111.4, 111.0, 66.0, 59.3, 58.7, 54.6, 54.5, 51.6, 37.8, 36.6, 33.2, 24.5, 23.3, 22.7; mass spectrum, *m/e* 356.2092 ($C_{21}H_{28}N_2O_3$ requires *m/e* 356.2100), 227, 226 (base), 208, 194, 166, 144, 130, 58, 44.

(±)-α-Yohimbine (4). To a solution of **65** (57 mg, 0.16 mmol) in EtOH (2.5 mL) was added $Hg(OAc)_2$/EDTA·2Na (1:1) (4.8 mL of a 0.1 M solution in H_2O, 0.48 mmol), and the resulting solution was heated at reflux for 3 h, whereupon the reaction mixture was cooled to 0–5 °C and 25% $HClO_4$ (5 mL) added. The aqueous mixture was extracted with $CHCl_3$ (4 × 10 mL), and the combined extracts were washed with brine (10 mL) and concentrated under reduced pressure. The residue of iminium salts was dissolved in MeOH/H_2O (9:1) (5 mL), the pH was adjusted to 6 with 5% $NaHCO_3$, $NaBH_4$ (50 mg) was added, and the reaction mixture was stirred for 1 h at 25 °C. The solvents were removed under reduced pressure, and the residue was partitioned between cold 10% NH_4OH (3 mL) and $CHCl_3$ (3 mL). The aqueous layer was extracted with $CHCl_3$ (3 × 5 mL), and the combined extracts were dried (Na_2SO_4) and evaporated under reduced pressure to give a mixture of products, which was separated by HPLC [hexanes/EtOAc (1:2) containing 1% NEt_3] to afford **4** (17.5 mg, 31%) as a light yellow foam and **67** (17.5 mg, 31%) as a pale yellow solid.

For (±)-α-yohimbine (4): as white crystals from EtOAc/hexanes, mp 233–235 °C (dec); hydrochloride (from MeOH) mp 262–264 °C (dec); IR ($CHCl_3$) ν 3580, 3460, 3380, 2800, 2755, 1725 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.77 (br s, 1 H), 7.45 (d, J = 7.8 Hz, 1 H), 7.27 (br d, J = 7.8 Hz, 1 H), 7.12 (dt, J = 1.1, 7.8 Hz, 1 H), 7.07 (dt, J = 1.1, 7.8 Hz, 1 H), 3.99 (dt, J = 4.4, 11.0 Hz, 1 H), 3.83 (s, 3 H), 3.13 (dd, J = 11.2, 2.1 Hz, 1 H), 2.90–3.00 (m, 2 H), 2.83 (dd, J = 11.4, 1.9 Hz, 1 H), 2.77 (br s, 1 H), 2.67 (m, 1 H), 2.58 (dd, J = 11.4, 3.0 Hz, 1 H), 2.56 (dd, J = 11.0, 4.5 Hz, 1 H), 2.52 (m, 1 H), 2.42 (ddt, J = 12.5, 3.5, 4.5 Hz, 1 H), 2.09 (dq, J = 13.0, 3.5 Hz, 1 H), 2.04 (dq, J = 13.0, 3.5 Hz, 1 H), 1.81 (m, 1 H), 1.70 (dt, J = 11.2, 12.5 Hz, 1 H), 1.61 (dt, J = 12.5, 3.5 Hz, 1 H), 1.54 (dq, J = 13.0, 3.5 Hz, 1 H), 1.35 (ddt, J = 11.0, 3.5, 13.0 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 174.6, 136.1, 134.6, 127.4, 121.4, 119.5, 118.1, 110.8, 108.6, 66.1, 60.6, 60.3, 54.9, 53.3, 51.8, 38.1, 36.7, 33.3, 27.8, 24.7, 21.8; mass spectrum, *m/e* 354.1937 ($C_{21}H_{26}N_2O_3$ requires *m/e* 354.1943), 353 (base), 336, 335, 295, 223, 184, 170, 169, 156, 144, 86, 82, 57, 43, 41.

For (±)-inside α-yohimbine (67): mp 236–237 °C (dec) (from EtOAc/hexanes); hydrochloride (MeOH) mp 266–268 °C (dec); IR ($CDCl_3$) ν 3560, 3460, 3340, 2800, 2750, 1725, 1630 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.88 (br s, 1 H), 7.46 (dd, J = 6.5, 1.8 Hz, 1 H), 7.33 (dd, J = 6.5, 1.8 Hz, 1 H), 7.00–7.20 (m, 2 H), 4.10 (m, 1 H), 3.77 (s, 3 H), 3.38 (br s, 1 H), 2.25–3.10 (m, 9 H), 1.45–2.10 (m, 5 H), 1.2–1.40 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 174.5, 136.3, 133.3, 127.4, 121.4, 119.4, 118.1, 110.8, 109.9, 66.3, 63.4, 56.1, 54.9, 53.2, 51.8, 39.8, 38.8, 32.9, 23.5, 21.7, 20.0; mass spectrum, *m/e* 354.1947 ($C_{21}H_{26}N_2O_3$ requires *m/e* 354.1943), 353, 336, 335, 197, 185, 184 (base), 170, 169, 156, 143, 130, 115.

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Supplementary Material Available: General information for the Experimental Section and experimental details including infrared, proton magnetic resonance, carbon magnetic resonance, and mass spectra together with physical constants for other new compounds not described in the present Experimental Section (13 pages). Ordering information is given on any current masthead page.

A Novel Pentacyclic Aromatic Alkaloid from an Ascidian[1]

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Abstract: 2-Bromoleptoclinidinone, a pentacyclic aromatic alkaloid, $C_{18}H_8N_3OBr$, possessing a new skeleton, was isolated from an ascidian, and its structure was determined by making extensive use of long-range proton–carbon couplings. The new alkaloid is toxic in cell culture to lymphocytic leukemia cells (PS).

Fused tetra- and pentacyclic aromatic alkaloids are rare among the wide variety of alkaloids isolated from marine organisms.[2] The only examples are the sponge metabolite amphimedine (**1**, Chart I),[3] an anemone pigment, calliactine, and a hydrolysis product thereof, neocalliactine.[4] These highly fused structures have proven to be challenging structure elucidation problems as is indicated by the fact that calliactine and neocalliactine have been known for many years and their structures are still ambiguous in spite of analysis by modern spectrometric methods. Extensive long-range heterocorrelation and carbon–carbon correlations were needed to resolve the structure of amphimedine. We report here the isolation of a new fused pentacyclic alkaloid, designated 2-bromoleptoclinidinone, from an ascidian tentatively identified as a *Leptoclinides sp.* The structure elucidation required extensive utilization of long-range H/C coupling data.

The formula $C_{18}H_8N_3OBr$, implying 17 degrees of unsaturation, was established for the new alkaloid by high-resolution mass spectrometry. Only aromatic type protons were observed in the ^1H NMR spectrum, and these could be assigned to one benzene and two pyridine rings substituted as shown in partial structures A (see H-1, H-3, and H-4, Table I), B (see H-6 and H-7), and C (see H-9, H-10, and H-11) (see Chart II for A–C). The presence of two pyridine rings was inferred from the low-field position of two protons, 9.15 and 9.24 ppm, which showed no coupling to each other but did each show 5–6-Hz ortho couplings typical of the α-proton on a pyridine ring.[5] Definitive evidence for partial structures A–C was derived from one-bond and long-range proton–carbon correlations; see partial structures A–C and Table I. Two- and three-bond couplings determined by selective ^1H decoupling using low power are indicated by solid

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0002-7863/87/1509-6134$01.50/0

Chart I

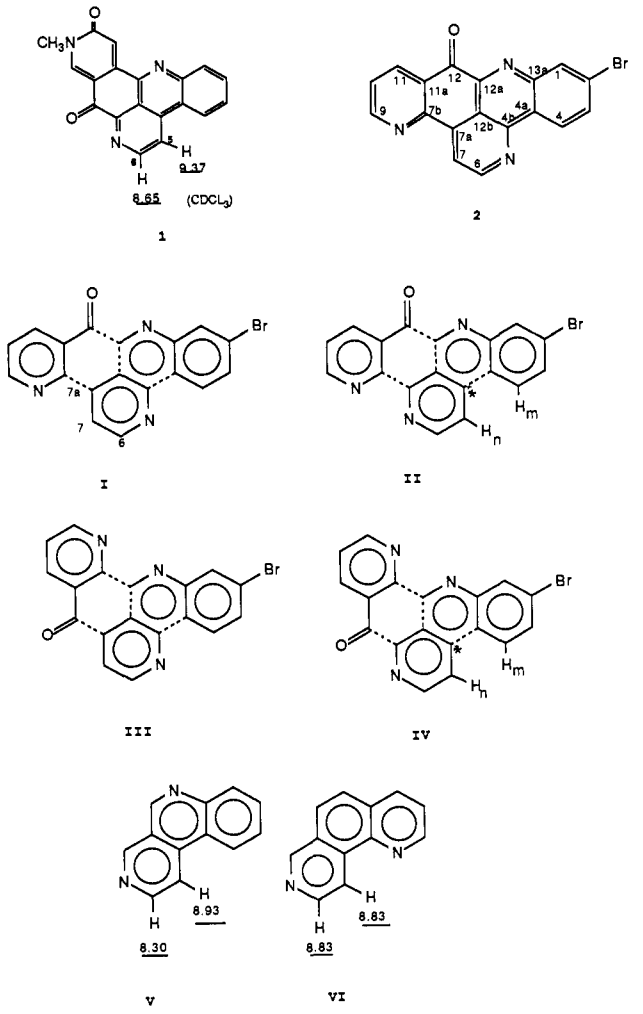
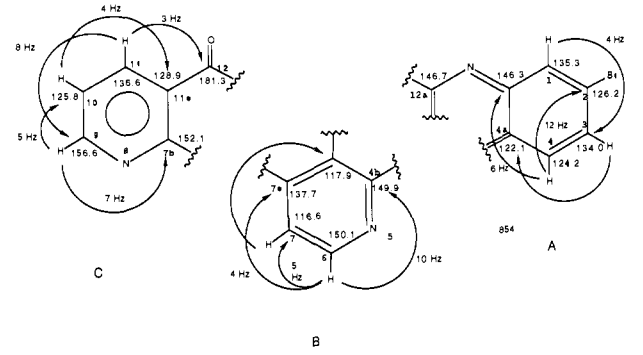


Chart II



curved arrows, while correlations established more clearly by long-range 2D correlations[6] (Figure 1) are indicated by dashed curved arrows.

Regarding partial structure A, the peak shape for the carbon absorbing at 122.1 ppm was changed upon irradiating H-3, but the correlation between these two signals was seen most clearly in a long-range H/C COSY experiment;[7] see Figure 1. In the 1D long-range H/C decoupling experiments, irradiation of the 8.73/8.76 ppm (H-1, H-11) proton signal sharpened the 122.1 ppm peak (C-4a), but since the environment around H-11 is completely defined (see partial structure C discussion), this coupling must be due to H-1/C-4a. This provided the final set of ortho H/C couplings needed to confirm the 6-membered ring of A. Comparison of the relative order of the chemical shifts for

Table I. NMR Data for **2**

C	δ(H) at C[a]	J, Hz (m)	δ(C)[b]	$^1J_{H-C}$, Hz	$^{2,3}J_{H,C}$,[c] Hz m	
1	8.73	2.1 (d)	135.3	170	d	4 (H-3)
2			126.2		dt	12(H-4), 3 (H-1, H-3)
3	7.98	8.7, 2.1 (dd)	134.0	175	d	4 (H-1)
4	8.49	8.7 (d)	124.2	164	s	
4a			122.1		m	
4b			149.9		bd	10 (H-6)
6	9.24	5.7 (d)	150.1	190	bs	
7	8.45	5.7 (d)	116.1	178	bd	5 (H-6)
7a			137.7		d[d]	4 (H-6)
7b			152.1		m	
9	9.15	4.6, 1.8 (dd)	155.6	191	bd	8 (H-11)
10	7.67	7.9, 4.6 (dd)	125.8	177	bd	5 (H-9)
11	8.76	7.9, 1.8 (dd)	136.6	173	d	4 (H-9)
11a			128.9		bd	4 (H-10)
12			181.3		d[e]	3 (H-11)
12a			146.7		s	
12b			117.9		d	6 (H-7)
13a			146.3		bd	8 (H-4)

[a] 300 MHz, $CDCl_3$. [b] 75 MHz, $CDCl_3$; assignments are based on single-frequency decoupling. [c] Assignments based on single-frequency decoupling and long-range H/C COSY. [d] Observable when H-1, H-7, or H-11 is irradiated. [e] Seen clearly in 1:1 $CDCl_3$–$CDCO_2D$.

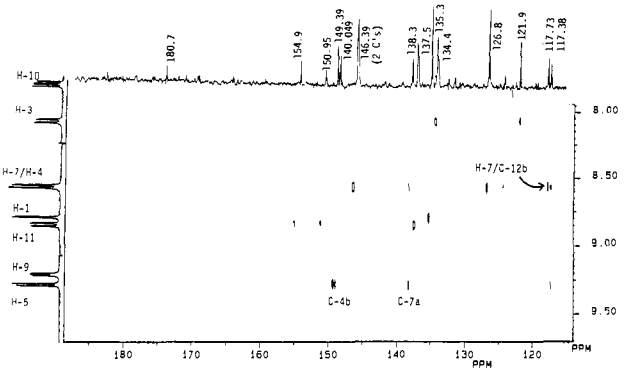


Figure 1. Long-range $^1H/^{13}C$ correlation plot for **2** from an experiment[7] with J set at 8 Hz in $CDCl_3$ with trifluoroacetic acid-d added.

carbons 1–3 of partial structure A with those of bromobenzene provides grounds for placing bromine at the 2-position.[8]

The four carbons adjacent to nitrogens in partial structures B and C can be unequivocally assigned since the large one-bond couplings of C-6 and C-9 are only compatible with C–H groups bonded to nitrogen,[8] and the locus of the two lowest field, non-protonated carbons in B and C were fixed by their 3-bond coupling to H-6 and H-9. The remaining two quaternary carbons absorbing at 146.3 and 146.7 ppm are joined to nitrogen in partial structure A on the basis of their chemical shifts. The lack of any observable coupling to the 146.7 ppm (C-12a) peak justifies placing this carbon at least four bonds away from any protons.

Partial structure C was completely defined by the long-range couplings shown. Although H-1 and H-11 are very similar in chemical shift, H-11 was resolved in the solvent used for the long-range H/C COSY experiment, and a clear correlation of H-11 with both C-9 and C-7b was seen (see Figure 1).

The coupling assinged to H-6/C-7a in partial structure B was not directly ascertainable by decoupling because in the fully coupled ^{13}C NMR spectrum the C-7a signal is accidentally coincident with one member of the C-11 dd (both in $CDCl_3$ and $CDCl_3/CD_3CO_2D$). However, upon low-power irradiation of H-11 or H-1, the one-bond coupling of C-11 is diminished slightly and C-7a can be seen clearly as a doublet, $J = 4$. This J could be assigned to H-6/C-7a since a clear correlation peak was observed between these signals in the long-range 2D H/C COSY

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(7) See, for example: Schoolery, J. N. *J. Nat. Prod.* **1984**, *47*, 726.
(8) Wehrli, F. W.; Wirthlin, T. *Interpretation of Carbon-13 NMR Spectra*; Heyden: London, 1976; Chapter 2.

experiment (see Figure 1). The remaining unassigned carbon signal, a doublet (6 Hz) at 117.9 ppm (C-12b), collapsed almost equally well upon ^1H irradiation at 8.45 or 8.49 ppm (H-7 and H-4, respectively), and since these signals are even less well resolved in the solvent used for the long-range 2D H/C COSY experiment, no definitive correlation could be made. However, since coupling between H-4 and the 149.9 ppm peak could be confirmed (see below), the correlation present in Figure 1 between the superimposed H-4, H-7 signals and the 117.9 ppm peak is taken as evidence for H-7/C-12b coupling. Also, in order to satisfy the requirement for a pyridine ring indicated by the 5–6 Hz ortho proton coupling in partial structure B, the 117.9 ppm absorbing carbon needs to be located in partial structure B as shown.

Since all three nitrogens are flanked by aromatic carbons, the carbonyl group of **2** (IR 1680 cm^{-1}; ^{13}C NMR 181.3 ppm) must be due to an unsaturated ketone and not to an amide group. Comparison of the IR absorption of **2** with data reported for ketone groups in fused aromatic compounds with 5-, 6-, and 7-membered rings suggests that the carbonyl group is incorporated in a 5- or 6-membered ring,[9] while the ^{13}C NMR chemical shift is more characteristic of a 6-membered-ring aromatic structure.[10]

Combination of partial structures A–C, keeping in mind the proviso that the carbonyl group be in a 6-membered ring, yields only the four possible structures I–IV. Of these, I is strongly indicated by long-range H/C decoupling data: ^1H irradiation at either 8.45 or 8.49 ppm sharpens the 149.9 ppm carbon signal (C-4b), but irradiation at 8.49 ppm gives the clearest sharpening. Likewise, irradiation at 8.45 ppm removes a small J from the 152.1 ppm signal better than irradiation at 8.49 ppm does. Structure III can be ruled out because the carbonyl resonance of **2** is a well-resolved doublet which collapses to a singlet upon ^1H irradiation at 8.76 ppm (H-11 of partial structure C), whereas in III the carbonyl carbon would be expected to show two similar three-bond couplings. Structures II and IV can be ruled out on the following grounds. First, the 137.7 ppm signal (C-7a in I) remains a doublet when H-7 is irradiated (the large J of the C-11 signal is also reduced slightly so as to allow the C-7a signal to be seen; cf. above), whereas in II or IV this signal (see starred carbons in formulas) would be expected to be a double doublet under these conditions due to two three-bond H/C couplings. Second, the downfield chemical shift position of H-6 (9.24 ppm) relative to H-7 (8.45 ppm) is more like the analogous protons H_a (8.83 ppm) and H_b (8.83 ppm) in compound VI[11] than like H_a (8.30 ppm) and H_b (8.93 ppm) in V[12] or H-6 (8.65 ppm) and H-5 (9.37) in amphimedine (**1**).[3] Third, when **2** was treated with Fe(II) solution, no colored complex formation was observed indicative of metal chelation characteristic[13] of the 1,10-phenanthroline partial structure present in II, III, and IV. Finally, no NOEs were observed upon irradiation of H-7 or H-4 under conditions where all of the NOEs expected for structure I were observed. In structures II and IV, the corresponding protons (labeled m and n) would be expected to show a significant NOE, as indeed the analogous protons in **1** do. Hence, the new alkalid is assigned structure **2**.

2-Bromoleptoclinidinone shows mild cytotoxicity, ED_{50}(PS = 0.4 $\mu g/mL$.[14]

Experimental Section[15]

Isolation of 2-Bromoleptoclinidinone (2). Ascidians were collected in Sept 1984 and Nov 1985 from Truk Lagoon and frozen for preservation. The initial collection was extracted several times with 1:1 chloroform–methanol, and the concentrated extracts were partitioned between dichloromethane and aqueous methanol (80:20). The second batch of specimens was freeze-dried, and the dried specimens (140 g) were soaked twice with hexane (3 h; 2 h), twice with dichloromethane [6 h; 12 h (combined yield, 1.8 g)], and twice with chloroform [24 h each (combined yield, 1.2 g)]. Vacuum flash chromatography[16] over silica gel of the dichloromethane solubles (900 mg from ~22 g of ascidian, dry weight) from the first extraction yielded a fraction (10:90 methanol–acetone elution) that was further resolved by preparative thin-layer chromatography (95:5 chloroform–methanol elution) to yield 3 mg of **2** as a yellow powder. Silica gel chromatography of the dichloromethane extractables of the freeze-dried specimens (dichloromethane–methanol gradient) yielded two fractions that contained **2**. Purification was achieved by adding methanol to a chloroform solution of these fractions, whereupon a precipitate of 30 mg of pure **2** was obtained as a yellow powder: mp >300 °C; IR (film from evaporation of a $CHCl_3$ solution on a NaCl plate) 3400 (br), 1680, 1600, 1580, 1415, 1270 cm^{-1}; UV (ethanol) λ_{max} (ϵ) 371 (21 000), 335 (18 500), 298 (30 600), 278 (32 000), 254 (sh, 27 300), 247 (27 800), 227 (151 600) nm; ^1H and ^{13}C NMR, see Table I; low-resolution mass spectrum (12 eV), m/z (relative intensity) 363.0 [M^+ + 2 (79)], 361.0 [M^+ (80)], 335.0 [M^+ + 2 – CO (47)], 333.0 [M^+ – CO (48)], 283.1 (19), 255.1 (33), 254 (M^+ – CO, Br (100)]; high-resolution mass spectrum, 362.98302 (98%), $C_{18}H_8O^{81}BrN_3$ (millimass error, –3.3), 360.98507 (100%), $C_{18}H_8O^{79}BrN_3$ (millimass error –3.7).

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Registry No. 2, 109802-17-7.

(9) Cf. Fluorenone, anthrone, and 5*H*-dibenzo[*a*,*d*]cyclohepten-5-one, respectively: 1700, 1660, 1650 cm^{-1}; see: Sadtler Standard Spectra; Sadtler Research Laboratories: Philadelphia, PA; Grating Spectra nos. 28034, 475, 1688, respectively.

(10) Cf. Fluorenone, 193.1 ppm; 2-ethylanthraquinone, 182.6 ppm; 5*H*-dibenzo[*a*,*d*]cyclohepten-5-one, 192.3 ppm. Sadtler Standard Spectra; Sadtler Research Laboratories: Philadelphia, PA; standard carbon-13 spectra nos. 1241C, 2102C, and 84906C, respectively.

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(14) Gueran, R. I.; Greenberg, N. H.; Macdonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep., Part 3* **1972**, *3*(2), 1–103. Effective doses (ED_{50}) in the tissue culture tests are expressed as concentrations ($\mu g/mL$) of test material in the growth medium that causes 50% inhibition of cell growth. "Active" materials display an ED_{50} 10 $\mu g/mL$. PS refers to in vitro lymphocytic leukemia.

(15) Melting points were taken on a Kofler hot stage apparatus and are uncorrected. Infrared spectra were taken on a Perkin-Elmer 298 spectrophotometer; ultraviolet spectra were taken in ethanol on a Perkin-Elmer Lambda 3 UV–Vis spectrophotometer. Low-resolution mass spectra were taken on a Hewlett-Packard 5985B GC/MS system, and high-resolution spectra were obtained on a VG ZAB E instrument. NMR spectra were acquired on a Varian XL-300 spectrometer in the solvents specified, except for one H/C COSY experiment that was carried out on a Bruker 400-MHz instrument. Signals are reported in parts per million downfield from internal tetramethylsilane. The chromatographic adsorbent used was Merck 60H TLC grade silica gel. Analtech Uniplate silica gel GF tapered plates were used for preparative layer chromatography.

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