Eudistones A and B: Two Novel Octacyclic Alkaloids from a Seychelles Tunicate, *Eudistoma* sp.

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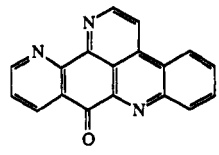
The known compound ascididemin (1) and two novel octacyclic aromatic alkaloids, eudistones A (2) and B (3), were isolated from the Seychelles tunicate *Eudistoma* sp. The structures of 2 and 3, which possess a unique carbon skeleton, were elucidated by extensive NMR analysis and chemical interconversion. The relative configuration was determined by comparison of NMR coupling constants with those predicted by molecular modeling.

The number of polycyclic aromatic alkaloids isolated from sessile marine invertebrates has increased rapidly in the last several years.[1] Their significant antitumor activities and remarkable structural features have attracted considerable attention from both synthetic chemists[2] and pharmacologists.[3] In this paper, we report the isolation and structural elucidation of two octacyclic alkaloids, eudistones A (2) and B (3), which are minor metabolites of the Seychelles tunicate *Eudistoma* sp.

The tunicate *Eudistoma* sp. was collected at Praslin Island, Seychelles. The frozen animal was extracted with methanol–dichloromethane, and the extracts were partitioned between butanol and water. Successive chromatography of the butanol-soluble material on Sephadex LH-20 and Spectral 40S yielded ascididemin (1, 0.26% dry wt), eudistone A (2, 0.0023% dry wt), and eudistone B (3, 0.0018% dry wt). Ascididemin (1) was previously isolated from the tunicate *Didemnum* sp.[4] and has been synthesized.[2a]

Eudistone A (2) was obtained as an amorphous yellow powder. The molecular formula, $C_{27}H_{19}N_5O$, which was determined by high-resolution mass measurement, implied 21 degrees of unsaturation. The ^{13}C NMR signal at δ 191.8 (s) and an IR band at 1660 cm^{-1} indicated the presence of an unsaturated ketone, and the broad IR bands at 3360 and 3220 cm^{-1} could be attributed to primary or secondary amines. An initial analysis of the ^1H NMR spectral data



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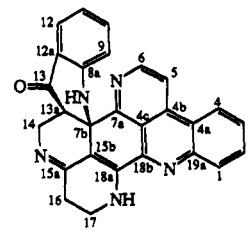

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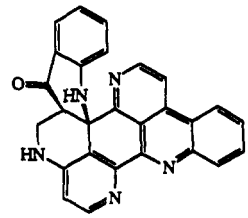

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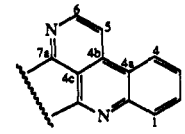
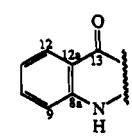
using a COSY experiment revealed three isolated spin systems in the aromatic region and two in the aliphatic region of the spectrum. The signals at δ 8.33 (br d, 1 H, J = 8.0 Hz), 8.01 (br t, 1 H, J = 7.5 Hz), 7.88 (br t, 1 H, J = 7.5 Hz), and 8.79 (br d, 1 H, J = 7.5 Hz) in the DMSO-d_6/CD_3OD spectrum were assigned to a 1,2-substituted benzene ring. A second system at δ 8.52 (d, 1 H, J = 5.5 Hz) and 8.48 (d, 1 H, J = 5.5 Hz) in the DMSO-d_6/$CDCl_3$ spectrum was indicative of a heteroatomic ring. Comparison of these data and selected ^{13}C NMR data with literature values suggested the presence of the familiar moiety a, which is common to many of the pentacyclic aromatic alkaloids such as segolines,[6] varamines,[7] and ascididemin (1),[4] the major component of this tunicate. The presence of fragment a was confirmed by an HMBC experiment in 2:1 DMSO-d_6/CD_3OD, optimized for J = 8 Hz, together with NOEDS measurements that showed the spatial proximity of H-4 (δ 8.72) and H-5 (δ 8.52).



a



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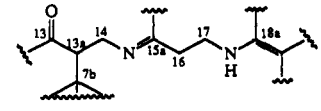

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Table I. The ^{1}H NMR Spectral Data and HMBC Correlations of Eudistone A (2)

H no.	chemical shift (mult, J in Hz) DMSO-d_6/CD_3OD (2:1)	chemical shift (mult, J in Hz) DMSO-d_6/$CDCl_3$ (2:1)	HMBC (J = 8 Hz) DMSO-d_6/CD_3OD (2:1)
1	8.33 (br d, 8.0)	8.20 (d, 8.0)	C-3, C-4a
2	8.01 (br t, 7.5)	7.93 (br t, 8.0)	C-4, C-19a
3	7.88 (br t, 7.5)	7.79 (br t, 7.5)	C-1
4	8.79 (br d, 7.5)	8.72 (br d, 8.0)	C-2, C-4b, C-19a
5	8.57 (s)	8.52 (d, 5.5)	C-4c, C-6
6	8.57 (s)	8.48 (d, 5.5)	C-4b, C-7a
9	6.32 (d, 8.5)	6.34 (d, 8.0)	C-11
10	7.11 (br t, 8.0)	7.04 (br t, 7.0)	C-8a, C-12
11	6.62 (br t, 8.0)	6.55 (br t, 5.5)	C-9, C-12a
12	7.68 (dd, 8.0, 1.0)	7.64 (br d, 7.5)	C-8a, C-10, C-13
13a	3.21 (dd, 12.0, 5.5)	2.92 (dd, 12.0, 5.5)	C-7a,[a] C-12a,[a] C-13, C-14
14ax	3.58 (br t, 13.0)	3.61 (dd, 16.5, 12.0)	C-7b, C-13, C-13a, C-15a
14eq	3.68 (dd, 14.0, 5.5)	~3.74 (m)	C-7b, C-13a, C-15a
16ax	3.03 (dt, 6.0, 15.5)	~2.69 (m)	C-15a, C-17
16eq	2.92 (br dd, 15.5, 5.5)	~2.69 (m)	C-15a
17ax	3.70 (dt, 5.5, 15.5)	3.59 (dt, 6.5, 16.0)	C-15a
17eq	3.99 (ddd, 15.5, 5.5, 2.0)	3.75 (dd, 16.0, 5.5)	C-15a, C-16, C-18a
NH-8	-	6.96 (br s, D_2O exchangable)	
NH-18	-	10.16 (broad, D_2O exchangable)	

[a] Correlations were shown by HMBC optimized for J = 6 Hz.

A third aromatic spin system at δ 6.32 (d, 1 H, J = 8.5 Hz), 7.11 (br t, 1 H, J = 8 Hz), 6.62 (br t, 1 H, J = 8 Hz), and 7.68 (dd, 1 H, J = 8, 1 Hz) in the DMSO-d_6/CD_3OD spectrum indicated the presence of the second 1,2-disubstituted benzene ring. The HMBC experiment showed a correlation between the carbonyl signal at δ 191.8 (s) and the ^{1}H NMR signal at 6.32 ppm that indicated the presence of an aromatic ketone. The downfield chemical shift of C-8a at δ 146.4, which was assigned from the HMBC experiment, suggested that an amino group was attached to that carbon, and this was confirmed by measurement of a significant NOE between NH-8 and H-9. Furthermore, the ^{1}H and ^{13}C NMR data for 2-aminoacetophenone[8] were very similar to those assigned to fragment **b**.

Analysis of the aliphatic portion of the ^{1}H NMR spectrum (see Table I) revealed the presence of two isolated spin systems that were assigned to $-CH_2CH_2-$ and $-CH_2CH<$ groups; the values of the coupling constants indicated that both were contained in six-membered rings. The chemical shifts of the H-14 signals at δ 3.68 and 3.58 and the H-17 signals at 3.99 and 3.70 required that both C-14 and C-17 be adjacent to nitrogen atoms. In DMSO-d_6/$CDCl_3$ solution, the H-17 signals were coupled to the NH-18 signal at δ 10.16. Once again the HMBC experiment (J = 8 Hz) provided crucial information about the connectivities defined in fragment c. Cross-peaks between both C-13 [δ 191.8 (s)] and C-14 [41.4 (t)] and H-13a (δ 3.21) indicated the attachment of C-13a to the carbonyl of the 2'-aminophenone moiety. The signal at δ 163.8 (s), which was assigned to an imine carbon (C-15a), showed two and three bond couplings to H-14, H-16, and H-17 signals, indicating that the two isolated system should be linked through an imine bond. Furthermore, a long-range correlation between the H-17$_{eq}$ and C-18a [δ 149.9 (s)] signals suggested that N-18 was attached to an olefinic carbon and a similar correlation between H-14 and the only aliphatic quaternary carbon at δ 55.0 (s) required that the aliphatic carbon atom (C-7b) be adjacent to C-13a and to a nitrogen atom, presumably N-8. The identity of fragment **c** and its connection to fragment **b** were therefore defined.

The structure of eudistone A (2) could be elucidated by combining partial structures **a**, **b**, and **c**, together with the two remaining carbon atoms that give rise to ^{13}C NMR signals at δ 114.9 (s) and 144.6 (s); these signals, which were not correlated to any proton signals in the HMBC spectra, were assigned to C-15b and C-18b, respectively. However, the data in hand did not eliminate the possibility that C-7a was connected to C-18a and C-7b was attached to C-18b. A second HMBC experiment, optimized for J = 6 Hz, clearly showed a weak correlation between H-13a and C-7a, eliminating the alternate structure. In a proton-coupled ^{13}C NMR spectrum, the signal at δ 144.6 was sharp and its chemical shift required attachment to a nitrogen atom while the signal at δ 114.9 was broadened due to the small long-range couplings between H-16 and C-15b.

There are two possible isomers of eudistone A (2), one having a *cis* ring junction at C-7b and C-13a and the other with a *trans*-diequatorial ring junction. Comparison of the observed spectral data with those predicted for the two isomers by using a computer modeling program[9] clearly supported a *cis* ring junction. The coupling constant between H-13a and C-7a was determined by a heteronuclear proton-decoupling experiment: irradiation of the H-6 signal caused the C-7a signal to appear as a doublet with J = 1.5 Hz. The calculated values[9,10] for this coupling constant are J = 2.6 Hz for the *cis* ring junction and J = 8.4 Hz for the *trans* ring junction. Furthermore, the observed coupling constants between the H-13a signal and the axial and equatorial H-14 signals agree well with those predicted for the *cis* ring junction.

Eudistone B (3) was obtained as a white amorphous powder. The molecular formula, $C_{27}H_{17}N_5O$, required one more degree of unsaturation than was present in eudistone A (2). Both the ^{1}H NMR spectral data [δ 8.30 (d, 1 H, J = 6 Hz) and 6.84 (d, 1 H, J = 6 Hz)] and ^{13}C NMR data [δ 146.9 (d) and 109.6 (d)] contained two new aromatic signals in place of the C-16 and C-17 methylene signals of 2. Eudistone B (3) is therefore a dehydrogenation product of eudistone A (2). Most of the ^{1}H and ^{13}C NMR signals were assigned by interpretation of the HMBC experiment

(8) Sadtler Research Laboratories, Sadtler Standard Spectra, ^{1}H NMR, p 4987; ^{13}C NMR, p 2242.

(9) The C-7a/C-13a dihedral angles were calculated for both the *trans* (θ = 166°) and *cis* (θ = 53°) ring junctions using PC Model version 2.03 (Serena software) and the expected coupling constants were calculated according to the equation $^3J_{C-H} = 4.26 - 1.00 \cos\theta + 3.56 \cos^2\theta$.[10]

(10) Marshall, J. L. *Methods in Stereochemical Analysis, vol. 2, Carbon-carbon and carbon-proton NMR couplings*; Verlag Chemie International: Deerfield Beach, FL, 1983; p 22.

Table II. The ^{1}H NMR Spectral Data and HMBC Correlations of Eudistone B (3)

H no.	chemical shift (mult, J in Hz) DMSO-d_6/CD_3OD (2:1)	chemical shift (mult, J in Hz) DMSO-d_6/$CDCl_3$ (2:1)	HMBC (J = 8 Hz) DMSO-d_6/CD_3OD (2:1)
1	8.37 (br d, 8.5)	8.35 (br d, 8.0)	C-3
2	7.97 (br t, 8.0)	7.89 (br t, 7.5)	C-4, C-19a
3	7.82 (br t, 8.0)	7.74 (br t, 7.5)	C-1, C-4a
4	8.77 (br d, 8.0)	8.68 (br d, 8.0)	C-2, C-4b, C-19a
5	8.59 (s)	8.53 (d, 6.0)	
6	8.59 (s)	8.50 (d, 6.0)	C-4b, C-5, C-7a
9	6.39 (d, 8.0)	6.35 (d, 8.0)	C-11
10	7.13 (br t, 7.5)	7.03 (br t, 7.0)	C-8a, C-12
11	6.65 (br t, 7.5)	6.56 (br t, 5.5)	C-9, C-12a
12	7.71 (dd, 8.0, 1.0)	7.66 (br d, 7.5)	C-8a, C-10, C-13
13a	3.32 (dd, 12.0, 5.5)	3.19 (dd, 12.0, 6.0)	C-13, C-14
14ax	3.43 (t, 12.0)	3.38 (t, 12.0)	C-13, C-13a
14eq	3.55 (dd, 12.0, 5.5)	3.42 (dd, 12.0, 6.0)	C-7b, C-13a, C-15a
16	6.84 (d, 6.0)	6.69 (d, 6.0)	C-15b
17	8.30 (d, 6.0)	8.32 (d, 6.0)	C-15a, C-16
NH-8	-	7.21 (br s, D_2O exchangable)	
NH-15	-	7.48 (braod, D_2O exchangable)	

Table III. The ^{13}C NMR Spectral Data of Eudistones A (2) and B (3)

C no.	chemical shift[a] (DMSO-d_6/CD_3OD, 2:1) 2	3	C no.	chemical shift[a] (DMSO-d_6/CD_3OD, 2:1) 2	3
1	131.0	130.7	11	117.9	118.17
2	132.4	132.2	12	126.3	126.2
3	130.5	129.5	12a	117.9	118.20
4	124.3	124.4	13	191.8	191.8
4a	123.6	123.0	13a	45.7	43.9
4b	137.8	137.9	14	41.4	40.1
4c	113.7	113.6	15a	163.8	153.4
5	116.7	116.7	15b	114.9	114.9
6	148.0	147.8	16	27.4	109.6
7a	157.9	157.7	17	38.2	146.9
7b	55.0	55.6	18a	149.9	149.3
8a	146.4	145.26*	18b	144.6	145.28*
9	116.0	116.2	19a	144.4	145.18*
10	135.4	135.3			

[a] Assignments marked with an asterisk (*) could be exchanged.

(J = 8 Hz), which supported the structural assignment. The synthesis of eudistone B (3) by air oxidation of eudistone A (2) confirmed its structure and stereochemistry.

In summary, we have isolated and identified two novel octacyclic aromatic alkaloids, eudistones A (2) and B (3), as minor metabolites from tunicate *Eudistoma* sp. The major metabolite, ascididemin (1), had previously been isolated from a tunicate of the genus *Didemnum* that is taxonomically distinct from *Eudistoma*. Specimens of a *Eudistoma* species from the Red Sea contained a quite different group of polycyclic aromatic alkaloids.[6] Examples of polycyclic aromatic alkaloids have been isolated from several invertebrate phyla; one compound, dercitamide, has even been isolated from both the sponge *Stelleta* sp.[5a] and an unidentified tunicate.[5b] The broad distribution of related polycyclic aromatic alkaloids has led to the suspicion that they might be produced by symbiotic microorganisms, but there is no direct evidence to support this hypothesis. We could not detect the photosynthetic pigments associated with symbiotic algae in our specimen of *Eudistoma* sp., but we could not rule out the presence of nonphotosynthetic symbionts.

Experimental Section

Collection, Extraction, and Isolation Procedures. The dark green tunicate, *Eudistoma* sp., was collected by SCUBA (−15 m) near Ave Maria Rocks on Praslin Island, Seychelles, and was stored frozen for 2 months. The chopped animal tissue (200 g dry wt) was extracted with a mixed solvent of CH_2Cl_2–MeOH (1:10, 2 × 800 mL), and, after evaporation of the solvent, the extracts were partitioned between butanol (2 × 250 mL) and water (200 mL). The butanol extract (2.6 g) was chromatographed on Sephadex LH-20 using methanol as the eluant to obtain the major product ascididemin (1, 512 mg, 0.26% dry wt) and a mixture of minor compounds. The mixture was further separated on Spectral 40S column using 15% aqueous methanol containing 0.01% NaCl as eluent to obtain eudistones A (2, 4.5 mg, 0.0023% dry wt) and B (3, 3.6 mg, 0.0018% dry wt).

Eudistone A (2): yellow amorphous powder; CD $\Delta\epsilon$ (MeOH, 23 °C) 209 (+4.8), 244 (−4.7), 319 (+5.1), 386 (−2.0), 450 nm (+1.1); UV (MeOH) 210 (ϵ 44 750), 238 (ϵ 48 475), 260 (sh, ϵ 24 670), 323 (ϵ 15 320), 338 (ϵ 15 480), 359 (sh, ϵ 13 060), 395 nm (ϵ 10 640); IR (neat, AgCl plate) 3360, 3220, 1660, 1595, 1535, 1200 cm^{-1}; ^{1}H NMR see Table I; ^{13}C NMR see Table III; HRFABMS, obsd m/z 430.1677, $C_{27}H_{19}N_5O$ requires m/z 430.1668.

Eudistone B (3): white amorphous powder; $[\alpha]_D$ −177.8° (c 0.036, MeOH); CD $\Delta\epsilon$ (MeOH, 23 °C) 206 (+35.8), 234 (−40.2), 264 (−22.2), 307 (+34.4), 363 nm (−17.2); UV (MeOH) 204 (ϵ 45 650), 239 (ϵ 47 365), 259 (ϵ 39 550), 324 nm (ϵ 14 670); IR (neat, AgCl plate) 3390, 1595, 1515, 1020 cm^{-1}; ^{1}H NMR see Table II; ^{13}C NMR see Table III; HRFABMS obsd m/z 428.1536, $C_{27}H_{17}N_5O$ requires m/z 428.1511.

Conversion of Eudistone A (2) into Eudistone B (3). A stream of air was bubbled through a solution of eudistone A (2, 1.2 mg) in DMSO (0.5 mL) at 60 °C for 48 h. The resulting solution was cooled and the solvent was evaporated under reduced pressure to obtain a residue that was purified by reversed-phase HPLC on a Dynamax C_8 column (30% aqueous MeOH) to obtain a major product (0.4 mg) that was identical with an authentic sample of eudistone B (3) by comparison of the TLC behavior and ^{1}H NMR spectra.

Acknowledgment. The tunicate was collected by Steve Bobzin, Brad Cartê, and Mary Kay Harper and identified by Dr. Françoise Monniot, Muséum National d'Histoire Naturelle, Paris. The CD spectrum was measured by Dr. Ted Molinski, U. C. Davis. This research was supported by grants from National Institutes of Health (CA 49084) and the California Sea Grant College Program (R/MP-46) and an unrestricted gift from SmithKline Beecham.