Further Minor Metabolites of Staurosporine Produced by a *Streptomyces longisporoflavus* Strain

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From the staurosporine producing strain R-19 *Streptomyces longisporoflavus* various minor metabolites were isolated: They include new compounds with a keto function at carbon 4' of staurosporine and several metabolites related to TAN-1030A. The new structures were elucidated by spectroscopic methods, mainly ^{1}H NMR and ^{13}C NMR and by comparison with TAN-1030A. The new compounds inhibited protein kinase C with IC_{50} values in the micromolar range with the exception of those compounds that are alkylated at the lactam nitrogen.

The family of protein kinase C (PKC) subtypes plays a key role in signal transduction and cellular regulation[1]. A variety of tumor promoting phorbol esters are able to bind to and activate PKC[2], suggesting that inhibitors of that enzyme are potentially useful as anticancer drugs. Staurosporine, isolated from *Streptomyces staurosporeus* as an alkaloidal antibiotic[3], was found to be the first compound that inhibited PKC in the low nanomolar range[4]. The absolute stereochemistry of staurosporine was determined only very recently[5]. Staurosporine binds not only to the ATP binding-site of various protein kinases but also blocks the autophosphorylation of neurotrophin receptors[6] and might interact with other ATP-dependent proteins. Staurosporine derivatives have attracted further interest, because they have been found to reverse multidrug resistance[7], presumably by direct interaction with the P-glycoprotein[8]. A semisynthetic derivative of staurosporine that shows a high degree of selectivity for PKC exerted strong antitumor activities in several animal models[9]. More simple synthetic analogues related to the aglycone of staurosporine are actively pursued by others[10].

In a previous publication we described several metabolites produced by *Streptomyces longisporoflavus* strain R-19, including a nitro analogue of staurosporine, that were isolated in course of the preparation of larger quantities of staurosporine[11]. In the present communication the production, isolation, physico-chemical data, structure elucidation and biological properties of further minor metabolites are described.

Results

Fermentation and Isolation

A 2000-liter fermentation and the isolation of the crude extract were performed as described earlier[11]. Silica gel chromatography yielded three fractions: an unpolar one, one containing staurosporine and a polar one containing mainly basic compounds[11]. The purification procedure is described in the Experimental part and summarized in Schemes 1 and 2.

Structure Elucidation

The absolute stereochemistry at centers 2' and 6'[††] of compounds **2**, **8** and **9** was shown to be the same as in TAN-1030A (**1**)[12] by measurement of CD spectra in ethanol and comparison with **1**.

7-Hydroxy Derivative of TAN-1030A (2)

The elementary composition was determined to be $C_{27}H_{22}N_4O_5$ by HRFAB-MS indicating one additional oxygen atom compared to TAN-1030A. Analysis of ^{1}H NMR (Table 1) and ^{13}C NMR (Table 2) and comparison to those of TAN-1030A (**1**)[12] and UCN-01 (**3**)[13] leads to structure **2** in a straight-forward way. CD-Spectra of **1**, **2**, **3** and staurosporine were recorded in an unsuc-

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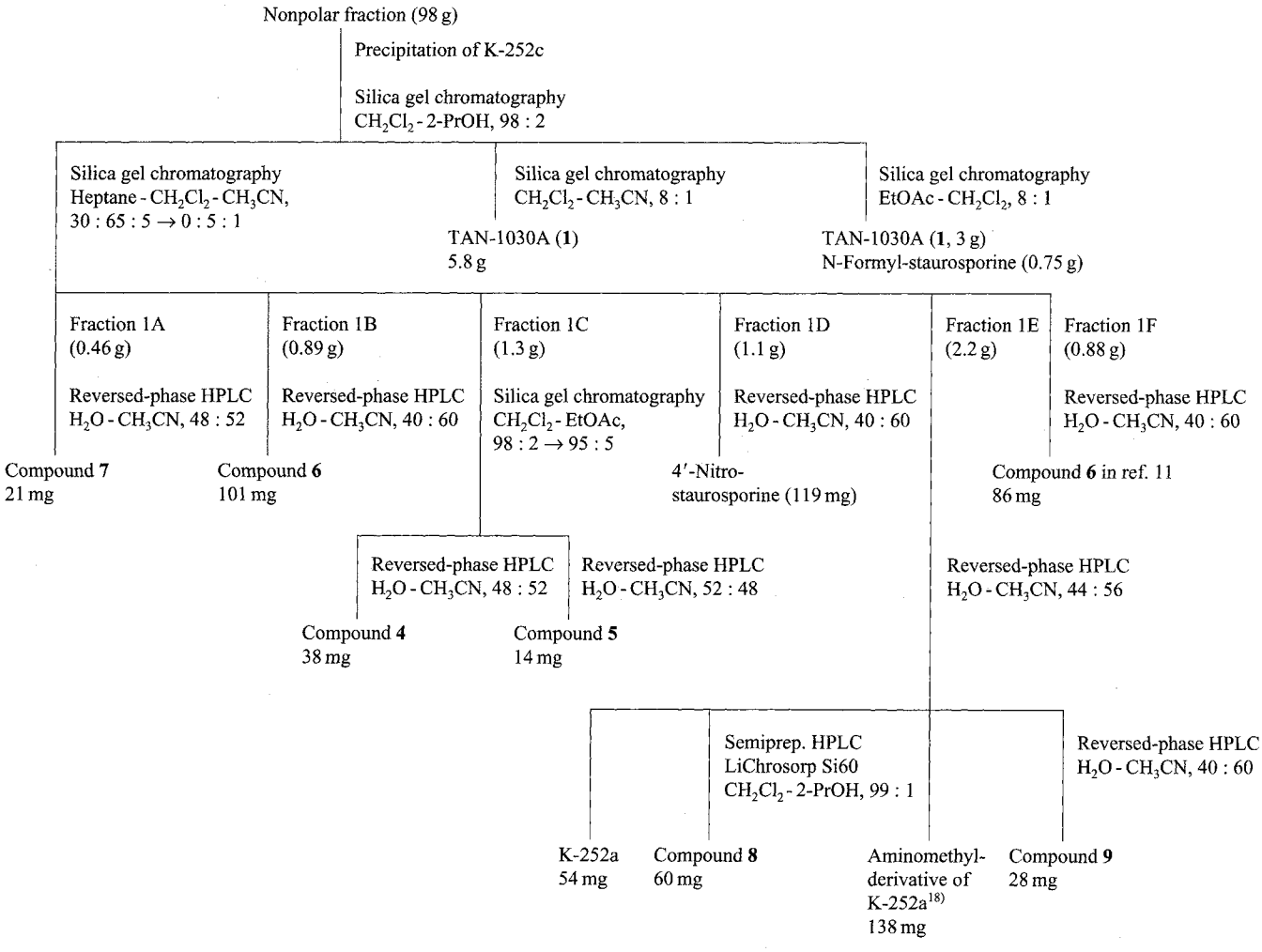
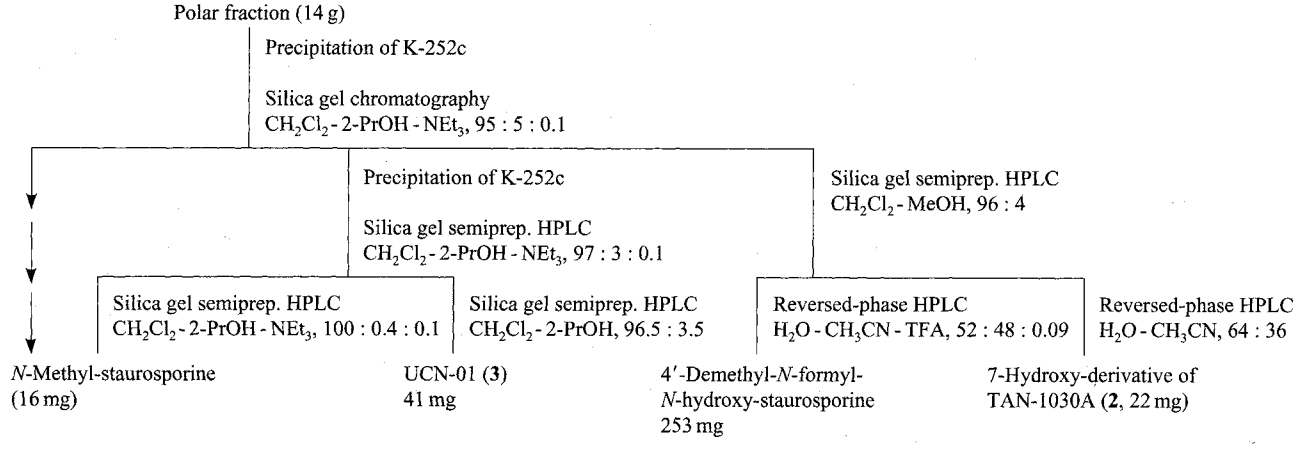
[††] Three different nomenclatures for staurosporine type alkaloids are used in the literature. We will use the numbering system for staurosporine assigning position 1' to the oxygen, since this metabolite has been described first and has been investigated most extensively.

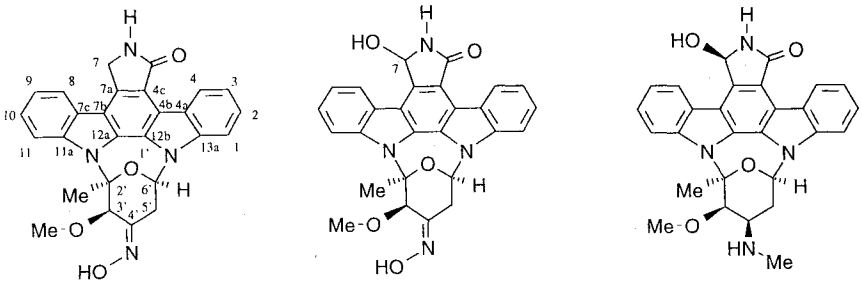
Scheme 1. Fractionation procedure of the nonpolar fraction.



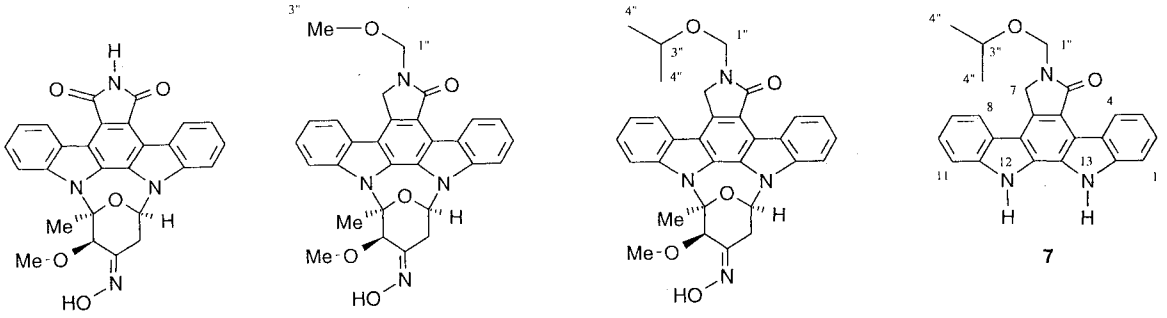
Scheme 2. Fractionation procedure of the polar fraction.



Scheme 3. Structure of minor metabolites **2**, **4** to **7** and of TAN-1030A (**1**) and UCN-01 (**3**).

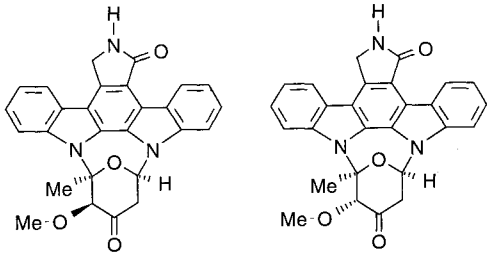


1 TAN-1030A **2** **3** UCN-01



4 **5** **6** **7**

Scheme 4. Structure of the 4'-oxo derivatives **8** and **9**.



8 **9**

cessful attempt to define the stereochemistry at C-7 (see Experimental part).

UCN-01 (**3**)

This compound was identified in comparison to semisynthetic material obtained from staurosporine[14]. The CD spectrum of **3** is included for reference purposes.

7-Oxo-derivative of TAN-1030A (**4**)

This compound shows the typical yellow fluorescence on TLC plates of 7-oxo-derivatives of the staurosporine chromophore. The elementary composition was determined to be $C_{27}H_{20}N_4O_5$ by HREI-MS demonstrating the presence of one additional oxygen and the lack of two hydrogens compared to TAN-1030A. Comparison of the ^1H NMR spectral data with **1** disclosed the absence of the H-7 methylene protons. The presence of a carbonyl group at C-7 was indicated by a second deshielded aromatic proton at 9.08 ppm. The ^{13}C NMR data were compared to those of **1** and 7-oxo-staurosporine[14] and proved structure **4** to be correct.

6-Alkylated Derivatives of TAN-1030A **5** and **6**

The two compounds exhibit all carbon and hydrogen signals of TAN-1030A in the NMR spectra with the exception of the lactam proton of nitrogen 6, suggesting a substitution at this center. Elemental composition determined by HR-MS shows that compound **6** has a C_4H_8O fragment in addition to TAN-1030A. Both compounds have singlet methylene protons which according to their chemical shifts (5.1 ppm in ^1H NMR, 65.5~73.9 ppm in ^{13}C NMR) should be bound to an oxygen and a nitrogen. Comparison of the ^1H and ^{13}C NMR data to compound **7** leads to the proposal of structure **6** in a straight-forward way. Compound **5** is a more simple analogue which has two carbons less than

Table 1. ^{1}H NMR chemical shifts (in ppm).

	TAN-1030A (1)		2		4		5		6		7		8		9	
1	7.71	d	7.72	d	7.80	d	7.73	d	7.75	d	7.72	d	7.72	d	7.69	d
2	7.49	t	7.52	t	7.63	t	7.51	t	7.52	t	7.43	t	7.53	t	7.53	t
3	7.29	t	7.29	t	7.35	t	7.31	t	7.33	t	7.22	t	7.35	t	7.32	t
4	9.28	d	9.24	d	9.22	d	9.25	d	9.28	d	9.19	d	9.31	d	9.26	d
6	8.58	s	8.84	s	11.10	s							8.62	s	8.62	s
7	4.95	s	6.41	d			5.08	d	5.12	s	5.09	s	4.98	s	4.97	s
8	7.96	d	8.42	d	9.08	d	8.00	d	8.03	d	8.07	d	7.99	d	8.00	d
9	7.30	t	7.32	t	7.43	t	7.32	t	7.35	t	7.30	t	7.35	t	7.41	t
10	7.43	t	7.43	t	7.51	t	7.45	t	7.46	t	7.47	t	7.47	t	7.51	t
11	8.00	d	8.00	d	8.03	d	8.02	d	8.03	d	7.78	d	8.02	d	8.07	d
12											11.76*	s				
13											11.57*	s				
3′	4.75	s	4.77	s	4.79	s	4.76	s	4.76	s			5.08	s	4.34	s
5′a	3.00	dd	3.04	dd	3.04	dd	3.00	dd	3.02	dd			3.99	dd	3.93	dd
5′b	3.61	d	3.66	d	3.66	d	3.62	d	3.64	d			2.67	d	2.55	d
6′	7.05	d	7.05	d	7.07	d	7.06	d	7.06	d			7.44	d	7.45	d
2′-CH_3	2.46	s	2.46	s	2.47	s	2.46	s	2.50	s			2.57	s	2.24	s
3′-OCH_3	3.41	s	3.46	s	3.41	s	3.40	s	3.42	s			3.42	s	3.44	s
4′=N–OH	10.42	s	10.54	s	10.54	s	10.44	s	10.42	s						
1″							5.12	s	5.15	d	5.14	s				
3″							3.29	s	3.80	m	3.80	m				
4″									1.17	d	1.14	d				
7-OH			6.53	d												
Some characteristic coupling-constants (Hz)																
1, 2	8		8		8		8		8		8		8		8	
3, 4	8		8		8		8		8		8		8		8	
8, 9	8		8		8		8		8		8		8		8	
10, 11	8		8		8		8		8		9		8		8	
5′a, 5′b	14		14		14.5		14		14.5				15		15	
5′a/b-6′	6		5.6		6		6		5.5				7		8	
1″a-1″b									11							
7-CH–OH			11													

Assignments with asterisks may be interchanged. Solvent: DMSO-d_6, temperature ambient.

6 and a methoxy group instead of the 2-propan-2-yl group in **6**.

6-Alkylated Derivative of K-252c (7)

EI-MS reveals a molecular weight of 383. In the ^{1}H NMR of **7** in DMSO-d_6 all signal of the aglycone of staurosporine, K-252c[15], were clearly observed with the exception of the lactam proton of nitrogen 6, suggesting a substitution at that position. In addition a $(CH_3)_2CH$–O and a singlet CH_2 at 5.14 were observed. These structural elements can be only combined as shown in structure **7**. The ^{13}C NMR data are well compatible with the proposed structure and the closely related compound **6**. As expected the signals of the carbons 4c, 5 and 7 and 7a are shifted by more than 2 ppm in comparison to K-252c or TAN-1030A (**1**).

4′-Oxo Derivatives 8 and 9

The ^{1}H NMR data of both compounds (Table 1) showed a striking resemblance to TAN-1030A (**1**) with the exception of 3′ and 5′ signals which were markedly shifted. The molecular formula determined by HR-MS shows one nitrogen and hydrogen less than **1** and suggests a ketone function at C-4′. The planar structures **8** and **9** are corroborated by typical ketone signals in IR (1730 cm^{-1}) and ^{13}C NMR spectroscopy (200.6 ppm, 204.1 ppm; Table 2).

The ^{13}C NMR spectra of the two compounds are quite similar although differences are observed for the 2′-methyl group and for carbons 3′ to 5′ of about 4 ppm suggesting epimeric compounds. NOE difference experiments in acetone-d_6 clarify that the two compounds have to be epimeric at center 3′: only in compound **8** irradiation of the axial proton 5′ gave rise to a NOE of 13% on 3′-H indicating that both protons are in an axial position. Thus the methoxy group has to be in an equatorial position leading to structure **8** and consequently to structure **9**. Irradiation on the 2′-methyl group provides additional evidence by causing a considerably more pronounced NOE on proton 3′ in compound **8** (13%)

Table 2. ^{13}C NMR chemical shifts (in ppm).

Carbon	TAN-1030A(1)	2	4[a]	5	6	8[a]	9	7[b]
1	109.0 d	108.9 d	110.0 d	109.7 d	109.7 d	110.0 d	109.7 d	112.0 d
2	125.3 d	125.5 d	127.9 d	125.9 d	125.9 d	127.2 d	126.2 d	125.1* d
3	119.6 d	119.7 d	121.9 d	120.3 d	120.2 d	121.6 d	120.6 d	119.0 d
4	125.7 d	125.5 d	126.4 d	126.0 d	125.9 d	128.1 d	126.3 d	124.9* d
4a	122.9 s	122.7 s	123.4 s	123.2 s	123.3 s	125.4 s	123.4 s	122.6 s
4b	115.0 s	115.2 s	121.7* s	115.5 s	115.5 s	117.8 s	116.2 s	115.5 s
4c	119.2 s	118.6 s	120.7* s	118.4 s	119.2 s	121.4 s	120.5 s	117.6 s
5	171.8 s	170.3 s	171.3* s	170.3 s	169.4 s	173.4 s	172.0 s	170.0 s
7	45.4 t	78.4 d	171.1* s	49.6 t	48.8 t	47.0 t	45.9 t	48.6 t
7a	132.3 s	134.4 s	118.0* s	130.9 s	130.8 s	134.4 s	133.5 s	130.8 s
7b	114.0 s	114.6 s	116.8* s	114.4 s	114.4 s	116.8 s	115.0 s	113.9 s
7c	123.9 s	123.4 s	124.5 s	124.2 s	124.3 s	126.0 s	124.6 s	122.4 s
8	120.8 d	122.8 d	125.8 d	121.3 d	121.1 d	122.5 d	122.4 d	121.1 d
9	120.2 d	119.8 d	121.3 d	120.9 d	120.9 d	122.1 d	121.6 d	120.0 d
10	124.7 d	124.8 d	127.2 d	125.4 d	125.4 d	126.4 d	126.1 d	125.1* d
11	115.7 d	115.4 d	116.6 d	116.3 d	116.3 d	117.5 d	113.7 d	111.4 d
11a	139.9 s	140.1 s	142.4 s	140.5 s	140.5 s	142.2 s	138.1 s	139.3* s
12a	128.1 s	128.2 s	130.9 s	128.9 s	128.8 s	129.2 s	127.7 s	128.2 s
12b	124.7 s	125.3 s	129.6 s	125.3 s	125.3 s	126.2 s	124.8 s	125.5 s
13a	136.1 s	136.4 s	138.9 s	136.7 s	136.6 s	138.1 s	136.9 s	139.1* s
2′	96.2 s	96.0 s	97.7 s	96.8 s	96.8 s	101.2 s	98.7 s	
3′	83.6 d	83.5 d	85.1 d	84.0 d	84.1 d	90.3 d	84.2 d	
4′	145.2 s	145.2 s	146.6 s	145.7 s	145.7 s	200.6 s	204.2 s	
5′	29.9 t	29.7 t	30.5 t	30.3 t	30.3 t	47.3 t	42.4 t	
6′	82.3 d	82.1 d	83.8 d	82.8 d	82.8 d	86.4 d	83.9 d	
2′-CH_3	28.7 q	28.8 q	29.6 q	29.2 q	29.2 q	30.9 q	25.3 q	
3′-OCH_3	58.4 q	58.5 q	58.9 q	58.9 q	58.9 q	60.2 q	58.7 q	
1″				73.9 t	65.5 t			69.5 t
3″				55.9 q	62.5 d			68.0 d
4″					26.0 q			22.3 q

Assignments with asterisks may be interchanged. Solvent: DMSO-d_6, 100°C, except for [a] solvent: acetone-d_6, temperature ambient, [b] temperature ambient.

Table 3. Enzyme inhibition of protein kinases.

Name	IC_{50} (μM)		
	PKC	PKA	PK
Staurosporine	0.006	0.015	0.003
1 TAN-1030A	1.17	1.4	0.04
2 7-Hydroxy-TAN-1030A	2.7	7.1	0.29
3 UCN-01	0.013	1.2	0.004
4 7-Oxo-TAN-1030A	0.65	1.1	0.21
6	32	170	0.76
8	0.86	1.5	0.02
9	0.26	1.7	0.07

PKC: protein kinase C.
PKA: c-AMP dependent protein kinase.
PK: phosphorylase kinase.

than in **9** (3%). The observed long range effects in the ^{13}C NMR are in agreement with the suggested structures.

Biological Properties

All compounds tested inhibit porcine PKC[9] in the nanomolar to micromolar range (Table 3). The compounds with a 4′-oxime or ketone function inhibit the protein kinases in a similar range like the known metabolite TAN-1030A, with the exception of compound **6** which is alkylated at the 7-nitrogen. A dramatic loss of activity upon substitution of that position has been shown before in the staurosporine series[14]. Therefore the PKC-inhibitory activity of the compounds **5** and **7** was not investigated. Most compounds are less active against PKC than staurosporine and are effective inhibitors of phosphorylase kinase.

Discussion

Compound **2** seems to be an oxidation product of TAN-1030A, just in the way UCN-01 (**3**) is a hydroxylated product of staurosporine. As both compounds were isolated as minor metabolites, it seems that the hydroxylating enzyme cannot discriminate between TAN-1030A and staurosporine. Further oxidation of **1** or **2** either during the fermentation or during workup leads to the yellow compound **4** with an imide function. Structure **4** is related to 7-oxo-staurosporine, which was found as an inhibitor of PKC produced by *Streptomyces*

platensis[16]. It can not be excluded that the 6-alkylated compounds **5**, **6**, and **7** are formed during workup, since methanol and 2-propanol have been used in the isolation procedure. It seems however, that at least the carbon 1″ which is a formaldehyde equivalent could be attached during the fermentation. In our present and earlier work[11] we have isolated several compounds which are formic acid derivatives or which, like compounds **5** to **7**, contain equivalents of formaldehyde and it can be speculated that such compounds might have a specific role: formylation at the 6- or at the 4′-nitrogen leads to metabolites which are much less toxic, as they inhibit many protein kinases at much higher IC_{50}-values (Table 3)[11]. They might protect the microorganism against its own metabolite, since staurosporine itself is toxic to the producing organism[17]. After completion of the biosynthesis formylated staurosporine might be transported to the outside of the cell and bound to the mycelium. Further protection would no longer be required and the formyl moieties could be cleaved off.

Compound **9** is epimeric to compound **8** at the center 3′ which is slightly acidic, being α to the 4′-ketone function. Whether compound **9** is genuinely produced by the microorganism or formed as an artefact is not completely clear, but we have not found any evidence for an epimerization of 3′ under isolation conditions. When the fermentation of *S. longisporoflavus* was monitored by HPLC, a very small peak with the retention time of **9** was observed.

Experimental

The following instruments were used in this study: CEC-121 B, VG 70-4SE (for HREI-MS) mass spectrometers, Varian VXR-400 S NMR spectrometer, Perkin Elmer Lamda 5 UV/VIS spectrophotometer, Perkin Elmer 241 polarimeter and Perkin Elmer 983G IR spectrophotometer.

General remarks: Melting points are uncorrected. Large scale liquid chromatography on silica gel was done using a medium pressure system equipped with a Büchi pump B-681, Büchi glass columns B-685 filled with LiChroprep Si60, 25~40 μm, a Kontron Uvikon 725 detector (1 mm pathlength) and a Büchi fraction collector B-684. All solvents for silica gel chromatography or HPLC were water-saturated except where stated otherwise. For HPLC a Spectra Physics SP8800 solvent delivery module with a Shimadzu SPD-6AV UV/VIS detector and Merck-Hitachi D-2500 integrator was used. For semipreparative HPLC, LiChrosorb Si60, 5 μm, 8×250 mm, and Nucleosil C_{18}, 5 μm, 16×250 mm, columns were used for normal and reversed-phase separations, respectively. Except where stated otherwise, for semipreparative reversed phase HPLC solvent A was water and solvent B was acetonitrile (CH_3CN) - water, 80 : 20, and the flow rate was set at 10 ml/minute.

Workup of fermentations on a 2000-liter scale yielded 480 g of an amorphous solid as described earlier[11]. Aliquots of such material (850 g in total) were separated into three fractions by silica gel chromatography (3 liters; 4 runs). The nonpolar fraction 1 was eluted with CH_2Cl_2 - 2-PrOH - AcOH, 95 : 4 : 1, (8 liters) giving 365 g of a brown oil. Fraction 2 contained staurosporine and was eluted first with CH_2Cl_2 - 2-PrOH, 96 : 4, (4 liters) and then with CH_2Cl_2 - 2-PrOH - triethylamine, 96 : 4 : 0.1, (6 liters). The polar fraction 3 was eluted with CH_2Cl_2 - 2-PrOH - triethylamine, 90 : 10 : 0.1, (4 liters) to give 76 g of a brown solid after solvent removal. Fraction 2 (383 g) was triturated in CH_2Cl_2 - MeOH, 10 : 2, (1200 ml) to give 143 g of staurosporine as yellowish crystals.

Preparative Separation of the Nonpolar Fraction (Scheme 1)

The insoluble K-252c (10 g) was removed by precipitation of the nonpolar fraction (450 g) in a mixture of CH_2Cl_2 - 2-PrOH, 1 : 1, (1.8 liters). Of that dried material 30 g was subjected to silica gel chromatography (920 ml; CH_2Cl_2, 1.8 liters; then CH_2Cl_2 - 2-PrOH, 98 : 2, 4 liters; 32 ml/minute) and separated into 3 fractions: Fraction 1 (8 g; 0.6~1.4 liters) was separated into several compounds as described below. Fraction 2 (4.1 g; 2.8~4.2 liters) contained TAN-1030A in 44% purity. Fraction 3 (2.8 g, 4.2~5.8 liters) was rechromatographed on silica gel (470 ml; CH_2Cl_2 - EtOAc, 8 : 1, 1.6 liters; 4 : 1, 3.1 liters; 2 : 1, 2 liters, dry solvents; 24 ml/minute) yielding TAN-1030A (2.2~3.0 liters; 0.94 g as colorless crystals from MeOH - EtOAc with mp 238~244°C; 62% overall recovery) and *N*-formyl-staurosporine (5.8~6.6 liters; 0.23 g; colorless crystals, mp 221~226°C from CH_2Cl_2 - EtOAc).

Fraction 1 (26 g of such material) was separated into the subfractions 1A to 1F on a silica gel column (920 ml; mixtures of heptane - CH_2Cl_2 - CH_3CN, 30 : 65 : 5, 2.5 liters; 20 : 75 : 5, 4 liters; 10 : 85 : 5, 2 liters; 0 : 95 : 5, 2 liters; 0 : 10 : 1, 1 liter; 0 : 5 : 1, 1.7 liters, dry solvents; 30 ml/minute; 2 runs): Fraction 1A (1.5~2.7 liters, 0.46 g), fraction 1B (3.2~3.7 liters, 0.89 g), fraction 1C (3.7~5.5liters, 1.3 g), fraction 1D (7.1~9.7 liters, 1.06 g), fraction 1E (9.7~12.1 liters, 2.23 g) and fraction 1F (12.5~13.2 liters, 0.88 g).

Fraction 1A was purified by reversed-phase HPLC (65% solvent B, isocratic; 320 nm; sample load 27 mg/run; Rt 9 minutes) yielding **7** as a yellowish solid (21 mg) after precipitation of the lyophilizate from a mixture of Et_2O - hexane (1 : 1). Fraction 1B was rechromatographed with reversed phase HPLC (75% solvent B (isocratic); 290 nm; 15 runs; Rt 13 minutes) yielding **6** (101 mg, 19% overall recovery).

Fraction 1C was rechromatographed on silica gel (920 ml; CH_2Cl_2 - EtOAc 98 : 2, 3.5 liters; then 95 : 5, 1.2 liters; 16 ml/minute) giving 2 subfractions. The first subfraction (3.5~4.1 liters, 179 mg) was purified with reversed-phase HPLC (65% solvent B (isocratic); 320 nm; 17 runs; Rt 7.6 minutes) yielding **4** (38 mg). The second subfraction (4.1~4.6 liters, 48 mg) was subjected to

reversed-phase HPLC (60% solvent B (isocratic); 290 nm; 8 runs; Rt 10 minutes) yielding **5** (14 mg).

Fraction 1D (1.06 g) was purified on a reversed-phase HPLC (75% solvent B (isocratic); 290 nm; 24 runs; Rt 14 minutes) giving 4'-demethylamino-4'-nitro-staurosporine after lyophilization and crystallization from CH_2Cl_2 (119 mg).

Fraction 1E (2.23 g) was subjected to preparative reversed-phase HPLC (70% solvent B (isocratic); 290 nm; 39 runs) yielding K-252a[15] (54 mg; Rt 16 minutes), crude **8** (113 mg; Rt 19 minutes), *N*-methyl-3'-deoxy-3'-amino-derivative of K-252a[18] (138 mg; Rt 23 minutes; white crystals with mp 152~155°C from CH_2Cl_2) and crude **9** (71 mg; Rt 26 minutes). The crude **8** was separated by semipreparative normal phase HPLC (CH_2Cl_2 - 2-PrOH, 99:1; 5 ml/minute; 300 nm; Rt 14 minutes; 22 runs) yielding **8** (60 mg). The crude **9** was finally purified by reversed-phase HPLC (75% solvent B (isocratic); 290 nm; Rt 12 minutes; 8 runs) yielding **9** (28 mg; 18% overall recovery). Fraction 1F was purified on a reversed-phase HPLC (75% solvent B (isocratic); 290 nm; 47 runs; Rt 10 minutes) to yield compound **6** of Ref. 11 (86 mg).

Preparative Separation of the Polar Fraction (Scheme 2)

Of the polar, basic fraction 13.7 g was separated into 3 subfractions on a silica gel column (920 ml; CH_2Cl_2 - 2-PrOH - triethylamine, 98:2:0.1, 4.7 liters; then 95:5:0.1, 2 liters; CH_3OH, 2.5 liters; 40 ml/minute): fraction 3A (2.9~3.7 liters, 0.92 g), fraction 3B (3.7~4.5 liters, 0.79 g), and fraction 3C (7.2~8.7 liters, 4.4 g).

After precipitation of K-252c (70 mg) with 90 ml of CH_2Cl_2 - 2-PrOH, 95:5, and solvent removal, fraction 3B was further separated by semipreparative HPLC (LiChrosorb-Si60, 10 μm; 8 × 250 mm; CH_2Cl_2 - 2-PrOH - triethylamine, 97:3:0.1; 5 ml/minute; 345 nm; 30 runs) yielding crude *N*-methyl-staurosporine (310 mg, Rt 4 minutes) and crude UCN-01 (**3**; 335 mg; Rt 17 minutes). Pure UCN-01 was obtained by semipreparative HPLC (Nucleosil 100 5 μm; 8 × 250 mm; CH_2Cl_2 - 2-PrOH, 96.5:3.5; 5 ml/minute; 310 nm; 40 runs; Rt 13 minutes) yielding 41 mg. Pure *N*-methyl-staurosporine[18] was obtained by semipreparative HPLC (LiChrosorb Si60, 10 μm; 8 × 250 mm; CH_2Cl_2 - 2-PrOH - triethylamine, 99.6:0.4:0.1; 5 ml/minute; 290 nm; 50 runs, Rt 10 minutes) yielding 10 mg of colorless crystals with mp 133~137°C from 2-PrOH - CH_2Cl_2). Additional 6 mg were isolated from fraction 3A in several chromatographic steps.

Fraction 3C was subjected to semipreparative HPLC (LiChrosorb-Si60, 5 μm; 8 × 250 mm; CH_2Cl_2 - MeOH, 96:4; 8 ml/minute; 300 nm; 120 runs) giving two fractions. The first fraction (626 mg, Rt 4.8 minutes) was subjected to reversed-phase HPLC (solvent A: water - TFA, 100:0.1; solvent B: CH_3CN - H_2O - TFA, 80:20:0.08; 60% solvent B (isocratic); 290 nm; 57 runs; Rt 10 minutes) yielding *N*-demethyl-*N*-formyl-*N*-hydroxy-staurosporine (253 mg, white lyophilizate, mp 220°C dec.)[11]. The second fraction (178 mg, Rt 7.8 minutes) was purified on reversed-phase HPLC (45% solvent B (isocratic); 5 ml/minute; 290 nm; 21 runs; Rt 22 minutes) yielding **2** (22 mg, white lyophilizate).

Data of **2**

White powder from CH_3CN - H_2O, mp 240°C (dec.); HRFAB-MS Found: *m/z* 483.1670 Calcd for $C_{27}H_{22}N_4O_5$ ($M+H^+$): 483.1668; IR (KBr) cm^{-1} 3390, 2920, 2830, 1690, 1580, 1460, 1390, 1370, 1350, 1320, 1130, 1120, 740; CD λ^{EtOH} nm (θ): 368 (3900), 350 (1950), 327 (850), 300 (17500), 262 (−9200), 239 (25500), 207 (−19500).

Data of UCN-01 (**3**)

White crystals from CH_2Cl_2 - 2-PrOH, mp 220°C (dec.); CD λ^{EtOH} nm (θ): 369 (2200), 353 (1700), 300 (24500), 265 (−7550), 255 (−1350), 247 (−9300), 233 (10200), 214 (−14700).

Data of **4**

Yellow powder from CH_2Cl_2 - 2-PrOH, mp 265~270°C (dec.); HREI-MS Found: *m/z* 480.1430 Calcd for $C_{27}H_{20}N_4O_5$: 480.1434; EI-MS: *m/z* 480 (12), 462 (11), 395 (40), 387 (28), 377 (46), 376 (75), 326 (73), 325 (100), 324 (46), 254 (56), 255 (35), 138 (48), 45 (70), 44 (46), 43 (50); IR (KBr) cm^{-1} 3240, 2930, 1740, 1690, 1630, 1570, 1490, 1470, 1460, 1410, 1340, 1320, 1280, 1220, 1120, 750, 740.

Data of **5**

White powder from CH_2Cl_2 - 2-PrOH, mp >300°C (dec.); HREI-MS Found: *m/z* 510.1904 Cacld for $C_{29}H_{26}N_4O_5$: 510.1903; EI-MS: *m/z* 510 (13), 494 (7), 492 (6), 479 (7), 407 (35), 406 (47), 376 (40), 375 (60), 366 (51), 355 (57), 325 (54), 324 (67), 323 (53), 311 (40), 296 (26), 282 (32), 269 (26), 268 (24), 255 (22), 138 (58), 125 (52), 113 (65), 112 (59), 111 (56), 109 (64), 98 (59), 97 (100) 95 (69); IR (KBr) cm^{-1} 3410, 2930, 1685, 1460, 1400, 1350, 1320, 1280, 1230, 1200, 1130, 1090, 740.

Data of **6**

Colorless crystals from CH_2Cl_2 - 2-PrOH, mp 266~272°C (dec.); HREI-MS Found: *m/z* 538.2207 Calcd for $C_{31}H_{30}N_4O_5$: 538.2218; EI-MS: *m/z* 538 (6), 479 (3), 366 (9), 323 (5), 270 (6), 256 (12), 213 (10), 129 (26), 97 (42), 85 (55), 83 (43), 71 (85), 69 (56), 59 (88), 57 (91), 55 (86), 46 (89), 45 (100), 44 (84), 43 (98); FAB-MS *m/z* 561 ($M+Na)^+$, 538 $(M)^+$; IR (KBr) cm^{-1} 3400, 2960, 2930, 1680, 1590, 1460, 1420, 1400, 1370, 1350, 1320, 1280, 1230, 1200, 1160, 1130, 1060, 740.

Data of **7**

Yellowish solid, mp 45~50°C; EI-MS: *m/z* 383 (0.2), 325 (0.5), 311 (0.1), 324 (0.2), 185 (16), 129 (30), 113 (28), 97 (38), 85 (32), 83 (62), 73 (64), 69 (58), 57 (100), 55 (93); IR (CH_2Cl_2) cm^{-1} 2925, 2850, 1650, 1590, 1490, 1460, 1410, 1400, 1340, 1330, 1270, 1240, 1120, 1050, 740.

Data of **8**

White crystals from CH_2Cl_2, mp 236~243°C; HRFAB-MS Found: *m/z* 452.1601 Calcd for $C_{27}H_{22}N_3O_4$ ($M+H^+$): 452.1610; EI-MS: *m/z* 451 (0.2), 364 (38), 311 (49), 140 (60), 122 (47), 82 (20), 71 (31), 69 (83), 57 (21), 55 (32), 44 (100); IR (KBr) cm^{-1} 3420, 2920, 1735, 1690, 1590, 1460, 1400, 1370, 1350, 1320, 1280, 1230, 1150, 1130, 1120, 770, 750; CD λ^{EtOH} nm (θ): 369 (19200), 351 (13200), 339 (6900), 304 (−10300), 290 (8100), 279 (−2200), 272 (0), 260 (−10500), 245 (25600), 209 (−25700).

Data of **9**

Colorless crystals from CH_2Cl_2, mp 171~176°C; FAB-MS *m/z* 452 ($M+H^+$); HREI-MS Found: *m/z* 451.1529 Calcd for $C_{27}H_{21}N_3O_4$: 451.1532; EI-MS: *m/z* 451 (0.2), 364 (7), 312 (12), 311 (50), 310 (14), 283 (22), 282 (34), 255 (14), 97 (17), 71 (22), 69 (38), 57 (45), 55 (56), 44 (100); IR (KBr) cm^{-1} 3420, 1730, 1680, 1590, 1460, 1400, 1340, 1320, 1240, 1230, 1150, 1120, 1110, CD λ^{EtOH} nm (θ): 369 (4100), 336 (10400), 309 (−8200), 276 (22700), 259 (6300), 250 (17000), 241 (−7100), 229 (14000), 207 (−35700).

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