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Anti-inflammatory activities of furanoditerpenoids and other constituents from *Fibraurea tinctoria*

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ABSTRACT

Five new furanoditerpenoids, *epi*-8-hydroxycolumbin (**1**), fibaruretin B (**2**), C (**3**), E (**5**), and F (**6**), were isolated from the stems of *Fibraurea tinctoria*, as well as fibaruretin D (**4**) from the natural source for the first time, and 39 known compounds. The structures (**1**–**6**) were elucidated on the basis of spectroscopic analysis. All the isolated furanoditerpenoids (**1**–**16**) were examined for their in vitro activity and some were in vivo anti-inflammatory activity. Compounds **8** and **9** showed significant anti-inflammatory action administered at a dose of 100 mg/kg of reducing carrageenan mice paw edema, whereas compound **7**, **9**, **10**, **14**, and **16** were more potent to inhibit NO production. The inhibitory effects of these compounds are dose-dependent (1–4 μg/ml).

1. Introduction

Fibraurea tinctoria Lour. (Menispermaceae) is a common dye-producing plant, and is widely distributed in Mainland China, Indonesia, Malaysia, Thailand, and Vietnam.[1] The stem bark of this species is used for the treatment of dysentery, and for analgesic, antipyretic, antidote, and diuretic effects.[2] A number of chemical constituents including protoberberine alkaloids and several furanoditerpenoids have been isolated from this species.[3,4] As a part of our continuing studies on bioactive constituents of natural medicines, the air-dried and powdered whole plants of *F. tinctoria* were extracted with methanol. The solvent was evaporated, and then the resulting residue was successively partitioned with $CHCl_3$, *n*-BuOH, and H_2O. The $CHCl_3$ layer was further portioned with 5% HCl and neutralized by NH_4OH to obtain the alkaloid fraction. As shown in Table 3, the crude methanol extract (FTM) and some subfractions (FTA, FTB) indicated significant inhibition on the edema induced by carrageenan. Therefore, this prompted us to undertake the chemical investigation of the stems of *F. tinctoria*.

As a result of further fractionation efforts on this plant, we isolated five new furanoditerpenoids termed *epi*-8-hydroxycolumbin (**1**), fibaruretin B (**2**), C (**3**), E (**5**), and F (**6**), including fibaruretin D (**4**) that was isolated from the natural source for the first time (Chart 1). The molecular structures of these furanoditerpenoids were established on the basis of extensive spectroscopic methods, including 1D and 2D NMR, and comparison of their NMR spectral data with those of related compounds. Moreover, 39 known compounds were also isolated from this plant. In this study we have attempted to find the active anti-inflammatory agents reducing carrageenan mice paw edema.

2. Chemistry

During this investigation, the chemical constituents of the stems of *F. tinctoria* grown in Vietnam were studied and afforded 45 compounds. The compounds **1**–**6** were identified by spectral methods (Chart 2).

epi-8-Hydroxycolumbin (**1**) was isolated as optically colorless needle, mp 210–211 °C. The HREIMS of **1** showed a peak at *m*/*z* 374.1364 corresponding to the molecular $C_{20}H_{22}O_7$. IR absorption bands at 1741 and 1721 cm^{-1} indicated the presence of lactone carbonyl groups, which were confirmed by carbon resonances at δ 177.9 and 175.7 in the ^{13}C NMR spectrum. The ^1H NMR spectrum showed a set of olefinic protons at δ 6.43 (1H, dd, J = 7.9, 5.3 Hz) and 6.29 (1H, dd, J = 7.9, 1.9 Hz), a signal at δ 5.30 (1H, dd, J = 5.3, 1.9 Hz), which are assignable to the protons of the lactone proton at C-1 and a double-bond at C-2 and C-3. In addition, signals at δ 6.40 (1H, dd, J = 1.7, 1.1 Hz), 7.44 (1H, dd, J = 1.7, 1.6 Hz), and 7.51 (1H, dd, J = 1.6, 1.1 Hz) were assignable to an α-substituted furan ring. Comparison of the NMR spectra of **1** with those of fibleucin (**11**)[4] revealed these compounds to be similar. It showed **1** lacked a set of olefinic protons at C-7 and C-8, but appeared the appearance of an additional signal at δ 74.4 in the ^{13}C NMR spec-

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Table 1
^{1}H NMR spectral data (δ_H, mult, J_{HH} in Hz) of **1**–**6**

Proton	**1** ($CDCl_3$)[a]	**2** (DMSO-d_6)[a]	**3** (DMSO-d_6)[b]	**4** ($CDCl_3$)[a]	**5** ($CDCl_3$)[a]	**6** ($CDCl_3$)[a]
1	5.30, dd (5.3, 1.9)	2.19, dd (13.0, 12.8) 1.70, d (13.0)	2.16, dd (13.4, 13.2) 1.73, m	2.80, ddd (20.5, 6.6, 3.1) 2.47, dd (20.5, 5.3)	4.48, dd (5.2, 4.7)	
2	6.43, dd (7.9, 5.3)	4.54, dd (6.0, 2.8)	4.53, d (5.7)	6.80, m	6.78, dd (10.1, 4.7)	6.75, d (10.3)
3	6.29, dd (7.9, 1.9)	4.13, d (6.0, 4.3)	4.11, dd (5.7, 4.3)	6.05, dd (10.3, 3.1)	6.10, d (10.1)	6.85, d (10.3)
6	1.67, m 1.26, dd (13.2, 9.3)	1.51, m 1.51, m	1.75, m 1.46, m	3.57, dd (21.2, 4.0) 1.95, dd (21.2, 4.0)	3.53, dd (21.2, 4.0) 2.00, dd (21.2, 4.0)	3.60, dd (21.2, 3.9) 2.08, dd (21.2, 4.2)
7	2.12, m 1.67, m	1.97, m 1.63, m	2.52, m 1.76, m	7.15, t (4.0)	7.16, t (4.0)	7.20, dd (4.2, 3.9)
8		2.95, dd (9.2, 8.9)				
10	1.40, br s	2.24, br s	2.57, m	2.02, d (6.6)	2.08, s	2.08, s
11	2.55, dd (14.3, 12.3) 1.85, dd (14.3, 4.4)	2.02, dd (14.5, 4.3) 1.61, dd (14.5, 12.0)	1.88, dd (13.6, 6.3) 1.70, m	2.21, dd (13.6, 3.4) 1.67, dd (13.6, 12.3)	2.33, dd (13.2, 3.3) 1.89, dd (13.2, 12.3)	2.19, dd (14.3, 3.2) 1.94, dd (14.3, 12.3)
12	5.52, dd (12.3, 4.4)	5.52, dd (12.0, 4.3)	5.89, dd (9.3, 7.8)	5.51, dd (12.3, 3.4)	5.48, dd (12.3, 3.3)	5.43, dd (12.3, 3.2)
14	6.40, dd (1.7, 1.1)	6.55, dd (1.8, 1.0)	6.53, dd (1.7, 1.1)	6.41, dd (1.8, 1.4)	6.41, dd (1.7, 1.1)	6.42, dd (1.8, 1.4)
15	7.44, dd (1.7, 1.6)	7.65, dd (1.8, 1.6)	7.65, dd (1.7, 1.6)	7.41, dd (1.8, 1.7)	7.41, dd (1.7, 1.6)	7.41, dd (1.8, 1.7)
16	7.51, dd (1.6, 1.1)	7.69, dd (1.6, 1.0)	7.74, dd (1.6, 1.1)	7.47, dd (1.7, 1.4)	7.47, dd (1.6, 1.1)	7.47, dd (1.7, 1.4)
19	1.21, s	1.37, s	1.46, s	1.33, s	1.51, s	1.41, s
20	1.46, s	0.82, s	0.84, s	0.99, s	0.87, s	1.00, s
OH-1					2.48, d (5.2)	
OH-3		6.02, d (4.3)	5.91, d (4.3)			
OH-4	3.57, s	5.56, s	5.45, s			
OH-8	3.12, s		5.94, s			

[a] Recorded in 400 MHz.
[b] Recorded in 500 MHz.

trum. Therefore, a hydroxyl group was positioned at C-8, based on HMBC correlations between H-11, H-10, and CH_3-20 with C-8 (Chart 2). Thus, the structure of **1** can be determined and confirmed. However, it was found the above spectral data of **1** were very similar to 8-hydroxycolumbin,[5,6] except for the configuration at C-12. The significant NOE correlations observed between CH_3-20 (δ 1.46) with H-12 (δ 5.52) and OH-8 (δ 3.12) indicated H-12, OH-8 are in β configuration (Chart 2). Thus, the structure of *epi*-8-Hydroxycolumbin was determined as **1** which is a stereoisomer of 8-hydroxycolumbin at the C-12 position.

Table 2
^{13}C NMR spectral data of **1**–**6**

Position	**1**[a,c]	**2**[b,c]	**3**[b,c]	**4**[a,c]	**5**[a,c]	**6**[a,d]
1	72.0	23.4	21.7	23.9	63.9	199.0
2	130.2	76.2	76.4	145.5	143.7	139.9
3	135.1	74.0	74.0	128.3	128.6	139.8
4	81.2	78.0	77.9	200.8	201.2	200.2
5	34.7	38.6	38.1	43.0	42.1	46.2
6	26.9	25.2	25.8	31.6	31.9	31.5
7	31.0	15.5	25.5	140.4	140.8	139.7
8	74.4	40.8	73.7	130.4	130.3	130.2
9	39.1	37.1	39.8	36.2	35.6	36.3
10	53.7	46.6	39.5	49.4	55.5	64.5
11	44.8	45.5	42.5	41.2	40.7	40.4
12	69.7	69.0	69.8	70.2	70.2	70.5
13	124.2	124.3	126.1	125.2	124.9	124.7
14	108.0	109.1	109.2	108.4	108.3	108.4
15	144.1	143.8	144.0	143.7	143.7	143.7
16	139.5	140.1	140.4	139.6	139.7	140.2
17	177.9	174.6	172.4	164.6	164.5	163.6
18	175.7	176.5	176.8			
19	26.8	27.0	25.8	29.2	32.5	31.3
20	20.5	22.3	21.3	22.0	21.5	23.1

[a] Recorded in $CDCl_3$.
[b] Recorded in DMSO-d_6.
[c] Recorded in 75 MHz.
[d] Recorded in 125 MHz.

Fibrauretin B (**2**) was obtained as optically colorless needle, mp 266–267 °C, and its pseudo-molecular formula was determined as $C_{20}H_{25}O_7$ in the HRFABMS. The IR spectrum displayed absorptions at 3563, 1781, and 1710 cm^{-1}, due to hydroxyl and lactone carbonyl groups. This was further supported by appearing two carbon resonances at δ 174.6 and 176.5 in the ^{13}C NMR spectrum. The ^{1}H NMR spectrum displayed the signals at δ 6.55 (1H, dd, J = 1.8, 1.0 Hz), 7.65 (1H, dd, J = 1.8, 1.6 Hz), 7.69 (1H, dd, J = 1.6, 1.0 Hz) revealed the presence of a furan ring. In addition, the ^{1}H NMR spectrum showed two tertiary methyl groups at δ 1.37 (3H, s) and a higher field methyl group at 0.82 (3H, s), indicating that both CH_3-20 and the furan ring are in a β-configuration.[7] The position of γ-lactone group was confirmed by the HMBC correlations observed from H-2 (δ 4.54) to C-18 (δ 176.5) and C-4 (δ 78.0); OH-4 (δ 5.56) to C-18 (δ 176.5) and C-4 (δ 78.0) in the HMBC spectrum (Chart 2). Moreover, an oxygenated methine proton signal at δ 4.13 (1H, dd, J = 6.0, 4.3 Hz, H-3) was also observed. Finally, the relative stereochemistry of **2** was determined by the analysis of its NOESY spectrum and proton coupling constants. A NOE correlation evident between H-12 and H-8, H-8, and H-10, H-10 and CH_3-19, H-10 and H-2, H-2, and H-3 indicated that H-2, H-3, H-8, H-10, H-12, and CH_3-19 are α-oriented (Chart 2). Thus, the structure of **2** was fully determined, and this compound has been named fibrauretin B (**2**).

Fibrauretin C (**3**) was assigned as a derivative of **2** on the basis of comparison of their ^{1}H and ^{13}C NMR spectra. From the ^{13}C NMR spectrum, it showed **3** bearing an additional hydroxyl group at C-8 (δ 73.7), which can be further identified by HMBC correlations of CH_3-20 (δ 0.84) and H-7 (δ 1.76) with C-8 (δ 73.7). However, the configuration of OH-8 could not be determined by NOESY spectroscopy (Chart 2) and was proposed as being β on the basis of ^{13}C NMR chemical shifts due to the steric effects and conformational

Table 3
Effects of FTM, FTA, FTB, and FTW on carrageenan-induced mice paw edema

(% of 0 h) mg/kg	Time (h)					
	0.5	1	2	3	4	5
Control	17.48 ± 1.54	16.03 ± 1.90	22.28 ± 1.63	24.55 ± 2.09	24.10 ± 0.91	24.28 ± 1.50
Indoemthacin	20.77 ± 4.92	21.48 ± 6.39	19.53 ± 6.70	18.52 ± 6.07	17.04 ± 6.47	16.30 ± 6.11
FTM						
50	33.45 ± 3.36	29.49 ± 4.84	27.70 ± 9.01	26.39 ± 7.54	22.78 ± 7.93*	19.87 ± 7.25
100	30.59 ± 9.10	17.21 ± 4.42	17.97 ± 1.75	16.01 ± 1.82	13.85 ± 1.61*	13.19 ± 0.40**
200	20.31 ± 2.34	18.29 ± 2.47	13.96 ± 1.63*	11.10 ± 2.85	11.52 ± 2.10*	11.86 ± 4.27
FTA						
50	28.56 ± 2.01	29.31 ± 4.59	25.27 ± 3.81	21.46 ± 3.68	21.61 ± 3.94	21.14 ± 3.76
100	17.67 ± 3.36	21.44 ± 5.44	19.24 ± 6.28	16.05 ± 6.64	17.69 ± 7.71	17.65 ± 7.46
200	23.52 ± 2.36	22.11 ± 1.29	19.36 ± 1.07	14.80 ± 1.53*	12.64 ± 0.63**	10.09 ± 1.59**
FTB						
50	15.94 ± 3.50	18.10 ± 1.98	21.93 ± 5.35	22.77 ± 7.84	22.01 ± 5.91	22.03 ± 10.44
100	18.11 ± 5.00	16.85 ± 6.06	11.40 ± 6.35	8.90 ± 6.06	9.01 ± 6.52	8.76 ± 6.63
200	13.46 ± 2.06	9.57 ± 0.10*	8.65 ± 0.99**	7.54 ± 1.21**	4.89 ± 1.12**	5.47 ± 2.17**
FTW						
50	20.06 ± 8.37	20.93 ± 7.35	20.63 ± 7.50	19.06 ± 8.16	19.31 ± 8.42	12.40 ± 5.34
100	15.65 ± 3.12	15.67 ± 4.25	14.12 ± 4.42	12.24 ± 5.37	13.34 ± 5.97	11.34 ± 4.30
200	12.95 ± 3.10	11.79 ± 3.22	10.17 ± 3.31	10.07 ± 3.32	11.29 ± 4.08	21.11 ± 8.32

FTM, crude methanol extract; FTA, alkaloids fraction; FTC, not dissolvable; FTB, *n*-BuOH fraction; FTW, H_2O fraction.
* $P<0.05$.
** $P<0.01$.

changes in the molecule.[8] Thus, the structure of **3** was determined as fibrauretin C (**3**).

Fibrauretin D (**4**) was isolated as optically colorless needle, mp 190–191 °C, and its molecular formula was determined as $C_{19}H_{20}O_4$ in the HREIMS. IR absorption bands at 1702 and 1667 cm^{-1} indicated the presence of lactone carbonyl and α,β-unsaturated carbonyl groups, which were confirmed by the presence of carbon signals at 164.6 and 200.8 in the ^{13}C NMR spectrum. The ^{1}H and ^{13}C NMR spectrum not only confirmed the presence of a furan ring but also provided the information of structure with only 19 carbons. Moreover, it is clearly evident that one lactone group was lost, but displayed the signal of a α,β-unsaturated carbonyl group at C-4 in the ^{13}C NMR spectrum, which was identified by HMBC correlations of H-6 (δ 3.57 and 1.95), H-10 (δ 2.02), and CH_3-19 (δ 1.33) with C-8 (δ 200.8) (Chart 2). In the previous studies, there is only one report about **4**, which was obtained from the decarboxylation of fibleucin.[9] However, the previous study only provided X-ray data of this compound without ^{1}H and ^{13}C spectral data. Therefore, **4** was isolated from the natural resource for the first time and the detailed spectral data of this compound was provided here.

Fibrauretin E (**5**) was isolated as optically colorless needle, mp 94–95 °C, its molecular formula $C_{19}H_{20}O_5$, and has strong absorptions at 3425, 1710, and 1681 cm^{-1} in the IR spectrum. The ^{13}C NMR spectrum of **4** and **5** were similar, except for the remarkable downfield shift at C-1 position. Moreover, a D_2O exchangeable signal occurred at δ 2.48 (1H, d), which assumed an additional hydroxyl group at C-1. The configuration of H-1 can be confirmed to be β form by the NOESY correlation between H-1 and CH_3-20 (Chart 2). Consequently, the structure of fibrauretin E was deduced as **5**.

Fibrauretin F (**6**) was isolated as optically yellow syrup, its molecular formula $C_{19}H_{18}O_5$, and has strong absorptions at 3021, 1712, and 1679 cm^{-1} in the IR spectrum. Compared ^{1}H and ^{13}C NMR spectral with **4**, they indicated the structure of **6** was similar to those of **5**, but appeared an additional carbonyl group at C-1 position, which was further supported by the HMBC correlations of H-10 (δ 2.69) and H-3 (δ 6.85) with C-1 (δ 199.0). Therefore, the structure of **6** was established as fibrauretin F (**6**) (Chart 2).

In addition, 39 known compounds were identified by comparison of their physical and spectral data with those reported in the literature. Among them, ten known furanoditerpenoids such as *epi*-12-palmatoside G (**7**),[10] floribundic ester (**8**),[11] fibraurin (**9**),[4] fibraurinoside (**10**),[4] fibleucin (**11**),[4] fibleucinoside (**12**),[4] chasmanthin (**13**),[12] fibrauretin A (**14**),[10] fibrauretinoside A (**15**),[10] and *epi*-fibrauretinoside A (**16**)[10] were also isolated from this plant (Chart 1). The others are palmatine (**17**)[13,14] jatrorrhizine (**18**),[13,14] columbamine (**19**),[15] stepharanine (**20**),[16] 8-trichloromethyl-7,8-dihydropalmatine (**21**),[17] mixture of *N*-(*p*-*trans*-coumaroyl)-tyramine (**22**) and *N*-(*p*-*cis*-coumaroyl)-tyramine (**23**),[18] *N*-*trans*-feruloyl tyramine (**24**),[19] *N*-*cis*-feruloyl tyramine (**25**),[19] corydaldine (**26**),[20] thalifoline (**27**),[21] *p*-hydroxybenzaldehyde (**28**),[22] vanillin (**29**),[23] syrigaldehyde (**30**),[24] methyl vanillate (**31**),[25] methyl syringate (**32**),[26] octadecyl (*E*)-ferulate (**33**),[27] vanillic acid (**34**),[28] syringic acid (**35**),[29] ferulic acid (**36**),[30] caffeic acid (**37**),[31] fibraurecdyside A (**38**),[10] makisterone A (**39**),[32] mixture of β-sitosterol (**40**) and sigmasterol (**41**),[33] β-sitosterol 3-*O*-β-D-glucopyranoside (**42**),[31] physcion (**43**),[34] dehydrogormouregine (**44**),[35] and thomasidioic acid dimethyl ester (**45**).[36]

3. Anti-inflammatory activities

3.1. Determination of anti-inflammation—Carrageenan-edema test

As shown in Table 3, the results indicated the *F. tinctoria* crude extract and subfractions (FTM, FTA, and FTB) significantly inhibited the edema induced by carrageenan. Some isolates from *F. tinctoria* were also investigated and given in Table 4. Compounds **8** and **9** show significant anti-inflammatory action administered at a dose of 100 mg/kg of reducing carrageenan mice paw edema.

3.2. Compounds 1–16 reduced NO production from macrophages

To determine NO production, the mouse macrophage (RAW 264·7) monolayer was treated with various constituents (2 μg/ml). After 24 h, the supernatant was analyzed for nitrite by Griess reaction. NO was produced by the cells stimulated with LPS at a final concentration of 2 μg/ml for 24 h. Sixteen furanoditerpenoids **1**–**16** (Chart 1) isolated from *F. tinctoria* were evaluated, and it showed that **7**, **9**, **10**, **14**, and **16** have inhibitory effect on NO pro-

Table 4
Effects of different compounds on carrageenan-induced mice paw edema

(% of 0 h) mg/kg	Time (h)					
	0.5	1	2	3	4	5
Control	12.47 ± 3.36	20.00 ± 0.88	21.91 ± 1.29	27.32 ± 1.26	29.01 ± 2.40	29.05 ± 2.66
2						
50	14.00 ± 0.78	20.10 ± 1.50	24.03 ± 1.75	23.43 ± 2.94	26.73 ± 5.15	28.34 ± 4.51
100	11.45 ± 0.37	19.17 ± 1.34	16.73 ± 0.29*	15.21 ± 2.63**	17.69±5.36	18.16 ± 6.99
4						
50	19.44 ± 5.99	24.17 ± 8.74	25.18 ± 9.33	21.55 ± 10.86	20.67 ± 11.04	20.50 ± 11.02
100	17.00 ± 5.03	20.91 ± 6.24	22.79 ± 5.88	21.47 ± 6.23	23.67 ± 5.43	23.41 ± 5.45
8						
50	16.27 ± 3.56	20.95 ± 3.40	21.51 ± 3.72	21.84 ± 3.58	21.85 ± 2.40	20.18 ± 2.25
100	13.07 ± 1.28	15.46 ± 2.39	16.78 ± 2.50	13.95 ± 1.22***	14.90 ± 2.61	13.07 ± 4.22*
9						
50	19.68 ± 2.58	27.17 ± 1.95	28.66 ± 2.53	30.50 ± 3.40	32.19 ± 3.49	33.57 ± 4.78
100	10.10 ± 2.40	12.44 ± 1.83	14.00 ± 2.67	10.85 ± 1.78	11.41 ± 1.74	10.61 ± 2.41
10						
50	17.12 ± 1.96	18.18 ± 2.85	18.99 ± 2.93	20.36 ± 2.30*	20.89 ± 2.68	18.48 ± 3.12
100	18.67 ± 3.64	23.03 ± 6.48	25.07 ± 7.98	25.03 ± 7.36	25.25 ± 7.38	24.80 ± 7.42
11						
50	11.80 ± 1.95	13.12 ± 1.71*	17.97 ± 1.32	21.61 ± 1.15	25.29 ± 1.31	25.60 ± 0.52
100	14.56 ± 1.07	17.79 ± 0.90	20.68 ± 1.88	24.44 ± 3.32	26.19 ± 2.49	26.02 ± 3.02
12						
50	15.64 ± 3.66	18.8 ± 4.21	24.26 ± 5.30	22.82 ± 6.53	24.01 ± 7.50	23.88 ± 10.16
100	14.91 ± 5.23	19.05 ± 4.84	19.82 ± 6.26	21.31 ± 6.01	22.01 ± 6.61	20.11 ± 5.96
13						
50	15.39 ± 0.83	18.46 ± 1.69	22.41 ± 2.13	23.99 ± 2.88	31.60 ± 0.19	32.49 ± 1.80
100	13.15 ± 6.92	18.75 ± 1.89	21.76 ± 3.02	21.23 ± 2.99	23.73 ± 4.45	20.71 ± 4.54

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

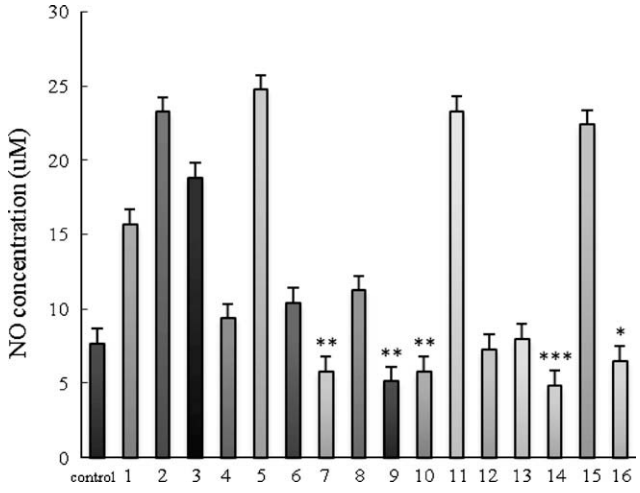

Figure 1. Effects of compounds **1**–**16** on NO production. RAW 264.7 macrophages (1×10^4 cells/well) were incubated with various compounds at a final concentration of 2 μg/ml for 24 h. Nitrite in the supernatant was determined after 24 h of incubation. LPS-treated cells served as control. Compounds **1**–**16** represented different compounds isolated from *F. tinctoria*. Vertical bars represent means and SE of eight separate experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with control group.

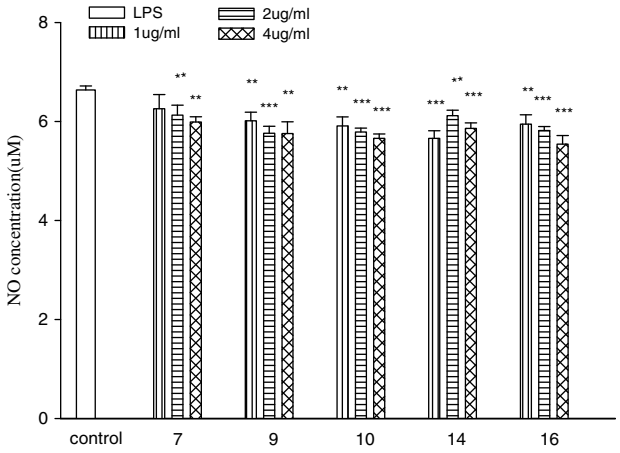

Figure 2. Effects of compounds **7**, **9**, **10**, **14**, and **16** on NO production. RAW 264.7 Macrophages (1×10^4 cells/well) were incubated with various compounds. At a final concentration of 1, 2, and 4 μg/ml for 24 h. Nitrite in the supernatant was determined after 24 h of incubation. LPS-treated cells served as control. Compounds **7**, **9**, **10**, **14**, and **16** represented different compounds isolated from *F. tinctoria*. Vertical bars represent means and SE of eight separate experiments. $^{**}P < 0.01$, $^{***}P < 0.001$ compared with control group.

duction in Figure 1. ($P < 0.05$–<0.001). Moreover, compound **7**, **9**, **10**, **14**, and **16** inhibited NO production in a dose-dependent manner as shown in Figure 2.

4. Conclusion

Five new furanoditerpenoids *epi*-8-hydroxycolumbin (**1**), fibaruretin B (**2**), C (**3**), E (**5**), F (**6**), and a first natural occurrence product fibaruretin D (**4**), together with thirty-nine known compounds, were isolated from the stems of *F. tinctoria*. According to the anti-inflammatory activity in vitro and in vivo essay, we found that compound **8** and **9** show significant anti-inflammatory action administered at a dose of 100 mg/kg of reducing carrageenan mice paw edema, whereas compound **7**, **9**, **10**, **14**, and **16** were more potent to inhibit NO production. The inhibitory effects of these compounds are dose-dependent (1–4 μg/ml).

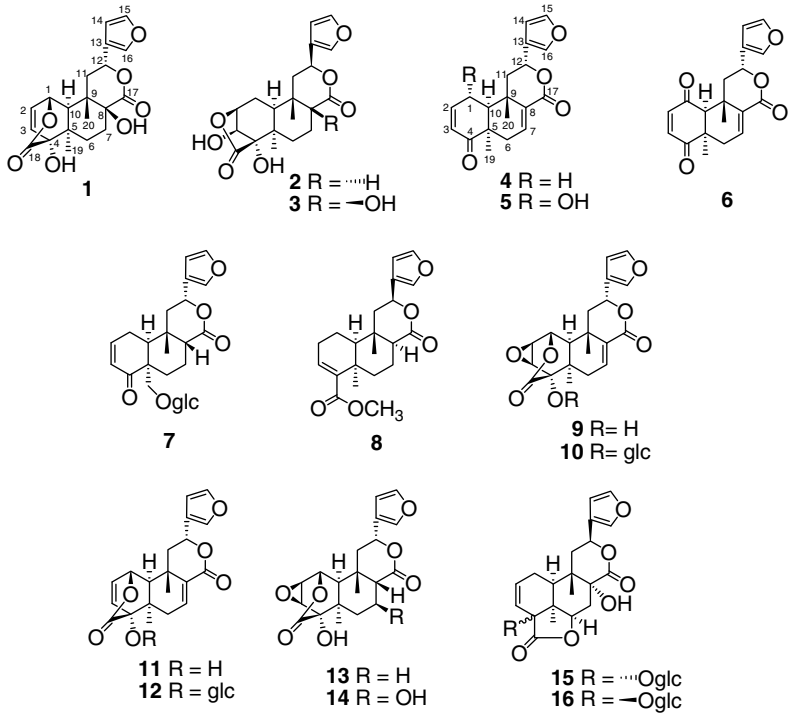

Chart 1. Structures of furanoditepenoids (**1**–**16**).

5. Experimental

5.1. General experimental procedures

Melting points were determined using Yanagimoto MP-S3 micro-melting point apparatus and were uncorrected. Optical rotations were measured using a JASCO DIP-370 digital polarimeter. UV spectra were recorded on a Hitachi U-3210 spectrophotometer, and IR spectra were recorded on a Shimadzu FT-IR Prestige-21 spectrophotometer. ^{1}H and ^{13}C NMR, COSY, HMQC, HMBC, and NOESY spectra were recorded on Bruker AVANCE-300, 500, and AMX-400 spectrometers, using tetramethylsilane (TMS) as internal standard. Standard pulse sequences and parameters were used for the NMR experiments and all chemical shifts were reported in parts per million (ppm, δ). FABMS were obtained on a JEOL JMS-700 spectrometer, and EIMS were obtained on a VG-70-250S spectrometer. Column chromatography was performed on silica gel (70–230 mesh, 230–400 mesh). Fractions were monitored by TLC (Merck precoated Si gel 60 F_{254} plates), using UV light. TLC was conducted on precoated Kieselgel 60 F_{254} plates (Merck) and the spots were detected either by examining the plates under a UV lamp or by treating the plates with a 10% methanolic solution of *p*-anisaldehyde acid followed by heating at 110 °C.

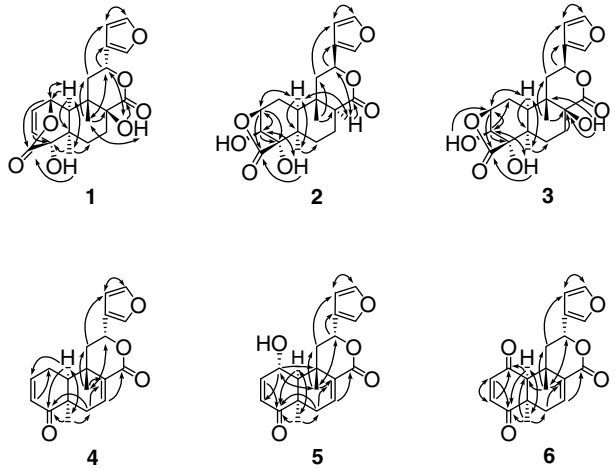

Chart 2. Major HMBC (→) and NOESY (↔) correlations of compound **1**–**6**.

5.2. Plant material

The whole plant of *F. tinctoria* (Menispermaceae) was collected near Hanoi in Vietnam on July 12 in 2004. The plant material was identified and authenticated by Assoc. Prof. Dr. Vu Xuan Phuong, Institute of Ecology and Biological Resources, Vietnamese Academy of Science and Technology. A voucher specimen (FT04011) has been deposited in the herbarium of the Institute of Ecology and Biological Resources, Vietnamese Academy of Science and Technology, Hanoi, Vietnam.

5.3. Extraction and isolation

The air-dried and powdered whole plant of *F. tinctoria* (10 kg) was extracted with MeOH (6× 20 L) under reflux for 8 h. The filtrate was concentrated under reduced pressure to obtain a dark crude extract (FTM, 1.6 kg), which was suspended in H_2O, then partitioned with $CHCl_3$ and *n*-BuOH to afford $CHCl_3$ layer (380 g), *n*-BuOH layer (FTB, 840 g), and H_2O layer (FTW, 335 g), respectively. The condensed $CHCl_3$ soluble was further subjected to alkaloids extraction using 5% HCl and 5% NH_4OH to yield the crude alkaloid extract (FTA, 40 g) and non-alkaloid extract (FTC, 340 g).

The crude alkaloid extract (40 g) was subjected to chromatography on a silica gel column using $CHCl_3/CH_3OH$ (39:1–0:1) as step gradient mixtures as eluents to afford six fractions. Fraction 2 was subjected to silica gel column chromatography using benzene/acetone (39:1–0:1) as step gradient mixtures as eluents to afford five fractions (2.1–2.5). Subfraction 2.1 formed a yellow precipitate with CH_3OH to yield **21** (21.2 mg). Fraction 3 was repeatedly column chromatographed over silica gel and eluted with *n*-hexane/ethyl acetate (1:2) to yield **25** (5.4 mg).

Fraction 4 was separated using a silica gel column, with $CHCl_3$/CH_3OH (29:1) as step gradient mixtures as eluents, to afford five fractions (4.1–4.5). Purification of the fraction 4.2 by silica gel with benzene/acetone (4:1) afforded four subfractions (4.2.1–4.2.4). Subfraction 4.2.1 was further separated by silica gel column chromatography and then further purified by preparative TLC with diisoproyl ether/CH_3OH (9:1) to obtain **26** (1.1 mg). Subfraction 4.2.3 was purified using a silica gel column with benzene-acetone (2:1) as solvent to give **24** (8.1 mg). Fraction 5 was subjected to silica gel column chromatography using $CHCl_3$/CH_3OH (29:1–0:1) as step gradient mixtures as eluents to afford seven fractions (5.1–5.7). Subfraction 5.4 was further separated by a silica gel column chromatography with $CHCl_3$/CH_3OH (5:1) as eluent to give **17** (200.0 mg) and **18** (12.3 mg).

The non-alkaloid extract (340 g) was subjected to silica gel column chromatography and eluted with gradients of $CHCl_3$ and CH_3OH (29:1–0:1) to obtain eight fractions. Fraction 1 was separated on a silica gel column chromatography using $CHCl_3$/CH_3OH (39:1–0:1) as step gradient mixtures as eluents to afford five fractions (1.1–1.5). Separation of fraction 1.3 on a silica gel column chromatography eluted with *n*-hexane/EtOAc (15:1) yielded **43** (2.9 mg). Purification of the fraction 1.4 by silica gel column chromatography with *n*-hexane/EtOAc (8:1) afforded **33** (1.2 mg). Fraction 2 was subjected to silica gel column chromatography using *n*-hexane/EtOAc (15:1–0:1) as step gradient mixtures as eluents to afford six fractions (2.1–2.6). Fraction 2.4 occurring the white precipitate was recrystallized by $CHCl_3$ to yield mixture of **40** and **41** (2.9 g). The remaining fraction 2.4 was further separated by a silica gel column chromatography and then further purified by preparative TLC with *n*-hexane/EtOAc (8:1) to obtain **31** (3.3 mg). Purification of the fraction 2.5 by a silica gel column chromatography with *n*-hexane/EtOAc (5:1) yielded **8** (170.1 mg). Subfraction 2.6 was separated on a silica gel column chromatography using benzene/acetone (19:1–0:1) as step gradient mixtures as eluents to afford five fractions (2.6.1–2.6.5). Subfraction 2.6.2 was further separated by silica gel column chromatography with benzene/acetone (29:1) to four fractions (2.6.2.1–2.6.2.4). Purification of subfraction 2.6.2.2 on a silica gel column chromatography with *n*-hexane/acetone (3:1) yielded four fractions (2.6.2.2.1–2.6.2.2.4). Subfraction 2.6.2.2.4 was separated on a silica gel column chromatography with *n*-hexane/EtOAc (3:1) to afford five fractions (2.6.2.2.4.1–2.6.2.2.4.5). Subfraction 2.6.2.2.4.5 occurring the white precipitate was recrystallized by CH_3OH to yield **4** (176.0 mg). Purification of subfraction 2.6.2.2.4.1 was further purified by preparative TLC with *n*-hexane/acetone (6:1) to furnish **29** (3.2 mg) and **31** (3.7 mg). Subfraction 2.6.2.2.4.3 was further purified by HPLC with CH_3OH/H_2O (50:50) to give **6** (0.8 mg). Subfraction 2.6.2.3 was separated on a silica gel column chromatography and further purified by preparative TLC with benzene/acetone (9:1) to obtain **30** (1.7 mg) and **45** (1.4 mg). Purification of subfraction 2.6.3 on a silica gel column chromatography and further separated by preparative TLC with benzene/diisopropyl ether (4:1) to obtain **44** (1.1 mg). Separation of fraction 2.6.4.3 was repeatedly column chromatographed on silica gel and further isolated by preparative TLC with benzene-acetone (9:1) to obtain **5** (3.1 mg). Recrystallization of fraction 3 with CH_3OH afforded **11** (52.3 g). Fraction 4 occurring the white precipitate recrystallized by CH_3OH to yield the pure **9** (43.2 g). Furthermore, the remaining fraction 4 was further separated using a silica gel column chromatography and eluted with gradients of $CHCl_3$ and CH_3OH (15:1–0:1) to afford four fractions (4.1–4.4). Purification of the fraction 4.3 by a silica gel column chromatography with benzene-acetone (9:1) afforded five fractions (4.3.1–4.3.5). Subfraction 4.3.1 was separated on a silica gel column chromatography and further purified by preparative TLC with *n*-hexane/EtOAc/acetone (5:1:1) to obtain **28** (1.3 mg). Fraction 4.3.2 yielded the white precipitate and was recrystallized by CH_3OH to yield the pure **13** (313.0 mg). Subfraction 4.3.3 was further separated on silica gel column chromatography with *n*-hexane/EtOAc (1:2) to afford **1** (20.5 mg). Fraction 5 was divided into four fractions (5.1–5.4) by a column chromatography on silica gel with gradient mixtures of *n*-hexane/EtOAc (4:1–0:1). Fraction 5.3 produced the white precipitate recrystallized by CH_3OH to yield **2** (1.2 g). Fraction 6 was purified on a silica gel column chromatography eluting with gradients of *n*-hexane/EtOAc (1:1–0:1) to afford five fractions (6.1–6.4). Fraction 6.3 was further purified on a silica gel column chromatography with *n*-hexane-acetone (3:1) to furnish **3** (15.2 mg). Fraction 7 was separated on a silica gel column chromatography using EtOAc/CH_3OH (1:1–0:1) as step gradient mixtures as eluents to afford four fractions (7.1–7.4). Purification of fraction 7.1 on a silica gel column chromatography using $CHCl_3$/CH_3OH (9:1) yielded the white precipitate and was recrystallized by CH_3OH to obtain **42** (120.0 mg).

The *n*-BuOH layer (840 g) was chromatographed over reversed-phase Diaion HP-20 gel using H_2O/CH_3OH gradients, and afforded six fractions. Fraction 1 formed a yellow precipitate with CH_3OH to yield **17** (140.3 g). Fraction 2 was subjected to silica gel column chromatography using $CHCl_3$/CH_3OH (5:1–0:1) as step gradient mixtures as eluents to afford five fractions (2.1–2.5). Purification of the fraction 2.1 by silica gel with $CHCl_3$/CH_3OH (3:1) afforded five subfractions (2.1.1–2.1.5). Subfraction 2.1.5 was further separated by aluminum oxide with $CHCl_3$/CH_3OH (4:1) as eluent to obtain **18** (120.5 mg) and **19** (2.1 mg). Fraction 2.3 was chromatographed on a RP-18 column and eluted with H_2O and CH_3OH to give five subfractions (2.3.1–2.3.5). Subfraction 2.3.2 was purified using a silica gel column with $CHCl_3$/CH_3OH (4:1) as solvent, to give **20** (10.3 mg). Separation of subfraction 2.3.4, with EtOAc/CH_3OH (8:1) as eluent yielded **38** (30.2 mg). Fraction 3 produced a white precipitate with CH_3OH to yield pure **39** (20.3 g). In turn, the filtrate of fraction 3 was separated on a silica gel column and eluted with $CHCl_3$ and CH_3OH (9:1–0:1), as step gradient mixtures, to afford eight fractions (3.1–3.8). Subfraction 3.1 was separated on a silica gel column chromatography and further purified by preparative TLC with $CHCl_3$/CH_3OH (5:1:1) to obtain **34** (3.4 mg), **35** (2.3 mg), **36** (1.1 mg), and mixture of **22** and **23** (1.4 mg). Fraction 3.7 was rechromatographed over a RP-18 column and eluted with mixture of H_2O and CH_3OH to yield **12** (223 mg) and **10** (120 mg). Fraction 3.8 was further purified using a Sephadex LH-20 column, eluted with H_2O and CH_3OH as eluents, to yield **15** (8.2 mg). Fraction 4 was separated using a silica gel column, with diisopropyl ether/CH_3OH (5:1) as eluent, to afford seven fractions (4.1–4.7). Subfraction 4.1 was separated on a silica gel column chromatography and further purified by preparative TLC with diisoproply ether/CH_3OH (15:1) to obtain **27** (0.9 mg). Purification of subfraction 4.4 with a silica gel column, using EtOAc/CH_3OH (15:1) as eluent, yielded **37** (7.2 mg). Separation of fraction 4.6 with a silica gel column, using EtOAc/CH_3OH (15:1) as eluent, yielded **14** (13.2 mg).

The H_2O layer (335 g) was chromatographed over a reversed-phase Diaion HP-20 gel, using H_2O/CH_3OH step gradient mixtures as eluents, and afforded five fractions. Fraction 3 was further chromatographed on a Sephadex LH-20 column, eluted with H_2O and CH_3OH, and then purified by preparative TLC with $CHCl_3$/CH_3OH (4:1) to yield **16** (3.6 mg). Fraction 4 was chromatographed on a RP-18 column, eluted with H_2O and CH_3OH, and then further purified by preparative TLC with $CHCl_3$/CH_3OH (5:1) to obtain **7** (3.1 mg).

5.3.1. *epi*-8-Hydroxycolumbin (1)

Colorless needle; mp 210–211 °C; α_D^{25} +110.0 (*c* 0.06, CH_3OH); UV (MeOH) λ_{max} (log ε) 230 (3.51), 205 (sh) (3.90) nm; IR (KBr) γ_{max}

3480, 2967, 1741, 1721, 1633, 1504 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 2; EIMS m/z 374 $[M]^+$ (6), 204 (23), 124 (100); HREIMS *m/z* 374.1364 (calcd for $C_{20}H_{22}O_7$, 374.1366).

5.3.2. Fibrauretin B (2)

Colorless needle; mp 266–267 °C; α_D^{25} +10.5 (*c* 0.20, CH_3OH); UV (MeOH) λ_{max} (log ε) 209 (3.35) nm; IR (KBr) γ_{max} 3563, 2940, 1781, 1710, 1602, 1506 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz), see Table 1; ^{13}C NMR (DMSO-d_6, 75 MHz), see Table 2; FABMS *m/z* 377 $[M+H]^+$ (41); HRFABMS *m/z* 377.1601 ($[M+H]^+$, calcd for $C_{20}H_{25}O_7$, 377.1600).

5.3.3. Fibrauretin C (3)

Colorless needle; mp 187–188 °C; α_D^{25} +45.6 (*c* 0.04, CH_3OH); UV (MeOH) λ_{max} (log ε) 209 (3.41) nm; IR (KBr) γ_{max} 3466, 2997, 1766, 1725, 1608, 1498 cm^{-1}; ^{1} H NMR (DMSO-d_6, 500 MHz), see Table 2; ^{13}C NMR (DMSO-d_6, 75 MHz), see Table 3; FABMS *m/z* 393 $[M+H]^+$ (5); HRFABMS *m/z* 393.1553 ($[M+H]^+$, calcd for $C_{20}H_{27}O_9$, 393.1549).

5.3.4. Fibrauretin D (4)

Colorless needle; mp 190–191 °C; α_D^{25} +115.0 (*c* 0.08, CH_3OH); UV (MeOH) λ_{max} (log ε) 216 (4.36) nm; IR (KBr) γ_{max} 3133, 2957, 1702, 1667, 1506 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 2; EIMS *m/z* 312 $[M]^+$ (100), 297 (41), 269 (52); EIMS *m/z* 312.1359 (calcd for $C_{19}H_{20}O_4$, 312.1362).

5.3.5. Fibrauretin E (5)

colorless needle; mp 94–95 °C; α_D^{25} +23.1 (*c* 0.02, CH_3OH); UV (MeOH) λ_{max} (log ε) 230 (sh) (4.96), 211 (5.23) nm; IR (KBr) γ_{max} 3425, 2963, 1710, 1681, 1645, 1504 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 2; EIMS *m/z* 328 $[M]^+$ (62), 295 (55), 216 (71); EIMS *m/z* 328.1313 (calcd for $C_{19}H_{20}O_5$, 328.1311).

5.3.6. Fibrauretin F (6)

Yellow syrup; α_D^{25} +136.0 (*c* 0.1, CH_3OH); UV (MeOH) λ_{max} (log ε) 217 (3.42) nm; IR (KBr) γ_{max} 3021, 2971, 1712, 1679, 1647, 1457 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2; EIMS *m/z* 326 $[M]^+$ (84), 215 (66); EIMS *m/z* 326.1157 (calcd for $C_{19}H_{18}O_5$, 326.1154).

5.4. Anti-inflammatory activities

5.4.1. Determination of anti-inflammation—Carrageenan-edema test

Male ICR mice, weighing about 30–35 g, were divided into several groups. Different concentrations (50, 100, 200 mg/kg) of *F. tinctoria* crude extracts (FTM, FTA, FTC, FTB, and FTW) were intraperitoneally given 30 min before carrageenan injection (1.0%, 50 μl). Two groups of mice were given normal saline as control group and indomethacin (4 mg/kg) as positive one, respectively. After carrageenan was injected into the planar surface of left hind paw, the swelling of the foot was measured. The changes in the swelling of the foot were detected every 30 min for 5 h. The determination of anti-inflammation of compounds **1**–**16** were applied as above statement. Different concentrations (50, 100 mg/kg) of compounds **1**–**16** were intraperitoneally given 30 min before carrageenan injection, and normal-saline group as control group.

5.4.2. Reduction of NO production from macrophages

Cell line and culture condition. Mouse macrophage cell line (RAW 264.7) was obtained from Bioresource Collection and Research Center (Hsinchu, Taiwan). The cells were cultured in 10-cm dish with RPMI (Gibco Laboratories, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS) (HyClone, Logan, UT, USA) at 37 °C under a 5% CO_2 atmosphere.

NO assay

The production of NO was determined by measuring the quantity of nitrite in the supernatant from the cells culture under different conditions by the Griess method,[37] using a standard curve constructed with nitrite ranging from 1 to 100 μM. *Escherichia coli* LPS (Sigma) at concentration of 2 μg/ml was used as a positive control.

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