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THREE β-COUMARANONES FROM *SPARAXIS TRICOLOR*

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Key Word Index—*Sparaxis tricolor*; Iridaceae; bulbs; β-coumaranones; aromatic compounds; sparanone A; sparanone B; sparanoside B.

Abstract—Three new β-coumaranones, sparanone A, sparanone B and sparanoside B, were isolated from bulbs of *Sparaxis tricolor*. Their stereostructures were established on the basis of chemical evidence and spectroscopic studies. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Phenolic compounds are distributed widely from microorganisms to higher plants and animals. Some antibiotics, such as tetracyclines and anthracyclinones, the important role in the oxidation-reduction systems of all kind of living things, such as quinones, and allelopathic effects, such as phenolcarboxylic acids, isoflavones and quinones, are well-known examples of the medicinal uses of phenolic compounds. *Sparaxis tricolor* (Fire King in Japan) is a plant which was introduced into Japan from South Africa and Williams *et al* [1]. have reported the presence of plumbagin (5-hydroxynaphthoquinone, **1**), as a constituent. As a continuation of our study to find phenolic compounds with biological activities from the plants of the Iridaceae, bulbs of *S. tricolor* were examined. The present paper deals with the isolation and structural elucidation of three new β-coumaranones, named sparanone A (**2**), sparanone B (**6**) and sparanoside B (**7**).

RESULTS AND DISCUSSION

From a methanol extract of the bulbs of *S. tricolor*, three new β-coumaranones named sparanone A (**2**), sparanone B (**6**) and sparanoside B (**7**) were isolated by the procedures described in the Experimental section. Sparanone A and sparanone B are genuine compounds, since these compounds (**2** and **6**) were also isolated from the chloroform extract of *S. tricolor*, as described in the Experimental section.

Sparanone A (**2**) was isolated as white yellow needles. Its molecular formula $C_{11}H_{12}O_5$ was determined by HREI mass spectrometry and elemental analysis, indicating the equivalent of six double bonds. Sparanone A gave a grey-green colour with ethanolic ferric chloride, suggesting the presence of a phenolic hydroxyl group in the molecule. The IR spectrum of **2** showed absorption bands due to phenolic hydroxyl, carbonyl and aromatic ring at 3400, 1700, 1690, 1650, 1603 and 1505 cm^{-1}. The ^1H NMR ($CDCl_3$) spectrum exhibited singlet signals at δ 7.65 (1H, HO-6, exchangeable with D_2O), 6.11 (1H, H-7), 5.32 (1H, H-2), 3.90 (3H, MeO-6), 3.61 (3H, MeO-2) and 1.98 (3H, Me-5). The ^{13}C NMR ($CDCl_3$) spectrum of **2**, which was assigned based on Distortionless Enhancement by Polarization Transfer (DEPT) and Heteronuclear Single Quantum Coherence (HSQC), exhibited the carbon signals of a *tert*-methyl at δ 6.8 (Me-5), two methines at δ 87.2 (C-2) and 103.6 (C-7), two methoxyls at δ 56.3 (MeO-2) and 56.8 (MeO-6), a carbonyl at δ 194.0 (C-3), and five qurternaly carbons at δ 102.0 (C-3a), 106.4 (C-5), 154.5 (C-4), 169.4 (C-6) and 170.3 (C-1a). These results indicated the presence of penta-substituted benzene ring in **2**.

Acetylation of **2** with acetic anhydride and pyridine gave the monoacetate (**2a**) $C_{13}H_{14}O_6$ and no hydroxyl group was evident in its IR spectrum. In the ^1H NMR spectrum (Table 1) of **2a**, an aromatic proton was

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Table 1. ^{1}H NMR spectral data of compounds **2**, **2a**, **2b**, **6**, **6a** and **6b** (δ, 400 MHz)

Proton No.	**2**	**2a**	**2b**	**6**	**6a**	**6b**
H-2	5.32	5.22	5.22	5.33	5.20	5.24
H-5				6.04	6.26	6.02
H-7	6.11	6.38	6.28			
MeO-2	3.61	3.52	3.60	3.63	3.54	3.58
MeO-4			4.05			3.95
MeCO-4		2.38			2.34	
Me-5	1.98	1.94	2.02			
MeO-6	3.90	3.90	3.90	3.88	3.88	3.87
Me-7				2.00	2.08	2.02

Spectra taken in $CDCl_3$ and showed singlet signals.

observed at lower field (δ 6.38) than the signal (δ 6.11) in **2**. This result indicated that the acetoxyl group is located at the *ortho* or *para* position to the aromatic proton. In the ^{13}C NMR spectrum (Table 2), the carbon signal at C-4 was observed at higher field (δ 114.7) than the signal (δ 154.5) in **2**, an esterification shift [2] of the acetyl group, suggesting the presence of a phenolic hydroxyl group at C-4. In the nuclear Overhauser effect Difference spectrum (NOEDS) of **2** and **2a**, when the signals at δ 6.11 (H-7) and 5.32 (H-2) of **2** and the signals at δ 6.38 (H-7) and 5.22 (H-2) of **2a** were irradiated, NOEDS were observed at δ 3.90 (MeO-6) and 3.61 (MeO-2) in **2** and at δ 3.90 (MeO-6) and 3.52 (MeO-2) in **2a**, respectively. As shown by the lines in [A] in Scheme 1, these results indicated that the methoxyl groups at δ 3.90 and 3.61 are located at C-6 and C-2 in **2**, respectively.

Methylation of **2** with CH_2N_2 gave a trimethoxy derivative (**2b**) $C_{12}H_{14}O_5$, with no OH absorption in the IR spectrum. In the ^{1}H NMR spectral data of **2a** and **2b**, the protons of the acetoxyl group (δ 2.38) and a methoxyl group (δ 4.05) were observed at lower field than usual (Table 1). These results suggested that the remaining carbonyl group is located at the *ortho* (C-3a) position to the hydroxyl group at C-4 in **2**. As shown by the arrows in [A] of Scheme 1, the linkage positions of the oxygen bonded by the methoxymethine group (O-CH-OMe) at C-7a and the methyl group at C-5 in **2** were characterized by means of a heteronuclear multiple bond correlation (HMBC)experiments. Namely, long-range correlations were observed between the following protons and carbons [δ_H7.65(HO-4) and δ_c 102.0(C-3a), 106.4(C-5), and 154.5(C-4); δ_H 6.11(H-7) and δ_c 102.0(C-3a), 106.4(C-5), 169.4(C-6) and 170.3(C-7a); δ_H 5.32(H-2) and δ_C 56.8(MeO-2), 170.3(C-1a); δ_H 3.90(MeO-6) and δ_C 169.4(C-6); δ_H 3.61(MeO-2) and δ_c 103.6(C-2); δ_H 1.98 (Me-5) and δ_c 106.4(C-5), 154.5(C-4) and 169.4(C-6)]. Consequently, the gross planar structure of sparanone A was examined as [A] (Scheme 1).

Table 2. ^{13}C NMR spectral data of compounds **2** and **6** (δ, 100 MHz)

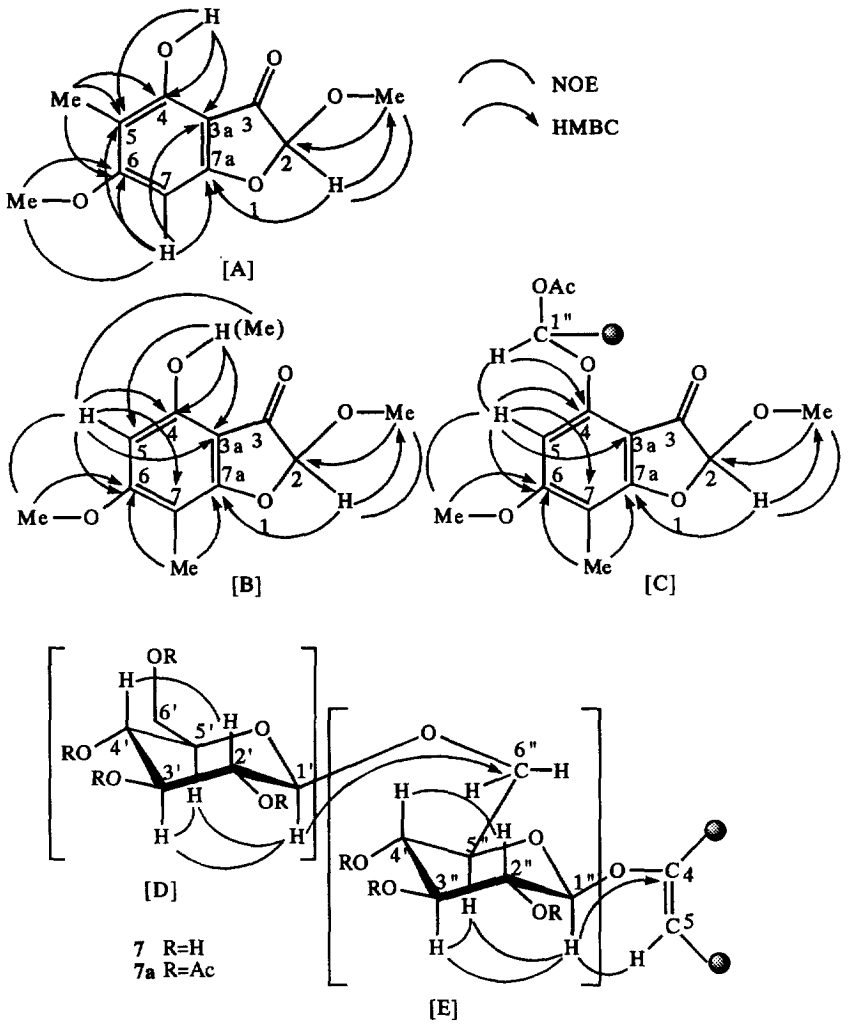
Carbon No.	**2**	**6**
C-1a	170.3 *s*	169.9 *s*
C-2	103.6 *d*	103.5 *d*
C-3	194.0 *s*	194.4 *d*
C-3a	102.0 *s*	101.8 *s*
C-4	154.5 *s*	156.4 *s*
C-5	106.4 *s*	92.1 *d*
C-6	169.4 *s*	168.0 *d*
C-7	87.2 *d*	101.9 *s*
MeO-2	56.8 *q*	56.9 *q*
Me-5	6.8 *q*	
MeO-6	56.3 *q*	56.4 *q*
Me-7		6.9 *q*

Spectra taken in $CDCl_3$.

The conformations of five-membered ring in indan-1-ones (**3c, 4c** and **5c**) constitute a homologous family of compounds very similar to that of the five-membered ring in the coumaran-3-one moiety, such as sparanone A(**2**) (Table 3). The absolute configurations at C-2 in **3c, 4c** and **5c**, were established on the basis of CD data which showed negative, positive and negative Cotton effects {**3c**: λext 320 nm ($\Delta\epsilon$ −4.79) [3], **4c**: λext 324 nm ($\Delta\epsilon$ +1.30) [4], **5c**: λext 320 nm ($\Delta\epsilon$ −0.97) [5]} for n−π^* trandision (R band), respectively. If the R band follows the same rule as for coumaran-3-ones substituted by a methoxyl group at C-2, the absolute configuration at C-2 was concluded to be *S* on the basis that the CD curve of sparanone A (**2**) showed a negative Cotton effect for the n−π^* transition at λext 311.6 nm ($\Delta\epsilon$ −4.53). Consequently, the stereostructure of sparanone A was established as 4-hydroxy-2(*S*),6-dimethoxy-5-methylcoumaran-3-one (**2**).

Sparanone B (**6**) was isolated as white yellow needles. The molecular formula $C_{11}H_{12}O_5$ was determined by HREI mass spectrometry and elemental analysis, indicating the equivalent of six double bonds. Sparanone B also gave a grey-green colour with ethanolic ferric chloride, suggesting the presence of a phenolic hydroxyl group in the molecule, and its IR spectrum showed absorption bands due to phenolic hydroxyl, carbonyl and aromatic ring groups at 3400, 1700, 1650, 1600 and 1510 cm^{-1}. The ^{1}H and ^{13}C NMR spectra of **6** (Tables 1 and 2) showed the presence of a methine (H-2), two methoxyls (MeO-2 and MeO-6), a ketone carbonyl (C-3) and five quarternary carbons. The relationship between protons and carbons was assigned by HSQC.

Treatment of **6** with acetic anhydride-pyridine and CH_2N_2 gave a monoacetate (**6a**) $C_{13}H_{14}O_6$ and a trimethoxy derivative (**6b**) $C_{12}H_{14}O_5$, respectively. The IR spectra of **6a** and **6b** showed no hydroxyl groups. The ^{1}H NMR spectral data (Table 1) of **6a** and **6b** showed the presence of a carbonyl group at the *ortho* (C-3a) position to the hydroxyl group at C-4 in **6**, using the same reasoning described in the case of **2a** and **2b**. In the NOE experiments of **6b**, NOEs were observed between H-2 and MeO-2, between H-5 and



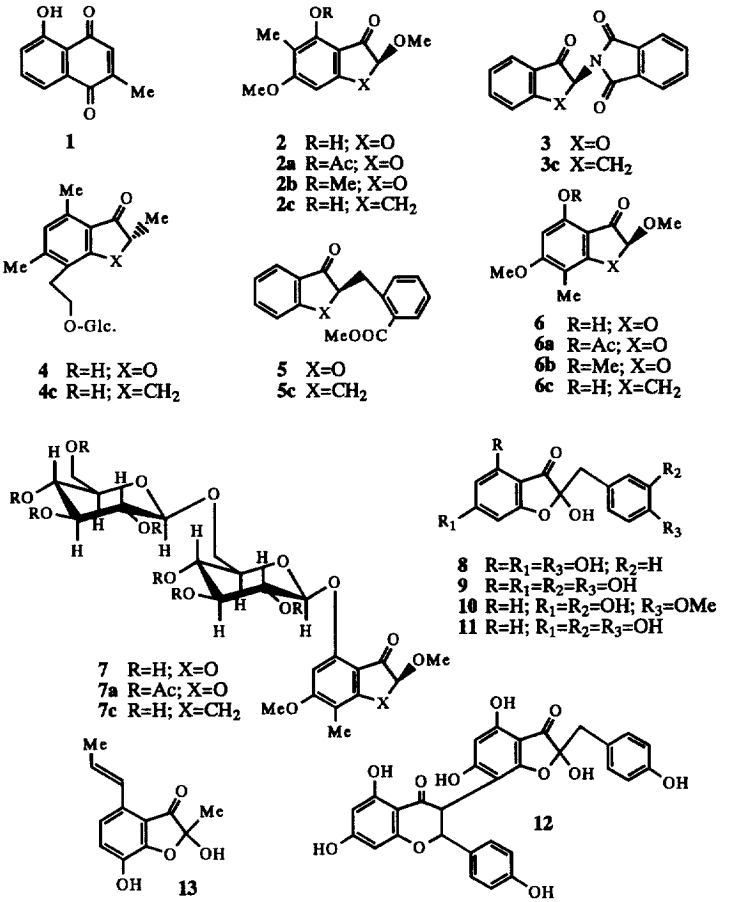
Scheme 1. NOE and HMBC cross-peaks.

MeO-6, and between H-5 and MeO-4, as described by the curved lines in[B] of Scheme 1. The gross planar structure of sparanone B (**6**) was confirmed as 4-hydroxy-2,6-dimethoxy-7-methylcoumaran-3-one from HMBC as shown by the arrows in [B] of Scheme 1.

The absolute configuration at C-2 of **6** was concluded to be *S* on the basis of the CD spectrum which showed a negative cotton effect at λ ext 317 nm ($\Delta\epsilon - 0.60$) for $n-\pi^*$ transition. Consequently, the stereostructure of sparanone B was established as 4-hydroxy-2(*S*),6-dimethoxy-7-methylcoumaran-3-one (**6**).

Sparanoside B (**7**) was obtained as pale yellow needles and showed absorption bands due to hydroxyl (3400 cm^{-1}), α,β-unsaturated carbonyl (1700 cm^{-1}) and an aromatic ring (1630, 1602, 1520 and 800 cm^{-1}) in its IR spectrum. In the FAB-mass spectrum a $[M+Na]^+$ was observed at *m/z* 571 and elemental analysis data was consistent with $C_{23}H_{32}O_{15}$. Methanolysis of **7** with 5% hydrogen chloride in anhydrous methanol gave methylglucopyranose. The ^1H and ^{13}C NMR spectral data of **7** (Tables 4 and 5) showed the presence of two glucopyranosyl groups, together with a coumaran-3-one compound with a secondary and five tertiary carbons of an aromatic group, a tertiary methyl group, a tertiary methoxyl group, a secondary methoxyl group and a secondary carbon as the aglycone part.

Acetylation of **7** with acetic anhydride and pyridine yielded a heptaacetate (**7a**) $C_{37}H_{46}O_{22}$ and no hydroxyl absorption was evident in its IR spectrum. As shown by the arrows and curved lines in [C] of Scheme 1, long-range correlations were observed between the following carbons and protons in **7a** (Me-7: C-7, C-6, C-7a; H-5: C-3a, C-4, C-6, C-7; MeO-6: C-6; H-2: MeO-2, C-7 a; H-1″: C-4), and NOE correlations were observed in the pairs of protons in **7a** (H-2 and MeO-2; H-5 and H-1″; MeO-6 and H-5). Consequently, the structure of the aglycone part of sparanoside B was deduced as sparanone B (**6**), with the glucose joined to the hydroxyl group at C-4. The absolute configuration at C-2 in **7** was also concluded to be *S* on



the basis of the CD data which showed a negative Cotton effect at λext 316.2 nm ($\Delta\epsilon -2.68$) for the $n-\pi^*$ transition. Methanolysis of **7** with 5% HCl-MeOH gave methylglucopyranose and enzymatic hydrolysis with β-glucosidase gave sparanone B (**6**), which was identified by comparison with the authentic material. The mass spectrum of **7a** showed the characteristic fragment peaks due to sparanone B (*m/z* 224, 209, 194, 181, 164, and 136) and sugar moieties (*m/z* 619, 331, 271, 198, 139, 169 and 105).

The bonding position of these moieties in **7** were deduced by Nuclear Overhauser and Exchange Spectroscopy (NOESY) and HMBC experiment of **7a** after assignments (Tables 4 and 5) of proton and carbon signals due to a sugar moiety by $^1H-^1H$ COSY, $^{13}C-^1H$ COSY, Relayed COSY, Homonuclear Hartmann–Hahn (HOHAHA) and decoupling NMR spectral data. As shown by the arrows and curved lines in [D and E] of Scheme 1, NOE correlations were observed between the following protons: H-1″ and H-3″; H-1″ and H-5″; H-1″ and H-5; H-2″ and H-4″; H-1′ and H-3′; H-1′ and H-5′; H-1′ and H-6″; H-2′ and H-4′, and long-range correlations were observed between the following protons and carbons: δ_H 4.50(H-1′) and δ_c 68.8(C-6″); δ_H 5.38(H-1″) and δ_c 154.1(C-4). These results indicated that the terminal glucose on [D] was located at C-6″ of the inner glucose moiety of [E], which was located at C-4 in the structure of [C] (Scheme 1). The stereochemistries of the glycoside linkages at C-1′ and C-1″ of the glucose moieties in **7** were deduced as β- and β-, respectively, from the large coupling constants [(H-1′:*d*, $J=7.8$ Hz) and (H-2″:*d*, $J=7.8$ Hz)] of the anomeric hydrogen signals in the ^1H NMR spectral data of **7** and **7a**. Consequently, the stereostructure of sparanoside **B** was established as sparanone B 4-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (**7**).

Compounds **2, 6** and **7** are the first examples of β-coumaranones without alkyl groups at C-2 from the Iridaceae, although the relatively rare coumaran-3-one compounds **8–12** [6–11] have been isolated from a small number of species from the Rhamnaceae and Anacardiaceae, together with cycloarthropsone (**13**), a fungal metabolite [12].

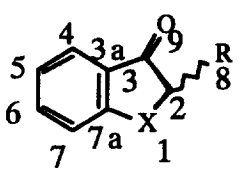
EXPERIMENTAL

Mps are uncorr. NMR spectra were measured on JEOL EX400 or a Bruker ARX 400 spectrometers and chemical shifts are given in δ values with TMS as int. standard. HPLC was performed using TSK gel ODS-120 T(21.5 mm i.d. × 300 mm) and Asahipak

Table 3. Dihedral angles (°) in five-membered ring for the most stable conformers of coumaran-3-ones (**2**–**7**) and indanones (**2c**, **3c**, **4c**, **5c**, **6c** and **7c**)

Compound	Dihedral angle C_2-C_3-C_{3a}-C_{7a}	C_2-X_1-C_{7a}-C_{3a}	O_9-C_3-C_{3a}-C_{7a}
Coumaran-3-one (X=O)			
2	6.4	−5.3	−174.0
3	7.3	−6.0	−171.3
4	−2.9	3.6	177.5
5	2.8	−2.7	−174.5
6	9.2	−7.4	−170.8
7	5.6	−2.4	−173.9
Indanone (X=CH_2)			
2c	6.8	−5.4	−179.0
3c	11.2	−13.9	−170.4
4c	−6.8	7.6	174.9
5c	5.6	−11.5	−173.7
6c	7.0	−10.5	−175.3
7c	1.9	−1.5	−174.8

Dihedral angles were deduced after optimizing the molecular geometry first using Augmented MM2 then using MOPAC with PM3 parameters of CAChe (CAChe Scientific Inc., Bearverton, Oregon, U.S.A).



coumaran-3-one (X=O)
indanone (X=CH_2)

ODP-90 (21.5 mm i.d. × 300 mm) columns. TLC, HPTLC and prep. TLC were conducted on Kieselgel 60 F254 (Merck), Kieselgel 60 HF254 (Merck) and 60 PF254 (Merck) plates, respectively; spots were located by UV or by spraying with 1% $Ce(SO_4)_2$ – 10% H_2SO_4 soln followed by heating.

Plant material

Bulbs of *S. tricolor* were collected at the Botanic Garden of Naruto University of Education, in May 1991. A voucher specimen is deposited at the Botanic Garden of the Faculty of Sciences, Naruto University of Education. Bulbs were purchased from Sakata Seed Corporation, Yokohama 224, Japan.

Extraction and isolation

Bulbs (725 g) were cut finely and extracted with MeOH in a Sohxlet apparatus for 10 hr. Evap of solvent under red. pres. gave the MeOH extract (68.8 g). A portion (35 g) was chromatographed over a silica gel (2 kg) column in $CHCl_3$-MeOH-H_2O (7:3:0.3), yielding 5 frs: fr. I (frs 1–5, 2.06 g), fr. II (fr. 6, 3.50 g), fr. III (frs 7–8), fr. IV (frs 9–18, 5.94 g) and fr. V (frs 19–26, 2.74 g) (100 ml frs 1). Fr. II (3.50 g) was purified by silica gel CC (benzene-Me_2CO, 50:1) and HPLC (Asahipac ODP-90, MeCN-H_2O, 1:1, 8 ml min^{-1}, 32 kg cm^{-2}, Rt 28 min) separation to give sparanone A (**2**, 26.2 mg). Fr. III (2.85 g) was purified by silica gel CC (benzene-Me_2CO, 10:1 ~ 5:1) and HPLC (Asahipac ODP-90, MeCN-H_2O), 1:1, 8 ml min^{-1}, 32 kg cm^{-2}, Rt 20 min] separation to give sparanone B (**6**, 18.5 mg). HPLC (TSKgel ODS-120T, MeOH – 1% HOAc of H_2O, 3:7, 3 ml min^{-1}, 15 kg cm^{-2}) separation of Fr. V (2.74 g) afforded four peaks (peak 1, R_t:110 min; peak 2, R_t:131 min; peak 3, R_t:174 min; peak 4, R_t:182 min). Peaks 3 and 4 was further purified by HPLC (TSKgel ODS-120T, MeOH – 1% HOAC

Table 4. ^{1}H NMR spectral data of compounds **7** and **7a** (δ, 400 MHz)

Proton (No)	**7**[a]	**7a**[b]	Proton (No)	**7**[a]	**7a**[b]
Aglycone moiety			Aglycone moiety		
H-2	5.61 *s*	5.21 *s*	MeCO-4		2.34 *s*
H-5	6.78 *s*	6.33 *s*	MeO-6	3.92 *s*	3.92 *s*
MeO-2	3.55 s	3.62 *s*	Me-7	2.07 *s*	2.06 *s*
Sugar moiety at C-4					
Terminal Glc			Inner Glc		
H-1′	5.00 *d* (7.8)	4.50 *d* (7.8)	H-1″	5.86 *d* (7.8)	5.38 *d* (7.8)
H-2′	4.00 *t-like* (8.3)	4.89 *dd* (9.8,7.8)	H-2″	4.31*	5.28*
H-3′	4.18 *t-like* (8.8)	5.14 *t* (9.8)	H-3″	4.35*	5.29*
H-4′	4.25 *t-like* (9.0)	5.02 *t* (9.8)	H-4″	4.16*	4.99 *t* (9.8)
H-5′	3.86 *ddd* (9.2,5.0,2.4)	3.64 *ddd* (9.8,4.9,2.4)	H-5″	4.29*	3.91 *ddd* (9.8,6.3,2.4)
H-6′	4.48 *dd* (11.8,2.4)	4.10 *dd* (12.2,2.4)	H-6″	4.48 *d-like* (9.7)	3.71 *dd* (11.2,6.3)
H-6′	4.36*	4.24 *dd* (12.2,4.9)	H-6″	4.32*	3.86 *dd* (11.2,2.4)

a) Spectrum taken in Pyridine-d_5.
b) Spectrum taken in $CDCl_3$.
* Overlapping with other signals.
Figures in parentheses are coupling constants (J) in Hz.

Table 5. ^{13}C NMR spectral data of compounds **7** and **7a** (δ, 100 MHz)

Carbon No.	**7**[a]	**7a**[b]	Carbon No.	**7**[a]	**7a**[b]
Aglycone moiety					
C-2	103.5 *s*	102.8 *s*	C-6	167.7 *s*	167.3 *s*
C-3	191.3 *s*	190.9 *s*	C-7	104.0 *s*	105.2 *s*
C-3a	103.1 *s*	103.7 *d*	C-7a	170.2 *s*	170.1 *s*
C-4	156.2 *s*	154.1 *s*	MeO-2	56.7 *q*	56.9 *q*
C-5	104.0 *s*	105.2 *s*	MeO-6	55.9 *q*	56.4 *q*
			Me-7	7.3 *q*	7.1 *q*
Sugar moiety at C-4					
Terminal Glc			Inner Glc		
C-1′	105.2 *d*	101.1 *d*	C-1″	101.9 *d*	99.6 *d*
C-2′	75.1 *d*	70.8 *d*	C-2″	74.2 *d*	70.6 *d*
C-3′	78.4 *d*	72.7 *d*	C-3″	77.8 *d*	72.5 *d*
C-4	71.5 *d*	68.2 *d*	C-4″	71.0 *d*	68.9 *d*
C-5′	78.4 *d*	72.1 *d*	C-5″	78.4 *d*	73.4 *d*
C-6′	62.6 *t*	61.8 *t*	C-6″	70.3 *t*	68.8 *t*
Others	20.4–20.7 (q, MeCO × 7)		169.3–170.5 (s, MeCO × 7)		

a) Spectrum taken in pyridine-d_5.
b) Spectrum taken in $CDCl_3$.

in H_2O, 3:7, 7 ml min^{-1}, 57 kg cm^{-2}, 8 recycles, Rt 87 min) separation to give sparanoside B (**7**, 37.8 mg).

Bulbs (530 g) were cut finely and extracted with $CHCl_3$ (1 l × 2) at 60° for 8 hr to give the $CHCl_3$ extract (2.9 g). Sparanone A (**2**, 10 mg) and sparanone B (**6**, 8.5 mg) were also isolated from the $CHCl_3$ extract (2.9 g) by the same procedures as described above.

Sparanone A (**2**)

Pale yellow needles from MeOH, mp 148–150°, $[\alpha]_D$-179.7° ($CHCl_3$; c 0.36). IR ν_{max}^{KBr} cm^{-1}:3400, 1700, 1690, 1650, 1603, 1505. CD(MeOH) λ_{ext} nm:345.8($\Delta\epsilon$ +2.36), 311.6($\Delta\epsilon$ −4.53), 226.2($\Delta\epsilon$ −2.85). UV λ_{max}^{MeOH} nm: 290.8 (log ϵ 4.20), 209.8(log ϵ 4.28). ^1H and ^{13}C NMR($CDCl_3$): Tables 1 and 2. EIMS *m/z* (rel. int): 224 $[M]^+$ (37), 209 $[M\text{-}Me]^+$ (12), 194 $[M\text{-}OCH_2]^+$ (47), 181 $[M\text{-}CHCH_2O]^+$ (100), 164 $[M\text{-}OCH_2\text{-}OCH_2]^+$(77), 136 $[M\text{-}OCH_2 \times 2\text{-}CO]^+$(77); HREIMS *m/z*: 224.0691 $[M]^+$, Calcd for $C_{11}H_{12}O_5$:224.0685.

Acetylation of Sparanone A (**2**)

Sparanone A (**2**, 20 mg) was acetylated with Ac_2O (0.5 ml) and pyridine (0.5 ml) to give monoacetyl sparanone A (**2a**, 19 mg) as colourless needles from $CHCl_3$-*n*-hexane, mp 116–119°. ^1H and ^{13}C NMR($CDCl_3$): Tables 1 and 2. EIMS *m/z* (rel. int):266 $[M]^+$ (10), 251 $[M\text{-}Me]^+$ (17), 236 $[M\text{-}OCH_2]^+$ (54), 224 $[M\text{-}CH_2\ CO]^+$ (100), 223 $[M\text{-}CH_2CHO]^+$ (42), 206 $[M\text{-}OCH_2 \times 2]^+$ (76), 178 $[M\text{-}OCH_2 \times 2\text{-}CO]^+$ (30); HREIMS *m/z*:266.0785 $[M]^+$, Calcd for $C_{13}H_{14}O_6$:266.0791.

Methylation of Sparanone A (**2**)

Sparanone A (**2**, 20 mg) was methylated with CH_2N_2 in MeOH (0.5 ml) to give monomethyl sparanone A (**2b**, 15 mg) as colorless needles from $CHCl_3$-*n*-hexane, mp 122–124°. ^1H and ^{13}C NMR($CDCl_3$): Tables 1 and 2. EIMS *m/z* (rel. int): 238 $[M]^+$ (100), 223 $[M\text{- }Me]^+$ (21), 208 $[M\text{-}OCH_2]^+$ (30), 195 $[M\text{-}CH_2CHO]^+$ (61), 178 $[M\text{-}OCH_2X2]^+$ (14), 150 $[M\text{-}OCH_2X2\text{-}CO]^+$ (49); HREIMS *m/z*:238.0809 $[M]^+$, Calcd for $C_{12}H_{14}O_5$:238.0842.

Sparanone B (**6**)

White yellow needles from EtOH-Et_2O, mp 157–158°, $[\alpha]_D$-50.0° ($CHCl_3$; c 0.35). IR ν_{max}^{KBr} cm^{-1}: 3400, 1700, 1650, 1600, 1510. CD λ_{max}^{MeOH} nm: 348.2 ($\Delta\epsilon$ +0.35), 317.4 ($\Delta\epsilon$ −0.60), 232.8 ($\Delta\epsilon$ −2.16). UV λ_{max}^{MeOH} nm: 294.6 (logϵ 4.26), 235.2sh (log ϵ 4.05), 209.2 (log ϵ 5.40). ^1H and ^{13}CNMR ($CDCl_3$): Tables 1 and 2. EIMS *m/z* (rel. int): 224 $[M]^+$ (39), 209 $[M\text{- }Me]^+$ (10), 194 $[M\text{-}OCH_2]^+$ (59), 181 $[M\text{-}CHCH_2O]^+$ (62), 164 $[M\text{-}OCH_2\text{-}OCH_2]^+$(53), 136 $[M\text{-}OCH_2 \times 2\text{-}CO]^+$ (100); HREIMS *m/z*: 224.0693 $[M]^+$, Calcd for $C_{11}H_{12}O_5$:224.0685.

Acetylation of Sparanone B (**6**)

Sparanone B (**6**, 15 mg) was acetylated with Ac_2O (0.5 ml) and pyridine (0.5 ml) to give monoacetyl sparanone A (**6a**, 15 mg) as an amorphous powder from $CHCl_3$-*n*-hexane. ^1H and ^{13}C NMR($CDCl_3$): Tables 1 and 2. EIMS *m/z* (rel. int): 266 $[M]^+$ (15), 251 $[M\text{-}Me]^+$ (13), 236 $[M\text{-}OCH_2]^+$ (57), 224 $[M\text{-}CH_2CO]^+$ (100), 223 $[M\text{-}CH_2\ CHO]^+$ (40), 206 $[M\text{-}OCH_2 \times 2]^+$

(80), 178 [M-OCH_2 × 2-CO]$^+$ (30); HREIMS *m/z*: 266.0815 [M]$^+$, Calcd for $C_{13}H_{14}O_6$:266.0791.

Methylation of Sparanone B (**6**)

Sparanone B (**6**), 18 mg) was methylated with CH_2N_2 in MeOH (0.5 ml) to give monomethyl sparanone B (**6b**, 15 mg) as colorless needles from $CHCl_3$-*n*-hexane, mp 160–161°. ^{1}H and ^{13}C NMR($CDCl_3$): Tables 1 and 2. EIMS *m/z* (rel. int): 238 [M]$^+$ (100), 223 [M- Me]$^+$ (16), 208 [M-OCH_2]$^+$ (54), 195 [M-CH_2 CHO]$^+$ (54), 195 [M-CH_2 CHO]$^+$ (46), 178 [M-OCH_2 × 2]$^+$ (13), 150 [M-OCH_2 × 2-CO]$^+$ (20); HREIMS *m/z*: 238.0839 [M]$^+$, Calcd for $C_{12}H_{14}O_5$:238.0842.

Sparanoside B (**7**)

White yellow needles from MeOH, mp 180–183°, $[\alpha]_D$-40.6°(MeOH; c 0.36). IR ν_{max}^{KBr} cm^{-1}: 3400, 1700, 1630, 1603, 1520. CD λ_{max}^{MeOH} nm: 347.8 ($\Delta\epsilon$ + 2.57), 316.2 ($\Delta\epsilon$ − 2.68), 233.2 ($\Delta\epsilon$ − 6.09). UV λ_{max}^{MeOH} nm: 295.4 (log ϵ 4.20), 235.4sh (log ϵ 4.06), 210.4 (log ϵ 4.27). ^{1}H and ^{13}CNMR($CDCl_3$): Tables 1 and 2. FAB-MS: *m/z* 571 [M + Na]$^+$.

Methanolysis of sparanoside B (**7**)

A soln of **7** (3.0 mg) in 9% HCl-dry MeOH (1.5 ml) was heated under reflux for 2 hr. The reaction mixt. was neutralized with Ag_2CO_3 and filtered. The filtrate was concd to dryness *in vacuo* and the residue identified as methyl-(α,β)-D-glucopyanosides by HPTLC [$CHCl_3$-MeOH-H_2O] (65:35:10, lower layer, *Rf* = 0.28 and 0.29).

Enzymatic hydrolysis of sparanoside B (**7**)

A mixt. of **7** (10.5 mg) and β-D-glucosidase (30 mg, Wako Chemical Co.,) in 0.1 M acetate buffer (pH 4.4, 2 ml) was stirred at 38° for 20 hr. The reaction mixt. was poured into ice-H_2O and extracted with EtOAC. This extract was washed with satd aq. NaCl soln, then dried ($MgSO_4$) and filtered. The filtrate was concd to dryness *in vacuo* and the residue purified by prep. TLC ($CHCl_3$, *Rf* = 0.40) to give **6**, which was identified as sparanone B (**6**) by TLC and spectral data.

Acetylation of sparanoside B (**7**)

Sparanoside B (**7**, 20 mg) was acetylated with Ac_2O (1.5 ml), 4-dimethylaminopyridine (43 mg) and pyridine (1.0 ml) to give heptaacetyl sparanoside B (**7a**, 11 mg) as an amorphous powder from Me_2CO. IR ν_{max}^{CCl4} cm^{-1}: 1760, 1700, 1630, 1586, 1540. ^{1}H and ^{13}C NMR($CDCl_3$): Tables 1 and 2. FAB-MS *m/z*: 865 [M + Na]$^+$; EIMS *m/z* (rel. int): 782 [M- MeCOOH]$^+$ (1.0), 618 [M- $C_{11}H_{12}O_5$]$^+$ (3), 331 [$C_{14}H_{19}O_9$ (sugar part)]$^+$ (33), 271 [$C_{14}H_{19}O_9$-MeCOOH]$^+$ (10), 229 [$C_{14}H_{19}O_9$-MeCOOH-CH_2CO]$^+$ (4), 224 [$C_{11}H_{12}O_5$ (sparanone B)-CH_2CO]$^+$ (23.3), 209 [$C_{11}H_{12}O_5$-Me]$^+$ (2.0), 194 [$C_{11}H_{12}O_5$-OCH_2]$^+$ (20), 181 [$C_{11}H_{12}O_5$-$CHCH_2O$]$^+$ (18), 169 [$C_{14}H_{19}O_9$-MeCOOHX2-CH_2CO]$^+$ (100), 169 [$C_{14}H_{19}O_9$-MeCOOHX3-CH_2CO]$^+$ (65), 164 [$C_{11}H_{12}O_5$-OCH_2-OCH_2]$^+$ (20), 136 [$C_{11}H_{12}O_5$-OCH_2X2-CO]$^+$ (21); HREIMS *m/z*: 782.2297 [M-MeCOOH]$^+$, calcd for $C_{35}H_{42}O_{20}$: 782.2272.

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