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BIOSYNTHESIS OF STAUROSPORINE, 1. ^{1}H- AND ^{13}C-NMR ASSIGNMENTS[1,2]

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ABSTRACT.—Complete and unambiguous assignments of the ^{1}H- and ^{13}C-nmr spectra of the potent antitumor antibiotic staurosporine [**1**] have been accomplished using a combination of one- and two-dimensional nmr techniques, including one-bond and long-range heteronuclear multiple-quantum coherence spectroscopy (HMQC). Staurosporine [**1**] was found to be highly cytotoxic in the KB and P-388 assays but was inactive in the microtubulin assembly assay.

Staurosporine [**1**], an indolo[2,3-*a*]carbazole alkaloid, was first isolated from *Streptomyces staurosporeus* Awaya (AM-2282) (1) and, subsequently, from other actinomycetes, e.g., *Streptomyces actuosus* (2), and *Streptomyces* species strain M-193 (3). The structure and stereochemistry were deduced by X-ray crystallography of its MeOH H_2O solvate, and the antibiotic was named staurosporine (4,5). Staurosporine possesses inhibitory activity against fungi and yeasts but has no significant effects on bacteria. The acute toxicity (LD_{50}) of its hydrochloride on intraperitoneal administration in mice is 6.6 mg/kg (1). Preliminary evidence has also shown that staurosporine possesses strong antihypertensive activity (6,7). Staurosporine has pronounced in vitro activity against a number of experimental tumors, e.g., a human neuroblastoma cell line (NB-1) (2), HeLa S3 cells, B16 melanoma cells, and P-388 leukemia cells (2,8,9). Most interestingly, it is a potent inhibitor of protein kinase C (8) and platelet aggregation (3).

The aglycone moiety **2** of staurosporine itself is important for biological activity (10), strongly suggesting that other indolocarbazole derivatives might also possess important biological activity. The occurrence of several unusual indolocarbazoles has recently been reported, e.g., the antibacterials arcyriaflavin B [**3**] and C [**4**], isolated from the slime mold *Arcyria denudata* (L.) Wettstein (11), the antitumor antibiotic rebeccamycin [**5**] isolated from *Nocardia aerocolonigenes* strain C38383-RK-2 (ATCC 39243) (12), and the protein kinase C inhibitors K-252a [**6**], b [**7**], c [**2**], and d [**8**], isolated from *Nocardiopsis* sp. strain K-252 (NRRL 15532) and strain K-290 and *Actinomadura* sp. SF-2370 (10, 13–15). The structures of these indolo[2,3-*a*]carbazole derivatives are summarized in Figure 1. Although the therapeutic potential of staurosporine and related compounds as antitumor antibiotic agents has not as yet been established in clinical practice, this agent is of potential interest in chemotherapy because of the manner in which it interacts with cancer cells (2,8,9,12).

Although three biogenetic syntheses of staurosporine aglycone [**2**] and related compounds have been completed (16–18), to date there have been no reports on the biosynthesis of these interesting metabolites; such knowledge might ultimately be applicable for the production of systematically modified staurosporine derivatives. As a working hypothesis, staurosporine is most likely constructed in nature from a tryptophan-derived aromatic unit and an amino sugar moiety which are connected stereospecifically by an unusual double *N*-glycosidic linkage. As the initial steps in a program to examine the biosynthesis of staurosporine, we report in this and the subsequent paper (19) exper-

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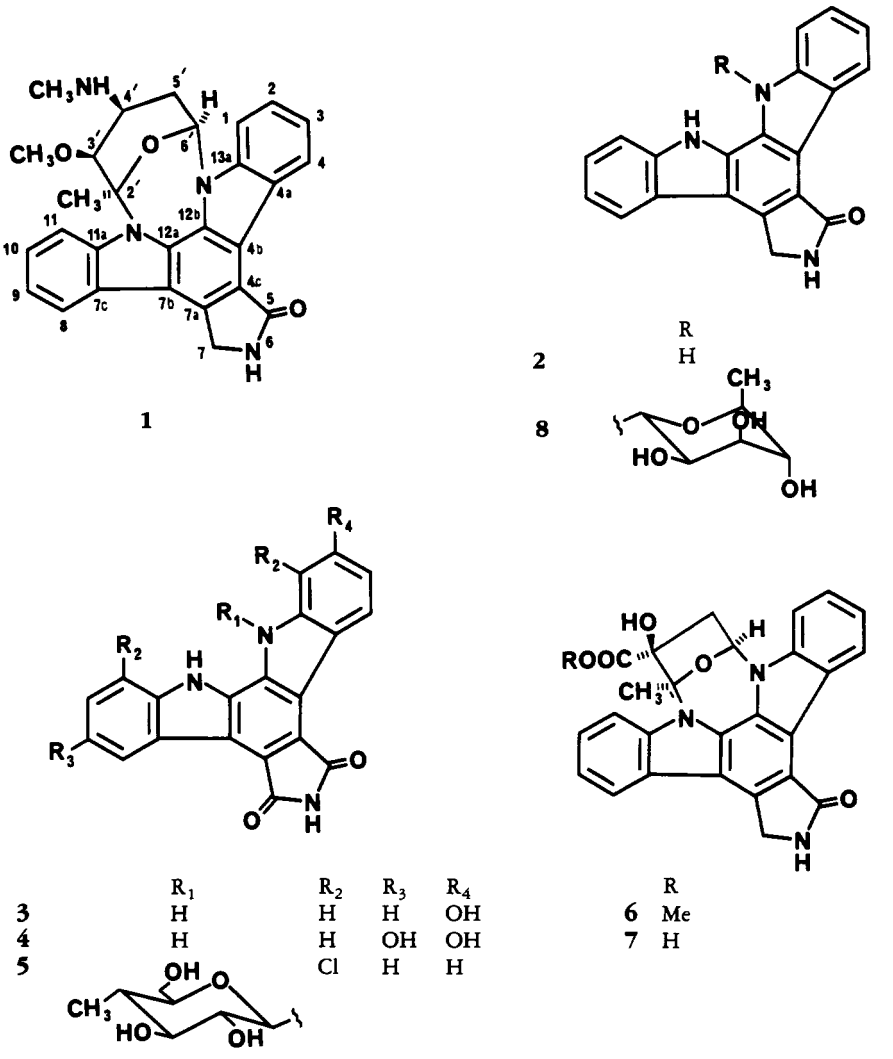

FIGURE 1. Structures of some indolo[2,3-*a*]carbazole alkaloids.

iments designed to establish the spectral parameters of staurosporine, a preliminary evaluation of the production of this metabolite and a demonstration of the aromatic building blocks involved in its biosynthesis.

Preliminary assignment of the ^{1}H-nmr chemical shifts, in different nmr solvents, of staurosporine aglycone [**2**] and SF-2370 or K-252a [**6**] (15,20), was achieved by homonuclear decoupling and nOe experiments and showed that there was a chemical shift difference between the two parts of the indolocarbazole nucleus (Table 1). Because complete and unambiguous determination of nmr spectral parameters is a normal prerequisite for stable isotope incorporation studies, we began our work by attempting to assign the ^{1}H-nmr spectrum of staurosporine.

Staurosporine is soluble in DMSO and DMF, slightly soluble in MeOH, EtOAc, and $CHCl_3$, and insoluble in H_2O (1,2). Previous nmr data of staurosporine and other indolocarbazole alkaloids were, therefore, mostly recorded in polar deuterated solvents, i.e., DMSO-d_6 (1,2,20), MeOH-d_4, and Me_2CO-d_6 (11). Because of the multiplicity of overlapping resonances in these solvents between signals of interest in the high-field region, especially the protons in the glycosidic moiety, it was decided to use $CDCl_3$ for

TABLE 1. Comparison of the Proton Assignments of Indolocarbazole Alkaloids [Chemical shifts in ppm (multiplicity, J, Hz)].

Proton	Compound			
	1	2	6	
	($CDCl_3$)[a]	(DMSO-d_6)[b]	($CDCl_3$)[c]	(DMSO-d_6)[b]
1	7.26 (t, 7.6)	7.73 (d, 8.3)	7.15 (d)	7.90 (d, 8.3)
2	7.46 (t, 7.6)	7.44 (br t)	7.23 (t, 8.1)	7.49 (br t)
3	7.35 (t, 7.6)	7.24 (br t)	7.03 (t, 7.0)	7.29 (br t)
4	9.42 (t, 7.6)	9.24 (d, 7.9)	8.70 (d, 7.7)	9.24 (d, 7.9)
6	6.81 (br s)	8.49 (br s)	5.62 (br s)	8.64 (br s)
7	4.99 (AB)	4.98 (s)	4.39 (d, 16.5)	5.04 (AB, 17.3)
			4.55 (d)	5.00
8	7.87 (d, 7.8)	8.05 (d, 7.8)	7.81 (d, 7.7)	8.05 (d, 7.8)
9	7.30 (t, 7.8)	7.31 (br t)	7.36 (t, 7.3)	7.36 (br t)
10	7.41 (t, 7.8)	7.48 (br t)	7.48 (t, 8.1)	7.49 (br t)
11	7.91 (d, 7.8)	7.79 (d, 8.1)	7.98 (d)	7.95 (d, 8.5)
12		11.56 (br s)		
13		11.38 (br s)		
1′			6.75 (dd, 7.7, 5.0)	7.15 (dd, 7.4, 4.9)
2′			3.46 (dd)	3.41 (dd, 14.0, 7.4)
			2.93 (dd, 14.3)	2.04 (dd, 14.0, 4.9)
3′	3.86 (d, 3.6)			
4′	3.33 (t, 3.6)			
5′	2.71 (dd, 14.7, 3.6)			
5′	2.39 (ddd, 14.7, 5.2, 3.6)			
6′	6.52 (d, 5.2)			
Me	2.33 (s)		2.16 (s)	2.16 (s)
OMe	3.37 (s)		4.05 (s)	3.94 (s)
NMe	1.54 (s)			
HO			5.41 (s)	

[a]Obtained at 360 MHz, $d_{TMS} = 0$ ppm.
[b]Data are from Meksuriyen and Cordell (19).
[c]Data are from Sezaki *et al.* (15).

nmr analysis. In the COSY spectrum (Figure 2), a doublet for H-3′ at 3.86 ppm was scalar coupled to a methine proton at 3.33 ppm, assigned to H-4′, which in turn showed scalar connectivity to only one of two nonequivalent geminal protons, H-5′, at 2.39 ppm, but not to the resonance at 2.71 ppm. However, both H-5′ protons showed scalar coupling to a downfield proton, H-6′, at 6.52 ppm. As noted from the X-ray crystal structure data, the indolo[2,3-*a*]carbazole aromatic system is slightly bent, and the two terminal benzene rings assume an angle of 7.5° with each other because of the steric hindrance between the methylamino and the aromatic system; the seven-membered heterocyclic ring has an envelope-like conformation while the six-membered pyran ring assumes a flattened chair conformation (5). Under these conditions H-4′ was disposed at 90° to the downfield H-5′ signal (2.71 ppm) and at an equal dihedral angle to the upfield H-5′ signal (2.39 ppm) and to H-3′. Consequently, the two nonequivalent geminal protons, H-5′β and H-5′α, could be assigned to the protons with the downfield and upfield chemical shifts, respectively. The substantial ^1H-^1H nOe observed for H-5′β on irradiation of the aminomethyl protons (1.54 ppm) in the nOe difference spectrum confirmed the above assignment. The unusual high field shift of the aminomethyl protons could possibly be due to steric and anisotropic effects from the aromatic system. The observation of cross peaks in the 2D-nOe spectrum between the

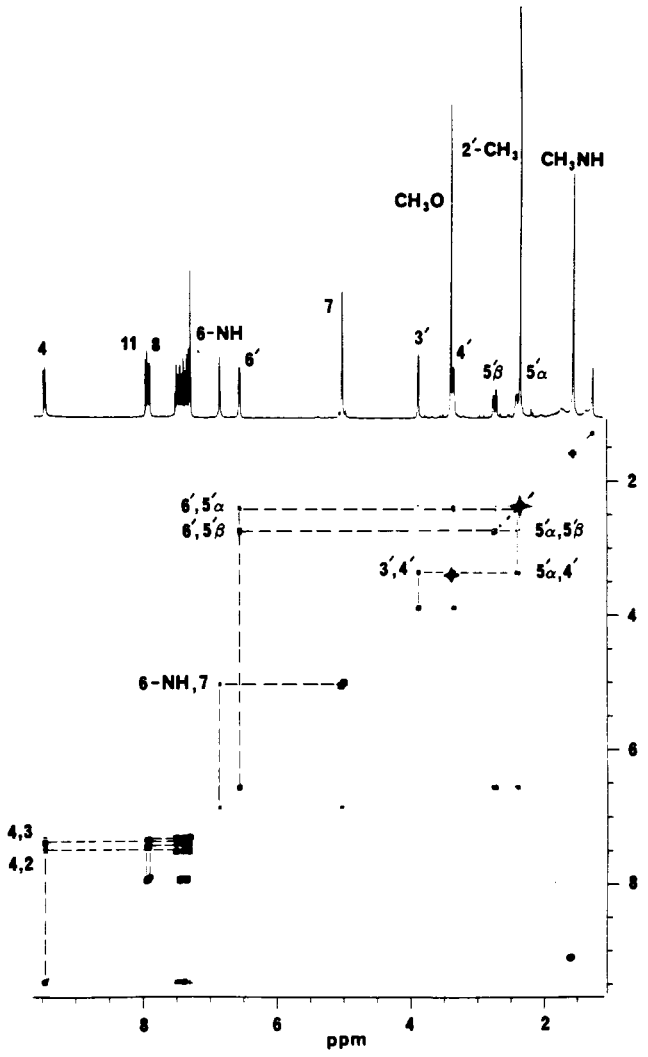

FIGURE 2. ^{1}H-^{1}H COSY spectrum of staurosporine [1].

Me-2′ and a resonance at 7.91 ppm and between the AB methylene protons (H-7) and a resonance at 7.87 ppm permitted the assignment of H-11 and H-8, respectively. The protons H-6′, H-8, H-11, and the downfield H-4 showed nOe cross peaks to resonances which, unfortunately, fell into a strongly crowded second order system of the remaining indolocarbazole protons equivocally identified as H-1, H-9, H-10, and H-3, respectively. Ambiguities concerning these assignments were resolved on the basis of long-range ^{1}H-^{13}C connectivity, as will be demonstrated.

Subsequent examination of both broad band proton-decoupled and attached proton test (APT) ^{13}C-nmr spectra did not permit complete and unambiguous resonance identification. In addition, because the similarity of a number of quaternary carbon signals was, unfortunately, reflected by overlap of their attached aromatic proton resonances in the ^{1}H-nmr spectrum, the selective INEPT and CSCM 1D techniques provided little additional information. Recent development of the double-quantum coherence nmr, 2D INADEQUATE experiment (21) allows determination of the carbon connectivity pattern through the observation of natural abundance carbon-carbon coupling, but typically a substantial quantity (0.5–1.0 g) of sample is required for spectral analysis (22).

On the other hand, the low solubility of staurosporine [**1**] in $CDCl_3$ made the use of regular heteronuclear ^1H-^{13}C and 2D INADEQUATE experiments difficult. This problem was overcome by the application of two new techniques of sensitivity-enhanced, ^1H-detected heteronuclear multiple-quantum coherence via direct coupling (HMQC) (23–27) for protonated carbons and via heteronuclear multiple-bond coupling nmr spectroscopy (HMBC) (28) for determination of three-bond proton-carbon connectivity. In this way, complete ^{13}C-nmr assignments of staurosporine [**1**] were achieved with only 20 mg of sample available (29).

With the proton assignments tentatively completed, attention focussed on the assignment of the protonated carbons, which could be obtained straightforwardly using the ^1H-detected HMQC for correlating ^1H and ^{13}C chemical shifts (Figure 3). The use

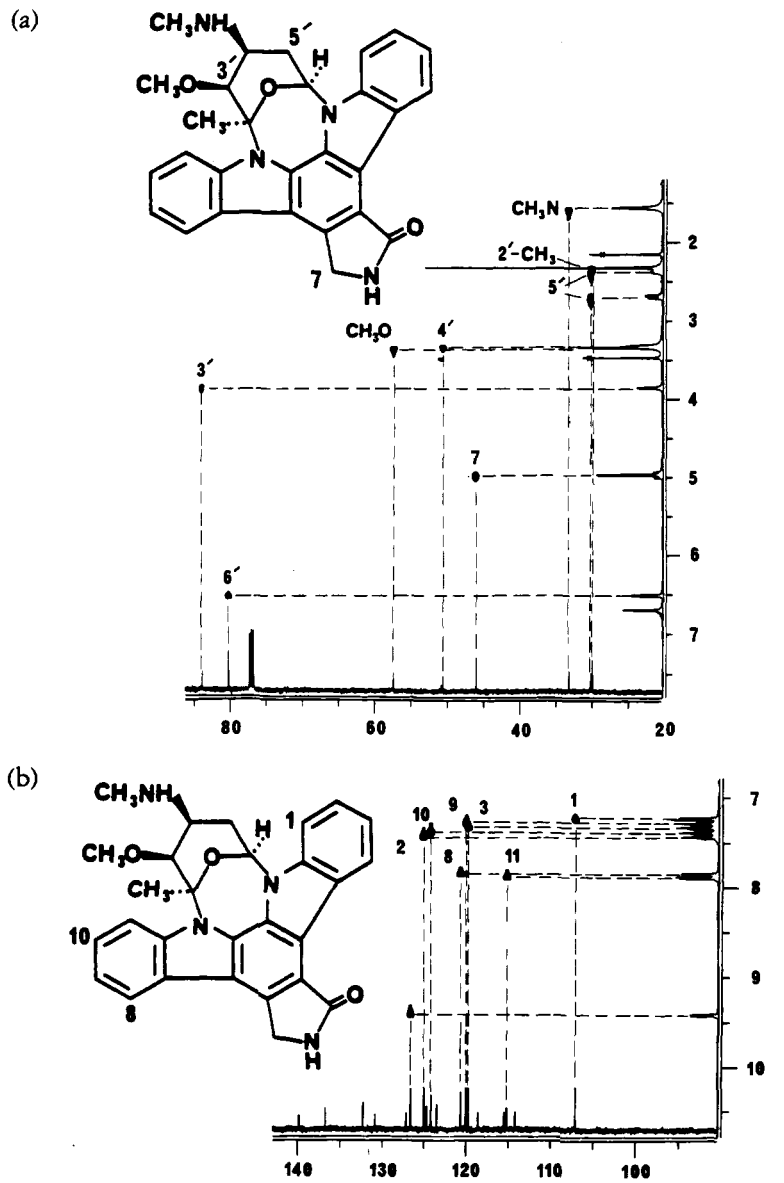

FIGURE 3. Heteronuclear multiple-quantum coherence spectrum of staurosporine [**1**], (a) high-field region, (b) low-field region.

of $^{2}J_{CH}$ and $^{3}J_{CH}$ to generate HMBC then provided a more sensitive alternative equivalent of a heteronuclear shift correlation spectrum via long-range couplings (COLOC) (30,31) which had been previously used to assign the quaternary carbons in K-252a [6] (20). Since two-bond J_{CH} couplings in aromatic systems are typically consistently smaller than the three-bond couplings (32), it was assumed that the correlation between the carbons and the indolocarbazole protons was principally due to three-bond connectivity (Figure 4). Connectivity of H-11 with C-9 and C-7c was observed, and H-8 showed three-bond connectivity to C-7b, C-10, and C-11a and two-bond connectivity to C-7c. Distinction between C-7b and C-11a was clarified by the observation of the connectivity of H-10 with C-8 and C-11a, removing the ambiguity between H-9 and H-10 in the 2D-nOe spectrum through the necessity of internal consistency. Connectivity of the remaining indolocarbazole carbons was established straightforwardly, i.e., H-2 was coupled to C-4 and C-13a, H-3 coupled to C-1 and C-4a, H-9 coupled to C-11 and C-7c, H-1 coupled to C-3 and C-4a, and H-6′ coupled to C-12b.

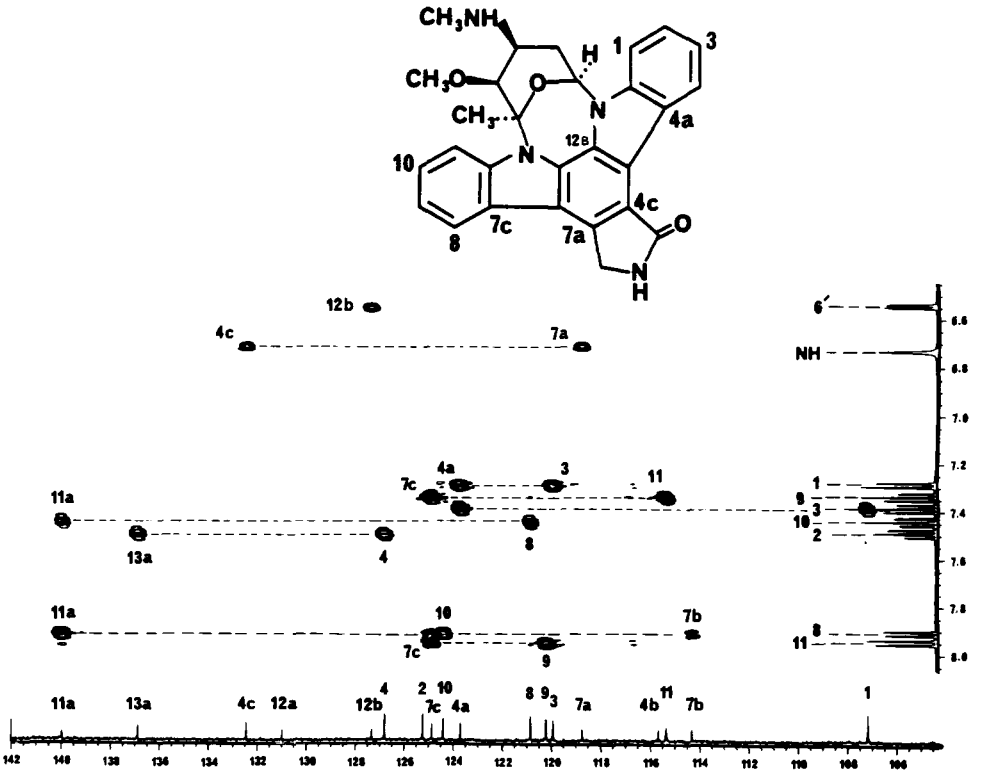

FIGURE 4. Heteronuclear multiple-bond coupling spectrum of staurosporine [1].

Advantageously, HMBC utilizes the full proton magnetization of NH-6 (29,33) providing the connectivity of C-7a and C-4c. Assignment of the latter was confirmed by the connectivity of H-7 with this resonance. The remaining quaternary carbons, C-4b and C-12a, were unambiguously assigned through the observation of the connectivity of H-4 to C-2, C-13a, and C-4b. No other unidentified resonances remained, and the complete proton and carbon assignments are summarized in Tables 1 and 2, respectively.

Staurosporine was evaluated for cytotoxicity in both the murine P-388 lymphocytic leukemia and human carcinoma KB test systems in vitro according to established protocols (34,35). Potent cytotoxic activity (i.e., $ED_{50} = 0.0024$ μg/ml for the KB sys-

TABLE 2. Comparison of the Carbon Assignments of Indolocarbazole Alkaloids (Chemical Shifts in ppm).

Carbon	Compound			
	1	2	6	
	($CDCl_3$)[a]	(DMSO-d_6)[b]	($CDCl_3$)[c]	(DMSO-d_6)[b]
1	106.90	112.0	107.2	109.0
2	124.96	125.1	120.8	125.4
3	119.66	119.0	119.3	119.4
4	127.06	125.4	125.9	125.6
4a	123.38	123.0	124.9	122.6
4b	115.34	115.7	116.5	115.8
4c	132.17	120.0	117.8	119.5
5	173.62	172.6	173.6	171.7
7	45.95	45.4	45.9	45.4
7a	118.43	133.0	122.2	132.9
7b	114.00	114.2	114.2	114.6
7c	124.57	122.7	123.9	124.1
8	120.57	121.2	120.5	121.2
9	119.94	120.0	125.5	120.4
10	124.12	125.1	125.1	125.0
11	115.13	111.4	114.8	114.7
11a	139.67	139.2	136.9	139.8
12a	130.69	128.0	129.0	128.3
12b	128.26	125.2	132.3	123.9
13a	136.58	139.3	140.3	136.8
1′			84.9	84.9
2′	91.09		42.4	42.5
3′	84.05		85.7	84.9
4′	50.35		99.5	99.3
5′	30.14			
6′	80.10			
Me	30.00		22.8	22.8
OMe	57.27		53.4	52.6
NMe	33.27			
COOMe			172.5	172.8

[a]Obtained at 90.8 MHz, $\delta_{TMS} = 0$ ppm.
[b]Data are from Meksuriyen and Cordell (19).
[c]Data are from Sezaki *et al.* (15).

tem and <0.08 μg/ml for the P-388 system) was observed. The antitumor agents, vincristine, vinblastine, and colchicine, produce a characteristic inhibition of cell mitosis in the metaphase by binding to tubulin and preventing its assembly to microtubuli (36–39). It therefore seemed appropriate to examine the effect of staurosporine on the in vitro microtubule-assembly system in order to explore the relationship between staurosporine and microtubules. The result of this study showed that staurosporine had no ability to inhibit the tubulin polymerization and did not disrupt microtubular function at concentrations of 6 and 32 μg/ml.

EXPERIMENTAL

GENERAL EXPERIMENTAL CONDITIONS.—*^{1}H-nmr spectra.*—One- or two-dimensional ^{1}H-nmr spectra were recorded on a Varian XL-300 (300 MHz) or a Nicolet NT-360 (360 MHz) spectrometer. Samples were dissolved in $CDCl_3$ and were transferred to 5-mm nmr tubes. TMS was used as an internal standard ($\delta_{TMS} = 0$ ppm), and chemical shifts are reported in ppm on the δ scale; coupling constants (J) in Hz, s = singlet, d = doublet, dd = doublet of doublets, t = triplet, m = multiplet, and br = broad signal. NOe difference spectra were recorded on a Varian XL300 instrument using standard Varian pulse se-

quences with an interpulse delay of 4 sec and an irradiation period of 2 sec. Two-dimensional nOe (NOESY) spectra were obtained on a Nicolet NT-360 instrument using the pulse sequence D1-90°-t_1-mix-90°-acquire. The acquisition time was 200 msec, and the mixing time was 81.25 μsec. The samples were degassed using a repeated freeze-pump-thaw cycle and then closed under N_2 (subaseal). Data sets of 16K covering a spectral width of 2 KHz were acquired. A 2.0-Hz line broadening was applied to the data prior to Fourier transformation.

^{13}C-nmr spectra.—One- or two-dimensional ^{13}C-nmr spectra were obtained using a Nicolet NT-360 instrument operating at 90.8 MHz using a ^{13}C-tuned probe accommodating a 5-mm sample tube or a Varian XL-300 (75.4 MHz) instrument. The CSCM 1D and selective INEPT experiments were performed on a Nicolet NT-360 spectrometer. Data sets of 16K covering a spectral width of 10,000 Hz were acquired. Proton pulse widths were calibrated using a sample of HOAc in 10% C_6D_6 ($^1J = 6.7$ Hz) in a 5-mm nmr tube (40). The radiofrequency field strength of the soft proton pulse was on the order of 25 Hz for these experiments. Two-dimensional, ^1H-detected, HMQC and HMBC experiments were performed on 20 mg of **1** using a General Electric GN-500 spectrometer (General Electric Corp.). The HMQC experiment described by Summers *et al.* (41) was used with the one-bond coupling optimized at 139 Hz. The final size of the data matrix was 1K × 1K. Gaussian apodization was used, and double quantum/zero quantum coherence was obtained after addition/subtraction of the two blocks. The total number of acquisitions was 8 for each t_1 with 4 dummy acquisitions also being acquired. The sample was not spun, and total accumulation time was 1.27 h. The HMBC sequence used was that described by Summers *et al.* (41). One-bond coupling was eliminated with a J-filter optimized to 139 Hz, while the long-range coupling was optimized for $J = 7$ Hz. The final size of the data matrix was 2K × 2K. The number of acquisitions at each t_1 was 32 with 4 dummy acquisitions also being acquired. The sample was not spun.

Staurosporine.—Staurosporine [**1**] was obtained from cultures of *S. staurosporeus* strains AM-2282 and NRRL 11182 and purified as described elsewhere (19). The physical and spectroscopic properties were identical with those published previously (2).

ASSAY FOR DRUG-TUBULIN INTERACTION.—*Preparation of microtubule protein.*—Microtubules were purified by the method of Weingarten *et al.* (37). Brains from freshly slaughtered hogs were supplied by American Meat Packing Co., Chicago, IL. The superficial blood vessels and meninges were removed, and the remainder was homogenized in 0.75 ml of purification buffer (0.1 M MES, 1 mM EGTA, 0.5 mM $MgCl_2$, 1 mM mercaptoethanol, 0.1 mM EDTA, and 1 mM GTP, pH adjusted to 6.4 with NaOH) per g of brain tissue. Homogenization was performed in a Waring Blendor at 4°. The homogenate was centrifuged at 45,000*g* for 2.5 h at 0–4° in a Beckman Type 42.1 rotor. The supernatant was removed and diluted 1:1 with purification buffer containing 8 M glycerol and warmed to 37° for 20 min to allow microtubule polymerization. This was centrifuged at 45,000*g* for 2.5 h at 25°, and the supernatants were discarded. The pellets were resuspended in purification buffer (0.2 ml/g of brain), chilled on ice for 30 min, and centrifuged at 75,000*g* for 75 min at 0–4°. The supernatants were diluted 1:1 with purification buffer containing 8 M glycerol and warmed to 37° for 20 min. This microtubule suspension was centrifuged at 75,000*g* for 75 min at 25°. The pellets, containing the purified microtubule protein, were suspended in cold purification buffer and were made up to 8 M glycerol by addition of pure glycerol, so that the final concentration of protein was 3.5–6 mg/ml.

The protein was stored at −20° and was used for 4–6 weeks after purification. Before every experiment, a suitable aliquot of the protein was removed and diluted 1:1 with reassembly buffer (0.1 M MES, 1mM EGTA, 0.5 mM $MgCl_2$, and 1 mM GTP, pH 6.4), and warmed to 37° for 20 min. The microtubules were pelleted by centrifugation at 100,000*g* for 45 min at 25°. Protein concentrations were determined by the method of Lowry *et al.* (42), using bovine serum albumin (BSA) as a standard.

Turbidity measurement.—Microtubule assembly was monitored by the turbidity assay (43). The microtubule pellets were resuspended in reassembly buffer to a final concentration of 1 mg of protein/ml, mixed over a vortex, and chilled on ice for 30 min. The ice-cold microtubule solution (1 ml) was transferred to a uv-cuvette and measurements were made every 15 sec at 350 nm on a Beckman model DU-7 spectrophotometer. Assembly of the microtubules was indicated by an increase in absorptivity. Test compounds were added to the cuvette containing microtubules (1 ml) either before or after assembly reached a stable condition. Colchicine and taxol were used as depolymerization and polymerization controls, respectively (38,39). $CaCl_2$ (4 mM) was added at 45 min to test the extent and reversibility of depolymerization.

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LITERATURE CITED

1. S. Omura, Y. Iwai, A. Hirano, A. Nakagawa, J. Awaya, H. Tsuchiya, Y. Takahashi, and R. Masuma, *J. Antibiot.*, **30**, 275 (1977).
2. H. Morioka, M. Ishihara, H. Shibai, and T. Suzuki, *Agric. Biol. Chem.*, **49**, 1959 (1985).
3. S. Oka, M. Kodama, H. Takeda, N. Tomizuka, and H. Suzuki, *Agric. Biol. Chem.*, **50**, 2723 (1986).
4. A. Furusaki, N. Hashiba, T. Matsumoto, A. Hirano, Y. Iwai, and S. Omura, *J. Chem. Soc., Chem. Commun.*, 800 (1978).
5. A. Furusaki, N. Hashiba, T. Matsumoto, A. Hirano, Y. Iwai, and S. Omura, *Bull. Chem. Soc. Jpn.*, **55**, 3681 (1982).
6. S. Omura, Y. Iwai, and A. Hirano, Ger. Offen. 2,754,326; *Chem. Abstr.*, **89**, 58348y (1978).
7. S. Omura, Y. Iwai, and A. Hirano, Japan. Kokai 78 73,501; *Chem. Abstr.*, **89**, 178086h (1978).
8. T. Tamaoki, H. Nomoto, I. Takahashi, Y. Kato, M. Morimoto, and F. Tomita, *Biochem. Biophys. Res. Commun.*, **135**, 397 (1986).
9. H. Morioka, H. Shibai, Y. Yokogawa, M. Ishihara, T. Kida, and T. Suzuki, Jpn. Kokai Tokkyo Koho JP 60,185,719; *Chem. Abstr.*, **104**, 18649u (1986).
10. S. Nakanishi, Y. Matsuda, K. Iwahashi, and H. Kase, *J. Antibiot.*, **39**, 1066 (1986).
11. W. Steglich, B. Steffan, L. Kopanski, and G. Eckhardt, *Angew. Chem., Int. Ed. Engl.*, **19**, 459 (1980).
12. D.E. Nettleton, W.T. Bradner, J.A. Bush, and T.W. Doyle, Eur. Pat. Appl. EP 115,350; *Chem. Abstr.*, **102**, 22793n (1985).
13. K. Yamada, H. Kase, and K. Kubo, *Jpn. J. Pharmacol.*, **40**, 265P (1986).
14. H. Kase, K. Iwahashi, and Y. Matsuda, *J. Antibiot.*, **39**, 1059 (1986).
15. M. Sezaki, T. Sasaki, T. Nakazawa, U. Takeda, M. Iwata, and T. Watanabe, *J. Antibiot.*, **38**, 1437 (1985).
16. B. Sarstedt and E. Winterfeldt, *Heterocycles*, **20**, 469 (1983).
17. S.M. Weinreb, R.S. Garigipati, and J.A. Gainor, *Heterocycles*, **21**, 309 (1984).
18. J. Bergman and B. Pelcman, *Tetrahedron Lett.*, **28**, 4441 (1987).
19. D. Meksuriyen and G.A. Cordell, *J. Nat. Prod.*, **51**, 893 (1988).
20. T. Yasuzawa, T. Iida, M. Yoshida, N. Hirayama, M. Takahashi, K. Shirahata, and H. Sano, *J. Antibiot.*, **39**, 1072 (1986).
21. A. Bax, R. Freeman, and T.A. Frenkiel, *J. Am. Chem. Soc.*, **103**, 2102 (1981).
22. R.N. Moore, G. Bigam, J.K. Chan, A.M. Hogg, T.T. Kakashima, and J.C. Vederas, *J. Am. Chem. Soc.*, **107**, 3694 (1985).
23. L. Muller, *J. Am. Chem. Soc.*, **101**, 4481 (1979).
24. M.R. Bendall, D.T. Pegg, and D.M. Doddrell, *J. Magn. Reson.*, **52**, 81 (1983).
25. A. Bax, R.H. Griffey, and B.L. Hawkins, *J. Magn. Reson.*, **55**, 301 (1983).
26. A. Bax, R.H. Griffey, and B.L. Hawkins, *J. Am. Chem. Soc.*, **105**, 7188 (1983).
27. D.H. Live, D.G. Davis, W.C. Agosta, and D. Cowburn, *J. Am. Chem. Soc.*, **106**, 6104 (1984).
28. A. Bax and M.F. Summers, *J. Am. Chem. Soc.*, **108**, 2093 (1986).
29. M.F. Summers, L.G. Marzilli, and A. Bax, *J. Am. Chem. Soc.*, **108**, 4285 (1986).
30. H. Kessler, C. Griesinger, J. Zarbock, and H. Loosli, *J. Magn. Reson.*, **57**, 331 (1984).
31. H. Kessler, W. Bermel, and C. Griesinger, *J. Am. Chem. Soc.*, **107**, 1083 (1985).
32. P.E. Hansen, *Prog. Nucl. Magn. Reson. Spectrosc.*, **14**, 175 (1981).
33. A. Bax, A. Aszalos, Z. Dinya, and K. Sudo, *J. Am. Chem. Soc.*, **108**, 8056 (1986).
34. R.I. Geran, N.H. Greenberg, M.M. MacDonald, A.M. Schumacher, and B.J. Abbott, *Cancer Chemother. Rep.*, **3**, 1 (1972).
35. M. Arisawa, J.M. Pezzuto, C. Bevelle, and G.A. Cordell, *J. Nat. Prod.*, **47**, 453 (1984).
36. L. Wilson, J.R. Bamburg, S.B. Mizel, L.M. Grisham, and K.M. Creswell, *Fed. Am. Soc. Exp. Biol.*, **33**, 158 (1974).
37. M.D. Weingarten, M.M. Suter, D.R. Littman, and M.W. Kirschner, *Biochemistry*, **13**, 5529 (1974).
38. L.D. Barnes and G.M. Roberson, *Arch. Biochem. Biophys.*, **196**, 511 (1979).
39. P.B. Schiff, J. Fant, and S.B. Horwitz, *Nature*, **277**, 665 (1979).
40. A. Bax, *J. Magn. Reson.*, **52**, 76 (1983).
41. M.F. Summers, L.G. Marzilli, and A. Bax, *J. Am. Chem. Soc.*, **108**, 4285 (1986).
42. O.H. Lowry, N.J. Rosebrough, A.L. Farr, and R.J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
43. F. Gaskin and C.R. Cantor, *J. Mol. Biol.*, **89**, 737 (1974).

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