Tetrahedron 67 (2011) 9193–9198

Contents lists available at SciVerse ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Sesquiterpenoids, alantolactone analogues, and *seco*-guaiene from the roots of *Inula helenium*

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ARTICLE INFO

Article history:
Received 28 June 2011
Received in revised form 7 September 2011
Accepted 16 September 2011
Available online 22 September 2011

Keywords:
Inula helenium
Dimeric eudesmanolide
Nor-eudesmanolide
seco-Guaiene
Anti-bacterial activity

ABSTRACT

Three new sesquiterpenoids, a new unusual dimeric eudesmanolide, bialantolactone, a new nor-eudesmanolide, trinoralantolactone, and a new *seco*-guaiene, 7*S*,1(10)*Z*-4,5-*seco*-guaia-1(10),11-diene-4,5-dioxo, together with 13 known sesquiterpenoids, were isolated from the roots of *Inula helenium*. Their structures were elucidated by comprehensive spectroscopic analyses. The absolute configurations of bialantolactone and 7*S*,1(10)*Z*-4,5-*seco*-guaia-1(10),11-diene-4,5-dioxo were defined via the experimental and computational optical rotation and CD data. The plausible biosynthetic pathways to bialantolactone and 7*S*,1(10)*Z*-4,5-*seco*-guaia-1(10),11-diene-4,5-dioxo are discussed. 16 compounds were evaluated for their anti-bacterial activities against six bacteria.

1. Introduction

Inula helenium L. (Family: Asteraceae) is a widely occurring perennial herb in East Asia, North American and Europe.[1] The roots of *I. helenium* are used as versatile medicine against fever, lung disorders, bronchitis, indigestion, chronic enterogastritis, and infectious diseases.[2,3] The volatile oil of *I. helenium* possesses strong anthelminthic, anti-tumor, and anti-bacterial activities, which have been demonstrated by pharmacological studies.[4–6] The investigations have revealed that the classes of most common secondary metabolites from the volatile oil, the aerial parts and the roots of *I. helenium* are eudesmanolides, germacranolides, and guaianolides.[6–8]

Discovery of new bioactive natural products from edible and medicinal plants is of particular interest to us. Sesquiterpenoids, especially eudesmane-type sesquiterpenoids from Asteraceae, which exhibited a wide range of biological activities and diverse architectures, were the most important part in our research.[9–11] The secondary metabolites from the roots of *I. helenium* have been investigated by us. The results reported below involve three new sesquiterpenoids (Fig. 1), a new dimeric eudesmanolide (**1**), a new nor-eudesmanolide (**2**), and a new *seco*-guaiene (**3**), together with 11 known eudesmanolides (**4**–**14**) and two known germacranolides (**15** and **16**). Their structures were elucidated by spectroscopic analysis. The absolute configurations of **1** and **3** were defined via the experimental and computational optical rotation and circular dichroism (CD) data. The plausible biosynthetic pathways of **1** and **3** are discussed. In addition, the anti-bacterial activities of compounds **1**–**16** were evaluated against six bacteria.

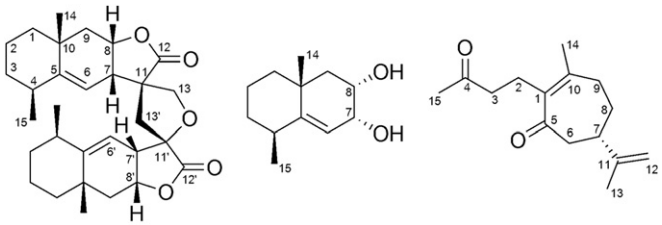

Fig. 1. Structures of compounds **1**–**3**.

2. Results and discussion

2.1. Structure elucidation

The petroleum ether (60–90 °C) extract of *I. helenium* was subjected to chromatographic purification over repeated normal phase silica gel and reverse phase C18 column chromatography to afford three new sesquiterpenoids (**1**–**3**) (Fig. 1). In addition, 13 known compounds were obtained and identified as alantolactone (**4**),[12] isoalantolactone (**5**),[12] 11α,13-dihydroisoalantolactone (**6**),[13] eudesm-4,11(13)-dien-12,8β-olide (**7**),[14] 4α,5α-epoxyalantolactone

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doi:10.1016/j.tet.2011.09.070

(**8**),[15] 4α,15-epoxyisoalantolactone (**9**),[16] macrophyllilactone (**10**),[17] 11α-hydroxyeudesm-5-en-12,8β-olide (**11**),[18] telekin (**12**),[19] 3α-hydroxyeudesm-4,11-dien-12,8β-olide (**13**),[20] 4,5-*seco*-eudesm-11(13)-en-4,5-dioxo-12,8β-olide (**14**),[21] 4α,5α-epoxygermacr-1(10),11(13)-dien-12,8β-olide (**15**),[22] and 5β-hydroxygermacr-1(10),4(15),11(13)-trien-12,8β-olide (**16**),[23] respectively, on the basis of their spectroscopic data and by comparison with literature data.

Bialantolactone (**1**) was obtained as a whiter amorphous powder. The molecular formula of the purified material was deduced to be $C_{30}H_{40}O_5$, 11° unsaturation, on the basis of the quasi-molecular ion peak at *m*/*z* 503.2755 $[M+Na]^+$ observed in the positive HR-ESI-MS. The ^{13}C NMR and DEPT spectra in chloroform-*d* (Table 1) were then used to identify 30 carbon units, which included four methyls, ten methylenes, eight methines (six sp^3- and two sp^2-hybridized), and eight quaternary carbons (four sp^3- and four sp^2-hybridized). In the upfield region of NMR spectra, there were four methyl signals (δ_C/δ_H 28.52/1.22 (3H, s, H-14), 28.35/1.21 (3H, s, H-14′), 23.08/1.09 (3H, d, *J*=7.6 Hz, H-15), and 22.76/1.14 (3H, d, *J*=7.6 Hz, H-15′)). In the downfield region, there were some characteristic signals at δ_C 151.86 (C-5), 115.34 (C-6), 153.50 (C-5′), 113.78 (C-6′), and δ_H 5.25 (1H, d, *J*=3.6 Hz, H-6), 5.03 (1H, d, *J*=3.2 Hz, H-6′) ascribed to two tri-substituted double bond functions, δ_C 176.21 (C-12) and 174.76 (C-12′) identified as two carbonyl groups, and δ_C/δ_H 75.75/4.88 (1H, m, H-8), 76.82/5.02 (1H, m, H-8′) determined as oxygen-bearing methines. Closed scrutiny of the above ^1H and ^{13}C NMR data revealed the structure of **1** to be quite similar to those of alantolactone (**4**), which suggested **1** could be an eudesmanolide.[12] Other characteristic signals at δ_C 87.12 (C-11′), 71.10 (C-13) and δ_H 4.19 (1H, d, *J*=9.2 Hz, H-13a), 4.07 (1H, d, *J*=9.6 Hz, H-13b) were assigned as oxygen-bonded quaternary carbon (C-11′) and oxygen-bearing methylene (CH_2-13). Taking into account the 11° unsaturation, compound **1** was a seven-cyclic structure with two carbonyl carbons and two double bonds. Along with the molecular formula, compound **1** was predicted as a dimeric eudesmanolide according to its ESI-MS data (*m*/*z* 233.2/255.2/271.2 ($[M_1+H]^+/[M_1+Na]^+/[M_1+K]^+$), 249.2/271.2 ($[M_2+H]^+/[M_2+Na]^+$), and 481.4/503.3/519.2 ($[M_1+M_2+H]^+/[M_1+M_2+Na]^+/[M_1+M_2+K]^+$)).

Extensive analyses of 1D and 2D NMR data of **1** in $CDCl_3$ (Table 1 and Fig. 2) led to two substructures. Substructure **1a** (5° unsaturation) was assembled on the basis of $^1H-^1H$ COSY correlations (H-1/H-2/H-3/H-4/H-15 and H-6/H-7/H-8/H-9) and key gHMBC correlations (H-6/C-4, 7, 8, 10, H-7/C-5, 6, 11, 12, H-8/C-10, H-13/C-7, 11, 12, H-14/C-1, 5, 9, 10, and H-15/C-3, 4). Substructure **1b** (5° unsaturation) was also deduced on the basis of $^1H-^1H$ COSY correlations (H-1′/H-2′/H-3′/H-4′/H-15′ and H-6′/H-7′/H-8′/H-9′) and key gHMBC correlations (H-6′/C-4′, 7′, 8′, 10′, H-7′/C-5′, 6′, 11′, 12′, H-8′/C-10′, H-13′/C-7′, 11′, 12′, H-14′/C-1′, 5′, 9′, 10′, and H-15′/C-3′, 4′). Taking into account the 11° unsaturation, there was another cycle in the structure of compound **1**. The two substructures (**1a** and **1b**) could be assembled into a structure by key gHMBC correlations of H-13/C-11′, 13′ and H-13′/C-11. They indicated that **1** had a C–C linkage between C-11 and C-13′ and a C–O–C linkage between C-13 and C-11′, and was resulted in a unique spiro-tetrahydrofuran skeleton.

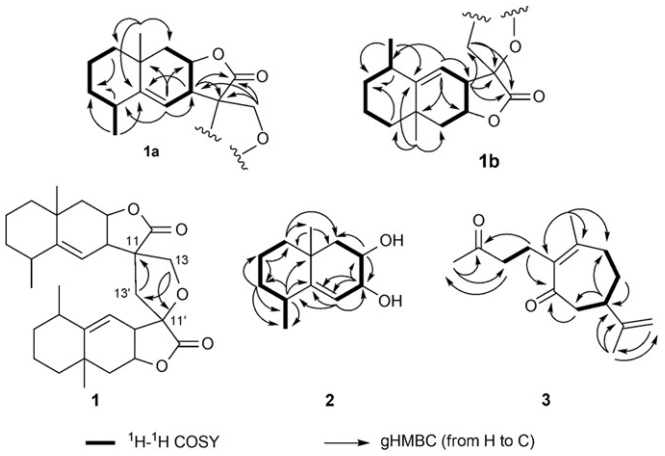

Fig. 2. Key $^1H-^1H$ COSY and gHMBC correlations of compounds **1**–**3**.

The relative configuration of the ring system in **1** was determined by its NOE experiments. Irradiating the proton at δ_H 2.19 (H-9b), significant NOE enhancements were obtained at δ_H 4.88 (H-8) and 1.22 (H-14). If CH_3-14 was appointed to β-orientation, the H-8 and H-9b were in β-orientations. Irradiating the proton δ_H 1.22 (H-14), the enhancement signal of the CH_3-15 was also yielded. In the same way, the same direction of H-8′, H-9′b, CH_3-14′, and CH_3-

Table 1
NMR spectroscopic data ($CDCl_3$) for **1** and **2**

1[c]						**2**		
Position	δ_H mult. (*J*)[a]	δ_C type[b]	Position	δ_H mult. (*J*)[a]	δ_C type[b]	Position	δ_H mult. (*J*)[a]	δ_C type[b]
1	1.17m	42.28 CH_2	1′	1.19m	42.15 CH_2	1	1.19m	40.69 CH_2
	1.64m			1.66m			1.55m	
2	1.42m	16.75 CH_2	2′	1.44m	16.77 CH_2	2	1.48m	17.97 CH_2
	1.83m			1.83m			1.82m	
3	1.52m	32.91 CH_2	3′	1.52m	32.85 CH_2	3	1.46m	33.37 CH_2
	1.55m			1.55m			1.52m	
4	2.51m	38.78 CH	4′	2.47m	38.30 CH	4	2.55m	37.97 CH
5		151.86 C	5′		153.50 C	5		149.94 C
6	5.25d (3.6)	115.34 CH	6′	5.03d (3.2)	113.78 CH	6	5.27d (3.6)	122.13 CH
7	2.78dd (3.6, 6.0)	42.97 CH	7′	2.86dd (3.6, 5.2)	44.85 CH	7	3.97dd (3.6, 7.2)	73.67 CH
8	4.88m	75.75 CH	8′	5.02m	76.82 CH	8	3.54ddd (4.0, 6.4, 7.2)	71.74 CH
9	1.59m H-9α	42.80 CH_2	9′	1.59m H-9′α	42.56 CH_2	9	1.63dd (4.0, 13.2),	45.34 CH_2
	2.19dd (3.0,12.0) H-9β			2.16dd (3.4, 12.0) H-9′β			2.34dd (6.4, 13.2)	
10		33.03 C	10′		32.98 C	10		38.79 C
11		55.68 C	11′		87.12 C	11		
12		176.21 C	12′		174.76 C	12		
13	4.19d (9.2) H-13a	71.10 CH_2	13′	2.69d (13.2) H-13′a	37.89 CH_2	13		
	4.07d (9.6) H-13b			2.36d (13.2) H-13′b				
14	1.22s	28.52 CH_3	14′	1.21s	28.35 CH_3	14	1.17s	29.07 CH_3
15	1.09d (7.6)	23.08 CH_3	15′	1.14d (7.6)	22.76 CH_3	15	1.16d (7.2)	21.15 CH_3

[a] Recorded at 400 MHz, *J* in Hz.
[b] Recorded at 100 MHz.
[c] The assignment was performed by $^1H-^1H$ COSY, HMBC, and NOE experiments.

15′ could be proved. Irradiating the proton δ_H 2.36 (H-13′b), strong NOE enhancements were obtained at δ_H 2.78 (H-7), 2.86 (H-7′), 4.88 (H-8), and 5.02 (H-8′), which showed the relative position of H-7, H-7′, H-8′, and CH_2-13′ as β-orientation.

The absolute configuration of **1** was determined by the experimental and computational optical rotation data. The B3LYP/6-31G method was used to confirm the absolute configuration.[24] Along with the relative configuration, it was possible to prune two candidate stereostructures, coded as 4*S*,7*S*,8*S*,10*R*,11*S*,4′*R*,7′*S*,8′*R*,10′*S*,11′*S*,5*Z*,5′*Z* (**1**) and 4*R*,7*R*,8*R*,10*S*,11*R*,4′*S*,7′*R*,8′*S*,10′*R*,11′*R*,5*Z*,5′*Z* (enantiomer of **1**) candidate. The sum of optical rotation data for candidate **1** is +48.35 and for its enantiomer of **1** is −51.68. The former orientation was same with the experimental +73, which suggested the configuration of compound **1**. Hence, compound **1** was elucidated unambiguously, and given a trivial name of bialantolactone that was a novel dimeric eudesmanolide.

A plausible biosynthetic pathway of bialantolactone (**1**) was discussed (Fig. 3). The natural occurring alantolactone (**4**) was also obtained from the species and it was considered to be the parent compound for the new dimeric eudesmanolide. First, there could be a 1,4-addition. The double bond (C-11═CH_2-13) of **4** is the reaction donor, the α,β-unsaturated carbonyl (CH_2-13′═C-11′−C-12′═O) of another **4** is the acceptor, and the key intermediate molecule **A** (**KIM A**) with a dihydropyran could be obtained. Then the double bond of the dihydropyran could be enzymatically oxygenated to yield an oxirane of **KIM B**. Last, two C−O bonds could be displaced each other to generate the tetrahydrofuran of bialantolactone (**1**).

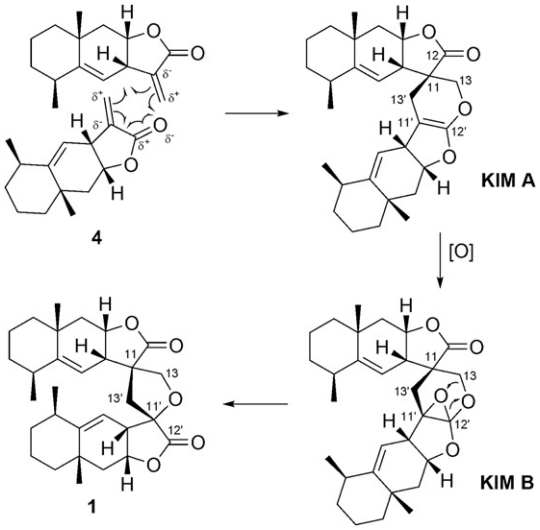

Fig. 3. Plausible biosynthetic pathway of **1**.

Trinoralantolactone (**2**) was isolated as colorless gum. The HR-ESI-MS spectrum displayed a quasi-molecular ion peak at *m*/*z* 219.1352 ([M+Na]$^+$) consistent with a molecular formula of $C_{12}H_{20}O_2$, inferring 3° unsaturation. The ^{1}H NMR spectrum clearly showed the presences of two methyls (δ_H 1.17 (3H, s, H-14) and 1.16 (3H, d, *J*=7.2 Hz, H-15)), two oxygen-bearing methine protons (δ_H 3.97 (1H, dd, *J*=7.2, 3.6 Hz, H-7) and 3.54 (1H, ddd, *J*=7.2, 6.4, 4.0 Hz, H-8)), and an olefinic proton (δ_H 5.27 (1H, d, *J*=3.6 Hz, H-6)). The ^{13}C NMR and DEPT 135 (Table 1) spectra of **2** displayed the existence of two methyls (δ_C 29.07 (C-14) and 21.15 (C-15)), four methylenes, four methines (two sp^3-hybridized oxygen-bearing carbons at δ_C 73.67 (C-7), 71.74 (C-8), and a sp^2-hybridized carbon at δ_C 149.94 (C-6)). It was revealed from analysis of NMR spectroscopic data of compound **2** that this compound was also an eudesmanolide with distinct similarities to that of alantolactone (**4**).[12] Based on the above spectroscopic data, compound **2** was considered to be a trinor-eudesmanolide (C-12−C-11−C-13) with a double bond and two hydroxyl groups. The structure was unambiguously deduced on the basis of ^{1}H−^{1}H COSY correlations (H-1/H-2/H-3/H-4/H-15 and H-6/H-7/H-8/H-9) and key gHMBC correlations (Fig. 2) (H-6/C-4, 5, 7, H-7/C-5, 6, 8, H-8/C-7, 9, H-14/C-1, 5, 9, 10, and H-15/C-3, 4, 5). The relative configuration of **2** could be determined on the basis of key NOESY correlations. The strong correlations from CH_3-14 to H-7, H-8, and CH_3-15 indicated that OH-7 and OH-8 were in α-orientation if CH_3-14 was assumed to β-orientation. Consequently, the molecular structure of **2** was elucidated and named trinoralantolactone.

7*S*,1(10)*Z*-4,5-*seco*-Guaia-1(10),11-diene-4,5-dioxo (**3**) was isolated as colorless oil. The molecular formula $C_{15}H_{22}O_2$ was deduced by its HR-ESI-MS and ^{13}C NMR spectra. The UV spectrum exhibited a strong absorption at 245 nm attributable to the conjugated α,β-unsaturated ketone. The ^{1}H NMR spectrum clearly showed three methyls connected with quaternary carbons (δ_H 1.62, 2.03, 2.11, each 3H, s) and two olefinic protons (δ_H 4.59, 4.69, each 1H, s). The ^{13}C NMR, DEPT 135, and HSQC spectra indicated 15 carbon units, including three methyls, six methylenes, one methine, five quaternary carbons (three olefinic carbons at δ_C 138.89, 146.47, 170.89, and two carbonyl carbons at δ_C 208.98, 209.42). Based on the above spectroscopic data, compound **3** was considered to be a sesquiterpene with a quasubstituted double bond, a disubstituted double bond, and two carbonyl groups.[25,26] The structure was unambiguously assembled as a *seco*-guaiene on the basis of ^{1}H−^{1}H COSY correlations (H-2/H-3 and H-6/H-7/H-8/H-9) and gHMBC correlations (H-2/C-4, 5, H-3/C-15, H-6/C-1, 5, H-7/C-6, 9, H-8/C-7, H-12/C-13, H-13/C-7, 11, 12, H-14/C-1, 9, 10, and H-15/C-3, 4) (Fig. 2).

There was a stereogenic carbon (C-7) in the structure of **3**. The absolute configuration was studied based on the experimental and electronic CD (ECD) data. This approach is emerging as a powerful tool in the absolute configuration analysis of natural products.[27,28] Corresponding to this UV maximum (245 nm), the CD spectrum of **3** (Fig. 4) showed a negative Cotton Effect (CE) centering at 237 nm ($\Delta\varepsilon_{max}$=−2.18) (π−π*) and a positive CE centering at 317 nm ($\Delta\varepsilon_{max}$=0.21) (n−π*) due to the chromophore of α,β-unsaturated ketone.

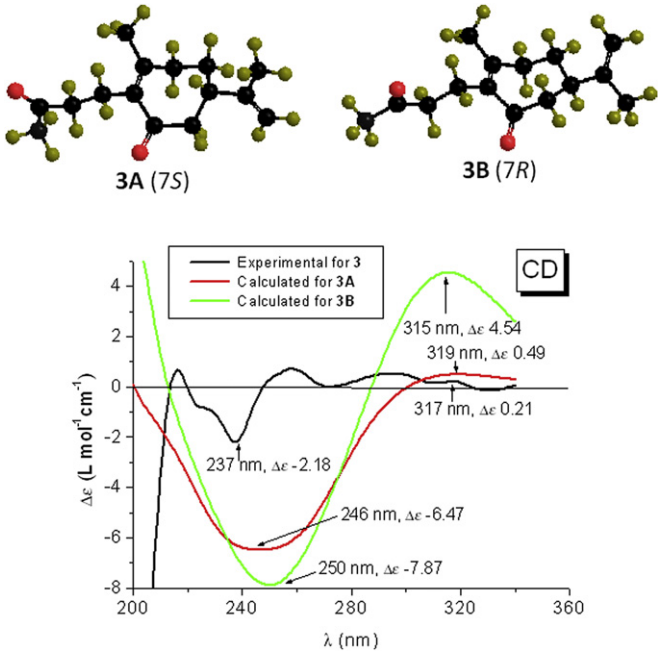

Fig. 4. Matrix of possible configurations and CD spectrum of **3**.

The helicity rule is applied to elucidation of the absolute configuraton of α,β-unsaturated ketones.[29] According to calculations utilizing simple Hückel type wave functions,[30] the negative CE centering at 237 nm of **3** means the orientation is in a counterclockwise manner from the plane of the carbonyl to that of double bond (α,β-unsaturated ketone). Given these conclusions, it was possible to prune two candidate stereostructures to just those assembled in Fig. 4, coded as **3A** and **3B**. Model building of them via Chem3D energy minimization calculations provided the valuable insights (Fig. 4). Initial qualitative evaluation of these structures suggested that **3A** and **3B** possessed significant negative torsion angle of α,β-unsaturated ketone.

Our next task is to evaluate the C-7 configuration of **3**, which was guided by comparing the actual ECD trace of **3** (**3A** and **3B**) with those predicted using time-dependent density functional theory (TD-DFT) calculations (Fig. 4).[31,32] **3A** and **3B** displayed negative CEs near 230–260 nm and positive CEs near 320 nm, obtained by calculation for the optimized geometries of each structure using the B3LYP/6-31G(d) forcefield annoted for methanol solution. According to Snatzke's sphere rule, when the substituent is not connected with the α-carbon of a chromophore, the chromophore contributes to the signs of CEs, and the substituent just affects the amplitudes.[33] **3A** and **3B** possessed similar wavelengths and same CD signs, but the D-value of the absorption coefficient ($\Delta\varepsilon$) was different, which showed different chiral carbons at the same ring with the chromophore.[34] The matchup between the experimental of **3** and that calculated for **3A** (7*S*) was excellent as can be seen from comparison to the predicted negative CE 246 nm ($\Delta\varepsilon_{max}$=− 6.47) and positive CE at 319 nm ($\Delta\varepsilon_{max}$=0.49), especially the latter pair. Thus, the absolute configuration was determined as 7*S*.

The absolute configuration of **3** was established using the experimental and computational optical rotation data. There is only one chiral carbon atom, it was possible to prune two candidate stereostructures, coded as 7*S* (**3A**) and 7*R* (**3B**) candidate. The sum of optical rotation data for candidate **3A** is −110.44 and for **3B** is +35.75. The former orientation was same with the experimental −25, which suggested the configuration of compound **3** is 7*S*. Hence, the absolute configuration was firmly determined and **3** was named as 7*S*,1(10)*Z*-4,5-*seco*-guaia-1(10),11-diene-4,5-dioxo.

A plausible biosynthetic pathway of 7*S*,1(10)*Z*-4,5-*seco*-guaia-1(10),11-diene-4,5-dioxo (**3**) from the parent compound 4α,5α-epoxygermacr-1(10),11(13)-dien-12,8β-olide (**15**)[22] was surmised (Fig. 5). First, the lactone group and the oxirane of **15** could be hydrogenated and the double bond of compound **15a** would be obtained. Then germacramane **15a** could be derived to guaiene **15c** after closed loop and dehydrogenated reaction. Last, the strong oxygenated reaction could be occurred at the double bond (C-4═C-5) of **15c** and *seco*-guaiene, 7*S*,1(10)*Z*-4,5-*seco*-guaia-1(10),11-diene-4,5-dioxo (**3**), could be derived.

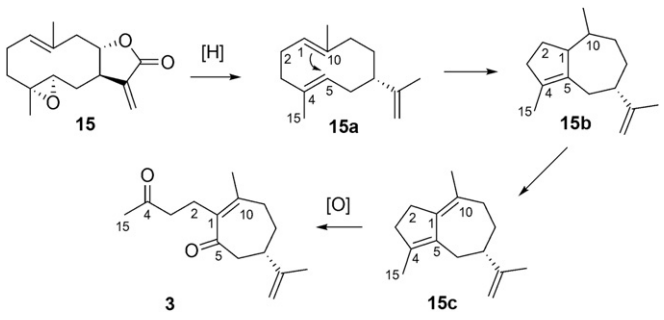

Fig. 5. Plausible biosynthetic pathway of **3**.

2.2. Anti-bacterial activity

The anti-bacterial activity of compounds **1**–**16** was assayed against *Escherichia coli*, *Bacillus cereus*, *Staphyloccocus aureus*, *Erwinia carotovora*, *Bacillus subtilis*, and *Pseudomonas aeruginosa* (Table 2) using the micro-broth dilution method.[35] Compound **8** exhibited stronger (MIC 15.5 μg/mL) activity to *B. cereus* than ampicillin, positive control, and **4** also showed moderate antibiosis to *B. cereus* (MIC 31.3 μg/mL). Several compounds had shown weak inhibition (MICs 62.5–125 μg/mL) to different bacteria, such as compounds **4**, **8**, **9**, **10**, **13**, and **14** to *E. coli*, **9**, **10**, **13**, and **14** to *B. cereus*, **4** to *S. aureus*, **4**, **9**, **13**, and **14** to *E. carotovora*, and **4** and **14** to *B. subtilis*. Unfortunately, the new compound **1** was not given characteristic data because of no solution in DMSO, H_2O, and ethanol in this experiment. And other compounds were inactive (MICs >250 μg/mL).

Table 2
Anti-bacterial activity data of compounds **4**, **8**–**10**, **13**, and **14**

Compounds	MIC (μg/mL)					
	E. coli[a]	*B. cereus*[b]	*S. aureus*[c]	*E. carotovora*[d]	*B. subtilis*[e]	*P. aeruginosa*[f]
4	125	31.3	125	62.5	62.5	>250
8	62.5	15.5	250	>250	>250	>250
9	125	62.5	>250	125	>250	>250
10	125	62.5	>250	>250	>250	>250
13	62.5	62.5	>250	62.5	>250	>250
14	62.5	125	>250	62.5	125	>250
Ampicillin[g]	100	25.0	25.0	100	12.5	100

[a] *Escherichia coli.*
[b] *Bacillus cereus.*
[c] *Staphyloccocus aureus.*
[d] *Erwinia carotovora.*
[e] *Bacillus subtilis.*
[f] *Pseudomonas aeruginosa.*
[g] Positive control.

3. Conclusion

Only a few dimeric eudesmane-type sesquiterpenoides exist in nature.[11] Bialantolactone (**1**) is the novel naturally occurring dimeric eudesmanolide with a rare carbon skeleton linked through a spiro-tetrahydrofuran ring. Tri-nor-eudesmanolide was rarely found as a natural product.[36,37] Lots of them were produced by chemical synthesist.[38,39] And the absolute configurations of **1** and **3** were determined via optical rotation and CD data. Plausible biosynthetic pathways of **1** and **3** had been discussed.

Results of bioassays indicate that bioactive molecules are characteristic of an eudesmane structure with α,β-unsaturated lactone and oxirane, explored the relationship between bioactivity and molecular structure.

4. Experimental

4.1. General experimental procedures

Optical rotations were measured on a Perkin–Elmer Model 341 polarimeter. NMR spectra were recorded on Varian INOVA-300 and Bruker AVANCE III-400 spectrometers with TMS as an internal standard in $CDCl_3$. HR-ESI-MS were obtained on a Bruker Daltonics APEX-II 47e spectrometer. ESI-MS was carried out on a VG ZABHS mass spectrometer. UV detection was measured on a Shimadzu UV-260 spectrophotometer. IR spectra were conducted on Nicolet FTIR-360 spectrometer. Analytical and preparative thin-layer chromatography (TLC) was performed on silica gel plates (GF_{254} 10–40 μm, Qingdao Marine Chemical Factory). Analytical TLC was provided to follow the separation and check the purity of isolated compounds. Spots on the plates were observed under UV light and visualized by

spraying them with 5% H_2SO_4 in EtOH (v/v), followed by heating. Column chromatography (CC) was performed on silica gel (200–300 mesh, Qingdao Marine Chemical Co.).

4.2. Plant material

The roots of *I. helenium* (tumuxiang in Chinese) were purchased from Huanghe Medicinal Material Market in Gansu in 2009 and were identified by Prof. Shi-Jian Xu, School of Life Science, Lanzhou University. A voucher specimen (No. Ih2009001) has been deposited in the State Key Laboratory of Applied Organic Chemistry, Lanzhou University, China.

4.3. Extraction and isolation

The air-dried and powdered roots of *I. helenium* (9.0 kg) were extracted with EtOH (3×3 h) at ca. 65 °C. The crude extract was mixed with H_2O (2 L) to form a suspension and partitioned successively with petroleum ether (60–90 °C), EtOAc, and *n*-BuOH. The petroleum ether soluble part (120 g) was subjected to silica gel column chromatography (CC) eluted with petroleum ether–EtOAc (30/1, 15/1, 8/1, 4/1, 2/1, 1/1, 0/100, v/v) and CH_3OH to give eight fractions A–H. Fractions B–E, composed of main sesquiterpenoids, were further chromatographed on silica gel CC with gradient petroleum ether–EtOAc system to give several subfractions. Further purification of Fr.B through repeated chromatography with petroleum ether–EtOAc (30/1), $CHCl_3$–EtOAc (30/1) and petroleum ether–acetone (25/1) as eluent yielded compounds **4** (4.2 g), **5** (21.0 mg), **7** (24.0 mg), and **15** (7.0 mg). Fr.C was separated by silica gel CC eluted repeatedly with petroleum ether–EtOAc (20/1, 10/1, and EtOAc), and by recrystallization to give compounds **6** (22.0 mg), **8** (9.0 mg), **9** (13.0 mg), **12** (9.0 mg), and **14** (5.0 mg), respectively. Likewise, a similar isolation procedure adopted for Fr.D afforded compounds **1** (10.0 mg), **3** (2.0 mg), **10** (3.0 mg), **13** (11.0 mg), and **16** (5.0 mg). And in the Fr.E we got compounds **2** (7.0 mg) and **11** (17.0 mg).

*4.3.1. Bialantolactone (**1**).* White amorphous powder ($CHCl_3$); $[\alpha]_D^{20}$ +73.0 (*c* 1.0, $CHCl_3$); IR (KBr) ν 1769.7 and 1635.6 cm^{-1}; for ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) spectroscopic data see Table 1; ESI-MS (positive ion mode) *m*/*z* 233.2 $[M_1+H]^+$, 249.2 $[M_2+H]^+$, 255.2 $[M_1+Na]^+$, 271.2 $[M_1+K]^+$ and/or $[M_2+Na]^+$, 481.4 $[M+H]^+$, 503.3 $[M+Na]^+$, and 519.2 $[M+K]^+$; HR-ESI-MS *m*/*z* 503.2755 $[M+Na]^+$ (calcd for $C_{30}H_{40}O_5Na$, 503.2768).

*4.3.2. Trinoralantolactone (**2**).* Colorless gum ($CHCl_3$); $[\alpha]_D^{20}$ +20.0 (*c* 0.5, $CHCl_3$); IR (KBr) ν 3368.6, 1652.2 and 1451.9 cm^{-1}; for ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) spectroscopic data see Table 1; HR-ESI-MS *m*/*z* 219.1352 $[M+Na]^+$ (calcd for $C_{12}H_{20}O_2Na$, 219.1356).

*4.3.3. 4,5-seco-Guaia-1(10),11-diene-4,5-dioxo (**3**).* Colorless oil ($CHCl_3$); $[\alpha]_D^{20}$ −25.0 (*c* 0.3, $CHCl_3$); for experimental and calculated CD (MeOH) data see Supplementary data Table S4; UV (MeOH) λ_{max} 245 nm; ^1H NMR ($CDCl_3$, 400 MHz) spectroscopic data: δ_H 2.29 (1H, m, H-2a), 2.34 (1H, m, H-2b), 2.27 (1H, m, H-3a), 2.36 (1H, m, H-3b), 2.20 (1H, m, H-6a), 2.25 (1H, m, H-6b), 2.30 (1H, m, H-7), 1.55 (1H, m, H-8a), 1.65 (1H, m, H-8b), 2.48 (1H, m, H-9a), 2.49 (1H, m, H-9b), 4.59 (1H, s, H-12a), 4.69 (1H, s, H-12b), 1.62 (3H, s, H-13), 2.03 (3H, s, H-14), 2.11 (3H, s, H-15); ^{13}C NMR ($CDCl_3$, 100 MHz) spectroscopic data: δ_C 138.89 (C-1), 34.24 (CH_2-2), 41.55 (CH_2-3), 208.98 (C-4), 209.42 (C-5), 27.70 (CH_2-6), 45.24 (CH-7), 26.30 (CH_2-8), 31.61 (CH_2-9), 170.89 (C-10), 146.47 (C-11), 112.41 (CH_2-12), 18.07 (CH_3-13), 17.53 (CH_3-14), 30.02 (CH_3-15); ESI-MS (positive ion mode) *m*/*z* 235.3 $[M+H]^+$, 257.2 $[M+Na]^+$, 273.2 $[M+K]^+$, and 491.0 $[2M+Na]^+$; HR-ESI-MS *m*/*z* 235.1700 $[M+H]^+$ (calcd for $C_{15}H_{23}O_2$, 235.1693).

4.4. Assay for anti-bacterial activity

Anti-bacterial activity was measured by the micro-broth dilution method in 96-well culture plates using the Mueller-Hinton (MH) broth (Hangzhou Microbial Reagent Co. Ltd, Hangzhou, China), according to the Standard of National Committee for Clinical Laboratory. The standard bacterial strains were obtained from the China General Microbiological Culture Collection Center. Ampicillin (Sigma, Shanghai, China) was used as positive control. The tested bacteria were incubated in the MH broth for 12 h at 30 °C at 190 rpm., and the spore concentration was diluted to approximately 1×10^5–1×10^6 CFU with MH broth. After incubation for 24 h at 30 °C, the MICs were examined.

Acknowledgements

This work was financially supported by the National Basic Research Program of China (973 Project, No. 2007CB108903), the Fundamental Research Funds for the Central Universities (LZUJBKY-2011-75), and Interdisciplinary Youth Innovation Fund of Lanzhou University (LZUJC2007005). We are grateful to Prof. Xuan Tian, State Key Laboratory of Applied Organic Chemistry, Lanzhou University, for assistance with the elucidation of absolute configuration.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2011.09.070. These data include MOL files and InChIKeys of the most important compounds described in this article.

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