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β-CARBOLINES FROM THE BLUE-GREEN ALGA *DICHOTHRIX BAUERIANA*

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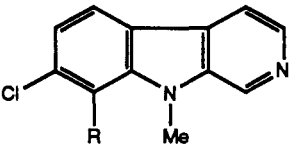
ABSTRACT.—Three new chlorine-containing β-carbolines, bauerines A–C (**1–3**), have been isolated from the terrestrial blue-green alga *Dichothrix baueriana* GO-25-2, and identified by mass and nmr spectral analysis. The alkaloids show activity against herpes simplex virus type 2.

Recently we reported preliminary results of screening lipophilic and hydrophilic extracts of over 500 strains of cultured cyanophytes for in vitro activity against three pathogenic viruses (1). Of the 529 strains that were evaluated for activity against herpes simplex virus type 2 (HSV-2), extracts of 10.2% of these blue-green algae were found to exhibit at least 25% less viral cytopathic effect (CPE) in a primary screen and 90% or more plaque reduction at a dose less than or equal to one-tenth the cytotoxic dose for uninfected host cells (mink lung) in a confirmation assay. In all cases, activity was associated with the lipophilic extract and only in a few instances was it found in the hydrophilic extract.

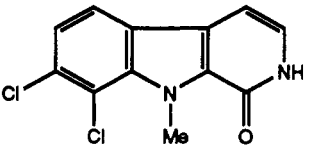
The lipophilic extract of *D. baueriana* (Grun.) Bornet & Flahault (UH isolate GO-25-2) was found to significantly inhibit CPE and reduce the number of plaques formed in infected mink lung cells by 97% at 33 μg/ml. The antiviral activity was concluded to be marginal, however, since this extract exhibited comparable cytotoxicity towards tumor cell lines such as LoVo (MIC 33 μg/ml) (2). In the diagnostic Corbett and Valeriote assays, the cytotoxicity of this extract was neither solid tumor-selective nor tumor-selective (3,4).

Successive bioassay-directed, reversed-phase chromatography and gel filtration of the extract yielded two fractions which possessed both anti-herpes activity and LoVo cytotoxicity. Hplc of the faster-moving fraction from the gel filtration column afforded two active compounds, 7-chloro-9-methyl-β-carboline (bauerine A, [**1**]) and 7,8-dichloro-9-methyl-β-carboline (bauerine B, [**2**]). The slower-moving fraction yielded a third active compound, 7,8-dichloro-1-hydroxy-9-methyl-β-carboline (bauerine C, [**3**]).

The structures of the bauerines were determined in a straightforward manner by spectral analysis. The uv spectra, which were similar to that of harman, indicated that the bauerines were β-carbolines, i.e., 9*H*-pyrido[3,4-*b*]indoles. The eims established the molecular formulas and showed the presence of one chlorine in **1** and two chlorines in **2** and **3**. Nmr analysis indicated that the chlorine was on the benzenoid ring and that a methyl group was on N-9 in all three compounds. Strong nOe signals were observed between the *N*-methyl protons and the H-1/H-8 protons. In the case of **1**, the nOe between the *N*-methyl protons and H-8 indicated that the chlorine substituent was on C-7. For **2** and **3**, an nOe could not be seen



1 R=H
2 R=Cl



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between the *N*-methyl protons and a benzenoid proton and doublets coupled by 8.5 Hz were present for two adjacent protons on the benzenoid ring (J=8.5 Hz), showing that the two chlorines were on C-7 and C-8. In the HMBC spectra, the signal for the *N*-methyl protons showed cross-peaks (couplings) with two non-protonated carbon signals. For example, these occurred at 143.3 and 138.3 ppm for **1**, and were assignable to C-8a and C-9a, respectively, since HMBC cross-peaks were also observed between the H-5 and C-8a signals and between the H-4 and C-9a signals.

Antiviral effects have been noted before with simple β-carbolines such as harmine and other harman derivatives, which inactivate murine cytomegalovirus and Sindbis virus in the presence of long wavelength uv radiation (5). Also, the eudistomins, compounds found in the marine tunicate *Eudistoma olivaceum*, exhibit moderate to potent activity against herpes simplex virus type 1 (6,7).

EXPERIMENTAL

SPECTRAL ANALYSIS.—Nmr spectra were determined on a 11.75 tesla instrument operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C. ^{1}H-Nmr chemical shifts are referenced in DMSO-d_6 to residual DMSO-d_5 (2.49 ppm) and in Me_2CO-d_6 to residual Me_2CO-d_5 (2.05 ppm); ^{13}C-nmr chemical shifts are referenced in DMSO-d_6 and Me_2CO-d_6 to the solvent (39.5 and 206.0/29.8 ppm, respectively). ^{1}H- and ^{13}C-nmr chemical shifts have been assigned on the basis of HMQC and HMBC experiments (8,9).

CULTURE CONDITIONS.—*Dichothrix baueriana* (Grun.) Bornet & Flahault, designated strain GO-25-2, was isolated from a soil sample collected in Hanakoa Valley on the Na Pali coast of Kauai, Hawaii, on 20 April 1988. Clonal cultures were prepared by repeated subculture on solidified media. The alga was cultured in 20-liter glass bottles containing a modification of inorganic medium BG-11 (1). Prior to autoclaving, the pH of the medium was adjusted to 7.0 with NaOH. Cultures were illuminated continuously at an incident intensity of 25 μmol photons PAR (photosynthetically active radiation) m^{-2} s^{-1} from banks of cool-white fluorescent tubes, aerated at a rate of 5 liters/min with a mixture of 0.5% CO_2 in air, and incubated at a temperature of 24±1°. After 28 days the alga was harvested by filtration and freeze-dried. Yields of lyophilized cells ranged from 0.4 to 0.5 g/liter.

ISOLATION OF BAUERINES A, B, AND C.—Freeze-dried alga (120.5 g) was extracted with 4×5 liter portions of EtOH-H_2O (7:3). The total extract (15.8 g) was subjected to step-gradient, reversed-phase flash chromatography on a C-18 column (140 g, YMC Gel ODS-A, 12 nm particle size) in batches of approximately 3 g each. For each batch, elution was carried out with successive 200-ml portions of the following solvents: MeOH-H_2O (2:8), MeOH-H_2O (1:1), MeOH-H_2O (7:3), MeOH-H_2O (9:1), MeOH, and EtOAc. Six fractions, each amounting to 5×200 ml in volume, were collected.

The material passing through the ODS column with MeOH-H_2O (9:1) (D, 289.5 mg) and MeOH (E, 982 mg) was separated further by gel filtration on a Sephadex LH-20 column (44.5 ×330.2 mm) using MeOH as the eluent. Fifty-four 20 ml fractions were collected from fractions D and E and analyzed by tlc on silica-254 with Me_2CO-EtOAc (1:1). Fractions 20–24 were combined and evaporated to give 38.7 and 20.8 mg of solid residues from D and E, respectively. Reversed-phase hplc of these solid residues over a 10×250 mm C-18 column (Alltech Econosil ODS, 10 μm, 2 ml/min flow rate) using MeOH-H_2O (17:3) led to totals of 13.2 mg of bauerine A (R_t 19 min, 7-chloro-9-methyl-β-carboline, [**1**]) and 16.8 mg of bauerine B (R_t 33 min, 7,8-dichloro-9-methyl-β-carboline, [**2**]). Fractions 26–30 were combined to give 11.5 and 6.2 mg of solid from D and E, respectively, which after further purification on the same ODS column with MeOH-H_2O (4:1) gave a total of 4.7 mg of bauerine C (R_t 29 min, 7,8-dichloro-1-hydroxy-9-methyl-β-carboline, [**3**]).

BAUERINE A [**1**].—Final purification of **1** was achieved by successive sublimation at 120–145° (0.01 mm) and crystallization from hexane/Me_2CO to give colorless needles, mp 109–110°; eims *m/z* 214 (7), 215 (89, loss of proton from *N*-methyl group), 216 (100, M^+), 217 (79, M^+ protonated on N-2), 218 (76), 219 (15); hreims *m/z* 216.0460 (calcd for $C_{12}H_9ClN_2$, −0.5 mmu); uv (EtOH) λ max (ε) 240 (28,700), 260 sh (9,600), 286 sh (6,790), 294 (11,200), 330 sh (1,500), 343 (2,700), 357 (3,300) nm; ^{13}C nmr (Me_2CO-d_6) δ in ppm 143.3 (s, C-8a), 140.2 (d, C-3), 138.3 (s, C-9a), 134.4 (s, C-7), 133.6 (d, C-1), 128.0 (s, C-4a), 123.8 (d, C-5), 120.7 (d, C-6), 120.6 (s, C-4b), 115.0 (d, C-4), 110.6 (d, C-8), 29.6 (q, NMe); ^{1}H nmr (Me_2CO-d_6) δ 9.02 (s, H-1), 8.43 (br d, J=4.8 Hz, H-3), 8.25 (d, J=8.5 Hz, H-5), 8.07 (d, J=4.8 Hz, H-4), 7.72 (d, J=1.6 Hz, H-8), 7.28 (dd, J=8.5 and 1.6 Hz, H-6), 4.04 (s, NMe). Antiviral activity: HSV-2 IC_{90} 2 μg/ml; cytotoxicity: LoVo IC_{50} 3 μg/ml.

BAUERINE B [**2**].—Final purification of **2** was achieved by successive sublimation at 120–145° (0.01 mm) and crystallization from MeOH/hexane to give a white solid, mp 163–164°; hr eims *m/z* 250.0078 (calcd for $C_{12}H_8Cl_2N_2$, −1.3 mmu); uv (EtOH) λ max (ε) 222 sh (18,500), 246 (38,500), 281 (8,200), 292 (12,300), 330 sh (2,500), 345 (4,500), 359 (5,500) nm; ^{13}C nmr (Me_2CO-d_6) δ in ppm 140.8 (d, C-3), 139.2 (s, C-9a), 138.8 (s, C-8a), 134.2 (d, C-1), 133.5 (s, C-7), 127.8 (s, C-4a), 123.7 (s, C-4b), 122.5 (d, C-6), 121.9 (d, C-5), 116.2 (s, C-8), 114.8 (d, C-4), 33.2 (q, NMe); ^1H nmr (Me_2CO-d_6) δ 9.04 (s, H-1), 8.46 (d, J=5.4 Hz, H-3), 8.18 (d, J=8.3 Hz, H-5), 8.05 (dd, J=5.4 and 1.0 Hz, H-4), 7.42 (d, J=8.3 Hz, H-6), 4.38 (s, NMe). Antiviral activity: HSV-2 IC_{90} 3 μg/ml; cytotoxicity: LoVo IC_{50} 5 μg/ml.

BAUERINE C [**3**].—Final purification of **3** was achieved by successive sublimation at 120–145° (0.01 mm) and crystallization from EtOH to give a white solid, mp >220° (dec); hreims *m/z* 266.0016 (calcd for $C_{12}H_8Cl_2N_2O$, −0.2 mmu); uv (EtOH) λ max (ε) 250 (43,900), 261 sh (23,300), 290 (4,370), 301 (2,740), 334 (4,040), 347 (6,000), 362 (4,630) nm; ^{13}C nmr (DMSO-d_5) δ in ppm 156.0 (s, C-1), 136.3 (s, C-8a), 130.6 (s, C-7), 128.0 (s, C-9a), 126.3 (d, C-3), 124.9 (s, C-4a), 122.9 (s, C-4b), 121.8 (d, C-6), 121.3 (d, C-5), 115.1 (s, C-8), 99.0 (d, C-4), 34.1 (q, NMe); ^1H nmr (DMSO-d_5) δ 11.61 (br s, H-N2), 8.09 (d, J=8.8 Hz, H-5), 7.44 (d, J=8.8 Hz, H-6), 7.15 (d, J=6.7 Hz, H-3), 7.02 (d, J=6.7 Hz, H-4), 4.63 (s, NMe). Antiviral activity: HSV-2 IC_{90} 2.5 μg/ml; cytotoxicity: LoVo IC_{50} 30 ng/ml.

ANTIVIRAL ASSAYS.—Clinical isolates of HSV-2 were obtained from Dr. Nyven Marchette, Department of Tropical Medicine, John A. Burns School of Medicine, University of Hawaii. Viral stock cultures were prepared and samples (extracts, fractions, pure compounds) were evaluated for antiviral activity as previously described (1).

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