Further Metabolic Studies of Indole and Sugar Derivatives Using the Staurosporine Producer *Streptomyces staurosporeus*†

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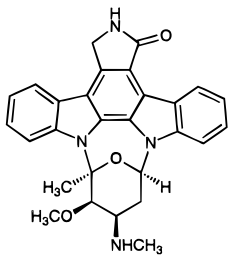
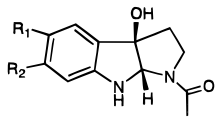
During our continuing studies of staurosporine (**1**) biosynthesis and the metabolism of indole derivatives, three additional novel metabolites, β-hydroxy-N_b-acetyltryptamine (**7**) and its 5- and 6-fluoro derivatives (**8** and **9**, respectively), were isolated, respectively, from the feeding of tryptamine and 5- and 6-fluorotryptamines using *Streptomyces staurosporeus*. In addition, two new 5-hydroxytryptamine (serotonin) metabolites, N_b-acetyltryptamine-5-*O*-β-D-quinovopyranoside (**11**) and N_b-acetyltryptamine-5-*O*-α-L-rhamnopyranoside (**12**), were isolated along with 5-hydroxy-N_b-acetyltryptamine (**10**). From a glucose containing medium, 6-*O*-acetylglucose and its ^{13}C uniformly labeled derivative were isolated from a culture of *S. staurosporeus* during biosynthetic studies of **1**. In 2-deoxyglucose metabolism studies, 6-*O*-acetyl-2-deoxyglucose was isolated and identified as a metabolic product of 2-deoxyglucose. A copper-enzyme inhibitor, *N*,*N*-diethyldithiocarbamic acid, was evaluated for the accumulation of staurosporine intermediates; however, no modified staurosporines were observed.

Streptomyces staurosporeus (AM-2282) (Streptomycetaceae) was found to produce the potent protein kinase C inhibitor staurosporine (**1**),[1] which has 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 the most important aspect of its biological profile.[4,5] Some staurosporine derivatives have the ability to reverse the MDR phenotype to sensitive cell lines[6−8] and could potentially serve as anticancer drugs or as therapeutic adjuncts. Some chemical modifications have been performed to lower the toxicity of **1**,[9−12] and biological modifications achieved through mutation,[13] genetic engineering, and precursor-directed biosynthesis are of some interest. From a biosynthetic view, tryptophan, methionine, and glucose have been identified as precursors of **1**.[14,15] Consequently, metabolic studies of indole and sugar derivatives may permit a deeper understanding of staurosporine biosynthesis or may directly produce novel staurosporine derivatives via precursor-directed biosynthesis.

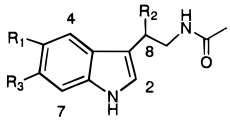
We reported previously that tryptamine and 5- and 6-fluorotryptamines could be transformed to N_b-acetyltryptamine, 5-fluoro-N_b-acetyltryptamine (**2**), and 6-fluoro-N_b-acetyltryptamine (**3**) and the novel metabolites (3a*R*,8a*S*)-1-acetyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-3a-ol (**4**) and 5-fluoro- and 6-fluoro-substituted alkaloids **5** and **6**, respectively.[16] Here, we report the isolation of three additional novel metabolites, β-hydroxy-N_b-acetyltryptamine (**7**), 5-fluoro-β-hydroxy-N_b-acetyltryptamine (**8**), and 6-fluoro-β-hydroxy-N_b-acetyltryptamine (**9**) from the EtOAc extract of *S. staurosporeus* cultures after tryptamine HCl, 5-fluorotryptamine HCl, and 6-fluorotryptamine feeding, respectively. In another feeding experiment with a 5-hydroxytryptamine (serotonin)−creatinine sulfate complex, we indicated that 5-hydroxytryptamine could not be transformed to the 5-hydroxy-substituted alkaloid **4**,[16] whereas it was principally biotransformed into 5-hydroxy-N_b-acetyltryptamine (**10**) and two novel alkaloids, N_b-acetyltryptamine-5-*O*-β-D-quinovopyranoside (**11**) and N_b-acetyltryptamine-5-*O*-α-L-rhamnopyranoside (**12**). We describe here the structure elucidation and the unambiguous assignment of **7**−**9**, **11**, and **12** by various NMR techniques, including 2-D-NOESY, HMQC, and HMBC techniques.



1 Staurosporine



4 $R_1 = R_2 = H$
5 $R_1 = F$; $R_2 = H$
6 $R_1 = H$; $R_2 = F$



2 $R_1 = F$; $R_2 = R_3 = H$
3 $R_1 = R_2 = H$; $R_3 = F$
7 $R_1 = R_3 = H$; $R_2 = OH$
8 $R_1 = F$; $R_2 = OH$; $R_3 = H$
9 $R_1 = H$; $R_2 = OH$; $R_3 = F$
10 $R_1 = OH$; $R_2 = R_3 = H$
11 R_1 = *O*-β-D-quinovose; $R_2 = R_3 = H$
12 R_1 = *O*-α-L-rhamnose; $R_2 = R_3 = H$

Sugar derivatives such as D-(+)-fucose, L-(−)-fucose, 2-deoxyglucose, and 3-*O*-methylglucose were evaluated for their ability to be incorporated into the pathway of staurosporine biosynthesis. The enzyme inhibitor, *N*,*N*-diethyldithiocarbamic acid, which has been used to alter the biosynthesis of violacein, a bisindole compound[17] whose structure is somewhat related to the aglycon of

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† Biosynthesis of Staurosporine, 6. For previous papers in the series see Cordell and co-workers.[14−16]

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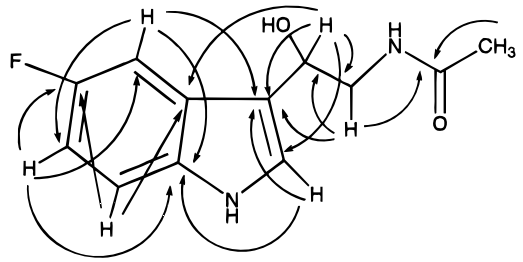


Figure 1. Long-range $^1H-^{13}C$ correlations (→) of compound **8** obtained from HMBC.

1, was evaluated for its ability to influence the biosynthesis of **1**.

Results and Discussion

During a continuing investigation of the metabolites from the feeding of tryptamine HCl, a low yield (0.4%) of an additional novel alkaloid **7** was discovered with a lower R_f value than compound **4**. Its corresponding 5-fluoro- and 6-fluoro-derivatives (**8**, 1.5% and **9**, 1%, respectively) were also found by TLC with a lower R_f value than alkaloids **5** and **6**, respectively. Because the yield of compound **8** was more substantial than that of **7** and **9**, it was examined initially for its structure. After isolation and purification by preparative TLC, the optically inactive alkaloid **8** was obtained as a colorless gum and showed a M^+ at m/z 236 in the EIMS. The molecular formula was determined as $C_{12}H_{13}O_2N_2F$ by HREIMS (obsd 236.0956 05; calcd 236.0961 06). The fragment ion m/z 218 in EIMS and an absorption at υ_{max} 3295 cm^{-1} in the IR spectrum indicated the existence of a hydroxy group. The UV spectrum of **8** showed characteristic indole absorption [λ 284 (sh), 279, and 275 nm (sh)]. In the aromatic region of the ^1H- and ^{13}C-NMR spectra of **8**, the chemical shifts of the aromatic protons and carbons were in accord with those of **2**, except that those of H-2 and H-4 were slightly shifted to δ 7.26 (s) and 7.35 (dd), respectively, suggesting that the fluoro-substituted indole moiety was retained. The ^1H-NMR spectrum of **8** showed the signals of an acetyl group (δ 1.93, s) and three aliphatic protons, including a hydroxyl methine proton (δ 5.02, dd, J = 8, 5 Hz) and two neighboring methylene protons (δ 3.63, dd, J = 13.5, 5 Hz; δ 3.50, dd, J = 13.5, 8 Hz), indicating the occurrence of hydroxy substitution on the side chain of **2**. Based on the ^1H and ^{13}C resonances (δ 67.7, d; 47.2, t) of the protons and carbons on the side chain, the hydroxy group was assigned to the β position.

The unambiguous assignments were achieved by $^1H-^1H$ COSY and HMBC. The proposed structure **8** was confirmed by HMBC, which correlated the β-hydroxymethine proton (H-8) to C-2, C-3, and C-3a on the indole ring (Figure 1). Therefore, alkaloid **8** was determined to be 5-fluoro-β-hydroxy-N_b-acetyltryptamine.

Alkaloid **7** was purified by preparative TLC and examined by ^1H NMR and EIMS. The molecular weight of **7** was observed in the EIMS at m/z 218, a fluorine unit different from **8**. In the ^1H NMR, the signals in the aromatic region of **7** showed a pattern similar to those of N_b-acetyltryptamine, whereas those in the aliphatic region were in accord with those of **8**. The proton signals were correlated and assigned by $^1H-^1H$ COSY analogously to the assigned structure **8**. Therefore, alkaloid **7** was assigned the structure β-hydroxy-N_b-acetyltryptamine.

Alkaloid **9** was obtained by the same method and examined by ^1H NMR and EIMS. The molecular weight of **9** was observed in the EIMS at m/z 236. In the ^1H NMR, the signals in the aromatic region of **9** showed a pattern similar to those of 6-fluoro-N_b-acetyltryptamine (**3**), whereas those in the aliphatic region were in accord with those of compounds **7** and **8**. Alkaloid **9** was therefore assigned the structure 6-fluoro-β-hydroxy-N_b-acetyltryptamine. The ^1H-NMR assignments of compounds **7**−**9** and the ^{13}C-NMR assignment of compound **8** are listed in Table 1. No Cotton effect was observed in the CD spectrum for alkaloids **7**−**9**, which also each showed an $[\alpha]^{25}_D$ of 0°. Hence, these metabolites probably exist in their racemic form.

5-Hydroxytryptamine creatinine sulfate complex (100 mg) was added to the fermentation medium (100 mL) of *S. staurosporeus*. The extract of the harvested culture broth was examined by TLC, and three metabolites not appearing in the normal culture were detected, isolated, and examined for their structures. Staurosporine production was not affected under these conditions. The major product was identified as 5-hydroxy-N_b-acetyltryptamine (**10**, 55%) based on ^1H NMR, ^{13}C NMR, and EIMS (see Experimental Section), and the other two products with lower R_f value compared to **10** possessed very close R_f values. The separation of these two minor metabolites was accomplished by preparative TLC with continuous development. Alkaloid **11**, which has a lower R_f in the solvent system $CHCl_3$−MeOH (10:3) value compared to alkaloid **12**, showed the M + 1 at m/z 365 in the CIMS, and the molecular formula, $C_{18}H_{24}N_2O_6$, was determined by HREIMS, (obsd m/z 364.163 58, calcd 364.163 44). UV absorption at λ 278 nm indicated retention of the indole moiety. The ^1H-NMR spectrum of **11** showed the additional signals of five hydroxylmethines with a methyl resonance (δ 1.32, d, J = 6.2 Hz), instead of a hydroxymethylene group as in glucose, and therefore resembled the sugar protons of a 6-deoxy sugar. Also, the observation of ions at m/z 163 ($C_6H_{11}O_5$) and 219 (tryptamine moiety) in the CIMS supported this assumption. The resonances of H-2, the side-chain protons (H_2-8 and H_2-9), and the methyl protons of *N*-Ac appeared at the same chemical shift as those of **10**. However, the resonances of H-4, H-6, and H-7 were shifted to δ 7.25 (d, J = 2.2 Hz), 6.92 (dd, J = 8.8, 2.2 Hz), and 7.23 (d, J = 8.8 Hz), respectively, indicating that glycosidic substitution had occurred at the 5-position. The ^{13}C-NMR spectrum of **11** displayed six additional carbon resonances, and the chemical shifts of C-4, C-5, and C-6 were shifted to δ 106.9, 152.8, and 114.4 ppm, respectively, due to C-5 glycoside substitution in support of this inference. The doublet signal of H-1′ (δ 4.84, J = 7.5 Hz) and the triplet signal of H-4′ (δ 3.09, J = 9 Hz) indicated that the protons H-1′−H-5′ are all *trans*-axial, although the resonances of H-2′, H-3′ and H-5′ overlapped with the H_2-9 (δ 3.44, t, J = 7.2 Hz). In addition, the carbon resonances in the ^{13}C-NMR spectrum for the sugar moiety matched those of known metabolites with a β-D-quinovose glycoside residue.[18] Therefore, the structure of **11** was determined as N_b-acetyltryptamine 5-*O*-β-D-quinovopyranoside. The connection of the quinovose and tryptamine moieties at the 5-position was further confirmed by NOESY and HMBC. The observation of a NOE between H-4 (δ 7.25) and H-1′ (δ 4.84) and H-6 (δ 6.92)

Table 1. ^{1}H NMR Data (in CD_3OD) of Alkaloids **7**−**9** and ^{13}C-NMR Spectral Data of **8**

carbon	^{1}H (**7**)	^{1}H (**9**)	^{1}H (**8**)	^{13}C (**8**)
2	7.23 (s)	7.21 (s)	7.26 (s)	125.0 (d)
3				117.5 (s, J_{C-F} = 4)
3a				127.8 (s, J_{C-F} = 9)
4	7.69 (d, 7.7)	7.64 (dd, 8.7, 5.3[a])	7.35 (dd, 10,[a] 2.5)	104.8 (d, J_{C-F} = 24)
5	7.00 (t, 7.6)	6.80 (ddd, 8.8,[a] 8.7, 2.3)		158.8 (s, J_{C-F} = 232)
6	7.09 (t, 8.1)		6.86 (ddd, 9,[a] 9, 2.5)	110.6 (d, J_{C-F} = 26)
7	7.33 (d, 8.2)	7.03 (dd, 10,[a] 2.3)	7.29 (dd, 9, 4.5[a])	113.1 (d, J_{C-F} = 10)
7a				135.0 (s)
8	5.07 (dd, 7.8, 5.0)	5.03 (dd, 7.8, 5)	5.02 (dd, 8, 5)	67.7 (d)
9	3.65 (dd, 13.5, 5.0) 3.50 (dd, 13.5, 7.8)	3.63 (dd, 13.5, 5) 3.50 (dd, 13.5, 7.8)	3.63 (dd, 13.5, 5) 3.50 (dd, 13.5, 8)	47.2 (t)
C=O				173.5 (s)
$COCH_3$	1.93 (s)	1.93	1.93 (s)	22.5 (q)

[a] Coupling constant due to fluorine substitution.

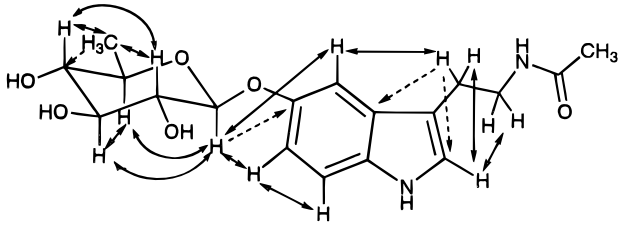


Figure 2. NOE correlations (double solid arrows) from NOESY and some important long-range ^{1}H−^{13}C correlations (single broken arrows) from HMBC in compound **11**.

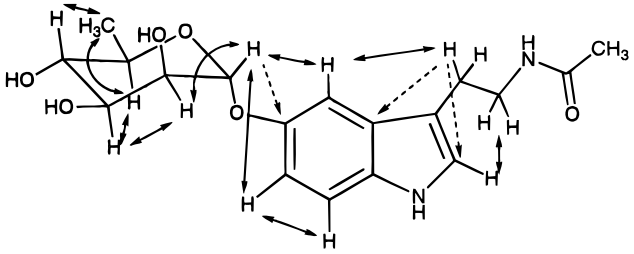


Figure 3. NOE correlations (double solid arrows) from NOESY and some important long-range ^{1}H−^{13}C correlations (single broken arrows) from HMBC in compound **12**.

and H-1′, and the long-range correlation of H-1′ to C-5 (δ 152.8), directly established the glyco-linkage between C-1′ and C-5. The important NOESY and selected HMBC correlations of **11** are summarized in Figure 2.

The other closely related product **12**, with a higher R_f value, showed identical physical data in the EIMS, UV, and IR spectra, indicating the existence of the same skeleton and functional groups as in **11**. Also, in the ^{1}H- and ^{13}C-NMR spectra, the resonances of the aglycon were in accord with those of **11**, except that a change was noted in the glycosidic unit, indicating the presence of a different 6-deoxy sugar substituent on the C-5 position. On the basis of the observation of the coupling constant of the anomeric proton (H-1′, δ 5.36, J = 1.8 Hz), and those of the other hydroxyl methine resonances (H-2′, δ 4.03, dd, J = 3.4, 1.8 Hz; H-3′, δ 3.89, dd, J = 9.5, 3.4 Hz; H-5′ δ 3.82, dd, J =9.5, 6.2 Hz), the proton configurations of the 6-deoxy sugar were determined as equatorial for H-1′ and H-2′, and axial for H-3′, H-4′, and H-5′, which were confirmed by NOE correlations between H-1′ and H-2′, H-2′ and H-3′, H_3-6′ and H-4′, and H-3′ and H-5′. Hence, the substituted 6-deoxy sugar was determined as 5-*O*-α-rhamnose, and alkaloid **12** was deduced to be N_b-acetyltryptamine 5-*O*-α-L-rhamnopyranoside. The ^{13}C-NMR spectrum of **12** supported the proposed structure by comparison with known glycosides.[19] The connection of the rhamnose and tryptamine moieties at the 5-position was also confirmed by NOESY and HMBC, through the observation of a NOE between H-4 and H-1′ and H-6 and H-1′, and the long-range correlation of H-1′ to C-5. The NOESY and selected HMBC correlations of **12** are summarized in Figure 3. The unambiguous assignment of the proton and carbon resonances of **11** and **12** were established by COSY, HMQC, HMBC, and NOESY two-dimensional techniques and are listed in Tables 2 and 3.

Table 2. ^{1}H NMR Data (in CD_3OD) of Alkaloids **11** and **12**

proton	**11**	**12**
2	7.06 (s)	7.06 (s)
4	7.25 (d, 2.2)	7.24 (d, 2.2)
6	6.92 (dd, 8.8, 2.2)	6.85 (dd, 8.8, 2.2)
7	7.23 (d, 8.8)	7.23 (d, 8.8)
8	2.88 (t, 7.2)	2.88 (t, 7.2)
9	3.44 (t, 7.2)	3.44 (t, 7.2)
COMe	1.91 (s)	1.91 (s)
1′	4.84 (d, 7.5)	5.36 (d, 1.8)
2′	3.45 (o)[a]	4.03 (dd, 3.4, 1.8)
3′	3.45 (o)[a]	3.89 (dd, 9.5, 3.4)
4′	3.09 (t, 9)	3.46 (o)[a]
5′	3.45 (m)[a]	3.82 (dd, 9.5, 6.2)
6′	1.32 (d, 6.2)	1.25 (d, 6.2)

[a] Overlapping signals.

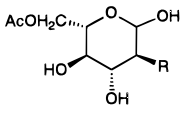
During staurosporine biosynthetic studies, 6-*O*-acetylglucose (**13**) was found in the medium (1 mg/100 mL), and in the incorporation experiment of [U-$^{13}C_6$]-D-glucose into **1**, uniformly labeled 6-*O*-acetylglucose, mixed with the natural abundance isotope, was found and directly established the biotransformation through glucose to 6-*O*-acetylglucose. It has been reported that there are enzymes (proteases) that exist in *Streptomyces* sp.[20] and *Bacillus megaterium*[21] which can selectively effect an acetylation reaction on glucose at C-6 rather than at the other hydroxyl group positions (C-2−C-4). Other sugar derivatives, including D(+)-fucose, L(−)-fucose, 2-deoxyglucose, and 3-*O*-methylglucose, were also evaluated for their efficacy in precursor-directed biosynthesis. However, no staurosporine derivatives with a modified glycone moiety were found in these experiments, and staurosporine biosynthesis was not inhibited. During these studies, 6-*O*-acetyl-2-deoxyglucose (**14**, 2%) was isolated as a metabolite of 2-deoxyglucose. Compound **14** has been enzymatically synthe-

Table 3. ^{13}C-NMR Data (in CD_3OD) and HMBC Correlations of Alkaloids **11** and **12**

carbon	**11**[a]	HMBC of **11**	**12**[a]	HMBC of **12**
2	124.6 (d)	H_2-8	124.6 (d)	H_2-8
3	113.2 (s)	H_2-9, H_2-8, H-2, H-4	113.1 (s)	H_2-9, H_2-8, H-2, H-4
3a	129.1 (s)	H-7, H_2-8, H-2	129.1 (s)	H-7, H_2-8, H-2
4	106.9 (d)	H-6	106.4 (d)	H-6
5	152.8 (s)	H-7, H-1′	151.5 (s)	H-7, H-1′
6	114.4 (d)	H-4	114.0 (d)	H-4
7	112.5 (d)		112.7 (d)	
7a	134.6 (s)	H-4, H-6, H-2	134.6 (s)	H-4, H-6, H-2
8	26.2 (t)	H-2, H-9	26.2 (t)	H-2, H-9
9	41.4 (t)	H_2-8	41.4 (t)	H_2-8
1′	104.2 (s)	H-5′	101.4 (s)	
2′	75.4 (d)		72.4 (d)	
3′	77.8 (d)	H-1′, H-4′, H-5′	72.4 (d)	H-1′, H-2′, H-5′
4′	77.0 (d)	H-2′, H_3-6′	74.1 (d)	H-2′, H-3′, H_3-6′
5′	73.4 (d)	H-1′, H_3-6′, H-4′	70.4 (d)	H-1′, H_3-6′, H-4′
6′	18.1 (q)	H-4′	18.2 (q)	H-4′
C=O	173.3 (s)	H_2-9, COCH_3	173.3 (s)	H_2-9, COCH_3
COCH_3	22.6 (q)		22.6 (q)	

[a] Multiplicity was determined by APT experiment.

sized from 2-deoxyglucose using lipase from *Candida antarctica*.[22]



13 R = OH
14 R = H

When *N*,*N*-diethyldithiocarbamate, a copper-enzyme inhibitor, was used as a copper chelator to block the biosynthetic pathway of violacein,[17] a bisindole derivative, a reduced form of violacein accumulated. Therefore, this inhibitor was considered as a possible modifier of staurosporine biosynthesis. When *N*,*N*-diethyldithiocarbamic acid was added (at 5−100 mg/mL) to cell cultures of *S. staurosporeus*, staurosporine biosynthesis was inhibited, and the organism failed to produce the bisindole (aglycon) derivative of **1** either in the growing medium or in still-washed cells of *S. staurosporeus* (see Experimental Section).

In conclusion, five novel alkaloids **7**−**9**, **11**, and **12** were obtained from biotransformations using *S. staurosporeus*. From these experiments, it is apparent that acyl transferases exist in *S. staurosporeus*, which trigger the most common, and first, reaction, selective acetylation of the hetero-atoms of hydroxy or amino groups on glucose, 2-deoxyglucose, and tryptamine and its substituted derivatives. Further metabolism may occur on the tryptamine side chain through β-hydroxylation, without stereoselectivity in the case of tryptamine and 5- and 6-fluorotryptamines, possibly through a tryptophan side-chain oxidase (or similar enzyme). Two novel metabolites were isolated and identified as N_b-acetyltryptamine 5-*O*-β-D-quinovopyranoside (**11**) and N_b-acetyltryptamine 5-*O*-α-L-rhamnopyranoside (**12**), which might be deactivation process products. No new staurosporine derivatives were found in these experiments, including the addition of a copper-enzyme inhibitor, indicating that the biosynthetic enzymes in staurosporine biosynthesis in *S. staurosporeus* are highly substrate specific and may not be inducible by other indole or sugar derivatives. Hence, using mutant species of *S. staurosporeus* might be an alternative method for the identification of modified derivatives of staurosporine.

Experimental Section

General Experimental Conditions. The detailed methods were described previously.[14,15] NOESY spectra were recorded on a Varian XL-300 instrument operating at 300 MHz. TMS or $CDCl_3$ were used as an internal standard (δ_{TMS} = 0 ppm; δ_{CDCl_3} = 7.24 ppm), and chemical shifts are reported in ppm on the δ scale; coupling constants (J) are given in Hz. HMQC and HMBC spectra were performed on a GE Omega 500 MHz instrument (499.9 MHz for ^{1}H NMR) with the standard programs. Glucose, 3-*O*-methylglucose, 2-deoxyglucose, tryptamine HCl, 5-fluorotryptamine HCl, 6-fluorotryptamine, 5-hydroxytryptamine−creatinine sulfate complex, and sodium *N*,*N*-diethyldithiocarbamate were purchased from Sigma. [U-$^{13}C_6$]-D-Glucose 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 Midwest Area National Center for Agricultural Utilization Research, ARS, USDA, Peoria, Illinois. The activation, maintenance, and fermentation of *S. staurosporeus* are the same as previously described.[15,16]

Tryptamine HCl, 5-Fluorotryptamine HCl, and 6-Fluorotryptamine Feeding Experiments. For these experiments, see the previous paper.[16]

Isolation and Purification of 8. Cultured fermentation broth containing mycelia was extracted with EtOAc (3 ×). The pooled organic phase was dried (Na_2SO_4) and evaporated under vacuum at 40 °C, and alkaloid **8** was isolated by preparative TLC. Developed chromatograms were visualized by fluorescence quenching under 254 nm UV light. Preparative TLC was carried out using $CHCl_3$−MeOH (10:1) systems. The yield of alkaloid **8** produced from *S. staurosporeus* was 1.5%.

***β*-Hydroxy-*N*$_b$-acetyltryptamine (7).** The same isolation procedures as those used for alkaloid **8** were applied. Alkaloid **7** was obtained as a colorless gum (0.3 mg/100 mg tryptamine HCl): $[\alpha]^{25}_D$ 0° (*c* 0.15, MeOH); EIMS *m*/*z* 218 $[M]^+$; UV (MeOH) λ_{max} (log ϵ) 289 (3.63), 280 (3.68), 273 (sh, 3.67), and 227 nm (3.89); IR ν_{max} 3295 (−OH), 1653, 1651, 1559, 1435, 1373, and 745 cm^{-1}; CD no curve observed; ^{1}H NMR, see Table 1.

5-Fluoro-β-hydroxy-N_b-acetyltryptamine (8): $[\alpha]^{25}_D$ 0° (*c* 0.15, MeOH); colorless gum; EIMS *m/z* 236 (77) $[M]^+$, 218 (77), 176 (65), 148 (100), and 126 (69); HREIMS, obsd *m/z* 236.0956 05; calcd 236.0961 06 for $C_{12}H_{13}N_2O_2F$; UV (MeOH) λ_{max} (log ϵ) 295 (sh, 3.65), 284 (sh, 3.75), 279 (3.75), 275 (sh), and 225 nm (4.04); IR ν_{max} 3295 (−OH), 1651, 1636, 1559, 1487, 1435, 1173, and 799 cm^{-1}; CD no Cotton effect observed; ^1H and ^{13}C NMR, see Table 1.

6-Fluoro-β-hydroxy-N_b-acetyltryptamine (9). The same isolation procedures as those used for alkaloid **8** were applied. Alkaloid **9** was obtained as a colorless gum (1 mg/100 mg 6-fluorotryptamine): $[\alpha]^{25}_D$ 0° (*c* 0.1, MeOH); EIMS *m/z* 236 $[M]^+$; UV (MeOH) λ_{max} (log ϵ) 281 (3.60), 278 (3.60), and 223 nm (4.02); CD no Cotton effect observed; ^1H NMR, see Table 1.

5-Hydroxytryptamine Feeding Experiment. 5-Hydroxytryptamine−creatinine sulfate complex (100 mg/100 mL) was added in a cold sterilized manner (filtration). All of the metabolites were detected by UV on either TLC or preparative TLC plates. Three metabolites (**10**−**12**) were isolated by preparative TLC developed with the solvent system $CHCl_3$−MeOH (1:1).

Isolation and Purification of Compounds 10−12. The EtOAc extract from cell broth was condensed and applied to the preparative TLC (Si gel) developed with $CHCl_3$−MeOH (10:2) three times. Alkaloid **10** was observed with R_f 0.7, whereas alkaloids **11** and **12** have R_f values 0.20 and 0.23, respectively. Alkaloids **10**−**12** were removed from the TLC plate individually and dissolved in Me_2CO. After evaporation of the solvent, the crude metabolites were purified by preparative TLC, using the same conditions as mentioned above.

5-Hydroxy-N_b-acetyltryptamine (10): CIMS *m/z* 219 (100) $[M + 1]^+$, 160 (22); ^1H NMR δ 7.14 (1H, d, J = 8.6, H-7), 6.99 (1H, s, H-2), 6.92 (1H, d, J = 2.3, H-4), 6.65 (1H, dd, J = 8.6, 2.3, H-6), 3.42 (2H, t, J = 7.3, H_2-9), 2.84 (2H, t, J = 7.3, H_2-8), 1.90 (3H, s, COCH_3); ^{13}C NMR δ 173.2 (s, *C*OMe), 151.1 (s, C-5), 133.1 (s, C-7a), 129.4 (s, C-3a), 124.2 (d, C-2), 112.7 (d, C-6), 112.5 (s, C-3), 112.3 (d, C-7), 103.5 (d, C-4), 41.4 (t, C-9), 26.2 (t, C-8), and 22.6 (q, CO*Me*).

N_b-Acetyltryptamine 5-*O*-β-D-quinovopyranoside (11): $[\alpha]^{25}_D$ −7.7° (*c* 0.3, MeOH), amorphous; CIMS *m/z* 365 (25) $[M + 1^+]$, 219 (100), and 163 (22); HREIMS, obsd *m/z* 364.16358; calcd 364.16344 for $C_{18}H_{24}N_2O_6$; UV (MeOH) λ_{max} (log ϵ) 218 (4.09), 226 (4.24), and 278 nm (3.72); IR ν_{max} 3385 (−OH), 1645, 1598, 1459, 1196, 1061, and 1028 cm^{-1}; CD λ ($\delta\epsilon$) 319 (0), 298 (−4.2), 272 (0), 238 (0), 228 (−7.82), 223 (−16.0), 221 (−15.9), 215 (−10.2), and 209 (−9.6) nm; ^1H NMR, see Table 2; ^{13}C NMR, see Table 3.

N_b-Acetyltryptamine 5-*O*-α-L-rhamnopyranoside (12): $[\alpha]^{25}_D$ −35° (*c* 0.33, MeOH), amorphous; CIMS *m/z* 365 (27) $[M + 1]^+$, 219 (100), and 163 (25); HREIMS, obsd *m/z* 364.16240; calcd 364.16344 for $C_{18}H_{24}N_2O_6$; UV (MeOH) λ_{max} (log ϵ) 218 (4.07), 227 (4.21), and 278 nm (3.72); IR ν_{max} 3385 (−OH), 1645, 1598, 1459, 1196, 1125, 1061, 1028, and 996 cm^{-1}; CD λ ($\delta\epsilon$) 316 (0), 306 (−6.8), 297 (−9.1), 288 (−8.3), 245 (0), 233 (−14.5), 229 (0), and 226 (+8.4) nm (positive tail); ^1H NMR, see Table 2; ^{13}C NMR, see Table 3.

Feeding Experiment with [U-$^{13}C_6$]-D-Glucose. For information on this experiment, see previous paper.[15]

Isolation of $^{13}C_6$ Uniformly Labeled 6-*O*-Acetylglucose (13). Labeled 6-*O*-acetyl-D-glucose was separated by preparative TLC and was detected by 20% H_2SO_4 following heating of the TLC plate in an oven for 10 min, which resulted in a visible black spot. Preparative TLC was carried out using an Me_2CO−MeOH (2:1) solvent system, the same as for analytical TLC, using a 1-mm plate (20 × 20 cm). After development, labeled 6-*O*-acetyl-D-glucose was removed, dissolved in Me_2CO, and further purified by preparative TLC using an Me_2CO−MeOH (1:1) solvent system. The yield of **13** produced from *S. staurosporeus* was around 0.5 mg in a 100-mL broth containing 3 g of glucose.

$^{13}C_6$ Uniformly Labeled 6-*O*-acetylglucose (13): ^{13}C-NMR (CD_3OD, δ 49.0) α rotamer 172.8 (s, O*C*OMe), 93.9 (s + d, J = 43, C-1), 74.7 (o, C-3), 73.7 (s + t, C-2), 71.7 (o, C-4), 70.5 (s + t, C-5), 65.0 (s + d, J = 45, C-6), and 20.7 (s, OCO*C*H_3); β rotamer: 172.9 (s, O*C*OMe), 98.2 (s + d, J = 45, C-1), 77.9 (s + t, C-3), 76.1 (s + t, C-5), 75,2 (s + t, C-2), 71.9 (o, C-4), 65.1 (s + d, J = 66, C-6), and 20.7 (s, OCO*C*H_3). Here, s, d, and t indicate ^{13}C−^{13}C coupling pattern, singlet, doublet, and triplet, respectively, but not multiplicity; o means overlapping. All the above ^{13}C signals were mixed with a corresponding singlet from the natural abundance isotope. The coupling constants of the triplets were not calculated due to some overlapping resonances and not enough resolution. The ^{13}C-NMR assignment of **13** was based on the comparison with spectra of standard glucose and published data.[23−25]

2-Deoxyglucose Feeding Experiment. 2-Deoxyglucose (300 mg/100 mL) was added to the culture medium before sterilizing. The same procedures of inoculation and cultivation were applied. The cultures were harvested after fully growing at around 4 days. The medium was extracted with EtOAc (3 × 100 mL) directly. The organic layer was dried by Na_2SO_4 and evaporated under vacuum. For analysis, the solvent system $CHCl_3$−EtOAc (1:1) was used to develop the EtOAc extract on TLC. Separated spots were detected by spraying with H_2SO_4 solution (20%) followed by heating during which the 2-deoxyglucose metabolites turned black. For preparative scale work, the crude extract was applied to preparative TLC (40 mg on 20 × 20, 1-mm preparative TLC plate) and developed with the solvent system $CHCl_3$−EtOAc (1:1). The band observed with H_2SO_4 at R_f 0.2 was removed and dissolved in Me_2CO−MeOH (3:1). After filtration, the organic solvent was removed by rotavapor, and the dried samples were further purified by preparative TLC with a $CHCl_3$−MeOH (10:1) system. The band observed with H_2SO_4 at R_f 0.3 was removed and dissolved in Me_2CO−MeOH (3:1). Compound **14** was obtained after filtration and evaporation.

6-*O*-Acetyl-2-deoxyglucose (α and β rotamers) (14): FAB *m/z* 207 $[M + 1]^+$; ^{13}C NMR (D_2O) α rotamer, δ 175.1 (s, *C*OMe), 92.3 (d, C-1), 71.9 (d, C-4), 70.5 (d, C-5), 68.7 (d, C-3), 64.4 (d, C-6), 38.1 (t, C-2), and 21.1 (q, CO*Me*); β rotamer, δ 175.1 (s, *C*OMe), 94.5 (d, C-1), 74.3 (d, C-5), 71.5 (d, C-4), 71.1 (d, C-3), 64.6 (d, C-6), 40.3 (t, C-2), and 21.1 (q, CO*Me*); ^1H NMR (D_2O) α rotamer, 5.24 (br s, H-1), 4.19 (m, H_2-6), 3.86 (m, H-5),

3.85 (m, H-3), 3.31 (t, J = 9.8 Hz, H-4), 2.14 (m, H-2β), 1.57 (td, J = 12.9, 3.7 Hz, H-2α); β rotamer, 4.82 (d, J = 9.8 Hz, H-1), 4.19 (m, H_2-6), 3.71 (m, H-3), 3.45 (m, H-5), 3.21 (t, J = 9.8 Hz, H-4), 2.07 (o, H-2β), 1.39 (quartet, J = 11.6 Hz, H-2α). The spectral assignment was based on published data.[22]

Addition of the Copper-Enzyme Inhibitor, *N*,*N*-Diethyldithiocarbamic Acid. Sodium *N*,*N*-diethyldithiocarbamic acid (5, 10, 20, 50, or 100 mg/100 mL) was added to the culture medium containing tryptophan (50 mg/100 mL) after 24 h of fermentation, and allowed to grow for 15 days. The washed cell system was also applied (see below). The washed cells were obtained from the harvested cells (3 × 100 mL), which were washed with saline solution, and then resuspended in three individual aqueous solutions (100 mL) with tryptophan (100 mg), K_2HPO_4 (100 mg), $MgSO_4 \cdot 7\ H_2O$ (20 mg), and sodium *N*,*N*-diethyldithiocarbamic acid (100, 200, or 300 mg, respectively). The mycelia were extracted with EtOAc, condensed, and then analyzed by TLC. No indole derivatives were detected or isolated.

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