한국영양식량학회지

The Structures of Two Diosgenin Glycosides Isolated from the Subterranean Parts of *Allium fistulosum*

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Abstract

The structures of dioscin(1) and saponin P-d(2), isolated from the subterranean part of *Allium fistulosum*, were determined as diosgenin 3-O-α-L-rhamnopyranosyl(1→2)-[α-L-rhamnopyranosyl(1→4)]-β-D-glucopyranoside(1) and diosgenin 3-O-α-L-rhamnopyranosyl(1→4)-α-L-rhamnopyranosyl(1→4)-[α-L-rhamnopyranosyl(1→2)]-β-D-glucopyranoside(2) by spectroscopic and chemical degradational methods. The prosapogenin(4), which was provided on partial hydrolysis of 2, was not reported in previous literature.

Key words : *Allium fistulosum*, liliaceae, dioscin, saponin P-d

INTRODUCTION

Allium fistulosum Linne (Liliaceae) is a perennial herb which is cultivated widely for food. The bulbs and roots of this plant have been used for various medicinal purposes[1,2]. Recently, we reported the isolation and characterization of three new yuccagenin glycosides, named fistulosides A, B and C together with isolation of dioscin (1) and saponin P-d (2)[3]. But we did not represent their spectral data as well as chemical evidences for supporting their structures. In this paper, we describe the structural determination of these two compounds (1, 2) in detail. Especially, the prosapogenin (4) of 2 is a new diosgenin glycoside, although it is not a natural product.

MATERIALS AND METHODS

Plant material and general experimental procedures

Plant material, instruments used and isolation procedure were as described previously[3].

Dioscin(1)

White needles from MeOH : mp 288~290°C, $[\alpha]_D^{23}$ =-110° (c=0.5, MeOH) ; Infrared spectrum (IR) (KBr, cm^{-1}) 3390, 1047, 982, 919, 900, 866[intensity 900>919, 25 (R)-spiroketal] ; Fast atomic bombardment mass spectrum (FAB MS) m/z (rel. int.) [M+Na]$^+$ 891 (2.5), [genin+H]$^+$ 415 (3.4) ; ^{1}H-nuclear magnetic resonance spectrum (^{1}H-NMR) see Table 1 ; ^{13}C-NMR see Table 2. Anal. calcd. for $C_{45}H_{72}O_{16}$ · 2 H_2O, C 59.71, H 8.46 ; found C 59.84, H 8.16.

Saponin P-d(2)

White needles from MeOH : mp 210~212°C, $[\alpha]_D^{21}$ =-118.6° (c=0.2, MeOH) ; IR (KBr, cm^{-1}) 3404, 10

Table 1. Partial ^{1}H-NMR spectral data for 1, 2 and 4 in pyridine-d$_5$[a]

Proton	1	2	4
18-Me	0.83, s	0.84, s	0.85, s
19-Me	1.04, s	1.06, s	1.06, s
21-Me	1.13, d (7.0)	1.15, d (6.8)	1.15, d (6.8)
27-Me	0.71, d (5.3)	0.71, d (5.6)	0.71, d (5.6)
H-6	5.31, br.d (4.1)	5.32, br.d (4.1)	5.34, br.d (4.1)
Rha-Me	1.61, d (6.2)	1.61, d (5.6)	1.60, d (5.8)
	1.75, d (6.2)	1.61, d (5.6)	1.68, d (6.0)
		1.78, d (6.1)	
Anomeric	4.80, d (7.6)	4.89, d (7.7)	4.87, d (7.8)
protons	5.81, s	5.85, s	5.85, s
	6.35, s	6.30, s	6.28, s
		6.41, s	

[a]Data are δ (ppm), multiplicity, and J (in parentheses) in Hz

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50, 982, 919, 900, 866 [intensity 900>919, 25 (R)-spiroketal] ; FAB MS m/z (rel. int.) [M+Na]$^+$ 1037 (2. 5), [genin+H]$^+$ 415 (1.5) ; ^{1}H-NMR see Table 1 ; ^{13}C-NMR see Table 2. Anal. calcd. for $C_{51}H_{82}O_{20} \cdot -3H_2O$, C 57.29, H 8.30 ; found C 57.45, H 8.02.

Acid hydrolysis of 1 and 2

A solution of each glycoside (30mg each) in 4N HCl-dioxane (1 : 1, 9ml) was refluxed for 30 min. Dilution of the reaction mixture liberated the precipitate, which was collected by filtration followed by recrystallization from MeOH to afford the common aglycone, diosgenin (3) as colorless needles, mp 205~207°, which was identified by direct comparison with an authentic sample (mmp, IR, MS, and NMR)[4]. Glucose (Rf 0.45) ; and rhamnose (Rf 0.66) were detected from the hydrolysate of 1 and 2 as the common sugar components by TLC (sol. pyridine-

3 R = H **1** R =

2 R =

4 R =

Fig. 1. Structures of compounds.

EtOAc-HOAc-H$_2$O = 36 : 36 : 7 : 21).

Partial hydrolysis of saponin P-d (2)

2 (100mg) was refluxed with 1% H$_2$SO$_4$ in MeOH (25ml) for 10 min. After cooling, the reaction mixture was diluted with ice water and the precipitate was collected by filtration, and dried. The precipitate (70 mg), which showed seven spots on TLC (solvent, EtOAc-MeOH-H$_2$O=100 : 16.5 : 13.5), was subjected to column chromatography over silica gel with hexane-EtOAc (8 : 5) and then EtOAc saturated with H$_2$O-MeOH (gradient, 0 to 5%) to afford diosgenin (3, 15mg), prosapogenins C (7mg), B (5mg), and A (8mg) of dioscin, 1 (11mg), 4 (10mg) and then recovered 2 (9mg) in the order of elution. Diosgenin (3) and recovered saponin P-d (2) were identified by comparison with the previously obtained samples. Four prosapogenins were identified by direct comparison - (co-TLC, mmp) with the authentic standards of prosapogenins C, B, and A of dioscin together with dioscin (1), respectively[4]. Compound 4 was crystallized from MeOH as colorless needles : mp 260~263°C, $[\alpha]_D$=-62° (c=0.09, pyridine) ; ^{1}H-NMR see Table 1 ; ^{13}C-NMR see Table 2. Compound 4 was hydrolyzed with acid in the same manner as 2, as described above, and glucose and rhamnose as sugar components were detected by TLC.
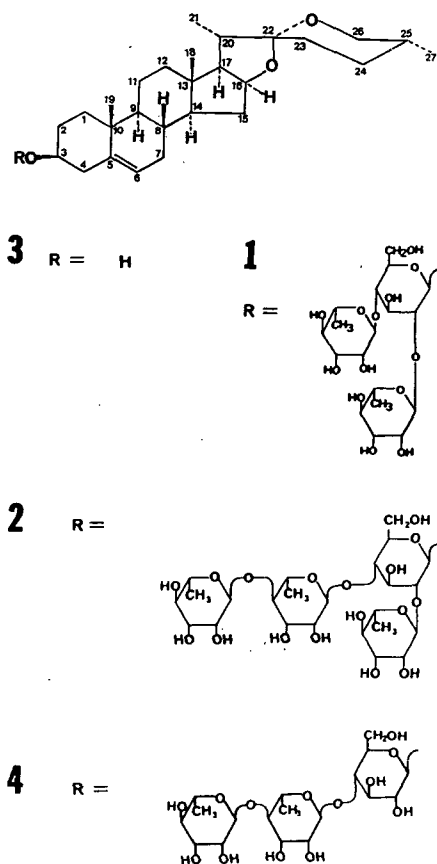
RESULTS AND DISCUSSION

Compound 1, mp 288~290°, was positive to the Liebermann–Burchard test, and showed characteristic absorption of the 25 (R)-spiroketal moiety at 981, 918, 899 and 865 cm^{-1} (intensity 899>918) in its IR spectrum[5]. Acid hydrolysis of 1 yielded rhamnose and glucose together with an aglycone (3), which was identified as diosgenin by direct comparisons (co-TLC, mmp) with an authentic sample. The FAB MS spectrum of 1 showed a cationized molecular ion [M+Na]$^+$ at m/z 891 and [genin+H]$^+$ ion at m/z 415, indicating that 1 was a diosgenin trisaccharide having one mole of glucose and 2 moles of rhamnose. The ^{1}H-NMR spectrum of 1 showed β configuration for one glucose unit and α configuration for two rhamnose units (see Table 1)[6]. On the basis of the

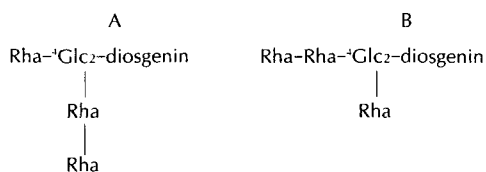**Table 2. ¹³C-NMR chemical shifts of diosgenin glycosides in pyridine-d₅[a]**

Carbon No.		1	2	4
	C- 1	37.4	37.5	37.4
	C- 2	30.0	30.2	30.2
	C- 3	78.0	78.1	78.2
	C- 4	38.8	39.0	39.1
	C- 5	140.7	140.8	140.9
	C- 6	121.7	121.8	121.8
	C- 7	32.1	32.2	32.1
	C- 8	31.6	31.7	31.8
	C- 9	50.2	50.3	50.3
	C-10	37.0	37.2	37.1
	C-11	21.0	21.1	21.1
	C-12	39.8	39.9	39.9
	C-13	40.4	40.5	40.5
	C-14	56.5	56.7	56.7
	C-15	32.2	32.3	32.2
	C-16	81.0	81.1	81.1
	C-17	62.8	62.9	62.9
	C-18	16.3	16.4	16.4
	C-19	19.3	19.4	19.4
	C-20	41.9	42.0	42.0
	C-21	14.9	15.0	15.0
	C-22	109.2	109.3	109.3
	C-23	31.7	31.8	31.8
	C-24	29.2	29.3	29.3
	C-25	30.5	30.6	30.6
	C-26	66.8	66.9	66.9
	C-27	17.2	17.3	17.3
Glc	C- 1	100.1	100.4	102.5
	C- 2	78.6	80.4	75.4
	C- 3	76.8	77.0	76.7
	C- 4	77.8[b]	77.7	78.2
	C- 5	77.7[b]	78.0	76.6
	C- 6	61.2	61.3	61.3
Rha	C- 1	101.9	102.2	
(→²Glc)	C- 2	72.2	72.8	
	C- 3	72.3	72.5	
	C- 4	74.0	74.0[b]	
	C- 5	70.3	69.6	
	C- 6	18.4	18.9	
Rha	C- 1	102.8	103.3	103.2
(→⁴Glc)	C- 2	72.6	72.8	72.9[b]
	C- 3	72.7	70.4	70.4
	C- 4	73.7	77.9	77.7
	C- 5	69.4	68.4	68.3
	C- 6	18.5	18.6	18.9
Rha	C- 1		102.2	102.2
(→⁴Rha)	C- 2		72.8	72.7[b]
	C- 3		72.6	73.0[b]
	C- 4		74.1[b]	73.4
	C- 5		69.6	70.3
	C- 6		18.4	18.5

[a]Chemical shifts are reported in ppm from TMS. Multiplicites were obtained by DEPT spectra
[b]Assignment may be reversed in each column

above observation, **1** was presumed to be dioscin. Furthermore, a direct comparison (co-TLC, mmp) of **1** with an authentic standard of dioscin revealed two compounds to be completely identical. The ¹³C-NMR spectral data of **1** were also in good agreement with those of dioscin[4].

Compound **2**, mp 210~212°, was positive in the Liebermann–Burchard test and gave quite similar IR spectrum to that of **1** suggesting it to be a 25 (R)–spirostanol derivative. It gave also diosgenin (**3**), glucose and rhamnose on acid hydrolysis. The FAB MS spectrum of **2** exhibited a cationized molecular ion [M+Na]⁺ at m/z 1037 and [genin+H]⁺ ion at m/z 415, indicating that **2** was a tetrasaccharide of diosgenin. The ¹H-NMR spectrum of **2** showed that **2** was comprised of one mole of glucose and 3 moles of rhamnose in its structure : one doublet at δ 4. 89 (J=7.7 Hz) and three singlets at δ 5.85, 6.30 and 6.41, demonstrating β–configuration for one glucose and α configurations for three rhamnoses[6]. Partial hydrolysis of **2** afforded five prosapogenins together with diosgenin (**3**). Among them, four prosapogenins were identified by direct comparison (co-TLC, mmp) with authentic samples as prosapogenins C, B, and A of dioscin[4], together with dioscin (**1**), respectively. On acid hydrolysis, remaining prosapogenin (**4**) gave glucose, rhamnose and diosgenin. The ¹H-NMR spectrum of **4** showed two rhamnosyl anomeric protons and one glucosyl anomeric proton. Based on the above evidence, the sugar moiety of **2** is to have the sequence either A or B (Formulae 1). The ¹³C-NMR spectrum of **4** exhibited signals due to a terminal rhamnopyranosyl moiety. Furthermore, the ¹³C-NMR signals due to the glucopyranosyl moiety of **4** were in good agreement with those of prosapogenin B of dioscin[4]. Therefore, the structure of **4** could be designated as Rha–Rha–⁴Glc³–diosgenin.

A   B

Rha–⁴Glc₂–diosgenin   Rha–Rha–⁴Glc₂–diosgenin
      | |
     Rha   Rha
      |
     Rha

Formulae 1

And the signals due to the inner rhamnopyranosyl moiety of **4** revealed significant glycosidation shifts at C-3 (-2.2ppm), C-4 (+3.8ppm) and C-5 (-1.2ppm) signals, compared to terminal one, indicating that the terminal rhamnose unit was attached at C-4 of inner rhamnoside. From the above observation, the structure of **4** was determined as diosgenin 3-O-α-L-rhamnopyranosyl(1→4)-α-L-rhamnopyranosyl(1→4)-β-D-glucopyranoside.

Finally, the structure of **2** was assigned to be diosgenin 3-O-α-L-rhamnopyranosyl(1→4)-α-L-rhamnopyranosyl (1→4)-[α-L-rhamnopyranosyl (1→2)]-β-D-glucopyranoside. Compound **2** was identical with saponin P-d (=Pb), which had previously been isolated from another plant, *Paris polyphylla*[7], and ¹H- and ¹³C-NMR spectral data of **2** were superimposable on those of saponin P-d. Especially, the prosapogenin (**4**) of saponin P-d (**2**) has not been reported previousely in the literature.

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파 지하부로부터 분리된 2종의 Diosgenin 배당체의 구조

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요 약

파 지하부로부터 분리된 2종의 diosgenin 배당체의 구조를 그의 분광학적 data와 화학적 실험 등의 방법으로 결정하였다. 그 결과, 이 두화합물의 구조는 diosgenin 3-O-α-L-rhamnopyranosyl (1→2)-[α-L-rhamnopyranosyl (1→4)]-β-D-glucopyranoside (1, dioscin)과 diosgenin 3-O-α-L-rhamnopyranosyl (1→4)-α-L-rhamnopyranosyl (1→4)-[α-L-rhamnopyranosyl (1→2)]-β-D-glucopyranoside (2, saponin P-d)로서 기지물질이나, saponin P-d를 부분가수분해하여 얻은 prosapogenin 중 compound 4는 문헌 미기재의 물질이다.