Metabolism Studies of Indole Derivatives Using a Staurosporine Producer, *Streptomyces staurosporeus*†

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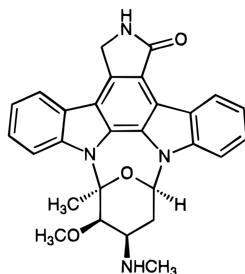
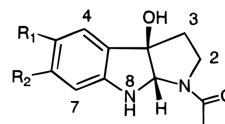
From a tryptophan metabolic study, 3-(hydroxyacetyl)indole, indole-3-carboxaldehyde, and *o*-aminobenzoic acid were obtained as tryptophan metabolites from a staurosporine (**1**) producer, *Streptomyces staurosporeus*. A new tryptamine metabolite, (3a*R*,8a*S*)-1-acetyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-3a-ol (**2**), was isolated along with N_b-acetyltryptamine using *S. staurosporeus* fed with tryptamine. The unusual compound **2** was proposed as a further metabolite of N_b-acetyltryptamine through an enzymatic oxidative cyclization. Examination of the metabolites from the feeding of 5- and 6-fluorotryptamines using the same microorganism afforded the two novel compounds **3** and **4** as the 5- and 6-fluoro derivatives of **2**. However, 5-hydroxytryptamine failed to generate the 5-hydroxy derivative of **2**. Indole-ring-substituted metabolites of staurosporine (**1**) were not observed.

Streptomyces staurosporeus (AM-2282) was found to produce the potent protein kinase C inhibitor staurosporine (**1**) in 1977.[1] Staurosporine has very interesting other biological activities, including antifungal,[1] hypotensive,[2] and platelet aggregation activities.[3] However, its cytotoxic activity against tumor cells, based on the inhibition of protein kinase C, is considered the most important aspect of its biological profile.[4,5] Some staurosporine derivatives have been found to be capable of reversing the MDR phenotype to sensitive cell lines;[6−8] therefore, indolocarbazole-type alkaloids could potentially serve as anticancer drugs or as therapeutic adjuncts. Some chemical modifications have been performed to lower its toxicity,[9−12] and biological modifications achieved through mutation,[13] genetic engineering, and precursor-directed biosynthesis are of some interest. From a biosynthetic view, tryptophan has been identified as a precursor of **1**;[14] therefore, metabolism studies of indole derivatives may develop an understanding of staurosporine biosynthesis or directly produce novel staurosporine derivatives via precursor-directed biosynthesis.

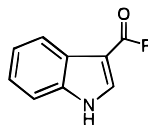


1 Staurosporine

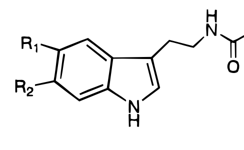
For these purposes, the metabolism of tryptophan and tryptamine were evaluated using *S. staurosporeus*. Tryptophan was principally metabolized to *o*-aminobenzoic acid (40%) along with two minor derivatives, 3-(hydroxyacetyl)indole (**5**, 4%) and indole-3-carboxaldehyde (**6**, 1%). Tryptamine, another precursor of staurosporine,[15] was principally biotransformed into N_b-acetyltryptamine (**7**, 50%) and a new minor metabolite (3a*R*,8a*S*)-1-acetyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-3a-ol (**2**, 8%). The structure elucidation and the unambiguous assignment of **2** by various NMR techniques, including HETCOR and HMBC, are described here. Tryptamine derivatives, including 5-fluorotryptamine, 6-fluorotryptamine, and 5-hydroxytryptamine, were also evaluated for their ability to produce derivatives of compound **2**.



2 $R_1 = R_2 =$ H
3 $R_1 =$ F; $R_2 =$ H
4 $R_1 =$ H; $R_2 =$ F



5 R= CH_2OH
6 R= H



7 $R_1 = R_2 =$ H
8 $R_1 =$ F; $R_2 =$ H
9 $R_1 =$ H; $R_2 =$ F

Alkaloid **2** was originally synthesized as a racemic mixture through the photooxygenation of N_b-acetyltryptamine (**7**) during a study of the absolute configuration of brevianamide E.[16] However, this is the first report of **2** as a fungal metabolite or from a natural source. Two new fluoro-substituted compounds **3** and **4** were also obtained by the feeding of 5-fluoro- and 6-fluorotryptamines, respectively, but the organism failed to metabolize 5-hydroxytryptamine into the corresponding hydroxy derivative of **2**.

For the purpose of the biological modification of staurosporine, several other indole derivatives, including indole-3-acetonitrile, indole-3-acetic acid, indole-3-acetamide, 5-fluorotryptophan, 6-fluorotryptophan, 5-hy-

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Table 1. ^{1}H NMR Spectral Data (in $CDCl_3$) of Derivatives **2**−**4**

proton	2	3	4
2	3.30 (ddd, 9, 9, 9)	3.27 (ddd, 9, 9, 9)	3.24 (ddd, 9, 9, 9)
	3.69 (ddd, 9, 9, 3)	3.66 (ddd, 9, 9, 3)	3.64 (ddd, 9, 9, 3)
3	2.43 (m)	2.41 (m)	2.38 (m)
	2.70 (m)	2.68 (m)	2.65 (m)
4	7.29 (bd, 6.9)	6.98 (dd, 8.0,[a] 2.6)	7.17 (dd, 8.3, 5.6[a])
5	6.79 (dd, 7.4, 6.9)		6.42 (ddd, 9.4,[a] 8.3, 2.3)
6	7.16 (dt, 7.4, 1.1)	6.84 (td, 8.7,[a] 2.6)	
7	6.61 (bd, 7.9)	6.51 (dd, 8.7, 4.1[a])	6.26 (dd, 9.8,[a] 2.3)
8a	5.29 (s)	5.30 (s)	5.28 (s)
$COCH_3$	2.01 (s)	1.95 (s)	1.94 (s)
−NH− (or OH)	5.25 (bs)	5.15 (bs)	5.41 (bs)

[a] Coupling constants are due to fluorine substitution via 3- or 4-bonds.

droxytryptophan, indole-3-pyruvate, and indole-3-propionic acid were also evaluated as potential precursors for the biosynthesis of staurosporine derivatives.

Tryptophan was added to the fermentation medium B. The extract of the harvested culture broth was examined by TLC, and the metabolites that did not appear in the culture without added tryptophan were isolated and examined for their structures. Three metabolites were isolated and identified as *o*-aminobenzoic acid (anthranilic acid), 3-(hydroxyacetyl)indole (**5**), and indole-3-carboxaldehyde (**6**). Anthranilic acid is the precursor of tryptophan biosynthesis[17] and has been found as a tryptophan degradation product in fungi.[18−20] Reports of the occurrence of compound **5** are limited. It has been found in liquid cultures of *Lactarius deliciosus*,[21] the sponge *Tedania ignis*,[22] and in marine red algae.[23] Compound **6** has been found in a variety of natural sources, including plants,[24] red algae,[25] and microorganisms.[18,19,26] *S. staurosporeus* is also able to transform indole-3-acetonitrile to **6** (4%).

In a preliminary examination of the ability of the organism to perform precursor-directed biosynthesis, a variety of indole derivatives including indole-3-acetonitrile, indole-3-acetic acid, indole-3-acetamide, 5- and 6-fluorotryptophans, 5-hydroxytryptophan, indole-3-pyruvate, indole-3-propionic acid, tryptamine, 5- and 6-fluorotryptamines, and 5-hydroxytryptamine were utilized. Although no bis-indole or staurosporine derivatives were found in these precursor feeding experiments, some novel metabolites were discovered in the tryptamine and 5-fluoro- and 6-fluorotryptamine feeding experiments.

When tryptamine hydrochloride was added to the fermentation medium, two major products were detected by TLC analysis, and staurosporine production was not affected. The major product, N_b-acetyltryptamine (**7**, 50%), was identified on the basis of a comparison of physical data (TLC, ^{1}H and ^{13}C NMR) with those of a synthetic sample. Alkaloid **2** (8%) was obtained as an optically active colorless gum. The EIMS showed a molecular ion peak at m/z 218, $C_{12}H_{14}N_2O_2$ (HREIMS, obsd m/z 218.10658; calcd 218.10553), one oxygen unit difference from N_b-acetyltryptamine. The UV (λ_{max} (log ϵ) 211 (4.40), 236 (4.13), 292 nm (3.58)) and IR spectra (ν_{max} 3379 (−NH), 1622, 1464 cm^{-1} (aromatic C=C)) suggested the presence of an indoline rather than an indole moiety. From the ^{1}H NMR spectrum, four contiguously coupled aromatic protons, a heteroatom-substituted methine proton (s, δ 5.29), a heteroatom-attached proton (bs, δ 5.25), four coupled aliphatic protons, and a singlet for the acetyl methyl protons (s, δ 2.01) were deduced. Compared to the ^{1}H NMR of N_b-acetyltryptamine, the aromatic singlet of H-2 (δ 7.03) in **7** was replaced by a signal at δ 5.29 (s), indicating that the double bond at the 2,3-position was modified. Four aliphatic protons were found to have the complex coupling patterns of nonmagnetically equivalent methylene protons, especially H_2-2 (δ 3.30 and 3.69), indicating that they lose the free rotation characteristic of **7**, and, in addition, the downfield shift of C-2 (δ 47.0) in **2** compared to C-9 (δ 39.8) in **7** indicated that cyclization had occurred. The four aromatic and four aliphatic protons (δ 3.69 and δ 3.30 (H_2-2), coupled to δ 2.43 and 2.70 (H_2-3)) were further correlated by $^{1}H-^{1}H$ DQF-COSY. The four aliphatic protons are on the C-ring from a HETCOR experiment that showed the correlation of δ 2.43 and 2.70 (H_2-3) to δ 36.4 (C-3) and of δ 3.30 and 3.69 (H_2-2) to δ 47.0 (C-2) and by long-range C−H coupling (selective INEPT and HMBC). The long-range correlations between H_2-2 and C-8a and H-8a and C-2 in the HMBC spectrum and the complexity of H_2-2 are the primary evidence for the cyclization. The ^{1}H resonance of H-8a in the NMR spectrum is also in accord with that reported previously for the synthesized racemate (other protons were not assigned specifically in the literature).[16] N_b-Substituted tryptamine has been used to synthesize related racemic compounds via photosensitized oxygenation.[16,27,28]

From this information, the structure of compound **2** was proposed as 1-acetyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-3a-ol, and the complete structure and unambiguous assignment of the ^{1}H and ^{13}C resonances were established by HETCOR, HMBC, and selective INEPT techniques. Long-range C−H correlations of compounds **2** and **3** obtained from HMBC are shown in Table 3, and the unambiguous NMR assignments of the three compounds (**2**−**4**) are shown in Tables 1 and 2. The stereochemistry of **2** was determined by circular dichroism (CD) and cyclization chemistry. The CD spectrum showed a positive Cotton effect (λ 209 ($\Delta\epsilon$ +11.8), 243 ($\Delta\epsilon$ +7.72), 301 ($\Delta\epsilon$ +2.01) nm); therefore, the absolute configuration was tentatively assigned as 3a(*R*) by a Dreiding model and comparison with nortryptoquivaline (negative $^{1}L_B$ band).[29] The absolute stereochemistry of nortryptoquivaline, which has the same substitutions at the benzylic carbon in the indoline ring, was determined as an opposite configuration to compound **2** by X-ray crystallography.[30] The 8a(*S*) configuration was determined on the basis of the *cis* configuration of the ring junction deduced from cyclization chemistry. When the deuterium-labeled precursor, [α-$^{2}H_2$]-tryptamine, was fed, both of the α-$^{2}H_2$ in tryptamine were retained in **2** and **7** according to the

Table 2. ^{13}C NMR Spectral Data (in $CDCl_3$) of Derivatives **2**–**4**

carbon	**2**[a,b]	**3**[a–c]	**4**[a,c]
2	47.0 (t)	47.0 (t)	47.0 (t)
3	36.4 (t)	36.7 (t)	36.7 (t)
3a	86.6 (s)	86.6 (s)	85.9 (s)
3b	129.1 (s)	131.0 (s)	124.5 (s, $J = 9$)
4	123.5 (d)	110.7 (d, $J = 24$)	124.8 (d, $J = 23$)
5	119.2 (d)	157.1 (s, $J = 236$)	105.6 (d, $J = 23$)
6	130.4 (d)	116.9 (d, $J = 23$)	164.8 (s, $J = 242$)
7	110.2 (d)	110.9 (d, $J = 8$)	97.5 (d, $J = 26$)
7a	149.4 (s)	145.3 (s)	151 (s, $J = 12$)
8a	81.4 (d)	82.4 (d)	81.9 (d)
C=O	170.8 (s)	170.9 (s)	170.7 (s)
CO*C*H$_3$	22.1 (q)	22.0 (q)	21.9 (q)

[a] Multiplicity determined by APT. [b] Assignments were made using the HETCOR, selective INEPT, and HMBC techniques. [c] Coupling constants (*J*, Hz) are due to carbon–fluorine coupling via 1-, 2-, or 3-bonds.

Table 3. Long Range $^1H-^{13}C$ Corelation Data from HMBC for Derivatives **2** and **3**

	2		**3**	
^1H	J_2	J_3	J_2	J_3
2	C-3	C-3a, 8a	C-3	C-3a, 8a
3	C-2, 3a	C-3b	C-2, 3a	C-3b
4	C-3b	C-3a, 6, 7a	C-5	C-3a, 6, 7a
5	C-4, 6	C-3b, 7		
6		C-4, 7a	C-5	C-4, 7a
7	C-6	C-3b, 5		C-3b, 5
8a	C-3a	C-2, 3b, 7a	C-3a	C-2, 3b, 7a
CO*C*H$_3$	*C*=O		*C*=O	

^1H-NMR spectra, which are described in the Experimental Section.

Fluoro-derivatives **3** (11%) and **4** (13%) along with the major products, fluoro-N_b-acetyltryptamines **8** (60%) and **9** (36%), were obtained from individual feeding experiments with 5-fluoro- and 6-fluorotryptamine. The resonances and coupling patterns of protons in the ^1H NMR spectra for the tetrahydropyrrole rings of **3** and **4** are identical to those of compound **2**. Protons in the aromatic region showed coupling with the fluorine, and the coupling constants are shown in Table 1. The ^{13}C NMR spectra were in agreement with the proposed structures. 5-Hydroxytryptamine (serotonin) could not be biotransformed to a corresponding tricyclic metabolite under these conditions. The biotransformation of 5-hydroxytryptamine by *S. staurosporeus* is reported elsewhere.

In conclusion, the attempts at precursor-directed biosynthesis of staurosporine analogues using various indole derivatives have not been successful thus far, indicating that the biosynthetic enzymes in staurosporine biosynthesis in *S. staurosporeus* are substrate specific and may not be inducible by other indole derivatives. Alternatively, the yield of any derivatives may be too low to be obtained preparatively. Tryptamine and 5-fluoro- or 6-fluorotryptamine were biotransformed stereospecifically to hexahydropyrrolo[2,3-*b*]indol-3a-ol-type compounds, probably through N_b-acetyltryptamine (or through 5-fluoro- or 6-fluoro-N_b-acetyltryptamine) by *S. staurosporeus.*

Experimental Section

General Experimental Conditions. One- and two-dimensional ^1H NMR spectra were recorded on either a Nicolet NT-360 instrument operating at 360 MHz or a Varian XL-300 instrument operating at 300 MHz. TMS or $CHCl_3$ residue, were used as an internal standards ($\delta_{TMS} = 0$ ppm; $\delta_{CHCl_3} = 7.24$ ppm), and chemical shifts are reported in ppm on the δ scale; ^{13}C NMR, APT, and selective INEPT experiments were conducted in $CDCl_3$ using a Nicolet NT-360 (360 MHz) spectrometer operating at 90.8 MHz. ^{13}C-NMR multiplicity was determined using APT experiments. HMBC was performed on a GE Omega 500 MHz instrument (499.9 MHz for ^1H NMR) with the standard program. Tryptophan, tryptamine HCl, 5-fluorotryptamine HCl, 6-fluorotryptamine, 5-hydroxytryptamine creatinine sulfate complex, and other indole derivatives were purchased from Sigma. Lithium aluminum deuteride was purchased from Cambridge Isotope Laboratories (Andover, MA).

Maintenance and Fermentation of *S. staurosporeus.* *S. staurosporeus* strain NRRL 11184 was generously supplied in the lyophilized form by Dr. J. L. Swezey of the National Center for Agricultural Utilization Research, ARS, USDA, Peoria, IL. The activation, maintenance, and fermentation of *S. staurosporeus* are the same as previously described.[14,31] Two medium systems with different sugar sources were used for the biotransformation of tryptophan and tryptamine. Medium A (3 g of glucose, 1.5 g of soybean, and 0.4 g of $CaCO_3$ in 100 mL of H_2O) was used for tryptamine and all other metabolism studies, and medium B (3 g of xylose, 1.5 g of soybean, and 0.4 g of $CaCO_3$ in 100 mL of H_2O) was used for tryptophan metabolism.

Tryptophan Feeding Experiment. Medium B was used in this experiment. Tryptophan (100 mg/100 mL) was added in a cold sterilized manner (filtration). The fermentation and purification methods are the same as described above. All of the metabolites could be detected by UV on either TLC or Prep-TLC plates. Three metabolites were isolated by preparative TLC with the eluent system $CHCl_3$–EtOAc (1:1).

Anthranilic acid (*o*-aminobenzoic acid): mp 132–140 °C; EIMS: M^+ *m/z* 137 (77) and 119 (100).

3-(Hydroxyacetyl)indole (5): ^{13}C NMR (360 MHz, in $CDCl_3$) δ 193.1 (s, C=O), 136.1 (s, C-7a), 130.6 (d, C-2), 125.0 (s, C-2a), 124.1 (d, C-4)[a], 123.1 (d, C-5)[a], 121.9 (d, C-6)[a], 114.3 (s, C-3), 111.6 (d, C-7), and 65.3 (t, CH_2OH) ([a]not unambiguously assigned); ^1H NMR (300 MHz, in $CDCl_3$) δ 8.26 (1H, m), 7.91 (1H, d, $J = 2.9$ Hz, H-2), 7.44 (1H, m), 7.32 (2H, m), and 4.77 (2H, s, CH_2OH); EIMS *m/z* [M^+] 175 (22) and 144 (100).

Indole-3-carboxaldehyde (6): ^1H NMR δ 10.08 (1H, s), 8.33 (1H, m), 7.84 (1H, d), 7.45 (1H, m), and 7.34 (1H, m); EIMS (M^+) *m/z* 145. The structure was confirmed by comparison with a standard compound.

Preliminary Evaluation of the Feeding of Indole Precursors. For the purpose of biological modification of staurosporine, the indole derivatives, including 5-fluoro- and 6-fluorotryptophans, 5-hydroxytryptophan, tryptamine HCl, 5-fluoro- and 6-fluorotryptamines HCl, indole-3-acetonitrile, indole-3-acetic acid, indole-3-acetamide, indole-3-pyruvate, and indole-3-propionic acid, were evaluated as potential precursors for the biosynthesis of staurosporine derivatives. All of the precursors were added at the beginning of cell inoculation at a concentration of 30 mg/100 mL individually into the fermentation medium. After growing for 48 h, another 70 mg of the precursors were added individually to the medium to achieve a higher concentration. After full

growth, the mycelium-containing metabolites was adjusted to pH 10 and extracted by EtOAc (3 × 100 mL). The evaporated extract was examined by TLC ($CHCl_3$–MeOH; 10:1). No bis-indole derivatives other than staurosporine were found on TLC detected by UV and Dragendorff's reagent. The metabolites were also isolated by preparative TLC, and the ^{1}H NMR data were examined for each band that showed fluorescent quenching on TLC. However, no staurosporine analogs or bis-indole derivatives were found in these experiments, and staurosporine biosynthesis was not inhibited in most of the precursor feedings, except that 6-fluorotryptophan and 5-fluorotryptamine could inhibit staurosporine biosynthesis. Indole-3-acetonitrile and 6-fluorotryptamine have inhibitory effects on cell growth.

Tryptamine, 5-Fluorotryptamine, and 6-Fluorotryptamine Feeding Experiments. Tryptamine hydrochloride (100 mg) was added to the fermentation medium A (100 mL) with the inoculation of seed medium (2 mL), and the culture was allowed to grow for an additional 5 days. The other tryptamine derivatives were evaluated by the same method as tryptamine feeding.

Isolation and Purification of 2. Culture fermentation broth (100 mL) containing mycelia was extracted with EtOAc (3 × 100 mL). The pooled organic phase was dried (Na_2SO_4) and evaporated to dryness under vacuum at 40 °C, and compound **2** was isolated by column or by preparative TLC. TLC was carried out using silica gel GF_{254} (Merck) plates developed with $CHCl_3$–MeOH (10:1). Developed chromatograms were visualized by fluorescence quenching under 254 nm UV light. Preparative TLC was carried out using two consecutive solvent systems, $CHCl_3$–EtOAc (1:1) and $CHCl_3$–MeOH (10:1). The yield of compound **2** produced from *S. staurosporeus* was 8% based on the added tryptamine (100 mg). Column chromatography was carried out using Si gel (60 mesh). Columns were slurry packed in $CHCl_3$ and eluted with a mixture of $CHCl_3$–MeOH (50:1).

(3a*R*,8a*S*)-1-Acetyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-3a-ol (2): colorless gum; $[\alpha]^{25}_D$ +72° (*c* 0.75, MeOH); CIMS [M$^+$ + 1] *m/z* 219; EIMS *m/z* [M$^+$] 218 (100), 201 (5), 190 (33), 175 (13), 148 (75), 130 (35), 120 (21); HREIMS obsd *m/z* 218.1066; calcd 218.1055 (calcd for $C_{12}H_{14}N_2O_2$); UV (MeOH) λ max (log ϵ) 211 (4.40), 236 (4.13), 292 nm (3.58); IR ν max 3379 (–NH), 1622, 1614, 1464, 1456, 1314, 1200, 1061, 750 cm^{-1}; CD λ ($\Delta\epsilon$) 209 (+2.65), 224 (+0.69), 243 (+1.73), 269 (0), 301 (+0.45), 344 (0) nm; ^{1}H-NMR, see Table 1; ^{13}C-NMR, see Table 2.

***N*$_b$-Acetyltryptamine (7):** FAB [M + 1]$^+$ *m/z* 203. The structure of *N*$_b$-acetyltryptamine was confirmed by comparison of the spectral data and the R_f value on TLC with a synthetic product obtained from the acetylation of tryptamine using acetic anhydride in pyridine.

[α-$^2H_2$]-Tryptamine. [α-$^2H_2$]-Tryptamine was synthesized by reduction of 3-indolyl-acetonitrile (250 mg) using lithium aluminum deuteride (125 mg) in anhydrous ethyl ether (10 mL) at room temperature for 3 days. The remaining lithium aluminum deuteride was decomposed by adding EtOAc and H_2O dropwise at 0 °C. The reaction mixture was evaporated to remove ethyl ether, and the dried residue was separated by Si gel chromatography. The yield of [α-$^2H_2$]-tryptamine was 30%, with 50% of the 3-indolylacetonitrile recovered. Deuterium-labeled tryptamine was stored in an amber bottle at –20 °C. The purified product (50 mg) was added to the fermentation medium A, and the further procedures are the same as described above.

[α-$^2H_2$]-*N*$_b$-Acetyltryptamine (7) and [2-$^2H_2$]-(3aR,-8aS)-1-Acetyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]-indol-3a-ol (2). The isolated deuterium-labeled products **2** and **7** were examined by ^{1}H-NMR. The ^{1}H NMR data in the indole region and acetyl methyl group of compound **7** were the same as those of the unlabeled metabolite except that the proton resonances of α-H_2 (H_2-9, δ 3.60) had disappeared and the signals of the β-H_2 (H_2-8, δ 2.98) had become a singlet (original, t, J = 6.7 Hz). The ^{1}H NMR data in the aromatic region and acetyl methyl group were the same as those of the unlabeled metabolite, except that the proton resonances of H_2-2 (δ 3.30 and δ 3.69) were diminished and the signals of H_2-3 (δ 2.43 and 2.70) became a pair of geminal doublets (J = 14.5 Hz).

5-Fluoro-*N*$_b$-acetyltryptamine (8): colorless gum; EIMS *m/z* 220; ^{1}H-NMR ($CDCl_3$) δ 8.99 (1H, brs, *N*H), 7.23 (1H, dd, J = 8.9, 4.5, H-7), 7.17 (1H, dd, J = 9.6, 2.1, H-4), 6.98 (1H, s, H-2), 6.88 (1H, td, J = 9.0, 2.3, H-6), 5.99 (1H, brs, NH), 3.50 (2H, q, J = 6.7, H_2-9), 2.86 (2H, t, J = 6.7, H_2-8), 1.92 (3H, s, $COCH_3$). ^{13}C NMR 170.6 (s, N*C*OCH_3), 157.7 (s, J_{C-F} = 233, C-5), 132.9 (s, C-7a), 127.6 (s, J_{C-F} = 9, C-3a), 124.0 (d, C-2), 112.5 (s, J_{C-F} = 5, C-3), 112.0 (d, J_{C-F} = 10, C-7), 110.2 (d, J_{C-F} = 26, C-6), 103.3 (d, J_{C-F} = 23, C-4), 39.8 (t, C-9), 25.1 (t, C-8), and 23.3 (q, CO*C*H_3).

(3a*R*,8a*S*)-5-Fluoro-1-acetyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-3a-ol (3): HREIMS obsd *m/z* 236.0958, calcd 236.0961 (calcd for $C_{12}H_{13}N_2O_2F$); ^{1}H NMR and ^{13}C NMR are shown in Tables 1 and 2, respectively; $[\alpha]^{25}_D$ 0° (*c* 0.9, MeOH); UV (MeOH) λ max (log ϵ) 223 (3.52, sh), 239 (3.56), 303 nm (3.28); IR ν max 3391, 1634, 1588, 1456, 1215, 758 cm^{-1}; EIMS *m/z* [M$^+$] 236 (100), 219 (3), 208 (19), 193 (9), 177 (10), 166 (73), 148 (18), 138 (15), 122 (7), 111 (6).

6-Fluoro-*N*$_b$-acetyltryptamine (9): EIMS *m/z* 220; ^{1}H NMR ($CDCl_3$) δ 8.90 (1H, brs, *N*H), 7.46 (1H, dd, J = 8.9, 5.3, H-4), 7.03 (1H, dd, J = 9.7, 2.2, H-7), 6.96 (1H, s, H-2), 6.85 (1H, td, J = 9.0, 2.2, H-5), 5.89 (1H, brs, NH), 3.55 (2H, q, J = 6.7, H-9), 2.93 (2H, t, J = 6.7, H-8), 1.93 (3H, s, $COCH_3$). ^{13}C NMR δ 170.4 (s, N*C*OCH_3), 159.9 (s, J_{C-F} = 235, C-6), 136.3 (s, J_{C-F} = 12, C-7a), 123.9 (s, C-3a), 122.3 (d, J_{C-F} = 6, C-2), 119.2 (d, J_{C-F} = 5, C-4), 112.7 (s, C-3), 108.0 (d, J_{C-F} = 25, C-7), 97.6 (d, J_{C-F} = 19, C-5), 39.9 (t, C-9), 25.2 (t, C-8), 23.3 (q, CO*C*H_3).

(3a*R*,8a*S*)-6-Fluoro-1-acetyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-3a-ol (4): HREIMS obsd *m/z* 236.0948, calcd 236.0961 (calcd for $C_{12}H_{13}N_2O_2F$); ^{1}H NMR and ^{13}C NMR are shown in Tables 1 and 2, respectively; $[\alpha]^{25}_D$ +14° (c 0.3, MeOH); UV (MeOH) λ max (log ϵ), 242 (3.93), 295 nm (3.46); IR ν max 3351, 1622, 1495, 1456, 1424, 1321, 1142, 1065 cm^{-1}; EIMS: *m/z* [M$^+$] 236 (100), 219 (3), 208 (29), 193 (16), 177 (10), 166 (77), 148 (22), 138 (23), 123 (3), 111 (7).

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References and Notes

(1) Omura, S.; Iwai, Y.; Hirano, A.; Nakagawa, A.; Awaya, J.; Tsuchiya, H.; Takahashi, Y.; Masuma, R. *J. Antibiot.* **1977**, *30*, 275–282.
(2) Omura, S., Iwai, Y., Hirano, A. Japan Kokai 78 73,501; *Chem. Abstr.* **1978**, *89*, 178086b.
(3) Oka, S.; Kodama, M.; Takeda, H.; Tomizuka, N.; Suzuki, H. *Agric. Biol. Chem.* **1986**, *50*, 2723–2727.
(4) Tamaoki, T.; Nomoto, H.; Takahashi, I.; Kato, Y.; Morimoto, M.; Tomita, F. *Biochem. Biophys. Res. Commun.* **1986**, *135*, 397–402.
(5) Yamada, S.; Hirota, K.; Chida, K.; Kuroki, T. *Biochem. Biophys. Res. Commun.* **1988**, *157*, 9–15.
(6) Wakusawa, S.; Inoko, K.; Miyamoto, K.; Kajita, S.; Hasegawa, T.; Harimaya, K.; Koyama, M. *J. Antibiot.* **1993**, *46*, 353–355.
(7) Miyamoto, K.; Inoko, K.; Ikeda, K.; Wakusawa, S.; Kajita, S.; Hasegawa, T.; Takagi, K.; Koyama, M. *J. Pharm. Pharmacol.* **1993**, *45*, 43–47.
(8) Akinaga, S.; Nomura, K.; Gomi, K.; Okabe, M. *Cancer Chemother. Pharmacol.* **1993**, *32*, 183–189.
(9) Murakata, C.; Saitho, Y.; Akinaga, S.; Okabe, M. PCT Int. Appl. WO 94 20,106, **1994**, 25 pp; *Chem. Abstr.* **1995**, *122*, 187953h.
(10) Caravatti, G.; Fredenhagen, A. Eur. Pat. Appl. EP 296110, 1988, 27 pp; *Chem. Abstr.* **1989**, *111*, 96977.
(11) Yuspa, S. H.; Dlugosz, A. A.; Hennings, H.; Strickland, J. U. S. Pat. Appl. US 677429 (91-677429), 1992, 57 pp; *Chem. Abstr.* **1992**, *117*, 20499.
(12) Regenass, U.; Caravatti, G.; Wacker, O. PCT Int. Appl. WO 95 32,974 (Cl. c07D498/22), 1995, 48 pp; *Chem. Abstr.* **1996**, *124*, 261440f.
(13) Hoehn-Thierry, P.; Ghisalba, O.; Moerker, T.; Peter, H. H. *J. Antibiot.* **1995**, *48*, 100–305.
(14) Meksuriyen, D.; Cordell, G. A. *J. Nat. Prod.* **1988**, *51*, 893–899.
(15) Yang, S.-W.; Cordell, G. A. Unpublished data.
(16) Kametani, T.; Kanaya, N.; Ihara, M. *J. Chem. Soc., Perkin Trans. 1* **1981**, 959–963.
(17) Voet, D; Voet, J. G. *Biochemistry: Amino Acid Metabolism*; John Wiley & Sons: New York, 1990; p 723.
(18) Fenn, P.; Durbin, R. D.; Kuntz, J. E. *Physiol. Plant Pathol.* **1978**, *12*, 197–309.
(19) Hoeregott, H. *Biochem. Physiol. Pflanz.* **1973**, *164*, 500–508.
(20) Bhaskaran, R. *Curr. Sci.* **1973**, *42*, 567–568.
(21) Ayer, W. A.; Trifonov, L. S. *J. Nat. Prod.* **1994**, *57*, 839–841.
(22) Dillman, R. L.; Cardellina, J. H., II. *J. Nat. Prod.* **1991**, *54*, 1056–1061.
(23) Bernart, M.; Gerwick, W. H. *Phytochemistry* **1990**, *29*, 3697–3698.
(24) Bourdoux, P.; Vandervorst, D.; Hootele, C. *Phytochemistry* **1971**, *10*, 1934–1935.
(25) Bano, S.; Bano, N.; Ahmad, V. U. *J. Nat. Prod.* **1986**, *49*, 549.
(26) Evidente, A; Surico, G. *J. Nat. Prod.* **1986**, *49*, 938–939.
(27) Nakagawa, M.; Yoshikawa, K.; Hino, T. *J. Am. Chem. Soc.* **1975**, *97*, 6496–6501.
(28) Nakagawa, M.; Okajima, H.; Hino, T. *J. Am. Chem. Soc.* **1977**, *99*, 4424–4429.
(29) Büchi, G.; Luk, K. C.; Kobbe, B.; Townsend, J. M. *J. Org. Chem.* **1977**, *42*, 244–246.
(30) Springer, J. P. *Tetrahedron Lett.* **1979**, 339–342.
(31) Yang, S.-W.; Cordell, G. A. *J. Nat. Prod.* **1996**, *59*, 823–833.

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