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Synthetic Glycosides of *ent*-Caurene Series Containing Substituents with Benzyl, Phenoxyl, and Uracyl Fragments

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Abstract—New 19-O esters of steviolobioside containing benzyl, phenoxyl, and 6-methyluracyl fragments were synthesized. Molecular structure of two glycosides was established by the X-ray diffraction analysis.

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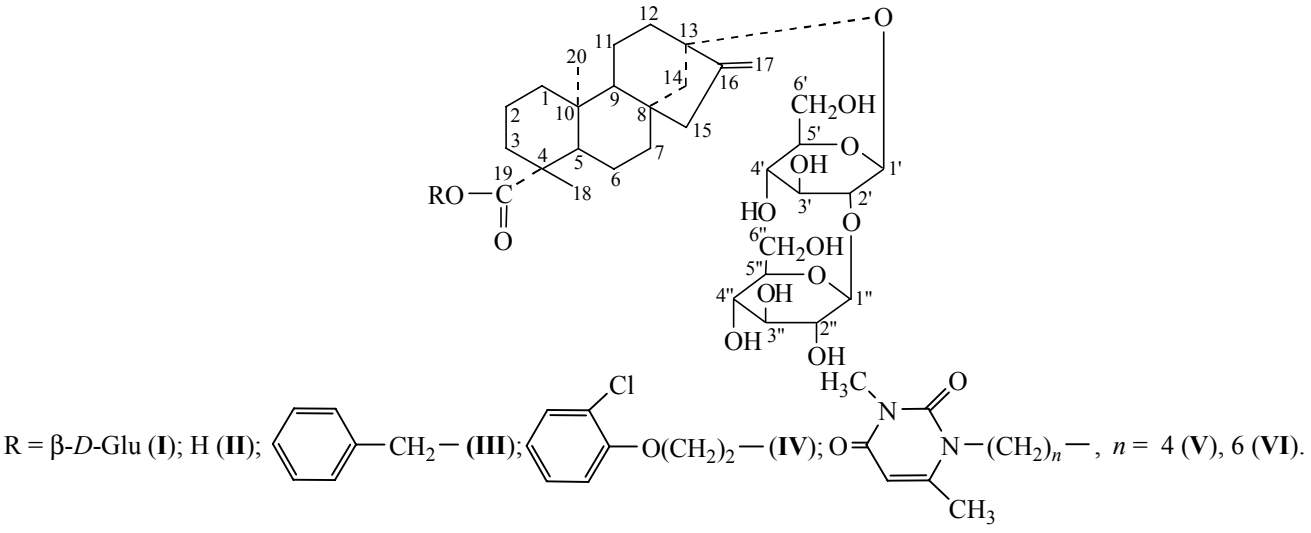
Natural glycosides including those on the basis of higher terpenoids are well known due to their biological activity [1–6]. For example, such glycoside as glycirrisinic acid exhibits the antiviral, antitumour, and gepatoprotective activity [2]. Stevioside **I** and steviolobioside **II**, the glycosides from *Stevia rebaundiana* demonstrate the insulinotropic and antihyperglycemic action [3, 4]. Large series of the synthetic derivatives of glycirrisinic acid is described [2], while in the case of the rebaudioside steviolobioside **II** only several esters [5] and amides [6] are known. It proved that they possess the antibacterial activity, hence the continuation of syntheses of the glycoside **II** derivatives presents great interest.

In this work the synthesis of four new 19-O-esters of steviolobioside containing the benzyl and uracyl fragments is reported. Compounds **III–VI** were obtained analogously to [7] by the reaction of steviolobioside **II** with benzyl bromide, *o*-chloro-2-bromoethoxybenzene, N^1-4-bromobutyl-3,.6-dimethyluracyl, and N^1-6-bromohexyl-3,6-dimethyluracyl in 20–45% yield after the column chromatography on silica gel.



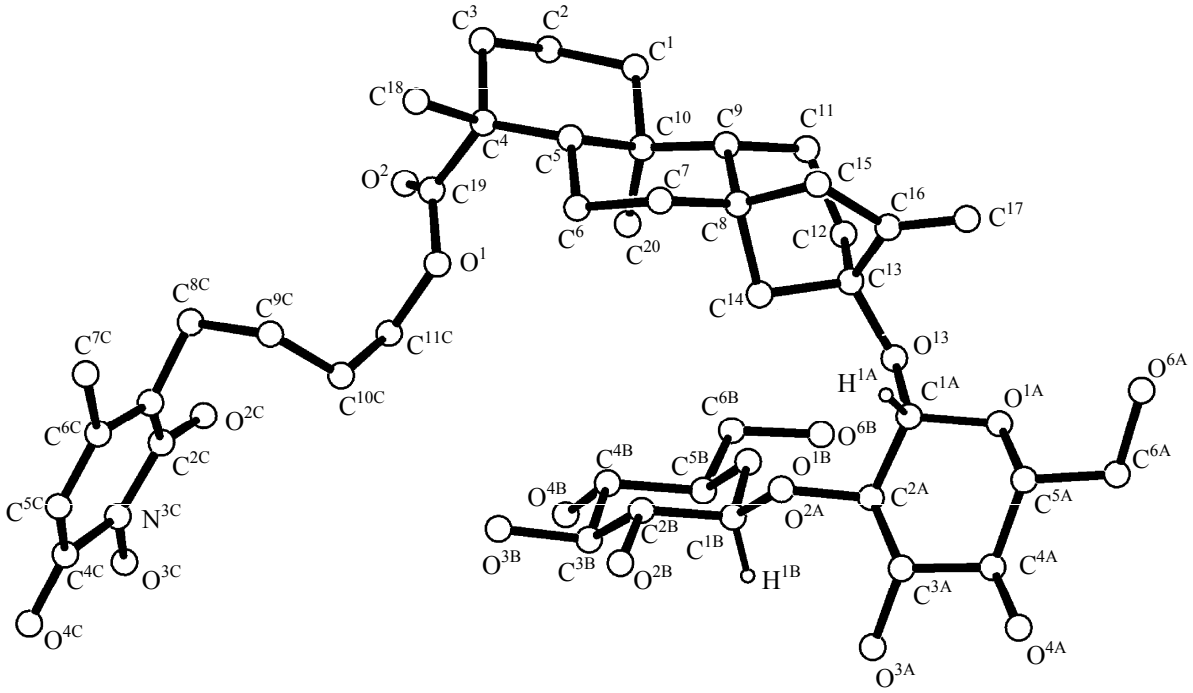


Fig. 1. Spatial arrangement of the glycoside **V** molecule in crystal (only the anomeric hydrogen atoms of the glucose residues from the soforosyl fragment are shown).

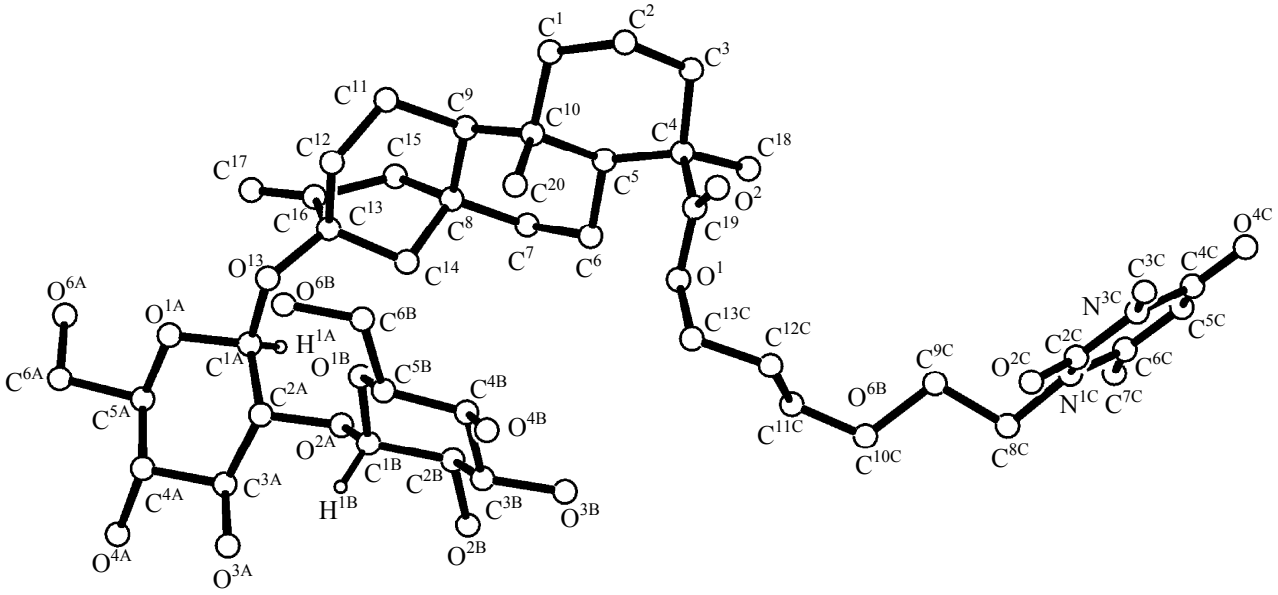


Fig. 2. Spatial arrangement of the glycoside **VI** molecule in crystal (only the anomeric hydrogen atoms from the glucose residues of the soforosyl fragment are shown).

Compounds **III–VI** are white crystalline substances well soluble in methanol, pyridine, DMSO, and DMF. Their structure is confirmed by spectral methods (IR, ^{1}H NMR), elemental analysis data, and MALDI mass spectra. Molecular structure of the glycosides **V, VI** established by XRD analysis is presented in Figs. 1, 2.

EXPERIMENTAL

^{1}H NMR spectra were taken on a Bruker Avance spectrometer (600 MHz) in C_5D_5N. IR spectra were recorded on a Bruker Vector-22 Fourier spectrometer from the suspensions in mineral oil. Mass spectra of

the matrix-activated laser desorption-ionization (MALDI) were measured on a time-of-flight Bruker Reflex III mass spectrometer in the mode of positive ions registration. For laser desorption the impulse UV laser was used (λ 337 nm). α-Cyano-4-hydroxycinnamic acid was used as a matrix. The samples were prepared by the "dried drop" method. A mixture of the saturated matrix solution in methanol and the methanolic solution of compound under study was applied on the sublayer and dried at 25°C.

X-ray analysis of compounds **V**, **VI** was carried out in the Department of X-ray studies of the Spectro-analytical Center of Collective Use on the basis of the laboratory of the diffractional methods of investigation of the Arbuzov Institute of Organic and Physical Chemistry of the Kazan Scientific Center of the Russian Academy of Sciences. Single crystals of compounds **V**, **VI** were prepared by crystallization from methanol. X-ray diffraction experiments were carried out on a Kappa Apex diffractometer [graphite monochromator λ(CuK_α) = 1.54184 Å, temperature 293(2) K, φ,ω-scanning]. Crystallographic data and main refining parameters for the structures **V**, **VI** are listed in the table. Semiempirical accounting for the extinction was carried out with the help of the SADABS program [9]. Structures were solved by the direct method. The positions and temperature parameters of nonhydrogen atoms were refined in isotropic and then in anisotropic approximation by the full-matrix route-mean-square method.

Water molecules were revealed in crystals of both compounds. For the crystal **V** the glycoside–water ratio is 1:3, and for the crystal **VI** it is 1:4. Hydrogen atoms were placed in the geometrically calculated positions and included in refining in the *rider* model. All calculations were carried out using SHELXL-97 complex of programs [10]. Figures 1 and 2 were drawn using the PLATON program [11]. Atomic coordinates of structures and their temperature parameters were deposited in the Cambridge Crystallostructural Data Center (http://www.ccdc.cam.ac.uk).

TLC was carried out on Silufol UV-254 plates (Chemapol, Czechia). Development of chromatograms was carried out by treating the plates with a mixture of the saturated potassium permanganate solution and acetic acid (1:1). Elution was carried out with 6:1 chloroform-methanol. Column chromatography was carried out on an Alfa Aesar Silica gel 60, 0.060–0.2 mm. Specific rotation was measured on a Perkin-Elmer M 341 polarimeter, cell 55 mm long. Melting points were measured on a Boetius apparatus. Steviolobioside **II** was prepared according to the reported procedure [8].

Commercial benzyl bromide from Alfa Aesar was used. *o*-Chloro-2-bromoethoxybenzene was prepared according to the procedure [12]. N^1-ω-bromouracyls

Crystallographic data and the parameters of the X-ray experiment

Parameter	Crystal of compound **V**	Crystal of compound **VI**
Formula	$C_{42}H_{62}N_2O_{15}\cdot 3\,H_2O$	$C_{44}H_{68}N_2O_{15}\cdot 4\,H_2O$
Molecular weight	888.98	937.07
Crystal system	Monoclinic	
Space group	$P2_1$	
a, Å	17.363(5)	16.2145(7)
b, Å	7.734(2)	7.6176(3)
c, Å	19.064(5)	21.1838(9)
β, deg	113.67(2)	108.850(3)
V, Å^3	2344.7(11)	2476.20(18)
Z	2	
d_{calc}, g cm^{-3}	1.259	1.257
μ, cm^{-1}	8.22	8.18
Scanning range	$2.53 \le \theta \ge 59.97$	$2.20 \le \theta \ge 59.98$
Number of reflections measured (R_{int})	10764 (0.1398)	15821 (0.0442)
Number of reflections with $I \ge 2\sigma(I)$	1589	3161
Number of refined parameters	571	591
$R_1[I \ge 2\sigma(I)]$	0.0847	0.0722
wR_2 (by all reflections)	0.2067	0.2266

were prepared by the reaction of sodium 3,6-dimethyluracyl with the 5-fold excess of 1,4- and 1,6-dibromoalkanes according to the reported procedures [13, 14].

General procedure for preparing compounds III–VI. A mixture of 0.09 g of KOH and 20 ml of DMSO was stirred for 1 h at 60–70°C, 0.5 g of steviolobioside **II** was added, and the resulting mixture was stirred for additional 30 min. After that 0.7 mmol of the corresponding alkylating agent was added dropwise, and the mixture obtained was stirred for 10 h. The reaction progress was monitored by TLC. After the completion of the reaction the mixture was diluted with water to 500 ml and extracted with butanol (4×100 ml). Joined extracts were dried with magnesium sulfate and concentrated in a vacuum. After chromatographing on silica gel (elution with chloroform–methanol) the products **III–VI** were obtained and crystallized from methanol.

13-*O*-[β-D-soforosyl]-benzyl-*ent*-cauren-19-oate (III). White powder, 0.18 g (32%) was isolated, mp 165°C, R_f 0.3. Elution with 10:3 chloroform-methanol. $[\alpha]_D^{20}$ –49° (*c* 0.3, MeOH). IR spectrum, ν, cm^{-1}: 1718 (C=O), 1660 (C=C), 1630, 1550, 1450, 754, 698 (all H_{Ph}). ^1H NMR spectrum (pyridine-d_5), δ, ppm (*J*, Hz): 0.71 m (1H, C^1H), 0.87 d (1H, C^9H, *J* 6.1), 0.97 s (3H, $C^{20}H_3$), 1.17 s (3H, $C^{18}H_3$), 1.29-2.18 m (14H, aglycone), 2.28 d (1H, C^3H, *J* 13.7), 2.48 d (1H, $C^{14}H$, *J* 11.2), 3.81–4.53 m (12H, soforosyl), 5.08 s (1H, $C^{17}H_2$), 5.74 s (1H, $C^{17}H_2$), 5.15–5.19 m [2H, C(O)–CH_2], 5.30 d (1H, $C^{1'}H_{anomer}$, *J* 7.6), 5.35 d (1H, $C^{1'}H_{anomer}$. *J* 7.6), 7.3–7.6 m (5H, Ar). Found, %: C 63.75; H 7.78. $C_{39}H_{56}O_{13}$. Calculated, %: C 63.90, H 7.71.

1,3-*O*-[β-D-soforosyl]-[2-(*o*-chlorophenoxy)ethyl]-*ent*-cauren-19-oate (IV). White powder, 0.12 g (20%) was isolated, mp 135°C. R_f 0.42. Elution with 10:2 chloroform–methanol. $[\alpha]_D^{20}$ –36° (*c* 0.4, MeOH). IR spectrum, ν, cm^{-1}: 1718 (C=O), 1660 (C–C), 1590, 1465, 891, 749 (all H_{Ph}). ^1H NMR spectrum (pyridine-d_5), δ, ppm (*J*, Hz): 0.69 m (1H, C^1H), 0.85 d (1H, C^9H, *J* 6.1), 0.96 d (1H, C^5H, *J* 12.2), 1.00 s (3H, $C^{20}H_3$), 1.19 s (3H, $C^{18}H_3$), 1.27–2.1 m (14H, aglycone), 2.27 d (1H, C^3H, *J* 13.7), 2.50 d (1H, $C^{14}H$ *J* 11.2), 3.74-4.66 m (12H, soforosyl), 4.6-4.7 m [2H, $C(O)CH_2$], 5.04 s (1H, $C^{17}H_2$), 5.7 s (1H, $C^{17}H_2$), 5.07 d (1H, $C^{1'}H_{anomer}$, *J* 7.6), 5.15 d (1H, $C^{1'}H_{anomer}$, *J* 7.6), 6.8–7.4 m (4H, Ar). Mass spectrum, *m/z* (I_{rel}, %): 821 (100) $[M + Na]^+$. Found, %: C 51.06; H 7.82; Cl 4.03. $C_{40}H_{57}O_{14}Cl{\cdot}8H_2O$. Calculated, %: C 51.02, H 7.83, Cl 3.76.

13-*O*-[β-D-soforosyl]-[4-N^1(3,6-dimethyluracyl)-butyl]-*ent*-cauren-19-oate (V). Needle-like crystals, 0.15 g (20%) were isolated, mp 115°C, R_f 0.4 Elution with 10:2 chloroform-methanol, $[\alpha]_D^{20}$ –35° (*c* 0.3, MeOH). IR spectrum, ν, cm^{-1}: 1714 (C=O), 1702 (C^2=O_{uracyl}), 1662 (C=C), 1653 (C^4=O_{uracyl}), 1614 (C–N). ^1H NMR spectrum (pyridine-d_5), δ, ppm (*J*, Hz): 0.73 m (1H, C^1H). 0.91 d (1H, C^9H, *J* 6.1), 1.01 s (3H, $C^{20}H_3$), 1.20 s 3H, $C^{18}H_3$), 1.4–2.23 m (14H, aglycone), 2.14 s (3H, $C^{6'}{}_{uracyl}H_3$), 2.31 d (1H, C^3H, *J* 13.7), 2.55 d (1H, $C^{14}H$, *J* 11.2), 3.37 s (3H, $N^3{}_{uracyl}H_3$), 3.78-4.51 m (12H, soforosyl), 3.87-3.9 m [2H, C(O)-CH_2], 5.09 s (1H, $C^{17}H_2$), 5.77 s (1H, $C^{17}H_2$), 5.17 d (1H, $C^{1'}H_{anomer}$, *J* 7.6), 5.29 d (1H, $C^{1'}H_{anomer}$, *J* 7.6), 5.68 s (1H, $C^5{}_{uracyl}H$). Mass spectrum. *m/z* (I_{rel}, %): 860(100) $[M + Na]^+$. Found, %: C 56.51, H 7.98, N 3.04. $C_{42}H_{64}O_{15}N_2{\cdot}2H_2O$. Calculated, %: C 56.62; H 7.93; N 3.14.

13-*O*-[β-D-soforosyl]-[6-N^1(3,6-dimethyluracyl)-hexyl]-*ent*-cauren-19-oate (VI). Needle-like crystals, 0.6 g (45%) were isolated, mp 132°C, R_f 0.38. Elution with 10:5 chloroform-methanol. $[\alpha]_D^{20}$ –44° (*c* 0.3, MeOH). IR spectrum, ν, cm^{-1}: 1718 (C=O), 1702 (C^2=O_{uracyl}), 1670 (C^4=O_{uracyl}). 1656 (C=C), 1615 (C–N). ^1H NMR spectrum (pyridine-d_5), δ, ppm (*J*, Hz): 0.74 m (1H, C^1H, 0.89 d (1H, C^9H, *J* 6.1), 1.02 s (3H, $C^{20}H_3$), 1.19 s (3H, $C^{18}H_3$), 1.3–2.1 m (14H, aglycone), 2.11 s (3H, $C^6{}_{uracyl}H_3$), 2,31 d (1H, C^3H, *J* 13.7), 2.54 d (1H, $C_{14}H$, *J* 11.2), 3.38 s (3H, $N^3{}_{uracyl}H$), 3.30–3.79 m [2H, C(O)–CH_2), 3.94-4.51 m (12H, soforosyl), 5.08 s (1H, $C^{17}H_2$), 5.75 s (1H, $C^5{}_{uracyl}H$). Mass spectrum, *m/z* (I_{rel}, %): 888 (100) $[M + Na]^+$. Found, %: C 56.63, H 8.12, N 3.07. $C_{44}H_{68}O_{15}N_2$. Calculated, %: C 56.38; H 8.19; N 2.98.

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