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CHEMICAL CONSTITUENTS OF LIGULARIA FRANCHETIANA COLLECTED IN YUNNAN PROVINCE OF CHINA

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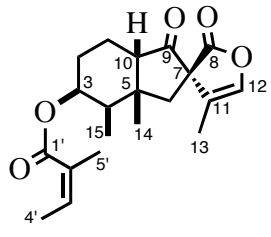
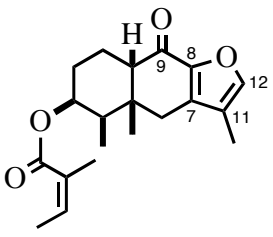
Abstract – Two new ketones, franchetianones A and B, were isolated from the root of *Ligularia franchetiana* collected in Yunnan province of China and their structures determined based on the spectroscopic analyses.

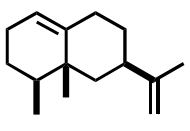
Ligularia (Compositae, Asteraceae) in the Hengduan Mountains region of China has been attracting scientific interest lately,[1] as it is highly diversified in the area and some species of *Ligularia* contain medicinal activities.[1] We have been studying chemical constituents and their diversity in *Ligularia* in Yunnan and Sichuan Provinces of China.[2-6] In the present study, we analyzed root chemicals and DNA sequences of *L. franchetiana* (Lével.) Hand.-Mass. The root of *L. franchetiana* is one of the Chinese herbal medicines collectively called "Shanziyuan" ("San-shion" in Japanese), which has been used against cough and asthma.[7,8] Here we report the structures of two new sesquiterpenoids, which must be biogenetically related to each other, as well as the base sequences of the ITS1-5.8S-ITS2 region of the nuclear ribosomal RNA gene and the *atpB-rbcL* intergenic region of the plastid genome. A 16 base-pair repeat, not previously seen in *Ligularia*, was found in the latter.



franchetianone A (**1**)



franchetianone B (**2**)



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Compounds in an extract of root of *L. franchetiana* were purified by a combination of column and high-performance liquid chromatography and compounds (**1**) and (**2**), as well as eremophila-1(10),11-diene (**3**), were isolated. The structures of the compounds were determined as follows.

The molecular formula of **1** was deduced to be $C_{20}H_{26}O_5$ by HRCIMS. The IR (1770, 1730, 1710 cm^{-1}) and ^{13}C-NMR (δ 166.6, 176.9, 206.4) spectra indicated the presence of three carbonyl groups. There were five methyl, three methylene, five methine, and seven quaternary carbons. Since there were two double bonds and three carbonyl groups, this molecule was deduced to be tricyclic. The COSY and HMBC spectra showed connectivities as illustrated in Fig. 1. It has a rearranged eremophilane skeleton, a bakkane-type skeleton,[9] with an angelate at the C-3 position. The stereochemistry was deduced from NOE correlations, important ones of which are shown in Fig. 1. NOE between H-14/H-10 indicated a *cis*-fused hydrindane skeleton and NOE between H-4/H-13 indicated the stereochemistry of a spiro carbon of C-7. At the same time the hydrindanone system was deduced to adopt a non-steroidal conformation in which the methyl group attached to the C-5 position was axial to the ring A (Figure 2a). Because H-3 appeared as a quartet with J = 3 Hz, the angelate group was β-axial. The structure was novel and the compound was named franchetianone A.

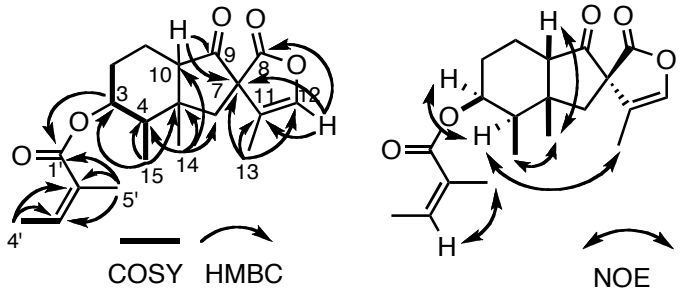

Figure 1. Selected HMBC, COSY and NOESY correlations for franchetianone A (**1**).

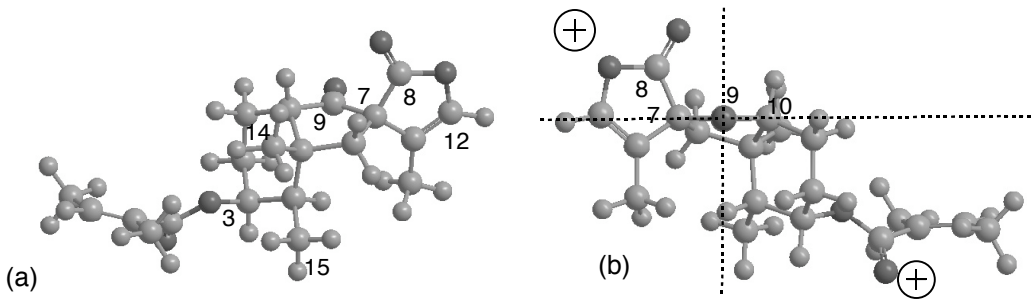

Figure 2. (a) The conformation of franchetianone A (**1**), (b) Back octant of the carbonyl at C-9.

The absolute configuration was determined by CD. A positive Cotton effect (Δε +7.04) at 304.7 nm was observed and the back octant as shown in Figure 2b. suggested the absolute configuration as depicted in the formula.

Table 1. ^{1}H- and ^{13}C-NMR Data for franchetianone B (**2**) and its isomer (**4**)

Position	franchetianone (**2**)[a] ^{1}H (mult., *J* in Hz)	franchetianone (**2**)[a] ^{13}C	compound (**4**)[b] ^{1}H (mult., *J* in Hz)
1	[c]	20.2	[d]
2	[c]	24.6	[d]
3	5.22 (dt, 9.5, 4.4)	71.7	4.74 (ddd)
4	[c]	41.0	[d]
5	-	42.0	[d]
6	α 2.74 (d, 17.2)	30.1	α 1.38 (d, 16)
	[c]	-	β 2.72 (d, 16)
7	-	135.2	[d]
8	-	146.2	[d]
9	-	185.6	[d]
10	2.22 (dd, 10.2, 4.0)	51.2	[d]
11	-	120.2	[d]
12	6.85 (s)	143.9	7.28 (q)
13	1.52 (s)	6.7	1.99 (d)
14	0.84 (s)	24.2	0.89 (s)
15	0.75 (d, 7.0)	8.4	0.96 (d)
1'	-	166.2	[d]
2'	-	128.0	[d]
3'	5.75 (qq, 7.3, 1.4)	136.8	[d]
4'	1.97 (dq, 7.3, 1.4)	15.0	[d]
5'	1.87 (quint, 1.4)	20.1	[d]

a) 400 MHz in C_6D_6 at 60˚C
b) 100 MHz in $CDCl_3$ (ref. 10)
c) could not be assigned
d) not described in the ref.

Compound (**2**) showed a quasi-molecular ion peak at *m/z* 331 and its molecular formula was deduced to be $C_{20}H_{27}O_4$ by HRCIMS. The IR spectrum indicated the presence of two carbonyl groups (1712 and 1700 cm^{-1}), which was supported by the ^{13}C-NMR (Table 1). The ^{1}H- and ^{13}C-NMR spectra at rt showed broad peaks. Therefore, the spectra were taken in C_6D_6 at 60˚C. They indicated the presence of an angelate group and a furan (Table 1). The HMBC spectrum showed the compound to be an eremophilane and the position of the angelate group to be at the C-3 position (Fig. 3). No correlation was observed from the ketone group (δ 185.5). The chemical shift at the C-7 position was δ 135.2 for compound (**2**), while the shift was normally observed around δ 115-120 in furans without the carbonyl group at the C-9 position.[1-6] Furthermore, H-10 coupled with only two protons at the C-1 position. Therefore, the position of the carbonyl group was deduced to be at C-9. NOE was observed between H-10/H-14 and H-3α/H-6α, which clearly indicated the *cis*-fused junction (Figure 3). This compound was named franchetianone B. Bohlmann et al. reported the isolation of compound (**4**), a *trans*-fused derivative of **2**, the spectral comparison was shown in the Table 1.[10]

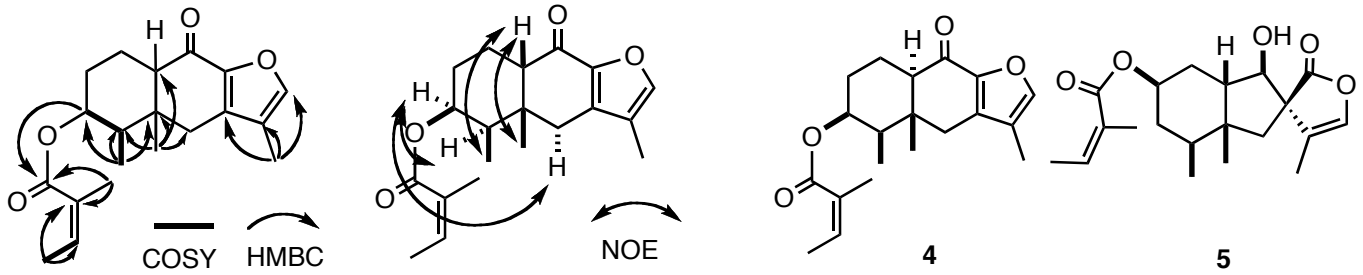

Figure 3. Selected HMBC, COSY and NOESY correlations for franchetianone B (**2**).

Bauer and his group isolated a similar compound (**5**), having a hydroxy group at the 9-position and an angelate at the C-2 position from *Petasites hybridus*.[11] They also reported the presence of 2b-angelate derivative of compound (**2**).[11] Compounds (**1**) and (**2**) must be biogenetically related each other as shown in Figure 4. Bakkanes and eremophilanes are sometimes isolated simultaneously.[11,12]

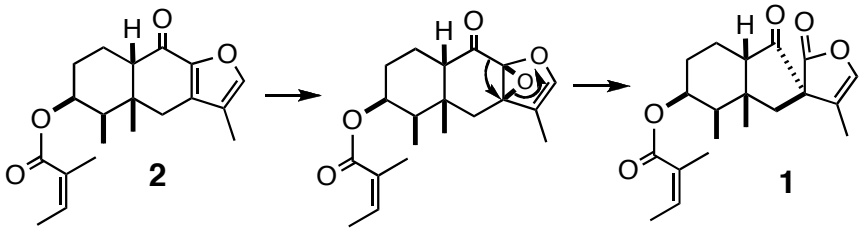

Figure 4. Plausible biogenetic pathway from franchetianones B (**2**) to A (**1**).

The base sequence of the ITS1-5.8S-ITS2 was determined and deposited to the database (**AB375311**). A database search retrieved the ITS1-5.8S-ITS2 sequence of *L. duciformis*, a species in the same Section Corymbosae Series Retusae as *L. franchetiana*, as the most similar. The sequence of the *atpB-rbcL* region was also determined (**AB375447**). Interestingly, a 16 base-pair tandem repeat was found in between the 438th and the 469th base position. Such a repeat had not been observed in the *Ligularia* species thus far studied[2-5] or in *L. duciformis* (**AB376962**).[6]

There have been reports on terpenoids from *L. duciformis*. From the plant, some guaianes and eudesmanes[13] have been isolated as well as coniferyl alcohol derivatives,[14] however, eremophilane derivatives have not been obtained. Guaianes[15] and sinapyl alcohols[16] have been isolated also from *L. nelumbifolia*, a species taxonomically close to both *L. duciformis* and *L. franchetiana*. Thus *L. franchetiana* appears unique among plants of the Section Corymbosae Series Retusae both in the chemical composition and in the *atpB-rbcL* sequence.

Some *Ligularia* species including *L. franchetiana*, *L. latihastata*, *L. sibirica*, and *L. hodgsonii*, are registered as "Shanziyuan" and the roots of these plants look alike.[7] We previously reported the benzofuran derivatives were the major components of *L. latihastata*.[17] Present results show that the terpenoid constituents of "Shanziyuan" depend on the species.

EXPERIMENTAL

Specific rotations and CD spectra were measured on a JASCO DIP-1000 and a JASCO J-725 auto recording polarimeter; IR spectra, on a JASCO FT/IR-5300 spectrophotometer; ^{1}H and ^{13}C NMR spectra, on a Varian Unity 600 (600 MHz and 150 MHz, respectively) and a JEOL ECP 400 (400 MHz and 100 MHz, respectively) spectrometer. Mass spectra, including high-resolution ones, were recorded on a JEOL JMS-700 MStation. Chemcopak Nucleosil 50-5 (4.6×250 mm) with a solvent system of hexane-ethyl acetate was used for HPLC (a JASCO pump system). Silica gel 60 (70-230 mesh, Fuji Sylisia) was used for column chromatography. Silica gel 60 F_{254} plates (Merck) were used for TLC. Ehrlich's test on TLC was carried out as previously described.[2-4] Polymerase chain reaction of the ITS1-5.8S-ITS2 and *atpB-rbcL* regions and purification of the products were carried out as described.[1-3]

DNA sequencing reactions were carried out with the BigDye Terminator Ver. 3.0 kit (Applied Biosystems) and the primers described previously[3,4] and analyzed on a 3130xl sequencer (Applied Biosystems).

Plant material. The plant was collected at Qiaozishan, north of Kunming, Yunnan in 2004 (voucher specimen, K-G-0487, was deposited in the Herbarium of Kunming Institute of Botany) and was identified by Xun Gong, one of the authors.

Extraction and isolation. The root of *L. franchetiana* was extracted with EtOAc to give an extract (1.0 g), which was separated by silica-gel column chromatography (hexane-EtOAc, in a gradient) followed by HPLC (hexane-EtOAc, 10%) to afford **1** (19.7mg), **2** (3.2mg), and **3** (10.6mg).

franchetianone A (1): $[\alpha]_D^{23}$ +197.1 (c 1.17, EtOH); FT-IR (KBr): 1770, 1730, 1710 cm^{-1}; EIMS *m/z* 346 $[M]^+$, 218, 108 (base), 83; EIHRMS *m/z* 346.1778 (Calcd for $C_{20}H_{26}O_5$ 346.1780); ^1H NMR (600 MHz, C_6D_6) δ 0.65 (3H, d, *J* = 6.9 Hz, H-15), 0.85 (H, qd, *J* = 6.9, 3.0 Hz, H-4), 1.08 (3H, d, *J* = 1.6 Hz, H-13), 1.10 (3H, s, H-14), 1.36 (H, dddd, *J* = 14.0, 11.3, 4.4, 2.7 Hz, H-2α), 1.46 (H, tt, *J* = 14.0, 4.4 Hz, H-1β), 1.64 (H, br d, *J* = 14.0 Hz, H-2β), 1.69 (H, d, *J* = 14.6 Hz, H-6α), 1.78 (3H, quint, *J* = 1.4 Hz, H-5'), 1.80 (H, d, *J* = 14.6 Hz, H-6β), 2.00 (3H, dq, *J* = 7.1, 1.4 Hz, H-4'), 2.65 (H, m, H-10), 5.02 (H, q, *J* = 3.0 Hz, H-3), 5.71 (H, qq, *J* = 7.1, 1.4 Hz, H-3'), 6.08 (1H, q, *J* = 1.6 Hz, H-12); ^{13}C NMR (150 MHz, C_6D_6) δ 9.9 (C-13), 12.0 (C-15), 15.0 (C-1), 15.8 (C-4'), 21.0 (C-5'), 22.8 (C-14), 26.4 (C-2), 38.1 (C-5), 38.8 (C-4), 42.3 (C-6), 55.5 (C-10), 62.9 (C-7), 72.1 (C-3), 118.7 (C-11), 119.2 (C-12), 128.3 (C-2'), 138.8 (C-3'), 166.6, (C-1'), 176.9 (C-8), 206.4 (C-9); CD (EtOH) Δε +16.2 (220.1 nm), -0.39 (255.8 nm), +7.04 (304.7 nm).

franchetianone B (2): FT-IR (KBr): 1712, 1700, 1670 cm^{-1}; CIMS *m/z* 331 $[M+H]^+$ (base), 247, 231, CIHRMS Obs. *m/z* 331.1891 $[M+H]^+$ (Calcd for $C_{20}H_{27}O_4$ 331.1909); ^1H and ^{13}C NMR (100 MHz, C_6D_6, 60˚C) (see Table 1).

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