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CEREBROSIDES FROM GREEN BRITTLE STAR *Ophiarachna incrassata*

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Echinoderms are rich sources of cerebrosides from marine invertebrates that differ in structure and biological properties from cerebrosides from terrestrial organisms. Unusual cerebrosides have been isolated from starfish, sea cucumbers, and sea urchins [1]. We have for the first time isolated cerebrosides from the green brittle star.

Ophiarachna incrassata was collected in January 2005 in the South China Sea (Van-fong Bay) at a depth of 3 m.

Green brittle star (180 g dry wt.) was extracted twice with ethanol. The extract (3 L) was concentrated in vacuo and chromatographed over a silica-gel column using a $CHCl_3 \rightarrow CHCl_3$:EtOH (1:1)$\rightarrow$EtOH gradient. The fraction eluted by $CHCl_3$:EtOH (1:1) was separated again over a silica-gel column using $CHCl_3$:EtOH:H_2O (65:25:2). The fraction containing cerebrosides was chromatographed over Sephadex LH-20 using $CHCl_3$:EtOH (1:1) to afford total cerebrosides (15 mg, 0.008% of dry animal wt.).

The structures of the cerebrosides were identified using spectral methods (NMR, MALDI-TOF-GC-MS) and chemical transformations.

The PMR and ^{13}C NMR spectra had signals typical of glycosylceramides such as a broad doublet for the amide proton at δ_H 8.64 ppm, *N*-substituted CH (δ_C 51.4 ppm), two types of terminal methyls in a normal chain (δ_H 0.88 ppm and δ_C 14.1 ppm) and an isopropyl group (δ_H 0.86 ppm and δ_C 22.6 ppm). The spectra also exhibited signals for methine/methylene groups bound to oxygen or nitrogen including an anomeric proton of a monosaccharide unit (δ_H 4.98 ppm) and an anomeric C atom (δ_C 105.3 ppm). Strong signals at δ_H 1.20-1.39 ppm and δ_C 29.8-30.5 ppm [$(CH_2)_n$] indicated that hydrocarbon radicals were present. Signals for a carbonyl C (δ_C 175.6 ppm) and carbonyl H (δ_H 4.62 ppm) were consistent with a 2-hydroxy fatty acid.

The chain length of the fatty acids and sphingosine bases were determined by methanolysis.

The results showed that the cerebrosides were *iso*- and *n*-$C_{16:0}$, -$C_{17:0}$-, -$C_{18:0}$-, and -$C_{19:0}$-phytosphingosines acylated at the amino group by 2-hydroxy C_{22}-C_{24}-saturated normal fatty acids and substituted at the hydroxyl in the 1-position by β-glucopyranose [2-5]. The MALDI-TOF mass spectra agreed completely with the proposed structures.

PMR spectrum (500 MHz, C_5D_5N, δ, ppm, J/Hz): 8.64 (d, J = 9.3, NH), 5.31 (overlaps exchangeable water and OH signal, H-2), 4.98 (d, J = 7.7, H-1″), 4.77 (dd, J = 6.6, 10.5, H-1a), 4.62 (dd, J = 3.7, 7.8, H-2′), 4.55 (dd, J = 4.3, 10.5, H-1b), 4.52 (dd, J = 2.6, 11.9, H-6″), 4.37 (dd, J = 6.7, 5.2, H-3), 4.35 (dd, J = 5.3, 11.9, H-6″), 4.17-4.28 (m, H-4, H-3″, H-4″), 4.04 (t, J = 8.0, H-2″), 3.91 (m, H-5″), 2.20 (m, H-3′), 1.98 (m, H-3′), 1.20-1.39 [$(CH_2)_n$], 0.88 (t, J = 7.0, –CH_3), 0.86 [d, J = 6.6, $CH(C\underline{H}_3)_2$].

^{13}C NMR spectrum (125 MHz, C_5D_5N, δ, ppm): 175.6 (C-1′), 105.3 (C-1″), 78.4 (C-3″), 78.2 (C-5″), 75.6 (C-3), 75.0 (C-2″), 72.3 (C-4), 72.2 (C-2′), 7.13 (C-4″), 70.7 (C-1), 62.4 (C-6″), 51.4 (C-2), 22.8 (–$\underline{C}H_2CH_3$), 29.8-30.5 [$(CH_2)_n$], 22.6 [–$CH(\underline{C}H_3)_2$], 14.1 (–CH_3).

MALDI-TOF MS (*m*/*z*, I_{rel}, %): 812 (18), 826 (16), 840 (29), 854 (20), 868 (17) $[M + Na]^+$.

Methanolysis of Cerebrosides Mixture. A mixture of cerebrosides (0.5 mg) was heated with HCl (1 mL, 5%) in MeOH for 4 h at 90°C and extracted with hexane. The hexane layer was concentrated to dryness in vacuo to produce a mixture of fatty acid methyl esters (FAME). The methanol layer that contained a mixture of sphingosine bases and methylglycosides

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was cooled with liquid N_2 and lyophilized. The solid was separated over a column of silica gel [$CHCl_3$→$CHCl_3$:MeOH (1:1)→$CHCl_3$:MeOH:NH_4OH (9:4:1)]. The mixture of methyl-α-glucopyranoside and methyl-β-glucopyranoside was eluted with $CHCl_3$:MeOH (1:1); sphingosine bases, by $CHCl_3$:MeOH:NH_4OH (9:4:1), acetylated by a mixture of acetic acid and pyridine (1:1), and chromatographed over a silica-gel column using a hexane→EtOAc gradient.

FAME. PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 4.19 (m, H-2), 3.79 (s, COOMe), 0.88 (t, J = 7.0, $–CH_3$).

GC—MS Analysis of FAME. Methyl esters were analyzed by GC—MS over a column (HP-5 MS, 30 m × 0.25 mm, Agilent, USA) with a temperature gradient 200-290°C at 2°C/min, vaporizer temperature 260°C, interface temperature 270°C. FAME were identified as 2OH-22:0 (62%), 2OH-23:0 (22%), and 2OH-24:0 (16%).

Sphingosine Base Acetates. MALDI-TOF mass spectrum (CCA matrix, *m*/*z*, I_{rel}, %): 480 (20), 494 (19), 508 (49), 522 (12) $[M + Na]^+$. PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.91 (1H, d, J = 9.4, NH), 5.10 (1H, dd, J = 3.2, 8.2, H-3), 4.93 (1H, dt, J = 3.2, 3.2, 9.9, H-4), 4.47 (1H, m, H-2), 4.29 (1H, dd, J = 4.7, 11.6, H-1), 4.00 (1H, dd, J = 3.1, 11.5, H-1), 2.08 (3H, s, NAc), 2.05 (6H, s, 2 OAc), 2.03 (3H, s, OAc), 1.35-1.2 (br.s), 0.88 (t, J = 7.0, $CH_2C\underline{H}_3$), 0.86 [d, J = 6.7, $CH(C\underline{H}_3)_2$].

Methyl-α-glucopyranoside [methyl-β-glucopyranoside]. PMR spectrum (500 MHz, D_2O, δ, ppm, J/Hz): 4.92 (d, J = 3.6, H-1) [4.49 (d, J = 8.2)], 3.98 (dd, J = 2.4, 12.3, H-6) [4.04 (dd, J = 2.4, 12.3)], 3.87 (dd, J = 5.5, 12.3, H-6) [3.86 (dd, J = 6.0, 12.3)], 3.78 (t, J = 9.4, H-3) [3.61 (t, J = 9.4)], 3.75 (m, H-5), [3.56 (m)], 3.66 (t, J = 3.8, H-2) [3.38 (t, J = 9.0)], 3.51 (s, H-4), [3.50 (s)].

Cerebrosides of this molecular type are widely distributed among marine invertebrates [1]. They possess wound-healing activity [6] and are enzyme inhibitors [4].

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REFERENCES

1. R. X. Tan and J. H. Chen, *Nat. Prod. Rep.*, **20**, 509 (2003).
2. K. Yamada, K. Sasaki, Y. Harada, R. Isobe, and R. Higuchi, *Chem. Pharm. Bull.*, **50**, 1467 (2002).
3. R. Higuchi, M. Kagoshima, and T. Komori, *Liebigs Ann. Chem.*, 659 (1990).
4. A. Loukaci, V. Bultel-Ponce, A. Longeon, and M. Guyot, *J. Nat. Prod.*, **63**, 799 (2000).
5. Y. Kawano, R. Higuchi, R. Isobe, and T. Komori, *Liebigs Ann. Chem.*, 19 (1988).
6. U. Venkannababu, S. P. S. Bhandari, and H. S. Garg, *Liebigs Ann. Recl.*, 1245 (1997).