Further Studies on New Furostanol Saponins from the Bulbs of *Allium macrostemon*

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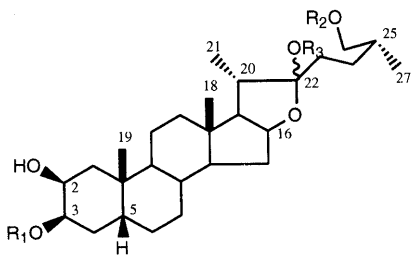
Further studies by means of preparative HPLC led to the isolation of two new furostanol saponins, macrostemonoside J (1) and L (3), along with an artifact, macrostemonoside K (2) from the bulbs of *Allium macrostemon*. On the basis of chemical evidence and spectral analysis (^{1}H-, ^{13}C-NMR and FAB-MS), the structure of 1 was elucidated to be 26-*O*-β-D-glucopyranosyl 2β,3β,22,26-tetrahydroxy-25(*R*)-5β-furostan 3-*O*-β-D-glucopyranosyl (1→2)-β-D-galactopyranoside. 3 was deduced to be 26-*O*-β-D-glucopyranosyl 2β,3β,26-trihydroxy-25(*R*)-5β-furostan-20(22)-ene 3-*O*-β-D-glucopyranosyl (1→2)-β-D-galactopyranoside.

Keywords *Allium macrostemon*; furostanol saponin; macrostemonoside J; macrostemonoside L

The structures of four new steroidal glycosides from small-headed garlic, the bulbs of *Allium macrostemon* BUNGE (Liliaceae), macrostemonoside A, D, E and F, have already been elucidated.[1,2] In further studies of the title plant, eight new furostanol glycosides, macrostemonosides B, C and G—L were isolated. The present paper briefly deals with the isolation and structure elucidation of furostanol glycosides, macrostemonoside J (**1**), K (**2**) and L (**3**) from the title plant.

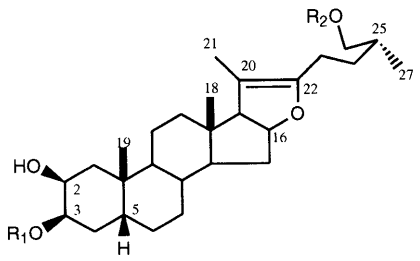
The glycoside fraction of small-headed garlic obtained previously[1] was repeatedly subjected to silica gel column chromatography and on reversed-phase highly porous polymer followed by a Lobar column (Rp-8), and preparative HPLC ($5C_{18}$ column) to give three glycosides **1**, **2** and **3**, respectively. After isolation by preparative HPLC, **1** easily became a mixture of two spots on HPTLC when it was allowed to stand in a methanol solution. The mixture conveniently converted to **1** when heated with 30% aqueous acetone at 100 °C for 4 h, and **1** easily turned to **2** in methanol at room temperature for 2 h, suggesting that **1** was a 22-hydroxy furostanol saponin, whereas **2** was its artifact, a 22-methoxy derivative.[3] Saponins **1**, **2** and **3** were hydrolysed with acid to afford the same aglycone, samogenin (**4**), which was identified by comparison with an authentic sample on TLC and galactose and glucose in a ratio of 1:2 as sugar residues. All of them reacted positively to Ehrlich's reagent. Enzymatic hydrolysis of **1** gave glucose and the corresponding spirostanol saponin (**5**) (1 mg), which was identified by IR spectrum. The IR spectrum of **5** showed that C-25 of the spirostanol saponin had an *R*-configuration based on the characteristic 25(*R*)-spiroketal IR absorption bands (intensity: 905 cm^{-1} > 930 cm^{-1}).[4] On sugar sequence analysis, each saponin afforded three partially methylated alditol acetates, 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylhexitol (**6**) and 1,2,5-tri-*O*-acetyl-3,4,6-tri-*O*-methylhexitol (**7**) in a 2:1 ratio on detection by gas chromatograph–mass spectrometry (GC-MS) (alditol acetate analysis).[5] The ^{13}C-NMR spectrum of **1** exhibited signals of 5β-furostanol saponin [δ 36.7 (C-5), 40.7 (C-9), 23.9 (C-19)]. Positive FAB mass spectrum showed ions at *m/z* 919 $(M+H-H_2O)^+$, 595 $(M+H-H_2O-Glc\times2)^+$, 433 $(aglycone+H-H_2O)^+$ and 415 $(aglycone+H-H_2O\times2)^+$. A carbon signal of C-2 ascribable to the aglycone of **1** shifted downfield by 40.7 ppm to δ 67.3, while the β-carbon signal (C-1) shifted to downfield by 9.4 ppm to δ 40.4 in comparison with that of macrostemonoside F.[2] With the C-19 methyl carbon unshifted, the free hydroxyl group can be assigned to the C-2 position rather than to C-1. It was not easy to determine which hydroxyl group between C-2 and C-3 was glycosylated. Since **1** was 5β-furostanol, comparison of the ^{13}C-NMR of **1** with those of three (5β)-2,3-dihydroxyl spirostanols [yonogenin: 5β, 25*R*, 2β, 3α $(OH)_2$; neoyonogenin: 5β, 25*S*, 2β, 3α $(OH)_2$ and samogenin: 5β, 25*R*, 2β, 3β $(OH)_2$][6] showed that carbon signals due to the aglycone and C-3 sugar moieties of **1** appeared at almost the same position as those of samogenin 3-*O*-β-D-glucopyranosyl (1→2)-β-D-galactopyranoside (**8**).[7] Thus, **1** was established to be (5β,25*R*)-2β,3β-dihydroxyl furostanol saponin.

It was observed that the glycosylation of the C-3 hydroxyl group resulted in a downfield shift (14.6 ppm) of α-carbon and upfield shifts of β-carbons (2.9 and 1.5 ppm for C-2 and C-4, respectively) compared with the ^{13}C-NMR spectrum of samogenin, while a downfield shift of the anomeric carbon of galactose in sugar moiety (+4.1 ppm) was noted. According to the glycosylation shift value of the 1,2-diol type saponin proposed by Tanaka,[8] the absolute stereochemistry of C-3 was deduced to have an *R*-configuration. Therefore, **1** was deduced to be 26-*O*-β-D-glucopyranosyl 2β,3β,22,26-tetrahydroxy-25(*R*)-5β-furostan 3-*O*-β-D-glucopyranosyl (1→2)-β-D-galactopyranoside. As mentioned above, **2** was easily converted from **1** in methanol at room temperature for 2 h. Assignments of ^{13}C-NMR signals are shown in Table I. On comparison of the NMR spectra of **2** with those of **1**, all signals appeared at almost the same positions with the exception of a methoxyl group [δ 3.28 (3H, s) in ^{1}H- and δ 47.3 in ^{13}C-NMR spectra]. Thus, **2** can be identified as 26-*O*-β-D-glucopyranosyl 2β,3β,26-trihydroxy, 22-methoxy-25(*R*)-5-furostan-3-*O*-β-D-glucopyranosyl (1→2)-β-D-galactopyranoside. Similarly, the ^{13}C-NMR spectrum of **3** showed the characteristic double bond between C-20 and C-22 (δ 103.7 and 152.4 ppm). The



1: R_1= -β-Gal $\xrightarrow{2}$ β-Glc R_2 = -β-Glc R_3 =H

2: R_1= -β-Gal $\xrightarrow{2}$ β-Glc R_2 = -β-Glc R_3 =CH_3



3: R_1= -β-Gal $\xrightarrow{2}$ β-Glc R_2 = -β-Glc

TABLE I. ^{13}C-NMR Data for **1**, **2**, **3** and the Related Compounds

C	Samogenin 5β,25*R* 2β,3β(OH)$_2$	**8**	**1**	**2**	**3**
1	39.2	40.5	40.4	40.1	40.1
2	70.2	67.2	67.3	67.3	67.3
3	67.5	81.7	82.1	82.0	82.1
4	33.5	31.9	32.0	32.0	31.9
5	36.1	36.5	36.7	36.7	36.6
6	26.3	26.3	26.3	26.3	26.3
7	26.8	26.8	26.8	26.8	26.9
8	35.7	35.6	35.6	35.6	35.3
9	41.5	41.4	40.7	40.6	41.4
10	37.0	37.1	37.2	37.1	37.1
11	21.3	21.3	21.4	21.3	21.5
12	40.4	40.3	40.7	40.6	40.6
13	40.9	40.8	41.2	41.2	43.9
14	56.5	56.4	56.3	56.3	54.7
15	32.1	32.1	32.4	32.1	31.5
16	81.1	81.2	81.2	81.5	84.6
17	63.1	63.2	64.1	64.5	64.7
18	16.6	16.5	16.7	16.5	14.5
19	24.1	23.9	23.9	23.9	23.9
20	42.0	42.0	41.5	41.4	103.7
21	14.9	15.0	16.5	16.3	11.9
22	109.1	109.1	110.7	112.7	152.4
23	31.8	31.9	37.2	30.9	34.4
24	29.2	29.3	28.4	28.3	23.7
25	30.6	30.6	34.3	34.3	33.5
26	66.8	66.9	75.3	75.2	75.0
27	17.3	17.3	17.5	17.2	17.4
OCH_3				47.3	
C-3 sugar part					
Gal-1		103.2	103.5	103.4	103.5
2		81.8	81.9	81.9	81.7
3		76.8	75.2	75.2	75.2
4		69.7	69.8	69.8	69.7
5		76.8	77.0	77.0	77.0
6		62.0[a]	62.0[a]	62.0[a]	62.0[a]
Glc-1		106.1	106.3	106.3	106.3
2		75.1	77.0	77.0	77.0
3		78.3[b]	78.1[b]	78.1[b]	78.0[b]
4		71.7	71.8	71.8	71.8
5		78.0[b]	78.5[b]	78.5[b]	78.5[b]
6		62.9[a]	62.9[a]	63.0[a]	62.9[a]
C-26 Sugar part					
Glc-1			105.0	105.0	104.9
2			75.2	75.2	75.2
3			78.7[b]	78.7[b]	78.6[b]
4			71.8	71.8	71.8
5			78.5[b]	78.5[b]	78.5[b]
6			62.9[a]	62.9[a]	62.9[a]

a, *b*) Signals may be interchangeable in each column.

remaining signals appeared at almost the same positions as those of **1** and **2**. Therefore, **3** can be deduced to be 26-*O*-β-D-glucopyranosyl 2β,3β,26-trihydroxy-25(*R*)-5β-furostan-20(22)-ene 3-*O*-β-D-glucopyranosyl (1→2)-β-D-galactopyranoside.

Experimental

General Procedure Melting points were determined on a Yanaco MP-S_3-melting point apparatus; the thermometer was uncorrected. IR spectra were recorded on an IR-27G spectrometer. GC was run on a HP-5890 Series II gas-chromatograph. EI-MS, GC-MS and FD-MS spectra were obtained with a JEOL-DX 300 mass spectrometer. Positive and negative FAB-MS spectra were measured on a JEOL JMS-DX 302 mass spectrometer. ^{1}H- and ^{13}C-NMR spectra were taken on a JEOL JNM-GX 400 spectrometer and on a JEOL JNM-GX 500 spectrometer using tetramethylsilane as an internal standard.

Isolation of 1, 2 and 3 The crushed bulbs (36 kg) of *A. macrostemon* BUNGE (Liliaceae) were extracted with 75% EtOH (80 l × 3). The combined extracts were concentrated *in vacuo*, and then suspended in H_2O, followed by extraction with $CHCl_3$, EtOAc and *n*-BuOH, successively. The *n*-BuOH layer was subjected to Diaion column chromatography, and the MeOH eluate was subjected to chromatography on silica gel using a $CHCl_3$–MeOH system. Further separation using Diaion, repeated Lobar column (Rp-8) and HPLC ($5C_{18}$) with the MeOH–H_2O system, was performed to obtain **1** (10 mg), **2** (18 mg) and **3** (14 mg), respectively.

Macrostemonoside J (1) White amorphous powder, mp 230—232 °C. **1** was positive to Ehrlich's reagent. FAB-MS (pos.) *m/z*: 919 $(M+H-H_2O)^+$, 595 $(M+H-H_2O-Glc\times2)^+$, 433 $(aglycone+H-H_2O)^+$, 415 $(aglycone+H-H_2O\times2)^+$, 287, 271, 253, 212, 185, 145, 115, 93, 73, 60, 53. **1**+Na: 959 $(M+Na)^+$, 941 $(M+Na-H_2O)^+$, 919 $(M+H-H_2O)^+$, 595, 331, 309, 177,155, 135, 119, 103, 85, 77, 73, 57. ^{1}H-NMR (400 MHz, ppm, in C_5D_5N): 0.87 (3H, s), 0.97 (3H, s), 0.99 (3H, d, J=6.6 Hz), 1.34 (3H, d, J=6.8 Hz), 4.84 (1H, d, J=7.8 Hz), 5.02 (1H, d, J=7.6 Hz), 5.30 (1H, d, J=7.6 Hz). ^{13}C-NMR (100.4 MHz, ppm, in C_5D_5N) are shown in Table I.

Macrostemonoside K (2) White amorphous powder, mp 219—221 °C. **2** exibited a purple coloration with Ehrlich's reagent. FAB-MS (neg.) *m/z*: 949 $(M-H)^-$, FAB-MS (pos.) *m/z*: 973 $(M+Na)^+$, 919 $(M+H-MeOH)^+$, 595 $(M+H-MeOH-Glc\times2)^+$, 453. ^{1}H-NMR (400 MHz, ppm, in C_5D_5N): 0.79 (3H, s), 0.96 (3H, s), 1.01 (3H, d, J=6.6 Hz), 1.19 (3H, d, J=7.0 Hz), 3.28 (3H, s, OCH_3), 4.88 (1H, d, J=7.8 Hz), 5.02 (1H, d, J=7.6 Hz), 5.30 (1H, d, J=7.6 Hz). ^{13}C-NMR spectral data (100.4 MHz, ppm, in C_5D_5N) are shown in Table I.

Macrostemonoside L (3) White amorphous powder, mp 206—208 °C. **3** showed a purple coloration with Ehrlich's reagent. FAB-MS (pos.) *m/z*: 941 $(M+Na)^+$. ^{1}H-NMR (500 MHz, ppm, in C_5D_5N): 0.68 (3H, s), 0.95 (3H, s), 1.02 (3H, d, J=6.7 Hz), 1.61 (3H, s), 4.81 (1H, d, J=7.8 Hz), 4.96 (1H, d, J=7.7 Hz), 5.23 (1H, d, J=7.7 Hz). ^{13}C-NMR (125 MHz, ppm, in C_5D_5N) spectral data are shown in Table I.

Acid Hydrolysis of Glycosides 1, 2 and 3 A mixture of **1**, **2** and **3** (a few mg, each) was heated with 2 N HCl–dioxane (1 : 1, 2 ml) in a sealed tube at 100 °C for 4 h. The reaction mixture was concentrated to dryness by blowing N_2 gas at r.t. For GC analysis, the residue was trimethylsilylated with hexamethyl disilazane and trimethylchlorosilane in a 2:1 ratio at r.t. GC: SE-30 capillary column (12 m × 0.22 mm i.d.), detector: FID (270 °C), column temperature, 150—210 °C, rate 5 °C/min,

carrier gas, N_2 (30 ml/min)

Enzymatic Hydrolysis of 1, 2 and 3 A phosphate buffer solution (pH 4—5, 40 ml) of each saponin (5 mg) and β-glucosidase from almond was incubated at 37 °C for 24 h. The precipitate on the reaction mixture was filtered and washed with hot water to give the product. Glucose was identified in the filtrate by silica gel TLC developed with $CHCl_3$–MeOH–H_2O (160 : 92 : 20) (*Rf* 0.31).

References and Notes

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