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Methyl 1,2-Shift Rearrangement on C-ring and Decarboxylation at C28 of Oleanolic Acid Derivatives

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A new oleanolic acid derivative with A-ring lactone, C-ring rearrangement and decarboxylation at C28 was synthesized, which was confirmed by HRMS, NMR and X-ray crystal structure. It is the first report about the methyl rearrangement on C-ring of oleanolic phenylmethyl ester, and the possible mechanism was proposed as the 1,2-methyl shift.

Keywords 1,2-methyl shift, trifluoroacetic acid, oleanolic acid

Introduction

The molecular diversity which arises from research into natural products represents a valuable tool for driving drug discovery.[1-4] Pentacyclic triterpenoids are currently regarded as one of the most important scaffolds for the drug development due to their biological and pharmacological activities, such as antitumor, antiviral, antiinflammatory, hepatoprotective, gastroprotective, antimicrobial, antidiabetic, and hemolytic properties.[5-11] As one of their representatives, oleanolic acid (**OA,** Figure 1a), widely present in natural plants in the form of free acids or aglycones, has been noted for its therapeutic effect on human liver disorders and antitumor-promotion.[12-14]

Our previous work showed that **OA**-heterocyclic conjugates have obvious antitumor effects through apoptosis and inhibiting the cell growth,[7,15] such as **OA**-pyrimidine and **OA**-oxadiazole (Figures 1b and 1c). Although lots of work has been done about its modification, there is no report about the methyl shift rearrangement on C-ring of **OA**. Herein, a new straightforward procedure was proposed to convert **OA** into A-ring lactone and C-ring rearrangement derivative with elimination 28-carboxylic benzyl ester by using trifluoroacetic acid. This is the first report about the double methyl 1,2-shifts on C-ring, and a possible shift mechanism was proposed according to analyzing the crystal structure and reaction condition.

Results and Discussion

The synthetic route of the new ring-opened compounds **5** and **6**, as well as the methyl-rearrangement product **7** is shown in Scheme 1. First, **OA** was oxidized to **2** by Jones' reagent after the protection of 28-carboxylic acid. Following treatment with *n*-butyl nitrite under strong basic condition, compound **3** was obtained as well as its reductive product **4** after reacting with sodium borohydride. Under the condition of *p*-toluenesulfonyl chloride, ring-opened derivatives **5** and its reductive product **6** were produced. Finally, with treatment of trifluoroacetic acid in autoclave, rearrangement product **7** was afforded in yield 56% (details see experimental part).

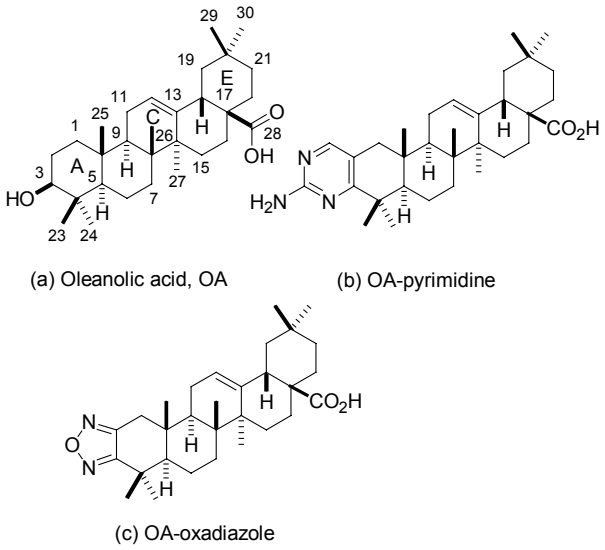

Figure 1 Structures of **OA**, **OA**-pyrimidine, and **OA**-oxadiazole.

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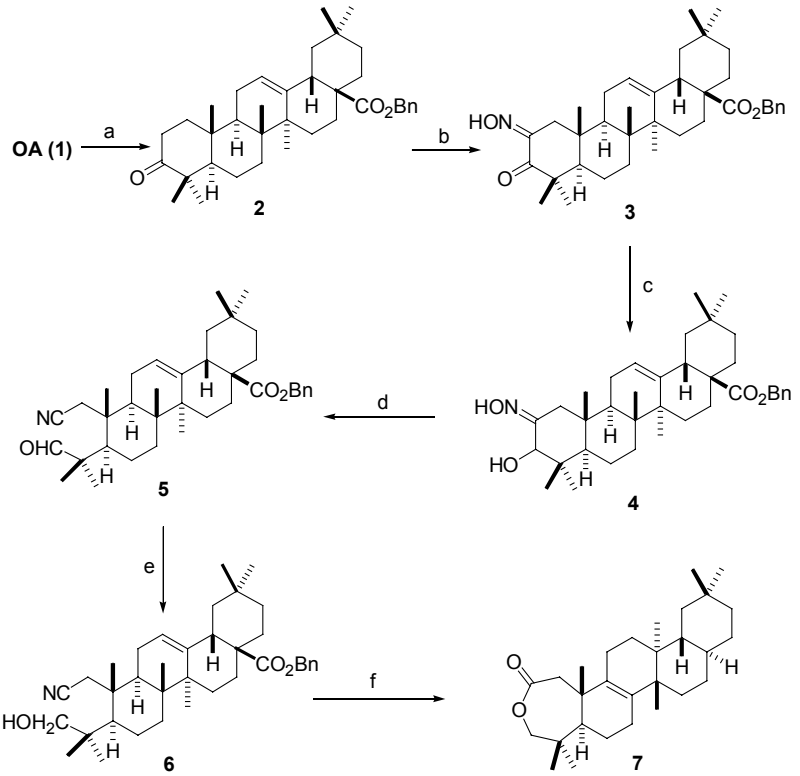
Scheme 1



Reagents and conditions: (a) i: Jones' reagent, ii: BnBr, K_2CO_3, 96%; (b) *n*-BuONO, *t*-BuOK, 76%; (c) $NaBH_4$, 94%; (d) *p*-toluenesulfonyl chloride, 67%; (e) $NaBH_4$, 86%; (f) trifluoroacetic acid (3 equiv.), THF, 56%.

At the beginning, our original plan is to synthesize the ring-opened compounds **5**, **6** and A-ring lactone derivative of **OA**. However, ^{1}H NMR spectrum of **7** indicated that there was no proton signal for C=CH on C-ring and the 28-carboxylic phenylmethyl ester, which was further confirmed by ^{13}C NMR spectrum. Meanwhile, through comparing its molecular weight (*m*/*z* 426, deduced by ESI-MS (+) spectrum which gave the signals of 427 $[M+H]^+$ and 449 $[M+Na]^+$), it indicated that the product **7** was different from the one we planned to synthesize, and it is believed that an unexpected reaction occurred. In order to figure out its structure, the crystal was grown in a mixed solvents of ethyl acetate and hexane (1∶4, *V*/*V*) (Figure 2). The X-ray crystallography showed that: (1) the A-ring lactone formed as expected; (2) the 28-carboxylic benzyl ester was eliminated; (3) the C=C double bond shifted from C12−C13 to C8−C9 in C-ring (bond length of C8−C9 is 1.33 Å which is shorter than the other C−C bonds as shown in Figure 2);[15,16] (4) two methyl groups on C8 and C14 shifted to C14 and C13 with keeping their stereo configuration respectively. For the formation of A-ring lactone, it can be easily understood since the cyano group can be converted to carboxylic acid under the acid condition, and then reacted with hydroxyl group to produce the lactone in A-ring; the 28-carboxylic phenylmethyl ester group was protonated under the acidic condition with the high temperature and pressure, followed by cleaving the benzyl alcohol and carbon dioxide moieties to afford the decarboxylation product.[17-21] However, for the rearrangements of two methyl groups and C=C double bonds in C-ring, it is believed that a shift reaction occurred. Through analyzing the crystal structure and the reaction condition, a possible rearrangement mechanism on C-ring was proposed in Figure 3.[22-26] Under the acidic condition, the tertiary carbocation (I) formed on C13, and by twice methyl 1,2-shifts, the other tertiary carbocation (II) was produced. Finally, the C=C double bond between C9 and C10 formed after leaving hydrogen-10. Moreover, when oleanolic acid was used under the same condition, no rearrangement product was obtained. In order to explain the difference, we have tried to get the intermediate structure, but it did not succeed so far. It is still not clear what induced the difference and what kinds of role the benzyl ester group played during this process.

Conclusions

A procedure to convert oleanolic acid into A-ring lactone and C-ring rearrangement derivative without 28-carboxyl acid was reported, and it is the first time to realize the shifts of methyl groups and C=C double bond on C-ring of oleanolic acid benzyl ester. According to analyzing the crystal structure of final product and the reaction condition, a possible mechanism of double methyl 1,2-shifts was proposed.

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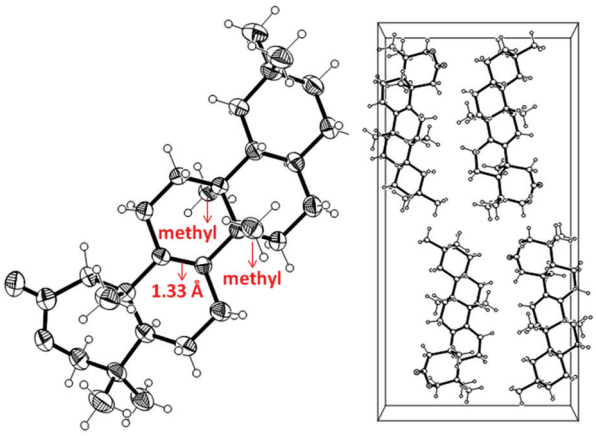

Figure 2 ORTEP drawing of $C_{29}H_{46}O_2$ (compound **7**) with 35% probability ellipsoids (left) and a packing view along *a* direction (right).

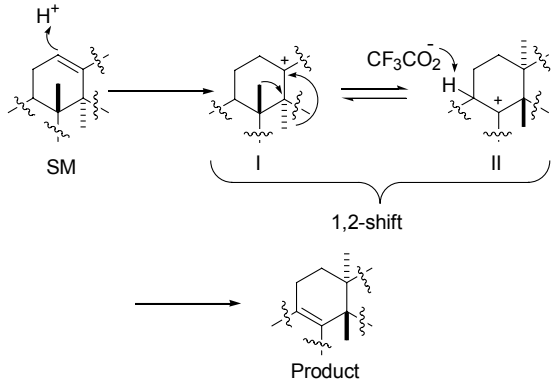

Figure 3 A possible mechanism of double methyl 1,2-shifts on C-ring.

Experimental

General procedure

^{1}H NMR and ^{13}C NMR spectra were obtained with a JEOL JNM-ECA 300/400 instrument. Electrospray ionization mass spectrometry (ESI-MS) was measured on Bruker ESQUIRE-LC spectrometer in positive/negative mode; IR spectra were recorded on Spectrum GX FT-IR spectrophotometer with KBr pellets; melting point data were obtained by X-4 micro melting point analyzer. The room temperature (294±1 K) single-crystal X-ray experiments were performed on a Bruker P4 diffractometer equipped with graphite monochromatized Mo Kα radiation. Chromatographic purifications were conducted by column chromatography with the use of 0.06−0.20 mm silica gel. TLC analysis was facilitated by the use of UV light (254 nm) with fluorescent-indicating plates. Chemicals and solvents were either purchased from commercial suppliers or purified by standard techniques.

2-Cyano-3-aldehyde-12-en-oleanolic phenylmethyl ester (5) Compound **4** (230 mg, 0.40 mmol) and *p*-toluenesulfonyl chloride (126 mg, 0.66 mmol) were dissolved in 10 mL anhydrous pyridine, and the mixture was stirred for 7 h at r.t. Then the mixture was poured into water, and the precipitate was filtered. The crude product was purified by flash chromatography (DCM∶Methanol=40∶1) to afford **5** as a white solid (149 mg, 67%). ESI-MS (+) *m/z*: 580.7 $[M+Na]^+$, 1138.5 $[2M+Na]^+$; ESI-MS (−) *m/z*: 573.2 $[M+OH]^-$; HRMS-ESI (+): calcd for $C_{37}H_{51}NO_3$: 580.3761; found 580.3758. m.p. 78−79 ℃; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.69 (s, 1H, CHO-3), 7.32 (br s, 5H, CH_2Ph), 5.29 (br s, 1H, H-12), 5.08 (d, J=12.3 Hz, 1H, CH_2Ph), 5.03 (d, J=12.3 Hz, 1H, CH_2Ph), 2.90 (d, J=13.2 Hz, 1H, H-14), 1.20 (s, 3H, CH_3), 1.16 (s, 3H, CH_3), 1.10 (s, 3H, CH_3), 0.95 (s, 3H, CH_3), 0.92 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 0.59 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 206.3 (CHO-3), 177.4 (C-28), 144.0 (C-13), 136.4, 128.5, 128.1, 128.0 (CH_2Ph), 121.4 (C-12), 118.1 (C-2), 66.0 (CH_2Ph), 50.8, 49.5, 46.8, 45.7, 42.4, 42.1, 41.7, 41.5, 39.3, 33.9, 33.1, 32.3, 31.9, 30.8, 29.7, 27.7, 25.4, 23.7, 23.6, 23.5, 20.2, 19.6, 18.2, 16.7.

2-Cyano-3-hydroxyl-12-en-oleanolic phenylmethyl ester (6) Sodium borohydride (120 mg, 3.15 mmol) was added slowly to a solution of **5** (172 mg, 0.31 mmol) in 5 mL methanol, and then the mixture was stirred for 3 h at r.t. The reaction solution was treated with diluted hydrochloric acid (1 mol/L), and extracted with ethyl acetate. The combined organic layer was washed with water, brine, dried by $MgSO_4$ and evaporated. Purification by flash chromatography (DCM∶Methanol=30∶1) afforded **6** as a white solid (149 mg, 86%). ESI-MS (+): *m/z*: 561.0 $[M+H]^+$, 582.6 $[M+Na]^+$, 598.4 $[M+K]^+$; HRMS-ESI (+) calcd for $C_{37}H_{53}NO_3$: 582.3918 $[M+Na]^+$; found 582.3913. m.p. 109−110 ℃; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.33 (br s, 5H, CH_2Ph), 5.31 (br s, 1H, H-12), 5.09 (d, J=12.3 Hz, 1H, CH_2Ph), 5.06 (d, J=12.3 Hz, 1H, CH_2Ph), 3.55 (d, J=9.9 Hz, 1H, H-3), 3.32 (d, J=9.9 Hz, 1H, H-3), 2.91 (d, J=13.2 Hz, 1H, H-14), 2.78 (d, J=17.7 Hz, 1H, H-1), 2.46 (d, J=17.7 Hz, 1H, H-1), 1.18 (s, 3H, CH_3), 1.11 (s, 3H, CH_3), 1.03 (s, 3H, CH_3), 0.95 (s, 3H, CH_3), 0.92 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 0.62 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 177.6 (C-28), 143.8 (C-13), 136.5, 128.5, 128.1, 128.0 (CH_2Ph), 121.8 (C-12), 119.2 (C-2), 72.1 (C-3), 66.0 (CH_2Ph), 48.3, 47.0, 45.9, 42.5, 42.2, 41.6, 40.4, 39.5, 34.0, 33.1, 32.6, 32.3, 30.8, 29.7, 27.9, 25.3, 24.6, 24.4, 23.7, 23.2, 21.7, 17.8, 16.9.

1,2-Methyl shift rearrangement derivative of oleanolic acid (7) Compound **6** (120 mg, 0.21 mmol) was dissolved in 1 mL THF in autoclave with a PTFE container inside, and trifluoroacetic acid (0.25 mmol) was added. After that, the reaction solution was left in the oven at 100 ℃ for 10 h. After transferring the autoclave to the room temperature and evaporating the solvent, the crude product was purified by flash chromatography (Hexane∶EtOAc=3∶1) to afford **7** as a white powder (50 mg, 56%). ESI-MS (+) *m/z*: 427.0 $[M+H]^+$, 449.5 $[M+Na]^+$; HRMS-ESI (+) calcd for $C_{29}H_{46}O_2$: 449.3390 $[M+Na]^+$; found 449.3392; m.p.

267—268 ℃; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.11 (d, J=12.72 Hz, 1H, CO_2CH_2), 3.77 (d, J=12.72 Hz, 1H, CO_2CH_2), 0.77 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 0.88 (s, 3H, CH_3), 0.94 (s, 3H, CH_3), 0.99 (s, 3H, CH_3), 1.02 (s, 3H, CH_3), 1.12 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 175.2 (CO_2CH_2), 137.6 (9-C), 131.2 (8-C), 77.58 ($OCOCH_2$), 42.5, 41.9, 40.3, 39.9, 39.2, 37.9, 36.6, 36.0, 33.9, 31.1, 31.0, 28.8, 28.4, 28.3, 28.2, 27.3, 25.1, 22.3, 21.8, 21.3, 20.9, 19.4, 14.9.

Supporting Information

Synthetic details of compounds **2**, **3** and **4**; ^{1}H NMR, ^{13}C NMR, IR and MS spectra of compounds **2**, **3**, **4**, **5**, **6** and **7**; the cif. file of compound **7**.

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