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# VERRUCOFORTINE, A MAJOR METABOLITE OF *PENICILLIUM VERRUCOSUM* VAR. *CYCLOPIUM*, THE FUNGUS THAT PRODUCES THE MYCOTOXIN VERRUCOSIDIN

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ABSTRACT.—Verrucofortine [**8**], an alkaloid derived from tryptophan and leucine, has been isolated from the fungus *Penicillium verrucosum* var. *cyclopium*. The structure and absolute configuration have been established by a combination of spectroscopic and chemical techniques. Its structure is unrelated to that of the other major metabolite of the organism, the highly toxic pyrone-type polyketide verrucosidin [**1**], which was previously reported to be a tremorgen. A second novel metabolite, normethylverrucosidin [**3**], has also been isolated and identified. Small quantities of several other secondary metabolites, ergosterol, cyclopenin [**4**], cyclopenol [**5**], and 3-*O*-methylviridicatin [**6**], were isolated. They are known fungal metabolites but had not previously been obtained from this fungus. Studies of verrucofortine toxicity in mice showed no apparent toxic effects at doses as high at 160 mg/kg ip.

Outbreaks of neurotoxicoses in cattle in middle Tennessee led to isolation of two tremorgen-producing fungi from mold-contaminated hay (1). One of these, identified as *Penicillium verrucosum* var. *cyclopium*, was shown to contain a novel pyrone, verrucosidin [**1**], which bears a structural relationship to citreoviridin [**2**] and other members of its class (2,3). The pyrone is a potent toxin ($LD_{50}$ in mice, 4 mg/kg, ip) and was initially described as causing sustained trembling in experimental animals (2). This finding was highly unusual because almost all tremorgenic mycotoxins hitherto isolated have been nitrogenous, usually tryptophan derivatives (4,5). In contrast, reported symptoms of citreoviridin intoxication include paralysis of limbs, convulsions, and respiratory arrest, but no mention has been made of sustained trembling (6,7). It is noteworthy that verrucosidin has epoxides at two locations where citreoviridin and related metabolites have only a double bond or a diol. However, repetition of the toxicity tests in our laboratory with carefully purified verrucosidin has failed to confirm the tremorgenic activity. Indeed, verrucosidin is a powerful paralytic toxin with a toxicity indistinguishable from that of citreoviridin. The earlier work was carried out with noncrystalline material, because verrucosidin is difficult to purify by chromatography to a stage where crystallization will occur. In view of this failure to reproduce the tremorgenic response with verrucosidin, a further search has been made for the causative metabolite.

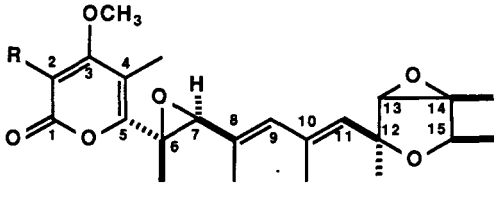
## RESULTS AND DISCUSSION

The fungus was grown for 2 weeks on a solid medium made up of instant potatoes, nonfat dry milk, sucrose, and $H_2O$. Si gel chromatography of an EtOAc extract of the mycelium yielded a series of metabolites that were further purified by chromatography and/or crystallization. Less polar fractions contained $C_{16}$ and $C_{18}$ fatty acids and a sterol identified as ergosterol. One component eluting from the column was identified as verrucosidin [**1**]. Burka *et al.* (2) established the structure of verrucosidin by X-ray crystallography, but a detailed analysis of the $^1$H-nmr spectrum had not been made. In the $^1$H-nmr spectrum of **1** the only large coupling observed is between 15-H and the 15-Me group. However, by decoupling experiments and COSY spectra, small long-range couplings could be detected: 7-H with 8-Me, 7-H with 9-H, 8-Me with 9-H, 9-H with 10-Me, 9-H with 11-H, and 10-Me with 11-H. The nOe's observed via nOe difference spectra confirmed the proton assignments for 7-H through 11-H. Other nOe's were observed, including 2-Me with 3-OMe, 3-OMe with 4-Me, 4-Me with 7-H, 11-

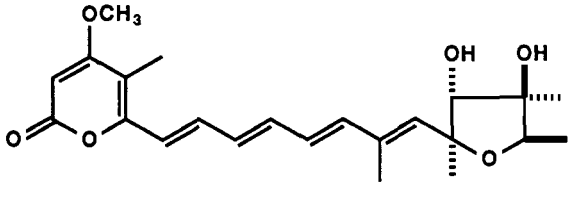
H with 12-Me, 11-H with 13-H, 12-Me with 13-H, 13-H with 14-Me, 14-Me with 15-H, and 14-Me with 15-Me. An experiment with Eu(fod)$_3$ shift reagent revealed that the pyrone methyl at C-2 shifted substantially more than the methyl at C-4 and more than any other signal in the spectrum, indicating that the pyrone carbonyl group is the primary site of Eu coordination.

Another metabolite that eluted from the column after verrucosidin was a structurally related compound that has been identified as the normethyl analog **3**. The deletion of one methyl group in **3** when compared with **1** was indicated by the mass spectrum showing an empirical formula of $C_{23}H_{30}O_6$ rather than $C_{24}H_{32}O_6$. The $^1$H nmr of **3** was similar to that of verrucosidin except that one of the pyrone methyl groups (near 2 ppm) in the spectrum of verrucosidin was missing, replaced by a vinylic proton signal at 5.51 in the spectrum of **3**. An Eu(fod)$_3$ shift experiment revealed that the new signal arises from an olefinic proton at C-2, inasmuch as the vinylic proton was much more sensitive to the shift reagent than the 4-Me group. The chemical shift of 2-H in **3** is similar to those reported for the corresponding protons in citreoviridin (8), citreomontanine (9), the aurovertins B and D (10,11), and asteltoxin (12), all of which lack a methyl group at the 2 position.

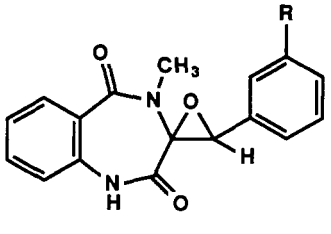
More polar fractions contained a poorly resolved group of metabolites that were separated by chromatography using a Chromatotron apparatus and further purified by preparative tlc or hplc. Three of the more polar metabolites were identified as cyclopenin [**4**], cyclopenol [**5**], and 3-*O*-methylviridicatin [**6**]. These compounds have been isolated previously from other fungi and are biosynthetically interrelated (13). Toxicological studies on these metabolites have not yet been reported. However, viridicatin



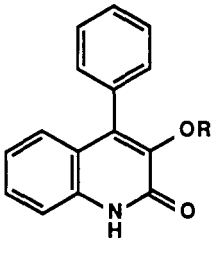
**1** R=Me verrucosidin
**3** R=H normethylverrucosidin



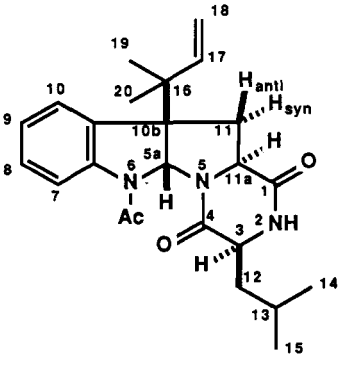
**2** citreoviridin



**4** R=H cyclopenin
**5** R=OH cyclopenol



**6** R=Me 3-*O*-methylviridicatin
**7** R=H viridicatin



**8** verrucofortine

[7], a rearrangement product of **4** (14,15), is reported to be a plant growth regulator and to have antibiotic activity against Gram-positive bacteria (16).

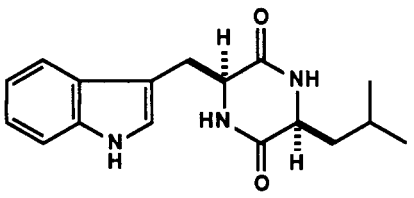
Another of the polar components was identified as the novel diketopiperazine alkaloid **8** and given the name verrucofortine. This compound was a glass-like material that could not be crystallized even after further purification by hplc. Powdered samples exhibited a gradual phase change between 90° and 165°, but subsequent analysis by tlc revealed no structural alteration. The empirical formula of **8** was assigned as $C_{24}H_{31}N_3O_3$ by hrms. Major fragmentation included loss of 69 ($C_5H_9$) and 42 ($C_2H_2O$) to give a base peak at *m/z* 298. The ir spectrum of **8** showed NH and amide carbonyl absorption bands at 3200 and 1660 $cm^{-1}$, respectively.

Insight into the structure of verrucofortine was obtained by mild acid hydrolysis (1 M methanolic HCl), which afforded a diketopiperazine **9** derived from L-tryptophan and L-leucine as the major degradation product. The structure of **9** was established by comparison of the $^1$H-nmr spectrum, mass spectrum, tlc behavior, and optical rotation with those of authentic material prepared from L-tryptophan and L-leucine by the method of Suzuki *et al.* (18). Treatment of synthetic **9** with triethylamine caused partial epimerization/racemization (19); the epimeric material was readily distinguished from **9** by hplc retention time and by nmr.

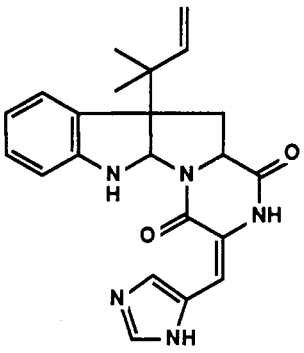
Further insight into the structure of **8** came primarily from nmr spectra. Fully decoupled and off-resonance $^{13}$C-nmr spectra showed the presence of three non-ketonic carbonyl groups (166.0, 169.0, and 170.1 ppm), all of which could be assigned as amides rather than esters or carboxylic acids because the molecule contains only three oxygen atoms. Eight additional trigonal carbons are present, but they do not represent the simple indole nucleus found in **9** because one of the carbon atoms (114.6 ppm) is a $=CH_2$ group. Two of the trigonal carbons (132.1 and 143.3 ppm) are fully substituted and the remaining five (119.2, 124.5, 124.5, 129.0, 143.1 ppm) are =CH– groups.

Proton spectra were well resolved at 400 MHz; a COSY spectrum and homonuclear decoupling experiments established that the $=CH_2$ lies in a $-CH=CH_2$ group isolated from other protons. A two-dimensional heterocorrelation spectrum identified the $-CH=CH_2$ carbon signal as the one at 143.1 ppm. The remaining six trigonal carbon atoms can reasonably be assigned to a benzene ring. The COSY spectrum and homonuclear decoupling experiments showed that the four protonated aromatic carbon atoms are contiguous with proton-proton coupling constants of 7–8 Hz.

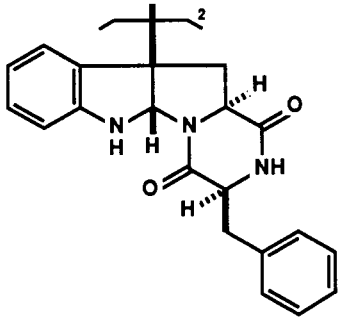
The presence of the leucine residue in **8** was confirmed from the COSY spectrum; the α proton signal appeared at 3.95 ppm and was coupled to the NH signal (ca. 6.40 ppm). The NH was exchangeable with $D_2O$, and its chemical shift was dependent on concentration and temperature. It was apparent from the $^1$H spectrum that the tryptophan component in **8** existed in a modified form; however, the tryptophan side chain was recognizable as an ABX system with the α proton (3.85 ppm) coupled to the β proton signals at 2.39 ppm ($J$ = 11.5 Hz) and 2.61 ppm ($J$ = 5.9 Hz), which were mutually coupled ($J$ = 12.5 Hz). The assignments of the signal at δ 2.39 as that of the proton *anti* to tryptophan α proton 11a-H and the one at δ 2.61 as *syn* were initially made by comparison to literature values (20,21) for proline-leucine and proline-proline diketopiperazines; the subsequent observation that the proton at δ 2.61 showed a greater nOe to 11a-H than did the one at δ 2.37 confirmed the assignment. There was no evidence of vicinal coupling of the tryptophan $CH_2$ group to other protons or of coupling of the α proton to an NH; however, weak coupling between the tryptophan and leucine α protons was detected in the COSY spectrum, and resolution enhancement of one-dimensional spectra by Gaussian multiplication revealed weak coupling between the signals ($J$ = ca. 1.0 Hz). Long-range coupling between amino acid α protons has previously been seen in ditryptophenaline [**10**], a dimeric pyrroloindole modification of the



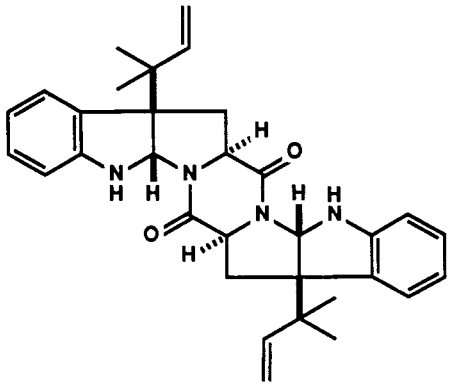
9



**11** roquefortine



**10** ditryptophenaline



**12** amauromine

diketopiperazine formed between L-tryptophan and L-phenylalanine; the coupling is attributed to a boat conformation of the diketopiperazine ring in which the two α protons are *syn* and pseudoaxial (22). On the basis of this unusual long-range coupling, verrucofortine was tentatively assigned as a diketopiperazine with annelation at the tryptophan amino group.

The remaining unidentified signals included three methyl singlets (0.91, 1.15, and 2.63 ppm) and a broad one-proton singlet (6.01 ppm). The facile loss of 42 mass units in the mass spectrum suggested an *N*-acetyl group to which the signal at 2.63 ppm was assigned; the other two were assigned as substituents on a quaternary carbon atom. The heterocorrelation spectrum showed that the signal for the carbon to which the 6.01-ppm proton was attached was at 79.5 ppm; the presence of two nitrogen substituents on the carbon was hypothesized in order to account for the deshielding of the proton and the carbon atom. Consequently, the proton was assigned as 5a-H. Two quaternary $sp^3$ carbon atoms (δ 40.4 and 60.8 ppm) are present. These can be assigned as the central atom of a 1,1-dimethylallyl moiety and as C-10b, respectively, on the basis of additivity rules (23).

The site of attachment of the acetyl group can be assigned as the nitrogen at position 6, because the one at position 2 has already been established to be protonated. Confirmatory evidence for the site of acetylation was derived from the observation that the $^1$H-nmr signals for the acetyl methyl group, the proton assigned as 5a-H, and one of the aromatic protons (8.00 ppm) that can now be assigned as 7-H are broad at 25° but sharpen significantly as the temperature is raised to 42°; the aromatic signal separates into a broadened doublet ($J = 7.3$ Hz). This effect on line shape is ascribed to *syn-anti* equilibration of the amide occurring at an intermediate rate on the nmr time scale. The nOe ex-

periments conducted at ambient temperature showed a 4% and a 14% enhancement of the signals for 7-H and 5a-H, respectively, when the acetyl methyl group was irradiated.

Dreiding models indicated that the pyrroloindole ring juncture was probably *cis* because the *trans* species would be highly strained. Confirmation was obtained from nOe experiments on verrucofortine, which established the relative configuration at 5a and 10b. Irradiation of the C-19 and C-20 methyl groups gave nOe's of 11% and 9%, respectively, at H-5a. The configuration of these centers relative to the previously established L-tryptophan methine proton (11a-H) was established by irradiation of 20-Me, which elicited a 5% nOe to *anti* 11-H and only a 2% nOe to *syn* 11-H. A NOESY experiment indicated nOe's from 20-Me to both methylene protons at C-11; the nOe for the *anti* proton at C-11 was of higher intensity, indicating that the 1,1-dimethylallyl group and, hence, the indoline proton are on the opposite face from 11a-H. In confirmation, a 7% nOe to the aromatic proton at position 10 was observed upon irradiation of *syn* 11-H. Examination of Dreiding models shows that this nOe would occur only for the stereoisomer having the 1,1-dimethylallyl group on the opposite face of the molecule from 11a-H. Therefore, the absolute configurational assignments for verrucofortine are complete.

Many diketopiperazine-type natural products are known, several of which are closely related to verrucofortine. Roquefortine [**11**] from *Penicillium roqueforti* (24), a fungus used in the production of blue cheese, comprises a similarly modified tryptophan, having a reverse isoprene substituent at the 3 position of the indoline nucleus plus a dehydrohistidine. Roquefortine was initially described as a neurotoxin (24), but this claim was later withdrawn (25). Amauromine[1] [**12**] is a symmetrical diketopiperazine derived from two modified tryptophans, each bearing a 1,1-dimethylallyl group (27); its structure has been confirmed by total synthesis (28). Amauromine has been reported to be a potent vasodilator (29). It is noteworthy that the methine proton signal 5a of verrucofortine [**8**] is a lower field (6.01 ppm) than that in roquefortine (5.70 ppm) (24) and amauromine (5.42 ppm) (27), due to acetylation of the indole in **8**.

The neurotoxicity of verrucofortine was examined in mice. It was our hypothesis that metabolites other than verrucosidin may be responsible for the reported tremorgenic activity, and verrucofortine [**8**] appeared to be a likely candidate. However, ip injections of **8** in dosages as high as 160 mg/kg caused only temporary distress, labored breathing, and ataxia, the ataxia lasting several hours. No tremorgenic activity was observed. Furthermore, crude extracts of the fungus also failed to produce tremors. Further studies will be required to identify the putative tremorgen.

## EXPERIMENTAL

Nmr spectra were recorded on JEOL FX-90Q, Bruker AM-400 and IBM NR-300 spectrometers. Ir spectra were obtained with a Perkin-Elmer 727 spectrophotometer or Nicolet 6000 Ft-ir; uv spectra were recorded on a Cary 14 uv spectrophotometer. Optical rotations were recorded at room temperature (22–24°) using a Rudolph Autopol III polarimeter. Cc and tlc were performed on Merck Si gel 60. Many separations were performed by flash chromatography (30) or on a Harrison Research 7924 Chromatotron using 1- and 2-mm plates coated with Merck Si gel PF-254 containing $CaSO_4$. High resolution mass spectra were obtained on a VG-Micromass 7070F spectrometer, while routine mass spectra were obtained on an LKB 9000 spectrometer. All mass spectra were obtained by electron impact. All melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Hplc analyses and separations were conducted on an IBM Instruments LC/9533 system equipped with a uv detector at 254 nm. Reversed-phase (Alltech $C_{18}$) analytical or preparative columns were used. A solution of $Eu(fod)_3$ (Aldrich) in $CDCl_3$ (0.14 M) was used for shift reagent studies. Toxicity studies were carried out on male ICR Swiss

[1]Amauromine was independently isolated and characterized by Laws and Mantle (26); the metabolite was assigned the name nigrifortine in their work.

mice weighing 25–35 g. Compounds tested were prepared in propylene glycol-DMSO (4:1) solutions (25) and administered ip in a volume of 0.1 ml.

CULTIVATION AND EXTRACTION OF FUNGAL PADS.—*P. verrucosum* var. *cyclopium* (deposited as PREM 47750 in the National Collection of Fungi, Plant Protection Research Institute, Pretoria, South Africa) was grown in 100 × 30 × 10 cm aluminum baking pans containing a medium consisting of sucrose (20 g), instant nonfat dry milk (30 g), instant potatoes (20 g), and gentamicin sulfate (10 mg) suspended in 1 liter of deionized $H_2O$. The pan was tightly covered with aluminum foil and autoclaved (121°, 2 × 1 h), then inoculated with a suspension of mycelium (grown on seed corn) in sterilized $H_2O$ containing 2 drops of Tween 80. The incubation mixture was allowed to stand at room temperature (24–25°) for 14 days.

ISOLATION OF METABOLITES.—The green fungal pads were collected, air-dried, chopped in a Waring blender, and extracted 12 h with $Et_2O$ in a Soxhlet apparatus. The extract was evaporated, and the residue (ca. 2 g from each pad), dissolved in a minimum amount of EtOAc-hexane (1:3), was partitioned by flash chromatography on a 5 × 15 cm Si gel column, eluted with an EtOAc/hexane gradient running from 25% to 100% EtOAc. A total of 53 2.5-ml fractions were collected.

Fractions 25–30 contained a single component (tlc $R_f$ 0.78, MeCN-$CH_2Cl_2$, 1:10) that was crystallized from EtOH to yield 16.9 mg of ergosterol as white flakes, mp 153–155° (authentic ergosterol mp 153–155°); $^1$H nmr, $^{13}$C nmr, and ms matched the authentic sample.

Fractions 34–40 contained one major component (tlc $R_f$ 0.67, MeCN-$CH_2Cl_2$, 1:5) that was further purified by flash chromatography (MeCN-$CH_2Cl_2$, 1:10) to give 90 mg of verrucosidin [**1**]. Recrystallization from $Et_2O$ gave colorless plates: mp 90–92°, lit. (2) mp 90–91°; $^1$H nmr ($CDCl_3$) δ 1.20 (3H, d, $J$=6.8 Hz, 15-Me), 1.43 (3H, s, 12-Me), 1.44 (3H, s, 6-Me), 1.48 (3H, s, 14-Me), 1.91 (3H, br s, 8-Me), 1.96 (3H, br s, 10-Me), 2.05 (6H, 2 × s, 2-Me and 4-Me), 3.45 (1H, s, 13-H), 3.50 (1H, br s, 7-H), 3.83 (3H, s, 3-OMe), 4.13 (1H, q, $J$=6.8 Hz, 15-H), 5.47 (1H, br s, 11-H), 5.81 (1H, br s, 9-H); ms *m/z* (rel. int.) [M]$^+$ 416 (4), 153 (12), 127 (100), 97 (16).

Fractions 43–47 were shown by tlc (MeCN-$CH_2Cl_2$, 1:5) to contain at least 6 components. The fractions were combined, evaporated, dissolved in a minimum of solvent (MeCN-$CH_2Cl_2$, 1:10), and repartitioned on Si gel with the Chromatotron. Elution was begun with 20 ml of the same solvent and polarity gradually increased to a 2:1 ratio. Fractions 5–7 gave 6 mg of normethylverrucosidin [**3**] as a yellow oil, $[\alpha]^{22}$D +31.6° (*c*=0.36, MeOH); uv λ max (EtOH) 283 (ε 6648), 238 (ε 11257); ir ($CDCl_3$) 1029, 1035, 1055, 1705, 2857, 2931, 2970 cm$^{-1}$; $^1$H nmr ($CDCl_3$) δ 1.20 (3H, d, $J$=6.8 Hz, 15-Me), 1.43 (6H, s, 6-Me and 12-Me), 1.48 (3H, s, 14-Me), 1.91 (3H, br s, 8-Me), 1.96 (3H, br s, 10-Me), 2.01 (3H, s, 4-Me), 3.43 (1H, s, 13-H), 3.50 (1H, br s, 7-H), 3.83 (3H, s, 3-OMe), 4.13 (1H, q, $J$=6.8 Hz, 15-H), 5.47 (1H, br s, 11-H), 5.50 (1H, s, 2-H), 5.87 (1H, br s, 9-H); $^{13}$C nmr δ 8.3 (q), 13.8 (q), 15.2 (q), 15.6 (q), 18.5 (q), 18.8 (q), 21.9 (q), 56.2 (q), 60.8 (s), 64.6 (d), 67.4 (s), 67.4 (d), 76.7 (d), 80.0 (s), 88.9 (s), 108.4 (s), 127.9 (s), 131.4 (d), 133.1 (d), 134.6 (s), 158.2 (s), 163.7 (s), 170.8 (s); ms *m/z* (rel. int.) [M]$^+$ 402 (1), 387 (7), 369 (4), 331 (3), 317 (3), 298 (10), 276 (5), 267 (4), 251 (4), 235 (4), 201 (7), 183 (13), 166 (12), 151 (13), 139 (36), 127 (100), 109 (13), 85 (28); hrms *m/z* [M−Me]$^+$ 387.1807 (calcd for $C_{22}H_{27}O_6$, 387.1807).

Fractions 13–16 from the Chromatotron separation contained a major component contaminated by a trailing impurity (tlc MeCN-$CH_2Cl_2$, 1:5). Further purification by flash chromatography (hexane-EtOAc-MeCN, 20:3:2) and then hplc (MeOH-$H_2O$, 2:1) gave 26 mg of verrucofortine [**8**] as a white glass-like material, $[\alpha]^{25}$D −116.2° (*c*=0.5, MeOH); uv λ max (EtOH) 247 (ε 9590), 277 (ε 1742), 285 (ε 1526); ir (KBr) 1420, 1660, 2860, 2940, 3075, 3200 cm$^{-1}$; $^1$H nmr ($CDCl_3$) δ 0.91 (3H, d, $J$=6.3 Hz, 14-Me), 0.99 (3H, d, $J$=6.3 Hz, 15-Me), 0.99 (3H, s, 19-Me), 1.15 (3H, s, 20-Me), 1.56 (1H, m, $J$=5.0, 14.5, 9.5 Hz, 12-CH), 1.72 (1H, m, $J$=5.0, 6.3, 10.0 Hz, 13-CH), 2.00 (1H, m, $J$=14.5, 10.0, 4.0 Hz, 12-CH′), 2.39 (1H, dd, $J$=12.5, 11.5 Hz, 11*anti*-CH), 2.61 (1H, dd, $J$=12.5, 5.9 Hz, 11*syn*-CH), 2.63 (3H, s, $COCH_3$), 3.85 (1H, m, $J$= 11.5, 5.9, 1.0 Hz, 11a-CH), 3.95 (1H, m, $J$=9.5, 4.0, 1.0 Hz, 3-CH), 5.11 (1H, d, $J$=17.5 Hz, 18-CH *trans*), 5.12 (1H, d, $J$=11.0 Hz, 18-CH *cis*), 5.80 (1H, m, $J$=17.5, 11.0 Hz, 17-CH), 6.01 (1H, br s, 5a-CH), 6.40 (1H, s, exchanges with $D_2O$, temperature and solvent dependent, 2-NH), 7.14 (1H, td, $J$=7.3, 1.2 Hz, 9-CH), 7.28 (1H, d, $J$=7.3 Hz, 10-CH), 7.32 (1H, t, $J$=7.5 Hz, 8-CH), 8.00 (1H, br s at 25°, br d at 42°, $J$=8.0, 7-CH); $^{13}$C nmr δ 21.1 (q, C-14), 22.3 (q, C-20), 23.18 (q, C-15), 23.23 (q, C-19), 23.5 (q, $COCH_3$), 24.4 (d, C-13), 35.8 (t, C-11), 38.9 (t, C-12), 40.4 (s, C-16), 53.2 (d, C-3), 59.0 (d, C-11a), 60.8 (s, C-10b), 79.5 (d, C-5a), 114.6 (t, C-18), 119.2 (d, C-7), 124.5 (d, C-9), 124.5 (d, C-10), 129.0 (d, C-8), 132.1 (s, C-10a), 143.1 (d, C-17), 143.3 (s, C-6a), 166.0 (s, C-4), 169.0 (s, C-1), 170.1 (s, $COCH_3$); hrms *m/z* (rel. int.) [M]$^+$ 409.2381 (25%), 367 (17), 340 (11), 298 (100), 270 (8), 157 (3), 130 (33), 86 (5) (calcd for $C_{24}H_{30}N_3O_3$, 409.2365).

Fractions 17–23 from the Chromatotron separation contained one major component contaminated with a minor amount of **8**. Purification by preparative tlc (MeCN-$CH_2Cl_2$, 1:5) followed by flash chromatography (hexane-EtOAc-MeCN, 10:3:2) gave cyclopenin [**4**] (11.3 mg), which was recrystallized

from MeOH: mp 182–184°, lit. (31) mp 183–184°; $^1$H nmr ($CDCl_3$) δ 3.24 (3H, s), 4.00 (1H, s), 6.6–6.7 (2H, m), 7.0–7.6 (7H, m), 8.83 (1H, br s); $^{13}$C nmr ($CDCl_3$) δ 31.4 (q), 65.0 (d), 70.4 (s), 121.0 (d), 125.2 (d), 126.1 (2×d), 127.2 (s), 128.2 (2×d), 129.1 (d), 130.3 (s), 131.6 (d), 132.5 (d), 134.6 (s), 166.0 (s), 167.7 (s); ms *m/z* (rel. int.) $[M]^+$ 294 (84), 237 (44), 236 (44), 161 (79), 146 (30), 119 (100).

Fractions 33–36 from the Chromatotron separation contained one component that was recrystallized (EtOH) to yield 3.2 mg of 3-*O*-methylviridicatin [**6**] mp 247–249°, lit. (32) mp 248–249°; $^1$H nmr ($CDCl_3$) δ 3.84 (3H, s), 7.13 (1H, t, $J$=8 Hz), 7.23 (1H, d, $J$=8 Hz), 7.39 (2H, d, $J$=9 Hz), 7.43–7.56 (5H, m), 11.07 (1H, br s); $^{13}$C nmr ($CDCl_3$) δ 60.4, 116.0, 121.0, 122.6, 126.5, 128.2, 128.4 (2), 128.8, 129.4 (2), 133.4, 135.4, 139.5, 145.2, 161.0; ms *m/z* (rel. int.) $[M]^+$ 251 (100), 233 (40), 223 (26), 220 (31), 208 (16), 180 (14), 165 (9), 152 (13); hrms *m/z* $[M]^+$ 251.0922 (calcd for $C_{16}H_{13}NO_2$, 251.0946).

Fractions 48 and 49 from the original flash chromatographic separation were shown by tlc (MeCN-$CH_2Cl_2$, 1:1) to contain four components of which the major one, cyclopenol [**5**] ($R_f$ 0.50) was separated by Chromatotron using the tlc solvent system above. The product (8.5 mg) was recrystallized (MeOH) to give clear needles mp 215–216° dec, lit. (31) mp 215° dec; $^1$H nmr ($d_6$-acetone) δ 3.14 (3H, s), 4.10 (1H, s), 6.10–6.26 (2H, m), 6.66–7.62 (6H, m), 8.43 (1H, br s), 9.70 (1H, br s); $^{13}$C nmr ($d_4$-MeOH) δ 31.7 (q), 65.9 (d), 71.7 (s), 113.9 (d), 117.1 (d), 118.5 (d), 122.3 (d), 126.1 (d), 128.0 (s), 130.3 (d), 132.2 (d), 133.5 (s), 134.0 (d), 136.4 (s), 158.4 (s), 168.4 (s), 168.7 (s); ms *m/z* (rel. int.) $[M]^+$ 310 (51), 253 (100), 252 (87), 196 (20), 161 (40), 146 (22), 119 (56). The other components of this fraction were minor and were not identified.

ACID HYDROLYSIS OF VERRUCOFORTINE.—Compound **8** (100 mg, 0.244 mmol) was refluxed for 3 h in 20 ml of 1 M methanolic HCl. Evaporation left a viscous oil that was subjected to flash chromatography ($CH_2Cl_2$-MeCN-$H_2O$, 14:7:1). A component giving a dark blue color with Ehrlich's reagent was collected and purified further by preparative hplc on a $C_{18}$ column (MeOH-$H_2O$, 3:2) to yield *cyclo*-L-tryptophan-L-leucine [**9**] (11.7 mg), which was recrystallized from MeOH/$H_2O$: mp 264–266° dec, lit. (17) mp 265–268° dec, lit. (18) 262–262° dec; $[\alpha]^{25}D$ +56.3° ($c$=0.17, HOAc), lit. (17) $[\alpha]^{25-28}D$ +48.0° ($c$=0.4, HOAc), lit. (18) $[\alpha]^{18-22}D$ +45.6° ($c$=1.0, HOAc); $^1$H nmr ($d_6$-acetone) δ 0.43 (1H, m), 0.59 (3H, d, $J$=6.5 Hz), 0.68 (3H, d, $J$= 6.5 Hz), 1.03 (1H, m), 1.36 (1H, m), 3.29 (2H, t, $J$=4 Hz), 3.67 (1H, dd, $J$=4.5 Hz), 4.23 (1H, t, $J$=4 Hz), 6.98–7.68 (5H, m); $^{13}$C nmr ($d_6$-acetone) δ 21.7, 23.2, 24.5, 30.5, 44.9, 54.0, 57.0, 110.2, 112.1, 119.9, 120.1, 122.2, 125.5, 125.7, 129.0, 168.1, 168.4; ms *m/z* (rel. int.) $[M]^+$ 299 (3), 170 (1), 156 (1), 143 (2), 130 (100).

SYNTHESIS OF *CYCLO*-L-TRYPTOPHAN-L-LEUCINE DIKETOPIPERAZINE [**9**].—Diketopiperazine **9** was prepared by the method of Suzuki *et al.* (18). Compound **9** was obtained as white flakes mp 265–267° dec; $[\alpha]^{25}D$ +52.4° ($c$=0.17, HOAc); $^1$H nmr and ms were identical to the material isolated from verrucofortine.

EPIMERIZATION OF **9**.—A solution of synthetic **9** (23 mg) in 2 ml of EtOH and 2 ml of $Et_3N$ was refluxed under $N_2$ for 6 days (19) at which time no further changes in the ratio of starting material to product could be observed by tlc (MeCN-$H_2O$, 9:1). The solution was evaporated and the residue fractionated by flash chromatography (MeCN-$H_2O$, 13:1) followed by preparative hplc on a $C_{18}$ column (MeOH-$H_2O$, 3:2) to yield two components: **9** (14.2 mg, 62%, retention time 8.7 min) and its epimer (2.2 mg, 10%, retention time 9.5 min). Compound **9** mp 262–268° dec, $[\alpha]^{25}D$ +53.2° ($c$=0.17, HOAc); $^1$H nmr and ms were identical with starting material. Epimerized **9** mp 199–205° dec; $[\alpha]^{25}D$ +76.3° ($c$=0.065, HOAc); $^1$H nmr ($d_6$-acetone) δ 0.74 (6H, dd, $J$=6.5 Hz), 1.27–1.87 (3H, m), 3.05–3.29 (3H, m), 4.19 (1H, t, $J$=4 Hz), 6.81–7.66 (5H, m). The NH protons were exchanged with HDO in the acetone-$d_6$.

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