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Revision of ^{13}C NMR Assignments of β-Sitosterol and β-Sitosteryl-3-O-β-D-glucopyranoside Isolated from *Plantago asiatica* Seed

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질경이씨로 부터 분리한 β-Sitosteryl-3-O-β-D-glucopyranoside 및 β-Sitosterol의 ^{13}C NMR

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β-Sitosteryl-3-O-β-D-glucopyranoside was isolated from the seeds of *Plantago asiatica* (Plantaginaceae). The assignments of ^{13}C NMR spectra of β-sitosterol and β-sitosteryl-3-O-β-D-glucopyranoside were made by comparing with ^{13}C NMR spectra of cholesterol and cholesteryl-3-O-β-D-glucopyranoside. Our data indicate that the revision of previous ^{13}C NMR spectral assignment is needed.

It was previously reported that seeds of *Plantago asiatica* exhibited liver-protective activity against an animal model of hepatic disease produced by CCl_4 intoxication.[1] There have been reported a few chemical investigations for chemical compositions of *Plantago asiatica*,[2,3] although a number of phytochemical studies with other Plantago species have been published.

In the course of our chemical investigation to isolate potential liver-protective substances from *Plantago asiatica* seeds, β-sitosteryl-3-O-β-D-glucopyranoside (II) was isolated. IR V_{max} (KBr) 3420, 2940cm^{-1} and m.p. 298-300°. [4] Upon the hydrolysis, compound II yielded β-sitosterol (I) and glucose which were identified in direct comparison with authentic samples.

The assignments of ^{13}C NMR spectra of I and II were made by comparing with those of cholesterol (III)[5] and cholesteryl-3-O-β-D-glucopyranoside[6,7] (IV) and chemical shifts calculations by the method of Lindeman and Adams.[8]

The assignments of ^{13}C NMR spectra of C-1~C-21 and C-1′~C-6′ of I and II appeared to be in good agreement with those of III and IV, respectively. The chemical shifts of C-22~C-29 of I and II were assigned and shown in Table I. The calculated values for six carbon atoms (C-24~C-29) by the method of Lindeman and Adams appeared to be in close agreement with the experimentally observed values within ±1 ppm range. The calculated values for remaining two carbon atoms (C-22 and C-23) were δ32.5 and 28.8. Near these chemical shifts, two triplet signals (δ34.2 and 26.4) remained, which were assigned to C-22 and C-23, respectively. Only one equivocal signal (δ28.5, t, 16-C) could

be distinguished from C-23 by PRFT measurement, which gave significantly higher signal than that at δ 26.4 due to slower segment movement.

Table I. ^{13}C-Chemical shifts (δ) of β-sitosterol (I) and its 3-O-β-D-glucoside (II) measured in pyridine-d_5

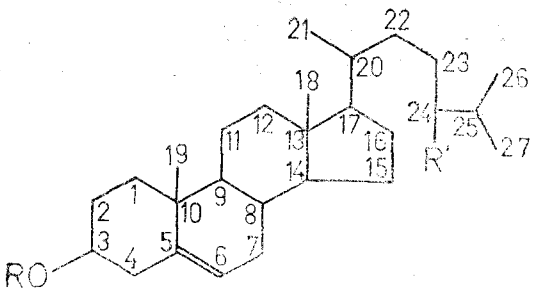
	I	II	$\Delta\delta$(G)[1]		I	II	Calcd.[2]
1	37.8	37.6		21	19.0[3]	19.2[4]	
2	32.2	30.3	−1.9	22	34.2	34.3	32.5
3	71.2	78.5	+7.3	23	26.4	26.5	28.8
4	43.2	39.3	−3.9	24	46.1	46.1	45.7
5	141.9	140.9	−1.0	25	29.4	29.5	30.5
6	121.1	121.9	+0.8	26	19.6[3]	19.4[4]	19.6
7	32.2	32.1		27	20.0[3]	20.0[4]	19.6
8	32.2	32.1		28	23.4	23.5	24.2
9	50.5	50.4		29	12.1	12.2	11.9
10	36.8	37.0					
11	21.4	21.4			C-1′	102.6	
12	40.1	40.0			2′	75.3	
13	42.5	42.5			3′	78.5	
14	56.9	56.9			4′	71.7	
15	24.5	24.6			5′	78.5	
16	28.5	28.5			6′	62.8	
17	56.3	56.3					
18	12.1	12.0					
19	19.3[3]	19.4[4]					
20	36.4	36.4					

1) Glucosylation shift, δ(II)-δ(I). Significant value (>0.5) only.
2) Calculated values by the method of Lindeman and Adams.
3), 4) These assignments may be interchanged.

^{13}C NMR spectra were recorded on a JEOL PFT-100 spectrometer at 25.15 MHz in pyridine-d_5 with TMS as an internal standard in 10mm spinning tube at 25°: concentrations were 0.2 mM for I and 0.08 mM for II. FT measurement conditions were: spectral width, 5KHz: pulse flipping angle, 45° for I, 90° for II: acquisition time, 0.4 sec: number of data points, 4192. PRFT measurement conditions for II were: pulse interval, 0.2 sec: pulse repetition time, 2 sec.

I; β-Sitosterol
R=H
R′=$\overset{28}{CH_2}\overset{29}{CH_3}$

II; β-Sitosteryl-3-O-β-D-glucopyranoside
R=β-D-glucose
R′=$\overset{28}{CH_2}\overset{29}{CH_3}$



III; Cholesterol
R=R′=H

IV; Cholesteryl-3-O-β-D-glucopyranoside
R=β-D-glucose
R′=H

It should be noted that the previous report on chemical shift values of C-23 and C-25 of I were reverse to our data shown in Table I.[9] The multiplicities of the peaks at δ26.4 and 29.4 in off resonance decoupled spectrum were triplet and doublet, respectively. This fact is enough to support our results. Moreover, in the previous report, resonance due C-24 was assigned to δ50.3 (overlapped with C-9). In both spectra of I and II, a clear signal at δ46.1 (d) appeared and the signal at δ50.4 (or 50.5) were not large enough to represent two carbons. Probably, inadequate measuring condition caused saturation at C-24 signal in the previous work.[9]

It should be noted that the stereochemistry of glucose unit was decided to be β-glucopyranoside by chemical shifts of C-1′ to C-6′, and to be D-type by the consideration of glucosylation shift (δ_I-δ_{II}) of C-2, 3, 4 and 1′ without measuring optical rotation.[6,7]

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