SUPPORTING INFORMATION

Amphimelibiosides A-F, Six New Ceramide Dihexosides, Isolated from a Japanese Marine Sponge *Amphimedon* sp.

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Isolation of Amphimelibiosides

Amphimedon sp.(145.8g, wet.)

- 1) Homogenation and extraction with EtOH
- 2) Concentration and Addition of H₂O
- 3) Extraction with Et₂O and *n*-BuOH

Et₂O ext.(842.4 mg)

n-BuOH ext.(189.2 mg)

- 1) Silica-gel CC
- 2) RP CC(C8)

Fr.3-3(29.6mg,Amphimelibiosides molecular species)

RP-HPLC(C18)

Column: Cosmosil AR-II
Solv.: 100% MeOH
Detect.: $\lambda_{210\text{nm}}$
Flow rate: 1.0mL/min

AM-C(0.72% yielded from Et₂O ext.)

General Experimental Procedures.

Column chromatography: Silica gel 60 (0.063-0.200 μm), LiChroprep RP-8 pre-packed column (40-63 μm , size B), Cosmosil 5C18 AR-II packed column (4.6 i.d. x 250 mm); TLC: Silica gel 60 F₂₅₄, and RP-8 F₂₅₄; HPLC apparatus: PU-980 HPLC pump (flow rate 1.0 mL/min.) with 7125 injector and 875-UV detector (detected at λ_{210} nm.); Optical rotations: DIP-370 digital polarimeter; FAB mass spectra: Jeol SX-102 mass spectrometer (xenon atom beam 5 kV; ion source accelerating potential 10 kV, matrix *m*-nitrobenzyl alcohol); NMR spectra: All spectra were recorded using a Varian Inova-600 spectrometer. The operating conditions were as follows: ¹H frequency 599.879 MHz, sweep width 8 kHz, sampling point 28 k, accumulation 64-512 pulses, temperature 30 °C. ¹³C frequency 150.856 MHz, sweep width 36 kHz, sampling point 160 k, accumulation 16000 pulses. Chemical shifts were referenced to TMS (0 ppm). Conventional pulse sequences were used for 1D-TOCSY, DQ-COSY, TOCSY, ROESY, HSQC, HMBC, and HSQC-TOCSY. The mixing times in the TOCSY and ROESY experiments were set to 90 and 300 ms. Water suppression was carried out by selective presaturation placing the carrier on the solvent; GC-MS: Shimadzu QP-5050, EI mode (ionizing potential of 70 eV, injector and separator temperature 250 °C, ion-source temperature 300 °C), and a capillary column (Neutra BOND-5, 30 m x 0.25 mm, 0.4 μm), carrier He (1.1 mL/min.), column temperature 150 -280 °C (rate of temperature, increase 5 °C /min.).

^1H NMR spectrum of Fr. 3-3

Pulse Sequence: presat
PULSE SEQUENCE presat
DATE Aug 4 2001
SOLVENT c5d5n
OBSERVE H1
FREQUENCY 599.880 MHz
SPECTRAL WIDTH 12001.2 Hz
ACQUISITION TIME 4.549 sec
RELAXATION DELAY 0.000 sec
PULSE WIDTH 9.4 usec
TEMPERATURE 30.0 deg. C.
NO. REPETITIONS 64
DOUBLE PRECISION ACQUISITION
DATA PROCESSING
RESOLUTION ENHANCEMENT -0.0 Hz
FT SIZE 65536
TOTAL ACQUISITION TIME 4 minutes

¹³C NMR spectrum of Fr. 3-3

Pulse Sequence: bilevel
PULSE SEQUENCE bilevel
DATE Aug 4 2001
SOLVENT c5d5n
OBSERVE C13
FREQUENCY 150.856 MHz
SPECTRAL WIDTH 36182.7 Hz
ACQUISITION TIME 0.453 sec
RELAXATION DELAY 2.547 sec
PULSE WIDTH 7.8 usec
TEMPERATURE 30.0 deg. C.
NO. REPETITIONS 0
DECOUPLE H1
HIGH POWER 40
DECOUPLER CONTINUOUSLY ON
GARP MODULATION
DOUBLE PRECISION ACQUISITION
DATA PROCESSING
LINE BROADENING 1.0 Hz
FT SIZE 65536
TOTAL ACQUISITION TIME 1 minutes

amphimelibioside-¹³C.presat
Pulse Sequence: presat
PULSE SEQUENCE presat
DATE May 15 2002
SOLVENT c5d5n
OBSERVE H1
FREQUENCY 599.880 MHz
SPECTRAL WIDTH 12001.2 Hz
ACQUISITION TIME 4.549 sec
RELAXATION DELAY 5.000 sec
PULSE WIDTH 10.0 usec
TEMPERATURE 30.0 deg. C.
NO. REPETITIONS 128
DOUBLE PRECISION ACQUISITION
DATA PROCESSING
RESOLUTION ENHANCEMENT -0.0 Hz
FT SIZE 65536
TOTAL ACQUISITION TIME 20 minutes

¹H NMR spectrum of Amphimelibioside C

^1H NMR spectrum of Amphimelibioside B

Pulse Sequence: presat
PULSE SEQUENCE presat
DATE Jun 24 2002
SOLVENT c5d5n
OBSERVE H1
FREQUENCY 599.880 MHz
SPECTRAL WIDTH 12001.2 Hz
ACQUISITION TIME 4.549 sec
RELAXATION DELAY 5.000 sec
PULSE WIDTH 10.0 μsec
TEMPERATURE 30.0 deg. C.
NO. REPETITIONS 256
DOUBLE PRECISION ACQUISITION
DATA PROCESSING
RESOLUTION ENHANCEMENT -0.0 Hz
FT SIZE 65536
TOTAL ACQUISITION TIME 40 minutes

^{13}C NMR spectrum of Amphimelibioside C

DQ-COSY spectrum of Amphimelibioside C

TOCSY spectrum of Amphimelibioside C

ROESY spectrum of Amphimelibioside C

HSQC spectrum of Amphimelibioside C

amphimelibioside-A, ghsqc

Pulse Sequence: ghsqc

HMBC spectrum of Amphimelibioside C

amphimelibioside-A,ghmqc

Pulse Sequence: ghmqc

HSQC-TOCSY spectrum of Amphimelibioside C

*Negative & Positive ion FABMS of Amphimelibioside C
(Matrix: m-NBA)*

Results of GC-MS analysis of Amphimelibioside C

スキャン番号 : (2931 - 2946) バックグラウンド : (2899 - 2918)
 ピーク数 : 327 保持時間 : (27.417 - 27.542)
 ベースピーク : 132.25 (386753)

1D TOCSY experiment of Amphimelibioside B and C

(a) 1D TOCSY spectrum of Amphimelibioside B

17 and 18-CH₃

(b) Normal ¹H NMR spectrum of Amphimelibioside B 17,18, and 22'-CH₃

(c) Normal ¹H NMR spectrum of Amphimelibioside C

18 and 22'-CH₃