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Anti-adipogenic Effect of Taurine-Carbohydrate Derivatives

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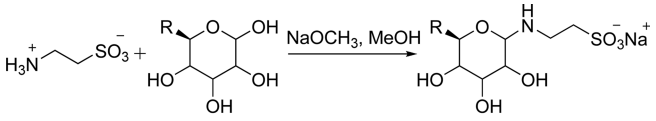
2-Aminoethanesulfonic acid, commonly known as taurine, is a β-amino acid.[1] It plays important roles in many physiological processes such as neuromodulation, osmoregulation, immune response, inflammatory response, brain development, retinal function, cell membrane stabilization, antioxidation, and detoxification.[2] Taurine has also attracted attention because of its effects on adult diseases such as diabetes, arteriosclerosis, hypertension, and heart failure.[3] However, regardless of its profound beneficial effects as a therapeutic agent, taurine has some disadvantages as well, such as poor absorption, unfavorable pharmacokinetics, high-dose requirement, and fast rate of extraction through urine. Therefore, the taurine framework needs to be modified for overcoming these disadvantages.

Numerous taurine derivatives have been reported in the literature, and some such as taltrimide, acamprosate, and tauromustine are commercially available.[4] Although many studies on the synthesis of taurine derivatives have been reported, only few describe the synthesis of taurine-carbohydrate derivatives. Heyns *et al.* were the first to report the synthesis of a taurine-carbohydrate derivative, 1-deoxy-1-(2-sulfoethylamino)-D-fructose.[5] The synthesis of 2-[[2-(D-glucopyranosyloxy)ethyl]amino]ethansulfonic acid and 4-nitrophenyl-6-deoxy-6-[(2-sulfoethyl)amino]-β-D-galactopyranoside was reported by Weingarten and Thiem.[6] Recently, a taurine-glucose derivative was synthesized by transglycation of glucose-ethylamine with taurine in an NMR tube.[7]

As a part of the systematic approach for the synthesis of new and effective taurine-carbohydrate derivatives, we prepared several taurine-aldopentose and taurine-aldohexose derivatives in a simple and efficient manner with the aim of 1) enhancing the absorption rate using carbohydrate transporters such as glucose transporter proteins and ribose transporter proteins;[8] and 2) improving the liposolubility and physiological activities of the taurine-carbohydrate derivatives.

The condensation was carried out under mild reaction conditions in a simple manner without using any protecting group. Because methanol showed a good solubility for the reactants and a poor solubility for the products, it drove the reaction to completion (Scheme 1). When the reaction temperature was increased to accelerate the reaction rate, the amount of brown side products also increased because of the Maillard reaction.[9] In the case of aldopentoses, the reactions proceeded well at a lower temperature because of their higher reactivity.[10] Therefore, the products from aldopentoses such as D-xylose, D-arabinose, D-ribose, and D-lyxose were obtained in pure form, *i.e.*, without any trace of Maillard products. In some cases, the products were purified by adding absolute ethanol to reduce the effect of water formed during the reaction and to precipitate more products from the solution by reducing the polarity of the solvent. In case of the products from xylose and arabinose, they were filtered immediately without addition of absolute ethanol since the products were obtained as crystals in the course of the reaction. In the discussion that follows, the terms αP, βP, αF, and βF designate α-pyranose, β-pyranose, α-furanose, and β-furanose structures, respectively.

The chemical structures of the taurine-carbohydrate derivatives were identified by NMR spectroscopic methods. The structure and conformation of the taurine-glucose (T-Glu) derivative were confirmed to be a β-pyranoside with the $^4C_1$ conformation in which the anomeric carbon is attached to the taurine, and the coupling constant between the H-1 and H-2 protons was 8.42 Hz. Thus, *N*-(β-D-glucopyranosyl)taurine salt was obtained from the condensation of taurine and D-glucose. The coupling constant between the H-1 and H-2 protons of the taurine-galactose (T-Gal) and taurine-xylose (T-Xyl) derivatives was 8.37 and 8.27 Hz, respectively, and thus, they seemed to have the $^4C_1$ confor-



Carbohydrate	*Compounds*	*R*
D-Glucose (**1**)	**1**βP	-CH_2OH
D-Galactose (**2**)	**2**βP	-CH_2OH
D-Xylose (**3**)	**3**βP	-H
D-Arabinose (**4**)	**4**βP	-H
D-Ribose (**5**)	**5**αP + **5**βP	-H
D-Lyxose (**6**)	**6**αP + **6**βP	-H

Scheme 1. Synthesis of taurine-carbohydrate derivatives.

mation, which is similar to that of the T-Glu derivative.[11] However, taurine-ribose (T-Rib) and taurine-lyxose (T-Lyx) derivatives were obtained as a mixture of two major products in nearly equal amounts; therefore, it was difficult to analyze the correct structure by NMR spectroscopic methods. Their structures were compared with the literature data. In the literature, the products from the direct condensation of primary amines and D-ribose mainly consisted of an αP structure with the $^1C_4$ conformation and a βP structure with the $^4C_1$ conformation.[12] Therefore, it may be concluded that the structures of the T-Rib and T-Lyx derivatives are a mixture of αP structure with the $^1C_4$ conformation and βP structure with the $^4C_1$ conformation.

Obesity is a modern lifestyle-related disease, and since the last decade, has become a global problem.[13] Cellular experiments were carried out to investigate the effectiveness of the taurine-carbohydrate derivatives synthesized in the present study on the prevention and treatment of this disease. Obesity is caused by the accumulation of excess fat of the body in the adipose tissue, which increases the number and volume of adipocytes.[14] Thus, we made human preadipocytes differentiate into adipocytes in the presence of taurine-carbohydrate derivatives for 14 days and investigated the anti-adipogenesis effect of these derivatives by oil red O staining. We found that the OD values of the taurine-treated (40 μg/mL), xylose-treated (40 μg/mL), and lyxose-treated (40 μg/mL) cells appeared at similar levels, whereas those of the ribose-treated (40 μg/mL) cells were expressed at a

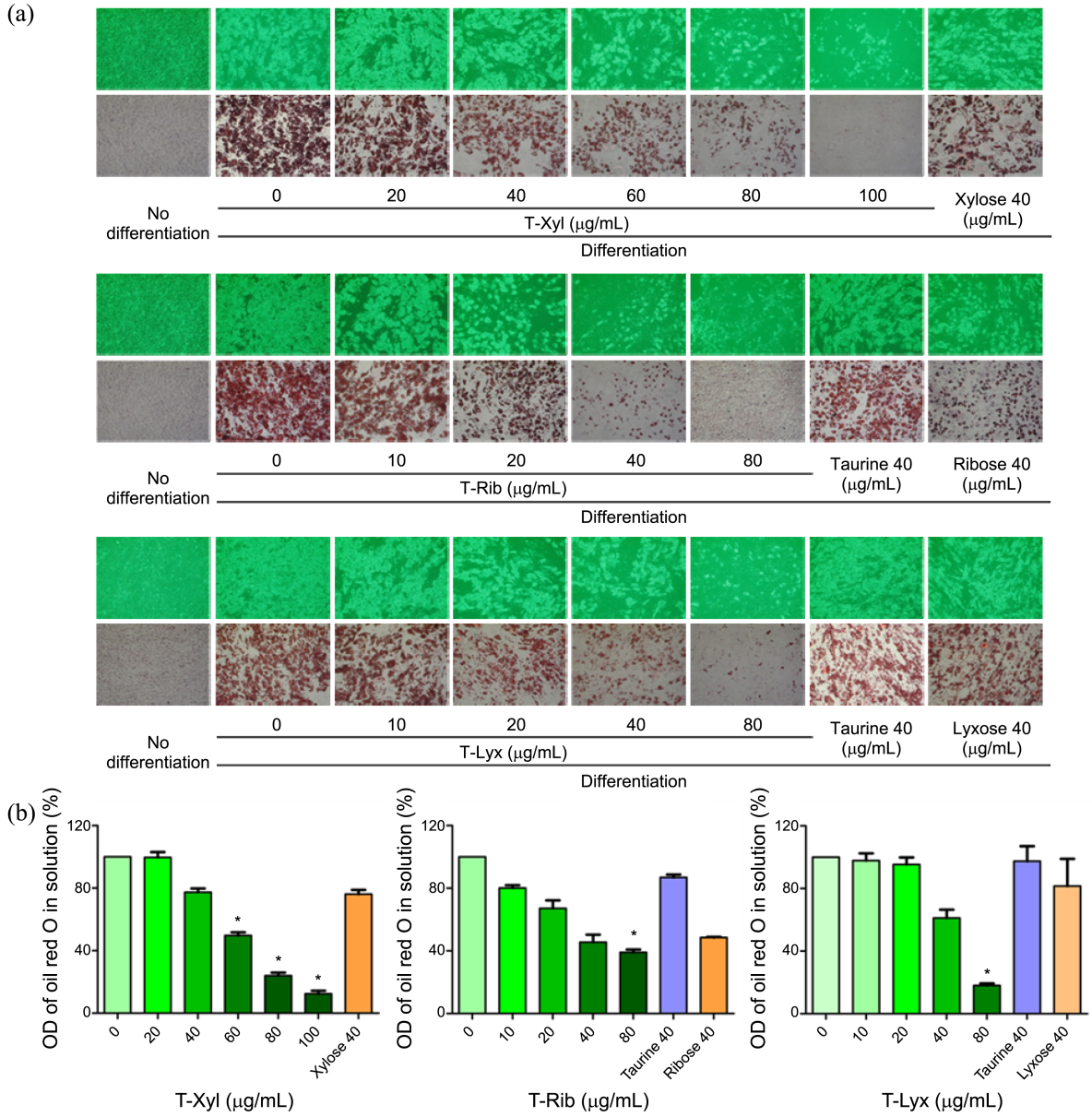


Figure 1. Effect of the T-Xyl, T-Rib, and T-Lyx derivatives on human adipocyte differentiation. Scale bar = 100 μm. Lipid accumulation was examined by oil red O staining. (a) Microscopic image of differentiated adipocytes before (top row) and after (bottom row) oil red O staining. (b) Optical absorbance at 500 nm of dye retained in adipocytes. Three independent experiments were performed. Values are expressed as mean ± SEM*: $P < 0.05$ *vs.* control.

Table 1. Reaction conditions for the synthesis of taurine-carbohydrate derivatives

Carbohydrate	Solvent (mL)[a]	Time (h)	Temp. (°C)	Purification method
D-Glucose	40	24	43	addition of absolute ethanol and filtration
D-Galactose	100	23	45	removal of half of the solvent, addition of absolute ethanol, and filtration
D-Xylose	33	48	15	filtration
D-Arabinose	40	48	15	filtration
D-Ribose	33	48	15	addition of absolute ethanol and filtration
D-Lyxose	25	24	15	addition of absolute ethanol and filtration

[a]Amount of solvent per 10 mmol carbohydrate

slightly low level compared to the control experiments. On the other hand, the OD values of the T-Xyl-treated, T-Rib-treated, and T-Lyx-treated cells appeared at low levels depending on their concentrations. Furthermore, based on the microscopic image of the differentiated adipocytes in the presence of the T-Xyl-treated, T-Rib-treated, and T-Lyx-treated cells, we confirmed that the adipocytes were significantly reduced (Figure 1). In other words, T-Xyl, T-Rib, and T-Lyx derivatives significantly inhibited the differentiation of adipocytes. In contrast, T-Glu, T-Gal, and T-Ara derivatives did not inhibit the differentiation.

The T-Xyl, T-Rib, and T-Lyx derivatives exhibited good anti-adipogenesis effect in the cellular experiments. Further *in vivo* studies on the anti-obesity effects using animal model, transportation mechanism, and other biological effects of these derivatives are under investigation.

Experimental

Condensation of Carbohydrates with Taurine. Sodium methoxide (28% in methanol, 2.23 g, 11.6 mmol) was added to taurine (1.38 g, 11.0 mmol) in methanol (20 mL) and sonicated for a few minutes. To the resulting solution, D-glucose (1.80 g, 10.0 mmol) in methanol (20 mL) was added, sonicated for a few minutes, and stirred at 43 °C for 24 h in an oil bath. After the reaction completion, absolute ethanol (approximately 40 mL) was added dropwise at 0 °C with vigorous stirring until the precipitation stopped. The precipitate was filtered, washed with absolute ethanol, and dried in a vacuum desiccator. The reactions of other carbohydrates (D-galactose, D-xylose, D-arabinose, D-ribose, and D-lyxose) with taurine were also carried out in the similar manner. The reaction conditions and purification methods are listed in Table 1.

***N*-(β-D-Glucopyranosyl)taurine Sodium Salt (1βP):** Yield: 80%; hygroscopic yellow powder, mp 76.0–91.1 °C (dec. 69.3 °C), $[\alpha]_D^{29} = -7.5°$ (c = 1.0, H_2O), ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.61 (t, J_1 = 6.4 Hz, J_2 = 6.6 Hz, 2H, SCH_2), 2.73–2.79 (m, 1H, NCH_2), 2.84 (td, J_1 = 3.2 Hz, J_2 = 8.6 Hz, 1H, H-2), 3.02 (br, 2H, H-4, H-5), 3.06–3.12 (m, 2H, NCH_2, H-3), 3.41–3.45 (m, 1H, H-6b), 3.62–3.66 (m, 2H, H-6a, H-1), 4.12 (q, J = 5.1 Hz, 1H, NH), 4.48 (t, J = 5.7 Hz, 1H, 6-OH), 4.65 (d, J = 3.7 Hz, 1H, 2-OH), 4.82 (br, 1H, 4-OH), 4.87 (d, J = 4.1 Hz, 1H, 3-OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 41.9 (NCH_2), 51.8 (SCH_2), 61.3 (C-6), 70.4 (C-5), 73.5 (C-2), 77.6 (C-3), 77.7 (C-4), 90.5 (C-1).

***N*-(β-D-Galactopyranosyl)taurine Sodium Salt (2βP):** Yield: 80%; hygroscopic yellow powder, mp 64.8–78.0 °C (dec. 66.9 °C), $[\alpha]_D^{31.8} = +5°$ (c = 1.0, H_2O), ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.61 (t, J_1 = 7.0 Hz, J_2 = 7.1 Hz, 2H, SCH_2), 2.72–2.80 (m, 1H, NCH_2), 3.04–3.12 (m, 1H, NCH_2), 3.17 (td, J_1 = 3.3 Hz, 1H, H-2), 3.23–3.28 (m, 2H, H-3, H-5), 3.41–3.46 (m, 1H, H-6b), 3.48–3.53 (m, 1H, H-6a), 3.58–3.63 (m, 2H, H-1, H-4), 4.12 (q, J = 5.2 Hz, 1H, NH), 4.27 (d, J = 4.6 Hz, 1H, 4-OH), 4.48 (d, J = 3.6 Hz, 1H, 2-OH), 4.55 (t, J = 5.5 Hz, 1H, 6-OH), 4.64 (d, J = 5.3 Hz, 1H, 3-OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 42.0 (NCH_2), 52.0 (SCH_2), 60.6 (C-6), 68.5 (C-4), 70.8 (C-2), 74.2 (C-3), 75.9 (C-5), 91.2 (C-1).

***N*-(β-D-Xylopyranosyl)taurine Sodium Salt (3βP):** Yield: 84%; hygroscopic white crystal, mp 131.8–132.8 °C (dec. 99.8 °C), $[\alpha]_D^{29.7} = -27.5°$ (c = 1.0, H_2O), ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.56 (t, J_1 = 6.7 Hz, J_2 = 6.6 Hz, 2H, SCH_2), 2.65–2.68 (m, 1H, NH), 2.71–2.77 (m, 1H, NCH_2), 2.82 (td, J_1 = 4.1 Hz, J_2 = 8.6 Hz, 1H, H-2), 2.96 (t, J = 10.8 Hz, 1H, H-5a), 2.97–3.03 (m, 1H, NCH_2), 3.06 (td, J_1 = 4.5 Hz, J_2 = 8.7 Hz, 1H, H-3), 3.19–3.26 (m, 1H, H-4), 3.59 (t, J = 8.1 Hz, 1H, H-1), 3.63 (dd, J_1 = 5.3 Hz, J_2 = 11.1 Hz, 1H, H-5b), 4.61 (d, J = 4.1 Hz, 1H, 2-OH), 4.87 (d, J = 4.3 Hz, 2H, 3-OH, 4-OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 41.9 (NCH_2), 51.9 (SCH_2), 66.7 (C-5), 69.9 (C-4), 73.4 (C-2), 77.4 (C-3), 91.5 (C-1).

***N*-(β-D-Arabinopyranosyl)taurine Sodium Salt (4βP):** Yield: 72%; hygroscopic white crystal, mp 114.9–115.7 °C (dec. 96 °C), $[\alpha]_D^{28.6} = -17.5°$ (c = 1.0, H_2O), ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.62 (t, J_1 = 6.4 Hz, J_2 = 6.3 Hz, 2H, SCH_2), 2.73–2.78 (m, 2H, NCH_2, NH), 3.01–3.07 (m, 1H, NCH_2), 3.24 (td, J_1 = 4.0 Hz, J_2 = 8.0 Hz, 1H, H-3), 3.32–3.35 (m, 2H, H-2, H-5), 3.62–3.65 (m, 3H, H-1, H-4, H-5), 4.48 (d, J = 3.9 Hz, 1H, 4-OH), 4.57 (d, J = 4.0 Hz, 1H, 3-OH), 4.71 (d, J = 5.2 Hz, 1H, 2-OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 42.0 (NCH_2), 52.0 (SCH_2), 65.6 (C-5), 67.9 (C-4), 70.8 (C-3), 73.2 (C-2), 91.0 (C-1).

***N*-(D-Ribopyranosyl)taurine Sodium Salt (5αP, 5βP):** Yield: 82%; hygroscopic white powder, mp 73.6–84.5 °C (dec. 66 °C), $[\alpha]_D^{30.4} = -10°$ (c = 1.0, H_2O), ^{1}H NMR (400 MHz, D_2O): δ ppm 3.00–3.09 (m, 3.5H), 3.10–3.15 (m, 5.5H), 3.17–3.25 (m, 1.5H), 3.28–3.34 (m, 1.5H), 3.43 (dd, J_1 = 2.9 Hz, J_2 = 8.4 Hz, 1H), 3.61 (dd, J_1 = 1.3 Hz, J_2 = 12.9 Hz, 1.5H), 3.62 (q, J = 10.8 Hz, 1.5H), 3.72 (dd, J_1 = 4.9 Hz,

J_2 = 10.9 Hz, 1H), 3.83–3.85 (m, 1.5H), 3.89 (m, 1.5H), 3.95 (dd, J_1 = 2.9 Hz, J_2 = 12.8 Hz, 1.5H), 4.13 (t, J = 2.8 Hz, 1H), 4.16 (s, 1.5H), 4.24 (d, J = 8.4 Hz, 1H); ^{13}C NMR (100 MHz, D_2O) δ 40.4, 40.7, 50.9, 51.0, 63.2, 66.8, 68.3, 68.6, 70.0, 70.1, 71.2, 86.6, 87.5.

***N*-(D-Lyxopyranosyl)taurine sodium salt (6αP, 6βP):** Yield: 78%; hygroscopic white powder, mp 77.8–89.2 °C (dec. 70.8 °C), $[\alpha]_D^{28.5}$ = –17.5° (c = 1.0, H_2O), ^{1}H NMR (400 MHz, D_2O) δ 2.97–3.04 (m, 2.5H), 3.06–3.11 (m, 4H), 3.13–3.28 (m, 3.5H), 3.55 (dd, J_1 = 3.4 Hz, J_2 = 9.6 Hz, 1H), 3.60–3.64 (m, 1H), 3.67 (dd, J_1 = 3.3 Hz, J_2 = 7.1 Hz, 1H), 3.75 (dd, J_1 = 5.4 Hz, J_2 = 9.8 Hz, 1H), 3.78–3.82 (m, 2H), 3.87–3.89 (m, 1H), 3.91 (d, J = 5.3 Hz, 1H), 4.17 (s, 1H), 4.28 (d, J = 7.0 Hz, 1H); ^{13}C NMR (100 MHz, D_2O) δ 40.5, 50.8, 50.8, 64.2, 66.2, 66.6, 68.6, 70.4, 70.7, 73.7, 87.2, 87.8.

Human Preadipocytes Culture and Differentiation into Adipocytes. Human preadipocytes were seeded into 6-well plates (1.8×10^5 cell per well in 2 mL of the medium) and cultured in 5% carbon dioxide at 37 °C until confluent. In order to make the preadipocytes differentiate into adipocytes, the culture medium was changed to an adipocyte differentiation medium, and the preadipocytes were cultured for two weeks by changing the medium every three days in the presence of taurine-carbohydrate derivatives at different concentrations (0-100 μg/mL).

Oil Red O Staining and Measurement of Optical Density Value. After the removal of the culture solution, the cultured cells were washed twice with phosphate-buffered saline and kept in 100 mL/L formaldehyde solution for 1 h. Then, the formaldehyde solution was removed, and the cells were rinsed twice with deionized water. Next, the cells were stained with oil red O solution (60% in isopropanol) for 20 min at room temperature. After removing the staining solution, the cultured cells were observed using an optical microscope and pictures were recorded. Next, the dye retained in the cells was eluted with isopropanol, and the OD values were measured at the optical absorbance of 500 nm using an E_{max} microplate reader.

Statistical Analysis. The data were analyzed using an SPSS 17.0 program and expressed as the mean ± standard error of the mean (SEM). One-way analysis of variance followed by Duncan's multiple range tests at $P < 0.05$ were used for the determination of significant differences.

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