Acetylated Aporphine Alkaloids from *Lysichiton camtschatcense*

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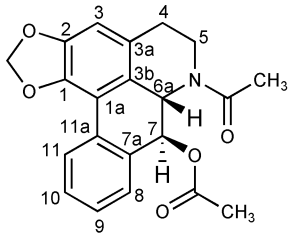
Two *N*,*O*-diacetylaporphine alkaloids, *N*,*O*-diacetylnoroliveroline (**1**) and *N*,*O*-diacetyl-(−)-nornuciferidine (**2**), have been isolated and characterized from the rhizomes of *Lysichiton camtschatcense*. Their structures were determined by spectroscopic data analysis. DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals were scavenged by compounds **1** and **2**, but with weak activity.

The skunk cabbage, *Lysichiton camtschatcense* (L.) Schott (Araceae), is distributed in marshes of eastern Siberia, the Kuril Islands, and northern Japan. As a traditional medicine for acute nephritis, perspiration, and constipation, the Ainu, an indigenous race who inhabit Hokkaido in northern Japan, have ingested extracts of the rhizomes of this plant for many years. Since it has been recognized that toxic reactive oxygen species (ROS) are implicated in inflammation, it is possible that the constituents of *L. camtschatcense* rhizomes may act as an anti-inflammation agent for ROS in nephritis. In fact, it was found that an extract of *L. camtschatcense* rhizomes shows an antioxidant effect on Fe^{2+}/ascorbate-induced lipidperoxidation of a homogenate of rat kidney.[1] Hence, the isolation and the structure determination of the active component as antioxidants were undertaken. It has been reported previously that the flavonoid kaempferol glycoside from the leaves[2] and several aporphine alkaloids, namely, liriodenine, nuciferine, and lysicamine, from the rhizomes are constituents of *L. camtschatcense*.[3] Moreover, aporphine alkaloids among the Araceae are present in only this plant. In a search for bioactive compounds from *L. camtschatcense*, two aporphine alkaloids, *N*,*O*-diacetylnoroliveroline (**1**) and *N*,*O*-diacetyl-(−)-nornuciferidine (**2**), with the mildly scavenging effect on DPPH radicals, were isolated from this plant in the present investigation.



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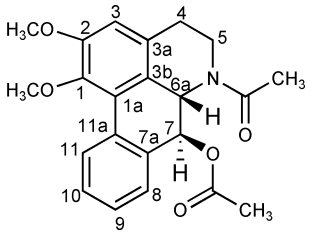

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N,*O*-Diacetylnoroliveroline (**1**) was obtained as a light brown powder. HRFABMS revealed the molecular formula to be $C_{21}H_{19}O_5N$ $[M + H]^+$. The specific rotation of **1** was levorotatory ($[\alpha]^{25}_D$ −221.3°, *c* 0.18, $CHCl_3$), and the IR spectrum of **1** exhibited absorption bands at 1743, 1240, and 1043 cm^{-1} (ester carbonyl groups), 1620, 1508, and 841 cm^{-1} (aromatic), and 1653 cm^{-1} (amide carbonyl), respectively. The UV spectrum of **1** showed absorptions at λ_{max} 319, 276, and 211 nm, which indicated the presence of an aporphine skeleton.[4] The ^1H NMR spectrum showed a signal at δ 8.09 for H-11 and a multiplet at δ 7.43−7.32 for H-8, H-9, and H-10, and a singlet at δ 6.61 for H-3, in the region for aromatic protons. These protons were correlated in the HSQC spectrum with carbon signals at δ 127.5, 128.4, 128.2, 123.7, and 108.2, respectively, and each position was determined by the CT-HMBC method[5] to improve the separation of cross-peaks. Two singlet peaks at δ 6.12 and 6.01 were assigned to the methylenedioxy protons from their J_{H-H} and J_{C-H} coupling constants of 1.2 and 173.8 Hz, respectively, and because they were related to the ^{13}C NMR signal appearing at δ 101.3 in the HSQC experiment. Signals at δ 6.25 and 4.83 for H-7 and H-6a indicated that they were adjacent to nitrogen or to oxygen atoms. The coupling constant between H-7 and H-6a was 12.3 Hz, and in several previous reports,[6,7] the coupling constant of 7-oxygenated aporphines, with a *trans* relationship between H-7 and H-6a, gave comparable values. Therefore, H-7 and H-6a were assigned with a *trans* relationship. Two acetyl methyl groups were assigned at δ 2.26 and 2.18, correlating in the HMBC spectrum to carbonyl carbons in the ^{13}C NMR spectrum at δ 170.6 and 170.0. The ^1H NMR spectrum displayed signals at δ 4.82, 2.76 for H-5 and δ 2.76, 2.56 for H-4. From the data mentioned above, this compound must possess structure **1**.

N,*O*-Diacetyl-(−)-nornuciferidine (**2**) was obtained as a yellow powder, and its molecular formula of $C_{22}H_{24}O_5N$ $[M + H]^+$ was determined by HRFABMS. The specific rotation of **2** was again levorotatory ($[\alpha]^{25}_D$ −227.7°, *c* 0.24, $CHCl_3$). The IR and the UV spectra of **2** closely resembled those of **1**. The ^1H, ^{13}C, and various 2D ($^1H-^1H$ COSY, $^1H-^{13}C$ HMBC, and HMQC) NMR spectra of **2** were also similar to those of **1**. However, in their ^1H NMR spectra, the two methylenedioxy peaks at δ 6.12 and 6.01 (each 1H, d) of **1** were replaced by two new signals at δ 3.90 (3H, s, OC*H*$_3$-2) and 3.67 (3H, s, OC*H*$_3$-1). In addition to these differences, the chemical shift of a methylenedioxy carbon in the ^{13}C NMR spectrum was replaced by two new signals at δ 60.0 (q) and 56.0 (q), due to methoxyl carbons. The coupling constant between H-7 and H-6a (J = 11.9 Hz) was also similar to that of **1**, so that these protons were

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assigned with a *trans* relationship. Thus, the structure of **2** was characterized as a new aporphine alkaloid, *N*,*O*-diacetyl-(−)-nornuciferidine.

These acetylated aporphine alkaloids were isolated and identified from *L. camtschatcense* for the first time, although the nonacetylated analogues, noroliveroline and (−)-nornuciferidine, were previously isolated from various plants.[8−11] Although compound **1** was prepared as a semisynthetic derivative from an isolate of *Polyalthia acuminata*,[7] our study is the first report of its isolation from nature. Therefore, *N*,*O*-diacetylnoroliveroline isolated from *L. camtschatcense* is a naturally occurring new compound.

Compounds **1** and **2** were assessed for DPPH radical scavenging activity. They had a slight antioxidant activity after 30 min incubation (both compounds, <10%). However, after 48 h incubation, the IC_{50} values of compounds **1** and **2** were 518 and 362 μM, respectively. Thus, two acetylated aporphine alkaloids had extremely mild antioxidant activity.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a JASCO DIP-1000 digital polarimeter. The UV and IR spectra were recorded on Shimadzu UV-1200 and JASCO FT/IR-5300 spectrophotometers, respectively. ^{1}H (500 MHz), ^{13}C (125 MHz), and 2D (gHMBC and gHMQC or gHSQC, COSY, NOESY) NMR spectra were recorded using JEOL JNM-A500 and/or Varian INOVA-500 spectrometers with tetramethylsilane (TMS) as an internal standard in $CDCl_3$. A JEOL JMS-700 instrument was used to obtain the FABMS and the HRFABMS. Silica gel 60 (Merck, 40−63 μm) column chromatography was used for the separations, and precoated silica gel plates (Merck, silica gel 60 F_{254}, 0.20 mm) were used for analytical TLC. The spots were visualized by spraying with 10% H_2SO_4 and then heating. Preparative HPLC was conducted using a reversed-phase ODS column (Senshu Scientific Co., PEGASIL ODS, 10 i.d. × 250 mm) with a UV detector (Soma Optics Co., S-310A model II), using MeOH−H_2O (70:30) as mobile phase and a flow rate of 3.0 mL/min.

Plant Material. The rhizomes of *L. camtschatcense* were collected at Memanbetsu, Japan, in August 2001. The plant was identified by Mr. K. Kojima, a member of the committee for "Mizubasho" (Japanese name for *L. camtschatcense*) in Memanbetsu city. A voucher specimen (No. 010819) is deposited at Shibaura Institute of Technology.

Extraction and Isolation. The dry, powdered rhizomes of *L. camtschatcense* (1.8 kg) were extracted repeatedly with 80% EtOH at room temperature. After partitioning with hexane, the extract was concentrated in vacuo. The residue obtained was partitioned between EtOAc and H_2O. The EtOAc layer was evaporated and subjected to column chromatography on silica gel using mixtures of $CHCl_3$−MeOH ($CHCl_3$, 100:1, 50:1, 10:1, 5:1, and MeOH) to give eight fractions (A to H). Fraction B was separated on a silica gel column again using a mixture of $CHCl_3$−MeOH ($CHCl_3$, 100:1, 50:1, 10:1, and MeOH) to obtain seven additional fractions (B1−B7). Fraction B3 was rechromatographed on an ODS column using acetonitrile as an eluent to yield a crude alkaloidal fraction. This fraction was further purified by a preparative HPLC with a ODS column (PEGASIL ODS, 10 i.d. × 250 mm) with UV detection (260 nm) to yield compounds **1** (8 mg) and **2** (8 mg).

***N*,*O*-Diacetylnoroliveroline (1):** light brown powder; $[\alpha]^{25}_D$ −221.3° (*c* 0.18, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 211 (4.36), 276 (3.98), 319 (3.44) nm; IR (KBr disk) ν_{max} 1743, 1653, 1620, 1508, 1366, 1240, 1043, 841 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.09 (1H, d, J = 7.6 Hz, H-11), 7.43−7.32 (3H, m, H-8, H-9, and H-10), 6.61 (1H, s, H-3), 6.25 (1H, d, J = 12.3 Hz, H-7), 6.12 and 6.01 (each 1H, d, J = 1.2 Hz, OCH_2O), 4.83 (1H, d, J = 12.3 Hz, H-6a), 4.82 and 2.76 (each 1H, m, H-5), 2.76 and 2.56 (each 1H, m, H-4), 2.26 (3H, s, OCOCH_3), 2.18 (3H, s, NCOCH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.6 (s, NCOCH$_3$), 170.0 (s, OCOCH$_3$), 147.8 (s, C-2), 142.9 (s, C-1), 135.3 (s, C-7a), 129.5 (s, C-11a), 129.4 (s, C-3a), 128.4 (d, C-10), 128.2 (d, C-9), 127.5 (d, C-11), 123.7 (d, C-8), 121.9 (s, C-3b), 116.3 (s, C-1a), 108.2 (d, C-3), 101.3 (t, OCH$_2$O), 71.3 (d, C-7), 57.6 (d, C-6a), 36.7 (t, C-5), 29.3 (t, C-4), 22.2 (q, NCOCH$_3$), 20.9 (q, OCOCH$_3$); FABMS *m/z* 366.2 [M + H]$^+$; HRFABMS *m/z* 366.1342 [M + H]$^+$ (calcd for $C_{21}H_{20}O_5N$, 366.1341).

***N*,*O*-Diacetyl-(−)-nornuciferidine (2):** yellow powder; $[\alpha]^{25}_D$ −227.7° (*c* 0.24, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 211 (4.39), 273 (4.00), 313 (3.40) nm; IR (KBr disk) ν_{max} 1743, 1655, 1625, 1510, 1366, 1240, 1043, 1028, 846 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.45 (1H, d, J = 7.9 Hz, H-11), 7.45−7.34 (3H, m, H8, H-9 and H-10), 6.70 (1H, s, H-3), 6.18 (1H, d, J = 11.9 Hz, H-7), 4.86 and 2.78 (each 1H, m, H-5), 4.71 (1H, d, J = 11.9 Hz, H-6a), 3.90 (3H, s, OCH_3-2), 3.67 (3H, s, OCH_3-1), 2.81 and 2.62 (each 1H, m, H-4), 2.26 (3H, s, OCOCH_3), 2.17 (3H, s, NCOCH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.6 (s, NCOCH$_3$), 170.1 (s, OCOCH$_3$), 152.9 (s, C-2), 145.5 (s, C-1), 136.2 (s, C-7a), 131.3 (s, C-3a), 130.3 (s, C-11a), 129.0 (d, C-11), 128.3 (d, C-10), 128.1 (d, C-9), 126.7 (s, C-1a), 122.9 (d, C-8), 121.9 (s, C-3b), 112.1 (d, C-3), 71.3 (d, C-7), 60.0 (q, OCH$_3$-2), 57.6 (d, C-6a), 56.0 (q, OCH$_3$-1), 36.4 (t, C-5), 29.4 (t, C-4), 22.2 (q, NCOCH$_3$), 20.9 (q, OCOCH$_3$); FABMS *m/z* 382.2 [M + H]$^+$; HRFABMS *m/z* 382.1646 [M + H]$^+$ (calcd for $C_{22}H_{24}O_5N$, 382.1654).

Antioxidant Activity of 1 and 2. The antioxidant activity of **1** and **2** was assessed using a DPPH scavenging assay as follows. Each diluted sample by EtOH was added into a 100 μM DPPH EtOH solution and incubated for 30 min or 48 h at room temperature, respectively. Each mixture was measured by a spectrophotometer at 517 nm. DPPH scavenging activity was evaluated by the ratio of the absorbance of samples to DPPH itself.

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