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The Constituents of *Ledebouriella seseloides* WOLFF. I. Structures of Three New Chromones

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Three new chromones (**8**, **9**, and **12**), named 3′-*O*-angeloylhamaudol, ledebouriellol, and 4′-*O*-β-D-glucosyl-5-*O*-methylvisamminol, respectively, were isolated from the root and rhizoma of *Ledebouriella seseloides* WOLFF (Umbelliferae), together with five known coumarins (**1**—**5**) and six known chromones (**6**, **7**, **10**, **11**, **13** and **14**). The structures of **8**, **9**, and **12** were elucidated as (3*S*)-3,4-dihydro-5-hydroxy-3-(2-methyl-2-butenyl)oxy-2,2,8-trimethyl-2*H*,6*H*-benzo[1,2-*b*: 5,4-*b*′]dipyran-6-one, (3*S*)-3,4-dihydro-2,2-dimethyl-5-hydroxy-8-hydroxymethyl-3-(2-methyl-2-butenyl)oxy-2*H*,6*H*-benzo[1,2-*b*: 5,4-*b*′]dipyran-6-one, and (2*S*)-2,3-dihydro-2-(1-β-D-glucopyranosyloxy-1-methylethyl)-4-methoxy-7-methyl-5*H*-furo[3,2-*g*][1]benzopyran-5-one, respectively, by chemical and spectral studies.

Keywords——*Ledebouriella seseloides*; Umbelliferae; chromones; coumarins; dihydropyranochromones; dihydrofuranochromones

The dried root and rhizoma of *Ledebouriella seseloides* WOLFF (Syn: *Siler divaricatum* BENTH. *et* HOOK, *Trinia seseloides* LEDEB., *Trinia dahurica* TURCZ., *Stenocoelium divaricatum* TURCZ., *Saposhinkovia divaricata* SCHISCHK.) (Umbelliferae) have been used a diaphoretic, an analgesic, and an antipyretic under the name of "Fang feng" in China (Japanese name: "Bohu" 防風).[1]

Since there have been no reports on the constituents of this crude drug, we now wish to report the structure elucidation of three new chromones (**8**, **9**, and **12**), isolated together with eleven known compounds from this crude drug.

The pulverized root and rhizoma of the plant were extracted with hexane and then methanol. The hexane extract was treated as described in the experimental section to afford five coumarins (**1**—**5**) and four chromones (**6**—**9**).

Compounds **1**, **2**, **3**, and **4** were identified as psoralen, bergapten, imperatorin, and phellopterin, respectively, by comparison with authentic samples [mixed mp and infrared (IR) spectrum].[2] Compound **5** was identified as deltoin[3] by comparison of the IR and proton nuclear magnetic resonance (^{1}H-NMR) spectral data with those of an authentic sample and the identification was confirmed by the formation of marmesin (**5a**) on alkaline hydrolysis with 5% KOH–MeOH.

Compounds **6**, **7**, **8**, and **9** gave a brown coloration with ferric chloride, indicating the presence of a phenolic hydroxyl, and were presumed to be chromones from their ultraviolet (UV)[4a] and IR spectral data[4] as well as ^{1}H- and carbon (^{13}C)-NMR spectral analysis. Compound **6**, mp 202—202.5°C, $[\alpha]_D$ −22.0° ($CHCl_3$), $C_{15}H_{16}O_5$, was identified as hamaudol by comparison with an authentic sample (mixed mp, IR, and $[\alpha]_D$).[5] Compound **7**, mp 129.5—130°C, $[\alpha]_D$ −28.4° ($CHCl_3$), $C_{17}H_{18}O_6$, showed an absorption band of an ester carbonyl at 1740 cm^{-1} in the IR spectrum (KBr), and exhibited signals of an acetyl group (δ 2.07, 3H, s) and a chelated hydroxy group (δ 13.02, 1H, s) in the ^{1}H-NMR spectrum (in $CDCl_3$). The ^{13}C-NMR spectrum (in $CDCl_3$) of **7** closely resembled that of **6**, except for the signals assignable to C-2′, -3′, and -4′. The C-3′ signal at δ 69.8 (d) showed a downfield shift by 0.9 ppm, and C-2′ (δ 76.8, s) and C-4′ (δ 22.6, t) showed upfield shifts by 1.8 and 2.9 ppm, respectively, compared with those of **6** (Table I). These observations could be interpreted as esterification shifts.[6] The signals at δ 21.0 (q) and δ 170.3 (s) were assignable to an acetyl group. All of the above data indicated that **7** might be 3′-*O*-acetylhamaudol.[7] On hydrolysis with 3% KOH–EtOH,

7 gave hamaudol (**6**). Compound **7** was thus identified as 3′-*O*-acetylhamaudol.

Compound **8** was obtained as colorless needles, mp 128—128.5°C, $[\alpha]_D$ −56.8° ($CHCl_3$), $C_{20}H_{22}O_6$. The IR spectrum (KBr) of **8** showed an absorption band at 1720 cm^{-1}, indicating the presence of an ester linkage in **8**. The ^1H- and ^{13}C-NMR spectra (in $CDCl_3$) of **8** revealed almost the same chemical shifts as those of **7**, except for signals due to the acyl moiety. The signals at δ 1.87 (3H, br s), 1.92 (3H, dq, J=7/1 Hz) and 6.07 (1H, q, J=7 Hz) in the ^1H-NMR spectrum, and those at 1.57 (q), 20.5 (q), 127.6 (s), 138.9 (d) and 166.9 (s) in the ^{13}C-NMR spectrum as well as the strong peaks at m/z 83 and 55 in the mass spectrum (MS) of **8** indicated the presence of an angeloyl group in **8**.[8] On the basis of the above observations and the fact that hydrolysis of **8** with 3% KOH–EtOH afforded **6**, the structure of **8** was elucidated as 3′-*O*-angeloylhamaudol.

Compound **9**, named ledebouriellol, mp 97—99°C, $[\alpha]_D$ −41.8° ($CHCl_3$), $C_{20}H_{22}O_7$, was inferred to be a hydroxylated derivative of **8** by comparison of the ^1H-NMR spectrum with that of **8** and from its molecular formula. The ^1H-NMR spectrum (in $CDCl_3$) of **9** showed a signal due to a hydroxymethyl at δ 4.53 (2H, br s) instead of the olefinic methyl signal (δ 2.33) observed in **8**. In the ^{13}C-NMR spectrum (in $CDCl_3$) of **9**, a signal attributable to a hydroxymethyl carbon was observed at δ 61.3 (t), but one of methyl signals observed at δ 20.5 (q) in **8** was absent. Other carbon signals were quite coincident with those of **8**, except for the C-2 and C-3 carbons (β- and γ-positions relative to OH), which absorbed at δ 168.4 (s) and 106.2 (d) in **9**, respectively. The β-carbon (C-2) appeared at lower field by 1.6 ppm and the γ-carbon (C-3) appeared at higher field by 2.2 ppm, compared with those of **8**. This indicated that the methyl group at C-2 of **8** was hydroxylated in **9**. The structure of ledebouriellol was thus elucidated as **9**. The absolute configuration at C-3′ of **9** was confirmed as *S* by comparison of the circular dichroism (CD) curve with those of **6** and **8** (Fig. 1).[9]

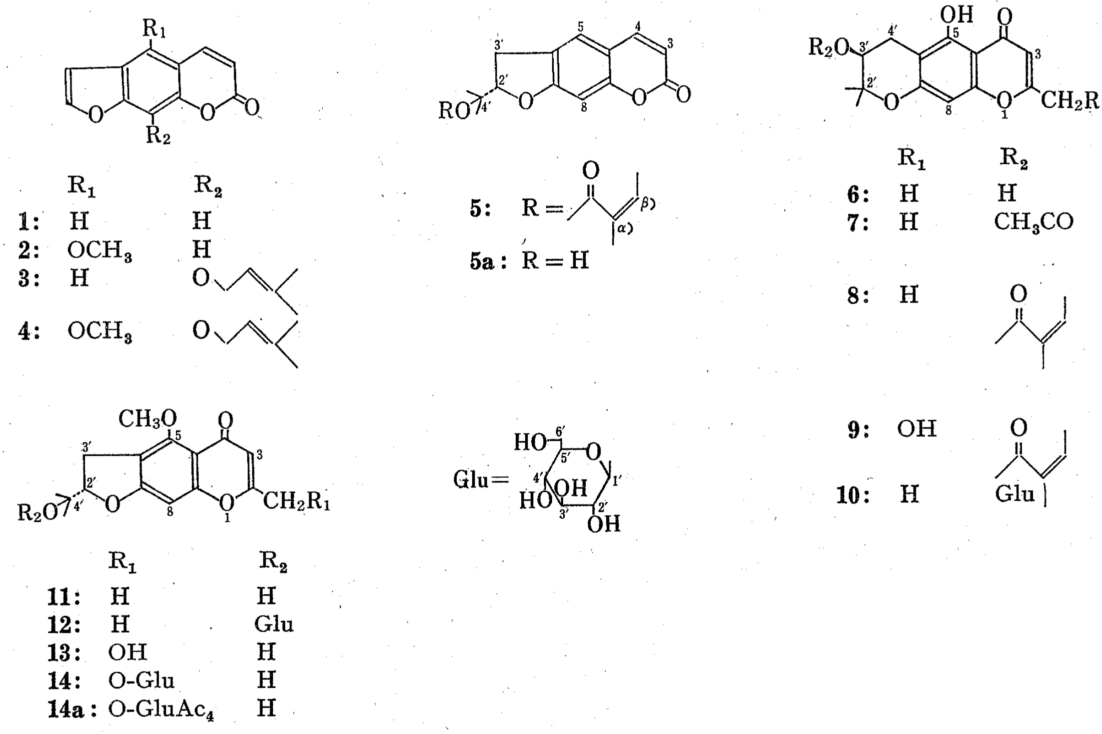

Chart 1

The methanolic extract was dissolved in water and extracted with EtOAc and then BuOH. The EtOAc extract was repeatedly subjected to silica gel column chromatography as described in the experimental section to furnish compounds **10**, **11**, and **13**. The BuOH extract was subjected to preparative high performance liquid chromatography (prep. HPLC) to give compounds **10**, **12**, **13**, and **14**.

Compound **11**, mp 141—142°C, $[\alpha]_D$ +91.8° ($CHCl_3$), $C_{16}H_{18}O_5$, was identified as 5-*O*-methylvisamminol on the basis of UV, IR, ^1H- and ^{13}C-NMR spectral analysis as well as comparison of physical constants with those reported in the literature.[10,11]

Compound **13**, mp 110—111°C, $[\alpha]_D$ +77.4° ($CHCl_3$), $C_{16}H_{18}O_6$, was identified as cimifugin by comparison with an authentic sample (mixed mp and IR).[12]

Compound **10**, mp 229—230°C, $[\alpha]_D$ −48.5° (MeOH), FD-MS, *m/z*: 438 (M^+), gave a brown coloration with ferric chloride, indicating the presence of a phenolic hydroxy group. The ^{13}C-NMR spectrum (Table I) exhibited six signals due to a *β*-D-glucopyranosyl moiety, and fifteen signals attributable to the aglycone moiety, which

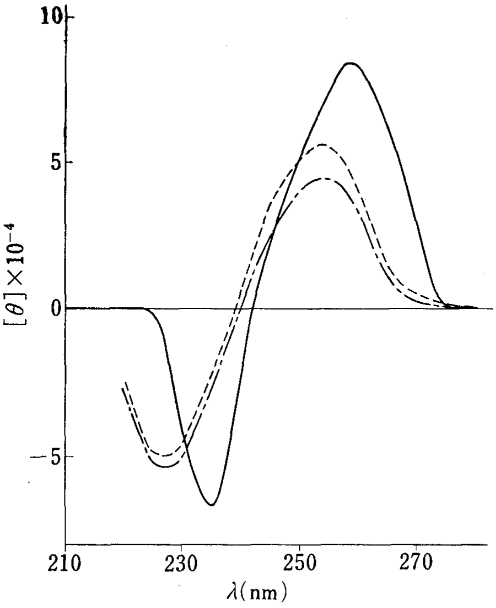

Fig. 1. CD Spectra of **6** (——), **8** (----), and **9** (–·–·–)

Table I. ^{13}C Chemical Shifts (δ ppm in $CDCl_3$, 20 MHz)[a]

Carbon	**5**	**5a**	Carbon	**6** (R_1=H)	**7** (R_1=H)	**8** (R_1=H)	**9** (R_1=OH)	**10** (R_1=H)
C-2 (s)	161.4	161.5	C-2 (s)	166.9	166.8	166.8	168.4	167.4
C-3 (d)	112.2	112.1	C-3 (d)	108.4	108.4	108.4	106.2	108.4
C-4 (d)	143.0	143.7	C-4 (s)	182.6	182.5	182.5	182.6	182.7
C-4a (s)	112.7	112.8	C-4a (s)	104.5	104.5	104.3	104.7	104.4
C-5 (d)	123.3	123.4	C-5[b] (s)	159.8	159.6	159.5	159.2	160.0
C-6 (s)	124.6	125.2	C-6 (s)	103.1	102.5	102.6	102.9	104.1
C-7 (s)	163.7	163.7	C-7[b] (s)	159.1	158.8	158.9	159.5	159.6
C-8 (d)	97.8	97.9	C-8 (d)	94.9	94.8	94.7	94.9	94.9
C-8a (s)	155.8	155.7	C-8a (s)	156.3	156.3	156.2	155.9	156.3
C-2′ (d)	89.2	91.2	C-2-CH_2R_1	20.6 (q)	20.5 (q)	20.5 (q)	61.3 (t)	20.1 (t)
C-3′ (s)	29.6	29.5	C-2′ (s)	78.6	76.8	76.9	76.7	78.4
C-4′ (s)	82.0	71.6	C-3′ (d)	68.9	69.8	69.7	69.5	74.3
gem-$(CH_3)_2$ (q)	21.4	24.5	C-4′ (t)					
(q)	22.3	26.0		25.5	22.6	22.7	22.7	22.3
			gem-$(CH_3)_2$ (q)	22.1	23.0	22.9	22.9	22.3
Angeloyl			(q)	25.0	24.7	24.9	24.9	25.7
α-CH_3 (q)	20.5		Others	Acetyl		Angeloyl		
β-CH_3 (q)	15.6			CH_3 (q)	21.0	α-CH_3 (q) 20.5	20.5	
C=C (s)	128.7			CO (s)	170.3	β-CH_3(q) 15.7	15.7	
(d)	137.5					C=C(s) 127.6	127.4	
C=O (s)	167.1					(d) 138.9	139.0	
						C=O(s) 166.9	167.0	
							Glu-1′ (d)	102.4
							Glu-2′ (d)	74.9
							Glu-3′ (d)	78.4
							Glu-4′ (d)	71.8
							Glu-5′ (d)	78.4
							Glu-6′ (t)	63.0

Multiplicity: s=singlet, d=doublet, t=triplet, q=quartet.

[a] Spectra were recorded at 25°C in 5 mm spinning tubes as 0.1—0.5 M solutions. Compound **10** was measured in C_5D_5N. FT-NMR conditions: spectral width, 5000 Hz; pulse flipping angle, *ca.* 45°; acquisition time, 1.638 s; number of data points, 16384.

[b] These assignments may be reversed.

Table II. ^{13}C Chemical Shifts (δ ppm C_5D_5N, 20 MHz)[a]

Carbon	**11** (R_1=H)	**12** (R_1=H)	**13** (R_1=OH)	**14** R_1=O-Glu)	**14a** (R_1=O-GluAc$_4$)
C-2 (s)	163.4	163.5	167.3	162.7	160.8
C-3 (d)	111.6	111.5	109.3	110.9	111.2
C-4 (s)	176.5	176.7	176.9	176.6	176.6
C-4a (s)	112.2	112.2	112.7	112.5	112.4
C-5[b] (s)	165.2	164.9	165.3	165.3	164.7
C-6 (s)	118.2	118.0	118.3	118.1	117.4
C-7[b] (s)	160.1	159.9	159.9	159.7	159.5
C-8 (d)	93.9	93.9	94.0	94.0	93.8
C-8a (s)	156.2	156.2	156.3	156.1	156.1
2-CH_2R_1	19.3(q)	19.4(q)	60.8(t)	66.4(t)	66.3(t)
C-2′ (d)	92.2	91.0	92.2	92.1	91.4
C-3′ (t)	27.9	28.2	28.0	27.9	27.8
C-4′ (s)	70.8	77.8	70.8	70.8	71.6
gem-$(CH_3)_2$ (q)	25.6	22.5	25.7	25.6	24.4
(q)	26.2	23.5	26.2	26.0	25.6
OCH_3 (q)	60.9	60.8	60.9	60.8	61.0
Others	Glu-1′ (d)	98.9		104.0	99.9
	Glu-2′ (d)	75.0		74.8	71.1
	Glu-3′ (d)	78.5		78.4	72.7
	Glu-4′ (d)	71.5		71.4	68.2
	Glu-5′ (d)	77.8		78.1	72.1
	Glu-6′ (t)	62.5		62.6	61.8
					Acetyl
					CH_3 (q) 20.6(2C)
					20.7(2C)
					CO (s) 169.3(2C)
					170.2
					170.6

a) Spectra were measured under the conditions described in the footnote to Table I. That of **14a** was taken in $CDCl_3$.

b) These assignments may be reversed.

bore a close resemblance to those of **6**. On the basis of the above observations and the fact that enzymatic hydrolysis of **10** furnished **6**, **10** was identified as *3′-O-β-*D-glucopyranosylhamaudol (*sec-O*-glucosylhamaudol).[11]

Compound **12**, mp 149—151°C, $[\alpha]_D$ +88.2° (MeOH), $C_{22}H_{28}O_{10}\cdot 1/2H_2O$, which was isolated here for the first time as a natural product, was assumed to be a glucoside of 5-*O*-methylvisamminol (**11**) on the basis of ^{1}H- and ^{13}C-NMR spectral analysis. The ^{1}H-NMR spectrum (in C_5D_5N) of **12** showed a singlet methyl signal (δ 1.53, 6H), a broad singlet due to an olefinic methyl (δ 2.05, 3H), a sharp singlet due to a methoxyl (δ 4.00, 3H), an olefinic proton signal as a broad singlet (δ 6.07, 1H), and an aromatic proton signal (δ 6.60, 1H, s). A doublet at δ 5.10 (1H, J=7.5 Hz) could be assigned to an anomeric proton of the *β*-D-glucopyranosyl moiety. The ^{13}C-NMR spectrum (in C_5D_5N) of **12** exhibited sixteen signals due to the aglycone moiety, which bore a close resemblance to those of **11**, and six signals arising from the *β*-D-glucopyranosyl moiety, among which the anomeric carbon signal appeared a considerably higher field (δ 98.9, d) than that of **10**.

Although the signals attributable to the aglycone moiety closely resembled those of **11**, the following features were different. Namely, the C-4′ carbon appeared at lower field (δ 77.8, s) in **12** by 7.0 ppm than in **11**, and the *gem*-dimethyl group absorbed at higher field (δ 22.5 and 23.5, each q) than in **11** (δ 25.6 and 26.2). These observations indicate that the *β*-D-glucosyl moiety is linked to a tertiary hydroxy group of the aglycone[13] and the structure of **12** was suggested to be *4′-O-β-*D-glucopyranosyl-5-*O*-methylvisamminol. Indeed, on hydrolysis with 10% HCl, **12** gave **11**. The structure of **12** was thus elucidated.

Compound **14**, $[\alpha]_D$ +12.6° (MeOH), FD–MS, *m/z*: 469 [(M+H)$^+$], was obtained as an amorphous solid and afforded a crystalline tetraacetate (**14a**), mp 189.5—190°C, $[\alpha]_D$ +6.7° ($CHCl_3$), $C_{30}H_{36}O_{15}$. The IR spectrum (KBr) of **14a** showed an absorption band at 3420 cm^{-1}, indicating the presence of a tertiary hydroxyl in **14**. The ^{13}C-NMR (in C_5D_5N) analysis of **14** suggested that **14** might be a glucoside of cimifugin (**13**). In fact, enzymatic hydrolysis of **14** afforded **13**. On the other hand, the ^{13}C-NMR spectrum of **14** showed downfield shifts of the C-2 hydroxymethyl carbon signal ($\Delta\delta$ +5.6 ppm) and the C-3 signal ($\Delta\delta$ +1.6 ppm), and an upfield shift of the C-2 signal ($\Delta\delta$ −4.6 ppm), compared with those of **13**, indicating that the glucosyl moiety is linked to the 2-hydroxymethyl group of **13**. Thus, **14** was elucidated as *prim-O*-glucosylcimifugin.[11]

Pharmacological studies on some of the chromones isolated from the plant were carried out. Compounds **10**, **11**, **12**, **13**, and **14** showed hypotensive activity in the guinea pig; **11** and **13** showed more potent activity than the others and also increased coronary flow in the isolated guinea pig heart as determined by the Langendorff method. Further pharmacological studies of **11** and **13** are in progress.

Experimental

All melting points were determined on a Yanagimoto micromelting point apparatus (a hot stage type) and are uncorrected. The IR spectra were recorded with a Hitachi EPI-G2 unit. The ^{1}H-NMR spectra were taken with a Varian T-60 spectrometer, and ^{13}C-NMR spectra were recorded with a Varian FT-80A spectrometer with tetramethylsilane as an internal standard. The mass spectra were measured with a JEOL JMS-DX 300 spectrometer. The specific rotations were recorded with a JASCO DIP-SL unit. The CD spectra were measured with a JASCO J-40A spectrometer. Prep. HPLC was carried out on a Waters Prep LC/System 500A apparatus and a JASCO Trirotar apparatus with a refractive index monitor. Thin-layer chromatography (TLC) was performed on Merck plates precoated with Kieselgel 60 F_{254} and preparative layer chromatography (PLC) was carried out on plates (20×20 cm, 0.75 mm thick) coated with Kieselgel PF_{254} (Merck).

Isolation of 1—9——The dried and pulverized root and rhizoma of *Ledebouriella seseloides* (1 kg, commercial crude drug) were extracted with hexane (3 l×3) and then with MeOH (3 l×4) under reflux. The hexane extract (62 g) was dissolved in hexane (200 ml) and extracted with 90% MeOH (150 ml×4). The 90% MeOH solution was evaporated to dryness and the residue (9.7 g) was chromatographed over silica gel (200 g), developing with a hexane–EtOAc solvent system, to give five main fractions A (5.6 g), B (1.1 g), C (1.0 g) [each eluted with hexane–EtOAc (4: 1)], D (0.58 g) [eluted with hexane–EtOAc (3: 1)], and E (0.57 g) [eluted with hexane–EtOAc (1: 1)].

Fractions C, D, and E were rechromatographed, independently, over silica gel with a hexane–ether solvent system to give **8** (from fraction C), **1**, **2**, **3**, **4**, **5**, and **7** (from fraction D), **6** and **9** (from fraction E).

Psoralen (**1**)——Colorless needles from hexane–EtOAc, mp 164—165°C. IR ν_{max}^{KBr} cm^{-1}: 1720, 1710, 1630, 1575. This compound was identified as psoralen by direct comparison with an authentic sample (mixed mp and IR). Yield 0.0005%.

Bergapten (**2**)——Colorless needles from EtOH, mp 187—189°C. IR ν_{max}^{KBr} cm^{-1}: 1730, 1620, 1605, 1580. This compound was identified as bergapten by direct comparison with an authentic sample (mixed mp and IR). Yield 0.001%.

Imperatorin (**3**)——Colorless prisms from EtOH–H_2O, mp 101—102°C. IR ν_{max}^{KBr} cm^{-1}: 1720, 1710, 1620, 1585. This compound was identified as imperatorin by direct comparison with an authentic sample (mixed mp and IR). Yield 0.001%.

Phellopterin (**4**)——Slightly yellow needles from EtOH, mp 103.5—104.5°C. IR ν_{max}^{KBr} cm^{-1}: 1740, 1665, 1605, 1590. This compound was identified as phellopterin by direct comparison with an authentic sample (mixed mp and IR). Yield 0.001%.

Deltoin (**5**)——Colorless prisms from ether–pet. ether, mp 106—107°C. $[\alpha]_D^{23}$ −42.3° (c=0.62, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 1725, 1710, 1625, 1570. UV λ_{max}^{EtOH} nm (log ε): 222 (4.30), 248 (3.73), 258 (3.63), 300 (sh 3.82), 333 (4.22). ^{1}H-NMR (δ in $CDCl_3$): 1.62 (6H, s, $(C\underline{H}_3)_2$–C–OH), 3.24 (2H, d, J=9 Hz, H-3′), 5.05 (1H, t, J=9 Hz, H-2′), 6.15 (1H, d, J=9.5 Hz, H-3), 6.66 (1H, s, H-5), 7.18 (1H, s, H-8), 7.55 (1H, d, J=9.5 Hz, H-4). Angeloyl moiety: 1.67 (3H, br s, α-CH_3), 1.87 (3H, d, J=8 Hz, β-CH_3), 5.94 (1H, q, J=8 Hz, β-H). The ^{13}C-NMR spectral data are given in Table I. High resolution MS (*m/z*), Calcd for $C_{19}H_{20}O_5$ (M$^+$): 328.1311. Found: 328.1311. MS, *m/z* (%): 328 (M$^+$, 6), 228 (41), 213 (100), 83 (51), 55 (52). The IR and ^{1}H-NMR spectra were identical with those of an authentic sample of deltoin. Yield 0.007%.

Hydrolysis of 5——A solution of **5** (46 mg) in 5% KOH–MeOH (6 ml) was kept at 65°C for 3 h, then diluted with H_2O (20 ml), neutralized with 10% HCl, and extracted with chloroform (15 ml × 3). The combined chloroform solution was washed with water, dried over Na_2SO_4 and concentrated to give a residue, which was purified by PLC [hexane–EtOAc (1: 1)] to afford **5a** (17.5 mg) as colorless prisms (from EtOAc), mp 190—191°C, $[\alpha]_D^{25}$ +25.6° (c=0.51, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3440, 1700, 1625, 1565. This compound (**5a**) was identified as marmesin by direct comparison with an authentic sample (mixed mp, IR, and $[\alpha]_D$).

Hamaudol (6)——Slightly yellow needles from EtOH, mp 202—202.5°C, $[\alpha]_D^{25}$ −22.0° (c=0.46, $CHCl_3$). This compound gave a brown coloration with 1% $FeCl_3$–EtOH on TLC. IR ν_{max}^{KBr} cm^{-1}: 3500, 1650, 1620, 1580. UV λ_{max}^{EtOH} nm (log ε): 230 (4.22), 252 (4.27), 259 (4.27), 299 (3.98). *Anal.* Calcd for $C_{15}H_{16}O_5$: C, 65.21; H, 5.84. Found: C, 65.47; H, 5.86. MS, m/z (%): 276 (M^+, 57), 217 (10), 205 (100), 176 (8), 123 (6). ^1H-NMR (δ in $CDCl_3$): 1.33, 1.38 (each 3H, s, *gem*-$(CH_3)_2$), 2.33 (3H, br s, 2-CH_3), 2.40 (1H, br s, 3′-OH, disappeared on addition of D_2O), 2.62, 3.03 (each 1H, dd, J=18/5.5 Hz, H-4′), 3.87 (1H, t, J=5.5 Hz, H-3′), 5.97 (1H, br s, H-3), 6.30 (1H, s, H-8), 12.93 (1H, br s, 5-OH, disappeared on addition of D_2O). CD (c=0.0101, MeOH) $[\theta]^{25}$ (nm): 85000 (258), 0 (243), −66000 (235). The ^{13}C-NMR spectral data are given in Table I. This compound was identified as hamaudol by direct comparison with an authentic sample (mixed mp, IR, and $[\alpha]_D$). Yield 0.001%.

3′-*O*-Acetylhamaudol (7)——Colorless needles from EtOH, mp 129.5—130°C, $[\alpha]_D^{25}$ −28.4° (c=0.88, $CHCl_3$). This compound gave a brown coloration with 1% $FeCl_3$–EtOH on TLC. IR ν_{max}^{KBr} cm^{-1}: 1740, 1645, 1630, 1585, 1580. UV λ_{max}^{EtOH} nm (log ε): 230 (4.27), 251 (4.30), 258 (4.29), 296 (3.98). *Anal.* Calcd for $C_{17}H_{18}O_6$: C, 64.14; H, 5.70. Found: C, 64.36; H, 5.81. ^1H-NMR (δ in $CDCl_3$): 1.35 (6H, s, *gem*-$(CH_3)_2$), 2.33 (3H, br s, 2-CH_3), 2.07 (3H, s, OAc), 2.70, 3.05 (each 1H, dd, J=18/5.5 Hz, H-4′), 5.10 (1H, t, J=5.5 Hz, H-3′), 6.00 (1H, br s, H-3), 6.33 (1H, s, H-8), 13.02 (1H, br s, 5-OH, disappeared on addition of D_2O). The ^{13}C-NMR spectral data are given in Table I. Yield 0.002%.

Hydrolysis of 7——A solution of **7** (15 mg) in 3% KOH–EtOH (4 ml) was kept at 70°C for 30 min. The reaction mixture was passed through an Amberlite IR-120B (H^+) column and concentrated to give a residue, which was purified by PLC (benzene–EtOAc (4: 1)) to furnish **6** (7 mg), slightly yellow needles from EtOH, mp 202—203°C. This compound (**6**) was identified as hamaudol by direct comparison with an authentic sample (mixed mp and IR).

3′-*O*-Angeloylhamaudol (8)——Colorless needles from EtOH, mp 128—128.5°C, $[\alpha]_D^{25}$ −56.8° (c=2.19, $CHCl_3$). This compound gave a brown coloration with 1% $FeCl_3$–EtOH on TLC. IR ν_{max}^{KBr} cm^{-1}: 1720, 1680, 1630, 1590. UV λ_{max}^{EtOH} nm (log ε): 225 (sh 4.37), 251 (4.28), 258 (4.27), 295 (3.95). High resolution MS (m/z), Calcd for $C_{20}H_{22}O_6$ (M^+): 358.1415. Found: 358.1409. MS, m/z (%): 358 (M^+, 8), 258 [$M^+-CH_3CH{=}C(CH_3)$–COOH, 46], 243 (258−CH_3, 100), 83 [$CH_3CH{=}C(CH_3)CO$, 40], 55 [$CH_3CH{=}C(CH_3)$, 73]. ^1H-NMR (δ in $CDCl_3$): 1.38 (6H, s, *gem*-$(CH_3)_2$), 2.33 (3H, br s, 2-CH_3), 2.73, 3.10 (each 1H, dd, J=18/5.5 Hz, H-4′), 5.18 (1H, t, J=5.5 Hz, H-3′), 5.98 (1H, br s, H-3), 6.32 (1H, s, H-8), 13.02 (1H, br s, 5-OH, disappeared on addition of D_2O). Angeloyl moiety: 1.87 (3H, br s, α-CH_3), 1.92 (3H, dq, J=7/1 Hz, β-CH_3), 6.07 (1H, q, J=7 Hz, β-H). CD (c=0.0092, MeOH) $[\theta]^{23}$ (nm): +56000 (254), 0 (238), −49000 (227). The ^{13}C-NMR spectral data are given in Table I. Yield 0.012%.

Hydrolysis of 8——A solution of **8** (38 mg) in 3% KOH–EtOH (5 ml) was kept at 70°C for 30 min, and then the reaction mixture was treated as described for the hydrolysis of **7** to give **6** (18 mg), slightly yellow needles from EtOH, mp 202°C. This compound (**6**) was identified as hamaudol by direct comparison with an authentic sample (mixed mp and IR).

Ledebouriellol (9)——Colorless needles from hexane–acetone, mp 97—99°C, $[\alpha]_D^{25}$ −41.8° (c=0.77, $CHCl_3$). This compound gave a brown coloration with 1% $FeCl_3$–EtOH on TLC. IR ν_{max}^{KBr} cm^{-1}: 3360, 1710, 1660, 1625, 1580. UV λ_{max}^{EtOH} nm (log ε): 227 (sh 4.41), 251 (4.31), 258 (4.30), 297 (3.99). High resolution MS (m/z), Calcd for $C_{20}H_{22}O_7$ (M^+): 374.1365. Found: 374.1350. MS, m/z (%): 374 (M^+, 2), 274 [$M^+-CH_3CH{=}C(CH_3)$–COOH, 31], 259 (274−CH_3, 100), 83 [$CH_3CH{=}C(CH_3)CO$, 13], 55 [$CH_3CH{=}C(CH_3)$, 36]. ^1H-NMR (δ in $CDCl_3$): 1.40 (6H, s, *gem*-$(CH_3)_2$), 2.70 (1H, br s, 2-$CH_2\underline{OH}$, disappeared on addition of D_2O), 2.74, 3.06 (each 1H, dd, J=18/5.5 Hz, H-4′), 4.53 (2H, br s, 2-$C\underline{H_2}OH$), 5.18 (1H, t, J=5.5 Hz, H-3′), 6.33 (2H, s, H-3 and H-8), 12.81 (1H, br s, 5-OH, disappeared on addition of D_2O). Angeloyl moiety: 1.88 (3H, br s, α-CH_3), 1.91 (3H, d, J=7 Hz, β-CH_3), 6.06 (1H, q, J=7 Hz, β-H). CD (c=0.0138, MeOH) $[\theta]^{25}$ (nm): +45000 (254), 0 (239), −54000 (227). The ^{13}C-NMR spectral data are given in Table I. Yield 0.001%.

Isolation of 10—14——The methanolic extract (172 g) was dissolved in H_2O (400 ml), defatted with ether (400 ml × 3), and extracted with EtOAc (40 ml × 3) and then with BuOH (400 ml × 3). The EtOAc extract (3.6 g) was subjected to column chromatography on silica gel with a chloroform–methanol solvent system to afford **10**, **13**, and a crude fraction containing **11**. The crude fraction of **11** was rechromatographed over silica gel with a mixture of benzene–acetone (3: 1) to give pure **11**.

The BuOH-soluble part of the methanolic extract was concentrated to afford a brown mass (29.5 g), a portion (18.5 g) of which was subjected to column chromatography on charcoal (30 g), eluting with H_2O (1.2 l), and then MeOH (2 l). The MeOH eluate (6.7 g) was subjected to prep. HPLC to give crude fractions of **12**, **13**, and **14**, and a small amount of pure **10**. HPLC conditions: column, prep PAK/C_{18} (5.7 cm i.d. × 30 cm, Waters Assoc.); solvent, H_2O–MeOH (13: 7) for elution of **12**, **13**, and **14**, and MeOH for elution of **10**;

flow rate, 150 ml/min; apparatus, Waters Prep LC/System 500A.

The crude fraction of **12** (1.2 g) was rechromatographed over silica gel with a chloroform–methanol solvent system to give pure **12**.

The crude fraction of **14** (2 g) was purified by silica gel column chromatography with a chloroform–methanol solvent system, followed by prep. HPLC to give pure **14**. HPLC conditions: column, μ-Bondapak C_{18} (8 mm i.d. × 30 cm, Waters Assoc.); solvent, H_2O–MeOH (2: 1); flow rate, 2 ml/min; apparatus, JASCO Trirotar.

***sec*-*O*-Glucosylhamaudol (10)**——Colorless needles from EtOH, mp 229—230°C, $[\alpha]_D^{26}$ −48.5° (c=0.68, MeOH). This compound gave a brown coloration with 1% $FeCl_3$–EtOH on TLC. IR ν_{max}^{KBr} cm^{-1}: 3700—3000, 1665, 1640, 1580. UV λ_{max}^{EtOH} nm (log ε): 230 (4.25), 252 (4.29), 259 (4.29), 299 (4.00). FD-MS, m/z: 438 (M^+). ^1H-NMR (δ in C_5D_5N): 1.48 (6H, s, *gem*-$(CH_3)_2$), 2.15 (3H, br s, 2-CH_3), 3.15 (2H, d, J=6 Hz, H-4′), 4.98 (1H, d, J=7.5 Hz, Glu-1′-H), 6.12 (1H, br s, H-3), 6.42 (1H, s, H-4), 13.58 (1H, br s, 5-OH, disappeared on addition of D_2O). The ^{13}C-NMR spectral data are given in Table I. Yield 0.07%.

Enzymatic Hydrolysis of 10——β-Glucosidase (Sigma Chem. Co., 15 mg) was added to a solution of **10** (26 mg) in a mixture of H_2O–DMSO (3: 1) (6 ml). The mixture was allowed to stand overnight at 37°C and then extracted with EtOAc. The EtOAc extract was dried over Na_2SO_4 and concentrated to give **6** (9 mg) as slightly yellow needles from EtOH, mp 200—201°C. This compound (**6**) was identified as hamaudol by direct comparison with an authentic sample (mixed mp and IR).

5-*O*-Methylvisamminol (11)——Colorless needles from hexane–EtOAc, mp 141—142°C, $[\alpha]_D^{25}$ +91.8° (c=0.90, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3360, 1655, 1625, 1610, 1595. UV λ_{max}^{EtOH} nm (log ε): 232 (4.27), 245 (4.26), 252 (4.22), 289 (4.08). High resolution MS (m/z), Calcd for $C_{16}H_{18}O_5$ (M^+): 290.1154. Found: 290.1168. MS, m/z (%): 290 (M^+, 100), 275 (14), 257 (21), 231 (95), 213 (99), 201 (48), 186 (35), 59 (96). ^1H-NMR (δ in $CDCl_3$): 1.23, 1.33 (each 3H, s, *gem*-$(CH_3)_2$), 2.25 (3H, br s, 2-CH_3), 3.23 (2H, d, J=9 Hz, H-3′), 3.93 (3H, s, OCH_3), 4.72 (1H, t, J=9 Hz, H-2′), 5.95 (1H, br s, H-3), 6.48 (1H, s, H-8). The ^{13}C-NMR spectral data are given in Table II. Yield 0.001%.

4′-*O*-β-D-Glucosyl-5-*O*-methylvisamminol (12)——Colorless needles from H_2O, mp 149—151°C, $[\alpha]_D^{25}$ +88.2° (c=1.66, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3700—3000, 1650, 1625, 1600, 1580. UV λ_{max}^{EtOH} nm (log ε): 232 (4.28), 244 (sh 4.26), 251 (4.21), 289 (4.07). FD-MS, m/z: 453 [$(M+H)^+$]. *Anal.* Calcd for $C_{22}H_{28}O_{10}\cdot 1/2H_2O$: C, 57.26; H, 6.33. Found: C, 57.15; H, 6.32. ^1H-NMR (δ in C_5D_5N): 1.53 (6H, s, *gem*-$(CH_3)_2$), 2.05 (3H, br s, 2-CH_3), 4.00 (3H, s, OMe), 4.95 (1H, dd, J=10/8Hz, H-2′), 5.10 (1H, d, J=7.5 Hz, Glu-1′-H), 6.07 (1H, br s, H-3), 6.60 (1H, s, H-8). The ^{13}C-NMR spectral data are given in Table II. Yield 0.15%.

Hydrolysis of 12——A solution of **12** (40 mg) in 5% HCl (4 ml) was kept at 90°C for 3 h, then neutralized with saturated $NaHCO_3$ solution, and extracted with EtOAc. The EtOAc extract was dried over Na_2SO_4 and concentrated to afford **11** (18 mg) as colorless needles (from EtOAc), mp 141—142°C, $[\alpha]_D^{24}$ +90.8° (c=0.88, $CHCl_3$). This compound was identified as 5-*O*-methylvisamminol (**11**) by direct comparison (mixed mp, IR, and $[\alpha]_D$).

Cimifugin (13)——Colorless needles from EtOH, mp 110—111°C, $[\alpha]_D^{25}$ +77.4° (c=1.07, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3360, 1655, 1620, 1580. UV λ_{max}^{EtOH} nm (log ε): 230 (sh 4.30), 244 (sh 4.28), 251 (sh 4.24), 291 (4.11). High resolution MS (m/z), Calcd for $C_{16}H_{18}O_6$ (M^+): 306.1102. Found: 306.1071. MS, m/z (%): 306 (M^+, 100), 291 (7), 273 (12), 247 (63), 229 (71), 217 (17), 205 (15), 202 (15), 59 (48). ^1H-NMR (δ in $CDCl_3$): 1.25, 1.37 (each 3H, s, *gem*-$(CH_3)_2$), 3.27 (2H, d, J=9 Hz, H-3′), 3.92 (3H, s, OMe), 4.47 (2H, br s, 2-$C\underline{H}_2OH$), 4.72 (1H, t, J=9 Hz, H-2′), 6.22 (1H, br s, H-3), 6.40 (1H, s, H-8). The ^{13}C-NMR spectral data are given in Table II. Yield 0.05%. This compound was identified as cimifugin by direct comparison with an authentic sample (mixed mp and IR).

***prim*-*O*-Glucosylcimifugin (14)**——Colorless amorphous solid, $[\alpha]_D^{25}$ +12.6° (c=1.03, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3700—3000, 1655, 1625, 1605. UV λ_{max}^{EtOH} nm (log ε): 231 (sh 4.23), 245 (sh 4.18), 253 (sh 4.09), 292 (4.02). FD-MS, m/z: 469 [$(M+H)^+$]. ^1H-NMR (δ in C_5D_5N): 1.40, 1.47 (each 3H, s, *gem*-$(CH_3)_2$), 4.02 (3H, s, OMe), 4.75 (2H, br s, 2-$C\underline{H}_2OH$), 6.57 (1H, br s, H-3), 6.68 (1H, s, H-8). The ^{13}C-NMR spectral data are given in Table II.

Acetylation of 14——A solution of **14** (29 mg) in Ac_2O and pyridine (each 0.5 ml) was allowed to stand overnight at room temperature. The reaction mixture was poured into ice-water extracted with EtOAc. The EtOAc extract was dried over Na_2SO_4 and concentrated to give a residue, which was purified by PLC (benzene–acetone (1: 1)) to afford the tetraacetate (**14a**, 33 mg) as a major product, and a minor product (5 mg).[14] Compound **14a** was obtained as colorless needles from hexane–EtOAc, mp 189.5—190°C, $[\alpha]_D^{25}$ +6.7° (c=0.75, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3420, 1745, 1670, 1625, 1605. UV λ_{max}^{EtOH} nm (log ε): 244 (sh 4.21), 251 (sh 4.16), 294 (4.05). *Anal.* Calcd for $C_{30}H_{36}O_{15}$: C, 56.60; H, 5.70. Found: C, 56.27; H, 5.68. ^1H-NMR (δ in $CDCl_3$): 1.23, 1.33 (each 3H, s, *gem*-$(CH_3)_2$), 2.00, 2.07 (each 6H, s, 4 × OAc), 3.25 (2H, d, J=9 Hz, H-3′), 3.5—3.9 (1H, m, Glu-5′-H), 3.93 (3H, s, OMe), 4.20 (center) (2H, m, Glu-6′-H), 4.57 (2H, br s, 2-CH_2-O-Glu), 4.73 (1H, t, J=9 Hz, H-2′), 4.5—5.3 (4H, m, Glu-1′,2′,3′,4′-H), 6.13 (1H, br s, H-3), 6.48 (1H, s, H-8). The ^{13}C-NMR spectral data are given in Table II.

Enzymatic Hydrolysis of 14——β-Glucosidase (24 mg) was added to a solution of **14** (52 mg) in H_2O (5 ml). The mixture was allowed to stand overnight at 37°C and then extracted with EtOAc. The EtOAc extract was dried over Na_2SO_4 and concentrated to give **13** (28 mg), colorless needles from EtOH, mp 110—

111°C, $[\alpha]_D^{24}$ $+71.2°$ ($c=0.67$, $CHCl_3$). This compound (**13**) was identified as cimifugin by direct comparison with an authentic sample (mixed mp, IR, and $[\alpha]_D$).

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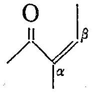
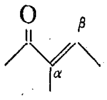
References and Notes

1) "Zhong Yao Zhi (中药志)," Vol. I, ed. by The Pharmaceutical Institute, Chinese Academy of Medical Science, Peking, 1961, p. 256.
2) H. Sasaki, H. Taguchi, T. Endo, and I. Yosioka, *Chem. Pharm. Bull.*, **28**, 1847 (1980).
3) G.K. Nikonov, *Doklady Akad. Nauk S.S.S.R.*, **156**, 1210 (1964) [*Chem. Abstr.*, **61**, 7000*h* (1964)]; B.E. Nielsen, P.K. Larsen, and J. Lemmich, *Acta Chim. Scand.*, **24**, 2863 (1970).
4) *a*) K. Hata and A. Nitta, *Yakugaku Zasshi*, **80**, 742 (1960); *b*) R.D.H. Murray and P.H. McCabe, *Tetrahedron*, **25**, 5819 (1969); R.D.H. Murray, P.H. McCabe, and T.C. Hogg, *ibid.*, **25**, 5839 (1969).
5) A. Nitta, *Yakugaku Zasshi*, **85**, 55 (1965); A. Nitta and H. Irie, *ibid.*, **88**, 819, 1168 (1968).
6) Y. Terui, K. Tori, and N. Tsuji, *Tetrahedron Lett.*, **1976**, 621; K. Yamasaki, R. Kasai, Y. Masaki, M. Okihara, and O. Tanaka, *ibid.*, **1977**, 1231.
7) K. Hata, M. Kozawa, Y. Ikeshiro, and K-Y. Yen, *Yakugaku Zasshi*, **85**, 656 (1965).
8) Ikeya *et al.* reported the structures of new lignans, angeloyl- and tigloylgomosin P, isolated from *Schizandra chinensis* and presented the following ^{1}H- and ^{13}C-NMR spectral data (δ in $CDCl_3$) for the angeloyl and tigloyl groups therein [Y. Ikeya, H. Taguchi, I. Yosioka, and H. Kobayashi, *Chem. Pharm. Bull.*, **28**, 3357 (1980)].



^{1}H-NMR: 1.80 (3H, s, α-CH_3), 1.87 (3H, dq, $J=7/1.5$ Hz, β-CH_3), 5.70 (1H, m, β-H).
^{13}C-NMR: 15.8 (q, β-CH_3), 20.8 (q, α-CH_3), 127.6 (s), 138.7 (d) (C=C), 166.4 (s, C=O).

angeloyl group



^{1}H-NMR: 1.38 (3H, dq, $J=7/1$ Hz, β-CH_3), 1.68 (3H, m, α-CH_3), 6.85 (1H, m, β-H).
^{13}C-NMR: 12.2 (q, α-CH_3), 14.4 (q, β-CH_3), 128.8 (s), 137.5 (d) (C=C), 166.6 (s, C=O).

tigloyl group

The chemical shifts in the ^{1}H- and ^{13}C-NMR spectra of **8** show that the acyl group in **8** is an angeloyl group on the basis of a comparison with the above spectral data. See also, R.R. Fraser, *Can. J. Chem.*, **38**, 549 (1960); M.D. Nair and R. Adams, *J. Am. Chem. Soc.*, **83**, 922 (1961); Y. Ikeya, H. Taguchi, I. Yosioka, and H. Kobayashi, *Chem. Pharm. Bull.*, **27**, 1576 (1979).

9) Recently, Baba *et al.* reported the isolation and the structure elucidation of a deacyl derivative of **9** from *Angelica japonica*, and they also concluded that the absolute configuration at the C-3′ asymmetric center was *S* by ORD spectral analysis. See ref. 11.
10) W. Bencze and H. Schmid, *Experientia*, **10**, 12 (1954); W. Bencze, J. Eisenbeiss, and H. Schmid, *Helv. Chim. Acta*, **39**, 923 (1956).
11) K. Baba, K. Hata, Y. Kimura, Y. Matsuya, and M. Kozawa, *Chem. Pharm. Bull.*, **29**, 2565 (1981).
12) Y. Kondo and T. Takemoto, *Chem. Pharm. Bull.*, **20**, 1940 (1972).
13) R. Kasai, M. Suzuo, J. Asakawa, and O. Tanaka, *Tetrahedron Lett.*, **1977**, 175.
14) The minor product has not been investigated in detail, because an insufficient amount was available.