Synthesis of *N*-[4-(α-D-Glucopyranosyloxy)-3-methoxybenzyl]nonanamide and Its β-Anomer

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New capsaicinoid glucosides [α- and β-anomers of *N*-[4-(D-glucopyranosyloxy)-3-methoxybenzyl]-nonanamide (α- and β-1)] were synthesized from *N*-vanillylnonanamide (2). The pungencies of α-1 [threshold value (*T*) = 0.13 ppm] and β-1 (*T* = 0.24 ppm) tend to be produced more slowly and are weaker than those of 2 (*T* = 0.054 ppm).

INTRODUCTION

Capsaicin (CAP) is a pungent principle of hot red peppers, which is used as an important spice for enhancing the palatability of food and medicinally as a counterirritant. There have been studies showing that CAP caused the induction of a warming action (Kawada et al., 1988), promotion of the peristaltic reflex (Schulze-Delrieu, 1985), and an increase in gastric acid secretion (Limlomwongse et al., 1979). Although CAP and its analogs have useful activity, their intense pungency limits their application as a food additive. *N*-Vanillylnonanamide (2) occurs in the *Capsicum* species and stimulates pungency. Changes in the 4-hydroxy-3-methoxy substitution on the aromatic moiety of CAP almost abolished the pungency stimulant property (Govindarajan et al., 1987). Yamamoto et al. (1990) reported that L-ascorbic acid released from 2-*O*-α-D-glucopyranosyl-L-ascorbic acid by α-glucosidase in the cell acts as an active moiety. In this study, we synthesized the α- and β-anomers of capsaicinoid glucoside (α-1 and β-1). The objectives of the synthesis of 1, to investigate the possibility of applying 1 to foods and beverages, were (1) to reduce the pungency of capsaicinoid, (2) to release capsaicinoid by glucosidase in the body, and (3) to increase the solubility of capsaicinoid in water.

MATERIALS AND METHODS

Infrared (IR) spectra were recorded on liquid films; absorptions are given in reciprocal centimeters. ^1H (400 MHz) and ^{13}C (100 MHz) NMR were obtained using a Bruker AM-400 spectrometer. Chemical shifts (δ) are expressed in parts per million downfield from the internal tetramethylsilane standard. Silica gel 60 (70–230 mesh) (Merck) was used for the column chromatography. Quantitative HPLC determinations were conducted with a Hewlett-Packard HP1090M [column, Shiseido Capcell Pak C18 SG120, 5 μm, 10 mm i.d. × 250 mm; solvent, CH_3CN–H_2O (3:2), 1 mL/min; detected by a variable-wavelength detector, UV 220 and 280 nm]. Two types of HPLC instruments were used for the preparative HPLC. (1) GPC: a Japan Analytical Industry LC-08; column, JAIGEL-1H, 20 mm i.d. × 300 mm × 2; solvent, $CHCl_3$, 3 mL/min; detected by a refractive index detector. (2) Reversed-phase HPLC: a Waters Model 600E; column, Tosoh TSK gel ODS-120T, 10 μm, 21.5 mm i.d. × 300 mm; solvent, MeOH–H_2O (7:3), 8 mL/min; detected by a 490E variable-wavelength detector, UV 220 and 280 nm.

Synthesis of *N*-[4-(Tetra-*O*-acetyl-β-D-glucopyranosyloxy)-3-methoxybenzyl]nonanamide (β-4). The O-glycosylation of 2 (0.29 g; 1 mmol) has been carried out using β-D-pentaacetylglucose (3; 2.34 g, 6 mol) in the presence of $SnCl_4$ (0.65 g, 2.5 mmol) in 5 mL of dichloromethane and stirred at room temperature for 2 h (see Figure 1). The reaction mixture was washed with 2.5 g of water. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure to give a crude brown oil. The crude oil was chromatographed on a silica gel [solvent, $CHCl_3$–EtOAc (1:1)] and further purified by recrystallization from hexane to give β-4 (208 mg, 33%): mp 93–96 °C; $[\alpha]^{24}_D$ −18° (*c* 1, $CHCl_3$); IR ν_{max} (KBr) 3270, 2930, 1745, 1640, 1260, 1230, 1040 cm^{-1}; FAB MS *m/z* 624 $[M + H]^+$. Anal. Found: C, 59.60; H, 7.25; N, 2.32. Calcd for $C_{31}H_{45}O_{12}N$: C, 59.70; H, 7.27; N, 2.25.

Synthesis of *N*-[4-(β-D-Glucopyranosyloxy)-3-methoxybenzyl]nonanamide (β-1). A solution of β-4 (0.77 g, 1.24 mmol) in acetone (40 mL) and 1 N NaOH (6 mL) was stirred at 20 ± 2 °C for 2 h. After neutralization with concentrated HCl, the reaction mixture was evaporated under reduced pressure. The precipitate was recrystallized from MeOH–H_2O (2:1) to give β-1 as colorless needles (450 mg, 0.99 mmol, 80%): mp 179–181 °C; $[\alpha]^{24}_D$ −45.8° (*c* 0.6, CH_3OH); IR ν_{max} (KBr) 3350, 3270, 2920, 2850, 1640, 1510, 1270, 1225, 1090, 1080 cm^{-1}; FAB MS *m/z* 456 $[M + H]^+$, 294, 154, 137. Anal. Found: C, 60.43; H, 8.28; N, 3.18. Calcd for $C_{23}H_{37}O_8N$: C, 60.64; H, 8.19; N, 3.07.

Synthesis of *N*-[4-(Tetra-*O*-acetyl-α-D-glucopyranosyloxy)-3-methoxybenzyl]nonanamide (α-4). The O-glycosylation of 2 (0.29 g, 1 mmol) has been carried out using β-D-pentaacetylglucose (3; 0.98 g, 2.5 mmol) in the presence of $SnCl_4$ (0.65 g, 2.5 mmol) in 2.5 mL of dichloromethane with stirring at 40 °C for 8 h. The reaction mixture was washed with 2.5 g of water. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure to give a crude brown oil (1.3 g). The crude oil was subjected to preparative HPLC (GPC and succeeding reversed-phase HPLC) to give α-4 (236 mg, 41%) as a colorless oil and β-4 (45 mg, 8%). α-4: $[\alpha]^{24}_D$ +110° (*c* 1, $CHCl_3$); IR ν_{max} (KBr) 2950, 2850, 1745, 1640, 1515, 1365, 1220, 1040 cm^{-1}; FAB MS *m/z* 624 $[M + H]^+$, 331. Anal. Found: C, 59.59; H, 7.47; N, 2.39. Calcd for $C_{31}H_{45}O_{12}N$: C, 59.70; H, 7.27; N, 2.25.

Synthesis of *N*-[4-(α-D-Glucopyranosyloxy)-3-methoxybenzyl]nonanamide (α-1). A solution of α-4 (62 mg, 0.1 mmol) in MeOH (3 mL) and 1 N NaOH (1 mL) was stirred at 20 ± 2 °C for 1.5 h. After neutralization with concentrated HCl, the reaction mixture was evaporated under reduced pressure and extracted with EtOAc. The EtOAc layer was dried over Na_2SO_4 and evaporated. The residue was subjected to preparative HPLC (reversed-phase HPLC) to give α-1 (26 mg, 57%): mp 174–177 °C; $[\alpha]^{24}_D$ +113.5° (*c* 0.8, CH_3OH); IR ν_{max} (KBr) 3300, 2900, 1640, 1515, 1275, 1230, 1090, 1030 cm^{-1}; FAB MS *m/z* 456 $[M + H]^+$, 294, 154, 137. Anal. Found: C, 59.74; H, 8.12; N, 2.97. Calcd for $C_{23}H_{37}O_8N$: C, 60.64; H, 8.19; N, 3.07. To illustrate the purity of α-1, full-scale ^1H and ^{13}C NMR spectra are presented as supplementary material.

Sensory Evaluation. Threshold values for α-1 and β-1 [>99.8% purity (HPLC)] were determined by the "2/5 test" (selecting the same two of five samples) employed by Amoore (1970); above these values individuals with normal acuity would readily perceive taste (recognition threshold). The ascending concentration series method is applied because of its advantages of overcoming gustatory fatigue. The panelists (10 males) ranged in age from approximately 25 to 29 years of age. They had

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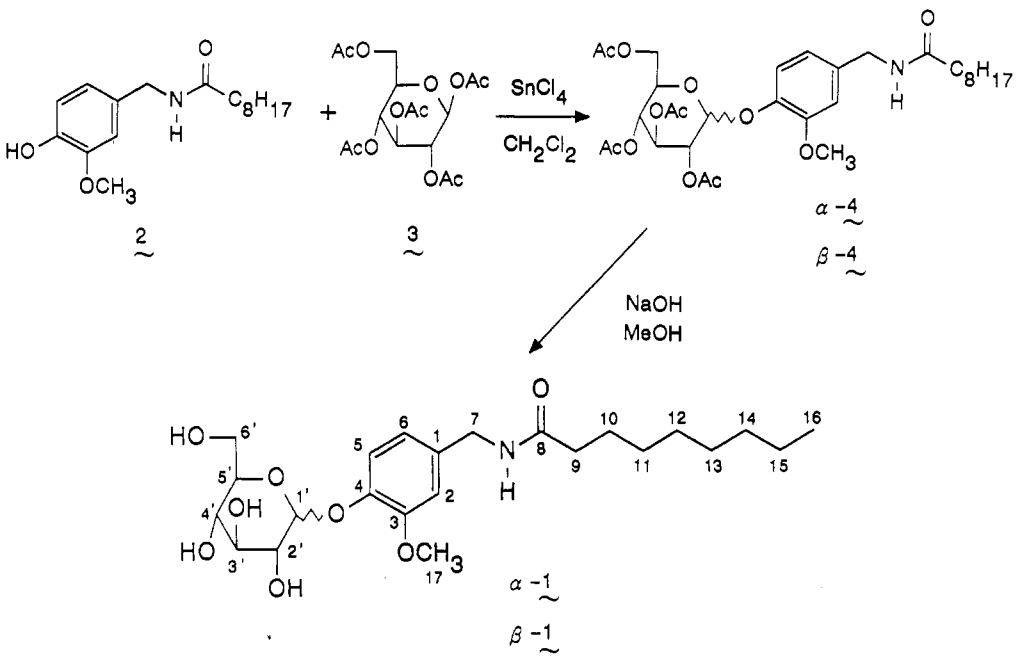

Figure 1.

Table I. ^{1}H NMR Data for 1 and 4[a]

H	α-1[b]	β-1[b]	α-4[c]	β-4[c]
aglycon				
2	6.94 (d, 2.0)	6.94 (d, 2.0)	6.84 (d, 2.0)	6.83 (d, 1.9)
5	7.14 (d, 8.2)	7.11 (d, 8.3)	6.96 (d, 8.1)	7.06 (d, 8.2)
6	6.81 (dd, 8.2, 2.0)	6.82 (dd, 8.3, 2.0)	6.77 (dd, 8.1, 2.0)	6.76 (dd, 8.2, 1.9)
7	4.29 s	4.29 s	4.35 (d, 5.7)	4.37 (d, 5.8)
9	2.21 (t, 7.5)	2.21 (t, 7.5)	2.21 (t, 7.7)	2.21 (t, 7.7)
10	1.62 m	1.62 m	1.64 m	1.65 m
11				
12				
13	1.25–1.38 m	1.25–1.38 m	1.21–1.37 m	1.21–1.35 m
14				
15				
16	0.89 (t, 6.8)	0.89 (t, 6.8)	0.87 (t, 7.0)	0.87 (t, 6.9)
17	3.84 s	3.84 s	3.80 s	3.80 s
NH	4.90[d]	4.91[d]	6.10 (t, 5.7)	5.92 (t, 5.8)
glucose				
1′	5.40 (d, 3.7)	4.85 (d, 7.3)	5.64 (d, 3.8)	4.93 (d, 7.6)
2′	3.51 (dd, 10.0, 3.7)		4.96 (dd, 10.2, 3.8)	5.27 m
3′	3.86 (t, 10.0)	3.36–3.50 m	5.72 (dd, 10.2, 9.7)	5.27 m
4′	3.42 (dd, 10.0, 9.0)		5.13 (dd, 10.3, 9.7)	5.15 m
5′	3.78–3.85 m		4.42 (ddd, 10.3, 4.7, 2.2)	3.76 (ddd, 10.0, 5.6, 2.7)
6′	3.69–3.71 m	3.66–3.70 m	4.26 (dd, 12.1, 4.7)	4.27 (dd, 12.0, 5.6)
		3.83–3.87 m	4.09 (dd, 12.1, 2.2)	4.15 (dd, 12.0, 2.7)
acetyl			2.05 s	2.04 s
			2.11 s	2.08 s

[a] Chemical shifts (δ) in ppm, multiplicities, and J values in Hz. [b] CD_3OD. [c] $CDCl_3$. [d] May be covered by solvent signal.

extensive experience and proven reliability in taste judgments. Daily midmorning sessions were conducted.

RESULTS AND DISCUSSION

The syntheses of *N*-[4-(α-D-glucopyranosyloxy)-3-methoxybenzyl]nonanamide and its β-anomer (α-1 and β-1) were according to the Helferich reactions (Honma et al., 1976) as shown in Figure 1. The reaction of 2 and 3 with stannic tetrachloride in dichloromethane at 20 °C for 2 h produced the tetraacetyl-β-glycoside (β-4) in 33% yield. An increase in the reaction times in conjunction with higher temperatures leads to increasing proportions of α-glycosides due to anomerization (Banoub and Bundle, 1979). The addition of 3 to 2 gave optimum yields (41%) of the tetraacetyl-α-glycoside (α-4) after only 8 h at 40 °C. The IR spectra of β-4 and α-4 indicated acetoxy (1745, 1240 cm^{-1}) and amide (1640 cm^{-1}) groups. A FAB MS spectrum suggested that 4 had a molecular formula of $C_{31}H_{45}O_{12}N$ [m/z at 624 $[M+H]^+$], and the base peak at m/z 331 was in accordance with a tetraacetylated glucosyl moiety. ^{1}H NMR showed four acetyl signals in a region between δ 2.04 and 2.11, seven protons attached to the sugar pyranose ring in the region between δ 3.76 and 5.72, and the trisubstituted benzene ring protons in the region between δ 6.76 and 7.06 (see Table I). ^{13}C NMR signals of 4 were assigned on the basis of their chemical shifts, DEPT, and ^{1}H–^{13}C COSY data (Table II). The ^{13}C NMR data of 4 showed six signals for glucose at δ 61.8–100.8 and four acetyl carbons at around δ 20 and 170. From this evidence, compound 4 is presumed to be the monoglucoside tetraacetate of 3. The anomeric configurations of 4 were confirmed by ^{1}H NMR ($J_{1',2'}$ of β-4 and α-4 were 7.6 and 3.8 Hz, respectively) by also comparing their $[\alpha]^{24}_D$ values [−18° (c 1, $CHCl_3$) and +110° (c 1, $CHCl_3$), respectively].

Table II. ^{13}C NMR Data for 1 and 4[a]

C	α-1[b]	β-1(DEPT)[b]	α-4[c]	β-4[c]
aglycon				
1	135.7	135.1 (C)	135.5	135.3
2	113.4	113.4 (CH)	112.6	112.6
3	151.9	150.8 (C)	151.2	150.8
4	146.7	147.1 (C)	144.6	145.4
5	119.9	118.3 (CH)	120.4	120.2
6	121.2	121.3 (CH)	120.0	119.9
7	43.8	43.8 (CH_2)	43.2	43.3
8	176.0	176.0 (C)	172.9	172.9
9	37.1	37.1 (CH_2)	36.7	36.7
10	27.0	27.0 (CH_2)	25.8	25.7
11	30.3[d]	30.3 (CH_2)[d]	29.3[d]	29.3[d]
12	30.3[d]	30.4 (CH_2)[d]	29.4[d]	29.4[d]
13	30.2[d]	30.2 (CH_2)[d]	29.1[d]	29.1[d]
14	32.9	32.9 (CH_2)	31.8	31.8
15	23.6	23.6 (CH_2)	22.6	22.6
16	14.4	14.4 (CH_3)	14.0	14.0
17	56.6	56.8 (CH_3)	55.9	56.1
glucose				
1′	101.0	103.0 (CH)	96.4	100.8
2′	73.7	74.9 (CH)	70.9	72.7[e]
3′	75.0	77.8 (CH)[e]	70.0	71.4[e]
4′	71.4	71.4 (CH)	68.7	68.6
5′	74.5	78.1 (CH)[e]	68.2	72.0
6′	62.3	62.5 (CH_2)	61.8	62.0
acetyl				
(C=O)			169.5	169.2
			169.8	169.3
			170.0	170.0
			170.3	170.4
(Me)			20.6	20.5
			20.6	20.6
			20.6	20.6

[a] Chemical shifts (δ) in ppm. [b] CD_3OD. [c] $CDCl_3$. [d,e] Interchangeable values in each column.

The acetylated α- and β-glycosides (α-4 and β-4) were de-O-acetylated to provide the corresponding *N*-[4-(D-glucopyranosyloxy)-3-methoxybenzyl]nonanamide (1). The IR spectra of 1 showed the absorption of hydroxyl groups (3300 cm^{-1}) and a pyranose ring (1100–1050 cm^{-1}). FAB MS showed the ion peaks of *m/z* 456 $(M + H)^+$, 294 (aglycone H^+) and 163 ($sugar^+$). The ^1H and ^{13}C NMR signals of 1 were reasonably assigned as shown in Tables I and II, respectively. This evidence supports 1 being a monoglucoside of 3. The anomeric configurations of 1 were confirmed by ^1H NMR ($J_{1',2'}$ of β-1 and α-1 were 7.3 Hz and 3.7 Hz, respectively) and $[\alpha]^{24}_D$ values [−45.8° (*c* 0.6, MeOH) and +113.5° (*c* 0.8, MeOH), respectively], in the same manner as already mentioned.

Compound 2 gives a quick and intense pungent sensation [threshold value (*T*) = 0.056 ppm], while its glucosides (α-1 and β-1) tend to produce a slow and weak pungency (*T* = 0.13 and 0.24 ppm, respectively). It takes several minutes to feel the pungency of 1.

Supplementary Material Available: Full-scale ^1H and ^{13}C NMR spectra of α-1 (2 pages). Ordering information is given on any current masthead page.

LITERATURE CITED

Amoore, J. E. *Molecular Basis of Odor*; Charles C. Thomas: Springfield, IL, 1970; pp 16–25.

Banoub, J.; Bundle, D. R. Stannic Tetrachloride Catalyzed Glycosylation of 8-Ethoxycarbonyloctanol by Cellobiose, Lactose, and Maltose Octaacetates; Synthesis of α- and β-Glycosidic Linkages. *Can. J. Chem.* **1979**, *57*, 2085–2090.

Govindarajan, V. S.; Rajalakshmi, D.; Chand, N. Capsium—Production, Technology, Chemistry, and Quality. Part V. Impact on Physiology, Pharmacology, Nutrition, and Metabolism; Structure, Pungency, Pain, and Desensitization Sequences. *Crit. Rev. Food Sci. Nutr.* **1987**, *25*, 185–282.

Honma, K.; Nakajima, K.; Uematsu, T.; Hamada, A. A Facile O-Glycosidation using Stannic Chloride. *Chem. Pharm. Bull.* **1976**, *24*, 394–399.

Kawada, T.; Sakabe, S.; Watanabe, T.; Yamamoto, M.; Iwai, K. Some Pungent Principles of Spices Cause the Adrenal Medulla to Secrete Catecholamine in Anesthetized Rats. *Proc. Soc. Exp. Biol. Med.* **1988**, *188*, 229–233.

Limlomwongse, L.; Chaitauchawong, C.; Tongyai, S. Effect of Capsaicin on Gastric Acid Secretion and Mucosal Blood Flow in the Rat. *J. Nutr.* **1979**, *109*, 773–777.

Schulze-Delrieu, K. Pepper and Peristalsis. *Gastroenterology* **1985**, *88*, 590.

Yamamoto, I.; Muto, N.; Murakami, K.; Suga, S.; Yamaguchi, H. L-Ascorbic Acid α-Gulucoside Formed by Regioselective Transglucosylation with Rat Intestinal and Rice Seed α-Gulcosidases: Its Improved Stability and Structure Determination. *Chem. Pharm. Bull.* **1990**, *38*, 3020–3023.

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Registry No. α-1, 143733-84-0; β-1, 143733-86-2; 2, 2444-46-4; 3, 604-69-3; α-4, 143733-85-1; β-4, 143733-87-3.