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Synthesis and revision of stereochemistry of rubescensin S†

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An effective two step transformation of oridonin to 15,16-seco-ent-kaurane skeleton is reported. We also achieved the conversion of one intermediate to natural product rubescensin S and revised its structure as a *13S* configuration although *13R* is reported in the literature.

Natural products have been a rich source of medicines. The great structural diversity of natural compounds at various levels has always served medicinal chemists as a source of inspiration in their search for new molecular entities with pharmacological activity.[1–3] Oridonin (Fig. 1) is an active ent-kaurane diterpenoid compound extracted from the medicinal herb *Rabdosia rubescens*, a perennial herb of the genus Isodon that is notably rich in ent-kaurane diterpenoids.[4,5] To date, more than 600 ent-kaurane diterpenoids have been isolated from the Isodon genus. These compounds displayed diverse biological activities, including anti-inflammation, antibacterial, antifeeding, antivirus and particularly antitumor activity.[6–9] The leaves of *Rabdosia rubescens* are still used by the local people in China for the treatment of respiratory and gastrointestinal bacterial infections, inflammation, and cancer. Oridonin is the main active ingredient of *Rabdosia rubescens*, and it is generally believed that the α,β-unsaturated ketone unit is the active center.[10,11]

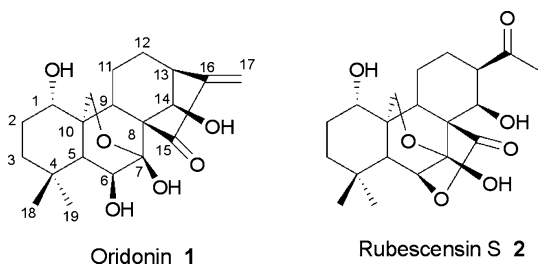

Fig. 1 Structures of oridonin and rubescensin S.

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† Electronic supplementary information (ESI) available: Characterization data including the crystallographic data, and copies of the ^{1}H and ^{13}C NMR spectra of the synthetic intermediates. CCDC reference numbers 822516–822519. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c1ob05611e

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In 2004, two novel 15,16-seco-ent-kauranoids named rubescensins S (Fig. 1) and T were reported by Sun *et al.*[12] These two unprecedented seco-ent-kauranoid diterpenoids both exhibited cytotoxic activities against K562 human leukemia cells (IC50 = 7.03 and 6.18 μg mL^{-1}). These unique 15,16-seco-ent-kauranoids were proposed to be generated from the major diterpenoid components involved in biogenesis, such as oridonin or lasiodonin, *via* an oxidation and ring-opening procedure at the C15–C16 bond. In fact, many similar "seco-skeleton" ent-kaurane diterpenoids with diverse oxygenation patterns and bioactivity have been found in the Isodon genus, therefore providing a huge, structurally diverse family of ent-kauranoid natural products.[13]

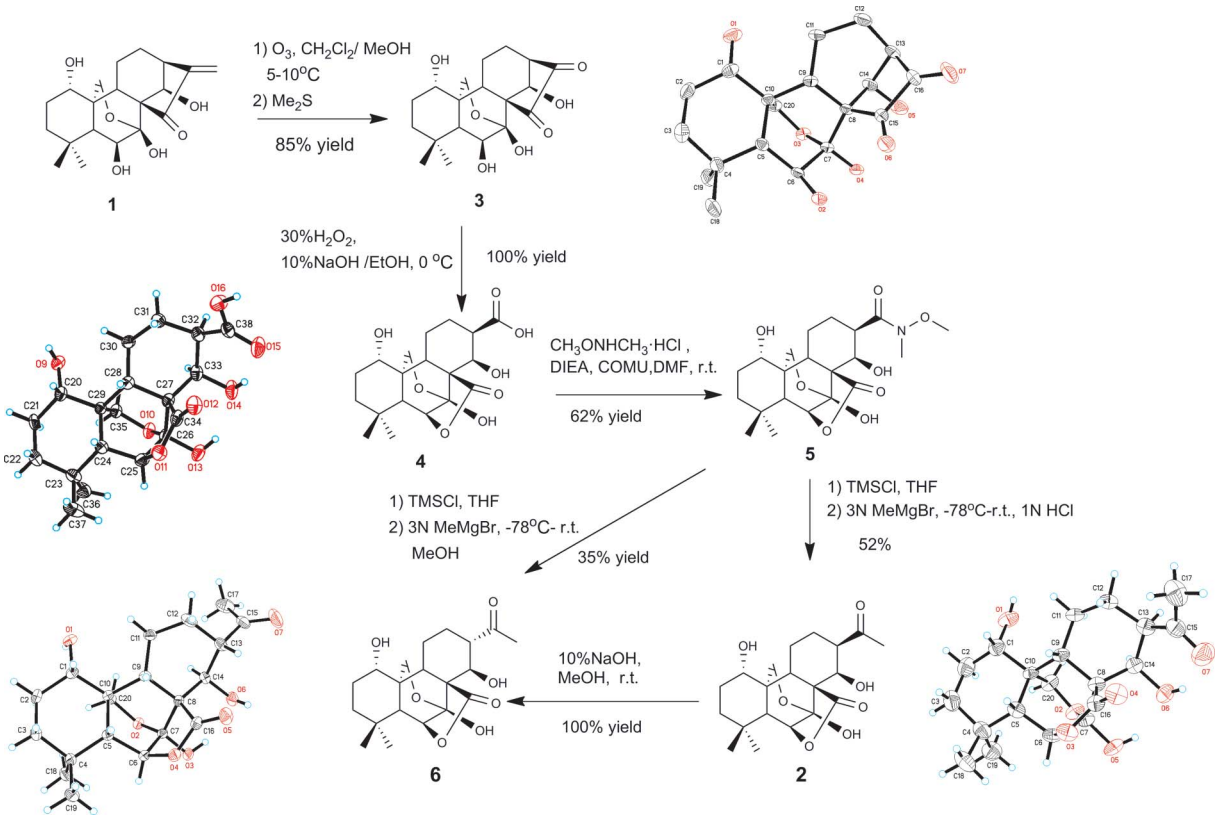
Inspired by these biogenetic chemical transformations, we selected oridonin, a naturally rich and readily available ent-kauranoid diterpenoid as the basis of a template to construct a natural product-like compound library through feasible chemical space exploration. In this paper, we report the effective transformation of oridonin to 15,16-seco-ent-kaurane skeleton through simple chemical conversions, as well as the first semisyntheses of rubescensin S and its isomer, and revision of the structures.

The successful conversion of oridonin **1** to rubescensin S **2** and its isomer **6** is outlined in Scheme 1.

Ozonolysis of **1** in CH_2Cl_2 and MeOH gave diketone **3** in 85% yield. The structure of **3** was assigned using spectral (^{1}H-NMR, ^{13}C-NMR) and single-crystal X-ray data. Compound **3** was oxidized in H_2O_2/NaOH to afford the key compound **4** in quantitative yield,[14] the structure of which was also determined using spectral data (^{1}H-NMR, ^{13}C-NMR) and crystal analysis. Compound **4** has a very similar skeleton to the natural product rubescensin S, encouraging us to further convert **4** to rubescensin S.

The acid **4** was converted into Weinreb amide **5** in 62% yield using 1-[(1-cyano-2-ethoxy-2-oxoethylideneaminooxy)-dimethylaminomorpholinomethylene)] methanaminium hexafluorophosphate (COMU) as a coupling agent.[15] A wide variety of coupling agents, such as DCC/HOBt or EDCI/HOBt, gave product in low yields only. Weinreb amide **4** was treated with TMSCl and MeMgBr,[16] then quenched with 1 N HCl solution to give rubescensin S **2**, with the structure originally assigned.[12] The ^{1}H NMR and ^{13}C NMR spectra of **2** (Table 1) were significantly different from those reported in the literature. The specific rotation value of **2** was also different from reported values ($[\alpha]_D^{20} = -41.7$, Ref. $[\alpha]_D^{20} = +8.0$).[12] Further single-crystal X-ray analysis of **2** supported the structure of compound **2** being as indicated in Scheme 1.

During the conversion of **5** to **2**, if the reaction was quenched with MeOH, instead of 1 N HCl, we obtained compound **6**, which



Scheme 1 Preparation of compounds **2** and **6**.

Table 1 ^{1}H- and ^{13}C-NMR data for compounds **2**, **6**, and rubescensin S.[a]Parameters: 300 MHz (^{1}H) and 75 MHz (^{13}C); solvent: C_5D_5N; δ in ppm, J in Hz

	2		**6**		rubescensin S	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
H_β-C(1)	73.2	3.73(t)	73.2	3.67(t)	73.2	3.70(t)
H_α-C(2)	30.0	1.86(m)	30.0	1.84(m)	29.9	1.87(m)
H_β-C(2)		1.89(m)		1.86(m)		1.89(m)
H_α-C(3)	38.4	1.40	39.0	1.34	38.9	1.36
H_β-C(3)		1.31		1.28		1.27
C(4)	32.8		33.0		33.0	
H_β-C(5)	57.4	1.56(s)	57.4	1.62(s)	57.3	1.59(s)
H_β-C(6)	80.6	4.53(s)	80.7	4.61(s)	80.6	4.61(s)
C(7)	99.9		101.2		101.1	
C(8)	49.9		50.2		50.1	
H_β-C(9)	46.7	2.23(m)	52.5	2.18(m)	52.4	2.14(m)
C(10)	42.2		41.3		41.3	
H_α-C(11)	20.8	2.79(m)	21.7	2.16(m)	21.6	2.17(m)
H_β-C(11)		2.98(m)		2.31(m)		2.31(m)
H_α-C(12)	19.3	2.84(m)	24.8	2.06(m)	24.8	2.03(m)
H_β-C(12)		2.28(m)		1.98(m)		1.92(m)
H_α-C(13)	48.2	3.35(m)	48.6	4.16(dt, J = 10.5, 3.3)	48.6	4.12(dt, J = 10.5, 2.5)
H_α-C(14)	66.5	5.52(d, J = 4.2)	66.8	5.29(d, J = 10.5)	66.7	5.26(d, J = 10.5)
C(15)	179.8		177.1		177.0	
C(16)	208.1		211.2		211.2	
H-C(17)	28.9	2.37(s)	30.3	2.22(s)	30.2	2.23(s)
Me(18)	31.6	0.93	31.7	0.97(s)	31.6	0.94(s)
Me(19)	21.0	0.99	21.3	1.03(s)	21.3	1.01(s)
H-C(20)	65.6	4.80(d, J = 10.2) 4.54(d, J = 10.2)	64.3	4.70(d, J = 10.2) 4.49(d, J = 10.5)	64.3	4.68(d, J = 10.5) 4.48(d, J = 10.5)

[a] Reported in reference 12

differed from compound **2**. The NMR spectra of **6** (Table 1) and specific rotation value ($[\alpha]_D^{20} = +12.3$, Ref. $[\alpha]_D^{20} = +8.0$) showed that it was rubescensin S reported earlier.[12] Furthermore, we obtained crystal structure data for compound **6**. Interestingly, we found compound **6** was actually an isomer of compound **2**, with the 13-position chiral center reversed, as indicated in Scheme 1. To confirm the possibility of configuration inversion from **2** to **6** during its biogenesis, we performed a conversion of **2** to **6** in alkaline alcohol solution, and found that **2** can be converted to **6** in quantitative yield. These results indicated that compound **6** possesses a more stable configuration than **2**, and might originate from less stable compound **2** in the biogenesis pathway.

In summary, we reported an effective two-step transformation of oridonin to the 15,16-seco-ent-kaurane skeleton and these key skeleton compounds provided the possibility for further construction of a natural product-like compound library. We also achieved the conversion of one intermediate to natural product rubescensin S and revised that its structure has a *13S* configuration although *13R* is reported in the literature.

Experimental section

Unless otherwise noted, all reagents were purchased from commercial suppliers and used without further purification. Tetrahydrofuran (THF) were distilled from sodium, DMF was distilled in a vacuum. The progress of reactions was monitored by silica gel thin layer chromatography (TLC) plates, colored using $KMnO_4/NaHCO_3$ solution followed by heating. ^1H (300 MHz) NMR spectra were recorded on a Varian Mercury-Vx 300 M Fourier transform spectrometer. The chemical shifts were reported in δ (ppm) using the δ7.58 signal of C_5D_5N (^1H NMR) as internal standards. Low-resolution mass data were obtained on an Agilent 6110 Single Quadrupole LC/MS System. Oridonin was purchased from Xi'an Haoxuan Biological Technology Co. Ltd (Xi'an, China).

Compound 3

Oridonin **1** (3 g, 8.2 mmol) was dissolved in CH_2Cl_2 (150 mL) and MeOH (20 mL) at 5~10 °C, then treated with ozone for about 90 min. Me_2S (3 mL) was added to the solution then allowed to warm to room temperature over night and evaporated to dryness. The resulting residue was recrystallized from acetone to afford diketone **3** as yellow needles (2.5 g, 85%). ^1H NMR (300 MHz, DMSO-d_6): δ 5.04 (s, 1H), 4.99 (d, J = 10.2 Hz, 1H), 4.46 (d, J = 5.1 Hz, 1H), 4.12(d, J = 10.2, 1H), 3.87(d, J = 10.2, 1H), 3.61 (dd, 1H), 2.92 (d, J = 10.2, 1H), 2.41(m, 1H), 1.92(m, 2H), 1.61(m, 1H), 1.49(m, 4H), 1.33(m, 1H), 1.21(m, 1H), 1.17(m, 1H), 1.02(s, 3H), 0.99(s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 205.9, 205.1, 97.1, 73.3, 71.7, 69.5, 63.1, 61.9, 58.9, 54.2, 49.8, 40.8, 38.3, 33.4, 32.8, 29.3, 25.5, 21.8, 19.6; LRMS (ESI): Mass calcd for $C_{19}H_{26}O_7$ $[M-H]^-$, 365.2. Found $[M-H]^-$, 365.2.

Compound 4

Compound **3** (1 g, 2.7 mmol) was dissolved in EtOH (100 mL) at 0 °C, 30%H_2O_2 (6 mL) was added to the solution, then added slowly 10% NaOH (6 mL) solution, the reaction mixture was stirred for 2 h at 0 °C and for a further 2 h at room temperature. Then 3 N HCl was added. After removal of the solvent and water under reduced pressure, CH_2Cl_2 and MeOH were added to remove the inorganic salts and the residue evaporated to afford acid **4** (1.03 g, ~100%). Recrystallization from CH_2Cl_2 and MeOH afforded a needle solid for X-ray crystallography. ^1H NMR (300 MHz, CD_3OD): δ 4.70 (d, J = 6.3 Hz, 1H), 4.22 (s, 1H), 4.21(s, 2H), 3.56(t, 1H), 3.07 (m, 1H), 2.19(m, 2H), 2.12(m, 1H), 2.08(m, 1H), 1.95(m, 1H), 1.66(m, 2H), 1.49(m, 1H), 1.47(s, 1H), 1.39(m, 1H), 1.09(s, 3H), 1.03(s, 3H); ^{13}C NMR (75 MHz, CD_3OD): δ 180.1, 178.1, 100.5, 81.7, 74.1, 66.9, 65.4, 58.1, 50.7, 50.3, 42.6, 40.4, 39.4, 33.6, 31.9, 30.6, 23.2, 21.4, 21.3; LRMS (ESI): Mass calcd for $C_{19}H_{26}O_8$ $[M-H]^-$, 381.2. Found $[M-H]^-$, 381.2.

Compound 5

N,O-dimethylhydroxylamine hydrochloride (56 mg, 0.575 mmol) was in DMF (3 mL) and redistilled diisopropylethylamine (0.2 mL, 1.2 mmol) was added at 0 °C under a nitrogen atmosphere. To the mixture was added compound **4** (200 mg, 0.523 mmol) followed by COMU (56 mg, 0.575 mmol). The solution immediately turned yellow and the solution was left stirring in the ice-bath for a further 20 min then allowed to reach ambient temperature overnight. The organic solution was concentrated to remove DMF. The crude product was purified by silica gel chromatograph eluting with CH_2Cl_2/MeOH (50 : 1). Compound **5** was obtained (140 mg, 62%) as a white solid: ^1H NMR (300 MHz, $CDCl_3$): δ 4.58 (d, J = 7.8 Hz, 1H), 4.22 (s, 1H), 4.21 (s, 2H), 3.7 (s, 3H), 3.62 (t, 1H), 3.47 (m, 1H), 3.23 (s, 1H), 2.21 (m, 1H), 2.12 (m, 2H), 1.95 (m, 2H), 1.66 (m, 2H), 1.51 (m, 1H), 1.49 (s, 1H), 1.39 (m, 2H), 1.09 (s, 3H), 1.03 (s, 3H); ^{13}C NMR (75 MHz, CD_3OD): δ 175.9, 174.7, 99.8, 79.7, 73.5, 66.2, 63.6, 561.6, 55.8, 50.0, 40.9, 38.5, 38.3, 32.7, 31.5, 29.4, 23.9, 21.6, 21.1, 18.7, 17.3; LRMS (ESI): Mass calcd for $C_{21}H_{31}NO_8$ $[M+H]^+$, 426.2. Found $[M+H]^+$, 426.2.

Compound 2

TMSCl (0.1 mL, 1.21 mmol) was added dropwise to a solution of compound **5** (130 mg, 0.305 mmol) in dry THF (5 mL) at −78 °C within 5 min under a nitrogen atmosphere. The mixture was then allowed to stir at −78 °C for 20 min. MeMgBr solution (3 N, 0.81 mL, 2.43 mmol) was added. The reaction mixture was allowed to warm to room temperature overnight and 1 N HCl (2 mL) added carefully, the product was extracted with ethyl acetate (3 × 20 mL). The combined organic phase were washed with water and brine, dried (Na_2S0_4), and evaporated to give a colorless oil. Purification by silica gel chromatograph eluting with CH_2Cl_2/MeOH (50 : 1) afforded compound **2** (60 mg, 52%) as a white solid. Recrystallization from petroleum ether and ethyl acetate provided X-ray quality crystals. $[\alpha]_D^{20} = -41.7$ (c = 0.30, acetone), ^1H- and ^{13}C-NMR: see the Table 1. LRMS (ESI): Mass calcd for $C_{20}H_{28}O_7$ $[M-H]^-$, 379.2. Found $[M-H]^-$, 379.2.

Compound 6

TMSCl (0.1 mL, 1.21 mmol) was added dropwise to a solution of compound **5** (130 mg, 0.305 mmol) in dry THF (5 mL) at −78 °C within 5 min under a nitrogen atmosphere. The mixture was then allowed to stir at −78 °C for 20 min. MeMgBr solution (3 N, 0.81 mL, 2.43 mmol) was added. The reaction mixture was allowed to warm to room temperature overnight and

MeOH (0.2 mL) added carefully. After removal of the solvent under reduced pressure the residue was purified by silica gel chromatograph eluting with CH_2Cl_2/MeOH (50 : 1). Compound **6** was obtained (40 mg, 35%) as a white solid. Recrystallization from acetone provided X-ray quality crystals. $[\alpha]_D^{20} = +12.3$ (c = 0.31, acetone), ^{1}H- and ^{13}C-NMR: see the Table 1. LRMS (ESI): Mass calcd for $C_{20}H_{28}O_7$ $[M-H]^-$, 379.2. Found $[M-H]^-$, 379.2.

Compound 6 can also be obtained: Compound **2** (5 mg) was in MeOH (2 mL), 10% NaOH solution (10 mL) was added, stirred at room temperature for 2 h, and 2 N HCl (0.2 mL) added, the residue was extracted with ethyl acetate (3 × 5 mL). The combined organic phase was washed with water and brine, dried (Na_2SO_4), and evaporated to give compound **6** (5 mg, 100%).

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