Pure & Appl. Chem., Vol. 69, No. 4, pp. 675–683, 1997.
Printed in Great Britain.

Improvement of taste of natural sweeteners

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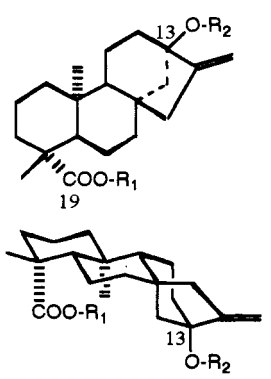
Abstract: Toward improvement of sweetness, studies on transglycosylation of steviol glycosides, stevioside from *Stevia rebaudinana*, rubusoside from *Rubus suavisimus* and their derivatives have been extensively carried out by a variety of enzyme systems. Together with rubusoside, a number of minor diterpene glycosides were isolated from leaves of *R. suavissimus*, being significant in view of structure-taste relationship of diterpene glycosides of this series. Monoglucuronide of glycyrrhetinic acid (MGGR) was found to be more than 5 times sweeter than glycyrrhizin. Quantitative production of MGGR from glycyrrhizin was achieved by selective removal of the terminal glucuronide unit of glycyrrhizin by an enzyme from *Cryptococcus magnus* MG-27 (yeast).

STEVIOL GLYCOSIDES

Abundant resorces

From leaves of *Stevia rebaudiana* (Compositae), several sweet glycosides of steviol (= 13-hydroxy-*ent*-kaur-16(17)-en-19-oic acid) have been isolated (Fig. 1) (refs. 1-5). Of these, the major glycoside, stevioside is used as a sweetener for seasonings, pickles and salted foods in Japan. The second major glycoside named rebaudioside A which is sweeter and more delicious than stevioside, is utilized in beverages. The improved plant species which contains more than 10% of steviol glycosides, are cultivated in China and other southeastern Asian countries, and exported to Japan and Korea.



steviol $R_1 = R_2 = H$

glycoside	R_1	R_2	RS	QT
stevioside *	-Glc	-Glc-(2-1)-Glc	143	0
dulcoside-A *	-Glc	-Glc-(2-1)-Rha	nd	-2
rebaudioside-A *	-Glc	-Glc-(2-1)-Glc \(3-1)-Glc	242	+2
rebaudioside-C * (=dulcoside-B)	-Glc	-Glc-(2-1)-Rha \(3-1)-Glc	nd	-1
rebaudioside-D*	-Glc-(2-1)-Glc	-Glc-(2-1)-Glc \(3-1)-Glc	221	+3
rebaudioside-E *	-Glc-(2-1)-Glc	-Glc-(2-1)-Glc	174	+1
rubusoside **	-Glc	-Glc	114	-2
steviolbioside (Sb) ***	-H	-Glc-(2-1)-Glc	<50	-3
steviolmonoside (Sm) ***	-H	-Glc	<50	-3

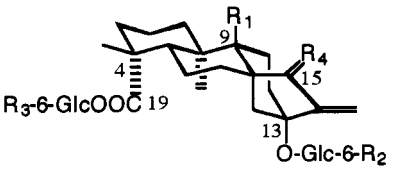
RS: relative sweetness to sucrose (on a weight basis), nd : not determined
QT: quality of taste, stevioside: 0, + better, - worse

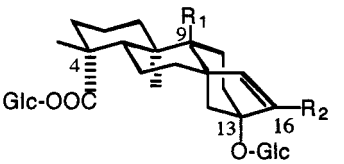
Fig. 1. Steviol glycosides from *Stevia rebaudiana** and *Rubus suavissimus*** and the derivatives ***

Abbreviation of sugar units: Fru : β-D-fructofuranoside, Gal : β-D-galactoside, α-Gal : α-D-galactoside, Glc : β-D-glucoside, α-Glc : α-D-glucoside, GlcA : β-D-glucuronide, Rha : α-L-rhamnoside

Very recently, long term toxicity test of stevioside including carcinogenesis was further conducted by a group organized by National Institute of Health Sciences, Japan, comfirming the safety.

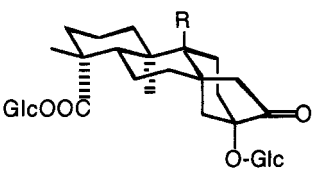
Rubus suavissimus (Rosaceae) grows in southern China (ref. 6). From leaves of this plant, a sweet steviol glycoside named rubusoside was isolated in a more than 5% yield (Fig. 1) (ref. 7). In our study on conversion of stevioside to rebaudioside A (ref. 8, 9), rubusoside had been prepared quantitively from stevioside by enzymic partial hydrolysis.



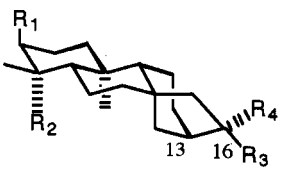


	R_1	R_2	R_3	R_4
++ suavioside-B	H	H	H	H_2
B suavioside-C1	OH	*p*-coumaroyl	H	H_2
N suavioside-C2	H	H	*p*-coumaroyl	H_2
N suavioside-D1	H	*p*-caffeoyl	H	H_2
N suavioside-D2	H	H	caffeoyl	H_2
++ 15β-OH-rubusoside	H	H	H	β-OH
++ 15-oxo-rubusoside	H	H	H	O
+++ suavioside-Q1	H	α-Glc	H	H_2
+++ suavioside-Q2	H	H	α-Glc	H_2
+++ suavioside-R1	H	α-Gal	H	H_2
+++ suavioside-R2	H	H	α-Gal	H_2
+ steviol-monoside	H	H	H	H_2

	R_1	R_2
+ suavioside-J	H	CH_2OH
N 9-OH-suavioside-J	OH	CH_2OH
+ suavioside-H	H	CHO
N 9-OH-suavioside-H	OH	CHO

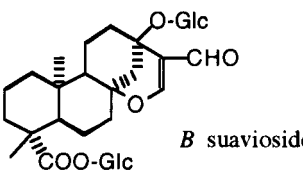


+ suavioside O R= H
N 9-OH-suavioside O R= OH

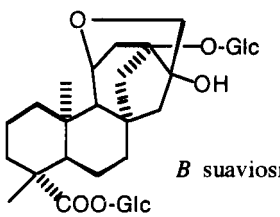


	R_1	R_2	R_3	R_4
N paniculoside-IV*	H	COO-Glc	CH_2OH	OH
+ suavioside-A	OH	CH_3	CH_2O-Glc	OH
N suavioside-E	H	COO-Glc	OH	CH_2OH
B suavioside-K	H	CH_2OH	CH_2O-Glc	OH
N sugeroside*	=O	CH_3	CH_2O-Glc	OH

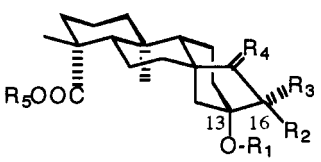
* first isolated from other plants



B suavioside-P



B suavioside-M



	R_1	R_2	R_3	R_4	R_5
B suavioside-F	Glc	CH_3	OH	H_2	H
+ suavioside-G	Glc	CH_3	OH	H_2	Glc
+ suavioside-I	H	CH_2OH	OH	H_2	Glc
+ suavioside-L	Glc	CH_2OH	H	H_2	Glc
B 15-oxo-suavioside-L	Glc	CH_2OH	H	O	Glc
B 15-oxo-16-epi- suavioside-L	Glc	H	CH_2OH	O	Glc
+ 16β-OH-suavioside-L	Glc	CH_2OH	OH	H_2	Glc
+ 16α-OH-suavioside-L	Glc	OH	CH_2OH	H_2	Glc

abbreviation for taste	
+++	very sweet (rubusoside: +++)
++	sweet
+	slightly sweet
N	tasteless
B	bitter

Fig. 2 Minor diterpene glycosides from leaves of *Rubus suavissimus*

A number of minor diterpene glycosides which are related to rubusoside, were isolated from the leaves (Fig. 2) (refs. 10, 11). On inspection of the taste of these glycosides, it was suggested that hydroxylation of the 16(17)-double bond (ref. 9) as well as C-9 (center of the skeleton) led to decrease or disappearance of sweetness. Acylation of sugar moiety also resulted in loss of sweetness. Recently, occurrence of anti-allergic ellagitannins in the leaves was reported and the extract of the leaves has attracted much attention as a health-giving food ingredient in Japan (refs. 12-15).

Trans-α-1,4-glucosylation by CGTase-starch system

Stevioside and rubusoside taste somewhat bitter, and show aftertaste. In order to improve of sweetness, modification of sugar moieties of both the glycosides were conducted by enzymic transglucosylation. Cyclomaltodextrin-glucanotransferase (CGTase) efficiently catalyzes intermolecular glycosylation to transfer α-glucosyl units from starch to 4-OH of a glucosyl moiety (trans-α-1,4-glucosylation) (ref. 16).

Stevioside was treated by this system, yielding a complex mixture of products which were mono-, di-, tri- and more glucosylated both at the 19-*O*-glucosyl unit and the terminal glucosyl unit of the 13-*O*-sophorosyl moiety (ref. 9). Separation and structure identification of all of the mono- (S1a and S1b), di- (S2a, S2b and S2c) and tri- (S3a, S3b, S3c and S3d) glucosylated products were achieved, and evaluation of intensity of sweetness and quality of taste were conducted (Fig. 3) (ref. 17).

Significant improvement in quality of taste was observed for most of the glucosylated products, especially for S1a and S2a which were mono- and di-glucosylated at the 13-*O*-sophorosyl moiety, respectively. Remarkable enhancement of intensity of sweetness was also observed for both the products, while glucosylation at the 19-*O*-glucosyl moiety resulted in decrease of intensity of sweetness.

Products S1a, S2a and S2c were also obtained from stevioside by pullulan and crude pullulanase from *Klebsiella* sp though yields were rather low (ref 18).

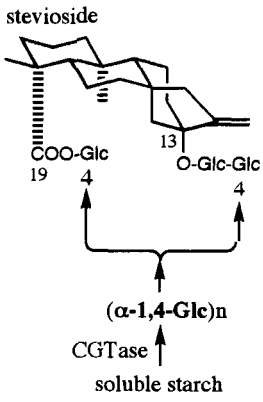
Rubusoside was also trans-α-1,4-glucosylated by the same enzyme system, and mono- (Ru1a and Ru1b), di- (Ru2a, Ru2b and Ru2c), tri- (Ru3a, Ru3b, Ru3c and Ru3d) and three of five tetra-glucosylated products (Ru4a, Ru4b and Ru4e) were separated and identified (Fig. 4) (refs. 19, 20). Two of tetraglucosylated products (Ru4c and Ru4d) were obtained as a mixture. As indicated in Fig. 4, strong enhancement of intensity of sweetness was observed for products (Ru2a, Ru3a, Ru3b and Ru4b) which were di- or tri-glucosylated at the 13-*O*-glucosyl moiety. On the other hand, tetraglucosylation at the 13-*O*-glucosyl moiety as well as glucosylation at the 19-*O*-glucosyl moiety led to decrease of intensity of sweetness.

These results strongly suggested that for enhancement of intensity of sweetness of steviol glycosides, the elongation of the 13-*O*-glucosyl moiety up to a total of four glucosyl units under suppression of glucosylation at 19-*O*-glucosyl moiety, may be desirable.

Shortening of long α-1,4-glucosyl chain of products by β-amylase β-Amylase hydrolyzes α-1,4-glucosyl chain from the non-reducing end to release maltose. By treatment with this enzyme, tri- and more α-1,4-glucosyl chains are converted into a mono- or di-α-1,4-glucosyl chain. Since decrease of sweetness was observed for products with a poly-α-1,4-glucosyl chain, treatment of glucosylated products with β-amylase resulted in the further improvement of sweetness. The transglucosylated stevioside is commercially sold after shortening the α-glucosyl chain by this treatment (ref. 9).

Selective syntheses of improved sweeteners (1) Trans-α-1,4-glucosylation of steviolmonoside (Sm) and steviolbioside (Sb) By alkaline saponification of 19-COO-β-Glc to 19-COOH, stevioside and rubusoside afforded steviolbioside (Sb) and steviolmonoside (Sm), respectively (Fig. 1). Transglucosylation of both the compounds by CGTase-starch system proceeded very slowly due to the low solubility in a buffer solution. It was disclosed that the solubility of both the compounds was remarkably increased in the

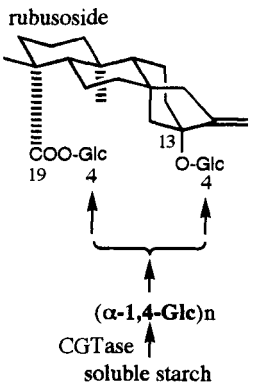
presence of γ-cyclodextrin (γ-CD) (Fig. 5). Trans-α-1,4-glucosylation of Sb and Sm by CGTase-starch system was carried out with the aid of γ-CD, and each product, after acetylation, was subjected to chemical β-glucosylation of 19-COOH to give a mixture of mono-, di-, tri- and more glucosylated stevioside or rubusoside (Fig. 5) (ref. 21). The reaction mixture was treated with β-amylase to give excellent sweeteners, S1a and S2a from Sb and Ru1a and Ru2a from Sm.



	19-*O*-glycosyl	13-*O*-glycosyl	RS	QT
S	-Glc	-Glc-Glc	160*	0
A	-Glc	-Glc-Glc \Glc	210*	+2
Ru	-Glc	-Glc	134*	-2
S1a	-Glc	-Glc-Glc-α-**Glc**	180	+4
S2a	-Glc	-Glc-Glc-α-**Glc-α-Glc**	205	+4
S3a	-Glc	-Glc-Glc-α-**Glc-α-Glc-α-Glc**	117	+3
S1b	-Glc-α-**Glc**	-Glc-Glc	133	+2
S2b	-Glc-α-**Glc**	-Glc-Glc-α-**Glc**	136	+1
S3b	-Glc-α-**Glc**	-Glc-Glc-α-**Glc-α-Glc**	146	0
S2c	-Glc-α-**Glc-α-Glc**	-Glc-Glc	136	0
S3c	-Glc-α-**Glc-α-Glc**	-Glc-Glc-α-**Glc**	150	+1
S3d	-Glc-α-**Glc-α-Glc-α-Glc**	-Glc-Glc	121	+3

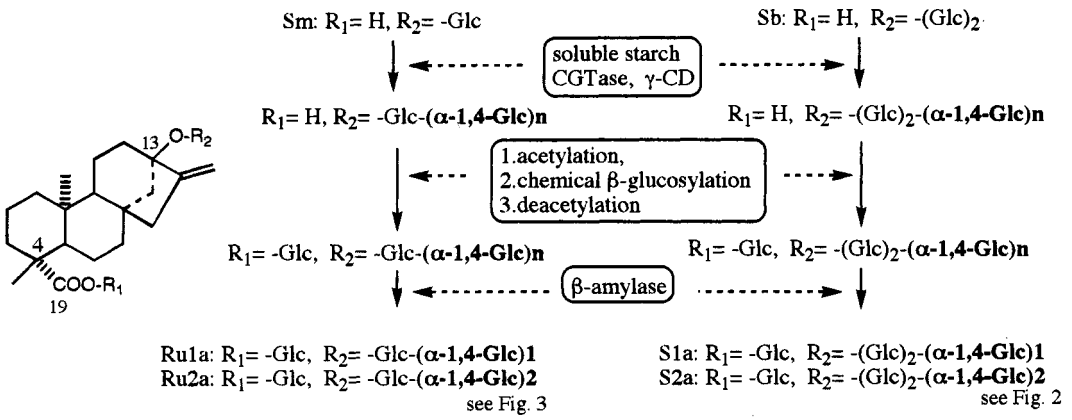
* Relative sweetness to sucrose (RS) was estimated by differnet panelers from those of Fig. 1, so that the value is not the same as that indicated in Fig. 1. A : rebudioside A, Ru : rubusoside
QT : quality of taste, stevioside: 0, + better, - worse

Fig. 3 Trans-α-1,4-glucosylation products from stevioside (S)



	19-*O*-glycosyl	13-*O*-glycosyl	RS
Ru1a	-Glc	-Glc-α-**Glc**	132
Ru2a	-Glc	-Glc-α-**Glc-α-Glc**	278
Ru3a	-Glc	-Glc-α-**Glc-α-Glc-α-Glc**	214
Ru4a	-Glc	-Glc-α-**Glc-α-Glc-α-Glc-α-Glc**	115
Ru1b	-Glc-α-**Glc**	-Glc	102
Ru2c	-Glc-α-**Glc**	-Glc-α-**Glc**	95
Ru3b	-Glc-α-**Glc**	-Glc-α-**Glc-α-Glc**	182
Ru4b	-Glc-α-**Glc**	-Glc-α-**Glc-α-Glc-α-Glc**	202
Ru2b	-Glc-α-**Glc-α-Glc**	-Glc	99
Ru3c	-Glc-α-**Glc-α-Glc**	-Glc-α-**Glc**	110
Ru4c	-Glc-α-**Glc-α-Glc**	-Glc-α-**Glc-α-Glc**	184
Ru4d	-Glc-α-**Glc-α-Glc-α-Glc**	-Glc-α-**Glc**	
Ru3d	-Glc-α-**Glc-α-Glc-α-Glc**	-Glc	58
Ru4e	-Glc-α-**Glc-α-Glc-α-Glc-α-Glc**	-Glc	49

Fig. 4 Trans-α-1,4-glucosylation products from rubusoside (Ru)



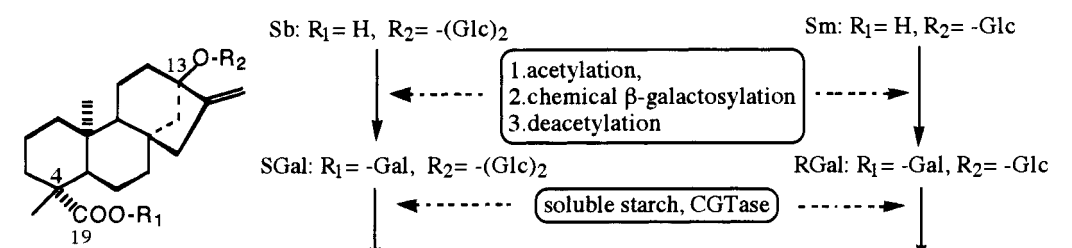
increase of solubility (mg/ml at 37°C) of Sm and Sb in acetate buffer (pH 5.4) by equimolar cyclodextrins (CD)		none	α-CD	β-CD	γ-CD
	steviolmonoside (Sm)	0.69	1.26	2.48	7.01
	steviolbioside (Sb)	0.21	0.28	0.90	> 10.0

Fig. 5 Trans-α-1,4-glucosylation of steviolbioside (Sb) and steviolmonoside (Sm)

Selective syntheses of improved sweeteners (2) Trans-α-1,4-glucosylation of 19-O-β-galactosyl esters

In the study on acceptor specificity of transglucosylation by CGTase-starch system, it was revealed that transglucosylation hardly occurs to galactose, mannose and ribose (ref. 22). The 19-*O*-glucosyl group of stevioside and rubusoside were chemically replaced by a β-D-galactosyl group (Fig. 5). Then, both the galactosyl esters (SGal and RGal) were subjected to trans-α-1,4-glucosylation by CGTase-starch sytem to give mono-, di-, tri- and tetra-glucosylated products; SGal-1, -2, -3 and -4 from SGal, and RGal-1, -2, -3 and -4 from RGal (ref. 23). Evaluation of sweetness is summarized in Fig. 6.

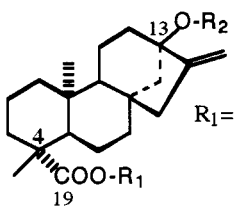
Structure-sweetness correlation was investigated for synthesized steviol glycosides, some of the glucosyl units of which were replaced by other sugar units (refs. 24, 25). Replacement of the 19-*O*-glucosyl group by a β-galactosyl group (SGal, RGal) led to change the taste for worse. Elongation of the 13-*O*-glycosyl moiety of SGal and RGal up to total of four glucosyl units led to improvement of the sweetness remarkably; SGal-1 and -2 from SGal, and RGal-1, -2 and -3 from RGal, while more transglucosylation resulted in change of taste for the worse (Fig. 6). The improved sweeteners, SGal-1 and -2 as well as RGal-1 and -2 were obtained by treatment of the reaction mixtures by β-amylase.



	RS	QT		RS	QT
SGal-1: R1= -Gal, R2= -(Glc)2-(α-1,4-Glc)1	236	+5	RGal-1: R1= -Gal, R2= -Glc-(α-1,4-Glc)1	167	-1
SGal-2: R1= -Gal, R2= -(Glc)2-(α-1,4-Glc)2	289	+4	RGal-2: R1= -Gal, R2= -Glc-(α-1,4-Glc)2	312	+3
SGal-3: R1= -Gal, R2= -(Glc)2-(α-1,4-Glc)3	143	-1	RGal-3: R1= -Gal, R2= -Glc-(α-1,4-Glc)3	203	+1
SGal-4: R1= -Gal, R2= -(Glc)2-(α-1,4-Glc)4	152	-3	RGal-4: R1= -Gal, R2= -Glc-(α-1,4-Glc)4	111	-3
SGal: R1= -Gal, R2= -(Glc)2	129	-2	RGal: R1= -Gal, R2= -Glc	104	-4
S: R1= -Glc, R2= -(Glc)2	143	0	Ru: R1= -Glc, R2= -Glc	114	-2

Fig. 6 Trans-α-1,4-glucosylation of β-D-galactosyl Esters

Selective syntheses of improved sweeteners (3) Protection of the 19-O-glucosyl group agaisnt trans-glucosylation For protection of appropriate position of the 19-*O*-glucosyl group against transglucosylation, transgalactosylation of rubusoside by a variety of α- (ref. 26) and β- (ref. 27) galactosidase has been investigated. It was found that treatment of rubusoside by *Bacillus circulans* β-glactosidase and lactose for 60 min afforded a desirable product (RGal-C1) which was formulated as 13-*O*-β-D-glucosyl-19-*O*-[β-D-galactosyl-(1-4)-β-D-glucosyl]-steviol (Fig. 6) together with a small amount of undesirable by-products. In this reaction, a yield of RGal-C1 reached maximum for nearly 60 min, and then decreased followed by increase of the by-products. RGal-C1 was subjected to trans-α-1,4-glucosylation followed by degalactosylation with β-galactosidase and subsequent treatment by β-amylase to give the improved sweeteners, Rula and Ru2a exclusively (Fig. 7) (ref. 27).



Ru: R_1= R_2= -Glc ⟶ RGal-C_1: R_1= -Glc-(4-1)-**β-Gal**, R_2= -Glc

lactose (Glc-(4-1)-**β-Gal**)
β-galactosidase for 60 min

soluble starch
CGTase

R_1= -Glc, R_2= -Glc-(**α-1,4-Glc**)**n** ⟵ R_1= -Glc-(4-1)-**β-Gal**, R_2= -Glc-(**α-1,4-Glc**)**n**

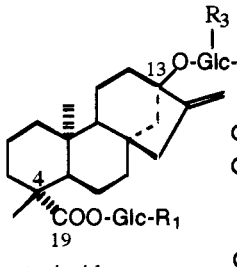
β-amylase ↓ β-galactosidase

Rula: R_1= -Glc, R2= -Glc-**α-Glc** (see Fig. 4)
Ru2a: R_1= -Glc, R2= -Glc-**α-Glc-α-Glc**

Fig. 7 Protection of 19-*O*-glucosyl group against transglucosylation

Transglycosylation by other enzyme systems

Trans-α-glucosylation by Biozyme L and glucosyltransferase from Streptococcus mutans Treatment of stevioside with maltose and Biozyme L (crude β-amylase preparation produced by *Aspergillus* spp.) afforded three products, A, B and C which were formulated as shown in Fig. 8, respectively (ref. 18). The relative intensity of sweetness of A and B was less than that of stevioside, while a remarkable improvement of quality of taste was observed for A. Product C tasted bitter. Treatment of stevioside with sucrose and glucosyltransferase from *Streptococcus mutans* (sero-type C) afforded A selectively in a better yield than that by the Biozyme L-maltose system.



stevioside
R_1= R_2 = R_3= H

○ maltose, Biozyme L (crude β-amylase from *Aspegillus* spp.) ⟶ A, B, C

A : R_1= -(6-1)-α-Glc, R_2= R_3= H RS: 110, QT: +2
B : R_1= R_3= H, R_2= -(6-1)-α-Glc RS: 40, QT: +1
C : R_1= R_3= H, R_2= -(3-1)-α-Glc bitter

○ sucrose, glucosyltransferase from *Streptococcus mutans* (sero-type C) ⟶ A

○ starch hydrolysate, dextrin dextranase from *Acetobacter capsulatus* ATCC 11894 ⟶ SG1a (= B), SG1b, SG2

SG1b : R_1= R_2= H, R_3= -(6-1)-α-Glc, SG2 : R_1= H, R_2= R_3= -(6-1)-α-Glc

○ curdlan (β-1,3-glucan), enzyme from *Streptomyces* sp. W19-1 ⟶ D, E, F

D : R_1= -(3-1)-β-Glc, R_2= R_3= H, E: R_1= R_3= H, R_2= -(3-1)-β-Glc
F : R_1= -(3-1)-β-Glc-(3-1)-β-Glc, R_2= R_3= H

○ crudlan, *Actynomycete*, strain K-128 ⟶ R_1= R_3= H, R_2= -(6-1)-β-Glc

Fig. 8 Transglucosylation of stevioside by various glucosidases

Trans-α-1,6-glucosylation by dextrin dextranase On treatment with starch hydrolysate (by isoamylase) and dextrin dextranase produced by *Acetobacter capsulatus* ATCC 11894, stevioside yielded three products tentaively designated as SG1a, SG1b and SG2 in remarkably high yields. Of these products, SG1a was identical with product B (*vide supra*, Fig. 8). SG1a and SG2 were respectively formulated as illustrated in Fig. 8 (ref. 28). Relative intensity of sweetness of these compounds are significantly lower than stevioside.

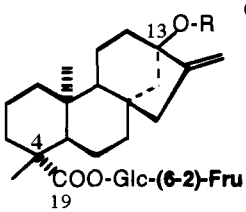
Trans-β-1,3-glucosylation Stevioside was treated with curdlan and the enzyme from *Streptomyces* sp. W19-1 to give three β-glucosylated products, D : 13-*O*-β-sophorosyl-19-*O*-β-laminaribiosyl-steviol, E : 13-*O*-[β-glucosyl-(1-3)-β-glucosyl-(1-2)-β-glucosyl]-19-*O*-β-glucosyl-steviol and F : 13-*O*-β-sophorosyl-19-*O*-β-laminaritriosyl-steviol (Fig. 8) (ref. 29). Improvement of quality of taste was noted for D and F as well as a whole mixture of the products, while the relative intensity of sweetness of these products was lower than stevioside. Evaluation of sweetness of E has not been conducted due to its low yield.

Treatment of stevioside by 1,3-glucan and an enzyme from *Streptomyces* sp. DIC-108 yielded rebaudioside A (Fig. 1), the better sweetener (Patent : JapanTokkyo Koho (B2) H6-73468, 1994).

Trans-β-1,6-glucosylation by cultivation with Actynomycete strain K-128 An *Actinomycete* strain K-128 isolated from soil was cultured in a medium containg stevioside and curdlan to give a trans-β-1,6-glucosylated product, 13-[β-glucosyl-(1-6)-β-glucosyl-(1-2)-β-glucosyl]-19-O-β-glucosyl-steviol (Fig. 8) (ref. 30). The evaluation of taste was not described.

Trans-β-2,6-fructofuranosylation Incubation of stevioside and rubusoside with sucrose and β-fructofuranosidase from *Arthrobactor* sp. K-1 afforded a product trans-β-2,6-fructofuranosylated at the 19-*O*-glucosyl moiety in a high yield (Fig. 9); S-F from stevioside and Ru-F from rubusoside (ref. 31). Relative intensity of sweetness was not enhanced, however, significant improvement of quality of taste was observed for both the compounds. Rebaudioside A was subjected to trans-β-2,6-fructofranosylation by sucrose and β-fructofuranosidase from *Microbacterium* sp. H-1 to give a product, RA-F which is β-2,6-fructofuranosylated at the 19-*O*-glucosyl group (Fig. 9) (ref. 32). In this case, relative intensity of sweetness and quality of taste were not significantly improved. It is noteworthy that the fructofuranosyl linkage is rather unstable, being hydrolyzed on standing in foods.

Treatment of S-F by CGTase-starch system yielded trans-α-1,4-glucosylated products (S-F-α-Glc) at the 13-*O*-sophorosyl moiety (ref. 12). Significant improvement both in relative intensity of sweetness and quality of taste was noted for the di-α-glucosylated product.



○ sucrose, β-fructofuranosidase from *Arthrobactor* sp. K-1

stevioside (S) ⟶ S-F : R= -(Glc)$_2$, RS: similar to S, QT: remarkably improved

↓ soluble starch, CGTase

S-F-α-Glc : R= -(Glc)2-(α-1,4-Glc)n RS, QT : greatly improved

rubusoside (Ru) ⟶ Ru-F: R= -Glc, RS: similar to Ru, QT: improved

○ sucrose, β-fructofuransidase from *Microbacterium* sp. H-1

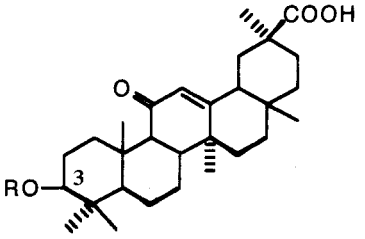
rebaudioside-A ⟶ RA-F: R= -(Glc)$_3$, RS: similar to RA, QT: litlle improved

Fig. 9 Trans-β-2,6-fructofuranosylation of Steviol Glycosides

GLYCOSIDES OF GLYCYRRHETINIC ACID

Relationship between structures of sugar moieties and sweetness

The major sweet principle of licorice root, glycyrrhizin [G, glucuronobioside of glycyrrhetinic acid (GA), content : nearly 4%], has been used as a sweetener and a flavor enhancer in foods and also as a medicine. Structure-sweetness relationship of glycyrrhizin derivatives has been studied (refs. 33, 34). As shown in Fig. 10, in this series, glycosides of a monosaccharide are generally sweeter than those of oligosaccharides, and sweetness varies depending on the type of bound sugar units. It was revealed that monoglucuronide of GA (MGGR) showed the intense sweetness relative to sucrose: x 941, being more than 5 times sweeter than G (ref. 33). This intense sweetener has already been isolated from rat liver as a metabolic intermediate of G (ref. 35) though the sweetness had not been reported.



glycyrrhetinitc acid (GA) R= H

	R	RS	QT*
glycyrrhizin (G)	-GlcA-(2-1)-GlcA	170	aftertaste
MGGR	-GlcA	941	less aftertaste
GA-Glc	-Glc	218	less aftertaste
GA-Gal	-Gal	110	less aftertaste
GA-Xyl	-β-xylopyanosyl	544	less aftertaste
GA-Ara	-α-L-arabinopyranosyl	33	more bitter
GA-Cell	-Glc-(4-1)-Glc	71	less aftertaste
MGGR-Glc	-GlcA-(4-1)-α-Glc	150	less aftertaste, better
MGGR-2Glc	-GlcA-[(4-1)-α-Glc]2	119	less aftertaste, better
G-Glc	-(GlcA)2-(4-1)-α-Glc	48	less aftertaste

* quality of taste relative to glycyrrhizin

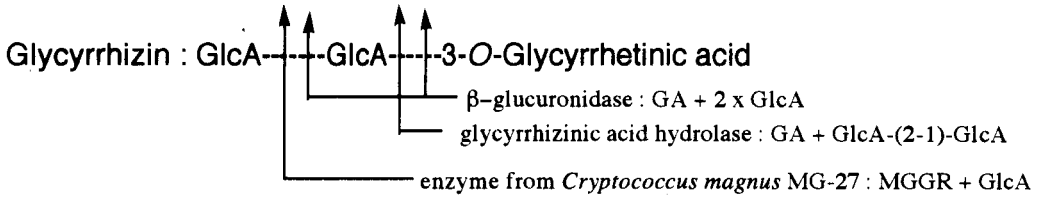


Fig. 10 Sweetness of glycosides of glycyrrhetinic acid (GA) and enzymic hydrolysis of glycyrrhizin (G)

Selective production of MGGR from glycyrrhizin

As to the enzymic hydrolysis of G, treatment of G with usual β-glucuronidase yielded GA and two mol of glucuronic acid, while an enzyme named glycyrrhizinic acid hydrolase from *Aspergillus niger* selectively hydrolysed G to yield GA and glucuronobiose (ref. 36, 37). Microorganisms which have potent activity to hydrolyze the terminal glucuronide unit of G, have been searched in soil collected in the factory of extraction of licorice roots. It was revealed that enzyme from an yeast identified as *Cryptococcus magnus* MG-27 selectively hydrolyzed the terminal glucuronide likage of G to yield MGGR and one mol of glucuronic acid almost quantitatively (ref. 38). By this enzyme, MGGR is now produced in an industrial scale, and commercially used as an improved sweetener and a flavor enhancer. Acute toxicity of MGGR was determined as LD50 > 5000mg/kg (*p.o.* in mice), and it showed no mutagenicity (by umu-test).

The potent inhibitory effect against two stage carcinogenesis was observed for GA and G. A new improved sweetener, MGGR exhibited stronger inhibitory effect against two stage skin carcinogenesis in mice and also against plumonary tumorigenesis in mice than GA and G (ref. 39).

REFERENCES

1. E. Mosettig, U. Beglinger, F. Dolder, H. Lichiti, P. Quitt and J. A. Waters. *J. Am. Chem. Soc.* **85**, 2305 (1963).
2. H. Kohda, R. Kasai, K. Yamasaki and O. Tanaka. *Phytochemistry* **15**, 981 (1976).
3. I. Sakamoto, K. Yamasaki and O.Tanaka. *Chem. Pharm. Bull.* **25**, 844 (1977).
4. I. Sakamoto, K. Yamasaki and O.Tanaka. *Chem. Pharm. Bull.* **25**, 3437 (1977).
5. M. Kobayashi, S. Horikawa, I. Degradi, J. Ueno and H. Mitsuhashi. *Phytochemistry* **16**, 1405 (1977).
6. S. Lee. *Guihaia* **1**, 17 (1981).
7. T. Tanaka, H. Kohda, O. Tanaka, F. Chen, W. Chou and J. Leu. *Agric. Biol. Chem.* **45**, 2165 (1981). In this paper, the plant was tentatively assigned as *R. chingii* Hu. However, later on, it was amended as shown in the text.
8. N. Kaneda, R. Kasai, K. Yamasaki and O. Tanaka. *Chem. Pharm. Bull.* **25**, 2466 (1977).
9. R. Kasai, N. Kaneda, O. Tanaka, K. Yamasaki, I. Sakamoto, K. Morimoto, S. Okada, S. Kitahata, H. Furukawa. *Nippon Kagakukaishi*, **1981**, 726.
10. S. Hirono, W. Chou, R. Kasai, O. Tanaka and T.Tada. *Chem. Pharm. Bull.* **38**, 1743 (1990).
11. K. Ohtani, Y. Aikawa, R. Kasai, W. Chou, K. Yamasaki and O. Tanaka. *Phytochemistry* **31**, 1553 (1992).

12. K. Hirai. *Jap. Food Sci.* **1995** (1), 57.
13. K. Nakahara and T. Tanaka. *Jap. Food Sci.* **1995** (10), 44.
14. K.Nakahara. *Food Chemicals* **1955** (9), 44.
15. Y. Ishikura, Y. Suwa, T. Okada and S. Kohno. *Ensho* **15**, 167 (1995).
16. The Amylase Research Society of Japan, ed. *Handbook of Amylases and Related Enzymes*, pp. 154-164. Pergamon Press, Oxford, U. K. (1988).
17. Y. Fukunaga, T. Miyata, N. Nakayasu, K. Mizutani R. Kasai and O. Tanaka. *Agric. Biol. Chem.* **53**, 1603.
18. S. V. Lobov, R. Kasai, K. Ohtani, O. Tanaka and K. Yamasaki. *Agric. Biol. Chem.* **55**, 2959 (1991).
19. M. Darise, K. Mizutani, R. Kasai, O. Tanaka, S. Kitahata, S. Okada, S. Ogawa, F. Murakami and F. Chen. *Agric. Biol. Chem.* **48**, 2483 (1984).
20. K. Ohtani, Y. Aikawa, H. Ishikawa, R. Kasai, S. Kitahata, K. Mizutani, S. Doi, M. Nakamura and O. Tanaka. *Agric. Biol. Chem.* **55**, 449 (1991).
21. K. Ohtani, Y. Aikawa, Y. Fujisawa, R. Kasai, O. Tanaka and K. Yamasaki. *Chem. Pharm. Bull.* **39**, 3172 (1991).
22. S. Kitahata, S. Okada and T. Fukui. *Agric. Biol. Chem.* **42**, 2369 (1978).
23. K. Mizutani, T. Miyata, R. Kasai, O.Tanaka, S. Ogawa, and S. Doi. *Agric. Biol. Chem.* **53**, 395 (1989).
24. S. Kamiya, F. Konishi and S. Esaki. *Agric. Biol. Chem.* **43**, 1863 (1979).
25. S. Esaki, R. Tanaka and S. Kamiya. *Agric. Biol. Chem.* **48**, 1831 (1984).
26. S. Kitahata, H. Ishikawa, T. Miyata and O. Tanaka. *Agric. Biol. Chem.* **53**, 2929 (1989).
27. S. Kitahata, H. Ishikawa, T. Miyata and O. Tanaka. *Agric. Biol. Chem.* **53**, 2923 (1989).
28. K. Yamamoto, K.Yoshikawa and S. Okada. *Biosci. Biotech. Biochem.* **58**, 1657 (1994).
29. S. Kusama, I. Kusakabe, Y. Nakamura, S. Eda and K. Murakami. *Agric. Biol. Chem.* **50**, 2445 (1986).
30. I. Kusakabe, S. Watanabe, R. Morita, M. Terahara and K. Murakami. *Biosci. Biotech. Biochem.* **56**, 233 (1992).
31. H. Ishikawa, S. Kitahata, K. Ohtani, C. Ikuhara and O. Tanaka. *Agric. Biol. Chem.* **54**, 3137 (1990).
32. H. Ishikawa, S. Kitahata, K. Ohtani and O. Tanaka. *Chem. Pharm. Bull.* **39**, 2043 (1991).
33. K. Mizutani, T. Kuramoto, Y. Tamura, N. Ohtake, S. Doi, M. Nakamura and O. Tanaka. *Biosci. Biotech. Biochem.* **58**, 554 (1994).
34. S. Esaki, F. Konishi and S. Kamiya. *Agric. Biol. Chem.* **42**, 1599 (1978).
35. M. Kanaoka, S. Yano, H. Kato and T. Nakada. *Chem. Pharm. Bull.* **34**, 4978 (1986).
36. T. Muro, T. Kuramoto, K. Imoto and S. Okada. *Agric. Biol. Chem.* **50**, 689 (1986).
37. Y. Sasaki, T. Morita, T. Kuramoto, K. Mizutani, R. Ikeda and O. Tanaka. *Agric. Biol. Chem.* **52**, 207 (1988).
38. T. Kuramoto, Y. Ito, M. Oda, Y. Tamura and S. Kitahata. *Biosci. Biotech. Biochem.* **58**, 455 (1994).
39. K. Mizutani. In *Food Phytochemicals for Cancer Prevention II, ACS Symosium Series 547* (C. Ho, T. Osawa, M. Huangand and R. T. Rosen, ed.), pp. 322-328. Am. Chem. Soc., Washington D. C. (1994).