

**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.

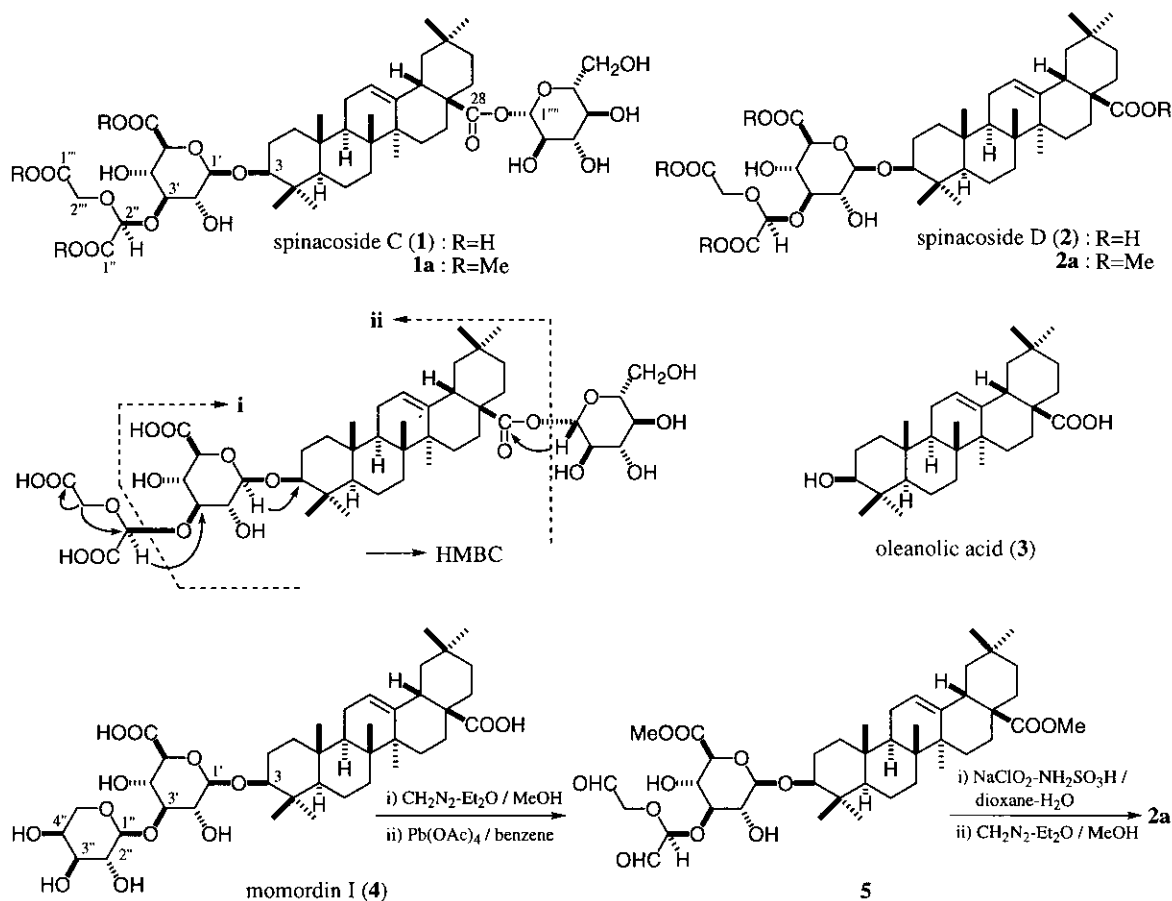
Abstract — Spinacosides C and D with a novel acetal-type substituent were isolated from the fresh aerial parts of *Spinacia oleracea* (Spinach) and *Basella rubra* (Indian spinach). Their absolute stereostructures were determined on the basis of physicochemical and chemical evidence, which included the 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 (H₂O→MeOH→CHCl₃) and silica gel (CHCl₃-MeOH-H₂O) 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–H₂O), $[\alpha]_D^{24} +10.2^\circ$ (MeOH), C₄₆H₇₀O₁₉,⁷ 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-C₄H₅O₅)⁻ and m/z 767 (ii, M-C₆H₁₁O₅)⁻. Acid hydrolysis of **1** with 5% aq. H₂SO₄-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 ¹³C-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'''-H₂); **1a** : δ 6.24 (s, 2''-H), 4.90, 5.05 (ABq, $J=16.2$ Hz, 2'''-H₂)] 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'-OCH₃), 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'''-H₂ and 1''', 2'''-C, 1'''-H and 28-C).

Table 1. ^{13}C -NMR Data for **1**,^a **1a**,^b and **2a** (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-H₂O), $[\alpha]_{\text{D}}^{25} +11.4^\circ$ (MeOH), C₄₀H₆₀O₁₄, 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 ¹H-NMR and ¹³C-NMR (Table 1) spectra¹⁰ 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'''-H₂); **2a** (CDCl₃) : δ 5.39 (s, 2''-H), 4.35, 4.52 (ABq, $J=16.5$ Hz, 2'''-H₂)] 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 diazomethane 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 NaClO₂ and NH₂SO₃H 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.

REFERENCES AND NOTES

1. a) A. Zane and S. H. Wender, *J. Org. Chem.*, 1961, **26**, 4718; b) R. Tschesche, H. Rehkämper, and G. Wulfe, *Liebigs Ann. Chem.*, 1969, **726**, 125; c) P. Da Re, P. Valenti, L. Cateni, and P. L. Barili, *Tetrahedron Lett.*, 1976, 71; d) H. Wagner, I. Maurer, L. Farkas, and J. Strelisky, *Tetrahedron*, 1977, **33**, 1405; e) M. Bathory, I. Toth, K. Szendrei, and J. Reisch, *Phytochemistry*, 1982, **21**, 236; f) M. Aritomi and T. Kawasaki, *ibid.*, 1984, **23**, 2043; g) M. Aritomi, T. Komori, and T. Kawasaki, *ibid.*, 1986, **25**, 231.
2. a) A. Takami, *Oita Daigaku Kyoikugakubu Kenkyo Kiyo, Shizenkagaku*, 1969, **3**, 53 [*Chem. Abstr.*, 1971, **74**, 108135q]; b) A. K. Banerjee, M. Jain, and V. Dubey, *Fitoterapia*, 1992, **63**, 377 [*Chem. Abstr.*, 1993, **118**, 109476r]; c) W. E. Glassgen, J. W., Metzger, S. Heuer, and D. Strack, *Phytochemistry*, 1993, **33**, 1525.
3. a) M. Yoshikawa, T. Murakami, H. Komatsu, N. Murakami, J. Yamahara, and H. Matsuda, *Chem. Pharm. Bull.*, 1997, **45**, 81; b) M. Yoshikawa, H. Shimada, M. Saka, S. Yoshizumi, J. Yamahara, and H. Matsuda, *ibid.*, 1997, **45**, 464; c) M. Yoshikawa, H. Shimada, H. Komatsu, T. Sakurama, N. Nishida, J. Yamahara, H. Shimoda, H. Matsuda, and T. Tani, *ibid.*, 1997, **45**, 877; d) M. Yoshikawa, H. Shimada, T. Morikawa, S. Yoshizumi, N. Matsumura, T. Murakami, H. Matsuda, K. Hori, and J. Yamahara, *ibid.*, 1997, **45**, 1300; e) M. Yoshikawa, T. Murakami, and H. Matsuda, *ibid.*, 1997, **45**, 2034; f) M. Yoshikawa, T. Murakami, H. Komatsu, J. Yamahara, and H. Matsuda, *Heterocycles*, 1998, **47**, 397; g) S. Yoshizumi, T. Murakami, M. Kadoya, H. Matsuda, J. Yamahara, and M. Yoshikawa, *Yakugaku Zasshi*, 1998, **118**, 188; h) M. Yoshikawa, T. Murakami, H. Komatsu, and H. Matsuda, *Chem. Pharm. Bull.*, 1998, **46**, 812; i) H. Komatsu, T. Murakami, H. Matsuda, and M. Yoshikawa, *Heterocycles*, 1998, **48**, 703.
4. a) M. Yoshikawa, T. Murakami, M. Kadoya, H. Matsuda, J. Yamahara, O. Muraoka, and N. Murakami, *Heterocycles*, 1995, **41**, 1621; b) M. Yoshikawa, T. Murakami, M. Kadoya, H. Matsuda, O. Muraoka, J. Yamahara, and N. Murakami, *Chem. Pharm. Bull.*, 1996, **44**, 1212; c) M. Yoshikawa, T. Murakami, M. Inaduki, K. Hirano, J. Yamahara, and H. Matsuda, *ibid.*, 1997, **45**, 561.
5. M. Iwamoto, H. Okabe, and T. Yamauchi, *Chem. Pharm. Bull.*, 1985, **33**, 1.
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.
8. S. Hara, H. Okabe, and K. Mihashi, *Chem. Pharm. Bull.*, 1986, **34**, 1843.
9. **1a**: colorless fine crystals, mp 158-160 °C (MeOH-H₂O), [α]_D²⁵ +4.9° (MeOH), C₄₉H₇₆O₁₉,⁶ IR (KBr): 3442, 1748, 1730, 1075 cm⁻¹. ¹H-NMR (pyridine-d₅) 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-H₃), 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'-OCH₃), 4.86 (1H, d, *J*=7.9, 1'-H), 4.90, 5.05 (2H, ABq, *J*=16.2, 2'''-H₂), 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 ¹H- and ¹³C-NMR spectra of **1**, **1a**, **2**, and **2a** were assigned with the aid of homo- and hetero-correlation spectroscopy (¹H-¹H, ¹H-¹³C COSY), distortionless enhancement by polarization transfer (DEPT), and heteronuclear multiple bond connectivity (HMBC) experiments.
11. **2a**: colorless fine crystals, mp 110-111 °C (MeOH-H₂O), [α]_D²⁸ +15.8° (CHCl₃), C₄₄H₆₈O₁₄,⁶ IR (KBr): 3471, 1751, 1735, 1088, 1048 cm⁻¹. ¹H-NMR (CDCl₃) δ : 0.72, 0.82, 0.90, 0.91, 0.93, 1.01, 1.12 (3H each, all s, *tert*-CH₃×7), 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) (COOCH₃×4), 4.35, 4.52 (2H, ABq, *J*=16.5, 2'''-H₂), 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)⁺.

Received, 1st June, 1998