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Letter

Preparation of a new pepper: chemoenzymatic synthesis of capsaicin oligosaccharide and 8-nordihydrocapsaicin oligosaccharide

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Abstract

Capsaicin and 8-nordihydrocapsaicin were readily converted into the corresponding monoglucosides (capsaicin β-D-glucopyranoside and 8-nordihydrocapsaicin β-D-glucopyranoside) with tetraacetyl-α-D-glucose fluoride (TAGF) in the presence of $BF_3{\cdot}OEt_2$. Furthermore, capsaicin oligosaccharides and 8-nordihydrocapsaicin oligosaccharides were synthesized from their monoglucosides by a cyclodextrin glucanotransferase (CGTase)-catalyzed glycosylation for the preparation of higher water-soluble capsaicinoids.

Keywords: Capsaicin; 8-Nordihydrocapsaicin; Oligosaccharide; Glycosylation; CGTase

The hot or chili peppers are members of the genus capsicum. The extremely pungent oleoresin in capsicum is capsaicin (about 0.02% in flesh fruit and 0.5–1% in the dried ripe fruit). Capsaicin is a fat-soluble phenol. It imparts a distinctly pungent taste to water even when diluted to one part in eleven million parts of water [1]. The irritant principle of hot peppers, capsaicin, possesses extensive neurological toxicity most pronounced in the developing nervous system and focused on substance P containing neurons. It also exhibits direct skin and mucous membrane irritant effects [2]. Furthermore, the excitatory effects of dihydrocapsaicin on nociceptive neurons in the medial thalamus were reported [3]. It was reported that, capsaicin reduced the perirenal adipose tissue weight and serum triglyceride concentration in rats by enhancing the energy metabolism through a β-adrenergic action [4,5]. In humans, it was reported that, the ingestion of chili sauce with meals resulted in a marked increase in energy metabolism [6]. Irrespective of such biological activities, the use of capsaicin and its derivative as food ingredients has been limited, because of its low solubility.

Glycosylation allows the conversion of water-insoluble organic compounds to the corresponding water-soluble one for improving its bio- and pharmacological

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properties [7,8]. For example, Kometani et al. demonstrated the preparation of capsaicin monoglucoside using a suspension culture of *Coffea arabica* [9].

In this paper, we report that the chemoenzymatic synthesis of the capsaicin oligosaccharides and 8-nordihydrocapsaicin oligosaccharide as new capsaicinoids which possess a higher water-solubility.

Capsaicin was purchased from Nacalai Tesque, Inc., Kyoto, Japan. 8-Nordihydrocapsaicin was purchased from Tokyo Kasei Kogyo Co. Ltd., Tokyo, Japan. Cyclodextrin glucanotransferase (CGTase from *Bacillus macerans*) was obtained from Amano Pharmaceutical Co. Ltd., Nagoya, Japan. All other reagents used were of analytical grade. ^{1}H NMR (400 MHz) spectra were recorded on a BRUKER AMX-R400 spectrometer in methanol-d_4 using tetramethylsilane (TMS) as the internal standard. The FAB-MS spectrum was measured on a JEOL, the MStation JMS-700 spectrometer. The molecular weight was estimated from the *m*/*z* value of the quasimolecular ion [M + Na] peak. Tetraacetyl-α-D-glucose fluoride (TAGF) was prepared according to the literature procedure [10]. Capsaicin (**1**) and 8-nordihydrocapsaicin (**3**) were readily converted to the corresponding monoglucoside (**2a**,**4a**) with TAGF in the presence of $BF_3{\cdot}OEt_2$ [11]. As a typical run, under a nitrogen atmosphere, $BF_3{\cdot}OEt_2$ (4.0 ml) was added to a mixture of TAGF (15 mmol), substrate (**1** or **3**, 10 mmol), and 1,1,3,3-tetramethylguanidine (TMG) (17 mmol) in dry acetonitrile (15 ml, distilled from CaH_2, and stored over MS 4A) at room temperature. After the reaction mixture was stirred for 3 h, saturated aqueous $NaHCO_3$ was added. The organic materials were extracted twice with AcOEt, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The viscous residue was dissolved into 20 ml of MeOH containing 400 mg of K_2CO_3. After stirring for 2 h at room temperature, the mixture was filtered and concentrated under reduced pressure. The crude residue was recrystallized twice from H_2O–MeOH to give a white powder (isolated yield: **2a**, 71%; **4a**, 78%). Selected NMR data: **2a**; ^{1}H NMR (400 MHz, CD_3OD): δ = 0.955 (d, 6H), 1.36 (m, 2H), 1.62 (m, 2H), 1.98 (q, 2H), 2.18 (t, 2H), 2.32 (m, 1H), 3.30–3.48 (m, 4H), 3.66 (dd, 1H), 3.69 (dd, 1H), 3.84 (s, 3H), 4.30 (d, 2H), 4.84 (br, 1H), 5.36 (d, 1H, anomeric H: J = 6.7 Hz), 5.37 (m, 2H), 6.82 (d, 1H), 6.94 (s, 1H), 7.11 (d, 1H), **4a**; ^{1}H NMR (400 MHz, CD_3OD): δ = 0.893 (t, 3H), 1.30 (m, 10H), 1.62 (m, 2H), 2.21(t, 2H), 3.29–3.51 (m, 4H), 3.66 (dd, 1H), 3.69 (dd, 1H), 3.84 (s, 3H), 4.29 (d, 2H), 4.64 (br, 1H), 4.85 (d, 1H, anomeric H: J = 7.6 Hz), 6.81 (dd, 2H), 6.93 (d, 1H), 7.11 (d, 1H). The FAB-MS spectrum of **2a** and **4a** gave a molecular ion at *m*/*z* 490 and 478 [M + Na], respectively. A large coupling constant (J = 6.6 and 7.6 Hz) of the anomeric proton in **2a** and **4a** suggested the β configuration for the anomeric center. Therefore, **2a** and **4a** could be identified as capsaicin monoglucoside (capsaicin β-D-glucopyraoside) and 8-nordihydrocapsaicin monoglucoside (8-nordihydrocapsaicin β-D-glucopyraoside), respectively (Fig. 1).

As the synthetic monoglucosides (**2a** and **4a**) prepared had a resemble water-solubility to those of **1** and **3** (data not shown), the further glucosylation to the sugar moiety in **2a** and **4a** was necessary to increase the solubility of their capsaisinoids. For the further glucosylation of the monoglucosides, we planed to use a cyclodextrin glucanotransferase (CGTase) which catalyzed the transfer reaction of sugar from "donor sugar" to "acceptor sugar" to form oligosaccharides [12,13].

Soluble starch (40 g) and $CaCl_2$ (20 mg) were added to 100 ml of distilled water (pH 6.0), and heated until the starch was completely dissolved (it became a viscous solution). The substrate (50 mg, **2a** or **4a**) and CGTase (1800 U) were added to the viscous starch solution. After stirring for 24 h at 55°C, the mixture was extracted twice with *n*-butanol, and concentrated under reduced pressure to give a white powder (Fig. 2).

The crude products were separated and purified using an HPLC equipped with a Crestpak C18S (JASCO, ϕ 4.6 mm × 150 mm, UV 280 nm, flow rate: 1.0 ml/min, mobile phase: CH_3CN:H_2O = 13:7, column temperature: 40°C). When we used α-, β- and γ-cyclodextrins as sugar donors, the further glucosylation for the monoglucosides (**2a** and **4a**) were not proceeded effectively by the CGTase-catalyzed reaction. The optimum temperature of the reaction was 55°C. The CGTase-catalyzed glucosylation toward **1** and **3** was not observed.

Five products were produced from **4a** as shown in Fig. 3. The FAB-MS analyses of isolated **4b–f** indicated the formation of 8-nordihydrocapsaicin oligosaccharide (**4b**; diglucoside, **4c**; triglucoside, **4d**;

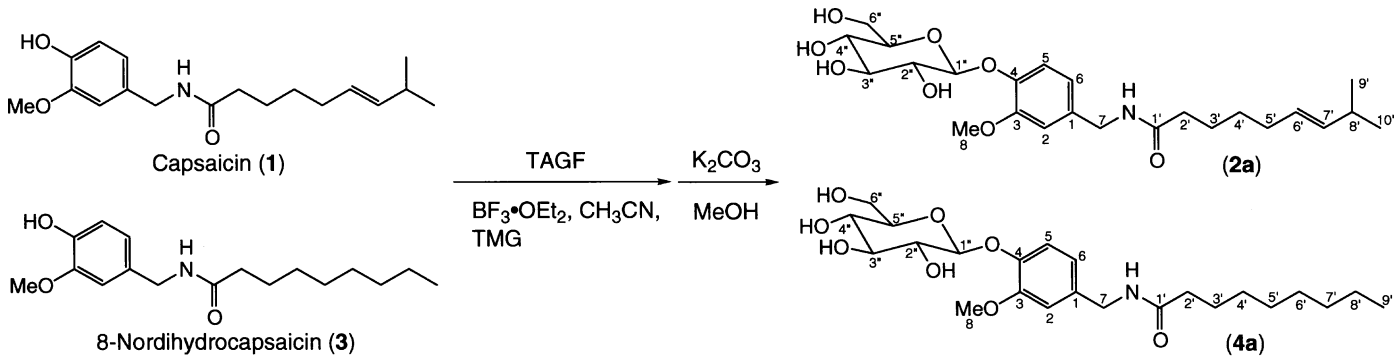

Fig. 1. Synthesis of capsaicin β-D-glucopyranoside (**2a**) and 8-nordihydrocapsaicin β-D-glucopyranoside (**4a**) from **1** or **3**.

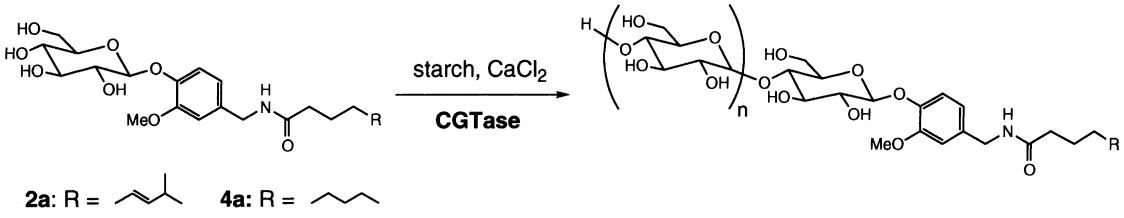

Fig. 2. Enzymatic synthesis of capsaicin oligosaccharides and 8-nordihydrocapsaicin oligosaccharides from the corresponding monoglucosides (**2a** and **4a**).

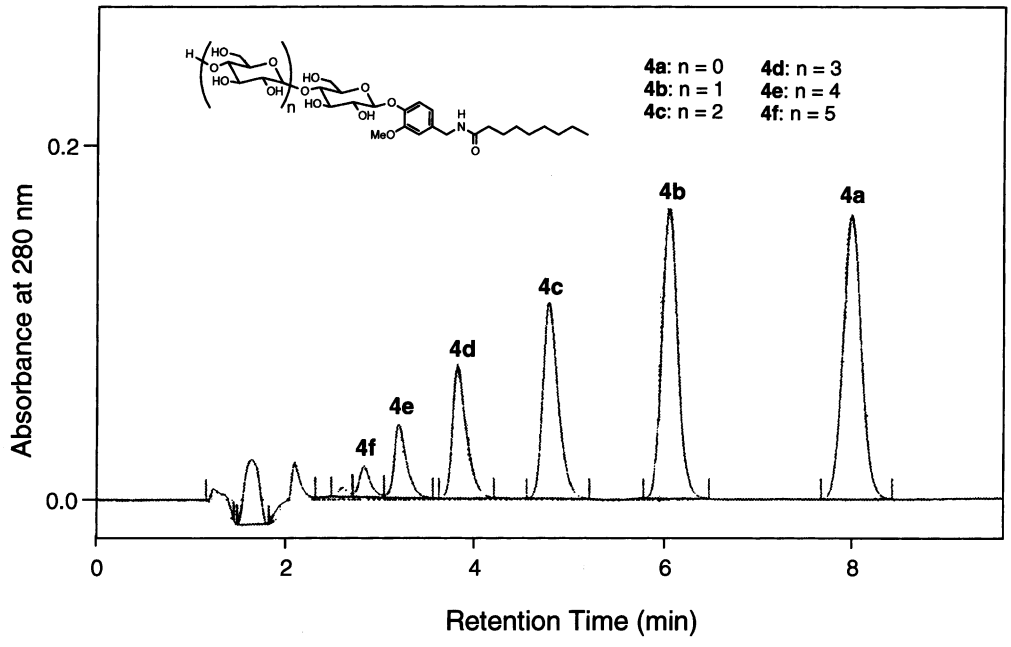

Fig. 3. The HPLC chromatogram of the products from **4a** by CGTase-catalyzed glycosylation.

tetraglucoside, **4e**; pentaglucoside, and **4f**; hexaglucoside), because the *m*/*z* values were 478, 640, 802, 964, 1126, and 1288, respectively. The isolated yields of products **4a, 4b, 4c, 4d, 4e,** and **4f** are 13, 12, 8, 6, 4, and 3 mg, respectively (from 50 mg of **4a**). In the same manner, four oligosaccharides (**2b**; diglucoside, **2c**; triglucoside, **2d**; tetraglucoside, **2e**; pentaglucoside) were produced from **2a** (chromatographic data not shown). The isolated yields of products **2a, 2b, 2c, 2d,** and **2e** are 16, 13, 10, 4, and 2 mg, respectively (from 50 mg of **2a**).

Thus, the enzymatic synthesis of new capsaicin and 8-nordihydrocapsaicin oligosaccharides was achieved by the CGTase-catalyzed glycosylation in the presence of starch as a sugar donor.

The application of this chemoenzymatic system to a large-scale (gram scale) production of the capsaicin oligosaccharides and their derivatives is currently under investigation and will be reported in a future paper.

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