JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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# Enzymatic Synthesis of Piceid Glucosides Using Maltosyltransferase from *Caldicellulosiruptor bescii* DSM 6725

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**ABSTRACT:** Piceid is widely used in food, cosmetics, and pharmaceuticals because of its therapeutic benefits. However, the use of piceid as a drug is limited because of its low solubility. To increase solubility, we synthesized piceid glucosides using maltosyltransferase from *Caldicellulosiruptor bescii*. The MTase gene was cloned and expressed in *Escherichia coli*. The enzyme had a unique transfer specificity to the transfer of maltosyl units. Four piceid transglycosylation products were present and identified by thin-layer chromatography and recycling preparative high-performance liquid chromatography. The major product was purified by $C_{18}$ and gel filtration chromatography, and its molecular structure was determined using nuclear magnetic resonance spectroscopy to be $\alpha$-D-maltosyl-(1→4)-piceid. The solubility of maltosyl piceid was $8.54 \times 10^3$ and $1.86 \times 10^3$ times those of natural resveratrol and piceid, respectively, suggesting that the transglycosylation greatly increased the water solubility. This suggests that dietary intake of this compound can enhance the bioavailability of resveratrol in the human body.

**KEYWORDS:** *maltosyltransferase, piceid, resveratrol, transglycosylation, Caldicellulosiruptor bescii*

## INTRODUCTION

In red wine, *trans*-resveratrol (*trans*-3,5,4′-trihydroxystilbene) is the most significant compound in terms of possible positive health benefits (Figure 1A).[1] Resveratrol has been reported to have potential cancer chemopreventive activities[2,3] and may exert a protective effect against atherogenesis through its antioxidant properties.[4] However, resveratrol mostly exists as its glycoside form, *trans*-piceid (5,4′-dihydroxystilbene-3-*O*-$\beta$-D-glucopyranoside), in wine (Figure 1B). After oral administration in humans, the *trans*-piceid is hydrolyzed by a $\beta$-glucosidase in the small intestine, and converted resveratrol is efficiently absorbed across intestinal Caco-2 cells and metabolized to glucuronides or sulfated derivatives.[5] *trans*-Piceid is not absorbed through the Caco-2 apical membrane via this transport system because of its lack of affinity with the lipid bilayer of the cell surface. Instead, the uptake of *trans*-piceid into Caco-2 cells occurs via the sodium-dependent active transporter SGLT1.

Although resveratrol as *trans*-piceid has beneficial health effects, poor water solubility limits its practical application. One way to enhance the water solubility is to add a sugar moiety to the compound by enzymatic glycosylation. Various phenolic compounds such as catechin,[6] hesperidin,[7,8] naringin,[9] and rutin[10,11] have been glycosylated using bacterial glycosidases and glycosyltransferases. Kometani et al.[8] reported the transglycosylation of neohesperidin and naringin by a reaction with cyclodextrin glucanotransferase (CGTase) from alkaliphilic *Bacillus* species. It was found that glycosyl neohesperidin and glycosyl naringin were less bitter or more soluble in water than the original compounds. Maltogenic amylases (MAases) from *Bacillus stearothermophilus* and *Thermus* sp. have also been used to modify bioactive compounds, such as ascorbic acid, naringin, and acarbose, by transferring maltosyl residues to the acceptor molecules to produce maltosyl transfer products.[9,12−14] A maltosyltransferase (MTase) from *Thermotoga maritima* MSB8 has also been used to produce maltosyl daidzin, which is 10000-fold more soluble than daidzin.[15]

*Caldicellulosiruptor bescii* is a Gram-positive anaerobic bacterium that grows optimally at 80 °C on a wide variety of biomass-related complex carbohydrates, including crystalline cellulose, hemicellulose, starch, and pectin, as well as on simple sugars.[16,17] The genome of *C. bescii* DSM 6725 has been sequenced,[18] and many CAZy (carbohydrate-active enzymes) related to starch utilization have been predicted: proteins with predominant hydrolytic activity which are able to cleave $\alpha$-1,4-linkages, $\alpha$-1,6-linkages, or both types of linkages.

In this study we identified the gene for MTase from *C. bescii* and used the maltosyl transfer activity of MTase to synthesize new, highly soluble piceid derivatives. The transglycosylation reaction was successfully conducted with piceid as the acceptor and maltotriose as the donor. The molecular structure of the major transglycosylation product of piceid was determined, and its water solubility was examined.

## MATERIALS AND METHODS

**Chemicals and Reagents.** *trans*-Piceid, resveratrol, and maltooligosaccharides were purchased from Sigma Chemical Co. (St. Louis, MO). Water and methanol (high-performance liquid chromatography (HPLC)-grade) were purchased from Burdick & Jackson (Morristown, NJ)

Received: May 17, 2012
Revised: July 22, 2012
Accepted: July 23, 2012
Published: July 23, 2012





dx.doi.org/10.1021/jf302127a | J. Agric. Food Chem. 2012, 60, 8183−8189

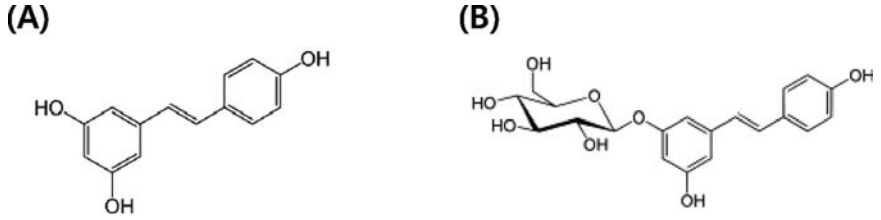


**Figure 1.** Chemical structures of resveratrol (A) and piceid (B).

for purification of transfer products. All other chemicals were of reagent grade and purchased from Sigma.

**Construction of MTase Gene Expression Vector in *Escherichia coli*.** To detect a potential MTase gene, *T. maritima* ORF TM0767 encoded MTase was compared with other genes through a BLAST search. *T. maritima* ORF TM0767 was highly homologous (94%) to a putative MTase gene (*cbes0143*) from *C. bescii* DSM 6725. Polymerase chain reaction (PCR) amplification of *cbes0143* was performed with *C. bescii* DSM 6725 genomic DNA as a template, using Takara prime STAR polymerase and the *cbes0143*-specific primers Cbes0143-Nco1-F (5′-AGAACCATGGCAGCTTTAAAA-AAGCTTGTT-3′) and Cbes0143-Xho1-R (5′-AGTGCCAATCTC-GAGTTTTACCTTTA-3′), which respectively contained *Nco*I and *Xho*I restriction enzyme sites (underlined). The *cbes0143*-specific internal primers were designed on the basis of the *C. bescii* putative MTase nucleotide sequence. The PCR conditions were as follows: 30 cycles at 98 °C for 10 s (denaturation), 55 °C for 5 s (annealing), and 72 °C for 2 min 20 s (extension). The 2084 bp product was purified with a PCR purification kit and then digested with *Nco*I and *Xho*I. This was then ligated into the expression vector pET24dBam (Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA) and digested with the corresponding restriction enzymes to generate pET24dBam-*cbes0143*. The nucleotide sequence of the PCR-generated gene was determined using a BigDye Terminator Cycle Sequencing Kit for ABI 3700 PRISM (Perkin-Elmer, Norwalk, CT). The resulting recombinant plasmid pET24 dBam-*cbes0143* was transformed into *E. coli* strain BL21 (DE3).

**Expression and Purification of Recombinant MTase.** The recombinant *E. coli* BL21(DE3) harboring pET24dBam-*cbes0143* was grown in Luria−Bertani (LB) medium containing kanamycin (30 μg/mL) overnight at 37 °C for small-scale culture. The culture was then transferred into fresh LB medium (1:100 dilution) at 37 °C, and the T7 promoter of the plasmid was induced with 0.1 mM isopropyl 1-thio-β-D-galactoside (IPTG) at an optical density (600 nm) of 0.5−0.6 for protein expression. After 4 h of IPTG induction, the cells were harvested by centrifugation at 8000*g* for 20 min at 4 °C and resuspended in 20 mM sodium phosphate buffer (pH 7.4). The harvested cells were disrupted by a 3-fold passage through a French pressure cell (American Instruments, Inc., Silver Spring, MD). The crude cell extract was centrifuged at 12000*g* for 30 min at 4 °C to collect the soluble fraction. The fraction was heat-treated at 65 °C for 30 min followed by centrifugation to remove thermolabile host proteins. The supernatant fraction was filtered with a 0.45 μm syringe filter (Sartorius, Goettingen, Germany) and then subjected to nickel−nitrilotriacetic acid (Ni−NTA) affinity chromatography (Qiagen, Hilden, Germany) equilibrated with 20 mM sodium phosphate (pH 7.4) including 0.5 M NaCl. After the column was washed with washing buffer (20 mM sodium phosphate, 0.5 M NaCl, pH 7.4, 40 mM imidazole), elution was carried out with elution buffer (20 mM sodium phosphate, 0.5 M NaCl, pH 7.4, 200 mM imidazole). Vivaspin 6 (GE Healthcare, Freiburg, Germany) was used to remove excess imidazole and concentrate the MTase in the protein solution. The purified enzymes were analyzed by 10% (w/v) sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE). SDS−PAGE was performed as described by Laemmli.[19] The protein concentration was determined using the Bradford method.[20]

**MTase Assay.** The MTase transglycosylation activity was determined as mentioned previously with minor modification by measuring the amount of maltooligosaccharides (≥G12) produced during incubation with maltotriose.[21] The standard assay mixture (0.1 mL) was assayed at 70 °C for 2 h in McIlvaine buffer (pH 6.0) containing 25 mM maltotriose as a substrate with 1.25 nM MTase. After enzyme reaction, the enzyme samples were mixed with 1 mL of diluted 0.2% Lugol solution ($I_2$/KI, 150 mg/mL) at room temperature, and then the reaction mixtures were placed on ice for 10 min. Color development was measured at 480 nm, and the specific activity was calculated using soluble starch as a standard. One unit was defined as the amount of enzyme that caused an increase in absorbance of 1.0 in 1 min under the standard assay condition. The influence of pH on the activity of MTase was measured at 70 °C in McIlvaine buffer at pH values from 4.0 to 8.0. The pH of each McIlvaine buffer was adjusted at 70 °C by using the standard enzyme assay conditions described above. The influence of temperature on the activity of MTase was examined in McIlvaine buffer (pH 6.0) in the range from 50 to 80 °C. All enzyme assays were performed by preincubation for 10 min before addition of enzyme. The substrate solution in McIlvaine buffer (pH 6.0) containing 25 mM maltooligosaccharides (G1−G7) was preincubated at 70 °C for 10 min, then MTase ($4 \times 10^{-4}$ U) was added, and the incubation of the reaction mixture (1 mL) was continued for 1 h. The reaction was stopped by boiling for 30 min and placing the mixture tube in ice. The amount of transfer products was determined by thin-layer chromatography (TLC) analysis.

**Enzymatic Synthesis of Piceid Transglycosylation Products.** The substrate solution in McIlvaine buffer (pH 6.0) containing 14 mM piceid and 6 mM maltotriose was preincubated at 70 °C for 10 min, then MTase ($4 \times 10^{-4}$ U) was added, and the incubation of the reaction mixture (1 mL) was continued for 10 min. The reaction was stopped by boiling for 30 min and placing the mixture tube in ice. The amount of transfer products was determined by TLC analysis.

**Purification of Piceid Transglycosylation Products.** The transfer products were separated by a $C_{18}$-T cartridge (100 mg/mL, Strata) and recycling preparative HPLC instrument equipped with a UV detector (JAI, Tokyo, Japan). A $C_{18}$-T cartridge, previously activated using methanol and water, was used to absorb the piceid glucosides in the transglycosylation reaction mixture and to remove any maltooligosaccharides and salts. The transglycosylation reaction mixture was filtered by a 0.45 μm syringe filter (Sartorius) and subjected to a $C_{18}$-T cartridge. After two washings, elution of transfer products was carried out with methanol. The main transfer products in methanol were purified using a combination W-252/W-251 polymeric gel filtration column (2 cm × 50 cm, JAI) in the recycling preparative HPLC instrument. The mobile phase was 100% methanol at a flow rate 3 mL/min. The fractions corresponding to the detected peaks were collected and freeze-dried. The purity of each sample was confirmed using TLC analysis.

**TLC Analysis.** The purified transfer products were spotted on Whatman K5F silica gel plates (Whatman, Maidstone, U.K.) activated at 110 °C for 30 min. The plates were developed in developing solution composed of 1-butanol/ethanol/water (5:3:2, v/v/v) for piceid glucosides or 1-propanol/ethyl acetate/water (6:1:3, v/v/v) for maltooligosaccharides. The developed TLC plates were dried completely at room temperature after one or two irrigations and visualized using a UV lamp in combination with a UV viewing box (Camag, Muttenz, Switzerland) at 254 nm. The maltooligosaccharides and carbohydrates were visualized by dipping in a solution containing 0.3% (w/v) *N*-(1-naphthyl)ethylenediamine and 5% (v/v) $H_2SO_4$ in methanol followed by heating at 110 °C for 10 min.

**Nuclear Magnetic Resonance Analysis.** The $^{1}H$ and $^{13}C$ nuclear magnetic resonance (NMR) spectra of piceid and purified maltosyl piceid were obtained with a Varian Inova AS 400 MHz NMR spectrometer (Varian, Palo Alto, CA). The sample was dissolved in DMSO-$d_6$ at 24 °C with tetramethylsilane (TMS) as the chemical shift reference. $^{1}H$ NMR (400 MHz, $CD_3OD$, $\delta_H$): 7.35 (2H, d, $J$ = 8.4 Hz, H-2′,6′), 6.99 (1H, d, $J$ = 16.4 Hz, H-$\beta$), 6.83 (1H, d, $J$ = 16.4 Hz, H-$\alpha$), 6.76 (1H, d, $J$ = 2.0 Hz, H-2), 6.75 (2H, d, $J$ = 8.4 Hz, H-3′,5′), 6.61 (1H, t-like, $J$ = 2.0 Hz, H-6), (1H, t-like, $J$ = 2.0 Hz, H-4), 5.21 (1H, d, $J$ = 4.0 Hz, H-1‴), 5.14 (1H, d, $J$ = 4.4 Hz, H-1⁗), 4.91 (1H, d, $J$ = 7.6 Hz, H-1″), 3.87−3.42 (18H, m, sugar moieties). $^{13}C$ NMR (100 MHz, $CD_3OD$, $\delta_C$): 160.4 (C-3), 159.6 (C-5), 158.5 (C-4′), 141.4 (C-1), 130.3 (C-1′), 130.0 (C-$\beta$′), 128.9 (C-2′), 126.6 (C-$\alpha$′), 116.5 (C-3′), 108.4 (C-6), 107.0 (C-4), 104.1 (C-2), 102.9 (C-1⁗), 102.7 (C-1″), 102.3 (C-1‴), 81.4 (C-4″), 81.0 (C-4‴), 77.8 (C-3″), 76.8 (C-5″), 75.1 (C-3‴), 74.9 (C-3⁗), 74.6 (C-5⁗), 74.3 (C-2⁗), 74.3 (C-2‴), 73.8 (C-2″), 73.4 (C-5‴), 71.5 (C-4⁗), 62.7 (C-6‴), 62.2 (C-6″), 62.1 (C-6⁗).

**α-Glucosidase Hydrolysis.** The purified transfer product was dissolved in McIlvaine buffer (pH 5.0). *Sulfolobus acidocaldarius* α-glucosidase (0.5 μg) was added to the solution containing 10 mM maltosyl piceid and the resulting solution incubated at 70 °C for 30 min. The reaction mixture was analyzed by TLC.

**Solubility Determination.** Excess piceid or piceid glucosides were suspended in 1 mL of distilled water in an Eppendorf tube at 25 °C. A JAC-4020 ultrasonic cleaner (Kodo, Hwaseong, Korea) was used to maximize the solubility of the piceid glucosides. After sonication at room temperature for 1 h with intermittent pauses, the sample was centrifuged at 12000*g* for 20 min. The supernatant of each sample was filtered through a 0.45 μm membrane filter (Millipore, Billerica, MA). The concentration of the compound in the supernatant, which is defined as water-soluble, was estimated by measuring its absorbance at 280 nm using a Gene Quant pro UV/vis spectrophotometer (Amersham Biosciences, Buckinghamshire, U.K.), and the absolute solubilities were calculated.

## RESULTS AND DISCUSSION

**Purification of Recombinant MTase.** The glycosyltransferase, designated MTase, of the *C. bescii* DSM 6725 was expressed in *E. coli* BL21 (DE3) cells harboring pET24 dBam-*cbes0143*. MTase was efficiently purified through heat treatment and Ni−NTA chromatography. Crude cell-free extract was heat-treated at 65 °C for 30 min to remove thermolabile host proteins, and then the heat-stable proteins were filtered and loaded onto a Ni−NTA affinity column. The size of the purified MTase estimated by SDS−PAGE was approximately 80 kDa, which is close to the expected molecular mass deduced from the amino acid sequence of *C. bescii* MTase (Figure 2). The yield of purified MTase was about 17.2%.

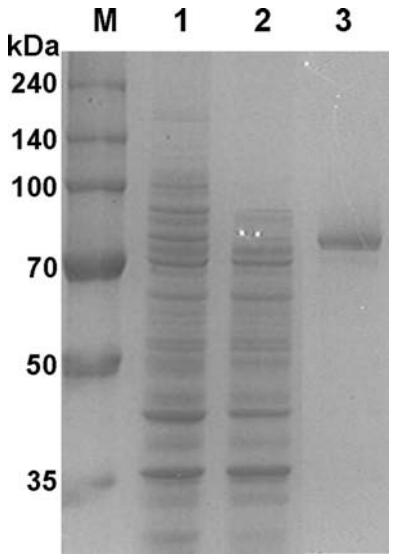


**Figure 2.** SDS−PAGE analysis of the purified MTase: lane M, protein size standards; lane 1, cell-free extracts of MTase; lane 2, proteins after heat treatment; lane 3, proteins after Ni−NTA affinity chromatography.

**Enzyme Properties of MTase.** To optimize transglycosylation conditions for synthesis of piceid glucosides, the pH range at which the recombinant MTase was active was determined using maltotriose as the substrate. The maximum activity was observed at pH 6.0. More than 80% of the maximum activity was obtained in the range between pH 4.5 and pH 7.0 (Figure 3A). The optimal temperature of MTase was 72 °C, and the activity was sharply decreased above 75 °C (Figure 3B). The effect of dimethyl sulfoxide (DMSO) on MTase activity was investigated because DMSO is normally used to dissolve the polyphenols, including resveratrol derivatives, because of their low water solubility. The transglycosylation reaction of MTase was not affected by a concentration of 5% DMSO in the reaction mixture, and more than 80% of the

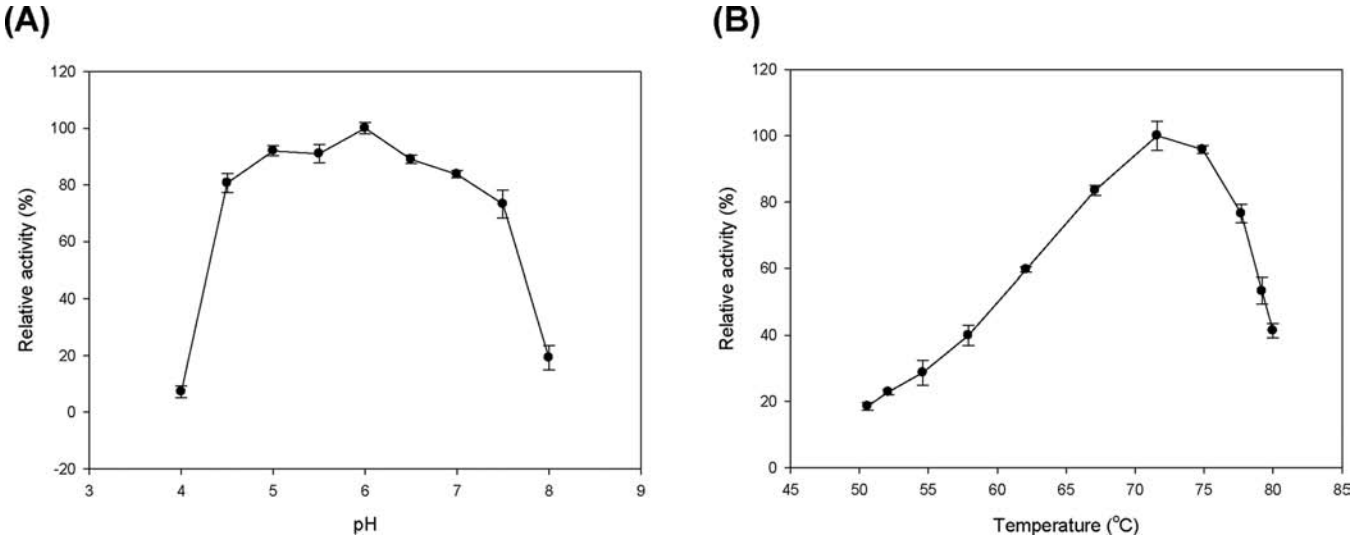


**Figure 3.** Effects of pH (A) and temperature (B) on the transglycosylation activity of MTase.


dx.doi.org/10.1021/jf302127a | J. Agric. Food Chem. 2012, 60, 8183−8189

maximum activity was maintained in the range between 5% and 15% DMSO. When more than 20% DMSO was present in the reaction mixture, MTase activity was reduced to below 50% of the maximum (data not shown). According to these results, the transglycosylation reaction was carried out at 70 °C and pH 6.0.

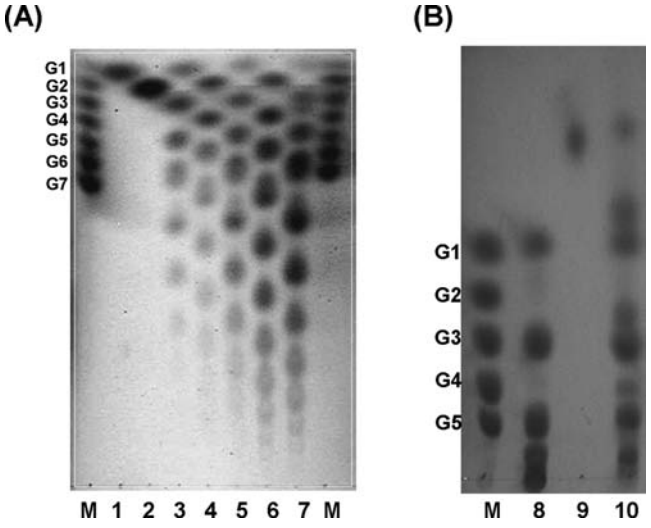


**Figure 4.** TLC analysis of the reaction products of MTase with maltooligosaccharide substrates (A) and piceid (B): lane M, maltooligosaccharide standard solution containing glucose (G1) through maltoheptaose (G7) (10 mM each); lanes 1−7, reaction products produced from the substrates glucose, maltose, maltotriose, maltotetraose, maltopentaose, maltohexaose, and maltoheptaose, respectively; lane 8, reaction products with maltotriose; lane 9, piceid; lane 10, reaction products with maltotriose and piceid.

Approximately 1.5% DMSO was used to increase the solubility of the piceid as an acceptor for synthesis of piceid glucosides.

MTase is known for having unique substrate specificity strictly to transfer maltosyl units and disproportionate maltooligosaccharides to form a set of maltosyl transfer products.[21] To confirm the mode of action of MTase, unbranched maltooligosaccharides (G1−G7) were used to examine the transglycosylation products. As shown in Figure 4A, MTase disproportionated maltooligosaccharides with degree of polymerization (DP) $X$ to products with a DP of $X \pm 2n$ (with $X \geq 3$ and $n = 0, 1, 2, ...$). Even-numbered substrates gave rise to only even-numbered oligosaccharides, and odd-numbered substrates gave rise to only odd-numbered products. This result indicates that MTase from *C. bescii* is indeed a MTase that transfers maltosyl units exclusively.

**Transglycosylation of Piceid by MTase.** MTase was reacted with piceid in the presence of maltotriose as a donor molecule. On the TLC plate, the spots corresponding to piceid were diminished, whereas other spots, possibly newly produced piceid glucosides, appeared after the MTase reaction (Figure 4B). These spots were detected under UV light (data not shown), implying that they originated from piceid, since piceid was the only chromophore in the reaction. Extra spots corresponding to odd-numbered maltooligosaccharides came from the disproportionation of maltotriose, as was confirmed in the reaction of MTase without piceid, which produced glucose, maltotriose, maltopentaose, and longer maltooligosaccharides (Figure 4B). TLC analysis of the MTase reaction with maltotriose and piceid suggested that the maltosyl transfer activity of MTase was successfully used to produce the piceid glucosides. Other polyphenols such as aesculin, arbutin, genistin, and

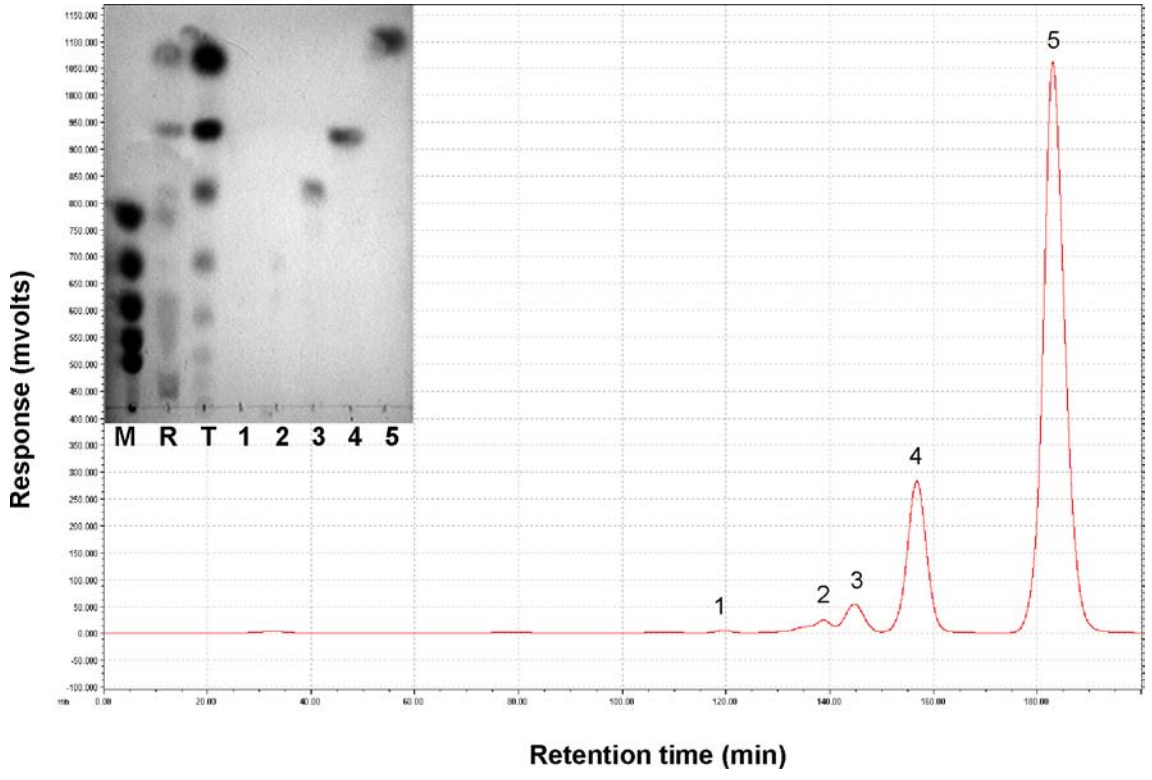


**Figure 5.** TLC and recycling preparative HPLC analysis of the piceid transfer products by the transglycosylation of MTase. The inset shows the purified transfer product. Inset: M, maltooligosaccharide standard containing glucose (G1) through maltopentaose (G5); R, piceid and maltotriose reaction products; T, after sugar removal by a $C_{18}$-T cartridge; 1−4, four piceid transfer products; 5, unreacted piceid.


dx.doi.org/10.1021/jf302127a | J. Agric. Food Chem. 2012, 60, 8183−8189

salicin were also good acceptors for transglycosylation by MTase (data not shown). When the MTase of *T. maritima* was used to transglycosylate daidzin, the major daidzin glycoside transfer product was determined to be maltosyl daidzin.[15] Likewise, newly produced piceid glucoside products observed in the MTase transglycosylation reactions here were believed to be a set of multiples of maltosyl units attached to piceid (Figure 4B).

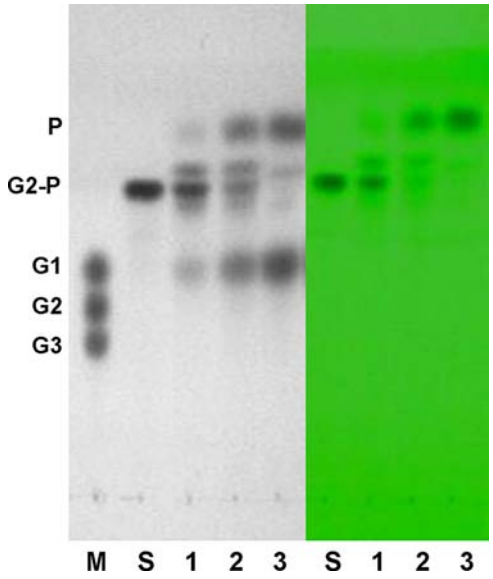


**Figure 6.** TLC analysis of the maltosyl piceid after $\alpha$-glucosidase treatment. The spots were visualized by (A) dipping solution and (B) UV at 254 nm. Lane M, maltooligosaccharide standard (G1, glucose; G2, maltose; G3, maltotriose); lane S, maltosyl piceid; lanes 1−3, enzyme treatment for 1, 5, and 10 min. P, piceid; G2-P, maltosyl piceid.

**Separation of Piceid Glucosides and Structural Analysis of the Major Piceid Glucoside.** The maltooligosaccharides included in the transglycosylation reaction mixture were first removed by a reversed-phase $C_{18}$-T column, and then the mixture of a series of piceid glycosides was separated by a W-251 and W-252 polymeric gel filtration column using a recycling preparative HPLC instrument. The presence of five piceid transglycosylation products was detected by a UV detector at 238 nm (Figure 5). TLC analysis of each peak fraction confirmed that each fraction was composed of a single compound. The molecular structures of the newly synthesized piceid glucosides were predicted on the basis of the transglycosylation mechanism of the MTase. The major compound among four piceid transfer products was isolated, and the molecular structure was confirmed by enzymatic digestion and $^{1}$H and $^{13}$C NMR analysis. $\alpha$-Glucosidase releases $\alpha$-glucose residues from the nonreducing end of a polysaccharide chain by the hydrolysis of the $\alpha$-1,4-glucosidic linkage. When the major transfer product was treated with $\alpha$-glucosidase, the compound was eventually broken down into piceid and glucose (Figure 6). This suggested that the glucose unit was attached to the glucose moiety of piceid and that the new linkage formed between the maltose and glucose of piceid would be an $\alpha$-1,4-glucosidic bond, because it was cleaved by $\alpha$-glucosidase. The detailed molecular structure of the major transfer product was identified

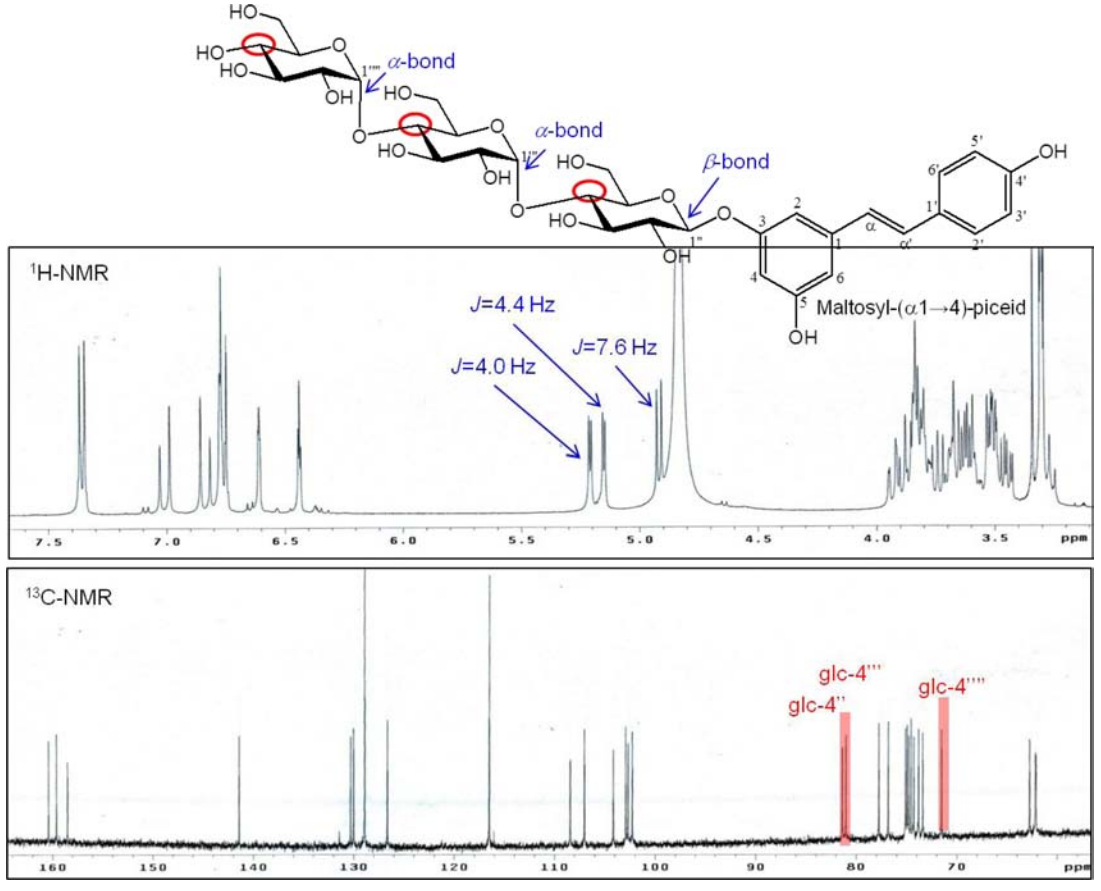


**Figure 7.** $^{1}$H and $^{13}$C NMR spectra of the major transfer product maltosyl piceid.


dx.doi.org/10.1021/jf302127a | J. Agric. Food Chem. 2012, 60, 8183−8189

using NMR. The $^{1}H$ and $^{13}C$ NMR spectra of the major transfer product were compared with those of piceid. The three anomer proton signals were detected, and the bonds between the three glucose molecules were determined to be $\alpha$-glucosidic linkages according to the coupling constant ($J$ = 4.0 and 4.4 Hz) in the $^{1}H$ NMR spectrum. The coupling constant value ($J$ = 7.6 Hz) of one anomeric proton signal indicates the $\beta$-linkage between the glucose moiety and aglycon resveratrol in piceid. The carbon signal of glc-4″, glc-4‴, and glc-4⁗ was 81.4, 81.0, and 71.5 ppm, respectively, for the major transfer product, confirming that the transferred maltosyl group was connected to C-4 of piceid (Figure 7). On the basis of the result, the major transfer product of piceid by MTase was defined to be an $\alpha$-D-maltosyl-(1→4)-piceid. Likewise, the other transfer products of piceid created by MTase were determined to be maltotetraosyl, maltohexaosyl, and maltooctaosyl piceid derivatives with $\alpha$-1,4-glucosidic linkage. The yield of maltosyl piceid was approximately 18.4% after the transglycosylation reaction for 1 h.

**Water Solubility of Maltosyl Piceid.** The solubility of maltosyl piceid in water was evaluated by comparing the water solubility of maltosyl piceid with those of resveratrol and piceid. The solubilities of resveratrol and piceid were determined to be 0.015 and 0.069 g/L, respectively. The water solubility of maltosyl piceid was 128.09 g/L, which was $8.54 \times 10^3$ and $1.86 \times 10^3$ times greater, respectively, than those of natural resveratrol and piceid. This implies that the attachment of a maltosyl residue to piceid by MTase greatly enhanced the water solubility of the original compound. This tendency is consistent with previous results obtained from the transglycosylation products of daidzin.[15] Although resveratrol and piceid have potent antitumor properties, serum levels of unmetabolized resveratrol are low because of poor absorption and ready metabolism to glucuronidated and sulfated compounds after oral or intraperitoneal administration.[22] To increase the potency of resveratrol, it is necessary to increase the amount of unmetabolized compound at the tumor site. A major obstacle to attaining this goal is the low solubility of the compound.

Much effort has been made to increase the solubility of natural polyphenols. One method has been to use cyclodextrin, which is widely employed as an excipient to increase the solubility and stability of drugs and chelate isoflavone. Gu[23] reported that a cyclodextrin−isoflavone complex increased solubility by 2−6 times.[23] However, the use of this complex is limited because the solutions are cloudy. Enzymatic synthesis using MTase is highly applicable for producing natural compound analogues with improved solubility. Furthermore, the $\alpha$-1,4-glycosidic linkage formed in the transglycosylation reaction is easily hydrolyzed by various glycosyl hydrolases, such as $\alpha$-glucosidase, as seen in Figure 6, implying that the human body metabolizes piceid glucosides in the same way as piceid or resveratrol itself. This suggests that the bioavailability of piceid glucosides would not change greatly. Therefore, enzymatic glycosylation of biologically active compounds such as polyphenols will be an alternative to pre-existing lipophilic forms to increase bioavailability and delay metabolism.

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### Funding

This work was supported, in part, by the Marine and Extreme Genome Research Center Program of the Ministry of Land, Transportation and Maritime Affairs and by grants from the Next-Generation BioGreen 21 Program (PJ008069), Rural Development Administration, Republic of Korea.

### Notes

The authors declare no competing financial interest.


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