Synthesis of α-Tocopheryl Disaccharides as Potential Antiallergic Agents

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α-Tocopheryl disaccharides such as β-gentiobioside, β-maltoside, β-cellobioside, β-lactoside, β-melibioside, and β-isomaltoside were synthesized by biological and chemical glycosylation, and their inhibitory effects on superoxide generation from rat neutrophils were examined.

α-Tocopherol (vitamin E), which has been known as an essential nutrient for reproduction since 1922,[1] is well documented for its antioxidant property, specifically against lipid peroxidation in biological membranes.[2] Recently, α-tocopherol has attracted considerable attention clinically because of its potential to be a very useful drug, and has been widely studied for its antiaging, anticancer, antiatherosclerosis, and anticarcinogenesis effects.[3] Irrespective of such pharmacological activities, the use of α-tocopherol as a drug is limited because of its water-insolubility and low absorbability after oral administration. Glycosylation allows the conversion of water-insoluble and unstable organic compounds into the corresponding water-soluble and stable ones to improve their bioavailability and pharmacological properties. Several attempts to synthesize α-tocopheryl glycosides, water-soluble vitamin E derivatives, by chemical glycosylation have been made, and some α-tocopheryl glycosides such as glucoside and mannoside have been reported to exhibit antiallergic activity.[4] On the other hand, there is evidence that superoxide anion (O_2^-), which is generated primarily through the activation of the plasma membrane-bound NADPH–oxidase system, is responsible for allergic reactions, and antiallergic drugs such as epinastine and mequitazine have been reported to inhibit O_2^- generation.[5] However, there are no reports on the inhibitory effects of α-tocopheryl glycosides on O_2^- generation. We report, herein, the synthesis of α-tocopheryl glycosides by either biological or chemical glycosylation, and the effect of α-tocopheryl glycosides on the O_2^- generation from rat neutrophils.

A total of 1 mmol of α-tocopherol (**1**, *RRR* form, purchased from Sigma-Aldrich Co.) was administered to ten 300-mL-conical flasks (0.1 mmol/flask) containing the suspension cultured cells of *N. tabacum* (fresh weight 50 g) and MS medium (100 mL, pH 5.7),[6] and the cultures were incubated at 25 °C for 5 days on a rotary shaker (120 rpm). After the incubation period, the cells were harvested and extracted (×3) by homogenization with MeOH and the extract was concentrated. The residue was partitioned between H_2O and EtOAc. The H_2O layer was applied to a Diaion HP-20 column and the column was washed with H_2O followed by elution with MeOH. The MeOH eluate was subjected to HPLC [column: YMC-Pack R&D ODS column (150 × 30 mm); solvent: MeOH–H_2O (9:11, v/v); detection: UV (280 nm); flow rate: 1.0 mL/min] to give two glycoside products. The isolated yield of the products was calculated on the basis of the peak area from HPLC using the calibration curves prepared by the HPLC analyses of authentic glycosides. The structure of the products[7] was identified using HR-FAB-MS, ^{1}H and ^{13}C NMR, H–H COSY, and C–H COSY as α-tocopheryl glucoside (**2**, 30%) and α-tocopheryl gentiobioside (**3**, 7%).

α-Tocopheryl glycosides, including β-maltoside **4**, β-cellobioside **5**, β-lactoside **6**, β-melibioside **7**, and β-isomaltoside **8**, were prepared by chemical syntheses in the presence of 1,1,3,3-tetramethylguanizine. A typical procedure is as follows. Hepta-*O*-acetyl-α-D-maltosyl fluoride was prepared from octaacetylated maltoside and hydrogen fluoride by modification of Noyori's method[8] in 86% yield. Under a nitrogen atmosphere, $BF_3\cdot OEt_2$ was added to a mixture of hepta-*O*-acetyl-α-D-maltosyl fluoride (1.5 mmol), α-tocopherol (1 mmol), and 1,1,3,3-tetramethylguanidine (3 mmol) in acetonitrile (20 mL) at room temperature. After the mixture had been stirred for 2 h, saturated aqueous sodium hydrogen carbonate was added. Organic materials were extracted twice with ethyl acetate, washed with saturated aqueous potassium hydrogen surfonate and brine, dried, and concentrated. The residue was chromatographed on silica gel to give α-tocopheryl hepta-*O*-acetyl-β-D-maltoside in 60% yield. Deacetylation of α-tocopheryl hepta-*O*-acetyl-β-D-maltoside with 3% potassium carbonate in methanol afforded α-tocopheryl β-maltoside (**4**) (total yield, 51%). The other glycosides, i.e., β-cellobioside (**5**, total yield, 55%), β-lactoside (**6**, 68%), β-melibioside (**7**, 65%), and β-isomaltoside (**8**, 69%), were prepared by the same procedure.

Some of α-tocopheryl glycosides exhibited strong suppressive action on IgE formation, e.g., the inhibitory action of glyco-

Table 1. Suppressive action of α-tocopheryl glycosides on IgE formation

Compound	IgE level[a]
2	195
3	337
4	358
5	369
6	377
7	170
8	228
Hydrocortisone	341

[a]The results were expressed as average of plasma IgE level of 5 rats administered a total of 10 mg/kg of each test compound.

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Table 2. Inhibitory action of α-tocopheryl glycosides on the O_2^- generation from rat neutrophils

Compound	%Inhibition[a]
2	60
3	22
4	15
5	15
6	14
7	66
8	41
Mequitazine	58

[a]%Inhibition $= [(P_c - P_t)/P_c] \times 100$, where P_t and P_c are the photon count of sample solutions with and without the test compound.

sides **2**, **7**, and **8** was much stronger than that of hydrocortisone (Table 1).[9] Next, inhibition on the O_2^- generation from rat neutrophils using cypridina luciferin analog-dependent chemiluminescence as a probe was examined.[10,11] As a result, α-tocopheryl glycosides **2**–**8** showed inhibitory effects on fMLP-induced O_2^- generation from rat neutrophils (Table 2). Particularly, β-glucoside **2**, β-melibioside **7**, and β-isomaltoside **8** exhibited significant inhibitory activity for O_2^- generation.

This is the first description of synthesis of α-tocopheryl β-gentiobioside by biological glycosylation with *N. tabacum* and syntheses of α-tocopheryl β-melibioside and β-isomaltoside by chemical glycosylation. It was found, for the first time, that α-tocopheryl glycosides show strong inhibitory effect on O_2^- generation. The α-tocopheryl glycosides, water-soluble vitamin E derivatives, obtained here can be of pharmacological interest and importance as potential antiallergic agents.

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7 Spectral data for selected products; product **3**: HR-FAB-MS: m/z 777.4770 [M + Na]$^+$; ^{1}H NMR (400 MHz, CD_3OD): δ 0.86 (6H, d, $J = 6.8$ Hz, 13-, 17-CH_3), 0.87 (6H, d, $J = 6.8$ Hz, 21-CH_3, H-22), 1.06–1.53 (21H, m, H-10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21), 1.22 (3H, s, 1-CH_3), 1.80 (2H, m, H-2), 2.05 (3H, s, 5-CH_3), 2.17 (3H, s, 8-CH_3), 2.21 (3H, s, 7-CH_3), 2.58 (2H, m, H-3), 3.14–3.53 (8H, m, H-2′, 2″, 3′, 3″, 4′, 4″, 5′, 5″), 3.64 (1H, dd, $J = 12.0$, 5.4 Hz, H-6a″), 3.74 (1H, dd, $J = 12.0$, 5.2 Hz, H-6a′), 3.84 (1H, dd, $J = 12.0$, 1.2 Hz, H-6b″), 4.03 (1H, dd, $J = 11.2$, 1.2 Hz, H-6b′), 4.23 (1H, d, $J = 7.6$ Hz, H-1″), 4.52 (1H, d, $J = 7.6$ Hz, H-1′); ^{13}C NMR (100 MHz, CD_3OD): δ 12.2 (5-CH_3), 13.3 (8-CH_3), 14.2 (7-CH_3), 20.2 (13-, 17-CH_3), 21.7 (C-3), 22.1 (C-11), 23.1 (C-22, 21-CH_3), 24.1 (1-CH_3), 25.4 (C-15), 25.9 (C-19), 29.1 (C-21), 32.7 (C-2), 33.9 (C-13, C-17), 38.3 (C-12, C-14, C-16, C-18), 40.5 (C-20), 40.9 (C-10), 62.8 (C-6″), 70.1 (C-6′), 71.5 (C-4″), 71.6 (C-4′), 75.1 (C-2′), 75.8 (C-2″), 76.9, 77.9 (C-3′, C-3″, C-5′, C-5″), 104.5 (C-1′), 105.8 (C-1″), 118.5 (C-4), 123.4 (C-5), 127.7 (C-7), 129.7 (C-8), 147.1 (C-6), 149.2 (C-9). Product **7**: HR-FAB-MS: m/z 777.4778 [M + Na]$^+$; ^{1}H NMR (400 MHz, CD_3OD): δ 0.76 (6H, d, $J = 6.2$ Hz, 13-, 17-CH_3), 0.78 (6H, d, $J = 6.8$ Hz, 21-CH_3, H-22), 0.95–1.50 (18H, m, H-11, 12, 13, 14, 15, 16, 17, 18, 19, 20), 1.12 (3H, s, 1-CH_3), 1.48–1.61 (3H, m, H-10, 21), 1.71 (2H, m, H-2), 1.94 (3H, s, 5-CH_3), 2.08 (3H, s, 8-CH_3), 2.12 (3H, s, 7-CH_3), 2.50 (2H, m, H-3), 3.21–3.79 (12H, m, H-2′, 2″, 3′, 3″, 4′, 4″, 5′, 5″, 6′, 6″), 4.40 (1H, d, $J = 8.0$ Hz, H-1″), 4.74 (1H, d, $J = 3.2$ Hz, H-1′); ^{13}C NMR (100 MHz, CD_3OD): δ 12.2 (5-CH_3), 13.3 (8-CH_3), 14.3 (7-CH_3), 20.2 (13-, 17-CH_3), 21.7 (C-3), 22.1 (C-11), 23.1 (C-22, 21-CH_3), 24.0 (1-CH_3), 25.5 (C-15), 25.9 (C-19), 29.2 (C-21), 32.7 (C-2), 33.9 (C-13, C-17), 38.4 (C-12, C-14, C-16, C-18), 40.5 (C-20), 41.1 (C-10), 62.7 (C-6″), 68.4 (C-6′), 70.4 (C-4″), 71.0 (C-4′), 71.5 (C-2″), 72.2 (C-3″), 75.8 (C-1, C-2′), 76.4 (C-3′, C-5″), 77.9 (C-5′), 100.5 (C-1′), 105.8 (C-1″), 118.5 (C-4), 123.3 (C-5), 128.0 (C-7), 129.7 (C-8), 147.2 (C-6), 149.2 (C-9). Product **8**: HR-FAB-MS: m/z 777.4769 [M + Na]$^+$; ^{1}H NMR (400 MHz, CD_3OD): δ 0.86 (6H, d, $J = 6.0$ Hz, 13-, 17-CH_3), 0.88 (6H, d, $J = 6.8$ Hz, 21-CH_3, H-22), 1.06–1.46 (18H, m, H-11, 12, 13, 14, 15, 16, 17, 18, 19, 20), 1.22 (3H, s, 1-CH_3), 1.49–1.53 (3H, m, H-10, 21), 1.78 (2H, m, H-2), 2.05 (3H, s, 5-CH_3), 2.18 (3H, s, 8-CH_3), 2.22 (3H, s, 7-CH_3), 2.56 (2H, m, H-3), 3.28–3.88 (12H, m, H-2′, 2″, 3′, 3″, 4′, 4″, 5′, 5″, 6′, 6″), 4.49 (1H, d, $J = 7.6$ Hz, H-1″), 4.83 (1H, d, $J = 2.4$ Hz, H-1′); ^{13}C NMR (100 MHz, CD_3OD): δ 12.2 (5-CH_3), 13.3 (8-CH_3), 14.2 (7-CH_3), 20.3 (13-, 17-CH_3), 21.7 (C-3), 22.1 (C-11), 23.1 (C-22, 21-CH_3), 24.0 (1-CH_3), 25.4 (C-15), 25.9 (C-19), 29.2 (C-21), 32.8 (C-2), 33.8 (C-13, C-17), 38.6 (C-12, C-14, C-16, C-18), 40.5 (C-20), 41.1 (C-10), 62.7 (C-6″), 68.5 (C-6′), 71.7 (C-4′), 72.0 (C-4″), 72.8 (C-5″), 73.4 (C-2″), 73.7 (C-3″), 75.2 (C-2′), 75.8 (C-1), 76.4 (C-3′), 77.9 (C-5′), 100.4 (C-1′), 106.0 (C-1″), 118.6 (C-4), 123.4 (C-5), 127.7 (C-7), 129.6 (C-8), 147.3 (C-6), 149.2 (C-9).
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10 Male Wistar rats, each weighing 250 to 300 g, were used. Under ether anesthesia, whole blood was collected from the carotid artery and diluted twice with Hanks' balanced salt solution (HBSS) (pH 7.4). Neutrophils were purified by Percoll density gradient centrifugation. O_2^- generation from rat neutrophils was measured by the cypridina luciferin analog-dependent chemiluminescence method.[11] An authentic antiallergic agent, mequitazine, was used as a positive control. Neutrophil suspensions (10^6 cells/mL) were incubated for 3 min in HBSS containing 0.4 mM of cypridina luciferin analog and 50 μM of α-tocopheryl glycoside or authentic antiallergic agent, which was a proper concentration for the present bioassay, at 37 °C in the dark. Five seconds later, *N*-formyl–Met–Leu–Phe (fMLP) (2.5 μM) was added into the assay mixture. Cypridina luciferin analog-dependent chemiluminescence was monitored with a Lumicounter ATP-237 (Advantec Co., Ltd.). The results are expressed in terms of the percentage reduction of the O_2^- generation from rat neutrophils at 5 min after the administration of fMLP by test compounds.
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