Antineoplastic agents. Part 395.[1] Isolation and structure of agelagalastatin from the Papua New Guinea marine sponge *Agelas* sp.

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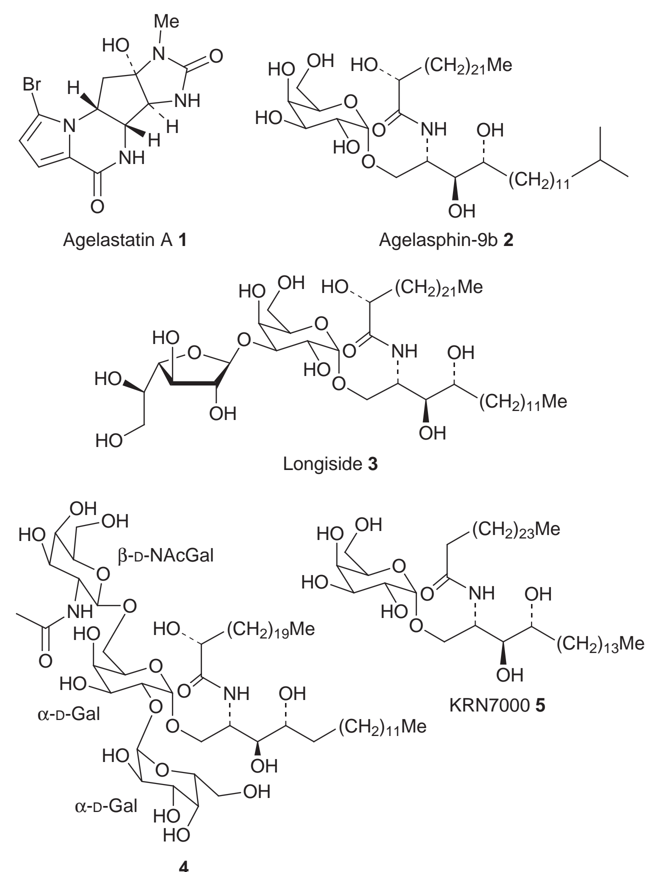
A human cancer cell line bioassay-directed investigation of the Western Pacific marine sponge *Agelas* sp. led to isolation of a trace (7.42 × 10^{-6}% yield) cancer cell growth inhibitor (lung NCI-H460 GI_{50} 0.77 μg m^{-1} to ovary OVCAR-3 GI_{50} 2.8 μg ml^{-1}) designated agelagalastatin 6; it is the first example of a natural product containing a digalactofuranosyl unit.

The marine porifera genus *Agelas* (class Demospongae, order Agelasida, family Agelasidae) has proven to be a rich source of new marine alkaloids[2] such as the cytotoxic (L1210 leukemia cell line) agelastatin A **1**[2a] and a series of glycosphingolipids[3]



(*cf.* **2**,[3a], **3**,[3b], **4**[3c], **5**[3d]). Some (*e.g.* **2** and **5**) of these have shown immunomodulating activity,[2a,3d] and a structural modification **5**[3g] has been considered for preclinical development as an anticancer (murine melanoma B16 *in vivo* active) and non-specific immunostimulating agent.[3a,g] Because glycosphingolipids (cerebrosides) are vitally important in a variety of biochemical processes ranging from antigenic specificity to cell–cell signaling and modulation of the immune response, discovery of new naturally occurring cancer cell growth inhibitory compounds is clearly necessary. We now report the isolation and structural elucidation of the new glycosphingolipid agelagalastatin **6** from *Agelas* sp. that was initially evaluated during our 1980 expedition to the southeast coast of Papua New Guinea and recollected in 1983.

Agelas sp. (450 kg wet wt.) was extracted with MeOH and the alcohol-soluble portion was successively partitioned between 1 : 1 CH_2Cl_2–MeOH and water followed by n-hexane and 9 : 1 MeOH–water and finally CH_2Cl_2 and 3 : 2 MeOH–water. The resulting CH_2Cl_2-soluble fraction (630.5 g) was separated (directed by human cancer cell line bioassays) by a series of gel permeation and partition chromatographic procedures on Sephadex LH-20 columns with the series MeOH → n-hexane–CH_2Cl_2–MeOH (8 : 1 : 1) → n-hexane–Pr^iOH–MeOH (8 : 1 : 1) → n-hexane–toluene–acetone (1 : 4 : 4) as eluents to afford a fraction inhibitory to a selection of cancer cells. The bioactive fraction was treated with MeOH to selectively isolate the more soluble active constituent herein named agelagalastatin (**6**, 6.5 mg, 7.42 × 10^{-6}% yield). The residual fraction was dissolved in CH_2Cl_2–MeOH (1 : 1) and subsequently identified (by NMR spectral analysis) as a mixture of monogalactosyl ceramides[4,5] related to agelasphin-9b **2**.

Agelagalastatin **6** was obtained as a colorless amorphous powder: $[\alpha]_D$ + 59 (*c* 0.65, CH_3OH) which showed a molecular ion peak in the HRFABMS spectrum at *m/z* 1192.7859 $[M+Na]^+$ (calc. 1192.7910) corresponding to molecular formula $C_{60}H_{115}NO_{20}$. When a 5.2 mg specimen of agelagalastatin was subjected to acid hydrolysis (15 h at 70 °C) with 1 M HCl–MeOH (8 : 91) followed by acetylation, methyl α,β-D-galactopyranoside tetracetate (identical with an authentic sample) and the sphinganines (2*S*,3*S*,4*R*)-2-amino-15-methyl-1,3,4-trihydroxyhexadecane and (2*S*,3*S*,4*R*)-2-amino-16-methyl-1,3,4-hydroxyheptadecane were identified by physical and spectral data. The FABMS spectrum afforded three fragment ion peaks at *m/z* 1030.5 $(M + Na + H - Gal)^+$, 868.5 $(M + Na + H\text{-}2Gal)^+$ and 706.5 $(M + Na + H - 3Gal)^+$, suggesting the trisaccharide unit [Gal-Gal-Gal].

Interpretation of the ^1H–^1H COSY and TOCSY-NMR spectra led to assignment of the proton relay signals corresponding to five spin systems. The HMBC and ROESY 2D NMR experiments supplied definitive structural information regarding connections to the five spin systems and allowed a view of the overall structure. Detailed data from HMQC and HMBC spectra suggested the ceramide unit was composed of two spin systems, namely 4-hydroxysphinganine and an α-hydroxy ester. The latter was shown by mass spectral analysis of the preceding methanolysis products to be primarily a (2*R*)-hydroxypentacosanoate with about 20% of the corresponding homologous (2*R*)-hydroxytetracosanoate. Furthermore, the HMBC correlation peaks of C-1* with NH, H-2 and H-2* indicated that the two segments were linked together through an amide bond and all the chemical shifts shown by the ceramide units were reminiscent of those known for related compounds.[3]

The three anomeric proton signals appeared as doublets at δ 5.47 (H-1′), 5.78 (H-1″) and 5.61 (H-1‴) and were a useful starting point for establishing an additional three spin systems. The heteronuclear chemical shift correlation (HMQC) spectrum was used to assign relationships between protons and carbon in the three carbohydrate units A, B, and C. The ^{13}C NMR chemical shift data and the proton coupling constants measured by 2D *J* resolution experiments revealed the inner galactose (A) unit to be a D-galactopyranoside. From consideration of the *J*

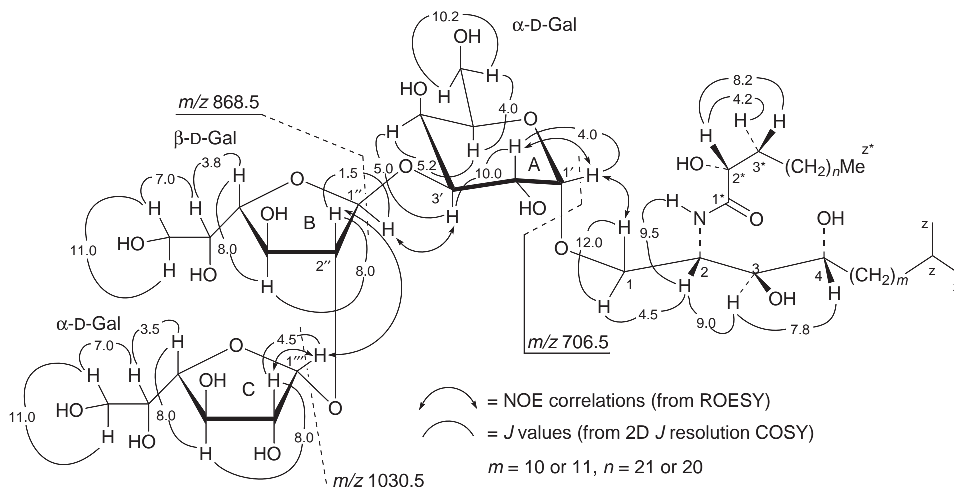


Fig. 1 NOE correlations and *J* values of agelagalastatin **6** (mass fragments include sodium).

value of the anomeric proton (H-1′, *J* 4.0 Hz) as well as the chemical shift of the corresponding carbon (C-1′, δ 101.31), the anomeric α-configuration was assigned. The HMBC correlations of H-1/C-1′ and H-1′/C-1 proved that the inner galactose segment (A) was directly connected with the C-1 ceramide hydroxy group by a glycosyl linkage.

Glycosylation shifts were observed at C-3′ (+8.42 ppm) and C-2′ (−2.36 ppm), along with HMBC correlations involving H-3′/C-1″ and H-1″/C-3′. Both results signified that C-3′ of galactose unit (A) was bonded through a glycoside link to the middle saccharide unit (B). The NOE correlation peaks of H-1/H-1′ and H-3′/H-1″ provided further evidence supporting two glycosyl linkages at two positions of the inner galactose section (A).

The two series of ^{13}C chemical shifts displayed by units B and C were both characteristic of a D-galactofuranoside.[6] Because of the five-membered ring, the ^{13}C chemical shifts of C-2″, C-3″ and C-4″ of unit B as well as those of C-2‴, C-3‴, and C-4‴ of unit C were significantly downfield compared with the corresponding data for D-galactopyranoside. Additional NMR data suggested that the two furanoside units (B and C) corresponded to 1,4-linked five-membered rings. Confirmatory evidence arose from the HMBC correlations of H-1″/C-4″, H-1‴C-4‴ and H-4‴/C-1‴, which exactly defined the furanosyl 1,4-linkages, and from 2D *J* resolution values attesting to the 2,3-diaxial (*J* 8.0 Hz) and 3,4-diaxial (*J* 8.0 Hz) relationships in the D-galactofuranoside (Fig. 1).

The chemical shifts of the anomeric carbons at δ 108.51 (C-1″) and 101.69 (C-1‴) together with the coupling constants of the anomeric protons (H-1″, *J* 1.5 Hz and H-1‴, *J* = 4.5 Hz) allowed the middle (B) and outer D-galactofuranosyl units (C) to be assigned β- and α-configurations,[6] respectively. Furthermore, the NOE relationship from H-1″ to H-3′ together with HMBC correlation between H-1″ and C-3′ showed the presence of a 1″–3′ glycosyl linkage between galactosyl sections A and B. The HMBC correlations of H-1‴/C-2″ and H-2″/C-1‴ combined with the NOE relationship of H-2″/H-1‴ indicated that the outer furanose unit (C) was joined to the C-2″ hydroxy group of the middle furanose (B). Thus, agelagalastatin was assigned structure **6** assuming that the overall stereochemistry and absolute configuration corresponds to that generally found for such glycosphingolipids.[3] To the best of our knowledge, agelagalastatin **6** is the first marine animal constituent found to possess a digalactofuranosyl unit.

Agelagalastatin **6** displayed significant *in vitro* activity against a portion of our minipanel (including brain SF-295, renal A498, colon KM20L2 and melanoma SK-MEL-5) of human cancer cell lines with GI_{50} values ranging from 0.77 μg ml^{-1} for lung NCI-H460 to 2.8 μg ml^{-1} for the ovarian OVCAR-3. Future research based on discovery of agelagalastatin will entail confirmation of the stereochemistry by total synthesis followed by detailed biological evaluation.

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