NOTES

SNF-4794: New Enzyme Inhibitors Produced by *Chaetomella* sp.

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Cell-cell interactions are very important in biological processes such as immune responses, metastasis of cancer cells, and viral infections[1]. It is well accepted that sugar chains of glycoproteins on the cell surface regulate cell-cell interactions. Since inhibition of glycosidase, which can synthesize and/or hydrolyze those sugar chains, affects cell-cell interactions, glucosidase inhibitors are expected to be useful pharmaceutical drugs.

In the course of screening for new α-glucosidase inhibitors from fungi, new mono sulfate analogues of A32724[2] and M-4854[3] have been isolated, together with known nonsulfate analogues. A32724A and M-4854 that have been isolated as α-glucosidase inhibitors from a eukaryotic fungus, *Chaetomella* sp., have the chemical structures of 5-alkylbenzene-1,3-disulfate derivatives with a hydroxyl and an acetoxyl group in the alkyl chain. However, to date, there is no report on the isolation of the monosulfate analogues of A-32724 and M-4854.

Here, we report the isolation, structural determination and biological activities of new α-glucosidase inhibitors.

The α-glucosidase inhibition screening was performed using yeast α-glucosidase (Funakoshi) and a synthetic substrate, *p*-nitrophenyl-α-D-glucopyranoside (Funakoshi). It was assayed in 750 μl of 0.1 M phosphate buffer (pH 7.0), 100 μl of a glucosidase (3.3 μg/ml), 100 μl of 2 mM substrate and 50 μl of sample (in MeOH). The reaction mixture was incubated at 37°C for 20 minutes in a glass tube or a 96-well microtiter plate (each 1/5 volume). The reaction was terminated on an ice bath and then amounts of liberated *p*-nitrophenol were determined at an absorbance of 410 nm with a photometer (HITACHI U-3210) and a microplate reader NJ-2000 (Inter Med).

The strain SNF-4794 was classified as *Chaetmella* sp. based on microscopy observations. The stock culture of the strain SNF-4794 was inoculated into a 500-ml Erlenmeyer flask containing 70 ml of a medium consisting of 2% glucose, 1% glycerol, 1% lactose, 1% sucrose, 3% soybean flour, 0.8% polypeptone, 0.2% sodium nitrate, 0.1% $MgSO_4 \cdot 7H_2O$, at pH 6.8. Incubation was carried out on a rotary shaker at 27°C for 2 days. Two ml of the seed culture was inoculated into each of one hundred 500-ml Erlenmeyer flasks containing 70 ml of the same medium. The flasks were incubated on a rotary shaker at 27°C for 7 days. The fermentation broth was centrifuged and the obtained supernatant was extracted twice with ethyl acetate. The ethyl acetate layer was concentrated under reduced pressure to remove ethyl acetate. The concentrate was dissolved in $CHCl_3$-MeOH=10:1, applied on a silica gel column (60 g, Kieselgel 60, 100~200 mesh, Merck) and eluted with $CHCl_3$-MeOH=10:1 and MeOH. The active fractions eluted with MeOH were precipitated from MeOH. The precipitate (4.5 g) was applied on a MCI GEL CHP20P column (2 i.d.×40 cm, 100 μm) and eluted with 10%~100% MeOH. The active fraction (450 mg) eluted with 80~100% MeOH was subjected to a preparative HPLC under the following conditions: column, Capcell Pak C_{18} (i.d. 20×250 mm, Shiseido Co.); mobile phase, CH_3CN-H_2O=90:10 (10 ml/minute) for SNF-4794-7, MeOH-H_2O=90:10 (13 ml/minute) for SNF-4794-9, and CH_3CN-10 mM phosphate buffer (pH 7.0)=70:30 (10 ml/minute) for SNF-4794-11 and -12; detection, UV 200 nm. The analytical HPLC was performed as follows: Capcell Pak C_{18} (i.d. 4.6×250 mm), CH_3CN-10 mM phosphate buffer (pH 7.0)=80:20 (1.5 ml/minute). Under these conditions, SNF-4794-12, -11, -9 and -7 were eluted in this order. They were concentrated and then desalted by ethyl acetate extraction to yield 57.0 mg of SNF-4794-7, 29.5 mg of SNF-4794-9, 23.6 mg of SNF-4794-11 and 30.8 mg of SNF-4794-12 as a white amorphous powder.

FAB/MS, FAB/MS^2 and FAB/MS^3 measurements were

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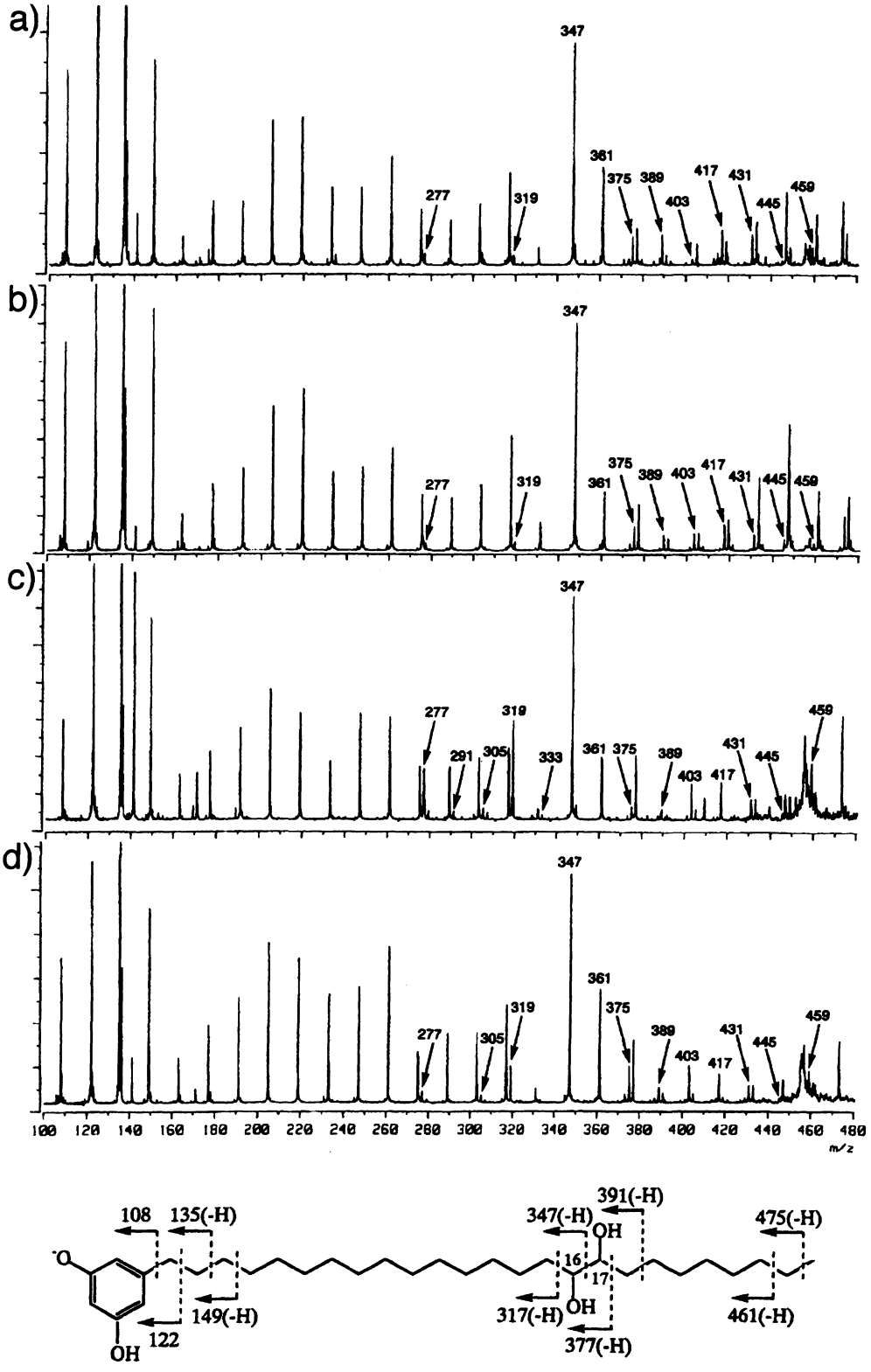
performed using a JEOL JMS-HX/HX110A four-sector (EBEB) tandem mass spectrometer equipped with an array detector. In the FAB/MS^3 experiments, the precursor ion formed in the ion source dissociates in the first field-free region between the ion source and the first electrostatic analyzer to generate the product ion. Then the product ion was scanned manually with a B/E constant (a manual B/E constant-linked scan unit was equipped with the first mass spectrometer) and passed into the collision cell located in the third field-free region between the first magnetic sector and the second electrostatic analyzer. The second-generation product ions formed by collision-induced dissociation (CID) were measured by the second mass spectrometer. Fast atom bombardment (FAB) was carried out using xenon as the primary beam with 6 keV energy and the ion acceleration voltage was 10 kV. High-energy CID was performed by introducing helium as collision gas until the intensity of the precursor ion decreased to 1/3 of the initial value. 3-Nitrobenzyl alcohol was purchased from Tokyo Kasei and used as the matrix without further purification. The ^1H and ^{13}C NMR spectra were measured using a JEOL GSX-500 and AL-400 instruments and the IR spectra were measured using a JASCO IR-810 instrument.

The physico-chemical properties of SNF-4794-7, -9, -11 and -12 were similar. In the obtained IR spectra, a strong absorbance at 1230~1240 cm^{-1} indicated the presence of sulfate residues of SNF-4794-11 and -12, and not those of SNF-4794-7 and -9, while an absorbance at 1670~1730 cm^{-1} indicated the presence of ester residues of SNF-4794-7 and -11, and not those of SNF-4794-9 and -12. The high-resolution FAB/MS $(M-H)^-$ shows the molecular formulae of SNF-4794-7 for $C_{33}H_{57}O_5$ (obs. 533.4201, calc. 533.4206), SNF-4794-9 for $C_{31}H_{55}O_4$ (obs. 491.4145, calc. 491.4120), SNF-4794-11 for $C_{33}H_{57}O_8S$ (obs. 613.3771, calc. 613.3774) and SNF-4794-12 for $C_{31}H_{55}O_7S$ (obs. 571.3668, calc. 571.3668). The ^1H and ^{13}C NMR spectra of SNF-4794-11 and -12 resembl those reported in the literature of the A-32724, alkylbenzene-1,3-disulfate analogue with a vicinal hydroxyl-acetoxyl group on the alkyl chain, except for the benzene-1,3-diol moiety. The benzene-1,3-diol moiety of SNF-4794-11 and -12 was similar to that of panosialin wA~wD[4,5], an alkylbenzene-1,3-diol monosulfate analogue, based on their ^1H and ^{13}C NMR spectra. That is, chemical shifts in ^{13}C NMR and chemical shifts and coupling constants in ^1H NMR of A32724 (disulfate type) and SNF-4794-11, -12 (monosulfate type) are comparable to those of panosialin D (disulfate type) and panosialin wD (monosulfate type). For example, compared to those of A32724, chemical shifts of 1, 3-C and 2, 4, 6-C on the benzene moiety in SNF-4794-11, -12 showed downfield shifts about 7 ppm and upfield shifts about 10 ppm, respectively. Otherwise, chemical shifts of 5-C is not so changed (2.1 ppm downfield shift). Similarly, chemical shifts of 2, 4, 6-H in the benzene moiety showed upfield shifts about 0.9 ppm. Coupling constants of them are not so changed (J=2.2 Hz). The position of the vicinal hydroxyl-acetoxyl or the dihydroxyl group in these compounds was determined from their FAB/MS^2 or MS^3 spectra. The acetoxyl and sulfate groups were easily dissociated under high-energy CID conditions. Therefore, the positions of the vicinal groups were deduced from the FAB/MS^2 spectrum of SNF-4794-9 lacking in such easily dissociated moiety and from the FAB/MS^3 spectra of SNF-4794-7 and -12 with the acetoxyl or the sulfate group. Moreover, the position of the vicinal groups in SNF-4794-11 with both the acetoxyl and sulfate groups was deduced from the FAB/MS^3 spectrum of its corresponding deacetylated derivative. Figure 1 shows FAB/MS^2 or MS^3 spectra, which indicate a charge-remote fragmentation pattern, of SNF-4794-7, -9, -11 and -12. Each position of the vicinal dihydroxyl or the hydroxyl-acetoxyl group in SNF-4794-7, -9, -11 and -12 was determined to correspond to that of C16-C17. In addition, the presence of the positional isomers as the minor components was shown by detailed analyses of the minor peaks in their FAB/MS^2 or MS^3 spectra. For example, in the MS^2 spectrum of SNF-4794-9 (Fig. 1(b)), the intense peak at m/z 347, which was derived from the main component, is assigned as the peak cleaved between the vicinal dihydroxyl groups. This shows that the vicinal dihydroxyl group in the main component is substituted at 16th and 17th carbons on the alkyl chain. In addition, the small peaks from m/z 277 to 459, which are indicated in the figure, are also assigned as the peaks cleaved between the vicinal dihydroxyl groups of the positionally isomeric minor components. Thus, the vicinal dihydroxyl groups of the minor isomers are distributed broadly from 11~12th carbons to 24~25th carbons. The oxidative cleavage of the vicinal dihydroxyl group with $NAIO_4$ followed by FAB/MS^2 measurements confirmed the presence of the minor positional isomers (data not shown). Figure 2 shows the structures of the main constituents of SNF-4794-7, -9, -11 and -12. Among these compounds, SNF-4794-11 and -12 are new monosulfate analogues of A32724 and probably M-4854.

The IC_{50} values of the compounds against α-glucosidase activity are 0.18 (SNF-4794-7), 1.20 (SNF-4794-9), 0.09 (SNF-4794-11), and 0.11 (SNF-4794-12) μg/ml. Among them, the monosulfate analogues exhibited higher inhibition activity than the nonsulfate analogues. It is very interesting to determine the role of the sulfate group

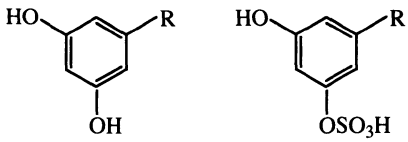
Fig. 1.



Negative FAB/MS^2 or MS^3 spectra of SNF-4794 and the assignment of the main constituent of the following common deacetylated and desulfated forms (*m/z* 491):

a) SNF-4794-7: MS^3 (*m/z* 533→491→)
b) SNF-4794-9: MS^2 (*m/z* 491→)
c) SNF-4794-11 (deacetylated): MS^3 (*m/z* 571→491→)
d) SNF-4794-12: MS^3 (*m/z* 571→491→)

Fig. 2. Structures of the main constituents of SNF-4794-7, -9, -11, and -12.



R=-$(CH_2)_{15}$-CH(X)-CH(Y)-$(CH_2)_7$-CH_3

SNF-4794-	M. F.	X	Y
7	$C_{33}H_{58}O_5$	OH(OAc)	OAc(OH)
9	$C_{31}H_{56}O_4$	OH	OH
11	$C_{33}H_{58}O_8S$	OH(OAc)	OAc(OH)
12	$C_{31}H_{56}O_7S$	OH	OH

in the interaction between the inhibitor and the enzyme.

References

1) VARKI, A.: Biological roles of oligosaccharides: all of the theories are correct. Glycobiology 3: 97~130, 1993
2) BERG, D. H. & M. M. HOEHN (Eli Lilly and Company): A-32724 antibiotics and process for production thereof. U.S. Patent 4375462, March 1, 1983
3) YAGINUMA, S.: Inhibition of cephalosporin β-lactamase by M4854-I and M4854-II. J. Antibiotics 33: 337~341, 1980
4) DOLAK, L. A.; E. P. SEEST, J. I. CIALDELLA, G. P. LI & M. J. BOHANON (The Upjohn Company): Compounds used for the inhibition of HIV-protease. PCT WO 93/04055, March 4, 1993
5) YAMADA, H.; K. SHIOMI, QI. XU, T. NAGAI, M. SHIBATA, I. OYA, Y. TAKAHASHI & S. OMURA: New glycosidases inhibitors, panosialin D and wD produced by *Streptomyces* sp. OH-5186. J. Antibiotics 48: 205~210, 1995