36 h (70%, 45% ee), 48 h (63%, 60% ee). Concentrated condition: 3 h (21%, 88% ee), 5 h (35%, 88% ee), 7 h (38%, 86% ee), 11 h (48%, 86% ee), 17 h (61%, 74% ee), 36 h (75%, 35% ee).

Acetonide of 2-(Methylthio)-3-phenylpropane-1,3-diol (4). To a suspensiom of 40 mg (1.16 mmol) of lithium aluminum hydride in 5 mL of ether was added a solution of 245 mg (1.51 mmol) of **2d** in 5 mL of ether at −78 °C under argon, and the mixture was stirred for 5 h. After reaction was quenched, the solution was dried, and the solvent was removed under reduced pressure to give an oily product. To a solution of this oil in 5 mL of CH_2Cl_2 was added 350 mg (3.0 mmol) of 2,2-dimethoxypropane and 5 mg of *p*-toluensulfonic acid under argon and stirred for 24 h. Crushed ice was added, and the resulting mixture was extracted with CH_2Cl_2. The extract was washed with brine, dried, and concentrated by evaporation. Distillation of the residue gave 4 in 87% yield as a colorless oil: bp 100 °C (2.5 mmHg), Kugelrohr. Other acetonides, **4**, were also prepared by the same method as previously described. Spectral data, each diastereomer of the acetonide **4** of ^1H NMR (200 MHz, δ, $CDCl_3$, J = Hz) and ^{13}C NMR (50 MHz, δ, $CDCl_3$, ppm) are summarized in Table V.

Acknowledgment. We are grateful to Professor Masanori Utaka of the Faculty of Engineering of Okayama University for helpful discussions throughout this work. We are also grateful to Mr. Noriyoshi Tateoka, Faculty of Education of Okayama University, for preparing the computer program for calculation of the E value. We also thank Amano Pharmaceutical Co., Ltd. and Meito Sangyo Co., Ltd. for providing lipases.

Cytotoxic Aromatic Alkaloids from the Ascidian *Amphicarpa meridiana* and *Leptoclinides* sp.: Meridine and 11-Hydroxyascididemin

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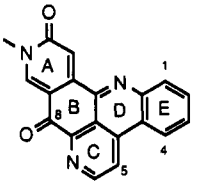
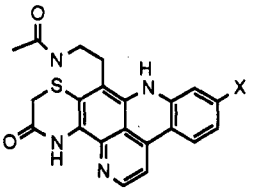
Three new pentacyclic aromatic alkaloids, 6–8, have been isolated from two ascidians. The structure of meridine, 6, obtained from *Amphicarpa meridiana* collected in South Australia, was determined by X-ray analysis while that of a relatively stable tautomer thereof, 7, was established by spectral analysis. The remaining alkaloid, 11-hydroxyascididemin (8), was isolated from a *Leptoclinides* sp. from Truk Lagoon. All three alkaloids are cytotoxic and one shows slight topoisomerase II activity. Limitations to the possible structures for neocalliactine acetate are discussed.

A series of structurally related and biologically active polycyclic aromatic alkaloids isolated from sponges, ascidians, and an anemone have been reported in the past few years.[1] Illustrative examples are amphimedine (**1**),[1a] shermilamines A and B (**2**, **3**),[1f,g] 2-bromoleptoclinidinone (**4**),[1b] and ascididemin (**5**),[1c] all of which have in common the tetracyclic moiety marked B–D in **1**. Cytotoxic activity has been reported for many of these metabolites. In our continuing search for cytotoxic compounds from marine organisms we have isolated three additional polycyclic aromatic alkaloids from two different ascidians. The ^{13}C NMR data confirmed for several of these alkaloids makes it possible to limit the possible structures proposed for neocalliactine acetate, a derivative of the anemone pigment calliactine. The structure of one of the new alkaloids, meridine (**6**), was confirmed by X-ray diffraction analysis.

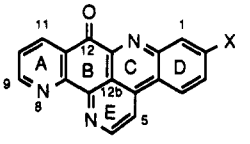
The source of two of the new alkaloids is the ascidian *Amphicarpa meridiana*, which was collected at Stenhouse Bay, South Australia, and frozen shortly after collection.



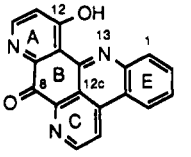
1



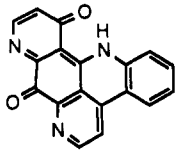
2, X = Br
3, X = H



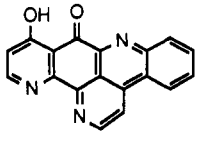
4, X = Br
5, X = H



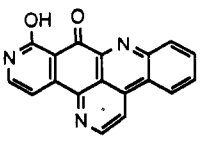
6



7



8



8'

The chloroform–methanol extracts yielded meridine (**6**) as a yellow, noncrystalline solid via conventional successive chromatographies on silica gel or via centrifugal countercurrent chromatography (CCCC) using a chloroform–methanol–5% aqueous HCl (5:5:3) solvent system. Meridine, $C_{18}H_9N_3O_2$ (M^+, 299.0693, +0.0305 amu), showed signals in the ^1H NMR spectrum for one exchangeable proton (15.26 ppm) and eight aromatic protons which were

(1) (a) Schmitz, F. J.; Agarwal, S. K.; Gunasekera, S. P.; Schmidt, P. G.; Shoolery, J. N. *J. Am. Chem. Soc.* **1983**, *105*, 4835. (b) De Guzman, F. S.; Schmitz, F. J. *Tetrahedron Lett.* **1989**, *31*, 1065. Bloor, S. J.; Schmitz, F. J. *J. Am. Chem. Soc.* **1987**, *109*, 6134. (c) Kobayashi, J.; Cheng, J.; Nakamura, H.; Ohizumi, Y.; Hirata, Y.; Sasaki, T.; Ohta, T.; Nozoe, S. *Tetrahedron Lett.* **1988**, *29*, 1177. (d) Kobayashi, J.; Cheng, J.; Walachli, M. R.; Nakamura, H.; Hirata, Y.; Sasaki, T.; Ohizumi, Y. *J. Org. Chem.* **1988**, *53*, 1800. (e) Molinski, T. F.; Fahy, E.; Faulkner, D. J.; van Duyne, G. D.; Clardy, J. *J. Org. Chem.* **1988**, *53*, 1340. (f) Cooray, N. M.; Scheuer, P. J.; Parkanyi, L.; Clardy, J. *J. Org. Chem.* **1988**, *53*, 4619. (g) Carroll, A. R.; Cooray, N. M.; Pointer, A.; Scheuer, P. J. *J. Org. Chem.* **1989**, *54*, 4231. (h) Gunawardana, G. P.; Hohmoto, S.; Gunasekera, S. P.; McConnell, O. J.; Koehn, F. E. *J. Am. Chem. Soc.* **1988**, *110*, 4856. (i) Charyulu, G. A.; McKee, T. C.; Ireland, C. M. *Tetrahedron Lett.* **1989**, *30*, 4210. (j) Molinski, T. F.; Ireland, C. M. *J. Org. Chem.* **1989**, *54*, 4256. (k) Inman, W. D.; O'Neill-Johnson, M.; Crews, P. *J. Am. Chem. Soc.* **1990**, *112*, 1. (l) Cimino, G.; de Rosa, S.; de Stefano, S.; Gavagnin, M.; Sodano, G. *Tetrahedron* **1987**, *43*, 4023.

0022-3263/91/1956-0804$02.50/0

Table I. NMR Data for 6, 7, and 8

C no.	$^1H^a$ δ, m (J, Hz)			$^{13}C^b$ δ, m (J, Hz)	
	6	7	8	6	8
1	8.23, d (8.1)	7.44, d (8.1)	8.64, dd (8.1, 1.8)	132.6, dd (166.6, 11.2)	133.3
2	7.97, dd (8.1, 7.8)	7.63, t (8.1)	8.06, dt (8.1, 1.2)	129.6, dd (161, 7)	132.1
3	7.86, dd (7.8, 8.3)	7.37, t (8.1)	8.00, dt (8.1, 1.8)	129.4, dd (161, 7)	131.5
4	8.64, d (8.3)	8.20, d (8.1)	8.73, dd (8.1, 1.2)	123.3, dd (164, 7)	123.1
4a				121.7, dd (14, 2.8)	*d*
4b				137.9, t (7)	137.8
5	8.66, d (5.6)	7.64, d (5.4)	8.58, d (5.7)	119.7, dd (162.4, 8.4)	117.2
6	9.38, d (5.6)	8.94, d (5.4)	9.31, d (5.7)	151.5, d (182)	150.1
7a				147.6,[c] d (12.6)	149.3
7b					154.1
8				180.3, s	
8a				148.9,[c] d (14)	
9			7.15, d (5.7)		156.7
10	8.79, d (5.5)	6.53, d (8.1)	8.89, d (5.7)	153.7, d (182)	114.8
11	7.25, d (5.5)	8.10, d (8.1)		116.8, dt (166.6, 7)	169.5
11a					115.6
12				167.3, t (8.4)	181.8
12a				116.4, d (2.8)	*d*
12b				152.1, s	117.7
12c				117.9, d (2.8)	
13a				142.6, m (7)	*d*
OH(NH)	15.26, s	12.51, s	13.06, s		

[a] $CDCl_3$, 300 MHz. [b] $CDCl_3$, 75.4 MHz. [c] Assignment confirmed by long-range H/C correlation (INAPT).[12] [d] Signal not observed.

arranged as three isolated spin systems as shown in rings A, C, and E in formula **6**. The presence of two α-pyrido protons was inferred from chemical shift, J_{HH} (see Table I), and J_{CH} values (182 Hz).[2] The proton chemical shift values for the C and E rings of the structure were virtually identical with those of the corresponding rings of amphimedine (**1**), and NOE enhancement was confirmed between the H-4 and H-5 protons. Although these proton signals were nearly overlapped in $CDCl_3$, they were sufficiently well-resolved for the NOE experiment in $CDCl_3$ containing a drop of CF_3CO_2D. Many of the ^{13}C chemical shifts of meridine were also the same as those of amphimedine. Irradiation of the exchangeable proton signal at 15.26 ppm ($CDCl_3$) produced a 4.9% NOE on the H-1 signal, providing evidence for the location of the OH group as shown in **6**. A fully coupled ^{13}C spectrum and the results of several long-range H/C correlation experiments led to the carbon assignments shown in Table I, all of which was consistent with structure **6** for meridine. Eventually crystals of a trifluoroacetate salt were obtained by slow evaporation at 5 °C of a solution of meridine in $CDCl_3$ plus a few drops of CF_3CO_2D. X-ray analysis confirmed structure **6** for meridine (see Figure 1).

During reisolation of meridine using the conventional silica gel chromatography, a second alkaloid, **7**, also having a molecular formula of $C_{18}H_9N_3O_2$ was isolated. The ^1H NMR spectrum of **7** contained signals identical in multiplicity and similar in chemical shift for rings C and E of meridine, but the chemical shifts of the signals for the remaining pair of ortho-coupled protons (8.10, 6.53 ppm) and an exchangeable proton (12.51 ppm) were quite different. However, after **7** had been stored in $CDCl_3$ for 1–2 days, the ^1H NMR spectrum of the sample was indistinguishable from that of meridine, indicating that the two alkaloids were tautomers. Three possible tautomeric structures were considered in which ring A was a pyridone and the exchangeable proton was located, respectively, at each of the different nitrogens. NOE's were observed between (a) the protons at 8.20 (H-4) and 7.64 ppm (H-5) and (b) between the exchangeable proton (12.51 ppm) and

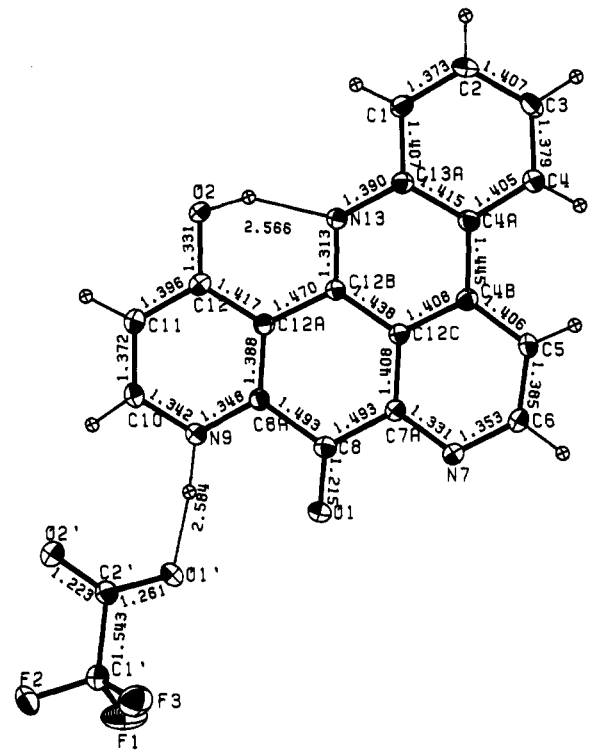


Figure 1. An ORTEP plot of meridine trifluoroacetate. Atom numbering and bond distances are shown. Dashed lines indicate hydrogen bonds; esd's for bond distances, 0.002–0.003 Å.

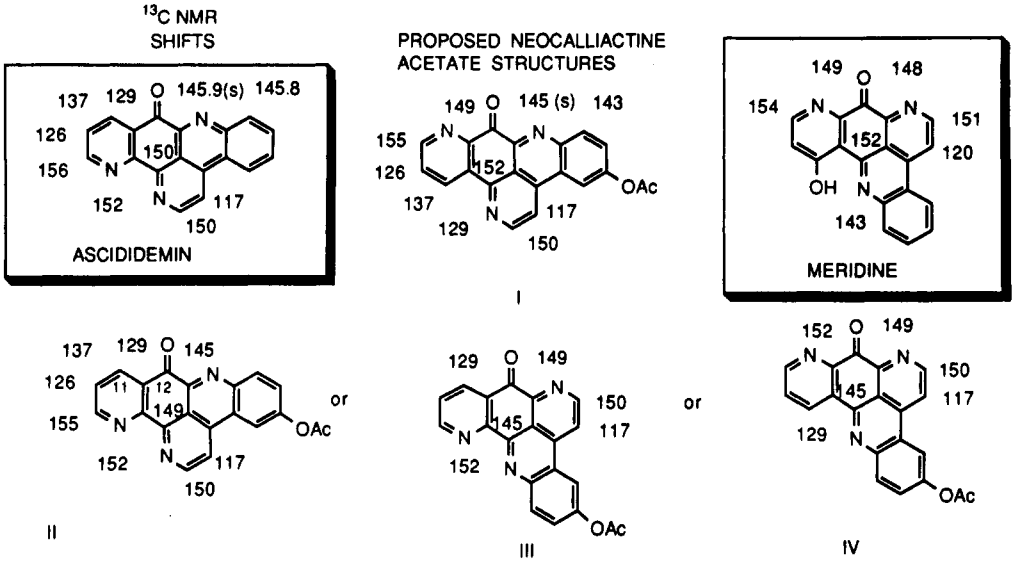
the proton resonating at 7.44 ppm (H-1) in the proton NMR spectrum of the tautomeric alkaloid. The latter is consistent only with structure **7** [meridin-12(13*H*)-one]. All relative proton assignments were confirmed by decoupling. The chemical shifts of H-10 and H-11 are consistent with those of 4-pyridone.[3]

In the course of reisolating 2-bromoleptoclinidinone (**4**) for biological testing purposes from a repeat collection of an ascidian, *Leptoclinides* sp., found in Truk Lagoon, Federated States of Micronesia, an additional alkaloid, **8**, $C_{18}H_9N_3O_2$ by HRMS, was obtained by conventional

(2) Cf.: Rahman, A. u. *Nuclear Magnetic Resonance*; Springer-Verlag: New York, 1986; Chapters 2 and 4.

(3) Vögeli, U.; Philipsborn, W. v. *Org. Magn. Reson.* **1973**, *5*, 551.

Chart I. Comparison of ^{13}C NMR Data for Possible Neocalliactine Structures I–IV, Ascididemin, and Meridine



chromatographic methods. Comparison of the proton and carbon-13 data of 8 and ascididemin (5) revealed that these two compounds had identical B–E-ring moieties. Differences between the compounds thus apparently was confined to ring A. The chemical shifts and small coupling constants of the pair of aromatic protons on this ring were indicative of the α,β-protons of a pyridine ring.[2] An INAPT experiment revealed that the proton signal at 8.89 ppm was long-range coupled to the hydroxyl-bearing carbon. Low-frequency hydroxyl absorption, 3071 cm^{-1}, characteristic of intramolecular hydrogen bonding,[4] and carbonyl absorption at 1674 cm^{-1} which is lower than that noted for 2-bromoleptoclinidinone, ascididemin, or meridine (1680 cm^{-1}) suggested hydrogen bonding between these groups. Furthermore, no NOE was observed between the exchangeable proton of 8 and any other signals, in contrast to the case for meridine, see above. Thus, it is concluded that the correct structure for this alkaloid is 8 (11-hydroxyascididemin), rather than the isomeric possibility, 8′. This is also consistent with the fact that 8 and 4 occur in the same organism.

Alkaloids **1**, **2**, **3**, **5**, and **7** all exhibit cytotoxicity to cultures of murine leukemia cells (P388) at 0.3–0.4 $\mu g/mL$. Compounds **3** and **5** inhibit toposiomerase II at 30 and 75 μM concentrations, respectively, whereas **1**, **2**, and **7** were inactive in this assay.[5]

Ascididemin and meridine embody the key features of the set of four alternative structures I–IV proposed for neocalliactine acetate.[6] Neocalliactine acetate is a derivative prepared from calliactine, a pigment isolated from the sea anemone *Calliactis parasitica*.[6] To the best of our knowledge no definitive structure has been determined for calliactine. Possible neocalliactine acetate structures I and II have the overall skeletal outline of ascididemin, while III and IV have the meridine ring array, and within each of these pairs the difference lies in the orientation of the left-most pyridine ring. Hence it appeared that the confirmed ^{13}C NMR data for meridine and ascididemin could be used to deduce an unambiguous structure assignment for neocalliactine acetate and, by inference, that of its precursor, calliactine.

Comparison of the ^{13}C NMR shifts of 11-hydroxyascididemin (8) with those of ascididemin, **5**, confirmed that the hydroxyl group has very little effect on the shifts of carbons meta to the hydroxyl-substituted position as might have been expected from data for simple substituted pyridines.[2] Hence the shifts of the carbons adjacent to the carbonyl group in meridine should be excellent models for any deoxy analogs thereof such as I and IV.

Chart I shows the key ^{13}C NMR chemical shifts for ascididemin, meridine, and I–IV. Shifts around structure I are those assigned in the literature (the 149 and 152 ppm assignments may be reversed). One *one* signal, 145 ppm, is reported to be a singlet. Shifts specified on structures II–IV are transpositions of the assigned shifts for I to like carbons. The data allow III and IV to be ruled out unequivocally. In IV the signals assignable to the quaternary carbons flanking the carbonyl group occur at quite different chemical shifts, and this is incompatible with the meridine model. Also, the 145 ppm signal should be a doublet.

In structure III the 145 ppm singlet signal must be assigned to the position "para" to the carbonyl group to avoid 3-bond couplings. This shift is inconsistent with either the ascididemin or meridine models. Also, the 117 and 150 ppm signals of neocalliactine acetate fit well for ring D of ascididemin but not the analogous ring E of meridine.

For both I and II the carbon-13 NMR shifts of rings A and B fit their respective models well, and hence no differentiation can be made from this data. The multiplicities of carbon signals 149 and 152 ppm (d, 10.8 Hz; mult, respectively) are in accord with expectations for structure II whereas in I the 149 ppm signal would be expected to be dd (or m). The coupling to the carbonyl carbon signal (3.5 Hz) is appropriate for the anticipated 3-bond coupling in II, but not in I as already noted in the literature.[6] Conversely, I, but not II, is compatibile with a NOE observed[6] between the signals assigned as H-6 and H-8. Although these data still do not allow an unequivocal choice to be made between I and II, we slightly prefer II, placing greater weight on the carbon coupling data than on the NOE result.

X-ray Results. A perspective ORTEP drawing of meridine trifluoroacetate is shown in Figure 1, along with the bond distances. The final atomic parameters of the non-

(4) Silverstein, R. M.; Bassler, G. C.; Morrill, T. C. *Spectrometric Identification of Organic Compounds*; John Wiley: New York, 1963; p 112.

(5) Private communication, Dr. John R. Westley, Smith, Kline & French Laboratories.

(6) Cimino, G.; Crispino, A.; De Rosa, S.; De Stefano, S.; Gavagnin, M.; Sodano, G. *Tetrahedron* **1987**, *43*, 4023.

Table II. Atomic Parameters (esd's Are within Parentheses)

atom	x	y	z	U(eq)[a]
C(1)	0.8446 (2)	-0.0463 (1)	0.2985 (1)	0.0184 (6)
C(2)	0.7425 (2)	-0.0544 (1)	0.3184 (3)	0.0196 (6)
C(3)	0.6754 (2)	0.0069 (1)	0.3012 (3)	0.0217 (7)
C(4)	0.7105 (2)	0.0754 (1)	0.2631 (3)	0.0193 (6)
C(4a)	0.8145 (1)	0.0849 (1)	0.2409 (3)	0.0168 (6)
C(4b)	0.8576 (1)	0.1552 (1)	0.2047 (3)	0.0162 (6)
C(5)	0.8026 (2)	0.2219 (1)	0.1922 (1)	0.0178 (6)
C(6)	0.8553 (2)	0.2862 (1)	0.1714 (3)	0.0195 (6)
N(7)	0.9571 (1)	0.28989 (9)	0.1555 (3)	0.0187 (5)
C(7a)	1.0080 (2)	0.2266 (1)	0.1582 (3)	0.0159 (6)
C(8)	1.1182 (2)	0.2305 (1)	0.1288 (3)	0.0176 (6)
C(8a)	1.1800 (1)	0.1609 (1)	0.1556 (3)	0.0154 (6)
N(9)	1.2822 (1)	0.1660 (1)	0.1455 (3)	0.0185 (5)
C(10)	1.3434 (2)	0.1061 (1)	0.1672 (3)	0.0199 (6)
C(11)	1.3047 (2)	0.0382 (1)	0.1967 (3)	0.0213 (6)
C(12)	1.1991 (1)	0.0309 (1)	0.2068 (3)	0.0174 (6)
C(12a)	1.1351 (1)	0.0944 (1)	0.1874 (3)	0.0156 (6)
C(12b)	1.0241 (1)	0.0919 (1)	0.2027 (3)	0.0147 (6)
C(12c)	0.9633 (1)	0.1583 (1)	0.1864 (3)	0.0159 (6)
N(13)	0.9854 (1)	0.02821 (9)	0.2380 (2)	0.0157 (5)
C(13a)	0.8815 (1)	0.0232 (1)	0.2579 (3)	0.0163 (6)
O(1)	1.1568 (1)	0.28634 (8)	0.0814 (2)	0.0248 (5)
O(2)	1.1615 (1)	-0.03494 (8)	0.2362 (2)	0.0209 (5)
C(1)′	0.5434 (2)	0.3508 (1)	0.2178 (4)	0.0250 (7)
C(2)′	0.4852 (2)	0.2768 (1)	0.1824 (3)	0.0220 (7)
O(1)′	1.3868 (1)	0.28472 (9)	0.1228 (3)	0.0320 (6)
O(2)′	0.5382 (1)	0.22108 (9)	0.2167 (3)	0.0306 (6)
F(1)	0.5227 (1)	0.39351 (8)	0.0564 (2)	0.0441 (6)
F(2)	0.6475 (1)	0.34441 (8)	0.2759 (3)	0.0563 (7)
F(3)	0.5132 (1)	0.38988 (7)	0.3599 (2)	0.0342 (5)

[a] $U(\text{eq}) = {}^1/_3\sum\sum U_{ij}a^*_i a^*_j(a_i \cdot a_j)$.

hydrogen atoms are listed in Table II. The crystal structure established the structure of meridine as **6** and resolved the ambiguity between **6** and any alternate structures with the ring A nitrogen at position 11. Meridine differs from other pentacyclic alkaloids like amphimedine[1a] and petrosamine,[1e] in having N(9) instead of N(10). In the crystal structure the compound exists as the trifluoroacetate salt with the monoprotonated meridine cation and $CF_3CO_2^-$ anion, held together by a strong N–H···O hydrogen bond: N(9)–H(9)···O′(1) = 2.584 (3) Å, N–H = 1.06 (4) Å, O′(1)···H = 1.53 (4) Å, and N–H···O angle of 175 (3)°. In this hydrogen bond, the N–H distance is longer and the O···H distance is much shorter than those seen in normal N–H···O hydrogen bonds. The cation as a whole can be considered to be planar, the rms deviation of the individual atoms from the least-squares plane through all 21 ring atoms is 0.045 Å. The individual deviations are shown in Figure 2. This planar structure is further stabilized by a strong intramolecular hydrogen bond: H(O2)···N(13) = 2.566 (3) Å, O(2)–H(O2) = 0.95 (2) Å, H(O2)···N(13) = 1.70 (3) Å, and angle O(2)–H(2)···N(13) of 150 (3)°. The bond distances and angles generally compare with other heterocyclic aromatic systems, in particular with those of petrosamine,[1e] the only other alkaloid with a similar ring skeleton whose solid-state structure is known.

Experimental Section

Merck silica gel 60H was used for flash chromatography. Chromatotron plates were made using EM Science Kieselgel 60 PF_{254}. All solvents used in the extraction and separations were distilled prior to use.

Isolation of Meridine (6). The ascidian *A. meridiana* was collected at Stenhouse Bay in South Australia and frozen shortly after collection. The frozen specimens were divided into two portions. The first portion (11 kg) was thawed, cut into very small pieces, and extracted four times with methanol–chloroform (1:1) at room temperature overnight. The first methanol–chloroform extract was concentrated to give 3.16 g of crude extract, which was then chromatographed using flash vacuum chromatography[7] on silica gel and eluting first with increasing concentrations of chloroform in hexane and then increasing amounts of methanol in chloroform. Fraction 11 contained impure meridine, and it was further purified either by chromatography on a chromatotron (silica gel plates, 5% MeOH in $CHCl_3$ as eluent) or a second flash chromatography on silica gel ($CHCl_3$ with increasing concentrations of MeOH) followed by preparative layer chromatography (PLC) (SiO_2; 5% MeOH in $CHCl_3$). A total of 21 mg of meridine was obtained from the 11 kg of wet specimens. Meridine (**6**): yellow amorphous solid, mp >250 °C; FT IR (thin film on KBr) 3444, 3071, 1692, 1605 cm^{-1}; LRMS (12 eV) *m/z* (%) 299.1 [M^+, (100)], 271.0 [M^+ – CO, (10.4)]; ^1H and ^{13}C NMR, see Table I.

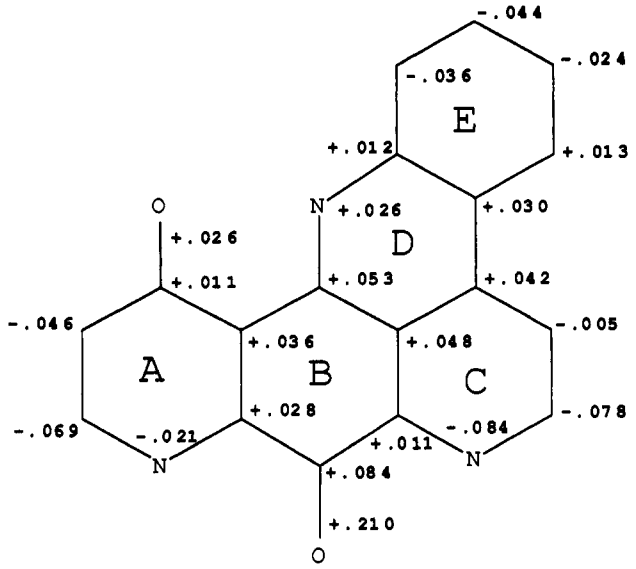


Figure 2. Deviations of individual atoms from the least-squares plane through all 21 ring atoms: $-0.9920x - 2.5873y - 6.5255z = 2.6298$.

The other methanol–chloroform extracts were combined and concentrated to give 28.21 g of crude extract. This extract was then fractionated by centrifugal countercurrent chromatography (CCCC) on an Ito multilayer coil[8] using the solvent system chloroform–methanol–5% aqueous HCl (5:5:3) with the lower organic layer as the mobile phase. Fractions (15 mL each) similar in color were combined. The third combined fraction (180 mL), which was violet, was evaporated to dryness, and the residue was partitioned between chloroform and 5% aqueous HCl. The aqueous layer was carefully neutralized with 1 N NaOH and reextracted with chloroform. Meridine (11.5 mg) was obtained after evaporation of the chloroform.

Crystallization of Meridine Trifluoroacetate. A solution of meridine (5–10 mg) in ~0.5 mL of $CDCl_3$ containing a drop or two of trifluoroacetic acid-*d* was made up for NMR analysis. This solution was transferred to a vial and diluted with about 1 mL of $CHCl_3$ rinse. The vial was capped and stored in a refrigerator at 5 °C for about a month, at which time it was noted that crystals had formed. The solvent was decanted and a crystal was taken for X-ray analysis, mp >250 °C.

Isolation of Meridin-12(13*H*)-one (7). Fractions 8 and 9 of the initial flash vacuum chromatography contained **7**. These combined fractions were vacuum flash chromatographed on a reverse-phase column (LRP-1) using methanol. The first fraction eluted was further purified by PLC [SiO_2; acetone–hexane (1:1)] to give 12 mg of **7**; ^1H NMR, see Table I. After standing for several days in $CDCl_3$, the same solution exhibited a proton NMR spectrum identical with that of meridine (**6**), and hence no other spectral properties were obtained for **7**.

Isolation of 11-Hydroxyascididemin (8). A batch of ascidian (521 g), *Leptoclinidines* sp., was collected in 1986, freeze-dried,

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then extracted with hexane, twice with chloroform, and finally with hot chloroform using a Soxhlet extractor. The first room temperature chloroform extract (0.89 g) was subjected to flash chromatography on silica gel using hexane, then chloroform, and finally, increasing methanol concentrations in chloroform. The fifth fraction from this chromatography was concentrated and then partitioned between chloroform and 5% HCl. The aqueous layer was neutralized with 1 N NaOH and reextracted with chloroform. Upon evaporation of chloroform, 1.4 mg of 11-hydroxyascididemin (8) was obtained. The second chloroform extract was then subjected to flash chromatography on silica gel. Fractions 9 and 10 from this chromatography were combined, concentrated, and then dissolved in chloroform. Methanol was slowly added to this chloroform solution to precipitate 2-bromoleptoclinidinone (4). Fractions 12–14 were combined, evaporated to dryness, and partitioned between chloroform and 5% aqueous HCl. The aqueous acid layer was carefully neutralized with 1 N NaOH and then reextracted with chloroform to give 28.8 mg of 8: yellow, amorphous solid; mp >250 °C; UV (MeOH) λ (nm) (ε), 203 (25 000), 227 (38 000), 275 (18 000), 285 (17 000), 370 (11 000); IR (film) ν max 3067, 1674 cm^{-1}; LR MS m/z (rel intensity), 299.0 [M^+, 100], 271.0 [$M^+ - H_2O$, 47]; HR FAB MS 300.0768 ($C_{18}H_{10}O_2N_3$, $M^+ + 1$, Δ −1.7 ppm).

X-ray Experimental Results. Deep brown, thick platy crystals were formed. Preliminary diffraction studies showed, however, that the crystals were twinned, and attempts to obtain a twin-free single crystal were unsuccessful even after recrystallization. On further analysis it was found that the twinning was simple, containing two individuals with a ratio of 60%:40%. The a^* and b^* axes are common to both parts of the twin, but the c^* axes are inclined to each other at an angle of $2(\beta - 90)°$. The twin axis is perpendicular to (001) with composition planes at zero and the 6th layer.

A crystal of size 0.21 × 0.17 × 0.08 mm was selected for all diffraction measurements. The unit cell parameters were obtained by a least-squares fit to ±2θ of 48 reflections measured at 163 K using Cu $K\alpha_1$ radiation. All X-ray measurements were carried out on an Enraf-Nonius CAD4 diffractometer equipped with a liquid N_2 low-temperture device.

Crystal data: meridine trifluoroacetate, $C_{18}H_{10}N_3O_2 \cdot C_2F_3O_2$, M_r = 413.3, monoclinic, $P2_1/c$, a = 12.995 (3) Å, b = 18.281 (3) Å, c = 6.902 (1) Å, β = 102.82 (2)°, V = 1598.8 Å^3, Z = 4, D_x = 1.716 g mL^{-1}, F(000) = 840, μ(Cu K$\bar{\alpha}$) = 11.3 cm^{-1}.

The intensity data of all the unique reflections within 2θ range 0–150° for one of the individuals of the twin (60%) were collected at 163 ± 2 K using Cu $K\bar{\alpha}_1$ radiation. Partial data were collected for the twin individual with 40%, to establish the scale factor needed in resolving the intensities of the reflections in the zero and the sixth layers. The intensities were collected in the ω − 2θ mode and for each reflection the entire peak profile was recorded. Three standard reflections were monitored every 2 h of X-ray exposure, and they showed no decay. The crystal orientation was checked regularly by three control reflections. A total of 3434 reflections were recorded, of which 3279 were unique. Diffraction data were reduced with a set of computer programs, DREAM,[9] which performed (i) integration of reflection intensity by least-squares analysis of peak profile widths, (ii) correction for Lorentz and polarization effects, (iii) interset scaling and averaging of equivalent data, and (iv) error estimates. The intensities of the reflections belonging to *hko* (292 reflections) and *hk*6 (289 reflections) layers were resolved into individual parts by applying an analytical procedure using the 2-fold symmetry along the twin axis. Fifty-three reflections in the 6th layer had negligible intensities, and these were left out of structure analysis and refinement.

The structure was solved by direct methods and the use of program MULTAN80[10] and refined by a full-matrix least-squares routine SHELX76[11] in which the quantity $\sum w(F_o - F_c)^2$ is minimized. All the hydrogen atoms were located from a difference Fourier map, and hydrogen parameters were refined. In the final stages of refinement, non-hydrogen atoms were given anisotropic thermal parameters. The refinement converged to a final R = 0.045, R_w = 0.051 for 2762 observed reflections ($I > 2\sigma(I)$), S = 2.8, Δ/σ = 0.01, electron density in the final difference map ±0.4 e/Å^3.

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Supplementary Material Available: Bond distances, bond angles, hydrogen atom parameters, hydrogen distances, and anisotropic thermal parameters for meridine trifluoroacetate (6 pages). Ordering information is given on any current masthead page.

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