ABSOLUTE STEREOSTRUCTURES OF SPINACOSIDES C AND D WITH A NOVEL ACETAL TYPE SUBSTITUENT FROM *SPINACIA OLERACEA* (SPINACH) AND *BASELLA RUBRA* (INDIAN SPINACH)†

Masayuki Yoshikawa,*,[a] Toshiyuki Murakami,[a] Kazuhiro Hirano,[a] Hisashi Matsuda,[a] Johji Yamahara,[b] Kazuhiro Ohtani,[c] Ryoji Kasai,[c] and Kazuo Yamasaki[c]

Kyoto Pharmaceutical University,[a] 5 Nakauchi-cho, Misasagi, Yamashina-ku, Kyoto 607-8414, Japan, Research Institute for Production Development,[b] 15 Shimogamo, Morimoto-cho, Sakyo-ku, Kyoto 606-0805, Japan, and Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine,[c] 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan.

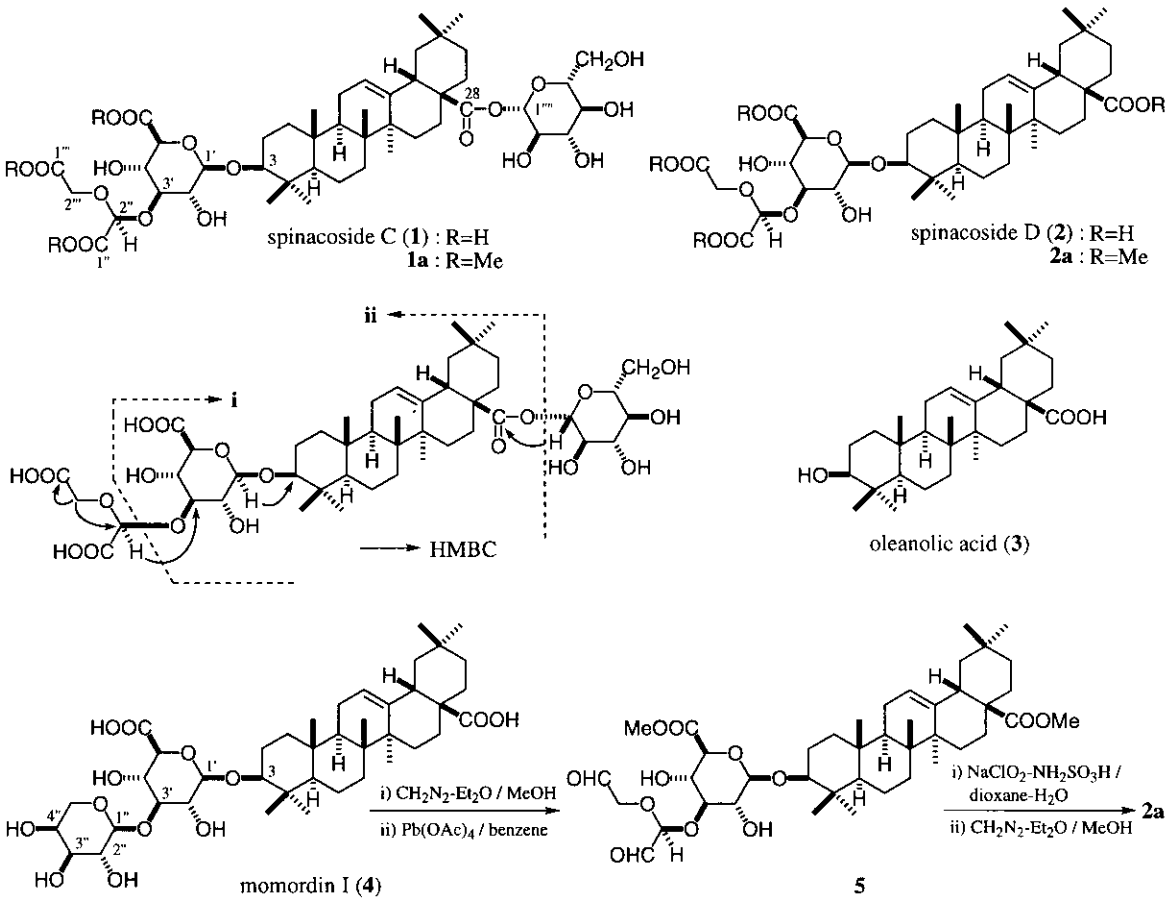
Abstract — Spinacosides C and D with a novel acetal-type substituent were isolated from the fresh aerial parts of *Spinacia oleracea* (Spinach) and *Basella rubra* (Indian spinach). Their absolute stereostructures were determined on the basis of physicochemical and chemical evidence, which included the conversion from the α-L-arabinopyranosyl moiety of a known saponin momordin I to the acetal-type substituent of spinacoside D.

Annual herbaceous plants *Spinacia oleracea* L. (Spinach, Japanese name "Hourenso," Chenopodiaceae) and *Basella rubra* L. (Indian spinach, Japanese name "Tsurumurasaki," Basellaceae) are extensively cultivated and their young aerial parts such as the leaves and stems are consumed as a vegetable and health food. As chemical constituents of these plants, saponins, sterols, and flavonoids have been characterized from *S. oleracea*,[1] whereas betacyanins, carotenoids, and organic acids were isolated from *B. rubra*.[2]

During the course of our characterization studies on bioactive constituents in medicinal foodstuffs,[3] we have recently isolated betavulgarosides with a unique substituent, which was presumed to be biosynthesized through an oxidative degradation process of terminal monosaccharide moiety in saponin, from the roots of *Beta vulgaris* L. (Sugar beet).[4] As a continuing study, we have found that the saponin fractions from the fresh aerial parts of *S. oleracea* and *B. rubra* showed inhibitory activity on sugar absorption. From both saponin fractions, we have isolated new saponins named spinacosides C (**1**) and D (**2**) having a novel acidic acetal-type substituent, which was also supposed to be generated by the oxidative degradation of monosaccharide. In this communication, we describe the isolation of spinacosides (**1**, **2**) from *S. oleracea* and *B. rubra* and the elucidation of their absolute stereostructures by means of chemical correlation with a known saponin momordin I (**4**).[5]

The methanolic extracts from the fresh aerial parts of *S. oleracea*[6] and *B. rubra* (cultivated in Kyoto Prefecture) were subjected to Diaion HP-20 ($H_2O \rightarrow MeOH \rightarrow CHCl_3$) and silica gel ($CHCl_3$-MeOH-$H_2O$) column chromatography and finally HPLC (YMC-Pack ODS-A, MeCN-1% aq. AcOH, MeCN-1% aq. trifluoroacetic acid) to give spinacosides C (**1**, 0.001% from the fresh aerial parts of *S. oleracea*, 0.017% from the fresh aerial parts of *B. rubra*) and D [**2**, 0.001% (*S. oleracea*), trace (*B. rubra*)].

† Dedicated to Dr. Bernhard Witkop in celebration of his 80th birthday.



Spinacoside C (**1**), colorless fine crystals of mp 220-222 °C (MeOH-H_2O), $[\alpha]_D^{24}$ +10.2° (MeOH), $C_{46}H_{70}O_{19}$,[7] showed absorption bands at 3426, 1735, 1728, and 1076 cm^{-1} due to hydroxyl, ester, and carboxyl functions in the IR spectrum. In the positive-ion FAB-MS of **1**, a quasimolecular ion peak was observed at *m/z* 949 $(M+Na)^+$, while the negative-ion FAB-MS of **1** exhibited a quasimolecular ion peak at *m/z* 925 $(M-H)^-$ in addition to fragment ion peaks at *m/z* 793 (**i**, M-$C_4H_5O_5)^-$ and *m/z* 767 (**ii**, M-$C_6H_{11}O_5)^-$. Acid hydrolysis of **1** with 5% aq. H_2SO_4-dioxane (1 : 1) liberated oleanolic acid (**3**) together with D-glucuronic acid and D-glucose, whose absolute configurations were characterized by GLC analysis of their TMS thiazolidine derivatives.[8] Methylation of **1** with diazomethane-etherate in MeOH gave the trimethyl ester (**1a**).[9]

The ^1H- and ^{13}C-NMR data (pyridine-d_5, Table 1) of **1** and **1a**, which were assigned by various NMR experiments,[10] showed the presence of an acetal-type substituent composed of glyoxylic acid (C-1'', 2'') and glycolic acid (C-1''', 2''') [**1** : δ 6.40 (s, 2''-H), 5.18, 5.37 (ABq, *J*=16.2 Hz, 2'''-H_2); **1a** : δ 6.24 (s, 2''-H), 4.90, 5.05 (ABq, *J*=16.2 Hz, 2'''-H_2)] together with the oleanolic acid 3-*O*-β-D-glucopyranosiduronic acid-28-*O*-β-D-glucopyranoside moiety [**1** : δ 3.35 (dd, *J*=3.9, 11.2 Hz, 3-H), 4.99 (d, *J*=7.6 Hz, 1'-H), 6.32 (d, *J*=7.6 Hz, 1''''-H); **1a** : δ 3.31 (dd, *J*=4.2, 11.5 Hz, 3-H), 3.56, 3.58, 3.70 (all s, 1''', 1'', 6'-OCH_3), 4.86 (d, *J*=7.9 Hz, 1'-H), 6.29 (d, *J*=7.9 Hz, 1''''-H)]. The plane structure of the acetal-type substituent bonded to the 3'-hydroxyl group of the 3-*O*-β-D-glucuronic acid moiety in **1** was clarified by the HMBC experiment. Namely, long-range correlations were observed between the following protons and carbons of **1** and **1a** (1'-H and 3-C, 2''-H and 3'-C, 2'''-H_2 and 1''', 2''-C, 1''''-H and 28-C).

Table 1. ^{13}C-NMR Data for **1**,[a] **1a**,[b] and **2**[a] (pyridine-d_5)

	1	**1a**	**2**		**1**	**1a**	**2**
C-1	38.6	38.7	38.6	C-26	17.5	17.5	17.4
C-2	26.6	26.6	26.6	C-27	26.1	26.1	26.2
C-3	89.3	89.5	89.3	C-28	176.4	176.4	180.1
C-4	39.5	39.5	39.6	C-29	33.1	33.2	33.2
C-5	55.7	55.8	55.8	C-30	23.6	23.7	23.8
C-6	18.5	18.5	18.5	3-GlcA-1'	106.9	106.8	106.8
C-7	33.1	33.2	33.2	2'	75.0	74.9	75.0
C-8	39.9	40.0	39.8	3'	85.7	84.3	85.6
C-9	48.0	48.1	48.0	4'	71.9	71.3	71.9
C-10	36.9	37.0	37.0	5'	77.6	76.8	77.6
C-11	23.4	23.5	23.8	6'	172.4	170.2	172.3
C-12	122.8	122.9	122.3	6'-OMe		52.1	
C-13	144.1	144.2	144.9	1''	171.7	168.1	171.9
C-14	42.1	42.2	42.2	1''-OMe		51.8	
C-15	28.3	28.3	28.4	2''	101.2	99.5	101.2
C-16	23.8	23.8	23.8	1'''	173.5	170.6	173.5
C-17	47.0	47.1	46.7	1'''-OMe		51.5	
C-18	41.7	41.7	42.1	2'''	64.7	63.2	65.0
C-19	46.2	46.3	46.6	28-Glc-1''''	95.8	95.8	
C-20	30.8	30.8	31.0	2''''	74.1	74.2	
C-21	34.0	34.1	34.3	3''''	78.9	79.0	
C-22	32.6	32.6	33.2	4''''	71.1	71.1	
C-23	28.1	28.2	28.2	5''''	79.3	79.3	
C-24	16.9	16.8	17.0	6''''	62.2	62.4	
C-25	15.5	15.5	15.5				

a : 67.5 MHz, b : 125.0 MHz

Spinacoside D (**2**), colorless fine crystals of mp 183-185 °C (MeOH-H_2O), $[\alpha]_D^{25}$ +11.4° (MeOH), $C_{40}H_{60}O_{14}$, IR (KBr) : 3453, 1736, 1638, 1070, cm^{-1}, showed a quasimolecular ion peak at *m/z* 787 $(M+Na)^+$ in the positive-ion FAB-MS. Diazomethane methylation of **2** provided the tetramethyl ester (**2a**).[11] The ^{1}H-NMR and ^{13}C-NMR (Table 1) spectra[10] of **2** and **2a** showed signals assignable to an acetal-type substituent [**2** (pyridine-d_5) : δ 6.34 (s, 2''-H), 5.08, 5.27 (ABq, *J*=16.5 Hz, 2'''-H_2); **2a** ($CDCl_3$) : δ 5.39 (s, 2''-H), 4.35, 4.52 (ABq, *J*=16.5 Hz, 2'''-H_2)] and the oleanolic acid 3-*O*-β-D-glucopyranosiduronic acid moiety [**2** : δ 3.34 (dd, *J*=4.0, 11.6 Hz, 3-H), 4.91 (d, *J*=7.9 Hz, 1'-H); **2a** : 3.16 (1H, dd-like, 3-H), 4.39 (1H, d, *J*=6.0, 1'-H),]. Finally, **2** was obtained by hydrolysis of **1** with 5% aq. NaOH. Consequently, the structures of **1** and **2** were characterized except for the stereostructure of the acetal-type substituent.

In order to determine the absolute stereostructure of the acetal-type substituent, we carried out the chemical correlation of spinacoside D (**2**) with momordin I (**4**), whose component monosaccharides were confirmed to be D-glucuronic acid and L-arabinose. Thus, momordin I (**4**) was subjected to diazomthane methylation and subsequent $Pb(OAc)_4$ degradation of the 2'', 3'', and 4''-triol moiety in the L-arabinose moiety to furnish an unstable dialdehyde (**5**). The dialdehyde (**5**) was immediately treated with $NaClO_2$ and NH_2SO_3H in 75% aq. dioxane followed by diazomethane methylation to give **2a**, which was identified with spinacoside D tetramethyl ester. On the basis of the above-mentioned evidence, the 2''*S* configuration of the acetal-type substituent was determined, so that the absolute stereostructures of spinacosides C (**1**) and D (**2**) were also characterized as shown.

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6. a) Spinacosides C and D were also isolated from the roots of *S. oleracea* as their methyl ester derivatives together with spinacosides A and B methyl esters;[6b] b) N. Tugimura, K. Ohtani, R. Kasai, and K. Yamasaki, Abstract Papers, 115th Annual Meeting of Pharmaceutical Society of Japan, Sendai, 1995, p. 254.
7. The molecular composition of the compound given with the chemical formula was determined by high-resolution FAB-MS.
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9. **1a** : colorless fine crystals, mp 158-160 °C (MeOH-H_2O), $[\alpha]_D^{25}$ +4.9° (MeOH), $C_{49}H_{76}O_{19}$,[6] IR (KBr) : 3442, 1748, 1730, 1075 cm^{-1}. ^{1}H-NMR (pyridine-d_5) δ : 0.83, 0.89, 0.90, 0.92, 1.08, 1.23, 1.27 (3H each, all s, 25, 24, 30, 29, 26, 23, 27-H_3), 3.19 (1H, dd, *J*=4.0, 13.5, 18-H), 3.31 (1H, dd, *J*=4.2, 11.5, 3-H), 3.56, 3.58, 3.70 (3H each, all s, 1'''', 1'', 6'-OCH_3), 4.86 (1H, d, *J*=7.9, 1'-H), 4.90, 5.05 (2H, ABq, *J*=16.2, 2''''-H_2), 5.41, (1H, br s, 12-H), 6.24 (1H, s, 2''-H), 6.29 (1H, d, *J*=7.9, 1''''-H). Positive-ion FAB-MS : *m/z* 991 $(M+Na)^+$.
10. The ^{1}H- and ^{13}C-NMR spectra of **1**, **1a**, **2**, and **2a** were assigned with the aid of homo- and hetero-correlation spectroscopy (^{1}H-^{1}H, ^{1}H-^{13}C COSY), distortionless enhancement by polarization transfer (DEPT), and heteronuclear multiple bond connectivity (HMBC) experiments.
11. **2a** : colorless fine crystals, mp 110-111 °C (MeOH-H_2O), $[\alpha]_D^{28}$ +15.8° ($CHCl_3$), $C_{44}H_{68}O_{14}$,[6] IR (KBr) : 3471, 1751, 1735, 1088, 1048 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ : 0.72, 0.82, 0.90, 0.91, 0.93, 1.01, 1.12 (3H each, all s, *tert.*-CH_3x7), 2.85 (1H, dd-like, 18-H), 3.16 (1H, dd-like, 3-H), 3.62, 3.76 (both 3H, s), 3.82 (6H, s) ($COOCH_3$x4), 4.35, 4.52 (2H, ABq, *J*=16.5, 2'''-H_2), 4.39 (1H, d, *J*=6.0, 1'-H), 5.28, (1H, dd-like, 12-H), 5.39 (1H, s, 2''-H). Positive-ion FAB-MS : *m/z* 843 $(M+Na)^+$.

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