[Chem. Pharm. Bull. 34(3)1067—1074(1986)]

Revised Structure of a Novel Disaccharide, Wilforibiose, Obtained from the Hydrolysate of *Cynanchum wilfordi* HEMSLEY Glycosides

SACHIKO TSUKAMOTO,[a] KOJI HAYASHI,*,[a] KOH KANEKO,[a]
HIROSHI MITSUHASHI,[b] and MOTOO SHIRO[c]

Faculty of Pharmaceutical Sciences, Hokkaido University,[a] Kita-12-jo, Nishi-6-chome, Kita-ku, Sapporo 060, Japan, Tsumura Institute,[b] Izumihon-cho, Komae, Tokyo 201, Japan, and Shionogi Research Laboratory,[c] Shionogi & Co., Ltd., Fukushima-ku, Osaka 553, Japan

(Received August 16, 1985)

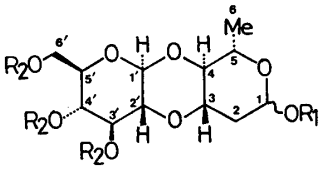
A novel disaccharide named wilforibiose (**1**) was isolated from the acidic hydrolysate of the crude glycoside of *Cynanchum wilfordi* HEMSLEY. The structure of **1** was deduced on the basis of chemical and spectral evidence, and was confirmed to be β-D-glucopyranose L-olivopyranose 1′,4:2′,3-dianhydride by X-ray analysis.

Keywords——wilforibiose; β-D-glucopyranose L-olivopyranose 1′,4:2′,3-dianhydride; *Cynanchum wilfordi*; Asclepiadaceae; nuclear Overhauser effect experiment; X-ray analysis

Cynanchum wilfordi HEMSLEY (Japanese name "Koikema," Asclepiadaceae) is widely distributed in the southern part of Japan and Korea. The dried root of this plant has been used as a substitute for a tonic, crude drug, Ka-shu-uh (何首烏), in Korea. Five aglycones were isolated from the aglycone fraction obtained by acidic hydrolysis, which was carried out in 1971, of the crude glycoside.[1] Eleven years later, we carried out the isolation and structure determination of a novel disaccharide named wilforibiose (**1**) from the sugar fraction of the hydrolysate.[2] In this paper, we wish to describe a revision of the structure of **1** based on data obtained after the publication of the preliminary communication.

Column chromatography of the crude sugar fraction on silica gel followed by crystallization from MeOH afforded **1** as colorless needles. It showed a positive Keller–Kiliani reaction,[3] which indicated the presence of 2-deoxysugar in the structure. The infrared (IR) spectrum of **1** showed the presence of hydroxyl functions. The elemental analysis and high-resolution electron-impact mass spectrometry (HR-EI-MS) of **1** gave the molecular formula $C_{12}H_{20}O_8$, which indicated the presence of three degrees of unsaturation.

Methylglycosylation of **1** provided a mixture of methyl α-(**1a**) and β-wilforibiosides (**1b**). The mixture consumed 1 mol of periodate, which suggested the presence of one vicinal glycol linkage in **1**. The methanolic solution of the mixture (262.8 mg) yielded **1b** (63.0 mg) as colorless needles and the mother liquor gave **1a** (36.4 mg) as colorless needles. The mixture (**1a**:**1b**=1:3) gave a triacetate mixture in the usual way. Acetylation of **1** and further purification by column chromatography afforded the α-tetraacetate (**1d**) and a very small amount of β-anomer, the IR spectrum of which lacked hydroxyl absorption bands but showed strong absorption bands due to acetoxyl functions at 1740 cm^{-1}. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1d** showed signals assignable to four acetoxyl groups and EI-MS gave the molecular ion peak at *m/z* 460 together with deacetylated ion peaks at *m/z* 400, 340, and 280. The proton signals of 3′-CH and 4′-CH of **1d** were assigned on the basis of a decoupling experiment. Irradiation of the proton signal at δ 3.41 (1H, dd, *J*=9.8, 7.8 Hz, 2′-CH) caused the triplet at δ 5.22 (1H, t, *J*=9.8 Hz) to collapse to a doublet



1 : $R_1 = R_2 = H$
1a: $R_1 = Me$, α, $R_2 = H$
1b: $R_1 = Me$, β, $R_2 = H$
1c: $R_1 = Me$, $R_2 = Ac$
1d: $R_1 = Ac$, α, $R_2 = Ac$

Chart 1

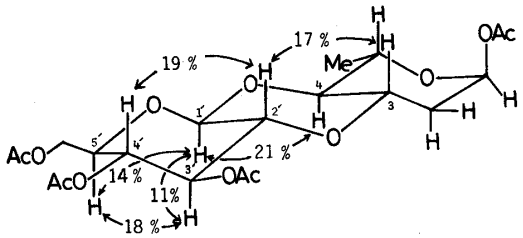


Fig. 1. NOE Findings for **1d** (200 MHz)

TABLE I. ^{1}H-NMR Chemical Shifts of **1d** (δ in ppm)

1.30 (3H, d, J=5.9 Hz, 6-Me)	3.78 (1H, ddd, J=11.7, 9.4, 4.9 Hz, 3-CH)
1.87 (1H, ddd, J=13.7, 11.7, 3.4 Hz, 2-CH_{ax})	3.85 (1H, ddd, J=9.8, 4.9, 2.5 Hz, 5-CH)
2.04 (3H, s, -OAc)	3.93 (1H, dq, J=9.8, 5.9 Hz, 5-CH)
2.07 (3H, s, -OAc)	4.17 (1H, dd, J=12.7, 2.5 Hz, 6′-CH)
2.09 (3H, s, -OAc)	4.25 (1H, dd, J=12.7, 4.9 Hz, 6′-CH)
2.10 (3H, s, -OAc)	4.53 (1H, d, J=7.8 Hz, 1′-CH)
2.14 (1H, ddd, J=13.7, 4.9, 1.4 Hz, 2-CH_{eq})	5.10 (1H, t, J=9.8 Hz, 4′-CH)
3.20 (1H, dd, J=9.8, 9.4 Hz, 4-CH)	5.22 (1H, t, J=9.8 Hz, 3′-CH)
3.41 (1H, dd, J=9.8, 7.8 Hz, 2′-CH)	6.20 (1H, dd, J=3.4, 1.4 Hz, 1-CH)

Measured in $CDCl_3$ with TMS as an internal standard.

TABLE II. ^{13}C-NMR Chemical Shifts of **1a**, **1b**, **1c**, **1d**, and **2** (δ in ppm)

	1d	**1c**		**1a**	**1b**	**2**	
		α	β				
C-1	91.8	98.5	101.0	98.7	101.2		
C-2	34.1	35.3	36.6	35.5	36.8		
C-3	73.2	73.7	75.7	73.4	75.5		
C-4	80.9	81.5	81.1	81.6	81.1		
C-5	68.7	66.0	69.6	66.3	70.0		
C-6	17.3	17.3	17.3	17.5	17.5		
1-OMe		54.5	56.2	54.6	56.3		
C-1′	99.0	99.0	99.0	99.9	99.8	C-1	105.4
C-2′	78.0	78.0	78.0	81.6	81.5	C-2	74.8
C-3′	72.4	72.4	72.4	74.8	74.7	C-3	78.1
C-4′	69.7	69.7	69.7	71.9	71.8	C-4	71.4
C-5′	74.0	73.9	73.9	80.4	80.4	C-5	78.1
C-6′	62.6	62.5	62.5	62.5	62.5	C-6	62.5
$-O\underline{C}OCH_3$	170.4	170.4	170.4			1-OMe	56.7
	170.3	170.2	170.2				
	169.9	169.9	169.9				
	169.1						
$-OCO\underline{C}H_3$	20.8	20.7	20.7				
	20.7	20.5×2	20.5×2				
	20.5×2						

Measured in C_5D_5N with TMS as an internal standard. **2**: methyl β-D-glucopyranoside.

assignable to 3′-CH, and therefore another triplet at δ 5.10 (1H, t, J=9.8 Hz) was assigned to 4′-CH. The analysis of the proton coupling constants of **1d** (Table I) indicated the presence of glucopyranose and olivopyranose (2,6-dideoxyglucose)[4] moieties in chair forms. The $^4C_1$

conformation of the glucose moiety was confirmed by the nuclear Overhauser effects (NOEs) denoted by the double-headed arrows in Fig. 1. Further, 21% enhancement was observed between 4-CH and 1′-CH and 17% enhancement between 3-CH and 2′-CH, which suggested a 1,4-dioxane linkage involving β-glucopyranose and olivopyranose.

The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of the mixture of **1a** and **1b** showed signals at δ 80.4 and 81.6 × 2 for **1a** and δ 80.4, 81.1, and 81.5 for **1b** (Table II). They were at rather lower field than the carbon signals of usual glucosides. All the carbon signals of both **1a** and **1b** were assigned on the basis of the examination described below. The carbon signal assignments of **1d** were made by means of selective proton decoupling (SEL)

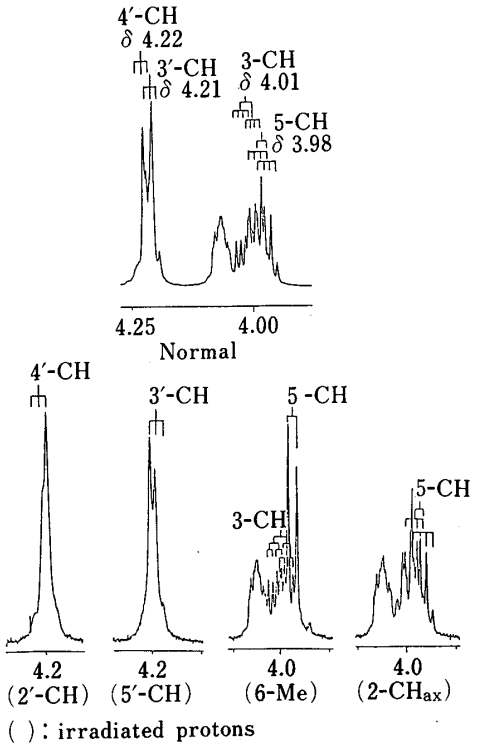


Fig. 2. Results of Decoupling Experiments with **1a** (500 MHz)

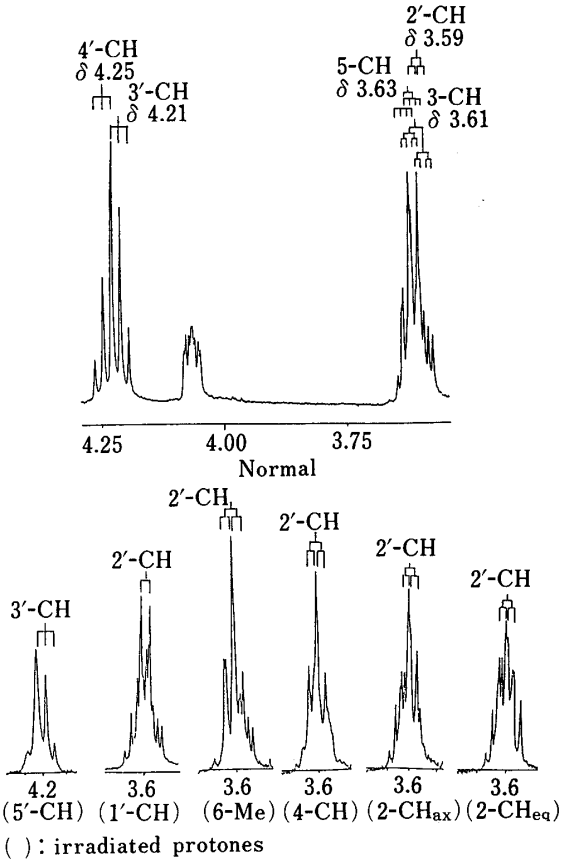


Fig. 3. Results of Decoupling Experiments with **1b** (500 MHz)

TABLE III. The Results of SEL Experiments with **1d**

Irradiated proton signals (ppm)	Irradiated carbon signals (ppm)
3.36 (4-CH)	80.9 d
3.78 (2′-CH)	78.0 d
3.98 (3-CH)	73.2 d
4.11 (5-CH)	68.7 d
4.27 (5′-CH)	74.0 d
5.53 (4′-CH)	69.7 d
5.80 (3′-CH)	72.4 d

Measured in C_5D_5N with TMS as an internal standard.

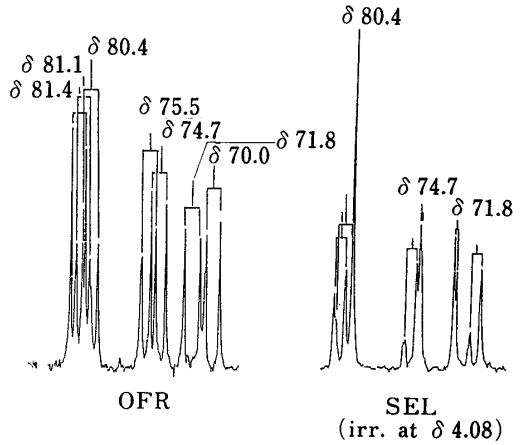


Fig. 4. Results of SEL Experiments with **1b**

TABLE IV. ^{1}H-NMR Chemical Shifts of **1a** (δ in ppm)

1.41 (3H, d, J=6.4 Hz, 6-Me)
1.86 (1H, dt, J=12.2, 3.7 Hz, 2-CH_{ax})
2.21 (1H, ddd, J=12.2, 4.9, 1 Hz, 2-CH_{eq})
3.26 (3H, s, 1-OMe)
3.32 (1H, t, J=9.2 Hz, 4-CH)
3.65 (1H, dd, J=9.5, 7.9 Hz, 2′-CH)
3.98 (1H, dq, J=9.2, 6.4 Hz, 5-CH)
4.01 (1H, ddd, J=12.2, 9.2, 4.9 Hz, 3-CH)
4.07 (1H, ddd, J=9.5, 5.5, 1.8 Hz, 5′-CH)
4.21 (1H, t, J=9.5 Hz, 3′-CH)
4.22 (1H, t, J=9.5 Hz, 4′-CH)
4.37 (1H, dd, J=11.9, 5.5 Hz, 6′-CH)
4.57 (1H, dd, J=11.9, 1.8 Hz, 6′-CH)
4.78 (1H, d, J=7.9 Hz, 1′-CH)
4.80 (1H, dd, J=3.7, 1 Hz, 1-CH)

Measured in C_5D_5N with TMS as an internal standard.

TABLE V. ^{1}H-NMR Chemical Shifts of **1b** (δ in ppm)

1.47 (3H, d, J=6.4 Hz, 6-Me)
1.89 (1H, dt, J=11.9, 9.5 Hz, 2-CH_{ax})
2.33 (1H, ddd, J=11.9, 4.3, 1.8 Hz, 2-CH_{eq})
3.29 (1H, t, J=9.2 Hz, 4-CH)
3.48 (3H, s, 1-OMe)
3.59 (1H, ddd, J=11.9, 9.2, 4.3 Hz, 3-CH)
3.62 (1H, dd, J=8.6, 7.9 Hz, 2′-CH)
3.63 (1H, dq, J=9.2, 6.4 Hz, 5-CH)
4.07 (1H, ddd, J=8.6, 5.5, 1.8 Hz, 5′-CH)
4.21 (1H, t, J=8.6 Hz, 3′-CH)
4.25 (1H, t, J=8.6 Hz, 4′-CH)
4.39 (1H, dd, J=11.9, 5.5 Hz, 6′-CH)
4.53 (1H, dd, J=9.5, 1.8 Hz, 1-CH)
4.58 (1H, dd, J=11.9, 1.8 Hz, 6′-CH)
4.76 (1H, d, J=7.9 Hz, 1′-CH)

Measured in C_5D_5N with TMS as an internal standard.

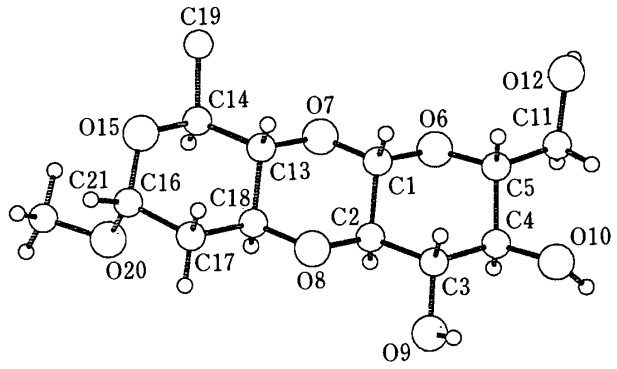


Fig. 5. Perspective Drawing of the Molecular Conformation of **1d**

experiments. The correlations between the irradiated proton signals and the collapsed carbon signals are summarized in Table III. Triacetates of the mixture of **1a** and **1b** (**1c**) were obtained in the usual way. The spectra of **1c** and **1d** coincided approximately (Table II). Further, the carbon signals at C-1—C-6 of both **1a** and **1b** exactly corresponded to those of **1c**, and the others were assigned by means of SEL experiments (Table II); the proton signal assignments of **1a** and **1b** were performed by means of proton decoupling experiments (Tables IV and V). The 2-CH_{ax}, 6-Me, 2′-CH, and 5′-CH protons of **1a** and the 2-CH_{ax}, 2-CH_{eq}, 4-CH, 6-Me, 1′-CH, and 5′-CH protons of **1b** were irradiated successively (Figs. 2 and 3). As for **1b**, when the signals overlapped with three protons at δ 3.61 were selectively decoupled, the splittings of the carbon signals at δ 70.0, 75.5, and 81.5 were eliminated at the same time. By comparison with the data of **1c** (Table II), the carbons at δ 70.0 and 75.5 were assigned to C-5 and C-3, respectively, so that the other carbon at δ 81.5 was assigned to C-2′. Irradiation of the proton signal at δ 4.08 caused the doublets at δ 71.8, 74.7, and 80.4 to collapse to a narrow doublet, a broad singlet, and a singlet, respectively, shown in Fig. 4. Therefore, based on the difference between the chemical shifts of the three protons and the irradiated chemical shifts, the carbons at δ 71.8, 74.7, and 80.4 were assigned to C-4′, C-3′, and C-5′, respectively. The assignments of the carbon signals of **1a** were made by comparison with those of **1b** and **1c** (α-anomer). The unusual carbon chemical shifts of C-1′—C-5′ of both **1a** and **1b** (Table II) seem to be due to the 1,4-dioxane structures.

Eventually, an X-ray analysis performed by using crystals of **1d** determined the structure and relative configuration unequivocally. The crystal of **1d** belongs to the monoclinic system with space group $P2_1$, and cell dimensions a=14.081 (2), b=10.446 (1), c=4.841 (1) Å, β=

TABLE VI. Atomic Fractional Coordinates with Their Estimated Standard Deviations in Parentheses

	X	*Y*	*Z*
C(1)	0.1494 (1)	−0.0103 (2)	0.7205 (3)
C(2)	0.2013 (1)	−0.1386 (2)	0.7483 (3)
C(3)	0.1358 (1)	−0.2445 (2)	0.6423 (3)
C(4)	0.0434 (1)	−0.2388 (2)	0.7966 (3)
C(5)	0.0002 (1)	−0.1037 (2)	0.7668 (3)
O(6)	0.0669 (1)	−0.0115 (2)	0.8805 (2)
O(7)	0.2070 (1)	0.0908 (0)	0.8218 (3)
O(8)	0.2844 (1)	−0.1349 (2)	0.5891 (2)
O(9)	0.1814 (1)	−0.3655 (2)	0.6791 (2)
O(10)	−0.0207 (1)	−0.3320 (2)	0.6855 (3)
C(11)	−0.0920 (1)	−0.0924 (2)	0.9146 (3)
O(12)	−0.1433 (1)	0.0191 (2)	0.8296 (2)
C(13)	0.2910 (1)	0.0949 (2)	0.6572 (3)
C(14)	0.3555 (1)	0.2042 (2)	0.7533 (5)
O(15)	0.4371 (1)	0.2025 (2)	0.5805 (3)
C(16)	0.4924 (1)	0.0888 (2)	0.5966 (3)
C(17)	0.4329 (1)	−0.0288 (2)	0.5202 (4)
C(18)	0.3436 (1)	−0.0315 (2)	0.6870 (3)
C(19)[a]	0.3112 (2)	0.3358 (2)	0.7116 (9)
O(20)	0.5345 (1)	0.0705 (2)	0.8590 (3)
C(21)	0.6042 (2)	0.1661 (3)	0.9234 (5)
H(C1)	0.126 (2)	0.007 (3)	0.506 (6)
H(C2)	0.212 (2)	−0.154 (3)	0.972 (5)
H(C3)	0.116 (2)	−0.228 (3)	0.437 (5)
H(C4)	0.057 (2)	−0.254 (3)	1.006 (6)
H(C5)	−0.016 (2)	−0.079 (3)	0.542 (6)
H(O9)	0.156 (2)	−0.410 (3)	0.532 (6)
H(O10)	−0.047 (2)	−0.379 (4)	0.854 (6)
H(C11)	−0.086 (2)	−0.094 (3)	1.141 (6)
H′(C11)	−0.124 (2)	−0.164 (4)	0.833 (6)
H(O12)	−0.162 (2)	0.057 (3)	0.963 (6)
H(C13)	0.273 (2)	0.106 (3)	0.455 (5)
H(C14)	0.376 (2)	0.196 (3)	0.944 (6)
H(C16)	0.541 (2)	0.106 (4)	0.462 (6)
H(C17)	0.412 (2)	−0.013 (4)	0.319 (6)
H′(C17)	0.468 (2)	−0.108 (4)	0.561 (6)
H(C18)	0.356 (2)	−0.044 (3)	0.899 (5)
H(C21)	0.642 (2)	0.141 (5)	1.099 (8)
H′(C21)	0.562 (3)	0.250 (4)	0.971 (8)
H″(C21)	0.637 (3)	0.175 (4)	0.772 (8)

a) The hydrogen atoms of the methyl group could not be located.

91.65 (1)°, $Z=2$. Three-dimensional intensity data were collected on a Rigaku AFC-5 diffractometer. The intensities of 1294 independent reflections with $\theta<65°$ were measured by using the θ–2θ scanning technique with graphite-monochromated Cu-$K\alpha$ radiation ($X=$ 1.54178 Å). The structure was solved by a conventional heavy-atom method and refined by a block-diagonal anisotropic least-squares technique to $R=0.038$ for 1210 reflections. A perspective drawing of the molecular conformation of **1d** is given in Fig. 5, atomic fractional coordinates in Table VI, anisotropic thermal parameters in Table VII, and bond lengths and angles in Table VIII. On the basis that the glucose existing in nature generally belongs to the D-series, the absolute configuration of **1** should be revised to β-D-glucopyranose L-

TABLE VII. Anisotropic Thermal Parameters ($\times 10^4$) with Their e.s.d. in Parentheses

	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
C(1)	21 (1)	37 (1)	320 (6)	−3 (1)	23 (3)	−1 (5)
C(2)	25 (1)	32 (1)	287 (6)	−4 (2)	5 (3)	−27 (5)
C(3)	27 (1)	36 (1)	245 (5)	−6 (1)	−24 (3)	−3 (4)
C(4)	28 (1)	42 (1)	262 (6)	−15 (2)	−19 (3)	−19 (5)
C(5)	24 (1)	47 (2)	264 (6)	−9 (2)	−14 (3)	1 (5)
O(6)	21 (1)	41 (1)	341 (5)	−5 (1)	33 (2)	−40 (4)
O(7)	22 (1)	37 (1)	444 (5)	−11 (1)	46 (2)	−59 (4)
O(8)	24 (1)	40 (1)	298 (4)	−4 (1)	14 (2)	−47 (4)
O(9)	35 (1)	33 (1)	335 (5)	3 (1)	−8 (3)	−29 (4)
O(10)	34 (1)	59 (1)	427 (6)	−35 (1)	−24 (3)	−79 (4)
C(11)	26 (1)	49 (2)	322 (6)	6 (2)	8 (3)	29 (5)
O(12)	36 (1)	60 (1)	326 (5)	23 (1)	10 (3)	30 (4)
C(13)	24 (1)	35 (1)	338 (6)	0 (2)	20 (3)	25 (5)
C(14)	25 (1)	38 (1)	557 (9)	−14 (2)	81 (4)	−36 (7)
O(15)	27 (1)	51 (1)	408 (5)	−4 (1)	55 (3)	61 (4)
C(16)	24 (1)	49 (1)	323 (6)	−4 (2)	23 (3)	−12 (5)
C(17)	25 (1)	54 (1)	342 (6)	−6 (2)	35 (4)	−53 (6)
C(18)	23 (1)	33 (1)	268 (6)	−7 (2)	−4 (3)	−35 (5)
C(19)	42 (1)	36 (2)	1334 (25)	−2 (2)	204 (8)	19 (11)
O(20)	33 (1)	60 (1)	365 (5)	−29 (1)	−24 (3)	49 (4)
C(21)	45 (1)	112 (3)	460 (9)	−84 (3)	−30 (5)	52 (8)

The temperature factor is of the form:

$$\exp[-(\beta_{11}h^2+\beta_{22}k^2+\beta_{33}l^2+\beta_{12}hk+\beta_{13}hl+\beta_{23}kl)]\,.$$

TABLE VIII. Bond Lengths (Å) and Angles (°) with Their e.s.d. in Parentheses

Bond length				Bond angle			
C(1)–C(2)	1.531 (3)	O(7)–C(13)	1.446 (3)	C(2)–C(1)–O(6)	110.0 (2)	C(2)–C(1)–O(7)	110.9 (2)
C(1)–O(7)	1.411 (3)	C(11)–O(12)	1.425 (3)	O(6)–C(1)–O(7)	106.8 (2)	C(1)–C(2)–C(3)	108.9 (2)
C(2)–O(8)	1.420 (3)	C(13)–C(18)	1.519 (3)	C(1)–C(2)–O(8)	109.1 (2)	C(3)–C(2)–O(8)	109.8 (2)
C(3)–O(9)	1.427 (3)	C(14)–C(19)	1.521 (5)	C(2)–C(3)–C(4)	109.0 (2)	C(2)–C(3)–O(9)	109.6 (2)
C(4)–O(10)	1.423 (3)	C(16)–C(17)	1.526 (3)	C(4)–C(3)–O(9)	111.2 (2)	C(3)–C(4)–C(5)	109.3 (2)
C(5)–C(11)	1.505 (3)	C(17)–C(18)	1.514 (3)	C(3)–C(4)–O(10)	109.3 (2)	C(5)–C(4)–O(10)	110.3 (2)
O(8)–C(18)	1.436 (3)			C(4)–C(5)–O(6)	108.9 (2)	C(4)–C(5)–C(11)	111.8 (2)
C(13)–C(14)	1.523 (3)			O(6)–C(5)–C(11)	109.1 (2)	C(1)–O(6)–C(5)	109.4 (2)
C(14)–O(15)	1.441 (3)			C(1)–O(7)–C(13)	107.6 (2)	C(2)–O(8)–C(18)	108.7 (2)
O(15)–C(16)	1.421 (3)			C(5)–C(11)–O(12)	111.3 (2)	O(7)–C(13)–C(14)	110.1 (2)
C(16)–O(20)	1.399 (3)			O(7)–C(13)–C(18)	109.0 (2)	C(14)–C(13)–C(18)	109.7 (2)
O(20)–C(21)	1.428 (4)			C(13)–C(14)–O(15)	107.0 (2)	C(13)–C(14)–C(19)	113.5 (2)
C(1)–O(6)	1.415 (3)			O(15)–C(14)–C(19)	105.4 (2)	C(14)–O(15)–C(16)	115.0 (2)
C(2)–C(3)	1.520 (3)			O(15)–C(16)–C(17)	111.3 (2)	O(15)–C(16)–O(20)	112.4 (2)
C(3)–C(4)	1.520 (3)			C(17)–C(16)–O(20)	109.0 (2)	C(16)–C(17)–C(18)	110.1 (2)
C(4)–C(5)	1.542 (3)			O(8)–C(18)–C(13)	110.2 (2)	O(8)–C(18)–C(17)	108.7 (2)
C(5)–O(6)	1.443 (3)			C(13)–C(18)–C(17)	110.1 (2)	C(16)–O(20)–C(21)	111.9 (2)

olivopyranose 1′,4:2′,3-dianhydride, as depicted in Chart 1.

Recently, we reported the structures of thirteen glycosides isolated from the fresh extract of this plant.[5] These glycosides contain no sugar unit such as **1**. Furthermore, **1** was found neither in the hydrolysate of the crude glycoside obtained from the fresh extract nor in the hydrolysate newly prepared from the crude glycoside which provided **1**. Consequently, it appears that **1** was an artifact produced during prolonged storage of the hydrolysate.

Experimental

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$ or MeOH with a JASCO DIP-4 digital polarimeter at room temperature. IR spectra were recorded in nujol or $CHCl_3$ on a JASCO A-102 spectrometer. ^{1}H-NMR spectra were run on JEOL GX-500 (500 MHz) and FX-200 (200 MHz) spectrometers in $CDCl_3$ or C_5D_5N, and ^{13}C-NMR spectra on JEOL FX-200 (50 MHz) and FX-100 (25 MHz) machines in C_5D_5N with tetramethylsilane (TMS) as an internal standard. EI-MS was carried out with a JEOL JMS-D-300 mass spectrometer and field-desorption mass spectrometry (FD-MS) with a JEOL JMS-01SG-2. Thin layer chromatography (TLC) was performed on Merck precoated plates (Kiesel gel 60 F_{254}) with the following solvent systems: Rf_1 H_2O–MeOH–$CHCl_3$ (1 : 3 : 9 (v/v), lower layer) and Rf_2 MeOH–acetone–hexane (1 : 4 : 4). Column chromatography was carried out on Wakogel C-200 (200 mesh) or Wakogel C-100 (100 mesh).

Isolation of Wilforibiose (1)——Extraction and acidic hydrolysis of the crude glycoside were described in the previous paper.[1] The crude sugar (61.50 g) was subjected to column chromatography on silica gel using solvents of increasing polarity from $CHCl_3$ to MeOH–$CHCl_3$ (2 : 8). Fraction S3 (12.99 g) eluted with MeOH–$CHCl_3$ (8 : 92) was rechromatographed on silica gel using MeOH–acetone–hexane (1 : 10 : 20) and H_2O–MeOH–$CHCl_3$ (1 : 3 : 9, lower layer) to give **1** (1.53 g) as a syrup, which crystallized as needles (597.9 mg) from MeOH.

1: Colorless needles. Rf_1 0.32, Rf_2 0.34. mp 189—191 °C. $[\alpha]_D^{15.5}$ −23.5° (c=0.98, MeOH). *Anal.* Calcd for $C_{12}H_{20}O_8$: C, 49.31; H, 6.90. Found: C, 49.05; H, 7.17. IR ν_{max}^{Nujol} cm^{-1}: 3400. FD-MS m/z: 292 (M^+). HR-EI-MS Calcd for $C_{12}H_{20}O_8$: 292.1158. Obsd: 292.1163.

Methyl α- (1a) and β-Wilforibioside (1b)——A solution of **1** (614.4 mg) in MeOH (10 ml) was allowed to react with 1% H_2SO_4–MeOH (10 ml) at 50 °C for 30 min, then H_2O (10 ml) was added and the reaction mixture was neutralized with satd. $Ba(OH_2)$. The precipitates were filtered off, and the filtrate was evaporated to give methyl glycosides of **1** (480.5 mg) as a syrup, which crystallized as needles (202.3 mg) from MeOH. From the mother liquor, methyl β-wilforibioside (**1b**, 63.0 mg) crystallized as needles, followed by the crystallization of methyl α-wilforibioside (**1a**, 36.4 mg).

1a: Colorless needles. Rf_1 0.50, Rf_2 0.48. mp 200—203 °C. $[\alpha]_D^{15}$ −70.0° (c=0.96, MeOH). *Anal.* Calcd for $C_{13}H_{22}O_8$: C, 50.97; H, 7.24. Found: C, 50.93; H, 7.12. FD-MS m/z: 307 (M^++H). ^{1}H-NMR (500 MHz, C_5D_5N) see Table IV. ^{13}C-NMR (50 MHz, C_5D_5N) see Table II.

1b: Colorless needles. Rf_1 0.50, Rf_2 0.48. mp 186—187 °C, $[\alpha]_D^{15}$ +36.3° (c=0.98, MeOH). *Anal.* Calcd for $C_{13}H_{22}O_8 \cdot H_2O$: C, 48.14; H, 7.46. Found: C, 48.19; H, 7.39. FD-MS m/z: 307 (M^++H). ^{1}H-NMR (500 MHz, C_5D_5N) see Table V. ^{13}C-NMR (50 MHz, C_5D_5N) see Table II.

Oxidation of a Mixture of 1a and 1b with Periodate——All operations were performed at 4 °C.

Blank Test: Satd. $NaHCO_3$ (10 ml), 0.01 M sodium arsenate (5 ml) and 20% KI (1 ml) were quickly added to a mixture of 0.02 M potassium periodate (1.5 ml) and 0.1 M acetate buffer (1.5 ml), and the solution was allowed to stand for 15 min. After adding 1% starch solution (1 ml), the solution was titrated with 0.01 M iodine until the violet color persisted for 10 s.

Tests: A mixture of **1a** and **1b** (30.6 mg) was dissolved in 0.1 M acetate buffer (20 ml) adjusted to pH 4.6 and 0.02 M potassium periodate (20 ml). Tests as follows were made after 15 min, 1, 3, and 24 h. An aliquot (3 ml) taken from the reaction mixture was treated with satd. $NaHCO_3$ (10 ml), 0.01 M sodium arsenite (5 ml), and 20% KI (1 ml). After 15 min, the solution was titrated with 0.01 M iodine in the presence of starch. These tests were carried out in duplicate. The periodate consumption was calculated based on the amount of iodine solution used in the titration.

Periodate Consumption: 15 min, 0.56 M; 1 h, 0.59 M; 3 h, 0.77 M; 24 h, 1.05 M.

Wilforibiose α-Tetraacetate (1d)——Compound **1** (191.3 mg) was dissolved in pyridine (2 ml), and acetic anhydride (1.6 ml) was added the solution. The reaction mixture was kept at room temperature for 18.5 h. Then H_2O (100 ml) was added, and the whole was extracted with $CHCl_3$ (30 ml × 3). The $CHCl_3$ layer was washed with 2 N HCl (20 ml × 3), satd. $NaHCO_3$ (20 ml × 3), and satd. NaCl (20 ml × 3), and dried over anhydrous sodium sulfate. Then, solvent was evaporated off to give a syrup (325.0 mg). After column chromatography of the product on silica gel with $CHCl_3$, the α-tetraacetate of **1** (44.0 mg, **1d**) crystallized as needles from MeOH–$CHCl_3$.

1d: Colorless needles. mp 206—210 °C. $[\alpha]_D^{19.5}$ −48.4° (c=0.98, $CHCl_3$). *Anal.* Calcd for $C_{20}H_{28}O_{12}$: C, 52.17; H, 6.13. Found: C, 51.99; H, 6.14. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1740. EI-MS m/z: 460 (M^+), 400 (M^+ −AcOH), 340 (M^+ −2AcOH), 280 (M^+ −3AcOH). ^{1}H-NMR ($CDCl_3$, 200 MHz) see Table I, (C_5D_5N, 500 MHz) δ: 1.30 (3H, d, J=6.1 Hz, 6-Me), 1.87 (1H, dt, J=12.2, 3.4 Hz, 2-CH_{ax}), 2.04, 2.07, 2.09, 2.10 (each 3H, s, -OAc), 2.14 (1H, ddd, J=12.2, 4.6, 1 Hz, 2-CH_{eq}), 3.20 (1H, t, J=9.5 Hz, 4-CH), 3.41 (1H, dd, J=9.8, 7.9 Hz, 2′-CH), 3.77 (1H, ddd, J=12.2, 9.2, 4.6 Hz, 3-CH), 3.85 (1H, ddd, J=9.8, 4.6, 2.1 Hz, 5′-CH), 4.17 (1H, dd, J=12.5, 2.1 Hz, 6′-CH), 4.53 (1H, d, J=7.9 Hz, 1′-CH), 5.10 (1H, t, J=9.8 Hz, 4′-CH), 5.22 (1H, t, J=9.8 Hz, 3′-CH), 6.19 (1H, dd, J=3.4, 1 Hz, 1-CH). ^{13}C-NMR (50 MHz, C_5D_5N) see Table II.

Triacetates of 1a and 1b (1c)——A mixture of **1a** and **1b** (**1a** : **1b** = 1 : 3, 19.7 mg) was acetylated by the procedure described above to provide the triacetates of **1a** and **1b** (21.4 mg, **1c**).

1c: A colorless syrup. FD-MS m/z: 432 (M^+). ^{1}H-NMR (200 MHz, $CDCl_3$) for β-anomer δ: 1.35 (3H, d, J=

6.3 Hz, 6-Me), 1.75 (1H, ddd, $J=13$, 12, 3.4 Hz, 2-CH_{ax}), 2.04, 2.06, 2.09 (each 3H, s, -OAc), 2.19 1(H, ddd, $J=12$, 4.5, 1.5 Hz, 2-CH_{eq}), 3.14 (1H, t, $J=9.3$ Hz, 4-CH), 3.49 (3H, s, 1-OMe), 3.85 (1H, ddd, $J=9.8$, 4.9, 2.4 Hz, 5′-CH), 4.16 (1H, dd, $J=12.7$, 2.4 Hz, 6′-CH), 4.26 (1H, dd, $J=12.7$, 4.9 Hz, 6′-CH), 4.52 (1H, d, $J=7.8$ Hz, 1′-CH), 4.45 (1H, dd, $J=9.3$, 2.0 Hz, 1-CH), 5.09 (1H, t, $J=9.8$ Hz, 4′-CH), 5.23 (1H, t, $J=9.8$ Hz, 3′-CH). For α-anomer δ: 1.30 (3H, d, $J=6.3$ Hz, 6-Me), 2.04, 2.06, 2.09 (each 3H, s, -OAc), 3.34 (3H, s, 1-OMe), 4.50 (1H, d, $J=7.8$ Hz, 1′-CH), 4.74 (1H, dd, $J=3.4$, 1 Hz, 1-CH). ^{13}C-NMR (25 MHz, C_5D_5N) see Table II.

Acknowledgements We are grateful to Prof. Dug-Ryong Hahn (Seoul) for his help in obtaining the plant. We thank Mr. K. Watanabe and Mr. M. Ikura of this university for FD-MS and the 500 MHz ^{1}H-NMR spectral measurements, respectively.

References

1) K. Hayashi and H. Mitsuhashi, *Chem. Pharm. Bull.*, **23**, 139 (1975).
2) S. Tsukamoto, K. Hayashi, and H. Mitsuhashi, *Tetrahedron Lett.*, **25**, 3595 (1984).
3) J. von Euw and T. Reichstein, *Helv. Chim. Acta*, **31**, 883 (1948).
4) Yu. A. Berlin, S. E. Esipov, M. N. Kolosov, M. M. Shemyakin, and M. G. Brazhnikova, *Tetrahedron Lett.*, **1964**, 1323; *idem, ibid.*, **1964**, 3513.
5) S. Tsukamoto, K. Hayashi, and H. Mitsuhashi, *Tetrahedron*, **41**, 927 (1985); *idem, Chem. Pharm. Bull.*, **33**, 2294 (1985).