Isolation and Structure of Korolkoside, a Bis-Iridoid Glucoside from *Lonicera korolkovii*

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A new bis-iridoid glucoside, korolkoside (**1**), was isolated from *Lonicera korolkovii*, and its structure and stereochemistry were determined by spectroscopic analysis and chemical derivatization. Korolkoside (**1**) consists of two secologanin moieties that are connected by an acetal linkage.

The chemical constituents of plants in the genus *Lonicera* have been studied, and several iridoid glycosides, such as secologanin, were isolated.[1−3] During the course of our search for the toxic constituents of *Lonicera* sp., we have investigated *L. korolkovii* Stapf (Caprifoliaceae), a cultivated plant in Japan, Western Europe, and North America, which showed acute toxicity against ddY mice. We report herein the isolation and structural determination of a new bis-iridoid glucoside, korolkoside (**1**).

The *n*-BuOH-soluble fraction of the MeOH extract of the aerial parts of the young plant *L. korolkovii* was partitioned, and the least lipophilic portion was chromatographed to provide the toxic fraction.[4] Further separation of this fraction using medium-pressure liquid chromatography (MPLC; ODS) and reversed-phase HPLC (ODS) gave korolkoside (**1**) (0.004% yield based on wet weight), which, however, showed little acute toxicity against ddY mice.

The molecular formula of **1** as determined by HRFABMS was $C_{36}H_{52}O_{20}$ (Δ +1.0 mmu). The NMR data for **1**, summarized in Table 1, showed the glycosidic nature of **1**. The ^{1}H NMR, ^{13}C NMR, and HSQC spectra of **1** indicated the presence of four methyl carbons, four sp^3 methylene carbons, 18 sp^3 methine carbons, eight olefinic carbons, and two carbonyl carbons. The ^{1}H and ^{13}C NMR spectra showed that most of the signals were observed in pairs having close chemical shifts and coupling constants, which suggested that **1** might have a dimeric structure possessing two sets of aglycons and sugars. A detailed analysis of the phase-sensitive DQF−COSY spectrum of **1** permitted four partial structures to be constructed, C-1$_a$ to C-10$_a$, C-1$_b$ to C-10$_b$, C-1′ to C-6′, and C-1″ to C-6″ (Figure 1). The remaining connectivities of the carbon framework were clarified by the HMBC correlations between H-3$_a$/C-4$_a$, C-5$_a$, C-11$_a$; H-5$_a$/C-4 $_a$; H-3$_b$/C-4$_b$, C-5$_b$, C-11$_b$; and H-5$_b$/C-4$_b$. Four methyl groups were determined to be those of a dimethyl acetal moiety and two methyl ester structures by the HMBC correlations shown in Figure 1. Furthermore, the HMBC correlations between H-1$_a$/C-3$_a$, C-1′; H-1$_b$/C-3$_b$, C-1″; H-7$_b$/C-4′, C-6′; and H-1″/C-5″ showed the existence of two dihydropyran rings, a tetrahydropyran ring, and a 1,3-dioxane ring and the connectivities among the previously mentioned four partial structures. On the basis of the molecular formula and the degree of unsaturation of **1**, the presence of another ether ring and six hydroxyl groups was suggested.

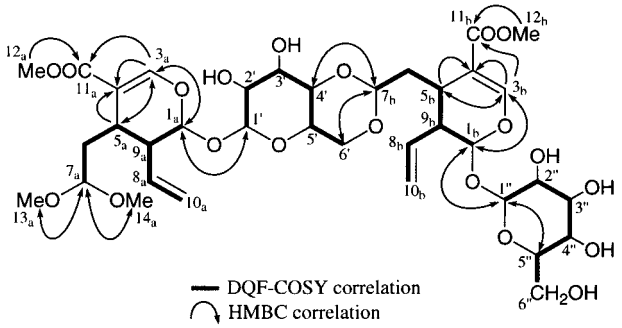

Figure 1. Gross structure of korolkoside (**1**) based on the 2D NMR correlations.

To obtain further information, **1** was acetylated to give the hexaacetate **2**, which showed no IR hydroxy absorption. The ^{1}H NMR signals of **2** were assigned on the basis of the 2D NMR spectra, and the proton chemical shifts of **1** and **2** were compared. Due to the solvent effect used for the NMR measurements (pyridine-d_5 and benzene-d_6), the protons unaffected by acetylation were shifted about 0.5 ppm upfield. On the other hand, the oxymethine protons of H-2′, H-3′, H-2″, H-3″, and H-4″ in **2** were shifted more than 1 ppm downfield from those in **1**, and the methylene protons of H-6″ in **2** were observed in the same area in **1**. These facts indicated that C-2′, C-3′, C-2″, C-3″, C-4″, and C-6″ in **1** possessed a hydroxyl group and that the remaining C-1′ and C-5′ were connected via an oxygen to form an ether bond. Therefore, the gross structure of korolkoside (**1**) was determined as shown in Figure 1.

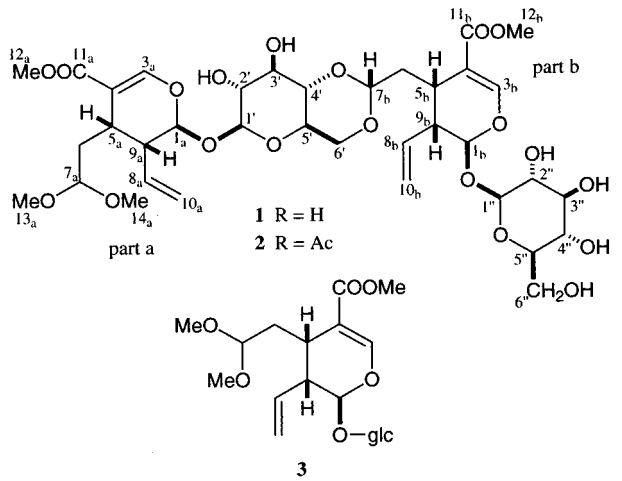

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Table 1. NMR Data for Korolkoside (**1**)[a]

position	^{13}C[b]	^{1}H[c]	position	^{13}C[b]	^{1}H[c]
1_a	97.3 d	5.75 d (5.5)	1_b	96.9 d	5.89 d (6.4)
3_a	152.2 d	7.68 br s	3_b	152.9 d	7.64 br s
4_a	110.8 s		4_b	110.4 s	
5_a	28.8 d	3.27 br ddd (7.8, 6.4, 5.4)	5_b	29.7 d	3.32 br ddd (6.8, 5.9, 5.7)
6_a	32.6 t	2.41 ddd (14.1, 7.0, 6.4)	6_b	35.2 t	2.18 m
		1.87 ddd (14.1, 7.8, 4.4)			2.09 m
7_a	103.1 d	4.70 dd (7.0, 4.4)	7_b	101.9 d	4.95 m
8_a	135.2[d] d	5.84 m	8_b	135.1[d] d	5.84 m
9_a	44.7[e] d	2.89 ddd (8.3, 5.5, 5.4)	9_b	44.5[e] d	2.83 ddd (7.3, 6.4, 5.7)
10_a	119.2[f] t	5.30−5.10 m	10_b	119.0[f] t	5.30−5.10 m
		5.30−5.10 m			5.30−5.10 m
11_a	167.3[g] s		11_b	167.2[g] s	
12_a	51.0[h] q	3.63 s 3 H	12_b	50.9[h] q	3.59 s 3 H
13_a	53.1 q	3.27 s 3 H			
14_a	51.9 q	3.27 s 3 H			
1′	101.1 d	5.28 d (7.9)	1″	100.5 d	5.40 d (7.9)
2′	75.1 d	3.94 dd (8.0, 7.9)	2″	74.6 d	4.01 m
3′	75.3 d	4.21 m	3″	78.4 d	4.23 m
4′	81.3 d	3.64 m	4″	71.4 d	4.20 m
5′	67.4 d	3.58 m	5″	79.0 d	4.02 m
6′	68.6 t	4.30 dd (10.3, 4.8)	6″	62.5 t	4.55 br d (11.1)
		3.71 dd (10.3, 10.3)			4.37 m

[a] Recorded in pyridine-d_5. [b] Recorded at 67.8 MHz. Multiplicity was based on the DEPT and HSQC spectra. [c]Recorded at 600 MHz. Coupling constants (Hz) are in parentheses. [d−h]Signals may be interchanged.

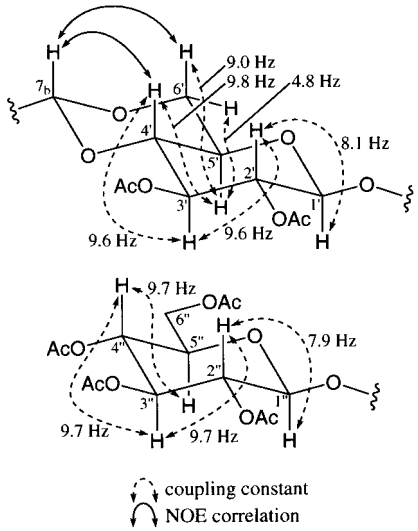

Figure 2. Stereochemistry of the sugar parts of hexaacetate **2**.

The relative stereostructure of korolkoside (**1**) was determined as follows. By comparison with the coupling constants in the ^{1}H NMR spectrum and the ^{13}C NMR chemical shifts of known iridoid glucosides, secologanol[5] and secologanin dimethyl acetal (**3**),[6] the configurations of H-1_a, H-5_a, H-9_a, H-1_b, H-5_b, and H-9_b were deduced to be the same as those of the known compounds. The stereochemistry of the two sugar parts in **1** was determined by the coupling constants of the hexaacetate **2** (Figure 2). Thus, the large *J* values showed that all the methine protons in the sugar moieties were oriented in *anti* arrangements, showing that the two sugars were β-glucose. The remaining problem was to establish the stereochemistry of the acetal methine proton (H-7_b). The NOESY correlations of H-7_b/H-4′ and H-7_b/*axial*-H-6′ in the hexaacetate **2** suggested that H-7_b was β with respect to the six-membered acetal ring with the chair conformation and that H-7_b and each of H-4′ and *axial*-H-6′ were in 1,3-diaxial arrangements (Figure 2). Therefore, the stereostructure of korolkoside was established as the acetal between secologanin and secologanin dimethyl acetal.

Korolkoside (**1**) consists of two secologanin units, and we wondered if this compound is an artifact produced from two molecules of secologanin or secologanin dimethyl acetal during extraction and isolation. Further extraction and separation of the aerial parts of *L. morrowii* without acidic conditions were performed, and we confirmed the existence of korolkoside (**1**) in the resultant extracts. This observation indicated that korolkoside (**1**), except for the dimethyl acelal moiety, is not an artifact produced by the acidic conditions during separation, but is a naturally occurring compound in *Lonicera* sp.

Compound **1** is structually related to caeruleosides A and B,[7a] two bis-iridoid glucosides isolated from *L. caerulea*, and caeruleoside C.[7b] Neither **1** (2 mg per mouse) nor **3** (38 mg per mouse) showed any lethality; however, 2 mg of **1** weakened a mouse for several hours. Because of the scarcity of a natural supply, we could not evaluate an LD_{50} value of **1**. It should be concluded that *L. korolkovii* possesses many kinds of weakly toxic compounds, such as **1**.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a JASCO DIP-1000 polarimeter. IR spectra were recorded on a JASCO FT/IR-230 spectrophotometer. The ^{1}H, ^{13}C, and 2D NMR spectra were recorded on JEOL JNM-EX270 and JNM-A600 spectrometers. The chemical shifts were referenced to the solvent peaks δ_H 8.71 and δ_C 149.8 for pyridine-d_5 and δ_H 7.16 for benzene-d_6. Mass spectra were determined on a JEOL JMS-L2000 spectrometer operating in the positive FAB mode (glycerol as a matrix).

Plant Material. The cultivated young plants of *L. korolkovii* were purchased from Nagoya Engei Co., Ltd., Aichi, Japan, in the summer of 1998, and identified by Dr. Fumihiro Konta, the National Science Museum. A voucher specimen (TNS 707750) is deposited in the herbarium of the National Science Museum, Tokyo, in Tsukuba, Japan.

Acute Toxicity. Lethality was examined by i.p. injection of 0.5 mL of sample solution into ddY male mice (Chubu Kagaku Shizai, Nagoya, Japan) weighing about 10 g. Deaths were scored 12 h after injection.

Extraction and Isolation. The aerial parts of the plants (760 g, wet weight) were crushed and extracted with MeOH (4 L) at room temperature for 7 days. The MeOH extract was filtered and concentrated to give a residue (40 g), which was suspended in H_2O (2.5 L) and extracted with EtOAc (3 × 2.5 L) and then with *n*-BuOH (3 × 2 L). The *n*-BuOH extracts

were concentrated and partitioned between aqueous 90% MeOH (1 L) and hexane (3 × 1 L). The aqueous 90% MeOH portion was partitioned between aqueous 60% MeOH (0.5 L) and CH_2Cl_2 (3 × 0.5 L). The material (5.6 g) obtained from the aqueous 60% MeOH portion showed an acute toxicity against ddY mice (lethality 15 min after injection of 100 mg of this sample), which was subjected to fractionation guided by the toxicity. ODS column chromatography [MeOH/H_2O (8:2→9:1) → MeOH] gave three fractions. The first of these fractions was separated by MPLC [(1) ODS, MeOH/H_2O (1:9→7:3) → MeOH, (2) ODS, MeOH/0.1 M aqueous TFA (2:8→8:2) → MeOH], affording four further fractions. One-sixth of the third fraction, which exhibited an acute toxicity (lethality 14−28 min after injection of 50 mg of this sample), was separated by reversed-phase HPLC [ODS, MeOH/H_2O (4:6) → MeOH] to give seven additional fractions (fractions I−VII). From fraction IV, secologanin dimethyl acetal (**3**) was obtained as an oil (81.5 mg, 0.13% yield based on wet weight). A half of fraction VI (8.7 mg) was purified by reversed-phase HPLC [ODS, MeOH/H_2O (6:4) → MeOH] to afford korolkoside (**1**) as a colorless oil (2.5 mg, 0.004% yield based on wet weight).

Korolkoside (1): oil; $[\alpha]^{28}_D$ −160° (*c* 0.097, MeOH); ^{1}H and ^{13}C NMR, see Table 1; FABMS *m*/*z* 827 $[M + Na]^+$; HRFABMS *m*/*z* 827.2960 (calcd for $C_{36}H_{52}NaO_{20}$, 827.2950).

Acetylation of Korolkoside (1). A solution of korolkoside (**1**) (1.0 mg, 1.2 μmol) in pyridine (0.4 mL) and Ac_2O (0.2 mL) was stirred at room temperature for 20 h and then concentrated in vacuo. The residue was chromatographed on Si gel using $CHCl_3$/EtOAc (2:1) and hexane/EtOAc (1:1) to yield the hexaacetate **2** (0.8 mg, 61%) as an amorphous powder: $[\alpha]^{28}_D$ −79° (*c* 0.016, MeOH); IR ($CHCl_3$) ν_{max} 1755, 1705, 1630, 1415, 1370, 1230, 1080, 1040, 930, 870 cm^{-1}; ^{1}H NMR (600 MHz, benzene-d_6) δ 7.46 (1H, br d, J = 1.7 Hz, H-3$_b$), 7.43 (1H, br d, J = 1.7 Hz, H-3$_a$), 5.55 (1H, m, H-8$_b$), 5.54 (1H, m, H-8$_a$), 5.46 (1H, dd, J = 9.6, 9.6 Hz, H-3′), 5.34 (1H, d, J = 4.4 Hz, H-1$_b$), 5.29 (1H, dd, J = 9.7, 9.7 Hz, H-3″), 5.26 (1H, dd, J = 9.6, 8.1 Hz, H-2′), 5.25 (1H, d, J = 4.0 Hz, H-1$_a$), 5.24 (1H, dd, J = 9.7, 7.9 Hz, H-2″), 5.23 (1H, dd, J = 9.7, 9.7 Hz, H-4″), 5.08 (1H, br dd, J = 10.6, 1.5 Hz, H-10$_b$), 5.07 (1H, br dd, J = 17.4, 1.5 Hz, H-10$_b$), 4.97 (1H, br dd, J = 10.8, 1.8 Hz, H-10$_a$), 4.87 (1H, br dd, J = 16.0, 1.8 Hz, H-10$_a$), 4.77 (1H, d, J = 8.1 Hz, H-1′), 4.70 (1H, d, J = 7.9 Hz, H-1″), 4.63 (1H, dd, J = 7.3, 4.6 Hz, H-7$_a$), 4.57 (1H, dd, J = 5.0, 5.0 Hz, H-7$_b$), 4.25 (1H, m, H-6″), 3.87 (1H, m, H-6″), 3.86 (1H, dd, J = 10.3, 4.8 Hz, H-6′), 3.35 (3H, s, H-12$_a$), 3.35 (3H, s, H-12$_b$), 3.26 (3H, s, H-13$_a$), 3.23 (1H, dd, J = 10.3, 9.0 Hz, H-6′), 3.19 (1H, m, H-5$_b$), 3.18 (1H, dd, J = 9.8, 9.6 Hz, H-4′), 3.16 (1H, m, H-5″), 3.16 (3H, s, H-14$_a$), 3.14 (1H, m, H-5$_a$), 2.94 (1H, ddd, J = 9.8, 9.0, 4.8 Hz, H-5′), 2.75 (1H, m, H-9$_b$), 2.73 (1H, m, H-9$_a$), 2.60 (1H, m, H-6$_a$), 2.48 (1H, m, H-6$_b$), 1.92 (1H, m, H-6$_b$), 1.89 (3H, s, O*Ac*), 1.82 (3H, s, O*Ac*), 1.77 (3H, s, O*Ac*), 1.72 (1H, m, H-6$_a$), 1.65 (3H, s, O*Ac*), 1.64 (3H, s, O*Ac*), 1.64 (3H, s, O*Ac*); FABMS *m*/*z* 1079 $[M + Na]^+$; HRFABMS *m*/*z* 1079.3600 (calcd for $C_{48}H_{64}O_{26}Na$, 1079.3584).

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