Studies on the Chemical Constituents of the Bulbs of *Fritillaria camtschatcensis*

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The fresh bulbs of *Fritillaria camtschatcensis* have yielded two phenolic glycosides, (2*S*)-1-*O*-*p*-coumaroyl-3-*O*-β-D-glucopyranosylglycerol (regaloside A) and 3,6′-*O*-diferuloylsucrose, and two steroidal alkaloids, solanidine 3-*O*-[α-L-rhamnopyranosyl(1→2)][β-D-glucopyranosyl(1→4)]-β-D-glucopyranoside and 2β,3α,6β-trihydroxy-5α-jervanin-12-ene (kuroyurinidine). Kuroyurinidine is the first example of the C-nor-D-homo steroidal alkaloid from *F. camtschatcensis*.

Keywords *Fritillaria camtschatcensis*; Liliaceae; jerveratrum alkaloid; steroidal alkaloid; kuroyurinidine; solanidine glycoside; phenolic glycoside; regaloside A; 3,6′-*O*-diferuloylsucrose; bulb

Fritillaria species have been extensively investigated and a large number of alkaloids have been isolated.[1] *Fritillaria camtschatcensis* (Japanese name, kuroyuri) grows in northern Japan. Mitsuhashi and his coworkers have isolated several steroidal alkaloids: hapepunine,[2,3] anrakorinine,[3] veralkamine,[4] solanidine,[3,5] camtschatcanidine,[4] solasodine and tomatidenol.[2,3] Their continuous studies have proven *F. camtschatcensis* to be devoid of the C-nor-D-homo steroidal alkaloid, that is, jerveratrum or ceveratrum type alkaloid. In the course of our phytochemical studies of the Liliaceae plants, examination has been made of the bulbs of *F. camtschatcensis* resulting in the isolation of a novel C-nor-D-homo steroidal alkaloid, named kuroyurinidine, a steroidal alkaloid glycoside and two phenolic glycosides. The structure of the new compound has been determined mainly by the use of the two-dimensional correlated spectroscopies and stereospecific spin-coupling constants. The present paper provides detailed evidence of the structure. A part of this work has been reported in a preliminary communication.[6]

The fresh bulbs of *F. camtschatcensis* were extracted with hot methanol. The crude extract was partitioned between chloroform and water, and the water phase was extracted with *n*-butanol. A series of chromatographic separations of the *n*-butanol soluble phase gave **1**—**4**.

Compounds **1** and **2** were obtained as pale-yellow amorphous powders. The structures were determined to be (2*S*)-1-*O*-*p*-coumaroyl-3-*O*-β-D-glucopyranosylglycerol (regaloside A)[7] and 3,6′-*O*-diferuloylsucrose[8] by the infrared (IR), proton nuclear magnetic resonance (^{1}H-NMR) spectra. Compound **3** was a more polar constituent than **1** and **2**, and obtained as colorless needles recrystallized from methanol, it decomposed at >250 °C without melting. It reacted positive to the Dragendorff reagent on thin-layer chromatography (TLC). The confirmative structure was assigned as solanidine 3-*O*-[α-L-rhamnopyranosyl(1→2)][β-D-glucopyranosyl(1→4)]-β-D-glucopyranoside by the secondary ion mass spectrum (SI-MS), IR, ^{1}H-NMR and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra. This compound was previously isolated from the aerial parts of *Fritillaria thunbergii*.[9]

Compound **4** was obtained as a white amorphous powder. A positive color reaction with Dragendorff reagent was suggestive of **4** being an alkaloid. The electron impact mass spectrum (EI-MS) showed a molecular ion peak at *m/z* 445. The accurate mass ion at *m/z* 445 was found to be 445.3142 by the high resolution MS, corresponding to the molecular formula, $C_{27}H_{43}NO_4$. The IR spectrum showed an absorption band of hydroxyl group(s) (3425 cm^{-1}). The ^{13}C-NMR spectrum showed a total of 27 carbons, and the various distortionless enhancement by polarization transfer (DEPT) spectra made it possible to assign all the signals as $CH_3 \times 4$, $CH_2 \times 8$, $CH \times 11$ and $C \times 4$. Signals at δ 142.7 and 127.2 were due to quaternary olefinic carbons. No signal for carbonyl function could be found. The ^{1}H-NMR spectrum showed the presence of two tertiary methyl groups [δ 1.85 (3H, s) and 1.72 (3H, br s)] and two secondary methyl groups [δ 1.12 (3H, d, J=7.4 Hz) and 0.83 (3H, d, J=6.6 Hz). Acetylation of **4** with acetic anhydride in pyridine yielded the corresponding *O*,*O*′,*O*″,*N*-tetraacetyl derivative (**4a**). The EI-MS of **4a** gave a molecular ion peak at *m/z* 613 and the IR spectrum showed no hydroxyl absorption. The prominent peaks at *m/z* 125, 124, 114 and 110 in the EI-MS of **4**, and peaks at *m/z* 156,

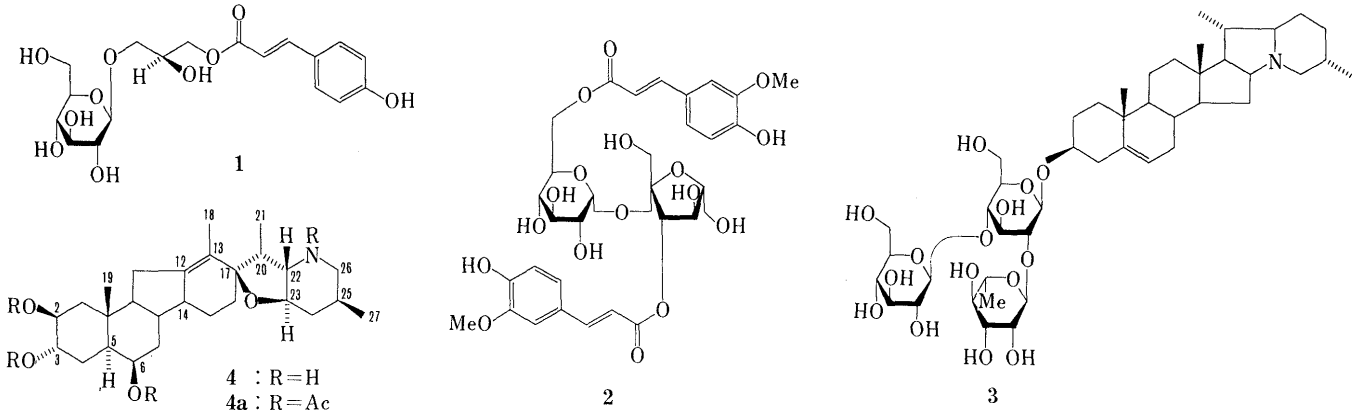

Chart 1

125, 124, 114 and 110 in that of **4a** seemed to correspond to the fragments of the E and F rings of the jervine derivatives.[10] From the spectral data and discussion referred to above, the fundamental structure of **4** appeared to be 5,6-dihydro-11-deoxojervine with three hydroxyl groups.

The ^{13}C-NMR spectrum of **4** exhibited six signals between 60—100 ppm [δ 85.6 (C), 75.4, 72.3, 72.0 × 2 and 66.6 (each CH)]. Three of them at δ 85.6, 75.4 and 66.6 were assigned to the C-17, C-23 and C-22 positions in the jervanin skeleton. The ^{1}H-NMR spin-coupling and the nuclear Overhauser effect (NOE) relationships of the E and F rings were revealed as shown in Fig. 1. Consequently, the remaining three signals at δ 72.3 and 72.0 × 2 were hydroxy methine carbons. In comparing the ^{13}C-NMR spectral data with those of the previously reported jervine derivatives,[10,11] the three hydroxyl groups were presumed to be localized at the A and/or B rings. The narrow half-height widths of the signals assignable to the hydroxy methine protons [δ 4.65, 4.57 and 4.24 (each 1H, br s, $W_{1/2}$ = 8—10 Hz) suggested that all the hydroxyl groups were present in the axial orientations. The ^{1}H–^{1}H two-dimentional correlation spectroscopy (^{1}H–^{1}H COSY) and the two-dimentional NOE correlation spectroscopy (NOESY) spectra were of particular help in elucidating the hydroxyl positions. In the ^{1}H–^{1}H COSY spectrum, the signal at δ 4.65 showed cross peaks at δ 4.57, 3.10 (1H, ddd, J = 13.3, 13.3, 2.2 Hz) and 2.01. The signals at δ 3.10 and 2.01 were due to a methylene, and both signals showed cross peaks not only at δ 4.65 but also at δ 2.35 (overlapping with other signals). A cross peak was observed between the signals at δ 2.35 and 4.24. Further, the NOESY spectrum showed the NOE correlation between the signals at δ 2.01 and 4.24. On the other hand, the signal at δ 4.57 exhibited cross peaks at δ 4.65, 2.17 and 2.03 (br d, J = 14.1 Hz) in the ^{1}H–^{1}H COSY spectrum. The signals at δ 2.17 and 2.03 were due to a methylene and both signals exhibited cross peaks at δ 4.57 only. The above data were shown in Fig. 2. Thus, the three hydroxyl groups were unequivocally concluded to be localized at the C-2β, C-3α and C-6β positions.

The NOE correlation between the H-4 axial proton and the H-19 methyl protons indicated that the ring junction was A/B *trans* situation. The above result was further reinforced by the ^{1}H-NMR chemical shift of the H-19 methyl function which was shifted extremely downfield (δ 1.85 in C_5D_5N) because of the 1,3-diaxial interaction with the β-axial hydroxyl groups at the C-2 and C-6 positions. Thus, the structure of **4** was formulated as 2β,3α,6β-trihydroxy-5α-jervanin-12-ene, designated as kuroyurinidine.

Phenylpropanoid glycerol glucosides, that is, regalosides, were first isolated from *Lilium regale*[7] and later from several *Lilium* plants.[12] We know of no other report on regalosides from a natural source. This time, regaloside A has been isolated from a good yield of *F. camtschatcensis*. The genus *Lilium* is taxonomically related to the genus *Fritillaria* in Liliaceae. Regalosides may be the specific constituents of the two genera. A survey of the phenolic glycosides in the *Fritillaria* plant is in progress in our laboratory. Solanidine had already been detected in the bulbs of *F. camtschatcensis*.[3,5] In this examination, we could isolate solanidine glycoside from the unhydrolysed methanolic bulb extract of *F. camtschatcensis* for the first time. Kuroyurinidine is unique in structure having three axial hydroxyl groups at the C-2, C-3 and C-6 positions on the steroid skeleton, and this is believed to be the first example of the C-nor-D-homo steroidal alkaloid from *F. camtschatcensis*.

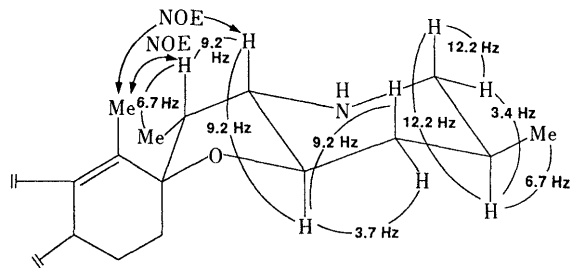

Fig. 1. ^{1}H-NMR Spin-Coupling Constants and NOE Correlation of the E and F Rings of **4** (^{1}H-NMR in CD_3OD, NOE in C_5D_5N)

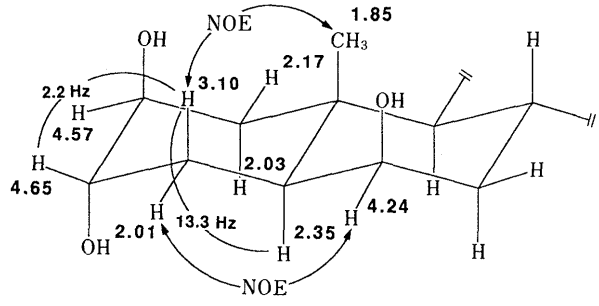

Fig. 2. ^{1}H-NMR Chemical Shifts (ppm), Spin-Coupling Constants and NOE Correlation of the A and B Rings of **4** (C_5D_5N)

Experimental

Melting point was determined on a Yazawa micro melting apparatus and is uncorrected. IR spectra were recorded on a Hitachi 260-30 or a Perkin-Elmer 1710 FTIR spectrometer and mass spectra (low and high resolution) on a Hitachi M-80 machine. Optical rotations were measured with a JASCO DIP-360 automatic polarimeter with concentrations of sample reported in grams/100 ml. NMR spectra were taken with a Bruker AM-400 instrument (400 MHz for ^{1}H-NMR and 100.6 MHz for ^{13}C-NMR). Chemical shifts were expressed in ppm (δ) values relative to the internal reference, tetramethylsilane (TMS), and the abbreviations used are as follows: s, singlet; d, doublet; dd, doublet of doublets; dq, doublet of quartets; m, multiplet; br, broad. All 1D and 2D pulse sequences were run using standard Bruker softwear. Column chromatographies were carried out on Fuji Davison silica gel BW-300 (200—400 mesh, Fuji Davison Co., Ltd.), Sephadex LH-20 (25—100 μm, Pharmacia Fine Chemicals Co., Ltd.) and DIAION HP-20 (Mitsubishi-kasei Co., Ltd.). TLC was performed on precoated Kieselgel 60 F_{254} plates (0.25 mm thick, Merck) and preparative TLC on precoated Kieselgel 60 F_{254} (0.5 mm thick, Merck). Spots were visualized under ultraviolet (UV) light (254 nm) irradiation and by spraying 10% H_2SO_4 solution followed by heating. Alkaloids were detected by spraying Dragendorff reagent.

Isolation The fresh bulbs of *F. camtschatcensis* (900 g) purchased from Heiwaen Co., Japan, were cut into pieces and exhaustively extracted with hot MeOH. The MeOH extract was concentrated under reduced pressure. The viscous concentrate was partitioned between H_2O and $CHCl_3$, and then between H_2O and *n*-BuOH. Each partition was repeated twice. The *n*-BuOH soluble fraction was chromatographed on silica gel with $CHCl_3$–MeOH (9 : 1) to give ten fractions (1—10).

Fraction 6 was applied to a silica gel column with $CHCl_3$–MeOH (6 : 1) and to a Sephadex LH-20 with MeOH to give **2**.

Fraction 7 was chromatographed on silica gel with $CHCl_3$–MeOH (6 : 1) and on Sephadex LH-20 with MeOH to give **1** with a few impurities. Final purification of **1** was carried out by silica gel column chromatography using $CHCl_3$–MeOH–H_2O (180 : 20 : 1).

Fractions 9 and 10 showed positive color spots to Dragendorff reagent on TLC (Rf 0.19 in 9; Rf 0.10 in 10) developed with $CHCl_3$–MeOH–H_2O

(100:40:1). Fraction 9 was subjected to a silica gel column with $CHCl_3$–MeOH–H_2O (100:40:1) and to a Sephadex LH-20 with MeOH. Fractions being positive to Dragendorff reagent were combined and further purified by silica gel column chromatography with $CHCl_3$–MeOH–H_2O (140:20:1) to yield **4**. Final purification of **4** was achieved by the preparative TLC with $CHCl_3$–MeOH–NH_3 (50:20:1). The most polar fraction, 10 was chromatographed on a silica gel with $CHCl_3$–MeOH–H_2O (125:50:2) and on a Sephadex LH-20 with MeOH as the eluents. Fractions containing alkaloid were further fractionated by DIAION HP-20 column chromatography with a H_2O/H_2O–MeOH/MeOH gradient system. Alkaloid was concentrated in the 60% MeOH/H_2O, 80% MeOH/H_2O and MeOH fractions, which were combined and subjected to silica gel column chromatography with $CHCl_3$–MeOH–H_2O (100:40:1) to furnish **3** as an almost pure compound. After being purified by the preparative TLC using EtOAc–MeOH–AcOH (30:10:1), **3** was recrystallized from MeOH.

(2*S*)-1-*O*-*p*-Coumaroyl-3-*O*-*β*-D-glucopyranosylglycerol (Regaloside A) (1) A pale-yellow amorphous powder, yield: 583 mg.

3,6′-*O*-Diferuloylsucrose (2) A pale yellow amorphous powder, yield: 408 mg.

Solanidine 3-*O*-[α-L-Rhamnopyranosyl(1→2)][*β*-D-glucopyranosyl(1→4)]-*β*-D-glucopyranoside (3) Colorless needles (MeOH), decomposed >250 °C, yield: 139 mg. SI-MS *m/z*: 867 $[M]^+$.

2*β*,3α,6*β*-Trihydroxy-5α-jervanin-12-ene (Kuroyurinidine) (4) A white amorphous powder, 20.3 mg, $[\alpha]_D^{25}$ −9.7° (*c*=0.44, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3425 (OH), 2910, 2850 (CH), 1445, 1420, 1370, 1290, 1250, 1165, 1150, 1110, 1090, 1065, 1020, 975, 965, 915, 890. EI-MS *m/z* (%): 445.3142 [M^+, Calcd for $C_{27}H_{43}NO_4$: 445.3194] (23), 430 (61), 332 (99), 314 (18), 299 (38), 173 (62), 159 (34), 145 (28), 125 (99), 124 (99), 114 (99), 110 (100), 83 (59). ^{1}H-NMR (C_5D_5N): δ 4.65 (1H, br s, $W_{1/2}$=8.0 Hz, H-3), 4.57 (1H, br s, $W_{1/2}$=10.0 Hz, H-2), 4.24 (1H, br s, $W_{1/2}$=8.0 Hz, H-6), 3.46 (1H, ddd, *J*=9.1, 9.1, 3.7 Hz, H-23), 3.17 (1H, dd, *J*=12.3, 3.6 Hz, H-26 equatorial), 3.10 (1H, ddd, *J*=13.3, 13.3, 2.2 Hz, H-4 axial), 2.85 (1H, dd, *J*=9.1, 9.1 Hz, H-22), 2.56 (1H, dq, *J*=9.1, 7.4 Hz, H-20), 2.03 (1H, br d, *J*=14.1 Hz, H-1 axial), 1.85 (3H, s, H-19), 1.72 (3H, br s, H-18), 1.27 (1H, ddd, *J*=11.3, 11.3, 9.1 Hz, H-24 axial), 1.12 (3H, d, *J*=7.4 Hz, H-21), 0.83 (3H, d, *J*=6.6 Hz, H-27). ^{1}H-NMR (CD_3OD): δ 3.89 (1H, br s, $W_{1/2}$=6.3 Hz, H-2, -3 or -6), 3.79 (2H, br s, H-2, -3 or -6), 3.37 (1H, ddd, *J*=9.2, 9.2, 3.7 Hz, H-23), 3.10 (1H, dd, *J*=12.2, 3.4 Hz, H-26 equatorial), 2.76 (1H, dd, *J*=9.2, 9.2 Hz, H-22), 2.51 (1H, dq, *J*=9.2, 6.7 Hz, H-20), 2.40 (1H, dd, *J*=12.2, 12.2 Hz, H-26 axial), 1.62 (3H, br s, H-18), 1.14 (3H, s, H-19), 0.98 (3H×2, d, *J*=6.7 Hz, H-21, -27). ^{13}C-NMR (C_5D_5N): δ 43.5 (C-1), 72.0 (C-2),* 72.0 (C-3),* 30.5 (C-4), 43.7 (C-5), 72.3 (C-6),* 39.4 (C-7), 40.1 (C-8), 57.2 (C-9), 36.9 (C-10), 29.3 (C-11), 127.4 (C-12), 142.7 (C-13), 48.8 (C-14), 25.1 (C-15), 32.3 (C-16), 85.6 (C-17), 13.5 (C-18), 17.6 (C-19), 40.8 (C-20), 11.3 (C-21), 66.6 (C-22), 75.4 (C-23), 40.0 (C-24), 31.1 (C-25), 54.6 (C-26), 18.9 (C-27). *: Assignments may be interchangeable.

Acetylation of 4 To a pyridine solution of **4** (3.2 mg) was added Ac_2O and it was left standing overnight at room temperature. The crude product was chromatographed on silica gel with *n*-hexane–Me_2CO (2:1) to furnish the corresponding tetraacetate (**4a**) (3.0 mg). A white amorphous powder. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3000, 2935, 2855 (CH), 1730 (C=O), 1635, 1430, 1370, 1235, 1175, 1105, 1025, 985, 950, 895. EI-MS *m/z* (%): 613 $[M]^+$ (weak), 553 (0.5), 458 (1), 398 (1.3), 397 (1.3), 396 (1.3), 369 (2.4), 277 (2), 263 (1), 261 (1.7), 249 (2), 223 (0.8), 209 (1.2), 167 (95), 156 (100), 152 (18), 142 (6), 125 (13), 124 (10), 114 (42), 110 (17). ^{1}H-NMR ($CDCl_3$): δ 5.00 (2H, br s, H-2, -3 or -6), 3.88 (1H, br s, $W_{1/2}$=7.5 Hz, H-2, -3 or -6), 2.10, 2.07×2, 2.01 (each 3H, s, Ac), 1.69 (3H, br s, H-18), 1.03 (3H, d, *J*=6.9 Hz, H-21), 1.01 (3H, s, H-19), 0.86 (3H, d, *J*=6.9 Hz, H-27).

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