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Nine 2-(2-Phenylethyl)chromone Derivatives from the Resinous Wood of *Aquilaria sinensis* and Their Inhibition of LPS-Induced NO Production in RAW 264.7 Cells

Dong Chen,[a] Zhengren Xu,[a] Xingyun Chai,[b] Kewu Zeng,[a] Yanxing Jia,[a] Dan Bi,[a] Zhizhong Ma,[a] and Pengfei Tu*[a]

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A phytochemical investigation of aquilariae lignum resinatum, the resinous wood of *Aquilaria sinensis* (Lour.) Gilg, led to the isolation of nine new 2-(2-phenylethyl)chromone derivatives, aquilarones A–I (**1**–**9**), together with two known analogues (**10** and **11**). Their structures were elucidated by extensive spectroscopic analyses, including UV, IR, NMR, and electronic circular dichroism (ECD) data, as well as by chemical methods. The absolute configuration of aquilarone A (**1**) was confirmed by single-crystal X-ray diffraction analysis of its acetonide derivative **1a**. All the compounds exhibited significant inhibition of the nitric oxide production in RAW 264.7 cells, with IC_{50} values in the range of 5.12–22.26 μM. In addition, an HPLC–UV comparison analysis of the resinous wood and resin-free wood is also included.

Introduction

Eaglewood, Chinese name "Chenxiang", termed aquilariae lignum resinatum (ALR) is derived from the diseased timber of the *Aquilaria* species of the family Thymelaeaceae. It is also called gaharu, jinko, or agarwood. Eaglewood has been used as incense, perfume, and traditional medicine in Asian countries. ALR, uniquely originating from the resinous wood of *Aquilaria sinensis* (Lour.) Gilg, is used as a sedative, analgesic, and digestive in traditional Chinese medicine.[1]

As well as being one of the most famous incense and Chinese medicinal materials, eaglewood is also used as the timber for expensive fine crafts and decorations all over the world. Currently, the price of high-quality eaglewood material reaches up to one hundred thousand U.S. dollars per kilogram.[2] However, lots of adulterated and inferior eaglewood, which is obtained by injecting perfume into the stems of *A. sinensis* or other resin-free woods, is traded in China and other Southeast Asian markets. Therefore, a simple and effective method, based on its characteristic constituents, is necessary and very important for the identification and quality evaluation of eaglewood.

Previous studies on eaglewood revealed the presence of its major components, which are sesquiterpenes and chromone derivatives,[3,4] and it exhibited biological activity as a laxative, sedative, antimicrobial, antitumor agent, antioxidant, anti-inflammatory activity, and anti-allergen.[5–12]

2-(2-Phenylethyl)chromones, one of the characteristic constituents of eaglewood, have been discovered only in *Aquilaria* plants so far. To the best of our knowledge, since the first isolation and characterization of 2-(2-phenylethyl)-chromones from *A. agallocha* in 1978,[13] only 57 congeners have been reported.[13–35] Also, little effort has been made to evaluate their biological activity, except for previous papers regarding cytotoxicity,[34] neuroprotective evaluation,[11,35] as well as a preliminary test of the anti-inflammatory activity of the EtOH extract of *A. sinensis* leaves.[8]

We systematically investigated the resinous wood of *A. sinensis* in order to find new and bioactive constituents and to provide chemical markers for its quality control. This led to the isolation and structural elucidation of nine new 2-(2-phenylethyl)chromones, namely, aquilarones A–I (**1**–**9**), along with two known ones (**10** and **11**) (Figure 1).[35] Their structures were elucidated by extensive UV, IR, NMR, and electronic circular dichroism (ECD) spectroscopic analysis as well as by chemical methods. Compounds **1**–**11** exhibited significant inhibition in an anti-inflammatory assay against nitric oxide (NO) production in RAW 264.7 cells. We describe herein the isolation, structural elucidation, and the anti-inflammation evaluation of these isolates.

[a] State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences,
Peking University Health Science Center,
No. 38 Xueyuan Road, Haidian District, Beijing 100191, China
Fax: +86-10-8280-2750
E-mail: pengfeitu@vip.163.com
[b] Modern Research Center for Traditional Chinese Medicine,
Beijing University of Chinese Medicine,
Beijing 100029, China

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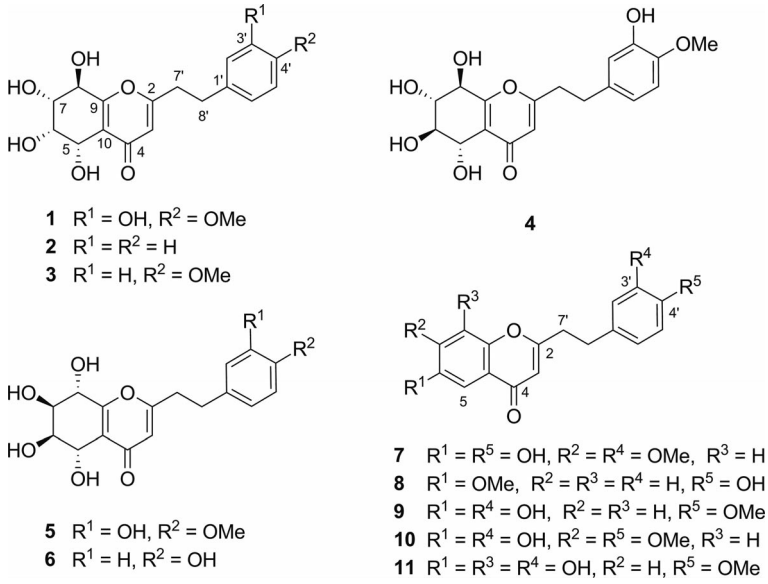


Figure 1. Structures of aquilarones A–I (**1**–**9**) and two congeners (**10**, **11**).

Results and Discussion

Aquilarone A (**1**) was obtained as a white amorphous powder. Its HRMS (ESI) gave an $[M + H]^+$ ion peak at m/z = 365.1230 (calcd. 365.1240), which corresponds to the molecular formula $C_{18}H_{20}O_8$. The IR absorptions suggested the presence of hydroxy groups (3359 cm^{-1}) and olefins (1656, 1589 cm^{-1}) in the molecule. The ^1H and ^{13}C NMR spectroscopic data (Tables 1 and 2) revealed the presence of a methoxy group, four consecutive methane groups [δ_H = 4.59/δ_C = 64.0 (C-5), δ_H = 4.36/δ_C = 70.0 (C-8), δ_H = 3.76/δ_C = 66.6 (C-6), and δ_H = 3.78/δ_C = 74.5(C-7) ppm], four hydroxy groups [δ_H = 6.07 (8-OH), 5.35 (7-OH), 5.05 (5-OH), and 4.90 (6-OH) ppm], two methylene groups [δ_H = 2.75 (m)/δ_C = 31.2 (C-7′), δ_H = 2.82 (m)/δ_C = 34.3 (C-8′) ppm], and a characteristic ABX coupling system [δ_H = 6.80 (d)/δ_C = 112.2 (C-5′), δ_H = 6.66 (d)/δ_C = 115.6 (C-2′), δ_H = 6.59/δ_C = 118.7 (C-6′) ppm]. Together, the ABX system, two methylene groups, and the methoxy group construct a partial (3′-hydroxy-4′-methoxyphenyl)ethyl structure. This is supported by HMBC spectroscopy (Figure 2), which correlates the methoxy protons to C-4′, and both 3′-OH (δ_H = 8.82 ppm) and 5′-H to C-3′. The establishment of consecutive methane groups was achieved from the ^1H–^1H COSY spectrum, which shows cross-peaks for 5-H through 8-H. Besides the presence of a carbonyl group (δ_C = 178.4 ppm, C-4), three sp^2 quaternary carbon atoms [of which two are oxygenated (δ_C = 146.3, 146.1 ppm)] were observed in the ^{13}C NMR spectrum. This combined with analysis of the remaining key HMBC correlations (Figure 2) from 2′-H and 6′-H to C-8′, from 7′-H and 8′-H to C-2, from 8-OH to C-9, from 5-H to C-9 and C-4, and from 3-H to C-10 and C-7′ established its planar structure as 5,6,7,8-tetrahydroxy-2-(3′-hydroxy-4′-methoxyphenylethyl)-5,6,7,8-tetrahydrochromone. It is a 2-(2-phenylethyl)chromone derivative similar to agarotetrol and isoagarotetrol.[13,16]

The relative stereochemistry of **1** was determined by analysis of the NOESY spectrum, in which a cross-peak of 5-H/7-H was observed (see Supporting Information), inferring their cofacial assignment. However, the absolute configuration was resolved only by a combination of CD spectroscopy (exciton chirality method) of its 5,6-di-*p*-methoxybenzoate derivative and X-ray diffraction analysis of the acetonide derivative of **1**, **1a**. The synthetic route for the derivatives of **1**, **1a**–**1d**, is depicted in Figure 3. Firstly, protection of the 1,2-diols with 2,2-dimethoxypropane in the presence of a catalytic amount of pyridinium *p*-toluenesulfonate (PPTS) afforded the acetonide derivative **1a**, which confirmed the *cis* arrangement of 5-OH and 6-OH. Then, acetylation of the free hydroxy groups and the phenol group with acetic anhydride produced the fully protected derivative **1b**, which upon treatment with trifluoroacetic acid (TFA) afforded the 1,2-diol derivative **1c**. Finally, esterification with *p*-methoxybenzoyl chloride yielded the desired 5,6-di-*p*-methoxybenzoate derivative **1d**.

In the CD spectrum of **1d**, a significant positive Cotton effect at 266 nm (benzoate groups) due to a $\pi \rightarrow \pi^*$ intramolecular charge-transfer transition was observed, which indicated the clockwise arrangement of the two *p*-methoxybenzoate groups as shown in **1d** (Figure 3), thus allowing the assignment of the absolute configuration of **1** as (5*S*,6*S*,7*S*,8*R*) (Figure 4).[37] Fortunately, a single-crystal of **1a** suitable for an X-ray diffraction experiment on a copper target [with its Flack coefficient of 0.0(2)] was obtained and provided unambiguous evidence for the same configuration assignments (Figure 4).[38] Consequently, the structure of **1** was elucidated as (5*S*,6*S*,7*S*,8*R*)-2-[2-(3′-hydroxy-4′-methoxyphenyl)ethyl]-5,6,7,8-tetrahydroxy-5,6,7,8-tetrahydrochromone.

Aquilarone B (**2**) was obtained as a pale yellow amorphous powder. Its molecular formula $C_{17}H_{18}O_6$ was determined from the HRMS (ESI) data. The ^1H NMR spectro-

Table 1. ^{1}H NMR spectroscopic data (in [D$_6$]dimethyl sulfoxide, DMSO) of compounds **1**–**6**,[a] δ in ppm (J in Hz).

	1	**2**	**3**	**4**	**5**	**6**
3-H	6.12 s	6.13 s	6.12 s	6.15 s	6.08 s	6.05 s
5-H	4.59 dd (5.5, 5.0)	4.58 dd (5.2, 5.0)	4.58 dd (5.5, 5.0)	4.47 dd (7.0, 3.0)	4.47 dd (5.0, 4.0)	4.47 dd, (4.5, 4.0)
5-OH	5.05 d (5.5)	5.04 d (5.2)	5.09 d (5.5)	5.14 d (3.0)	5.18 d (5.0)	5.17 d (4.5)
6-H	3.76 ddd (6.0, 5.0, 2.0)	3.76 ddd (5.6, 5.0, 2.0)	3.76 ddd (6.0, 5.0, 2.0)	3.49 ddd (7.0, 4.5, 4.5)	3.74 ddd (4.0, 4.0, 2.0)	3.74 ddd (4.0, 4.0, 2.0)
6-OH	4.90 d (6.0)	4.90 d (5.6)	5.09 d (6.0)	5.34 d (4.5)	4.96 d (4.0)	4.96 d (4.0)
7-H	3.78 ddd (5.0, 4.8, 2.0)	3.78 ddd (4.8, 4.0, 2.0)	3.78 ddd (5.0, 4.8, 2.0)	3.43 ddd (7.0, 4.5, 4.5)	3.84 ddd (7.5, 6.0, 2.0)	3.83 ddd (7.5, 6.0, 2.0)
7-OH	5.35 d (5.0)	5.35 d (4.8)	5.30 d (5.0)	5.21 d (4.5)	5.08 d (6.0)	5.10 d (6.0)
8-H	4.36 dd (6.5, 4.8)	4.36 dd (6.0, 4.0)	4.36 dd (6.5, 4.8)	4.32 dd (7.0, 6.5)	4.32 dd (7.5, 6.5)	4.31 dd (7.5, 6.5)
8-OH	6.07 d (6.5)	6.00 d (6.0)	5.90 d (6.5)	5.83 d (6.5)	5.80 d (6.5)	5.81 d (6.5)
2′-H	6.66 d (2.0)	–	7.15 d (8.4.)	6.66 d (2.0)	6.68 d (2.0)	7.01 d (8.0)
3′-H	–	–	6.83 d (8.4)	–	–	6.66 d (8.0)
4′-H	–	7.16–7.26 (m, 5 H)	–	–	–	–
5′-H	6.80 d (8.0)	–	6.83 d (8.4)	6.81 d (8.0)	6.81 d (8.5)	6.66 d (8.0)
6′-H	6.59 dd (8.0, 2.0)	–	7.15 d (8.4)	6.61 dd (8.0, 2.0)	6.61 dd (2.0, 8.5)	7.01 d (8.0)
7′-H	2.75 m	2.75 m	2.75 m	2.76 m	2.76 m	2.77 m
8′-H	2.82 m	2.82 m	2.82 m	2.83 m	2.83 m	2.83 m
OCH$_3$	3.71 s	–	3.70 s	3.72 s	3.72 s	–
OH	8.82 br. s	–	–	8.84 br. s	8.82 br. s	–

[a] Compounds **2** and **3** measured at 400 MHz, others at 500 MHz.

Table 2. ^{13}C NMR spectroscopic data (in [D$_6$]DMSO) for compounds **1**–**6**, δ in ppm.[a]

No.	**1**	**2**	**3**	**4**	**5**	**5**[b]	**6**
C-2	168.5	168.2	168.5	168.8	167.9	171.4	168.0
C-3	112.8	112.8	112.9	112.7	112.6	114.1	112.7
C-4	178.4	178.2	178.4	179.4	178.4	182.0	178.5
C-5	64.0	64.0	64.0	68.8	64.7	66.7	64.7
C-6	66.6	66.6	66.7	73.2	72.7	74.0	72.7
C-7	74.5	74.5	74.6	73.5	70.6	72.5	70.7
C-8	70.0	70.0	70.1	70.0	68.4	70.1	68.4
C-9	162.2	162.2	162.2	161.6	163.1	165.3	163.1
C-10	121.4	121.4	121.4	120.1	120.7	121.8	120.7
C-1′	132.5	139.9	131.8	132.5	132.6	134.1	130.1
C-2′	115.6	128.2	129.3	115.6	115.6	116.5	129.2
C-3′	146.3	128.3	113.8	146.3	146.3	147.7	115.1
C-4′	146.1	126.1	157.7	146.0	146.0	147.6	155.6
C-5′	112.2	128.3	113.8	112.2	112.2	113.0	115.1
C-6′	118.7	128.2	129.3	118.7	118.7	120.6	129.2
C-7′	31.2	31.7	31.0	31.1	31.1	33.1	31.2
C-8′	34.3	34.0	34.4	34.3	34.3	36.4	34.5
OCH$_3$	55.6	–	55.0	55.6	55.6	56.5	–

[a] Compounds **2** and **3** measured at 100 MHz, others at 125 MHz. [b] Measured in CD$_3$OD.

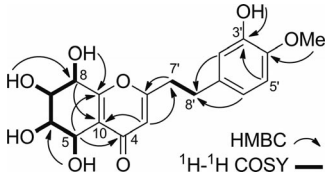


Figure 2. Key HMBC and ^{1}H–^{1}H COSY correlations of **1**.

scopic data of **2** (Tables 1 and 2) are very similar to those of **1**, suggesting their similarity in structure, except for the disappearance of the ABX coupling system and the methoxy signal, and the appearance of symmetrical phenyl multiplets at δ_H = 7.16–7.26 ppm (m, 5 H), consistent with differences in its ^{13}C NMR spectroscopic data. Identical data for the chromone moiety as well as the same sign of the optical rotation value showed that **2** has the same relative and absolute configurations of all four of its hydroxy groups as those of **1**. Thus, **2** was identified as (5*S*,6*S*,7*S*,8*R*)-2-(2-phenylethyl)-5,6,7,8-tetrahydroxy-5,6,7,8-tetrahydrochromone.

Analysis of the HRMS (ESI) data of aquilarone C (**3**) gave a molecular formula of $C_{18}H_{20}O_7$. Comparison of its ^{1}H and ^{13}C NMR spectroscopic data (Tables 1and 2) with those of **1** and **2** implied that they were highly related in structure, including their stereochemistry, with the exception of the phenylethyl part. The ^{1}H NMR spectroscopic data showed the presence of an AA′BB′ coupling system [δ_H = 7.15 (d, J = 8.4 Hz, 2 H, 2′,6′-H), 6.83 (d, J = 8.4 Hz, 2 H, 3′,5′-H) ppm] and a methoxy signal at δ_H = 3.70 ppm, which corresponds to a 4′-methoxyphenyl group. This assignment was confirmed by analysis of the HMBC correlations. Thus, compound **3** was determined as (5*S*,6*S*,7*S*,8*R*)-2-[2-(4′-methoxyphenyl)ethyl]-5,6,7,8-tetrahydroxy-5,6,7,8-tetrahydrochromone.

The molecular formula of aquilarone D (**4**) was determined as $C_{18}H_{20}O_8$ from its HRMS (ESI) data, m/z = 365.1230 [M + H]$^+$ (calcd. 365.1239). The ^{1}H and ^{13}C NMR spectroscopic data of **4** (Tables 1 and 2) are highly similar to those of **1**, suggesting that they have the same (3′-hydroxy-4′-methoxyphenyl)ethyl unit with only minor differences in the 5,6,7,8-tetrahydrochromone part. Detailed comparison of their ^{13}C NMR spectroscopic data revealed that the only difference is the stereochemistry of 6-OH. This conclusion was consistent with the different coupling constants of $J_{5,6}$ (7.0 Hz), $J_{5,5\text{-OH}}$ (3.0 Hz), $J_{6,7}$ (7.0 Hz), $J_{6,6\text{-OH}}$ (4.5 Hz) in **4**, and its comparatively large coupling constants require the presence of 6α-H, instead of 6β-H as in **1**. The deduction was supported further by comparing the ^{1}H and ^{13}C NMR spectroscopic data and optical rotation value (–58.0 for **4**, +18.0 for **1**) with that of isoagarotetrol (–58.6),[16,27] which confirms the 6α-H (6*R*) configuration for this molecule. Therefore, **4** was elucidated as (5*S*,6*R*,7*S*,8*R*)-2-[2-(3′-hydroxy-4′-methoxyphenyl)ethyl]-5,6,7,8-tetrahydroxy-5,6,7,8-tetrahydrochromone.

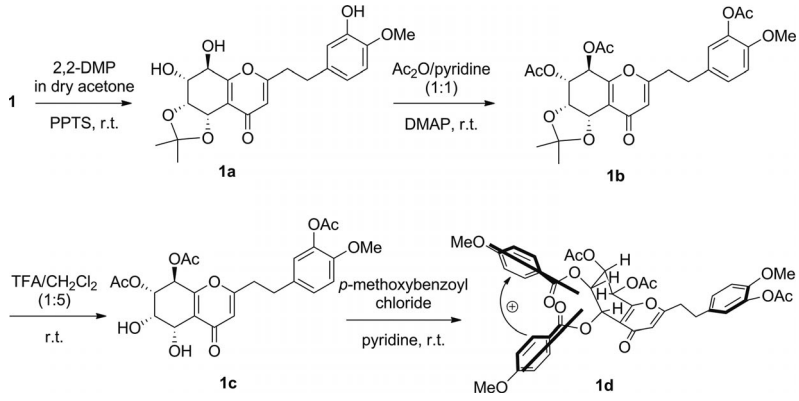
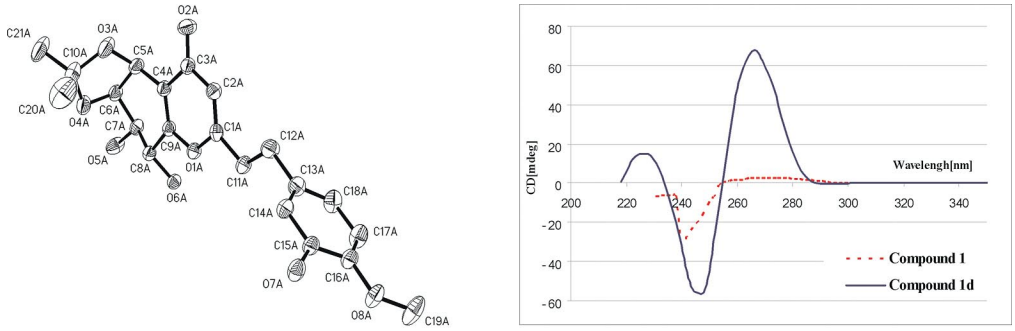


Figure 3. Synthetic route for derivatives **1a**–**1d** from **1**.



1a **1d**

Figure 4. X-ray structure of **1a** and CD spectrum of **1d**.

Aquilarone E (**5**) was obtained as white needle-type crystals. Its molecular formula $C_{18}H_{20}O_8$ was assigned from its HRMS (ESI) data. Its IR, ^{1}H NMR, and ^{13}C NMR spectroscopic data (Tables 1 and 2) exhibited close similarities with those of **1**, implying that they share the same 2-[2-(4′-hydroxyphenyl)ethyl]-5,6,7,8-tetrahydroxy-5,6,7,8-tetrahydrochromone planar structure, which was supported by heteronuclear single quantum coherence (HSQC) and HMBC spectra. Differences in the stereochemistry of 5-OH and 6-OH were revealed after a careful comparison of their ^{1}H and ^{13}C NMR spectroscopic data, with different data of δ_C = 72.7 (C-6), 70.6 (C-7), and 68.4 (C-8) ppm compared to those of **1**. A detailed analysis and comparison of the ^{1}H and ^{13}C NMR spectroscopic data, including coupling constants, with those of agarotetrol,[13,16] especially for the 5,6,7,8-tetrahydrochromone part, suggested their identical stereochemistry. Thus, the structure of **5** was elucidated as (5*S*,6*R*,7*R*,8*S*)-2-[2-(3′-hydroxy-4′-methoxyphenyl)ethyl]-5,6,7,8-tetrahydroxy-5,6,7,8-tetrahydrochromone.

Analysis of the HR mass (ESI) spectrum of aquilarone F (**6**) gave its molecular formula as $C_{17}H_{18}O_7$. The overall appearance of its ^{1}H and ^{13}C NMR spectroscopic data (Tables 1 and 2) suggests it is structurally related to **5**, except for differences in the 2-(3′-hydroxy-4′-methoxyphenyl)-ethyl part. Interpretation of its MS, ^{1}H NMR, and ^{13}C NMR data, in combination with HMBC correlations, established its structure as (5*S*,6*R*,7*R*,8*S*)-2-[2-(4′-hydroxyphenyl)ethyl]-5,6,7,8-tetrahydroxy-5,6,7,8-tetrahydrochromone (Figure 1).

Aquilarone G (**7**) has the molecular formula $C_{19}H_{18}O_6$ as determined from the HRMS (ESI) ion peak at *m*/*z* = 343.1176 $[M + H]^+$ (calcd. 343.1181). The presence of hydroxy (3415 cm^{-1}) and α,β-unsaturated ketone (1636 cm^{-1}) moieties was evident from the IR spectrum. The ^{1}H and ^{13}C NMR spectroscopic data (Table 3) showed signals for an ABX coupling system, two methylene groups, and a methoxy group, indicating the presence of a (3-methoxy-4-hydroxyphenyl)ethyl structure, which was supported by analysis of the HMBC correlations (Figure 5). Similarly, another set of signals of two aromatic singlets at δ_H = 7.23 (5-H) and 7.11 (8-H) ppm, one olefinic proton at δ_H = 6.03 (3-H) ppm, another methoxy group, together with an α,β-unsaturated carbonyl group (C-4) implied the presence of a chromone derivative unit with tetrasubstitution on the aromatic ring [δ_H = 7.23 (s)/δ_C = 107.2(C-5), δ_H = 7.11 (s)/δ_C = 100.3(C-8), δ_C = 144.9 (C-6), δ_C = 153.4 (C-7) ppm]. Assignments of the chromone part, the substitution positions of the methoxy and hydroxy groups, as well as the complete structure of **7** were determined by detailed analysis of the HMBC correlations (Figure 5). Finally, **7** was elucidated as 4′,6-dihydroxy-3′,7-dimethoxy-2-(2-phenyl)ethylchromone.

The molecular formula of aquilarone H (**8**) was established as $C_{18}H_{16}O_4$ from the HRMS (ESI) ion peak at *m*/*z*

Table 3. ^{1}H and ^{13}C NMR spectroscopic data (in [D$_6$]DMSO) of compounds **7**–**9**, δ in ppm (J in Hz).

No.	**7**[a]		**8**[a]		**9**[b]	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2	–	167.7	–	168.7	–	168.4
3	6.03 s	108.7	6.14 s	108.9	6.10 s	108.6
4	–	176.0	–	176.5	–	176.6
5	7.23 s	107.2	7.36 d (2.0)	104.7	7.24 d (2.0)	107.4
6	–	144.9	–	156.3	–	154.6
7	–	153.4	7.34 dd (8.8, 2.0)	122.9	7.18 dd (8.8, 2.0)	122.7
8	7.11 s	100.3	7.56 d (8.8)	119.7	7.47 d (8.8)	119.4
9	–	150.9	–	150.6	–	149.6
10	–	116.5	–	123.7	–	123.9
1′	–	130.8	–	130.0	–	132.5
2′	6.78 d (2.0)	112.5	7.01 d (8.5)	129.2	6.64 d (2.0)	115.6
3′	–	147.4	6.65 d (8.5)	115.1	–	146.3
4′	–	144.8	–	155.7	–	146.0
5′	6.64 d (8.0)	115.3	6.65 d (8.5)	115.1	6.78 d (8.2)	112.2
6′	6.60 dd (8.0, 2.0)	120.4	7.01 d (8.5)	129.1	6.58 dd (8.2, 2.0)	118.7
7′	2.87 m	31.8	2.88 m	31.3	2.88 m	31.4
8′	2.87 m	35.2	2.87 m	35.2	2.85 m	35.0
6-OCH$_3$	–	–	3.82 s	55.6	–	–
7-OCH$_3$	3.88 s	56.1	–	–	–	–
3′-OCH$_3$	3.67 s	55.4	–	–	–	–
4′-OCH$_3$	–	–	–	–	3.69 s	55.6
3′-OH	–	–	–	–	8.79 br. s	–
4′-OH	8.72 br. s	–	9.20 s	–	–	–
6-OH	9.71 br. s	–	–	–	10.29 br. s	–

[a] Measured at 500/125 MHz. [b] Measured at 400/100 MHz.

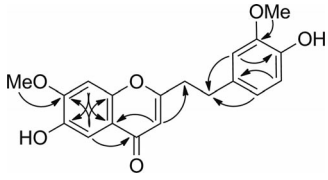


Figure 5. Selected HMBC correlations of **7**.

= 297.1121 [M + H]$^+$ (calcd. 297.1130). The ^{1}H NMR spectroscopic data (Table 3) showed the signals ascribed to an ABX system [δ_H = 7.56 (d, 1 H, J = 8.8 Hz), 7.36 (d, 1 H, J = 2.0 Hz), 7.34 (dd, 1 H, J = 8.8, 2.0 Hz) ppm] and an AA′BB′ system [δ_H = 7.01 (d, 2 H, J = 8.5 Hz), 6.65 (d, 2 H, J = 8.5 Hz) ppm], one methoxy group at δ_H = 3.82 ppm, and a broad signal due to one hydroxy group at δ_H = 9.20 ppm, suggesting a 2-(2-phenylethyl)chromone derivative with one hydroxy and one methoxy substituent. HMBC correlations from methoxy protons to C-6 determined its attachment at C-6 (δ = 156.3 ppm). Consequently, **8** was elucidated as 4′-hydroxy-6-methoxy-2-(2-phenylethyl)chromone.

Aquilarone I (**9**) has the molecular formula $C_{18}H_{16}O_5$ as established from its HRMS (ESI) data. Interpretation of its ^{1}H and ^{13}C NMR spectroscopic data (Table 3) allowed us to assign **9** also as a 2-(2-phenylethyl)chromone derivative, substituted by a methoxy and two hydroxy groups. Analysis of the HMBC correlations established the methoxy group (δ_H = 3.69 ppm) at C-4′, and two hydroxy groups (δ_H = 8.79 and 10.29 ppm) at C-6 (δ_C = 154.6 ppm) and C-3′ (δ_C = 146.3 ppm), respectively. Eventually, **9** was elucidated as 3′,6-dihydroxy-4′-methoxy-2-(2-phenylethyl)chromone.

HPLC Comparison Analysis

2-(2-Phenylethyl)chromone derivatives are considered special, not only for their limited distribution in *Aquilaria* (only 57 congeners were previously reported), but even more for the fact that they uniquely exist in the resinous wood and are believed to be produced either by decay or injury of the stems. None of the 2-(2-phenylethyl)chromone derivatives were discovered in the resin-free woods of *Aquilaria* species. An HPLC comparison of the resin-deposited and resin-free woods provided further proof for this hypothesis (Figure 6). Therefore, to some extent, the 2-(2-phenylethyl)chromone derivatives in *Aquilaria* plants are probably produced to fight against decay or disease. Validation of this hypothesis will be an interesting issue, although the details of the resin deposition process in *Aquilaria* species is still not clearly understood.

Biological Activities

Inspired by the report that the EtOH extract of *A. sinensis* leaves exhibited significant anti-inflammatory activity,[8] and the fact that NO plays an important role in the inflammatory process, inhibitors of NO release may be considered as therapeutic agents for inflammatory diseases.[39,40] The inorganic free radical NO is synthesized by a family of enzymes termed NO-synthases (NOS), and its cytotoxic properties are part of a host defense mechanisms against pathogenic microorganisms.[39] However, excess production of NO, due to the reaction with superoxide in biological systems, gives rise to various conditions such as inflammation, carcinogenesis, and atherosclerosis.[40] There-

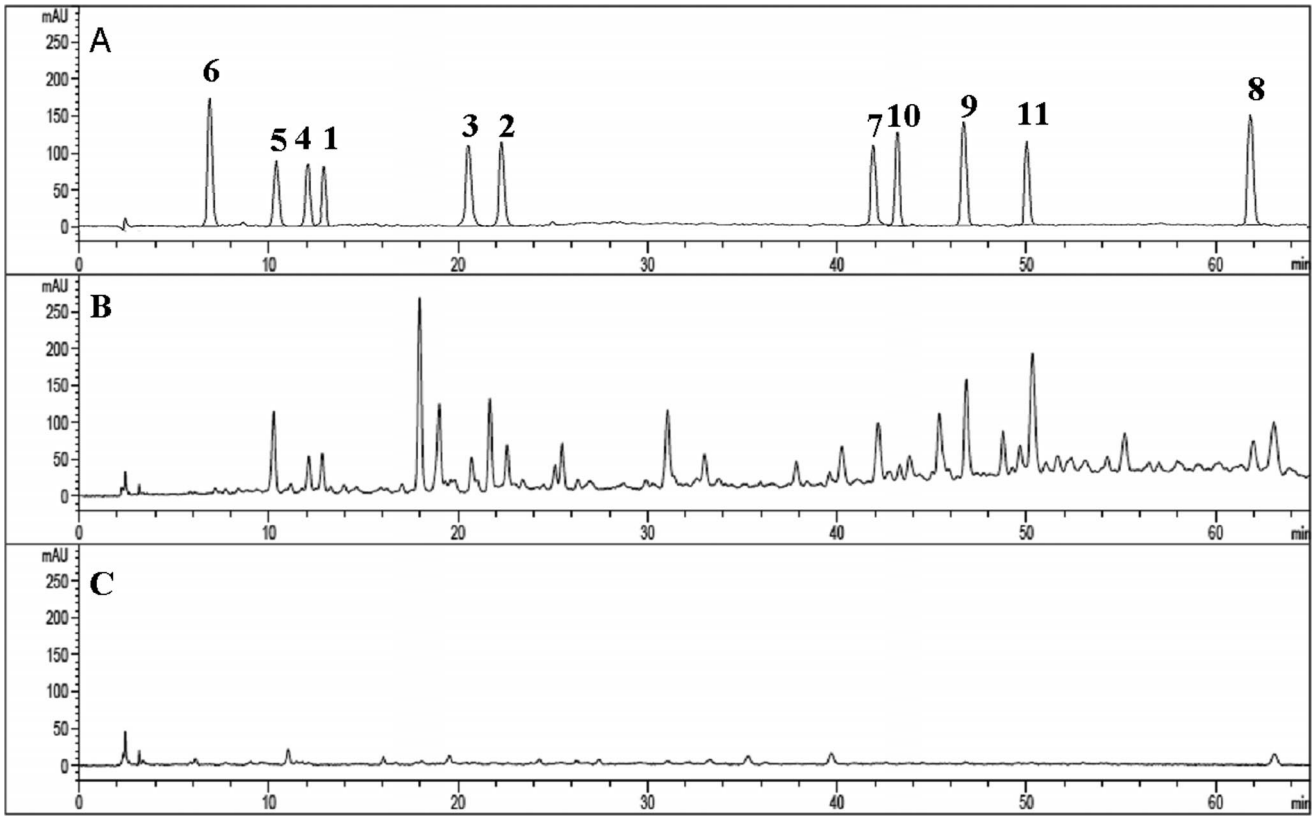


Figure 6. HPLC chromatograms of reference standards (A), ALR (B) and the stems of *A. sinensis* (C).

fore, inhibition of NO production is important to control infection and NO-dependent inflammatory diseases. Based on the above consideration, all compounds were tested for their inhibitory effects against lipopolysaccharide (LPS) induced NO production in RAW 264.7 macrophages and anti-COX-2 activity. The results showed that all assayed compounds exhibit potent inhibitory activity against NO production in RAW 264.7 cells, with IC_{50} values of 5.12–22.26 μM; by comparison the positive control ibuprofen, a clinical anti-inflammatory agent, has an IC_{50} value of 94.12 μM (Table 4). However, these compounds did not exhibit obvious inhibition against COX-2.

Table 4. Inhibition against LPS-induced NO production in RAW 264.7 for **1**–**11**.[a]

Compound	IC_{50} [μM]		IC_{50} [μM]
1	9.03	**7**	7.94
2	5.12	**8**	5.95
3	7.71	**9**	7.59
4	7.49	**10**	7.94
5	22.26	**11**	6.59
6	13.09	Ibuprofen	94.12

[a] Data are presented as mean values based on three experiments.

Strcuture–Activity Relationship

No obvious structure–activity relationship was found for these compounds. Both 2-(2-phenyl)ethyltetrahydrochromone derivatives (**1**–**6**) and 2-(2-phenyl)ethylchromones (**7**–**11**) exhibited considerable activity at the same level. And neither the different methoxy and hydroxy substitution patterns, nor their different substitution sites has a significant impact on the activity.

Conclusions

Nine 2-(2-phenylethyl)chromone derivatives, namely, aquilarones A–I (**1**–**9**), along with two known congeners (**10** and **11**) were isolated from the resin-deposited wood of *Aquilaria sinensis* (Lour.) Gilg. The structures of the new compounds were elucidated by means of NMR spectroscopy and MS techniques. The absolute configuration of aquilarone A (**1**) was achieved by chemical methods and was confirmed by single-crystal X-ray diffraction analysis of its acetonide derivative **1a**. An anti-inflammatory assay showed that these 2-(2-phenylethyl)chromone derivatives exhibit significant inhibitory effects against NO production in RAW 264.7 cells. Also included is a comparison analysis by HPLC–UV for the resin-free and resinous woods of *Aquilaria sinensis*, which – to some extent – provided important information in understanding the biosynthesis of the 2-(2-phenylethyl)chromone derivatives in eaglewood.

Experimental Section

General Experimental Procedures: Optical rotation measurements were carried out with a Perkin–Elmer 243B digital polarimeter. UV

spectra were measured with a Shimadzu spectrometer and IR spectra were measured with a Nicolet Avatar 360 FTIR spectrometer as KBr pellets. CD spectra were recorded with a JACSO J-815 spectrometer. HRMS (ESI) were measured with an Auto Spec Ultima-TOF mass spectrometer. NMR spectra were measured with a Varian 500 apparatus at 500 MHz (^{1}H) or 125 MHz (^{13}C) and a Bruker apparatus at 400 MHz (^{1}H) or 100 MHz (^{13}C) with tetramethylsilane (TMS) as an internal standard. Preparative HPLC was performed with a Waters 2487 instrument and an ODS column (Allsphere, 250 mm × 10 mm, i.d. 5 μm). Column chromatography was performed with silica gel (100–200 and 200–300 mesh, Qingdao Haiyang Chemical Co. Ltd., China), Sephadex LH-20 (40–70 μm; Amersham Pharmacia Biotech AB, Uppsala, Sweden), and Rp C_{18} gel (Merck, Darmstadt, Germany). The chemical ibuprofen (purity ≥ 98.0%) was purchased from Sigma.

Plant Material: Aquilariae lignum resinatum was collected in September 2009 in Hainan Province, China. The identification of the material was performed by Professor Peng-Fei Tu. A voucher specimen (KDC 20090901) is deposited at the Herbarium of the Modern Research Center for Traditional Chinese Medicine, Peking University Health Science Center.

Extraction and Isolation: The ALR material (3 kg) was exhaustively extracted with $CHCl_3$ (2 × 10 L) and 95% EtOH (2 × 10 L) by successive soxhlet extractions. The $CHCl_3$ extract (140 g) was subjected to a silica gel chromatography column (CC) (10 × 120 cm, 100–200 mesh) and eluted with a gradient of petroleum ether (PE)/EtOAc (50:1–1:1, v/v) to yield eight fractions (C1–C8). Fraction C5 (10 g) was applied to a silica gel CC (8 × 70 cm, 200–300 mesh) and eluted with PE/acetone (4:1) to give fractions C5-1–C5-8. Fraction C5-6 (1.2 g) was then separated by using semipreparative HPLC (MeOH/H_2O, 75:25, 2 mL/min) to yield **7** (4.4 mg, t_R 22.0 min). Fraction C6 (5 g) was subjected to a silica gel CC (5 × 50 cm, 200–300 mesh) and eluted with PE/acetone (4:1–1:1, 4 L) to give eight fractions (C6-1–C6-8). Fraction C6-4 (1.2 g) was purified by semipreparative HPLC (MeOH/H_2O, 60:40, 2 mL/min) to give **8** (225 mg, t_R = 34.2 min). Fraction C6-5 (0.8 g) was purified by using an octadecylsilane (ODS) CC (2 × 50 cm, 20 g) and eluted with MeOH/H_2O (60:40) to give **9** (16 mg). The 95% EtOH extract (150 g) was subjected to a silica gel CC (10 × 120 cm, 100–200 mesh) and eluted with a gradient of $CHCl_3$/MeOH (30:1–2:1, v/v) to yield six fractions (E1–E6). Fraction E4 (11 g) was subjected to an ODS CC (4 × 40 cm) and eluted with a gradient of MeOH/H_2O (1:4–1:1, 3.0 L) to give 58 fractions (E4-1–E4-58). A portion of combined fractions E4-17–E4-32 (2.4 g) was purified by semipreparative HPLC (ACN/H_2O, 12:88, 2 mL/min) to give **2** (17 mg, t_R 24 min) and **6** (5 mg, t_R 32 min). Fraction E5 (10 g) was subjected to a silica gel CC (8 × 70 cm, 100–200 mesh) and eluted with a gradient of $CHCl_3$/MeOH (10:1–6:1, 8.0 L) to give 62 fractions (E5-1–E5-62). Fractions E5-22–E5-48 were combined (3.2 g) and purified by using semipreparative HPLC (MeOH/H_2O, 25:75, 2 mL/min) to give **2** (60 mg, t_R = 32 min), **4** (70 mg, t_R = 23 min), and **1** (60 mg, t_R = 40 min). Fractions E5-49–E5-57 were combined (0.5 g) and purified by using semipreparative HPLC (MeOH/H_2O, 25:75, 2 mL/min) to yield **5** (36 mg, t_R = 60 min). The purities of these compounds are above 98% as determined by HPLC.

Aquilarone A (1): White amorphous powder. $[\alpha]_D^{20}$ = +18.0 (*c* = 1.0, MeOH). UV (MeOH): λ_{max} (log ε) = 253 (3.87), 207 (4.22) nm. IR (KBr): $\tilde{\nu}_{max}$ = 3359, 1656, 1589, 1515, 1446 cm^{-1}. ^{1}H and ^{13}C NMR: Tables 1 and 2. HRMS (ESI): calcd. for $C_{18}H_{21}O_8$ [M + H]$^+$ 365.1240; found 365.1230.

Aquilarone B (2): Pale yellow amorphous powder. $[\alpha]_D^{20}$ = +13.1 (*c* = 1.0, MeOH). UV (MeOH): λ_{max} (log ε) = 252 (4.07), 209 (4.24) nm. IR (KBr): $\tilde{\nu}$ = 3356, 1659, 1602, 1496, 1450 cm^{-1}. ^{1}H and ^{13}C NMR: Tables 1 and 2. HRMS (ESI): calcd. for $C_{17}H_{19}O_6$ [M + H]$^+$ 319.1174; found 319.1176.

Aquilarone C (3): Pale yellow amorphous powder. $[\alpha]_D^{20}$ = +12.0 (*c* = 1.0, MeOH). UV (MeOH): λ_{max} (log ε) = 252 (4.17), 209 (4.27) nm. IR (KBr): $\tilde{\nu}_{max}$ = 3405, 3309, 1662, 1578, 1514, 1451 cm^{-1}. ^{1}H and ^{13}C NMR: Tables 1 and 2. HRMS (ESI): calcd. for $C_{18}H_{21}O_7$ [M + H]$^+$ 349.1291; found 349.1281.

Aquilarone D (4): White needle-type crystals. M.p. 190–191 °C. $[\alpha]_D^{20}$ = –58.0 (*c* = 1.0, MeOH). UV (MeOH): λ_{max} (log ε) = 253 (4.04), 213 (4.19) nm. IR (KBr): $\tilde{\nu}_{max}$ = 3340, 1657, 1589, 1515 cm^{-1}. ^{1}H and ^{13}C NMR: Tables 1 and 2. HRMS (ESI): calcd. for $C_{18}H_{21}O_8$ [M + H]$^+$ 365.1239; found 365.1230.

Aquilarone E (5): White needle-type crystals. M.p. 212–213 °C. $[\alpha]_D^{20}$ = –16.0 (*c* = 1.0, MeOH). UV (MeOH): λ_{max} (log ε) = 281 (4.12), 252 (4.23), 212 (4.04) nm. IR (KBr): $\tilde{\nu}_{max}$ = 3357, 1656.9, 1590, 1514, 1447 cm^{-1}. ^{1}H and ^{13}C NMR: Tables 1 and 2. HRMS (ESI): calcd. for $C_{18}H_{21}O_8$ [M + H]$^+$ 365.1233; found 365.1230.

Aquilarone F (6): Pale yellow amorphous powder. $[\alpha]_D^{20}$ = –15.0 (*c* = 1.0, MeOH). UV (MeOH): λ_{max} (log ε) = 252 (4.07), 213 (4.23) nm. IR (KBr): $\tilde{\nu}_{max}$ = 3381, 1657, 1597, 1515, 1445 cm^{-1}. ^{1}H and ^{13}C NMR: Tables 1 and 2. HRMS (ESI): calcd. for $C_{17}H_{19}O_7$ [M + H]$^+$ 335.1125; found 335.1124.

Aquilarone G (7): Pale yellow amorphous powder. UV (MeOH): λ_{max} (log ε) = 210 (4.54), 231 (4.43), 281 (4.02), 322 (3.99) nm. IR (KBr): $\tilde{\nu}_{max}$ = 3415, 1636, 1567, 1518, 1459 cm^{-1}. ^{1}H and ^{13}C NMR: Table 3. HRMS (ESI): calcd. for $C_{19}H_{19}O_6$ [M + H]$^+$ 343.1181; found 343.1176.

Aquilarone H (8): White amorphous powder. UV (MeOH): λ_{max} (log ε) = 226 (4.43), 240 (4.41), 328 (3.74) nm. IR (KBr): $\tilde{\nu}_{max}$ = 3339, 1633, 1606, 1592, 1482 cm^{-1}. ^{1}H and ^{13}C NMR: Table 3. HRMS (ESI): calcd. for $C_{18}H_{17}O_4$ [M + H]$^+$ 297.1130; found 297.1121.

Aquilarone I (9): Yellow amorphous powder. UV (MeOH): λ_{max} (log ε) = 226 (4.46), 274 (3.92), 328 (3.78) nm. IR (KBr): $\tilde{\nu}_{max}$ = 3061, 1628, 1584, 1505, 1475 cm^{-1}. ^{1}H and ^{13}C NMR: Table 3. HRMS (ESI): calcd. for $C_{18}H_{17}O_5$ [M + H]$^+$ 313.1075; found 313.1070.

Acetonide Derivative of 1 (1a): To a suspension of **1** (9.4 mg, 0.026 mmol) in dry acetone (2.1 mL) and 2,2-dimethoxypropane (0.7 mL) was added a catalytic amount of PPTS (1.0 mg, 0.004 mmol), and the resultant reaction mixture was stirred at room temperature until the starting material was consumed (monitored by TLC). The solvent was removed under reduced pressure. Purification by using preparative TLC afforded **1a** as a colorless oil (10.4 mg), which was recrystallized from MeOH to give colorless prismatic crystals. $[\alpha]_D^{20}$ = +18.0 (*c* = 1.0, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ_H = 6.03 (s, 1 H, 3-H), 5.14 (d, *J* = 5.5 Hz, 1 H, 5-H), 4.55 (dd, *J* = 5.5, 2.5 Hz, 1 H, 6-H), 3.72 (dd, *J* = 8.5, 2.5 Hz, 1 H, 7-H), 4.60 (d, *J* = 8.5 Hz, 1 H, 8-H), 6.61 (d, *J* = 2.0 Hz, 1 H, 2′-H), 6.76 (d, *J* = 8.4 Hz, 1 H, 5′-H), 6.55 (dd, *J* = 8.4, 2.0 Hz, 1 H, 6′-H), 2.82 (m, 4 H, 7′,8′-H), 1.32 (s, 3 H, CH_3), 1.17 (s, 3 H, CH_3), 3.73 (s, 3 H, OCH_3) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ_C = 171.2 (C-2), 114.4 (C-3), 181.0 (C-4), 71.1 (C-5), 76.6 (C-6), 72.9 (C-7), 68.3 (C-8), 164.3 (C-9), 120.6 (C-10), 134.0 (C-1′), 116.4 (C-2′), 147.6 (C-3′), 147.6 (C-4′), 120.5 (C-5′), 112.8 (C-6′), 33.0 (C-7′), 36.3 (C-8′), 56.4 (4′-OCH_3), 110.8, 26.1, 27.7 [$C(CH_3)_2$] ppm. HRMS (ESI): calcd. for $C_{21}H_{25}O_8$ [M + H]$^+$ 405.1541; found 405.1544.

Compound 1b: A solution of **1a** (10.1 mg) and 4-(dimethylamino)-pyridine (0.9 mg) in pyridine (1.0 mL) and acetic anhydride

(1.0 mL) was stirred at room temperature for 5 h. The solvent was removed under reduced pressure. The residue was purified by preparative TLC (CH_2Cl_2/MeOH, 15:1) to give **1b** (9.6 mg) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H = 6.11 (s, 1 H, 3-H), 5.30 (d, J = 5.5 Hz, 1 H, 5-H), 4.67 (dd, J = 5.5, 2.0 Hz, 1 H, 6-H), 5.32 (dd, J = 6.0, 2.0 Hz, 1 H, 7-H), 6.18 (dd, J = 6.0, 2.0 Hz, 1 H, 8-H), 6.82 (d, J = 2.4 Hz, 1 H, 2′-H), 6.87 (d, J = 8.4 Hz, 1 H, 5′-H), 6.94 (dd, J = 8.4, 2.0 Hz, 1 H, 6′-H), 2.75–2.85 (m, 4 H, 7′-H, 8′-H), 1.34, 1.25 [s, 6 H, $C(CH_3)_2$], 2.18 (s, 6 H, $COCH_3$), 2.30 (s, 3 H, $COCH_3$), 3.80 (s, 3 H, OCH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ_C = 170.3 (C-2), 114.2 (C-3), 177.5 (C-4), 70.3 (C-5), 72.7 (C-6), 69.8 (C-7), 65.6 (C-8), 156.4 (C-9), 120.5 (C-10), 131.6 (C-1′), 122.6 (C-2′), 139.7 (C-3′),149.8 (C-4′), 126.3 (C-5′), 112.6 (C-6′), 31.5 (C-7′), 35.2 (C-8′), 56.4 (OCH_3), 110.8, 25.9, 27.5 [$C(CH_3)_2$], 169.7, 20.8 ($COCH_3$), 168.9, 20.6 ($COCH_3$), 168.7, 20.5 ($COCH_3$) ppm. HRMS (ESI): calcd. for $C_{27}H_{31}O_{11}$[M + H]$^+$ 531.1867; found 531.1860.

Compound 1c: To a solution of **1b** (6.8 mg) in dichloromethane (1.0 mL), TFA (0.2 mL) was added dropwise at 0 °C. The reaction mixture was then stirred at room temperature until the starting material was consumed (monitored by TLC). The solvent was removed under reduced pressure, and the residue was purified by preparative TLC (CH_2Cl_2/MeOH, 15:1) to give **1c** (5.8 mg) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H = 6.09 (s, 1 H, 3-H), 4.96 (dd, J = 3.6, 2.0 Hz, 1 H, 5-H), 4.35 (dd, J = 3.6, 2.0 Hz, 1 H, 6-H), 5.23 (dd, J = 8.8, 2.0 Hz, 1 H, 7-H), 6.22 (dd, J = 8.8, 2.0 Hz, 1 H, 8-H), 6.81 (d, J = 2.0 Hz, 1 H, 2′-H), 6.87 (d, J = 8.4 Hz, 1 H, 5′-H), 6.94 (dd, J = 8.4, 2.0 Hz, 1 H, 6′-H), 2.85 (m, 4 H, 7′-H, 8′-H), 2.12 (s, 6 H, $COCH_3$), 2.29 (s, 3 H, $COCH_3$), 3.79 (s, 3 H, OCH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ_C = 170.2 (C-2), 113.5 (C-3), 181.0 (C-4), 67.5 (C-5), 69.2 (C-6), 71.3 (C-7), 66.2 (C-8), 157.2 (C-9), 119.2 (C-10), 131.5 (C-1′), 122.6 (C-2′), 139.8 (C-3′),149.9 (C-4′), 126.2 (C-5′), 112.6 (C-6′), 31.5 (C-7′), 35.2 (C-8′), 55.9 (OCH_3), 169.7, 20.8 ($COCH_3$), 168.9, 20.6 ($COCH_3$), 168.7, 20.5 ($COCH_3$) ppm. HRMS (ESI): calcd. for $C_{24}H_{27}O_{11}$ [M + H]$^+$ 491.1558; found 491.1547.

Compound 1d: *p*-Methoxybenzoyl chloride (50.0 mg) was slowly added to a pyridine solution (1.0 mL) of **1c** at 0 °C, and the resulting reaction mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure, and the residue was purified by preparative TLC (CH_2Cl_2/MeOH, 15:1) to give **1d** (3.1 mg) as a colorless oil. $[\alpha]_D^{20}$ = +120.0 (c = 1.0, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ_H = 6.12 (s, 1 H, 3-H), 7.82 (d, J = 8.4 Hz, 4 H), 7.79 (d, J = 8.4 Hz, 4 H), 6.00 (d, J = 5.5 Hz, 1 H, 5-H), 5.56 (dd, J = 5.5, 2.4 Hz, 1 H, 6-H), 5.70 (dd, J = 4.8, 2.4 Hz, 1 H, 7-H), 6.10 (d, J = 4.8 Hz, 1 H, 8-H), 6.87 (d, J = 2.0 Hz, 1 H, 2′-H), 6.94 (d, J = 8.4 Hz, 1 H, 5′-H), 6.94 (dd, J = 8.4, 2.0 Hz, 1 H, 6′-H), 2.88 (m, 4 H, 7′-H, 8′-H), 2.01 (s, 3 H, $COCH_3$), 2.09 (s, 3 H, $COCH_3$), 2.15 (s, 3 H, $COCH_3$), 3.72 (s, 3 H, OCH_3), 3.76 (s, 3 H, OCH_3), 3.77 (s, 3 H, OCH_3) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ_C = 171.2 (C-2), 113.8 (C-3), 179.1 (C-4), 68.4 (C-5), 68.9 (C-6), 70.2 (C-7), 63.0 (C-8), 160.3 (C-9), 122.6 (C-10), 132.8 (C-1′), 122.6 (C-2′), 141.1 (C-3′), 151.2 (C-4′), 127.6 (C-5′), 114.7 (C-6′), 32.5 (C-7′), 35.8 (C-8′), 56.4 (OCH_3), 56.0 (OCH_3) 171.3, 20.8 ($COCH_3$), 170.9, 20.5 ($COCH_3$), 170.2, 20.5 ($COCH_3$), 166.5, 165.4, 133.4, 114.8, 123.1, 133.4, 114.8 (Ph), 166.3, 165.3, 132.8, 114.7, 123.1, 132.8, 114.7 (Ph) ppm. HRMS (ESI): calcd. for $C_{40}H_{39}O_{15}$ [M + H]$^+$ 759.2285; found 759.2283.

X-ray Crystallographic Analysis of 1a: Crystal data were obtained with a Rigaku MicroMax 002+ CCD single-crystal diffractometer with Cu-K_α radiation operating in the ω- and κ-scan mode. The structure was solved by direct methods (SHELXS-97) and refined by using full-matrix least-squares difference Fourier techniques. All non-hydrogen atoms were refined anisotropically, placed in idealized positions, and refined as riding atoms with relative isotropic parameters. Crystal data of **1a**: Monoclinic, $C_{21}H_{24}O_8$, space group $P2_1$, a = 8.120(3) Å, b = 19.967(4) Å, c = 12.413(3) Å, β = 91.14(1)°, V = 2012.3(1) Å^3, Z = 4, $D_{calcd.}$ = 1.335 g/cm^3, crystal size 0.12 × 0.14 × 0.36 mm, 7028 independent reflections. The final indices were R_1 = 0.0519, wR_2 = 0.1303, S = 1.014. CCDC-867726 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

HPLC Procedure: The analyses were performed with an Agilent Eclipse XDB C_{18} Column (250 × 4.6 mm, i.d. 5 μm) at a column temperature of 30 °C. The mobile phase consisted of 0.1% phosphoric acid in water (A) and acetonitrile (B) by using a gradient program of 10–20% B in 0–20 min, 20–25% B in 20–35 min, 25–35% B in 35–50 min, 35% B in 50–65 min. The flow rate was 1.0 mL/min, and the diode array detector (DAD) was set at 240 nm for acquiring chromatograms.

Preparation of Sample Solutions: The dried material was pulverized to 80 mesh. The powder (ca. 1.0 g) was suspended in methanol (10 mL) in a conical flask, accurately weighed, then sonicated for 1 h; the resultant suspension was cooled to room temperature. A certain amount of methanol was added to the original weight extract. The final solution was filtered through a 0.22 μm filter, and the primary filtrate was discarded. The subsequent filtrate was stored as the sample solution until analysis.

Preparation of Standard Solutions and the Identification of Components: These reference compounds were accurately weighed and dissolved in methanol and then diluted to appropriate concentration. The mixed stock solution of standards, containing compound **1** (0.226 mg mL^{-1}), **2** (0.265 mg mL^{-1}), **3** (0.262 mg mL^{-1}), **4** (0.230 mg mL^{-1}), **5** (0.236 mg mL^{-1}), **6** (0.342 mg mL^{-1}), **7** (0.264 mg mL^{-1}), **8** (0.285 mg mL^{-1}), **9** (0.274 mg mL^{-1}), **10** (0.264 mg mL^{-1}), and **11** (0.265 mg mL^{-1}), was prepared with methanol. Peaks were identified by comparison of retention time and DAD spectra with those of the corresponding standards. All stock and working standard solutions were stored at 4 °C until used for analysis.

Assay for Inhibition against LPS-Induced NO Production: RAW 264.7 cells grown on a 100 mm culture dish were harvested and seeded in 48-well plates at 6 × 10^4 cells/well for NO assay. The cells were pretreated with various concentrations of samples for 30 min and then incubated for 24 h with or without 1 μg/mL of LPS. The nitrite concentration in the culture supernatant was measured by the Griess reaction. Cell viability was measured by an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay (Sigma–Aldrich).[36]

Supporting Information (see footnote on the first page of this article): 1D, 2D NMR and HR mass (ESI) spectra for **1**–**9** and **1a**–**1d**.

Acknowledgments

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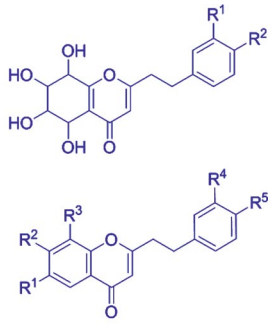
Natural Products

Aquilariae Lignum Resinatum



Nine new 2-(2-phenylethyl)chromone derivatives were isolated from the resin-deposited wood of *Aquilaria sinensis*. Their structures were elucidated by means of NMR spectroscopy, mass spectrometry, X-ray diffraction, and chemical methods. These compounds exhibit significant inhibition of LPS-induced NO production in RAW 264.7 cells.

Keywords: Biological activity / Chromones / Configuration determination / Anti-inflammatory agents / Eaglewood / Natural products

