4-(Aminomethyl)-5-[3-(dimethylamino)propyl]-2-methyl-3-pyridinol (3). At room temperature with stirring Zn dust (74 mg) was added to a solution of **13** (44 mg, 0.185 mmol) in glacial acetic acid (2 mL). Stirring was continued for another 15 min. The reaction mixture was then filtered through a sintered-glass filter under nitrogen pressure. The filtrate was concentrated on a rotary evaporator and the residue was chromatographed (5:1 followed by 10:3 of CH_2Cl_2/MeOH–NH_3) to afford 37 mg (0.166 mmol) of **3** as an off-white solid:[17] ^1H NMR ([$^2H_4$]methanol) δ 7.55 (s, 1 H), 4.10 (s, 2 H), 2.58 (t, J = 7.8 Hz, 2 H), 2.39 (t, J = 7.7 Hz, 2 H), 2.33 (s, 3 H), 2.27 (s, 6 H), 1.70 (br quintet, J = 7.8 Hz, 2 H); ^{13}C NMR ([$^2H_4$]methanol, with the central line of the solvent heptet set to 49.0 ppm as ref) δ 146.15, 136.85, 134.79, 130.81, 125.36, 59.32, 44.77, 39.54, 28.68, 28.18, 18.04.

Acknowledgment. We thank the Swedish Natural Science Research Council for support.

Registry No. **1**, 85-87-0; **2**, 96825-29-5; **3**, 143732-82-5; **4**, 96806-37-0; **5**, 96806-38-1; **6**, 101348-88-3; **7**, 6560-65-2; **8**, 21331-80-6; *cis*-**9**, 143732-83-6; *trans*-**9**, 143732-83-6; **10**, 143732-84-7; **11**, 143732-85-8; **12**, 143732-86-9; **13**, 143732-87-0; pyruvic acid, 127-17-3; transaminase, 9031-66-7.

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11-Hydroxystaurosporine: A Highly Cytotoxic, Powerful Protein Kinase C Inhibitor from a Tunicate

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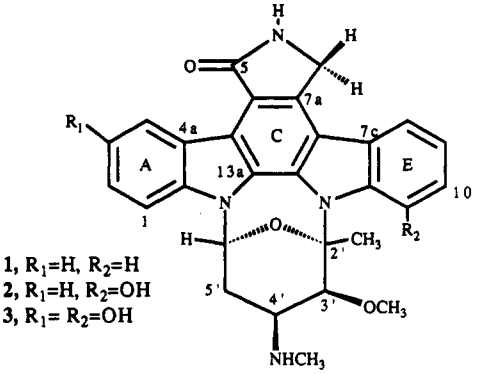
Ascidians, the largest class in the subphylum urochordata (tunicates), are highly developed sessile animals often rich in bioactive secondary metabolites.[2] Noteworthy examples are the didemnins, depsipeptides that show considerable promise as antitumor agents,[3] the antiviral β-carbolines such as the eudistomins,[4] cytotoxic iminoquinone pigments like wakayin[5] and the discorhabdins,[6] and the pentacyclic alkaloid shermilamine.[7] The indolo[2,3-*a*]carbazole ring system is not represented among alkaloids isolated from marine sources, although numerous indolocarbazoles are known from terrestrial microorganisms, slime molds,[8] and more recently, from fresh water blue-green algae.[9] We report here the isolation and structure proof of 11-hydroxystaurosporine (**2**), the first indolo[2,3-*a*]carbazole to be isolated from a marine organism. A dihydroxy derivative, 3,11-dihydroxystaurosporine (**3**), was also isolated but was incompletely characterized due to lack of material. Both are active against human nasopharyngeal cancer cells, and **2** is more potent than staurosporine (**1**) in protein kinase C (PKC) inhibition.



A brown tunicate, *Eudistoma* sp. (family Polycitoridae) was collected in June 1990 in Sapwale Bay, Pohnpei (Federated States of Micronesia), by snorkeling at −1 to −2 m. It was frozen and then lyophilized. Assays of both lipophilic and hydrophilic extracts revealed potent cytotoxic activity, which suggested either a number of active metabolites or a few compounds of intermediate polarity. Extraction of the freeze-dried animal with methanol, followed by solvent partition, then silica gel and RP-18 flash chromatography, and finally HPLC afforded 3.4 mg (0.013%) of **2** as a pale yellow amorphous solid, which discolored slowly in contact with air.

The UV spectrum suggested an extended aromatic chromophore. Since it was unaffected by the addition of acid but underwent reversible bathochromic–hyperchromic shifts of the maxima at 248 and 254 nm (but not those at 292 and 300 nm) upon addition of base, a phenol is likely present.

A combination of ^{13}C NMR spectroscopy and high-resolution, fast atom bombardment mass spectrometry (HRFABMS) established a molecular formula of $C_{28}H_{26}N_4O_4$. The ^1H NMR spectrum revealed the presence of seven aromatic protons, including a very low field doublet at 9.24 ppm. These protons were on two rings, 1,2-di- and 1,2,3-trisubstituted. ^{13}C NMR resonances were observed for 18 aromatic carbons, one carbonyl, and nine other carbons. Most striking were the three resonances between 82 and 100 ppm, which at first suggested the presence of acetylenic, carbinolamine or acetal carbons.

A literature search uncovered two compounds with the same composition, UCN-01 and UCN-02, epimeric 7-hydroxy derivatives of staurosporine.[10] Comparison of ^{13}C and ^1H NMR spectra and the UV spectrum of staurosporine with those of **2** revealed that the two compounds were very similar. Staurosporine (**1**) has the molecular formula $C_{28}H_{26}O_3N_4$; hence **2** has one more oxygen. Since there is no proton at C-11, but there is an exchangeable proton, the phenolic group must be present at that position. Comparison of the calculated ^{13}C and ^1H chemical shifts in ring E (using staurosporine as the model) with those observed in **2** reveals excellent agreement. In addition, **2** forms a diacetate, which provides chemical evi-

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Table I. ^1H and ^{13}C NMR Data for 11-Hydroxystaurosporine (2)

position[a]	δ H[b]	J (Hz)	δ C	HMBC[d]
1	7.39 dt	8.2, small	109.4 d[c]	C3, C4a
2	7.45 ddd	1.2,8.2,8.2	126.5 d	C1, C3, C4, C23a
3	7.27 ddd	1.0, 8.2, 8.0	120.8 d	C1, C2, C3, C4a
4	9.24 ddd	8.0, 1.2, .7	127.1 d	C2, C4b, C23a
4a			124.7 s	
4b			127.0 s	
4c			129.5 s	
5			175.5 s	
6	8.45[e] br s			C4c, C5, C7, C7a
7	5.01, 4.95 AB q	-17.7	47.3 t	C4, C5, C7a, C7b
7a			134.4 s	
7b			116.0 s	
7c			128.6 s	
8	7.52 dd	7.7, 1.0	113.9 d	C7b, C7c, C10, C11a
9	7.20 t	7.7	123.0 d	C7c, C8, C10, C11
10	6.95 dd	7.7, 1.0	113.3 d	C8, C11, C11a
11			144.1 s	
11-OH	10.6[e] br s			
11a			129.6 s	
12a			132.3 s	
12b			127.3 s	
13a			138.9 s	
2′			96.3	
3′	4.48 dd	1.7, 1.5	82.7 d	C3′-OCH_3, C4′, C2′-CH_3
4′	3.38 ddd	1.7, 8.0, 11.7	55.4 d	
5′α	3.01 dddd	-14.5, 9.2, 8.0, 1.5	{30.4 t}	C3′
5′β	2.39 ddd	-14.5, 11.7, 4.6		C3′, C4′
6′	6.56 dd	9.2, 4.6	83.5 d	C2′, C4′, C12b, C13a
2′-CH_3	2.39 s		30.4 q	C3′
3′-OCH_3	3.06 s		60.6 q	C3′
4′-NCH_3	2.55 s		33.0 q	C4′
4′-NH	1.55[e] br s			

[a] Numbered according to ref 7. [b] Shifts in ppm relative to the solvent peak. Unless otherwise noted, spectra were determined in methanol-d_4. [c] Measured by HMQC. [d] See: *One-dimensional and Two-dimensional NMR Spectra by Modern Pulse Techniques*; Nakanishi, K., Ed.; University Science Books: Mill Valley, CA, 1990; pp 168–171. [e] Determined in DMSO-d_6.

dence for the secondary amine and the phenol.

Initially, the upfield resonances and couplings for **2** were puzzling, since they did not correspond well with those originally reported for staurosporine. A more recent report[11] indicates that staurosporine hydrochloride has very different coupling constants for the hydrogens on the amino sugar moiety than the free base, and the authors conclude that the ring is in the boat form. The coupling constants for **2** are in close agreement with those observed for staurosporine hydrochloride, although the chemical shift for H4′ in **2** makes it clear that the free base is being observed in **2**. Furthermore, the *W*-coupling observed between H3′ and H5′α can only be observed if the amino sugar in **2** is a boat. Nuclear Overhauser (NOE) experiments lend further support. Irradiation of H6′ enhances the signal at H5′α, which suggests that the dihedral angle between these two protons is small. Furthermore, irradiation at 2.39 ppm (both 5′α and 2′CH_3) affords NOE enhancements at H3′, and H5′β, and H4′, as well as H6′. These NOE data fit best when the amino sugar ring is in the boat form.

Two factors may contribute to the greater stability of the boat form in **2**. First, examination of a Dreiding model reveals that the oxygen at the 3′-position in the boat form is within hydrogen bonding distance of the phenolic OH. The axial NHMe at C-4′ in staurosporine already destabilizes the chair form, and hydrogen bonding between the ether oxygen and the phenolic OH may provide the extra stability for the boat form. Second, steric crowding between Me-2′ and the phenolic OH in the chair form in **2** is relieved when the sugar ring is in the boat form. Whatever the cause, it is clear from the work of Davis et al.[11] that the energy differences between the chair and the boat forms are much smaller than expected for a cyclohexane ring, and relatively minor effects may alter the stability of one or the other.

The strong similarity between the CD spectrum of **2** (see the supplementary material) and those of staurosporine and RK-286,[12] a related indolocarbazole, supports the conclusion that **2** has the same absolute configuration as staurosporine, and is as shown.

Compound **2** is strongly cytotoxic. In the KB assay, approximately 50% of the cells were killed by 0.7 nM concentrations of **2**. In the LoVo assay **2** is active (~75%) at 0.03 μM. On the other hand, **2** showed no activity in any of the usual antibiotic screens.

Staurosporine and its derivatives are powerful inhibitors of protein kinase C (PKC). They have been used to explore the role of PKC in cell metabolism, in addition to being tested for use in anticancer chemotherapy.[13] The PKC inhibition IC_{50} for **2**, determined by Dr. Robert Bishop of the Schering-Plough Research Institute according to published[14] procedures, is 2.2 nM, about 30% more active than staurosporine.

By HPLC separation of the more polar fraction from the RP-18 flash chromatography, 0.7 mg (0.002%) of a second substance was isolated. Study of the ^1H NMR spectrum revealed that the upfield portion was identical to that from **2**, while there is one less proton in the aromatic region.

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Decoupling experiments demonstrate that ring A is now 1,2,4-trisubstituted, and H-4 is shifted to 8.68 ppm with a very small coupling constant. The changed polarity, the molecular formula (by HRFABMS) that includes one more oxygen, and the changes in the ^{1}H NMR spectrum make it clear that this compound is 3,11-dihydroxystaurosporine (**3**). This substance was also very active against KB cells, but no good quantitative data could be obtained.

Experimental Section

General Procedures. All solvents were distilled in glass prior to use. Melting points are uncorrected.

Isolation of 11-Hydroxystaurosporine (2). Freeze-dried tunicate (53.3 g) was extracted with 550 mL of MeOH/H_2O (4.5:1) to give 11.0 g of crude material. The residual tunicate was reextracted with 350 mL of MeOH, which afforded 3.6 g of dark green waxy solid. Extraction of this with 60 mL of CH_2Cl_2/EtOH (1:1) and then evaporation of half of this solution yielded 0.459 g of dark foamy gum. As much as possible was dissolved in hexane/EtOAc (1:1), and the solution was subjected to flash vacuum chromatography on silica. Elution with hexane/EtOAc (7:3) afforded 47.4 mg of a mixture of sterols; elution with MeOH gave 349 mg of a dark yellow solid. Flash vacuum chromatography of this material on RP-18 in two stages gave several fractions, totaling 27 mg, which were eluted with MeOH/H_2O (75:25). Preparative HPLC (Rainin Amino column, $CHCl_3$/MeOH (20:1)) of these fractions afforded 3.4 mg (.013%) of 11-hydroxystaurosporine, which exhibited a distinctive long-wavelength active spot on TLC, as a pale yellow amorphous solid, $[\alpha]_D$ +10.3 (c = 0.3, MeOH): see Table I for NMR data; UV (MeOH) 212 (29 200), 246 (25 600), 256 (24 500), 292 (50 500), 300 (46 400), 334 (sh) (10 500), 356 (9800), 374 (11 100) nm; IR (KBr) 3400 (br), 1660, 1572, 1453, 1340, and 745 cm^{-1}; HRFABMS 483.2045 calcd for $C_{28}H_{27}N_4O_4$ (MH^+) 483.2032 (Δmmu −2.3); CD 372 nm (Δε, −5.97), 355 (−4.33), 339 (−3.48), 300 (+8.12), 277 (−13.67), 247 (−6.05), 232 (+1.39), 223 (−9.09).

Acetylation of 1.0 mg of **2** with 10 μL of Ac_2O in 0.1 mL of pyridine (overnight) gave a 90% yield of the diacetate after removal of the solvent under a stream of nitrogen, followed by extraction with chloroform and workup under the usual conditions. The diacetate had two new methyl peaks at 2.3 and 2.4 ppm in the ^{1}H NMR spectrum.

3,11-Dihydroxystaurosporine. Preparative HPLC (Amino column, $CHCl_3$/MeOH, 10:1) of the MeOH/H_2O (60:40) fraction from the RP-18 flash chromatography above afforded 0.7 mg (0.0002%) of 3,11-dihydroxystaurosporine as an off-white amorphous solid: ^{1}H NMR (MeOH-d_4) δ 8.69 (1 H, d, J = 2.7), 7.53 (1 H, dd, J = 7.7, 1.3), 7.25 (1 H, d, J = 8.5), 7.21 (1 H, t, J = 7.7), 7.01 (1 H, dd, J = 8.5, 2.4), 6.95 (1 H, dd, J = 7.7, 0.8), 6.54 (1 H, dd, J = 9.1, 4.0), 5.01 and ~4.93 (2 H, AB q, the fourth signal is obscured by the solvent), 4.54 (1 H, br s), 3.62 (1 H, br multiplet), 3.15 (1 H, multiplet, partially obscured by solvent), 3.04 (3 H, s), 2.71 (3 H, br s), 2.46 (1 H, dt, J = 12.5, 4.5), 2.41 (3 H, s); HRFABMS 499.1973 (MH^+) calcd for $C_{28}H_{27}N_4O_5$ 499.1981 (Δmmu = −0.8).

Acknowledgment. We thank Jay Corgiat, Mark Hamann, and Toshio Ichiba for collecting the tunicate, Professor Claude Monniot for identifying the rather poorly preserved tunicate, Ryuichi Sakai and the University of Illinois for the mass spectra, Professor Gordon Gribble for useful discussions, and Wesley Yoshida for his interest and his knowledgeable capabilities with measuring NMR spectra. R.B.K. thanks Hamilton College for a generous sabbatical leave, and we are grateful for continuing support from the PharmaMar Co.

Supplementary Material Available: ^{1}H and ^{13}C NMR spectra, HMBC data, and a CD curve of **2** and a ^{1}H NMR spectrum of **3** (5 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

Asymmetric Epoxidation of Allylic Alcohols Catalyzed by Titanium Alkoxide–Peptide and –α-Amino Acid Complexes Anchored by Phenolic Schiff Base

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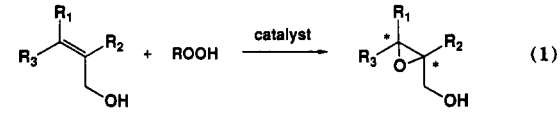
It has become important in synthetic organic chemistry to design enantioselective catalysts that yield optically active compounds with high asymmetric induction.[2] Our recent studies concerning the design of asymmetric catalyst have revealed that several metal complexes of peptide and α-amino acid derivatives anchored by phenolic Schiff bases are quite efficient catalysts for a variety of asymmetric synthetic reactions.[3] During our examination of the scope and limitations of this novel catalyst system, our interest became extended to the asymmetric epoxidation of allylic alcohols. This process has been demonstrated by Katsuki and Sharpless using titanium alkoxide–dialkyl tartrate based catalysts.[4] We wish to report the asymmetric epoxidation of allylic alcohols catalyzed by the peptide and the amino acid complexes of titanium(IV) alkoxides anchored by a phenolic Schiff base.[5]



(1)

The epoxidation of nerol (**1a**) by *tert*-butyl hydroperoxide (TBHP) was performed in methylene chloride at −20 °C in the presence of 10 mol % of a complex formed by mixing equimolar amounts of Ti(O^{i}Pr)$_4$ and Nap-*S*-Val-*S*-Phe-OMe (**2**), which has exhibited high enantioselectivity in the asymmetric syntheses of cyanohydrins,[3b] to afford the corresponding (2*S*,3*R*)-2,3-epoxynerol in 13% ee. The enantioselectivity could be increased to 48% ee by using a salicylal moiety of peptide (**3**) and cumene hydroperoxide (CHP) as the oxidant. It should be noted that the Schiff base of α-amino acid, Dbs-*S*-Val (**4**), also provided similar enantioselectivity (50% ee). This reaction using Dbs-*R*-Val inverted the facial selectivity to afford (2*R*,3*S*)-2,3-epoxynerol in 85% yield with an ee of 46%. In contrast, the use of Dbs-*S*-Valinol (**5**) and Dbs-*S*-Val-NHCy (**6**) lowered the enantioselectivity of the process. It was also observed that the use of a variety of organic hydroperoxides as oxidants influenced the stereoselectivity as well as the reactivity considerably.[6] Sterically hindered hydroperoxides such as 1,1,3,3-tetramethylbutyl hydroperoxide and

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