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Synthesis and Bioactivity of Isosteviol Derivatives: A Facile Method for Preparation of *Ent*-16α-hydroxy-15β-hydroxymethylbeyeran-19-oic Acid

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Abstract: In this paper, two new compounds, *ent*-16α-hydroxy-15β-hydroxymethylbeyeran-19-oic acid **4** and its ethyl ester **5** were synthesized in good yield with a facile method that has never been reported. The mechanism of this reaction was discussed and the mono-hydroxymethylation of carbonyl compound with two α-hydrogens *via* Tollens' reaction was firstly achieved. Compounds **2**-**5** were tested for activity of inhibition against glycosidases, among which **5** displays good inhibition of β-glucosidase and α-mannase , respectively.

Keywords: Isosteviol, 1,3-diol, hydroxylation.

Isosteviol (*ent*-16-ketobeyeran-19-oic acid **2**) is a tetracyclic diterpenoid with a beyerane skeleton, obtained by acid hydrolysis of stevioside **1**, a constituent of *Stevia rebaudiana*, which is commonly used as a noncaloric sugar substitute[1]. In recent years, the biological activity of tetracyclic diterpenoids, especially the kaurenes, has attracted extensive attention[2]. As proposed for the Rabdosia diterpenoids[3], hydroxyl groups may play an important role in binding to some receptors. Therefore, we attempt to introduce hydroxyl groups to isosteviol and expect to enhance the biological activity or lead to new biological activity.

Although some hydroxylation of isosteviol in different rings has been studied by biotransformation[3, 4], there are few reports on chemical hydroxylation of this compound. Our present work is to develop a chemical method for synthesizing some hydroxyl group-containing tetracyclic diterpenoids with beyerane skeleton. Herein, we report a facile method for preparing the *ent*-16α-hydroxy-15β-hydroxymethylbeyeran-19-oic acid **4** and its ethyl ester **5** in high yield. The mechanism was proposed as a one-pot "Aldol-Cannizzaro reation" process.

The synthetic route of the title compound is depicted in **Scheme 1**. Isosteviol **2** was obtained by hydrolysis of stevioside **1** with dilute sulfuric acid[5]. Proton and carbon assignments of isosteviol were identical with that in literature[3]. The ethyl ester of isosteviol **3**[6] (92%, m.p.125-127℃; literature, m.p.126-127℃) was obtained according to the literature mothod[7].

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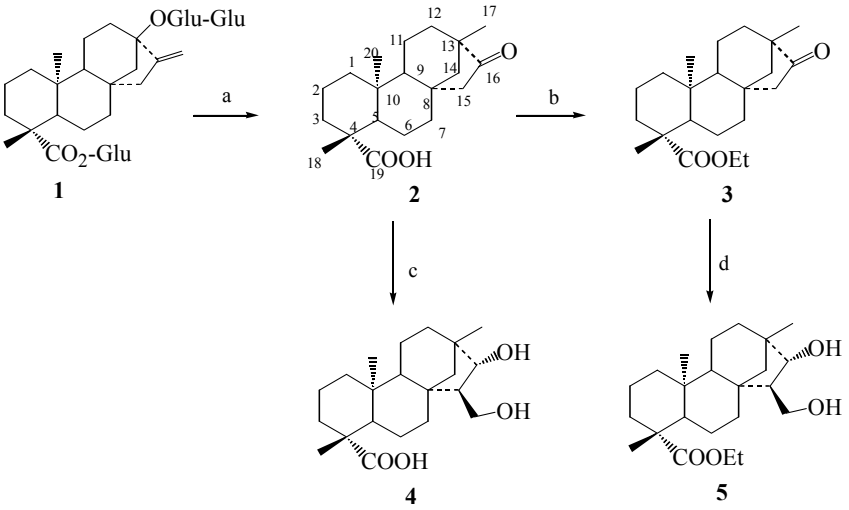
Scheme 1 Synthesis of compounds **4** and **5**



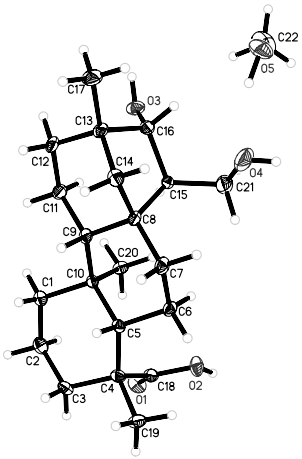
Reagents and conditions: (a) 10% H_2SO_4, 70℃, 4h, 65%; (b) EtBr, DMSO, KOH, r. t., 3h, 92%; (c) HCHO, aq. NaOH, C_2H_5OH, 60℃, 1h, 95%; (d) HCHO, C_2H_5ONa, C_2H_5OH, 60℃, 10h, 90%.

General procedure for preparation of 1,3-diol: To a solution of 0.01 mol of **2** in 4 mL of ethanol and 0.4 g of NaOH in water (6 mL), excessive amount of aqueous formaldehyde (37%, 2 mL) was added dropwise. After stirring for 1 h at 60℃, the reaction mixture was acidified with dilute HCl, giving a flocculent precipitate. Filtration and then recrystallization of the crude product from methanol afforded a colorless needle crystal in high yield (**4**[8]: 95%, m.p.233-235℃; **5**[9]: 87%, m.p.139-141℃). The products were characterized by HRMS, IR, NMR and the absolute configuration of compound **4** was confirmed by single crystal X-ray analysis (**Figure 1**). Compound **5** was synthesized from **3** in ethanol with sodium ethoxide as base to avoid hydrolysis of carboxylate group.

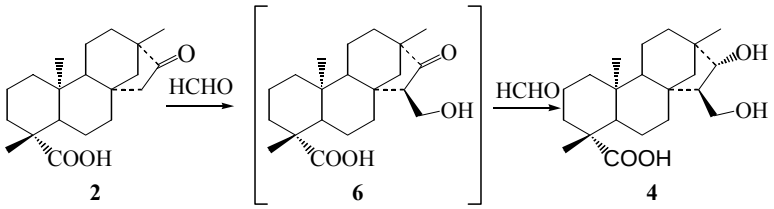
As it was known, in Tollens' reaction, two or three hydroxymethyl groups would be introduced to the α-position of the carbonyl group if there are two or three α-hydrogens in a carbonyl compound before the carbonyl group is reduced[10]. However, in our present study, although there are two hydrogens α to the carbonyl group in compounds **2** and **3**, only 1,3-diols were obtained, nothing of dihydroxymethyl substituted compounds were found.

This contradiction interested us. In order to make sure of the mechanism of the reaction, we quenched the reaction of the conversion from **2** to **4** with dilute HCl after the reaction was preceeded for 0.5 h. From the MS spectrum of the reaction mixture we found the existence of compound **6**, which probably was the intermediate from **2** to **4** (**Scheme 2**). From **6** we could propose that the carbonyl compound with hydrogen(s) α to the carbonyl group could be carried out crossed Cannizzaro reaction under special conditions.

Figure 1 Single crystal X-ray analysis of compound **4**



Scheme 2 Proposed process of formation of compound **4**



In addition, compounds **2**-**5** were assayed for activity of inhibition of glycosidase and, to our inspiration, compound **5** displayed selective inhibition against β-glucosidases and α-mannase (61.29% and 63.20% at 0.1 mmol/L, respectively[11]).

In conclusion, we have accomplished the hydroxylation of isosteviol and its ethyl ester. This method can be applied to synthesize more compounds for the studies of structure-activity relationship of this type of compounds. And we firstly achieved mono-hydroxymethylation of carbonyl compound with two α-hydrogens *via* Tollens' reaction. The interesting results impelled our further work, including effects of hindrance and skeleton on Tollens' reaction and detailed biological studies, to be progressed.

Acknowledgment

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References and Notes

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6. Compound **3**: IR (neat, cm^{-1}) υ: 2957, 2926, 2847, 1730, 1693, 1451, 1227, 1146; ^1H NMR ($CDCl_3$, 400 MHz, δ ppm): 4.10 (q, 2H, J=7.2Hz), 2.96 (dd, 1H, J=18.4, 2.8 Hz), 2.17 (br d, 1H, J=11.2 Hz), 1.99 (d, 1H, J=18.8 Hz), 1.88-1.58 (m, 8H), 1.47-1.40 (m, 5H), 1.26 (t, 3H, J=7.2 Hz), 1.32-1.19 (m, 3H), 1.18 (s, 3H), 1.07-0.84 (m, 4H), 0.77 (s, 3H).
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8. Compound **4**: IR (neat, cm^{-1}) υ: 3462, 2945, 2927, 2846, 1696, 1456, 1072, 1052; ^1H NMR (acetone-d_6, 400 MHz, δ ppm): 3.83 (q, 1H, J=5.2 Hz), 2.62 (d, 1H, J=4.8 Hz), 3.50 (t, 1H, J=9.6 Hz), 3.30 (br s, 2H), 2.12-1.99 (m, 2H), 1.95-1.70 (m, 6H), 1.56-1.51 (m, 1H), 1.44-1.34 (m, 2H), 1.17 (s, 3H), 1.15-0.90 (m, 6H), 0.88 (s, 3H), 0.87 (s, 3H).; HRMS (ESI) calcd. for $C_{21}H_{34}O_4Na$ ($[M+Na]^+$) *m/z* 373.2355. Found: 373.2358.
9. Compound **5**: IR (neat, cm^{-1}) υ: 3435, 2940, 2838, 1720, 1458, 1378, 1234, 1179, 1153,1123; ^1H NMR ($CDCl_3$, 400 MHz, δ ppm): 4.09 (q, 2H, J=7.2 Hz), 3.99 (q, 1H, J=4.7 Hz), 3.63 (d, 1H, J=4.7 Hz), 3.56 (t, 1H, J=10.3 Hz), 2.16 (br d, 1H, J=13.0 Hz) 2.05 (m, 1H), 1.83-1.56 (m, 12H), 1.43-1.37 (m, 2H), 1.26 (t, 3H, J=7.2 Hz), 1.22-1.19 (m, 1H), 1.16 (s, 3H), 1.08-0.95 (m, 4H), 0.94 (s, 3H), 0.88-0.86 (m, 1H), 0.78 (s, 3H); Anal. Calcd. for $C_{23}H_{38}O_4$: C, 72.98; H, 10.12. Found: C, 72.74; H, 10.38; HRMS (ESI) calcd. for $C_{23}H_{38}O_4Na$ ($[M+Na]^+$) *m/z* 401.2668. Found: 401.2670.
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11. Inhibition ratio was determined at 37℃ in 0.08 mol/L citric acid /Na_2HPO_4 buffer (pH 4.2). The enzymatic reaction was started after incubation of the enzyme for 30min in the presence of the inhibitor by the addition of substrate. The mixture was incubbated at 37℃ for 5min, and the reaction was quenched by 0.25 mol/L borate buffer (pH 9.8). The absorption at 405nm was measured immediately and taken as the relative rate for the hydrolysis of substrate.

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