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Chemical Studies on Sweet Diterpene-Glycosides of *Stevia rebaudiana*: Conversion of Stevioside into Rebaudioside-A

By means of enzymatic and chemical procedures, stevioside (**1**), the major sweet glycoside of *Stevia rebaudiana* was converted efficiently into another constituent of this plant, rebaudioside-A (**2**) which tastes sweeter and more pleasant than **1**.

Keywords——natural sweetener; *ent*-kaurene type diterpene glucoside; stevioside; rebaudioside-A; enzymatic hydrolysis; Takadiastase Y; glucosylation by *ortho*-ester; ^{13}C NMR spectra; *Stevia rebaudiana*; Compositae

From leaves of *Stevia rebaudiana* Bertoni (Compositae), a wild herb of Paraguay, stevioside (**1**) has been isolated as the major sweet glycoside in a yield of 5—10%.[1] In the search for new natural sweeteners, the present authors have investigated constituents of this plant, isolating other new sweet glycosides named rebaudiosides-A (**2**),[2a] -C, -D, and -E.[2b] Since **2** tastes sweeter (about 1.2—1.5 times as much as **1**) and more pleasant than **1**, much industrial interest has been shown recently in production of **2** rather than **1**. In this regard, the present authors have investigated efficient conversion of **1** into **2**.

Selective hydrolysis of the terminal glucosyl linkage of the β-sophorosyl moiety of **1** is the key step of the present study. This was achieved by the hydrolysis with Takadiastase Y, the crude preparation of amylase prepared from *Aspergillus oryzae*.[3] Incubation of **1** with this enzyme mixture at 37° in McIlvain buffer (pH 4.0) for 80 hr yielded a sweet desgluco-compound (**3**), colorless prisms, mp 178—182° (from MeOH), $[\alpha]_D^{20}$ −29.3° (c=0.3, MeOH) in a quantitative yield. The structure of **3** was substantiated by ^1H and ^{13}C nuclear magnetic resonance (NMR)[2,4,5] (see Table I). Saponification of **3** by refluxing with 5% NaOH in MeOH for 3 hr afforded steviolmonoside (**4**)[4] almost quantitatively, which was converted into 4′,6′-benzylidene derivative (**5**) by the action of benzaldehyde in 98% HCOOH at room temperature for 15 min (yield 87%); colorless needles, mp 167—170° (from MeOH), $[\alpha]_D^{20}$ −54.4° (c=0.25, MeOH). The ^1H and ^{13}C NMR spectra of **5** indicated that no migration of the double bond took place during the process of this benzylidene fromation in the acidic medium.

Previously, the ester glucoside was prepared from Ag-salt of *ent*-kaur-16-en-19-oic acid by the condensation of acetobromoglucose followed by mild deacetylation.[4] Whereas, treatment of Ag-salt of steviol (**6**, the common aglycone of **1** and **2**) in the same way afforded only trace of the desired ester glucoside (**7**), unexpectedly. Preparation of **7** in a high yield was furnished by glucosylation with the orthoester; refluxing of a solution of **6** and 3,4,6-tri-O-acetyl-α-D-glucopyranose 1,2-(*tert*-butyl orthoacetate)[6] (**8**) in chlorobenzene followed by deacetylation with BaO in MeOH at 5° gave **7**, colorless needles mp 185—187° (from MeOH–H_2O), $[\alpha]_D^{20}$ −31.0° (c=0.1, MeOH) in a yield of 70%. The structure of **7** was confirmed by IR, ^1H and ^{13}C NMR (see Table I) and mass spectrometry of its trimethylsilyl ether. The

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3) The crude enzyme was kindly supplied by Dr. A. Endo, Institute of Sankyo Co. Ltd., to whom authors' thanks are due.
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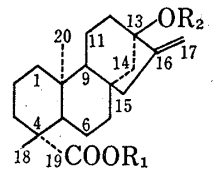
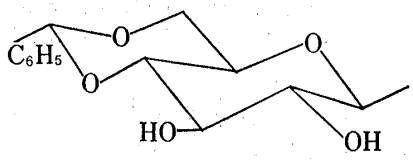
Table I. ^{13}C Chemical Shifts[a]

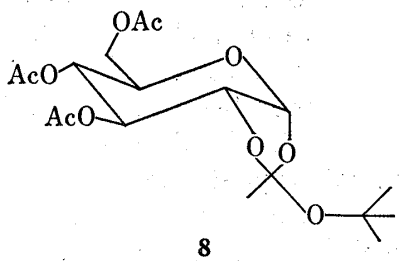
	3	4	7
C-1	40.7	41.0	40.7
2	19.3	19.7	19.4
3	38.2	38.4	38.2
4	44.0	43.8	44.0
5	57.3	56.9	57.4
6	22.0	22.5	22.1
7	41.6	41.6	41.7
8	42.4	42.1	41.7
9	53.9	54.1	54.2
10	39.8	39.7	39.7
11	20.7	20.6	20.7
12	37.1	38.4	40.7
13	85.8	86.4	79.7
14	44.4	44.6	47.2
15	47.8	48.2	48.0
16	154.3	153.7	157.5
17	104.5	104.9	102.8
18	28.2	29.2	28.4
19	176.9	180.0	176.8
20	15.5	15.7	15.6
G′-1[b]	95.7		95.7
2	73.8		73.9
3	78.9		79.1
4	70.9		71.0
5	78.9		79.1
6	61.9		62.0
G″-1[c]	99.5	99.4	
2	75.2	75.3	
3	78.5[d]	78.5[e]	
4	72.1	71.5	
5	77.8[d]	77.9[e]	
6	62.9	62.5	



	R_1	R_2
1	-Glc	-Glc (2-1 Glc)
2	-Glc	-Glc (2-1 Glc, 3-1 Glc)
3	-Glc	-Glc
4	H	-Glc
5	H	



6	H	H
7	-Glc	H



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a) δ ppm from internal TMS in C_5D_5N. Taken at 25° with JEOL JNM-PFT 100 NMR spectrometer at 25.15 MHz, computer limited resolution: ± 0.1 ppm.
b) Corresponding to ester glucose (R_1).
c) Corresponding to 13-O-glucose (R_2).
d,e) Values may be interchanged.
-Glc:-D-glucopyranosyl

benzylidene derivative (**5**) was subjected to glucosylation in the same way; a solution of **5** and excess of **8** in chlorobenzene was refluxed for 2 hr. The product was desbenzalated with 30% AcOH at 80° for 15 min and then deacetylated with 0.5 N BaO in MeOH at 0° for 2 hr, affording a tetraglucoside, colorless needles, mp 242—244° (from MeOH), $[\alpha]_D^{20}$ −19.5° (c=0.2, MeOH) in a yield of 75%, which was proved to be identical with an authentic sample of natural **2** by comparison of thin-layer chromatogram, ^{13}C NMR,[2,5] and other physical constants.

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