THE STRUCTURES OF THE NOVEL PROTEIN KINASE C INHIBITORS K-252a, b, c AND d

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The structures of four new protein kinase C inhibitors of microbial origin, K-252a, b, c and d were determined by spectral studies and chemical conversion.

K-252a (**1**)[1], b (**2**), c (**3**) and d (**4**)[2] are inhibitors of protein kinase C produced by *Nocardiopsis* sp. K-252 and sp. K-290, respectively. The production and isolation of these compounds has been reported by KASE *et al.*[1,2]. In this paper we wish to report their complete structures.

K-252a (**1**) was obtained as pale yellow crystals by recrystallization from acetone - methanol. It gave positive tests to anisaldehyde, Ehrlich and Dragendorf reactions and was negative to Ninhydrin and ferrous chloride reactions. Physico-chemical properties of **1** are listed in Table 1.

The molecular formula of **1** was determined as $C_{27}H_{21}N_3O_5$ by high resolution electron impact mass spectrum and elemental analysis data. The IR spectrum indicated the existence of NH and OH (3420 cm^{-1}), ester (1730 cm^{-1}) and amide (1663 cm^{-1}) groups. The UV spectrum of **1** was quite similar to that of staurosporine[3], which suggested the same chromophore in both compounds.

^1H and ^{13}C NMR data are summarized in Tables 2 and 3. The data show the presence of one secondary amide group, one methoxy carbonyl group, one tertiary hydroxyl group, eighteen sp^2 carbons, one quaternary carbon, one methine, two methylenes and one methyl group. Proton homo decoupling, ^1H-^{13}C selective decoupling and ^1H-^{13}C long range selective decoupling experiments revealed four structural moieties; two 1,2-disubstituted benzenes (**I**, **II**), a γ-lactam (**III**) and a sugar moiety (**IV**). The γ-lactam (**III**) contained two quaternary carbons (δ 119.5 and 132.9). Another two quaternary carbons (δ 114.6 and 115.8) are found in the partial structures **I** and **II**, respectively, and a quaternary

Fig. 1. ^1H-^{13}C Long range coupling and NOE experiment of K-252a (**1**).

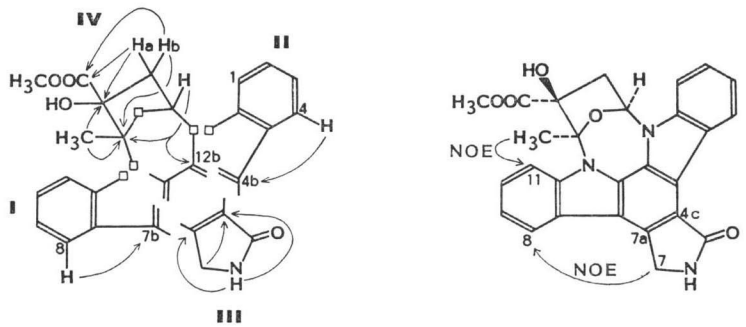


Table 1. Physico-chemical properties of K-252a (**1**), b (**2**), c (**3**) and d (**4**).

	1	**2**	**3**	**4**
Appearance	Pale yellow crystals	Pale yellow powder	Pale yellow needles	Pale yellow needles
MP	262~273°C (dec)	262~266°C (dec)	>300°C	240~245°C (dec)
$[\alpha]_D^{20}$	−23° (*c* 0.5, $CHCl_3$), +52° (*c* 0.1, MeOH)	+97° (*c* 0.6, DMF)		+35° (*c* 0.4, MeOH)
Molecular formula	$C_{27}H_{21}N_3O_5$	$C_{26}H_{19}N_3O_5$	$C_{20}H_{13}N_3O$	$C_{26}H_{23}N_3O_5$
Anal Calcd	C 69.37, H 4.52, N 8.98	C 68.87, H 4.22, N 9.27		
Found	C 69.08, H 4.47, N 8.79	C 68.71, H 4.35, N 9.01		
Mass (*m/z*)	467 (M^+), 424, 406, 364, 337, 321	453 (M^+)	311 (M^+), 282, 255, 155, 141, 127	458 $(M+1)^+$, 440, 354, 340, 311, 282, 268, 255
HR-MS Calcd	467.1482		311.1058	457.1636
Found	467.1462		311.1079	457.1650
UV λ_{max} nm (ε)	367 (21,000), 350 (19,000), 333 (26,000), 320 (sh, 19,000), 290 (102,000), 280 (sh, 68,000), 264 (sh, 44,000), 248 (41,000), 226 (42,000)[a]	371 (13,000), 353 (13,000), 337 (16,000), 323 (12,000), 291 (62,000), 280 (sh, 42,000), 268 (sh, 29,000), 246 (28,000), 232 (29,000)[b]	358 (11,000), 341 (16,000), 331 (20,000), 320 (sh, 16,000), 287 (86,000), 257 (sh, 29,000), 246 (sh, 28,000), 238 (sh, 34,000), 230 (37,000)[a]	364 (13,000), 347 (16,000), 335 (23,000), 322 (17,000), 290 (95,000), 280 (sh, 61,000), 268 (sh, 38,000), 260 (sh, 34,000), 248 (sh, 31,000), 242 (sh, 36,000), 223 (40,000)[a]
IR (KBr) cm^{-1}	3420, 1730, 1663, 1630, 1590, 1450, 1250, 1130, 950, 740	3430, 1720, 1665, 1632, 1591, 1460, 1313, 1279, 1235, 1133, 740	3590, 3440, 3320, 3200, 1648, 1585, 1453, 1410, 1330, 1239, 1108, 1030, 1011, 778, 737	3340, 1650, 1605, 1584, 1454, 1402, 1330, 1246, 1112, 1045, 744

[a] Measured in MeOH. [b] Measured in H_2O.

Table 2. ^{1}H NMR data of K-252a (**1**), b (**2**), c (**3**) and d (**4**) (400 MHz, DMSO-d_6).

Position	Chemical shift[a] (*J*, Hz)			
	1	**2**	**3**	**4**
1	7.90 (d, 8.3)	7.91 (d, 8.4)	7.73 (d, 8.1)	7.69 (d, 8.3)
2	7.49 (br t)[b]	7.48 (br t)[b]	7.44 (br t)	7.49 (br t)
3	7.29 (br t)	7.28 (br t)	7.24 (br t)	7.27 (br t)
4	9.24 (d, 7.9)	9.22 (d, 7.8)	9.24 (d, 7.9)	9.47 (d, 7.7)
6	8.64 (br s)	8.62 (br s)	8.49 (br s)	8.54 (br s)
7	5.04, 5.00 (AB, 17.3)	5.03, 5.04 (AB, 17.3)	4.98 (s)	5.01 (br s)
8	8.05 (d, 7.8)	8.05 (d, 7.8)	8.05 (d, 7.8)	8.07 (d, 7.8)
9	7.36 (br t)	7.36 (br t)	7.31 (br t)	7.31 (br t)
10	7.49 (br t)[b]	7.48 (br t)[b]	7.48 (br t)	7.50 (br t)
11	7.95 (d, 8.5)	7.98 (d, 8.5)	7.79 (d, 8.1)	7.60 (d, 8.1)
12			11.56 (br s)	11.68 (br s)
13			11.38 (br s)	
1′	7.15 (dd, 7.4, 4.9)	7.12 (dd, 7.3, 4.9)		6.40 (br d, 9.5)
2′	3.41 (dd, 14.0, 7.4) 2.04 (dd, 14.0, 4.9)	3.36 (dd, 13.8, 7.3) 1.98 (dd, 13.8, 4.9)		*ca.* 4.5 (m)
3′				4.18 (br q)[c]
4′				4.05 (br t)[c]
5′	2.16 (s)	2.22 (s)		4.48 (br q, 7.6)
6′				1.70 (d, 7.2)
2′-OH				*ca.* 5.0[d]
3′-OH				5.40 (d, 3.6)
4′-OH				6.69 (br s)
$COOCH_3$	3.94 (s)			

[a] Chemical shift in ppm from TMS as an internal reference.
[b] These signals are overlapped.
[c] Coupling constants are *ca.* 3 Hz.
[d] This signal is overlapped with 7-H_2 (δ 5.01).

carbon (δ 123.9) is connected to the sugar moiety (**IV**) through a hetero atom. It is apparent that these five quaternary carbons and a remaining quaternary carbon (δ 128.3) constitute another aromatic ring system. Observation of the nuclear Overhauser effect (NOE) (7.0%) of the methylene protons (δ 5.04, 5.00) to H-8 indicates the position of methylene to be at 7, thus confirming the chromophore of **1**, which is identical with that of staurosporine.

Correlation spectroscopy *via* long range couplings (COLOC)[4] is an useful tool to determine a structure of an aliphatic system containing a quaternary carbon or a hetero atom. The long range coupling pattern of the sugar moiety found by the COLOC spectrum (Fig. 2) is shown in Fig. 1. From the observation of long range coupling: The 2′-methylene protons to the 3′, 4′ and carbonyl carbons; the methyl proton to the 3′ and 4′ carbons; and the 1′ proton to the 4′ carbon, it is obvious that the methoxy carbonyl group is bonded to the 3′-position and the methyl group is at 4′. The 1′ and 2′ positions were determined as methine and methylene, respectively, by proton homo decoupling experiment. These facts indicate the 2-deoxyfuranoside structure (**IV**).

Further observations of long range coupling between H-1′ and C-12b, and the NOE (9.1%) between the 6′-methyl and 11-protons indicate 1′ is bonded to N-13 and 4′ to N-12. From these findings, the structure of **1** was determined as shown in Fig. 3. The complete structure with the 3′-configulation was confirmed by single crystal X-ray analysis (Fig. 4)[5].

Table 3. ^{13}C NMR data of K-252a (**1**), b (**2**), c (**3**) and d (**4**) (100 MHz, DMSO-d_6).

Position	1	2	3	4
1	109.0	109.0	112.0	109.7
2	125.4	125.3	125.1	125.1
3	119.4	119.4	119.0	119.2
4	125.6	125.6	125.4	125.5
4a	122.6	122.6	123.0	122.2
4b	115.8	115.7	115.7	117.4
4c	119.5	119.5	120.0	118.5
5	171.7	171.8	172.6	172.2
7	45.4	45.4	45.4	45.1
7a	132.9	132.9	133.0	133.8
7b	114.6	114.5	114.2	114.8
7c	124.1	124.1	122.7	121.8
8	121.2	121.2	121.2	121.1
9	120.4	120.3	120.0	119.7
10	125.0	124.9	125.1	125.0
11	114.7	114.8	111.4	111.1
11a	139.8	139.9	139.2	138.9
12a	128.3	128.3	128.0	127.5
12b	123.9	123.9	125.2	124.4
13a	136.8	136.8	139.3	140.1
1′	84.95	85.0		77.1
2′	42.5	42.5		66.8
3′	84.92	84.4		71.6
4′	99.3	99.2		71.4
5′	22.8	22.8		76.4
6′	172.8	174.0		15.3
$COOCH_3$	52.6			

Chemical shift in ppm from TMS as an internal reference.

Fig. 2. COLOC spectrum of K-252a (**1**).

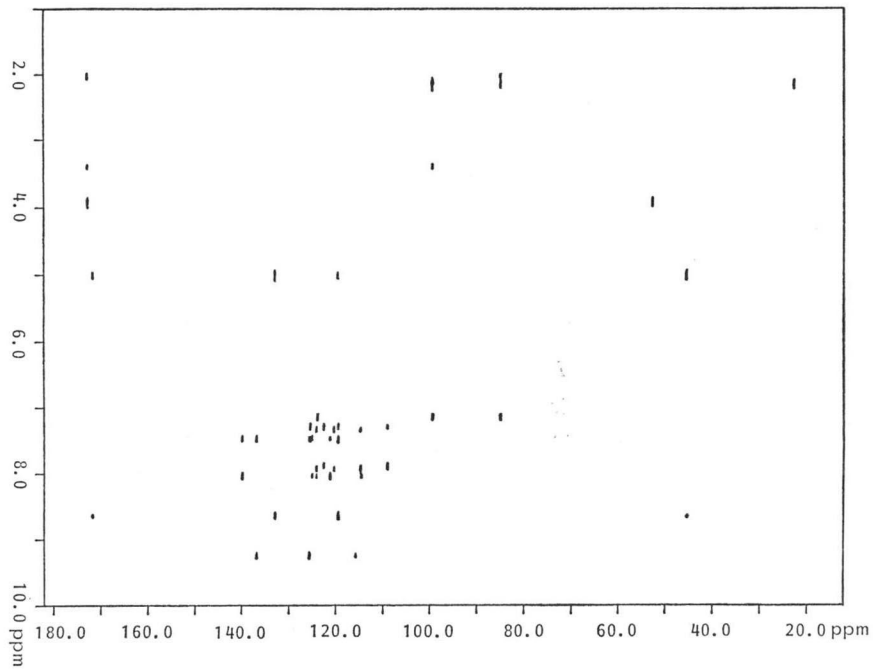
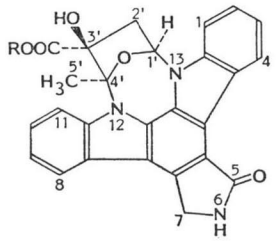
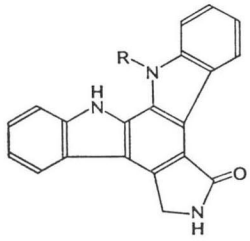


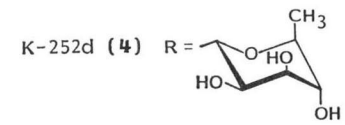
Fig. 3. Structures of K-252a (**1**), b (**2**), c (**3**) and d (**4**).



K-252a (**1**) R = CH_3
K-252b (**2**) R = H



K-252c (**3**) R = H

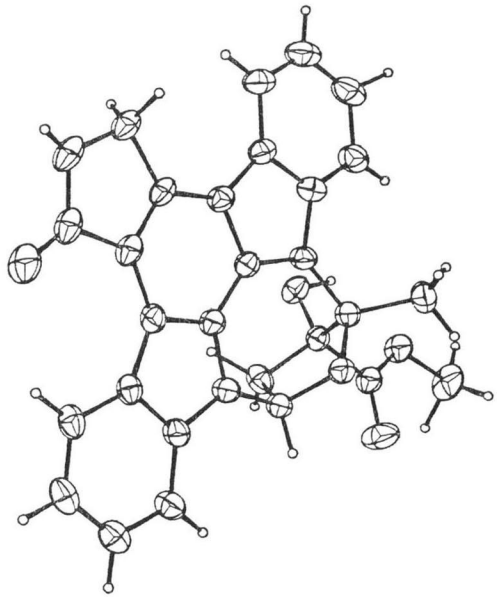


K-252d (**4**) R =

As K-252b (**2**), c (**3**) and d (**4**) show similar UV spectra to that of K-252a (**1**), they seem to have the same indolo[2,3-a]carbazole chromophore. ^1H and ^{13}C NMR spectra of **2** (Tables 2 and 3) are similar to those of **1** except for the disappearance of the methoxycarbonyl methyl signal. The compound obtained by hydrolysis of **1** was identical with **2** and methylation of **2** by diazomethane gave **1**, confirming the 3′-carboxyl free structure of **2**.

The molecular formula of **3**, $C_{20}H_{13}N_3O$, implies lack of the sugar moiety ($C_7H_8O_4$) in comparison with that of **1**. ^1H NMR of **3** (Table 2) shows two NH protons (δ 11.56 and 11.38) and the disappearance of protons assigned to the sugar moiety in **1**. Hydrolysis of **1** under drastic conditions gave **3**, which was thus confirmed as the aglycone of **1**.

Fig. 4. An ORTEP drawing of the molecule of K-252a (**1**).



In the ^1H NMR of **4** (Table 2), one NH proton (δ 11.68) as well as protons assigned to a sugar moiety were noted in addition to those of the aglycone. This suggests that **4** is an *N*-glycoside of **3**. The high resolution electron impact mass spectrum reveals the elemental composition of the sugar moiety of **4** as $C_6H_{11}O_4$. Hydrolysis of **4** gave a water soluble substance which was identical with rhamnose by comparison of ^1H NMR spectra[6] and gas-liquid chromatography (GLC) of their TMS derivatives[7]. The rotation, $[\alpha]_D^{20}$ +212°, and CD $[\theta]_{236}$ +110,000 (positive max), $[\theta]_{241}$ −34,000 (negative max) of the *p*-nitrobenzoate (**6**) of methyl α-rhamnopyranoside (**5**)[8] obtained from **4** are identical to those of an authentic sample synthesized from L-rhamnose. In the ^1H and ^{13}C NMR spectra of **4**, long range ^1H-^1H coupling between H-1′ and H-1, and ^1H-^{13}C coupling between H-1′ and C-12b were observed. A large coupling constant $J_{1',2'}$ (9.5 Hz) and a small coupling constant $J_{1'-C1'}$ (159 Hz) were observed but there was no large

coupling constant found for $J_{2',3'}$, $J_{3',4'}$ and $J_{4',5'}$. The structure of **4** is determined, therefore, as 13-*N*-(α-L-rhamnopyranosyl)-K-252c, where the rhamnopyranosyl moiety takes the $^1C_4$ conformation.

Experimental

NMR spectra were measured on a Jeol FX100 and Bruker AM400 spectrometer. Mass spectra were obtained on a Hitachi M-80B spectrometer at 70 eV. IR spectra were measured with a Shimadzu IR-27G spectrometer. UV spectra were taken with a Hitachi 200-20 spectrometer. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. CD spectra were taken on a Jasco J-500A spectropolarimeter and a Jasco DP-501 data processor. Melting points were taken with a Yanagimoto micro melting point apparatus and were not corrected. GLC was carried out on a Hitachi 263-70 gas chromatograph. Thin-layer chromatography (TLC) was performed on pre-coated plates, Merck Kieselgel 60 F_{254} and detected with UV light (254 nm) and 1% $Ce(SO_4)_2$ - 10% H_2SO_4 reagent.

Hydrolysis of 1

A suspension of K-252a (**1**, 20 mg) in MeOH (10 ml) and 1 M NaOH solution (1 ml) was stirred for 5 hours at room temp. The reaction mixture was concentrated *in vacuo* to remove MeOH. After acidification with 2 M HCl, the solution was extracted with EtOAc. EtOAc solution was washed with brine, dried over $MgSO_4$ and evaporated to give **2** (18 mg, 93%).

Methylation of 2

To a suspension of K-252b (**2**, 2.0 mg) in MeOH (3 ml), diazomethane in ether (1 ml) was added and stirred for 4 hours at room temp. The reaction mixture was evaporated to give **1** (2.0 mg, 97%).

Hydrolysis of 4

K-252d (**4**, 10 mg) was suspended in 2 M hydrochloric acid and heated to reflux for 2 hours. The reaction mixture was filtered to remove the solid (**3**, 5 mg) and washed with H_2O. The filtrate and washings were combined and evaporated to give a colorless solid, 2.5 mg (77%), which was identical with rhamnose by ^1H NMR spectroscopy. Rhamnose was further identified by GLC of its TMS derivative as follows.

GLC Analysis of TMS Derivative of Rhamnose

Rhamnose (1 mg), given by hydrolysis of **4**, was dissolved in TMS-Pz (Tokyo Kasei, T0623, 0.1 ml) and held for 15 minutes. The reaction mixture was diluted with 10 volumes of acetone and subjected to GLC using a silicone OV-101 25 m (0.25 mm i.d.) FS-WCOT capillary column, detection: FID, flow rate: N_2 15 ml/minute, temp: 210°C (injection), 180°C (column), 210°C (detection), t_R: α-TMS-rhamnose, 8'12''; β-TMS-rhamnose, 10'24'', which were identical with an authentic sample prepared from L-rhamnose in a similar manner.

Preparation of 7

Rhamnose (1.5 mg), given by the hydrolysis of **4**, was dissolved in 17% HCl in MeOH (1 ml) and heated to reflux for 1 hour. The reaction mixture was evaporated to give methyl α-rhamnopyranoside (**5**, 1.6 mg)[6], which was dissolved in dry pyridine (0.5 ml), treated with *p*-nitrobenzoyl chloride (20 mg) and stirred for 2 hours under N_2 atmosphere at room temp. The reaction mixture was evaporated to give a residue, which was chromatographed on a silicagel column, giving a colorless powder (**6**, 4 mg). Recrystallization from hexane - EtOAc gave colorless needles: MP 103~104°C; $[\alpha]_D^{20}$ +212° (*c* 0.2, $CHCl_3$); CD (dioxane) $[\theta]_{266}$ +110,000 (positive max), $[\theta]_{251}$ 0, $[\theta]_{241}$ −34,000 (negative max), $[\theta]_{223}$ 0, $[\theta]_{220}$ +5,000; ^1H NMR (100 MHz, $CDCl_3$) δ 1.41 (3H, d, J=6.1 Hz), 3.54 (3H, s), 4.25 (1H, dq, J= 9.3, 6.1 Hz), 4.93 (1H, d, J=1.5 Hz), 5.5~5.9 (3H, m), 7.9~8.4 (12H).

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