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A NEW NEOCLERODANE DITERPENOID FROM *SCUTELLARIA HEMATOCHLORA*

Yoshio Takeda,[a,*] Satomi Tateoka,[a] Toshiya Masuda,[a] Hideaki Otsuka,[b] Gisho Honda,[c] Yoshihisa Takaishi,[d] Michiho Ito,[c] Olimjon K. Khodzhimatov,[e] and Ozodbek A. Ashurmetov[e]

Faculty of Integrated Arts and Sciences, The University of Tokushima,[a] Tokushima 770-8502, Japan, Institute of Pharmaceutical Sciences, Hiroshima University Faculty of Medicine,[b] Minami-ku, Hiroshima 734-8551, Japan, Graduate School of Pharmaceutical Sciences, Kyoto University,[c] Sakyo-ku, Kyoto 606-8501, Japan, Faculty of Pharmaceutical Sciences, The University of Tokushima,[d] Tokushima 770-8505, Japan, Institute of Botany and Botanical Garden of Uzbek, Academy of Sciences,[e] Tashkent 700143, Uzbekistan

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] apigenin[5] and sitosterol 3-*O*-glucoside.[6] This paper describes the structure elucidation of the new compound.

Hematochloridin (**1**), $[\alpha]_D$ -15.0° ($CHCl_3$) was obtained as an amorphous powder and the molecular formula was determined as $C_{25}H_{36}O_7$ 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), δc 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 oxyrane 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 ^{13}C-NMR spectrum (Table 1) further showed the presence of five methylene groups, a methine

Table 1. ^{13}C-NMR data for hematochloridin (**1**) in $CDCl_3$ (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 ^{1}H-^{1}H and ^{1}H-^{13}C COSY spectra, three partial structures a) C-10(CH)～C-1(CH_2)～C-2(CH_2)-C-3(CH_2), b) C-6(CH)～C-7(CH) and c) C-11(CH_2)～C-12(CH_2) 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-tigloyloxyclerodan-15,16-olide.

The relative stereochemistry was determined as shown based on the results (Figure 2) of

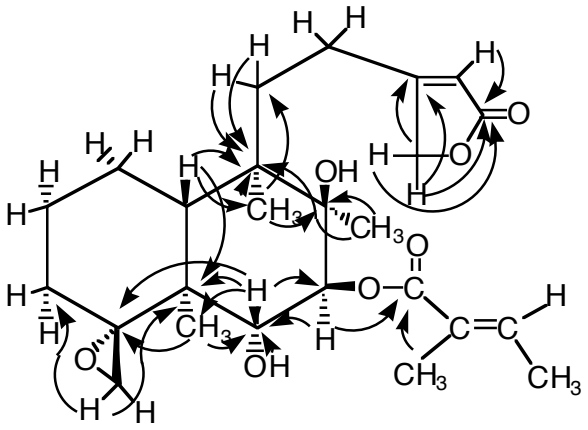


Figure 1 Selected data from the HMBC spectrum for **1** (*J*=8Hz)

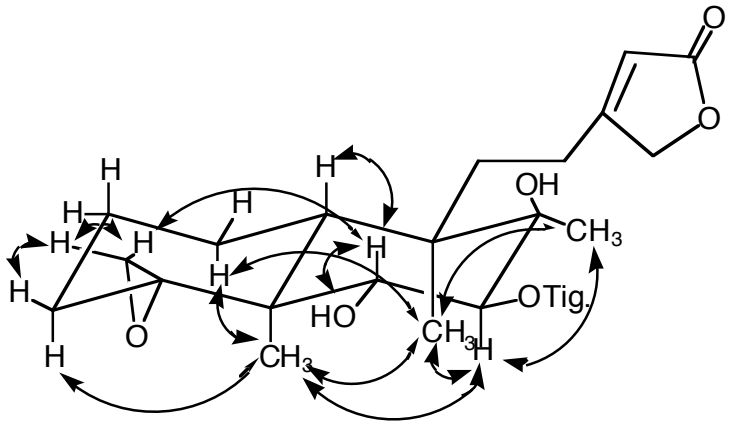
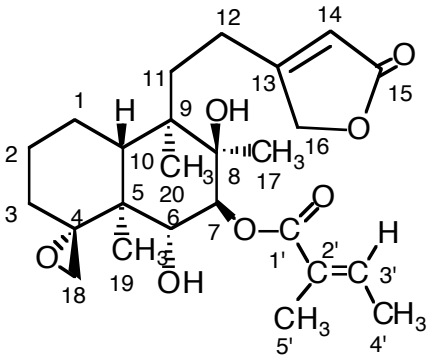
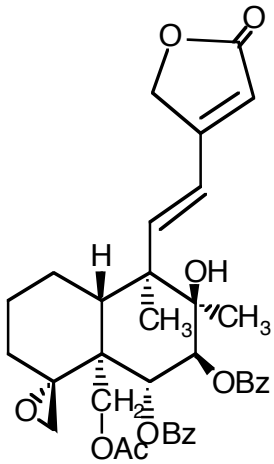


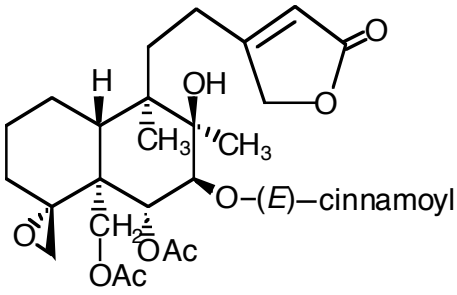
Figure 2 The results of phase sensitive NOESY for **1**



(**1**)



(**2**)



(**3**)

phase sensitive NOESY spectrum. Although the absolute stereochemistry was not ascertained, it may be suggested that hematochloridin (**1**) belongs to the neoclerodane series by comparisons of the sign of optical rotation with those of scutalpin K (**2**){ $[\alpha]_D$ -27.3° ($CHCl_3$)}[1c] and scutorientalin E (**3**) {$[\alpha]_D$ -9.6° ($CHCl_3$)}.[1d]

EXPERIMENTAL

The following apparatus were used to take spectral data. NMR: ^1H -(400 MHz) and ^{13}C-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 F_{254} 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, Usbekistan Republic in June, 1997 and identified as *Scutellaria hematochlora* 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. hematochlora* 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 H_2O (1 L) and the suspension was extracted with EtOAc (1 L x 3). The EtOAc layer was washed with H_2O (500 mL), dried over anhyd. $MgSO_4$ and evaporated *in vacuo* to give a residue (33.5 g) which was chromatographed over silica gel (800 g) with a mixture of $CHCl_3$ and Me_2CO with increasing amount of Me_2CO content. Each 6 liters of 0, 5, 10, 20 and 30% Me_2CO in $CHCl_3$ were eluted succesively, collecting 500 mL fractions. Fraction Nos. 7-8 gave a residue (1.24 g) which was repeatedly separated by silica gel chromatography (solvent: Et_2O and $CHCl_3$) and finally preparative TLC (solvent: *n*-hexane - Et_2O 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 $CHCl_3$ and then Et_2O to give hematochloridin (**1**)(324 mg)[*Rf* 0.51 ($CHCl_3$-Me_2CO 7 : 3) and 0.16 (Et_2O) on TLC]. Fraction Nos. 12-15 gave a residue (2.87 g) which was chromatographed over silica gel (100 g) with Et_2O as eluent. The residue (718 mg) from the faster eluate was washed well with $CHCl_3$ 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**): $[\alpha]^{25}_{D}$ -15.0° (*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}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}C- NMR: see Table 1; FABMS (negative) *m/z* 447.2371 $[M-H]^-$ (calcd for $C_{25}H_{35}O_7$: 447.2383).

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