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PREPARATION OF SYNTHONS FOR THE SYNTHESIS OF PROTEIN KINASE C INHIBITORS FROM REBECCAMYCIN.

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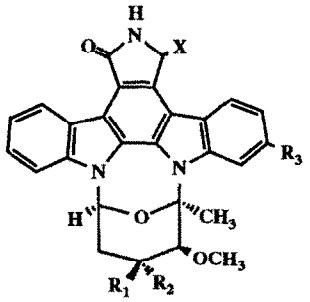
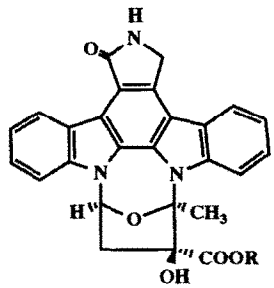
Abstract : An efficient process to obtain aglycons, useful for the preparation of protein kinase C inhibitors, was developed from Rebeccamycin, an antitumor antibiotic isolated from *Saccharotrix aerocolonigenes.*

The activation of protein kinase C is one of the earliest events in the cascade of signal transduction pathways leading to a large variety of cellular responses among which are gene expression and proliferation and muscle contraction[1]. Therefore, PKC inhibitors may be useful as drugs in the treatment of cardiac and vascular diseases or cancers. Among the PKC inhibitors which interact with the adenosine triphosphate binding site are microbial metabolites possessing an indolocarbazole unit as staurosporine[2], UCN-01 and 02[3], RK-286C[4], K-252 a,b[5], TAN-999 and 1030A[6] (figure 1) isolated from different strains of *Streptomyces* or *Nocardiopsis species.* Their inhibitory activity is shown in table I.

Figure 1





Staurosporine : $X=R_2=R_3=H$; $R_1=NHCH_3$
TAN-999 : $X=R_2=H$; $R_1=NHCH_3$; $R_3=OCH_3$
TAN-1030A : $X=R_3=H$; $R_1,R_2= =NOH$
RK-286C : $X=R_2=R_3=H$; $R_1=OH$
UCN-01 and 02 : $X=OH$; $R_2=R_3=H$; $R_1=NHCH_3$

K-252 a : $R = CH_3$
K-252 b : $R = H$

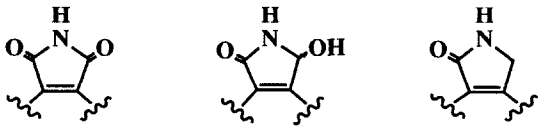
Although most of them are very efficient PKC inhibitors, with IC_{50} values in the nanomolar region, their lack of selectivity and availability in only small quantities from cultures (from 0.3 mg to 10 mg / liter of culture) are major problems.

Table I: Inhibition of PKC and PKA by microbial indolocarbazoles IC_{50} (μM)

Compounds	PKC	PKA
Staurosporine[3]	0.0027	0.0082
K-252a[5]	0.033	n.d.
K-252b[5]	0.038	n.d.
RK-286C[7]	3.0	n.d.
UCN-01[3]	0.0041	0.042
UCN-02[3]	0.062	0.25

In their search for accessible potent and selective PKC inhibitors, several groups have tried to synthesize new inhibitors in these series[8-13] directing their efforts, first of all, towards structurally related aglycons[14-19] with varying functions on the upper heterocycle (figure 2). These aglycones could, after substitution on the indolic NH or coupling of the two indole moieties with heterocycles[9,10], lead in only one step to a variety of protein kinase C inhibitors.

Figure 2



We report here an efficient method for the preparation of the aglycon **3** (figure 3) from Rebeccamycin **1**, an antitumor antibiotic isolated from cultures of *Saccharotrix aerocolonigenes* (ATCC 39243). This antibiotic, in contrast to other indolocarbazoles, could be obtained by fermentation in relatively large quantities (663 mg / liter of culture)[20].

Since the cleavage of the N-glycosidic bond did not occur significantly in refluxing 6N HCl, it was necessary to employ drastic conditions. A 1 hour reflux of **1** in 70% aqueous $HClO_4$ gave the imide **2** [21] in 80 % yield. Dechlorination was effected quantitatively by heating in formic acid / dimethyl formamide in the presence of catalytic amounts of palladium on carbon according to the method described by Pandey and Purkayastha (1982)[22] for the hydrodehalogenation of haloaromatic compounds.

The aglycon **3**[23], which was obtained in an excellent overall yield in only two steps , can be used directly for the preparation of new protein kinase C inhibitors possessing the imidic five membered ring system or can be converted to the UCN 01 and 02 aglycons **4**[24] by reduction with lithium aluminium hydride or to the staurosporine aglycon **5** by Clemmensen reduction with zinc amalgam[12] (figure 3).

This method from Rebeccamycin provides a rapid access to structures **3**, **4** and **5** from which new PKC inhibitors can be synthesized.

Although these synthetic intermediates were not expected to have an important biological activity, their inhibitory capacities towards PKC and PKA were however tested with histones III_S and II_A respectively as substrates, according to the method used by Ricouart *et al* [25]. IC_{50} values determinated both for PKC and PKA are reported in table II. The isoquinoline sulfonamide H-7 was tested as reference[25-26].

Figure 3

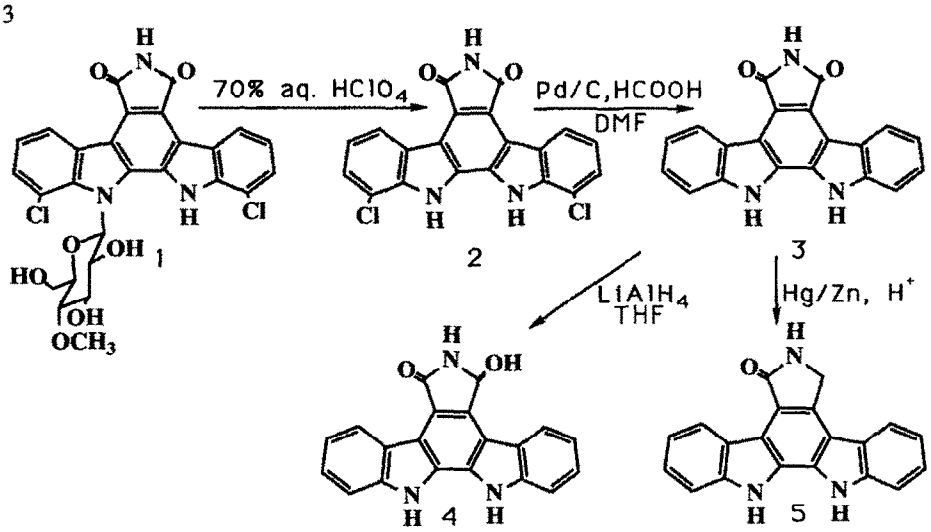


Table II: Inhibition potencies for compounds **1-5** compared to that of H-7[27]. IC_{50} (μM)

Compounds	PKC	PKA
H-7	9.1	3.3
1	> 100	n.d.
2	> 50	> 100
3	44.7	60
4	22.1	34
5	2.45	25.7

The amide **5** exhibit a significant inhibitory activity and seems to be the best aglycon to use for further substitutions on the indolic nitrogens.

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References and Notes

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21. Selected spectroscopic data for compound **2** : ^{1}H NMR (300 MHz Me_2SO-d_6) δ : 7.31 (2H, t, J = 7.7 Hz), 7.60 (2H, d, J = 7.7 Hz), 8.82 (2H, d, J = 7.7 Hz), 11.20 (s, 1H), 11.80 (s, 2H). ^{13}C NMR (75.47 MHz, Me_2SO-d_6) δ : 115.69, 120.76, 121.31, 123.19, 125.98, 128.76, 136.66, 170.77. MS m/z : 393.0051 (M^+ ; calcd for $C_{20}H_9Cl_2N_3O_2$: 393.0065).
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23. Selected spectroscopic data for compound **3** : ^{1}H NMR (300 MHz acetone-d_6) δ : 7.37 (2H, t, J=7.6 Hz), 7.58 (2H, t, J = 7.6 Hz), 7.79 (2H, d, J = 8.4 Hz), 9.01 (2H, d, J = 8.4 Hz), 11.01 (1H, s), 12.26 (2H, s). ^{13}C NMR (75.47 MHz, Me_2SO-d_6) δ : 111.86, 115.37, 119.81, 120.11, 121.52, 124.28, 126.71, 129.14, 140.15, 171.32. MS m/z : 325.0845 (M^+ ; calcd for $C_{20}H_{11}N_3O_2$: 325.0851).
24. Selected spectroscopic data for compound **4** : ^{1}H NMR (300 MHz acetone-d_6) δ : 6.55 (1H, s), 7.27 (2H, 2t, J = 7.5 Hz), 7.43 (2H, 2t, J = 7.5 Hz), 7.61 (1H, d, J = 8.5 Hz), 7.68 (1H, d, J = 8.5 Hz), 7.98 (1H, s), 8.35 (1H, d, J = 8.5 Hz), 9.29 (1H, d, J = 8.5 Hz), 11.02 (1H, s), 11.21 (1H, s). ^{13}C NMR (75.47 MHz, acetone-d_6) δ : 80,9 (CH-OH), 108.5, 110.9, 112.1, 112.2, 119.5, 120.2, 121.9, 122.3, 122.8, 126.8, 127.4, 128.2, 137.1, 137.2, 137.5, 146.6, 173.6 (C=O). MS m/z : 326.0905 (M-H^+; calcd for $C_{20}H_{12}N_3O_2$: 326.0929).
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27. Histone III_S and II_A, phosphatidylserine, diacylglycerol and PKA were purchased from Sigma. [γ^{32}P] ATP was from Amersham. PKC was from Calbiochem. PKC phosphorylation assays were performed in a reaction mixture (80 µl) containing histone III_S (2,4 mg/ml), $MgCl_2$ (10mM), $CaCl_2$ (0.1 mM), phosphatidylserine (10mg/ml), diacylglycerol (10 mg/ml), Tris/HCl buffer (pH 7.5), ATP (10 µM,1000-2000 cpm/pmol), PKC (0.5 µg/ml). Stock solutions of inhibitors were prepared in DMSO. In each assay, data points were determinated in triplicate.