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The Crystal and Molecular Structure of Staurosporine, a New Alkaloid from a *Streptomyces* Strain

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The molecular sturcture of staurosporine, an alkaloid from a *Streptomyces* strain, has been determined by means of an X-ray crystallographic analysis of its methanol solvate ($C_{28}H_{26}N_4O_3 \cdot CH_3OH$). The crystals are monoclinic, with four formula units in a unit cell with dimensions of $a=23.487(6)$, $b=7.636(3)$, $c=15.638(4)$ Å, and $\beta=116.71(5)°$; the space group is C2. 2532 unique intensity data were collected on a four-circle diffractometer with LiF-monochromated Cu $K\alpha$ radiation. The structure was solved by the Monte Carlo direct method, using the 20 strongest reflections as the starting set; the 72nd random phase set led to the correct solution. The R value reached 0.047 by block-diagonal least-squares refinements. Staurosporine has been found to contain a unique dimeric indole structure. Because of the steric hindrance, the indolo[2,3-*a*]carbazole aromatic system in the molecule is slightly bent; the two terminal benzene rings make an angle of 7.5° with each other.

Staurosporine (**1**), formerly referred to as AM-2282, is an alkaloid isolated from *Streptomyces staurosporeus* Awaya, Takahashi, and Ōmura, nov. sp., and shows strong hypotensive activity as well as antimicrobial activity against fungi and yeast.[1] Since **1** exhibits characteristic ultraviolet absorption at 292 nm, it was compared with other known antibiotics (*e.g.*, oxamicetin[2] and pyridomycin[3,4]) which absorb in a region near 292 nm. However, **1** could not be structurally correlated to these known compounds. Therefore, we have tried to determine the molecular structure of **1** by means of X-ray analysis of its methanol solvate. The results have already been reported in a preliminary form.[5] The present paper will describe the details of the structure determination and molecular geometry of this novel alkaloid, **1**.

Experimental

Pale-yellow plate-like crystals, mp 270 °C (decomp), were obtained from a methanol solution of **1**. A single crystal with dimensions of about $0.5\times0.5\times0.2$ mm^3 was used for the X-ray measurement. The crystal data are summarized in Table 1. The unit-cell dimensions and reflection intensities were measured on a Rigaku four-circle diffractometer with LiF-monochromated Cu $K\alpha$ radiation ($\lambda=1.5418$ Å). The θ-2θ continuous-scan technique was applied at a 2θ scan rate of 1° min^{-1}; the background was measured for 30—60 s at each end of the scan range. Three standard reflections, measured at intervals of every 29 reflections, showed no significant decrease in intensity during the course of data collection. The intensities were corrected for the Lorentz and polarization factors, but not for the absorption or the extinction effect. In the range of 2θ values up to 140°, 2532 independent structure factors above the $\sigma(F)$ level were selected for the structure determination.

Structure Determination

The structure was elucidated by the Monte Carlo direct method,[6] using the 20 reflections with the greatest $|E|$ values as the starting set. In order to extend each of the tentative-phase sets derived from random numbers, 12 cycles of the tangent procedure were carried out using 487 $|E|$ values above 1.30. The 72nd phase set which showed a low R_K value of 0.274 ($R_K=\sum||E_o|-k|E_c||/\sum|E_o|$) and a nonzero q value of 0.400 ($q=1-\sum_h|\langle E_h\rangle|(|\sum_k E_kE_{h-k}|/\sum_k|E_kE_{h-k}|)/\sum_h|\langle E_h\rangle|$)[7] led to the correct solution; an E-map based on 458 phases afforded 33 of the 37 independent non-hydrogen atoms. The remaining four atoms were located in a difference Fourier map. After several cycles of the least-squares refinement using the carbon atomic scattering factors for all the non-hydrogen atoms, the nitrogen and oxygen atoms were assigned by taking account of the values of isotropic temperature factors as well as the interatomic distances. The structure thus obtained was refined by the block-diagonal least-squares method with anisotropic thermal parameters. After 25 hydrogen atoms had been located in a second difference Fourier map, further least-squares refinements were performed including the hydrogen atoms with isotropic temperature factors. The weighting scheme used was as follows:

$$w = 1/\{\sigma(F)^2 \exp(c_1X^2+c_2Y^2+c_3XY+c_4X+c_5Y)\},$$

where $X=|F_o|$ and $Y=\sin\theta/\lambda$. The c_1, c_2, c_3, c_4, and c_5 coefficients were evaluated from the $(\Delta F)^2$ distribution: $c_1=-0.552\times10^{-3}$, $c_2=39.5$, $c_3=0.0541$, $c_4=0.0528$, and $c_5=-44.7$. In this manner, the R value reached 0.047. The final atomic parameters are listed in Table 2.[8]

The calculations were done on a FACOM 230-75 computer at the Hokkaido University Computing Center, using our own programs. The atomic scattering

Table 1. The crystal data

$C_{28}H_{26}N_4O_3 \cdot CH_3OH$	$F.W.=498.58$
Crystal system	Monoclinic
Space group	C2
Cell dimensions	$a=23.487(6)$ Å
	$b=7.636(3)$ Å
	$c=15.638(4)$ Å
	$\beta=116.71(5)°$
V	2505.4 Å^3
Z	4
D_c	1.322 g cm^{-3}
μ(Cu $K\alpha$)	6.86 cm^{-1}

TABLE 2. THE FINAL ATOMIC PARAMETERS AND ESTIMATED STANDARD DEVIATIONS
The atomic coordinates are multiplied by 10^4.

Atom	x	y	z	$B_{eq}^{a)}$/Å^2	Atom	x	y	z	$B_{eq}^{a)}$/Å^2
C(1)	2119(2)	1027(4)	5023(2)	4.10	C(20)	3591(1)	1190(5)	4997(2)	4.38
C(2)	1612(2)	937(5)	5240(2)	4.83	C(21)	3090(1)	1679(4)	4012(2)	3.27
C(3)	1002(2)	1431(5)	4565(2)	4.69	C(22)	2427(1)	1861(3)	3678(2)	2.95
C(4)	880(1)	2024(5)	3659(2)	4.06	C(23)	2010(1)	1611(4)	4109(2)	3.18
C(5)	1393(1)	2078(4)	3424(2)	3.10	C(24)	883(1)	3122(4)	1662(2)	3.38
N(6)	1420(1)	2575(3)	2579(1)	2.95	C(25)	715(1)	5082(5)	1637(2)	4.01
C(7)	2048(1)	2455(3)	2739(2)	2.73	C(26)	1166(1)	6348(4)	1492(2)	3.86
C(8)	2341(1)	2859(3)	2152(1)	2.73	C(27)	1220(1)	5751(4)	602(2)	3.75
N(9)	2077(1)	3518(3)	1238(1)	3.00	C(28)	1404(1)	3841(4)	633(2)	3.28
C(10)	2558(1)	3829(3)	973(2)	3.06	O(29)	1042(1)	2685(3)	903(1)	3.50
C(11)	2510(2)	4535(4)	122(2)	3.76	O(30)	4480(1)	1845(5)	3709(2)	5.25
C(12)	3079(2)	4746(4)	55(2)	4.13	N(31)	1777(1)	6406(3)	2347(2)	3.84
C(13)	3664(2)	4278(5)	805(3)	4.31	C(32)	2191(3)	7798(6)	2331(3)	6.37
C(14)	3707(1)	3570(4)	1644(2)	3.68	O(33)	669(1)	5439(4)	2497(2)	4.72
C(15)	3143(1)	3322(3)	1736(2)	3.11	C(34)	362(3)	7063(9)	2478(4)	7.65
C(16)	3005(1)	2689(3)	2487(2)	2.85	C(35)	294(1)	2004(6)	1437(2)	5.12
C(17)	3372(1)	2054(4)	3429(2)	3.20	O(M)	4222(4)	−1024(9)	2286(8)	14.00
C(18)	4065(1)	1747(5)	3984(2)	4.04	C(M)	3885(6)	−2014(10)	2566(11)	12.99
N(19)	4166(1)	1291(5)	4870(2)	4.92					

a) $B_{eq}=8\pi^2(u_1^2+u_2^2+u_3^2)/3$, where u_i is the root-mean-square deviation in the i-th principal axis of the thermal ellipsoid.

factors were taken from the International Tables.[9]

Results and Discussion

Molecular Structure. The molecular skeleton of **1** obtained is illustrated in Fig. 1, while the bond distances and angles are given in Table 3. From these results, it is concluded that **1** has the **1a** structure containing a unique dimeric indole skeleton.

As Fig. 2 shows, the aromatic system composed of the A-, B-, C-, D-, and E-rings is slightly bent; the terminal A- and E-rings deviate from the mean plane of the central C-ring onto the side of the N(31)H–C(32)H_3 methylamino group, and make an angle of 7.5° with each other. Of the C–C bond distances in the aromatic system, the C(17)–C(21), C(1)–C(2), and C(13)–C(14)

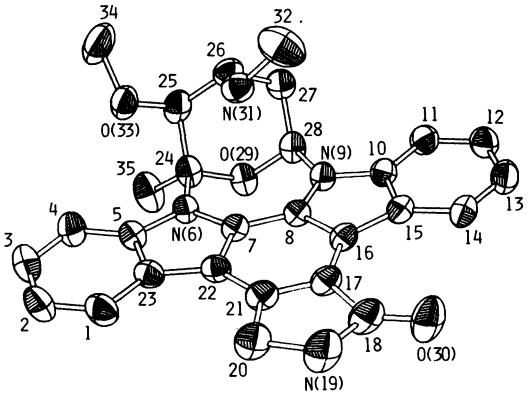
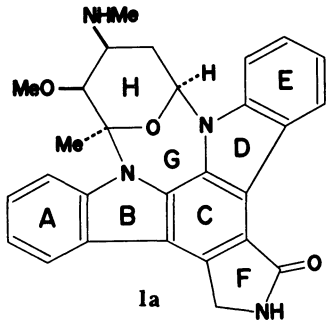


Fig. 1. A perspective view of the staurosporine molecule, showing the crystallographic numbering. Each non-hydrogen atom is represented as a thermal ellipsoid enclosing a 50% probability. The symbol C for carbon atoms is omitted.



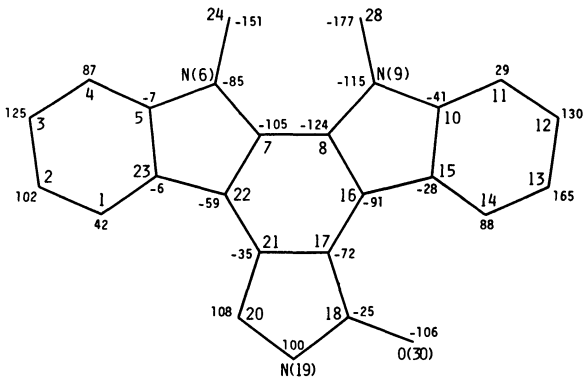
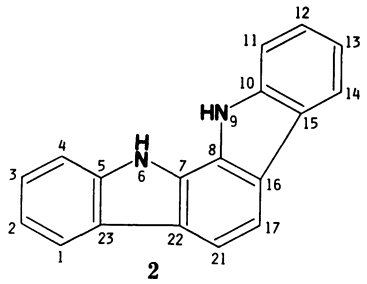


Fig. 2. The deviations ($d\times 10^3$/Å) of the atoms from the mean plane of the A–B–C–D–E aromatic system. This mean plane is represented by the following equation: $0.1522X+0.9328Y+0.3266Z-3.200=0$, where X, Y, and Z are rectangular coordinates measured in Å units along the a*, b, and c axes respectively.

distances are appreciably shorter than the others. These bond shortenings may be explained on the basis of the π-bond-order distribution in the carbazole molecule[10] as follows: when each of the A–B–C and C–D–E ring

Table 3. The bond distances (l/Å) and angles (ϕ/°), with their standard deviations
The e.s.d.'s given in parentheses refer to the last decimal position.

C(1)–C(2)	1.380(6)	C(26)–N(31)	1.458(3)	C(15)–C(16)–C(17)	135.3(2)
C(1)–C(23)	1.407(4)	C(27)–C(28)	1.516(4)	C(16)–C(17)–C(18)	131.3(3)
C(2)–C(3)	1.395(5)	C(28)–O(29)	1.416(4)	C(16)–C(17)–C(21)	120.7(2)
C(3)–C(4)	1.389(5)	N(31)–C(32)	1.448(6)	C(18)–C(17)–C(21)	108.0(2)
C(4)–C(5)	1.407(5)	O(33)–C(34)	1.429(7)	C(17)–C(18)–N(19)	106.5(3)
C(5)–N(6)	1.404(4)	C(M)–O(M)	1.31(2)	C(17)–C(18)–O(30)	128.3(3)
C(5)–C(23)	1.406(3)	C(2)–C(1)–C(23)	118.8(3)	N(19)–C(18)–O(30)	125.2(2)
N(6)–C(7)	1.383(3)	C(1)–C(2)–C(3)	120.4(4)	C(18)–N(19)–C(20)	114.3(2)
N(6)–C(24)	1.482(2)	C(2)–C(3)–C(4)	122.1(4)	N(19)–C(20)–C(21)	101.2(3)
C(7)–C(8)	1.408(4)	C(3)–C(4)–C(5)	117.8(3)	C(17)–C(21)–C(20)	109.9(2)
C(7)–C(22)	1.406(3)	C(4)–C(5)–N(6)	131.3(2)	C(17)–C(21)–C(22)	121.0(2)
C(8)–N(9)	1.372(3)	C(4)–C(5)–C(23)	120.2(3)	C(20)–C(21)–C(22)	129.1(3)
C(8)–C(16)	1.409(3)	N(6)–C(5)–C(23)	108.5(2)	C(7)–C(22)–C(21)	119.6(3)
N(9)–C(10)	1.388(4)	C(5)–N(6)–C(7)	108.0(2)	C(7)–C(22)–C(23)	106.8(2)
N(9)–C(28)	1.451(3)	C(5)–N(6)–C(24)	127.5(2)	C(21)–C(22)–C(23)	133.6(2)
C(10)–C(11)	1.393(4)	C(7)–N(6)–C(24)	124.6(2)	C(1)–C(23)–C(5)	120.6(3)
C(10)–C(15)	1.409(3)	N(6)–C(7)–C(8)	131.5(2)	C(1)–C(23)–C(22)	132.1(2)
C(11)–C(12)	1.396(6)	N(6)–C(7)–C(22)	109.4(2)	C(5)–C(23)–C(22)	107.3(2)
C(12)–C(13)	1.394(4)	C(8)–C(7)–C(22)	119.1(2)	N(6)–C(24)–C(25)	113.2(2)
C(13)–C(14)	1.381(5)	C(7)–C(8)–N(9)	129.4(2)	N(6)–C(24)–O(29)	108.1(2)
C(14)–C(15)	1.409(4)	C(7)–C(8)–C(16)	121.4(2)	N(6)–C(24)–C(35)	110.7(2)
C(15)–C(16)	1.436(4)	N(9)–C(8)–C(16)	109.2(2)	C(25)–C(24)–O(29)	111.3(2)
C(16)–C(17)	1.417(3)	C(8)–N(9)–C(10)	109.0(2)	C(25)–C(24)–C(35)	109.9(2)
C(17)–C(18)	1.480(3)	C(8)–N(9)–C(28)	126.3(2)	O(29)–C(24)–C(35)	103.1(2)
C(17)–C(21)	1.377(4)	C(10)–N(9)–C(28)	124.7(2)	C(24)–C(25)–C(26)	115.6(3)
C(18)–N(19)	1.342(4)	N(9)–C(10)–C(11)	128.7(2)	C(24)–C(25)–O(33)	106.8(3)
C(18)–O(30)	1.232(4)	N(9)–C(10)–C(15)	108.4(2)	C(26)–C(25)–O(33)	111.8(3)
N(19)–C(20)	1.452(4)	C(11)–C(10)–C(15)	123.0(3)	C(25)–C(26)–C(27)	107.2(2)
C(20)–C(21)	1.507(3)	C(10)–C(11)–C(12)	116.6(2)	C(25)–C(26)–N(31)	110.3(2)
C(21)–C(22)	1.409(3)	C(11)–C(12)–C(13)	121.4(3)	C(27)–C(26)–N(31)	113.2(3)
C(22)–C(23)	1.430(4)	C(12)–C(13)–C(14)	121.6(4)	C(26)–C(27)–C(28)	113.5(2)
C(24)–C(25)	1.544(5)	C(13)–C(14)–C(15)	118.6(2)	N(9)–C(28)–C(27)	113.3(2)
C(24)–O(29)	1.434(3)	C(10)–C(15)–C(14)	118.8(3)	N(9)–C(28)–O(29)	108.9(2)
C(24)–C(35)	1.526(4)	C(10)–C(15)–C(16)	107.0(2)	C(27)–C(28)–O(29)	114.1(2)
C(25)–C(26)	1.523(5)	C(14)–C(15)–C(16)	134.2(2)	C(24)–O(29)–C(28)	121.1(2)
C(25)–O(33)	1.423(4)	C(8)–C(16)–C(15)	106.5(2)	C(26)–N(31)–C(32)	113.5(3)
C(26)–C(27)	1.524(5)	C(8)–C(16)–C(17)	118.2(2)	C(25)–O(33)–C(34)	113.5(3)



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systems is taken as the carbazole skeleton, all the above short bonds correspond to the carbazole C_3–C_4 or C_5–C_6 bond having the greatest π-bond order; especially the shortest C(17)–C(21) bond corresponds exactly to such bonds in both A–B–C and C–D–E carbazole skeletons. In order to estimate the π-bond orders in **1**, we performed a Hückel MO calculation on the indolo[2,3-*a*]carbazole (**2**) molecule, assuming the C_{2v} symmetry. The results given in Table 4 show a good correlation between the calculated π-bond orders and the observed C–C bond distances; as has been expected, the C(17)–C(21) bond

Table 4. The π-bond orders in indolo[2,3-*a*]carbazole

Bond[a]	π-Bond order[b]	Distance[c] l/Å
C_1–C_2	0.691	1.381
C_2–C_3	0.642	1.395
C_3–C_4	0.683	1.393
C_4–C_5	0.627	1.399
C_5–C_{23}	0.584	1.408
C_1–C_{23}	0.601	1.408
C_7–C_8	0.592	1.408
C_7–C_{22}	0.599	1.408
C_{22}–C_{23}	0.421	1.433
C_{17}–C_{21}	0.716	1.377
C_{21}–C_{22}	0.579	1.413
C_5–N_6	0.337	1.396
N_6–C_7	0.334	1.378

a) For the atomic numbering, see the **2** formula. b) The parameters used for nitrogen atoms were as follows: $\alpha_N = \alpha_C + 1.5\beta_{CC}$; $\beta_{CN} = 0.8\beta_{CC}$. c) The equivalent bond distances were averaged, the C_{2v} symmetry being assumed for the aromatic system in **1**.

Table 5. The torsion angles (ϕ/°) for the G- and H-rings

C(24)–N(6)–C(7)–C(8)	3.3
N(6)–C(7)–C(8)–N(9)	0.8
C(7)–C(8)–N(9)–C(28)	−4.7
C(8)–N(9)–C(28)–O(29)	−27.1
N(9)–C(28)–O(29)–C(24)	86.1
C(28)–O(29)–C(24)–N(6)	−85.8
O(29)–C(24)–N(6)–C(7)	27.4
C(24)–C(25)–C(26)–C(27)	52.9
C(25)–C(26)–C(27)–C(28)	−53.1
C(26)–C(27)–C(28)–O(29)	48.3
C(27)–C(28)–O(29)–C(24)	−41.6
C(28)–O(29)–C(24)–C(25)	39.2
O(29)–C(24)–C(25)–C(26)	−45.5

has the greatest π-bond order, and the C(1)–C(2) and C(13)–C(14) bonds have the next greatest.

The γ-lactam F-ring is almost planar, and lies approximately in the mean plane of the A–B–C–D–E aromatic system. In *cis*-peptides[11] as well as in *trans*-peptides,[12] usually the N–C=O bond angle is larger than the C_α–C=O angle, but the relation is reversed in the present amide (125.2 and 128.3°). This bond-angle deformation may result from the steric hindrance between the O(30) atom and the C(14)H(14) grouping: O(30)···C(14), 3.192(4); O(30)···H(14), 2.47(4) Å. In this connection, the C(16)–C(17)–C(18) bond angle is also found to be larger than the C(20)–C(21)–C(22) angle by 2.2°. Further, the O(30) atom and the C(14)-H(14) group become more distant from each other by deviating slightly from the mean plane of the aromatic system to the opposite sides (see Fig. 2).

The torsion angles for the G- and H-rings are given in Table 5. The seven-membered G-ring has an envelope-like conformation, while the six-membered H-ring takes a flattened chair conformation. All the N(6), N(9), and N(31) atoms are axially placed on the same side of the H-ring. As the result, the N(31) atom approaches the planar N(6)–C(7)–C(8)–N(9) segment very closely: C(7), 3.087(4); C(8), 3.089(4) Å. This close contact results in a slight deviation of the C(24) and C(28) atoms from the N(6)–C(7)–C(8)–N(9) plane. Since the three N–C bonds at each of the N(6) and N(9) atoms are found to be coplanar, this deviation must arise from pure twistings about the N(6)–C(7) and N(9)–C(8) bonds. These twistings can be expanded into the bending deformation of the indolo[2,3-*a*]-carbazole moiety. Thus, the bending of the aromatic system can be regarded as due mainly to the severe steric hindrance between the methylamino group and the aromatic system.

As will be seen from the C(27)–C(26)–N(31)–C(32) and C(25)–C(26)–N(31)–H(31) torsion angles of 70.2 and −56° respectively, the N(31) atom directs its lone pair approximately toward the C(8) atom, and donates its H(31) proton to the O(33) methoxyl oxygen atom, possibly forming a weak hydrogen bond of the five-membered-ring type: N(31)···O(33), 2.814(4); H(31)··· O(33), 2.41(6) Å; H(31)···O(33)–C(25), 79(1); H(31)··· O(33)–C(34), 96(1)°. The C(34) methoxyl carbon atom is *trans* to the C(24) atom; this corresponds exactly to the most stable of the three staggered conformations about the C(25)–O(33) bond. The O(33) and C(35) atoms are very close to the A-ring: O(33)···C(4), 3.088(4); O(33)···C(5), 3.059(4); C(35)···C(4), 3.108(4) Å. These steric repulsions may explain the following facts: (1) the N(6)–C(24) and C(24)–C(25) bond distances are longer than the N(9)–C(28) and C(27)–C(28) distances by 0.031 and 0.028 Å respectively; (2) the O(29)–C(24)–C(35) bond angle (103.1°) is considerably smaller than the tetrahedral angle; (3) the C(4)–C(5)–N(6) and C(5)–N(6)–C(24) bond angles are larger than the N(9)–C(10)–C(11) and C(10)–N(9)–C(28) angles by 2.6 and 2.8° respectively.

Crystal Structure. The molecular arrangement viewed along the b axis is drawn in Fig. 3. The methanol

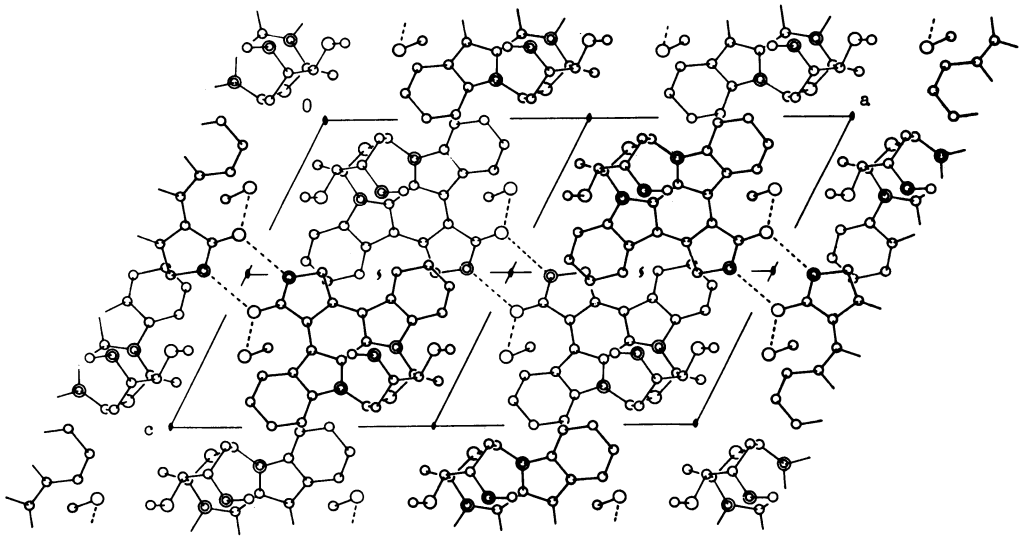

Fig. 3. The crystal structure viewed along the b axis. The samll single circles represent carbon atoms, the large single circles oxygen atoms, and the double circles nitrogen atoms.

molecule is attached to the staurosporine molecule by the O(M)–H···O(30) hydrogen bond with the O···O distance of 2.98(1) Å. The staurosporine molecules related by the two-fold rotation axis are connected by a pair of intermolecular hydrogen bonds, N(19)–H···O(30′) and N(19′)–H···O(30), forming a hydrogen-bonded dimer; the N···O distances are 2.979(3) Å. The N(19), O(30), N(19′), and O(30′) atoms deviate tetrahedrally from the planar arrangement; their deviations from the mean plane are 0.211 Å. These hydrogen-bonded dimers are further held together mainly by the van der Waals interactions. It is interesting to note that the two H-rings, placed approximately perpendicular to the (010) plane, face each other about the two-fold rotation axis.

References

1) S. Ōmura, Y. Iwai, A. Hirano, A. Nakagawa, J. Awaya, H. Tsuchiya, Y. Takahashi, and R. Masuma, *J. Antibiot.*, **30**, 275 (1977).

2) M. Konishi, M. Kimeda, H. Tsukiura, H. Yamamoto, T. Hoshiya, T. Miyaki, K. Fujisawa, H. Koshiyama, and H. Kawaguchi, *J. Antibiot.*, **26**, 752 (1973).

3) K. Maeda, H. Kosaka, Y. Okami, and H. Umezawa, *J. Antibiot., Ser. A*, **6**, 140 (1953).

4) H. Ogawara, K. Maeda, G. Koyama, H. Naganawa, and H. Umezawa, *Chem. Pharm. Bull.*, **16**, 679 (1968).

5) A. Furusaki, N. Hashiba, T. Matsumoto, A. Hirano, Y. Iwai, and S. Ōmura, *J. Chem. Soc., Chem. Commun.*, **1978**, 800.

6) A. Furusaki, *Acta Crystallogr., Sect. A*, **35**, 220 (1979).

7) W. Hoppe, J. Gassmann, and K. Zechmeister, "Crystallographic Computing," ed by F. R. Ahmed, Munksgaard, Copenhagen (1970), p. 26.

8) The tables of the anisotropic thermal parameters and hydrogen parameters and the F_o-F_c table are kept a tthe Chemical Society of Japan (Document No. 8253).

9) "International Tables for X-Ray Crystallography," The Kynoch Press, Birmingham (1974), Vol. IV.

10) H. C. Longuet-Higgins and C. A. Coulson, *Trans. Faraday Soc.*, **43**, 87 (1947).

11) G. N. Ramachandran and V. Sasisekharan, *Adv. Protein Chem.*, **23**, 288 (1968).

12) R. E. Marsh and J. Donohue, *Adv. Protein Chem.*, **22**, 249 (1967).