Biosynthesis of Staurosporine: Incorporation of Glucose

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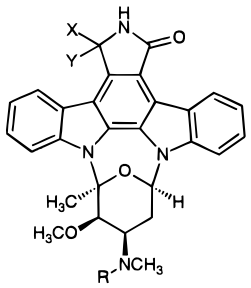
Previously, it was disclosed that tryptophan provides two units of the basic building blocks involved in the biosynthesis of the indolo[2,3-*a*]carbazole unit of staurosporine (**1**). In this paper we describe the incorporation of [1-^{13}C]-D-glucose and [U-$^{13}C_6$]-D-glucose into staurosporine, through which it was established that the amino-sugar moiety is derived from glucose, based on the observation of the direct incorporation of uniformly labeled ^{13}C glucose, and that glucose is converted via glycolysis and the shikimic acid pathway to tryptophan and hence into **1**. Deuterium-labeled glucose derivatives, including [6-$^2H_2$]-D-glucose, [2-^{2}H]-D-glucose, and [U-$^2H_7$]-D-glucose, were used to determine the biogenetic origin of the protons in staurosporine (**1**). From uniformly deuterium-labeled glucose up to seven deuterium atoms were incorporated into **1** according to EIMS. Probably, five protons were incorporated into the glycon moiety, and two protons into the aglycon unit based on an analysis of the ^{1}H-NMR spectrum and the fragment peaks in the EIMS.

Staurosporine (**1**), an indolo[2,3-*a*]carbazole alkaloid, was first isolated from *Streptomyces staurosporeus* (AM-2282) in 1977,[1] and its absolute configuration was recently determined by X-ray crystallographic analysis.[2] The total synthesis of **1** has also been successfully completed.[3] It has been found that **1** possesses very interesting biological activities, including antifungal,[1] hypotensive,[4] and platelet aggregation activities,[5] and cell cytotoxicity.[6] Its cytotoxic activity against tumor cells, based on the inhibition of protein kinase C (PKC), is the most important aspect of its biological profile.[6,7] Hence, several derivatives have been isolated or semisynthesized in order to increase its selectivity against PKC and to eliminate its toxicity to normal cells.[8−17]

UCN-01 (**2**), UCN-02 (**3**),[10,11] 7-*O*-methyl-UCN-01 (**4**),[16] and CGP41 251 (**5**),[17] which are staurosporine derivatives, are selective PKC inhibitors. UCN-01 and UCN-02, two 7-hydroxystaurosporine epimers, isolated from *Streptomyces* sp., show enhanced selective inhibition against PKC and possess anticancer activity both in vivo and in vitro.[18] NA-382 (**6**), a 7-oxostaurosporine (**7**) derivative, not only exhibits higher selectivity against PKC, which leads to a decrease in cytotoxicity, but also shows the effect of intracellular accumulation of vinblastine in Adriamycin-resistant P-388 cells, which have the MDR phenotype.[19,20] The combination of UCN-01 and mitomycin C was active against MMC-insensitive tumors, and this has resulted in a clinical trial.[21] Because the only difference between UCN-01 (**2**) and staurosporine (**1**) is 7-hydroxy substitution, the 7-position has become an important target for the modification of the structure to improve the specificity against PKC. For instance, 7-*O*-methyl-UCN-01 (**4**) shows more selectivity against PKC than does UCN-01, without losing activity.[16] From this recent progress, it has become apparent that a PKC inhibitor may well become a candidate for a new class of clinically useful antineoplastic drugs or as an adjunct against clinically resistant tumor cells.



1 Staurosporine X= Y= H; R= H
2 UCN-01 X= α-OH; Y= H; R= H
3 UCN-02 X= H; Y= β-OH; R= H
4 7-*O*-Methyl UCN-01 X= α-OMe; Y= H; R= H
5 CGP41-251 X= Y= H; R= Benzoyl
6 NA-382 X= Y= O; R= $COOCH_2CH_3$
7 7-Oxo-staurosporine X= Y= O; R= H

It has been established by our group that the indolocarbazole unit of staurosporine is derived from two intact units of tryptophan,[22,23] probably by way of tryptamine, and that the *O*- and *N*-methyl groups are derived from L-methionine.[24] We have now successfully incorporated [1-^{13}C]-D-glucose and [U-$^{13}C_6$]-D-glucose into the amino sugar moiety of staurosporine. From the ^{13}C-NMR spectra of the resulting labeled staurosporine derivatives, we have identified that glucose is the biosynthetic precursor of staurosporine glycon, and that tryptophan derived from glucose can incorporate into the aglycon unit in **1**. From additional deuterium glucose feeding experiments, including the use of [6-$^2H_2$]-D-glucose, [2-^{2}H]-D-glucose, and [U-$^2H_7$]-D-glucose, we can identify that (a) 6-H_2 and 1-H in glucose are retained on 2′-Me and 6-H in **1**, respectively, (b) that 2-H in glucose labels the glycon of **1**, and (c) that one proton from glucose is transferred to the 2′-Me during the replacement of the 6-OH of glucose during staurosporine biosynthesis. In addition, the protons at C-6 in glucose are also capable of labeling the indolocarbazole unit, although the specific labeled positions are not yet identified.

Results and Discussion

A number of different sugar sources were examined in order to evaluate whether *S. staurosporeus* could

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continue to produce staurosporine from those sugars in the absence of glucose. Compared to a glucose standard, L-arabinose and xylose could also produce staurosporine in a similar yield when the cell system was fully grown. Other sugars, including ribose, D-mannitol, L-(−)-sorbose, methyl α(D)-glucopyranoside, calcium D-glucuronic acid, and 2-deoxyglucose, as well as sodium pyruvate, yielded less or no staurosporine. Xylose and arabinose are five-carbon sugars requiring bioconversion to a six-carbon sugar before incorporation into staurosporine. Hence, glucose was regarded as the most probable precursor of staurosporine glycon.

A high rate (70%) was observed for the incorporation of [1-^{13}C]-D-glucose into **1**, indicating that the major carbon source of the amino sugar moiety is derived from glucose. Minor incorporation was observed to occur at another position (20%) (C-6′ in **1**) through glycolysis and gluconeogenesis. A similar phenomenon, which showed ^{13}C enhancement of both C-1 and C-6 by [1-^{13}C]-D-glucose, was observed during glucose metabolism studies in the insect *Manduca sexta*.[25] Direct glucose incorporation was established by the subsequent [U-$^{13}C_6$]-D-glucose incorporation experiment (see below).

[U-$^{13}C_6$]-D-Glucose has been applied in biosynthetic studies of pactamycin,[26] streptonigrin,[27] and lincomycins.[28,29] The experiments using [U-$^{13}C_6$]-D-glucose in combination with the analysis of the ^{13}C−^{13}C spin-coupling patterns in these antibiotics permits the determination of those carbon atoms that remain conjoined during the biosynthetic process. The direct incorporation of intact [U-$^{13}C_6$]-D-glucose into **1** was observed based on the appearance of the resonances of C-2′ to C-5′, which have similar intensity in their triplet satellites.[30] The patterns (doublet mixed with triplet) and intensities (doublets for C-2′ and C-5′, weak; doublets for C-3′ and C-4′, strong) in the ^{13}C spectrum of carbon atoms C-2′ to C-5′ in **1** correspond to the pattern of labeling observed for glycolytic cleavage (62%).[31] Strong doublet signals of C-3′ and C-4′ and their ^{13}C−^{13}C couplings to C-2′ ($J_{3',2'} = 46$) and C-5′ ($J_{4',5'} = 35$), respectively, indicate that bond cleavage between C-3 and C-4 in glucose occurred to form two three-carbon units via glycolysis, and then the two units recombined to form glucose via gluconeogenesis before incorporation into **1**. The ^{13}C chemical shifts, one-bond coupling patterns and their relative distributions, and the ^{13}C−^{13}C coupling constants in **1** are listed in Table 1. The direct incorporation rate of an intact glucose unit into **1** was 38%.[31] The coupling constants and the relative intensities of the doublets of C-2′ ($J_{2',2'Me} = 43$, 15%) and C-5′ ($J_{5',6'} = 38$, 29%) indicate minor bond breakage between C-2′ and C-3′ and C-4′ and C-5′ (cleavage percentages: 21% and 46%, respectively). The calculated percentages of glycolytic cleavage are shown in Scheme 1. This results in the enhancement of the center peak of C-3′ and C-4′ due to enrichment by ^{13}C of a one carbon unit rejoined into glucose via gluconeogenesis, especially the resonance of C-4′, which shows a strong center peak relative to its satellites (see Figure 1). The major incorporations from alternative pathways are shown in Scheme 2.

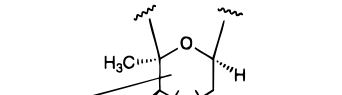
From the same experiment, enriched ^{13}C from [U-$^{13}C_6$]-D-glucose was also incorporated into the aglycon moiety, which indicates that tryptophan production from glucose is predominant, although soybean meal could be also a tryptophan source. Tryptophan is known to be derived from glucose through the shikimic acid pathway.[32] The incorporation of intact units from glucose to tryptophan and the coupling patterns in the resulting ^{13}C-NMR spectrum were described by Gould and Cane in their biosynthetic studies of streptonigrin.[27] The observation and calculation of the coupling pattern and coupling constants of the ^{13}C resonances in the indolocarbazole region are in accord with the incorporation of intact two- and four-carbon units, shown in Scheme 3. The coupling patterns, relative distribution, and coupling constants are shown in Table 1. Owing to the symmetric structure of the indolocarbazole, the coupling patterns

Table 1. Chemical Shifts, Coupling Patterns, and Coupling Constants in [U-$^{13}C_6$]-D-glucose labeled Staurosporine (**1**)

carbon	chemical shift, δ, ppm (in $CDCl_3$)[a]	multiplicity[c] and relative intensity[d]	J_{cc}, Hz
1	106.9	s, 89; br d, 11	$J_{1,2} = 56$
2	125.0	s, 48; d, 27, dd 25	$J_{2,1} = 56$; $J_{2,3} = 56$[e]
3	119.7	s, 54; d, 10; dd 36	$J_{3,2} = 56$; $J_{3,4} = 56$[e]
4	127.1	s, 58; br d, 42	$J_{4,3} = 57$
4a	123.4	s, 50; d, 50	$J_{4a,13a} = 55$
4b	115.3	s, 61; d, 39	$J_{4b,12b} = 61$
4c	118.4[b]	s, 66; d, 33	$J_{4c,5} = 66$
5	173.6	s, 70; d, 30	$J_{5,4c} = 66$
7	45.9	s, 73; d, 27	$J_{7,7a} = 41$
7a	132.2[b]	s, 66; d, 34	$J_{7a,7} = 41$
7b	114.0	s, 62; d, 38	$J_{7b,12a} = 61$
7c	124.6	s, 48; d, 52	$J_{7c,11a} = 56$
8	120.6	s, 63; br d, 37	$J_{8,9} = 54$
9	119.9	s, 55; d, 13; dd, 32	$J_{9,8} = 54$; $J_{9,10} = 56$[e]
10	124.1	s, 67; d, 18; dd, 15	$J_{10,9} = 55$; $J_{10,11} = 56$[e]
11	115.1	s, 86; br d 14	$J_{11,10} = 56$
11a	139.7	s, 54; d, 46	$J_{11a,7c} = 56$
12a	130.7	s, 61; d, 39	$J_{12a,7b} = 61$
12b	127.1	s, 60; d, 40	$J_{12b,4b} = 61$
13a	136.6	s, 54; d, 46	$J_{13a,4a} = 55$
2′	91.1	s, 30; d, 15; dd, 55	$J_{2',2'-Me} = 43$ $J_{2',2'-Me} = 43$; $J_{2',3'} = 46$[e]
3′	84.1	s, 37; d, 39; dd, 24	$J_{3',2'} = 46$ $J_{3',2'} = 46$; $J_{3',4'} = 38$[e]
4′	50.4	s, 58; d, 25; dd, 16	$J_{4',5'} = 35$ $J_{4',5'} = 35$; $J_{4',3'} = 38$[e]
5′	30.1	s, 35; d, 29; dd, 36	$J_{5',6'} = 38$ $J_{5',6'} = 38$; $J_{5',4'} = 35$[e]
6′	80.1	s, 47; d, 53	$J_{6',5'} = 38$
2′-Me	30.0	s, 47; d, 53	$J_{2'-Me',2'} = 43$
OMe	57.3	s, 100	
NMe	33.3	s, 100	

[a] Obtained at 90.8 MHz. Chemical shifts of the singlets are listed. Unambiguous assignments were made by comparison with the reported spectrum.[22] [b] The chemical shifts of C-4c and C-7a were revised from Meksuriyen and Cordell[22] based on the coupling constants of $J_{4c,5}$ and $J_{7,7a}$. [c] Coupling patterns: s, singlet; d, doublet; br d, broad doublet; dd, doublet of doublets (doublet of doublets were observed as a triplet). [d] The calculation of relative distribution for the singlet is based on the height of the center peak over the height of the center peak plus their satellites, as well as doublet and doublet of doublets. [e] The indirect calculation of the coupling constants for the doublet of doublets (dd) is based on the neighboring doublet coupling constant and the unambiguous assignment.[22]

Scheme 1. Calculated Percentages of Glycolytic Cleavage in the Glycon Unit of **1** from [U-$^{13}C_6$]-D-Glucose Feeding Experiment



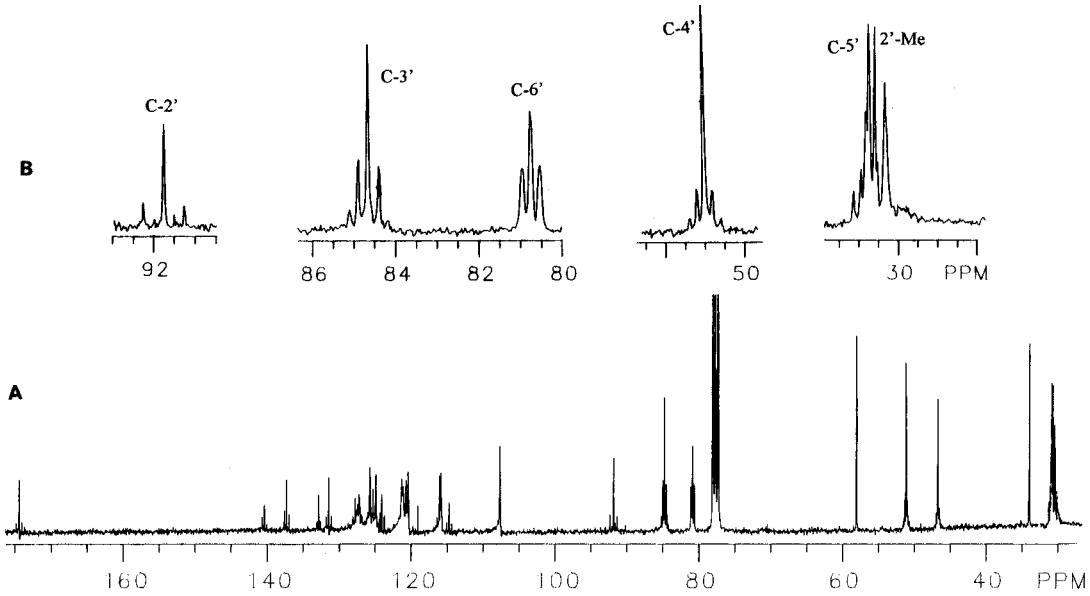
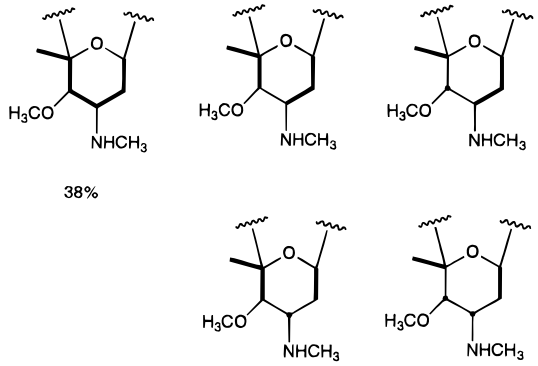


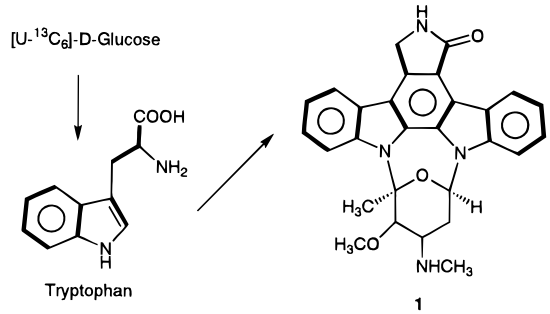
Figure 1. 90.8 MHz Broad band decoupled ^{13}C NMR of [U-$^{13}C_6$]-D-glucose labeled staurosporine (**1**); 6 mg in 0.5 mL of $CDCl_3$. (A) Full ^{13}C-coupled NMR spectrum; (B) expansion spectrum of (A) showing signals of aminosugar, C-2′ (s + d + dd), C-3′ (s + d + dd), C-4′ (s + d + dd), C-5′ (s + d + dd), C-6′ (s + d), and 2′Me (s + d).

Scheme 2[a]



[a] The major intact incorporation from alternative carbon units are shown. Thickened solid bonds indicate intact carbon units derived from intact [U-$^{13}C_6$]-D-glucose of via glycolysis, whereas the dots indicate the enriched one-carbon unit rejoined to glucose through gluconeogenesis.

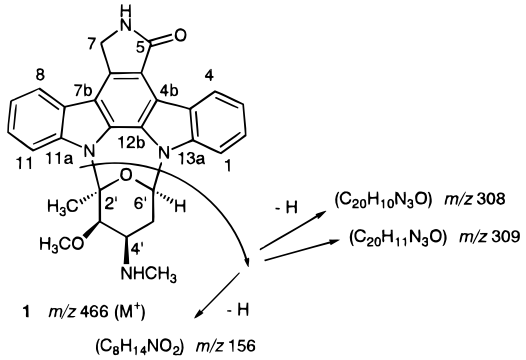
Scheme 3. Proposed Biogenetic Origin Leading from Glucose through Tryptophan to the Indolocarbazole Unit of Staurosporine[a]



[a] Bold lines represent intact carbon units retaining $^{13}C-^{13}C$ coupling.

and relative distributions between two corresponding carbon atoms (e.g., C-1 vs. C-11; C-4a vs. C-7c) show a similarity. Artificial ^{13}C enrichment of the indolocarbazole could be eliminated by adding tryptophan into the medium. This method could also be applied to indirectly identify precursors or intermediates by adding them with uniformly labeled ^{13}C-glucose.

Scheme 4. Fragment Ions of Staurosporine (**1**) in the EIMS



To further understand the mechanism of glucose incorporation, deuterium-labeled glucose was used as a precursor. [6-$^2H_2$]-D-Glucose, [2-^{2}H]-D-glucose, and [U-$^2H_7$]-D-glucose, without natural glucose being added, were fed to the microorganism individually, and no significant isotope effect was observed. From the EIMS of the deuterium-labeled staurosporine obtained from the [6-$^2H_2$]-D-glucose feeding experiment, predominantly four deuterium atoms were incorporated into the molecule **1**, due to the observation of a molecular ion at *m/z* 470 (MW 466 for unlabeled **1**) . The glycon fragment peak was observed at *m/z* 158 instead of at *m/z* 156 in the EIMS of unlabeled **1**, which indicates that two deuterium atoms were incorporated into the glycon moiety and the other two deuterium atoms were incorporated into the aglycon unit (fragment peak at *m/z* 311 compared to the original peaks at *m/z* 308 and 309). The fragmentation of **1** in the EIMS is summarized in Scheme 4. It was further confirmed by ^1H NMR that two deuterium atoms were retained at the 6-position (2′-Me in **1**). The intensity of the 2′-Me resonance (δ 2.33, s) was significantly decreased, and the integration was around 1.2 protons compared to a single proton (3′-H). The pathway of forming the 6-deoxy sugar is therefore probably through a dehydration reaction.[33] The deuterium incorporation into the indolocarbazole residue could not be observed in the ^1H NMR.

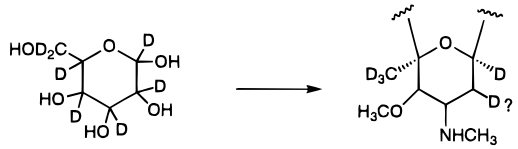
Table 2. List of the Major Fragment Ion Peaks and Relative Intensities (in parentheses) of Naturally Occurring and Deuterium-Labeled Staurosporine

precursor fed	natural glucose	[6-$^2H_2$]-D-glucose	[2-^{2}H]-D-glucose	[U-$^2H_7$]-D-glucose
molecular ion	466 (78) 467 (26)	467 (38) 468 (73) 469 (83) 470 (59)	466 (100) 467 (86)	467 (15) 468 (32) 469 (55) 470 (73) 471 (86) 472 (87) 473 (74) 474 (56)
unknown fragment ions	337 (59) 338 (18)	337 (95) 338 (100) 339 (66)	337 (92) 338 (65)	337 (46) 338 (86) 339 (100) 340 (93) 341 (76) 342 (50)
aglycon (indolocarbazole)	308 (8) 309 (6)	309 (25) 310 (28) 311 (36) 312 (24)	308 (17) 309 (16)	310 (26) 311 (32) 312 (28) 313 (22)
glycon (2,6-dideoxy sugar)	156 (100) 157 (9)	156 (37) 157 (27) 158 (77) 159 (36)	156 (61) 157 (41)	157 (27) 158 (45) 159 (51) 160 (60) 161 (50) 162 (36)
unknown fragment ions	87 (15) 88 (15)	87 (82) 88 (66) 89 (51)	87 (44) 88 (47)	87 (60) 88 (72) 89 (73) 90 (47) 91 (22)

In the [2-^{2}H]-D-glucose feeding experiment, deuterium was incorporated into **1** to a limited extent due to the glycolysis effect. The molecular ion at *m/z* 467 (86% intensity of 100% at *m/z* 466) and the glycon fragment peak at *m/z* 157 (41% compared to 61% of *m/z* 156) were observed, and no incorporation into the aglycon moiety was observed (fragment peaks at *m/z* 308 or 309). The calculated incorporation rate of 36% resembles that calculated from the direct incorporation rate (38%) from the [U-$^{13}C_6$]-D-glucose feeding experiment owing to the 2-H in glucose, which is lost when glucose-6-phosphate is converted to fructose-6-phosphate during glycolysis. Unfortunately, we could not determine the specific labeled position and stereochemistry due to the low incorporation and the overlapping signal of the 5′α-H (δ 2.39, ddd).

In the [U-$^2H_7$]-D-glucose feeding experiment, there were primarily seven deuterium atoms incorporated into the molecule of **1**, according to the observation of a shift from *m/z* 466 to 473 of M^+. The intensities and relative fragment peaks may be found in Table 2. The proposed glycon fragment peak was observed at *m/z* 157−161, which indicates that up to five deuterium atoms labeled the glycon unit and that the other two deuterium atoms were incorporated into the aglycon (fragment peak at *m/z* 311). The two deuterium atoms that labeled the indolocarbazole were probably from the C-6 position in glucose based on the [6-$^2H_2$]-glucose feeding experiment. From the ^{1}H-NMR spectrum of this labeled staurosporine, the signals of the 2′-Me (δ 2.33, s) and the 6′-H (δ 6.52, d) were significantly decreased due to high deuterium incorporation from 1-^{2}H and 6-$^2H_2$ in [U-$^2H_7$]-D-glucose. High deuterium incorporation at the C-6′ position leads to a simplified coupling pattern (doublet of doublets) for 5′α-H (originally, ddd). It is believed that H-2 in glucose (i.e., 5′-H in **1**) is retained at either the α or β position during 2,6-dideoxyhexose biosynthesis.[34−38] Again, the incorporation of 2-^{2}H in glucose was not observed in the ^{1}H-NMR spectrum.

Scheme 5. Deuterium Atoms Retained and Transferred from [U-$^2H_7$]-D-Glucose to the 2,6-Dideoxyamino Sugar Unit of Staurosporine[a]



[a] Question mark represents the specific incorporation position is not identified yet.

The integration of the 2′-Me group in **1** obtained from [U-$^2H_7$]-D-glucose was small (about half) compared with that obtained from the [6-$^2H_2$]-D-glucose feeding experiment, which was consistent with the 4,6-dehydration pathway previously described in two different pathways by Liu and Floss.[33,34] The hydrogen on C-4 is transferred to C-6 intramolecularly in glucose, and the migrating hydrogen replaces the 6-hydroxyl group with inversion of configuration in the enzyme studies of dTDP-glucose oxidoreductase and in the biosynthetic studies of antibiotic granaticin.[34,38] In the biosynthetic studies of all deoxyhexose sugars reported thus far, deoxygenation occurs at the sugar nucleotide level and is initiated by 4,6-dehydratases (oxidoreductases), which catalyze the irreversible conversion of the hexose nucleotide into a 4-keto-6-deoxyhexose moiety by way of a 4,6-dehydration pathway.[33] From our deuterium labeling experiments, the results did not conflict with those studies of 2,6-dideoxy sugar biosynthetic pathways. It is possible that *Streptomyces staurosporeus* shares the same biosynthetic pathway with those of the studied 2,6-dideoxy sugars. The overall fate of deuterium atoms in the conversion of uniformly labeled glucose into the 2,6-dideoxy-amino sugar moiety in **1** is shown in Scheme 5.

Further studies are underway to (a) determine whether the 7-H_2 in staurosporine is retained from tryptophan/tryptamine, (b) establish whether substituted tryptophans can serve as precursors of modified staurosporine derivatives, and (c) determine the overall sequence of the biosynthetic pathway, including the sequence of attachment of the sugar moiety to the indole nitrogen atoms.

Experimental Section

General Experimental Conditions. 1D and 2D ^{1}H-NMR spectra were recorded on either a Nicolet NMC 360 instrument operating at 360 MHz or a Varian XL-300 instrument operating at 300 MHz. TMS or $CDCl_3$ was used as an internal standard ($\delta_{TMS} = 0$ ppm; $\delta_{CDCl_3} = 7.24$ ppm), and chemical shifts are reported in ppm on the δ scale; coupling constants (*J*) in Hz, s = singlet, d = doublet, dd = double doublet, and t = triplet. ^{13}C-NMR spectra were obtained using a Nicolet NT-360 (360 MHz) spectrometer operating at 90.8 MHz or a Varian XL-300 (75.4 MHz) instrument. A ^{13}C-tuned probe accommodating a 5-mm NMR tube with the samples

dissolved in $CDCl_3$ was used. ^{13}C-NMR spectra of ^{13}C-enriched staurosporine were obtained on the Nicolet NMC 360 instrument. In the [1-^{13}C]-D-glucose feeding experiment, the incorporation rate of 70% was calculated from the height of enriched C-6′ compared to the corresponding resonance in unlabeled staurosporine. The spectra of labeled and unlabeled samples were run under the same instrument conditions, and C-3′ was used as a relative standard peak. The incorporation rate of 2′-CH_3 (20%) was calculated in the same way. [1-^{13}C]-D-glucose (99%), [U-$^{13}C_6$]-D-glucose (98%), [6-$^2H_2$]-D-glucose (98%), [2-^2H]-D-glucose (98%), and [U-$^2H_7$]-D-glucose (98%) were purchased from Cambridge Isotope Laboratories. Tryptophan, 2-deoxyglucose, sugar derivatives, and sodium pyruvate were purchased from Sigma Chemicals.

Maintenance 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, IL. The activation and maintenance of *S. staurosporeus* are the same as previously described.[22,23]

Fermentation. The fermentation methods are the same as those previously described.[22,23]

Isolation and Purification of Staurosporine (1). The isolation and separation methods were modified and described as below. Culture fermentation broth (100 mL) containing mycelia was adjusted to pH 10 with NH_4OH and 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 the staurosporine was isolated by column chromatography or by preparative TLC. TLC was carried out using Si gel GF_{254} (Merck) plates developed with $CHCl_3$−MeOH (10:1). Staurosporine gave an R_f of 0.3 in this system. Developed chromatograms were visualized by fluorescence quenching under 254 nm UV light and by spraying with Dragendorff's reagent. Preparative TLC was carried out using two solvent systems. First, the chromatogram was developed in $CHCl_3$−EtOAc (1:1) and then developed in $CHCl_3$−MeOH (10:1). After developing, staurosporine was detected by UV, and removed and dissolved in $CHCl_3$−MeOH (10:2) or Me_2CO. The yield of staurosporine produced from *S. staurosporeus* was around 1.2−1.5 mg/100 mL. Column chromatography was carried out using Si gel (60 mesh). Columns were slurry packed in $CHCl_3$, and eluted with a mixture of $CHCl_3$−EtOAc (1:1), then eluted with a mixture of $CHCl_3$−MeOH (50:1), while fractions were collected.

Characterization of Staurosporine (1). The physical data, including melting point, UV, IR, $[\alpha]_D$, EIMS, and ^1H-NMR, were described in the previously published papers.[22,23] For ^{13}C-NMR (90.8 MHz, $CDCl_3$) and C−C one-bond coupling constants ($^1J_{CC}$), see Table 1. EIMS of different deuterium-labeled staurosporines are summarized in Table 2.

Initial Carbon Source Test as Precursor of Staurosporine. Several different sugars were fed to evaluate whether they could act as precursors of staurosporine. Xylose, L-arabinose, ribose, D-mannitol, L-sorbose, methyl α(D)-glucopyranoside, D-glucuronic acid, sodium pyruvate, and 2-deoxyglucose were tested. The concentration of all sugars was 3%. Fermentation and isolation procedures were the same as previously described. The crude extracts were brought to the same volume (0.5 mL in Me_2CO) and applied to the TLC in the same volume. TLC plates were developed and visualized by UV or by spraying with Dragendoff's reagent.

Feeding Experiments with Stable Isotopes. Purities of ^{13}C-precursors were confirmed by NMR spectral analysis before they were used. ^{13}C-Labeled glucose, [1-^{13}C]-D-glucose (8 × 125 mg with 3 g glucose in 100 mL medium) and [U-$^{13}C_6$]-D-glucose (8 × 62 mg with 2 g glucose/100 mL medium), was added, respectively, into the fermentation medium before sterilization by autoclave. For the feeding experiment with [6-$^2H_2$]-D-glucose, [2-^2H]-D-glucose, and [U-$^2H_7$]-D-glucose, labeled glucose was used at a concentration of 1 g/50 mL fermentation medium in a plugged 250-mL Erlenmeyer flask, and no natural glucose was added. Cells were harvested after 72 h, and the subsequent isolation and separation procedures (preparative TLC) were the same as described previously. The yields of staurosporine production were 0.5−0.6 mg/50 mL in all experiments.

Feeding Experiments with [U-$^{13}C_6$]-D-Glucose and Tryptophan. This experiment was performed to eliminate the incorporation of enriched ^{13}C to the indolocarbazole. The procedure was the same as above except that tryptophan (50 mg/100 mL) was added with [U-$^{13}C_6$]-D-glucose after 24 h of fermentation by Millipore filtration (0.2 μm). The isolation and separation procedures (preparative TLC) were the same as the methods described previously. The yield of staurosporine production was 1.2 mg/100 mL.

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References and Notes

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(30) The incorporation rate is not given due to the long range couplings of two or three bonds that exist on the satellites, and thus the ratio of the height between the satellite and center peak is not proportional to the ratio of the number of the ^{13}C atom. Triplet here actually means doublet of doublets.
(31) The cleavage rate is calculated based on the ratio of the intensity distribution between the doublet and the triplet for C-3′ and C-4′, respectively [39/(39 + 24) × 100% = 62% or 25/(25 + 16) × 100% = 61%]. Hence, the direct incorporation rate is calculated from 24/(39 + 24) × 100% = 38% or 16/(25 + 16) × 100% = 39%. The error may result from the different concentrations and the difference of the coupling constants over two or three bonds and was ignored.
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