—Articles—

Evaluation on Biological Activity of Highly Functionalized Synthetic Cycloalkenoids; Trichodenone and Pericosine Analogues

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α-Glucosidase inhibitory activity of highly functionalized synthesized cycloalkenoides, which are trichodenones and pericosine analogues, was evaluated by the PNP (*para*-nitrophenol) procedure along with *in vitro* cytotoxic activity against murine P-388 cell line. It was found that (–)-trichodenone A, (+)-dechlorinated trichodenone B, and methyl (1*R*,2*S*,3*S*,4*S*,5*R*)-1,2,3,4,5-pentahydroxycyclohexane-1-carboxylate showed significant α-glucosidase inhibitory activity.

Key words——carbasugar; synthesis; cytotoxicity; α-glucosidase inhibitor; tricodenone; pericosine analogue

INTRODUCTION

Carbasugars are in the group of organic compounds in which an oxygen atom in the hexose or pentose ring is substituted by a carbon atom.[1] To date a lot of important carbasugars were isolated from the natural substances. For example, a potent α-glucosidase inhibitor acarbose was isolated as a fungal metabolite of *Actinoplanes* strain SE 50/110.[2] And well known antiinfluenza drugs, Tamiflu®[3] and Relenza®[4] are synthetic carbasugars. Since they are expected to exhibit some biological activities such as antitumor, antiviral, antifungal, or glucosidase inhibitory activities, various studies on carbasugars have still continued in the natural product chemistry and synthetic organic chemistry.[5] In the course of searching antitumor natural or synthetic organic compounds, we have studied syntheses of highly functionalized natural cycloalkenonid,[6] trichodenones A–C[7-9] (**1**–**3**) and pericosines A–D[11-20] (**4**–**7**) in order to determine the relative or the absolute configurations. These unique natural substances were isolated as cytotoxic fungal metabolites from the marine sources and their structures are presented in Fig. 1. More noteworthy is that natural **1**, **5**, and **6** exist as enantiomeric mixtures.[11,21] As described above, these synthesized compounds are the kinds of carbasugars with some biological activities. Here we report the cytotoxicity against murine P-388 cell line and α-glucosidase inhibitory activity of the compounds synthesized in our laboratory before 2005[13,14] (Fig.2).

RESULTS AND DISCUSSION

Cytotoxicity against murine P-388 lymphocytic leukemia cell line was evaluated about the compounds **1**–**3**, **6**, **10**, **11** which appeared in the previous literatures using 5-fluorouracil (5-FU) as a positive control.[22] Results were summarized in Table 1. All of the tested compounds showed significant cytotoxicity. It is

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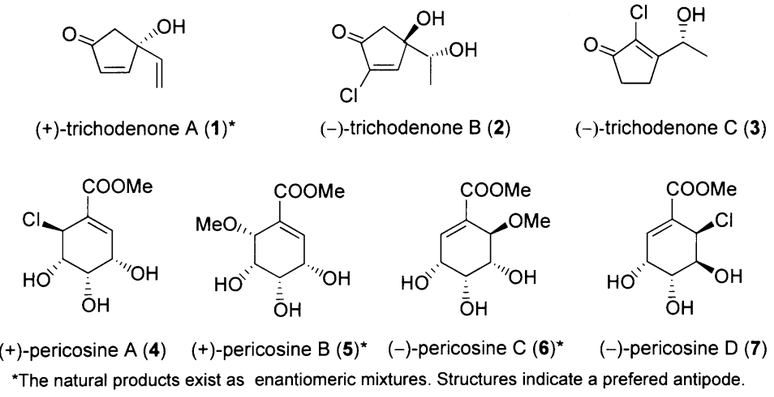

Fig. 1. Structures of natural trichodenones and pericosines

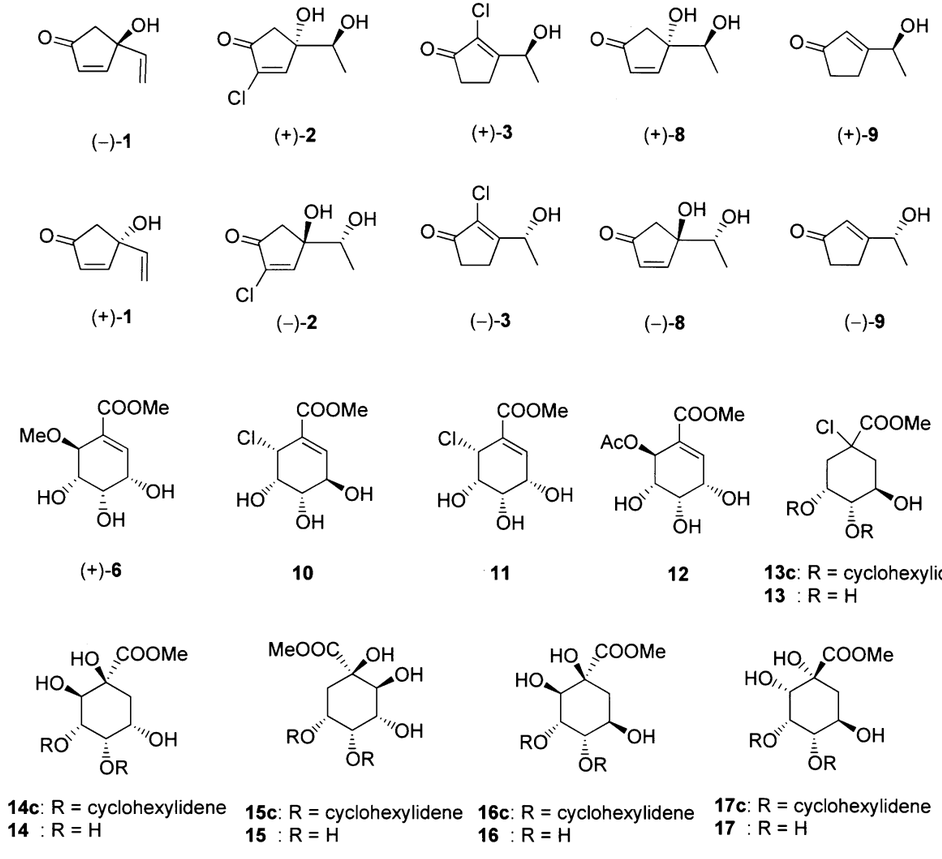

Fig. 2. Structures of tested compounds

Table 1. Cytotoxicity against murine P388 cell lline

Compound	ED_{50} (μg/ml)
(+)-**1**	0.23
(−)-**1**	0.21
natural **1**	0.22[a]
(+)-**2**	1.46
(−)-**2**	1.21[a]
(+)-**3**	14.0
(−)-**3**	1.45[a]
(+)-**6**	17.8
(−)-**10**	30.0
(+)-**11**	33.0
5-FU	0.06

a. reference 7

interesting that both of the enantiomers of **1** showed almost the same potency although **1** exists as an enantiomeric mixture in nature. Similarly the ED_{50} values of both of the enantiomers of **2** were almost the same. In case of **3**, difference between each enantiomer was observed and the naturally occurring enantiomer (−)-**3** showed more potent activity. All of the three tested pericosine analogues **6**, **10**, and **11** showed weak activity compared to the natural **1**, **2** and **3**.

As mentioned above, carbasugars are expected to exhibit glycosidase inhibitory activity. α-Glucosidase inhibition by the PNP (*para*-nitrophenol) procedure[23] was examined against tricodenones and pericosine analogues with the five new highly oxygenated carbohydrates **13**–**17** derived from the known compounds **13c**–**17c**, which are the corresponding cyclohexylidene derivatives,[13,14,24] respectively. *p*-Nitrophenyl-α-D-glucopyranoside was used as the substrate of the enzymatic assay. Compounds **13**–**17** exist in chair conformation based on the ^{1}H-NMR data and they were attempted to imitate the hexopyranose conformation. Chair conformation of **17** was shown in Fig. 3. On the other hand, **6** and **10**–**12** were attempted to imitate the oxocarbenium ion like the transition structure in the cleavage of sugar-sugar linkage.[1] Results of preliminary inhibitory rate assay and IC_{50} determination were summarized in Table 2. 1-Deoxynojirimycin (DNJ), a well-known potent α-glucosidase inhibitor, was appeared in Fig. 3 and Table 2 as the positive control of this enzyme inhibitory assay.

Among the tested compounds, (−)-**1**, (+)-**8**, and **17** exhibited the significant inhibitory rate over 50%. It is interesting that great difference of potency of each enantiomer in trichodenones was observed whereas there wasn't any cytotoxic activity. These three

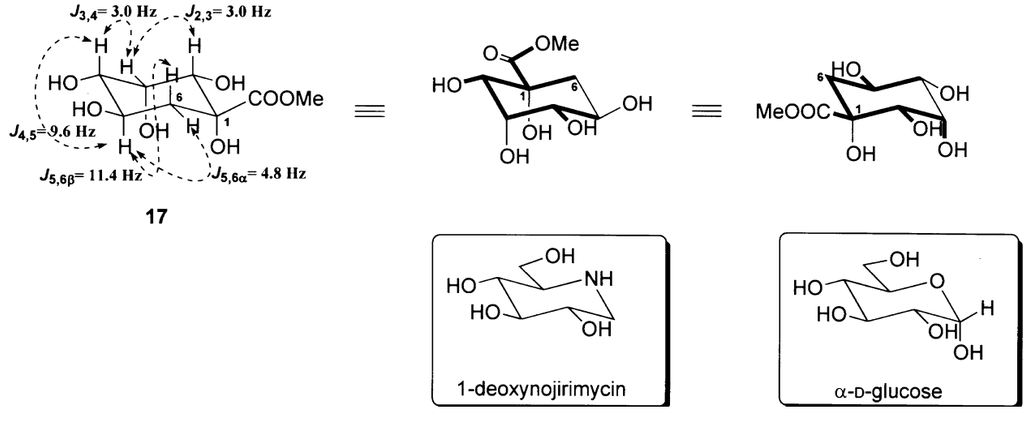

Fig. 3. Conformation of **17** and reference compounds

Table 2. α-glucosidase inhibitory activity

Compound	IC_{50} (M)	Concentration[b] (μg/ml)	
(+)-**1**	NI[a]	0.4	(H_2O)
(−)-**1**	3.8 x 10^{-3}	2.19	(H_2O)
(+)-**2**	NI	2.11	(DMSO)
(−)-**2**	NI	2.57	(DMSO)
(+)-**3**	NI	2.00	(H_2O)
(−)-**3**	NI	2.15	(H_2O)
(+)-**8**	3.0 x 10^{-3}	2.76	(DMSO)
(−)-**8**	NI	0.2	(DMSO)
(+)-**9**	NI	2.15	(H_2O)
(−)-**9**	NI	2.02	(H_2O)
(+)-**6**	NI	2.15	(H_2O)
(−)-**10**	NI	2.79	(H_2O)
(+)-**11**	NI	2.1	(H_2O)
(+)-**12**	NI	3.2	(H_2O)
(−)-**13**	NI	2.79	(H_2O)
(−)-**14**	NI	3.44	(H_2O)
(−)-**15**	NI	1.9	(H_2O)
(−)-**16**	NI	3.44	(H_2O)
(−)-**17**	9.0 x 10^{-4}	2.40	(H_2O)
DNJ	9.3 x 10^{-4}		

a. NI: No inhibition means the inhibitory rate was below 50% in the preliminary assay. b. Concentration of the tested compounds in the preliminary assay.

candidates were examined further in order to determine their IC_{50} values. The values were 3.8 x 10^{-3}, 3.0 x 10^{-3}, and 9.0 x 10^{-4} M for (–)-**1**, (+)-**8**, and **17**, respectively as seen in Table 2. Compound **17** had comparable activity as 1-DNJ.

CONCLUSION

We evaluated cytotoxic activity aginst P-388 cell line for synthetic trichodenones and the pericosine analogues and found that all the tested trichodenones showed significant activity whatever the chiralities were. And the pericosine analogues showed weak activities. On the other hand, synthesized (–)-trichodenone A (**1**), (+)-dechlorinated trichodenone B (**8**) showed potent α-glucosidase inhibitory activity and between each enantiomers the significant difference of potency was observed. Of all tested componds, methyl (1*R*,2*S*,3*S*,4*S*,5*R*)-1,2,3,4,5-pentahydroxycyclohexane-1-carboxylate (**17**) showed the remarkable α-glucosidase inhibitory activity almost as potent as 1-DNJ.

EXPERIMENTAL

IR spectra were obtained with a Perkin Elmer FT-IR spectrometer 1720X. HRMS was determined with a JEOL JMS-700 (2) or a Hitachi 4000H mass spectrometer. NMR spectra were recorded at 27˚C on Varian UNITY INOVA-500 and Mercury-300 spectrometers in $CDCl_3$ with tetramethylsilane (TMS) as internal standard. Specific rotations were measured on a JASCO ORD/UV-5 spectropolarimeter and $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. Liquid column chromatography was conducted over silica gel (Nacalai, silica gel 60, mesh 70–230 or 230–400). Analytical TLC was performed on precoated Merck glass plates (silica gel 60 F_{254}) and compounds were detected by dipping the plates in an ethanol solution of phosphomolybdic acid, followed by heating. Dry THF was distilled over sodium benzophenone ketyl under nitrogen atmosphere.

5-Fluorouracil was obtained from Tokyo Chemical Industry Co. Ltd. (TCI, Tokyo, Japan). MTT for the *in vitro* cytotoxicity assay was obtained from Aldrich Chemical Company (St. Louis, U.S.A.). α-Glucosidase (from Bakers yeast, lot 83H8000) was also obtained from Sigma Chemical Company (St. Louis, U.S.A.), *p*-nitrophenyl-α-D-glucopyranoside from Nacalai Tesque, Inc. (Osaka, Japan), and 1-deoxynojirimycin from Funakoshi Company (Tokyo, Japan).

Synthesis of new carbasugars

Methyl 3β,4α,5α-1-chloro-3,4,5-trihydroxycyclohexane-1-carboxylate 13

Chloride **13c** (116.4 mg) was dissolved in MeOH (2.5 ml) and TFA (2.5 ml, excess). After stirring overnight at 70˚C, the reaction mixture was allowed to return to room temperature and condensed under the reduced pressure to afford a crude residue that was purified by silica gel column chromatography (eluent: 10% MeOH in CH_2Cl_2) to give **13** (41.1 mg, 66%). **13**: Oil; $[\alpha]_D^{26}$ –40.2 (c 1.3, EtOH); IR (liquid film) ν_{max} 3392 (OH), 1737 (C=O) cm^{-1}; ^{1}H-NMR (CD_3OD) δ 1.88 (1H, dd, *J* = 12.8, 10.8 Hz, H-2β), 2.06 (1H, dd, *J* = 13.7, 2.3 Hz, H-6β), 2.79 (1H, ddd, *J* = 12.8, 4.6, 3.2 Hz, H-2α), 2.86 (1H, ddd, *J* = 13.7, 4.3, 3.2 Hz, H-6α), 3.37 (1H, dd, *J* = 9.2, 3.2 Hz, H-4), 3.73 (3H, s, COOMe), 3.90 (1H, ddd, *J* = 10.8, 9.2, 4.6 Hz, H-3), 4.01 (1H, ddd, *J* = 4.3, 3.2, 2.3 Hz, H-5); ^{13}C-NMR (CD_3OD) δ 43.7 (t), 44.9 (t), 53.2 (q), 65.3 (s), 68.8 (d), 70.5 (d), 76.7 (d), 174.6 (s); HRMS *m/z* calcd for $C_8H_{14}O_5Cl$ $(M+H)^+$ 225.0529, found 225.0532.

Methyl (1*S*,2*R*,3*S*,4*S*,5*S*)-1,2,3,4,5-pentahydroxycyclohexane-1-carboxylate 14

Using the same procedure described above, triol **14c** (47.2 mg) was converted into **14** (25.4 mg, 74%). **14**: Oil; $[\alpha]_D^{26}$ –37.7 (c 1.7, MeOH); IR (liquid film) ν_{max} 3390 (OH), 1735 (C=O) cm^{-1}; ^{1}H-NMR (acetone-d_6) δ 1.78 (1H, ddd, *J* = 12.8, 4.8, 1.4 Hz, H-6_A), 2.22 (1H, dd, *J* = 12.8, 12.1 Hz, H-6_B), 3.68 (1H, dd, *J* = 9.8, 2.7 Hz, H-3), 3.74 (3H, s, COOMe), 3.95 (1H, ddd, *J* = 12.8, 4.8, 2.7 Hz, H-5), 4.05 (1H, d, *J* = 9.8 Hz, H-2), 4.08 (1H, td, *J* = 2.7, 1.4 Hz, H-4); ^{13}C-NMR (acetone-d_6) δ 36.7 (t), 52.8 (q), 67.2 (d), 72.2 (d), 73.1 (d), 74.3 (d), 77.5 (s), 175.1 (s); HRMS *m/z* calcd for $C_8H_{15}O_7$ $(M+H)^+$ 223.0817, found 223.0811.

Methyl (1*R*,2*S*,3*R*,4*R*,5*R*)-1,2,3,4,5-pentahydroxycyclohexane-1-carboxylate 15

Using the same procedure described above, triol **15c** (61.5 mg) was converted into **15** (32.1 mg, 73%). **15**: Oil; $[\alpha]_D^{25}$ –27.2 (c 1.7, MeOH); IR (liquid film) ν_{max} 3362 (OH), 1729 (C=O) cm^{-1}; ^{1}H-NMR (acetone-d_6) δ 1.74 (1H, ddd, *J* = 12.8, 4.6, 1.2 Hz, H-6_A), 2.20 (1H, dd, *J* = 12.8, 11.9 Hz, H-6_B), 3.62 (1H, dd, *J* = 9.6, 2.8 Hz, H-4), 3.74 (3H, s, COOMe), 3.92 (1H, ddd, *J* = 11.9, 4.6, 2.8 Hz, H-5), 4.00 (1H, d, *J* = 2.5 Hz, H-2), 4.01 (1H, m, *J* = 2.7, 1.4 Hz, H-3); ^{13}C-NMR (acetone-d_6) δ 37.0 (t), 52.6 (q), 67.4 (d), 72.4 (d), 73.3 (d), 74.2 (d), 77.4 (s), 175.5 (s); HRMS *m/z* calcd for $C_8H_{15}O_7$ $(M+H)^+$ 223.0817, found 223.0816.

Methyl (1*S*,2*R*,3*S*,4*S*,5*R*)-1,2,3,4,5-pentahydroxycyclohexane-1-carboxylate 16

Triol **16c** (41.6 mg) was treated in similar condition as

described above at 60˚C to afford **16** (25.5 mg, 85%). **16**: Oil; $[\alpha]_D^{25}$ –94.9 (c 1.4, MeOH); IR (liquid film) ν_{max} 3390 (OH), 1733 (C=O) cm^{-1}; ^{1}H-NMR (acetone-d_6) δ 1.87 (1H, ddd, *J* = 14.4, 3.0, 1.4 Hz, H-6_A), 2.33 (1H, dd, *J* = 14.4, 3.4 Hz, H-6_B), 3.74 (3H, s, COOMe), 3.96–3.99 (2H, m, H-3, H-5), 4.04 (1H, td, *J* = 3.0, 1.4 Hz, H-4), 4.09 (1H, d, *J* = 9.6 Hz, H-2); ^{13}C-NMR (acetone-d_6) δ 33.8 (t), 52.9 (q), 69.9 (d), 71.1 (d), 73.2 (d), 74.1 (d), 80.1 (s), 174.9 (s); HRMS *m/z* calcd for $C_8H_{15}O_7$ $(M+H)^+$ 223.0817, found 223.0815.

Methyl (1*R*,2*S*,3*S*,4*S*,5*R*)-1,2,3,4,5-pentahydroxycyclohexane-1-carboxylate 17

Using the same procedure described above, triol **17c** (47.7 mg) was transformed into **17** (20.7 mg, 60%). The reaction was completed in 2 hours. **17**: Oil; $[\alpha]_D^{26}$ –18.6 (c 1.4, MeOH); IR (liquid film) ν_{max} 3389 (OH), 1735 (C=O) cm^{-1}; ^{1}H-NMR (acetone-d_6) δ 1.72 (1H, dd, *J* = 13.5, 11.4 Hz, H-6_A), 2.04 (1H, dd, *J* = 13.5, 4.8 Hz, H-6_B), 3.39 (1H, dd, *J* = 9.6, 3.0 Hz, H-4), 3.73 (3H, s, COOMe), 3.93 (1H, d, *J* = 3.0 Hz, H-2), 3.95 (1H, ddd, *J* = 11.4, 9.6, 4.8 Hz, H-5), 4.04 (1H, br t, *J* = 3.0 Hz, H-3); ^{13}C-NMR (acetone-d_6) δ39.9 (t), 52.8 (q), 67.1 (d), 71.9 (d), 75.6 (d), 76.2 (d), 80.2 (s), 174.7 (s); HRMS *m/z* calcd for $C_8H_{15}O_7$ $(M+H)^+$ 223.0817, found 223.0815.

Biological assays of synthesized compounds

In vitro cytotoxic activity against murine P-388 lymphocytic leukemia cell line and α-glucosidase inhibitory assay were evaluated along the literatures.[22,23]

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