Further Studies on Steroidal Glycosides from Bulbs, Roots and Leaves of *Allium sativum* L.

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Received March 22, 1989

A new furostanol glycoside (2), named sativoside-B1, was isolated from garlic, bulbs of *Allium sativum* L., along with proto-desgalactotigonin (3). The structure of 2 was established to be (25*R*)-26-*O*-*β*-D-glucopyranosyl-22-hydroxy-5*α*-furostane-3*β*,6*β*,26-triol 3-*O*-*β*-D-glucopyranosyl-(1→3)-*O*-*β*-D-glucopyranosyl-(1→2)-*O*-[*β*-D-glucopyranosyl-(1→3)]-*O*-*β*-D-glucopyranosyl-(1→4)-*O*-*β*-D-galactopyranoside.

From roots of this plant, two new steroidal glycosides, named sativoside-R1 (16) and sativoside-R2 (15) were isolated and their structures were determined to be (25*R*)-26-*O*-*β*-D-glucopyranosyl-22-hydroxy-5*α*-furostane-3*β*,26-diol 3-*O*-*β*-D-glucopyranosyl-(1→3)-*O*-*β*-D-glucopyranosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-*O*-*β*-D-glucopyranosyl-(1→4)-*O*-*β*-D-galactopyranoside (16) and its corresponding spirostanol glycoside (15). Besides these glycosides, three known glycosides, 3, desgalactotigonin (13) and F-gitonin (14) were isolated and identified.

In a glycoside fraction of the leaves of *A. sativum*, steroidal glycosides were not detected by thin layer chromatography analysis.

Keywords *Allium sativum*; garlic; Liliaceae; steroidal glycoside; sativoside-B1; sativoside-R1; sativoside-R2; proto-desgalactotigonin; desgalactotigonin; F-gitonin

The structure of a new furostanol glycoside from garlic, bulbs of *Allium sativum* L., proto-eruboside-B (**1**), has already been elucidated.[1] In further studies of the glycosides of the *Allium* family, several steroidal glycosides from bulbs of *A. ampeloprasum* (elephant garlic) and *A. chinense* were reported.[2] The present paper deals with the further isolation and structure elucidation of two furostanol glycosides from garlic and also the glycoside composition of roots and leaves of *A. sativum*.

The glycoside fraction of garlic obtained previously[1] was subjected to repeated column chromatography on silica gel and on reversed-phase highly porous polymer, followed by heating in aqueous acetone to give two glycosides, **2** and **3**, in yields of 0.003% and 0.001%, respectively.

A new glycoside (**2**), $C_{63}H_{106}O_{35}\cdot 4H_2O$, named sativoside-B1, is positive to the Ehrlich reagent on thin layer chromatography (TLC).[3] On standing in methanol, **2** gave a glycoside (**4**), which showed a methoxyl signal at 3.26 ppm in the proton nuclear magnetic resonance (^{1}H-NMR) spectrum. In the carbon-13 NMR (^{13}C-NMR) spectrum of **2**, carbon signals due to the aglycone moiety appeared at almost the same positions as those of **1**, indicating that **2** is a glycoside of (25*R*)-5*α*-furostane-3*β*,6*β*,22,26-tetraol having sugar units at the 3- and 26-hydroxyl groups. On acid hydrolysis, **2** afforded galactose and glucose, and the anomeric carbon signals of **2** indicated the presence of six monosaccharide units. Enzymatic hydrolysis of **2** with *β*-glucosidase gave a glycoside (**5**), $C_{57}H_{94}O_{29}\cdot 5H_2O$, and glucose. On sugar sequence analysis, **5** afforded four partially methylated alditol acetates, 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylhexitol (**6**), 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methylhexitol (**7**), 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methylhexitol (**8**) and 1,2,3,5-tetra-*O*-acetyl-4,6-di-*O*-methylhexitol (**9**) detected by gas chromatography-mass spectrometry (GC-MS) (alditol acetate analysis).[4] Partial hydrolysis of **5** with aqueous sulfuric acid yielded two tetraglycosides of *β*-chlorogenin, **10** and **11**, of which the former was identical with eruboside-B.[1,5] A comparison of the ^{13}C-NMR spectrum of **5** with that of **10** revealed an additional set of signals due to a *β*-glucopyranosyl unit in the spectrum of **5**. This evidence coupled with these results indicated that the sugar sequence of **5** should be formulated as either glc(1→3)glc(1→2)[glc(1→3)]glc(1→4)gal or glc(1→3)glc(1→3)[glc(1→2)]glc(1→4)gal. In the ^{13}C-NMR spectrum of **11**, a carbon signal at 85.8 ppm assignable to C-2 of one of the *β*-glucopyranosyl units[6,7] was observed, suggesting that the structure of **11** is *β*-chlorogenin 3-*O*-*β*-D-glucopyranosyl(1→3)-*O*-*β*-D-glucopyranosyl-(1→2)-*O*-*β*-D-glucopyranosyl-(1→4)-*O*-*β*-D-galactopyranoside.

Since **2** is a furostanol glycoside corresponding to **5**, it was established to be (25*R*)-26-*O*-*β*-D-glucopyranosyl-22-hydroxy-5*α*-furostane-3*β*,6*β*,26-triol 3-*O*-*β*-D-glucopyranosyl-(1→3)-*O*-*β*-D-glucopyranosyl-(1→2)-*O*-[*β*-D-glucopyranosyl-(1→3)]-*O*-*β*-D-glucopyranosyl-(1→4)-*O*-*β*-D-galactopyranoside.

The glycoside (**3**) is assumed to be a furostanol glycoside on the basis of positive coloration with the Ehrlich reagent and appearance of a methoxyl signal at 3.28 ppm in the ^{1}H-NMR spectrum of the glycoside (**12**) formed by the reaction of **3** with methanol. The inspection of the anomeric carbon signals of **3** revealed the presence of five monosaccharide units. Enzymatic hydrolysis of **3** with *β*-glucosidase gave glucose and a glycoside (**13**), which is identical with desgalactotigonin.[8] Therefore, **3** was identified as proto-desgalactotigonin, previously isolated from berries of *Solanum nigrum*.[9]

A number of steroidal glycosides have been isolated from roots and leaves of many medicinal plants.[10] We have carried out a comparative study of the glycosides of roots and leaves of *A. sativum*.

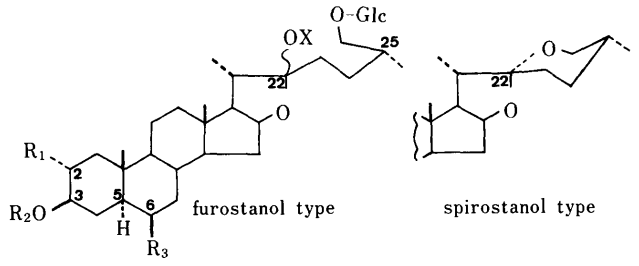
A crude glycoside fraction of roots of *A. sativum* was subjected to chromatography on silica gel and on reversed-phase highly porous polymer to give **3**, **13** and three glycosides (**14**—**16**) in yields of 0.02%, 0.04%, 0.03%, 0.03% and 0.05%, respectively. The glycoside (**14**) was identical with F-gitonin[8,11] based on an analysis of ^{13}C-NMR spectrum, the products of partial hydrolysis of **14** and physical properties.

A new glycoside (**16**), $C_{62}H_{104}O_{33}\cdot 2H_2O$, named sativoside-R1, showed a purple coloration with the Ehrlich

reagent on TLC. On heating in methanol, **16** gave a glycoside (**17**), which exhibited a methoxyl signal at 3.27 ppm in the ^{1}H-NMR spectrum. In the ^{13}C-NMR spectrum of **16**, six anomeric carbon signals were observed, suggesting that **16** is a furostanol hexaglycoside. On enzymatic hydrolysis with β-glucosidase, **16** liberated glucose and a new glycoside, which is identical with **15**, $C_{56}H_{92}O_{27}\cdot 4H_2O$, named sativoside-R2. On acid hydrolysis, **15** gave tigogenin (**18**) as the aglycone and galactose, glucose and xylose, and the ^{13}C-NMR spectrum of **15** indicated the presence of five monosaccharide units. The electron impact mass spectrum (EI-MS) of acetylated **15** showed fragment ions at *m/z* 619 $[(\text{hexosyl-hexose})Ac_7]^+$, 331 $[(\text{terminal-hexose})Ac_4]^+$ and 259 $[(\text{terminal-pentose})Ac_3]^+$. On alditol acetates analysis, **15** afforded five partially methylated alditol acetates, **6**, **7**, **8**, **9** and 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylpentitol (**19**). Partial hydrolysis of **15** yielded **13**. On comparison of the ^{13}C-NMR spectrum of the sugar moiety of **15** with that of **13**, an additional set of signals due to a β-glucopyranosyl unit appeared in the spectrum of **15**, leading to the formulation of **15** as tigogenin 3-*O*-β-D-glucopyranosyl-(1→3)-*O*-β-D-glucopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-galactopyranoside.

Since **16** is a furostanol glycoside corresponding to **15**, it was determined to be (25*R*)-26-*O*-β-D-glucopyranosyl-22-hydroxy-5α-furostane-3β,26-diol 3-*O*-β-D-glucopyranosyl-(1→3)-*O*-β-D-glucopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-galactopyranoside.

On TLC analysis of the crude glycoside fraction and its hydrolysate from leaves of *A. sativum*, no corresponding glycosides and aglycones could be detected.



furostanol type		R$_1$	R$_2$	R$_3$	spirostanol type
1: X=H		H	-Gal^{4}-Glc^{2}(Glc, ^{3}Glc)	OH	**10**
2: X=H	**4**: X=CH$_3$	H	-Gal^{4}-Glc^{2}(Glc^{3}Glc)	OH	**5**
3: X=H	**12**: X=CH$_3$	H	-Gal^{4}-Glc^{2}(Glc, ^{3}Xyl)	H	**13**
		H	-Gal^{4}-Glc^{2}-Glc^{3}-Glc	OH	**11**
		OH	-Gal^{4}-Glc^{2}(Glc, ^{3}Xyl)	H	**14**
16: X=H	**17**: X=CH$_3$	H	-Gal^{4}-Glc^{2}(Glc^{3}Glc, ^{3}Xyl)	H	**15**

Gal: β-galactopyranosyl Glc: β-glucopyranosyl Xyl: β-xylopyranosyl

Chart 1

Experimental

General Procedure The NMR spectra were taken on a JEOL JNM GX-270 spectrometer (^{1}H 270 MHz, ^{13}C 67.8 MHz) in pyridine-d_5 using tetramethylsilane as an internal standard. The MS were recorded on a JEOL JMS DX-300 mass spectrometer. Gas liquid chromatography (GLC) was run on a Shimadzu GC-9AM gas chromatograph. Reagents for chromatography: see the previous paper.[1]

Identification of monosaccharides obtained by acid hydrolysis was carried out as described in the previous paper.[1]

Extraction and Isolation of 2, 3, 13, 14, 15 and 16 The crude glycoside fraction of garlic (see the previous paper)[1] was subjected to repeated column chromatography on reversed-phase highly porous polymer, MCI gel CHP20P (solvent: 70% aqueous MeOH), and on silica gel (solvent: $CHCl_3$–MeOH–H_2O (7:4:0.6, homogeneous), followed by heating in 30% aqueous acetone at 100 °C for 4 h, to give **2** and **3** in yields of 0.003% and 0.001%.

Glycoside (**2**): White powder (from aqueous acetone), $[\alpha]_D^{26}$ −40.0° (*c*= 0.39, H_2O). *Anal.* Calcd for $C_{63}H_{106}O_{35}\cdot 4H_2O$: C, 50.59; H, 7.68. Found: C, 50.30; H, 7.91. ^{1}H-NMR δ: 0.91 (3H, s), 0.99 (3H, d, *J*=6.3 Hz), 1.24 (3H, s), 1.35 (3H, d, *J*=6.6 Hz), 4.85 (1H, d, *J*=7.7 Hz), 4.95 (1H, d, *J*= 7.3 Hz), 5.17 (1H×2, d, *J*=7.3 Hz), 5.25 (1H, d, *J*=7.3 Hz), 5.57 (1H, d, *J*=6.4 Hz). ^{13}C-NMR δ: (aglycone C-1—C-27) 38.8, 30.0, 78.0[c], 32.8, 48.0, 70.8, 40.3, 30.6, 54.7, 36.2, 21.3, 40.9, 41.2, 56.3, 32.5, 81.1, 64.0, 16.7, 16.4, 40.7, 16.0, 110.7, 37.2, 28.4, 34.3, 75.3, 17.5; (aglycone-^{3}-)galactose C-1—C-6) 102.3, 73.2, 75.5[a], 80.1, 75.5[a], 60.7; ((galactose-^{4}-)glucose C-1—C-6) 104.1[b], 80.9, 88.6, 70.9, 78.0[c], 62.0[d]; ((glucose-^{2}-)glucose C-1—C-6) 104.5[b], 74.7[a], 87.5, 69.2, 77.5[c], 62.3[d]; ((glucose-^{2}-glucose-^{3}-)glucose C-1—C-6) 104.8[b], 75.2[a], 78.6[c], 71.5[e], 78.6[c], 62.5[d]; ((glucose-^{3}-)glucose C-1—C-6) 105.0[b], 75.2[a], 78.5[c], 71.5[e], 78.5[c], 62.8[d]; ((aglycone-^{26}-)glucose C-1—C-6) 105.3[b], 75.2[a], 78.6[c], 71.7[e], 78.5[c], 62.8[d] (a—e may be reversed).

Glycoside (**3**): White powder (from aqueous acetone), $[\alpha]_D^{26}$ −42.7° (*c*= 0.39, pyridine). *Anal.* Calcd for $C_{56}H_{94}O_{28}\cdot 4H_2O$: C, 52.25; H, 7.99. Found: C, 52.27; H, 8.09.

Frozen roots of *A. sativum*, 300 g (collected in our plant garden, Hiroshima), were crushed in MeOH and twice extracted with hot MeOH. A suspension of the MeOH extract in H_2O was applied to a column of MCI gel CHP20P (stepwise elution with H_2O, 20% aqueous MeOH, and MeOH). The crude glycoside fraction (2.7 g) eluted with MeOH was separated by repeated chromatography on silica gel (solvent: $CHCl_3$–MeOH–H_2O (7:3:0.5)) to give **13** (yield: 0.04%), **14** (yield: 0.03%) and **15** (yield: 0.03%), and two glycoside fractions. These fractions were further purified by reversed-phase chromatography (MCI gel CHP20P, solvent: 75% aqueous MeOH), followed by heating in 30% aqueous acetone at 100 °C for 4 h, to afford **3** and **16** in yields of 0.02% and 0.05%.

Glycoside (**13**): Colorless microcrystals (from EtOH), mp 282—287 °C (dec.) (lit.[8] 284—286 °C (dec.)), $[\alpha]_D^{26}$ −57.9° (*c*=0.61, pyridine).

Glycoside (**14**): Colorless needles (from 1-BuOH saturated with H_2O), mp 255—260 °C (dec.) (lit.[8] 252—255 °C (dec.)), $[\alpha]_D^{26}$ −75.8° (*c*=0.38, pyridine).

Glycoside (**15**): Colorless microcrystals (from MeOH–$CHCl_3$), mp 265—270 °C (dec.), $[\alpha]_D^{26}$ −51.5° (*c*=0.51, pyridine). *Anal.* Calcd for $C_{56}H_{92}O_{27}\cdot 4H_2O$: C, 52.99; H, 7.94. Found: C, 53.07; H, 8.19. ^{1}H-NMR δ: 0.63 (3H, s), 0.70 (3H, d, *J*=4.8 Hz), 0.83 (3H, s), 1.15 (3H, d, *J*= 7.0 Hz), 4.89 (1H, d, *J*=7.3 Hz), 5.14 (1H, d, *J*=7.3 Hz), 5.17 (1H, d, *J*= 8.1 Hz), 5.20 (1H, d, *J*=8.1 Hz), 5.55 (1H, d, *J*=7.3 Hz). ^{13}C-NMR δ: (aglycone C-1—C-27) 37.2, 29.9, 77.5[c], 34.8, 44.7, 28.9, 32.4, 35.3, 54.4, 35.8, 21.3, 40.2, 40.8, 56.5, 32.1, 81.1, 63.0, 16.6, 12.3, 42.0, 15.0, 109.2, 31.8, 29.3, 30.6, 66.9, 17.3; ((aglycone-^{3}-)galactose C-1—C-6) 102.4, 73.1, 75.4[a], 79.7, 75.6[a], 60.7; ((galactose-^{4}-)glucose C-1—C-6) 104.9[b], 80.7, 86.8, 70.7[d], 77.6[c], 62.1[e]; ((glucose-^{2}-)glucose C-1—C-6) 104.9[b], 74.8[a], 87.5, 69.4, 78.0[c], 62.5[e]; ((glucose-^{3}-)glucose C-1—C-6) 104.0[b], 75.1[a], 78.3[c], 70.4[d], 78.1[c], 63.0[e]; ((glucose-^{3}-)xylose C-1—C-5) 105.4[b], 75.5[a], 78.5[c], 71.5, 67.3 (a—e may be reversed).

Glycoside (**16**): White powder (from aqueous acetone), $[\alpha]_D^{26}$ −45.0° (*c*=0.59, pyridine). *Anal.* Calcd for $C_{62}H_{104}O_{33}\cdot 2H_2O$: C, 52.68; H, 7,70. Found: C, 52.40; H, 8.03. ^{1}H-NMR δ: 0.65 (3H, s), 0.88 (3H, s), 0.99 (3H, d, *J*=6.6 Hz), 1.34 (3H, d, *J*=6.6 Hz), 4.82 (1H, d, *J*=7.7 Hz), 4.89 (1H, d, *J*=7.3 Hz), 5.12 (1H, d, *J*=8.7 Hz), 5.15 (1H, d, *J*=8.7 Hz), 5.18 (1H, d, *J*=8.3 Hz), 5.54 (1H, d, *J*=7.4 Hz). ^{13}C-NMR δ: (aglycone C-1—C-27) 37.2, 29.9, 77.6[c], 34.8, 44.7, 29.0, 32.4, 35.3, 54.5, 35.8, 21.3, 40.2, 41.1, 56.4, 32.4, 81.1, 64.0, 16.7, 12.3, 40.7, 16.4, 110.6, 37.2, 28.4, 34.3, 75.3, 17.5; ((aglycone-^{3}-)galactose C-1—C-6) 102.5, 73.1, 75.4[a], 79.6, 75.6[a],

60.7; ((galactose$\frac{4}{1}$)glucose C-1—C-6) 104.9[b], 80.8, 86.8, 70.6[d], 78.0[e], 62.2[e]; ((glucose$\frac{2}{1}$)glucose C-1—C-6) 104.9[b], 74.7[a], 87.4, 69.4, 78.2[c], 62.5[e]; ((glucose$\frac{3}{1}$)glucose C-1—C-6) 104.0[b], 75.2[a], 78.5[c], 70.4[d], 78.5[c], 62.9[e]; ((glucose$\frac{3}{1}$)xylose C-1—C-5) 105.4[b], 75.4[a], 78.6[c], 71.5[d], 67.3; ((aglycone$\frac{26}{1}$)glucose C-1—C-6) 104.9[b], 75.2[a], 78.6[c], 71.7[d], 78.5[c], 62.9[e] (a—e may be reversed).

Formation of 4, 12 and 17 A methanol solution of each glycoside, **2**, **3** and **16**, was heated in a sealed tube at 70 °C for 2 h and then concentrated to dryness to give **4**, **12** and **17**, respectively. **4**: White powder (from MeOH–AcOEt), $[\alpha]_D^{27}$ −37.0° (c=0.25, MeOH). *Anal.* Calcd for $C_{64}H_{108}O_{35}\cdot 5H_2O$: C, 50.32; H, 7.79. Found: C, 50.65; H, 7.70. ^{1}H-NMR δ: 3.26 (3H, s), ^{13}C-NMR δ: 47.3 (OCH_3). **12**: White powder (from MeOH–AcOEt), $[\alpha]_D^{27}$ −45.5° (c=0.25, MeOH). ^{1}H-NMR δ: 3.28 (3H, s), ^{13}C-NMR δ: 47.3 (OCH_3). **17**: White powder (from MeOH–AcOEt), $[\alpha]_D^{27}$ −48.4° (c=0.27, MeOH). *Anal.* Calcd for $C_{63}H_{106}O_{33}\cdot 4H_2O$: C, 51.70; H, 7.85. Found: C, 51.49; H, 8.00. ^{1}H-NMR δ: 3.27 (3H, s), ^{13}C-NMR δ: 47.3 (OCH_3).

Acid Hydrolysis of 15 A glycoside (**15**, 52 mg) was heated with 5% sulfuric acid aqueous solution–EtOH (1 : 1, 5 ml) at 100 °C for 6 h. After cooling, the reaction mixture was diluted with H_2O and applied to a column of MCI gel CHP20P (solvent: H_2O and then MeOH). The fraction eluted with MeOH was chromatographed on silica gel (solvent: $CHCl_3$–MeOH (50 : 1)) to give **18** (15 mg). **18**: Colorless needles (from aqueous MeOH) mp 197—200 °C (lit.[12] 203 °C). $[\alpha]_D^{26}$ −58.0° (c=0.34, $CHCl_3$).

Enzymatic Hydrolysis of 2, 3 and 16 A mixture of each of **2** (130 mg), **3** (30 mg) and **16** (50 mg) with β-glucosidase from almond (Cooper Biomedical) in acetate buffer solution (pH 4.1) was incubated at 37 °C for 2 h. The reaction mixture was diluted with H_2O and applied to a column of MCI gel CHP20P. The column was washed with H_2O and then eluted with MeOH. The fraction eluted with MeOH was chromatographed on silica gel (solvent: $CHCl_3$–MeOH–H_2O (7 : 3 : 0.5)) to afford **5** (68 mg), **13** (18 mg) and **15** (33 mg), while glucose was identified by TLC in the fraction eluted with H_2O.

Glycoside (**5**): Colorless microcrystals (from MeOH), mp 252—256 °C (dec.). $[\alpha]_D^{26}$ −47.3° (c=0.35, pyridine). *Anal.* Calcd for $C_{57}H_{94}O_{29}\cdot 5H_2O$: C, 51.34; H, 7.86. Found: C, 51.00; H, 7.94. ^{1}H-NMR δ: 0.69 (3H, d, J=5.5 Hz), 0.87 (3H, s), 1.15 (3H, d, J=6.6 Hz), 1.22 (3H, s), 4.92 (1H, d, J=7.7 Hz), 5.13 (1H, d, J=7.3 Hz), 5.15 (1H, d, J=7.3 Hz), 5.24 (1H, d, J=7.7 Hz), 5.55 (1H, d, J=6.6 Hz). ^{13}C-NMR δ: (aglycone C-1—C-27) 38.8, 30.0, 77.9[c], 32.7, 47.9, 70.8, 40.2, 30.6, 54.6, 36.1, 21.2, 40.9, 40.9, 56.4, 31.8, 81.1, 63.1, 16.6, 16.0, 42.0, 15.0, 109.2, 32.2, 29.2, 30.6, 66.9, 17.3; ((aglycone$\frac{3}{1}$)galactose C-1—C-6) 102.3, 73.1, 75.5[a], 80.0, 75.5[a], 60.7; ((galactose$\frac{4}{1}$)glucose C-1—C-6) 104.0[b], 80.9, 88.4, 70.7, 77.9[c], 62.0[d]; ((glucose$\frac{2}{1}$)glucose C-1—C-6) 104.4[b], 74.7[a], 87.5, 69.2, 77.5[c], 63.1[d]; ((glucose$\frac{2}{1}$-glucose$\frac{3}{1}$)glucose C-1—C-6) 104.7[b], 75.5[a], 78.5[c], 71.5, 78.3[c], 62.3[d]; ((glucose$\frac{3}{1}$)glucose C-1—C-6) 105.2[b], 75.5[a], 78.3[c], 71.5, 78.0[c], 62.5[d] (a—d may be reversed).

Partial Hydrolysis of 5 and 15 The glycoside (**5**) (57 mg) was heated with 5% aqueous sulfuric acid–EtOH (1 : 1, 5 ml) in a sealed tube at 95 °C for 1 h. After cooling, the reaction mixture was diluted with H_2O and applied to a column of MCI gel CHP20P (stepwise elution with H_2O and MeOH). The fraction eluted with MeOH was separated by silica gel chromatography (solvent: $CHCl_3$–MeOH–H_2O (7 : 3 : 0.5)) to afford **10** (7.0 mg) and **11** (1.6 mg). **10**: White powder (from EtOH–AcOEt), $[\alpha]_D^{27}$ −59.0° (c=0.20, $CHCl_3$–MeOH (10 : 1)). **11**: FD-MS m/z: 1103 $(M+Na)^+$. ^{13}C-NMR δ: (aglycone C-1—C-27) 38.9, 30.0, 78.1[c], 32.9, 48.0, 70.9, 40.3, 30.6, 54.7, 36.2, 21.3, 40.9, 40.9, 56.5, 31.9, 81.2, 63.1, 16.6, 16.1, 42.1, 15.0, 109.3, 32.3, 29.3, 30.6, 66.9, 17.4; ((aglycone$\frac{3}{1}$)galactose C-1—C-6) 102.4, 73.2, 75.5[a], 80.9, 75.5[a], 60.6; ((galactose$\frac{4}{1}$)glucose C-1—C-6) 105.0[b], 85.8, 78.1[c], 71.8[e], 78.3[c], 61.5[d]; ((glucose$\frac{2}{1}$)glucose C-1—C-6) 105.5[b], 75.2[a], 87.3, 68.7, 78.4[c], 62.6[d]; ((glucose$\frac{3}{1}$)glucose C-1—C-6) 106.2[b], 75.7[a], 78.5[c], 71.6[e], 78.7[c], 63.2[d] (a—d may be reversed).

Similarly, **15** (30 mg) was heated at 85 °C for 2.5 h to give **13** (2.5 mg) and tigogenin 3-*O*-β-D-glucopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside (**20**),[13] the prosapogenin of **13** (8.0 mg). **20**: white powder (from EtOH–AcOEt), $[\alpha]_D^{27}$ −42.5° (c=0.20, pyridine).

Permethylation Followed by Alditol Acetate Analysis of 5 and 15 According to Hakomori's method,[14] **5** (2 mg) and **15** (2 mg) were methylated with NaH and DMSO, and CH_3I, respectively. The resulting permethylated ethers of **5** and **15** were converted to alditol acetates according to the previous paper.[15] GC-MS conditions: 1.5% OV-210 on Chromosorb-W; glass column 2 mm × 2 m; carrier gas, He (50 ml/min); column temperature a) 195 °C, t_R (min): **6** (3.8), **7** (6.0), **8** (7.2), **9** (11.1); b) 180 °C, t_R (min): **6** (5.2), **7** (8.5), **8** (10.5), **9** (16.8), **19** (3.7).

Acknowledgement The authors are grateful to Prof. O. Tanaka, Hiroshima University, and Dr. T. Etoh, Kagoshima University, for valuable advice. Thanks are also due to Mr. Y. Saruwatari of our institute for supplying roots of *A. sativum*.

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