Studies on the Chinese Crude Drug "Shoma." VIII. Two New Triterpenol Bisdesmosides, 3-Arabinosyl-24-*O*-acetylhydroshengmanol 15-Glucoside and 3-Xylosyl-24-*O*-acetylhydroshengmanol 15-Glucoside, from *Cimicifuga dahurica*

Nobuko SAKURAI, Mamoru KOEDA, Takao INOUE, and Masahiro NAGAI*

Faculty of Pharmaceutical Science, Hoshi University, Ebara 2–4–41, Shinagawa-ku, Tokyo 142, Japan.
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Two new triterpenol glycosides were isolated from the rhizomes of *Cimicifuga dahurica* (Ranunculaceae): 3-arabinosyl-24-*O*-acetylhydroshengmanol 15-glucoside (1), $C_{43}H_{70}O_{16}$, mp 222—223 °C, $[\alpha]_D$ +21.0° and 3-xylosyl-24-*O*-acetylhydroshengmanol 15-glucoside (2), $C_{43}H_{70}O_{16}$, mp 208—210 °C, $[\alpha]_D$ +9.5°. On acidic hydrolysis, 1 afforded cimigenol (3) as an aglycone, and glucose and arabinose as sugars. On enzymatic hydrolysis with molsin, 1 afforded 24-*O*-acetylhydroshengmanol 15-*O*-glucoside (4). On the basis of chemical and spectral data, the structure of 1 was proposed to be (23*R*,24*S*)-24-acetoxy-3-*O*-α-L-arabinopyranosyloxy-16,23-epoxy-9,19-cyclolanostane-15α,16ξ,25-triol 15-*O*-β-D-glucopyranoside.

The other glycoside (2) showed, in its ^{13}C-NMR spectrum, a pattern of chemical shifts very similar to that of 1. On acidic hydrolysis, 2 afforded cimigenol (3), xylose and glucose. On enzymatic hydrolysis with molsin, 2 afforded 4. From these results, the structure of 2 was proposed to be (23*R*,24*S*)-24-acetoxy-3-*O*-β-D-xylopyranosyloxy-16,23-epoxy-9,19-cyclolanostane-15α,16ξ,25-triol 15-*O*-β-D-glucopyranoside.

Keywords *Cimicifuga dahurica*; 24-*O*-acetylhydroshengmanol diglycoside; 9,19-cyclolanostanol; Ranunculaceae

The rhizoma of the genus *Cimicifuga* (Ranunculaceae) have been used as an antipyretic and an analgesic remedy in Japanese and Chinese traditional medicine. Some pharmacological studies have been reported.[1] Recently, Yamahara *et al.* reported that cimigenol xyloside from *C. dahurica* has a detoxifying effect.[2]

The rhizoma of *Cimicifuga* spp. are known to contain highly oxygenated 9,19-cyclolanostane triterpenol glycosides such as acetylshengmanol xyloside,[3] 27-deoxyactein[4] and 7,8-didehydro-24-*O*-acetylhydroshengmanol-3-xyloside.[5] We isolated some xylosides, shengmanol xyloside, acetylshengmanol xyloside and 24-*O*-acetylhydroshengmanol xyloside, which are key intermediates of the biosynthetic precursors of some *Cimicifuga* glycosides such as cimigenol xyloside, 25-*O*-methylcimigenol xyloside and cimigol xyloside.[6]

The reinvestigation of the rhizoma extract of *C. dahurica* has now led to the isolation of two new bisdesmosides, 3-arabinosyl-24-*O*-acetylhydroshengmanol 15-glucoside (**1**) and 3-xylosyl-24-*O*-acetylhydroshengmanol 15-glucoside (**2**). The isolation and purification of the compounds are described in detail in the experimental section.

Compound **1** was obtained as colorless needles, mp 222—223 °C, $[\alpha]_D$ +21.0°. The molecular formula of **1** was determined as $C_{43}H_{70}O_{16}$ on the basis of elemental analysis, the FAB-MS and the ^{13}C-NMR spectrum. The IR spectrum of **1** showed absorption at 3500—3400 (OH), 1720 and 1260 cm^{-1} (acetoxyl). The ^{1}H-NMR spectrum exhibited the presence of the cyclopropane methylene at δ 0.32 and 0.63 (each 1H, d, J=4.1 Hz, 19-H_2), an acetyl methyl group at 2.29, a secondary and six tertiary methyl groups at 0.95—1.54 ppm and two anomeric protons at 4.79 (1H, d, J=7.5 Hz) and 5.02 ppm (1H, d, J=7.6 Hz). The ^{13}C-NMR spectrum showed the signals due to a cyclopropane methylene at δ_C 31.2 (C-19), methine carbons bearing oxygen at 76.2 (C-23), 78.3 (C-24) and 88.7 (C-3), two quarternary carbons at 72.6 (C-25) and 103.1 (C-16), an acetyl group at 21.1 and 171.2, and two anomeric carbons at 105.5 (Glc-1) and 107.4 (Ara-1). It showed a very similar ^{13}C-NMR spectrum to that of 24-*O*-acetylhydroshengmanol xyloside (**6**),[7] except for the signals due to C-15 and the sugar moieties. The above evidence suggested that **1** was a 9,19-cyclolanostane triterpenol diglycoside monoacetate.

On hydrolysis with 5% sulfuric acid in aqueous methanol under heating, **1** afforded cimigenol (**3**) as an aglycone which was identified by direct comparison with an authentic sample (TLC, mixed melting point determination and IR and ^{1}H-NMR spectra). We have reported that some 9,19-cyclolanostane type triterpenoids, such as acetylshengmanol and 24-*O*-acetylhydroshengmanol, from *Cimicifuga* plants are convertible to cimigenol (**3**) under acidic conditions. So it was suggested that the

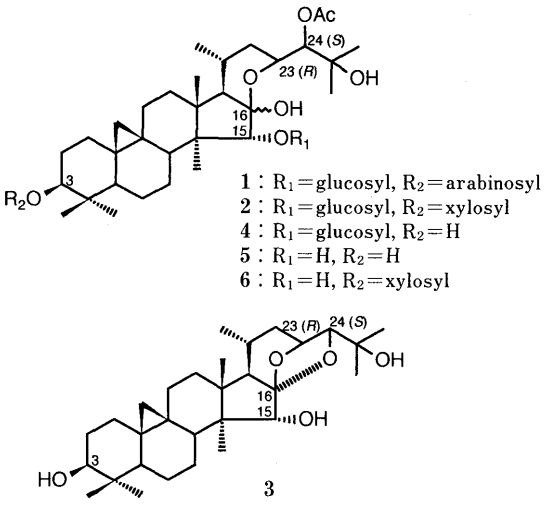

Chart 1

TABLE I. ^{13}C-NMR Chemical Shifts of **1**, **2** and **4** in Pyridine-d_5

	1	2	4
1	32.6	32.7	32.0
2	30.1	30.2	29.9
3	88.7	88.6	78.0
4	41.4	41.4	41.0
5	47.8	47.8	47.7
6	21.4	21.1	21.9
7	26.4	26.4	26.1
8	49.3	49.3	49.3
9	20.4	20.3	20.2
10	26.8	26.8	26.7
11	25.8	25.8	25.8
12	32.9	32.9	32.2
13	42.2	42.2	42.1
14	47.8	47.7	47.6
15	95.4	95.4	95.1
16	103.1	103.0	102.9
17	59.8	59.8	59.6
18	20.3	20.3	20.2
19	31.2	31.2	31.2
20	27.1	27.1	27.0
21	23.1	23.1	23.0
22	32.4	32.4	32.8
23	76.2	76.2	76.1
24	78.3	78.4	78.3
25	72.6	72.6	72.6
26	25.8	25.8	25.8
27	27.0	27.0	26.9
28	12.8	12.7	12.7
29	28.4	28.4	28.3
30	15.4	15.4	14.8
Acetate	171.2	171.3	171.1
	21.1	21.5	21.1
Glu-1	105.5	105.5	105.4
2	75.4	75.5	74.8
3	78.7	78.3	78.2
4	70.3	70.3	69.2
5	78.5	78.6	78.4
6	61.7	61.7	61.5
Ara-1	107.4		
2	72.9		
3	74.6		
4	69.4		
5	66.6		
Xyl-1		107.5	
2		75.4	
3		78.7	
4		71.2	
5		67.1	

Glu, β-D-glucopyranosyl; Xyl, β-D-xylopyranosyl; Ara, α-L-arabinopyranosyl.

genuine aglycone of **1** was not cimigenol or cimigenol acetate. The sugar moieties were detected as arabinitol acetate and glucitol acetate by GLC. The ^{13}C-NMR spectrum of **1** gave more detailed information: five oxygenated carbons were assignable to those of a α-L-arabinopyranose [δ 107.4 (C-1), 72.9 (C-2), 74.6 (C-3), 69.4 (C-4), 66.6 (C-5)] and six others to a β-D-glucopyranose [δ 105.5 (C-1), 75.4 (C-2), 78.7 (C-3), 70.3 (C-4), 78.5 (C-5), 61.7 (C-6)].

The ^{1}H–^{1}H shift correlation spectroscopy (COSY) of **1** showed cross peaks between a signal due to 24-H at δ_H 5.05 and a methine signal due to 23-H at 4.55 ppm, and between the signal at δ_H 4.55 and the methylene signals due to 22-H_2 at 2.08 and 1.48 ppm. These findings indicated that **1** has a partial structure A (Fig. 1). The

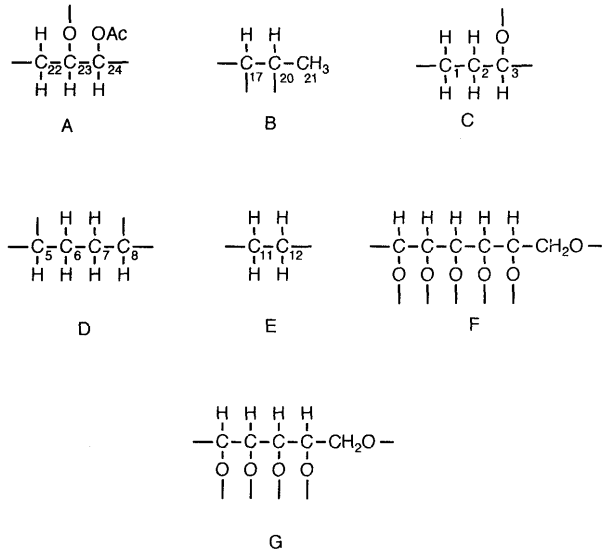

Fig. 1. Partial Structures of Compound **1**

C-22 in the structure A was connected further to C-20 in a partial structure B (Fig. 2). Five other partial structures C to G, shown in Fig. 1, were suggested by the ^{1}H–^{1}H COSY spectrum. The partial structure C is C-1, -2 and -3, D is C-5, -6, -7 and -8, and E is C-11 and -12 in the structure of **1**. An additional isolated methine signal bearing the oxygen atom was observed as a singlet at δ 4.18 (C-15). The partial structures F and G are those of glucose and arabinose.

A significant difference in the ^{13}C-NMR spectrum of the aglycone moiety of **1** compared with that of cimigenol (**3**) was the observation of a hemiketal carbon at δ_C 103.1, instead of a ketal carbon at 111.8 ppm. The genuine aglycone of **1** has a structure that converts readily to cimigenol (**3**) under acidic conditions. We reported that acetylshengmanol or 24-*O*-acetylhydroshengmanol (**5**) is easily convertible to cimigenol (**3**) under the same conditions.[3b] Therefore the aglycone of **1** must have the same stereochemistry as **3** to C-3, -15, -16, -23 and -24. From the above evidence, the genuine aglycone of **1** was supposed to be 24-*O*-acetylhydroshengmanol (**5**).

Enzymatic hydrolysis of **1** with molsin yielded a monoglycoside (**4**), $C_{38}H_{62}O_{12}$, mp 198—201 °C. On comparison of the ^{13}C-NMR spectra of **1** and **4**, the signal due to C-3 showed an upfield shift from δ 88.7 in **1** to δ 78.0 in **4**. The ^{13}C-NMR spectrum of **4** showed the existence of β-D-glucopyranose. We further compared the ^{13}C-NMR spectrum of **4** with that of the aglycone **5**, and found that the signal due to C-15 showed an upfield shift from δ 95.1 in **4** to δ 82.3 in **5**. On the basis of these results, the structure of **4** was determined to be 24-*O*-acetylhydroshengmanol 15-*O*-β-D-glucopyranoside.

Based on these findings, the structure of **1**, named 3-arabinosyl-24-*O*-acetylhydroshengmanol 15-glucoside, was determined to be (23*R*,24*S*)-24-acetoxy-3-*O*-α-L-arabinopyranosyloxy-16,23-epoxy-9,19-cyclolanostane-15α,16ξ,25-triol 15-*O*-β-D-glucopyranoside.

The other new compound (**2**), mp 208—210 °C, $[\alpha]_D$ +9.5°, showed, in its ^{13}C-NMR spectrum, a pattern of chemical shifts very similar to that of **1**, except for the

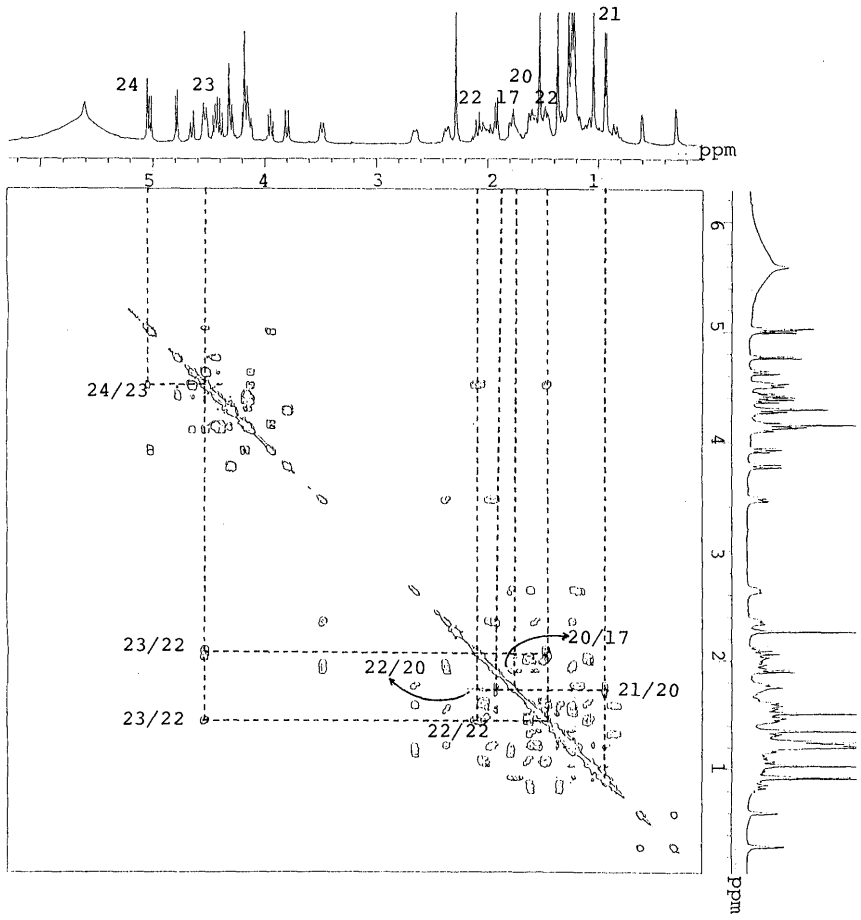

Fig. 2. ^{1}H–^{1}H COSY Spectrum of Compound **1** in Pyridine-d_5

signals due to sugar moieties. Its ^{13}C-NMR spectrum showed the existence of β-D-glucopyranose and β-D-xylopyranose units. Enzymatic hydrolysis of **2** with molsin yielded the monoglycoside (**4**), 24-*O*-acetylhydroshengmanol 15-*O*-glucoside described above. On acidic hydrolysis of **2** with 5% sulfuric acid in aqueous methanol under heating, **2** afforded cimigenol (**3**) as an aglycone, and xylose and glucose as sugars.

On the basis of these results, **2** was established as (23*R*,24*S*)-24-acetoxy-3-*O*-β-D-xylopyranosyloxy-16,23-epoxy-9,19-cyclolanostane-15α,16ξ,25-triol 15-β-D-glucopyranoside and named 3-xylosyl-24-*O*-acetylhydroshengmanol 15-glucoside.

Assignments of the ^{1}H- and ^{13}C-NMR signals of **1** and **2** shown in Tables I and II were confirmed by ^{1}H–^{1}H and ^{13}C–^{1}H COSY methods.

The triterpenol glycosides so far isolated from *Cimicifuga* genus are all monoglycosides, except for 15-*O*-acetylcimigenol glucosyl-arabinoside, a diglycoside, isolated from *C. dahurica* by Kondo *et al.*[8] This is the first report of the isolation of bisdesmoside-type triterpenoids from the *Cimicifuga* genus. It is interesting that the aglycone of the two bisdesmosides is 24-*O*-acetylhydroshengmanol, which is a biosynthetic precursor of the genins of some *Cimicifuga* glycosides.

Experimental

General Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-181 automatic polarimeter. NMR spectra were recorded with JEOL JMN GX-270 and JEOL JMN GX-400 spectrometers. Tetramethylsilane was used as the internal standard. Chemical shifts are given on the δ scale (ppm). The following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet. Coupling constants (*J* values) are given in hertz (Hz). Mass spectra (MS) were recorded with a JEOL JMS D-300 spectrometer. FAB-MS were recorded with a JEOL JMS SX-102 spectrometer. Gas-liquid partition chromatography (GLC) was run on a Shimadzu GC-4A chromatograph with a hydrogen flame ionization detector. Silica gel 60 F_{254} (Merck) precoated TLC plates were used, and detection was carried out by spraying 10% H_2SO_4 followed by heating.

Isolation of Compounds 1 and 2 An EtOAc-soluble fraction (80 g) of the MeOH extract (258 g) from the rhizomes (5 kg) of *C. dahurica* was subjected to column chromatography on silica gel with EtOAc–MeOH (98:2—0:1) to give fr. A_1—A_4. Fraction A_4, eluted with MeOH, was rechromatographed on silica gel with $CHCl_3$–MeOH (9:1—0:1) to give fr. B_1—B_3. Fraction B_3 was rechromatographed on silica gel with EtOAc–acetone–MeOH–H_2O (20:3:1:1) to afford **1** (32.1 mg) and **2** (13.8 mg).

Properties of 3-Arabinosyl-24-*O*-acetylhydroshengmanol 15-Glucoside (1) Colorless needles (MeOH), mp 222—223 °C, optical rotatory dispersion (ORD) (c=0.85, $CHCl_3$–MeOH, 1:1), $[\alpha]^{15}$ nm; +21.0° (589), +22.1° (577), +24.8° (546), +39.9° (435), +56.2° (365). *Anal.* Calcd for $C_{43}H_{70}O_{16}\cdot 3H_2O$: C, 57.57; H, 8.53. Found: C, 57.77; H, 8.49. Pos. FAB-MS *m/z*: 865 $[M+Na]^+$. Neg. FAB-MS *m/z*: 841 $[M-H]^-$. IR ν_{max}^{KBr} cm^{-1}: 3500—3400 (br OH), 1720, 1260 ($OCOCH_3$). TLC *Rf* value: 0.37 ($CHCl_3$–MeOH, 5:1). ^{1}H-NMR and ^{13}C-NMR, see Tables I and II.

Acid Hydrolysis of 1 A solution of **1** (5 mg) in 50% MeOH containing 5% H_2SO_4 (10 ml) was refluxed for 4 h. The MeOH was removed *in vacuo* and the residue was diluted with water, and extracted with EtOAc. The EtOAc layer was washed with water, dried over anhydrous Na_2SO_4

TABLE II. ^{1}H-NMR Chemical Shifts[a] of **1** and **2** in Pyridine-d_5

	1	2
1	1.22, 1.56	1.23, 1.56
2	1.90, 2.36	1.95, 2.32
3	3.49 dd (10.7, 4.4)	3.49 dd (10.7, 4.0)
4		
5	1.32	1.32
6	0.88, 1.60	0.88, 1.61
7	1.07, 2.63	1.11, 2.61
8	1.80	1.78
9		
10		
11	1.05, 1.08	1.05, 1.10
12	1.48, 1.61	1.50, 1.63
13		
14		
15	4.18 s	4.17 s
16		
17	1.89	1.93
18	1.24	1.23
19	0.32 d (4.1), 0.63 d (4.1)	0.32 d (4.2), 0.63 d (4.2)
20	1.78	1.78
21	0.95 d (6.7)	0.95 d (6.3)
22	1.48, 2.08	1.46, 2.08
23	4.55 br d (7.8)	4.54 br d (8.5)
24	5.05 d (1.4)	5.05 d (1.7)
25		
26	1.26 s	1.28 s
27	1.38 s	1.38 s
28	1.28 s	1.29 s
29	1.54 s	1.54 s
30	1.06 s	1.09 s
Acetate	2.29 s	2.29 s
Glu-1	5.02 d (7.6)	5.02 d (7.8)
2	3.95 dd (7.6, 7.9)	3.95 dd (9.0, 7.8)
3	4.17	4.22
4	4.43 dd (7.2, 8.3)	4.38
5	4.16	4.18
6	4.54 dd (9.8, 3.1), 4.66 dd (9.8, 2.1)	4.52, 4.64 br d (9.5)
Ara-1	4.79 d (7.5)	
2	4.42 dd (7.8, 7.5)	
3	4.16	
4	4.29	
5	3.80 br d (10.3) 4.28 dd (10.3, 1.5)	
Xyl-1		4.85 d (7.6)
2		4.03 dd (7.9, 7.6)
3		4.20
4		4.22
5		3.76 t (10.5), 4.36

a) Signal assignments were done based on ^{1}H–^{1}H COSY spectra.

and concentrated. The residue was chromatographed over silica gel. Elution with benzene–EtOAc (5 : 2) gave cimigenol (**3**), mp 226—227 °C, which was identical with an authentic sample by mixed melting point determination, and comparisons of TLC behavior, and IR and ^{1}H-NMR spectra. The water-soluble fraction was treated with Amberlite MB-3, and concentrated under reduced pressure. The residue was treated with $NaBH_4$ (*ca.* 2 mg) at room temperature for 1 h. The reaction mixture was passed through an Amberlite MB-3 column and concentrated to dryness. Boric acid was removed by co-distillation with MeOH. The residue was acetylated with acetic anhydride (10 ml) and C_5H_5N (5 ml) at room temperature. The reagents were evaporated *in vacuo*. Alditol acetate was detected by GLC. GLC conditions: column, 2% OV-17, 3 mm × 2 m; column temperature, 200 °C; carrier gas, N_2. t_R, 17.7 min (glucitol acetate), 6.4 min (arabinitol acetate).

Enzymatic Hydrolysis of 1 A solution of **1** (15.1 mg) in a mixture of EtOH (10 ml) and 0.2 M Na_2HPO_4–0.1 M citric acid buffer (pH 4.0) (20 ml) was treated with molsin (*Aspergillus saitoi*) (16 mg) in H_2O (10 ml), and the total mixture was kept for 43 d with gentle stirring at 37 °C. Usual work-up afforded **4** (5 mg), colorless needles, mp 198—201 °C (EtOH). IR ν_{max}^{KBr} cm^{-1}: 3500—3400 (OH), 1720, 1260 ($OCOCH_3$). ^{13}C-NMR, see Table I.

Properties of 3-Xylosyl-24-*O*-acetylhydroshengmanol 15-Glucoside (2) Colorless needles (MeOH), mp 208—210 °C, $[\alpha]_D$ +9.5° (c=0.98, $CHCl_3$–MeOH, 1 : 1). Pos. FAB-MS *m/z*: 843 $[M+H]^+$, 865 $[M+Na]^+$. Neg. FAB-MS *m/z*: 841 $[M-H]^-$. IR ν_{max}^{KBr} cm^{-1}: 3500—3400 (OH), 1730, 1250 ($OCOCH_3$). TLC *Rf* value: 0.40 ($CHCl_3$–MeOH, 5 : 1). ^{1}H-NMR and ^{13}C-NMR, see Tables I and II.

Acid Hydrolysis of 2 A solution of **2** (5 mg) in 50% MeOH containing 5% H_2SO_4 (5 ml) was refluxed for 4 h. The MeOH was removed *in vacuo*. The resulting solution was treated in the same way as described for **1**, to afford cimigenol (**3**) as colorless needles from EtOAc, mp 226—228 °C. The water-soluble fraction was treated in the same way as described for **1**. Alditol acetate was detected by GLC. GLC conditions: column, 3% SE-30, 3 mm × 1.5 m; column temperature, 190 °C; carrier gas, N_2. t_R, 9.3 min (glucitol acetate), 4.1 min (xylitol acetate).

Enzymatic Hydrolysis of 2 A solution of **2** (4.6 mg) in a mixture of EtOH (3 ml) and 0.2 M Na_2HPO_4–0.1 M citric acid buffer (pH 4.0) (10 ml) was treated with molsin (*Aspergillus saitoi*) (5 mg) in H_2O (3 ml), and the mixture was kept at 37 °C for 43 h with gentle stirring. Then the solution was treated in the same way as described for **1**. The crude product afforded 24-*O*-acetylhydroshengmanol 15-*O*-glucoside (**4**) as colorless needles from EtOH, mp 197—199 °C. Identity of **4** with **4**, the partial hydrolysis product of **1** above, was shown by mixed melting point determination and comparisons of TLC behavior and ^{1}H-NMR spectra. ^{1}H-NMR (pyridine-d_5) δ: 5.05 (1H, d, J=7.9 Hz, Glc-1), 5.09 (1H, d, J=1.8 Hz, H-24), 4.20 (1H, s, H-15), 2.30 (3H, s, $OCOCH_3$).

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