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FURANODITERPENE GLUCOSIDES FROM *FIBRAUREA TINCTORIA*

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Key Word Index—*Fibraurea tinctoria*; Menispermaceae; furanoditerpenes; diterpeneglucosides; NMR; tinophylloloside; fibleucinoside; fibraurinoside; bitter principle.

Abstract—Two known furanoditerpenes, fibleucin and fibraurin, and three new furanoditerpene glucosides, tinophylloloside, fibleucinoside and fibraurinoside were isolated as bitter principles from the non-alkaloidal fraction of *Fibraurea tinctoria*. The structures were established by chemical and spectroscopic methods.

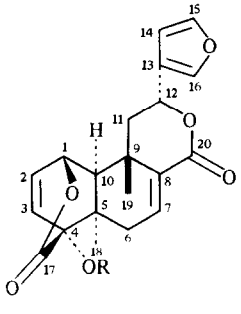
INTRODUCTION

The rhizomes of *Fibraurea tinctoria* Lour. are one of the important crude drugs in Chinese medicine, and have been used from ancient times as an antipyretic, antidote, diuretic and cathartic [1, 2]. Studies on the chemical constituents of *Fibraurea* plants afforded bitter-tasting alkaloids such as palmatine, jatrorrhizine and columbamine [1, 2], and diterpenes such as fibleucin (**1**) and fibraurin (**2**) [3, 4]. We have now isolated a further three novel bitter-tasting diterpene glucosides which we have named tinophylloloside (**3**), fibleucinoside (**4**) and fibraurinoside (**5**). This paper describes the structural elucidation of these new bitter principles.

RESULTS AND DISCUSSION

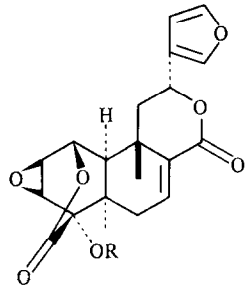
The aqueous solution of the methanol extract from the rhizomes of *F. tinctoria* was partitioned successively with chloroform and ethyl acetate, and each extract fractionated into its bitter-tasting components. When the chloroform extract was subjected to silica gel column chromatography, compounds **1** and **2** were obtained. Reversed column chromatography of the ethyl acetate extract gave three new bitter principles (**3**–**5**). Compounds **1** and **2** were identified as fibleucin and fibraurin respectively by comparison of their physical and spectral properties with those reported in the literature for these two diterpenes [3–7]. The structures were also supported by the ^1H and ^{13}C NMR spectral data (Tables 1 and 2). Each signal was assigned by known chemical shift rules, decoupling experiments and off-resonance decoupling techniques, etc. [8, 9].

Tinophylloloside (**3**), $C_{27}H_{36}O_{11}$, colourless powder, mp 155–158°, $[\alpha]_D^{21}$ −19.2° showed an $[M+1]^+$ ion at *m/z* 537 on secondary ion mass spectrometry (SIMS). It IR spectrum showed the presence of hydroxyl groups (3440 cm^{-1}), a furan ring (3160, 1510, 875 cm^{-1}), a δ-lactone and an α,β-unsaturated ester carbonyl group (1730 and 1715 cm^{-1}). The ^1H NMR spectrum suggested the presence of β-substituted furan ring [δ6.51 (1H, *dd*, *J* = 0.7 and 1.7 Hz), 7.51 (1H, *t*, *J* = 1.7 Hz) and 7.59 (1H, *t*, *J* = 0.7 Hz)], two tertiary methyl groups (δ0.97 and 1.41), one methoxy-methyl group (δ3.75) and one olefinic proton [δ6.45 (1H, *d*, *J* = 3.8 Hz)]. The signal pattern was similar to that of tinophyllone (**6**) [10, 11], however, the olefinic proton at δ6.45 was coupled (*J* = 3.8 Hz) with the proton at δ4.69 (1H, *dt*, *J* = 3.8 and 8.4 Hz), which was further coupled (*J* = 8.4 Hz) with the C-1 protons at δ2.31. In addition glucosyl proton signals [δ3.15–3.87 and



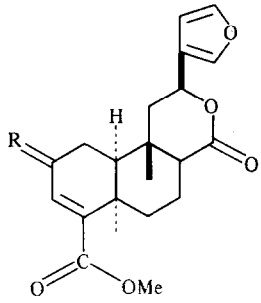
R

1 H Fibleucin
4 O - Glc Fibleucinoside



R

2 H Fibraurin
5 O - Glc Fibraurinoside



R

3 H
O - Glc Tinophylloloside
6 O Tinophyllone

Table 1. ^{1}H NMR spectral data of the furanoditerpenes **1**–**5** [400 MHz, DMSO-d_6 (**1** and **2**) or CD_3OD (**3**–**5**), TMS as int. standard J (Hz) in parentheses]

H	1	2	3	4	5
1	5.36 (*dd*, 5.0, 2.0 Hz)	5.08 (*dd*, 3.0, 0.8 Hz)	1.26–1.40 (*m*)	5.37 (*dd*, 5.1, 1.5 Hz)	5.09 (*d*, 2.9 Hz)
			2.23–2.39 (*m*)		
2	6.53 (*dd*, 9.0, 5.0 Hz)	3.85 (*dd*, 4.5, 3.0 Hz)	4.69 (*td*, 8.4, 3.8 Hz)	6.64 (*dd*, 8.4, 5.1 Hz)	3.89 (*dd*, 4.5, 2.9 Hz)
3	6.34 (*dd*, 9.0, 2.0 Hz)	3.65 (*dd*, 4.5, 0.8 Hz)	6.45 (*d*, 3.8 Hz)	6.85 (*dd*, 8.4, 1.5 Hz)	4.26 (*d*, 4.5 Hz)
6	1.87 (*dd*, 16.0, 3.0 Hz)	1.64 (*dd*, 16.0, 2.5 Hz)	1.26–1.40 (*m*)	2.10 (*dd*, 16.3, 2.8 Hz)	1.98 (*dd*, 16.3, 3.0 Hz)
	2.42 (*dd*, 16.0, 8.0 Hz)	2.32 (*dd*, 16.0, 8.0 Hz)	2.57 (*dd*, 14.7, 3.0 Hz)	2.73 (*dd*, 16.3, 8.3 Hz)	2.59 (*dd*, 16.3, 8.4 Hz)
7	7.26 (*dd*, 8.0, 3.0 Hz)	7.23 (*dd*, 8.0, 2.5 Hz)	1.75 (*dd*, 13.4, 2.9 Hz)	7.43 (*dd*, 8.3, 2.8 Hz)	7.41 (*dd*, 8.4, 3.0 Hz)
			2.23–2.39 (*m*)		
8			2.78 (*dd*, 11.4, 2.9 Hz)		
10	1.67 (*s*)	1.77 (*s*)	1.90 (*br d*, 4.7 Hz)	1.77 (*s*)	1.91 (*s*)
11	1.98 (*dd*, 14.0, 12.0 Hz)	1.95 (*dd*, 14.0, 12.0 Hz)	1.99 (*dd*, 14.1, 11.4 Hz)	2.00 (*dd*, 14.0, 12.0 Hz)	2.00 (*dd*, 14.0, 12.0 Hz)
	2.34 (*dd*, 14.0, 2.0 Hz)	2.30 (*dd*, 14.0, 2.8 Hz)	2.14 (*dd*, 14.1, 5.8 Hz)	2.40 (*dd*, 14.0, 2.2 Hz)	2.37 (*dd*, 14.0, 1.8 Hz)
12	5.65 (*dd*, 12.0, 2.0 Hz)	5.64 (*dd*, 12.0, 2.8 Hz)	5.50 (*dd*, 11.4, 5.8 Hz)	5.72 (*dd*, 12.0, 2.2 Hz)	5.72 (*dd*, 12.0, 1.8 Hz)
14	6.62 (*dd*, 1.5, 1.0 Hz)	6.62 (*dd*, 1.5, 1.0 Hz)	6.51 (*dd*, 1.7, 0.7 Hz)	6.57 (*dd*, 1.8, 0.8 Hz)	6.57 (*dd*, 1.8, 0.8 Hz)
15	7.66 (*t*, 1.5 Hz)	7.66 (*t*, 1.5 Hz)	7.51 (*t*, 1.7 Hz)	7.51 (*t*, 1.8 Hz)	7.52 (*t*, 1.8 Hz)
16	7.74 (*dd*, 1.5, 1.0 Hz)	7.75 (*dd*, 1.5, 1.0 Hz)	7.59 (*t*, 0.7 Hz)	7.63 (*t*, 0.7 Hz)	7.63 (*t*, 0.8 Hz)
18	1.18 (*s*)	1.18 (*s*)	1.41 (*s*)	1.30 (*s*)	1.30 (*s*)
19	0.92 (*s*)	1.08 (*s*)	0.97 (*s*)	1.12 (*s*)	1.26 (*s*)
Ac			3.75 (*s*)		
anomeric H			4.45 (*d*, 7.8 Hz)	4.73 (*d*, 7.2 Hz)	4.82 (*d*, 7.3 Hz)

Table 2. ^{13}C chemical shifts of furanoditerpenes **1**–**5** [101 MHz, DMSO-d_6 (**1** and **2**) or CD_3OD (**3**–**5**), TMS as int. standard]

C	1	2	3	4	5
1	73.72 (*d*)	69.63 (*d*)[c]	27.07 (*t*)	75.45 (*d*)	71.36 (*d*)[i]
2	130.57 (*d*)	49.37 (*d*)	72.93 (*d*)	132.19 (*d*)	50.18 (*d*)
3	137.03 (*d*)	51.44 (*d*)	138.00 (*d*)	133.61 (*d*)	51.45 (*d*)
4	80.17 (*s*)	79.88 (*s*)	142.60 (*s*)	87.37 (*s*)	87.70 (*s*)
5	35.59 (*s*)	35.17 (*s*)	37.70 (*s*)	37.22 (*s*)	36.80 (*s*)
6	31.06 (*t*)	31.07 (*t*)	36.54 (*t*)	32.57 (*t*)	32.58 (*t*)
7	142.46 (*d*)	141.82 (*d*)	21.05 (*t*)	145.01 (*d*)	144.39 (*d*)
8	134.31 (*s*)	133.39 (*s*)	49.00 (*d*)	135.70 (*s*)	134.75 (*s*)
9	42.36 (*s*)	44.75 (*s*)	38.73 (*s*)	45.35 (*s*)	47.59 (*s*)
10	55.88 (*d*)	54.01 (*d*)	56.59 (*d*)	57.78 (*d*)	56.62 (*d*)
11	41.92 (*t*)	41.95 (*t*)	46.19 (*t*)	44.26 (*t*)	43.96 (*t*)
12	69.58 (*d*)	70.53 (*d*)[c]	71.60 (*d*)	71.42 (*d*)	72.29 (*d*)[i]
13	124.97 (*s*)	125.00 (*s*)	126.14 (*s*)	126.47 (*s*)	126.43 (*s*)
14	109.05 (*d*)	108.95 (*d*)	109.84 (*d*)	109.67 (*d*)	109.63 (*d*)
15	140.44 (*d*)	140.31 (*d*)	141.37 (*d*)	141.50 (*d*)	141.49 (*d*)
16	143.80 (*d*)	143.77 (*d*)	145.03 (*d*)	145.01 (*d*)	145.04 (*d*)
17	174.49 (*s*)[a]	171.33 (*s*)[d]	177.48 (*s*)[f]	174.87 (*s*)[g]	171.68 (*s*)[j]
18	26.36 (*q*)[b]	24.93 (*q*)[e]	34.04 (*q*)	27.18 (*q*)[h]	25.82 (*q*)[k]
19	20.31 (*q*)[b]	20.68 (*q*)[e]	23.54 (*q*)	21.36 (*q*)[h]	21.67 (*q*)[k]
20	163.07 (*s*)[a]	163.02 (*s*)[d]	170.18 (*s*)[f]	166.22 (*s*)[g]	166.15 (*s*)[j]
1′			103.66 (*d*)	101.54 (*d*)	101.80 (*d*)
2′			75.19 (*d*)	75.31 (*d*)	75.34 (*d*)
3′			78.21 (*d*)	78.41 (*d*)	78.41 (*d*)
4′			71.90 (*d*)	72.24 (*d*)	72.19 (*d*)
5′			78.03 (*d*)	78.15 (*d*)	78.41 (*d*)
6′			62.93 (*t*)	62.71 (*t*)	62.83 (*t*)
Ac			52.23 (*q*)		

[a–k]Assignments may be reversed.

δ4.45 (1H, *d*, J = 7.8 Hz, anomeric H)] were present. Hydrolysis of **3** gave glucose (identified by GLC). From the above results, it is reasonable to conclude that **3** was tinophyllol 2-β-glucoside. This structure was also supported from the ^{13}C NMR data (Table 2).

Fibleucinoside (**4**), $C_{26}H_{30}O_{11}$, colourless needles, mp 187.5–189.0°, $[\alpha]_D^{21}$ +1.0°; SIMS *m/z*: 519 $[M+1]^+$, showed hydroxyl (3420 cm^{-1}), two δ-lactone carbonyl (1760 and 1710 cm^{-1}) and furan ring (3160, 1620, 1510 and 875 cm^{-1}) bands in the IR spectrum. Most of the ^1H and ^{13}C NMR signals of **4** were the same as to those of **1**, however, the C-4 carbon signal of **1** at δ80.17 was shifted downfield to δ87.37 and six additional carbon signals due to glucose were present. The coupling constant of the anomeric proton at δ4.73 was 7.2 Hz. Hydrolysis of **4** furnished compound **1** and glucose, which were identified by TLC and GLC. On the basis of the above data, **4** was determined as fibleucin 4-β-glucoside.

Fibraurinoside (**5**), $C_{26}H_{30}O_{12}$, colourless prism, mp 212–214.5°, $[\alpha]_D^{21}$ −8.7°; SIMS *m/z*: 535 $[M+1]^+$. Its IR spectrum was similar to that of **4** and indicated the presence of a furanoditerpene glucoside. The ^1H and ^{13}C NMR spectra data due to the B and C furan rings and glucose moiety of **5** were similar to those of **4**, however, the olefinic proton and carbon signals of ring A at δ6.64, 6.85, 132.19 and 133.61 in **4** were shifted to δ3.89, 4.26, 50.18 and 51.45 in **5**. These findings suggested that **5** was 2,3-epoxy-fibleucinoside. Hydrolysis of **5** gave compound **2** and glucose (TLC or GLC). When the ^{13}C chemical shifts of **5** were compared with those of **2**, the C-4 signal of **2** at δ79.88 was found to have been shifted downfield to δ87.70 in **5**. Consequently, **5** was confirmed to be fibraurin 4-β-glucoside.

The new furanoditerpene glucosides, **3**, **4** and **5**, were much more bitter than the aglycones, **1** and **2**.

EXPERIMENTAL

General procedures. Mps (micro melting point apparatus): uncorr; ^1H (400 MHz) and ^{13}C NMR (101 MHz): DMSO-d_6 or CD_3OD, with TMS as the int. standard; CC: silica gel (Wakogel C-200 or Kieselgel 60) at amounts equivalent to 50–100 times the sample amount; prep. HPLC: glass 22 mm i.d. × 30 cm CIG column (Kusano Scientific Co., Tokyo) packed with Wakogel LC-50H (50 μm, silica gel) or RP-18 (25–40 μm); TLC: 0.25 mm silica gel ($60F_{254}$, Merck) or RP-18 plates (F_{254s}, Merck). Spots were detected by UV light (254 nm) and Ehrlich reagent.

Plant material. The rhizomes of *F. tinctoria* used in this experiment were purchased in Hong Kong, July 1983. A small number of them are kept in our laboratory. The material was identified as *F. tinctoria* by comparing the TLC pattern of a MeOH extract of the rhizomes with those of authentic herbarium specimens of *F. tinctoria* and *Arcangelisia flava*, which had been cultivated at Tsukuba Medicinal Plant Research Station (Botanical garden director, Dr. M. Satake), National Institute of Hygienic Science, Japan. The TLC systems used were silica gel C_6H_6–EtOAc–*n*-PrOH–MeOH–$EtNH_2$ (8:4:2:1:1) [12] MeOH–H_2O–NH_4OH 25% (8:1:1), EtOAc–*iso*-PrOH–H_2O

(40:40:25) and *n*-BuOH–AcOH–H_2O (4:1:1) [13]. The spots were detected under UV light (254 and 366 nm) and by spraying with Dragendorff's reagent.

Extraction and isolation. The crude drug (1.0 kg) was cut into pieces and extracted with MeOH (2 × 3 l.). The following isolation was made with the guidance of the bitter taste. The concd MeOH extract (91.2 g) was partitioned (× 2) between H_2O (1.5 l.) and $CHCl_3$ (1.5 l.) and then (× 2) between H_2O and EtOAc (1.5 l.). The $CHCl_3$ extract (19.6 g) was subjected to CC on silica gel eluted with $CHCl_3$–MeOH. The fraction eluted with $CHCl_3$–MeOH (99:1) was subjected to HPLC on silica gel eluted with *n*-hexane–EtOAc (1:1). This gave 634 mg of **1**. Repeated recrystallizations of the $CHCl_3$–MeOH (49:1) eluate furnished 1031 mg of **2**. The EtOAc extract (2.3 g) was subjected to HPLC on silica gel using $CHCl_3$–MeOH (9:1) and C_6H_6–EtOAc–MeOH (3:2:1), and then HPLC on RP-18 using MeOH–H_2O (7:3). When the procedures were repeated a few times, 26.9 mg of **3**, 32.7 mg of **4** and 48.6 mg of **5** were isolated.

Compound **1**. Colourless prisms, mp 172–174° (from $CHCl_3$–MeOH), $[\alpha]_D^{19}$ −5.2° (*c* 0.5; C_5H_5N); Calc. for $C_{20}H_{20}O_6 \cdot 1/5H_2O$: C, 66.73; H, 5.71; O, 27.55. Found: C, 66.92; H, 5.66; O, 27.42%; EIMS *m/z* (rel. int.): no parent ion, 312 (6), 279 (3), 218 (43), 203 (100), 159 (27), 108 (44), 81 (33); IR ν_{max}^{KBr} cm^{-1}: 3570 (OH), 3160, 1610, 1510, 875 (furan bands), 1740, 1720 (C=O), 1635 (C=C); UV λ_{max}^{EtOH} nm (ε): 208 (10500), 230 (7350).

Compound **2**. Colourless prisms, mp 282.0–282.5° (dec. from MeOH), $[\alpha]_D^{19}$ −32.8° (*c* 0.5; C_5H_5N); EIMS *m/z* (rel. int.): 372 $[M]^+$ (54), 327 (2), 261 (9), 229 (8), 203 (22), 133 (76), 94 (100), 81 (59); HRMS: Calc. 372.1208. Found 372.1235; IR ν_{max}^{KBr} cm^{-1}: 3490 (OH), 3160, 1605, 1510, 880 (furan bands), 1770, 1695 (C=O), 1635 (C=C); UV λ_{max}^{EtOH} nm (ε): 209 (9300), 230 (6420).

Compound **3**. Colourless powder, mp 135–138° (from H_2O), $[\alpha]_D^{21}$ −19.2° (*c* 0.25, MeOH); Calc. for $C_{27}H_{36}O_{11} \cdot H_2O$: C, 58.47; H, 6.90; O, 34.61. Found: C, 58.67; H, 6.73; O, 34.60%; SIMS *m/z*: 537 $[M+1]^+$; EIMS *m/z* (rel. int.): no parent ion, 374 (98), 358 (39), 342 (100); IR ν_{max}^{KBr} cm^{-1}: 3440 (br OH), 3160, 1510, 875 (furan bands), 1730, 1715 (C=O); UV λ_{max}^{MeOH} nm (ε): 210 (10620).

Compound **4**. Colourless needles, mp 187.5–189.0° (from H_2O), $[\alpha]_D^{21}$ +1.0° (*c* 0.2; MeOH); Calc. for $C_{26}H_{30}O_{11} \cdot H_2O$: C, 58.20; H, 6.01; O, 35.78. Found: C, 58.15; H, 5.96; O, 35.89%; SIMS *m/z*: 519 $[M+1]^+$; EIMS *m/z* (rel. int.): no parent ion, 312 (8), 297 (3), 203 (23), 201 (28), 131 (37), 108 (100), 94 (33), 81 (48); IR ν_{max}^{KBr} cm^{-1}: 3420 (br OH), 3160, 1620, 1510, 875 (furan bands), 1760, 1710 (C=O), 1635 (C=C); UV λ_{max}^{MeOH} nm (ε): 207 (9350), 229 (6110).

Compound **5**. Colourless prisms, mp 212.0–214.5° (from $CHCl_3$–MeOH), $[\alpha]_D^{21}$ −8.7° (*c* 0.28; MeOH); Calc. for $C_{26}H_{30}O_{12} \cdot 3/4CHCl_3$: C, 51.48; H, 4.96; O, 30.76; Cl, 12.78. Found: C, 51.49; H, 5.05; O, 30.87; Cl, 12.59%; SIMS *m/z*: 535 $[M+1]^+$; EIMS *m/z* (rel. int.): 372 (48), 327 (3), 261 (12), 229 (8), 203 (24), 133 (91), 94 (100), 81 (79); IR ν_{max}^{KBr} cm^{-1}: 3420 (br OH), 3160, 1510, 878 (furan bands), 1775, 1703 (C=O), 1635 (C=C); UV λ_{max}^{MeOH} nm (ε): 212 (8710), 230 (6760).

Hydrolysis of furanoditerpene glucosides. The sample (3 mg) was hydrolysed with 1 ml 10% HCl at 90° for 4 hr, then cooled. The reaction mixture was concd under a stream of N_2 and the residue was employed for TLC and GLC. TLC of the hydrolysate of **4** on silica gel developed with $CHCl_3$–MeOH (19:1) showed the presence of **1** (R_f 0.75) whilst **5** gave **2** (R_f 0.70). TLC of the hydrolysates of **3**, **4** and **5** on silica gel developed with *n*-BuOH–Me_2CO–H_2O (4:5:1) gave glucose (R_f 0.34). The residue was trimethylsilylated and subjected to GLC, which showed the presence of glucose (GLC conditions: 1.5% silicone SE-30, 3 mm × 2 m, column temp. 150°, N_2 1.0 kg/cm^2; R_t 10.4 min).

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