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CHROMONES FROM *HARRISONIA PERFORATA*

TOMOKO TANAKA, KAZUO KOIKE,* KATSUYOSHI MITSUNAGA, KEIKO NARITA, SEIKO TAKANO, AKIKO KAMIOKA, EMI SASE, YISHAN OUYANG and TAICHI OHMOTO

Department of Pharmacognosy, School of Pharmaceutical Sciences, Toho University, 2-2-1 Miyama, Funabashi, Chiba 274, Japan

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Key Word Index—*Harrisonia perforata*; Simaroubaceae; perforatin C; perforatin D; perforatin E; perforatin F; perforatin G.

Abstract—Five new chromones, perforatins C–G, together with 10 known compounds were isolated from the wood of *Harrisonia perforata*.

INTRODUCTION

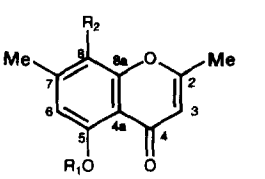
Harrisonia perforata (Blanko) Merr. is widely distributed in southeast Asia and southeast China. The root of this plant is used in a folk medicine in south China for the prevention and treatment of malaria and boils [1, 2]. Previously, five chromones [3, 4] and two limonoids [5, 6] have been reported from this plant. In this paper, we report on the isolation and structural elucidation of five new chromones and 10 known compounds from the wood of this plant, which was collected in Hainan, China.

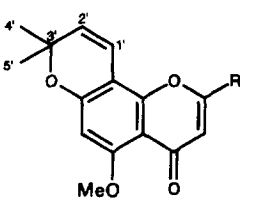
RESULTS AND DISCUSSION

A combination of column chromatography on silica gel and Diaion HP-20, and preparative HPLC of the methylene chloride and *n*-butanol extracts of *H. perforata* gave five new chromones, perforatins C–G (**1**–**5**) and six known chromones, heteropeucenin-7-methyl ether (**6**) [3], heteropeucenin-5-methoxy-7-methyl ether (**7**) [3], 2-hydroxymethylallopataeroxylin-5-methyl ether (**8**) [7], perforatin A (**9**) [8], perforatic acid (**10**) [4] and perforatic acid methyl ester (**11**) [4], three coumarins, scopoletin (**12**) [9], cedrelopsin (**13**) [10] and xanthoxyletin (**14**) [11], and a phenyl propanoid, coniferyl aldehyde (**15**) [12].

The chromone chromophore in **1**–**5** was readily identified by its characteristic UV absorption bands [3, 4]. In the ^{1}H NMR (Table 1) and ^{13}C NMR (Table 2) spectra of **1**–**5** most of the signals of the chromone nucleus were comparable with those of heteropeucenin or alloptaeroxylin type chromones. For each compound, the ^{1}H and ^{13}C NMR spectra revealed the chromone to contain a C_5 side chain, which could, in each case, be placed at C-8. This was established from HMBC spectra, which revealed the $^3J_{CH}$ correlation of H-2′ to C-8 and the $^2J_{CH}$ correlation of H-1′ to C-8.

Perforatin C (**1**) was assigned the molecular formula $C_{16}H_{20}O_6$ by HR mass spectrometry (MS). The IR spectrum showed absorption bands for hydroxyl (3483 cm^{-1}), chromone carbonyl (1657 cm^{-1}) and conjugated olefin (1618 cm^{-1}). The ^{1}H NMR spectrum (Table 1) showed the presence of an olefin methyl (δ2.36), an aromatic methoxyl (δ3.91), two singlet aromatic protons (δ6.03 and 6.41) and a chelated phenolic hydroxyl proton (δ12.80). The presence of an oxymethine carbon (δ78.4), a quaternary carbon bearing oxygen (δ72.9), a methylene (δ25.4), and two methyl groups (δ23.6 and 26.2) were evident from the ^{13}C NMR spectrum (Table 2). This was proved by the ^{13}C–^{1}H COSY in





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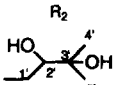
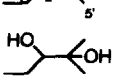
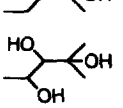
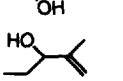
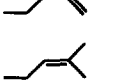
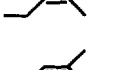
1	H	
2	Me	
3	Me	
4	Me	
6	H	
7	Me	

	R
5	CH_2O-β-D-glc
8	CH_2OH
9	Me
10	COOH
11	COOMe

*Author to whom correspondence should be addressed.

Table 1. ^{1}H NMR spectral data for compounds **1**–**6** and **8**

H	1*	2†	3†	4*	6*	5†	8†
3	6.03 *s*	6.00 *s*	6.03 *d* (0.7)	6.00 *d* (0.7)	6.00 *s*	6.38 *s*	6.22 *t* (1.0)
6	6.41 *s*	6.60 *s*	6.63 *s*	6.40 *s*	6.36 *s*	6.40 *s*	6.40 *s*
1′	2.86 *dd* (14.0, 10.1) 2.95 *dd* (14.1, 2.7)	2.92 *m*	5.37 *d* (6.2)	2.99 *dd* (13.6, 8.1) 3.08 *dd* (13.8, 5.0)	3.38 *d* (7.2)	6.75 *d* (10.1)	6.73 *d* (10.1)
2′	3.59 *dd* (10.1, 2.7)	3.59 *dd* (8.8, 4.0)	3.86 *d* (6.2)	4.29 *m*	5.51 *d* (7.2)	5.67 *d* (10.1)	5.68 *d* (10.1)
4′	1.31 *s*	1.27 *s*	1.07 *s*	1.85 *s*	1.67 *s*	1.47 *s*	1.46 *s*
5′	1.32 *s*	1.28 *s*	1.18 *s*	4.80 *d* (1.5) 4.88 *d* (0.9)	1.79 *s*	1.47 *s*	1.46 *s*
CH_3-2	2.36 *s*	2.35 *s*	2.37 *d* (0.7)	2.29 *d* (0.7)	2.36 *s*		
CH_2-2						4.64 *d* (14.8) 4.80 *d* (14.8)	4.47 *d* (1.0)
OH-5	12.80 *s*				12.77 *s*		
CH_3O-5		3.93 *s*	3.95 *s*	3.97 *s*		3.89 *s*	
CH_3O-7	3.91 *s*	3.97 *s*	4.01 *s*	3.96 *s*	3.88 *s*		
Glc-1						4.44 *d* (7.7)	

*In $CDCl_3$.
†In CD_3OD.
Coupling constants (J) in Hz are given in parentheses.

Table 2. ^{13}C NMR spectral data for compounds **1**–**6** and **8**

C	1*	2†	3†	4*	6*	5†	8†
2	166.8	166.6	166.5	162.8	166.7	164.5	169.1
3	108.5	111.2	111.5	111.5	108.2	111.2	110.4
4	182.9	180.8	180.4	177.9	183.0	179.8	180.8
4a	104.9	108.6	108.9	108.6	104.6	109.2	109.9
5	161.1	160.8	162.0	159.8	160.4	161.7	162.7
6	95.2	93.1	93.5	91.4	95.0	97.8	98.7
7	163.0	163.9	163.7	161.4	162.6	159.8	160.7
8	105.0	109.3	112.0	106.6	107.6	103.7	104.6
8a	155.3	158.7	158.3	157.2	154.7	155.2	156.1
1′	25.4	26.3	66.7	29.6	21.5	115.7	116.5
2′	78.4	78.9	79.9	75.5	122.0	128.9	129.8
3′	72.9	74.1	73.5	147.1	131.6	79.4	80.2
4′	23.6	25.4	25.6	17.9	25.7	28.5	29.2
5′	26.2	25.6	26.3	110.6	17.7	28.5	29.2
CH_3-2	20.5	19.8	19.8	19.8	20.6		
CH_2-2						67.1	62.0
CH_3O-5		56.5	56.6	55.9		56.6	57.4
CH_3O-7	56.3	56.6	56.8	56.3	55.9		
Glc-1						104.0	
Glc-2						74.8	
Glc-3						78.0	
Glc-4						71.4	
Glc-5						77.8	
Glc-6						62.6	

*In $CDCl_3$.
†In CD_3OD.
Assignments were confirmed by the ^{13}C–^{1}H COSY and HMBC spectra.

which all protons could be correlated to the respective carbons. Therefore, the C_5 side chain in **1** had to be a 2,3-dihydroxy-3-methylbutyl group. In the HMBC spectrum of **1**, a singlet methyl proton at δ2.36 showed a $^2J_{CH}$ correlation with a quaternary carbon at δ166.8 (C-2) and a $^3J_{CH}$ correlation with an olefinic carbon at δ108.5 (C-3), and furthermore a methoxyl proton at δ3.91 was correlated with a quaternary carbon at δ163.0 (C-7). The difference NOE experiments showed NOEs between CH_3-2 and H-3 (6%), and CH_3O-7 and H-6 (15%). These confirmed the co-identity of this compound with the heteropeucenin-7-methyl ether (**6**) system. From the above results, the structure of **1** was deduced to be 5-hydroxy-7-methoxy-2-methyl-8-(2,3-dihydroxy-3-methylbutyl)-chromone.

Perforatin D (**2**) was assigned the molecular formula $C_{17}H_{22}O_6$ by HRMS. The ^{1}H and ^{13}C NMR spectra of **2** were very similar to those of **1**. However, there was no chelated hydroxyl proton signal at C-5, but there was an additional methoxyl signal present (^{1}H at δ3.93; ^{13}C at δ56.5). HMBC correlations from the methoxyl protons to C-5 (δ160.8) defined the regiochemical placement of this substituent. These data established that **2** is the C-5 methyl ether derivative of **1**. Methylation of **1** with diazomethane gave **2**. Thus, the structure of **2** was determined to be 5,7-dimethoxy-2-methyl-8-(2,3-dihydroxy-3-methylbutyl)-chromone.

Perforatin E (**3**) was assigned the molecular formula $C_{17}H_{22}O_7$ by FAB-MS and from the ^{1}H and ^{13}C NMR spectra. The molecular formula of **3** contains one oxygen atom more than that of **2**, which suggested that **3** had an extra hydroxyl group in the C_5 side chain. In **3** the presence of two oxymethine carbons (δ66.7 and 79.9), a quaternary carbon bearing oxygen (δ73.5) and two methyl groups (δ25.6 and 26.3) were evident from the ^{13}C NMR spectrum. In the HMBC spectrum, the geminal methyl protons (δ1.07 and 1.18) showed long-range coupling with a quaternary carbon (δ79.9). This required that in **3** the C_5 side chain was the 1,2,3-trihydroxy-3-methylbutyl moiety. Thus, **3** is 5,7-dimethoxy-2-methyl-8-(1,2,3-trihydroxy-3-methylbutyl)-chromone.

Perforatin F (**4**) was shown to have the molecular formula $C_{17}H_{20}O_5$ by FAB-MS and from the ^{1}H and ^{13}C NMR spectra. In the HMBC spectrum of **4**, the methyl protons at δ1.85 (H-4′) showed a $^2J_{CH}$ correlation with an olefinic quaternary carbon at δ147.1 (C-3′) and a $^3J_{CH}$ correlation with an exomethylene carbon at δ110.6 (C-5′). The downfield methine proton at δ4.29 (H-2′) was in turn coupled with C-5′. From the above results, the side chain had to be a 2-hydroxy-3-methyl-3-butenyl group. Thus, **4** is 5,7-dimethoxy-2-methyl-8-(2-hydroxy-3-methyl-3-butenyl)-chromone.

Perforatin G (**5**), $[\alpha]_D$ − 67.7° (pyridine), was assigned the molecular formula $C_{22}H_{26}O_{10}$ (EIMS, FABMS, and ^{1}H and ^{13}C NMR). A major fragment ion at *m/z* 287 was a typical fragment due to the loss of a hexose moiety. The ^{1}H NMR spectrum of **5** clearly showed an anomeric doublet at δ4.44 (J = 7.7 Hz) indicative of the presence of a β-linked sugar. Acid hydrolysis of **5** afforded **8** and a component sugar; the latter was identified as D-glucose by GC as its trimethylsilyl derivative. Therefore, **5** was determined to be 2-hydroxymethylalloptaeroxylin-5-methyl ether β-D-glucopyranoside.

EXPERIMENTAL

General. Mps: uncorr.; IR: KBr pellets; UV: MeOH; EIMS: JEOL D-300; HR-MS and FAB-MS: JEOL DX-303; ^{1}H, ^{13}C and 2D NMR: 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR, TMS as int. standard; CC: Silica gel 60 (Merck), Diaion HP-20 (Mitsubishi Kasei) and ODS (Fuji Silysia); HPLC: ODS [SG-120, Shiseido, 10 × 250 mm; detector UV, 254 nm; solvent system, H_2O–MeOH (1:1); flow rate, 2.0 ml min^{-1}].

Extraction and isolation. Dried wood (8.7 kg) of *H. perforata* collected in Hainan, China, in August 1992, was extracted with CH_2Cl_2 (115 l) and MeOH (107 l) under reflux conditions for 3 hr. The CH_2Cl_2 and MeOH extracts were concd under red. pres. to give residues of 110 and 200 g, respectively. The MeOH extract was mixed with H_2O and extracted with *n*-BuOH. The *n*-BuOH extract was concd under red. pres. to give a residue (70 g). The CH_2Cl_2 extract was subjected to repeated CC to give **1** (11.4 mg), **2** (9.3 mg), **3** (10.8 mg), **4** (9.6 mg), **6** (9.89 g), **7** (3.33 g), **8** (13.4 mg), **9** (0.72 g), **11** (35.0 mg), **12** (9.2 mg), **13** (10.9 mg), **14** (43.0 mg) and **15** (5.9 mg). The *n*-BuOH extract was subjected to repeated CC to give **5** (6.2 mg) and **10** (9.01 g),

Perforatin C (**1**). Needles, mp 157°, $[\alpha]_D^{24}$ 0.0° ($CHCl_3$; *c* 1.00). UV λ_{max}^{MeOH} nm (log ε): 250sh (4.19), 258 (4.20), 292 (3.52), 324 (3.43); IR ν_{max}^{KBr} cm^{-1}: 3483, 1657, 1618, 1585, 1267, 1203, 1119, 1080; HR-MS *m/z* 308.1266 (calc. for $C_{16}H_{20}O_6$, 308.1254); EI-MS *m/z* (rel. int.): 308 $[M]^+$ (8), 249 (10), 219 (100), 207 (9), 189 (11), 176 (3), 149 (5); ^{1}H NMR: Table 1; ^{13}C NMR: Table 2.

Perforatin D (**2**). Needles, mp 115°, $[\alpha]_D^{25}$ − 1.5° (MeOH; *c* 0.92). UV λ_{max}^{MeOH} nm (log ε): 246sh (4.28), 254 (4.31), 288 (3.76), 310 (3.75); IR ν_{max}^{KBr} cm^{-1}: 3421, 1658, 1603, 1277, 1211, 1119, 1093; HR-MS *m/z* 322.1458 (calc. for $C_{17}H_{22}O_6$, 322.1410); EI-MS *m/z* (rel. int.): 322 $[M]^+$ (26), 263 (17), 247 (6), 233 (100), 219 (3), 203 (19), 189 (13), 163 (8), 149 (3), 133 (5), 103 (4), 43 (30); ^{1}H NMR: Table 1; ^{13}C NMR: Table 2.

Methylation of **1**. A soln of **1** (1 mg) in MeOH (0.5 ml) was treated with CH_2N_2 for 3 hr. The reaction mixt. was evapd to give needles of **2** (1 mg). This compound was identified as perforatin D by direct comparison with an authentic sample (TLC, IR, ^{1}H NMR and MS).

Perforatin E (**3**). Needles, mp 171–173°, $[\alpha]_D^{25}$ + 1.8° (MeOH; *c* 1.00). UV λ_{max}^{MeOH} nm (log ε): 230 (4.46), 246 (4.44), 254 (4.45), 288 (4.07), 310 (3.91); IR ν_{max}^{KBr} cm^{-1}: 3429, 1658, 1601, 1215, 1117; EI-MS *m/z* (rel. int.): 249 (100); FAB-MS *m/z*: 339 $[M + H]^+$; ^{1}H NMR: Table 1; ^{13}C NMR: Table 2.

Perforatin F (**4**). Needles, mp 196°, $[\alpha]_D^{25}$ −0.4° (MeOH; *c* 0.55). UV λ_{max}^{MeOH} nm (log ε): 249 (3.69), 257sh (4.29), 291 (4.26), 311 (4.20); IR ν_{max}^{KBr} cm^{-1}: 3424, 1662, 1602, 1124; EI-MS *m/z* (rel. int.): 233 (100); FAB-MS *m/z*: 305 $[M + H]^+$; ^{1}H NMR: Table 1; ^{13}C NMR: Table 2.

Perforatin G (**5**). Needles, mp 155°, $[\alpha]_D^{26}$ −67.7° (pyridine; *c* 0.62). UV λ_{max}^{MeOH} nm (log ε): 240 (4.66), 265 (4.95), 300 (4.03), 332 (4.00); IR ν_{max}^{KBr} cm^{-1}: 3406, 1662, 1604, 1122, 1088; EI-MS *m/z* (rel. int.): 450 $[M]^+$ (19), 435 (100), 421 (44), 273 (36), 243 (24), 217 (26), 203 (9), 43 (33); FAB-MS *m/z*: 451 $[M+H]^+$; ^{1}H NMR: Table 1; ^{13}C NMR: Table 2.

Acid hydrolysis of **5**. Compound **5** (1 mg) was dissolved in 2 M HCl (1 ml) and heated at 80° for 2 hr in a hot-water bath. The reaction mixt. was extracted with EtOAc, then EtOAc layer was evapd to dryness *in vacuo* after being washed with H_2O. The EtOAc extract was identified as 2-hydroxymethylalloptaeroxylin-5-methyl ether (**8**) by HPLC. The aq. layer was neutralized with Amberlite MB-3 and evapd to dryness *in vacuo*. The residue was trimethylsilylated with *N*-trimethylsilylimidazole (0.2 ml) at room temp. for 1 hr. The reaction mixt. was added to H_2O and extracted with *n*-hexane, and the *n*-hexane layer was washed with H_2O. The *n*-hexane soln was subjected to GC for identification of the sugar moiety. The TMSi derivative was identified as D-glucose. The GC conditions were as follows: column, 2% SE-30 (3 mm × 1 m); column temp., 165°; injection port temp., 310°; carrier gas, N_2 (45 ml min^{-1}).

Heteropeucenin-7-methyl ether (**6**). Needles, mp 104–106°. UV λ_{max}^{MeOH} nm (log ε): 253sh (4.72), 258 (4.74), 295 (4.04), 327 (3.98); IR ν_{max}^{KBr} cm^{-1}: 2900, 1650, 1620, 1585, 1260; EI-MS *m/z* (rel. int.): 274 $[M]^+$ (56), 259 (100), 206 (53); ^{1}H NMR: Table 1; ^{13}C NMR: Table 2.

Heteropeucenin-5-methoxy-7-methyl ether (**7**). Prisms, mp 154°. UV λ_{max}^{MeOH} nm (log ε): 246sh (4.86), 255 (4.89), 287 (4.26), 313 (4.28); IR ν_{max}^{KBr} cm^{-1}: 1650, 1600, 1380, 1320, 1090; EI-MS *m/z* (rel. int.): 288 $[M]^+$ (100), 259 (63), 242 (25).

2-Hydroxymethylalloptaeroxylin-5-methyl ether (**8**). Needles, mp 195°, UV λ_{max}^{MeOH} nm (log ε): 238 (4.69), 262sh (4.93), 293 (3.98), 330 (3.96); IR ν_{max}^{KBr} cm^{-1}: 3360, 1655, 1600, 1560, 1310, 1120; EI-MS *m/z* (rel. int.): 288 $[M]^+$ (20), 273 (100): ^{1}H NMR: Table 1; ^{13}C NMR: Table 2.

Perforatin A (**9**). Needles, mp 159°. UV λ_{max}^{MeOH} nm (log ε): 264 (4.51), 300 (3.56), 330 (3.56); IR ν_{max}^{KBr} cm^{-1}: 1666, 1600, 1086; EI-MS *m/z* (rel. int.): 272 $[M]^+$ (35), 257 (100).

Perforatic acid (**10**). Powder, mp > 300°. UV λ_{max}^{MeOH} nm (log ε): 225 (3.91), 264 (4.00); IR ν_{max}^{KBr} cm^{-1}: 3383, 1612, 1203, 1159, 1115, 1065; EI-MS *m/z* (rel. int.): 302 $[M]^+$ (27), 301 (100), 287 (31).

Perforatic acid methyl ester (**11**). Needles, mp 223°. UV λ_{max}^{MeOH} nm (log ε): 280 (4.32), 332sh (3.65); IR ν_{max}^{KBr} cm^{-1}: 1743, 1658, 1570, 1254, 1136; EI-MS *m/z* (rel. int.): 316 $[M]^+$ (13), 301 (100).

Scopoletin (**12**). Needles, mp 202–204°. IR ν_{max}^{KBr} cm^{-1}: 3338, 1703. EI-MS *m/z* (rel. int.): 192 $[M]^+$ (100), 177 (60).

Cedrelopsin (**13**). Needles, mp 172–175°. IR ν_{max}^{KBr} cm^{-1}: 3352, 1707; EI-MS *m/z* (rel. int.): 260 $[M]^+$ (79), 204 (100), 176 (33).

Xanthoxyletin (**14**). Needles, mp 136°. IR ν_{max}^{KBr} cm^{-1}: 1709, 1568, 1290; EI-MS *m/z* (rel. int.): 258 $[M]^+$ (22), 243 (100).

Coniferyl aldehyde (**15**). Needles, mp 70–71°. IR ν_{max}^{KBr} cm^{-1}: 3338, 1647, 1591, 1514; EI-MS *m/z* (rel. int.): 178 $[M]^+$ (100), 147 (48).

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