Five New Alkaloids from the Tropical Ascidian, *Lissoclinum* sp. Lissoclinotoxin A is Chiral†

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Five new alkaloids, lissoclin A (**1**), lissoclin B (**2**), lissoclin C (**3**), lissoclinotoxin C (**5**), and the dimeric lissoclinotoxin D (**6**), were isolated along with the known compounds lissoclinotoxin A (**4**), 2-phenylethylamine (**11**), and 6-bromotryptamine (**12**) from *Lissoclinum* sp. collected from the Great Barrier Reef, Australia. Lissoclin A (**1**) undergoes photorearrangement to a new benzo-1,3-oxathiazoline **10**. The ^{13}C NMR spectrum of **4** is reported for the first time and the structure independently assigned by conversion to the known varacin *N*-trifluoroacetamide with diazomethane and [^{13}C]diazomethane. Compound **4** is chiral and exhibits unusual stereoisomerism due to *restricted inversion* about the benzopentathiepin ring. Lissoclinotoxin A (**4**) and D (**6**) exhibit antifungal activity against *Candida albicans*.

Since the discovery of amphimedine by Schmitz and co-workers in 1983,[1] pyridoacridine alkaloids have emerged as a class of alkaloids from sponges and ascidians with significant antifungal, cytotoxic, and DNA binding properties. Dercitin,[2,3] kuononiamine D,[4,5] and neoamphimedine[6] were shown to intercalate DNA while neoamphimedine, but not dercitin, inhibits topoisomerase II.[6] Kuanoniamine D[5] and 2-bromoleptoclinidone[7] chelate transition metal ions with high binding constants, a property that may play a role in their cytotoxic effects. Meridine, isolated independently from the ascidian *Amphicarpa meridiana*[8] and sponge *Corticum* sp.,[9] displays significant antifungal activity against *Candida albicans*, a property that is related to inhibition of nucleic acid synthesis.[9] The antineoplastic tetracyclic alkaloids varamine A and B[10] and diplamine[11] are examples of a less common group of sulfur-containing pyridoacridines. β-Carbolines and tetrahydro-β-carbolines, including the eudistomins[12] isolated from several species of tropical ascidians, have shown activity as antitumor and antiviral agents. In this report, we demonstrate secondary metabolism from three distinct biosynthetic pathways within one organism, the blue-white colonial ascidian *Lissoclinum* sp. from the Great Barrier Reef and the isolation of five new alkaloids; the pyridoacridines, lissoclins A (**1**) and B (**2**), the indole alkaloid, lissoclin C (**3**), and lissoclinotoxins C (**5**) and D (**6**). The known polysulfide, lissoclinotoxin A (**4**), and putative alkaloid precursors, 2-phenylethylamine (**11**) and 6-bromotryptamine (**12**), were also found in this organism. Alkaloids **4** and **6** exhibited potent antifungal activity against *Candida albicans*.

Specimens of *Lissoclinum* sp. were collected from the Great Barrier Reef in May of 1991, lyophilized, and exhaustively extracted with methanol. The methanol extract was found to inhibit the growth of *C. albicans* and Gram positive bacteria, but did not exhibit ergosterol-sensitive antifungal activity.[13] Bioassay-guided solvent separation of the extract by Sephadex LH-20 chromatography and reversed phase (RP) C_{18} flash chromatography gave several fractions containing alkaloids. Crystallization of one chromatography fraction gave orange needles of **1** (mp 202–204 °C, MeOH) as the major pyridoacridine component. Examination of the mother liquors by analytical HPLC with diode array UV detection revealed the presence of several minor components with the same UV-visible spectrum (λ 263 nm (ε 28 000), 300 (17 000), 383 (6800), 450 (4900)). Semipreparative RP C_{18} HPLC purification of the mother liquors provided additional **1** (total 0.039% of dry weight) and one additional analog **2** (0.001%) in sufficient quantity for characterization.

Compounds **1** and **2** were shown to be analogs of diplamine (**7**), an iminoquinone first isolated from a Fijian *Diplosoma* sp.[11] The new compounds differ from **7** only in substitution of the acyl group on the *N*-ethylamino side chain. High resolution FAB mass spectroscopic measurements of **1** showed a prominent MH + 2 ion (*m/z* 408, 100%), characteristic of iminoquinones, and provided the molecular formula $C_{23}H_{23}N_3O_2S$ (FABMS MH^+ 406.1575, Δmmu −1.4). The IR spectrum revealed signals characteristic of amide and quinone functional groups (ν 3450, 1665, 1605 cm^{-1}). The ^{13}C NMR and DEPT spectra showed 22 carbon signals, nineteen of which matched those of **7** exactly, including the charac-

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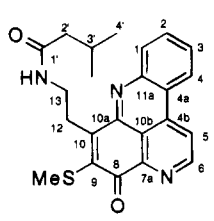
teristic methylthio group (17.9 q, $^1J_{CH}$ = 141.8 Hz). The side chain, however, was different and was assigned as an (*N*-3-methylbutanamido)ethyl group. Examination of the COSY spectrum revealed two degenerate methyl signals (0.80, d, 6H, J = 6 Hz, H4′) coupled to a methine signal (1.94, m, 1H, H3′) which overlapped with a vicinal methylene signal (1.94, m, 2H, H2′). The structure of **1** was fully consistent with this data and all other signals were readily assigned by comparision with those of **7** or the related alkaloids, varamines A (**8**) and B (**9**).[10]



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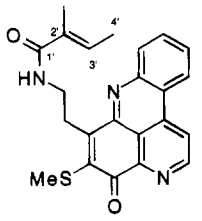

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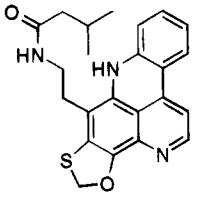

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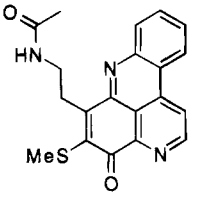

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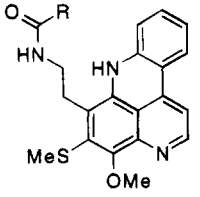
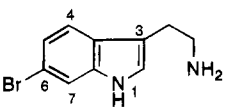


8 R = Et
9 R = Me

Iminoquinone **2**, with the formula $C_{23}H_{21}N_3O_2S$ (MH^+ *m/z* 404.1432, Δmmu 0.1), has one degree of unsaturation more than **1**. The signals of the isobutyl group in **1** were replaced by two vinylic methyl groups in **2**, one coupled to a vinyl proton (1.50, dd, 3H, J = 6.9, 0.9 Hz; 6.17, qd, 1H, J = 6.9, 0.9 Hz) and the other attached to a quaternary sp^2 carbon (1.57, br s, 3H), indicating the presence of a 1-methyl-1-propenyl group. Insufficient quantities of **2** were available to obtain a ^{13}C NMR spectrum, but the side-chain double bond geometry could be assigned as *E*, consistent by comparison of ^1H NMR data with that for cystodytin B.[14]

Each of **1** and **2**, upon exposure to light in aqueous MeOH, slowly produced a purple pigment. HPLC purification of the pigment produced by **1** gave an isomeric pure compound, **10** as a red solid ($C_{23}H_{23}N_3O_2S$, FABMS *m/z* 406.1586, Δmmu 0.3). Unlike **1**, the color of **10** was dependent on pH. Neutral or basic solutions of **10** were red-orange and in acidic solution the color changed to purple, an observation that correlates with a reduced pyridoacridine chromophore and appearance of a new aromatic $\pi-\pi^*$ absorption band in the UV-visible spectrum (λ 550 nm). Examination of the ^1H NMR spectrum ($CDCl_3$) of **10** showed that the methylthio signal of **1** (2.66, s, 3H) had been replaced by a downfield methylene singlet (5.85, s, 2H) and a broad NH signal (10.05, br s, 1H). All other signals were essentially the same as those of **1**. The pH dependence of the UV-visible spectrum of **10**, that was highly reminiscent of basic pyridoacridines such **8** and **9**,[10] together with the appearance of an exchangeable NH signal, allowed assignment of the new isomer as a benzooxathiazoline **10**. Benzooxathiazoline **10** is a product formed by intramolecular photoreduction of iminoquinone **1**, a rearrangement that has precedence in the photochemistry of α-alkyl-substituted quinones.[15] The unusual *O*-methyl aryl ether obtained by Kobayashi *et al.* upon prolonged treatment of the iminoquinone, cystodytin A, with diazomethane,[14] is probably formed by methylation of an incipient hydroiminoquinone photoreduction product.



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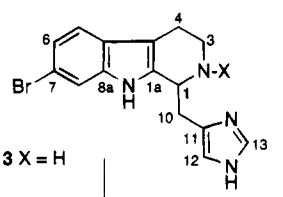
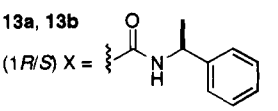


3 X = H

a



13a, 13b
(1*R*/*S*) X =

Figure 1. Conditions: (a) (*S*)-1-phenylethyl isocyanate, py, CH_2Cl_2, rt, 20 h.

The antifungal activity of the original extract resided in two water soluble fractions obtained by Sephadex LH-20 column chromatography. Further purification of the first fraction produced 2-phenylethylamine hydrochloride (**11**, 0.084% dry weight), lissoclin C (**3**, 0.035%), lissoclinotoxin C (**5**, 0.0009%), and lissoclinotoxin D (**6**, 0.0028%). The second fraction gave lissoclinotoxin A (**4**, 0.037%) and 6-bromotryptamine (**12**, 0.005%).[16]

The formula of $C_{15}H_{15}N_4Br$ for lissoclin C (**3**, Figure 1), provided by high resolution FABMS (*m/z* 331.0553, Δmmu 0.5), indicated 10 degrees of unsaturation. The UV spectrum (λ_{max} 231 nm, ϵ 21 900; 285, 4170) indicated an indole alkaloid[16] while ^1H NMR and ^{13}C NMR confirmed a substituted β-carboline substituted by bromine at C6 or C7 (β-carboline numbering). The location of the bromine was shown to be at C7, rather than C6, by phase sensitive NOESY. The methylene protons (3.08, m, 2H) gave a strong NOE cross peak to H5 (7.44, d, J = 8.4 Hz, 1H). Full ^1H NMR and ^{13}C NMR assigments of **3** were provided by interpretation of COSY, HMQC, and HMBC experiments (see Table 1).

Lissoclin C was found along with significant amounts of its putative precursor, 6-bromotryptamine (**12**), that presumably engaged a suitable aldehyde or β-keto acid in a Pictet–Spengler condensation to give a tetrahydro-β-carboline ring system. From biosynthetic considerations, **3** is formally obtained from condensation of 6-bromotryptamine with C2 rather than C1 of 4-imidazolylpyruvic acid, probably derived by transamination from histidine, followed by loss of C1 (Figure 2). Although this sequence of events is unexceptional for plant

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Table 1. ^{1}H NMR and ^{13}C NMR Assignments of Lissoclin C (3) (CD_3OD)

no.	^{13}C NMR[a]	mult[b]	^{1}H NMR, δ (mult, J, integ)[c]	HMBC[c]	NOESY[d]
1	53.5	d	5.09 (br dd, J = 9.0, 5.0 Hz, 1H)		H12
1a	129.4	s			
3	42.6	t	3.50 (m, 1H) 3.70 (m, 1H)		
4	19.3	t	3.08 (m, 2H)	C3, C4a, C1a	H5
4a	108.7	s			
5	120.9	d	7.44 (d, J = 8.4 Hz, 1H)	C8a, C6	H4
5a	126.3	s			
6	124.1	d	7.20 (dd, J = 8.4, 1.6 Hz, 1H)	C5a, C8	
7	117.3	s			
8	115.5	d	7.58 (d, J = 1.6 Hz, 1H)	C7, C6, C5a	
8a	139.2	s			
10	28.5	t	3.43 (br dd, J = 15.9, 9.0 Hz, 1H)	C11, C12	H12
			3.73 (m, 1H)	C11, C12	
11	128.8	s			
12	119.6	d	7.38 (br s, 1H)	C13	H1, H10a
13	136.2	d	8.90 (br s, 1H)	C11, C12	

[a] ^{1}H NMR and ^{13}C NMR shifts are referenced to CHD_2OD (δ 3.30 ppm) and CD_3OD (δ 49.00 ppm), respectively. [b] ^{13}C multiplicities were assigned by DEPT experiments. [c] HMBC was optimized for $^nJ_{CH}$ = 7 Hz. [d] Mixing time (t_m) for NOESY = 1.5 s.

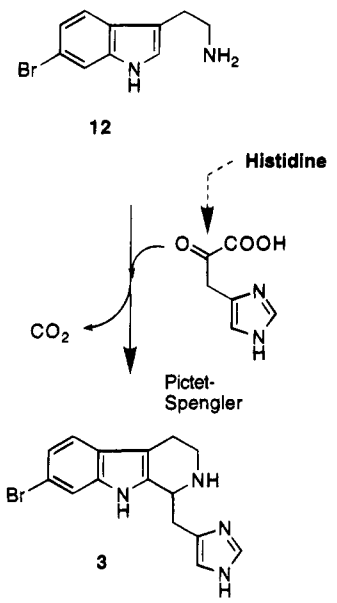

Figure 2. Proposed biosynthesis of lissoclin C (**3**).

alkaloids, it contrasts with all other known *marine* tetrahydro-β-carbolines in which condensation occurs with retention of C1 of the amino acid precursor.[12]

Compound **3** is chiral, but the optical activity of our sample was almost zero. Both the specific rotation and circular dichroism spectrum (CD) of the TFA salt of **3** were neglible; thus the compound was racemic. Brossi[17] and Cook[18] have pointed out that C1-substituted tetrahydro-β-carbolines readily racemize in acidic solution, a property that is enhanced with substitution at C6 (indole numbering) with electron-donating groups. Native **3** may be optically active, but racemization probably occurred during purification, particularly upon exposure to aqueous TFA–MeOH (pH *ca.* 4.5). Compound **3** was converted to the diastereomeric (*S*)-1-phenethyl ureas **13a** and **13b** (Figure 1) and separated on HPLC (diode array detection). Two closely eluting peaks of equal intensity were observed, each with UV spectra consistent with ureas **13a** and **13b**. Although the mechanism of racemization is not clear, a possible explanation for loss of optical activity **3** would involve protonation of the piperidine ring followed by reversible ring opening to the achiral intermediate **3a**, stabilized by electron donation from the 7-bromo substituent (Figure 3).

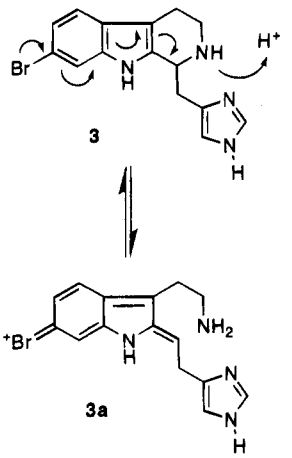

Figure 3. Mechanism of acid-catalyzed racemization of **3**.

The major antifungal component was isolated as its trifluoroacetate salt and shown to be lissoclinotoxin A (**4**). Compound **4** was reported by Guyot *et al.* with an incorrect trithiane structure **14**,[19] but this was revised recently.[20] The lack of published data for the natural product **4**, particularly ^{13}C NMR, frustrated our attempts to identify our sample by simple comparison of NMR data and prompted us to confirm the structure independently. In contrast to the first report,[19] positive ion FABMS of **4** provided a clear parent ion (MH^+ 326, 15%; HRMS 325.9469, Δmmu 0.3) and supported the formula $C_9H_{11}NO_2S_5$. We could find no evidence for spontaneous fragmentation of the *pentathiepin trifluoroacetate salt* of **4** to a trithiane. The FABMS spectrum of the *N*-trifluoroacetamide **15** prepared from **4** (TFAA, CH_2Cl_2, 100 °C, 10 min, quantitative), showed prominent loss of S_2 (*m/z* 357, 36%) from the parent ion, which further supported a pentathiepin. Treatment of **15** with CH_2N_2 provided pure varacin *N*-trifluoroacetamide **16** (quantitative), identical by ^{1}H NMR, MS, and HPLC retention time with a sample prepared from authentic **17**;[21-23] however, the question of location of the CH_3O group remained. Application of NOEDS and HMBC to **4** failed to unambiguously distinguish between the two possible 3- and 4-OCH_3 regioisomers, and a sample of **15** (*ca.* 1 mg) was converted to 4-*O*-$^{13}CH_3$ varacin *N*-trifluoroacetamide (**18**)

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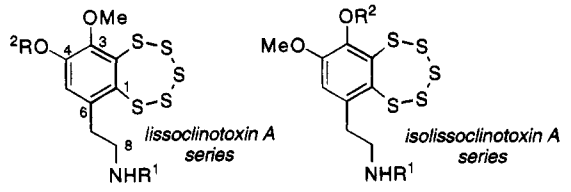
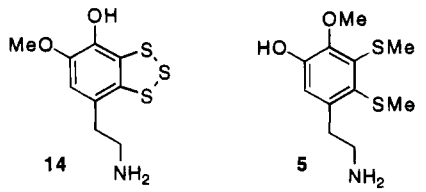
(23) We thank Dr. Brad Davidson of the University of Hawaii for generous gifts of synthetic varacin and isolissoclinotoxin A TFA salts.

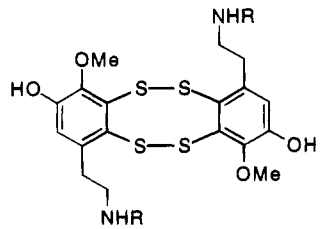
by treatment with $^{13}CH_2N_2$. The ^1H NMR spectrum in $CDCl_3$ revealed one CH_3O singlet (3.865, s, 3H) and a $^{13}CH_3O$ doublet (3.89, d, 3H, $^1J_{CH}$ 145.4 Hz) while the ^{13}C NMR spectrum was dominated by one enhanced carbon signal (56.2, q). Conversely, reaction of $^{13}CH_2N_2$ with isolissoclinotoxin A *N*-trifluoroacetamide (**20**, prepared from isolissoclinotoxin A, **19**,[23,24] and TFAA) gave varacin *N*-trifluoroacetamide (**21**) labeled in the alternate C3 OCH_3 group (3.863, d, 3H, ^1J 145.2 Hz; 61.9, q). The ^{13}C NMR signals of varacin (**17**), in particular the two OCH_3 signals (δ 56.9, q, 4-OCH_3; 62.2, q, 3-OCH_3), have been rigorously assigned.[21] Thus it follows that **18** derives from **15** by isotopic labeling at the C4-OH, and lissoclinotoxin A must have structure **4**. The ^{13}C NMR spectrum of **4** was completely assigned by HMBC and comparison with that of varacin (**17**).



4 R^1 = H R^2 = H
15 R^1 = $COCF_3$ R^2 = H
16 R^1 = $COCF_3$ R^2 = CH_3
18 R^1 = $COCF_3$ R^2 = $^{13}CH_3$
17 R^1 = H R^2 = CH_3

19 R^1 = H R^2 = H
20 R^1 = $COCF_3$ R^2 = H
21 R^1 = $COCF_3$ R^2 = $^{13}CH_3$





6 R = H
22 R = $COCF_3$

The trifluoroacetate salt of lissoclinotoxin C (**5**) was obtained as a colorless amorphous solid which showed a structural relationship to **4**. FAB HRMS provided a formula of $C_{11}H_{17}NO_2S_2$ (*m/z* 260.0799, MH^+, Δmmu 2.0), revealing only two sulfurs and two extra carbons. Comparison of the ^1H NMR spectrum of **5** with that of **4** showed two SCH_3 signals (δ CD_3OD, 2.28, s, 3H; 2.47, s, 3H, *c.f.* **1**, 2.66, s, 3H) and absence of diastereotopic methylene signals (3.10–3.40, m). Based on these data, structure **5** is assigned to lissoclinotoxin C.

A second, more polar ninhydrin-positive component, lissoclinotoxin D was isolated by HPLC as the TFA salt and assigned the symmetrical dimeric structure **6**. Although the ^1H NMR spectrum was almost identical with that of **4**, slight differences were evident in chemical shift for the aryl proton (6.97, s, 1H, $\Delta\delta$ 0.06 ppm) and the methoxyl group (3.83, $\Delta\delta$ 0.01 ppm). Lissoclinotoxin D (**6**) eluted much earlier on RP C_{18} HPLC than either **4** or **5**, consistent with double charges on the two aminoethyl groups in HPLC buffer. Mass spectroscopic analysis of the natural product failed to give a molecular ion, but treatment with TFAA under standard conditions gave an *N,N*-bis(trifluoroacetamide), **22**, that exhibited parent ions in both positive ion (MH^+ 651) and negative ion (M – H^-, 649) FABMS spectra. Thus, **22** has the formula $C_{22}H_{20}F_6N_2O_6S_4$. The possibility of **22** being an artifact is eliminated by the lack of similar dimer formation during FABMS measurements of **15**. A single aryl ^1H NMR signal (6.47, s, 1H) for **22** also rules out the possibility of a heterodimer of **4** and **19**, the latter as yet undetected as a natural product. We propose, instead, the homodimer **22** with the same aryl substitution pattern found in lissoclinotoxin A. Dibenzopolysulfur heterocycles are known as products in the synthesis of pentathiepins from dimercaptobenzenes.[25] The dibenzotetrathiepin **6**, together with **4** and **5**, can be viewed as natural products derived from an overlapping manifold of tyramine biosynthesis coupled with hypovalent sulfur metabolism. Polysulfur heterocyclic chemistry, although previously observed in ascidians, is more commonly associated with anaerobic thermophilic archaebacteria.[26] We have assigned the energetically more favorable C_{2v} dimeric "head to tail" structure **6** to lissoclinotoxin D; however, we cannot exclude the alternative "head to head" dimer. Treatment of **22** with CH_2N_2 lead only to complex mixtures, and insufficient material remained to characterize further this intriguing molecule.

Lissoclinotoxin A (**4**) is chiral. The ^1H NMR spectrum of **4** show diastereotopic signals for the side chain methylene groups, similar to that observed by Davidson and Ford for varacin (**17**).[22,27] The anisochronous chemical shifts of the CH_2 proton are most noticeable in the ^1H NMR spectrum of the corresponding *N*-trifluoroacetamide **15**. The benzylic H_2-7 signals (3.20, ddd, 1H, J = 13.4, 8.4, 6.4 Hz; 3.28, ddd, 1H, J = 13.4, 8.2, 6.4 Hz) and the vicinal H_2-8 signals (3.55, m, 1H; 3.62, m, 1H) of **15** were resolved and interpreted as a first order system at 500 MHz (Figure 4). The four mutually coupled protons do not give rise to signals with the commonly expected AA'XX' spin coupling pattern, but appear as an ABXY pattern with four distinct chemical shifts and geminal scalar couplings (^1J 13.4 Hz).

The observation of diastereotopism leads us to conclude that **4** and **15** are chiral, at least on the NMR time scale. Restricted rotation about the phenethylamine side chain has been suggested to account for the diastereotopism of **17**;[22] however, we do not see this in **5** or in other simple substituted phenethylamines. The origin of the chirality in **4** and its derivatives is best explained by slow inversion of the benzopentathiepin ring due to an exceptionally high barrier (*ca.* 29 kcal mol^{-1}) to ring inversion as has been noted previously in simple benzopentathiepins.[28] The reason for the high barrier to inversion in **4** and its analogs lies, presumably, in unfavorable eclipsing between the sulfur 3sp^3 lone pair orbitals when

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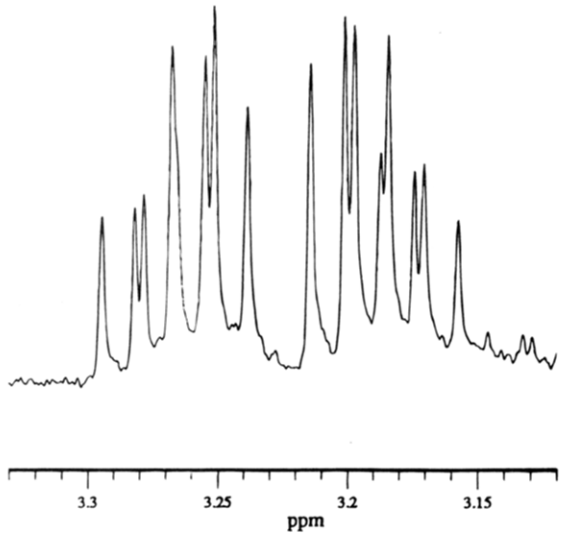

Figure 4. Expanded ^1H NMR spectrum ($CDCl_3$, 500 MHz) of lissoclinotoxin A *N*-trifluoroacetamide (**15**).

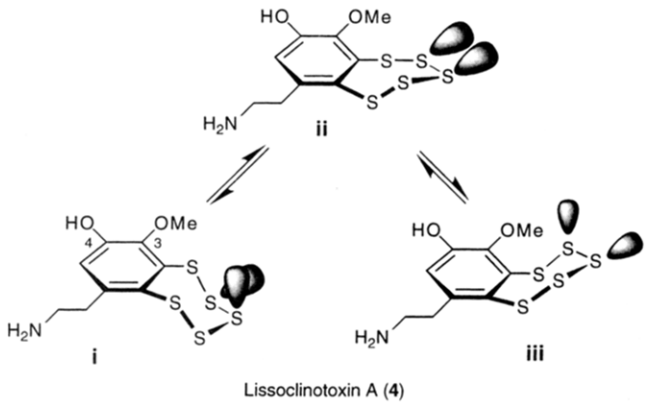

Figure 5. Ring inversion of lissoclinotoxin A (**4**) proceeds through a strained transition state (**ii**) requiring eclipsed sulfur lone pairs (**ii**). For clarity, only two $3sp^3$ lone pair orbitals are shown.

passing through the half-chair transition state during ring inversion (Figure 5).

It now seems certain that a rigid chiral substituted benzopentathiepin ring is also responsible for diastereotopism in the ^1H NMR spectrum of **17**.[22] In principle, this barrier should be sufficiently high to allow for chiral discrimination of the enantiomers of lissoclinotoxins A (**4**) and its analogues during enzymatic biosynthesis; however, in our hands neither **4** nor **6** showed any optical activity and, therefore, were racemic. Optical activity may be present in **4** within the intact ascidian and lost during isolation. Lack of freshly collected organism prevented us from examining this possibility.

Bioactivity. Of the compounds isolated only **4** and **6** exhibited significant activity in the agar disk diffusion assay against *C. albicans* but showed no ergosterol dependence.[13] Lissoclinotoxin A (**4**) gave the following zones of inhibition with the corresponding loadings on 6.5 mm paper disks: 40 μg/disk (27 mm), 10 μg /disk (19 mm), and 1 μg/disk (8 mm). Lissoclinotoxin D (**6**) gave 40 μg/disk (19 mm) and 10 μg/disk (15 mm). Interestingly, lissoclinotoxin C (**5**), lacking the benzopentathiepin ring, was devoid of activity at up to 40 μg/disk. All of the remaining compounds were inactive at up to 40 μg/disk.

Conclusion

Four new alkaloids and one photorearrangement product have been isolated from *Lissoclinum* sp. collected from the Great Barrier Reef. Two of these compounds, lissoclinotoxin A (**4**) and D (**6**), were found to be active against *C. albicans*. Compound **4** exhibits unusual stereoisomerism due to restricted inversion about the benzopentathiepin ring. The benzopentathiepin ring was found to be essential for antifungal activity against *C. albicans*. Lissoclins A (**1**) and B (**2**) are new pyridoacridines related to diplamine (**7**), and lissoclin C (**3**) is a new bromotetrahydro-β-carboline derived from Pictet–Spengler condensation of 6-bromotryptamine with a carbonyl precursor derived from histidine (Figure 2). Lissoclin C (**3**), although chiral, is optically inactive and probably racemizes during isolation. Lissoclinotoxin A (**4**) is a racemic chiral pentathiepin, characterized completely for the first time here, while lissoclinotoxin D (**6**) contains a dimeric "head to tail" lissoclinotoxin A skeleton.

Experimental Section

General. NMR spectra were recorded at 300, 400, or 500 MHz for ^1H, and 75 or 100 MHz for ^{13}C. ^1H NMR and ^{13}C NMR are referenced to solvent signals at 3.30 ppm and 49.00 ppm for CD_3OD, or at 7.26 and 77.00 ppm for $CDCl_3$, respectively. HPLC was carried out on semipreparative reversed phase C_{18} columns (10 × 250 mm) by monitoring UV single wavelength or UV-visible diode array detection. Other general experimental procedures are listed elsewhere.[29]

Collection and Extraction. The ascidian *Lissoclinum* sp. (91-02-028) was collected in 1991 by hand using SCUBA at a depth of −15 m from Michelmas Reef, Great Barrier Reef, Australia, and frozen until used. Lyophilized animals (42.4 g) were extracted with MeOH (3 × 250 mL). The combined extracts were concentrated to approximately 150 mL and successively extracted using a modified Kupchan partition as follows. The water content (% v/v) of the MeOH extract was adjusted prior to sequential partitioning against *n*-hexane (10% v/v H_2O), CCl_4 (20%), and $CHCl_3$ (40%). The aqueous phase was concentrated to remove MeOH and then extracted with *n*-BuOH. The $CHCl_3$ and *n*-BuOH extracts (135 mg and 210 mg) were combined and this material was eluted through a column of Sephadex LH-20 (105 cm × 2.5 cm) with MeOH to afford two antifungal fractions as brown oils (110 mg and 59.4 mg). Purification of the first fraction by flash chromatography (C_{18} bonded silica, methanol/water 80:20, to 100% MeOH) followed by HPLC (Dynamax C_{18}, 10 mm × 250 mm, 70:30 MeOH/water) and flash chromatography of the separated fractions (silica gel, pipet tube, chloroform/methanol 95:5), gave the pyridoacridines **1** (7.4 mg, 0.017%) and **2** (0.4 mg, 0.001%) as orange solids.

Purification of the first fraction from the LH-20 column by repeated HPLC (RP C_{18}, 10 mm × 250 mm, MeOH/0.1% aqueous TFA) gave 2-phenylethylamine (**11**, 35.5 mg, 0.084% dry weight), lissoclin C (**3**, 14.7 mg, 0.035%), lissoclinotoxin D (**6**, 1.2 mg, 0.0028%), and lissoclinotoxin C (**5**, 0.4 mg, 0.0009%).

Purification of the second fraction by repeated HPLC (RP C_{18}, 10 mm × 250 mm, MeOH/0.1% TFA aqueous) gave lissoclinotoxin A (**4**, 15.8 mg, 0.037% dry weight) and 6-bromotryptamine (**12**, 2.3 mg, 0.005% dry weight).

Lissoclin A (1): orange needles; mp 202–204 °C (from MeOH); $C_{23}H_{23}N_3O_2S$; UV (MeOH) λ_{max} 264 nm (ϵ 29 100), 301 (17 200), 382 (7000), 450 (5000); IR ($CHCl_3$) υ_{max} 3450, 3000, 1665, 1605 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.80 (d, J = 6.0 Hz, 6H), 1.94 (m, 3H), 2.66 (s, 3H), 3.76 (m, 4H), 6.18 (br s, 1H), 7.82 (td, J = 8.0, 1.2 Hz, 1H), 7.93 (td, J = 8.0, 1.2 Hz, 1H), 8.29 (dd, J = 8.0, 1.2 Hz, 1H), 8.48 (d, J = 5.5 Hz, 1H), 8.53 (dd, J

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= 8.0, 1.2 Hz, 1H), 9.17 (d, J = 5.5 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 17.9 (q, $^1J_{CH}$ = 141.8 Hz), 22.4 (2 × q), 25.9 (d), 29.9 (t), 39.6 (t), 46.1 (t), 117.3 (s), 119.2 (d), 121.5 (s), 122.9 (d), 129.7 (d), 131.7 (d), 131.8 (d), 136.9 (s), 143.3 (s), 145.5 (s), 146.8 (s), 149.7 (s), 150.0 (d), 151.6 (s), 172.5 (s), 179.6 (s); FABMS *m/z* 408 ($MH^+ + H_2$, 100%), 406 (MH^+, 75); HR FABMS found 406.1575 (MH^+), $C_{23}H_{24}N_3O_2S$ requires 406.1589.

Lissoclin B (2): orange solid; $C_{23}H_{21}N_3O_2S$; UV (MeOH) λ_{max} 263 nm (ϵ 28 000), 300 (17 000), 383 (6800), 450 (4900); IR ($CHCl_3$) ν_{max} 3000, 1665, 1630, 1605 cm^{-1}; ^{1}H NMR ($CDCl_3$/CD_3OD) δ 1.50 (dq, J = 6.9, 0.9 Hz, 3H); 1.57 (br s, 3H), 2.57 (s, 3H), 3.72 (m, 4H), 6.17 (qq, J = 6.9, 0.9 Hz, 1H), 6.78 (br s, 1H), 7.78 (td, J = 8.0, 1.3 Hz, 1H), 7.87 (td, J = 8.0, 1.2 Hz, 1H), 8.23 (dd, J = 8.0, 1.2 Hz, 1H), 8.53 (d, J = 5.5 Hz, 1H), 8.56 (dd, J = 8.0, 1.2 Hz, 1H), 9.13 (d J = 5.5 Hz, 1H); FABMS *m/z* 406 ($MH^+ + H_2$, 65%), 404 (MH^+, 100); HR FABMS found 404.1432 (MH^+), $C_{23}H_{22}N_3O_2S$ requires 404.1433.

Lissoclin C (3) TFA salt: colorless amorphous solid; $C_{15}H_{15}N_4Br$; $[\alpha]_D$ +5.2° (c = 0.97, MeOH); UV (MeOH) λ_{max} 231 nm (ϵ 21900), 285 (4170); IR (film) ν_{max} 3500–2500 br, 1675, 1460, 1435, 1200, 1140, 840, 815, 725 cm^{-1}; ^{1}H NMR (CD_3OD) and ^{13}C NMR (CD_3OD) see Figure 1; FABMS *m/z* 333, 331 (MH^+, 100%), 251 (40), 249 (45); HR FABMS found 331.0553 (MH^+), $C_{15}H_{15}N_4{}^{79}Br$ requires 331.0558.

Lissoclinotoxin A (4) TFA salt: light brown glass; $C_9H_{11}NO_2S_5$; UV (MeOH) λ_{max} 213 nm (ϵ 14 100); IR (film) ν_{max} 3500–2500 br, 1674, 1539, 1419, 1257, 1201, 1140, 615 cm^{-1}; ^{1}H NMR (CD_3OD) δ 3.10 (m, 3H), 3.24 (m, 1H), 3.81 (s, 3H), 6.91 (s, 1H); ^{13}C NMR (CD_3OD) δ 34.8 (t, C7), 41.7 (t, C8), 62.6 (q, 3-OCH_3), 121.3 (d, C5), 135.1 (s, C1), 140.4 (s, C6), 142.4 (s, C2), 150.3 (s, C3), 154.8 (s, C4); FABMS *m/z* 326 (MH^+, 15%); HR FABMS found 325.9469 (MH^+), $C_9H_{12}NO_2S_5$ requires 325.9472; HPLC (55:45 MeOH, 0.1% TFA aqueous, RP C18, 3.0 μm, 4.7 × 100 mm) lissoclinotoxin A (**4**), rt 9.5 min, *c.f.* isolissoclinotoxin (**19**)[23,24] 6.0 min.

Lissoclinotoxin C (5) TFA salt: colorless amorphous solid; $C_{11}H_{17}NO_2S_2$; ^{1}H NMR (CD_3OD) δ 2.32 (s, 3H), 2.48 (s, 3H), 3.10–3.40 (m, 4H), 3.82 (s, 3H), 6.80 (s, 1H); ^{1}H NMR (CD_3CN) δ 2.28 (s, 3H), 2.47 (s, 3H), 3.16 (br s, 4H), 3.81 (s, 3H), 6.89 (s, 1H); FABMS *m/z* 260 (MH^+, 48%), 243 (41), 228 (10), 212 (23); HR FABMS found 260.0799 (MH^+), $C_{11}H_{18}NO_2S_2$ requires 260.0779.

Lissoclinotoxin D (6) Bis-TFA salt: colorless amorphous solid; $C_{18}H_{22}N_2O_4S_4$ ^{1}H NMR (CD_3OD) δ 3.10–3.30 (m, 8H), 3.83 (s, 6H), 6.97 (s, 2H). Lissoclinotoxin D (**6**, *ca.* 0.5 mg) was converted (TFAA, CH_2Cl_2, 100 °C, 10 min) to lissoclinotoxin D *N,N'*-bis(trifluoroacetamide) (**22**): FABMS (+ve ion) *m/z* 651 (MH^+, 18%), 621.5 (MH^+ − MeO, 100), 619 (M^+ − MeO, 63), 618 (M^+ − MeOH, 63), 587 (31), 586.1 ($M^+ - S_2$, 72), 531.4 (43); FABMS (−ve ion) 649.0 (M^- − H, 69%), 617 (M^- − MeOH, 100), 585 ($M^- - S_2$ − H, 96); HR FABMS found 586.0664 ($M^+ - S_2$), $C_{22}H_{20}N_2O_6F_6S_2$ requires 586.0667.

Photoreduction-Rearrangement Product, 10, from Lissoclin A (1). Irradiation of a methanol solution of **1** (*ca.* 1 mg in MeOH, 1.0 mL, quartz cuvette) with a portable UV lamp (3 h) gave a purple pigment. Evaporation of the solvent and separation of the residue by flash chromatography (silica gel, pipet tube, $CHCl_3$:MeOH 95:5) gave the rearranged product **10** as a red-orange solid (0.5 mg). The same compound could be isolated from samples of **1** that had been standing for some time on the benchtop: $C_{23}H_{23}N_3O_2S$; UV (neutral, MeOH) λ_{max} 222 nm (ϵ 31 000), 265 (28 000), 275 (sh), 300 (sh), 311 (sh), 373 (sh), 392 (4800), 480 (4100); UV (80:20 MeOH–0.1% TFA, aqueous) 223 nm, 236(sh), 282, 295 (s), 310 (sh), 365 (sh), 384, 550; ^{1}H NMR ($CDCl_3$) δ 1.00 (d, J = 6.2 Hz, 6H), 2.14 (d, J = 6.6 Hz, 2H), 2.19 (m, 1H), 2.88 (m, 2H), 3.31 (m, 2H), 5.85 (s, 2H), 6.11 (br s, 1H), 6.99 (td, J = 8.0, 2.0 Hz, 1H), 7.10 (d, J = 5.1 Hz, 1H), 7.38 (m, 2H), 7.80 (d, J = 8.0 Hz, 1H), 8.46 (d, J = 5.1 Hz, 1H), 10.05 (br s, 1H); FABMS *m/z* 406 (MH^+, 12%), 307 (24), 289 (10); HR FABMS found 406.1586 (MH^+), $C_{23}H_{24}N_3O_2S$ requires 406.1589.

Preparation of (*S*)-1-Phenylethyl Ureas 13a and 13b. A solution of **3** (0.9 mg) in CH_2Cl_2 (1 mL) and pyridine (0.1 mL) was treated with (*S*)-1-phenylethyl isocyanate (30 μL) at room temperature for 20 h. After evaporation of the solvent, the mixture was analyzed by HPLC (85:15 MeOH–0.1% TFA aqueous, RP C_{18}, 3 μm, 4.7 × 100 mm, 1.0 mL/min, UV diode array detection) and showed two new major peaks (rt 4.07 min, 4.30 min): UV (MeOH–H_2O) identical for both peaks, λ_{max} 232, 286 nm.

Conversion of Lissoclinotoxin A (4) to Varacin *N*-Trifluoroacetamide (16). Lissoclinotoxin A (**1**) was dissolved in 1:1 CH_2Cl_2:TFAA and heated at 100 °C in a sealed tube for 10 min. Evaporation of the volatiles gave pure **15** as a colorless glass, $C_{11}H_{10}NF_3O_3S_5$; ^{1}H NMR ($CDCl_3$) δ 3.20 (ddd, 1H, J = 13.4, 8.4, 6.4 Hz, 1H), 3.28 (ddd, 1H, J = 13.4, 8.2, 6.4 Hz, 1H), 3.55 (m, 1H), 3.62 (m, 1H), 3.89 (s, 3H), 6.47 (br s, 1H), 7.14 (s, 1H); FABMS (+ve ion) *m/z* 422 (MH^+, 9%), 357 ($M^+ - S_2$, 36); FABMS (−ve ion) *m/z* 420 (M^- − H, 27%), 356 (M^- − H − S_2, 22); HR FABMS found 421.9297 (MH^+), $C_{11}H_{11}NF_3O_3S_5$ requires 421.9295.

To a solution of **15** (*ca.* 0.4 mg) in methanol (0.4 mL) at 0 °C was added an excess of ethereal CH_2N_2 and the solution allowed to stand for 2 h. Evaporation and then filtration through silica gel (pipet tube, 1% MeOH–$CHCl_3$) gave a quantitative yield of varacin *N*-trifluoroacetamide (**16**), identical with an authentic sample by ^{1}H NMR, FABMS, and HPLC retention time.[23]

Conversion of Lissoclinotoxin A (4) to 4-*O*-$^{13}CH_3$ Varacin *N*-Trifluoroacetamide (18). Treatment of **15** with $^{13}CH_2N_2$ generated from Diazald (*N*-$^{13}CH_3$) as above gave 4-*O*-$^{13}CH_3$ varacin *N*-trifluoroacetamide (**18**): ^{1}H NMR ($CDCl_3$) δ 3.86 (s, 3H, 3-OCH_3), 3.89 (d, 3H, 4-OCH_3, ^1J = 145.2 Hz); 6.48 (br s, 1H), 6.75 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 56.2 (q, 4-OCH_3); FABMS *m/z* 436 (M^+, 10%), 372 ($M^+ - S_2$, 100%); HR FABMS found 435.9400 (M^+), $C_{11}{}^{13}CH_{12}NO_3S_5F_3$ requires 435.9406.

Conversion of Isolissoclinotoxin A (19) to 3-*O*-$^{13}CH_3$ Varacin *N*-Trifluoroacetamide (21). Conversion of isolissoclinotoxin A (**19**, *ca.* 0.2 mg) to the corresponding *N*-trifluoroacetamide **20** followed by treatment with $^{13}CH_2N_2$ as above gave 3-*O*-$^{13}CH_3$ varacin *N*-trifluoroacetamide (**21**): ^{1}H NMR ($CDCl_3$) 3.86 (d, 3H, 3-OCH_3, ^1J = 145.2 Hz), 3.89 (s, 3H, 4-OCH_3), 6.48 (br s, 1H), 6.75 (s, 1H); ^{13}C NMR ($CDCl_3$) 61.9 (q, 3-OCH_3); FABMS *m/z* 436 (M^+, 15%), 372 ($M^+ - S_2$, 90%); HR FABMS found 435.9414 (M^+), $C_{11}{}^{13}CH_{12}NO_3S_5F_3$ requires 435.9406.

6-Bromotryptamine TFA salt (12):[16] colorless amorphous solid; $C_{10}H_{11}N_2Br$; ^{1}H NMR (CD_3OD) δ 3.08 (t, J = 7.0 Hz, 2H), 3.21 (t, J = 7.0 Hz, 2H), 7.15 (dd, J = 8.5, 1.7 Hz, 1H), 7.18 (s, 1H), 7.48 (dd, J = 8.5, 0.4 Hz, 1H), 7.53 (dd, J = 1.7, 0.4 Hz, 1H); ^{13}C NMR (CD_3OD) δ 24.35 (t), 41.19 (t), 115.48 (d), 120.35 (d), 123.29 (d), 125.23 (d), remaining peaks below detection level; FABMS *m/z* 241 (MH^+ + 2, 34%), 239 (MH^+, 35), 224 (18), 222 (16); HR FABMS found 239.0166 (MH^+), $C_{10}H_{12}N_2{}^{79}Br$ requires 239.0184.

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Supplementary Material Available: Copies of the ^{1}H NMR spectra of **1-6**, **10**, **15**, **18**, and **21**, ^{13}C-NMR spectra of **1**, **3**, **4**, **18**, **21**, PS NOESY, COSY, and HMBC of **3**, and FABMS spectra of **4**, **15**, and **22** can be found in the supplementary material (16 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of this journal, and can be ordered from the ACS; see any current masthead page for ordering information.