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AGELASPHINS, NOVEL α-GALACTOSYLCERAMIDES FROM THE MARINE SPONGE *AGELAS MAURITIANUS*

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Abstract: New glycosphingolipids, agelasphins (1-4), have been isolated from the marine sponge *Agelas mauritianus*, and their structures were determined. Agelasphins having antitumor activity are the first examples of galactosylceramides containing an α-galactosyl linkage.

In our quest for bioactive substances from marine organisms, we found novel glycosphingolipids possessing an α-galactosylceramide structure from an extract of the sponge *Agelas mauritianus* Carter (1883).[1] To the best of our knowledge, this is the first report on the isolation of α-galactosylceramides from natural sources. We herein describe the isolation and the structure elucidation of these novel substances, named agelasphins (AGLs).

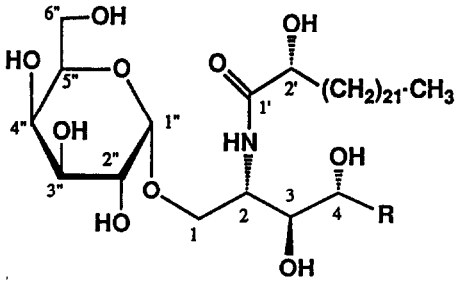
A lyophilized sample of the sponge was extracted with 1:1 $CHCl_3$-MeOH. The residue of the extract was partitioned between EtOAc and water. The EtOAc layer was washed with 30% aq. MeOH to remove polar constituents containing agelasines.[2] The remaining lipophilic portion was repeatedly chromatographed on silica gel to give a fraction which was shown to be virtually a mixture of homologous glycosphingolipids as demonstrated by a series of molecular ion peaks at *m/z* 816, 830, 844, 858, and 872 in the negative FABMS. The IR spectrum of the fraction exhibited strong absorption bands for hydroxyl and amide groups. The ^{13}C NMR spectrum (C_5D_5N) showed, in addition to the amide functionality (δ 175.0, 50.6), the presence of an anomeric carbon (δ 101.2) and a number of aliphatic carbons, suggesting the major portion of the fraction being cerebrosides. The presence of galactose as the common sugar moiety was shown by methanolysis of the fraction followed by acetylation and analysis of the resulting sugar.[3] Optical rotation ($[\alpha]_D^{23}$ +142.3° → +78.2°, H_2O, 24 h) of the free sugar revealed that it was D-galactose. Furthermore, a signal at δ 5.58 (d, J = 3.7Hz) in the ^1H NMR spectrum of the intact fraction indicated the stereochemistry of the anomeric position to be α.

The fraction could be separated using reversed-phase HPLC (ODS, MeOH) to give six major constituents designated AGL-7a (1), -7b, -9a (2), -9b (3), -11 (4), and -13 in 0.0022, 0.0027, 0.0019, 0.0080, 0.0096, and 0.0009% yield, respectively, along with some minor ones. The structures of four (1-4) of the AGLs are reported in this paper. All the compounds gave essentially the same IR absorptions (3400, 2950, 2870, 1645, 1535, 1475, and 1080 cm^{-1}) and ^1H and ^{13}C NMR data except for minor differences.[4] Thus, structure elucidation is described for agelasphin-9b (3) as a representative.

Agelasphin-9b (3) was analyzed for $C_{48}H_{94}NO_{10}$ [$(M-H)^-$ *m/z* 844.6948, Δ6.5mmu] by negative HR FABMS. In the ^1H NMR spectrum in C_5D_5N 3 showed eight exchageable proton signals due to one NH [δ 8.50 (d, J = 9.2 Hz)] and seven OH (broad singlets or doublets in the range of δ 6 - 7.5). The anomeric proton was observed as a doublet (J = 3.7 Hz) at δ 5.59. The coupling constant clearly indicated its β-orientation in the galactose moiety. The NMR data for the core portion of the molecule were assigned by 2D NMR experiments.[4] In methanolysis with aq. HCl-MeOH[5] 3 gave 1-*O*-methylgalactose, methyl 2-(*R*)-hydroxytetracosanoate (5), and a long-chain base. The structure of the ester 5 which could be separated from the reaction mixture by extraction with hexane was identified by spectroscopic data[6] and confirmed including the stereochemistry by synthesis.[7] The mixture of base and sugar was separated on an

Amberlite CG-400 column by eluting with MeOH. The base (phytosphingosine) was acetylated and identified by spectroscopic data to be 2-acetoamino-1,3,4-tri-*O*-acetyl-16-methyl-1,3,4-hexadecanetriol (6),[8] The stereochemistry of 6 was also established by synthesis.[7] The overall structure of 3 was unambiguously established by a total synthesis.[7] The structures for the remaining AGLs were determined by spectroscopic comparison with 3 and methanolytic analysis.

Although a number of β-galactosylceramides have been known, AGLs are the first cerebrosides having an α-galactosyl linkage. All AGLs showed antitumor activity.[9]



	R
Agelasphin-7a (1)	-$(CH_2)_{11}$-CH_3
Agelasphin-9a (2)	-$(CH_2)_{12}$-CH_3
Agelasphin-9b (3)	-$(CH_2)_{11}$-$CH(CH_3)_2$
Agelasphin-11 (4)	-$(CH_2)_{11}$-$CH(CH_3)$-C_2H_5

References and Notes

1. The sponge was collected in Okinawa in 1988 and identified by Dr. Ole Tendal.
2. H. Nakamura, H. Wu, Y. Ohizumi, and Y. Hirata, *Tetrahedron Lett.*, 25, 2989 (1984).
3. Y. Kawano, R. Higuchi, R. Isobe, and T. Komori, *Liebigs Ann. Chem.*, 19 (1988).
4. Full ^1H and ^{13}C NMR data are given for 1, while only signals different from 1 are listed for the rest.
   AGL-7a (1): $[\alpha]_D^{24}$ +52.3° (*c* 1.0, Pyr); mp 193.5-195.0 °C; HR FABMS *m/z* 816.6619 [(M-H)$^-$, calcd. for $C_{46}H_{90}NO_{10}$ 816.6557]; ^1H NMR (500 MHz, C_5D_5N) δ 8.49 (1H, d, *J* = 9.2 Hz, NH), 7.53 (1H, bs, OH), 7.04 (1H, bs, OH), 6.71 (1H, d, *J* = 6.7 Hz, OH), 6.68 (1H, bs, OH), 6.52 (1H, bs, OH), 6.32 (1H, bs, OH), 6.09 (1H, d, *J* = 6.1 Hz, OH), 5.58 (1H, d, *J* = 3.7 Hz, H1"), 5.26 (1H, m, H2), 4.62 (2H, m, H2', H2"), 4.57 (1H, m, H1a), 4.52 (1H, bs, H4"), 4.48 (2H, m, H5", H3"), 4.37 (1H, m, H3), 4.34 (2H, m, H1b, H6"a), 4.32 (1H, m, H6"b), 4.26 (1H, m, H4), 2.28 (1H, m), 2.18 (1H, m), 1.98 (1H, m), 1.87 (2H, m), 1.73 (1H, m), 1.66 (2H, m), 1.10-1.40 (56H, m), and 0.85 (6H, t, *J* = 7.3 Hz); ^{13}C NMR (125 MHz, C_5D_5N) δ 175.0 (s, C1'), 101.2 (d, C1"), 76.5 (d, C3), 73.0 (d, C5"), 72.4 (d, C2'), 72.3 (d, C4), 71.6 (d, C3"), 70.9 (d, C4"), 70.1 (d, C2"), 68.1 (t, C1), 62.6 (t, C6"), 50.4 (d, C2), 35.5 (t), 34.4 (t), 32.1 (t), 30.3 (t), 30.1 (t),30.0 (t), 29.9 (t), 29.5 (t), 26.4 (t), 25.8 (t), 22.9 (t), and 14.2 (q).
   AGL-9a (2): $[\alpha]_D^{24}$ +49.9° (*c* 1.0, Pyr); mp 201.0-203.5 °C; HR FABMS *m/z* 830.6747 [(M-H)$^-$, calcd. for $C_{47}H_{92}NO_{10}$ 830.6726]; ^1H NMR same to 1 except for the integration for the CH_2 signal (δ 1.10-1.40); ^{13}C-NMR same to 1.
   AGL-9b (3): $[\alpha]_D^{24}$ +55.5° (*c* 1.0, Pyr); mp 211.0-212.0 °C; HR FABMS 844.6948 [(M-H)$^-$, calcd. for $C_{48}H_{94}NO_{10}$ 844.6883]; ^1H NMR δ 1.46 (2H, m), 0.84 (3H, t, *J* = 6.1 Hz), and 0.84 (6H, d, *J* = 6.7 Hz); ^{13}C NMR δ 50.5 (d, C2), 39.2 (t), 28.2 (d), 27.7 (t), and 22.7 (q).
   AGL-11 (4): $[\alpha]_D^{24}$ +51.9° (*c* 1.0, Pyr); mp 189.5-190.5 °C; HR FABMS 858.7032 [(M-H)$^-$, calcd. for $C_{49}H_{96}NO_{10}$ 858.7040]; ^1H NMR δ 0.88 (9H, m); ^{13}C NMR (55 °C) δ (ppm) 176.4 (s, C1'), 51.5 (d, C2), 37.4 (t), 35.1 (d), 33.6 (t), 19.9 (q), and 12.0 (q).
5. R. C. Gaver and C. C. Sweeley, *J. Am. Oil Chem. Soc.* ,42, 294 (1965). R. Higuchi, T. Natori and T. Komori, *Liebigs Ann. Chem.*, 51 (1990).
6. 5: $[\alpha]_D^{23}$ -2.4° (*c* 3.0, $CHCl_3$); FDMS *m/z* 398 (M^+); ^1H NMR (500 MHz, $CDCl_3$) δ 4.19 (1H, dd, *J* = 4.3, 7.3 Hz, H2), 3.79 (3H, s, OCH_3), 2.74 (1H, d, OH), 1.78 (1H, m), 1.64 (1H, m), 1.18-1.50 (40H, m), and 0.88 (3H, t, *J* = 7.3 Hz, CH_3).
7. See accompanying paper.
8. 6: $[\alpha]_D^{23}$ +23.4° (*c* 0.3, $CHCl_3$); FDMS *m/z* 486 [(M+H)$^+$]; ^1H NMR (500 MHz, $CDCl_3$) δ 5.97 (1H, d, *J* = 9.2 Hz, NH), 5.10(1H, dd, *J* = 8.5, 3.1 Hz, H3), 4.93 (1H, dt, *J* = 9.8, 3.1 Hz, H4), 4.47 (1H, m, H2), 4.29 (1H, dd, *J* = 11.6, 4.3 Hz, H1a), 4.00 (1H, dd, *J* = 11.6, 3.1 Hz, H1b), 2.08 (3H, s, 3-OAc), 2.05 (6H, s, 1-OAc, 4-OAc), 2.03 (3H, s, NHAc), 1.12-1.70 (23H, m), and 0.86 (6H, d, *J* = 6.7 Hz).
9. Antitumor activity will be reported elsewhere.

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