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Synthetic Glycosides Containing Two Isosteviol Fragments Functionalized with D-Glucopyranose

B. F. Garifullin, R. R. Sharipova, I. Yu. Strobykina, V. M. Babaev, and V. E. Kataev

Arbuzov Institute of Organic and Physical Chemistry, Kazan Scientific Center, Russian Academy of Sciences, ul. Arbuzova 8, Kazan, 420088 Tatarstan, Russia
e-mail: kataev@iopc.ru

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Abstract—The synthesis of isosteviol (16-oxo-*ent*-beyeran-19-oic acid) glycosides in which two isosteviol fragments functionalized with tetra-*O*-acetyl-D-glucopyranose are linked through a diester spacer is described for the first time.

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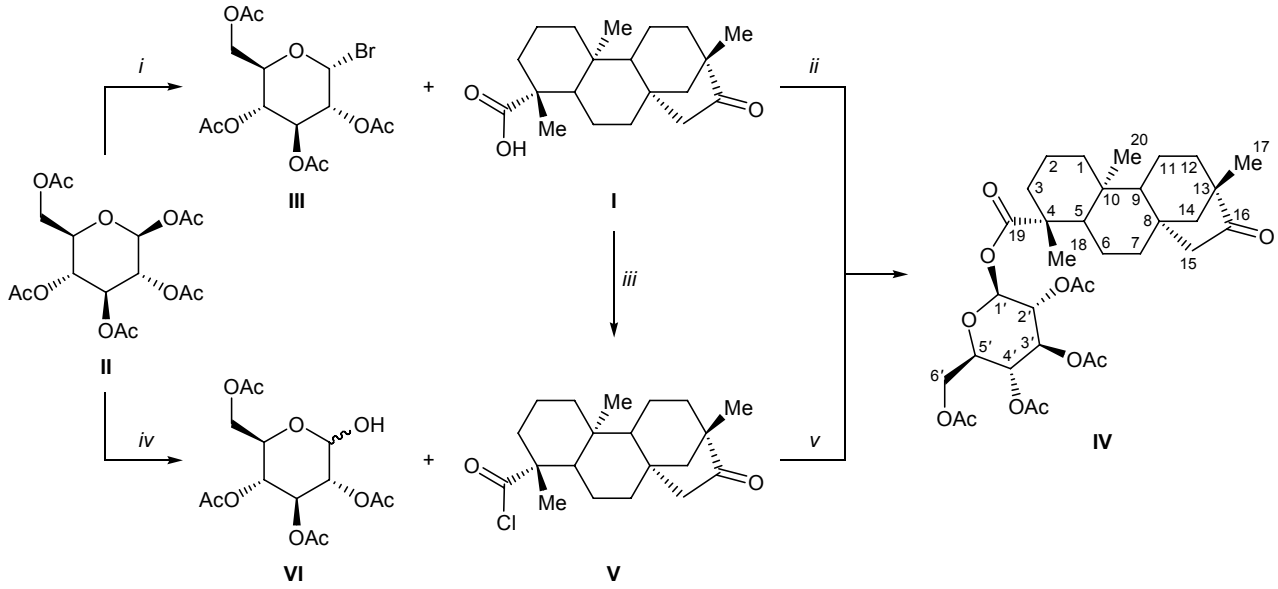
Diterpene glycosides occur in natural sources in different amounts. The most abundant (about 70) are *ent*-kaurane glycosides [1–3], next follow (more than 20) glycosides of the pimarane and isopimarane series [4–6], the third place (>10) is occupied by labdane derivatives [7, 8], and the fourth, by several cembrane [9, 10] and abietane glycosides [11, 12]. Among diterpene glycosides, the most common are *ent*-kaurane glycosides isolated from *Stevia rebaudiana* (they are more often referred to as rebaudiosides), in which the aglycone is diterpenoid steviol (13-hydroxy-*ent*-kaur-16-en-19-oic acid) [13]. The reason is that rebaudiosides are 250–300 times sweeter than sucrose; therefore, they are used in China, Japan, and Southeast Asian countries for large-scale manufacture of low-calorie sweeteners. It should be noted that one step in the development of the production of these sweeteners involves chemical and enzymatic modification of rebaudiosides with a view to enhance their taste characteristics. Therefore, it is not surprising that a large number of various glycosides have been synthesized where the aglycone is an *ent*-kaurane diterpenoid, steviol [14]. On the other hand, only one natural glycoside is known for its isomer, *ent*-beyerane diterpenoid isosteviol (**I**, 16-oxo-*ent*-beyeran-19-oic acid) [15], which is readily obtained by acid hydrolysis of rebaudiosides via skeletal rearrangement of steviol; this glycoside was also isolated from *Stevia rebaudiana* [16]. Although glycosylation of natural biologically active non-glycoside compounds has long been proposed as a tool for tuning their activity [17], among more than 150 known isosteviol derivatives [18, 19] only three ones are synthetic glycosides [20–22].

In the present article we describe the synthesis of new isosteviol glycosides. In the first step of our study we compared the efficiencies of two procedures for the synthesis of isosteviol glycoside **IV**. The first procedure is based on the Koenigs–Knorr reaction [21], i.e., the reaction of isosteviol (**I**) with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**III**) prepared from 1,2,3,4,6-pentaacetyl-β-D-glucose (**II**). The second procedure involved reaction of acid chloride **V** with 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose (**VI**) (Scheme 1). The yield of **IV** was 66% in the first case and 4% in the second.

In the second step, 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**III**) was brought into reaction with dihydroisosteviol **VII** prepared by chemoselective reduction of isosteviol (**I**) with sodium tetrahydridoborate in methanol according to the procedure described in [23]. Phase-transfer catalysis with tetrabutylammonium bromide ensured glycosylation to occur exclusively at the carboxy group of **VII**, while the 16-hydroxy group remained intact (Scheme 2). The addition of the D-glucopyranose fragment to the carboxy group of **VII** followed from the disappearance of the IR absorption band at 1690 cm^{-1} (COOH) and conservation of the band at 3470 cm^{-1}, corresponding to stretching vibrations of the 16-OH group.

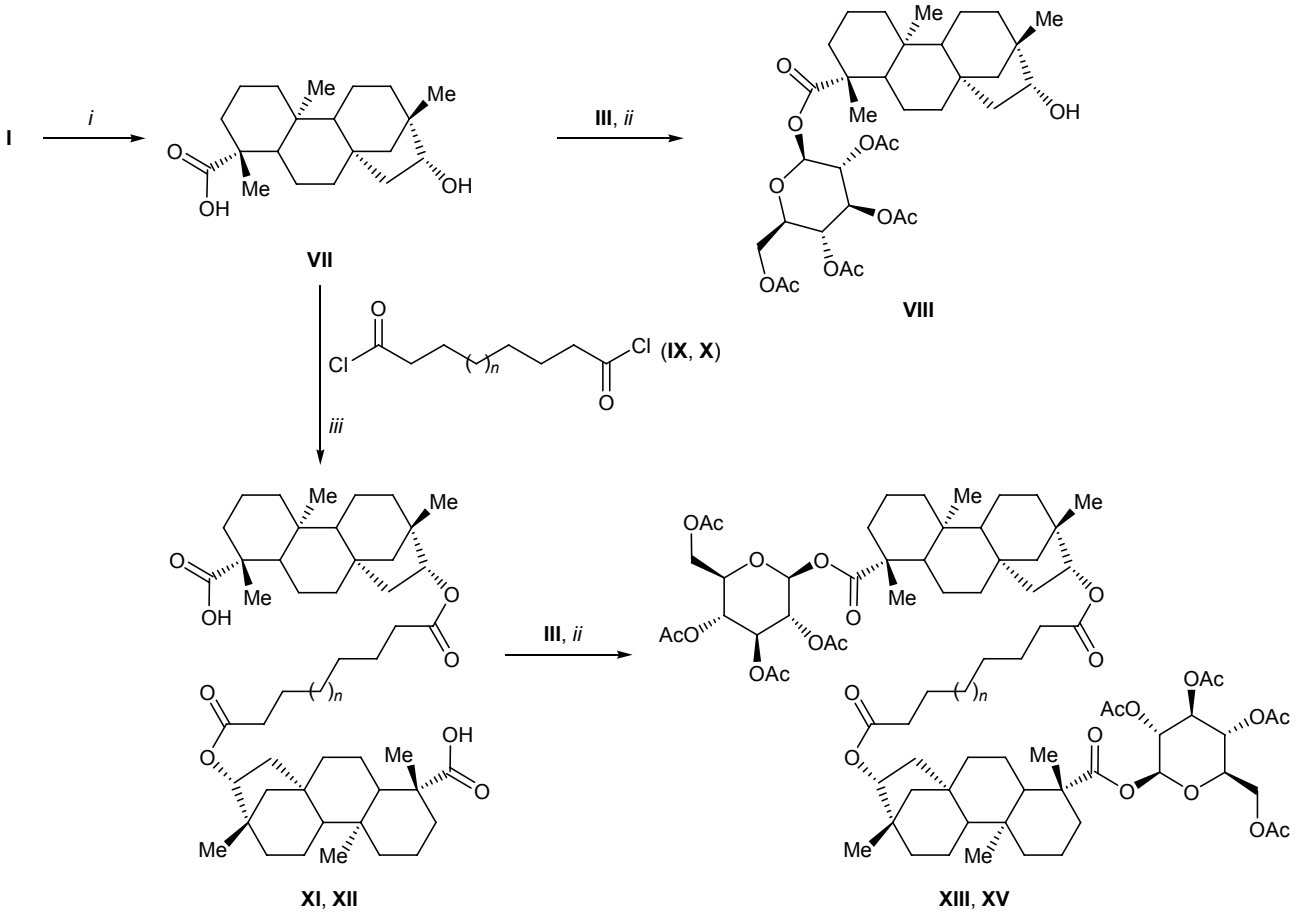
In the third step, we synthesized dinuclear isosteviol derivatives **XI** and **XII** by reaction of dihydro-

Scheme 1.



i: 33% HBr/AcOH, 0°C; *ii*: $Bu_4N^+Br^-$, K_2CO_3, CH_2Cl_2–H_2O, reflux; *iii*: $SOCl_2$, 50°C; *iv*: $FeCl_3 \cdot 6H_2O$, MeCN, 70°C; *v*: CCl_4, reflux.

Scheme 2.



i: $NaBH_4$, MeOH; *ii*: $Bu_4N^+Br^-$, K_2CO_3, CH_2Cl_2–H_2O, reflux; *iii*: CH_2Cl_2, DMAP, pyridine.
IX, **XI**, **XIII**, $n = 1$; **X**, **XII**, **XIV**, $n = 3$.

isosteviol **VII** with octane- and decanedioyl dichlorides. Reactions of **XI** and **XII** with bromide **III** gave dinuclear isosteviol glycosides **XIII** and **XIV** in 29 and 58% yield, respectively (Scheme 2).

The anomeric protons in glycosides **IV**, **VIII**, **XIII**, and **XIV** resonated in the ^{1}H NMR spectra as doublets at δ 5.68 (broadened, $^3J_{1,2}$ = 7.9 Hz), 5.69 (broadened, $^3J_{1,2}$ = 8.2 Hz), 5.68 ($^3J_{1,2}$ = 7.6 Hz), and 5.68 ppm ($^3J_{1,2}$ = 7.4 Hz), respectively. These values considerably differ from the corresponding data for 1,2,3,4,6-penta-*O*-acetyl-α-D-glucopyranose (δ 6.33 ppm, d, $^3J_{1,2}$ = 3.5 Hz) but approach those typical of its β-anomer (δ 5.71 ppm, d, $^3J_{1,2}$ = 8.3 Hz) [24]. Therefore, compounds **IV**, **VIII**, **XIII**, and **XIV** were unambiguously assigned the β-glycoside structure.

All known diterpene glycosides [1–12], including isosteviol glycosides [16, 20–22], contain only one aglycone (monoglycosides). The described synthesis of dinuclear glycosides with two natural diterpenoid fragments as aglycones pioneers a new line in synthetic organic chemistry.

EXPERIMENTAL

The ^{1}H NMR spectra were recorded on a Bruker Avance-400 spectrometer at 400 MHz. The mass spectra (MALDI) were obtained on a Bruker UltraFlex III TOF/TOF mass spectrometer (a.m.u. range 200–3000). The data were processed using FlexAnalysis 3.0 program. Given below are *m*/*z* values for monoisotopic ions. Samples were dissolved in methylene chloride to a concentration of 10^{-3} mg/mL. *p*-Nitroaniline used as matrix was dissolved in methanol to a concentration of 5 mg/mL. Samples were applied by the dried drop method. A 0.3-μL portion of the matrix solution was applied onto a Bruker Anchor Chip target; after evaporation of the solvent, 0.5 μL of a 1:1 mixture of an analyte and calibration mixture was applied thereonto. The IR spectra were recorded from films on a Bruker Vector 22 spectrometer with Fourier transform. The progress of reactions and the purity of products were monitored by TLC on Sorbfil plates; spots were detected by treatment with 5% H_2SO_4, followed by heating to 120°C. The products were isolated by flash chromatography using dry columns charged with KSKG silica gel with a grain size of less than 0.063 mm.

Isosteviol (**I**) [25], bis-acids **XI** and **XII** [26, 27], 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose (**VI**) [28], and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopiranosyl bromide (**III**) [29] were synthesized according to known methods. 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopiranosyl acetate and tetrabutylammonium bromide were commercial products (from Acros Organics). Isosteviol glycoside **IV** was synthesized by reaction of isosteviol (**I**) with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**III**) by analogy with the procedure described in [21], as well as by reaction of acid chloride **V** with 2,3,4,6-tetraacetyl-β-D-glucopyranose (**VI**).

2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl 16-oxo-*ent*-beyeran-19-oate (IV). A mixture of 0.78 g (2.4 mmol) of isosteviol (**I**) and 1 mL of thionyl chloride was heated for 2 h at 50°C. Excess thionyl chloride was removed under reduced pressure (water-jet pump). The residue was treated with carbon tetrachloride, and the solvent was removed under reduced pressure; this procedure was repeated several times until the thionyl chloride odor disappeared. The product, chloride **V**, was dissolved in 15 mL of carbon tetrachloride, a solution of 0.85 g (2.4 mmol) of 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose in 10 mL of carbon tetrachloride was added, and the mixture was heated for 48 h under reflux. The solvent was removed under reduced pressure, and the residue was subjected to chromatography on silica gel using petroleum ether–ethyl acetate (4:1) as eluent. Yield 4%, amorphous powder, $[\alpha]_D^{20}$ = –44° (*c* = 0.4, CH_2Cl_2). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 0.60–1.90 m (19H, *ent*-beyerane), 0.69 s (3H, $C^{20}H_3$), 0.96 s (3H, $C^{17}H_3$), 1.19 s (3H, $C^{18}H_3$), 2.00 s (3H, CH_3CO), 2.02 s (3H, CH_3CO), 2.03 s (3H, CH_3CO), 2.06 s (3H, CH_3CO), 2.50 d.d (1H, 15α-H, *J* = 18.4, 3.6 Hz), 3.73–3.80 m (1H, 5′-H), 4.02 d.d (1H, 6′-H, *J* = 12.4, 1.9 Hz), 4.31 d.d (1H, 6′-H, *J* = 12.4, 4.7 Hz), 5.06–5.13 m (1H), 5.15–5.23 m (2H), 5.68 br.d (1H, 1′-H, *J* = 7.9 Hz). Mass spectrum, *m*/*z*: 671.34 $[M + Na]^+$, 687.26 $[M + K]^+$. $C_{34}H_{48}O_{12}$. Calculated: $[M + Na]^+$ 671.30, $[M + K]^+$ 687.27.

Isosteviol glycosides VIII, XIII and XIV (*general procedure*). A solution of 2.4 mmol of potassium carbonate in 5 mL of water was added to a solution of 1 mmol of compound **VII** or 0.5 mmol of bis-acid **XI** or **XII**, 1 mmol of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**III**), and 0.015 g of tetrabutylammonium bromide in methylene chloride, and the mixture was heated for 30 h under reflux. The organic phase was separated and washed with water and brine, the aqueous phase was extracted with methylene chloride, and the extract was combined with the organic phase and dried over anhydrous Na_2SO_4. The

products were isolated by chromatography on a dry column charged with silica gel using first petroleum ether–ethyl acetate (4 : 1) and then ethyl acetate as eluents.

2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl 16-hydroxy-*ent*-beyeran-19-oate (VIII). Yield 47%, amorphous powder, $[\alpha]_D^{20} = -32°$ (c = 0.33, CH_2Cl_2). IR spectrum, ν, cm^{-1}: 3470 (OH), 1755 (C=O), 1226 (C–O). ^1H NMR spectrum ($CDCl_3$), δ, ppm: 0.70–1.80 m (19H, *ent*-beyerane), 0.71 s (3H, $C^{20}H_3$), 0.89 s (3H, $C^{17}H_3$), 1.18 s (3H, $C^{18}H_3$), 2.00 s (3H, CH_3CO), 2.02 s (3H, CH_3CO), 2.03 s (3H, CH_3CO), 2.07 s (3H, CH_3CO), 2.15 d (1H, 3-H_{eq}, J = 13.1 Hz), 3.74–3.80 m (1H, 5′-H), 3.81–3.85 m (1H, 16-H), 4.02 d.d (1H, 6′-H, J = 12.3, 2.2 Hz), 4.32 d.d (1H, 6′-H, J = 12.4, 4.7 Hz), 5.06–5.17 m (1H), 5.18–5.21 m (1H), 5.69 br.d (1H, 1′-H, J = 8.2 Hz). Mass spectrum, m/z: 673.35 $[M + Na]^+$, 689.34 $[M + K]^+$. $C_{34}H_{50}O_{12}$. Calculated: $[M + Na]^+$ 673.32, $[M + K]^+$ 689.29.

Bis[19-*nor*-4α-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyloxycarbonyl)-*ent*-beyeran-16-yl] octanedioate (XIII). Yield 29%, amorphous powder, $[\alpha]_D^{20} = -39°$ (c = 0.28, CH_2Cl_2). ^1H NMR spectrum ($CDCl_3$), δ, ppm: 0.60–1.90 m [46H, *ent*-beyerane, $(CH_2)_4$], 0.65 s (6H, $C^{20}H_3$), 0.88 s (6H, $C^{17}H_3$), 1.15 s (6H, $C^{18}H_3$), 1.96 s (6H, CH_3CO), 1.98 s (6H, CH_3CO), 1.99 s (6H, CH_3CO), 2.03 s (6H, CH_3CO), 2.14 d (2H, 3-H_{eq}, J = 13.8 Hz), 2.24 t [4H, $C(O)CH_2$, J = 7.4 Hz], 3.74–3.80 m (2H, 5′-H), 3.97–4.04 m and 4.25–4.29 m (2H each, 6′-H), 4.63–4.74 m (2H, 16-H), 5.03–5.13 m (2H), 5.14–5.23 m (2H), 5.66 d (2H, 1′-H, J = 7.6 Hz). Mass spectrum, m/z: 1461.75 $[M + Na]^+$, 1477.73 $[M + K]^+$. $C_{76}H_{110}O_{26}$. Calculated: $[M + Na]^+$ 1461.72, $[M + K]^+$ 1477.69.

Bis[19-*nor*-4α-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyloxycarbonyl)-*ent*-beyeran-16-yl] decanedioate (XIV). Yield 58%, amorphous powder, $[\alpha]_D^{20} = -41°$ (c = 0.44, CH_2Cl_2). ^1H NMR spectrum ($CDCl_3$), δ, ppm: 0.6–1.9 m [50H, *ent*-beyerane, $(CH_2)_6$], 0.67 s (6H, $C^{20}H_3$), 0.90 s (6H, $C^{17}H_3$), 1.17 s (6H, $C^{18}H_3$), 1.98 s (6H, CH_3CO), 1.99 s (6H, CH_3CO), 2.01 s (6H, CH_3CO), 2.05 s (6H, CH_3CO), 2.14 d (2H, 3-H_{eq}, J = 13.5 Hz), 2.26 t [4H, $C(O)CH_2$, J = 7.4 Hz], 3.73–3.83 m (2H, 5′-H), 4.00–4.07 m (2H, 6′-H), 4.30 d.d (2H, 6′-H, J = 12.3, 4.7 Hz), 4.68 d.d (2H, 16-H, J = 10.4, 3.2 Hz), 5.07–5.15 m (2H), 5.16–5.24 m (2H), 5.68 d (2H, 1′-H, J = 7.4 Hz). Mass spectrum, m/z: 1489.94 $[M + Na]^+$, 1505.90 $[M + K]^+$; m/z 1505.724 $[M + K]^+$. $C_{78}H_{114}O_{26}$. Calculated: $[M + Na]^+$ 1489.750, $[M + K]^+$ 1505.724.

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