSCCC SER #401.

Collection, extraction and isolation - The sponge (425gr, wet weight) *Erylus* sp. was collected off Dahlak archipelago, the Red Sea, and kept frozen until used. The sponge was tentatively identified as *Erylus cf. lendenfeldi*. It does not match the description of *E. proximus*, the second known *Erylus* known from the Red Sea, and it differs slightly from *E. lendenfeldi* by the appearance of the surface. A voucher specimen is kept at the Zoological Museum, Tel Aviv University (ZMTAU E172). The sponge was extracted with MeOH:EtOAc (1:1). After evaporation of the solvent, the residue (2.5 gr out of 25 gr),

Table 1: NMR Data for Erylusamine TA (8)[a]

No.	^{13}C	^{1}H	COSY (H to H)	HMBC (H to C)	No.	^{13}C	^{1}H	COSY (H to H)	HMBC (H to C)
1	174.8s								
2	35.7t	2.17(t,7.5)	3	1,3,4	3A	82.7d	3.73(brd,9.6)	2A,4A	
3	25.3t	1.55m	2,4	1	4A	67.9d	4.08(brd,2.8)	3A,5A	
4-11	28.8t	1.30brs			5A	71.4d	3.73m	4A,6A	
12	23.2t	1.47m	11,13	14	6A	63.5t	4.27m	5A	
13	42.1t	2.43(t,7.5)	12	11,12,14	6A Ac	20.0q,	2.06s		
14	213.0s					171.0s			
15	42.1t	2.43(t,7.5)	16	14,16,17					
16	23.2t	1.47m	15,17	14	1B	103.8d	4.53(d,7.5)	2B	3A
17-19	28.8t	1.30s			2B	71.0d	3.60(dd,8.0,9.5)	1B,3B	
20	31.2t[b]	1.33m, 1.47m	19,21		3B	73.0d	3.45(dd,3.5,9.5)	2B,4B	
21	73.0d	3.52m	20,22		4B	68.4d	3.86brs	3B,5B	
22	86.5d	3.32m	21,23		5B	74.7d	3.52m	4B,6B	
23	31.5t[b]	1.47m, 1.60m	22,24		6B	60.7t[d]	3.73m	5B	
24-26	28.8t	1.30brs							
27	14.0q	0.88(t,7.0)	26	25/26	1C	103.0d	4.73(d,8.0)	2C	2A
1NH		7.8brs[c]			2C	73.6d	3.16(t,8.0)	1C,3C	
1′	37.6t	3.20(t,6.5)	2′	1,2′/3′	3C	76.2d	3.32(t,8.8)	2C,4C	
2′	23.2t	1.47m	1′		4C	68.7d	3.34(t,9.2)	3C,5C	
3′	23.2t	1.47m	4′	5′	5C	76.2d	3.32(brd,10.5)	4C,6C	
4′	22.6t	1.64m	3′,5′		6C	60.6t[d]	3.37m, 3.86m	5C	
5′	57.2t	2.98(t,7.5)	4′	3′/4′,5′NMe_2					
5′NMe_2	42.4q	2.67s		5′					
1A	102.5d	4.42(d,8.0)	2A	22					
2A	77.6d	3.86(t,9.2)	1A,3A						

a) CD_3OD:$CDCl_3$ (1:1); coupling constants, in parenthesis, are given in Hz.

b,d) Values can be interchanged.

c) Value in DMSO-d_6.

Table 2: NMR Data for Erylusine (9)[a]

No.	^{13}C	^{1}H	COSY (H to H)	HMBC (H to C)	No.	^{13}C	^{1}H	COSY (H to H)	HMBC (H to C)
1	172.6s				1A	102.6d	4.33(d,7.6)	2A	22
2	35.5t	2.05(t,7.4)	3	1,3,4	2A	77.0d	3.68m	1A,3A	
3	25.4t	1.45m	2,4	1,2	3A	81.7d	3.66m	2A,4A	
4-11	29.0t	1.21brs			4A	61.8d[c]	3.84brs	3A,5A	
12	23.4t	1.42m	11,13	13	5A	71.6d	3.66m	4A,6A	
13	41.9t	2.36(t,7.1)	12	11,12,14	6A	63.9t	4.05m,4.10m	5A	
14	210.9t				6AAc	20.7q,	1.98s		
15	41.9t	2.36(t,7.1)	16	14,16,17		171.0s			
16	23.4t	1.42m	15,17	15					
17-19	29.0t	1.21brs			1B	104.2d	4.43(d,7.5)	2B	3A
20	31.1t[b]	1.40m, 1.50m	19,21		2B	71.1d	3.35m	1B,3B	
21	71.6d	3.38m	20,22		3B	73.5d	3.25m	2B,4B	
22	85.0d	3.25m	21,23		4B	68.2d[c]	3.64m	3B,5B	
23	31.5t[b]	1.30m, 1.40m	22,24		5B	75.5d	3.32m	4B,6B	
24-26	29.0t	1.21brs			6B	60.4t	3.48m	5B	
27	14.0q	0.83(t,7.0)	26	25/26					
1NH		7.9(t,5.1)			1C	102.8d	4.61(d,7.7)	2C	2A
1′	36.0t	3.08m	2′	1,2′,3′	2C	74.1d	2.91m	1C,3C	
2′	24.2t	1.77(quintet,6.5)	1′,3′	1′,3′	3C	76.7d	3.15m	2C,4C	
3′	53.2t	2.91m	2′		4C	70.1d	3.05m	3C,5C	
4′	54.4t	2.94m	5′	5′/6′	5C	76.7d	3.10m	4C,6C	
5′	21.4t	1.69brs	4′	7′	6C	61.1t	3.46m,3.66m	5C	
6′	21.4t	1.69brs	7′	7′					
7′	56.0t	2.98m	6′	5′/6′,7′NMe_2					
3′NMe	38.8q	2.60s		3′,4′					
7′NMe_2	42.2q	2.66s		7′					

a) DMSO-d_6; coupling constants, in parenthesis, are given in Hz.

b,c) Values can be interchanged

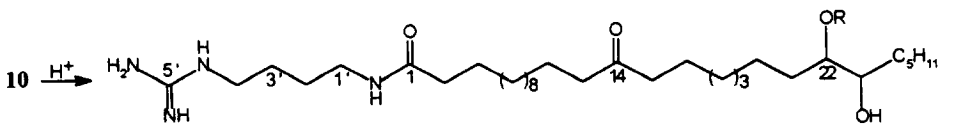
Table 3: NMR Data for Erylusidine (10)[a]

No.	^{13}C	^{1}H	COSY (H to H)	HMBC (H to C)	No.	^{13}C	^{1}H	COSY (H to H)	HMBC (H to C)
1	174.8s				3"	25.3d	2.10m	2",4",5"	4",5"
2	35.6t	2.20(t,7.4)	3	1,3,4	4",5"	21.4q	0.99(d,6.6)	3"	2",3"
3	25.3t	1.60m[b]	2.4	1					
4-11	29.0t	1.30brs			1A	101.9d	4.49(d,7.8)	2A	23
12	23.2t	1.55m	11,13		2A	75.4d	3.88(t,7.6)	1A,3A	
13	42.0t	2.45(t,7.2)	12	14	3A	83.8d	3.79(dd,3.1,9.6	2A,4A	
14	213.0s				4A	67.7d	4.17brs	3A,5A	
15	42.0t	2.45(t,7.2)	16	14,16,17	5A	73.8d	3.55m	4A,6A	
16	23.2t	1.55m	15,17		6A	60.9t[d]	3.77m	5A	
17-20	29.0t	1.30brs							
21	29.7t	1.55m	20,22		1B	103.9d	4.57(d,7.7)	2B	3A
22	73.9d	4.97(brtd, $\Delta w_{1/2}$=9.2)	21,23	1"	2B	71.1d	3.64(dd,6.4, 10.0)	1B,3B	
23	80.1d	3.67m	22,24		3B	73.2d	3.48(dd,3.5,9.5)	2B,4B	
24	31.1t	1.50m,1.55m	23,25		4B	68.5d	3.88brs	3B,5B	
25-27	29.0t	1.30brs			5B	75.0d	3.55m	4B,6B	
28	14.0q	0.91(t,7.0)	27	26/27	6B	60.2t[d]	3.77m	5B	
1NH		7.82brt[c]	4'						
1'	37.8t	3.25m	2'	1,2'/3'	1C	102.1d	4.84(d,8.0)	2C	2A
2'	25.3t[b]	1.60m	1'		2C	74.0d	3.13(t,8.6)	1C,3C	
3'	25.4t[b]	1.60m	4'		3C	76.6d	3.36m	2C,4C	
4'	40.3t	3.24m	3'	2'/3',5'	4C	70.6d	3.19(t,9.3)	3C,5C	
5'	156.7s				5C	76.1d	3.32m	4C,6C	
4'NH		7.60brt[c]			6C	62.0t	3.60(dd,7.1,11.9)	5C	
5'NHNH$_2$		6.8-7.5br					3.86(d,11.9)	5C	
1"	173.0s								
2"	43.0t	2.25(d,5.7)	3"	1",4",5"					

a) $CD_3OD:CDCl_3$ (2:1); coupling constant, in parenthesis, are given in Hz.

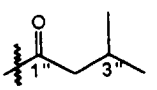
b,d) Values can be interchanged

c) Value in DMSO-d_6



Scheme II

11 R=H : **12** R=



was partitioned between aq. MeOH and petroleum ether, CCl_4 and $CHCl_3$. The $CHCl_3$ soluble (70 mg) was subjected to counter current chromatography three times under different conditions a) with $CHCl_3$-MeOH-H_2O (5:5.7:3) to give erylusamine TA (**8**, 6 mg), b) with $CHCl_3$-MeOH-nPrOH-H_2O (5:6:1:4) to give erylusine (**9**, 10 mg) out of the stationary phase, and c) with $CHCl_3$-MeOH-nPrOH-H_2O (5:6:1:4, containing 0.1% NH_4OH) to give erylusidine (**10**, 7 mg).

Erylusamine TA (8) - Colorless oil, $[\alpha]_D^{25}$+ 2.8^0 (c=2.9, MeOH); IR(neat): ν_{max} 3359, 2920, 2851, 1736, 1704, 1642, 1554, 1467, 1413, 1375, 1264, 1156, 1125, 1076, 1037 cm^{-1}.

FABMS: *m/z* 1097 $(M+H)^+$, 569, 551, 467, 437, 423, 409, 395, 381, 367, 353, 325, 311, 297, 283, 269, 255, 241, 227, 213, 199, 185, 171, 157, 100, 86, 72, 58, 44.

Erylusine (9) - Colorless oil, $[\alpha]_D^{25}$+ 1.9^0 (c=4.3, MeOH); IR(neat): ν_{max} 3384, 2921, 2852, 1736, 1705, 1642, 1556, 1467, 1413, 1372, 1264, 1156, 1125, 1075, 1038 cm^{-1}.

FABMS: *m/z* 1154 $(M+H)^+$, 129, 100, 86, 72, 58, 44.

Erylusidine (10) - Colorless oil, $[\alpha]_D^{25}$- 4.1^0 (c=4.7, MeOH); IR(neat): ν_{max} 3358, 2927, 2855, 1736, 1708, 1654, 1560, 1459, 1371, 1164, 1137, 1081, 1056 cm^{-1}.

FABMS: *m/z* 1153 $(M+H)^+$, 325, 311, 297, 283, 269, 255, 241, 227, 213, 199, 185, 172, 157, 130, 114, 100, 86, 72, 58, 57.

Acid hydrolysis of Erylusidine (10): Erylusidine (**10**, 3 mg) was dissolved in 2N HCl-MeOH (3 mL) and the mixture was heated at refluxing temperature for 2h. The reaction mixture was neutralized with NH_4OH(aq) and then evaporated to dryness and partitioned between H_2O and EtOAc. The EtOAc layer was evaporated and subjected to vacuum liquid chromatography over silica gel (eluted with MeOH-EtOAc 1:9) to afford a mixture of **11** and **12** (0.5 mg, which weren't separated).

11+12: ^{1}H NMR (DMSO-d_6) δ 0.84 (3H,t, J=7.0Hz), 0.90 (6H, d, J=6.6 Hz), 1.23brs, 1.43br, 2.00 (1H,m), 2.03(2H,t, J=7.4 Hz), 2.16(2H,d, J=7.2Hz), 2.36(4H,t, J=7.4Hz), 3.05(2H,m), 3.10 (2H,m), 4.10(1H,m), 4.55(1H,t, J=5.2Hz), 4.61(1H,d, J=5.3Hz), 4.72(1H,m), 7.40(3H,br), 7.68(1H,brt), 7.81(1H,brt).

FABMS: *m/z* 667 $(M+H)^+$, 583 $(M+H)^+$, 481, 451, 437, 423, 409, 395, 381, 367, 353, 325, 311, 297, 283, 269, 255, 241, 227, 213, 199, 185, 172, 158, 130, 114, 100, 86, 72, 58, 57.

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