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Novel α-Glucosidase Inhibitors, CKD-711 and CKD-711a Produced by *Streptomyces* sp. CK-4416

III. Physico-chemical Properties and Structure Elucidation

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We have isolated two novel α-glucosidase inhibitors, *O*-[4-deoxy-4-(2,3-epoxy-3-hydroxymethyl-4,5,6-trihydroxycyclohexane-1-yl-amino)-α-D-glucopyranosyl]-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-α-D-glucopyranose (named CKD-711) and its hexameric analog CKD-711a, from the fermentation broth of *Streptomyces* sp. CK-4416. HRFAB-MS and NMR analyses reveal that molecular formulae of CKD-711 and CKD-711a are $C_{25}H_{43}NO_{20}$ and $C_{37}H_{63}NO_{30}$, respectively with the latter containing two more glucose moieties than the former. Detailed chemical structures of both compounds have been characterized by high-resolution two-dimensional NMR methods.

Diabetes mellitus (DM) is a major chronic disease caused by an improper balance of glucose homeostasis[1]. Two types of DM are currently known, one being insulin-dependent diabetes mellitus, IDDM and the other being non-insulin-dependent diabetes mellitus, NIDDM[2]. While the IDDM can be effectively controlled by proper administration of insulin, it is difficult to find an effective treatment for NIDDM due to its non-insulin-dependent nature, which consists of more than 90% of diabetic population. As of today, the number of individuals affected by NIDDM is well over 100 million. The human small intestine can absorb only the monomeric forms of saccharides such as glucose and fructose that are produced when starch or sucrose, the primary carbohydrate components of the human diet, are hydrolyzed by α-amylase or α-glucosidase[3,4]. A sudden rise in blood glucose level due to the rapid degradation of carbohydrates by α-glucosidases in the brush-border membrane of the small intestine causes postprandial hyperglycemia in NIDDM patients. Currently available therapeutics for hyperglycemia include insulin sensitizers (or inducers) as well as α-glucosidase inhibitors[5].

Since α-glucosidases are key enzymes responsible for hydrolysis of carbohydrates into glucose, retardation of carbohydrate absorption by blocking α-glucosidase offers a potential therapeutic method for the management of DM. Even though α-glucosidase inhibitors such as acarbose[6] produced by *Actinoplanes* sp. and voglibose[7] obtained semisynthetically from valiolamine are commercially available. These compounds possess adverse effects such as flatulence, meteorism and diarrhea. While screening for bioactive metabolites from the *Streptomyces* sp. CK-4416 found in the soil of Jeju Island in Korea, we have discovered two new potent α-glucosidase inhibitors, CKD-711 and CKD-711a. The pattern of enzyme inhibitions with these compounds may offer a pharmacological advantage[8]. Relevant taxonomy and fermentation process of CK-4416 as well as isolation procedures[9], and biological activity[8] of

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CKD-711 and CKD-711a were described in the previous papers. In this paper, we report the physico-chemical properties and structure elucidation of CKD-711 and CKD-711a.

Experimental Procedures

Infrared spectra of CKD-711 and CKD-711a were measured on a Shimadzu IR 435 spectrophotometer with KBr pellet samples and UV spectra were measured on a Shimadzu UV-160A UV spectrophotometer. High-Resolution Fast Atom Bombardment (HRFAB)-MS and FAB-MS experiments were done on a JEOL JMS-DX 300 FAB-mass spectrometer while electrospary (ES)-MS analysis was carried out using a Hewlett-Packard HP5989B ESI-mass spectrometer. For NMR studies samples were prepared in 90% H_2O/10% ^2H_2O or in 100% ^2H_2O. NMR experiments were carried out on a Bruker DPX 400, a Varian UNITY 500 and on a Varian UNITY INOVA 600 spectrometers. In order to obtain unambiguous resonance assignment several NMR techniques such as two-dimensional ^1H homonuclear COSY, TOCSY, NOESY as well as ^1H-^{13}C heteronuclear two-dimensional HETCOR, HMQC, HMBC and HMQC-TOCSY were used in addition to ^1H and ^{13}C one-dimensional methods including DEPT. Typical 2D data consist of 2048 complex points in the t_2 dimension with 256 complex t_1 increments.

Results and Discussion

Physico-chemical Properties

Table 1 summarizes various physico-chemical properties of CKD-711 and CKD-711a. Both compounds were obtained as white powders that are highly soluble in water, but not in chloroform and *n*-hexane. Neither CKD-711 nor CKD-711a exhibited any appreciable UV absorption at wavelength higher than 200 nm. Both CKD-711 and CKD-711a gave positive color reactions with $AgNO_3$ and $KMnO_4$, but negative reactions to Sakaguchi reagent. The Rf values of CKD-711 and CKD-711a on a thin layer chromatography using the solvent system of EtOAc-MeOH-H_2O (5 : 3 : 2) were 0.3 and 0.2, respectively.

Structure Elucidation

Even though physico-chemical properties of CKD-711 and CKD-711a are quite similar the former shows better biological activities than the latter[8]. Recently, the former was shown to be formed when the latter is biotransformed by porcine pancreatic amylase (Data not shown), suggesting that the two compounds should be structurally related. Therefore, structural characterization has been performed for both compounds. Table 2 summarizes the NMR chemical shift assignments of CKD-711 and CKD-711a. The complete structures of CKD-711 and CKD-711a are shown in Fig. 1. Shown in Fig. 2 is the HMBC connectivity

Table 1. Physico-chemical properties of CKD-711 and CKD-711a.

	CKD-711	CKD-711a
Appearance	White powder	White powder
Solubility	Soluble in water Insoluble in *n*-hexane,$CHCl_3$	Soluble in water Insoluble in *n*-hexane,$CHCl_3$
Molecular formula	$C_{25}H_{43}NO_{20}$	$C_{37}H_{63}NO_{30}$
FAB-MS (m/z)	678.30 $(M+H)^+$	1002.30 $(M+H)^+$
HRFAB-MS (m/z)	678.2460 $(M+H)^+$	Not determined
Calcd :	678.2457 for $C_{25}H_{44}NO_{20}$	
UV $\lambda_{max}^{H_2O}$ nm (ε)	End absorption	End absorption
IR ν_{max}^{KBr} cm^{-1}	3300, 2900, 1650, 1400, 1250, 1150, 950	3380, 2920, 1640, 1400, 1360, 1250, 1150, 1020
TLC (R_f) (EtOAc:MeOH:H_2O)	0.3	0.2
Color reaction		
$AgNO_3$-NaOH	Positive	Positive
$KMnO_4$	Positive	Positive
Sakaguchi	Negative	Negative

Table 2. ^{1}H-NMR and ^{13}C-NMR chemical shift assignments of CKD-711 and CKD-711a.

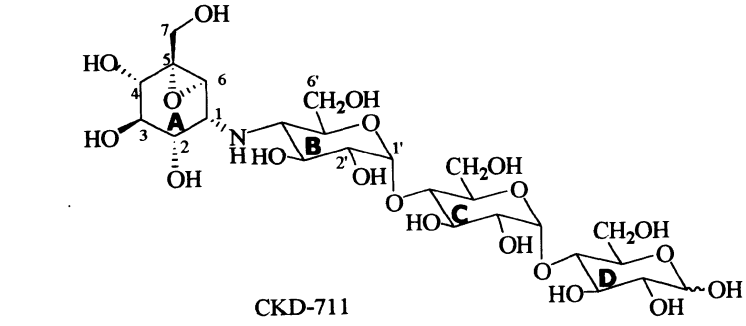
CKD-711				CKD-711a			
Atom	^{13}C	DEPT	^{1}H (δ)	Atom	^{13}C (δ)	DEPT	^{1}H (δ)
A1	56.74	CH	3.46	A1	56.84	CH	3.55
2	73.36	CH	3.41	2	73.60	CH	3.52
3	73.22	CH	3.40	3	73.49	CH	3.51
4	73.86	CH	3.84	4	74.13	CH	3.94
5	68.24	C		5	68.50	C	
6	61.85	CH	3.56	6	62.13	CH	3.65
7	62.96	CH_2	3.54, 3.91	7	63.24	CH_2	3.67, 4.01
B1	102.81	CH	5.29	B1	103.07	CH	5.40
2	75.29	CH	3.51	2	75.55	CH	3.63
3	76.21	CH	3.62	3	76.45	CH	3.74
4	61.98	CH	2.69	4	62.23	CH	2.78
5	75.77	CH	3.65	5	76.03	CH	3.77
6	63.73	CH_2	3.83	6	63.99	CH_2	3.91, 3.94
C1	102.24	CH	5.32	C1	102.72	CH	5.41
2	74.34	CH	3.53	2	74.57	CH	3.66
3	76.07	CH	3.87	3	76.33	CH	3.98
4	79.78	CH	3.56	4	80.02	CH	3.68
5	73.98	CH	3.75	5	74.22	CH	3.86
6	63.24	CH_2	3.76	6	63.45	CH_2	3.82, 3.88
(α)D1	94.67	CH	5.15	D1	102.68	CH	5.41
2	74.05	CH	3.49	2	74.57	CH	3.66
3	75.95	CH	3.87	3	76.33	CH	3.98
4	79.83	CH	3.56	4	80.02	CH	3.68
5	72.70	CH	3.85	5	74.22	CH	3.86
6	63.32	CH_2	3.72	6	63.45	CH_2	3.82, 3.88
(β)D1	98.54	CH	4.57	E1	102.47	CH	5.42
2	76.75	CH	3.20	2	74.50	CH	3.65
3	78.93	CH	3.69	3	76.33	CH	3.98
4	79.70	CH	3.58	4	79.89	CH	3.66
5	77.31	CH	3.51	5	74.20	CH	3.85
6	63.45	CH_2	3.81	6	63.24	CH_2	3.67, 4.01
				(α)F1	94.93	CH	5.26
				2	74.31	CH	3.60
				3	76.22	CH	3.97
				4	80.05	CH	3.68
				5	72.97	CH	3.96
				6	63.49	CH_2	3.84
				(β)F1	98.80	CH	4.68
				2	77.02	CH	3.30
				3	79.19	CH	3.79
				4	79.95	CH	3.68
				5	77.56	CH	3.62
				6	63.71	CH_2	3.78, 3.92

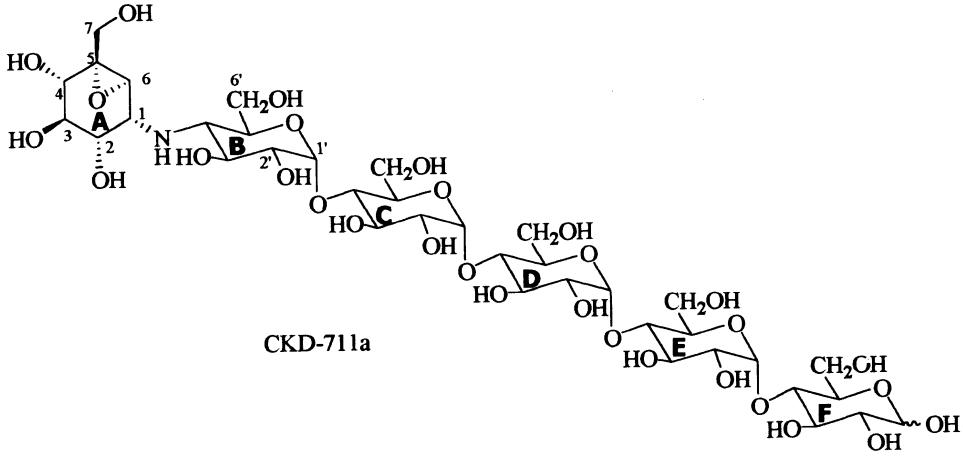
for the core portion of CKD-711/711a.

(A) CKD-711a

The ^{13}C-NMR and DEPT spectra of CKD-711a shows 38 carbon resonances, implying that the terminal glucose unit (ring F in Table 2) exists in two anomeric forms, α and β. Two anomeric proton peaks are observed at 4.68 (β) and 5.26 (α) ppm in the ^{1}H-NMR spectrum. Two corresponding carbon signals are detected at 98.80 (β) and 94.93 (α) ppm, respectively.[10] The presence of α/β-anomers in the NMR spectra is consistent with the HPLC analysis where two anomeric forms of CKD-711a eluted at different retention times of 21.4 (β) and 23.2 (α) minutes. Interestingly, no methyl group is present within the B ring of

Fig. 1. Structure of CKD-711 and CKD-711a.

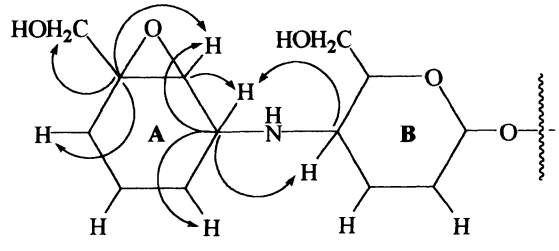




CKD-711/711a, which is present in other α-glucosidase inhibitors such as acarbose[6] and NS-520[11].

Tandem mass spectrum and NMR spectra showed that CKD-711a contains four more glucoses (rings B, C, D and E in Table 2) other than the terminal glucose unit. Furthermore, one-bond coupling constant of $^1J_{CH}=172$ Hz suggests that these five glucose moieties are connected by α-linkage ($^1J_{CH}=172$ Hz) rather than β-linkage[12] ($^1J_{CH}=162$ Hz). The evidence that the glucoses of CKD-711 and CKD-711a are D-form rather than L-form is that both hydrogens at H5 and H4 are axial position according to the high coupling constant of those in the ^{1}H-NMR spectrum. And the other evidence is that both compounds were produced much higher in the medium with maltose or dextrin existing as D-forms in nature than the medium with glucose only. The ring B is connected to the pseudosaccharide cyclitol ring A by a nitrogen atom *via* 4′-CH. The molecular formula showed seven unsaturation equivalents consisting of seven rings including five glucoses with no olefinic double bonds. The presence of an epoxy ring on the cyclitol ring A was confirmed by an IR spectrum at 1250 cm^{-1}. And ^{13}C-NMR, DEPT and HMBC spectra show that the epoxy ring is formed between C-5 and C-6.

Fig. 2. HMBC connectivity for the core portion of CKD-711 and CKD-711a.



(B) CKD-711

The ^{13}C-NMR and DEPT spectra of CKD-711 shows 35 carbon resonances, indicating that CKD-711 also contains a terminal glucose unit (ring D in Table 2), which exists in two anomeric forms; the one at 4.57 ppm (^{1}H)/98.54 ppm (^{13}C) (β form) and the other at 5.15 ppm (^{1}H)/94.67 ppm (^{13}C). In the HPLC analysis two anomers eluted at retention times of 9.7 (β) and 11.3 (α) minutes, respectively.

Tandem mass spectrum and NMR spectra showed that CKD-711 consists of three glucose units including the terminal glucose (ring D), which are connected by α-linkage ($^{1}J_{CH}$=172 Hz) as in the case of CKD-711a. The connectivity pattern between the B ring glucose and the cyclitol ring A is the same as that found in CKD-711a. The molecular formula showed five unsaturation equivalents consisting of five rings including three glucoses with an epoxy ring present in the cyclitol ring A.

The stereochemistry of the epoxy ring in CKD-711/711a was determined using the three-bond coupling constant, $^{3}J_{H1\text{-}H6}$ of 4.2 Hz, in the cyclitol ring A, which suggests that the epoxy ring assumes *cis* configuration rather than *trans*[11] with respect to two hydrogens. The core portion of CKD-711 and CKD-711a is quite similar to that of NS-520[11]. However, the chemical moiety containing the C-6 on the B ring CKD-711/711a is CH_2OH that is different from the CH_3 group present in NS-520.[11] In the case of NS-520 the cyclitol ring is connected by oligosaccharide *via* the C-4 carbon. In contrast, the C-4 carbon in the cyclitol ring A of CKD-711/711a is not connected by glucose or oligosaccharide. The structural similarity of both CKD-711 and NS-520 suggests that the biosynthetic pathway of *m*-C_7N unit of two compounds is similar. The biosynthetic studies of CKD-711 are in progress.

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