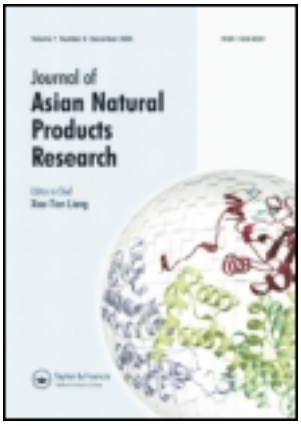

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Sesquiterpenoids and other constituents from the flower buds of *Tussilago farfara*

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One new norsesquiterpenoid, namely tussfarfarin A (**1**), and four new artifacts resulting from extraction procedure, namely tussfarfarin B (**2**), 6-(1-ethoxyethyl)-2,2-dimethylchroman-4-ol (**3**), 5-ethoxymethyl-1H-pyrrole-2-carbaldehyde (**4**), and 3β-hydroxy-7α-ethoxy-24β-ethylcholest-5-ene (**5**), along with 18 known compounds, were isolated from the flower buds of *Tussilago farfara*. Their structures were elucidated by extensive spectroscopic analysis.

Keywords: *Tussilago farfara*; Asteraceae; sesquiterpenoids; oplopananes

1. Introduction

Sesquiterpenoids are widely found in the Asteraceae family and exhibit interesting chemical diversities and important biological properties, which make them attractive targets for phytochemical, pharmacological, and synthetic purposes [1]. As a result of a thorough study on the important active compositions from the Asteraceae, some structurally novel sesquiterpenoids have been reported by our group [2,3].

Tussilago farfara L., belonging to the Asteraceae, is a perennial herb blooming in winter, which is widely spread in China, North Africa, and Europe. The flower buds of *T. farfara*, recorded in Chinese Pharmacopoeia (named Kuan Dong Hua), are used as an important traditional Chinese medicine (TCM) for the treatment of cough, bronchitis, and asthmatic disorders [4]. Previous phytochemical and pharmacological studies on *T. farfara* have resulted in the isolation of a series of active sesquiterpenoids, such as tussilagone and bisabolene epoxide derivatives [5]. The major constituent, tussilagone, showed potent inhibition against microsomal diacylglycerol acyltransferase 1 derived from rat liver and human hepatocellular carcinoma HepG2 cells, and significantly inhibited triglyceride synthesis by suppressing the incorporation of label [^{14}C] acetate or [^{14}C] glycerol into triglycerides in HepG2 cells, as well as by displaying potent inhibitory activity on platelet-activating factors and stimulating activity on the cardiovascular and respiratory systems [4]. In continuation of our study on the chemical constituents of TCM, we have recently examined the flower buds of *T. farfara* and obtained 23 compounds, including five new ones, tussfarfarins A (**1**) and B (**2**), 6-(1-ethoxyethyl)-2,2-dimethylchroman-4-ol (**3**), 5-ethoxymethyl-1H-pyrrole-2-carbaldehyde (**4**), and 3β-hydroxy-7α-ethoxy-24β-ethylcholest-5-ene (**5**).

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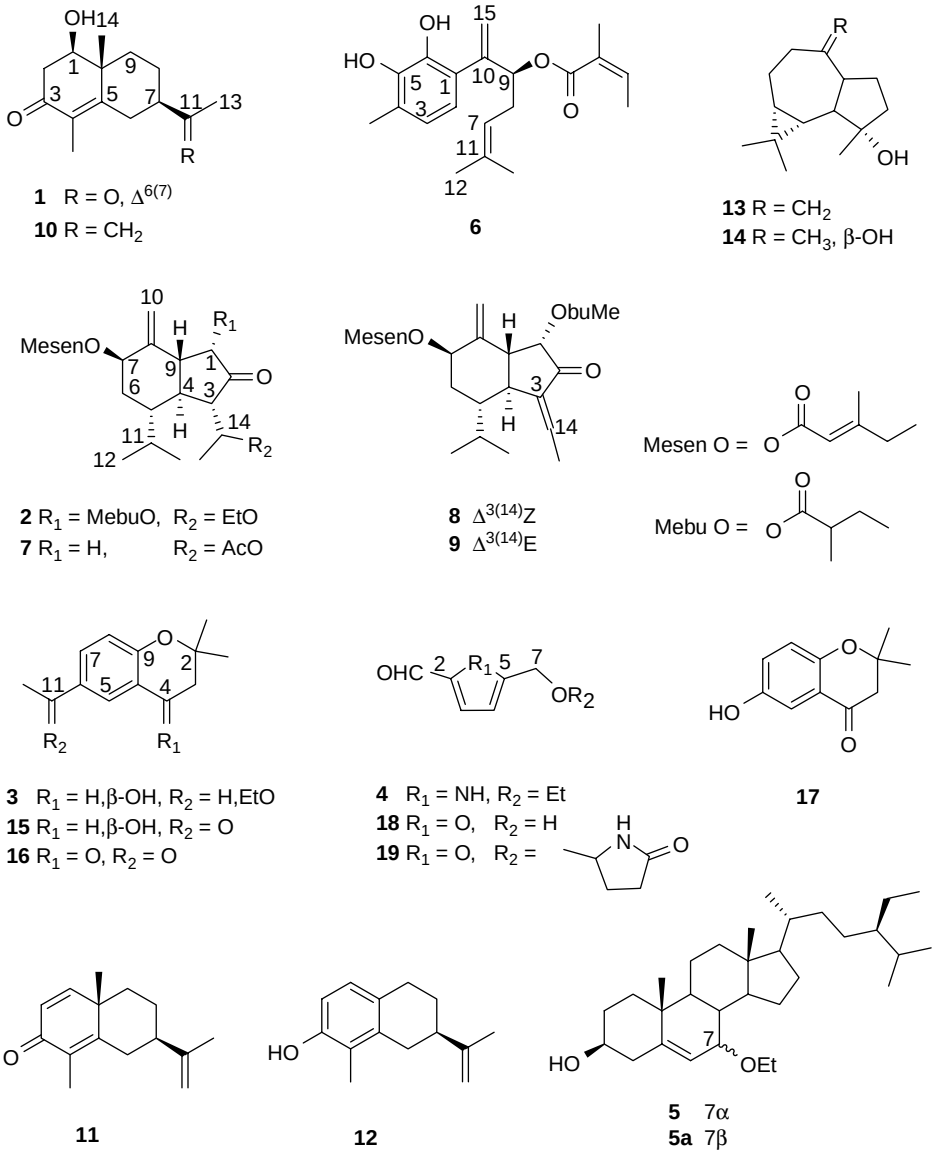

Figure 1. Structures of compounds **1**–**19**.

Herein, we report the isolation and the structural elucidation of these compounds (Figure 1).

2. Results and discussion

Tussfarfarin A (**1**) was isolated as a colorless gum, and the molecular formula was established as $C_{14}H_{18}O_3$ from HR-ESI-MS ($[M + H]^+$ at *m/z* 235.1322) and ^{13}C NMR spectra. Its IR spectrum showed the presence of OH (3436 cm^{-1}) and α,β-unsaturated ketone (1771, 1655, and 1576 cm^{-1}). Analysis of its ^1H NMR spectrum (Table 1) indicated three methyl singlets appearing at δ1.05 (3H, s, CH_3-14), 1.95 (3H, s, CH_3-15), and 2.43 (3H, s, CH_3-13), and one oxygenated methine at δ3.88 (1H, dd, $J = 12.4$, 5.6 Hz, H-1). The ^{13}C NMR and DEPT spectra (Table 1) exhibited 14 carbons, assigned to three methyls,

Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectroscopic data and key HMBC correlations for compounds **1** and **2** ($CDCl_3$).

	1			**2**		
Position	δ_H (J in Hz)	δ_C	HMBC for **1** (H to C)	δ_H (J in Hz)	δ_C	HMBC for **2** (H to C)
1	3.88 dd (12.4, 5.6)	73.0	2, 3, 9, 10, 14	5.38 d (4.0)	72.3	1″, 2, 3, 4
2	2.69 m	42.5	1, 3, 4, 10		209.0	
3		197.6		2.16 m	58.5	2, 4, 14, 15
4		133.8		2.52 m	42.5	3, 5, 8
5		152.3		1.84 m	44.0	
6	7.45 d (2.4)	132.0	4, 5, 7, 8,10, 11	1.51 m, 2.01 m	30.2	4, 7, 8
7		143.9		5.55 t (2.8)	73.6	1′, 5, 6, 8, 9, 10
8	2.78 m, 2.33 m	20.5	6, 7, 9, 10, 11		140.9	
9	2.28 m, 1.37 ddd (12.4, 12.4, 5.2)	31.7	1, 5, 7, 8, 10, 14	2.55 dquint (12.4, 4.0, 2.0, 1.6)	46.4	4, 8, 10
10		38.7		4.80 s, 5.18 s	112.9	7, 8, 9
11		198.9		2.14 m	27.5	4,6,12, 13
12				0.96 d (6.8)	21.4	5, 11, 13
13	2.43 s	25.9	6, 7, 11	0.85 d (6.8)	15.4	5, 11, 12
14	1.05 s	15.2	1, 5, 9, 10	3.80 dq (6.4, 1.6)	74.9	1‴, 2, 3, 4, 15
15	1.95 s	10.8	3, 4, 5	1.34 d (6.4)	17.3	3, 14
1′					165.7	
2′				5.63 d (1.6)	114.5	1′, 3′, 4′, 6′
3′					161.6	
4′				2.16 m	33.6	2′, 3′, 5′, 6′
5′				1.07 t (6.4)	11.7	3′, 4′
6′				2.15 s	18.7	2′, 3′, 4′
1″					175.1	
2″				2.36 ddd (14.0, 7.2, 6.8)	40.8	1″, 3″, 4″, 5″
3″				1.45 m, 1.73 m	26.2	1″, 2″, 4″, 5″
4″				0.88 t (7.2)	11.3	2″, 3″
5″				1.14 d (7.2)	16.3	1″, 2″, 3″
1‴				3.33 m, 3.58 m	63.9	2‴, 14
2‴				1.16 t (7.6)	15.4	1‴

three methylenes, two methines, two carbonyl carbons, and four quaternary carbons, suggesting that **1** should be a bicyclic norsesquiterpenoid. In the HMBC spectrum, the long-range correlations (Table 1) between CH_3-14 with C-1, C-5, C-9, and C-10 and between CH_2-8 with C-6, C-7, C-9, C-10, and C-11 verified that **1** was an eudesmane framework possessing a hydroxy group at C-1. In the ^{1}H NMR spectrum, J values of 12.4 and 5.6 Hz between H-1 and H-2 indicated an α-orientation of H-1. In the NOESY experiment, the interaction between CH_3-14 and H-8_{ax} (δ2.33), together with no correlation between CH_3-14 and H-1 (δ3.88), suggested a β-orientation of CH_3-14 with axial bond. Thus, the structure of **1** was confirmed and named tussfarfarin A.

Tussfarfarin B (**2**) was obtained as yellow oil. The molecular formula was deduced as $C_{28}H_{44}O_6$ by the pseudo-molecular ion peak in HR-ESI-MS at *m/z* 499.3038 $[M + Na]^+$, corresponding to seven degrees of unsaturation. Its IR spectrum showed typical absorptions for C=O (1755, 1740, and 1713 cm^{-1}) and C=C (1649 cm^{-1}) groups. The NMR spectral data of **2** indicated the presence of three side chains, 2-methyl butyryloxyl (MebuO), 3-ethyl-*cis*-crotonoyloxyl (MesenO), and EtO group, respectively, which were linked to C-1, C-7, and C-14 by HMBC correlations (Table 1) between H-1 and C-1″ (δ175.1), H-7 and C-1′ (δ165.7), and H-14 and C-1‴ (δ63.9). Apart from three side chains, the remaining ^{13}C NMR signals consisted of three methyls, two methylenes (including one olefinic carbon appearing at δ112.9), eight methines (including three oxygenated carbons at δ72.3, 73.6, and 74.9), two unsaturated quaternary carbons at δ209.0 and 140.9. As five degrees of unsaturation were assigned to two double bonds and three carbonyls, the remaining two indicated that compound **2** should be a bicyclic sesquiterpene bearing one terminal C=CH_2 and one C=O. And then, the planar structure of tussfarfarin B (**2**) was further determined as an oplopanane sesquiterpene by the analysis of the ^{1}H–^{1}H COSY (Figure 2(a)), HSQC, and HMBC (Table 1) correlations. The relative configuration was elucidated by the analysis of its NOESY experiment (Figure 2(b)). NOESY correlations of H-9 with H-1 and H-5 and of H-4 with CH_3-13 and CH_3-15 showed that H-1, H-3, H-5, and H-9 were all β-oriented, assuming that H-4 was α-oriented. The absence of NOESY correlations of H-7 with H-5 and H-9 showed an α-orientation of H-7, and this was further determined by comparing the chemical shift of C-7 in **2** (δ73.6) with those of compounds **7**–**9** (δ73.0, 73.2, and 72.3) [6]. The CD spectrum of **2** exhibited two negative Cotton effects ($\Delta\varepsilon_{211\,nm} = -3.55$ and $\Delta\varepsilon_{320\,nm} = -5.84$), and displayed almost identical Cotton effects as tussilagone (**7**) [6,7]. Finally, the absolute configurations of tussfarfarin B (**2**) were assigned as 1*S*, 3*S*, 4*S*, 5*S*, 7*R*, 9*R*, and 14*R*.

Compound **3** had a molecular formula $C_{15}H_{22}O_3$ determined by HR-ESI-MS at *m/z* 233.1532 $[M + H–H_2O]^+$. The IR spectrum showed the presence of hydroxy

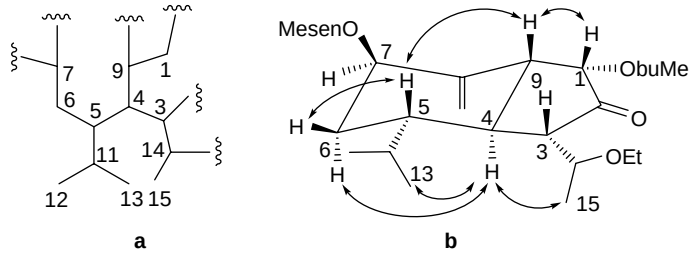

Figure 2. Key ^{1}H–^{1}H COSY (a) and NOESY (b) correlations for compound **2**.

Table 2. ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz), and HMBC spectroscopic data for compound **3** and ^{1}H NMR (400 MHz) spectroscopic data of **6**.

	3			**6**	
Position	δ_H[a] (*J* in Hz)	δ_C[a]	HMBC for **3** (H to C)	δ_H[a] (*J* in Hz)	δ_C[b] (*J* in Hz)
2		75.2		6.48 d (8.0)	6.87 d (8.0)
3	2.17 dd (13.2, 6.0), 1.87 dd (13.2, 10.0)	42.7	2, 4, 10, 13, 14	6.63 d (8.0)	6.94 d (8.0)
4	4.85 t (7.2)	63.7	2, 3, 9, 10		
5	7.38 brs	125.5	4, 7, 9, 11		
6		135.9			
7	7.13 dd (8.4, 2.8)	127.0	5, 9, 11	5.10 m	5.35 t (6.8)
8	6.76 d (8.4)	117.1	4, 6, 9, 10	2.41 dd (7.2, 7.6)	2.69 m, 2.59 m
9		152.5		5.10 m	6.38 dd (4.4, 2.4)
10		124.0			
11	4.36 q (6.4)	77.3	1′, 5, 6, 7, 12		
12	1.41 d (6.4)	24.0	6, 11	1.67 s	1.63 s
13	1.32 s	26.0	2, 3, 14	1.58 s	1.51 s
14	1.46 s	28.9	2, 3, 13	2.26 s	2.50 s
15				5.15 d (0.8), 5.31 s	5.46 s, 5.61 s
1′	3.34 q (7.2)	63.6	2′, 11		
2′	1.17 t (7.2)	15.4	1′		
3′				6.24 dq (7.2, 1.6)	6.00 dq (7.2, 1.2)
4′				2.06 dd (7.2, 1.6)	2.03 dd (7.2, 1.2)
5′				1.96 t (1.6)	1.96 d (1.2)
5-OH				5.83 s	
6-OH				8.98 s	

[a] Acquired in $CDCl_3$.
[b] Acquired in pyridine-d_5.

(3422 cm^{-1}) and phenyl (1616 and 1584 cm^{-1}) groups. The ^{1}H NMR spectrum of **3** (Table 2) displayed an EtO group at δ3.34 (2H, q, $J = 7.2$ Hz) and 1.17 (3H, t, $J = 7.2$ Hz), and a 1,3,4-trisubstituted benzene ring appearing as ABX system at δ 7.13 (1H, dd, $J = 8.4$, 2.8 Hz), 6.76 (1H, d, $J = 8.4$ Hz), and 7.38 (1H, brs). The ^{13}C NMR and DEPT spectra gave 15 carbons: four methyls, two methylenes, five methines, and four quaternary carbons. Its NMR spectral data (Table 2) were similar to those of compound **15** [8], except for the presence of the EtO group at C-11. This was verified by HMBC correlations of H-11 with C-1′, C-5, C-6, C-7, and C-12, and of H-1′ with C-2′ and C-11. Compound **3** must be a derivative from **15** in the extraction procedure, so it was assigned as 6-(1-ethoxyethyl)-2,2-dimethylchroman-4-ol.

Compound **4** was obtained as yellow oil. The molecular formula was determined as $C_8H_{11}NO_2$ by its HR-ESI-MS ($[M + H]^+$, at *m/z* 154.0861) and ^{13}C NMR spectra. The IR spectrum showed absorptions at 3389 and 1650 cm^{-1} for a pyrrole NH and a conjugated aldehyde group. In the low-field region of ^{1}H NMR spectrum, two triplet protons at δ6.89 (1H, t, $J = 3.0$ Hz) and 6.19 (1H, t, $J = 3.0$ Hz) suggested the presence of a 2,5-disubstituted pyrrole ring. The ^{13}C NMR and DEPT spectra indicated the presence of an EtO group (δ66.4 and 15.1), a conjugated aldehyde carbon (δ179.7), and four aromatic carbons (δ132.6, 121.3, 109.2, and 137.9). The NMR spectral data of **4** resembled those of 5-methoxymethyl-1H-pyrrole-2-carbaldehyde [9], except for the fact that an EtO group instead of a MeO group was attached at C-7. Therefore,

compound **4** was determined as 5-ethoxymethyl-1H-pyrrole-2-carbaldehyde.

Compound **5** was obtained as colorless needles. Its EI-MS gave a molecular ion peak at *m/z* 458, indicating the molecular formula $C_{31}H_{54}O_2$. Unfortunately, the molecular ion peak could not be observed from its HR-ESI-MS experiment. The IR absorptions indicated the presence of OH (3365 cm^{-1}) and C=C (1662 cm^{-1}) groups. The structure of **5** was deduced as a stigmastane skeleton by comparing its NMR spectral data with those of (24*S*)-stigmast-5-en-7β-ethoxy-3β-ol (**5a**) [10]. The ^{13}C NMR and DEPT spectra of **5** displayed 31 carbons, including seven methyls, 11 methylenes, 10 methines (including two oxygenated carbons at δ72.4 and 71.4, and one olefinic carbon at δ121.9), and three quaternary carbons (including one olefinic carbon at δ145.3), indicating that compound **5** possessed one OH group at C-3, an EtO group at C-7, and one C=C group between C-5 and C-6. The connections of the key structure units were determined by $^1H-^1H$ COSY and HMBC correlations as mentioned in Table 3. Chemical shifts of C-7 in compounds **5** (δ72.4) and **5a** (δ81.0) suggested an α-orientation of the EtO group. When comparing with its isomer **5a**, the resonance of C-5 was shifted upfield from δ145.3 to δ143.3, and resonances of C-7, C-9, and C-14 were shifted downfield from δ72.4, 42.1 and 49.0 to δ81.0, 48.5, and 55.5, respectively. Based on the above evidence, **5** was elucidated as 3β-hydroxy-7α-ethoxy-24β-ethylcholest-5-ene.

Compound **6** was isolated as colorless oil, and the structure was determined as altaicalarin B by the analysis of the HR-ESI-MS, IR, UV, and NMR (Table 2) spectra [11]. Proton signals for H-9 and H-7 were overlapped at δ 5.10, so the ^1H NMR and $^1H-^1H$ COSY experiments of **6** were recorded in both $CDCl_3$ and C_5D_5N to determine the position of OAng group. The C_5D_5N solvent resulted in the proton signals for H-9 and H-7 shifted from δ5.10 in $CDCl_3$ to δ6.38 and 5.35, respectively. And then, one spin coupling unit, —CH—CH_2—CH=C, was deduced from the ^1H NMR and $^1H-^1H$ COSY spectra. The methine proton signal of H-9, which appeared at δ6.38, indicated that the OAng group was attached at C-9. The CD exciton chirality method was applied to determine the absolute configuration [12]. The CD spectrum of **6** showed positive chirality resulting from exciton coupling of two chromophores for the OAng moiety and the tetra-substituted benzene ring.

Table 3. ^1H NMR and HMBC spectroscopic data for **5** and **5a** ($CDCl_3$).

	5	**5a**	
Position	δ_H[a] (*J* in Hz)	δ_H[b] (*J* in Hz)	HMBC for **5** (H to C)
3	3.61 m	3.54 m	
6	5.68 d (4.0)	5.42 br s	4, 7, 8, 10
7	3.41 m	3.43 br d (4.4)	5, 6, 8, 9
18	0.67 s	0.69 s	12, 13, 14, 17
19	0.99 s	1.04 s	1, 5, 9, 10
21	0.94 d (6.4)	0.95 d (7.8)	17, 20, 22
26	0.82 d (7.2)	0.82 d (6.6)	24, 25, 27
27	0.85 d (7.2)	0.83 d (6.9)	24, 25, 26
29	0.86 t (6.4)	0.86 t (7.2)	24, 28
1′	3.72 m, 3.37 m	3.64 dq (6.9, 8.1), 3.29 dq (6.9, 8.1)	2′, 7
2′	1.18 t (6.8)	1.17 t (6.9)	1′

[a] Run on 400 MHz.
[b] Run on 300 MHz.

The positive chirality suggested that the transition dipole moments of two chromophores were oriented in a clockwise manner, and the absolute configuration of **6** was elucidated as 9*S*.

A series of HPLC experiments determined that compounds **2**–**5** were the artifacts from the extraction process with EtOH. The structures of 17 known compounds were determined by comparing their spectroscopic data with the literature data. They were tussilagone (**7**) [4,6], 7β-[3′-ethyl-*cis*-crotonoyloxy]-1α-[2′-methylbutyryloxy]-3,14-dehydro-*Z*-notonipetranone (**8**) [6], 7β-[3′-ethyl-*cis*-crotonoyloxy]-1α-[2′-methylbutyryloxy]-3,14-dehydro-E-notonipetranone (**9**) [6], ligucyperonol (**10**) [13], (−)-1,2-dehydro-α-cyperone (**11**) [14], ligudentatol (**12**) [13], spathulenol (**13**) [15], (1βH, 5αH)-aromadendrane-4α,10β-diol (**14**) [16], 1-(4-hydroxy-2,2-dimethyl-chroman-6-yl)-ethanone (**15**) [8], 2,2-dimethyl-6-acetyl chromanone (**16**) [6], 6-hydroxy-2,2-dimethylchroman-4-one (**17**) [17], 2-formyl-5-hydroxymethyl-furan (**18**) [18], sessiline (**19**) [19], *n*-butyl-α-D-fructofuranoside (**20**) [20], β-sitosterol (**21**), isobauerenol (**22**) [21], and bauer-7-ene-3β,16α-diol (**23**) [22].

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a Perkin-Elmer 341 polarimeter. IR spectra were obtained on a Nicolet NEXUS 670 FT-IR spectrometer. UV spectra were measured using a Perkin-Elmer Lambda 950 UV/VIS spectrometer. The CD spectra were recorded on an Olis RSM 1000 Rapid-Scanning Monochromator instrument. NMR spectra were recorded on a Bruker Avance III-400 NMR spectrometer with tetramethylsilane as an internal standard and $CDCl_3$, DMSO-d_6, and C_5D_5N as the solvents. The EI-MS were measured on a Trace DSQ GC Ultra instrument at 70 eV. The HRESIMS were carried out on a Bruker APEX II mass spectrometer with glycerol as the matrix. HPLC experiments were carried out on an Agilent Technologies 1200 series instrument. Column chromatography (CC) was conducted on silica gel (200–300 mesh, Qingdao Marine Chemical Factory, Qingdao, China), and octadecylsilica (40–60 mesh, YMC, Kyoto, Japan). Thin layer chromatography (TLC) was performed on silica gel GF_{254} (Qingdao Marine Chemical Factory), and was detected at 254 nm, and by heating after spraying with 5% H_2SO_4 in EtOH (V/V).

3.2 Plant material

The flower buds of *T. farfara* were purchased from Gansu Huanghe Market for TCM in May 2007. They were identified by adjunct Prof. Huan-yang Qi, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences. A voucher specimen for this material (No. 2007T05) has been deposited in the Key Laboratory for Natural Medicine of Gansu Province, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, China.

3.3 Extraction and isolation

The air-dried flower buds (5.5 kg) were pulverized and extracted with 95% EtOH (10 liters × 3) at 50° for 3 h each time. The combined extracts were evaporated to dryness under reduced pressure. The resulting residue (535 g) was suspended in H_2O (2 liters), and extracted with petroleum ether (PE), EtOAc, and *n*-BuOH, respectively. The PE extract (132 g) was subjected to silica gel CC (1000 g) with a gradient system of PE–EtOAc (40:1, 20:1, 15:1, 10:1, 8:1, 5:1, 2:1, and 1:1) to afford eight fractions (F_1–F_8) after TLC analysis. Fractions F_2 (5 g) and F_3 (7 g) were reapplied to silica gel CC (70 and 105 g, respectively) using PE–EtOAc (20:1) to give two (F_{2-1}, F_{2-2}) and three subfractions (F_{3-1}–F_{3-3}), respectively. F_{2-1} (460 mg) was further separated by silica gel CC (15 g) eluting with PE–acetone

(10:1) followed by preparative TLC (PE–acetone, 12:1) to obtain **6** (16.1 mg). F_{2-2} (2.4 g) was subjected to silica gel CC (50 g) with PE–acetone (25:1) and yielded compounds **8** (300 mg) and **9** (42 mg). F_{3-1} was separated by silica gel CC (30 g) using PE–EtOAc (15:1) and $CHCl_3$–PE (5:2) to give **7** (250 mg). F_4 (6.1 g) was separated by silica gel CC (110 g) eluting with PE–EtOAc (10:1) to yield three fractions (F_{4-1}–F_{4-3}). F_{4-1} (1.1 g) was purified by repeated silica gel CC with PE–acetone (15:1) to obtain **11** (39 mg) and **22** (100 mg), and F_{4-2} (1.2 g) was crystallized in acetone to yield **21** (200 mg). F_5 (4.6 g) was rechromatographed on silica gel CC (90 g) using PE–acetone (10:1) to obtain F_{5-1}, F_{5-2}, and F_{5-3}. F_{5-1} (1.3 g) yielded compounds **2** (100 mg), **3** (13.3 mg), and **5** (6.3 mg), after using silica gel CC (35 g) with $CHCl_3$–EtOAc (18:1). Then, a similar purification procedure on fractions F_{5-2} and F_{5-3} yielded **10** (25 mg), **12** (21 mg), **13** (42 mg), **15** (13 mg), **16** (31 mg), **17** (1.8 mg), and **23** (13 mg). The EtOAc extract (123 g) was subjected to silica gel CC (900 g) eluting with PE–acetone (from 30:1 to 1:1) to give seven fractions (M_1–M_7). Fraction M_2 (2.4 g) was subjected to CC on silica gel (60 g) with PE–acetone (15:1) to afford impure **4**, which was purified by repeated silica gel CC using $CHCl_3$–EtOAc (25:1) to obtain **4** (10 mg). M_3 (4.4 g) was further separated by silica gel CC (90 g) using $CHCl_3$–EtOAc (20:1) to give two fractions (M_{3-1} and M_{3-2}); then M_{3-1} (250 mg) was subjected to silica gel CC (8 g) eluting with PE–acetone (12:1) to yield pure compounds **1** (23.5 mg) and **14** (15 mg). Fraction M_{3-2} (1.4 g) was subjected to CC on silica gel (40 g) with PE–acetone (15:1) to afford subfraction M_{3-2-1}. Then, compound **18** (53 mg) was isolated after CC on silica gel (15 g) with $CHCl_3$–acetone (18:1). The *n*-BuOH extract (153 g) was subjected to silica gel CC (1000 g) eluting with $CHCl_3$–MeOH, with gradually increasing volumes of MeOH to obtain six fractions: D_1 (30:1), D_2 (20:1), D_3 (10:1), D_4 (7:1), D_5 (5:1), and D_6 (2:1). Fraction D_2 (2.1 g) was further fractionated on silica gel CC (28 g) eluting with $CHCl_3$–acetone (10:1) to give one subfraction, which gave **19** (10 mg) after preparative TLC ($CHCl_3$–acetone, 7:3). D_3 (812 mg) was further purified by repeated silica gel CC using $CHCl_3$–MeOH (20:1) to obtain impure compound **20**, then a RP-18 silica gel column (60:40 → 80:20) to yield pure **20** (32 mg).

3.3.1 *Tussfarfarin A (**1**)*

Colorless gum; $R_f = 0.6$ ($CHCl_3$/acetone, 7:2); $[\alpha]_D^{20} + 11$ ($c = 0.10$, CH_3OH); UV (CH_3OH) λ_{max} (log ϵ): 306 (4.02) nm; IR (KBr, disk) ν_{max}: 3436, 2969, 2926, 2879, 1771, 1655, 1576, 1425, 1357, 1252, 1205, 1191, 1088, 1068, 1038, 890, 770, and 593 cm^{-1}; for ^1H and ^{13}C NMR spectral data, see Table 1; HR-ESI-MS: *m/z* 235.1322 $[M + H]^+$ (calculated for $C_{14}H_{19}O_3$, 235.1329).

3.3.2 *Tussfarfarin B (**2**)*

Yellow oil; $R_f = 0.5$ (PE–acetone, 7:1); $[\alpha]_D^{20} - 10$ ($c = 0.02$, CH_3OH); UV (CH_3OH) λ_{max} (log ϵ): 219 (4.22) nm; IR (film) ν_{max}: 2968, 2936, 2877, 1755, 1740, 1713, 1649, 1461, 1378, 1218, 1144, 1080, 994, 970, 917, 867, and 756 cm^{-1}; CD ($c = 0.2$, MeOH): $\Delta\varepsilon_{211\,nm} = -3.55$, $\Delta\varepsilon_{320\,nm} = -5.84$; for ^1H and ^{13}C NMR spectral data, see Table 1; HR-ESI-MS: *m/z* 499.3038 $[M + Na]^+$ (calculated for $C_{28}H_{44}NaO_6$, 499.3030).

3.3.3 *6-(1-Ethoxyethyl)-2,2-dimethylchroman-4-ol (**3**)*

Colorless gum; $R_f = 0.4$ ($CHCl_3$/acetone, 4:1); UV (CH_3OH) λ_{max} (log ϵ): 281 (3.21) nm; IR (KBr, disk) ν_{max}: 3422, 2972, 2931, 2869, 1691, 1616, 1584, 1491, 1458, 1370, 1258, 1190, 1128, 1081, 1033, 936, 913, and 827 cm^{-1}; for ^1H and ^{13}C NMR spectral data, see Table 2; HR-ESI-MS:

m/z 233.1532 $[M - H_2O + H]^+$ (calculated for $C_{15}H_{21}O$, 233.1536).

3.3.4 5-Ethoxymethyl-1H-pyrrole-2-carbaldehyde (**4**)

Yellow oil; $R_f = 0.5$ ($CHCl_3$/acetone, 2:1); UV (CH_3OH) λ_{max} (log ϵ): 291 (3.44) nm; IR (film) ν_{max}: 3389, 3262, 2928, 2856, 1722, 1650, 1374, 1244, 1184, 1096, 1041, and 778 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, ppm): δ 9.47 (1H, s, H-6), 6.89 (1H, t, $J = 3.0$ Hz, H-3), 6.19 (1H, t, $J = 3.0$ Hz, H-4), 4.53 (2H, s, H-7), 3.55 (2H, q, $J = 6.8$ Hz, H-1′), and 1.25 (3H, t, H-2′); ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): δ179.7 (CH, CHO), 137.9 (C, C-5), 132.6 (C, C-2), 121.3 (CH, C-3), 109.2 (CH, C-4), 66.4 (CH_2, C-1′), 65.2 (CH_2, C-7), and 15.1 (CH_3, C-2′); HR-ESI-MS: *m/z* 154.0861 $[M + H]^+$ (calculated for $C_8H_{12}NO_2$, 154.0863).

3.3.5 3β-Hydroxy-7α-ethoxy-24β-ethylcholest-5-ene (**5**)

Colorless needles; $R_f = 0.6$ ($CHCl_3$/-acetone, 7:2); $[\alpha]_D^{20} - 6$ ($c = 0.1$, CH_3OH); IR (KBr, disk) ν_{max}: 3365, 2955, 2931, 2866, 1662, 1461, 1378, 1328, 1118, 1062, 956, and 772 cm^{-1}; ^1H NMR spectral data see Table 3; ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): δ 145.3 (C, C-5), 121.9 (CH, C-6), 72.4 (CH, C-7), 71.4 (CH, C-3), 64.5 (CH_2, C-1′), 55.5 (CH, C-17), 49.0 (CH, C-14), 45.9 (CH, C-24), 42.6 (CH_2, C-4), 42.3 (C, C-13), 42.1 (CH, C-9), 39.0 (CH_2, C-12), 37.3 (CH, C-8), 37.3 (C, C-10), 36.7 (CH_2, C-1), 36.3 (CH, C-20), 34.0 (CH_2, C-22), 31.5 (CH_2, C-2), 29.2 (CH_2, C-25), 28.3 (CH_2, C-16), 26.2 (CH_2, C-23), 24.5 (CH_2, C-15), 23.1 (CH_2, C-28), 20.8 (CH_2, C-11), 19.8 (CH_3, C-26), 19.0 (CH_3, C-27), 18.8 (CH_3, C-21), 18.2 (CH_3, C-19), 16.0 (CH_3, C-2′), 12.0 (CH_3, C-29), and 11.5 (CH_3, C-18); EI-MS (probe) 70 eV: *m/z* 458 $[M]^+$(9.7), 441 $[M - OH]^+$(9.5), 412 $[M - EtOH]^+$(100), 398 (29.6), 316 (13.7), 253 (12.8), 180 (20.3), 11.2 (80.2).

3.3.6 (9S)-Altaicalarin B (**6**)

Colorless oil; $[\alpha]_D^{20} + 12$ ($c = 0.02$, CH_3OH); UV (CH_3OH) λ_{max} (log ϵ): 219 (4.38), 283 (3.27) nm; IR (film) ν_{max}: 3270, 2923, 2858, 1687, 1642, 1578, 1461, 1386, 1337, 1254, 1165, 1042, 920, 850 and 766 cm^{-1}; CD ($c = 0.2$, MeOH): $\Delta\varepsilon_{225nm} = +8.36$; ^1H and ^{13}C NMR spectral data see Table 2; HR-ESI-MS: *m/z* 353.1726 $[M + Na]^+$ (calculated for $C_{20}H_{26}O_4Na$, 353.1723).

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