(-)-Agelasidine C and (-)-Agelasidine D, Two New Hypotaurocyamine Diterpenoids from the Caribbean Sea Songe Agelas clathrodes

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J. Nat. Prod., **1992**, 55 (3), 389-394• DOI:
10.1021/np50081a019 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 55, No. 3, pp. 389-394, March 1992

(−)-AGELASIDINE C AND (−)-AGELASIDINE D, TWO NEW HYPOTAUROCYAMINE DITERPENOIDS FROM THE CARIBBEAN SEA SPONGE *AGELAS CLATHRODES*[1]

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ABSTRACT.—Two new diterpene derivatives of hypotaurocyamine, (−)-agelasidine C [**3**] and (−)-agelasidine D [**5**], have been isolated from the Caribbean sea sponge *Agelas clathrodes*. The structures of **3** and **5** have been established by interpretation and comparison of their spectral data to those of the known antipode (+)-agelasidine C [**1**].

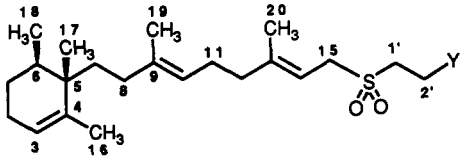
Several bioactive metabolites having a polar functionality such as a guanidine, sulfone, or methyladeninium unit appended to a terpenoid moiety have been reported from Okinawan (1–5) and Pacific (6) marine sponges of the genus *Agelas*. Included among these are the agelasidines A, B, and C, diterpene derivatives of hypotaurocyamine isolated by Nakamura and co-workers from the Okinawan sea sponge *Agelas nakamurai* (4). The agelasidines have been shown to possess significant inhibitory effects on growth of a variety of microorganisms, contractile responses of smooth muscle and enzymic reactions of Na,K-ATPase (4). As part of our ongoing search for physiologically active substances in marine invertebrates from Puerto Rico, we now report the isolation and structural elucidation of two new diterpene derivatives of hypotaurocyamine, named (−)-agelasidine C [**3**] and (−)-agelasidine D [**5**], from the Caribbean sea sponge *Agelas clathrodes* (Schmitt) (family Agelasidae). The structures of these new bioactive metabolites were elucidated by interpretation of spectral data and comparison of their physical and chemical properties with those of the known antipode (+)-agelasidine C [**1**] (4).

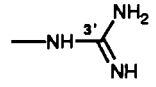
A specimen of *A. clathrodes* was collected near Desecheo Island, off the Northwest coast of Puerto Rico, lyophilized, and stored temporarily at −10°. The $CHCl_3$ solubles obtained from the MeOH extracts of the sponge were chromatographed on a Si gel column with $CHCl_3$–*n*-BuOH–HOAc–H_2O (3:12:2:2) as eluent to give two fractions: a complex mixture of sterols and a brown oily residue that showed at least three spots upon Si gel tlc analysis. The brown oily mixture was purified further on a Si gel column using CH_2Cl_2-MeOH-NH_4OH (9:2:0.5) as eluent to yield homogeneous (−)-agelasidine C [**3**] and (−)-agelasidine D [**5**] as light-yellow oils. Both **3** and **5** were shown to be guanidine derivatives by positive coloration with Sakaguchi reagent (7) and by their ^{13}C-nmr signals [δ 158.83 (s) for **3** and 158.58 (s) for **5**]. This observation was confirmed chemically by their conversion with 2,4-pentanedione to the corresponding 3,5-dimethylpyrimidine derivatives **4** and **6**. Upon derivatization of a mixture of **3** and **5**, the overall molecular polarity of the natural products was decreased and they could now be easily separated by cc on a Si gel column with C_6H_6-Me_2CO (9:1) as eluent. The molecular formulae of **4** and **6** were determined as $C_{28}H_{45}N_3SO_2$ and $C_{28}H_{45}N_3SO_3$, respectively, by lreims and ^{13}C-nmr analyses.

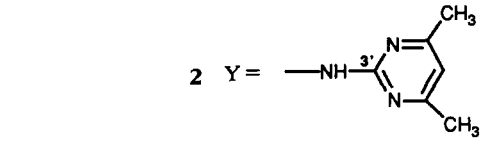
The hrfabms of **3** showed an $[M+H]^+$ molecular ion at *m/z* 424.3002, which

[1]Presented at the Second Panamerican Chemical Congress, San Juan, Puerto Rico, September 23–27, 1991.

[2]Graduate student sponsored by the NIH-MBRS Program of Puerto Rico.



1 Y = 



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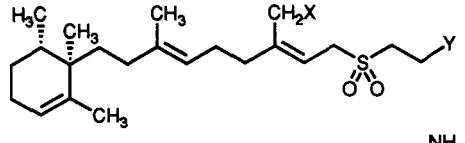
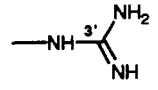
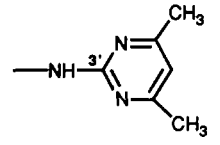
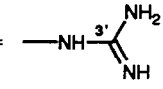
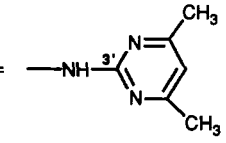
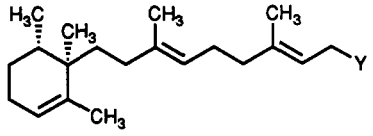


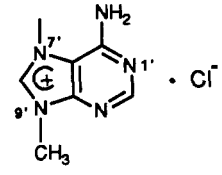
3 X = H, Y = 

4 X = H, Y = 

5 X = OH, Y = 

6 X = OH, Y = 



7 Y = 

was consistent with a molecular formula $C_{23}H_{41}N_3SO_2$. A careful comparison of the ^{1}H- and ^{13}C-nmr, uv, ir, and ms spectra of **3** (and **4**) with those reported for **1** (and **2**) revealed that **3** and **1** have the same elemental composition and structural formula. Indeed, the virtual identity of the ^{13}C-nmr spectra of **3** and **1** (see Table 1) can only be explained if they are optical antipodes. Comparison of the [α]D values of **1** (+8.5°) and **3** (−5.6°) and of **2** (+9.1°) and **4**

TABLE 1. ^{13}C-nmr Spectral Data (75 MHz, CD_3OD) for Agelasidines C [**1**, **3**], and D [**5**].

Carbon	(+)-Agelasidine C [**1**][a]	(−)-Agelasidine C [**3**][b,c]	(−)-Agelasidine D [**5**][b,c]
C-1	26.4 (t)	26.50 (t)	26.28 (t)
C-2	28.0 (t)	28.17 (t)	27.98 (t)
C-3	124.0 (d)	125.08 (d)	124.93 (d)
C-4	140.5 (s)	140.72 (s)	140.63 (s)
C-5	41.4 (s)	41.57 (s)	41.38 (s)
C-6	34.4 (d)	34.56 (d)	34.39 (d)
C-7	35.3 (t)	35.40 (t)	35.21 (t)
C-8	36.4 (t)	36.50 (t)	36.37 (t)
C-9	137.6 (s)	137.80 (s)	137.80 (s)
C-10	124.0 (d)	124.08 (d)	123.97 (d)
C-11	27.2 (t)	27.29 (t)	27.36 (t)
C-12	40.8 (t)	40.82 (t)	36.30 (t)
C-13	148.0 (s)	148.24 (s)	150.68 (s)
C-14	110.6 (d)	110.75 (d)	112.70 (d)
C-15	54.5 (t)	54.66 (t)	53.96 (t)
C-16	19.4 (q)	19.45 (q)	19.37 (q)
C-17	16.2 (q)	16.19 (q)	16.13 (q)
C-18	21.4 (q)	21.48 (q)	21.43 (q)
C-19	16.3 (q)	16.34 (q)	16.38 (q)
C-20	17.0 (q)	17.05 (q)	60.71 (t)
C-1′	51.4 (t)	51.44 (t)	51.60 (t)
C-2′	36.0 (t)	36.07 (t)	36.13 (t)
C-3′	158.5 (s)	158.83 (s)	158.58 (s)

[a]Values recorded in CD_3OD by Nakamura *et al.* (4).

[b]Chemical shifts are reported in ppm downfield from TMS.

[c]Resonance multiplicities were determined using the APT experiment and are denoted as s, d, t and q for singlet, doublet, triplet and quartet, respectively. Assignments were aided by ^{1}H-^{1}H COSY and ^{1}H-^{13}C COSY experiments, and comparisons to known models.

(−11.6°) confirmed this fact. Therefore, the absolute configuration of the substituents on the cyclohexene ring in (−)-agelasidine C [**3**] is 5*R*, 6*S* and is the same as that of the known monocyclic diterpene ageline A [**7**] (6).

(−)-Agelasidine D [**5**] showed a number of spectral features in common with **3**, which suggested a close structural relationship. The hrfabms analysis exhibited an $[M+H]^+$ molecular ion at *m/z* 440.2947, revealing the molecular formula $C_{23}H_{41}N_3SO_3$. A careful comparison of the ^{1}H- and ^{13}C-nmr and ir spectra of (−)-agelasidine D [**5**] with those of **3** indicated that both compounds contained a common unit $-S(O)_2-CH_2-CH_2-NH-C(=NH)-NH_2$. The ^{1}H-nmr spectra of **3** and **5** were superimposable because of the common unit. Therefore, the remaining portion of **5** was composed of a diterpene alcohol unit $C_{20}H_{33}O$.

The ^{1}H-nmr spectrum of (−)-agelasidine D [**5**] contained two proton signals due to three trisubstituted olefins at δ 5.38 (H-3 and H-14, overlapping triplets) and 5.08 (H-10, broad triplet). In general, the vinylic region of the ^{1}H-nmr spectrum of **5** was almost identical with that of (−)-agelasidine C [**3**], with the exception that one of the overlapping signals at δ 5.38 (H-14) in the spectrum of **5** occurs at δ 5.26 (broad triplet, $J=7.6$ Hz) in (−)-agelasidine C [**3**]. Furthermore, one of the high-field vinyl methyl signals of **3** (δ 1.73, Me-20) was no longer present in the ^{1}H-nmr spectrum of **5**; instead, a new proton signal at δ 4.14 (s, 2H) was observed. Moreover, a new carbinol signal (δ 60.71, t, C-20) and only four high-field methyl singlets (δ 19.37 Me-16, 16.13 Me-17, 21.43 Me-18, and 16.38 Me-19) were observed in the ^{13}C-nmr spectrum of (−)-agelasidine D [**5**]. On the basis of

these spectroscopic grounds together with a careful comparison of the ^{13}C-nmr chemical shift values of the present agelasidines (Table 1), we conclude that (−)-agelasidine D is the hydroxylated derivative of (−)-agelasidine C and that the new allylic hydroxyl functionality must be located at position C-20 as depicted in structure **5**.

Further comparison of the ^{13}C-nmr spectral data of (−)-agelasidine D [**5**] with those of **3** (Table 1) confirmed the relative stereochemistry of the monocyclic ring system as well as the orientation of the methyl groups around the Δ^9 and Δ^{13} trisubstituted double bonds as shown in structure **5**. The [α]D values of **5** (−3.6°) and **6** (−9.0°) suggest that the absolute configuration of (−)-agelasidine D is probably identical to that of (−)-agelasidine C (and hence ageline A [**7**]) since their specific rotations are similar both in sign and order of magnitude and because in both instances their optical rotation can be attributed to the same isolated monocyclic chiral unit. In an effort to provide additional evidence on the absolute stereostructure of **5** we undertook a series of experiments to correlate the structure of (−)-agelasidine D with that of (−)-agelasidine C. However, attempts to convert **6** into the synthetic pyrimidine derivative **4** via conversion of the allylic hydroxyl function into a suitable leaving group followed by hydride displacement resulted in either destruction of the starting material [(a) MsCl, Py, THF, 0° then $LiAlH_4$/THF, 25°] or in its recovery as unchanged material [(b) Py·SO_3/THF, 0° then $LiAlH_4$/THF, 0°] (8).

The new agelasidines reported here have been screened for antimicrobial activity and cytotoxicity. With use of the standard disk assay, (−)-agelasidine D [**5**] inhibited the growth of *Staphylococcus aureus, Escherichia coli, Hafnia alvei, Klebsiella pneumoniae,* and *Proteus vulgaris*. On the other hand, (−)-agelasidine C [**3**] showed inhibitory activity only against *S. aureus, K. pneumoniae* and *P. vulgaris* at concentrations of 1.9 mg/ml, the only concentration tested. Both (−)-agelasidine C and (−)-agelasidine D inhibited significantly the growth of CHO-K1 cells (respective ED_{50}'s = 5.70 and 2.21 μg/ml). The corresponding 3,5-dimethylpyrimidine derivatives were not examined for biological activity.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir and uv spectra were recorded on Nicolet 600 FT-IR and Hewlett-Packard Chem Station 8452A spectrophotometers, respectively. ^{1}H- and ^{13}C-nmr spectra were recorded on a General Electric Multinuclear QE-300; ^{1}H-nmr chemical shifts are recorded with respect to TMS (δ 0.0). Optical rotations were determined on a Perkin-Elmer Polarimeter Model 243B. Lreims were recorded on a Hewlett-Packard 5995A spectrometer. Cc was performed on Analtech Si gel (35–75 mesh), and tlc analyses were carried out using Analtech glass packed precoated Si gel plates. All solvents used were either spectral grade or were distilled from glass prior to use.

COLLECTION AND EXTRACTION OF *AGELAS CLATHRODES*.—*A. clathrodes*, a massive yellow-orange sponge, was collected by the authors near Desecheo Island, Puerto Rico on March, 1990 using scuba (−20 to −30 m); a voucher specimen is stored at the Chemistry Department of the University of Puerto Rico. The collection was stored at 0° for a few hours prior to freezing and lyophilization. The freeze-dried sponge was extracted with MeOH (3 × 1 liter) to give, after filtration, concentration, and storage under vacuum, 46.22 g of a dark orange semisolid. The residue was suspended in H_2O and extracted with $CHCl_3$ (3 × 250 ml). The $CHCl_3$-soluble material (13.8 g) was chromatographed successively on Sephadex LH-20 column [$CHCl_3$-MeOH (1:1)] and a portion (2.5 g) of the material eluted (9.3 g) on a Si gel (90 g) column using $CHCl_3$–*n*-BuOH–HOAc–H_2O (3:12:2:2) as eluent. Combination of like fractions on the basis of tlc analyses afforded two principal fractions: a complex mixture of sterols (340 mg) and a brown oily residue (1.6 g) that produced three spots on tlc when eluted with CH_2Cl_2-MeOH-NH_4OH (9:2:0.5). The brown oily residue was purified further over a Si gel column (60 g) using CH_2Cl_2-MeOH-NH_4OH (9:2:0.5) to yield pure (−)-agelasidine C [**3**] (115.4 mg) and (−)-agelasidine D [**5**] (147.5 mg).

(−)-*Agelasidine C* [**3**].—A light-yellow oil; $[\alpha]^{29}_D$ −5.6° (*c* = 7.2, MeOH); ir (neat) 3337 (broad), 3155 (broad), 2962, 2923, 1672, 1644, 1573, 1450, 1409, 1377, 1295, 1250, 1121

cm^{-1}; ^1H nmr (300 MHz, MeOH-d_4) δ 0.81 (s, 3H, Me-17), 0.82 (d, 3H, J = 6.7 Hz, Me-18), 1.57 (br s, 6H, Me-16 and Me-19), 1.73 (br s, 3H, Me-20), 1.30–2.20 (br m, 13H), 3.27 (t, 2H, J = 6.5 Hz, H-1′), 3.67 (t, 2H, J = 6.5 Hz, H-2′), 3.86 (d, 2H, J = 7.9 Hz, H-15), 5.06 (br s, 1H, H-10), 5.26 (br t, 1H, J = 7.6 Hz, H-14), 5.38 (br s, 1H, H-3); ^{13}C nmr (75 MHz, MeOH-d_4) see Table 1; hrfabms m/z $[M+H]^+$ 424.3002 (38%) ($C_{23}H_{42}N_3SO_2$ requires 424.2997), 303 (68), 262 (37), 238 (35), 182 (100).

(−)-*Agelasidine D* [**5**].—A light-yellow oil; $[\alpha]^{29}D$ −3.6° (c = 2.75, MeOH); ir (neat) 3351 (broad), 3182 (broad), 2962, 2925, 1658, 1631, 1450, 1377, 1296, 1120, 757 cm^{-1}; ^1H nmr (300 MHz, MeOH-d_4) δ 0.81 (s, 3H, Me-17), 0.82 (d, 3H, J = 6.7 Hz, Me-18), 1.55 (br s, 3H), 1.57 (br s, 3H), 1.30–2.25 (br m, 13H), 3.32 (t, 2H, J = 6.5 Hz, H-1′), 3.67 (t, 2H, J = 6.5, H-2′), 4.00 (d, 2H, J = 8.0 Hz, H-15), 4.14 (s, 2H, H-20), 5.08 (br t, 1H, H-10), 5.38 (br t, 2H, H-3 and H-14); ^{13}C nmr (75 MHz, MeOH-d_4) see Table 1; hrfabms m/z $[M+H]^+$ 440.2947 (100%) ($C_{23}H_{42}N_3SO_3$ requires 440.2946), 316 (16), 234 (23), 152 (88).

CONVERSION OF (−)-AGELASIDINE C [**3**] TO ITS 3,5-DIMETHYLPYRIMIDINE DERIVATIVE **4**.—A mixture of (−)-agelasidine C (35.6 mg), pyridine (0.23 ml) and 2,4-pentanedione (0.23 ml) was heated overnight at 125°–128° (about 19 h). The solvent was removed under reduced pressure and the residue obtained was chromatographed over a Si gel column with C_6H_6-Me_2CO (9:1) to give **4** (21.7 mg) as a light-yellow oil: $[\alpha]^{25}D$ −11.6° (c = 0.8, MeOH); uv (MeOH) 236 (ε 20,000), 294 nm (ε 6300); ir (neat) 3377 (broad), 3263 (broad), 2961, 2922, 2856, 1587, 1567, 1444, 1381, 1358, 1339, 1299, 1119 cm^{-1}; ^1H nmr (300 MHz, $CDCl_3$) δ 0.84 (s, 3H), 0.84 (d, 3H, J = 6.5 Hz), 1.58 (br s, 6H), 1.68 (br s, 3H), 2.27 (s, 6H), 1.32–2.35 (br m, 13H), 3.27 (t, 2H, J = 6.5 Hz), 3.74 (d, 2H, J = 7.3 Hz), 3.90 (q, 2H, J = 6.5 Hz), 5.04 (br s, 1H), 5.28 (t, 1H, J = 7.7 Hz), 5.40 (br s, 1H), 5.49 (t, NH, J = 6.5 Hz), 6.36 (s, 1H); ^{13}C nmr (75 MHz, $CDCl_3$) δ 15.82 (q), 16.22 (q), 16.83 (q), 19.14 (q), 21.04 (q), 23.84 (q), 25.50 (t), 26.23 (t), 27.06 (t), 33.25 (d), 34.23 (t), 35.27 (t), 35.56 (t), 39.79 (t), 40.41 (s), 51.16 (t), 54.18 (t), 110.16 (d), 110.52 (d), 122.59 (d), 124.05 (d), 136.87 (s), 139.64 (s), 146.33 (s), 161.73 (s), 167.60 (s); lreims m/z (% rel. int.) $[M]^+$ 487 (7), 364 (22), 282 (10), 216 (36), 151 (100), 81 (43).

CONVERSION OF (−)-AGELASIDINE D [**5**] TO ITS 3,5-DIMETHYLPYRIMIDINE DERIVATIVE **6**.—The procedure described above was followed using (−)-agelasidine D [**5**] (93.1 mg). After chromatography over a Si gel column using C_6H_6-Me_2CO (9:1), the 3,5-dimethylpyrimidine derivative **6** was isolated pure as a light-yellow oil: $[\alpha]^{25}D$ −9.0° (c = 3.4, MeOH); uv (MeOH) 236 (ε 14,000), 296 nm (ε 3400); ir (neat) 3394 (broad), 2960, 2926, 2858, 1589, 1569, 1539, 1458, 1340, 1296, 1119 cm^{-1}; ^1H nmr (300 MHz, $CDCl_3$) δ 0.83 (s, 3H), 0.83 (d, 3H, J = 6.9 Hz), 1.57 (br s, 6H), 2.28 (s, 6H), 1.34–2.32 (br m, 13H), 3.32 (t, 2H, J = 6.5 Hz), 3.88 (q, 2H, J = 6.5 Hz), 3.96 (d, 2H, J = 8.1 Hz), 4.13 (s, 2H), 5.05 (t, 1H, J = 7.1 Hz), 5.38 (br t, 2H), 5.64 (t, NH, J = 6.5 Hz), 6.37 (s, 1H); ^{13}C nmr (75 MHz, $CDCl_3$) δ 15.78 (q), 16.21 (q), 19.10 (q), 20.99 (q), 23.74 (q), 25.46 (t), 26.43 (t), 27.01 (t), 33.19 (d), 34.18 (t), 35.20 (t), 35.89 (t), 35.89 (t), 40.35 (s), 51.60 (t), 53.33 (t), 60.72 (t), 110.70 (d), 112.40 (d), 122.45 (d), 124.03 (d), 137.06 (s), 139.58 (s), 149.77 (s), 161.55 (s), 167.74 (s); lreims m/z (% rel. int.) $[M]^+$ 503 (7), 446 (4), 380 (11), 298 (11), 216 (26), 151 (100), 81 (28).

ACKNOWLEDGMENTS

The authors thank Dr. Vance Vicente from the United States Department of the Interior Fish and Wildlife Service for identification of the marine sponge and Mr. M.A. Medina, L.A. Fontán, and G. Cabrera for their kind assistance during collections. Special thanks are extended to Prof. A. Báez and Miss M.E. Pimentel for performing the CHO-K1 cytotoxicity tests and to Miss María L. Martínez for performing the antimicrobial bioassays. This work was funded in part by grants from the National Science Foundation EPSCoR Program (Grant No. R118610677), the NIH-MBRS Program (Grant No. S06 RR08102-17), the NSF-MRCE Program (Grant No. R11-8802961) and the University of Puerto Rico FIPI Program. High-resolution mass spectra determinations were performed by the Midwest Center for Mass Spectrometry, a National Science Foundation Regional Facility (Grant No. CHE8211164).

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Received 22 July 1991