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New Synthesis of Diterpenoid (16*S*)-Dihydrosteviol

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Abstract—A new procedure has been developed for the synthesis of diterpenoid (16*S*)-dihydrosteviol [(16*S*)-13-hydroxy-*ent*-kauran-19-oic acid] by acid hydrolysis (0.7% hydrochloric acid) of SWETA food sweetener and subsequent reduction of the resulting mixture of caurenoids with hydrazine hydrate over Raney nickel. The molecular geometry of (16*S*)-dihydrosteviol, as well as of Δ^{15}-steviol (13-hydroxy-*ent*-kaur-15-en-19-oic acid), was determined for the first time by X-ray analysis.

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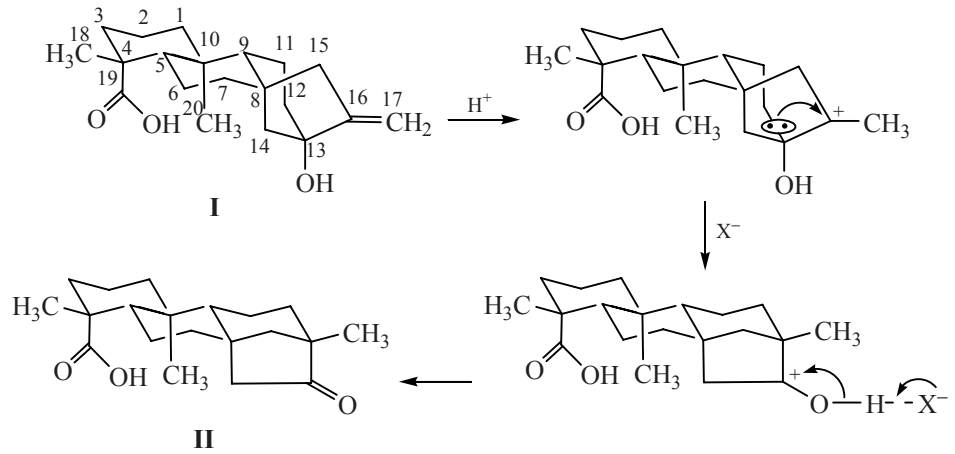
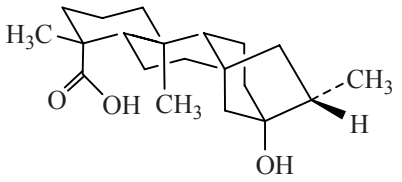
Steviol (**I**, 13-hydroxy-*ent*-kaur-16-en-19-oic acid) is a diterpenoid of the kaurane series [1, 2]; it is an aglycone in glycosides isolated from *Stevia rebaudiana* Bertoni, which are widely used as low-calorie sweeteners both in the form of individual substances (e.g., stevioside is sweeter than saccharose by a factor of 300 [3, 4]) and as enzymatic treatment product (SWETA [5]). Like most higher terpenoids, steviol is a biologically active compound. It inhibits glucose absorption [6], ADP/ATP exchange [7], and oxidative phosphorylation [8] and exhibits bactericidal [9], anti-inflammatory, and immunomodulating activity [10]. However, steviol (**I**) has found no application in medicinal chemistry as precursor of new synthetic biologically active diterpenoids of the kaurane series. A probable reason is that it undergoes Wagner–Meerwein rearrangement in acid medium to give isomeric diterpenoid isosteviol (**II**, 16-oxo-*ent*-beyeran-19-oic acid) [11, 12].

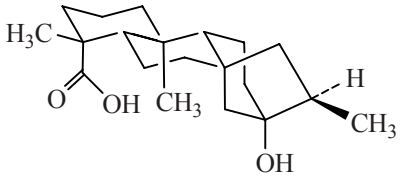
The rearrangement is promoted by the presence of exocyclic double C=C bond in molecule **I**. Therefore, to obtain precursors of new steviol derivatives with acid-resistant *ent*-kaurane skeleton it is necessary to modify the unsaturated moiety. The simplest way to do this is hydrogenation of the double bond with formation of dihydrosteviol (**III**). We have found only one published procedure for the synthesis of dihydrosteviol (**III**) via hydrogenation of steviol (**I**) with hydrogen over platinum or palladium [9], which leads to the formation of a mixture of epimers **IIIa** and **IIIb**.



I **II**



IIIa



IIIb

We used a more convenient procedure (from the viewpoints of stereoselectivity and reagent cost), namely hydrogenation of the double bond in steviol (**I**) with hydrazine hydrate over Raney nickel, in keeping with the data of [13]. This reaction should be stereospecific (*syn*-addition at the less sterically loaded side [13]). The IR spectrum of the reduction product lacked absorption band at 1660 cm^{-1}, which is typical of stretching vibrations of the double C=C bond in steviol (**I**). In the ^1H NMR spectrum of this compound we observed no signals assignable to protons at a double C=C bond (δ 4.82 and 4.98 ppm in the spectrum of **I**), signals from protons in the methyl groups were located at δ 0.93 ($C^{20}H_3$) and 1.22 ppm ($C^{18}H_3$), and a doublet appeared at δ 0.97 ppm (^3J = 7 Hz) due to newly formed $C^{17}H_3$ group. The electron impact mass spectrum of the reduction product contained the molecular ion peak with *m*/*z* 320. These data indicated formation of dihydrosteviol (**III**). The specific optical rotation and melting point of the product {$[\alpha]_D^{20}$ = –40° (*c* = 0.38, $CHCl_3$), mp 190°C} were consistent with published data for (16*S*)-epimer **IIIa** {$[\alpha]_D^{20}$ = 41.5° (*c* = 0.51) [14], $[\alpha]_D^{20}$ = –28.2° (*c* = 0.43, $CHCl_3$); mp 190–192°C [9]}. According to [9], (16*R*)-epimer **IIIb** is characterized by essentially different parameters: $[\alpha]_D^{20}$ = –110.9° (*c* = 0.33, $CHCl_3$), mp 216–218°C; therefore, the reduction product was assigned structure **IIIa**.

It should be noted that our assignment was based on the data of [15], where the isolated dihydrosteviol epimers were assigned structures **IIIa** and **IIIb** on the basis of the chemical shifts of C^{13}. Taking into account that carbon atoms involved in stronger van der Waals interactions with atoms in the nearby molecular fragments [16], the upfield signal (δ_C 41.5 ppm) was assigned [15] to C^{17} of epimer **IIIa**, and the downfield signal (δ_C 54.5 ppm), to C^{17} of epimer **IIIb**. The results of X-ray analysis performed in the present work confirmed the above assignment: the reduction product has the structure of (16*S*)-dihydrosteviol (**IIIa**) (Fig. 1).

As expected, dihydrosteviol (**IIIa**) turned out to be stable in acid medium: it remained unchanged after heating in boiling 20% sulfuric acid. However, the described procedure for the synthesis of dihydrosteviol (**III**) cannot be regarded as effective, for the initial compound is pure steviol (**I**). The reported synthesis of steviol (**I**) is based on oxidative hydrolysis of stevioside (which is specially isolated for this purpose from *Stevia rebaudiana* Bertoni), and it requires a large amount of sodium periodate [9]. We made an attempt

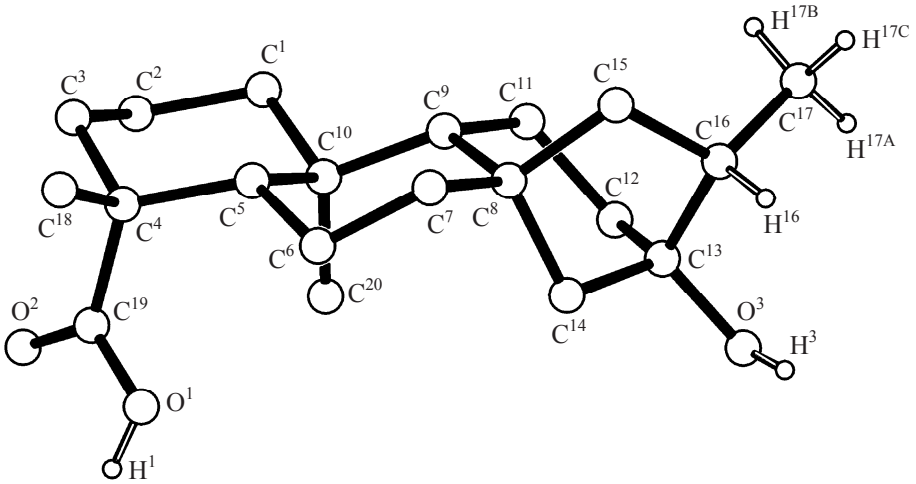
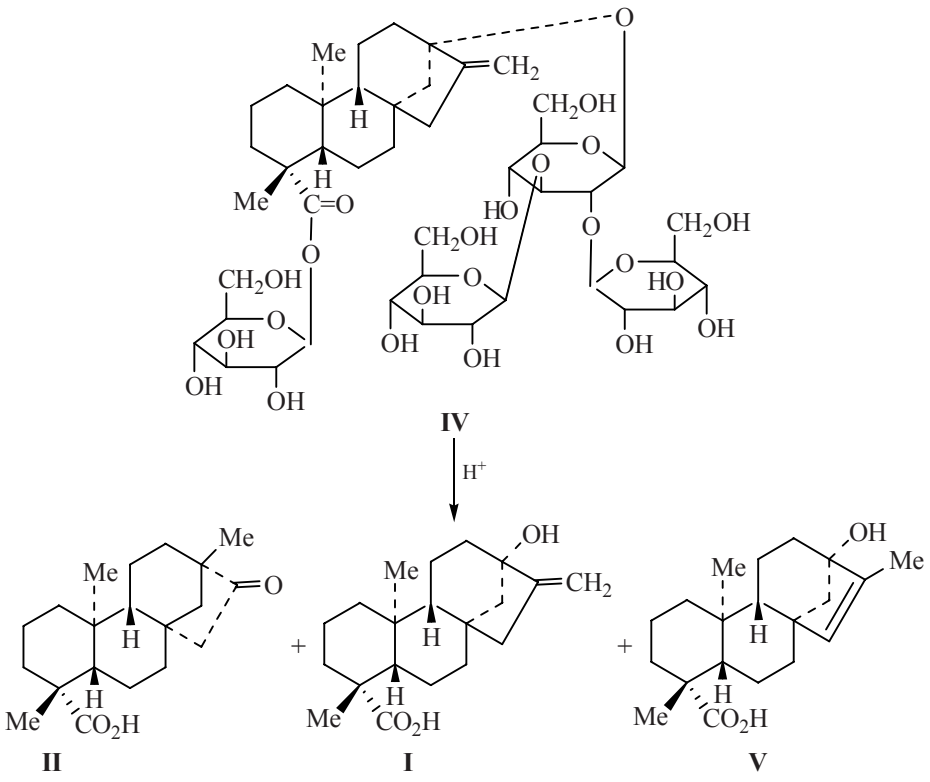


Fig. 1. Structure of the molecule of (16S)-dihydrosteviol (**IIIa**) according to the X-ray diffraction data. Some hydrogen atoms are shown.

to obtain dihydrosteviol (**III**) directly from the commercial food sweetener SWETA which is prepared by enzymatic treatment of a mixture of glycosides isolated from *Stevia rebaudiana* Bertoni. The major component of that mixture is rebaudioside A (**IV**) [3]. However, reduction of the glycoside double bonds with hydrazine hydrate over Raney nickel gave (after hydrolysis with 0.7% hydrochloric acid) a mixture of products, from which we isolated by column chromatography isosteviol (**II**) (yield 36%) and a mixture of steviol (**I**) and its Δ^{15}-isomer **V** at a ratio of 2:3 (8%). The product structure was determined by IR and ^{1}H NMR spectroscopy, the structure of Δ^{15}-steviol (**V**) was proved by X-ray analysis, and the ratio of kaurenoids **I** and **V** was estimated from signal intensities of the ^{1}H NMR spectrum of their mixture. No dihydrosteviol (**III**) was detected among the products.



An analogous mixture of isosteviol (**II**), steviol (**I**), and Δ^{15}-steviol (**V**) was obtained previously by acid hydrolysis of stevioside [17]. The authors failed to separate a mixture of steviol (**I**) with its Δ^{15}-isomer **V**, and the corresponding methyl esters were isolated and characterized [17]. We succeeded in isolating single crystals of hydroxy acid **V** from a solution of its mixture with steviol (**I**) in chloroform. The structure of molecule **V**, determined by X-ray analysis, is shown in Fig. 2.

Isomer mixture **I**/**V** was subjected to reduction with hydrazine hydrate over Raney nickel. We thus obtained 57% of a product which was identical to (16*S*)-dihydrosteviol (**IIIa**) synthesized by reduction of steviol (**I**). We can conclude that the proposed procedure ensures stereoselective reduction of both exocyclic double C=C bond in steviol (**I**) and endocyclic double bond in its Δ^{15}-isomer **V** with formation of (16*S*)-dihydrosteviol (**IIIa**).

Thus we have developed a new procedure for the synthesis of (16*S*)-dihydrosteviol (**IIIa**) from the commercial sweetener SWETA (maufactured by Stevian Biotechnology Corp.) instead of difficultly accessible stevioside [9]. Unlike known methods implying the use of the H_2–Pd/C system, the procedure proposed by us requires less expensive reagents (hydrazine hydrate over Raney nickel) and ensures stereoselective formation of (16*S*)-dihydrosteviol (**IIIa**) as the only product.

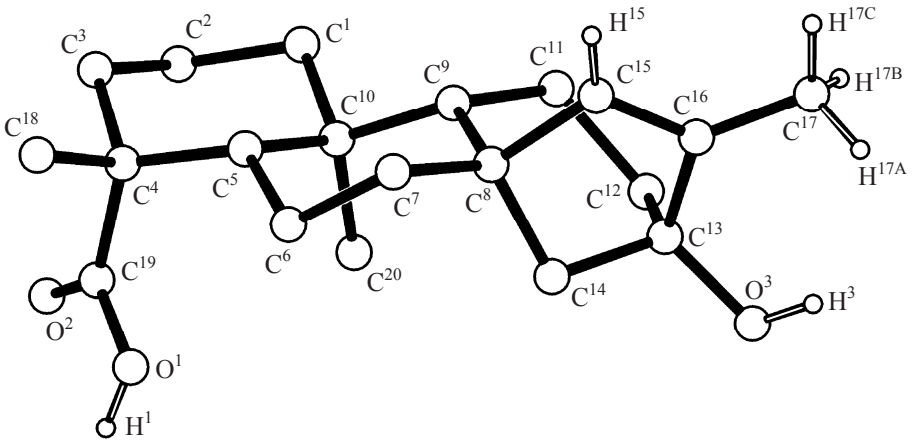


Fig. 2. Structure of the molecule of Δ^{15}-steviol (**V**) according to the X-ray diffraction data. Some hydrogen atoms are shown.

EXPERIMENTAL

The IR spectra were recorded in the frequency range from 400 to 4000 cm^{-1} on a Bruker Vector 22 spectrometer with Fourier transform. Samples were examined as KBr pellets. The mass spectra (electron impact, 70 eV) were obtained on a TRACE MS Finnigan MAT spectrometer with direct sample admission into the ion source heated to 200°C; the batch inlet probe temperature was programmed from 25 to 150°C at a rate of 35 deg min^{-1}; the data were processed using Xcalibur program. The ^1H NMR spectra were measured on a Bruker Avance-600 instrument (600 MHz). Column chromatography was performed on silica gel (Chemapol). The reaction mixtures were analyzed by thin-layer chromatography on Silufol UV-254 plates.

X-Ray analysis was performed at the X-Ray Department, Spectral Analytical Collective Use Center of the Russian Foundation for Basic Research. The X-ray diffraction data were acquired on a SMART Apex diffractometer at 20°C (MoK_α irradiation, multiscan mode). The structures were solved by the direct method using SIR program [18] and were refined first in isotropic and then in anisotropic approximation. The positions of hydroxy hydrogen atoms were determined from difference electron density series; hydrogen atoms attached to carbon atoms were localized on the basis of geometry considerations, and their positions were refined according to the rider model. The structures were refined using SHELXL97 [18] and WinGX software [19]; Figures 1 and 2 were plotted using PLATON program [22].

(16*S*)-Dihydrosteviol [(16*S*)-13-hydroxy-*ent*-kauran-19-oic acid] (IIIa), 2:1 solvate with chloroform. $2(C_{20}H_{30}O_3)\cdot CHCl_3$; *M* 756.25; rhombic crystals, space group $P2_12_12_1$; unit cell parameters: a = 12.122(5), b = 15.660(5), c = 21.746(5) Å; V = 4128(2) $Å^3$.

Δ^{15}-Steviol (13-hydroxy-*ent*-kaur-15-en-19-oic acid) (V), 2:1 solvate with chloroform. $2(C_{20}H_{32}O_3)\cdot CHCl_3$; *M* 760.25; rhombic crystals, space group $P2_12_12_1$; unit cell parameters: a = 12.2875(17), b = 15.566(2), c = 21.581(3) Å; V = 4127.7(10) $Å^3$.

Steviol (I, 13-hydroxy-*ent*-kaur-16-en-19-oic acid). A sample of SWETA (Stevian Biotechnology Corp.), 3 g, was dissolved in 900 ml of water, and 5.4 g of sodium periodate was added under stirring. The mixture was left to stand for 2 days at 20°C in the dark, 90 g of potassium hydroxide was added, and the mixture was heated for 1.5 h at the boiling point, cooled, and extracted with diethyl ether (5×25 ml). The extracts were combined, washed with 100 ml of water, dried over $MgSO_4$, and evaporated, and the residue was recrystallized from methanol. Yield 0.15 g (16%), mp 215–217°C; published data: mp 212–213°C [2], 211–213°C [9], 213–216°C [22]. IR spectrum, ν, cm^{-1}: 1691 s (C=O), 1028 m (C–OH), 1660 m (C=CH_2), 3460 m (O–H), 1188 m (COC). ^1H NMR spectrum ($CDCl_3$), δ, ppm: 0.8–2.2 m (32H), 0.96 s (3H, $C^{20}H_3$), 1.23 s (3H, $C^{18}H_3$), 4.82 s and 4.98 s (1H each, $C^{17}H_2$). Mass spectrum, *m*/*z* (I_{rel}, %): 318 (65) $[M]^+$, 300 (44) $[M - H_2O]^+$, 285 (28) $[M - H_2O - CH_3]^+$, 272 (22) $[M - H_2O - C_2H_4]^+$, 260 (43) $[M - (CH_3)_2CO]^+$. Found, %: C 76.30; H 9.84. $C_{20}H_{30}O_3$. Calculated, %: C 76.50; H 9.60.

Acid hydrolysis of SWETA. A 10-g portion of SWETA was added to 100 ml of 0.7% hydrochloric acid, and the mixture was heated for 8 h under reflux, cooled, and extracted with chloroform (5×50 ml). The

extracts were combined, washed with 100 ml of water, and dried over $MgSO_4$. The solvent was removed, and the residue was subjected to chromatography on silica gel to isolate isosteviol (**II**) and a mixture of steviol (**I**) and its Δ^{15}-isomer **V**.

Isosteviol (II, 16-oxo-*ent*-beyeran-19-oic acid). Eluent chloroform, yield 1.15 g (36%), mp 234–235°C; published data: mp 231–234°C [2], 228–230°C [14], 234–235°C [22]. IR spectrum, ν, cm^{-1}: 1180 m (COC), 1695 s (C=O, acid), 1740 s (C=O, ketone). ^1H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 0.6–2.0 m (36H), 0.80 s (3H, $C^{20}H_3$), 0.99 s (3H, $C^{17}H_3$), 1.26 s (3H, $C^{18}H_3$), 2.18 d (1H, 3-H_{eq}, J = 13.4), 2.65 d.d (1H, 15α-H, J = 18.7, 3.7). Found, %: C 76.01; H 9.90. $C_{20}H_{30}O_3$. Calculated, %: C 75.83; H 9.51.

Mixture of steviol (I) and Δ^{15}-steviol (V, 13-hydroxy-*ent*-kaur-15-en-19-oic acid). Eluent chloroform–methanol, 10:1. Yield 0.25 g (8%). ^1H NMR spectrum of **V** ($CDCl_3$), δ, ppm: 0.97 s (3H, $C^{20}H_3$), 1.22 s (3H, $C^{18}H_3$), 1.65 s (3H, $C^{17}H_3$), 5.1 s (1H, 15-H).

(16*S*)-Dihydrosteviol [IIIa, (16*S*)-13-hydroxy-*ent*-kauran-19-oic acid]. Solid potassium hydroxide was added without external cooling to a suspension of 0.3 g of Ni–Al alloy (containing 30 to 50% of Al) in 5 ml of water until gas no longer evolved (about 4 h). The mixture was heated for 30 min at 70°C on a water bath, and the precipitate was filtered off and washed with water (3×5 ml) and methanol (2×5 ml). A solution of 0.14 g of steviol (or its mixture with Δ^{15}-isomer **V**) in 20 ml of 1.5% aqueous potassium hydroxide was added to the freshly prepared catalyst, the mixture was heated to 80°C, and 10 ml of 95% hydrazine hydrate was added dropwise under stirring over a period of 8 h. The mixture was diluted with water, excess alkali was neutralized with acetic acid, the product was extracted into diethyl ether, the extract was dried over $MgSO_4$, the solvent was removed, and the residue (a white powder) was recrystallized from methanol. Yield 0.08 g (57%), $[\alpha]_D^{20}$ = –40° (c = 0.38, $CHCl_3$), mp 190°C; published data [9]: $[\alpha]_D^{20}$ = –28.2 (c = 0.43, $CHCl_3$), mp 190–192°C. IR spectrum, ν, cm^{-1}: 1693 s (C=O), 1025 m (C–OH), 1190 m (COC). ^1H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 0.79–2.15 m (32H), 0.93 s (3H, $C^{20}H_3$), 0.97 d (3H, $C^{17}H_3$, J = 7), 1.22 s (3H, $C^{18}H_3$). Mass spectrum, m/z (I_{rel}, %): 320 (35) $[M]^+$, 302 (9) $[M - H_2O]^+$, 277 (99), 123 (100).

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