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Additional minor Diterpene Glycosides from *Stevia rebaudiana*

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Abstract: *From an extract of the leaves of Stevia rebaudiana, seven new steviol glycosides were isolated. Their structures were elucidated based on extensive spectroscopic (NMR and MS) studies.*

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I. Introduction

*Stevia rebaudiana*Bertoni is a perennial shrub of the Asteraceae (Compositae) family native to Brazil and Paraguay, which is often referred to as "the sweet herb of Paraguay". The major constituents in the leaves of *S. rebaudiana* are the potently sweet diterpenoid glycosides, such asstevioside, rebaudiosides A, D, M; and other minor glycosides [7, 8]. These compounds, which are known as stevia sweeteners, are glycosides of the diterpene steviol (ent-13-hydroxykaur-16-en-19-oic acid) [1]. Steviol glycosides in Stevia leaf extracts are approved as sweeteners in all major markets and approved as a natural sweetener by several regulatory authorities. As a part of our continuing research to discover natural sweeteners [2 - 5], we have reported several diterpene glycosides from the commercial extract of *S. rebaudiana*. In this article, we present the isolation and structure elucidation based on extensive spectroscopic data (1D and 2D NMR and MS) of the new diterpenoid glycosides, identified from a subfraction of the stevia extract (SG-95) obtained from PureCircle (Kuala Lumpur, Malaysia).

II. Material And Methods

The extract with Lot No: WIP A95 27A was obtained from Pure Circle, Malaysia

General Methods:NMR spectra were acquired on a Bruker Avance DRX 500 MHz instrument with a 5 mm inverse detection probe using standard pulse sequences. The spectra were referenced to the residual solvent signal (δ_H 8.71, δ_C 149.9 for pyridine-d_5, δ_H 3.30, δ_C 49.0 for methanol-d_4), chemical shifts are given in δ (ppm), and coupling constants are reported in Hz. Mass spectra (MS) were generated in the negative-ion mode using a mass spectrometer (Shimadzu 2020 single quadrupole) equipped with an electrospray ionization source coupled to a Shimadzu HPLC LC-30AD Prominence.

Isolation and Purification: Isolation of the pure compounds 1–2 was carried out by repeated recrystallization and chromatography (MPLC and reversed-phase, normal phase and HILIC HPLC) of extract WIP A95 27A.

Recrystallization: The extract was recrystallized in 100 g batches by dissolving the extract in ethanol/water 70/30 (750 mL) at 65 °C. The milky solution was allowed to cool down to room temperature in a water bath and then filtered through a suction filter. The collected crystals were washed with ethanol, dried and stored. Mother liquor and wash solution were combined and the respective solvent was removed *in vacuo* yielding 30-33 g of enriched compounds 1-5 per recrystallization batch.

MPLC: The respective sample (15 g) is dissolved in methanol, celite (30 g) is added and the solvent is removed by a rotary evaporator. The immobilized sample is transferred into a glass column and installed as the injection column in the MPLC system. A time-based fractionation leads to 18 fractions (4 min each).

Reversed-phase HPLC (RP-HPLC): The respective sample (up to 3.5 g) is dissolved in methanol, C-18 RP material is added and the solvent is removed by a rotary evaporator. The immobilized sample is transferred into a column and installed as the injection column in the HPLC system. A time-based fractionation leads to 120 fractions, which are combined based on the UV and ELSD data generated during fractionation. Resulting fractions are analyzed by LC-MS.

Normal phase HPLC: The respective sample (20 g) is dissolved in methanol, silica (40 g) is added and the solvent is removed by a rotary evaporator. The immobilized sample is transferred into a glass column and installed as the injection column in the HPLC system. Air is removed from the transfer column by washing with

ethyl acetate/methanol 1:1. A time-based fractionation leads to 90 fractions (0.5 min each), which are combined based on the UV and ELSD data generated during fractionation. Resulting fractions are analyzed by LC-MS.

HILIC-HPLC: The respective sample is dissolved in 2 mL of a 3:1 mixture of solvents A and B. Sample injection takes place after 9.95 min. A time-based fractionation leads to 96 fractions (43 sec each, starting after 18 min), which are combined based on the UV and ELSD data generated during fractionation. Resulting fractions are analyzed by LC-MS.

Compound **1**:Recrystallized material (15 g) was fractionated by MPLC using gradient 2 in Table S1. Fraction 14 (t_R = 52-56 min) yielded 3.3 g of enriched minor components. A further reduction of REBD and REBM was accomplished during the preparation of the material for the next HPLC step as the major compounds tended to crystallize from a methanolic solution. Fraction 14 was thereby split into a crystalized fraction containing REBD and REBM mainly (1.74 g) and a methanolic solution of enriched minor components (1.56 g), which were fractionated using reversed-phase HPLC using gradient 3 in Table S2. Fractions 17-21 yielded 5.8 mg of enriched compound **1**.

In parallel, 33.2 g of recrystallized material were fractionated using normal phase chromatography. Fractions 61-70 (t_R = 30-35 min) yielded 1.5 g of further enriched minor components, which were further fractionated by reversed-phase HPLC using gradient 3 in Table S2. Fractions 14-17 yielded 20.2 mg of enriched compound **1**.

The material yielded by both pathways as described above was combined and purified by HILIC-chromatography. Fractions 68-71 yielded 1.8 mg of compound **1** (~95% pure).

Compound **2**:The extract (15 g) was fractionated by MPLC using gradient 1 in Table S1. Fraction 13 (t_R = 48-52 min) yielded 2.56 g of enriched minor compounds. The material was fractionated using RP-HPLC with gradient 5 in Table S2 Fractions 85-86 were combined, yielding 18.6 mg of compound **2**. Residual formate due to the solvent system used was removed by SPE yielding 14 mg of compound **2** (~85% pure).

Compound **3**:Recrystallized material (32.2 g) was fractionated using normal phase chromatography. During fraction 71 at 76% B, a rupture of the stationary phase was observed and the gradient stopped. Remaining material was eluted with 400 mL of solvent B followed by 400 mL acetone/water 1/1. The solvent B fraction contained 4.39 g of enriched minor components. The acetone/water fraction (2.2 g) was further fractionated by RP- HPLC using gradient 7 in Table S2. Fractions 60-69 yielded 437 mg of enriched minor compounds. The obtained material was further fractionated by HILIC chromatography. Fractions 48-52 yielded 2.9 mg of compound **3** (>95% pure).

Compound **4**:Recrystallized material (7.5 g) was fractionated by RP-HPLC using gradient 2 in Table S2 in three subsequent HPLC runs of 2.5 g each. Fractions 58 and 59 were combined yielding 395.6 mg of enriched minor compounds. The material was fractionated again by RP-HPLC using gradient 4 in Table S2 and fractions 39-46 were combined yielding 138 mg of enriched minor compounds.

In parallel, extract (20 g) was fractionated by normal phase chromatography. Fractions 35-43 yielded 0.74 g of enriched minor components. The material was fractionated by RP-HPLC using gradient 1 in Table S2. Fraction 61 yielded 6.8 mg of enriched minor compounds.

In a third batch, extract (15 g) was fractionated by MPLC using gradient 1 in Table S1. Fraction 13 (t_R = 48-52 min) yielded 2.56 g of enriched minor compounds. The material was fractionated by RP-HPLC using gradient 5 in Table S2. Fractions 72-77 were combined yielding 464 mg of enriched minor compounds. The obtained material was further fractionated by RP-HPLC with gradient 6 in Table S2. Fractions 55-57 were combined yielding 5 mg of enriched compound **4**.

The material from all three fractionation pathways described above was combined and purified by HILIC-HPLC. Fractions 31-34 yielded 14 mg of compound **4** (>95% pure).

Compound **5**: Recrystallized material (7.5 g) was fractionated by RP-HPLC using gradient 2 in Table S2 in three HPLC runs of 2.5 g each. Respective fractions 68-75 were combined yielding 564 mg of enriched minor components.Recrystallized material (15 g) was fractionated by MPLC using gradient 2 in Table S1.Fraction 6 (t_R = 20-24 min) yielded 2.53 g of enriched minor compounds.The material was fractionated using RP-HPLC with gradient 8 in Table S2. Fractions 83-88 yielded 184 mg of enriched minor compounds.Fraction 8 (t_R = 28-32 min) yielded 2.40 g of enriched minor compounds.The material was fractionated using RP-HPLC with gradient 8 in Table S2. Fractions 83-87 yielded 106.4 mg of enriched minor compounds.Fraction 9 (t_R = 32-36 min) yielded 1.75 g of enriched compounds #7-#23. The material was fractionated using RP-HPLC with gradient 8 in Table S2. Fractions 94-97 yielded 10.7 mg of minor compounds. Fractions 82-88 yielded 184 mg of enriched minor compounds.Fractions 94-97 yielded 10.7 mg of minor compounds which went directly to HILIC purification. Fraction 10 (t_R = 36-40 min) yielded 1.20 g of enriched minor compounds.The material was fractionated using RP-HPLC with gradient 8 in Table S2. Fractions 61-68 yielded 176.5 mg of enriched minor compounds.Fractions 11 and 12 (t_R = 40-48 min) yielded 1.65 g enriched minor compounds. The material was fractionated using RP-HPLC with gradient 8 in Table S2. Fractions 71-73 yielded 7.4 mg of enriched minor compounds, which went directly to HILIC purification.All obtained enriched fractions, except

those chosen for direct HILIC purification, were combined and further fractionated by RP-HPLC using gradient 3 in Table S2. Fractions 55-60 yielded 15 mg of enriched minor compounds.Recrystallized material (15 g) was fractionated using normal-phase chromatography. The pre-fractions collected during equilibration of the injection column with acetone/ethyl acetate (1:1) yielded 3.85 g of enriched minor compounds, which were further fractionated by RP-HPLC using gradient 3 in Table S2. Fractions 54-59 yielded 37 mg of enriched minor compounds.Fractions 49-60 yielded 1.32 g of enriched minor compounds, which were further fractionated by RP-HPLC using gradient 9 in Table S2. Fractions 69-71 yielded 15 mg of enriched minor compounds.Fractions 61-71 yielded 1.55 g of enriched minor compounds, which were further fractionated by RP-HPLC using gradient 9 in Table S2. Fractions 51-53 yielded 8 mg of enriched minor compounds.All fractions containing minor compounds collected during the steps above were combined and purified by HILIC-chromatography. Fractions 46 and 47 yielded 5.4 mg of compound **5** (~90% pure).

Compound **6**:Recrystallized material (15 g) was fractionated by MPLC using gradient 2 in Table S1.Fractions 10 and 11 (t_R = 34-41 min) yielded 6.5 g of enriched minor components. Fractionation of 1.96 g of the material by RP-HPLC using gradient 8 yielded a sample of enriched minor compounds (fractions 93-96, 33 mg). The material was further purified by diol-HPLC, yielding two samples of compound **6** (fractions 43-45, 4.2 mg,>95% pure and fractions 46-47, 0.8 mg, 85-90% pure).

Compound **7**:Recrystallized material (15 g) was fractionated by MPLC using MPLC gradient 2. MPLC-Fraction 6 (t_R = 20-24 min) yielded 2.53 g of enriched minor components. The material was further fractionated using RP-HPLC with gradient 8. Fractions 83-88 yielded 184 mg of enriched minor components.MPLC-Fraction 8 (t_R = 28-32 min) yielded 2.40 g of enriched minor components.The material was further fractionated using RP-HPLC with gradient 8. Fractions 83-87 yielded 106.4 mg of enriched minor components.MPLC-Fraction 9 (t_R = 32-36 min) yielded 1.75 g of enriched minor compounds. The material was further fractionated using HPLC gradient 8 Fractions 82-88 yielded 184 mg of enriched minor components. Fractions 94-97 yielded 10.7 mg of enriched minor compounds which went directly to final purification.MPLC-Fraction 10 (t_R = 36-40 min) yielded 1.20 g of enriched minor components.The material was further fractionated using RP-HPLC with gradient 8. Fractions 61-68 yielded 176.5 mg of enriched minor components.MPLC-Fractions 11 and 12 (t_R = 40-48 min) yielded 1.65 g enriched minor components. The material was further fractionated using HPLC gradient 8. Fractions 71-73 yielded 7.4 mg of enriched minor compounds which went directly to final purification.Recrystallized material (7.5 g) was fractionated by reversed-phase HPLC using HPLC gradient 2 in three subsequent HPLC runs of 2.5 g each. Respective fractions 68-75 were combined yielding 564 mg of enriched minor components. The obtained material was combined with the material from MPLC fractions (see above) and further fractionated using reversed-phase HPLC using HPLC gradient 3. Fractions 55-60 yielded 66 mg of enriched minor compounds.Recrystallized material (15 g) was fractionated using normal phase chromatography.The pre-fractions collected during equilibration of the injection column with acetone/ethyl acetate (1:1) yielded 3.85 g of enriched minor components which were further fractionated by reversed-phase HPLC using gradient 2. Fractions 54-59 yielded 37 mg of enriched minor compounds.NP-Fractions 49-60 yielded 1.32 g of enriched minor components which were further fractionated by RP HPLC, using gradient 9. Fractions 69-71 yielded 15 mg of enriched minor compounds.NP-Fractions 61-71 yielded 1.55 g of enriched minor components which were further fractionated by RP-HPLC using gradient 9. Fractions 51-53 yielded 8 mg of enriched minor compounds.The combined material generated by prefractionations as described above was purified in a final purification step by HILIC-chromatography. Fractions 53 and 54 yielded 8.4 mg of compound **7** (90% pure).

III. Result and Discussions:

Purification of the commercial extract of rebaudioside M obtained from the leaves of *S. rebaudiana* from Pure Circle, Malaysia (lot WIP A95 27A) resulted in the isolation of the seven new diterpenoid glycosides (Figure 1).

Compound **1** was obtained as a dry film and its molecular formula was deduced as $C_{55}H_{88}O_{32}$ from its ESI mass spectrum in the negative mode, which showed a $(M-H)^-$ ion at m/z 1259.6. The ^{1}H-NMR and HSQC spectraof **1** showed the presence of two methyl signals resonating at δ 1.26 and 0.97 as singlets, two olefinic proton singlets at δ 4.85 and 5.25 corresponding to an exocyclic double bond, nine methylene and two methine protons between δ 0.75–2.70, characteristic for the ent-kaurane diterpenoids isolated earlier from the genus Stevia [2-5]. The presence of six sugar units in its structure was evident by the presence of the anomeric protons resonating at δ 4.35, 4.76, 4.78, 4.83, 4.90, and 5.53 ppm in its ^{1}H-NMR and HMBC spectra. The most deepfield shifted anomeric proton observed at δ 5.53 showed an HMBC correlation to C-19, which indicated that it corresponds to the anomeric proton of Sugar I. Similarly, the anomeric proton observed at δ 4.76 showed an HMBC correlation to C-13 allowing it to be assigned as the anomeric proton of Sugar II. The sequence in sugar units and the assignments for C-1 through C-6 (respectively C-5 in the pentose) of all six sugars were made using the ^{1}H-NMR, COSY, HSQC and HMBC data. A close comparison of the 1D- and 2D-NMR

spectraof **1** with rebaudioside A suggested that compound **1** is also a steviol glycoside, which has three glucose residues that are attached at the C-13 hydroxyl as a 2,3-branched β-D-glucotriosyl substituent and another glucose moiety in the form of an ester at C-19 leaving the assignment of one additional glucose and one pentose moiety. The anomeric proton of the additional glucose resonates at 4.90 ppm (correlation at 103.1 ppm in HSQC, in ^{1}H-NMR overlapping with the water signal). The remaining anomeric proton (4.35 ppm) belongs to the pentose. Assignment of all ^{1}H-NMR signals of the pentose were done by HH-COSY. H-4 resonates as a broad singlet, which is the typical pattern for the equatorial H-4 in arabinopyranose. The arabinose is attached to C-6 of sugar I resulting in a downfield shift of H-6 and C-6 of sugar 1 (67.4 ppm instead of 62.5 for steviol glycosides with unsubstituted C-6 at sugar I, e.g. rebaudioside A). All anomeric protons of the sugars are showing large coupling constants (ca. 7.5 Hz), typical for ß-D-glucopyranosides and α-L-arabinopyranosides.

Compound **2** was obtained as a dry film and its molecular formula was deduced as $C_{49}H_{78}O_{27}$ from its ESI mass spectrum in the negative mode, which showed a $(M-H)^-$ ion at m/z 1097.6. The NMR signals of the aglycone are very similar to those of compound **1**. Mass spectrometric data together with five anomeric protons (recorded in pyridine-d_5: 6.28, 5.56, 5.32, 5.39, and 5.10 ppm) hints to a steviol glycoside with four hexoses and one pentose. All anomeric protons show large coupling constants of ca. 7.5 Hz, which is typical for ß-pyranosides. The linkages were deduced from the following HMBC correlations: H-1 of the xylose (5.32 ppm) to C-2 of sugar I (81.8 ppm), 5.56 ppm to C-2 (80.9 ppm) of sugar II, and 5.39 to C-3 (88.2 ppm) of sugar II. Thus, compound **2** has one glucose moiety less than another minor steviol glycoside we have published in 2013 [5].

Compound **3** was obtained as a dry film and its molecular formula was deduced as $C_{49}H_{78}O_{27}$ from its ESI mass spectrum in the negative mode, which showed a $(M-H)^-$ ion at m/z 1097.7. The NMR signals of the aglycone are very similar to those of compound **1**. Mass spectrometric data together with five anomeric protons (5.55, 4.70, 4.86, 4.76, and 4.87 ppm) hint to a steviol glycoside with four hexoses and one pentose. In contrast to compound **1**, C-6 of all four glucose moieties resonate between 61.4 and 62.0 ppm typical for unsubstituted O-6. Additionally, there are no sugar linkages at O-3 because the HSQC did not show signals around 85 ppm typical for oligosaccharides linked via O-3. By comparison of NMR data with those of rebaudioside E, the additional glucose has to be linked via C-4 of sugar I.

Compound **4**was obtained as a dry film and its molecular formula was deduced as $C_{44}H_{70}O_{22}$ from its ESI mass spectrum in the negative mode, which showed a $(M-H)^-$ ion at m/z 949.6. The NMR signals of the aglycone are very similar to those of compound **1**. Mass spectrometric data together with four anomeric protons (5.60, 5.30, 4.61, and 4.60 ppm) hints to a steviol glycoside with three hexoses and one deoxy-hexose. The typical pattern (broad singlet) and deepfield shift of the anomeric proton at 5.30 is typical for a rhamnose moiety. Comparison of NMR data with those of rebaudioside S [6] suggested that **4** has identical sugar linkage, but in **4**due to the large coupling constants of 7.5 Hz all three glucoses are bound via β linkage whereas rebaudioside S has one α-glucose moiety.

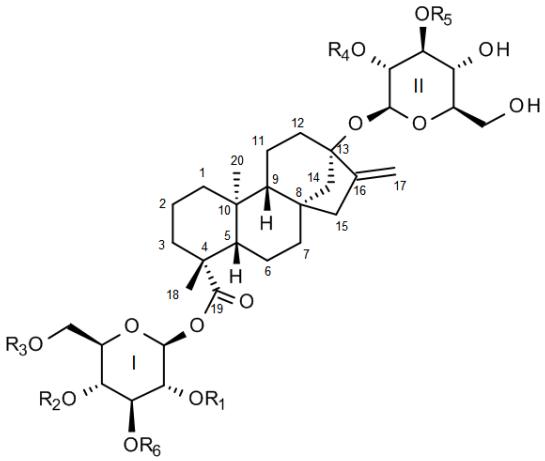
Compound **5** was obtained as a dry film and its molecular formula was deduced as $C_{50}H_{80}O_{27}$ from its ESI mass spectrum in the negative mode, which showed a $(M-H)^-$ ion at m/z 1111.3. The NMR signals of the aglycone are very similar to those of compound 1. Mass spectrometric data together with five anomeric protons (5.44, 5.31, 4.61, 4.52, and 4.39 ppm) hints to a steviol glycoside with four hexoses and one deoxy-hexose. The typical pattern (broad singlet) and deepfield shift of the anomeric proton at 5.44 is typical for a rhamnose moiety. A close comparison of the ^{1}H- and 2D-NMR spectraof **5** with rebaudioside C suggested that **5** is also a steviol glycoside, which has two glucose residues and one rhamnose that are attached at the C-13 hydroxyl as a glucosyl-(2-rhamnosyl)-3-glucosyl substituent and another glucose moiety in the form of an ester at C-19 leaving the assignment of one additional glucose moiety. The chemical shift of C-6 (downfield shifted to 68.5 ppm in HSQC) and H-6 (downfield shifted to 3.83 and 4.17 ppm) of sugar I showed that the additional glucose is attached to O-6 of sugar I.

Compound **6** was obtained as a dry film and its molecular formula was deduced as $C_{49}H_{78}O_{27}$ from its ESI mass spectrum in the negative mode, which showed a $(M-H)^-$ ion at m/z 1097.6. The NMR signals of the aglycone are very similar to those of compound **1**. The presence of five sugar units in its structure was evident by the presence of the anomeric protons resonating at δ 4.33, 4.64, 4.70, 4.87, and 5.40 ppm in its ^{1}H-NMR and HMBC spectra. The most deepfield shifted anomeric proton observed at δ 5.40 showed an HMBC correlation to C-19, which indicated that it corresponds to the anomeric proton of Sugar I. Similarly, the anomeric proton observed at δ 4.64 showed an HMBC correlation to C-13 allowing it to be assigned as the anomeric proton of Sugar II. The sequence in sugar units and the assignments for C-1 through C-6 (respectively C-5 in the pentose) of all five sugars were made using the ^{1}H-NMR, COSY, HSQC and HMBC data. A close comparison of the 1D- and 2D-NMR spectraof **1** with rebaudioside A suggested that compound **6** is also a steviol glycoside, which has three glucose residues that are attached at the C-13 hydroxyl as a 2,3-branched β-D-glucotriosyl substituent and another glucose moiety in the form of an ester at C-19 leaving the assignment of the pentose moiety. The remaining anomeric proton (4.33 ppm) belongs to the pentose. Assignment of all ^{1}H-NMR signals of the

pentose were done by HH-COSY. H-4 resonates as a broad singlet, which is the typical pattern for the equatorial H-4 in arabinopyranose. The arabinose is attached to C-6 of sugar I resulting in a downfield shift of H-6 and C-6 of sugar I (67.9 ppm instead of 62.5 for steviol glycosides with unsubstituted C-6 at sugar I, e.g. rebaudioside A). All anomeric protons of the sugars are showing large coupling constants (ca. 7.5 Hz), typical for ß-D-glucopyranosides and α-L-arabinopyranosides.

Compound **7** was obtained as a dry film and its molecular formula was deduced as $C_{55}H_{88}O_{32}$ from its ESI mass spectrum in the negative mode, which showed a $(M-H)^-$ ion at m/z 1259.8. The NMR signals of the aglycone are very similar to those of compound 1. The presence of six sugar units in its structure was evident by the presence of the anomeric protons resonating at δ 5.28, 5.33, 5.48, 5.50, 5.81 and 6.41 ppm in its ^{1}H-NMR and HMBC spectra. The most deepfield shifted anomeric proton observed at δ 6.41 showed an HMBC correlation to C-19, which indicated that it corresponds to the anomeric proton of Sugar I. Similarly, the anomeric proton observed at δ 5.48 showed an HMBC correlation to C-13 allowing it to be assigned as the anomeric proton of Sugar II. The sequence in sugar units and the assignments for C-1 through C-6 (respectively C-5 in the pentose) of all six sugars were made using the ^{1}H-NMR, COSY, HSQC and HMBC data. HMBC correlations of anomeric protons to C2 and C3 of sugar I from the anomeric protons observed at δ 5.81 and δ 5.28 respectively, indicate that compound **7** is a steviol glycoside, which has three glucose residues that are attached at the C-19 in the form of an ester as a 2,3-branched β-D-glucotriosyl substituent. This leaves two glucoses and a xylose which are attached to C13 hydroxyl. HMBC correlations to C2 and C3 of sugar II originating from the anomeric protons at δ 5.33 and 5.50 respectively show that the glucose is attached to position 2 of sugar II and the pentose being attached to position 3 of sugar II

Supplementary Materials: The following are available online at www.mdpi.com/link, Figure S1(a) through Figure S1(g): ^{1}H-NMR spectra of compounds 1-7, respectively. Figure S2(a) through Figure S2(g): LC-MS spectra of compounds 1-7, respectively. Figure S3: Structures of compound 1-7. Table S1: MPLC Gradients, Table S2: RP-HPLC gradients, Table S3 NP-HPLC gradients and Table S4 HILIC gradients.



	1	**2**	**3**	**4**	**5**	**6**	**7**
R_1	β-D-Glc	β-D-Xyl	β-D-Xyl	α-L-Rha	H	H	β-D-Glc
R_2	H	H	β-D-Glc	H	H	H	H
R_3	α-L-Ara	H	H	H	β-D-Glc	α-L-Ara	H
R_4	β-D-Glc	β-D-Glc	β-D-Glc	β-D-Glc	α-L-Rha	β-D-Glc	β-D-Glc
R_5	β-D-Glc	β-D-Glc	H	H	β-D-Glc	β-D-Glc	β-D-Xyl
R_6	H	H	H	H	H	H	β-D-Glc

Figure 1: Structures of steviol glycosides **1-7**

Table 1.[13]C-NMR chemical shift values for **1–7** isolated from *Stevia rebaudiana*Bertoni recorded in methanol-d_4 (**1, 3-6**) or pyridine-d_5 (**2,7**)

Carbon	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1	40.7	40.5	40.7	40.9	40.7	40.6	40.0
2	19.2	19.7	19.7	19.2	19.1	19.1	19.4
3	37.0	37.8	37.8	37.0	37.8	37.4	37.9
4	43.5	43.9	44.0	43.5	44.0	43.5	43.9
5	57.5	57.6	58.2	57.5	57.6	57.5	57.0
6	21.8	22.4	22.2	21.8	22.2	21.9	23.1
7	41.9	42.0	41.7	41.7	41.7	41.7	41.8
8	54.0	54.1	54.1	54.0	54.1	54.0	53.8
9	54.1	54.3	54.0	54.1	54.0	54.2	54.0
10	39.0	40.5	40.5	39.0	40.5	39.0	38.8
11	19.8	20.3	20.2	19.8	20.5	19.6	19.7
12	37.5	38.2	37.4	37.5	37.3	38.0	38.0
13	87.6	86.2	88.0	87.6	87.9	87.6	87.4
14	44.2	43.9	44.8	44.6	44.8	44.2	42.8
15	47.1	47.6	48.1	47.7	48.1	47.5	45.8
16	152.1	154.2	154.6	152.5	154.6	152.5	152.7
17	104.3	104.9	105.0	104.7	104.9	104.7	104.2
18	28.1	29.0	28.1	28.4	28.3	27.7	27.9
19	177.1	175.9	175.9	177.3	175.9	177.3	176.6
20	16.1	16.6	16.3	16.1	15.7	15.4	16.3
Sugar I	β-D-Glc	β-D-Glc	β-D-Glc	β-D-Glc	β-D-Glc	β-D-Glc	β-D-Glc
1	93.4	93.6	94.1	93.5	94.3	94.5	94.3
2	78.6	81.8	78.2	77.7	74.2	73.2	76.4
3	76.7	78.2	86.2	78.2	77.7	77.5	88.2
4	69.8	70.8	70.4	70.1	69.7	69.7	70.2
5	76.6	78.7	77.3	77.4	77.0	76.6	77.4
6	68.4	62.4	61.8	62.3	68.5	67.9	61.7
Sugar II	β-D-Glc	β-D-Glc	β-D-Glc	β-D-Glc	β-D-Glc	β-D-Glc	β-D-Glc
1	95.8	97.8	96.3	96.5	96.8	96.5	95.7
2	79.5	80.9	79.4	81.2	77.2	79.0	81.7
3	86.5	88.2	77.9	79.2	88.1	86.7	87.3
4	69.2	70.1	70.2	70.7	69.8	69.6	70.5
5	77.1	77.5	77.5	77.2	77.0	77.5	77.5
6	61.7	61.8	61.9	62.0	61.7	61.7	61.3
R_1	β-D-Glc	β-D-Xyl	β-D-Xyl	α-L-Rha	H	H	β-D-Glc
1	103.1	106.5	104.2	100.9	-	-	103.7
2	74.5	75.7	74.8	71.0	-	-	74.9
3	77.4	78.4	77.5	71.1	-	-	77.4
4	71.0	71.1	69.0	72.7	-	-	70.3
5	76.6	67.4	65.4	69.3	-	-	78.0
6	61.7	-	-	17.1	-	-	61.3
R_2	H	H	β-D-Glc	H	H	H	H
1	-	-	102.9	-	-	-	-
2	-	-	75.0	-	-	-	-
3	-	-	77.3	-	-	-	-
4	-	-	70.4	-	-	-	-
5	-	-	77.0	-	-	-	-
6	-	-	62.0	-	-	-	-
R_3	α-L-Ara	H	H	H	β-D-Glc	α-L-Ara	H
1	103.7	-	-	-	103.6	103.7	-
2	73.2	-	-	-	74.1	71.5	-
3	76.9	-	-	-	77.3	73.0	-
4	68.3	-	-	-	70.3	68.5	-
5	65.4	-	-	-	76.5	65.5	-
6	-	-	-	-	61.7	-	-
R_4	β-D-Glc	β-D-Glc	β-D-Glc	β-D-Glc	α-L-Rha	β-D-Glc	β-D-Glc
1	102.9	104.7	103.0	104.4	101.4	103.0	104.9
2	74.3	76.4	74.2	75.0	71.0	74.8	75.1
3	77.7	78.6	77.4	77.0	71.0	77.1	77.8
4	71.5	72.3	70.6	70.7	72.6	71.2	72.6
5	76.4	78.1	77.0	76.5	69.0	77.1	77.1
6	61.9	63.3	61.4	61.8	17.1	61.8	61.2
R_5	β-D-Glc	β-D-Glc	H	H	β-D-Glc	β-D-Glc	β-D-Xyl
1	103.7	104.9	-	-	103.4	103.4	103.5
2	74.3	75.4	-	-	74.4	74.5	75.0
3	77.1	79.1	-	-	76.7	77.3	77.1
4	70.9	71.3	-	-	69.9	70.2	71.0

5	76.9	78.8	-	-	77.0	76.7	66.4
6	62.1	62.2	-	-	61.8	61.3	-
R_6	H	H	H	H	H	H	β-D-Glc
1	-	-	-	-	-	-	103.7
2	-	-	-	-	-	-	74.9
3	-	-	-	-	-	-	78.0
4	-	-	-	-	-	-	70.2
5	-	-	-	-	-	-	78.0
6	-	-	-	-	-	-	61.9

Table 2:^{1}H-NMR chemical shift values for **1–7** isolated from *Stevia rebaudiana*Bertonirecorded in methanol-d_4 (**1, 3-6**) or pyridine-d_5 (**2, 7**) (based on HH-COSY, HSQC and HMBC experiments)

Proton	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1	0.85/*m* 1.88/*m*	0.72/*m* 1.69/*m*	0.88/*m* 1.88/*m*	0.83/*m* 1.85/*m*	0.88/*m* 1.88/*m*	0.87/*m* 1.90/*m*	0.77/*m* 1.74/*m*
2	1.42/*m* 1.91/*m*	1.40/*m* 2.14/*m*	1.45/*m* 1.99/*m*	1.40/*m* 1.92/*m*	1.45/*m* 1.99/*m*	1.46/*m* 1.96/*m*	1.35/*m* 2.27/*m*
3	1.55/*m* 1.96/*m*	1.05/*m* 2.55/*m*	1.55/*m* 2.25/*m*	1.55/*m* 1.96/*m*	1.55/*m* 2.15/*m*	1.56/*m* 1.98/*m*	1.01/*m* 2.28/*m*
4	-	-	-	-	-	-	-
5	1.07/*m*	0.99/*m*	0.99/*m*	1.07/*m*	1.15/*m*	1.15/*m*	1.06/*m*
6	1.86/*m* 1.90/*m*	1.90/*m* 2.21/*m*	1.87/*m* 1.90/*m*	1.86/*m* 1.90/*m*	1.87/*m* 1.90/*m*	1.88/*m* 2.03/*m*	2.26/*m* 2.40/*m*
7	1.46/*m* 1.59/*m*	1.29/*m* 1.44/*m*	1.46/*m* 1.58/*m*	1.43/*m* 1.54/*m*	1.46/*m* 1.58/*m*	1.48/*m* 1.60/*m*	1.40/*m* 1.83/*m*
8	-	-	-	-	-	-	-
9	0.98/*m*	0.88/*m*	1.01/*m*	0.98/*m*	1.01/*m*	1.00/*m*	0.91/*m*
10	-	-	-	-	-	-	-
11	1.63/*m* 1.80/*m*	1.63/*m* 1.68/*m*	1.65/*m* 1.79/*m*	1.63/*m* 1.80/*m*	1.65/*m* 1.79/*m*	1.67/*m* 1.80/*m*	1.63/*m* 1.67/*m*
12	1.09/*m* 2.26/*m*	1.87/*m* 2.27/*m*	1.10/*m* 2.25/*m*	1.07/*m* 2.27/*m*	1.10/*m* 2.25/*m*	1.08/*m* 2.17/*m*	1.79/*m* 2.65/*m*
13	-	-	-	-	-	-	-
14	1.53/*d* (11.9) 2.26/*d* (11.9)	1.79/*d* (11.9) 2.52/*d* (11.9)	1.52/*d* (11.9) 2.26/*d* (11.9)	1.50/*d* (11.9) 2.24/*d* (11.9)	1.52/*d* (11.9) 2.26/*d* (11.9)	1.59/*d* (11.6) 2.25/*d* (11.6)	2.07/*d* (11.6) 2.77/*d* (11.6)
15	2.09/*d* (15.9) 2.15/*d* (15.9)	2.00/*d* (15.9) 2.05/*d* (15.9)	2.13/s; (2H)	2.04/*d* (15.9) 2.13/*d* (15.9)	2.13/s; (2H)	2.07/*d* (15.9) 2.16/*d* (15.9)	1.86/*d* (15.9) 2.03/*d* (15.9)
16	-	-	-	-	-	-	-
17	4.85/*br s* 5.25/*br s*	4.98/*br s* 5.65/*br s*	4.90/*br s* 5.19/*br s*	4.84/*br s* 5.18/*br s*	4.90/*br s* 5.19/*br s*	4.90/*br s* 5.26/*br s*	4.88/*br s* 5.73/*br s*
18	1.26/*s*; 3H	1.37/*s*; 3H	1.27/*s*; 3H	1.25/*s*; 3H	1.24/*s*; 3H	1.24/*s*; 3H	1.32/*s*; 3H
19	-	-	-	-	-	-	-
20	0.97/s; 3H	1.17/s; 3H	0.97/s; 3H	0.93/s; 3H	1.00/s; 3H	0.99/s; 3H	1.34/s; 3H
Sugar I	β-D-Glc	β-D-Glc	β-D-Glc	β-D-Glc	β-D-Glc	β-D-Glc	β-D-Glc
1	5.53/*d*; 7.2	6.28/*d*; 7.9	5.55/*d*; 7.2	5.60/*d*; 7.2	5.31/*d*; 7.2	5.40/*d*; 8.4	6.41/*d*; 7.8
2	3.81/*m*	4.23/*m*	3.82/*m*	3.60/*m*	3.35/*m*	3.37/*m*	4.52/*m*
3	3.62/*m*	4.36/*m*	3.89/*m*	3.57/*m*	3.39/*m*	3.47/*m*	5.13/*m*
4	3.49/*m*	4.01/*m*	3.46/*m*	3.40/*m*	3.45/*m*	3.45/*m*	4.18/*m*
5	3.59/*m*	4.09/*m*	3.47/*m*	3.39/*m*	3.58/*m*	3.58/*m*	3.89/*m*
6	3.75/*m* 4.10/*m*	4.13/m 4.28/m	3.68/*m* 3.82/*m*	3.70/*m* 3.79/*m*	3.83/*m* 4.17/*m*	3.86/*m* 4.09/*m*	4.20/*m* 4.35/*m*
Sugar II	β-D-Glc	β-D-Glc	β-D-Glc	β-D-Glc	β-D-Glc	β-D-Glc	β-D-Glc
1	4.76/*d*; 7.6	5.10/*d*; 7.7	4.70/*d*; 7.6	4.61/*d*; 7.6	4.61/*d*; 7.7	4.64/*d*; 8.4	5.48/d; 7.8
2	3.62/*m*	4.29/*m*	3.59/*m*	3.47/*m*	3.60/*m*	3.67/*m*	4.06/*m*
3	3.99/*m*	4.42/*m*	3.60/*m*	3.57/*m*	3.74/*m*	3.78/*m*	5.00/*m*
4	3.40/*m*	4.04/*m*	3.41/*m*	3.31/*m*	3.40/*m*	3.38/*m*	4.08/*m*
5	3.35/*m*	3.66/*m*	3.45/*m*	3.25/*m*	3.38/*m*	3.41/*m*	3.81/*m*
6	3.69/*m* 3.85/*m*	4.13/*m* 4.30/*m*	3.69/*m* 3.86/*m*	3.63/*m* 3.83/*m*	3.66/*m* 3.84/*m*	3.68/*m* 3.93/*m*	4.11/*m* 4.32/*m*
R_1	β-D-Glc	β-D-Xyl	β-D-Xyl	α-L-Rha	H	H	β-D-Glc
1	4.90/*d*; 7.6	5.32/*d*; 7.1	4.86/d; 7.1	5.30/*br s*	-	-	5.81/*d*; 7.8
2	3.25/*m*	4.08/*m*	3.35/*m*	3.90/*m*	-	-	4.22/*m*
3	3.31/*m*	4.14/*m*	3.30/*m*	3.61/*m*	-	-	4.34/*m*
4	3.29/*m*	4.23/*m*	3.59/*m*	3.37/*m*	-	-	4.03/*m*
5	3.32/*m*	3.68/*m* 4.35/*m*	3.22/*m* 3.91/*m*	3.75/*m*	-	-	3.98/*m*
6	3.63/*m* 3.92/*m*	-	-	1.24/*d* 6.7; (3H)	-	-	4.07/*m* 4.28/*m*
R_2	H	H	β-D-Glc	H	H	H	H
1	-	-	4.76/*d*; 7.6	-	-	-	-

2	-	-	3.30/*m*	-	-	-	-
3	-	-	3.38/*m*	-	-	-	-
4	-	-	3.33/*m*	-	-	-	-
5	-	-	3.31/*m*	-	-	-	-
6	-	-	3.66/*m* 3.88/*m*	-	-	-	-
R_3	α-L-Ara	H	H	H	β-D-Glc	α-L-Ara	H
1	4.35/*d*; 7.1	-	-	-	4.39/d; 7.6	4.33/*d*; 8.4	-
2	3.61/*m*	-	-	-	3.24/*m*	3.60/*m*	-
3	3.67/*m*	-	-	-	3.32/*m*	3.58/m	-
4	3.85/*br s*	-	-	-	3.28/*m*	3.83/*br s*	-
5	3.55/*m* 3.88/*m*	-	-	-	3.27/*m*	3.53/m 3.89/m	-
6	-	-	-	-	3.70/*m* 3.88/*m*	-	-
R_4	β-D-Glc	β-D-Glc	β-D-Glc	β-D-Glc	α-L-Rha	β-D-Glc	β-D-Glc
1	4.78/*d*; 7.6	5.56/*d*; 7.6	4.87/*d*; 7.6	4.60/*d*; 7.6	5.44/*br s*	4.87/d; 8.4	5.33/*d;* 7.8
2	3.30/*m*	4.13/*m*	3.28/*m*	3.24/*m*	4.00/*m*	3.23/m	4.15/*m*
3	3.33/*m*	4.19/*m*	3.36/*m*	3.35/*m*	3.73/*m*	3.29/m	4.17/*m*
4	3.13/*m*	4.13/*m*	3.29/*m*	3.21/*m*	3.42/*m*	3.19/m	4.10/*m*
5	3.30/*m*	3.84/*m*	3.24/*m*	3.21/*m*	4.08/*m*	3.26/m	3.68/*m*
6	3.67/*m* 3.86/*m*	4.34/*m* 4.49/*m*	3.70/*m* 3.88/*m*	3.64/*m* 3.84/*m*	1.25/*d* 6.3; (3H)	3.66/m 3.89/m	4.35/*m* 4.16/*m*
R_5	β-D-Glc	β-D-Glc	H	H	β-D-Glc	β-D-Glc	β-D-Xyl
1	4.83/*d*; 7.6	5.39/*d*; 7.8	-	-	4.52/*d*; 7.6	4.70/*d*; 8.4	5.50/*d*; 7.8
2	3.33/*m*	4.03/*m*	-	-	3.29/*m*	3.30/*m*	3.97/*m*
3	3.40/*m*	4.14/*m*	-	-	3.32/*m*	3.33/*m*	4.56/*m*
4	3.26/*m*	4.08/*m*	-	-	3.32/*m*	3.28/*m*	4.15/*m*
5	3.37/*m*	3.94/*m*	-	-	3.41/*m*	3.35/*m*	3.39/*m* 4.28/*m*
6	3.63/*m* 3.89/*m*	4.21/*m* 4.43/*m*	-	-	3.64/*m* 3.92/*m*	3.67/m 3.88/m	-
R_6	H	H	H	H	H	H	β-D-Glc
1	-	-	-	-	-	-	5.28 *d*; 7.8
2	-	-	-	-	-	-	3.93/*m*
3	-	-	-	-	-	-	4.03/*m*
4	-	-	-	-	-	-	4.01/*m*
5	-	-	-	-	-	-	3.93/*m*
6	-	-	-	-	-	-	4.10/*m* 4.30/*m*

Detailed investigation of minor diterpene glycosides from stevia yielded some more compounds closely related to the already more than 50 known steviol glycosides [7,8]. Re-isolation is in progress to get sufficient material to taste the new compounds for their sweetness.

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