Avrainvilloside, a 6-Deoxy-6-aminoglucoglycerolipid from the Green Alga *Avrainvillea nigricans*

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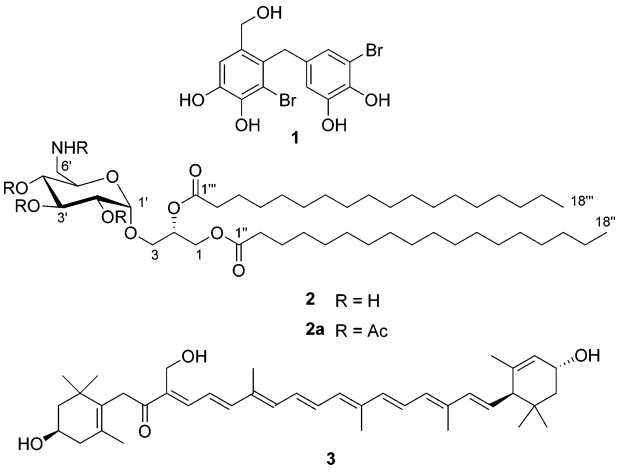
Fractionation of the organic extract obtained from the Dominican green alga *Avrainvillea nigricans* led to the isolation of avrainvilloside (**2**), a new glycoglycerolipid bearing the extremely rare 6-deoxy-6-aminoglucose moiety. The structure of avrainvilloside has been established on the basis of spectroscopic data and methanolysis/GC-MS analysis.

Marine Chlorophytes (green algae) are well known for the production of linear oxygenated terpenes; however, members of the genus *Avrainvillea* (Udoteaceae) have been shown to elaborate characteristic feeding-deterrent[1] brominated diphenylmethane derivatives endowed with HMG-CoA reductase (potential cholesterol-lowering)[2] and IMP dehydrogenase (potential anticancer and immunosuppressive)[3] inhibiting activities. Recently, the parent compound of this class, avrainvilleol, has been shown to possess also a strong antioxidant activity.[4]

In the course of our chemical screening of Caribbean marine organisms, we have undertaken the analysis of the green alga *Avrainvillea nigricans* Decaisne, for which a single report in the literature described the isolation of 5′-hydroxyisoavrainvilleol (**1**),[5] a member of the diphenylmethane class. The present note deals with the isolation and the structural elucidation of a major constituent of the organic extract, named avrainvilloside (**2**), a glycoglycerolipid bearing the extremely rare 6-deoxy-6-aminoglucose. During the isolation procedure, the carotenoid siphonaxanthin (**3**), which is considered a taxonomic marker of siphonaceous algae (as *A. nigricans*),[6] was also obtained.

Specimens of *A. nigricans* were collected along the coasts of Dominica, frozen on site, and transported to Vancouver over dry ice. Thawed samples (700 g wet wt) were cut in small pieces and extracted multiple times with fresh MeOH at room temperature. The combined MeOH extracts were concentrated in vacuo to a brown-colored gum, which was successively partitioned against *n*-hexane, CCl_4, $CHCl_3$, and *n*-butanol, following a modified Kupchan procedure.[7] The combined CCl_4 and $CHCl_3$ fractions were chromatographed over a Sephadex LH-20 column eluted with MeOH; selected fractions were fractionated by sequential application of reversed-phase flash (gradient elution from 7:3 CH_3CN/H_2O to CH_3CN in 5% increments) and reverse-phase high-performance liquid chromatographies to give pure samples of avrainvilloside (**2**, 16 mg) and siphonaxanthin (**3**, 5 mg). This carotenoid was identified by comparison of its spectroscopic data with those reported in the literature.[8]

Avrainvilloside (**2**), a white amorphous solid with $[\alpha]_D^{25}$ +25° (*c* 0.3, MeOH), has a molecular weight of 785, as indicated by the pseudomolecular ion $(M + Na)^+$ at *m/z*



808 in the ESI (positive ion) mass spectrum. The HR-FABMS of **2** also presented a pseudomolecular ion peak at *m/z* 786.6469 $[M + H]^+$ (calcd 786.6459), which was consistent with the molecular formula $C_{45}H_{87}NO_9$. Analysis of the ^1H and ^{13}C NMR data (in DMSO) of **2**, guided by $^1H-^1H$ COSY and HMQC spectra, allowed the assignment of all the ^{13}C and ^1H NMR signals for three spin systems, as shown in Table 1. The first spin system was assigned to a glycerol moiety (δ_H 4.34 and 4.12, δ_C 62.3; δ_H 5.10, δ_C 69.9; δ_H 3.87 and 3.40, δ_C 65.4); the second group of signals were attributable to two long-chain saturated fatty acids, while the third spin system indicated the presence of a glycosyl moiety. Hence, when the anomeric proton at δ_H 4.56 (δ_C 98.3) was used as a starting point, a sequence of four oxymethines and one deshielded methylene was identified. The large coupling constants observed for H-2′/H-3′, H-3′/H-4′, and H-4′/H-5′ vicinal couplings ($J = 9.6$ Hz) and the relatively small coupling constant of H-1′/H-2′ ($J = 3.6$ Hz) indicated the α-glucopyranose nature of this sugar. The relatively upfield shifted ^1H NMR resonances of H-6′a and H-6′b (δ_H 2.87 and 2.56) and the ^{13}C NMR resonance of C-6′ (δ_C 54.5) were strongly indicative of the presence of an amino group at this position. The complete pattern of ^{13}C NMR resonances (Table 1) confirmed the assignment of the sugar unit as 6-deoxy-6-amino-α-glucopyranose. This residue should be linked to the C-3 of the glycerol, as indicated by the HMBC correlation between the anomeric proton H-1′ and C-3, while the HMBC cross-peaks H-2/C-1‴, H_2-2‴/C-1‴, H_2-1/C-1″, and H_2-2″/C-1″

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Table 1. ^{13}C and ^{1}H NMR Data for Avrainvilloside (**2**)[a]

pos.	δ_C	δ_H (int., mult., *J* in Hz)
1a	62.3, CH_2	4.34 (1H, dd, 10.0, 2.0)
1b		4.12 (1H, dd, 10.0, 7.4)
2	69.9, CH	5.10 (1H, m)
3a	65.4, CH_2	3.87 (1H, dd, 10.0, 5.8)
3b		3.40 (1H, dd, 10.0, 3.5)
1′	98.3, CH	4.56 (1H, d, 3.6)
2′	72.0, CH	3.18 (1H, dd, 9.6, 3.6)
3′	72.6, CH	3.35 (1H, t, 9.6)
4′	74.1, CH	2.91 (1H, t, 9.6)
5′	68.2, CH	3.77 (1H, ddd, 9.6, 6.2, 4.0
6′a	54.5, CH_2	2.87 (1H, dd, 14.0, 4.0)
6′b		2.56 (1H, dd, 14.0, 6.2)
1″	172.4, C	
1‴	173.0, C	
2″−2‴	40.3, CH_2	2.28 (4H, t, 6.5)
3″−3‴	29.5, CH_2	1.49 (4H, t, 6.5)
4″ to 17″		1.12−1.30 (56H, m)
4‴ to 17‴		
18″−18‴	19.2, CH_3	0.90 (6H, t, 7.3)

[a] Recorded in DMSO-d_6.

indicated the attachment of acyl groups at the positions 1 and 2 of the glycerol moiety.

Standard acetylation of avrainvilloside gave the tetra-acetylated derivative **2a**, thus giving support to the 1,2-diacyl-3-*O*-(6-deoxy-6-amino-α-glucopyranosyl)glycerol structure deduced for **2**. According to the molecular formula, the acyl moieties of **2** should account for 36 carbon atoms. To infer the exact nature of the fatty acids, avrainvilloside (**2**) was treated with NaOMe/MeOH. After partitioning, the apolar organic extract was analyzed by GC-MS and only methyl stearate could be detected. This result indicates that the two acylating fatty acids at C-1 and C-2 of the glycerol are both stearic acid (C18). The MeOH phase was subjected to acid methanolysis, and after HPLC purification, 1.4 mg of methyl 6-deoxy-6-amino-α-glucopyranoside was obtained. The D configuration of this sugar was deduced by comparing its optical rotation ($[\alpha]_D^{25}$ +131.0° in H_2O) with that reported in the literature for an authentic sample of methyl 6-deoxy-6-amino-α-D-glucopyranoside ($[\alpha]_D^{25}$ +147.0° in H_2O).[9] The CD spectrum of avrainvilloside (**2**) exhibited a negative Cotton effect at λ_{max} 228 nm, and this is considered to be indicative of *sn*-1, *sn*-2 diacylglycosylglycerolipid;[10] thus, the stereostructure of avrainvilloside was completely defined as reported in **2**.

Glycoglycerolipids are common in red, brown, and green algae,[11] and also their analogues containing 2-deoxy-2-amino (or 2-acetamido) sugars have wide distribution among marine organisms. On the other hand, the 6-deoxy-6-amino glycosides are extremely rare. To our knowledge, avrainvilloside (**2**) represents only the third example in nature of the very small class of 6-deoxy-6-amino glycoglycerolipids, which apparently seem to be restricted to plants and algae. The first members of this class were strangulatosides, isolated in 2001 from rhizomes of the terrestrial plant *Serratula strangulata* (Compositae)[12] and reported to possess mild antibacterial activity. The second example, which differs from **2** in bearing an additional acyl group at C-6 of the sugar, has been very recently isolated from an unidentified marine alga,[13] and remarkably, it has been shown to potently and selectively inhibit Myt1 kinase, an enzyme involved in the cell cycle, whose inhibitors are expected to kill rapidly proliferating cells and abrogate normal cell cycle checkpoints.

Avrainvilloside (**2**) was inactive in preliminary cytotoxicity assays (IC_{50} >10 μg/mL on WEHI 164 cells, murine fibrosarcoma); however, its biological activity profile is worthy of further investigation.

Experimental Section

General Experimental Procedures. Optical rotations were measured in MeOH on a Perkin-Elmer 192 polarimeter equipped with a sodium lamp (λ_{max} = 589 nm) and a 10 cm microcell. CD spectra (MeOH) were recorded on a JASCO 500A polarimeter. UV spectra were obtained in MeOH using a Beckman DU70 spectrophotometer. ^{1}H (500 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on Bruker AMX-500 and AM-400 spectrometers, respectively; chemical shifts were referenced to residual solvent signals (DMSO-d_6: δ_H = 2.50, δ_C = 40.0; $CDCl_3$: δ_H = 7.26). ^{13}C NMR resonances were assigned to CH_3, CH_2 or CH by using DEPT experiments. Homonuclear ^{1}H connectivities were determined by using COSY experiments. One-bond heteronuclear $^{1}H-^{13}C$ connectivities were determined with the HMQC experiment using a BIRD pulse of 0.50 s (interpulse delay set for $^{1}J_{CH}$ = 135 Hz). Two- and three-bond $^{1}H-^{13}C$ connectivities were determined by HMBC experiments optimized for a $^{2,3}J$ of 10 Hz. Low-resolution electrospray (positive ions) mass spectra were performed with a LCQ Finnigan MAT mass spectrometer; low- and high-resolution FAB mass spectra (CsI ions, glycerol matrix) were performed on a VG Prospec Fisons mass spectrometer. Column chromatography was performed using Sigma-Lipophilic Sephadex LH-20 or Merck RP18 as stationary phase. High-performance liquid chromatography (HPLC) separations in isocratic mode were achieved using a Waters 600 pump and Alltech columns (250 μ 4.6 mm) and monitored by a RI detector (Waters 486 tunable absorbance detector). GC-MS experiments were performed on a Hewlett-Packard 5890 gas chromatograph with a MSD HP 5790 MS mass-selective detector. A fused-silica column (25 m × 0.20 mm HP-5; cross-linked 25% Ph-Me-silicone, 0.33 mm film thickness) was used with a helium carrier flow of 10 mL/min and a temperature gradient of 100 to 300 °C, 3 °C/min.

Plant Material, Extraction, and Isolation. Specimens of *Avrainvillea nigricans* Ducaisne were collected in summer 2001 in Prince Rupert Bay of Portsmouth (Dominica) at a depth of 25 m, immediately frozen, and transported to Vancouver at 0 °C. A voucher specimen (ref. no. 01-101f) is deposited at the Department of Earth and Ocean Sciences, University of British Columbia, Vancouver. Thawed samples (700 g wet wt) were cut in small pieces and extracted multiple times with fresh MeOH at room temperature. The obtained extract was dissolved in 9:1 MeOH−H_2O and then partitioned against *n*-hexane (3 × 500 mL) to yield an apolar extract weighing 800 mg. Successively, the water content of the hydromethanolic phase was adjusted to 20% (v/v) and 40% (v/v), and the solutions were partitioned against CCl_4 (3 × 500 mL) and $CHCl_3$ (3 × 500 mL), respectively, affording a carbon tetrachloride (1.2 g) and a chloroform (1.2 g) extract. Finally, all the MeOH was evaporated from the hydromethanolic layer, and the water solution thus obtained was partitioned against *n*-BuOH. The combined CCl_4 and $CHCl_3$ fractions were chromatographed over a Sephadex LH-20 column eluted with MeOH, obtaining 200 fractions of 20 mL. Fractions 40 to 60 were combined (75 mg) and fractionated by reversed-phase flash chromatography (gradient elution from 7:3 CH_3CN/H_2O to CH_3CN in 5% increments), yielding pure siphonaxanthin (**3**, 5 mg) and a crude fraction that was further purified by reversed-phase HPLC, using 1:1 CH_3CN/H_2O as eluant, to obtain pure avrainvilloside (**2**, 16 mg).

Avrainvilloside (2): white amorphous solid; $[\alpha]_D^{25}$ +25.0° (*c* 0.3, MeOH); CD (MeOH) λ_{max} 228 nm ($\Delta\epsilon$ − 6.5); ^{1}H (DMSO-d_6, 500 MHz) and ^{13}C (DMSO-d_6, 100 MHz) NMR spectra, see Table 1; ESIMS (positive ion) *m/z* 808 $[M + Na]^+$; FABMS (positive ion) *m/z* 786 $[M + H]^+$; HRFABMS *m/z* 786.6469 $[M + H]^+$ (calcd for $C_{45}H_{88}NO_9$, 786.6459, $\Delta\mu$ 1.3 ppm).

Acetylation of Avrainvilloside. Compound **2** (3.8 mg) was dissolved in dry pyridine (0.6 mL) and treated with Ac_2O (0.6

mL). After standing overnight, the reaction was worked up by addition of a few drops of methanol to destroy the excess Ac_2O, water (ca. 1 mL), and EtOAc (ca. 3 mL). The organic phase was washed sequentially with 2 N H_2SO_4, saturated $NaHCO_3$, and brine. After drying (Na_2SO_4) and removal of the solvent, the residue was purified by HPLC (*n*-hexanes/EtOAc, 8:2) to afford 3.3 mg of the tetraacetate 2a.

Avrainvilloside tetraacetate (2a): amorphous solid; $[\alpha]_D^{25}$ +14.5° (*c* 0.1, MeOH); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 5.21 (1H, t, J = 9.6 Hz, H-3′), 5.13 (1H, m, H-2), 4.86 (1H, d, J = 3.6 Hz, H-1′), 4.78 (1H, dd, J = 9.6, 3.6 Hz, H-2′), 4.71 (1H, t, J = 9.6 Hz, H-4′), 4.34 (1H, dd, J = 10.0, 2.0 Hz, H-1a), 4.12 (1H, overlapped, H-1b), 4.09 (1H, overlapped, H-5′), 4.02 (1H, dd, J = 10.0, 6.0 Hz, H-3a), 3.50 (1H, dd, J = 14.0, 4.0 Hz, H-6′a), 3.48 (1H, dd, J = 10.0, 4.0 Hz, H-3b), 3.33 (1H, submerged by residual water signal, H-6′b), 2.28 (4H, t, J = 6.5 Hz, H_2-2″ and H_2-2‴), 1.99 (3H, s, OCOCH_3), 1.98 (3H, s, OCOCH_3), 1.97 (3H, s, OCOCH_3), 1.95 (3H, s, OCOCH_3), 1.49 (4H, m, H_2-3″ and H_2-3‴), 1.28−1.12 (56 H, m, H_2-4″ to H_2-17″ and H_2-4‴ to H_2-17‴), 0.90 (6H, t, J = 7.3 Hz, H_3-18″ and H_3-18‴); FABMS (positive ion) *m/z* 954 $[M + H]^+$.

Methanolysis of Avrainvilloside. Avrainvilloside (**2**, 12.0 mg) was dissolved in 1.5 mL of methanol and treated with 2% NaOMe/MeOH solution (1.5 mL) under stirring at room temperature for 4 h. The reaction mixture was neutralized with AG 50W-X8 (BIO-RAD, H^+ form), the resin removed by filtration, and the filtrate dried under reduced pressure and partitioned between *n*-hexane and MeOH. The *n*-hexane phase was then subjected to GC-MS. Methyl stearate was detected on the basis of its retention time, MS data [EIMS *m/z* 298 $(M)^+$, 255, 213, 199, 0143, 87, 74], and comparison with an authentic sample of methyl stearate. The MeOH phase was dissolved in 1 N HCl in 85% MeOH (3.0 mL), and the solution was kept at 70 °C in a sealed tube for about 8 h. After being cooled, the reaction mixture was neutralized with Ag_2CO_3 and centrifuged, and the supernatant was evaporated to dryness under N_2. This was then purified by HPLC (MeOH/H_2O, 8:2) to afford 1.4 mg of methyl 6-deoxy-6-amino-α-D-glucopyranoside ($[\alpha]_D^{25}$ +131.0° in H_2O).

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