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 1H NMR and 13C NMR and by comparison with TAN-1030A.
The new compounds inhibited protein kinase C with IC50 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 regulation1). A variety of tumor promoting phorbol esters are able to
bind to and activate PKC2), suggesting that inhibitors of that enzyme are potentially useful as anticancer drugs.

Staurosporine, isolated from Streptomyces staurosporeus
as an alkaloidal antibiotic3), was found to be the first
compound that inhibited PKC in the low nanomolar
range4). The absolute stereochemistry of staurosporine
was determined only very recently5). Staurosporine binds
not only to the ATP binding-site of various protein
kinases but also blocks the autophosphorylation of
neurotrophin receptors6) and might interact with other
ATP-dependent proteins. Staurosporine derivatives have
attracted further interest, because they have been found
to reverse multidrug resistance7), presumably by direct
interaction with the P-glycoprotein8). A semisynthetic
derivative of staurosporine that shows a high degree of
selectivity for PKC exerted strong antitumor activities in
several animal models9). More simple synthetic analogues
related to the aglycone of staurosporine are actively
pursued by others10).

In a previous publication we described several metab-
olites 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 staurosporine11). In the present communica-
tion 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 earlier11). Silica gel
chromatography yielded three fractions: an unpolar one,
one containing staurosporine and a polar one containing
mainly basic compounds11). 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
C27H22N4O5 by HRFAB-MS indicating one additional
oxygen atom compared to TAN-1030A. Analysis of 1H
NMR (Table 1) and 13C 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-
Scheme 1. Fractionation procedure of the nonpolar fraction.

Nonpolar fraction (98 g)

Precipitation of K-252c

Silica gel chromatography
\(\text{CH}_2\text{Cl}_2 \cdot 2\text{PrOH}, 98 : 2\)

Silica gel chromatography
\(\text{CH}_2\text{Cl}_2 \cdot \text{CH}_3\text{CN}, 8 : 1\)

Silica gel chromatography
\(\text{EtOAc} \cdot \text{CH}_2\text{Cl}_2, 8 : 1\)

Silica gel chromatography
\(\text{Heptane} \cdot \text{CH}_2\text{Cl}_2 \cdot \text{CH}_3\text{CN}, 30 : 65 : 5 \to 0 : 5 : 1\)

TAN-1030A (1) 5.8 g

N-Formyl-staurosporine (0.75 g)

Fraction 1A (0.46 g)
Reversed-phase HPLC
\(\text{H}_2\text{O} \cdot \text{CH}_3\text{CN}, 48 : 52\)

Fraction 1B (0.89 g)
Reversed-phase HPLC
\(\text{H}_2\text{O} \cdot \text{CH}_3\text{CN}, 40 : 60\)

Fraction 1C (1.3 g)
Silica gel chromatography
\(\text{CH}_2\text{Cl}_2 \cdot \text{EtOAc}, 98 : 2 \to 95 : 5\)

Fraction 1D (1.1 g)
Reversed-phase HPLC
\(\text{H}_2\text{O} \cdot \text{CH}_3\text{CN}, 40 : 60\)

Fraction 1E (2.2 g)
Reversed-phase HPLC
\(\text{H}_2\text{O} \cdot \text{CH}_3\text{CN}, 40 : 60\)

Fraction 1F (0.88 g)

Compound 7
21 mg

Compound 6
101 mg

4'-Nitro-staurosporine (119 mg)

Compound 6 in ref. 11
86 mg

Reversed-phase HPLC
\(\text{H}_2\text{O} \cdot \text{CH}_3\text{CN}, 48 : 52\)

Reversed-phase HPLC
\(\text{H}_2\text{O} \cdot \text{CH}_3\text{CN}, 48 : 52\)

Reversed-phase HPLC
\(\text{H}_2\text{O} \cdot \text{CH}_3\text{CN}, 52 : 48\)

Reversed-phase HPLC
\(\text{H}_2\text{O} \cdot \text{CH}_3\text{CN}, 44 : 56\)

Compound 4
38 mg

Compound 5
14 mg

Semiprep. HPLC
LiChrosorb Si60
\(\text{CH}_2\text{Cl}_2 \cdot 2\text{PrOH}, 99 : 1\)

K-252a
54 mg

Compound 8
Aminomethyl-derivative of K-252a \(^{10}\)
73 mg

Compound 9
28 mg

Reversed-phase HPLC
\(\text{H}_2\text{O} \cdot \text{CH}_3\text{CN}, 40 : 60\)

Scheme 2. Fractionation procedure of the polar fraction.

Polar fraction (14 g)

Precipitation of K-252c

Silica gel chromatography
\(\text{CH}_2\text{Cl}_2 \cdot 2\text{PrOH} \cdot \text{NEt}_3, 95 : 5 : 0.1\)

Precipitation of K-252c

Silica gel semiprep. HPLC
\(\text{CH}_2\text{Cl}_2 \cdot 2\text{PrOH} \cdot \text{NEt}_3, 97 : 3 : 0.1\)

Silica gel semiprep. HPLC
\(\text{CH}_2\text{Cl}_2 \cdot 2\text{PrOH} \cdot \text{NEt}_3, 100 : 0.4 : 0.1\)

Silica gel semiprep. HPLC
\(\text{CH}_2\text{Cl}_2 \cdot 2\text{PrOH} \cdot \text{NEt}_3, 96.5 : 3.5\)

Reversed-phase HPLC
\(\text{H}_2\text{O} \cdot \text{CH}_3\text{CN} \cdot \text{TFA}, 52 : 48 : 0.09\)

Reversed-phase HPLC
\(\text{H}_2\text{O} \cdot \text{CH}_3\text{CN} \cdot \text{MeOH}, 64 : 36\)

N-Methyl-staurosporine (16 mg)

UCN-01 (3)
41 mg

4'-Demethyl-N-formyl-
\(\text{N}-\text{hydroxy-staurosporine}\)
253 mg

7-Hydroxy-derivative of

TAN-1030A (2, 22 mg)
Successful 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\(^1\). 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\(_4\)O\(_5\) by HREI-MS demonstrating the presence of one additional oxygen and the lack of two hydrogens compared to TAN-1030A. Comparison of the \(^{1}\)H 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\(^1\) 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\(_4\)H\(_8\)O fragment in addition to TAN-1030A. Both compounds have singlet methylene protons which according to their chemical shifts (5.1 ppm in \(^{1}\)H NMR, 65.5 - 73.9 ppm in \(^{13}\)C NMR) should be bound to an oxygen and a nitrogen. Comparison of the \(^{1}\)H and \(^{13}\)C NMR data to compound 7 leads to the proposal of structure 6 in a straightforward way. Compound 5 is a more simple analogue which has two carbons less than
Table 1. $^1$H NMR chemical shifts (in ppm).

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Some characteristic coupling-constants (Hz)

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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$_3$)$_2$CH-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%) of 3' and 5' signals which were markedly shifted.
Table 2. $^{13}$C NMR chemical shifts (in ppm).

| Carbon | TAN-1030A(1) | 2 | 4$\alpha$ | 5 | 6 | 8$\alpha$ | 9 | 7$\beta$
|--------|-------------|---|-----------|---|---|--------|---|---
| 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     | 115.9 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  | 71.1* 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 s     | 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 | 127.5 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  |           |         |         |         |         |
| 3'      | 55.9 q      | 62.5 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.

Table 3. Enzyme inhibition of protein kinases.

<table>
<thead>
<tr>
<th>Name</th>
<th>IC$_{50}$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PKC</td>
</tr>
<tr>
<td>Staurosporine</td>
<td>0.006</td>
</tr>
<tr>
<td>1 TAN-1030A</td>
<td>1.17</td>
</tr>
<tr>
<td>2 7-Hydroxy-TAN-1030A</td>
<td>2.7</td>
</tr>
<tr>
<td>3 UCN-01</td>
<td>0.013</td>
</tr>
<tr>
<td>4 7-Oxo-TAN-1030A</td>
<td>0.65</td>
</tr>
<tr>
<td>6</td>
<td>32</td>
</tr>
<tr>
<td>8</td>
<td>0.86</td>
</tr>
<tr>
<td>9</td>
<td>0.26</td>
</tr>
</tbody>
</table>

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$^{39}$ 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*
platenis\textsuperscript{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\textsuperscript{13} 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\textsubscript{50}-values (Table 3)\textsuperscript{11}. They might protect the microorganism against its own metabolite, since staurosporine itself is toxic to the producing organism\textsuperscript{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 \(\alpha\) 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 \textit{S. longisporoflavaus} 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 70-45E (for HREI-MS) mass spectrometers, Varian VX4-400 NMR spectrometer, Perkin Elmer Lambda 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 \(\times\) 250 mm, and Nucleosil C18, 5 μm, 16 \(\times\) 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\textsubscript{3}CN)-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\textsuperscript{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\textsubscript{2}Cl\textsubscript{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\textsubscript{2}Cl\textsubscript{2}-2-PrOH, 96:4, (4 liters) and then with CH\textsubscript{2}Cl\textsubscript{2}-2-PrOH-triethylamine, 96:4:0.1, (6 liters). The polar fraction 3 was eluted with CH\textsubscript{2}Cl\textsubscript{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\textsubscript{2}Cl\textsubscript{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\textsubscript{2}Cl\textsubscript{2}-2-PrOH, 1:1, (1.8 liters). Of that dried material 30 g was subjected to silica gel chromatography (920 ml; CH\textsubscript{2}Cl\textsubscript{2}, 1.8 liters; then CH\textsubscript{2}Cl\textsubscript{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 recrchromatographed on silica gel (470 ml; CH\textsubscript{2}Cl\textsubscript{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\textsubscript{2}Cl\textsubscript{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\textsubscript{2}Cl\textsubscript{2}-CH\textsubscript{3}CN, 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 (3.7-5.5 liters, 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\textsubscript{2}O-hexane (1:1). Fraction 1B was recrchromatographed 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 recrchromatographed on silica gel (920 ml; CH\textsubscript{2}Cl\textsubscript{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; 14 minutes) giving 4'-demethylamino-4'-nitro-staurosporine after lyophilization and crystallization from CH$_2$Cl$_2$ (119 mg).

Fraction 1E (2.23 g) was subjected to preparative reversed-phase HPLC (70% solvent B (isocratic); 290 nm; 39 runs) yielding K-252c$^{15}$ (54 mg; Rt 16 minutes), crude 8 (113 mg; Rt 19 minutes), N-methyl-3'-deoxy-3'-amino-derivative of K-252c$^{18}$ (138 mg; Rt 23 minutes; white crystals with mp 152~155°C from CH$_2$Cl$_2$) and crude 9 (71 mg; Rt 26 minutes). The crude 8 was separated by semipreparative normal phase HPLC (CH$_2$Cl$_2$ -2-PrOH, 96.5:3.5; 5 ml/minute; 310 nm; 40 runs; Rt 13 minutes) yielding 41 mg. Pure 7-N-methyl-staurosporine$^1$ was obtained by semipreparative HPLC (Nucleosil 100 5/μm; 8x250mm; CH$_2$Cl$_2$-2-PrOH-triethylamine, 97 : 3 : 0.1; 5 ml/minute; 345 nm; 30 runs) yielding crude 7-N-methyl-staurosporine (310 mg, 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$_2$Cl$_2$ -2-PrOH - triethylamine, 98 : 2 : 0.1; 5 ml/minute; 345 nm; 39 runs) yielding K-252a$^{18}$ (138 mg; Rt 23 minutes), 7-N-methyl-3'-deoxy-3'-amino-derivative of K-252c$^{18}$ (138 mg; Rt 23 minutes; white crystals with mp 152~155°C from CH$_2$Cl$_2$) and crude 9 (71 mg; Rt 26 minutes). The crude 8 was separated by semipreparative normal phase HPLC (CH$_2$Cl$_2$ -2-PrOH - triethylamine, 97 : 3 : 0.1; 5 ml/minute; 345 nm; 39 runs) yielding K-252a$^{18}$ (54 mg; Rt 16 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; 57 runs; Rt 10 minutes) to yield compound 6 of Ref. 11 (86 mg).

Data of 2

White powder from CH$_3$CN - H$_2$O, mp 240°C (dec.); HRFAB-MS Found: m/z 483.1670 Calcd for C$_{27}$H$_{22}$N$_4$O$_5$ (M+H$^+$): 483.1668; IR (KBr) cm$^{-1}$ 3390, 3290, 2830, 1690, 1580, 1460, 1390, 1370, 1350, 1320, 1130, 1120, 740; CD $^\text{EtOH}$ nm (0): 368 (3900), 350 (1950), 327 (850), 300 (17500), 262 (~9200), 239 (25500), 207 (~19500).

Data of UCN-01 (3)

White crystals from CH$_2$Cl$_2$ - 2-PrOH, mp 220°C (dec.); CD $^\text{EtOH}$ nm (0): 368 (2200), 350 (1700), 327 (850), 267 (~7550), 255 (~1350), 247 (~9300), 233 (4000), 214 (~14700).

Data of 4

Yellow powder from CH$_2$Cl$_2$ - 2-PrOH, mp 265~270°C (dec.); HREI-MS Found: m/z 480.1430 Calcd for C$_{27}$H$_{22}$N$_4$O$_5$: 480.1434; EI-MS: m/z 480 (12), 462 (11), 395 (40), 387 (28), 377 (46), 376 (75), 325 (73), 324 (100), 254 (56), 255 (35), 138 (48), 45 (70), 44 (46), 43 (50); IR (KBr) cm$^{-1}$ 3240, 2930, 2820, 2690, 2550, 1850, 1690, 1630, 1570, 1490, 1470, 1460, 1410, 1340, 1320, 1280, 1220, 1120, 750, 740.

Data of 5

White powder from CH$_2$Cl$_2$ - 2-PrOH, mp >300°C (dec.); HREI-MS Found: m/z 510.1904 Calcd for C$_{29}$H$_{26}$N$_4$O$_5$: 510.1903; EI-MS: m/z 510 (13), 494 (7), 492 (6), 479 (7), 470 (35), 406 (47), 376 (40), 357 (60), 366 (51), 355 (57), 325 (54), 324 (67), 323 (53), 311 (49), 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$_2$Cl$_2$ - 2-PrOH, mp 266~272°C (dec.); HREI-MS Found: m/z 538.2207 Calcd for C$_{29}$H$_{26}$N$_4$O$_5$: 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$_2$Cl$_2$) cm$^{-1}$ 3292, 2850, 1650, 1590, 1490, 1460, 1410, 1400, 1340, 1330, 1270, 1240, 1120, 1050, 740.
Data of 8
White crystals from CH₂Cl₂, mp 236 ~ 243°C; HRFAB-MS Found: m/z 452.1601 Calcd for C₁₇H₁₂₀N₃O₄ (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, 1730, 1680, 1590, 1460, 1370, 1350, 1230, 1280, 1230, 1150, 1130, 770, 750; CD XEtOH nm (9): 369 (4100), 336 (10400), 309 (-8200), 297 (13200), 272 (0), 260 (-10500), 245 (25600), 209 (-25700).

Data of 9
Colorless crystals from CH₂Cl₂, mp 171 ~ 176°C; FAB-MS m/z 452 (M + H⁺); HREI-MS Found: m/z 451.1529 Calcd for C₁₇H₁₂₀N₃O₄: 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, 1100, 770, 750; CD XEtOH nm (6): 369 (19200), 351 (370), 283 (12), 282 (34), 255 (14), 97 (17), 71 (22), 69 (38), 57 (45), 55 (56), 44 (100); IR (KBr) cm⁻¹ 3420, 2920, 1735, 1690, 1590, 1510, 1460, 1400, 1370, 1350, 1320, 1280, 1230, 1150, 1130, 770, 750; CD XEtOH nm (9): 369 (4100), 336 (10400), 309 (-8200), 297 (13200), 272 (0), 260 (-10500), 245 (25600), 209 (-25700).

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