Notes

A NEW INDOLOCARBAZOLE ANTIBIOTIC, RK-286D

HIROYUKI OSADA, MASAYUKI SATAKE, HIROYUKI KOSHINO, RIE ONOSE and KIYOSHI ISONO*

Antibiotics Laboratory, RIKEN, The Institute of Physical and Chemical Research, Wako, Saitama 351-01, Japan

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In a previous paper[1], we reported that *Streptomyces* sp. RK-286 produced a novel inhibitor of protein kinase C (PKC), RK-286C (4'-demethylamino-4'-hydroxystaurosporine). In the course of the fermentation, a new PKC inhibitor designated RK-286D was isolated as a minor component from the mycelial cake of *Streptomyces* sp. RK-286. In this paper, we report that RK-286D is a novel indolocarbazole antibiotic structurally related to RK-286C.

The producing strain was inoculated into a 500-ml cylindrical flask containing 70 ml of seed medium (glucose 2%, soybean meal 2.5%, soluble starch 1%, meat extract 0.1%, dried yeast 0.4%, K_2HPO_4 0.005%, and NaCl 0.2%, adjusted to pH 7.3), and cultured at 28°C for 3 days on a rotary shaker (250 rpm). The seed culture was transferred to the production medium (18 liters, glucose 0.1%, soybean meal 1%, malt extract 1%, oatmeal 0.5%, RPMI-1640 medium Nissui 0.25%, K_2HPO_4 0.1%, and $MgSO_4$ 0.01%, adjusted to pH 7.2) in a 30-liter jar fermenter. The fermentation was carried out at 28°C for 7 days under agitation (250 rpm) and aeration (15 liters/minute).

The acetone extract of mycelial cake was concentrated under reduced pressure and the resulting aqueous solution was extracted with EtOAc. After concentration of the EtOAc layer, the oily residue was applied to a silica gel column (packed in $CHCl_3$-MeOH, 20:1). Active fractions were eluted with $CHCl_3$-MeOH (10:1). The fractions contained staurosporine[2], RK-286C[3] and RK-286D. RK-286D enriched fractions were collected and concentrated under reduced pressure. The residue was added to a Sephadex LH-20 column equilibrated with 100% MeOH, and developed with the same solvent system. Active fractions were combined and concentrated to yield a yellow powder. The powder was finally purified by HPLC on a reverse-phase column (Capcell Pak C_{18}, MeOH-0.1% NH_4OH-H_2O, 80:10:10). Four mg of pure RK-286D were obtained from 18-liter fermentation broth.

RK-286D is a pale yellow powder with mp 225~230°C (dec). It is optically active, $[\alpha]_D^{20}$ −60.0° (*c* 0.13, MeOH). The molecular formula was established as $C_{26}H_{23}N_3O_4$ based on HREI-MS (M^+ *m/z* 441.1691, Δ+0.4 mmu). FD-MS gave a parent peak at 442 $(M+H)^+$. UV data of RK-286D are similar to those of RK-286C; UV λ_{max}^{MeOH} nm (ε) 364 (11,466), 347 (13,230), 335 (20,286), 320 (sh), 290 (98,785), 278 (sh), 233 (39,690). RK-286D showed positive color reactions to Rydon-Smith and anisaldehyde-H_2SO_4 tests, and negative to ninhydrin and ferric chloride reagents.

^1H NMR data are summarized in Table 1 and the assignments are in accord with those for RK-286C and staurosporine[3]. Decoupling experiment (Fig. 1) and the NOE difference spectrum revealed that the sugar moiety of RK-286D is digitoxose, attached to N-13 of the chromophore (Fig. 2). The digitoxopyranosyl moiety is in the $^1C_4$ conformation, which is presumably due to the steric effect of the bulky aglycone moiety. The absolute stereochemistry remains to be determined. However, it is considered that RK-286D is a shunt metabolite

Table 1. ^1H NMR data for RK-286D with assignments (MeOH-d_4).

Position	δ_H (ppm, *J* in Hz)
1	7.72 (d, 8.1)
2	7.50 (ddd, 8.1, 7.0, 1.1)
3	7.28 (ddd, 7.7, 7.0, 0.7)
4	9.42 (d, 7.7)
7	5.04 (s)
8	8.00 (d, 7.6)
9	7.30 (ddd, 7.6, 7.2, 0.7)
10	7.46 (ddd, 8.1, 7.2, 1.1)
11	7.70 (d, 8.1)
1'	6.61 (dd, 11.7, 2.9)
$2'_{eq}$	2.04 (ddd, 12.5, 5.1, 2.9)
$2'_{ax}$	2.69 (ddd, 12.5, 11.7, 11.7)
3'	4.48 (ddd, 11.7, 5.1, 2.9)
4'	4.04 (dd, 2.9, 1.8)
5'	4.66 (dq, 1.8, 7.3)
6'	1.63 (d, 7.3)

Fig. 1. Coupling constants between protons of the sugar moiety of RK-286D.

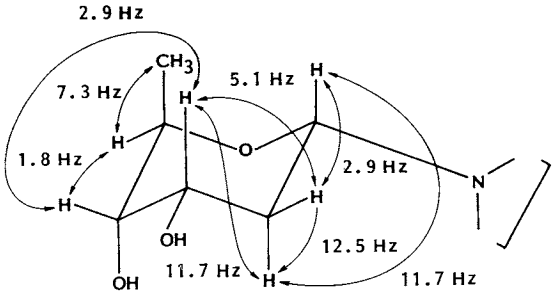

Fig. 2. ^{1}H NMR assignments and NOE observed in the NOE difference spectrum of RK-286D in MeOH-d_4.

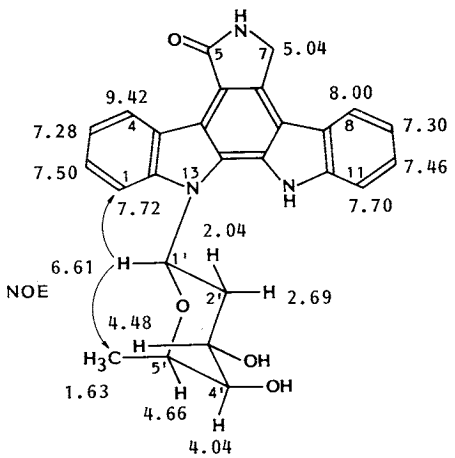

Table 2. Inhibitory activities against bleb formation of K-562 cells and PKC.

	IC_{50} (μM)	
	Bleb-suppression	PKC inhibition
RK-286D	20	10
RK-286C	3	1
Staurosporine	0.003	0.003

PKC activity was measured with an assay kit from Amersham.

in the biosynthesis of staurosporine and RK-286C (4′-demethylamino-4′-hydroxystaurosporine).

Inhibitory activities of RK-286D against bleb formation[4] induced by PDBu and *in vitro* PKC activity were compared with RK-286C and staurosporine (Table 2). Staurosporine showed the strongest inhibiton in both assays, and RK-286D showed the weakest inhibition. Since the chromophore is the same in the three compounds, the results suggest that the sugar moiety of indolocarbazole group antibiotics is important for their biological activity. Antimicrobial activity of RK-286D was not observed at a concentration of 100 μg/ml against bacteria, fungi, and yeast.

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