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

Hiromichi Matsushita,* Tsuyoshi Ushiroguchi, Yôichi Itakura and Toru Fuwa

Central Research Laboratories, Wako Sigma Pharmaceutical Co., Ltd., 1624 Shimokotachi, Koda-cho, Takata-gun, Hiroshima 729-64, Japan. Received March 22, 1989

A new furostanol glycoside (2), named sativoside-B1, was isolated from garlic, bulbs of *Allium sativum* L., along with proto-desgalactotigotin (3). The structure of 2 was established to be (25R)-26-O-β-D-glucopyranosyl-22-hydroxy-5x-furostan-3β,6β,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)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 (25R)-26-O-β-D-glucopyranosyl-22-hydroxy-5x-furostan-3β,26-diol-3-O-β-D-glucopyranosyl(1→3)-O-β-D-glucopyranosyl(1-2)-O-[β-D-xylpyranosyl(1→3)]O-β-D-glucopyranosyl(1→4)-O-β-D-galactopyranoside (16) and its corresponding spirosotranol glycoside (15). Besides these glycosides, three known glycosides, 3, desgalactotigotin (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-desgalactotigotin; desgalactotigotin; F-gitonin

The structure of a new furostanol glycoside from garlic, bulbs of *Allium sativum* L., proto-eruboside-B (1), has already been elucidated. In further studies of the glycosides of the *Allium* family, several steroidal glycosides from bulbs of *A. ampeoloprasum* (elephant garlic) and *A. chinense* were reported. 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 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), C₉₅H₁₈₀O₃₅•4H₂O, named sativoside-B1, is positive to the Ehrlich reagent on thin layer chromatography (TLC). On standing in methanol, 2 gave a glycoside (4), which showed a methoxyl signal at 3.26 ppm in the proton nuclear magnetic resonance (1H-NMR) spectrum. In the carbon-13 NMR (13C-NMR) spectrum at 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 (25R)-5x-furostan-3β,6β,22,26-tetraol having sugar units at the 3- and 26-hydroxy groups. On acid hydrolysis, 2 afforded galactose and glucose, and the anemic carbon signals of 2 indicated the presence of six monosaccharide units. Enzymatic hydrolysis of 2 with β-glucosidase gave a glycoside (5), C₇₅H₁₂₀O₃₅•5H₂O, and glucose. On sugar sequence analysis, 5 afforded four partially methylated alditol acetates, 1, 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). Partial hydrolysis of 5 with aqueous sulfuric acid yielded two teglycosides of β-chlorogenin, 10 and 11, of which the former was identical with eruboside-B.

A comparison of the 13C-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 glucosyl(1→2)glucosyl(1→2)glucosyl(1→4)gal or glucosyl(1→3)glucosyl(1→2)glucosyl(1→4)gal. In the 13C-NMR spectrum of 11, a carbon signal at 85.8 ppm assignable to C-2 of one of the β-glucopyranosyl units 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 (25R)-26-O-β-D-glucopyranosyl-22-hydroxy-5x-furostan-3β,6β,26-triol-3-O-β-D-glucopyranosyl(1→3)-O-β-D-glucopyranosyl(1→2)-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 1H-NMR spectrum of the glycoside (12) formed by the reaction of 3 with methanol. The inspection of the anemic 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 desgalactotigotin. Therefore, 3 was identified as proto-desgalactotigotin, previously isolated from berries of *Solanum nigrum*.

A number of steroidal glycosides have been isolated from roots and leaves of many medicinal plants. 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 based on an analysis of 13C-NMR spectrum, the products of partial hydrolysis of 14 and physical properties.

A new glycoside (16), C₉₅H₁₈₀O₃₅•2H₂O, 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 anomic carbon signals were observed, suggesting that 16 is a furanoside glycoside. On enzymatic hydrolysis with $\beta$-glucosidase, 16 liberated glucose and a new glycoside, which is identical with 15, C$_{30}$H$_{43}$O$_{14}$, named sativoseide-R2. On acid hydrolysis, 15 gave tgtogenin (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 [(hexosyl-hexose)Ac$_2$]$^+$, 331 [(terminal-hexose)Ac$_2$]$^+$ and 259 [(terminal-pentose)Ac$_2$]$^+$. 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 $\beta$-glucopyranosyl unit appeared in the spectrum of 15, leading to the formulation of 15 as tgtogenin 3-O-$\beta$-D-glucopyranosyl(1→3)-O-$\beta$-D-glucopyranosyl(1→2)-O-$\beta$-D-glucopyranosyl(1→3)-O-$\beta$-D-glucopyranosyl(1→4)-O-$\beta$-D-galactopyranoside.

Since 16 is a furanoside glycoside corresponding to 15, it was determined to be (2S,6S)-26-O-$\beta$-D-glucopyranosyl-22-hydroxy-5z-furostan-3$\beta$-26-diol 3-O-$\beta$-D-glucopyranosyl-(1→3)-O-$\beta$-D-glucopyranosyl-(1→2)-O-$\beta$-D-glucopyranosyl-(1→3)-O-$\beta$-D-glucopyranosyl-(1→4)-O-$\beta$-D-galactopyranoside.

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

**Experimental Procedure**
The NMR spectra were taken on a JEOL JNM GX-270 spectrometer (H 270 MHz. $^{13}$C 70.78 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.

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

**Extraction and Isolation of 2, 3, 13, 14, 15 and 16** The crude glycoside fraction of garlic (see the previous paper) was subjected to repeated column chromatography on reversed-phase highly porous polymer, MCI gel CH2PO2 (solvent: 70% aqueous MeOH), and on silica gel (solvent: CHCl$_3$-MeOH-H$_2$O (4:4:6, homogenous), followed by 50% 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), $\left[\alpha\right]_D^{20} = -40.0^\circ$ (c = 0.39, H$_2$O). Anal. Calcd for C$_{30}$H$_{43}$O$_{13}$: 49.0H; C: 52.55; H: 6.99. 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$_2$O was applied to a column of MCI gel CH2PO2 (stepwise elution with H$_2$O, 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$_2$O (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 CH2PO2, solvent: 75% aqueous MeOH), followed by heating in 30% aqueous acetone at 100°C for 4 h, to afford 2 and 3 in yields of 0.02% and 0.05%.

**Glycoside (13): Colorless microcrystals (from EtOH), mp 282—287°C (dec.) (lit. $^{49}$ 284—286°C (dec.)), $\left[\alpha\right]_D^{20} = -57.9^\circ$ (c = 0.61, pyridine).

**Glycoside (14): Colorless needles (from BuOH saturated with H$_2$O), mp 245—250°C (dec.) (lit. $^{49}$ 252—255°C (dec.)), $\left[\alpha\right]_D^{20} = -75.8^\circ$ (c = 0.38, pyridine).

**Glycoside (15): Colorless microcrystals (from MeOH-CHCl$_3$), mp 265—270°C (dec.), $\left[\alpha\right]_D^{20} = -51.5^\circ$ (c = 0.51, pyridine). Anal. Calcd for C$_{30}$H$_{42}$O$_{14}$: 49.0H; C: 52.99; H: 7.94. Found: C: 53.07; H: 8.19. $^{13}$NMR $\delta$: 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 $\delta$: (aglycone C-1→C-27) 37.2, 29.9, 77.5, 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 $^{2-}$-galactose C-1→C-6) 102.4, 73.1, 75.4, 79.5, 76.5, 60.7; (aglycone $^{3-}$-glucose C-1→C-6) 104.9, 80.7, 86.8, 70.7, 77.6, 62.1; (aglycone $^{2-}$-glucose C-1→C-6) 104.9, 74.8, 87.5, 69.4, 78.0, 62.5; (aglycone $^{3-}$-glucose C-1→C-6) 104.9, 75.1, 78.3, 70.4, 78.1, 63.0; (aglycone $^{4-}$-xylose C-1→C-5) 105.4, 75.5, 78.1, 67.5, 63.1 (a-e may be reversed).

**Glycoside (16): White powder (from aqueous acetone), $\left[\alpha\right]_D^{20} = -45.0^\circ$ (c = 0.59, pyridine). Anal. Calcd for C$_{30}$H$_{42}$O$_{13}$: 49.0H; C: 52.68; H: 7.70. Found: C: 52.20; H: 8.03. $^{13}$NMR $\delta$: 0.65 (3H, s), 0.88 (3H, s), 0.99 (3H, d, J = 6.6 Hz), 1.34 (3H, J = 6.6 Hz), 4.82 (1H, J = 7.7 Hz), 4.89 (1H, J = 7.3 Hz), 5.12 (1H, J = 8.7 Hz), 5.15 (1H, J = 8.7 Hz), 5.18 (1H, J = 8.3 Hz), 5.54 (1H, d, J = 7.4 Hz). $^{13}$C-NMR $\delta$: (aglycone C-1→C-27) 37.2, 29.9, 77.5, 34.3, 40.7, 29.0, 32.4, 35.3, 54.5, 35.8, 21.3, 56.4, 32.4, 81.1, 64.0, 16.7, 12.3, 40.7, 14.6, 110.6, 37.2, 28.4, 34.3, 75.3, 17.5; (aglycone $^{2-}$-galactose C-1→C-6) 102.5, 73.1, 75.4, 79.6, 75.6.
Acid Hydrolysis of 15 (52 mg) was heated with 5% aqueous sulfuric acid solution-EtOH (1:1; 5 ml) at 100 °C for 6 h. After cooling, the reaction mixture was diluted with H₂O and applied to a column of MCI gel CHP20P (solvent: H₂O and then MeOH). The fraction eluted with MeOH was chromatographed on silica gel (solvent: CHCl₃-MeOH-H₂O (7:3:0.5)) to afford 5 (68 mg). 13 (18 mg) and 15 (33 mg), while glucosidase was identified by TLC in the fraction eluted with H₂O.

Glycoside (5), Colorless microcrystals (from MeOH), mp 252-256 °C (dec.), [α]D = -47.3 (c = 0.55, pyridine). Anal. Calc. for C₁₉H₁₆O₁₂: C, 51.4; H, 7.94; N, 0.69 (H₂O, d, J = 5.5 Hz), 0.87 (H₂O, d, J = 6.6 Hz), 1.22 (H₂O, 4.92 (H₂O, d, J = 7.7 Hz), 5.13 (H₂O, d, J = 7.3 Hz), 5.15 (H₂O, d, J = 7.3 Hz), 5.24 (H₂O, d, J = 7.3 Hz), 5.55 (H₂O, d, J = 6.6 Hz). 13C-NMR δ (Tetrahydrofuran-2,2-dimethyl-4,4-dioxide 4): 38.8, 30.0, 77.9, 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, (galactose-1-galactose-1-C-6) 104.0, 80.9, 88.4, 70.7, 77.9, 62.0; (glucose-1-galactose-1-C-6) 104.0, 74.7, 87.5, 69.2, 75.5, 63.1; (glucose-1-galactose-1-C-6) 104.0, 75.5, 75.5, 75.5, 75.5, 62.5; (galactose-1-galactose-1-C-6) 105.2, 75.5, 78.3, 75.5, 78.3, 62.5 (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 H₂O and applied to a column of MCI gel CHP20P (stepwise elution with H₂O and MeOH). The fraction eluted with MeOH was separated by silica gel chromatography (solvent: CHCl₃-MeOH-H₂O (7:3:0.5)) to afford 10 (7.0 mg) and 11 (1.6 mg). 10: White powder (from EtOH-EtOH-OAc), [α]D = -59.0° (c = 0.20, CHCl₃-MeOH (10:1)). 11: FD-MS m/z: 1103 (M + Na)+. 12C-NMR δ (pyridine-d₅): 38.9, 30.0, 77.1, 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, 16.6, 16.1, 42.1, 15.0, 109.3, 32.3, 29.3, 30.6, 66.9, 17.4, (galactose-1-galactose-1-C-6) 102.4, 73.2, 75.5, 80.9, 75.5, 60.6, (galactose-1-galactose-1-C-6) 105.0, 85.8, 78.1, 71.8, 78.3, 61.5; (glucose-1-galactose-1-C-6) 105.2, 75.2, 87.3, 68.7, 78.4, 62.6; (galactose-1-galactose-1-C-6) 106.2, 75.2, 78.5, 51.6, 78.7, 62.3. (a-d may be reversed). Similarly, 15 (30 mg) was heated at 95 °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), the prosapogenin of 13 (8.0 mg). 20: White powder (from EtOH-AcOEt), [α]D = -42.5° (c = 0.20, pyridine).

Permethylation Followed by Alditol Acetate Analysis of 5 and 15 According to Hakomori’s method, 14, 15 (2 mg) and 15 (2 mg) were methylated with NaI and DMSO, and CH₃I, respectively. The resulting permethyalted 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 mm; carrier gas, He (50 ml/min); column temperature a) 195 °C, tR (min): 6 (3.8), 7 (6.0), 8 (7.2), 9 (11.1); b) 180 °C, tR (min): 6 (5.2), 7 (8.5), 8 (10.5), 9 (11.6), 19 (17.9).

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. Suvartari of our institute for supplying roots of A. sativum.

References and Notes
7) This assignment was further supported by the finding that the 13C-NMR spectrum of 20 exhibited a carbon signal at 86.1 ppm assignable to C-2 of the inner β-glucopyranosyl unit.