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Five new sesquiterpenoids from the agarwood of *Aquilaria sinensis*

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Abstract

Aquisinenoids F–G (**1**–**5**), five new eudesmane-type sesquiterpenoids, and five known compounds (**6**–**10**) were isolated from the agarwood of *Aquilaria sinensis*. Their structures, including absolute configurations were unambiguously identified by comprehensive spectroscopic analyses and computational methods. Inspiring by our previous study on the same kind of skeletons, the bioactive studies of all new compounds were carried towards anti-cancer and anti-inflammatory activity. The results do not show any activity but it revealed the structure-activity relationships (SAR).

Keywords

Aquilaria sinensis; agarwood; sesquiterpenoids; SAR studies

Introduction

Agarwood is the resinous wood of the *Aquilaria* species of the Thymelaeaceae family [1]. It is a precious traditional Chinese medicinal material and a kind of natural fragrance, which is widely distributed in China, India, the Middle East, and the Southeast Asia [2]. Agarwood has been considered as important role in both traditional Chinese medicine and Ayurvedic medicine in the treatment of stomach disorders, coughs, asthma, sedation, analgesia, and antiemetic [3]. Previous studies have shown that 2-(2-phenylethyl)chromones and sesquiterpenes are the characteristic and main bioactive components of agarwood [4]. Various bioactivities, including neuroactive, gastrointestinal modulation, cytotoxic, analgesic, anti-bacterial, anti-fungal, anti-inflammatory, anti-asthmatic, anti-diabetic, and anti-oxidant activities have been reported for

the extracts of agarwood [5]. Our group recently reported five structurally intriguing and biologically active sesquiterpene dimers [6], which attract our interest to gain a deep insight into the novel molecules with effective bioactivities from agarwood. Therefore, the continue study on *Aquilaria sinensis* has led to the isolation of ten sesquiterpenoids including five new eudesmane-type sesquiterpenoids (Fig 1). Herein, we have performed the isolation, structural elucidation, and bioactivity evaluation of the new compounds are described.

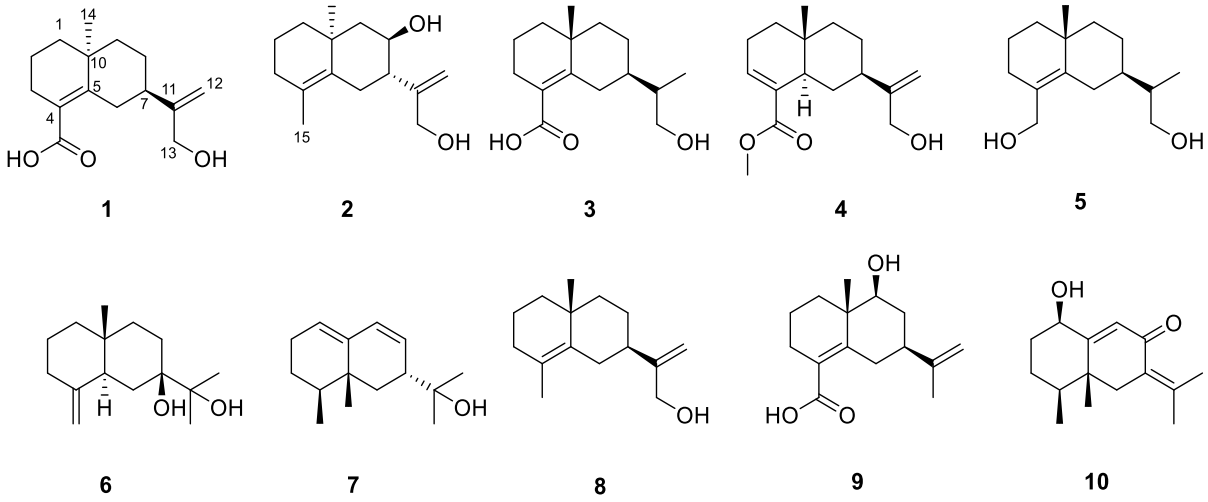

Figure 1. The structures of compounds **1**–**10**.

Results and Discussion

The dried and powdered agarwood sample was extracted by percolating with 95% EtOH to afford a crude extract, which was suspended in water followed by partition with EtOAc to afford an EtOAc soluble extract. Further, several methods used to purify the extract, such as MCI gel CHP 20, silica gel column, vacuum liquid chromatography, and semi-preparative HPLC purification to obtain the pure compounds. A total of ten compounds including five new

eudesmane-type sesquiterpenoids (**1**–**5**), and five known compounds were identified. In which, the known compounds are readily identified as eudesm-4(15)-ene-7*β*,11-diol (**6**) [7], *rel*-(2*R*,8*S*,8a*R*)-2-(1,2,6,7,8,8a-Hexahydro-8,8a-dimethyl-2-naphthyl)-propan-2 (**7**) [8], *γ*-Costol (**8**) [9], (+)-Methyl 9-hydroxyselina-4,11-dien-14-oate (**9**) [10] and 1*β*-hydroxyeremo-phila-7(11)9-dien-8-one (**10**) [11] by a comparison of their spectroscopic data with those reported in the literature (Fig 1), and the new derivatives were characterized as explained bellow.

Compound **1** was obtained as pale yellow gums, its molecular formula inferred from the positive HRESIMS at *m/z* 273.1461 $[M + Na]^+$ (calcd for $C_{15}H_{22}O_3Na$, 273.1461), ^{13}C NMR, and DEPT spectra, indicating 5 degrees of unsaturation. The ^1H NMR spectrum of **1** (Table 1) shows one methyl group at δ_H 1.16 (3H, s), two olefinic protons [δ_H 5.05 (1H, d, J = 1.4 Hz), δ_H 4.05 (1H, d, J = 1.4 Hz)], and an oxygenated methylene at δ_H 4.06 (2H, s). The ^{13}C NMR and DEPT (table 1) spectra indicate 15 carbons, including one methyl, eight methylenes (one sp^2), one methine, and four nonprotonated carbons (including three sp^2 and one sp^3). The planar structure of **1** was mainly constructed by 2D NMR analysis. Firstly, the ^1H-^1H COSY spectrum (Fig 2) displays the correlations of H-1/H-2/H-3 and H-6/H-7/H-8/H-9, suggesting the existence of two spin systems. Then, the HMBC correlations of (Fig 2) of H-1/C-5 (δ_C 148.6), H-3/C-4 (δ_C 126.8), C-5, and H_3-14/C-1 (δ_C 40.6), C-5, C-10 (δ_C 36.2) indicate the presence of a six-membered ring. Additional HMBC correlations of H-6/C-4, C-5, H-7/C-5, and

H_3-14/C-9 (δ_C 43.0) allow to assign another six-membered ring as shown in Fig 2. Furthermore, the positions of a methyl group at C-10 was clarified by the HMBC correlations of H_3-14/C-1, C-5, C-9, C-10. Besides, the HMBC correlations of H-3/C-15 (δ_C 174.9), H-6/C-4 indicate the presence of a carboxyl group at C-4. Finally, the HMBC correlations of H-12/C-7 (δ_C 44.1), C-11 (δ_C 154.7), C-13 (δ_C 65.2) demonstrate that C-7 (δ_C 43.9) is connected with acryl alcohol. Thus the planar structure of **1** was assigned (Fig 1). However, the ROSEY data cannot provide the correlation of H_3-14/H_2-6 (Fig 3), which result in the ambiguity of the relative configuration assignment of **1**. Thus, the NMR chemical shifts calculations and ECD calculations were used to confirm the relative and absolute configuration of **1**. In more specific, NMR calculations were carried out at the PCM/mPW1PW91/6-311+G(d,p) level for (7*R**,10*S**)-**1** (**1a**) and (7*S**,10*S**)-**1** (**1b**), which are possible diastereomers of **1**. The results reveal that **1a** has the highest probability score. Following that, ECD calculations on (7*R*,10*S*)-**1** and (7*S*,10*R*)-**1** were conducted according to the results obtained from NMR calculations. It was found that the CD spectrum well matches with the calculated ECD spectrum of **1a** (Fig 4), reveling the absolute configuration of **1** to be 7*R*,10*S*, and named as aquisinenoid F.

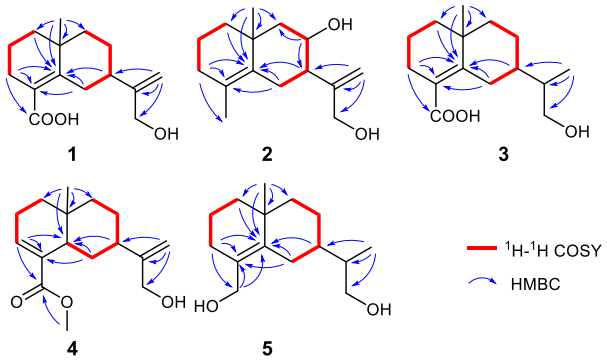

Figure 2. Key ^{1}H−^{1}H COSY and HMBC correlations for **1**–**5**.

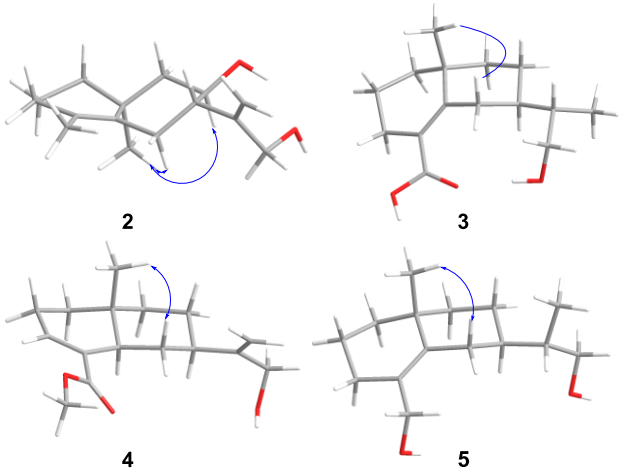

Figure 3. Key ROESY correlations for **2**–**5**.

Compound **2** was isolated as pale yellow gums, was assigned a molecule formula $C_{15}H_{24}O_2$ as inferred from the HRESIMS *m/z* 259.1671 $[M + Na]^+$ (calcd 259.1669). The^{13}C NMR and DEPT spectra (Table 1) of **2** indicate 4 degrees of unsaturation. Compound **2** is similar structure to **1** by a careful analysis of their NMR data. There are two differences between **2** and **1**. One is that C-4 in **2** is a methyl group instead of a carboxyl group in **1**, which is supported by the HMBC correlations of H-3/C-4 (δ_C 126.6), C-5 (δ_C 134.4), C-15 (δ_C 19.5). The other difference is that the existence of 8-OH in **2**, which is confirmed by down filed chemical shifts at δ_H 3.88 and δ_C 71.0 and the key HMBC correlations of H-8/C-7, C-9. Hence, the planer structure

of **2** was accomplished (Fig 1). The relative configuration of **2** was established by careful interpretation of ROESY correlations (Fig 3). The ROESY correlations of H-8/H_3-14, Ha-6/H_3-14 and the coupling constant of H-7 ($J_{H\text{-}7,\ Ha\text{-}6}$ = 3.8 Hz) suggesting the relative configurations (7S*,8*R**,10*S**)-**2** (**2a**) or (7*R**,8*S**,10*R**)-**2** (**2b**). To assign the absolute configuration of **2**, ECD calculations were performed for (7S,8*R*,10*S*)-**2** and (7*R*,8*S*,10*R*)-**2**. The result shows that the calculated ECD spectrum of **2a** is in accordance with that of experimental one (Fig 4). The absolute configuration of **2** was eventually clarified to be 7S,8*R*,10*S*, and named as aquisinenoid G.

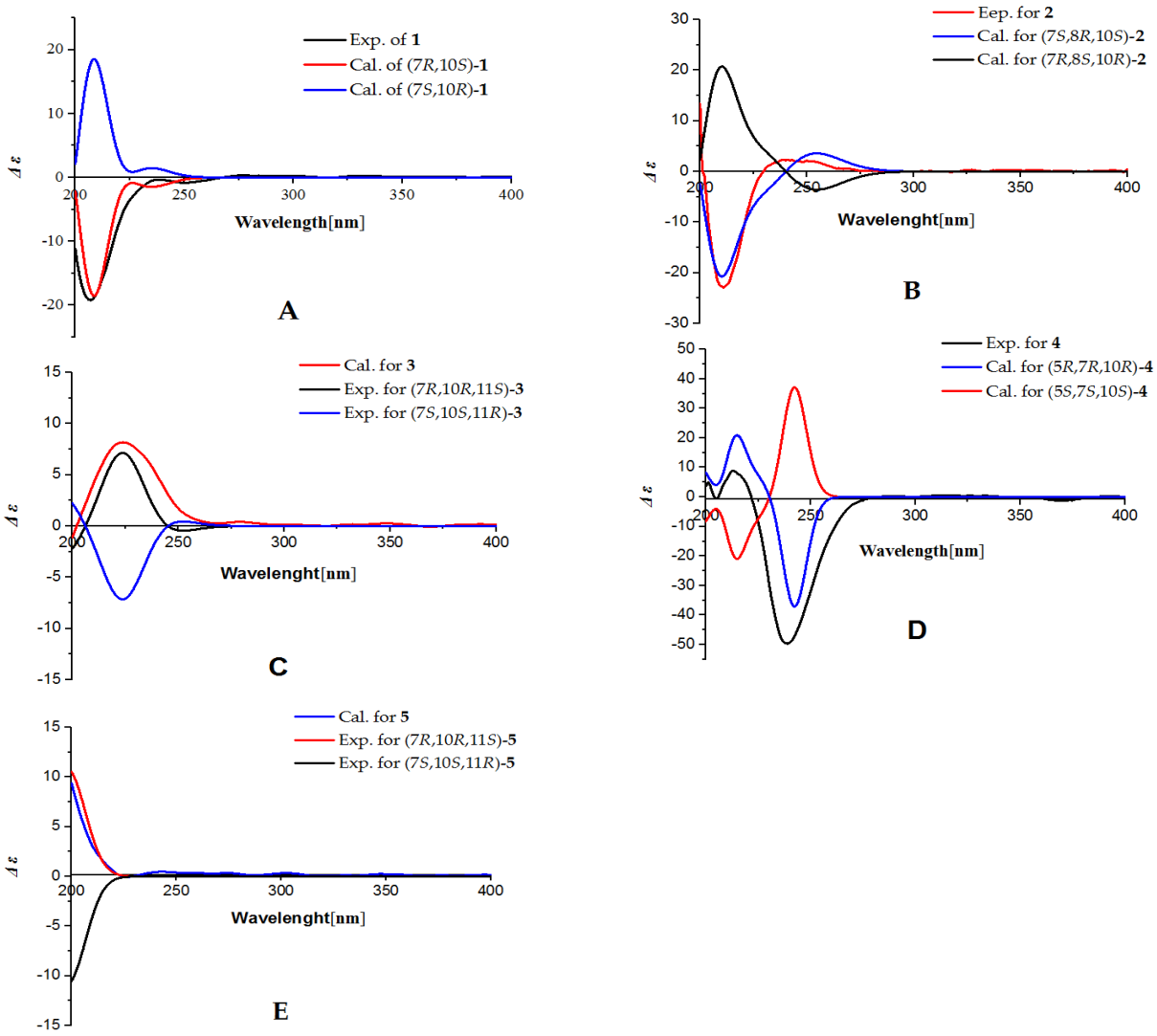

Figure 4. The calculated and experimental ECD spectra of **1**–**5.**

Compound **3** was isolated as pale yellow gums, whose molecular formula was determined to be $C_{15}H_{24}O_3$ based on its HRESIMS *m/z* 275.1622 $[M + Na]^+$ (calcd for $C_{15}H_{24}O_3Na$, 275.1618), ^{13}C NMR and DEPT spectra (Table 2), indicating 4 degrees of unsaturation. Detailed NMR interpretation implies that the data of **3** is similar to **1**. The only difference is the C-11double bond in **1** was saturated in **3**, which was verified by the HMBC correlations (Fig 2) of H-12/C-7 (δ_C 41.9), C-11 (δ_C 42.6), C-12 (δ_C 13.8). Thus the planar structure of **3** was assigned (Fig 1).

Table 1. ^1H (500 MHz) and ^{13}C (125 MHz) NMR data of **1** and **2** in MeOD.

	1			**2**	
no.	**δ_C**	**δ_H**, mult (*J* in Hz)	**no.**	**δ_C**	**δ_H**, mult (*J* in Hz)
1	40.6, CH_2	Ha: 1.59 (m)	1	41.3, CH_2	Ha: 1.53 (m)
		Hb: 1.39 (m)	2		Hb: 1.35 (m)
2	19.4, CH_2	1.67 (m)		19.9, CH_2	Ha: 1.64 (m)
3	29.0, CH_2	2.26 (m)			Hb: 1.53 (m)
4	126.8, C		3	33.9, CH_2	Ha: 2.00 (overlap)
5	148.3, C				Hb: 1.91 (m)
6	34.4, CH_2	Ha: 2.96 (m)	4	126.6, C	
		Hb: 2.03 (m)	5	134.4, C	
7	43.9, C	2.02 (m)	6	32.3, CH_2	Ha: 2.58 (dd, 14.3, 3.8)
8	28.9, CH_2	Ha: 1.69 (m)			Hb: 2.00 (overlap)
		Hb: 1.67 (m)	7	52.3, CH	1.80 (m)
9	43.0, CH_2	Ha: 1.66 (m)	8	71.0, CH	3.88 (ddd 11.3, 10.3, 4.3)
		Hb: 1.41 (ddd, 16.1, 7.5, 4.2)	9	51.8, CH_2	Ha: 1.80 (m)
10	36.2, C				Hb: 1.18 (m)
11	154.7, C		10	36.8, C	
12	108.1, CH_2	Ha: 5.05 (d 1.4)	11	152.6, C	
		Hb: 4.95 (d 1.4)	12	110.6, CH_2	Ha: 5.17 (d 1.5)
13	65.0, CH_2	4.06 (s)			Hb: 5.02 (d 1.5)
14	25.2, CH_3	1.14 (s)	13	65.9, CH_2	4.10 (dt 4.4, 1.3)
15	174.9, C		14	25.9, CH_3	1.11 (s)
			15	19.5,CH_3	1.61 (s)

Further analysis of coupling constants of H-7 ($J_{H\text{-}7,\ Ha\text{-}6}$ = 3.3 Hz and $J_{H\text{-}7,\ Hb\text{-}6}$ = 2.8 Hz) and the ROESY correlation (Fig 3) of H_3-14/Hb-6 allow concluding that H_3-14 and H-7 are in the opposite orientation. Thus, the relative configurations

of chiral centers in **3** apart from C-11 were assigned. In order to determine the configuration of C-11, we performed NMR calculations. The results disclose that **3** is likely the configuration of (7*R**,10*R**,11*S**)-**3** based on the DP4+ probability analysis and the correlation coefficient. To clarify the absolute configuration of **3**, the ECD calculations were carried out. It was found that the spectrum of (7*R*,10*R*,11*S)*-**3** (Fig 4) was agreed with the experimental one, suggesting the 7*R*,10*R*,11*S* configuration of **3**. As a result, the structure of **3** was determined as shown (Fig 1) and named aquisinenoid H.

Compound **4**, obtained as white powder, possesses a molecular formula of $C_{16}H_{24}O_3$ (5 degrees of unsaturation) derived from its HRESIMS (*m/z* 287.1614, calculated 287.1618 $[M + Na]^+$). Comparing the NMR data of **1** with those of **4** indicates that the $\Delta^{4,5}$ double bond migrates to $\Delta^{3,4}$ and the carboxylic acid group become a methyl ester derivative, which were confirmed by ^1H-^1H COSY correlations (Fig 2) of H-5/H-6/H-7 and the HMBC correlations (Fig 2) of H-3/C-4 (δ_C 135.1), C-15 (δ_C 170.3), H-16/C-15, as well as characteristic chemical shifts at C-3 (δ_C 138.6). Thus the planar structure of **4** was assigned. The opposite orientation of H-5 and Hb-6, as well as Hb-6 and H-7were proved by the coupling constants of Ha-6 ($J_{H\text{-}5,\ Hb\text{-}6}$ =12.3 Hz and $J_{H\text{-}7,\ Hb\text{-}6}$ =12.3 Hz), and the combined of ROESY (Fig 3) correlation of H_3-14/Hb-6 indicate its relative configurations were (5*R**,7*R**,10*R**)-**4**. In order to elucidate its absolute configuration, the CD spectrum was determined and compared with the calculated one. The results show that the calculated ECD spectrum of (5*R**,7*R**,10*R**)-**4** (Fig 4) matches well with the experimental one, suggesting

the absolute configuration of **4** is 5*R*,7*R*,10*R*. As a result, the absolute configuration of **4** was finally confirmed and named as aquisinenoid I.

Table 2. ^{1}H (500 MHz) and ^{13}C (150 MHz) NMR data of **3** and **4** in MeOD.

	3			**4**	
no.	**δ_C**	**δ_H**, mult (*J* in Hz)	**no.**	**δ_C**	**δ_H**, mult (*J* in Hz)
1	40.8, CH_2	Ha: 1.54 (m)	1	37.8, CH_2	Ha: 1.44 (dd 13.3, 6.2)
		Hb: 1.35 (m)			Hb: 1.37 (m)
2	19.6, CH_2	1.64 (m)	2	24.2, CH_2	Ha: 2.27 (m)
3	29.1, CH_2	2.23 (m)			Hb: 2.18 (m)
4	127.7, C		3	138.6, CH	6.58 (m)
5	146.5, C		4	135.1, C	
6	31.2, CH_2	Ha: 2.80 (dd 13.8, 3.3)	5	45.1, CH	2.22 (m)
		Hb: 1.78 (tt 13.8, 2.8)	6	29.7, CH_2	Ha: 2.17 (m)
7	41.9, CH	1.44 (m)			Hb: 1.12 (q 12.3)
8	27.4, CH_2	Ha: 1.53 (m)	7	43.1, CH	2.10 (m)
9	43.1, CH_2	Ha: 1.61(m)	8	28.5, CH_2	1.65 (m)
		Hb: 1.37(m)	9	41.1, CH_2	Ha: 1.51 (dt 12.9, 3.9)
10	36.1, C				Hb: 1.31 (m)
11	42.6, CH	1.51 (m)	10	33.5, C	
12	13.8, CH_3	0.92 (d 6.9)	11	155.3, C	
13	66.3, CH_2	3.57 (dd 10.8, 5.5)	12	108.2, CH_2	Ha: 5.03 (q 1.6)
		3.38 (dd 10.8, 4.2)			Hb: 4.85 (q 1.3)
14	25.1, CH_3	1.11 (s)	13	65.2, CH_2	4.06 (s)
15	176.3, C		14	16.0, CH_3	0.87 (s)
			15	170.3, C	
			16	51.8, CH_3	3.68 (s)

Compound **5**, obtained as colorless gums, with a molecular formula of $C_{15}H_{26}O_2$ (3 degrees of unsaturation) derived from its HRESIMS (*m/z* 261.1825, calculated 261.1825 $[M + Na]^+$). The 1D NMR spectra of **5** exhibits a pattern analogous to that of **3**. The difference is that the carboxylic acid at C-4 in **3** undergoes reduction to form methyl hydroxide at C-4 in **5**. This alteration gains supported from the analysis of the HMBC correlations (Fig 2) of H-3/C-4 (δ_C 128.6), C-15 (δ_C 63.5), and H-15/C-4, C-5 (δ_C 141.9). Thus the planar structure of **5** was assigned. By the analysis of coupling constants of H-7 ($J_{H\text{-}7,\ Ha\text{-}6}$ = 2.6 Hz) and the ROESY (Fig 3) correlations of H_3-14/Hb-6 shows that H_3-14 and H-7 are in the opposite orientation. The NMR calculations were performed to clarify the relative configuration at C-11. Finally, the relative configurations of **5** was assigned as (7*R**,10*R**,11*S**)-**5** by conducting NMR calculations at the

PCM/mPW1PW91/6-311+G(d,p) level, calculated for (7*S**,10*S**,11*S**)-**5** (**5a**) and (7*R**,10*R**,11*S**)-**5** (**5b**) having the correlation coefficient and DP4+ probability analysis. The absolute configuration of **5** was subsequently assigned by direct ECD calculation of (7*R*,10*R*,11*S*)-**5**. The results show that the spectrum of **5b** agrees well with the experimental spectrum of **5** (Fig 4), showing the absolute configuration of **5** to be 7*R*,10*R*,11*S*. Thus, the structure of **5**, named as aquisinenoid J, was finally identified.

Table 3. ^{1}H (500 MHz) and ^{13}C (150 MHz) NMR data of **5** in $CDCl_3$.

			5		
no.	**δ_C**	**δ_H**, mult (*J* in Hz)	**no.**	**δ_C**	**δ_H**, mult (*J* in Hz)
1	40.1, CH_2	Ha: 1.52 (m)	9	42.3, CH_2	Ha: 1.56 (m)
		Hb: 1.31 (m)			Hb: 1.34 (m)
2	19.3, CH_2	1.60 (m)	10	25.0, C	
3	30.2, CH_2	Ha: 2.13 (m)	11	40.3, CH	1.63 (m)
		Hb: 2.09 (m)	12	12.9, CH_3	0.89 (d 6.9)
4	128.6, C		13	66.2, CH_2	Ha: 3.61 (dd, 10.9, 6.9)
5	141.9, C				Hb: 3.53 (dd, 10.9, 6.9)
6	27.1, CH_2	Ha: 2.53 (dt 13.6, 2.6)	14	25.0, CH_3	1.05 (s)
		Hb: 1.67 (m)	15	63.5, CH_2	Ha: 4.12 (d, 11.8)
7	41.3, CH	1.47 (m)			Hb: 3.99 (d, 11.8)
8	26.9, CH_2	1.44 (m)			

According to our previous studies, the components from *A. sinensis* possess various attractive bioactivities, such as anti-inflammatory, anti-cancer, and anti-Ach, which motivate us assume that the compounds with the similar skeleton may have the same bioactivities, therefore, the new compounds were evaluated for their anti-inflammatory and anti-cancer potential using the same method as described previously [6, 12], and the cell viability was determined by CCK-8 assay (Fig A, B, Supporting Information File 1). Unfortunately, we could not distinguish any one of both activities for all the new derivatives. Further, we recall the skeletons in our current study and our previously reported ones [6,

12], which revealed the SAR. Moreover, eudesmane-type sesquiterpenes constructed with aldehyde groups are more active even though in the form of dimers. Present study described the skeletons with 1°-alcohols and/or acid groups suppress the activity and conclude eudesmane-type sesquiterpenes constructed with aldehyde groups are more active than alcohol or acids.

Conclusion

In summary, five new eudesmane-type sesquiterpenes compounds (**1**–**5**) and five known compounds (**6**–**10**) were reported from agarwood of *A. sinensis*. The discovery of these new compounds enrich the structural diversity and complexity of sesquiterpenes derived from agarwood. Unfortunately, none of the new compounds exhibit biological activity on LPS-induced inflammation in Raw264.7 cells and human break cancer cells. But, we have proven a good conclusion for SAR studies based on current study and our previous study. These compounds were isolated by other researchers in the future, who could consider our conclusions and choose other aspects of biological activity.

General Experimental Procedures

NMR spectra were recorded on a Bruker AV-500 or AV-600 spectrometer with TMS as an internal standard. Silica gel (200–300 mesh; Qingdao Marine Chemical Inc., Qingdao, China), RP-18 silica gel (40–60 μm; Daiso Co., Tokyo, Japan), MCI gel CHP 20P (75–150 μm, Mitsubishi Chemical Industries, Tokyo, Japan) were used for column chromatography. Optical rotations were

measured on an Anton Paar MCP-100 digital polarimeter. UV and CD spectra were obtained on a Jasco J−815 circular dichroism spectrometer (JASCO, Tokyo, Japan). Semi-preparative HPLC was carried out using an Agilent 1260 chromatograph with a 250 mm × 10 mm, i.d., 5 μm, SEP Basic 120 C18. HRESIMS were measured on a SCIEX X500R QTOF MS spectrometer (Shimadzu Corporation, Tokyo, Japan).

Plant Material

The resinous wood of *Aquilaria sinensis* was purchased from Hainan Xiangshu Agarwood Industry Groud Co., Ltd, July 2018. The material was identified by the Gansu Institute for Drug Control, and a voucher specimen (CHYX0642) was deposited at School of Pharmaceutical Sciences, Shenzhen University, P.R. China.

Extraction and Isolation

The dried and powdered agarwood sample (15 kg) was extracted by percolating with 95% EtOH to afford a crude extract, which was suspended in water followed by partition with EtOAc to afford an EtOAc soluble extract (1.7 kg). The EtOAc extract was separated by a MCI gel CHP 20P column eluted with gradient aqueous MeOH (50%–100%) to provide nine portions (Fr.1–Fr.9). Fr.6 (144.0 g) was separated into fourteen fractions (Fr.6.1–Fr.6.14) by silica gel column with petroleum ether–EtOAc (50:1–0:1). Fr.6.3 (1.3 g) was further divided into four parts (Fr.6.3.1–Fr.6.3.4) by a vacuum liquid chromatography (VLC) on silica gel column with petroleum ether–acetone (50:1–3:7) as solvents.

Fr.6.3.1 (808.5 mg) was subjected to preparative thin layer chromatography (PTLC) (dichloromethane) to give Fr.6.3.1.1–Fr.6.3.1.7, of which Fr.6.3.1.4 (255.9 mg) was purified by preparative HPLC on YMC-Pack-ODS-A with aqueous MeCN (63%), and then purified by semi-preparative HPLC on YMC-Pack-ODS-A with aqueous MeOH (82%) to afford **7** (36.1 mg, t_R = 21.3 min; flow rate: 3 mL/min). Fr.6.5 (9.3 g) was further fractionated into ten parts (Fr.6.5.1–Fr.6.5.10) by silica gel column washed with petroleum ether–EtOAC (50:1–1:1). Among them, Fr.6.5.7 (355.7 mg) was subjected to PTLC (petroleum ether:acetone–5:1) to give Fr.6.5.7.1–Fr.6.5.7.7. Fr.6.5.7.1 (34.3 mg) was purified by semi-preparative HPLC on SEP Basic 120 C18 (aqueous MeOH, 65%) to give compound **9** (4.9 mg, t_R = 9.4 min, flow rate: 3 mL/min). Fr.3.3.8 (787.3 mg) was separated by VLC on silica gel eluted with petroleum ether–acetone (25:1–1:1) to provide six portions (Fr.6.5.8.1–Fr.6.5.8.6). Fr.6.5.8.4 (91.0 mg) was further purified by semi-preparative HPLC on SEP Basic 120 C18 with aqueous MeCN (46%) to afford **10** (4.5 mg, t_R = 24.9 min; flow rate: 3 mL/min). Fr.6.6 (9.1 g) was further divided into fifteen parts (Fr.6.6.1–Fr.6.6.15) by YMC-ODS column (MeOH, 45%–80%). Fr.6.6.10 (747.8 g) was separated by silica gel column washed by petroleum ether-acetone (1:9–6:4) to yield thirteen portions (Fr.6.6.10.1–Fr.6.6.10.13). Fr.6.6.11(333.0 mg) was separated by preparative TLC with petroleum ether-acetone (3:1) to get five fractions (Fr.6.6.11.1–Fr.6.6.11.5). compound **6** (7.9 mg, t_R =17.4 min, flow rate: 3 mL/min) were obtained by semi-preparative HPLC on SEP Basic 120 C18 (MeCN, 50%) from Fr.6.6.11.2 (60.5 mg) and compound **4** (11.5 mg, t_R = 20.6 min, flow rate: 3 mL/min) were obtained by semi-preparative HPLC on SEP Basic 120 C18 (aqueous MeOH, 73%) from

Fr.6.6.11.3 (60.4 mg). Fr.6.7 (7.1 g) was further divided into ten parts (Fr.6.7.1–Fr.6.7.10) by YMC-ODS column (MeOH, 45%–70%). Fr.6.7.9 (4.9 g) was separated into eight fractions (Fr.6.7.9.1–Fr.6.7.9.8) by silica gel eluted with petroleum ether–acetone (6:94–55:45). Fr.6.7.9.5 (2.3 g) was further fractionated into seventeen parts (Fr.6.7.9.5.1–Fr.6.7.9.5.17) by a silica gel column washed with petroleum ether–acetone (6:94–55:45). Fr.6.7.9.5.14 (296.5 mg) was separated by PTLC (Petroleum ether:isopropyl alcohol–10:1) to afford eight fractions (Fr.6.7.9.5.14.1–Fr.6.7.9.5.14.8). Of which, Fr.6.7.9.5.14.3 (25.4 mg) was further purified by semi-preparative HPLC on YMC-PACK-ODS-A (aqueous MeCN, 65%) to yield **1** (12.7 mg, t_R = 28.1 min, flow rate: 3 mL/min). Fr.6.7.9.5.14.5 (53.5 mg) was further purified by semi-preparative HPLC on SEP Basic 120 C18 (aqueous MeCN, 65%) to yield **3** (1.8 mg, t_R = 18.0 min, flow rate: 3 mL/min). Fr.6.9 (7.7 g) was further divided into thirteen parts (Fr.6.9.1–Fr.6.9.13) by YMC-ODS column (MeOH, 45%–85%). Fr.6.9.7 (2.8 g) was separated into seven fractions (Fr.6.9.7.1–Fr.6.9.7.7) by using a silica gel column with petroleum ether–acetone (5:95–1:1). Fr.6.9.7.5 (559.6 mg) was separated by preparative TLC (petroleum ether:acetone–3:1) to give Fr.6.9.7.5.1–Fr.6.9.7.5.5, of which Fr.6.9.7.5.5 (35.1 mg) was purified by semi-preparative HPLC on SEP Basic 120 C18 (aqueous MeOH, 65%) to afford compound **2** (7.6 mg, t_R = 17.3 min, flow rate: 3 mL/min). Fr.6.11(2.8 g) was gel filtrated over Sephadex LH-20 (MeOH) to afford eight parts (Fr.6.11.1–Fr.6.11.8). Fr.6.11.2 (792.9 mg) was separated by VLC on silica gel eluted with petroleum ether–acetone (9:91–60:40) to provide seven portions (Fr.6.11.2.1–Fr.6.11.2.7). Of which, Fr.6.11.2.3 (46.2 mg) was further purified by semi-preparative HPLC (aqueous MeCN, 42%) to yield **8** (2.6 mg, t_R = 25.5 min, flow

rate: 3 mL/min). Fr.6.11.1 (660.0 mg) was separated by VLC on silica gel eluted with petroleum ether–acetone (10:90–55:45) to provide nine portions (Fr.6.11.1.1–Fr.6.11.1.9). Fr.6.11.1.5 (142.2 mg) was separated by preparative TLC (petroleum ether:acetone–3:1) to give Fr.6.11.1.5.1–Fr.6.11.1.5.4. The last part (63.6 mg) was submitted to semi-preparative HPLC on YMC-Pack-ODS-A (aqueous MeCN, 43%) to produce compound **5** (11.6 mg, t_R = 25.6 min, flow rate: 3 mL/min).

Compound Characterization Data

Compound **1**: Pale yellow gums. $[\alpha]^{20}_D$ +33.33 (*c* 0.3, MeOH); CD (MeOH) $\Delta\varepsilon_{201}$ −2.01, $\Delta\varepsilon_{208}$ −3.21; UV (MeOH) λ_{max} (logε) 200 (2.89) nm, 222 (2.73) nm; HRESIMS: *m/z* 273.1464 $[M + Na]^+$ (calcd for $C_{15}H_{22}O_3Na$, 273.1461). ^1H and ^{13}C NMR data, see Table 1.

Compound **2**: Pale yellow gums. $[\alpha]^{20}_D$ +18.25 (*c* 0.4, MeOH); CD (MeOH) $\Delta\varepsilon_{200}$ +2.99, $\Delta\varepsilon_{211}$ −5.12, $\Delta\varepsilon_{239}$ +0.53; UV(MeOH) λ_{max} (logε) 200 (3.02) nm, 246 (2.11) nm; HRESIMS: *m/z* 259.1671 $[M + Na]^+$ (calcd for $C_{15}H_{24}O_2Na$, 259.1669). ^1H and ^{13}C NMR data, see Table 1.

Compound **3**: Pale yellow gums. $[\alpha]^{20}_D$ +26.00 (*c* 0.5, MeOH); CD (MeOH) $\Delta\varepsilon_{202}$ −0.16, $\Delta\varepsilon_{224}$ +1.31; UV(MeOH) λ_{max} (logε) 223 (2.34) nm; HRESIMS: *m/z* 275.1622 $[M + Na]^+$ (calcd for $C_{15}H_{24}O_3Na$, 275.1618). ^1H and ^{13}C NMR data, see Table 2.

Compound **4**: White powder. $[\alpha]^{20}_D$ +20.99 (*c* 0.3, MeOH); CD (MeOH) $\Delta\varepsilon_{203}$ −0.10, $\Delta\varepsilon_{211}$ −1.43, $\Delta\varepsilon_{238}$ −7.89; UV (MeOH) λ_{max} (logε) 218 (2.85) nm;

HRESIMS *m/z* 287.1614 $[M + Na]^+$ (calcd for $C_{16}H_{24}O_3Na$, 287.1618). ^1H and ^{13}C NMR data, see Table 2.

Compound **5**: Colorless gums. $[\alpha]^{20}_D$ +74.00 (*c* 0.5, MeOH); CD (MeOH) $\Delta\varepsilon_{201}$ +2.69, $\Delta\varepsilon_{227}$ −0.05; UV (MeOH) λ_{max} (logε) 200 (2.82) nm; HRESIMS: *m/z* 261.1825 $[M + Na]^+$ (calcd for $C_{15}H_{26}O_2Na$, 261.1825). ^1H and ^{13}C NMR data, see Table 3.

Calculation of NMR Spectra

Using the density functional theory (DFT) and B3LYP/6-31G(d, p) levels in the Gaussian 09 software package, the obtained minimum energy conformation of the force field was optimized. DFT completes the calculation of Gauge-Independent Atomic Orbital for ^1H and ^{13}C NMR chemical shifts using the PCM solvent model in the Gaussian 09 software [13]. The NMR chemical shift was corrected by the isotope shift of TMS. The calculated ^{13}C NMR chemical shift was analyzed by regression with the experimental one. Boltzmann weighing of the predicted chemical shift of each isomer, the DP4+ parameters were calculated using the excel file provided by Ariel M. Sarotti [14].

Calculation of ECD Spectra

Using the Spartan'14 software package (Wavefunction Inc., Irvine, CA, USA) and the Gaussian 09 software package, the conformational search is performed using the molecular Merck force field (MMFF) under software CONFLEX 7.0 [15]. Generally, the next calculation is performed by selecting the energy difference less than 10 kcal/mol. ECD calculations were further conducted at

the B3LYP/6-31G(d,p) level with the PCM in MeOH. The SpecDis 1.62 was used to compared the calculated curves and experimental CD spectra.

Supporting Information

Supporting Information File 1

MS, UV, and NMR spectra of compounds **1**–**5**, NMR and ECD calculations, and bioactivity assay date.

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