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# FIRST DERIVATIVE OF THE *Stevia rebaudiana* GLYCOSIDE STEVIOLBIOSIDE CONTAINING THIAZOLYLHYDRAZONE MOIETIES

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*A derivative of the* Stevia rebaudiana *glycoside steviolbioside in which two molecules containing thiazolylhydrazone groups on C-16 atoms were connected through a polymethylene linker to their carboxylic functionalities was synthesized for the first time.*

**Keywords:** steviolbioside, *Stevia rebaudiana*, thiazoles, hydrazones, thiosemicarbazones.

*Stevia rebaudiana* glycosides, primarily the dominant glycoside stevioside **1**, are used for a large-scale production of calorie-free sugar substitutes [1]. Stevioside **1** with sophorosyl and glucopyranosyl residues has been extensively modified chemically and enzymatically to change the number and nature of carbohydrate residues [1]. The only goal was to design sweeter glycosides without unpleasant aftertastes. Steviolbioside **2** is a promising scaffold with respect to designing new therapeutic agents. First, it itself is biologically active and exhibits insulinotropic [1, 2], antihyperglycemic [2, 3], and hypoglycemic activity [4]. Second, it has a carboxylic group and a double bond that are available for functionalization. Extensive experience with the chemistry of natural compounds indicates that chemical modification of natural metabolites either enhances their already existing activity or alters it [5–13]. Thus, synthesized amides, esters, and hydrazides of steviolbioside exhibited anticancer, antibacterial, and antituberculosis activity [14–18]. Only a carboxylic group of steviolbioside was functionalized in all these studies.

We reported previously [19, 20] on the oxidation of the double bond and preparation of a ketone, oxime, and thiosemicarbazone of steviolbioside in addition to the synthesis of macrocycles containing two or four steviolbioside molecules connected through diester and dihydrazonohydrazide spacers. Herein, functionalization of the double bond of steviolbioside, which is used for the first time to prepare derivatives with a thiazolylhydrazone moiety, is reported.

The starting material (**2**) was obtained via alkaline hydrolysis of stevioside **1** [21]. The reaction of **2** with 1,8-dibromooctane in KOH–DMSO superbase solution produced diglycoside **3**, the sophorosyl hydroxyls of which were protected by acylation with $Ac_2O$ in Py [18]. The double bonds of resulting diglycoside **4** were then oxidized by aqueous $OsO_4$ (4%) in THF–$H_2O$ as before [19, 20] (Scheme 1). Diketone **5** was obtained in 45% yield and was used for the reaction with thiosemicarbazide in EtOH with $H_2SO_4$ solution (20%) (Scheme 1).

The MALDI mass spectrum of the product (78% yield) indicated that dithiosemicarbazone **6** had formed. Its PMR spectrum showed two singlets at 0.89 and 1.19 ppm for the two C-20 methyls and the two C-18 methyls of two *ent*-kaurane skeletons, a doublet at 2.26 ppm for the C-14 protons, two singlets at 6.45 and 7.33 ppm for four protons of two $NH_2$ groups, and a singlet at 8.24 ppm corresponding to the resonance of two NH protons.

Next, the reaction of dithiosemicarbazone **6** with 2-bromo-4′-chloroacetophenone in refluxing EtOH afforded in 20% yield (after flash chromatography over a dry column) diglycoside **7** in which two steviolbioside thiazolylhydrazone molecules were connected by a polymethylene linker through the carboxylic functionalities. The PMR spectrum of **7** contained two singlets at 0.89 and 1.19 ppm for $CH_3$-20 and $CH_3$-18 of two *ent*-kaurane skeletons, a singlet at 6.82 pm for the protons of two thiazole rings, two doublets at 7.30 and 7.83 ppm for an $A_2B_2$ spin system of two aromatic rings, and a singlet at 9.92 ppm corresponding to the resonance of two hydrazone protons. The MALDI mass spectrum showed a molecular ion corresponding to diglycoside **7**.

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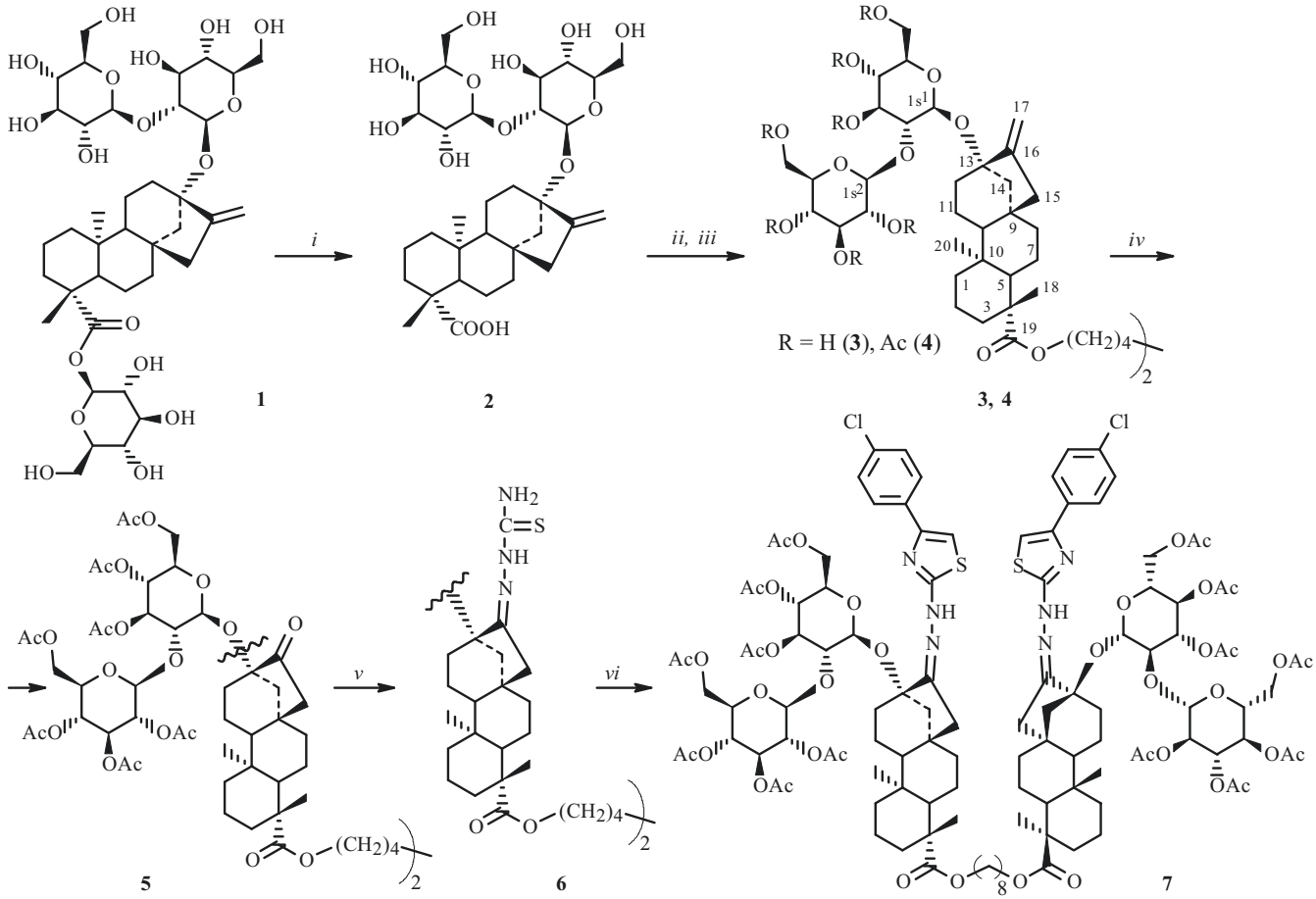


*i.* 10% KOH; *ii*. $Br(CH_2)_8Br$, KOH–DMSO, 50°C, 24 h; *iii.* $Ac_2O$–Py; *iv*. 4 % $OsO_4$–$H_2O$, $NaIO_4$, THF, 20°C, 24 h; *v*. $NH_2NHC(S)NH_2$, 20% $H_2SO_4$, EtOH, room temp., 24 h; *vi.* 2-bromo-4′-chloroacetophenone, EtOH, 68°C, 24 h

Scheme 1

Thus, derivative **7** of steviolbioside in which two molecules containing thiazolylhydrazone groups on C-16 that were connected through a polymethylene linker and the carboxylic functionalities was synthesized for the first time. The biological activity of the synthesized glycoside will be studied.

## EXPERIMENTAL

PMR spectra were recorded on an Avance-400 spectrometer (Bruker, Germany, 400 MHz). MALDI mass spectra were obtained in an UltraFlex III TOF/TOF time-of-flight mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany) in linear mode. A Nd:YAG laser had $\lambda$ = 355 nm. Data were processed using the FlexAnalysis 3.0 program (Bruker Daltonik GmbH, Bremen, Germany). The *m*/*z* range 200–6000 was scanned. Positively charged ions were detected using a metal target. The matrices were 2,5-dihydroxybenzoic acid (DHB) and *p*-nitroaniline (*p*-NA). Samples were dissolved in $CH_2Cl_2$–DMSO at a concentration of $10^{-3}$ mg/mL. A solution of the matrix in MeCN at a concentration of 10 mg/mL was prepared. Samples were deposited by the dried-drop method using a pipette to place matrix solution (0.5 μL) on an Anchor Chip target (Bruker Daltonik GmbH, Bremen, Germany). A solution of the analyte (0.5 μL) was placed on the target after the solvent evaporated. IR spectra were recorded in the range 400–4000 $cm^{-1}$ on a Vector 22 FT-IR (Bruker). Samples were studied as films. The course of reactions and purity of products were monitored by TLC on Sorbfil plates (OOO Imid, Krasnodar, Russia). Compounds were detected by $H_2SO_4$ solution (5%) followed by heating to 120°C.

*n*-Dibromooctane (Lancaster Synthesis) and $OsO_4$ aqueous solution (4%) and sodium periodate (Acros Organics) were purchased.

**19-β-D-Glucopyranosyl-13-*O*-β-D-sophorosyl-*ent*-kaur-16-ene (1)** was obtained from Stevioside sweetener (OOO Travy Baikala, Irkutsk, Russia) by column chromatography ($CHCl_3$–MeOH eluent, 10:0.1), mp 201–203°C (MeOH), $[\alpha]_D^{20}$ –33.7° (*c* 6.6, $H_2O$). $^1H$ NMR spectrum (400 MHz, $C_5D_5N$, δ, ppm, J/Hz): 1.23 (3H, s, $H_3$-20), 1.29 (3H, s, $H_3$-18), 2.34 (1H, d, J = 13, $H_{eq}$-3), 2.70 (1H, d, J = 11.95, H-14α), 3.59–4.55 (18H, m, H (sophorosyl)), 5.05 (1H, s, $H_A$-17), 5.68 (1H, s, $H_B$-17), 5.13 (1H, d, J = 7.97, H-1s$^1$ (anomer.)), 5.27 (1H, d, J = 7.97, H-1s$^2$ (anomer.)), 6.08 (1H, d, J = 7.97, H-1s$^3$ (anomer.)).

**13-*O*-β-D-Sophorosyl-*ent*-kaur-16-en-19-oic acid (2)** was prepared from stevioside (**1**) by the literature method [21], mp 190°C (MeOH), $[\alpha]_D^{20}$ –32.5° (*c* 0.2, MeOH). IR spectrum (mineral oil), ν, cm$^{-1}$): 3400 (OH), 1691 (C=O), 1662 (C=C), 1245 (C–O). $^1H$ NMR spectrum ($C_5D_5N$, δ, ppm, J/Hz): 1.20 (3H, s, $H_3$-20), 1.31 (3H, s, $H_3$-18), 2.45 (1H, d, J = 13, $H_{eq}$-3), 2.56 (1H, dd, J = 12.8, 1.7, H-14α), 3.69–4.53 (12H, m, H (sophorosyl)), 5.10 (1H, s, $H_A$-17), 5.76 (1H, s, $H_B$-17), 5.17 (1H, d, J = 7.8, H-1s$^1$ (anomer.)), 5.30 (1H, d, J = 7.8, H-1s$^2$ (anomer.)).

**Octane-1,8-diyl-*bis*[13-*O*-(β-D-sophorosyl)-*ent*-kaur-16-en-19-oate] (3).** KOH (0.18 g, 3.2 mmol) and DMSO (20 mL) were stirred for 20 min at 20°C, treated with **2** (0.5 g, 0.7 mmol), stirred another 30 min at 50°C, treated dropwise with 1,8-dibromooctane (0.1 g, 0.38 mmol), stirred for 23 h at 50°C, diluted with distilled $H_2O$ (40 mL), and neutralized with glacial AcOH (0.7 mL). The resulting precipitate was filtered off and chromatographed over a dry column (silica gel, $CH_2Cl_2$–MeOH eluent, 5:0.5 and then 5:1). Diglycoside **3** was isolated as a white powder, 0.22 g (20%), mp 168°C, $[\alpha]_D^{20}$ –45° (*c* 0.1, $CH_3OH$). IR spectrum (mineral oil, ν, cm$^{-1}$): 3364 (OH), 1719 (OC=O), 1662 (C=$CH_2$). $^1H$ NMR spectrum ($CD_3OD$ + $CDCl_3$, δ, ppm, J/Hz): 0.62–2.02 [48H, m, *ent*-kaurane skeletons and spacer $(CH_2)_4$], 0.66 (6H, s, $H_3$-20, 20′), 0.99 (6H, s, $H_3$-18, 18′), 3.02–3.65 (28H, m, sophorosyls and spacer 19, 19′-(O)O-$CH_2C\underline{H}_2$), 3.77–3.93 (4H, m, 19, 19′-(O)O-$CH_2$), 4.39 (2H, d, J = 7.7, H-1s$^1$, 1s$^2$), 4.45 (2H, d, J = 7.7, H-1s$^3$, 1s$^4$), 4.69 (2H, s, $H_A$-17, 17′), 4.95 (2H, s, $H_B$-17, 17′). MALDI mass spectrum, *m/z*: 1418.3 $[M + Na]^+$, 1434.3 $[M + K]^+$. Calcd: $[M + Na]^+$ 1418.7, $[M + K]^+$ 1434.8. $C_{72}H_{114}O_{26}$.

**Octane-1,8-diyl-*bis*[13-*O*-(hepta-*O*-acetyl-β-D-sophorosyl)-*ent*-kaur-16-en-19-oate] (4).** Diglycoside **3** (0.2 g, 0.14 mmol) was dissolved in anhydrous Py (1.2 mL), cooled to 4°C, treated dropwise with $Ac_2O$ (0.86 g, 8.4 mmol), stirred at 20°C for 20 h, warmed to 60°C, stirred for another 4 h, poured into AcOH solution (30 mL, 1%), and extracted with $Et_2O$ (3 × 15 mL). The organic phase was washed with ice water (3 × 30 mL), HCl solution (5%, 3 × 30 mL), saturated $NaHCO_3$ solution (3 × 30 mL), and saturated $Na_2SO_4$ solution (30 mL) and dried over anhydrous $Na_2SO_4$. The solvent was removed at reduced pressure to afford heptaacetylated diglycoside **4** as a white powder, 0.22 g (79%), mp 115°C, $[\alpha]_D^{20}$ –31.4° (*c* 0.7, $CH_2Cl_2$). IR spectrum (mineral oil, ν, cm$^{-1}$): 1755 [OC(O)$CH_3$], 1720 [C(O)OC], 1663 (C=$CH_2$). $^1H$ NMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.79–2.19 (48H, m, *ent*-kaurane skeletons and spacer $(CH_2)_4$), 0.86 (6H, s, $H_3$-20, 20′), 1.17 (6H, s, $H_3$-18, 18′), 1.98–2.08 (42H, m, 14 Ac), 3.64–5.19 (32H, m, sophorosyls and spacer 19, 19′-(O)O-$CH_2C\underline{H}_2$), 4.61 (2H, d, J = 7.8, H-1s$^1$, 1s$^2$), 4.69 (2H, d, J = 7.8, H-1s$^3$, 1s$^4$), 4.80 (2H, s, $H_A$-17, 17′), 5.11 (2H, s, $H_B$-17, 17′). MALDI mass spectrum, *m/z:* 2006.9 $[M + Na]^+$, 2022.9 $[M + K]^+$. Calcd: $[M + Na]^+$ 2006.9, $[M + K]^+$ 2022.9. $C_{100}H_{142}O_{40}$.

**Octane-1,8-diyl-*bis*[13-*O*-(hepta-*O*-acetyl-β-D-sophorosyl)-16-oxo-*ent*-kauran-19-oate] (5).** A solution of **4** (0.22 g, 1.1 mmol) in THF (7 mL) and $H_2O$ (5.2 mL) was treated with aqueous $OsO_4$ (4%, 1 mL, 0.22 mmol), stirred for 15 min at 20°C, treated with $NaIO_4$ (1.74 g, 8.1 mmol), stirred for 24 h at 20°C, and washed with EtOAc (3 × 20 mL). The organic phases were combined, dried over anhydrous $MgSO_4$, and evaporated. Diketone **5** was isolated by chromatography over a dry column (silica gel, petroleum ether–EtOAc eluent, 1:1, then EtOAc) as a white powder, 0.1 g (45%), mp 117°C, $[\alpha]_D^{20}$ –31.6° (*c* 0.6, MeOH). IR spectrum (mineral oil, ν, cm$^{-1}$): 1755 [OC(O)$CH_3$], 1747 (C=O), 1730 [C(O)OC]. $^1H$ NMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.82–1.94 (48H, m, *ent*-kaurane skeletons and spacer $(CH_2)_4$), 0.90 (6H, s, $H_3$-20, 20′), 1.19 (6H, s, $H_3$-18, 18′), 1.97–2.09 (42H, m, 14 Ac), 2.20 (2H, d, J = 12.7, H-3, 3′), 2.39 (2H, dd, J = 13.9, 2.5, H-14α, 14α′), 3.60–5.18 (32H, m, sophorosyls and spacer 19, 19′-(O)O-$C\underline{H}_2C\underline{H}_2$), 4.77 (2H, d, J = 7.6, H-1s$^1$, 1s$^2$), 4.88 (2H, d, J = 7.6, H-1s$^3$, 1s$^4$). MALDI mass spectrum, *m/z*: 2010.9 $[M + Na]^+$, 2025.9 $[M + K]^+$. Calcd: $[M + Na]^+$ 2010.9, $[M + K]^+$ 2025.8. $C_{98}H_{138}O_{42}$.

**Octane-1,8-diyl-*bis*[13-*O*-(hepta-*O*-acetyl-β-D-sophorosyl)-16-thiosemicarbazono-*ent*-kauran-19-oate] (6).** A mixture of **5** (0.17 g, 0.085 mmol) and thiosemicarbazide (0.031 g, 0.34 mmol) was dissolved in EtOH (15 mL), treated dropwise with $H_2SO_4$ solution (3 drops, 20%), and stirred for 24 h at 20°C. The solvent was distilled off. Unreacted thiosemicarbazide was removed by rinsing with hot water (3 × 30 mL). Diglycoside **6** was isolated as a white powder, 0.14 g (78%), mp 155°C. $^1H$ NMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.77–1.96 (48H, m, *ent*-kaurane skeletons and spacer $(CH_2)_4$), 0.89 (6H, s, $H_3$-20, 20′), 1.19 (6H, s, $H_3$-18, 18′), 1.99–2.08 (42H, m, 14 Ac), 2.16–2.23 (2H, m, H-3, 3′), 2.26 (2H, d, J = 11.5, H-14α, 14α′), 3.61–5.20 [36H, m, sophorosyls and spacer 19, 19′-(O)O-$C\underline{H}_2C\underline{H}_2$, H-1s$^1$, 1s$^2$, 1s$^3$, 1s$^4$], 6.45 (2H, s, $NH_{2A}$, $NH_{2'A}$), 7.33 (2H, s, $NH_{2B}$, $NH_{2'B}$), 8.24 (2H, s, NH, NH′). $C_{100}H_{144}O_{40}N_6S_2$. MALDI mass spectrum, *m/z*: 2132.9 $[M]^+$. Calcd: $[M]^+$ 2132.9. $C_{100}H_{144}O_{40}N_6S_2$.

**Octane-1,8-diyl-*bis*{13-*O*-(hepta-*O*-acetyl-β-D-sophorosyl)-16-[(4′-chlorophenylthiazol-2′-yl)hydrazono]-*ent*-kauran-19-oate} (7).** Dithiosemicarbazone **6** (0.062 g, 0.03 mmol) was dissolved in anhydrous EtOH (10 mL), treated with 2-bromo-4′-chloroacetophenone (0.014 g, 0.06 mmol), refluxed for 24 h, cooled, and treated with ice water (25 mL) and $NaHCO_3$ solution (2%, 3 drops). The resulting precipitate was filtered off to isolate diglycoside **7** as a yellow powder, 20%, mp 123°C. $^1$H NMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.82–2.57 (52H, m, *ent*-kaurane skeletons and spacer $(CH_2)_4$), 0.89 (6H, s, $H_3$-20, 20′), 1.19 (6H, s, $H_3$-18, 18′), 1.90–2.10 (42H, m, 14 Ac), 3.62–5.38 (36H, m, sophorosyls and spacer 19, 19′-(O)O-C$\underline{H}_2$C$\underline{H}_2$, H-1s$^1$, 1s$^2$, 1s$^3$, 1s$^4$), 6.82 [2H, s, CH(thiazole), CH′(thiazole)], 7.30 (4H, d, J = 8.4, Ar, Ar′), 7.83 (4H, J = 8.4, Ar, Ar′), 9.92 (2H, s, NH, NH′). $C_{116}H_{150}O_{40}N_6S_2Cl_2$. MALDI mass spectrum, *m/z*: 2403.4 $[M]^+$. Calcd: $[M]^+$ 2403.8. $C_{116}H_{150}O_{40}N_6S_2Cl_2$.


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