

**A NEW NEOCLERODANE DITERPENOID FROM
*SCUTELLARIA HEMATOCHLORA***

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Abstract - From the aerial parts of *Scutellaria hematochlora*, a new neoclerodane diterpenoid, hematochloridin (**1**) was isolated together with acetovanillon, vanillin, apigenin and sitosterol 3-*O*-glucoside. The structure of the new compound was elucidated by spectral analyses.

The genus *Scutellaria* (Labiatae) has been known to contain neoclerodane and seco-abietane diterpenoids,¹ flavonoids and phenylethanoids.² During the course of our studies on the constituents of medicinal plants grown in Uzbekistan Republic, we examined the diterpenic constituents of *Scutellaria hematochlora* (Uzbek name: Kukamaran) which was used in Uzbekistan Republic as a remedy for allergy.³ From the EtOAc soluble fraction of the methanolic extract of the aerial parts of *S. hematochlora*, we isolated a new neoclerodane type diterpenoid, hematochloridin (**1**), together with acetovanillon,⁴ vanillin,⁵ apigenin⁵ and sitosterol 3-*O*-glucoside.⁶ This paper describes the structure elucidation of the new compound.

Hematochloridin (**1**), [α]_D -15.0° (CHCl₃) was obtained as an amorphous powder and the molecular formula was determined as C₂₅H₃₆O₇ based on its negative ion high resolution

FABMS. The spectral data suggested that hematochloridin (**1**) contained an α,β -unsaturated γ -lactone group [UV λ_{\max} (MeOH) 218 nm; δ_{H} 5.81 (1H, dd, $J=1.7$ and 1.7 Hz), 4.73 (2H, d, $J=1.6$ Hz), δ_{C} 73.2, 114.4, 171.5, 174.1], a tigloyl ester group [δ_{H} 1.82 (3H, br d, $J=7.1$ Hz), 1.58 (3H, br s), 6.92 (1H, m); δ_{C} 12.3, 14.5, 128.3, 137.9, 167.3],¹ three tertiary methyl groups [δ_{H} 0.95, 1.12, 1.34 (each 3H, s)], a hydroxyl group [δ_{H} 3.22 (1H, s)], disubstituted oxirane ring [δ_{H} 2.66 (1H, d, $J=3.0$ Hz), 3.45 (1H, dd, $J=3.0$ and 2.8 Hz); δ_{C} 53.3 (t), 69.0 (s)] and two secondary carbinyl functions [δ_{H} 3.92, 5.32 (each 1H, d, $J=10.0$ Hz)], which are *trans*-oriented each other and adjacent to quaternary carbon atoms. The ¹³C-NMR spectrum (Table 1) further showed the presence of five methylene groups, a methine

Table 1. ¹³C-NMR data for hematochloridin (**1**) in CDCl₃ (100 MHz)

C		C	
1	21.5 (CH ₂)	14	114.4 (CH)
2	24.6 (CH ₂)	15	171.5 (C)
3	31.1 (CH ₂)	16	73.2 (CH ₂)
4	69.0 (C)	17	21.5 (CH ₃)
5	41.6 (C)	18	53.3 (CH ₂)
6	72.0 (CH)	19	14.8 (CH ₃)
7	75.5 (CH)	20	21.7 (CH ₃)
8	79.1 (C)	1'	167.3 (C)
9	43.1 (C)	2'	128.3 (C)
10	43.0 (CH)	3'	137.9 (CH)
11	35.0 (CH ₂)	4'	12.3 (CH ₃)
12	25.1 (CH ₂)	5'	14.5 (CH ₃)
13	174.1 (C)		

group and three quaternary carbon atoms, one (δ_{C} 79.1) of which has an oxygen atom on it. Based on the results of ¹H-¹H and ¹H-¹³C COSY spectra, three partial structures a) C-10(CH) ~ C-1(CH₂) ~ C-2(CH₂)-C-3(CH₂), b) C-6(CH) ~ C-7(CH) and c) C-11(CH₂) ~ C-12(CH₂) were elucidated in addition to the above mentioned partial structures. The results of HMBC spectrum (Figure 1) clearly showed the connectivities between quaternary carbon atoms and the partial structures mentioned above. Thus the planar structure of hematochloridin has been elucidated to be 6,8-dihydroxy-4,18-epoxy-7-tigloyloxycloerodan-15,16-olide. The relative stereochemistry was determined as shown based on the results (Figure 2) of

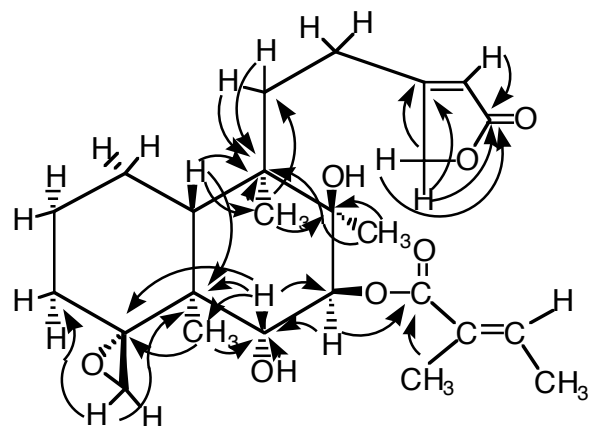


Figure 1 Selected data from the HMBC spectrum for **1** ($J=8\text{Hz}$)

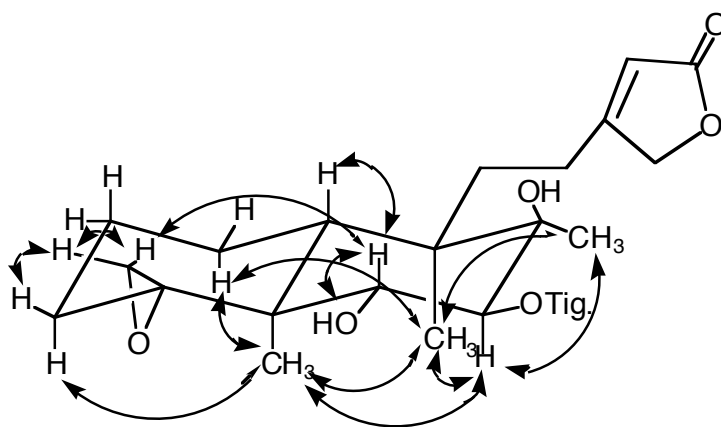
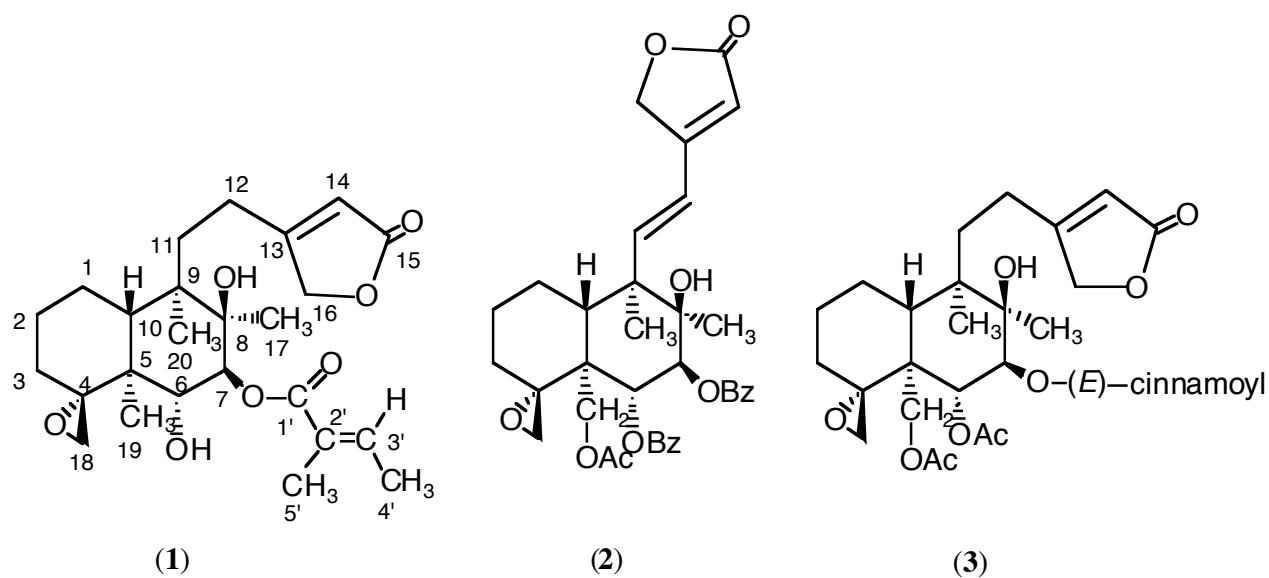


Figure 2 The results of phase sensitive NOESY for **1**



phase sensitive NOESY spectrum. Although the absolute stereochemistry was not ascertained, it may be suggested that hematocloridin (**1**) belongs to the neoclerodane series by comparisons of the sign of optical rotation with those of scutalpin K (**2**) { $[\alpha]_D$ -27.3° (CHCl₃) }^{1c} and scutorientalin E (**3**) { $[\alpha]_D$ -9.6° (CHCl₃) }.^{1d}

EXPERIMENTAL

The following apparatus were used to take spectral data. NMR: ¹H -(400 MHz) and ¹³C-NMR (100 MHz), JEOL JNM EX-400 and α -400 spectrometers; UV: JASCO V-530 SR spectrophotometer; IR: Shimadzu IR-400 spectrophotometer; MS: JEOL JMS SX-102 spectrometer. FABMS were recorded using PEG-400 as a calibration matrix; optical rotation: JASCO DIP-360 digital polarimeter. For purification, Kieselgel 60 Silica Gel (Merck, 230-400 mesh) and precoated silica gel 60 F₂₅₄ TLC plates (Merck, 0.25 and 0.5 mm in thickness) were used.

Plant Material The plant material was collected in the suburbs of Tashkent, Uzbekistan Republic in June, 1997 and identified as *Scutellaria hematoclora* Juz. by Dr. F. Khassanof of the Institute of Botany and Botanical Garden of Uzbek, Academy of Sciences, Uzbekistan Republic. A voucher specimen (97A 017) is deposited in the Herbarium of the Graduate School of Pharmaceutical Sciences, Kyoto University.

Isolation Dried aerial parts (2.2 kg) of *S. hematoclora* were extracted with MeOH (18 L x 2) at rt for two weeks. The combined MeOH extracts were concentrated *in vacuo* and the residue (210 g) was dissolved in 90% aqueous MeOH (1 L). The solution was washed with *n*-hexane (1 L x 3) and the aqueous MeOH layer was concentrated *in vacuo*. The residue (190 g) was suspended in H₂O (1 L) and the suspension was extracted with EtOAc (1 L x 3). The EtOAc layer was washed with H₂O (500 mL), dried over anhyd. MgSO₄ and evaporated *in vacuo* to give a residue (33.5 g) which was chromatographed over silica gel (800 g) with a mixture of CHCl₃ and Me₂CO with increasing amount of Me₂CO content. Each 6 liters of 0, 5, 10, 20 and 30% Me₂CO in CHCl₃ were eluted successively, collecting 500 mL fractions. Fraction Nos. 7-8 gave a residue (1.24 g) which was repeatedly separated by silica gel chromatography (solvent: Et₂O and CHCl₃) and finally preparative TLC (solvent: *n*-hexane - Et₂O 6 : 4, developed six times) to give vanillin (8.7 mg) and acetovanillon (21.0 mg). Fractions Nos. 10-11 gave a residue (778 mg) which was chromatographed over silica gel with CHCl₃ and then Et₂O to give hematocloridin (**1**)(324 mg)[*R*_f 0.51 (CHCl₃-Me₂CO 7 : 3) and 0.16 (Et₂O) on TLC]. Fraction Nos. 12-15 gave a residue (2.87 g) which was chromatographed over silica gel (100 g) with Et₂O as eluent. The residue (718 mg) from the faster eluate was washed well with CHCl₃ and the insoluble material (=apigenin)(110 mg) was collected by

filtration. Fraction Nos. 73-79 gave a residue (14.1 g) which was repeatedly separated by silica gel chromatography with a mixture of CHCl_3 and MeOH to give sitosterol 3-*O*-glucoside (49.3 mg).

Known compounds isolated were identified by comparison of spectral data with those authentic sample or those reported.

Hematochloridin (**1**): $[\alpha]_D^{25} -15.0^\circ$ ($c=1.09$, CHCl_3). UV λ_{max} (MeOH) nm (ϵ): 218 (12960); IR ν_{max} (CHCl_3): 3475, 1740, 1705, 1635, 1250, 1115 cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3): δ 0.95 (3H, s, H_3 -20), 1.03 (1H, m, H_a -3), 1.12 (3H, s, H_3 -17), 1.34 (3H, s, H_3 -19), 1.40 (1H, m, H_a -2), 1.45 (1H, m, H_a -1), 1.53 (1H, m, H_1 -11), 1.58 (3H, br s, H_3 -5'), 1.60 (1H, m, H_b -1), 1.82 (3H, br d, $J=7.1$ Hz, H_3 -4'), 1.88 (3H, br s, H_3 -5'), 1.97 (1H, dd, $J=2.7$ and 12.4 Hz, H-10), 2.07 (1H, m, H_b -2), 2.20 (1H, m, H_b -3), 2.24 (1H, m, H_a -12), 2.66 (1H, d, $J=3.0$ Hz, H_a -18), 3.05 (1H, m, H_b -12), 3.22 (1H, s, OH), 3.45 (1H, dd, $J=3.0$ and 2.8 Hz, H_b -18), 3.92 (1H, d, $J=10.0$ Hz, H-6), 4.73 (2H, d, $J=1.6$ Hz, H_2 -16), 5.32 (1H, d, $J=10.0$ Hz, H-7), 5.81 (1H, dd, $J=1.7$ and 1.7 Hz, H-14), 6.92 (1H, m, H-3'); $^{13}\text{C-NMR}$: see Table 1; FABMS (negative) m/z 447.2371 [M-H] $^-$ (calcd for $\text{C}_{25}\text{H}_{35}\text{O}_7$: 447.2383).

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