Regulosides A–C: Glycosphingolipids from the Starfish *Pentaceraster regulus*[*]

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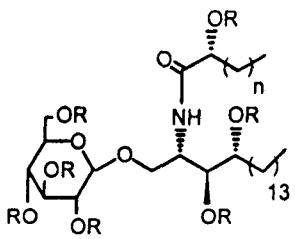
The glycosphingolipid (GSL) composition of the starfish *Pentaceraster regulus* has been studied. The structure of the major component, a new cerebroside named Reguloside A (**1**) and two minor homologues, Regulosides B (**1a**) and C (**1b**), are described.

Monoglycosyl phytosphingosine-type ceramides containing branched and unbranched long chain bases (LCB) of C-16 to C-22 chain length with galactose and glucosamine as sugar resicue have been reported from the sponges *Agelas mauritiana*[1] and *Amphimedon virdis*[2], respectively. The β-glucopyranosides of the same LCB have also been reported from the starfish *Asterias rubens*[3], *Asteriana pectinifera*[4] and *Acanthaster planci*[5]. Glucopyranosides of phytosphingosine-type ceramides with unbranched C-18 LCB, and 2-hydroxy docosanoic acid as *N*-acyl moiety, appear not to have been reported previously.

We report for the first time a new cerebroside named reguloside A, from *Pentaceraster regulus* found along the Indian coast. Reguloside A has shown moderate wound-healing activity[**].



	R	n
Reguloside A (**1**)	H	19
Reguloside B (**1a**)	H	20
Reguloside C (**1b**)	H	21
2	Ac	19

The methanolic extract was fractionated with *n*-butyl alcohol to give *n*-butyl alcohol soluble and insoluble fractions. The *n*-butyl alcohol soluble fraction was then treated with acetone to remove non-polar lipids. Column chromatography of the acetone-insoluble part yielded the cerebroside mixture PR-1, which on further purification by reversed phase chromatography afforded compound **1** as a colourless amorphous powder. The negative-ion FABMS of **1** displayed a molecular-ion peak at *m/z* 816 $[M - H]^+$, consistent with the molecular formula $C_{46}H_{91}NO_{10}$. The IR spectrum shows absorption bands at 3400, 1040 (hydroxy), 1642, 1538 (amide), 2922 (aliphatic) and 1074 cm^{-1} (glycosidic), which are characteristic features of glycosphingolipids. The fatty acid amide nature of **1** was also evidenced by the ^{1}H-NMR spectrum, which showed an NH doublet at δ = 8.58 (J = 8.2 Hz), and signals for the aliphatic methylene protons at δ = 1.25 as a broad singlet and the methyl protons as a triplet, resonating at δ = 0.85 (J = 7.8 Hz).

The ^{13}C-NMR spectrum of **1** further supported the presence of the amide functionality, as shown by the carbon signals resonating at δ = 175.75 and 51.67. In addition, a signal of an anomeric carbon appears at δ = 106.16 and the corresponding proton signal is observed as doublet at δ = 4.89 in its ^{1}H-NMR spectrum, indicating that the compound is a cerebroside.

The presence of hexose as a sugar residue in **1** is suggested by the fragment ion peak at *m/z* 654 $[(M - H)^+ - 162]$, which arises due to loss of a hexose unit from the molecular ion peak at *m/z* 816 $[M - H]^+$. Moreover, in the ^{13}C-NMR spectrum of **1** the carbon signals resonating at δ = 106.16, 75.26, 77.11 (2 C), 70.29 and 62.42 suggest that the hexose has β-gluco configuration[6], This is further supported by the coupling constant of 1-H″ (δ = 4.89 d, J = 8.0 Hz) and by NOE experiments. Carbon signals characteristic of a phytosphingosine-type ceramide[7] containing a 2-hydroxy fatty acid are observed at δ = 70.60, 51.67, 75.84, 72.80 (C-1 to C-4) and 175.75, 72.59 (C-1′, C-2′). Acetylation of **1** with Py/Ac_2O led to the acetyl derivative **2**. This showed a molecular ion peak at 1110 $[M - H]^+$, suggesting the presence of seven free hydroxyl groups in **1**. Out of seven free hydroxyl groups in **1**, four are accounted for by hexose and three by the ceramide part.

The position of the free hydroxyl groups in the ceramide part was assigned by ^{1}H–^{1}H COSY and DQF COSY experiments. The NH doublet appears at δ = 8.58 and shows coupling with the multiplet at δ = 5.28 assigned to

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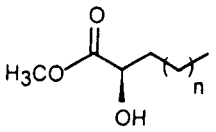
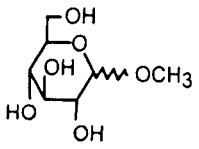
2-H protons, and the latter in turn couples with the triplet at δ = 4.30. This triplet and the two double doublets at δ = 4.48 and δ = 4.78 are attributed to 3-H, 1-Ha and 1Hb protons, respectively. Further the carbinol proton signal at δ = 4.30 (3-H) shows correlations with 2-H and another carbinol proton at δ = 4.21 assigned to 4-H; the latter protons also show coupling with the multiplet at δ = 1.70, which is assigned to the methylene protons at C-5. The remaining carbinol protons resonating at δ = 4.56 did not show correlations with any of the above-mentioned carbinol protons, but the cross peaks observed between δ = 4.56 and δ = 1.90 (m) suggest that the hydroxy group is probably present in the *N*-acyl moiety as a part of the hydroxy fatty acid.



3	n
a	18
b	19
c	20



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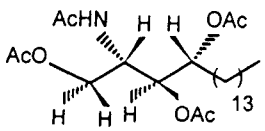
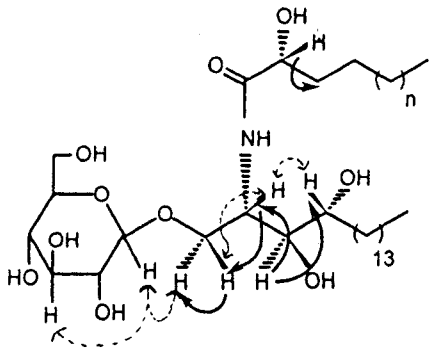


LCB (5)

Methanolysis of **1** by the Gaver–Sweeley method[8] afforded fatty acid methyl ester(s) (FAME), methyl glucoside and a long chain base (LCB). The FAME (m.p. 54°C, $[\alpha]_D^{27}$ = −2.9) was analysed by GC/MS and found to be methyl (2*R*)-2-hydroxydocosanoate (**3a**) as the major component, with minor homologues by spectral data[9] and optical rotation[4]. The methyl glucoside was identified as 1-*O*-methyl-β-D-glucopyranoside (**4**) by COTLC, m.p. 193–194°C, $[\alpha]_D^{27}$ = +72.0, and the spectral data are identical with an authentic sample[6]. The LCB fraction was acetylated with Py/Ac_2O to obtain a tetra-*O*-acetyl derivative as a colourless oil $[\alpha]_D^{27}$ = +25.6. The positive FABMS of the LCB shows the molecular ion peak at *m/z* 486 $[M + H]^+$, consistent with the molecular formula $C_{26}H_{47}NO_7$, and it was identified as 2-acetamino-1,3,4-tri-*O*-acetyloctadecane[4] by comparing the spectroscopic data of 2-acetamino-1,3,4-triacetoxyhexadecane[10]. The stereochemistry at C-2, C-3, C-4 of (LCB)[5] was unambiguously assigned as D-(+)-*ribo*-(2*S*,3*S*,4*R*) by comparing the optical rotation[11] and spectroscopic data[12] reported for the D-(+)-*ribo* isomer of 2-acetamino-1,3,4-tri-*O*-acetoxyhexadecane.



^1H-^1H COSY (↷) and NOE (⤳) correlations of **1**

Thus, the structure of **1** was deduced to be 1-*O*-β-D-glucopyranosyl-D-(+)-*ribo*-(2*S*,3*S*,4*R*)-2-[(2′*R*)-2′-hydroxydocosanoyl)amido]-1,3,4-octadecanetriol.

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Experimental Section

IR: Perkin-Elmer 881. – Mass spectra: JEOL JMS-D-300 (70 eV). – ^1H, ^{13}C NMR: Bruker WM 400 MHz – ^1H–^1H COSY, DQF COSY, HMQC: Bruker DRX 300 MHz equipped with an Aspect 2000 computer, using TMS as internal reference. – Optical rotations: Autopol-III automatic polarimeter in $CHCl_3$ or pyridine. – Flash chromatography: EE 10 (EYELA) A.S. C. equipment with fraction collector using silica gel (230–400 mesh).

The animal material (2 kg) was collected at Rameshwaram on the southern coast of India in 1989, and was brought to the laboratory dipped in methanol. The specimens of starfish *P. regulus* were dried in the shade and extracted with methanol (2 × 2.5 l) to yield a methanolic extract (D001, 100 g). The *n*-butyl alcohol soluble fraction (20 g) obtained from the methanolic extract was treated with acetone to remove non-polar lipids; the insoluble part (3.5 g) thus obtained was subjected to column chromatography over silica gel (60–120 mesh), eluting with $CHCl_3$/MeOH (9:1–7:3) and 100-ml fractions were collected. Similar fractions were combined after monitoring by TLC [solvent system: $CHCl_3$/MeOH (20%)] to obtain a crude cerebroside mixture PR-1 (1.10 g), which on further purification by flash chromatography followed by reversed phase chromatography over C_{18} silica gel [solvent system: MeOH–H_2O (2%)] afforded compound **1** as a colourless amorphous powder (700 mg), m.p. 218–219°C, $[\alpha]_D^{27}$ +10.0 (*c* = 0.05, pyridine). – IR (KBr): $\tilde{\nu}$ = 3400 cm^{-1}, 2922, 1642, 1538, 1460, 1074, 1040 cm^{-1}. – Negative FABMS: *m/z* 816 (molecular ion peak, $[M - H]^+$, 654 $[(M - H)^+ - 162]$, 395, 338, 198. – ^1H and ^{13}C NMR (C_5D_5N): δ = see Table 1. – $C_{46}H_{91}NO_{10} \cdot 2\,H_2O$ (854.2): calcd. C 64.68, H 11.21, N 1.64; found C 64.59, H 11.06, N 1.61.

Acetylation of **1**: 100 mg of **1** was acetylated with dry pyridine (1 ml) and Ac_2O (1.5 ml), refluxed for 3 h at 80°C and left for about 12 h. After usual workup the crude reaction product was purified on a silica-gel column [solvent system: hexane/ethyl acetate

Table 1. ^1H-[a] and ^{13}C[b]-NMR spectral data of **1** in C_5D_5N

Position	$^1H(\delta)$ (J in Hz)	$^{13}C(\delta)$	COSY correlations
Long chain Base (LCB)			
1a	4.48 (dd, 1H, J = 6.4, 10.4)	70.60	1b, H-2
1b	4.78 (dd, 1H, J = 6.4, 10.5)		1a, H-2
2	5.28 (m, 1H)	51.67	1a, 1b, H-3, N-H
3	4.30 (t, 1H, J = 8.0)	75.84	H-2, H-4
4	4.21 (dt, 1H, J = 8.6, 3.2)	72.80	H-3, H-5
5	1.70 (m, 2H)	35.59	H-4
CH_3	0.85 (t, 3H, J = 7.8)	14.33	–
NH	8.58 (d, J = 8.2)		H-2
N-Acetyl moiety			
1′	–	175.75	
2′	4.56 (dd, 1H, J = 3.6, 8.04)	72.59	H-3′
3′	1.90 (m, 2H)	39.33	H-2′
Sugar moiety			
1″	4.89 (d, 1H, J = 8.0)	106.16	H-2″
2″	4.0 (dd, 1H, J = 8.0, 9.2)	75.26	H-1″, H-3″
3″	4.40 (dd, 1H, J = 8.8, 9.2)	77.11	H-2″, H-4″
4″	4.52 (dd, 1H, J = 8.8, 9.2)	70.29	H-3″, H-5″
5″	3.85 (ddd, 1H, J = 9.2, 5.3, 2.5)	77.11	H-4″, H-6″a,6″b
6″a	4.53 (dd, 1H, J = 5.3, 11.6)	64.42	H-5″, H-6″b
6″b	4.11 (dd, 1H, J = 2.5, 11.8)		H-5″, H-6″a

[a] Assigned by $^1H-^1H$ COSY, HMQC. – [b] Assignments were made by DEPT spectroscopy.

(9:1–8:2)] to yield the peracetyl derivative **2** as a colourless syrup, $[\alpha]_D^{27}$ = +18 (c = 0.05, $CHCl_3$). – Negative FABMS: 1110 (M – H)$^+$, 780 [(M – H)$^+$ – 331]. – ^1H NMR ($CDCl_3$): δ = 0.85 (t, J = 7.2 Hz, CH_3), 1.30 (m, 30 × CH_2), 1.60 (m, 5-H), 1.85 (m, 3′-H), 2.0–2.30 (s, 7 × $COCH_3$), 3.68 (dd, J = 10.5 and 3.3 Hz, 1-H), 3.86 (dd, J = 10.5 and 2.8 Hz, 1-H), 3.92 (m, 5″-H), 4.12 (dd, J = 4.6, 11.8 Hz, 6″-H), 4.26 (dd, J = 1.7, 11.8 Hz, 6″-H), 4.29 (m, 2-H), 4.43 (d, J = 7.8 Hz, 1″-H), 4.89 (m, 4-H), 5.01 (dd, J = 8.6, 9.2 Hz, 4″-H), 5.07 (m, 2′-H), 5.10 (dd, J = 7.8 and 9.2 Hz, 2″-H), 5.16 (dd, J = 9.0 and 3.3 Hz, 3-H), 5.38 (dd, J = 8.6 and 9.2 Hz, 3″-H), 6.85 (d, J = 8.0 Hz, NH). – ^{13}C NMR ($CDCl_3$): δ = 14.01 (CH_3), 66.20 (t, C-1), 48.03 (d, C-2), 71.88 (d, C-3), 73.14 (d, C-4), 27.38 (t, C-5), 100.58 (d, C-1″), 68.84 (d, C-2″), 70.73 (d, C-3″), 66.92 (d, C-4″), 70.73 (d, C-5″), 61.01 (t, C-6″), 73.84 (d, C-2′); 29.33 (t, C-3′), 171.03 (s, C-1′), 169.37–170.22 (s, 7 × $COCH_3$).

Methanolysis of **1**: 100 mg of **1** was refluxed[8] in 0.9 N HCl with 82% aq. MeOH (10 ml) for 18 h. The reaction mixture was extracted with n-hexane; the hexane layer thus obtained was concentrated and chromatographed on a silica-gel column [solvent system: hexane/ethyl acetate (7:3)] to yield the fatty acid methyl ester(s) (FAME) **3** (26 mg). The GC-MS of the FAME showed three peaks in a ratio of 9:0.5:0.5 [M$^+$ 370 (R_t = 30.02 min), M$^+$ 384 (R_t = 31.75 min), M$^+$ 398 (R_t = 33.48 min)]. – The preparative TLC of **3** on HPTLC plates [solvent system: MeOH/H_2O (4%)] afforded three fatty acid esters **3a–c**. The aqueous methanolic layer was neutralized with Ag_2CO_3 and evaporated in vacuo to give a brown syrup, which was purified by column chromatography on silica gel (solvent system: $CHCl_3$/MeOH/H_2O (7:3:0.5)] to yield a mixture of α- and β-anomers of methyl glucopyranoside **4** [R_f = 0.65 (β), 0.60 (α), TLC solvent system $CHCl_3$/MeOH/H_2O (12:7:1)], $[\alpha]_D^{27}$ = +72.0 (c = 0.1, MeOH). – EIMS: m/z 194 (M$^+$). The LCB was acetylated with dry pyridine (0.5 ml) and Ac_2O (1.0 ml), then heated at 80°C for 3 h to give the peracetyl derivative of LCB 5.

Methyl (2R)-2-Hydroxydocosanoate (**3a**): Colourless flakes, m.p. 52–53°C, $[\alpha]_D^{27}$ = –3.2 (c = 0.01, $CHCl_3$). – EIMS: m/z 370 (M$^+$), 311 [M – 59 ($OCOCH_3$)]$^+$, 97, 83, 44. – ^1H NMR ($CDCl_3$): δ = 0.87 (t, J = 6.9 Hz, 3H, CH_3), 1.24 (br. s, methylene groups), 3.78 (s, 3H, OCH_3), 4.19 (t, J = 7 Hz, 1H, 2-H). – ^{13}C NMR ($CDCl_3$): δ = 14.03 (q), 22.66 (t), 24.77 (t), 29.32 (t), 29.45 (t), 29.69 (t), 31.92 (t), 34.44 (t), 37.00 (t), 52.31 (q, CH_3), 70.73 (d), 175.81 (s).

Methyl (2R)-2-Hydroxytricosanoate (**3b**): Colourless solid, m.p. 56°C, $[\alpha]_D^{27}$ = –2.8 (c = 0.01, $CHCl_3$). – EIMS: m/z 384 (M$^+$), 325 [M – 59 ($OCOCH_3$)]$^+$, 97, 83, 44. – ^1H NMR ($CDCl_3$): δ = Same as above.

Methyl (2R)-2-Hydroxytetracosanoate (**3c**): Colourless flakes, m.p. 55°C, $[\alpha]_D^{27}$ = –3.1 (c = 0.01, $CHCl_3$). – EIMS: m/z 398 (M$^+$) 111, 97, 83, 44. – ^1H NMR ($CDCl_3$): δ = Same as above.

(2S,3S,4R)-2-Acetamino – 1,3,4-triacetoxyoctadecane (**5**): Colourless oil, $[\alpha]_D^{27}$ 25.6 (c = 0.05, $CHCl_3$). – FABMS: 486 (M + H)$^+$. – ^1H NMR ($CDCl_3$): δ = 0.85 (t, J = 7 Hz, CH_3), 2.1 (s, 3H, $COCH_3$), 2.25 (s, 6H, 2 × $COCH_3$), 2.31 (s, 3H, $COCH_3$), 3.98 (dd, 1H, J = 10.8 and 3 Hz, 1-H), 4.29 (dd, 1H, J = 11 and 4.1 Hz, 1-H), 4.49 (m, 1H, 2-H), 4.93 (m, 1H, 4-H), 5.10 (dt, J = 8 and 2 Hz, 5-H), 6.01 (d, J = 7.8 Hz, NH).

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