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NEW CYTOTOXIC β-CARBOLINE ALKALOIDS FROM THE MARINE BRYOZOAN, *CRIBRICELLINA CRIBRARIA*

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ABSTRACT.—Bioactivity-directed separations led to the isolation of the new alkaloid, 1-vinyl-8-hydroxy-β-carboline [**1**], as the major cytotoxic component of the marine bryozoan *Cribricellina cribraria*. Another new β-carboline alkaloid **2** with the novel sulfone structure was isolated, together with a number of known β-carboline compounds. Cytotoxicity and antimicrobial effects are reported for these compounds and for other synthesized β-carbolines.

Although the simple β-carboline alkaloids are well known from terrestrial plants (1), reports of their occurrence in the marine environment are scarce. The first report of their isolation from a marine organism was from the dinoflagellate *Noctiluca miliaris* (2), and subsequent reports were for the isolation of the eudistomins from the tunicates *Eudistoma olivaceum* (3) and *Ritterella sigillinoides* (4), the manzamines from several sponges (5–8), and brominated β-carbolines from the hydroid *Aglaophenia pluma* (9). Simple β-carboline alkaloids have also been isolated from the related Tasmanian bryozoan, *Costaticella hastata* (10).

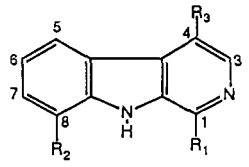
RESULTS AND DISCUSSION

In the present work, the crude MeOH/toluene extract of the New Zealand bryozoan *Cribricellina cribraria* (order Cheilostomata, suborder Ascophora, family Mucronellidae) exhibited strong cytotoxic P-388 activity and cytotoxicity against a BSC cell line. Bioactivity-directed partitioning of the crude extract by cc on C18 reversed-phase material led to the isolation of the new compound, 1-vinyl-8-hydroxy-β-carboline [**1**], as the major cytotoxic component. 1-Ethyl-4-methylsulfone-β-carboline [**2**] was also isolated as a minor component of the bryozoan, along with the known compounds harman [**3**], 1-ethyl-β-carboline [**4**], and pavettine [**5**].

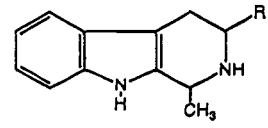
The structure of **1** was determined from the hreims, which revealed a molecular formula of $C_{13}H_{10}N_2O$, and from a series of 1D and 2D nmr spectroscopic experiments. The ^{1}H-nmr spectrum of **1** contained signals for eight non-exchangeable protons, of which five were for aromatic protons while the other three were in a pattern characteristic of a vinyl group. The ^{13}C-nmr spectrum of **1** contained twelve carbon resonances, of which seven arose from protonated carbons. The chemical shift data, together with the results of COSY, HETCOR, and XCORFE nmr experiments, suggested a β-carboline nucleus with a vinyl substituent at C-1 and a substituent, X, at either C-5 or C-8. A series of nOe experiments determined the site of substitution as C-8, and from the molecular formula and the presence of an hydroxyl stretching band in the ir spectrum of **1**, this substituent must have been an -OH group.

During the course of isolation of **1**, the presence of another compound, fluorescent under uv light and of similar R_f to **1** on Si gel tlc, was noted. A ^{1}H-nmr spectrum of the appropriate fraction revealed that this was also a β-carboline alkaloid. Semi-preparative reversed-phase hplc led to the isolation of **2** as a pale green oil, which fluoresced blue-purple in EtOH solution.

The structure of **2** was established through a combination of nmr and ms. An hreims of **2** showed an $[M]^+$ ion at 274.0737 with an $[M+2]^+$ peak of the appropriate intensity for one sulfur atom. These data indicated a molecular formula $C_{14}H_{14}N_2O_2S$. A cims obtained using ND_3 as reagent gas showed a peak at 277, confirming the presence of one exchangeable proton (11). The ^{1}H-nmr spectrum of **2** indicated the pres-



	R_1	R_2	R_3
1	$CH=CH_2$	OH	H
2	Et	H	$S(O_2)Me$
3	Me	H	H
4	Et	H	H
5	$CH=CH_2$	H	H
6	$CH=CH_2$	OMe	H
7	Et	OH	H
8	$CH=CH_2$	OAc	H
9	Et	OMe	H
10	H	H	H
11	$(CH_2)Me$	H	H
12	$CH(Me)_2$	H	H
13	$(CH_2)_5Me$	H	H
14	C_6H_5	H	H



15 $R=CO_2H$
16 $R=CO_2Me$

ence of a β-carboline nucleus, with substituents at C-1 and either C-3 or C-4. ^1H and ^{13}C resonances characteristic of an ethyl group at C-1 accounted for one of these substituents, leaving a fragment CH_3O_2S to be accounted for from the molecular formula. Fragmentations in the eims confirmed these groupings of atoms, with ions at 246 $[M-CH_2-CH_2]^+$, 194 $[M-H-CH_3SO_2]^+$ and 167 $[M-CH_3SO_2-CH_2CH_3]^+$. The singlet at 3.27 ppm in the ^1H-nmr spectrum and a ^{13}C resonance at 43.24 ppm were consistent with a methyl group attached to a sulfur atom, in turn attached to one or more oxygen atoms. NOe experiments in C_6D_6 permitted the resolution and separate irradiation of the *S*-methyl singlet and the methylene quartet of the ethyl group. Irradiation of the H-5 signal produced enhancement of the *S*-methyl signal and vice versa, thus locating the S-containing substituent at C-4.

A number of features in the spectra of **2** indicated that this substituent was a methyl sulfone rather than the alternative sulfinate ester. First, the ^{13}C chemical shifts for C-4 and C-3 were inconsistent with the direct attachment of an O atom at C-4 as required for the sulfinate ester. The chemical shifts of C-4 and C-3 were more in keeping with values expected for a methyl sulfone, as found from a comparison of shifts in toluene (12) and methyl 4-tolyl sulfone (13). Second, the ir spectrum of **2** had two strong bands at 1335 and 1160 cm^{-1}, typical of a sulfone (14), while in the uv spectrum of **2** in MeOH, some bands were shifted to longer wavelength than in 1-ethyl-β-carboline, in keeping with the extended conjugation offered by the sulfone grouping. Finally, sulfinate esters are known to be very unstable and are readily hydrolyzed under both acidic and basic conditions (15). Hydrolysis was attempted by dissolving a sample of **2** in $CDCl_3/D_2O$-TFA, and the reaction (if any) was monitored by ^1H-nmr spectroscopy. No evidence for a reaction was observed, which again strongly favored the sulfone structure.

Reversed-phase cc also led to the isolation of the known β-carboline alkaloids harman [**3**], 1-ethyl-β-carboline [**4**], and pavettine [**5**] as minor components of the extract. These alkaloids were identified by nmr, ms, and tlc comparison with authentic samples.

Homarine was isolated as the major H_2O-soluble component of the bryozoan, while the sterol mixture was isolated by normal phase cc. Cholesterol and cholest-4-en-3-one

were identified as major components of this mixture by ^{13}C-nmr spectroscopy (16), and this identification was confirmed by subsequent gc-ms analysis.

During these investigations, nmr spectroscopic experiments on 1-ethyl-β-carboline suggested that some of the previously published ^{13}C-nmr assignments for β-carbolines (17) should be revised. The ^{1}H- and ^{13}C-nmr spectra of 1-ethyl-β-carboline [**4**] have been rigorously assigned with the aid of COSY and HETCOR nmr experiments, which show that the chemical shifts of C-6 and C-7 are at ca. 120 and ca. 128 ppm, respectively, the reverse of those reported earlier.

The β-carboline alkaloids isolated from *C. cribraria* differed markedly in their degree of biological activity in the P-388 cytotoxicity assay. 1-Vinyl-8-hydroxy-β-carboline and pavettine both had IC_{50} values of 100 ng/ml against P-388 (Table 1), harman and 1-ethyl-β-carboline both had IC_{50} values of 25,000 ng/ml, while the IC_{50} value of 1-ethyl-4-methylsulfone-β-carboline was greater than 12,500 ng/ml. These results suggested that the vinyl group might be important for P-388 cytotoxicity, as

TABLE 1. P-388 and Antiviral/Cytotoxicity Assay Results for β-Carboline Alkaloids **1–16**.

Compound	IC_{50}[a]	HSV1[b]	PV1[c]	Cyt[d]	μg/disc[e]
1	100	?[f]	?	ww	2
		1+	1+	2+	0.5
		—	—	—	0.4
2	>12 500	—	—	—	20
3	25 000	—	—	—	20
4	25 000	—	—	—	20
5	100	?	?	ww	2
		1+	1+	2+	0.5
		—	—	—	0.4
6	100	1+	1+	2+	2
		1+	1+	1+	0.5
		—	—	—	0.4
7	>12 500	—	—	—	20
8	650	?	?	ww	20
		1+	1+	2+	5
		—	—	—	1
9	>12 500	—	—	—	20
10	25 000	—	—	—	20
11	25 000	—	—	—	20
12	13 000	—	—	—	20
13	9 000	1+	—	1+	40
		—	—	1+	10
14	90 000	—	—	—	20
15	>125 000	—	—	—	20
16	120 000	—	—	—	20

[a]The concentration of sample in ng/ml required to reduce the cell growth of the P-388 leukemia cell line (ATCC CCL 46) by 50%.

[b]*Herpes simplex* Type I (strain F, TCC VR 733) virus grown on the BSC cell line (ATCC CCL 26).

[c]*Polio* virus (Pfizer vacine strain) grown on the BSC cell line.

[d]Cytotoxicity to BSC cells.

[e]Loading of test sample on disc.

[f]?, Indeterminate activity; —, No discernable antiviral or cytotoxic effects; ±, Minor effects located under the disc; 1+, antiviral/cytotoxic zone 1–2 mm excess radius from disc edge; 2+, antiviral/cytotoxic zone 2–4 mm excess radius from disc edge; 3+, antiviral/cytotoxic zone 4–6 mm excess radius from disc edge; ww, antiviral/cytotoxic zone over whole well.

the alkaloids with a 1-vinyl substituent exhibited much greater activity than those with a 1-alkyl substituent. In order to verify this assertion, several derivatives of **1** were prepared.

Methylation of **1**, using CH_2N_2 in Et_2O, resulted in the formation of a complex mixture of products, the major component of which was obtained by reversed-phase cc and identified as 1-vinyl-8-methoxy-β-carboline [**6**]. The ^1H- and ^{13}C-nmr spectra of **6** in $CDCl_3$ were consistent with the formation of a methoxyl at C-8, while the eims of **6** contained ions at 224.0949 $[M]^+$, 223 $[M-H]^+$, 209 $[M-Me]^+$, and 194 $[M-CH_2O]^+$. 1-Ethyl-8-hydroxy-β-carboline [**7**] was prepared by hydrogenation of **1**. The ^1H- and ^{13}C-nmr signals from the vinyl group in **1** were replaced by signals appropriate for the ethyl group, while the ms showed the gain of 2 mass units. Acetylation of **1** in pyridine/Ac_2O yielded 1-vinyl-8-acetoxy-β-carboline [**8**], whose ^1H- and ^{13}C-nmr spectra were consistent with the formation of a monoacetate at position 8. The hreims contained ions at 252.0895 (assigned to $[M]^+$), 210.0806 $[M-C_2H_2O]^+$, 195.0910 $[M-C_2H_2O]^+$, and 168.0655 $[M-C_4H_4O_2]^+$.

Assay of the alkaloids **6–8** against P-388 confirmed that the presence of the vinyl group is essential for these alkaloids to exhibit any significant cytotoxic properties. The IC_{50} value of compound **6** was determined to be 100 ng/ml, while that of compound **8** was 670 ng/ml. The IC_{50} values of 1-ethyl-8-hydroxy-β-carboline [**7**] and 1-ethyl-8-methoxy-β-carboline [**9**] were both greater than 12,500 ng/ml. This trend was further confirmed by measuring the IC_{50} values of a number of synthetic β-carboline alkaloids, namely norharman [**10**], 1-propyl-β-carboline [**11**], 1-isopropyl-β-carboline [**12**], 1-hexyl-β-carboline [**13**], and 1-phenyl-β-carboline [**14**], for all of which the activities

TABLE 2. Antimicrobial/Antifungal Activities of β-Carboline Alkaloids **1–16**.[a]

Compound	Organism[b]					
	Ec	Bs	Pa	Ca	Tm	Cr
1	>60	7.5–15	>60	15–30	0.45–0.9	3.7–7.5
2	>60	30–60	>60	>60	30–60	>60
3	60–120	7.5–15	>60	1.9–3.8	3.7–7.5	15–30
4	>120	7.5–15	>60	1.9–3.8	1.9–3.8	30–60
5	30–60	1.9–3.8	>60	1.9–3.8	0.1–0.2	0.9–1.9
6	>60	7.5–15	>60	7.5–15	0.45–0.9	15–30
7	30–60	30–60	>60	30–60	30–60	>60
8	>60	7.5–15	>60	15–30	0.9–1.9	>60
9	>60	>60	>60	7.5–15	7.5–15	>60
10	3.7–7.5	7.5–15	>60	1.9–3.8	0.9–1.9	15–30
11	>120	>120	>60	>120	15–30	>120
12	>120	7.5–15	>60	1.9–3.8	0.45–0.9	15–30
13	>60	>60	>60	>60	1.9–3.8	7.5–15
14	>60	>60	>60	>60	>60	>60
15	>60	>60	>60	>60	>60	>60
16	>60	>60	>60	7.5–15	>60	>60
	9[c]	12–14[d]	8[c]	8–10[d]	4–6[e]	12–14[e]

[a]Expressed as minimum inhibitory concentraton (MIC) in μg/disc.

[b]Ec = *Escherichia coli*, Bs = *Bacillus subtilis*, Pa = *Pseudomonas aeruginosa*, Ca = *Candida albicans*, Tm = *Trichophyton mentagrophytes*, Cr = *Cladisporum resinae*. (All strains developed and held in Plant and Microbial Sciences Department, University of Canterbury, 1984).

[c]Inhibition zone as excess radius from disc containing 10 μg gentamicin.

[d]Inhibition zone as excess radius from disc containing 30 μg chloramphenicol.

[e]Inhibition zone as excess radius from disc containing 100 units nystatin.

were found to be much less than those of the vinyl alkaloids. Norharman and 1-propyl-β-carboline had IC_{50} values of 25,000, 1-isopropyl-β-carboline 3000, 1-hexyl-β-carboline 9000, and 1-phenyl-β-carboline 90,000 ng/ml. A similar trend has been observed for the antibiotics, ravidomycin and gilvocarcin V, in that the vinyl group appeared to be necessary for antitumor activity. For these compounds, replacing the vinyl group with an ethyl group significantly lowered the activity (18,19).

In the antiviral assays a 1-vinyl group again proved necessary for activity to be observed, but the antiviral potential of β-carbolines **1**, **5**, **6**, and **8** would have to be described as modest, as any viral inhibition was accompanied by significant cytotoxicity against the host cell line (Table 1).

The same trend, however, was not observed in the antimicrobial and antifungal properties of the β-carboline alkaloids (Table 2). The β-carboline alkaloids **1–9** and synthetic alkaloids **10–16** were assayed against two Gram-negative bacteria, (*Pseudomonas aeruginosa* and *Escherichia coli*), a Gram-positive bacterium (*Bacillus subtilis*) and three fungi (*Candida albicans, Trichophyton mentagrophytes,* and *Cladisporum resinae*). Trends observed in the antimicrobial and antifungal properties of the alkaloids varied, depending on the organism tested against, but were different from the trend in P-388/cytotoxic activity of the β-carboline alkaloids. The vinyl alkaloids were not necessarily much more active than the other alkaloids (Table 2), although pavettine was generally the most active compound. It is likely that the solubility of the alkaloids is of greater significance here.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Eims and cims were obtained on a Kratos MS80RFA mass spectrometer. Ir spectra were obtained using a Pye Unicam SP-300 infrared spectrophotometer. Uv spectra were obtained as MeOH solutions using a Varian DMS 100 spectrophotometer. ^{13}C-nmr spectra at 75 MHz and ^{1}H-nmr spectra at 300 MHz were recorded on a Varian XL-300 spectrometer, as were the 2D correlation spectra. Difference nOe spectra were obtained with the low power cycling method (20). Flash cc was performed using Si gel (Davisil 35-70 μ) and C18 reversed-phase material prepared by coating the Si gel with *n*-octadecyltrichlorosilane after the method of Evans *et al.* (21). Hplc was performed on a Varian 5000 liquid chromatograph using a Varian UV-50 variable wavelength detector and a Hewlett Packard 3390A integrating recorder. Semi-preparative reversed-phase chromatography was carried out on an Alltech cyanopropyl column (250 × 10 mm). All solvents used were either spectral grade or distilled prior to use.

COLLECTION AND EXTRACTION OF BRYOZOAN.—*Cr. cribraria* (1 kg wet) was collected by scuba diving in April 1987, from Sugar Loaf, Kaikoura, off the South Island of New Zealand. A voucher specimen, 87K15-02, is held at the Chemistry Department, University of Canterbury. The bryozoan was stored frozen. In a typical extraction, 200 g was left overnight in MeOH-toluene (4:1) (250 ml) followed by blending three times in the same solvent mixture. The combined extracts were filtered under vacuum, the filtrates combined, and the solvent removed under reduced pressure.

1-VINYL-8-HYDROXY-β-CARBOLINE [**1**].—The resulting extract (7.6 g) was chromatographed on a reversed-phase C18 column (50 g), using a H_2O/MeOH to MeOH/CH_2Cl_2 gradient to give 13 fractions. Alkaloids were shown to be concentrated in fractions 6 and 7 by tlc on Si gel [EtOAc-Me_2CO (1:1), fluorescent spots under uv light] and by the P-388 cytotoxicity assay. These two fractions (116 mg) were recombined and rechromatographed on a reversed-phase C18 column (10 g) using the same H_2O/MeOH/CH_2Cl_2 gradient as previously. Alkaloids were concentrated in fraction 4 as shown by tlc and the P-388 assay. A further reversed-phase C18 column on fraction 4 (37 mg) led to the isolation of 1-vinyl-8-hydroxy-β-carboline [**1**] (20 mg) as a yellow oil: ms *m/z* found $[M]^+$ 210.0787 (4) ($C_{13}H_{10}N_2O$ requires 210.0793), 209.0702 (2) ($C_{13}H_9N_2O$ requires 209.0715), 184.0636 (0.4) ($C_{11}H_8N_2O$ requires 184.0637); ^{1}H nmr (CD_3OD) 8.47 (1H, d, J = 5.2 Hz, H-3), 8.13 (1H, dd, J = 7.9, 0.9 Hz, H-5), 7.87 (1H, d, J = 5.2 Hz, H-4), 7.52 (2H, m, H-7, -8), 7.29 (1H, m, H-6), 7.22 (1H, dd, J = 17.5, 11.1 Hz, H-1′), 6.39 (1H, dd, J = 17.5, 1.3 Hz, H-2′a), 5.67 (1H, dd, J = 11.1, 1.3 Hz, H-2′b); ($CDCl_3$) 8.9 (1H, br s, H-9); ^{13}C nmr (CD_3OD) 147.15 (C-8), 141.23 (C-1), 138.48 (C-3), 135.24 (C-8b), 133.71 (C-8a, C-1′), 132.27 (C-4a), 124.12 (C-4b), 122.27 (C-6), 119.65 (C-2′), 115.66 (C-4), 114.47 (C-7), 112.71 (C-5); nOe's [H-4]–H-3, H-5; [H-5]–H-4, H-6; [H-6]–H-5; [H-1′]–H-2′b; XCORFE C-1′–H-1′, H-2′a, H-2′b;

C-1′–H-2′a; C-3–H-4; C-4–H-3; C-4a–H-3, H-5, H-6, H-1′; C-5–H-7; C-7–H-5; C-8–H-7; C-8a–H-5, H-7; C-8b–H-4; λ max (MeOH) 369, 296, 244, 230 nm (log ε 3.93, 3.97, 4.09, 4.06), λ max (NaOH/MeOH) 386, 287, 262, 240 nm (log ε 3.82, 4.03, 4.06, 4.07); ν max (smear) 3590 br, 3140, 2990, 1630, 1570, 1510, 1480, 1430, 1280, 1230, 780 cm^{-1}.

1-ETHYL-4-METHYLSULFONE-β-CARBOLINE [**2**].—Fractions from the flash reversed-phase columns on the extract were shown to contain a component fluorescent under uv light at $R_f = 0.9$ on tlc [Si gel, EtOAc-Me_2CO (1:1)]. Reversed-phase hplc of these fractions, with flow rate 5 ml/min, MeOH-H_2O (2:3), and uv detection at 220 nm, led to the isolation of 4 mg of **2** as a pale green oil: ms *m/z* found $[M]^+$ 274.0777 (100) ($C_{14}H_{14}N_2O_2S$ requires 274.0776), 246.0470 (12) ($C_{12}H_{10}N_2O_2S$ requires 246.0463), 195.0931 (13) ($C_{13}H_{11}N_2$ requires 195.0922), 168.0734 ($C_{11}H_8N_2$ requires 168.0687); ^{1}H nmr ($CDCl_3$) 8.98 (1H, s, H-3), 8.78 (1H, d, $J = 8.0$ Hz, H-5), 8.7 (1H, br s, H-9), 7.61 (2H, m, H-7, H-8), 7.38 (1H, m, H-6), 3.27 (3H, s, H-4′), 3.22 (2H, q, $J = 7.6$ Hz, H-1′), 1.50 (3H, t, $J = 7.6$ Hz, H-2′); ^{13}C nmr ($CDCl_3$) 152.70 (C-1), 140.57 (C-8a), 139.88 (C-3), 133.71 (C-8b), 129.77 (C-7), 126.67 (C-4b), 125.86 (C-5), 121.67 (C-6), 119.21 (C-4), 111.87 (C-8), 43.24 (C-4′), 27.54 (C-1′), 12.22 (C-2′); λ max (MeOH) 350, 304, 299, 258, 250, 213 nm (log ε 2.62, 3.03, 2.94, 3.32, 3.29, 3.43); ν max (smear) 3390, 2990, 1650, 1600, 1570, 1485, 1430, 1335, 1160, 1010, 785 cm^{-1}.

ISOLATION OF HARMAN [**3**].—A fraction (21 mg) from the second reversed-phase chromatography column on the extract was subjected to further chromatography on C18 reversed-phase material using an H_2O to MeOH gradient to yield 15 fractions. Tlc analysis revealed a spot fluorescent under uv light was present in several fractions, and fractions 9 and 10 from the column were essentially pure by ^{1}H-nmr spectroscopy in $CDCl_3$. These were combined to give a yellow oil (6 mg), the ^{1}H nmr, ^{13}C nmr, and ms of which were identical with literature values for harman (17,22).

IDENTIFICATION OF 1-ETHYL-β-CARBOLINE [**4**].—Some of the column fractions containing harman by tlc analysis contained another spot at higher R_f on Si gel, also fluorescent under uv light. The ^{1}H-nmr spectra of these fractions in $CDCl_3$ showed no signals in addition to those of harman, apart from a mutually coupled quartet and triplet ascribed to an ethyl group. This component had the same R_f value on Si gel as a synthetic sample of 1-ethyl-β-carboline, and the eims was identical to that reported in the literature for 1-ethyl-β-carboline (23).

ISOLATION OF PAVETTINE [**5**].—Signals characteristic of a vinyl group as in compound **1** were seen in the ^{1}H-nmr spectrum in $CDCl_3$ of one column fraction (2 mg) which also contained harman and 1-ethyl-β-carboline. The sample was dissolved in $CHCl_3$ and streaked along the baseline of a Si gel tlc plate (6 × 10 cm). The plate was developed in EtOAc-Me_2CO (1:1) and visualized under uv light. The uv-active band at highest R_f was scraped from the plate, and the Si gel was eluted with EtOAc-Me_2CO and filtered to yield <1 mg of a yellow-green oil. This was identified as pavettine from its ^{1}H nmr and eims (24) and by tlc comparison with an authentic sample.

HOMARINE.—Fraction 2 from the initial reversed-phase chromatography column on the crude extract contained a pale lemon precipitate (117 mg), which was identified as homarine by ^{13}C-nmr spectroscopy (25).

STEROLS.—Fraction 8 from the initial reversed-phase chromatography column on the extract (141 mg) was further partitioned by cc on Si gel, using a petroleum ether to EtOAc gradient to yield 10 fractions. Fraction 5 from the column (19 mg) was determined to contain cholest-4-en-3-one as a major component by ^{13}C-nmr spectroscopy (16). Fraction 7 from the column (82 mg) was determined to contain predominantly cholesterol by ^{13}C-nmr spectroscopy (15), while fraction 6 (25 mg) was a mixture of sterols. Fractions 5–7 were combined (127 mg) and analyzed by gc-ms to determine the sterol composition. Twelve sterols were identified, of which cholesterol was the most abundant (28%), followed by 24-methylcholesta-5,22-dien-3β-ol (18%), cholestanol (13%), cholest-4-en-3-one (10%), cholesta-5,22-dien-3β-ol (9%), 24-ethylcholesterol (6%), 24-methylenecholesterol (4%), 24-ethylcholesta-5,22-dien-3β-ol (4%), and 24-methyl-26,27-bisnorcholesta-5,22-dien-3β-ol (3%). The remaining 5% of the sterol mixture could not be identified.

1-VINYL-8-METHOXY-β-CARBOLINE [**6**].—1-Vinyl-8-hydroxy-β-carboline [**1**] (15 mg) was dissolved in MeOH (1 ml), and CH_2N_2 in Et_2O was added to the sample, which was then left for 3 h. After this time, tlc and ^{1}H-nmr spectroscopic analysis showed the formation of a complex mixture with 1-vinyl-8-methoxy-β-carboline [**6**] as a major component. This was purified by reversed-phase cc to yield 4 mg of a yellow-green oil: ms *m/z* found $[M]^+$ 224.0949 (76) ($C_{14}H_{12}N_2O$ requires 224.0949), 223.0848 (35) ($C_{14}H_{11}N_2O$ requires 223.0872), 209.0729 (45) ($C_{13}H_9N_2O$ requires 209.0715), 194.0832 (13) ($C_{13}H_{10}N_2O$ requires 194.0844); ^{1}H nmr ($CDCl_3$) 8.5 (1H, br s, H-9), 8.44 (1H, d, $J = 5.2$ Hz, H-3), 7.84 (1H, d, $J = 5.2$ Hz, H-4), 7.70 (1H, d, $J = 7.9$ Hz, H-5), 7.22 (1H, t, $J = 7.9$ Hz, H-6), 7.22 (1H,

dd, J = 17.5, 11.1 Hz, H-1'), 7.00 (1H, d, J = 7.9 Hz, H-7), 6.41 (1H, dd, J = 17.5, 1.4 Hz, H-2'a), 5.71 (1H, dd, J = 11.1, 1.4 Hz, H-2'b), 4.03 (3H, s, H-8'); ^{13}C nmr ($CDCl_3$) 146.20 (C-8), 140.12 (C-1), 139.19 (C-3), 133.24 (C-8b), 132.98 (C-1'), 130.85 (C-8a), 130.11 (C-4a), 122.77 (C-4b), 120.78 (C-6), 119.09 (C-2'), 114.32 (C-4), 113.86 (C-5), 107.95 (C-7), 55.62 (C-8'); λ max (MeOH) 363, 285, 267, 249, 220 nm (log ε 3.23, 3.43, 3.69, 3.93, 3.96); ν max (smear) 3550, 3050, 2925, 1625, 1575, 1420, 1310, 1260, 1240, 1050 cm^{-1}.

1-ETHYL-8-HYDROXY-β-CARBOLINE [**7**].—1-Vinyl-8-hydroxy-β-carboline [**1**] (4 mg) in MeOH (1 ml) was stirred with PtO_2 (0.5 mg) under H_2 until absorption of H_2 had ceased. After filtration of the solution through a bed of celite in MeOH to remove the catalyst, 1-ethyl-8-hydroxy-β-carboline [**7**] (4 mg) was isolated as a pale yellow oil; ms *m/z* found $[M]^+$ 212.0948 (100) ($C_{13}H_{12}N_2O$ requires 212.0950), 211.0865 (99) ($C_{13}H_{11}N_2O$ requires 211.0871), 184.0647 (17) ($C_{11}H_8N_2O$ requires 184.0636); ^{1}H nmr (CD_3OD) 8.14 (1H, d, J = 5.5 Hz, H-3), 7.85 (1H, d, J = 5.5 Hz, H-4), 7.61 (1H, d, J = 7.8 Hz, H-5), 7.06 (1H, t, J = 7.8 Hz, H-6), 6.94 (1H, d, J = 7.8 Hz, H-7), 3.19 (2H, q, J = 7.6 Hz, H-1'), 1.41 (3H, t, J = 7.6 Hz, H-2'); ^{13}C nmr (CD_3OD) 149.11 (C-1), 145.69 (C-8), 138.06 (C-3), 135.64 (C-8b), 132.97 (C-8a), 130.93 (C-4a), 124.65 (C-4b), 121.78 (C-6), 114.48 (C-4), 113.70 (C-5), 113.46 (C-7), 28.13 (C-1'), 14.05 (C-2'); λ max (MeOH) 341, 287, 278, 243, 219 nm (log ε 2.98, 3.25, 3.20, 3.88, 3.52); λ max (NaOH/MeOH) 358, 288, 279, 253, 226 nm (log ε 2.91, 3.18, 3.26, 3.77, 3.64); ν max (smear) 3390 br, 3005, 2950, 1640, 1590, 1490, 1440, 1385, 1245, 1230 cm^{-1}.

1-VINYL-8-ACETOXY-β-CARBOLINE [**8**].—1-Vinyl-8-hydroxy-β-carboline [**1**] (5 mg) was dissolved in pyridine-Ac_2O (1:1) (1 ml) and left stirring overnight. Solvent was removed, and 2 ml of H_2O was added. Extraction with EtOAc (3 × 1 ml) led to the isolation of 3 mg of **8** as a yellow oil: ms *m/z* found $[M]^+$ 252.0895 (35) ($C_{15}H_{12}N_2O_2$ requires 252.0899), 210.0806 (100) ($C_{13}H_{10}N_2O$ requires 210.0794), 195.0910 (17) ($C_{13}H_{11}N_2$ requires 195.0923), 168.0655 (14) ($C_{11}H_8N_2$ requires 168.0688); ^{1}H nmr (CD_3OD) 8.30 (1H, d, J = 5.3 Hz, H-3), 8.07 (1H, dd, J = 7.5, 1.8 Hz, H-5), 8.01 (1H, d, J = 5.3 Hz, H-4), 7.47 (1H, dd, J = 17.2, 11.0 Hz, H-1'), 7.31 (1H, dd, J = 7.5, 1.8 Hz, H-2'a), 5.66 (1H, dd, J = 11.0, 1.8 Hz, H-2'b), 2.45 (3H, s, H-8'), ($CDCl_3$) 8.5 (1H, br s, H-9); ^{13}C nmr (CD_3OD) 164.28 (C-8'a), 147.09 (C-8), 141.56 (C-1), 139.48 (C-3), 139.04 (C-8a), 136.45 (C-8b), 132.64 (C-1'), 132.48 (C-4a), 122.54 (C-7), 122.40 (C-4b), 121.45 (C-5), 120.63 (C-6), 120.08 (C-2'), 115.82 (C-4), 21.26 (C-8'b); λ max (MeOH) 349, 298 sh, 287, 272, 244, 221 nm (log ε 2.79, 2.91, 3.13, 3.19, 3.57, 3.58), λ max (NaOH-MeOH) 373, 288, 254, 237, 233 nm (log ε 2.61, 3.18, 3.45, 3.54, 3.54); ν max (smear) 3370, 2950, 1740, 1630, 1590, 1440, 1270, 1240, 1210, 1105, 1040, 800 cm^{-1}.

1-ETHYL-8-METHOXY-β-CARBOLINE [**9**].—1-Vinyl-8-methoxy-β-carboline [**6**] (2 mg) in MeOH (1 ml), was stirred with PtO_2 (0.5 mg) under H_2 for 30 min. Tlc analysis on Si gel [EtOAc-Me_2CO (1:1), fluorescent spot under uv light] indicated that a single product of lower R_f had formed. After filtration of the solution through a bed of celite in MeOH to remove the catalyst, 1-ethyl-8-methoxy-β-carboline [**9**] was isolated as a pale yellow oil, the ^{1}H nmr and ms of which were consistent with literature values (26).

SYNTHESES OF β-CARBOLINE ALKALOIDS: GENERAL SYNTHETIC METHOD.—In a typical preparation L-tryptophan (500 mg) was dissolved in H_2O (125 ml) to which concentrated H_2SO_4 (0.5 ml) had been added. Freshly distilled aldehyde (1–2 ml) was added, and the solution refluxed for 10 min. After the reflux period a 10% K_2CrO_7 solution (25 ml) was slowly added. Following a reaction period of 5 min the solution was reheated to boiling and allowed to cool before SO_2 gas was bubbled into the solution to destroy the excess oxidizing agent. Alternatively, Na_2SO_3 was added rather than using SO_2. In each case the solution was finally made basic by addition of solid Na_2CO_3 and extracted with CH_2Cl_2 (4 × 75 ml). The organic partition was dried over Na_2SO_4 and filtered, and the solvent was removed. The resulting crude product was purified by reversed-phase flash cc on C18 material using an H_2O to MeOH gradient. Column fractions were analyzed by tlc and ^{1}H nmr spectroscopy. Those fractions that contained the pure β-carboline alkaloids were combined and characterized (24,27).

NORHARMAN [**10**].—Yield 7%. Recrystallized as colorless needles from petroleum ether/EtOAc, mp 198° [lit. (28) 199–201°]. The ^{1}H-nmr (29), ^{13}C-nmr (17), and uv (30) spectra were consistent with literature values.

1-ETHYL-β-CARBOLINE [**4**].—Yield 12%. Recrystallized from $CHCl_3$/MeOH to yield clear, cubic crystals, mp 194° [lit. (31) 194–195°]. ^{1}H nmr (23), ^{13}C nmr (17), uv (23), and ms (23) were consistent with literature values.

1-PROPYL-β-CARBOLINE [**11**].—Yield 15%. The uv (32), ir, and ms (33) were consistent with literature data.

1-ISOPROPYL-β-CARBOLINE [**12**].—Yield 9%. The ir and ms were consistent with literature data (33).

1-HEXYL-β-CARBOLINE [**13**] AND 1-PHENYL-β-CARBOLINE [**14**].—A modification of the general procedure was used: L-tryptophan (500 mg) was dissolved in H_2O (30 ml) to which concentrated H_2SO_4 (0.1 ml) had been added. The freshly distilled aldehyde (1–2 ml) was added, and the solutions were refluxed overnight for 1-hexyl-β-carboline and 4 h for 1-phenyl-β-carboline. The remainder of the preparation procedure was carried out as above.

1-HEXYL-β-CARBOLINE [**13**].—Yield 1% of a clear oil. The uv and ms were consistent with literature data (32).

1-PHENYL-β-CARBOLINE [**14**].—Yield 19%. Uv and ir spectra were consistent with literature values (30).

1-METHYL-1,2,3,4-TETRAHYDRO-β-CARBOLINE-3-CARBOXYLIC ACID [**15**].—L-Tryptophan (300 mg) was dissolved in hot H_2O (10 ml), the solution cooled, and freshly distilled acetaldehyde (1 ml) added with stirring. The stirring was continued overnight at room temperature. A thick white precipitate formed, and removal of solvent under reduced pressure yielded the crude product (350 mg), which was purified by reversed-phase flash cc on C18 material to give 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid [**15**] (198 mg) as a white powder (34). Yield 51%. The ms was consistent with literature data (35).

CONVERSION OF **15** TO HARMAN [**3**].—1-Methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid [**15**] (145 mg) was dissolved in H_2O (40 ml) and heated to reflux. A 10% solution of K_2CrO_7 (7.5 ml) and HOAc (1.5 ml) was added, and refluxing continued for 1 min, followed by cooling. Na_2SO_3 solution (1 M, 100 ml) was slowly added, and the solution was then made basic by addition of solid Na_2CO_3 and extracted with CH_2Cl2 (3 × 75 ml) followed by EtOAc (3 × 75 ml). The organic solvents were combined, dried over Na_2SO_4, filtered, and the solvent removed to give semicrystalline harman [**3**] (69 mg), which was purified by reversed-phase cc. Yield 60%. The ^1H nmr, ^{13}C nmr (17), and ms (22) of the synthetic product were consistent with literature values.

METHYL 1-METHYL-1,2,3,4-TETRAHYDRO-β-CARBOLINE-3-CARBOXYLATE [**16**].—A sample of compound **15** (20 mg) was dissolved in the minimum amount of EtOH, and an excess of CH_2N_2 in Et_2O added. The resulting product was purified by flash cc on C18 reversed-phase material, using an H_2O to MeOH gradient, to yield methyl 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylate [**16**] (15 mg) as a white powder. The ms was consistent with literature data (35).

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