# DNA-Damaging Steroidal Alkaloids from *Eclipta alba* from the Suriname Rainforest[1]

Maged S. Abdel-Kader,[†] Brian D. Bahler,[†] Stan Malone,[‡] Marga C. M. Werkhoven,[§] Frits van Troon,[⊥] David,[⊥,∥] Jan H. Wisse,[▽] Isia Bursuker,[○] Kim M. Neddermann,[○] Stephen W. Mamber,[○] and David G. I. Kingston*[,†]

*Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061-0212, Stichting Conservation International Suriname, Gravenstraat 17, Paramaribo, Suriname, The National Herbarium of Suriname, P.O. Box 9212, Paramaribo, Suriname, Saramaka Village, Brownsweg, Kadju, Suriname, Bedrijf Geneesmiddelen Voorziening Suriname, Commissaris Roblesweg, Geyersvlijt, Suriname, and Bristol-Myers Squibb, Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, Connecticut 06492-7660*

*Received December 16, 1997*

Bioassay-guided fractionation of the MeOH extract of *Eclipta alba* using three yeast strains (1138, 1140, and 1353) resulted in the isolation of eight bioactive steroidal alkaloids (**1**−**8**), six of which are reported for the first time from nature. The major alkaloid was identified as (20*S*)(25*S*)-22,26-imino-cholesta-5,22(*N*)-dien-3$\beta$-ol (verazine, **3**), while the new alkaloids were identified as 20-*epi*-3-dehydroxy-3-oxo-5,6-dihydro-4,5-dehydroverazine (**1**), ecliptalbine [(20*R*)-20-pyridyl-cholesta-5-ene-3$\beta$,23-diol] (**4**), (20*R*)-4$\beta$-hydroxyverazine (**5**), 4$\beta$-hydroxyverazine (**6**), (20*R*)-25$\beta$-hydroxyverazine (**7**), and 25$\beta$-hydroxyverazine (**8**). Ecliptalbine (**4**), in which the 22,26-imino ring of verazine was replaced by a 3-hydroxypyridine moiety, had comparable bioactivity to verazine in these assays, while a second alkaloid (**8**) showed good activity against *Candida albicans*. All the alkaloids showed weak cytotoxicity against the M-109 cell line.

In continuation of our search for anticancer and other bioactive agents from the Suriname rainforest,[2,3] an extract of the leaves and bark of *Eclipta alba* (L.) Haask. (Asteraceae) was found to show reproducible activity in a mechanism-based bioassay utilizing genetically engineered mutants of the yeast *Saccharomyces cerevisiae*.[4] This plant is known as "totobia" in the Saramacca language and "luisawiwiri" in Sranan Tongo; it is used in Surinamese traditional medicine, and its collection was made as part of an ethnobotanical collection strategy.[5] *E. alba* is also used in Indian traditional medicine for the treatment of leukoderma, night blindness, and catarrhal jaundice; as an emetic, a purgative, and an antiseptic; and to treat hepatic and spleen enlargements. It is reported to improve hair growth and color.[6]

## Results and Discussion

A sample of the leaves of *E. alba* was subjected to bioassay at Virginia Tech and found to be active against the three yeast strains *S. cerevisiae* 1138, 1140, and 1353. Activities in these assays are recorded as $IC_{12}$ values, which are the concentrations (in $\mu$g/mL) required to give an inhibition zone 12 mm in diameter around a 100-$\mu$L well in a 4-mm agar layer plated with the yeast strain in question. Topoisomerase 1 inhibitors show inhibition of strains 1138 and 1140 only, topoisomerase 2 inhibitors show activity against strain 1138 only, while general DNA-damaging agents and antifungal agents show activity against all three strains. A methanol extract of *E. alba* leaves gave $IC_{12}$ values of 16, 26, and 9 $\mu$g/mL against the strains 1138, 1140, and 1353, respectively, and this extract was thus selected for fractionation studies as a potential DNA-damaging agent or as an antifungal agent. Fractionation was carried out by a combination of liquid−liquid partition and chromatography as described in the Experimental Section and yielded the eight bioactive compounds, **1**−**8**.

The major active compound was identified as verazine (**3**) [(20*S*,25*S*)-22,26-iminocholesta-5,22(*N*)-dien-3$\beta$-ol] by comparing its spectral data (see Experimental Section and Table 2) with the literature data.[7] Among flowering plants, the occurrence of verazine is restricted to *Veratrum* species in the family Liliaceae,[8] although it has also been isolated from the fern *Zygadenus sibiricus*.[9] This is the first report of the isolation of verazine from the Asteraceae. The (20*R*) epimer of verazine (**2**) was isolated as a minor component and identified by comparing its spectral data (see Experimental Section and Table 2) with the literature data.[7]

Reduction of verazine (**3**) with $NaBH_4$ resulted in the formation of veramiline (**9**), identified by comparing its spectral data (see Experimental Section) with the literature data.[10] Its $^{13}C$ NMR spectrum and assignments (Table 2) are reported for the first time.

Compound **1** was assigned the structure shown based on a comparison of its $^{13}C$ NMR chemical shifts with those of 3- and 6-oxo steroids[11,12] and those of verazine (**3**), as well as on the basis of the observation of HMBC correlations from H-4 to C-2, C-6, and C10 at $\delta_C$ 34.0, 32.9, and 38.6, respectively. The $^1H$ NMR chemical shift of $CH_3$-21 at $\delta_H$ 0.97 ppm indicated that **1** is the 20(*R*) epimer. The 20(*S*) epimer of compound **1** has previously been prepared from **3**,[8a] but it has not previously been isolated from a natural source. The absolute stereochemistry of **1** and of the other alkaloids described below is assigned on the basis of their assumed relationships to verazine (**3**).

Compound **4** had a composition of $C_{27}H_{39}NO_2$ as shown by HREIMS. The $^1H$ and $^{13}C$ NMR chemical shifts of its steroidal skeleton (Tables 1 and 2) showed a close similarity to those of **2** and **3**, indicating it to be a member of the same class of compounds. In contrast to the tetrahydro-

* To whom enquiries should be addressed: Tel.: (540) 231-6570. Fax: (540) 231-7702. E-mail: dkingston@vt.edu.
[†] Virginia Polytechnic Institute and State University.
[‡] Conservation International Suriname.
[§] The National Herbarium of Suriname.
[⊥] Saramaka Village.
[∥] Deceased April 11, 1994.
[▽] Bedrijf Geneesmiddelen Voorziening Suriname.
[○] Bristol-Myers Squibb, Pharmaceutical Research Institute.

10.1021/np970561c CCC: $15.00 
Published on Web 09/25/1998

**Table 1.** Selected $^1$H NMR Data ($\delta$ values) for Compounds **1**−**9**[a]

| position | **1** | **2** | **3** | **4** | **5** | **5a** |
|---|---|---|---|---|---|---|
| 3 | | 3.48 (m) | 3.05 (m) | 3.38 (m) | 3.52 (dt, $J$ = 3.3, 12.2 Hz) | 4.73 (dt, $J$ = 3.3, 12.2 Hz) |
| 4 | 5.71 (br s) | | | | 4.09 (d, $J$ = 2.8 Hz) | 5.49 (d, $J$ = 2.7 Hz) |
| 6 | | 5.33 (br d, $J$ = 5.3 Hz) | 5.32 (br d, $J$ = 5.0 Hz) | 5.22 (br d, $J$ = 4.9 Hz) | 5.62 (br d, $J$ = 3.7 Hz) | 5.80 (br d, $J$ = 3.6 Hz) |
| 18 | 0.74 (s) | 0.70 (s) | 0.69 (s) | 0.73 (s) | 0.69 (s) | 0.61 (s) |
| 19 | 1.16 (s) | 0.98 (s) | 0.99 (s) | 0.92 (s) | 1.15 (s) | 1.11 (s) |
| 21 | 0.97 (d, $J$ = 6.9 Hz) | 0.97 (d, $J$ = 7.8 Hz) | 1.07 (d, $J$ = 6.7 Hz) | 1.14 (d, $J$ = 6.6) | 0.96 (d, $J$ = 6.9 Hz) | 1.14 (d, $J$ = 6.9 Hz) |
| 22 | | | | | | |
| 23 | | | | | | 5.11 (t, $J$ = 3.4) |
| 24 | | | | 6.79 (br s) | | |
| 26ax | 2.92 (m) | 2.93 (m) | 2.96 (m) | 7.71 (br s) | 2.92 (m) | |
| 26eq | 3.69 (m) | 3.69 (m) | 3.65 (dd, $J$ = 6.7, 16.6 Hz) | | 3.68 (m) | |
| 27 | 0.91 (d, $J$ = 6.6 Hz) | 0.90 (d, $J$ = 6.6 Hz) | 0.88 (d, $J$ = 6.7 Hz) | 2.12 (s) | 0.90 (d, $J$ = 6.6 Hz) | 0.96 (d, $J$ = 6.6 Hz) |
| $CH_3CO$ | | | | | | 2.00 (s), 2.06 (s), 2.15 (s) |

| position | **6** | **6a** | **7** | **7a** | **8** | **8a** | **9** |
|---|---|---|---|---|---|---|---|
| 3 | 3.55 (dt, $J$ = 3.6, 11.6 Hz) | 4.74 (dt, $J$ = 3.6, 11.6 Hz) | 3.34 (m) | 4.61 (m) | 3.34 (m) | 4.62 (m) | 3.52 (m) |
| 4 | 4.11 (d, $J$ = 3.2 Hz) | 5.49 (d, $J$ = 2.8 Hz) | | | | | |
| 6 | 5.65 (br d, $J$ = 3.5 Hz) | 5.80 (m) | 5.20 (d, $J$ = 4.9 Hz) | 5.48 (br d, $J$ = 5.0 Hz) | 5.20 (d, $J$ = 4.9 Hz) | 5.37 (br d, $J$ = 4.8 Hz) | 5.33 (br d, $J$ = 4.7 Hz) |
| 18 | 0.69 (s) | 0.67 (s) | 0.62 (s) | 0.63 (s) | 0.60 (s) | 0.69 (s) | 0.68 (s) |
| 19 | 1.17 (s) | 1.13 (s) | 1.05 (s) | 1.28 (s) | 0.88 (s) | 1.25 (s) | 1.00 (s) |
| 21 | 1.07 (d, $J$ = 6.6 Hz) | 1.12 (d, $J$ = 5.7 Hz) | 0.91 (d, $J$ = 6.9 Hz) | 1.18 (d, $J$ = Hz) | 1.01 (d, $J$ = 6.8 Hz) | 1.11 (d, $J$ = Hz) | 0.85 (d, $J$ = 6.4 Hz) |
| 22 | | | | | | | 2.65 (br d, $J$ = 11.4 Hz) |
| 23 | | 5.18 (m) | | 5.09 (t, $J$ = 3.1) | | 5.16 (t, $J$ = 3.2) | |
| 24 | | | | | | | |
| 26ax | 2.97 (m) | | 3.23 (m) | | 3.23 (m) | | 2.26 (m) |
| 26eq | 3.66 (m) | | 3.23 (m) | | 3.23 (m) | | 3.22 (br d, $J$ = 11.4 Hz) |
| 27 | 0.90 (d, $J$ = 6.9 Hz) | 0.94 (d, $J$ = 6.6 Hz) | 0.91 (s) | 1.00 (s) | 1.08 (s) | 1.02 (s) | 1.03 (d, $J$ = 6.7 Hz) |
| $CH_3CO$ | | 2.01 (s), 2.06 (s), 2.14 (s) | | 2.01 (s), 2.22 (s) | | 2.02 (s), 2.20 (s) | |

[a] Measured in $CDCl_3$; chemical shifts in ppm from internal TMS, coupling constants in Hz.

pyridine rings of compounds **1**−**3**, however, compound **4** contained a substituted pyridine ring, as evidenced by the appearance of two broad proton singlets at $\delta_H$ 6.79 and 7.71 ppm, a singlet for an aromatic methyl group at $\delta_H$ 2.12 ppm (assigned to 27-$CH_3$), and its UV spectra in neutral and alkaline media ($\lambda_{max}$ 290, 307 nm). The $^{13}$C NMR chemical shifts for C-22 to C-26 (Table 2) and the presence of diagnostic peaks at $m/z$ 136, 137, and 150 all indicated the presence of a 3-hydroxy-5-methylpyridine moiety.[13,14] The position of the hydroxyl and methyl groups were further confirmed by a NOEDIFF experiment in which irradiation of $CH_3$-27 at $\delta_H$ 2.12 ppm resulted in 17 and 9% enhancements of the signals for H-26 and H-24 at $\delta_H$ 7.71 and 6.79 ppm, respectively. The HMBC correlations of H-24 and H-26 (Figure 1) were in complete agreement with the proposed structure.

Compounds **5** and **6** were isomers, with compositions of $C_{27}H_{43}NO_2$ as determined by HREIMS. Their $^1$H NMR spectra (Table 1) were essentially identical except for the chemical shift of $CH_3$-21 ($\delta_H$ 0.96 and 1.07 ppm), which indicated that they are (20*R*) and (20*S*) epimers. Their $^{13}$C NMR spectra (Table 2), in comparison with those of **2** and **3**, revealed the replacement of the $CH_2$ group at $\delta_C$ 42.3 ppm in **2** and **3** with an oxygenated CH at $\delta_C$ 77.2 ppm in **5** and **6**, consistent with the additional oxygen atom required by their composition. The presence of the additional oxygen atom was confirmed by the formation of the triacetyl derivatives **5a** and **6a** of both **5** and **6** upon acetylation. HMQC and HETCOR experiments for **5** and **6** correlated the CH group at $\delta_C$ 77.2 ppm to the protons at $\delta_H$ 4.09 (d, $J$ = 2.8 Hz) and 4.11 (d, $J$ = 3.2 Hz) in **5** and **6**, respectively. The COSY correlation of these protons with H-3 at 3.52 (dt, $J$ = 3.3, 12.2 Hz) in **5** and 3.55 (dt, $J$ = 3.6, 11.6 Hz) in **6** and the coupling pattern of H-3 and H-4 supported the assignment of the additional OH group to C-4. HMBC correlations of H-4 with C-2, C-6, and C-10 at $\delta_C$ 25.3, 128.3, and 36.0 ppm were in complete agreement with that assignment.

The $^1$H NMR chemical shift of $CH_3$-19 at $\delta_H$ 1.15 in **5** and 1.17 ppm in **6** (0.17 and 0.16 ppm downfield in comparison with the corresponding signals in **2** and **3**) indicated that the C-4 OH group has the axial, $\beta$-configuration.[15] Both $^1$H and $^{13}$C NMR chemical shifts of CH-3 and CH-4 were in complete agreement with those of 3$\beta$,4$\beta$-dihydroxy steroids,[16,17] confirming the structural and stereochemical assignments as indicated.

The HREIMS of **8** showed a molecular ion at $m/z$ 413.329, consistent with the molecular formula $C_{27}H_{43}NO_2$, indicating that **8** is a structural isomer of **5** and **6**. The position of the second OH group was assigned to C-25 based

**Table 2.** $^{13}C$ NMR Data ($\delta$ values) for Compounds **1**−**9**[a]

| carbon | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 35.7 | 37.3 | 37.3 | 37.0 | 37.0 | 37.0 | 36.9 | 37.0 | 37.2 |
| 2 | 34.0 | 31.6 | 31.6 | 31.4 | 25.3 | 25.3 | 31.0 | 31.1 | 29.7 |
| 3 | 199.7 | 71.7 | 71.6 | 71.2 | 72.2 | 72.3 | 71.1 | 71.1 | 71.7 |
| 4 | 123.7 | 42.3 | 42.3 | 41.7 | 77.2 | 77.2 | 41.8 | 41.8 | 42.3 |
| 5 | 171.6 | 140.8 | 140.9 | 140.7 | 142.8 | 142.8 | 140.5 | 140.6 | 140.7 |
| 6 | 32.9 | 121.6 | 121.5 | 121.4 | 128.3 | 128.3 | 121.1 | 121.1 | 121.6 |
| 7 | 32.0 | 31.8 | 31.8 | 31.6 | 32.0 | 32.0 | 31.7 | 31.7 | 31.8 |
| 8 | 35.6 | 31.9 | 31.8 | 31.7 | 31.9 | 31.8 | 31.5 | 31.6 | 31.8 |
| 9 | 53.8 | 50.1 | 50.1 | 49.9 | 50.2 | 50.3 | 49.9 | 49.9 | 50.0 |
| 10 | 38.6 | 36.5 | 36.5 | 36.3 | 36.0 | 36.0 | 36.3 | 36.3 | 36.4 |
| 11 | 21.0 | 21.1 | 21.0 | 20.9 | 20.5 | 20.5 | 20.1 | 20.1 | 21.1 |
| 12 | 37.9 | 38.1 | 39.7 | 39.5 | 38.0 | 39.6 | 39.7 | 39.7 | 39.7 |
| 13 | 42.3 | 42.2 | 42.4 | 42.2 | 42.2 | 42.4 | 42.2 | 42.3 | 42.2 |
| 14 | 55.4 | 56.3 | 56.4 | 56.2 | 56.4 | 56.7 | 56.0 | 56.3 | 56.4 |
| 15 | 24.0 | 24.0 | 24.3 | 24.0 | 24.0 | 24.3 | 23.8 | 24.1 | 24.2 |
| 16 | 27.8 | 26.4 | 27.7 | 27.2 | 27.3 | 27.2 | 26.7 | 26.8 | 27.9 |
| 17 | 53.5 | 53.6 | 53.1 | 29.6 | 53.5 | 53.1 | 53.2 | 52.9 | 53.9 |
| 18 | 11.8 | 11.8 | 12.0 | 12.0 | 11.8 | 12.0 | 11.3 | 11.6 | 11.6 |
| 19 | 17.4 | 19.4 | 19.3 | 19.3 | 20.1 | 20.9 | 19.1 | 19.1 | 19.4 |
| 20 | 46.6 | 46.6 | 47.0 | 54.3 | 46.3 | 46.8 | 46.1 | 46.3 | 40.3 |
| 21 | 18.0 | 18.1 | 18.3 | 19.3 | 18.1 | 18.3 | 17.5 | 17.6 | 13.6 |
| 22 | 174.4 | 174.5 | 175.3 | 151.7 | 175.0 | 175.5 | 178.1 | 178.2 | 59.6 |
| 23 | 26.4 | 27.3 | 26.5 | 151.7 | 26.5 | 26.6 | 31.7 | 31.7 | 31.7 |
| 24 | 27.4 | 27.8 | 27.2 | 122.9 | 27.8 | 27.6 | 30.8 | 30.9 | 33.4 |
| 25 | 27.7 | 27.6 | 27.4 | 130.9 | 27.6 | 27.4 | 65.1 | 65.4 | 31.6 |
| 26 | 56.9 | 56.9 | 56.4 | 139.9 | 56.6 | 56.3 | 59.1 | 59.1 | 53.9 |
| 27 | 19.5 | 19.5 | 19.1 | 30.6 | 19.4 | 19.1 | 26.6 | 26.7 | 19.2 |

[a] Obtained in $CDCl_3$. Assignments made by a combination of DEPT, HETCOR, or HMQC, HMBC data and comparison with the spectrum of verazine (**3**).[6]

**Table 3.** Antifungal and Cytotoxic Activities of Compounds **1**−**6**, **8**, **9**

| | $IC_{12}$ values ($\mu$g/mL) in *S. cerevisiae* strain: | | | MIC ($\mu$g/mL) in strain | | |
|---|---|---|---|---|---|---|
| compound | 1138 | 1140 | 1353 | *S. cerevisiae* Sc7 | *C. Albicans* SC5314 | $IC_{50}$ values ($\mu$g/mL) in the M-109 cell line |
| **1** | 14 | 45 | 50 | NT[a] | NT[a] | 16.8 |
| **2** | 0.88 | 1.12 | 0.69 | 6.25 | 12.5 | NT[a] |
| **3** | 0.14 | 0.25 | 0.14 | 6.25 | 25 | 12.5 |
| **4** | 0.04 | 0.70 | 0.40 | 20 | 20 | >20 |
| **5** | 10 | 23 | 24 | 200 | 400 | 10.5 |
| **6** | 12 | 10 | >50 | 100 | 200 | 15.5 |
| **8** | 0.35 | 2.5 | 4 | 6.25 | <3.1 | 31.2 |
| **9** | 3 | 3 | 4 | NT[a] | NT[a] | NT[a] |
| amphotericin B | 8.1 | 15.6 | 25 | 12.5 | 1.6 | 7.5 |
| ketoconozale | 2.5 | 4.8 | 9.4 | 1.6 | <0.8 | 17 |

[a] NT = not tested.

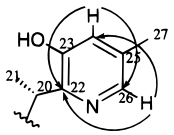



**Figure 1.** HMBC correlations of H-24 and H-26 in **4**.

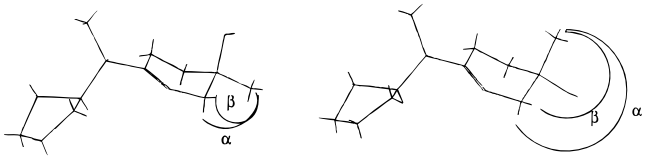
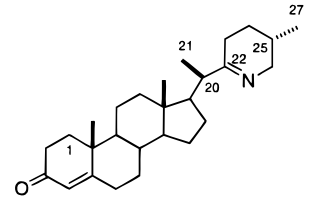



**Figure 2.** Partial structures of compound **8** (left) and its C-27 epimer (right) showing the $CH_3$-27 to H-$26_{ax}$ and $CH_3$-27 to H-$26_{eq}$ distances for both isomers.

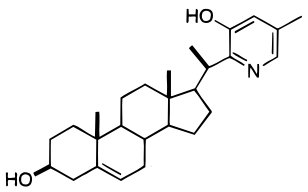
on the appearance of $CH_3$-27 as a singlet at $\delta_H$ 1.08 ppm, along with a quaternary carbon resonance at $\delta_C$ 65.4 ppm assigned to the oxygenated C-25. Additional evidence was derived from an HMBC experiment ($J$ = 9 Hz), where the H-26 proton at $\delta_H$ 3.23 ppm showed two bond correlations to C-25. Although the two H-26 protons were overlapped at 3.23 ppm when the spectrum was recorded in $CDCl_3$ (Table 1), the two resonances were well resolved when the spectrum was measured in pyridine-$d_5$ (see Experimental Section). The two doublets at $\delta_H$ 3.76 and 3.97 ppm were assigned to H-$26_{ax}$ and H-$26_{eq}$, respectively, based on comparison with the $^1H$ NMR spectra of **2** and **3**[7] as well as the $^1H$ NMR spectrum of **3** in pyridine (see Experimental Section). In a NOESY experiment, the $CH_3$-27 at $\delta_H$ 1.08 ppm showed a strong NOE correlation with the α-oriented H-$26_{ax}$ and a weaker correlation with the $\beta$-oriented H-$26_{eq}$. Molecular modeling (Figure 2) showed that an α-equatorial $CH_3$ group would be close enough to both H-26 protons to show NOE effects. Thus, distances of 2.55, 3.05, and 3.76 Å between the protons of $CH_3$-27 and H-$26_{ax}$ and distances of 2.67, 3.22, and 3.81 Å between the protons of $CH_3$-27 and H-$26_{eq}$ were calculated for one conformation of $CH_3$-$27_{eq}$, while similar calculations for $CH_3$-$27_{ax}$ gave distances of 3.79, 4.34, and 3.83 for H-$26_{ax}$ and 2.58, 3.79, and 2.97 for H-$26_{eq}$. The results of this experiment thus suggested that **8** had an α-equatorial $CH_3$ group and a $\beta$-axial OH group. Upon acetylation **8** produced the diacetyl derivative **8a**.

In addition to pure compound **8**, an epimeric mixture of compounds **7** and **8** was also obtained. This mixture could not be separated by normal chromatographic methods, but the acetylated mixture of **7a** and **8a** could be separated by

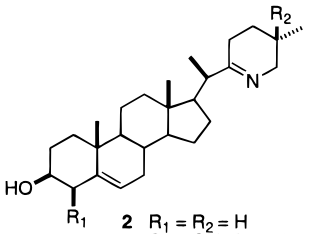
HPLC using a $C_{18}$ Si gel column. The $^1H$ and $^{13}C$ NMR spectra of **7** (Tables 1 and 2) were obtained by subtracting the resonances due to compound **8** from the spectra of the mixture. A comparison of the $^1H$ NMR data for **7**, **7a**, **8**, and **8a** indicated that **7** and **8** are epimeric at C-20, with **7** being the (20*R*) epimer and **8** being the (20*S*) epimer.



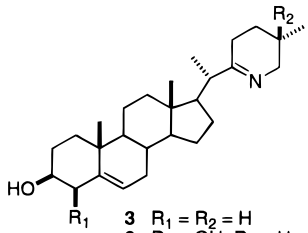
**1**



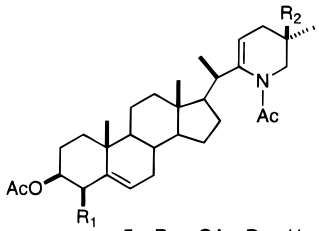
**4**



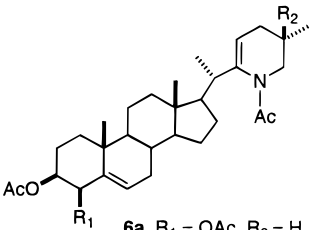
**2** $R_1 = R_2 = H$
**5** $R_1 = OH, R_2 = H$
**7** $R_1 = H, R_2 = OH$



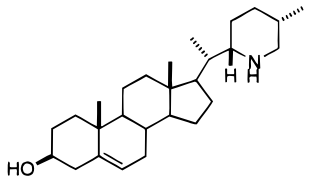
**3** $R_1 = R_2 = H$
**6** $R_1 = OH, R_2 = H$
**8** $R_1 = H, R_2 = OH$



**5a** $R_1 = OAc, R_2 = H$
**7a** $R_1 = H, R_2 = OH$



**6a** $R_1 = OAc, R_2 = H$
**8a** $R_1 = H, R_2 = OH$

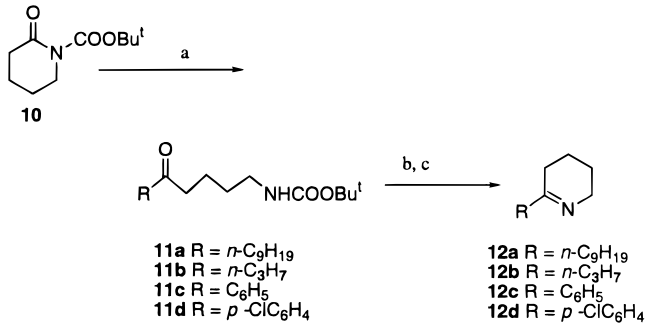


**9**

The activities of compounds **1**−**6** and **8** in the three yeast assays and against the Sc7 yeast strain[18] are shown in Table 3. The most active compounds are **2**−**4** and **8**, with the pyridine-containing alkaloid **4** being the most active in the yeast assay. Interestingly, compound **8** showed good activity against *Candida albicans*, with an MIC value only slightly less than those of the clinically used antifungal drugs amphotericin B and ketoconazole. These activities apparently do not translate well into cytotoxicity, however, because all the compounds showed $IC_{50}$ values of >10 $\mu$g/mL against the M-109 cell line, and this suggests that the primary activity of the alkaloids is as antifungal agents. Regrettably, the slight but real cytotoxicity of these compounds was sufficient to preclude further development as antifungal agents. Verazine (**3**) has previously been reported to have antifungal activity[19] and to inhibit DNA formation by hepatoma and S-180 cells.[20]

The observation that steroidal alkaloids such as verazine (**3**) showed good activity in our yeast bioassay was intriguing, for there seemed to be no obvious structural feature of these compounds that would suggest such activity. The only obviously reactive group was the imino function. In the initial stages of this work the pyridine alkaloid **4** had not been isolated, and so we carried out the synthesis of the model compounds **12a**−**d** to determine whether such simple analogues might show a similar bioactivity. The syntheses were carried out by literature procedures[21] and involved the addition of a Grignard reagent to the lactam 1-(*tert*-butoxycarbonyl)-1-azacyclohexan-2-one (**10**) to give the ketoamides **11a**−**d** (Scheme 1). These amides were then cyclized by treatment with TFA and deprotected to give the imines **12a**−**d**. These compounds were, however, completely inactive, indicating that the steroidal moiety is necessary for bioactivity.

**Scheme 1.** Synthesis of Cyclic Imines **12a**−**d** a) RMgBr, b) TFA, 3 h, c) NaOH.




All the acetylated derivatives were inactive. The large decrease in activity by small modifications in the steroidal skeleton, as in **1**, **5**, and **6** (Table 4), indicate that this skeleton with a 3$\beta$-OH is required. Introduction of an OH group at C-25 as in **8** or reduction of the double bond between C-22 and C-26 as in **9** resulted in less dramatic loss of activity. Finally, conversion of the imine to a pyridine moiety as in **4** resulted in an increase of both activity and selectivity in comparison with verazine (**3**).

## Experimental Section

**General Experimental Procedures.** NMR spectra were recorded in $CDCl_3$ on a Varian Unity 400 NMR instrument at 399.951 MHz for $^1H$ and 100.578 MHz for $^{13}C$, using standard Varian pulse sequence programs. LRMS and exact mass measurements were taken on a VG 7070 E-HF at the Virginia Polytechnic Institute and State University. Molecular modeling studies to calculate the internuclear distances discussed for compound **8** were carried out using the MacSpartan program (Wavefunction, Inc., Irvine, CA). Other conditions were as previously described.[22]

**Yeast Bioassay.** The yeast bioassay was carried out using the following strains: 1140 = JN392 = *ise1rad52*; 1353 = JN2−291 = ISE$^+$*rad52top1*; 1138 = JN394 = *a*ISE2*ura3-51leu2-3,112trp1-289 rad52*::LEU2. All strains were obtained from Dr. Nitiss through one of us (S. M.). Cultures were grown to stationary phase in YEPD broth (Difco), centrifuged, resuspended in 0.9% saline to 25% light transmission at 600 nm, and refrigerated at 4 °C. The yeasts were grown on YEPD Agar (Difco) as base agar with an overlay of 0.8% Noble Agar (Scott); plates were prepared and flooded with 2.5 mL of inoculum. After a brief interval the excess inoculum was pipeted off the plates (approximately 2 mL is removed) and the plates allowed to dry under a biological hood leaving a uniform lawn of yeast cells. After drying, wells of 6−7-mm diameter were cut in the agar layer, and samples to be tested dissolved in DMSO−MeOH (1:1) and added to the wells in 100-mL aliquots. Nystatin (Sigma) was used as a positive control at 20 $\mu$g/mL. The plates were incubated at 30 °C for 36−48

**Table 4.** $^1$H and $^{13}$C NMR Data ($\delta$ values) for Compounds **12a**−**d**[a]

| | **12a** | | **12b** | | **12c** | | **12d** | |
|---|---|---|---|---|---|---|---|---|
| position | $^1$H | $^{13}$C | $^1$H | $^{13}$C | $^1$H | $^{13}$C | $^1$H | $^{13}$C |
| 2 | | | | 170.8 | | 165.7 | | 164.4 |
| 3 | 2.06 (4H, m) | 21.8 | 2.01 (4H, m) | 21.7 | 2.60 (2H, m) | 21.8 | 2.56 (2H, m) | 21.7 |
| 4 | 1.49 (4H, m) | 28.9 | 1.49 (4H, m) | 28.8 | 1.65 (2H, m) | 27.0 | 1.64 (2H, m) | 26.8 |
| 5 | 1.60 (2H, m) | 19.5 | 1.60 (2H, m) | 19.6 | 1.81 (2H, m) | 19.6 | 1.81 (2H, m) | 19.6 |
| 6 | 3.49 (2H, m) | 49.0 | 3.42 (2H, m) | 48.9 | 3.81 (2H, m) | 49.7 | 3.80 (2H, m) | 49.9 |
| 1′ | 2.06 (4H, m) | 41.1 | 2.06 (4H, m) | 42.9 | | 130.4 | | 135.5 |
| 2′ | 1.49 (4H, m) | 31.9 | 1.49 (4H, m) | 19.4 | 7.35−7.69 (5H, m) | 128.1 | 7.68 (2H, d, $J$ = 8.6 Hz) | 128.3 |
| 3′ | 1.21 (6H, b) | 29.5 | | 13.7 | 7.35−7.69 (5H, m) | 125.8 | 7.31 (2H, d, $J$ = 8.6 Hz) | 127.2 |
| 4′ | 1.21 (6H, b) | 29.5 | | | 7.35−7.69 (5H, m) | 129.4 | | 138.6 |
| 5′ | 1.21 (6H, b) | 29.5 | | | 7.35−7.69 (5H, m) | 125.8 | 7.31 (2H, d, $J$ = 8.6 Hz) | 127.2 |
| 6′ | 1.21 (6H, b) | 29.2 | | | 7.35−7.69 (5H, m) | 128.1 | 7.68 (2H, d, $J$ = 8.6 Hz) | 128.3 |
| 7′ | 1.21 (6H, b) | 28.9 | | | | | | |
| 8′ | 1.21 (6H, b) | 22.6 | | | | | | |
| 9′ | 0.82 (3H, t, $J$ = 7.0 Hz) | 14.0 | | | | | | |

[a] Obtained in $CDCl_3$. [b] $J$ = Hz. [c] Carbon type as determined by DEPT spectra: 0 = quaternary, 1 = methine, 2 = methylene, 3 = methyl.

h, and the resulting zones of inhibition were measured in millimeters. Activity was determined from a dose−response curve and is reported as an $IC_{12}$ value, which is the dose (in $\mu$g/mL) required to produce a zone of inhibition 12 mm in diameter.

Antifungal assays against *S. cerevisiae* Sc7 and *Candida albicans* SC5314 and cytotoxicity assays against the M-109 cell line were performed at Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, CT, using standard protocols.

**Plant Materials.** The leaves of *E. alba* (L.) Hassk. (Asteraceae) were collected from Akisiamau, near Asindopo, Suriname, on June 11, 1994. A herbarium specimen was deposited in the National Herbarium of Suriname under voucher number CI 0018.

**Plant Extraction.** *E. alba* leaves were extracted with EtOAc and MeOH at Bedrijf Geneesmiddelen Voorziening Suriname (BGVS). Extraction of 1 kg of dried plant material with MeOH yielded 63 g of extract as BGVS M-940116.

**Isolation of Bioactive Alkaloids.** The MeOH extract of *E. alba* was active against the mutant strains 1138, 1140, and 1353. The three strains were used to determine the $IC_{12}$ values of the total extract and pure isolates, while the activity was monitored during the fractionation procedures using the 1138 mutant strain. The bioactive MeOH extract ($IC_{12}$, 9,16, 26 $\mu$g/mL on the mutant yeast strains 1353, 1138, and 1140, respectively, 63.5 g) was fractionated between BuOH and $H_2O$. The $H_2O$ fraction (18.5 g) was inactive. The active BuOH (44.9 g) fraction was dissolved in 80% aqueous MeOH and extracted with hexane (750 mL × 3). The activity was retained in the aqueous MeOH fraction (33.7 g), which was diluted with $H_2O$ to 60% MeOH and fractionated with $CHCl_3$ (750 mL × 3). The $CHCl_3$ fraction (20 g) was the most active, showing an $IC_{12}$ 3.5 $\mu$g/mL, while the 60% aqueous MeOH fraction was much weaker: $IC_{12}$ 114 $\mu$g/mL. The $CHCl_3$ fraction (20 g) was purified by chromatography on Sephadex LH-20 (5 × 100 cm) using the solvents hexane−$CH_2Cl_2$ (1:4, 3 L), $CH_2Cl_2$−$Me_2CO$ (3:2, 3 L), $CH_2Cl_2$−$Me_2CO$ (4:1, 3 L), and MeOH (2 L). The active fraction ($IC_{12}$ 2.8 $\mu$g/mL, 15.0 g), eluted with hexane−$CH_2Cl_2$ (1:4), was separated on 500 g Si gel using the VLC technique eluting with $CHCl_3$−$Me_2CO$ mixtures.

The active fractions (7−9) eluted with 30% $Me_2CO$ in $CHCl_3$ (5.8 g) were refractionated using flash Si gel column (200 g, $CHCl_3$−$Me_2CO$ mixtures), and 30 fractions were collected. Fractions 5−7 (0.2 g), eluted with 20% $Me_2CO$ in $CHCl_3$, were further purified on a Si gel column (15 g) eluting by $CHCl_3$ followed by preparative TLC ($C_{18}$ Si gel, 10% $H_2O$ in MeOH) to afford 20-*epi*-3-dehydroxy-3-oxo-5,6-dihydro-4,5-dehydroverazine (**1**, 10 mg, $R_f$ 0.64). Fraction 9 (100 mg), eluted with 25% $Me_2CO$ in $CHCl_3$, was again purified on a Si gel column (10 g) eluting by 3% MeOH in $CHCl_3$ followed by preparative TLC (Si gel, hexane−$Me_2CO$−MeOH, 60:40:4, 5 developments) to obtain 20-epiverazine (**2**, 11 mg, $R_f$ 0.78) and verazine (**3**, 23 mg, $R_f$ 0.75). Fractions 10−14 (1.5 g), eluted with 25% $Me_2CO$ in $CHCl_3$, were purified on a Si gel column (50 g) eluting by 3% MeOH in $CHCl_3$ followed by crystallization from MeOH to afford verazine (**3**, 230 mg). The more polar active fractions (18−20, 1.6 g), eluted with 35% $Me_2CO$ in $CHCl_3$, were rechromatographed on a Si gel column (50 g) eluting with 10% MeOH in $CHCl_3$. The active fractions (200 mg) were subjected to preparative TLC (Si gel, 8% MeOH in $CHCl_3$, 5 developments) to afford **4** (2 mg, $R_f$ 0.81), **5**, (18 mg, $R_f$ 0.68), and **6** (30 mg, $R_f$ 0.65). The most polar active fractions (23−25, 0.3 g), eluted with 50% $Me_2CO$ in $CHCl_3$, were further purified on a Si gel column (10 g) eluting by 10% MeOH in $CHCl_3$ followed by preparative TLC (Si gel, 10% MeOH in $CHCl_3$, 5 developments) to afford a mixture of **7** and **8** (7 mg, $R_f$ 0.62) and pure **8** (20 mg, $R_f$ 0.59).

**20-*epi*-3-Dehydroxy-3-oxo-5,6-dihydro-4,5-dehydroverazine [(20*R*,20*S*)-3-oxo-22,26-iminocholesta-4,22(*N*)-diene] (1):** colorless amorphous matrix, $[\alpha]^{26}_D$ +68° (*c* 1.0, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 240 (5.1), 209 (4.9) nm; IR (film) $\nu_{max}$ 2946, 1668 (CO), 1614, 1455, 1229, 753 cm$^{-1}$; $^1$H NMR and $^{13}$C NMR, see Tables 1 and 2; EIMS *m*/*z* 395 ($M^+$, 10), 381 (12), 366 (6), 272 (4), 164 (18), 152 (29), 140 (37), 125 ($C_8H_{15}N$, 100), 111 (64), 91 (48), 81 (47), 67 (35), 55 (75); HREIMS *m*/*z* 395.317 ($M^+$), calcd for $C_{27}H_{41}NO$ 395.318.

**20-*epi*-Verazine [(3*S*,20*R*,25*S*)-22,26-iminocholesta-5,22(*N*)-dien-3-ol] (2):** colorless amorphous matrix, $[\alpha]^{26}_D$ +6.5° (*c* 1.9, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 206 (4.8) nm; IR (film) $\nu_{max}$ 3328 (OH), 2936, 1653 (C=N), 1569, 1415, 1377, 1123 cm$^{-1}$; $^1$H NMR and $^{13}$C NMR, see Tables 1 and 2; $^{13}$C NMR, see Table 2; EIMS *m*/*z* 397 ($M^+$, 10), 382 ($M^+ - CH_3$, 11), 379 (9), 364 (13), 164 (15), 125 ($C_8H_{15}N$, 100), 111 (55), 91 (20), 81 (15), 55 (30).

**Verazine [(3*S*,20*S*,25*S*)-22,26-iminocholesta-5,22(*N*)-dien-3-ol] (3):** colorless needles, mp 175−176 °C (MeOH), lit. mp 176−178 °C,[8] $[\alpha]^{26}_D$ −65° (*c* 1.5, MeOH), [lit. $[\alpha]^{26}_D$ −89.7° (EtOH)];[8] UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 206 (4.6) nm; IR (film) $\nu_{max}$ 3336 (OH), 2928, 1659 (C=N), 1567, 1413, 1370, 1118 cm$^{-1}$; $^1$H NMR in $CDCl_3$, see Table 1; $^1$H NMR (pyridine-$d_5$) $\delta$ 0.67 (3H, s, $CH_3$-18), 0.82 (3H, d, $J$ = 6.6 Hz, $CH_3$-27), 1.05 (3H, s, $CH_3$-19), 1.14 (3H, d, $J$ = 6.9 Hz, $CH_3$-21), 3.08 (1H, dd, $J$ = 9.8, 16.8 Hz, H-26$_{ax}$), 3.83 (1H, dd, $J$ = 4.4, 23.9 Hz, H-26$_{eq}$), 3.84 (1H, m, H-3), 5.41 (1H, bd, $J$ = 4.8 Hz, H-6); $^{13}$C NMR, see Table 2; EIMS *m*/*z* 397 ($M^+$, 12), 382 ($M^+ - CH_3$, 13), 379 (12), 364 (10), 164 (11), 125 ($C_8H_{15}N$, 100), 111 (60), 91 (13), 81 (12), 55 (25).

**Ecliptalbine [(3*S*,20*R*)-20-(3-hydroxy-5-methyl-2-pyridyl)cholest-5-ene-3,23-diol] (4):** white powder, mp 275−278 °C (MeOH), $[\alpha]^{26}_D$ −77° (*c* 0.41, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 290 (3.8), 203 (4.1) nm, (NaOH) $\lambda_{max}$ (log $\epsilon$) 307 (4.1), 243 (4.1), 201 (4.5) nm; $^1$H NMR and $^{13}$C NMR, see Tables 1 and 2; $^{13}$C NMR (pyridine-$d_5$) $\delta$ 122.7 (C-24), 131.1 (C-25), 140.8 (C-26), 151.1 (C-22), 153.1 (C-23); EIMS *m*/*z* 409 ($M^+$, 20), 394 ($M^+ - CH_3$, 21), 150 ($C_9H_{12}NO$, 11), 138 (95), 137 ($C_8H_{11}NO$, 100), 136 ($C_8H_{10}NO$, 38), 117 (39), 91 (50), 77 (30), 67 (25), 55 (25); HREIMS *m*/*z* 409.297 ($M^+$), calcd for $C_{27}H_{39}NO_2$ 409.298.

**20-*epi*-4*β*-Hydroxyverazine [(3*S*,4*R*,20*R*,25*S*)-22,26-iminocholesta-5,22(*N*)-diene-3,4-diol] (5):** white powder, mp 107−109 °C (MeOH), $[\alpha]^{26}_D$ −20.5° (*c* 2.0, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 206 (3.4) nm; IR (film) $\nu_{max}$ 3316 (OH), 2933, 1657 (C═N), 1572, 1424, 1380, 1067, 841 cm$^{-1}$; $^1$H NMR and $^{13}$C NMR, see Tables 1 and 2; EIMS *m*/*z* 413 ($M^+$, 14), 395 ($M^+ - H_2O$, 11), 380 ($M^+ - H_2O - CH_3$, 10), 164 (18), 150 (20), 139 (15), 125 ($C_8H_{15}N$, 100), 121 (42), 111 (53), 93 (19), 91 (17), 79 (21), 67 (21), 55 (40); HREIMS *m*/*z* 413.329 ($M^+$), calcd for $C_{27}H_{43}NO_2$ 413.329.

**Acetylation of 20-*epi*-4*β*-Hydroxyverazine (5) to 20-*epi*-4*β*-Hydroxyverazine Triacetate (5a).** Compound **5** (2.0 mg) in pyridine (0.5 mL) was treated with $Ac_2O$ (0.2 mL) for 24 h at room temperature. Evaporation of the resulting solution under a stream of argon yielded chromatographically homogeneous **5a** (2.0 mg): colorless amorphous matrix; UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 228 (3.7), 206 (3.7) nm; $^1$H NMR, see Table 1.

**4*β*-Hydroxyverazine [(3*S*,4*R*,20*S*,25*S*)-22,26-iminocholesta-5,22(*N*)-diene-3,4-diol] (6):** colorless crystals, mp 163−165 °C (MeOH), $[\alpha]^{26}_D$ −12.8° (*c* 0.7, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 207 (3.4) nm; IR (film) $\nu_{max}$ 3319 (OH), 2939, 1654 (C═N), 1569, 1430, 1377, 1062, 838 cm$^{-1}$; $^1$H NMR and $^{13}$C NMR, see Tables 1 and 2; EIMS *m*/*z* 413 ($M^+$, 4), 395 ($M^+ - H_2O$, 8), 380 ($M^+ - H_2O - CH_3$, 8), 164 (14), 150 (19), 125 ($C_8H_{15}N$, 100), 121 (45), 111 (75), 91 (28), 79 (26), 67 (26), 55 (53); HREIMS *m*/*z* 413.329 ($M^+$), calcd for $C_{27}H_{43}NO_2$ 413.329.

**Acetylation of 4*β*-Hydroxyverazine (6) to 4*β*-Hydroxyverazine Triacetate (6a).** Compound **6** (4.0 mg) in pyridine (0.5 mL) was treated with $Ac_2O$ (0.2 mL) for 24 h at room temperature. Evaporation of the resulting solution under a stream of argon yielded chromatographically homogeneous **6a** (4.0 mg): colorless crystals, mp 97−98 °C (MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 228 (3.7), 207 (3.7) nm; $^1$H NMR, see Table 1.

**20-*epi*-25*β*-Hydroxyverazine [(3*S*,20*R*,25*R*)-22,26-iminocholesta-5,22(*N*)-diene-3,25-diol] (7):** compound **7** could not be separated from compound **8**, and the $^1$H NMR and $^{13}$C NMR data for **7** (Tables 1 and 2) were thus obtained by subtraction of the data for **8** from the corresponding spectrum of the mixture of **7** and **8**.

**20-*epi*-25*β*-Hydroxyverazine Diacetate (7a).** A mixture of **7** and **8** (4.0 mg) in pyridine (0.5 mL) was treated with $Ac_2O$ (0.2 mL) for 24 h at room temperature. After evaporation of the resulting solution the mixture was separated by HPLC ($C_{18}$ Si gel, MeOH−$H_2O$, 90:10) to afford 1.5 mg **7a** and 2 mg **8a**.

**Compound 7a:** amorphous matrix, $[\alpha]^{26}_D$ −134° (*c* 1.5, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 232 (4.6), 207 (4.6) nm; IR (film) $\nu_{max}$ 3408 (OH), 2962, 1713 (C═O), 1669, 1385, 1239 cm$^{-1}$; $^1$H NMR, see Table 1; EIMS *m*/*z* 497 ($M^+$, 35), 483 ($M^+ - CH_3$, 13), 437 (25), 420 (15), 375 (25), 360 (19), 183 (57), 168 (50), 141 (54), 121 (51), 91 (100), 79 (77), 67 (65), 55 (92); HREIMS *m*/*z* 497.352 ($M^+$), calcd for $C_{31}H_{47}NO_4$ 497.351.

**25*β*-Hydroxyverazine [(3*S*,20*S*,25*R*)-22,26-iminocholesta-5,22(*N*)-diene-3,25-diol] (8):** colorless crystals, mp 179−182 °C (MeOH), $[\alpha]^{26}_D$ −28° (*c* 0.5, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 211 (3.6) nm; IR (film) $\nu_{max}$ 3315 (OH), 2930, 1653 (C═N), 1569, 1415, 1377, 1123 cm$^{-1}$; $^1$H NMR ($CDCl_3$), see Table 1; $^1$H NMR (pyridine-$d_5$) $\delta$ 0.68 (3H, s, $CH_3$-18), 1.04 (3H, s, $CH_3$-19), 1.19 (3H, d, $J$ = 6.6 Hz, $CH_3$-21), 1.36 (3H, s, $CH_3$-27), 3.76 (1H, d, $J$ = 17.3 Hz, H-26$_{ax}$), 3.85 (1H, m, H-3), 3.97 (1H, d, $J$ = 17.3 Hz, H-26$_{eq}$), 5.40 (1H, br d, $J$ = 4.8 Hz, H-6); $^{13}$C NMR, see Table 2; EIMS *m*/*z* 413 ($M^+$, 5), 398 ($M^+ - CH_3$, 3), 142 ($C_8H_{16}NO$, 47), 141 ($C_8H_{15}NO$, 100), 127 (94), 121 (51), 91 (31), 79 (33), 67 (34), 55 (58); HREIMS *m*/*z* 413.328 ($M^+$), calcd for $C_{27}H_{43}NO_2$ 413.329.

**Acetylation of 25*β*-Hydroxyverazine to 25*β*-Hydroxyverazine Diacetate (8a).** Compound **8** (5.0 mg) in pyridine (0.5 mL) was treated with $Ac_2O$ (0.2 mL) for 24 h at room temperature. Evaporation of the resulting solution under a stream of argon yielded chromatographically homogeneous **8a** (5.0 mg), which was identical with **8a** obtained by HPLC separation of the acetylated mixture of **7** and **8**: colorless needle crystals, mp 160−163 °C (MeOH); $[\alpha]^{26}_D$ +16° (*c* 1.0, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 232 (4.6), 209 (4.6) nm; IR (film) $\nu_{max}$ 3423 (OH), 2939, 1723 (C═O), 1639, 1385, 1246, 1030, 753 cm$^{-1}$; $^1$H NMR, see Table 1.

**Reduction of Verazine (3) to Veramiline (9).** Compound **3** (15 mg) in 0.5 mL MeOH was treated with $NaHB_4$ (75 mg) for 30 min at room temperature; $H_2O$ (5 mL) was added to the reaction mixture, which then was extracted with $CHCl_3$ (4 × 5 mL). The reaction gave **9** (11 mg) as single product: colorless crystals, mp 199−202 °C ($Me_2CO$) [lit. 198−200 °C];[10] $[\alpha]^{26}_D$ −43° (*c* 0.7, MeOH) (lit. $[\alpha]^{26}_D$ −49°);[10] $^1$H NMR and $^{13}$C NMR, see Tables 1 and 2; EIMS *m*/*z* 156 (7), 99 (15), 98 ($C_6H_{12}N$, 100); CIMS *m*/*z* 400 ($M^+ + 1$, 20), 398 ($M^+ - 1$, 11), 98 ($C_6H_{12}N$, 100).

**General Procedure for the Synthesis of Cyclic Imines.** A solution of 2-piperidone (990 mg, 10 mmol) in dry THF (30 mL) was cooled to −78 °C, then BuLi (2.0 M, 4 mL, 10 mmol) was added dropwise with stirring, and after 30 min di-*tert*-butyl dicarbonate (2.2 g, 10 mmol) in dry THF (10 mL) was added slowly and the stirring was continued for 3 h at −78 °C. A solution of the chosen Grignard reagent (15 mmol) was then added over 15 min, and the mixture was stirred at −78 °C for 3 h more. The reaction mixture was then quenched with 2N HCl (10 mL) and extracted with ether (4 × 60 mL). The combined organic phase was washed with 10% aqueous $NaHCO_3$, then with brine, and finally dried over $Na_2SO_4$. The residue left after evaporation of the ether was purified by crystallization from MeOH. TFA (2 mL) was added slowly at 0 °C with stirring to each of the pure crystalline products **11a**−**d** (100 mg), and after 3 h 30% aqueous NaOH was added to pH 10−11, and the reaction mixture was extracted with ether (4 × 15 mL). The final product was obtained after evaporation of the ether under atmospheric pressure.

**2-Nonyl-3,4,5,6-tetrahydropyridine (12a):** colorless oil; $^1$H and $^{13}$C NMR, see Table 4; CIMS *m*/*z* 210 ($M^+ + H$, 22), 173 (19), 172 (24), 144 (23), 100 (24), 97 (21), 71 (45), 57 (100); EIMS *m*/*z* 144 (23), 143 (32), 124 ($C_8H_{14}N$, 14), 110 ($C_7H_{12}N$, 15), 97 ($C_7H_{13}$, 100).

**2-Propyl-3,4,5,6-tetrahydropyridine (12b):** colorless oil; $^1$H and $^{13}$C NMR, see Table 4.

**2-Phenyl-3,4,5,6-tetrahydropyridine (12c):** colorless oil; $^1$H and $^{13}$C NMR, see Table 4.

**2-(*p*-Chlorophenyl)-3,4,5,6-tetrahydropyridine (12d):** colorless crystals, mp 54−55 °C, (lit. mp 54 °C);[23] $^1$H and $^{13}$C NMR, see Table 4.

**Acknowledgment.** This work was supported by an International Cooperative Biodiversity Group Award, number U01 TW/CA-00313, from the Fogarty Center, NIH, and this financial support, together with support and encouragement from Dr. Joshua Rosenthal of the Fogarty Center, is gratefully acknowledged. Mass spectra were obtained by Mr. Kim Harich of Virginia Polytechnic Institute and State University. Ms. Yvette Merton, Mr. Reggy Nelson, and Mr. Richard Autar of Conservation International Suriname and Ms. Lisa Famolare and Ms. Regina de Souza of Conservation International USA are thanked for their administrative assistance and support.

## References and Notes

(1) Biodiversity Conservation and Drug Discovery in Suriname, Part 3. For Part 2, see Abdel-Kader et al.[3]
(2) Zhou, B.-N.; Baj, N. J.; Glass, T. E.; Malone, S.; Werkhoven, M. C. M.; van Troon, F.; David, M.; Wisse, J. H.; Kingston, D. G. I. *J. Nat. Prod.* **1997**, *60*, 1287−1293.
(3) Abdel-Kader, M. S.; Wisse, J. H.; Evans, R.; van der Werff, H.; Kingston, D. G. I. *J. Nat. Prod.* **1997**, *60*, 1294−1297.
(4) Nitiss, J.; Wang, J. C. *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 7501−7505.
(5) Plant collections in Suriname were made on a "random" basis by the Missouri Botanical Garden and on an ethnobotanical basis by Conservation International. This collection was made by Conservation International.
(6) (a) Kritikar, K. R.; Basu, B. D. *Indian Medicinal Plants*, Chronica Botanica: New Delhi, 1975. (b) Chopra, R. N.; Nayar, S. L.; Chopra, I. C. *Glossary of Indian Medicinal Plants*. C.S.I.R.: New Delhi, 1955.
(7) Han, X.; Ruegger, H. *Planta Med.* **1992**, *58*, 449−453.
(8) (a) Adam, G.; Schreiber, K.; Tomko, J.; Vassova, A. *Tetrahedron* **1967**, *23*, 167−171. (b) Bondarenko, N. V. *Khim. Prir. Soedin.* **1973**, 132. (c) Bondarenko, N. V. *Khim. Prir. Soedin.* **1979**, 415. (d) Bondarenko, N. V. *Khim. Prir. Soedin.* **1984**, 263. (e) Bondarenko, N. V. *Khim. Prir. Soedin.* **1984**, 801−2. (f) Zhao, W.; Tezuka, Y.; Kikuchi, T.; Chen, J.; Guo, Y. *Chem. Pharm. Bull.* **1989**, *37*, 2920−2928.

(9) Taskhanova, E. M.; Shakirov, R.; Yunusov, S. Y. *Chem. Nat. Comp.* **1985**, *21*, 343−344.
(10) Vassova, A.; Voticky, Z.; Tomko, J. *Collect. Czech. Chem. Commun.* **1977**, *42*, 3643−3645.
(11) Reich, H. J.; Jautelat, M.; Messe, M. T.; Weigert, F. J.; Roberts, J. D. *J. Am. Chem. Soc.* **1969**, *91*, 7445−7454.
(12) Hanson, J. R.; Siverns, M. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1956−1958.
(13) Ali, E.; Chakravarty, A. K.; Dhar, T. K.; Pakrashi, S. C. *Tetrahedron Lett.* **1978**, 3871−3874.
(14) Mosher, H. S. In *Heterocyclic Compounds*; Elderfield, R. C., Ed.; John Wiley & Sons: New York, 1959; Vol. 1, p 442.
(15) Zurcher, R. F. *Helv. Chim. Acta* **1963**, *46*, 2054−2088.
(16) Albrecht, H. P.; Reichling, P. *Liebigs Ann. Chem.* **1975**, 2211−2215.
(17) Gong, Y.-Z. *$^{13}$C NMR Spectra of Natural Products*; Yun-Nan Press of Sciences & Technology: Yun-Nan, Peoples' Republic of China, 1985; pp 243−244; the assignments of C-3 and C-4 chemical shifts in this reference should be interchanged.
(18) This strain was selected for screening and assay purposes because it demonstrated hypersensitivity to a variety of known antitumor and antifungal agents relative to that of other *S. cerevisiae* strains evaluated. (S. W. Mamber, Bristol-Myers Squibb Pharmaceutical Research Institute, personal communication.).
(19) Kusano, G.; Takahashi, A.; Sugiyama, K.; Nozoe, S. *Chem. Pharm. Bull.* **1987**, *35*, 4862−4867.
(20) Lisheng, L.; Xianhua, X.; Longdi, Z.; Rongliang, Z.; Zhongjian, J.; Yu, L.; Wenkai, L.; Liming, Y. *Lanzhou Daxue Xuebao, Ziran Kexueban* **1985**, *21*, 86−90.
(21) Giovannini, A.; Savoia, D.; Umani-Ronchi, A. *J. Org. Chem.* **1989**, *54*, 228−234.
(22) Valente, L. M. M.; Gunatilaka, A. A. L.; Kingston, D. G. I.; Patitucci, M. L.; Pinto, A. C. *J. Nat. Prod.* **1997**, *60*, 478−481.
(23) Salathiel, R.; Burch, J. M.; Hixon, R. M. *J. Am. Chem. Soc.* **1937**, *59*, 984−986.

NP970561C