EurJOC European Journal of Organic Chemistry

SHORT COMMUNICATION

DOI: 10.1002/ejoc.201200572

Antifungal Diterpene Alkaloids from the Caribbean Sponge *Agelas citrina*: Unified Configurational Assignments of Agelasidines and Agelasines

E. Paige Stout,[a] Lily C. Yu,[a] and Tadeusz F. Molinski*[a,b]

Dedicated to Professor Ernesto Fattorusso on the occasion of his 75th birthday

Keywords: Natural products / Alkaloids / Circular dichroism / Terpenoids

Three new diterpene alkaloids – the hypotaurocyamines, (–)-agelasidines E and F (**5**, **6**), and an adeninium salt, agelasine N (**9**) – were isolated from the Caribbean sponge *Agelas citrina* along with six known natural products: agelasines B–E (**7**, **10**–**12**), 2-oxo-agelasine B (**8**), and (–)-agelasidine C (**3**). The chemical structures of **5**, **6**, and **9** were elucidated by NMR spectroscopy and mass spectrometry. This represents the first report of natural products from the sponge *A. citrina*. Unified assignment of the absolute configurations of the new compounds and known compounds was achieved by chemical correlation, quantitative measurements of molar rotations, and comparative analysis by van't Hoff's principle of optical superposition. (–)-Agelasidine C (**3**) exhibited potent antifungal and modest cytotoxic activity against human chronic lymphocytic leukemia (CLL) cells.

Introduction

A growing class of nitrogenous diterpenes that include co-occurring hypotaurocyamines (e.g., **1**–**4**) and N^9-adeninium alkaloids from marine sponges of the genus *Agelas* have been shown to exhibit a wide range of biological activities, including antineoplastic,[1] antimicrobial,[2] and Na^+,K^+-adenosine triphosphatase (ATPase) inhibitory properties.[3] As a part of ongoing investigations into bioactive chemical constituents of Caribbean sponges, we explored the constituents of the potently antifungal extracts of the marine sponge *Agelas citrina*, which inhibited growth of several strains of *Candida* and the pernicious pathogens *Cryptococcus* var *grubii* and *Cryptococcus* var *gattii*. Here we report the isolation and structure elucidation of three new and six known alkaloid diterpenes – two hypotaurocyamine diterpenes, agelasidines E and F (**5**, **6**), one N^9-methyladeninium diterpene, agelasine N (**9**) – in addition to known compounds agelasines B–E (**7**, **10**–**12**),[3] 2-oxo-agelasine B (**8**),[4] and (–)-agelasidine C (**3**)[5] (Figure 1). Compounds **5**, **6**, and **9** expand the observed range of new oxidation motifs in the diterpenoid scaffold of the hypotaurocyamine and adeninium alkaloids, respectively, and are the first natural products reported from *A. citrina*, a species endemic to the Caribbean Sea. A unifying chiroptical analy-

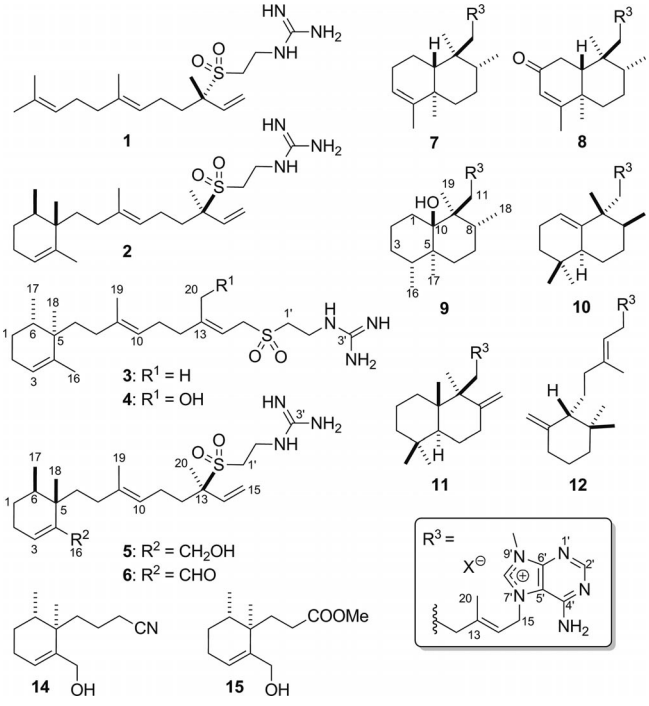


Figure 1. Agelasidines A–F (**1**–**6**); agelasines B (**7**), C (**10**), D (**11**), and E (**12**), (–)-2-oxo-agelasine B (**8**), and (+)-agelasine N (**9**); and synthetic compounds **14** and **15**.[15a] Guanidinium groups are depicted as a free-base.

[a] Department of Chemistry and Biochemistry, University of California,
San Diego, 9500 Gilman Drive MC0358, La Jolla, CA 92093-0358, USA
Fax: +1-858-822-0386
E-mail: tmolinski@ucsd.edu
[b] Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California,
San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0358, USA
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.201200572.

sis of the natural products by application of van't Hoff's principle of optical superposition, completed assignment of their absolute configurations.

Results and Discussion

A MeOH extract of the Caribbean sponge *A. citrina* was subjected to solvent partitioning and chromatography to yield compounds **3** and **5**–**12** (Figure 1), which represent the first natural products reported from *A. citrina*.

Compound (–)-**3** {HRMS (ESI): *m*/*z* = 424.2993 [M + H]$^+$, $C_{23}H_{41}N_3O_2S$} displayed ^{1}H NMR chemical shifts identical to the first reported values for (+)-agelasidine C [(+)-**3**],[2a] but the sign of the specific rotation {$[\alpha]_D^{25}$ = –12 (*c* = 2.0, MeOH)} was opposite {$[\alpha]_D^{25}$ = +8.5 (*c* = 2.0, MeOH)}.[2a] Conversion of (–)-**3** into the corresponding 4,6-dimethylpyrimidine derivative by reaction of the guanidine (pentane-2,4-dione, pyridine; Supporting Information, Scheme S1), followed by reductive ozonolysis according to the procedure of Nakamura and co-workers[2a] gave derivatives from (–)-**3** (Supporting Information) that were enantiomeric with those obtained from (+)-**3**.[2a] Consequently, the (5*R*,6*S*) configuration was assigned to the sample of (–)-**3** obtained from *A. citrina*.

Mass spectrometric measurements of agelasidine E (**5**), isolated as a colorless oil, gave HRMS (ESI): *m*/*z* = 440.2940 [M + H]$^+$, and suggested a molecular formula of $C_{23}H_{41}N_3O_3S$; one oxygen more than agelasines B and C (**2**, **3**). Analysis of the ^{1}H and ^{13}C NMR spectra of **5** (Supporting Information, Table S3) identified a terminal methylene at H_2-15 (δ_H = 5.50, 5.57 ppm) and a quaternary C-13 (δ_C = 68.9 ppm), which supported the branched hypotaurocyamine moiety similar to **2**. Lack of one of the methyl singlets and appearance of low-field diastereotopic methylene proton signals (δ_H = 3.97, 4.07 ppm) were consistent with an oxidation product of **2** formed by the replacement of a CH_3 group with a CH_2OH group. The location of the OH group in **5** was secured through detailed examination of the TOCSY and HMBC spectra (Figure 2). Interpretation of collective long-range ^{1}H–^{13}C correlations from H_2-16 to C-3 (δ_C = 124.7 ppm) and C-4 (δ_C = 143.6 ppm); H_3-18 (δ_H = 0.95 ppm) to C-4, C-5 (δ_C = 40.4 ppm), C-6 (δ_C = 34.4 ppm), and C-7 (δ_C = 36.1 ppm); and H-3 (δ_H = 5.79 ppm) to C-2 (δ_C = 25.7 ppm) and C-5 supported the placement of the hydroxy group at C-16 (δ_C = 63.1 ppm).

Unlike **5**, which showed no absorption in the UV/Vis spectrum above 210 nm, agelasidine F (**6**) contained a chromophore (λ_{max} = 240 nm). Analysis of the HRMS (ESI) data (*m*/*z* = 438.2786 [M + H]$^+$, $C_{23}H_{39}N_3O_3S$) and ^{1}H NMR signals of **6** (Supporting Information, Table S3) suggested the presence of a conjugated olefin associated with a new downfield proton signal (δ_H = 9.32 ppm, s) that was readily assigned to an α,β-unsaturated aldehyde (δ_C = 196.8 ppm). Differences in the NMR chemical shifts between **5** and **6** near the cyclohexene ring (Supporting Information, Table S3) and similarities of the remaining COSY and HMBC correlations allowed placement of the CH=O group at C-16.

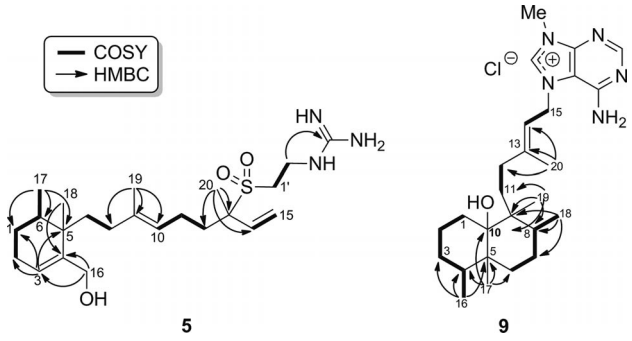
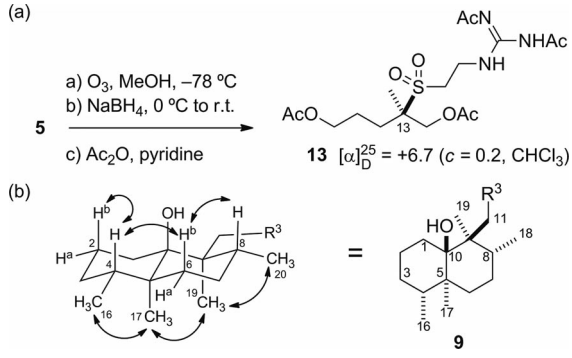


Figure 2. Key ^{1}H–^{1}H COSY/TOCSY (bold line) and HMBC (arrow) correlations for agelasidine E (**5**) and agelasine N (**9**).

Stereochemical assignments at C-13 for **5** and **6** were made by chemical degradation and comparison of the products with known compounds (Scheme 1a). Ozonolysis of either **5** or **6** (O_3, –78 °C, MeOH), followed by reductive workup ($NaBH_4$) and acetylation (Ac_2O, pyridine), gave tetraacetyl derivative (+)-**13** {$[\alpha]_D^{25}$ = +6.7 (*c* = 0.2, $CHCl_3$)}, reported earlier from degradation of **2**.[2a] Consequently, both **5** and **6** are (13*R*).



Scheme 1. (a) Chemical degradation of agelasidine E (**5**) to (*R*)-**13**.[2a] (b) Key NOEs (double-headed arrows) are shown for (+)-agelasine N (**9**).

The configurations of C5 and C6 in the cyclohexene segments of **5** and **6** were solved independently by global estimations of molar rotation, [*M*], by employing the principle of optical superposition, first proposed by van't Hoff[12] based on canonical observations by Le Bel. The power of the method has been nicely demonstrated by Wipf and Beratan in applications to natural product assignments of absolute and relative configurations of relatively complex natural products.[13a,13c,14] In its simplest interpretation, the molar rotation is the summation of independent atomic contributions to optical rotatory power – both positive and negative angles – from isolated centers of asymmetry, which in turn are dependent upon polarizability. Molar rotations can be calculated by DFT methods,[13b] but it is not trivial and requires formidable computational power. More conveniently, global contributions to molar rotation $[M]_D$ measured at the sodium D-line emission, can be approximated from the measured molar rotations of simpler analogs {derived from $[\alpha]_D$ according to Equation (1), where *M* is the molar mass}, each containing isolated chiral molecular

segments. In practice, this empirical approach is valid and van't Hoff's rule can be applied if stereocenters are separated by one or more methylene units and non-bonded interactions are negligible.[13a]

$$[\Phi]_D = \frac{[\alpha]_D \times M}{100} \quad (1)$$

To test the applicability of the method in the context of **5** and **6**, we calculated the values of all four stereoisomers of agelasidine B (**2**; Table 1, Entries 6–9) by superposition of combinations of both enantiomers of **1** and **3** and compared them to the measured value (Table 1, Entry 2; $[\Phi]_D$ = –11.5 deg mol^{-1} dm^3 cm^{-1}). A good fit of the calculated value (Table 1, Entry 9; $[\Phi]_D$ = –19.7) was obtained only for the known (5*S*,6*R*,13*R*) configuration of (–)-**2**, independently derived by Nakamura by using chemical degradation.[2a]

Table 1. Measured and Calculated molar rotations[a] $[\Phi]_D$ (deg mol^{-1} dm^3 cm^{-1}) by van't Hoff's principle of optical superposition.[12,13]

Entry	Compound	$[\Phi]_{D\ meas.}$[b]	$[\Phi]_{D\ calcd.}$
1	(+)-(13*S*)-**1**	74.9	
2	(–)-(5*S*,6*R*,13*R*)-**2**	–11.5	
3	(–)-(5*R*,6*S*)-**14**[b]	–15.9	
4	(–)-(5*R*,6S,13*R*)-**5**	–44.3	
5	(–)-(5*R*,6S,13*R*)-**6**	–59.7	
6	(5*R*,6*S*,13*S*)-**2a**		+19.7
7	(5*R*,6*S*,13*R*)-**2b**		–130.1
8	(5*S*,6*R*,13*S*)-**2c**		+130.1
9	(5*S*,6*R*,13*R*)-**2d**		–19.7
10	(5*R*,6*S*,13*S*)-**5a**		+56.3
11	(5*R*,6*S*,13*R*)-**5b**		–93.4
12	(5*S*,6*R*,13*S*)-**5c**		+93.4
13	(5*S*,6*R*,13*R*)-**5d**		–56.3

[a] Calculated from $[\alpha]_D$ values (MeOH, see Table 2) of the corresponding Cl^- salts. [b] See ref.[15a]

The method was applied next to agelasidine E [(–)-**5**] by combinations of $[\Phi]_D$ for **1** and **15** (Figure 1, $[\alpha]_D$ = –8.2) prepared in optically pure form reported by Paquette and co-workers as an intermediate in the synthesis of (+)-cleomeolide A.[15] Again, the best fit for the measured molar rotation of (–)-**5** ($[\Phi]_D$ = –44.3) was obtained for the calculated value (**5d**, $[\Phi]_D$ = –56.3) of the (5*S*,6*R*,13*R*) configuration of the natural product. This also matches agelasidine F [(–)-**6**] ($[\Phi]_D$ = –59.7) although, strictly, the molecular structure comparison here is not as valid as with (–)-**5**. In any case, as co-occurrence of (–)-**5** with (–)-**6** likely results from the latter being the oxidation product of the former; both are expected to share the same absolute configuration. Comparisons of $[\Phi]_D$ by using a different synthetic analog **14**[15a] and a synthetic compound[15b] lacking the allylic OH group [cf., (–)-**3**] gave a poor fit ($[\Phi]_D$ = –9.4 and +1.8, respectively; Supporting Information, Table S1). Despite the levorotatory nature of (–)-**3**, (–)-**5**, and (–)-**6** obtained from the same sample of *Agelas citrina*, the configurations at C5 and C6 of the latter two are *opposite* to the former, which reveals the dominant rotatory power of the allylic C-13 quaternary hypotaurocyamine group. These examples serve to illustrate the power of optical superposition and molar rotations[13] and underscore a reminder of the inherent risk of over-reliance on the sign of $[\alpha]_D$ alone.[4]

Agelasine N (**9**), an amorphous white solid, revealed a formula of $C_{26}H_{42}N_5O$ from the HRMS (ESI) data (*m*/*z* = 440.3386 $[M + H]^+$) and upon comparison with those of known agelasines **7** and **10**–**12**, indicating the addition of H_2O. The ^1H NMR spectroscopic data of **9** were consistent with a hydroxy-substituted clerodane ring system. HMBC correlations from both H_3-17 (δ_H = 0.94 ppm) and H_3-19 (δ_H = 0.89 ppm) to C-10 (δ_C = 80.6 ppm) supported a tertiary alcohol by placement of the OH group at C-10 (Figure 2). NOE correlations of **9** allowed assignment of the relative configuration at all stereogenic centers in a *trans*-fused clerodane bicyclic ring system through observation of *syn*-facial NOEs (Scheme 1b). The paucity of available sample (≈0.5 mg, **9**) precluded determination of absolute configuration; consequently, the depicted configuration of **9** is arbitrary.

The reported absolute configurations of agelasines A–F were determined through stepwise chemical degradation, followed by comparison of the products to known compounds by optical rotation and CD.[3] Total syntheses of agelasines A,[6] B,[7] D,[8] and E[9] supported the assignments, or – in the case of the synthesis of agelasine C (**10**)[10] – led to revision of the originally assigned absolute configuration. Subsequent configurational assignments of new agelasines were heavily reliant upon comparisons of $[\alpha]_D$ values with those of known agelasines (Table 2), despite significant structural differences, including variable counterions (HCO_2^-, $CF_3CO_2^-$, Cl^-) that result from purification of quaternary N^9-methyl adeninium or guanidinium salts under different protocols, or the use of different solvents for optical rotation measurements.[4,11] The inherent danger in the incorrect assignment of the absolute configuration through reliance on optical rotation alone is well known but may be compounded further by variables in measurement of *α*, especially highly charged or polar compounds. For example, the $[\alpha]_D$ of sceptrin, a pyrrole-aminoimidazole alkaloid from several different *Agelas* spp., was shown to be highly variable and dependent – both in sign and magnitude – on concentration and, particularly, on the nature of the counterion.[16]

To resolve lingering concerns of assignments of configuration in the agelasine/agelasidine family of adeninium alkaloids based on optical activity, we undertook measurements of $[\alpha]_D$ of (–)-agelasine B (**7**) and (–)-agelasidine C (**3**) after ion exchange with Cl^- or $HCOO^-$ while using a fixed concentration (*c*) close to the literature reported values[2,3] or varying *c* over a range of values (Supporting Information). Neither the counterion (Cl^-, $HCOO^-$) or variation of *c* across the range 0.03–3.0 g/100 mL appeared to affect the sign or magnitude of $[\alpha]_D$, within experimental error (Supporting Information, Table S2).

Nevertheless, an examination of the reported specific rotations of the same compound (Table 2) isolated from different *Agelas* specimens from different geographic locations reveals variable optical activity.

Table 2. $[\alpha]_D$ (MeOH) of salts of agelasines A–N and agelasidines A–F.

Entry	Compound		$[\alpha]_D$	Counterion	c (g/100 mL)	Ref
1	agelasine A		–31.3	Cl^-	0.59	[3a]
2	agelasine B	(–)-**7**	–21.5	Cl^-	1.0	[3a]
3		(–)-**7**	–20	HCO_2^-	1.5	[a]
4	2-oxo-**7**	(–)-**8**	–6.1	HCO_2^-	0.3	[4]
5			–7.4	HCO_2^-	0.5	[a]
6	agelasine C	(–)-**10**	–55.1	Cl^-	2.04	[3a]
7	agelasine D	(+)-**11**	+10.4	Cl^-	1.1	[3a]
8		(–)-**11**	–5.1	HCO_2^-	1.0	[a]
9	agelasine E	(–)-**12**	–17.1	Cl^-	1.88	[3b]
10		(+)-**12**	+1.7	HCO_2^-	1.7	[a]
11	agelasine F[b]		–5.5	Cl^-	2.55	[3b]
12	agelasine G		–85	Cl^-	0.02	[1]
13	agelasine H		–63.9	HCO_2^-	0.36	[11a]
14	agelasine I		–2.5	HCO_2^-	0.2	[11a]
15	agelasine J		+14	HCO_2^-	0.46	[11b]
16	agelasine K		+60	HCO_2^-	0.11	[11b]
17	agelasine L		–3.2	HCO_2^-	1.0	[11b]
18	agelasine M		+208	HCO_2^-	0.8	[4]
19	agelasine N	(+)-**9**	+6.4	Cl^-	0.5	[a]
20		(+)-**9**	+5.6	HCO_2^-	0.5	[a]
21	agelasidine A	(+)-**1**	+19.1	Cl^-	1.0	[2a]
22	agelasidine B	(–)-**2**	–2.5	Cl^-	0.43	[2a]
23	agelasidine C	(+)-**3**	+8.5	Cl^-	2.0	[2a]
24		(–)-**3**	–5.6	Cl^-	7.2	[5]
25		(–)-**3**	–12.2	Cl^-	2.0	[a]
26		(–)-**3**	–11.8	HCO_2^-	2.0	[a]
27	agelasidine D	(–)-**4**	–3.6	Cl^-	2.75	[5]
28	agelasidine E	(–)-**5**	–9.3	Cl^-	1.0	[a]
29	agelasidine F	(–)-**6**	–12.6	HCO_2^-	2.0	[a]

[a] This report. [b] = ageline A.

Agelasines B (**7**) and C (**10**) and 2-oxo-agelasine B (**8**) from *Agelas citrina* displayed specific rotations consistent with earlier reports;[3a,4] however, agelasines D (**11**) and E (**12**) were both opposite in sign with different magnitudes (Table 2, Entries 7/8 and 9/10, respectively), and consequently, are antipodal to the originally reported natural products. It is also of interest to note that the $[\alpha]_D$ value of (–)-agelasidine C (**3**) originally reported from *A. clathrodes*[5] has approximately half the magnitude of our observed value for (–)-**3** (Table 2, Entries 22 and 23, respectively) suggesting not only the occurrence of enantiomeric modifications from different geographically disparate sources but the possibility of heterogeneous composition (% *ee*) as non-racemic mixtures of enantiomers.

However, enantiomeric heterogeneity is still rare among marine natural products. The origins of optical purity in marine natural products have been the subject of discussion and speculation.[17,18] One explanation for the biosynthesis of antipodal C5, C6 configurations between (–)-**3** and (–)-**5/6** is that both enantiomers of **3** may be formed with less stringent asymmetric control (a promiscuous terpene cyclase, or two independent cyclases, as seen with independent α- and β-pinene biosynthesis in *Salvia officinalis*[19]) and that **5** and **6** arise through an independent oxidoreductase that effects kinetic resolution during allylic hydroxylation under more stringent control.

Natural products **3**, **5**–**8**, **10**, and **12** were tested for activity against the fungal pathogen *Candida albicans* and human chronic lymphocytic leukemia (CLL) cells. (–)-Agelasidine C (**3**) exhibited modest cytotoxicity against CLL (IC_{50} = 10 μM) and potent antifungal activity (MIC = 0.5 μg/mL). Other agelasidines showed less potent antifungal activity (**5**, 8.0 and **6**, 4.0 μg/mL), whereas the other compounds were less active (**8** and **10** >32; **12**, 16.0 μg/mL).

Conclusions

In conclusion, we describe three new alkaloid diterpenes, (–)-agelasidines E and F (**5**, **6**) and (+)-agelasine N (**9**), providing the first report of natural products from the Caribbean sponge *Agelas citrina*. Experimental evidence suggests that the counterion and the concentration have negligible effects on the sign and magnitude of $[\alpha]_D$ for members in this family of nitrogenous diterpenoid natural products.

Experimental Section

General Experimental Procedures: General experimental procedures are described elsewhere.[17a]

(–)-Agelasidine C (3): Pale yellow oil. $[\alpha]_D^{25}$ = –12 (c = 2.0, MeOH). FTIR (ATR, ZnSe): $\tilde{\nu}$ = 3234 (br.), 2966, 2935, 1633, 1572, 1450, 1380, 1297, 1120 cm^{-1}. HRMS (ESI): m/z = 424.2993 $[M + H]^+$ (calcd. for $C_{23}H_{42}N_3O_2S$, 424.2998); NMR spectroscopic data are in agreement with the literature;[2a,5] $[\alpha]_D$ is opposite in sign to the enantiomer reported by Nakamura and co-workers from *Agelas mauritiana*,[2a] but the same as that reported by Rodriguez[5] from a sample of *A. clathrodes* (see Table 2).

(–)-Agelasidine E (5): Colorless oil. $[\alpha]_D^{25}$ = –9.3 (c = 1.0, MeOH). FTIR (ATR, ZnSe): $\tilde{\nu}$ = 3350 (br.), 3173 (br.), 2958, 2928, 1672, 1633, 1458, 1373, 1281, 1135, 1082, 1005 cm^{-1}. HRMS (ESI): m/z = 440.2940 $[M + H]^+$ (calcd. for $C_{23}H_{42}N_3O_3S$, 440.2941). NMR spectroscopic data, see the Supporting Information.

(–)-Agelasidine F (6): Colorless oil. $[\alpha]_D^{25}$ = –12.6 (c = 2.0, MeOH). UV (MeOH): λ ($\log\varepsilon$) = 229 (4.60), 273 (3.60) nm. CD (MeOH): λ = 320 ($\Delta\varepsilon$ +0.78), 220 ($\Delta\varepsilon$ –4.4). FTIR (ATR, ZnSe): $\tilde{\nu}$ = 3350 (br.), 3165 (br.), 2965, 2928, 1680, 1625, 1572, 1458, 1373, 1343, 1289, 1135 cm^{-1}. HRMS (ESI): m/z = 438.2786 $[M + H]^+$ (calcd. for $C_{23}H_{40}N_3O_3S$, 438.2785).

(–)-Agelasine B (7): White amorphous powder. $[\alpha]_D^{25}$ = –20 (c = 1.5, MeOH). NMR spectroscopic data are in agreement with the literature.[3a]

(–)-2-Oxo-agelasine B (8): Pale yellow oil. $[\alpha]_D^{25}$ = –7.4 (c = 0.5, MeOH). NMR spectroscopic data are in agreement with the literature.[4]

(+)-Agelasine N (9): Amorphous white solid. $[\alpha]_D^{25}$ = +5.6 (c = 0.5, MeOH). UV (MeOH): λ (ε) = 210 (4.03), 273 (3.67) nm. FTIR (ATR, ZnSe): $\tilde{\nu}$ = 2952, 2925, 2357, 1658, 1586, 1462, 1384, 1344 cm^{-1}. HRMS (ESI): m/z = 440.3386 $[M + H]^+$ (calcd. for $C_{26}H_{43}N_5O$, 440.3389). NMR spectroscopic data, see Supporting Information.

(–)-Agelasine C (10): Pale yellow oil. $[\alpha]_D^{25}$ = –12 (c = 1.0, MeOH). NMR spectroscopic data are in agreement with the literature.[3a]

(–)-Agelasine D (11): Amorphous solid. $[\alpha]_D^{25}$ = –5.1 (*c* = 1.0, MeOH). NMR spectroscopic data are in agreement with the literature.[3a]

(+)-Agelasine E (12): Amorphous solid. $[\alpha]_D^{25}$ = +1.7 (*c* = 2.0, MeOH). NMR spectroscopic data are in agreement with the literature.[3b]

Supporting Information (see footnote on the first page of this article): 1D and 2D NMR spectra of **5**, **6**, and **9**, along with key experimental details for degradation correlation of **3** and **5**.

Acknowledgments

We are grateful for funding from the National Institutes of Health (NIH AI 100776, AI039987 to T. F. M) and a Ruth L. Kirschstein National Research Service Award (NIH T32 CA009523 to E. P. S.). The 500 MHz NMR spectrometer was purchased with a grant from National Science Foundation (NSF) (CRIF, CHE0741968). We thank Y. Su (UCSD) for HRMS measurements, D. Dalisay and R. Andersen (University of British Columbia) for antifungal assays, M. Choi and J. Castro (UCSD) for CLL cell cytotoxicity assays, and J. Pawlik (UNC Wilmington) together with the captain and crew of the RV Walton Smith for logistical support during collecting expeditions and in-field assays.

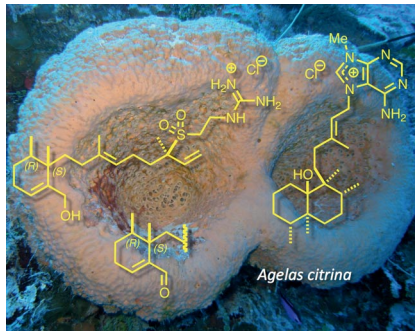
[1] K. Ishida, M. Ishibashi, H. Shigemori, T. Sasaki, J. Kobayashi, *Chem. Pharm. Bull.* **1992**, *40*, 766–767.
[2] a) H. Nakamura, H. Wu, J. Kobayashi, M. Kobayashi, Y. Ohizumi, Y. Hirata, *J. Org. Chem.* **1985**, *50*, 2494–2497; b) F. Yang, M. T. Hamann, Y. Zou, M. Y. Zhang, X. B. Gong, J. R. Xiao, W. S. Chen, H. W. Lin, *J. Nat. Prod.* **2012**, *75*, 774–778.
[3] a) H. Nakamura, H. M. Wu, Y. Ohizumi, Y. Hirata, *Tetrahedron Lett.* **1984**, *25*, 2989–2992; b) H. Wu, H. Nakamura, J. Kobayashi, Y. Ohizumi, Y. Hirata, *Tetrahedron Lett.* **1984**, *25*, 3719–3722.
[4] L. Calcul, K. Tenney, J. Ratnam, J. H. McKerrow, P. Crews, *Aust. J. Chem.* **2010**, *63*, 915–921.
[5] J. J. Morales, A. D. Rodriguez, *J. Nat. Prod.* **1992**, *55*, 389–394.
[6] E. Piers, M. L. Breau, Y. X. Han, G. L. Plourde, W. L. Yeh, *J. Chem. Soc. Perkin Trans. 1* **1995**, 963–966.
[7] E. Piers, J. Y. Roberge, *Tetrahedron Lett.* **1992**, *33*, 6923–6926.
[8] B. T. Utenova, L. L. Gundersen, *Tetrahedron Lett.* **2004**, *45*, 4233–4235.
[9] A. K. Bakkestuen, L. L. Gundersen, *Tetrahedron* **2003**, *59*, 115–121.
[10] I. S. Marcos, N. Garcia, A. J. Sexmero, P. Basabe, D. Diez, J. G. Urones, *Tetrahedron* **2005**, *61*, 11672–11678.
[11] a) X. Fu, F. J. Schmitz, R. S. Tanner, M. Kelly-Borges, *J. Nat. Prod.* **1998**, *61*, 548–550; b) J. Appenzeller, G. Mihci, M.-T. Martin, J.-F. Gallard, J.-L. Menou, N. Boury-Esnalllt, J. Hooper, S. Petek, S. Chevalley, A. Valentin, A. Zaparucha, A. Al-Mourabit, C. Debitus, *J. Nat. Prod.* **2008**, *71*, 1451–1454.
[12] J. H. van't Hoff, *Die Lagerung der Atome im Raume*, Vieweg, Braunschweig **1908**, ch. 8, pp. 95–97.
[13] a) R. K. Kondru, S. Lim, P. Wipf, D. N. Beratan, *Chirality* **1997**, *9*, 469–477; b) R. K. Kondru, P. Wipf, D. N. Beratan, *Science* **1998**, *282*, 2247–2250; c) R. K. Kondru, C. H. T. Chen, D. P. Curran, D. N. Beratan, P. Wipf, *Tetrahedron: Asymmetry* **1999**, *10*, 4143–4150.
[14] For a review of examples of modern structure elucidation by integrated spectroscopic, degradative, and chiroptical analysis, see B. I. Morinaka, T. F. Molinski, *Tetrahedron* **2012**, DOI: 10.1016/j.tet.2011.12.070.
[15] a) L. A. Paquette, T.-Z. Wang, C. M. G. Philippo, S. Wang, *J. Am. Chem. Soc.* **1994**, *116*, 3367–3374; b) Á. Proszenyák, M. Brændvang, C. Charnock, L.-L. Gundersen, *Tetrahedron* **2009**, *65*, 194–199.
[16] P. S. Baran, K. Li, D. R. O'Malley, C. Mitsos, *Angew. Chem.* **2006**, *118*, 255; *Angew. Chem. Int. Ed.* **2006**, *45*, 249–252.
[17] a) E. P. Stout, Y.-G. Wang, D. Romo, T. F. Molinski, *Angew. Chem. Int. Ed.* **2012**, *51*, 4877–4881; b) E. P. Stout, B. I. Morinaka, Y.-G. Wang, D. Romo, T. F. Molinski, *J. Nat. Prod.* **2012**, *75*, 527–530; c) P. A. Searle, N. M. Jamal, G. M. Lee, T. F. Molinski, *Tetrahedron* **1994**, *50*, 3879–3888; d) P. Horton, W. D. Inman, P. Crews, *J. Nat. Prod.* **1990**, *53*, 143–151.
[18] The trivial possibility of contamination of moderately levorotatory samples of **3** by small quantities of highly dextrorotatory impurities cannot be excluded.
[19] H. Gambliel, R. Croteau, *J. Biol. Chem.* **1984**, *259*, 740–748.

Received: May 1, 2012
Published Online: ■

SHORT COMMUNICATION

Marine Natural Products

Chiroptical methods, including molar rotations and application of van't Hoff's principle of optical superposition, were applied to solve the configurations of two new diterpenoid alkaloids isolated from *Agelas citrina*, a sponge endemic to the Bahamas.



Keywords: Natural products / Alkaloids / Circular dichroism / Terpenoids

