New Spirostanol Steroids and Steroidal Saponins from Roots and Rhizomes of *Dracaena angustifolia* and Their Antiproliferative Activity

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Received January 31, 2001

The MeOH extract of Nam ginseng (roots and rhizomes of *Dracaena angustifolia*) afforded nine new compounds, including three spirostanol sapogenins, named namogenins A−C (**1**−**3**), four spirostanol saponins, named namonins A−D (**4**−**7**), a furostanol saponin, named namonin E (**8**), and a pregnan glycoside, named namonin F (**9**), along with another eight known steroidal saponins (**10**−**17**). Their structures were determined on the basis of spectral analyses and chemical methods. All compounds were tested for their antiproliferative activity against murine colon 26-L5 carcinoma, human HT-1080 fibrosarcoma, and B-16 BL6 melanoma cells. Compounds **4**, **5**, and **10** showed potent antiproliferative activity against HT-1080 fibrosarcoma cells, having IC_{50} values of 0.2, 0.3, and 0.6 μM, respectively, comparable to that of doxorubicin.

Ginseng refers to the roots and rhizomes of plants of *Panax* species (Araliaceae), such as *P. ginseng* (ginseng), *P. notoginseng* (notoginseng), *P. quinquefolium* (American ginseng), *P. japonicus* (Japanese ginseng), and *P. vietnamensis* (Vietnamese ginseng). They are widely used in Asian countries as a tonic for increasing mental efficiency, recovering physical balance, and stimulating metabolic function.[1] In Vietnam, along with *P. vietnamensis* (Vietnamese ginseng), plants of other families are also used for the same purpose. *Dracaena angustifolia* Roxb. (Dracaenaceae), with a local name Nam ginseng (ginseng from the South), is one such plant, and its underground parts are used as a tonic and for treatment of leukemia.[2]

In our continuing studies on Vietnamese medicinal plants,[3] we have previously reported hepatoprotective triterpene saponins from Vietnamese ginseng (*P. vietnamensis*, a real ginseng).[4] We have now examined the constituents of Nam ginseng (*D. angustifolia*) and isolated three new spirostanol steroids and six new steroidal saponins, together with eight known steroidal saponins. This paper reports the isolation and structure elucidation of the new compounds, together with antiproliferative activity of all compounds isolated.

Results and Discussion

Air-dried roots and rhizomes of *D. angustifolia* were extracted successively by refluxing MeOH, 50% aqueous MeOH, and water to give MeOH, MeOH−H_2O, and H_2O extracts, respectively. Primary screening revealed the MeOH extract to have the most potent antiproliferative activity against the human HT-1080 fibrosarcoma cell line, with an IC_{50} value of 66 μg/mL, whereas the MeOH−H_2O and H_2O extracts were inactive. Thus, the MeOH extract was subjected to Diaion HP-20 column chromatography (CC). The MeOH eluate was further separated by a combination of silica gel and ODS column chromatographies, and normal- and reversed-phase preparative TLC, to afford nine new compounds, named namogenins A−C (**1**−**3**) and namonins A−F (**4**−**9**), together with eight known steroidal saponins (**10**−**17**) (Chart 1).

Negative-ion HRFABMS of **1** displayed a quasi-molecular ion at *m*/*z* 461.2859, indicating the molecular formula $C_{27}H_{42}O_6$. The ^1H NMR spectrum of **1** showed signals ascribable to two tertiary methyls and three secondary methyls, while the ^{13}C NMR spectrum of **1** showed 35 signals (Table 1). Analysis of the COSY and HMQC spectra, together with the molecular formula, suggested **1** to be a spirostane-type steroid, similar to the aglycon of the known saponins **10**−**16**, but the ^1H and ^{13}C NMR signals ascribable to ring F appeared as pairs of signals, indicating that **1** was a C-25 epimeric mixture. Since its isolation was very difficult, as reported for similar epimeric mixtures,[5] and could not be done, the structure of **1** was elucidated by spectroscopic analysis of the epimeric mixture. Analysis of the COSY and HMQC spectra indicated the disappearance of the methine carbons assignable to C-14 and C-17, but instead of them, the ^{13}C NMR spectrum showed signals of two quaternary carbons at δ 88.2 and 91.2. Thus, C-14 and C-17 seemed to have hydroxyl groups, which were confirmed by the HMBC correlations of H_3-21 and H-16 with the quaternary carbon at δ 91.2 (C-17) and of H_3-18 with both quaternary carbons at δ 91.2 (C-17) and 88.2 (C-14). The α-orientation of 14-OH and 17-OH was deduced by a comparison of the ^{13}C NMR data with that of (25*R*)-spirost-5-en-3β,14α,17α-triol (ophiogenin).[6] Thus, **1** was determined to be a mixture (1:1) of (25*R*)- and (25*S*)-spirost-5-en-1β,3β,14α,17α-tetrol, which were named as (25*R*)- and (25*S*)-namogenin A, respectively.

Negative-ion HRFABMS of **2** indicated the molecular formula $C_{27}H_{42}O_5$, one oxygen atom less than **1**. The ^1H and ^{13}C NMR spectra of **2** were similar to those of **1**, indicating **2** also to be a spirostane-type steroid. However, the signals ascribable to ring F protons and carbons appeared as only one set, and the chemical shifts of H_3-27 (δ 1.06) and of C-23 to C-27 (Table 1) suggested **2** to be a 25*S*-spirostane-type steroid.[7] The ^{13}C NMR spectrum of **2** revealed a high-field shift (δ 59.9) of the oxygenated quaternary carbon assigned to C-17 in **1**. Thus, C-17 was considered to be a methine group, which was confirmed by the ^1H−^1H connectivity deduced by the analysis of the COSY and HMQC spectra and the HMBC correlations of

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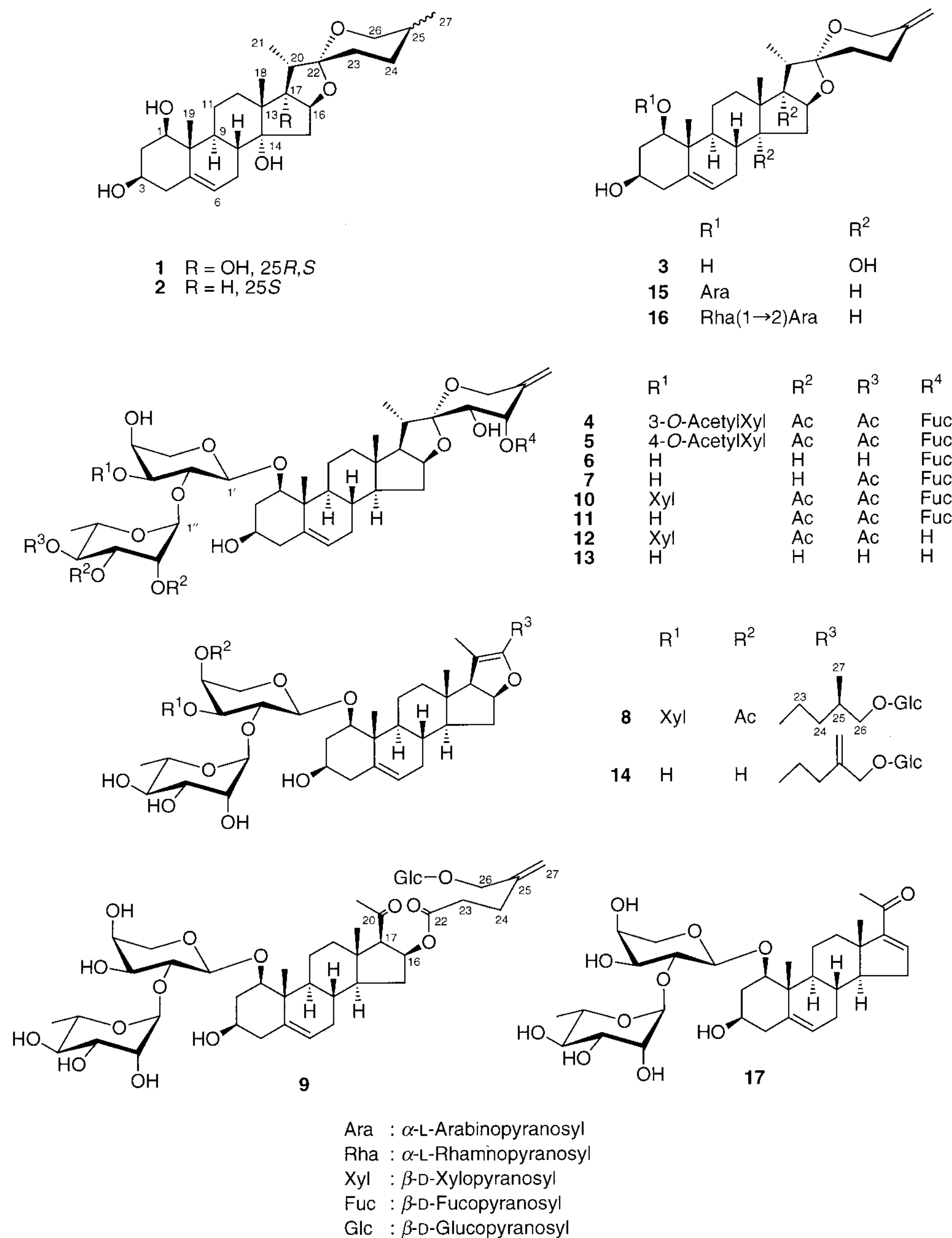
10.1021/np0100385 CCC: $20.00 © 2001 American Chemical Society and American Society of Pharmacognosy
Published on Web 06/02/2001

Chart 1



the methine carbon at δ 59.9 (C-17) with H_3-21 (δ 1.17), H_3-18 (δ 1.18), and H-16 (δ 5.10) and of the quaternary carbon at δ 86.8 (C-14) with H_3-18 (δ 1.18). Thus, namogenin B was determined to be (25*S*)-spirost-5-en-1β,3β,14α-triol (**2**).

The molecular formula of namogenin C (**3**) was determined by negative-ion HRFABMS to be $C_{27}H_{40}O_6$, two hydrogen atoms less than **1**. The ^{1}H and ^{13}C NMR spectra of **3** were almost the same as those of **1** (Table 1), except for the appearance of signals for an exo-olefin (δ_H 4.79, 2H; δ_C 144.2, 108.8) and the disappearance of the signals of a secondary methyl (CH_3-27) and a methine (CH-25). Thus, **3** was considered to be a 25,27-dehydro derivative of **1**, which was supported by the HMBC correlations of the exo-olefinic protons (δ 4.79, H_2-27) with C-24 (δ 28.7) and C-26 (δ 64.9). Thus, namogenin C was determined to be spirosta-5,25(27)-dien-1β,3β,14α,17α-tetrol (**3**).

HRFABMS data indicated that **4** and **5** have the same molecular formula, $C_{57}H_{84}O_{26}$. Their ^{1}H and ^{13}C NMR spectra were nearly identical (Tables 1, 2) and closely related to those of **10**, but they were characterized by the presence of signals of four acetyl groups, instead of three as in **10**. The ^{13}C NMR chemical shifts of the sugar portion of **4** and **5** and GC analysis of chiral derivatives of sugars in acid hydrolysates of **4** and **5** showed the presence of L-arabinose, D-xylose, L-rhamnose, and D-fucose.[8] Careful analysis of the COSY and HMQC spectra of **4** revealed a downfield shift of H-3 (δ 5.54) in the xylopyranosyl unit, compared to that of **10** (δ 3.97), while that of **5** showed a downfield shift of H-4 (δ 5.16) in the xylopyranosyl unit, compared to that of **10** (δ 3.95), suggesting the additional acetyl group of **4** and **5** to be at C-3 and at C-4 of the xylopyranosyl units, respectively. This was further supported by the HMBC correlation of the acetyl carbonyl carbon with H-3 and with H-4 in the xylopyranosyl units of **4** and **5**, respectively. Thus, namonins A and B were determined to be (23*S*,24*S*)-spirosta-5,25(27)-dien-1β,3β,-23,24-tetrol 1-*O*-{[2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-

Table 1. ^{13}C NMR Data (δ) for the Aglycon Part of Compounds **1**–**9** in Pyridine-d_5

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	78.2	78.2	78.2	83.7	84.0	83.5	83.5	83.6	83.4
2	43.6	43.6	43.7	37.5	37.7	37.4	37.4	37.1	37.3
3	68.1	68.1	69.1	67.9	67.9	68.2	68.0	68.1	68.2
4	44.0	44.0	44.1	43.8	43.9	43.8	43.8	43.7	43.9
5	139.8	139.8	139.8	139.3	139.4	139.7	139.6	139.2	139.8
6	124.9	125.0	124.9	124.9	125.0	124.6	124.8	124.8	124.4
7	26.4	26.9	26.4	31.9	31.9	31.9	32.0	31.9	31.5
8	37.5	36.8	37.5	32.9	33.0	33.0	33.0	32.9	32.4
9	44.4	44.5	44.4	50.2	50.2	50.3	50.3	50.3	50.4
10	43.9	43.9	43.9	42.8	42.8	42.9	42.9	42.7	42.8
11	23.2	23.6	23.2	23.9	24.0	23.9	23.9	24.5	23.7
12	27.2	32.7	27.2	40.4	40.4	40.4	40.4	40.2	38.7
13	48.1	44.8	48.2	40.7	40.7	40.7	40.7	43.2	42.1
14	88.2	86.8	88.3	56.6	56.7	56.7	56.7	55.2	54.2
15	40.6	40.1	40.6	32.3	32.4	32.3	32.4	34.7	35.6
16	90.5	81.9	90.8	82.9	82.9	82.9	82.9	84.5	74.8
17	91.2	59.9	91.2	61.4	61.5	61.5	61.5	64.7	66.8
18	21.0	20.4	21.0	16.8	16.8	16.8	16.8	14.5	14.2
19	14.0	13.9	14.0	14.8	14.9	15.1	14.9	15.0	15.1
20	45.2,[a] 45.7[b]	42.5	45.1	37.4	37.5	37.4	37.5	103.8	105.3
21	9.9,[a] 9.5[b]	15.2	9.9	14.8	14.8	14.8	14.8	11.8	30.7
22	109.6,[a] 110.0[b]	110.0	109.8	111.7	111.7	111.7	111.8	152.3	172.7
23	32.2,[a] 26.7[b]	26.5	33.6	70.3	70.3	70.2	70.3	23.7	32.7
24	28.9,[a] 25.8[b]	26.3	28.7	82.1	82.1	82.2	82.1	31.5	28.5
25	30.4,[a] 27.4[b]	27.6	144.2	143.9	143.9	143.9	143.9	33.5	145.3
26	66.8,[a] 65.0[b]	65.0	64.9	61.5	61.5	61.5	61.5	75.0	71.8
27	17.3,[a] 16.3[b]	16.3	108.8	113.7	113.8	113.7	113.8	17.3	111.8

[a,b] Data for the 25*R*- and 25*S*-epimers, respectively.

(1→2)]-[3-*O*-acetyl-β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranoside} 24-*O*-β-D-fucopyranoside (**4**) and (23*S*,24*S*)-spirosta-5,25(27)-dien-1β,3β,23,24-tetrol 1-*O*-{[2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)]-[4-*O*-acetyl-β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranoside} 24-*O*-β-D-fucopyranoside (**5**), respectively.

The negative-ion HRFABMS of namonin C (**6**) indicated the molecular formula $C_{44}H_{68}O_{18}$. The ^{1}H and ^{13}C NMR spectra of **6** were similar to those of **13** (Tables 1, 2), but they showed the presence of one more 6-deoxyhexopyranosyl unit than **13**. Detailed analysis of the COSY and HMQC spectra of **6** indicated the 6-deoxyhexopyranose to be a fucopyranose, which was confirmed by GC analysis of chiral derivatives of sugars in the acid hydrolysate. The GC analysis also confirmed the presence of D-fucose, L-rhamnose, and L-arabinose. The fucopyranosyl unit was determined to be at C-24, based on a glycosylation shift of C-24 (**6**, δ 82.2; **13**, δ 74.1). This was further supported by the HMBC correlation between H-1 of the fucopyranosyl unit and C-24 of the aglycon. Thus, namonin C was determined to be (23*S*,24*S*)-spirosta-5,25(27)-dien-1β,3β,-23,24-tetrol 1-*O*-{[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranoside} 24-*O*-β-D-fucopyranoside (**6**).

Negative-ion HRFABMS of **7** displayed a quasi-molecular ion at *m*/*z* 925.4443 to indicate the molecular formula $C_{46}H_{70}O_{19}$. The ^{1}H and ^{13}C NMR spectra of **7** resembled those of **6** and **11** (Tables 1, 2), but they showed the presence of one acetyl group more than **6** and two acetyl groups less than **11**. GC analysis of chiral derivatives of sugars in the acid hydrolysate showed the presence of L-rhamnose, L-arabinose, and D-fucose. Analysis of the COSY and HMQC spectra of **7** revealed a downfield shift of H-4 of the rhamnopyranosyl unit, compared to that of **6**, and highfield shifts of H-2 and H-3 of the rhamnopyranosyl unit, compared to those of **11**. Thus, the acetyl group in **7** was placed at C-4 of the rhamnopyranosyl unit, and this was confirmed by the HMBC correlation between H-4 of the rhamnopyranosyl unit and the acetyl carbonyl carbon. Thus, namonin D was determined to be (23*S*,24*S*)-spirosta-5,25(27)-dien-1β,3β,23,24-tetrol 1-*O*-{[4-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranoside} 24-*O*-β-D-fucopyranoside (**7**).

Analysis of the COSY and HMQC spectra indicated **8** ($C_{51}H_{80}O_{22}$) to be a furostanol saponin rather than a spirostanol saponin, and it contained a $CH-CH_3$ group instead of an exo-olefin in **14** (Table 1). The structure of the aglycon was determined by acid hydrolysis of **8** to be (25*R*)-spirost-5-en-1β,3β-diol (ruscogenin).[9] The sugar part of **8** was determined to be a combination of L-rhamnose, L-arabinose, D-xylose, and D-glucose, i.e., one D-xylopyranosyl unit more than **14**, from the ^{13}C NMR data and GC analysis of chiral derivatives of the sugars. The ^{1}H and ^{13}C NMR spectra of **8** also indicated the presence of one more acetyl group than in **14** (Table 2). The locations of the additional xylopyranosyl and acetyl groups were determined to be at C-3 and at C-4 of the arabinopyranosyl unit, on the basis of a glycosylation shift of C-3 (**8**, δ 82.5; **14**, δ 75.8) and an acylation shift of H-4 (**8**, δ 5.44; **14**, δ 4.16) of the arabinopyranosyl unit, respectively. This was confirmed by the HMBC correlations between H-4 of the arabinopyranosyl unit and the acetyl carbonyl carbon and between H-1 of the xylopyranosyl unit and C-3 of the arabinopyranosyl unit. In addition, HMBC correlations between H-1 of the arabinopyranosyl unit and C-1 of the aglycon, between H-1 of the rhamnopyranosyl unit and C-2 of the arabinopyranosyl unit, and between H-1 of the glucopyranosyl unit and C-26 of the aglycon confirmed the connectivities of the sugars. From these data, namonin E was determined to be (25*R*)-furosta-5,20(22)-dien-1β,3β,26-triol 1-*O*-{α-L-rhamnopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-4-*O*-acetyl-α-L-arabinopyranoside} 26-*O*-β-D-glucopyranoside (**8**).

The negative-ion HRFABMS of namonin F (**9**) indicated the molecular formula $C_{44}H_{68}O_{19}$. The ^{1}H and ^{13}C NMR spectra of **9** also resembled those of **14** (Table 1, 2), but they were characterized by the lack of the signals of the tetrasubstituted olefin (δ 104.1, 151.0) in **14** and the presence of the signals of a ketone (δ 205.3) and an ester (δ 172.7) carbonyl carbon. Analysis of the COSY and HMQC spectra showed low-field shifts of H-16 (**9**, δ 5.60;

Table 2. ^{1}H and ^{13}C NMR Data for Sugar Parts of Compounds **4**−**9** in Pyridine-d_5

	4		5		6		7		8		9	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
Ara 1′	100.0	4.59 d (7.5)	100.3	4.57 d (7.6)	100.3	4.68 d (7.5)	100.4	4.68 d (8.0)	99.8	4.86 d (7.0)	100.3	4.72 d (7.0)
2′	72.9	4.47 dd (8.0, 7.5)	72.9	4.50 m	75.1	4.55 m	74.2	4.52 m	76.4	4.21 m	75.2	4.58 m
3′	84.6	3.98 m	84.9	3.95 m	75.8	4.11 m	76.2	4.12 m	82.5	4.32 m	75.7	4.15 m
4′	69.7	4.33 m	69.9	4.31 m	70.0	4.13 m	70.3	3.88 m	71.2	5.44 dt (6.3, 9.0)	70.0	4.15 m
5′	67.4	4.19 m	67.6	4.20 m	67.3	4.24 d (11.7)	67.7	4.27 m	63.3	4.28 m	67.2	4.24 m
		3.64 m		3.60 m		3.64 d (11.7)		3.65 m		3.67 m		3.66 d (11.8)
4′-Ac									21.0	2.05 s		
									170.1			
Rha 1″	97.5	6.35 d (1.2)	97.7	6.47 br s	101.6	6.31 br s	100.9	6.39 br s	101.9	6.31 br s	101.7	6.29 br s
2″	70.0	5.99 dd (3.4, 1.2)	70.1	6.08 dd (3.2, 1.5)	72.5	4.70 m	73.3	4.67 m	72.4	4.78 m	72.5	4.70 m
3″	70.3	5.86 dd (10.2, 3.4)	70.6	5.89 dd (10.2, 3.2)	72.6	4.59 m	70.0	4.72 m	72.5	4.54 dd (9.0, 3.5)	72.7	4.61 dd (9.1, 3.1)
4″	71.8	5.55 t (10.2)	71.9	5.63 m	74.2	4.28 m	76.4	5.80 t (9.6)	74.1	4.28 m	74.2	4.26 m
5″	66.4	4.93 m	66.4	4.97 m	69.3	4.80 m	66.5	4.90 m	69.9	4.70 m	69.4	4.80 m
6″	18.1	1.39 d (6.1)	18.3	1.42 d (6.1)	19.0	1.70 d (5.6)	18.3	1.41 d (6.3)	19.2	1.74 d (6.3)	19.0	1.71 d (6.2)
2″-Ac	20.6[a]	1.99 s	20.7[b]	1.86 s								
	170.4		170.3									
3″-Ac	20.8	2.03 s	20.8	1.92 s								
	170.5		170.5									
4″-Ac	20.7	2.12 s	20.7[b]	2.11 s			21.0	1.98 s				
	170.3		170.3				170.7					
Xyl 1‴	105.8	4.80 d (7.3)	106.3	4.78 d (7.3)					105.4	4.97 d (7.2)		
2‴	72.2	3.76 m	74.7	3.70 t (7.3)					75.2	3.97 m		
3‴	79.0	5.54 t (9.2)	74.5	4.09 t (7.3)					78.4	4.09 t (8.6)		
4‴	69.0	4.05 m	72.8	5.16 m					71.0	4.14 m		
5‴	66.6	4.20 m	63.2	4.16 m					67.3	4.30 m		
		3.60 m		3.47 d (10.9)						3.63 m		
3‴-Ac	21.0[a]	1.92 s										
	170.4											
4‴-Ac			20.9	2.03 s								
			170.4									
Fuc 1⁗	106.2	5.12 d (8.1)	106.3	5.15 d (7.8)	106.2	5.15 d (7.8)	106.3	5.17 d (7.9)				
2⁗	73.0	4.38 dd (10.6, 8.1)	73.1	4.38 t (7.8)	73.0	4.38 t (7.8)	73.1	4.38 t (7.9)				
3⁗	75.3	4.03 m	75.4	4.04 dd (7.8, 3.4)	75.3	4.04 dd (7.8, 2.9)	75.4	4.05 dd (7.9, 3.3)				
4⁗	72.8	3.96 m	72.9	3.94 m	72.8	3.96 m	72.8	3.97 m				
5⁗	71.5	3.75 m	71.6	3.74 m	71.5	3.75 q (6.4)	71.6	3.76 m				
6⁗	17.2	1.46 d (6.4)	17.3	1.46 d (6.3)	17.3	1.47 d (6.4)	17.3	1.48 d (6.5)				
Glc 1⁗									104.9	4.83 d (8.0)	103.8	4.86 d (7.7)
2′′′′′									75.1	4.03 m	75.1	4.03 t (7.7)
3⁗									78.6	4.24 m	78.6	4.20 m
4⁗									71.8	4.22 m	71.7	4.19 m
5⁗									78.5	3.94 m	78.5	3.92 m
6⁗									62.9	4.57 dd (10.8, 4.5)	62.8	4.53 d (11.8)
										4.40 dd (10.8, 2.0)		4.37 dd (11.8, 5.3)

14, δ 4.78) and H_3-21 (**9**, δ 2.08; **14**, δ 1.68), while the HMBC spectrum showed correlations of the ketone carbonyl carbon (δ 205.3) with H-17 and H_3-21 and of the ester carbonyl carbon (δ 172.7) with H_2-23. Thus, C-20 and C-22 of **9** should be the carbonyl groups, instead of the olefinic group in **14**. On acid hydrolysis, **9** gave D-glucose, L-rhamnose, and L-arabinose, which were identified by GC analysis of chiral derivatives of the hydrolysate. Thus, namonin F was 16β-*O*-(4-methylidene-5-*O*-β-D-glucopyranosylpentanoyl)pregn-5-en-1β,3β,16β-triol-20-one 1-*O*-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranoside (**9**). This compound would be biosynthesized from **14** through an oxidative cleavage of the olefin between C-20 and C-22 and would give **17** through an elimination of the ester group at C-16.

The known saponins were identified by spectroscopic analysis and comparison with published data to be (23*S*,24*S*)-spirosta-5,25(27)-dien-1β,3β,23,24-tetrol 1-*O*-{[2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)]-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranoside} 24-*O*-β-D-fucopyranoside (**10**),[10] (23*S*,24*S*)-spirosta-5,25(27)-dien-1β,3β,23,24-tetrol 1-*O*-[2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside] 24-*O*-β-D-fucopyranoside (**11**),[11] (23*S*,24*S*)-spirosta-5,25(27)-dien-1β,3β,23,24-tetrol 1-*O*-{[2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)]-[β-D-xylopyranosyl-(1→3)]}-α-L-arabinopyranoside (**12**),[10] (23*S*,24*S*)-spirosta-5,25(27)-dien-1β,3β,23,24-tetrol 1-*O*-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranoside (**13**),[11] furosta-5,20(22),25(27)-trien-1β,3β,26-triol 1-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside] 26-*O*-β-D-glucopyranoside (**14**),[12] spirosta-5,25(27)-dien-1β,3β-diol 1-*O*-α-L-arabinopyranoside (**15**),[13] spirosta-5,25(27)-dien-1β,3β-diol 1-*O*-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranoside (**16**),[12] and pregna-5,16-dien-1β,3β-diol 1-*O*-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranoside (**17**).[14]

All the isolated compounds were tested for antiproliferative activity against human HT-1080 fibrosarcoma, murine colon 26-L5 carcinoma, and B-16 BL6 melanoma cell lines (Table 3). The results indicate that the spirostanol saponins possess more potent antiproliferative activity than their furostanol counterparts and that the acetyl group in the sugar moiety plays a crucial role in the activity, in accord with the reported results.[15] Compounds **4**, **5**, **10**, and **11** showed potent antiproliferative activities against HT-1080 fibrosarcoma cells with IC_{50} values of 0.2, 0.3, 0.6, and 3.8 μM, respectively, comparable to that of the positive control doxorubicin (IC_{50} 0.2 μM). On the other hand, against murine colon 26-L5 carcinoma and B-16 BL6 melanoma cells, they showed only mild activity. Similar selectivity toward HT-1080 fibrosarcoma cells was observed also in the case of compounds **6**, **7**, **8**, **12**, **15**, and **16**, although their activities were weaker. Thus, the 24-*O*-

Table 3. Antiproliferative Activity of Compounds Isolated from *D. angustifolia* (IC_{50} Values; μM)[a]

compound	colon 26-L5	HT-1080	B-16 BL6
4	26.6	0.2	9.7
5	27.7	0.3	11.8
6	>100	27.7	>100
7	97.4	21.6	42.4
8	>100	21.8	>100
10	22.1	0.6	11.9
11	30.2	3.8	20.9
12	76.6	11.1	28.4
13	5.3	56.5	4.2
15	77.2	40.0	47.9
16	95.3	42.1	41.2
5-fluorouracil	0.5	1.5	0.6
doxorubicin HCl	0.1	0.2	0.2

[a] Compounds having IC_{50} values larger than 100 μM against three cell lines are not included in the table.

fucopyranosyl and xylopyranosyl units seemed to be important for the cytotoxic activity against HT-1080 fibrosarcoma cells. Interestingly, compound **13**, without the fucopyranosyl, xylopyranosyl, and acetyl groups, showed significant activity against colon 26-L5 carcinoma and B-16 BL6 melanoma cells with IC_{50} values of 5.3 and 4.2 μM, respectively. The other compounds were inactive against all three cell lines (IC_{50} > 100 μM).

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-140 digital polarimeter at 25 °C. NMR spectra were recorded on a JEOL JNM-LA400 spectrometer in pyridine-d_5, using TMS as an internal reference. FABMS and HRFABMS was performed using a JEOL JMS-700T mass spectrometer, and glycerol was used as matrix.

Plant Material. Nam ginseng (roots and rhizomes of *D. angustifolia*) were collected in Quangnam Province, Vietnam, in November 1998, and the voucher sample (TMPU 010776) is preserved in the Museum for Materia Medica, Research Center for Ethnomedicines, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, Toyama, Japan.

Extraction and Isolation. Air-dried roots and rhizomes of *D. angustifolia* (440 g) were extracted by refluxing with MeOH, MeOH−H_2O, and H_2O successively to give MeOH (78 g), MeOH−H_2O (77 g), and H_2O (5.5 g) extracts, respectively. Part of the MeOH extract (70 g) was subjected to Diaion HP-20 CC and eluted with H_2O and then MeOH to give a MeOH fraction (7.2 g). The MeOH fraction was then chromatographed on silica gel with $CHCl_3$−MeOH−H_2O (14:6:1) to give seven fractions. Fraction 1 (1.5 g) was again chromatographed on silica gel to give three subfractions. Subfraction 2 (520 mg) was separated on normal-phase ($CHCl_3$−MeOH−H_2O, 14:6:0.5) and reversed-phase (MeOH−MeCN−H_2O, 2:2:1) PTLC to afford **1** (10 mg), **2** (11.6 mg), **3** (1.6 mg), **4** (11 mg), **5** (5 mg), **11** (50 mg), **12** (6 mg), and **18** (10 mg). Subfraction 3 (65 mg) was purified on normal-phase PTLC ($CHCl_3$−MeOH−H_2O, 14:6:0.5) to give **15** (1.7 mg). Fractions 2 (364 mg) and 3 (330 mg) were separated on normal-phase PTLC ($CHCl_3$−MeOH−H_2O, 14:6:1) to give **10** (90 mg) and **16** (76 mg), respectively. Fraction 4 (0.9 g) was separated by silica gel CC, followed by reversed-phase PTLC (MeOH−MeCN−H_2O, 1:1:1), to give **6** (19 mg), **7** (3.4 mg), and **13** (5.4 mg). Compounds **8** (21 mg) and **14** (50 mg) were obtained by normal-phase PTLC ($CHCl_3$−MeOH−H_2O, 14:6:1) of fraction 5 (195 mg). Fraction 6 (1.3 g) was chromatographed on ODS to give four subfractions, and subfraction 2 (500 mg) was again purified by ODS CC, followed by reversed-phase PTLC (MeOH−MeCN−H_2O, 1:1:1.5), to give **9** (2.9 mg) and **17** (1.3 mg).

A Mixture (1:1) of (25*R*)- and (25*S*)-Namogenin A (1): colorless amorphous solid; $[\alpha]_D^{25}$ −69.2° (*c* 0.6, MeOH); ^{1}H NMR (C_5D_5N) δ 5.70 (1H, d, J = 5.4 Hz, H-6), 4.78 (1H, m, H-16), 4.03 (1H, dd, J = 10.1, 2.7 Hz, H-26 of 25*S*-isomer), 3.93 (1H, m, H-3), 3.85 (1H, dd, J = 11.7, 4.2 Hz, H-1), 3.49 (2H, m, H_2-26 of 25*R*-isomer), 3.28 (1H, br d, J = 10.1 Hz, H-26 of 25*S*-isomer), 2.23 (3H, d, J = 7.1 Hz, H_3-21), 1.42 (3H, s, H_3-19), 1.20 (3H, s, H_3-18), 1.06 (3H, d, J = 7.1 Hz, H_3-27 of 25*S*-isomer), 0.68 (3H, d, J = 5.4 Hz, H_3-27 of 25*R*-isomer); ^{13}C NMR, see Table 1; FABMS *m/z* 461.3 $[M - H]^-$; HRFABMS *m/z* 461.2859 (calcd for $[M - H]^-$ 461.2904).

Namogenin B (2): colorless amorphous solid; $[\alpha]_D^{25}$ −74.5° (*c* 0.8, MeOH); ^{1}H NMR (C_5D_5N) δ 5.71 (1H, d, J = 5.5 Hz, H-6), 5.10 (1H, m, H-16), 4.05 (1H, dd, J = 10.8, 2.6 Hz, H-26), 3.33 (1H, br d, J = 10.8 Hz, H-26), 3.92 (1H, m, H-3), 3.84 (1H, dd, J = 11.5, 4.0 Hz, H-1), 2.82 (1H, m, H-17), 1.43 (3H, s, H_3-19), 1.18 (3H, s, H_3-18), 1.17 (3H, d, J = 7.2 Hz, H_3-21), 1.06 (3H, d, J = 7.0 Hz, H_3-27); ^{13}C NMR, see Table 1; FABMS *m/z* 445.3 $[M - H]^-$; HRFABMS *m/z* 445.2956 (calcd for $[M - H]^-$ 445.2954).

Namogenin C (3): colorless amorphous solid; $[\alpha]_D^{25}$ −29.8° (*c* 0.6, MeOH); ^{1}H NMR (C_5D_5N) δ 5.72 (1H, d, J = 5.1 Hz, H-6), 4.83 (1H, t, J = 6.4 Hz, H-16), 4.79 (2H, br s, H_2-27), 4.46 (1H, d, J = 11.9, H-26), 3.97 (1H, d, J = 11.9, H-26), 3.93 (1H, m, H-3), 3.88 (1H, dd, J = 11.7, 4.1 Hz, H-1), 2.43 (1H, q, J = 7.3 Hz, H-20), 2.34 (1H, dt, J = 11.9, 4.6 Hz, H-9), 2.17 (1H, dt, J = 11.5, 4.6 Hz, H-8), 1.44 (3H, s, H_3-19), 1.21 (3H, s, H_3-18), 1.20 (3H, d, J = 7.3 Hz, H_3-21); ^{13}C NMR, see Table 1; FABMS *m/z* 459.3 $[M - H]^-$; HRFABMS *m/z* 459.2751 (calcd for $[M - H]^-$ 459.2746).

Namonin A (4): colorless amorphous solid; $[\alpha]_D^{25}$ −65.7° (*c* 0.5, MeOH); ^{1}H NMR (C_5D_5N) δ 5.61 (1H, d, 5.4 Hz, H-6), 5.23 (1H, br s, H-27), 5.10 (1H, br s, H-27), 4.82 (1H, d, J = 10.0 Hz, H-26), 4.78 (1H, d, J = 3.9 Hz, H-24), 3.94 (1H, d, J = 3.9 Hz, H-23), 4.49 (1H, m, H-16), 3.96 (1H, m, H-26), 3.87 (1H, m, H-3), 3.77 (1H, m, H-1), 1.32 (3H, s, H_3-19), 1.07 (3H, d, J = 6.1 Hz, H_3-21), 0.95 (3H, s, H_3-18) and Table 2; ^{13}C NMR, see Tables 1 and 2; FABMS *m/z* 1183.7 $[M - H]^-$; HRFABMS *m/z* 1183.5182 (calcd for $[M - H]^-$ 1183.5172).

Namonin B (5): colorless amorphous solid; $[\alpha]_D^{25}$ −68.8° (*c* 0.8, MeOH); ^{1}H NMR (C_5D_5N) δ 5.61 (1H, d, J = 5.5 Hz, H-6), 5.23 (1H, br s, H-27), 5.08 (1H, br s, H-27), 4.82 (1H, d, J = 11.7 Hz, H-26), 4.77 (1H, d, J = 2.7 Hz, H-24), 4.60 (1H, m, H-16), 3.96 (1H, m, H-26), 3.95 (1H, m, H-23), 3.84 (1H, m, H-3), 3.76 (1H, m, H-1), 1.69 (1H, dd, J = 9.5, 6.8 Hz, H-17), 1.34 (3H, s, H_3-19), 1.06 (3H, d, J = 6.1 Hz, H_3-21), 0.95 (3H, s, H_3-18) and Table 2; ^{13}C NMR, see Tables 1 and 2; FABMS *m/z* 1183.7 $[M - H]^-$; HRFABMS *m/z* 1183.5162 (calcd for $[M - H]^-$ 1183.5172).

Namonin C (6): colorless amorphous solid; $[\alpha]_D^{25}$ −109.7° (*c* 0.9, MeOH); ^{1}H NMR (C_5D_5N) δ 5.56 (1H, d, J = 5.2 Hz, H-6), 5.24 (1H, br s, H-27), 5.09 (1H, br s, H-27), 4.82 (1H, d, J = 11.5 Hz, H-26), 4.78 (1H, d, J = 3.2 Hz, H-24), 4.59 (1H, m, H-16), 3.98 (1H, d, J = 11.5 Hz, H-26), 3.96 (1H, d, J = 3.2 Hz, H-23), 3.85 (1H, m, H-3), 3.81 (1H, m, H-1), 1.40 (3H, s, H_3-19), 1.06 (3H, d, J = 7.1 Hz, H_3-21), 0.93 (3H, s, H_3-18) and Table 2; ^{13}C NMR, see Tables 1 and 2; FABMS *m/z* 883.6 $[M - H]^-$; HRFABMS *m/z* 883.4349 (calcd for $[M - H]^-$ 883.4327).

Namonin D (7): colorless amorphous solid; $[\alpha]_D^{25}$ −58.7° (*c* 0.3, MeOH); ^{1}H NMR (C_5D_5N) δ 5.63 (1H, d, J = 5.5 Hz, H-6), 5.25 (1H, br s, H-27), 5.10 (1H, br s, H-27), 4.83 (1H, d, J = 11.8 Hz, H-26), 4.80 (1H, m, H-24), 4.57 (1H, m, H-16), 4.01 (1H, d, J = 11.8 Hz, H-26), 3.97 (1H, m, H-23), 3.85 (1H, m, H-3), 3.83 (1H, dd, J = 11.6, 3.6 Hz, H-1), 1.38 (3H, s, H_3-19), 1.07 (3H, d, J = 7.0 Hz, H_3-21), 0.95 (3H, s, H_3-18) and Table 2; ^{13}C NMR, see Tables 1 and 2; FABMS *m/z* 925.6 $[M - H]^-$; HRFABMS *m/z* 925.4443 (calcd for $[M - H]^-$ 925.4433).

Namonin E (8): colorless amorphous solid; $[\alpha]_D^{25}$ −49.4° (*c* 0.5, MeOH); ^{1}H NMR (C_5D_5N) δ 5.59 (1H, d, J = 5.2 Hz, H-6), 4.78 (1H, m, H-16), 3.93 (1H, m, H-26), 3.83 (1H, m, H-3), 3.75 (1H, dd, J = 11.0, 3.6 Hz, H-1), 3.62 (1H, m, H-26), 1.68 (3H, s, H_3-21), 1.37 (3H, s, H_3-19), 1.02 (3H, d, J = 6.5 Hz, H_3-27), 0.80 (3H, s, H_3-18) and Table 2; ^{13}C NMR, see Tables 1 and Table 2; FABMS *m/z* 1067.8 $[M + Na]^+$; HRFABMS *m/z* 1067.5052 (calcd for $[M + Na]^+$ 1067.5038).

Namonin F (9): colorless amorphous solid; $[\alpha]_D^{25}$ −18.4° (*c* 0.1, MeOH); ^{1}H NMR (C_5D_5N) δ 5.60 (1H, m, H-16), 5.58 (1H,

d, $J = 5.5$ Hz, H-6), 5.31 (1H, br s, H-27), 4.98 (1H, br s, H-27), 4.53 (1H, d, $J = 12.5$ Hz, H-26), 4.26 (1H, m, H-26), 3.87 (1H, m, H-3), 3.83 (1H, dd, $J = 11.4$, 3.7 Hz, H-1), 2.08 (3H, s, H_3-21), 1.42 (3H, s, H_3-19), 1.21 (3H, s, H_3-18) and Table 2; ^{13}C NMR, see Tables 1 and 2; FABMS *m*/*z* 899.6 $[M - H]^-$; HRFABMS *m*/*z* 899.4252 (calcd for $[M - H]^-$ 899.4276).

Sugar Analysis of 4−7 and 9.[8] Each compound (1 mg) was hydrolyzed with 1 M HCl (H_2O−dioxane, 1:1; 0.5 mL) at 80 °C for 3 h. The reaction mixture was neutralized with a small column of Amberlite IRA67 (OH^- form), and the filtrate was concentrated to dryness in vacuo. The residue was dissolved in 0.1 mL of pyridine, to which L-cysteine methyl ester hydrochloride in pyridine (0.1 M, 0.1 mL) was added. The mixture was heated at 60 °C for 2 h. Then, trimethylsilylimidazole (0.1 mL) was added, and the mixture was heated at 60 °C for 1.5 h. The reaction mixture was partitioned between hexane and water (0.1 mL each), and the hexane layer was analyzed on Shimadzu GC-14AH gas chromatograph; column, DB-5MS 30 m × 0.32 mm (J&W Scientific Inc.); column temperature, 210 °C; detector temperature, 270 °C; injection temperature, 270 °C. Standard sugars gave peaks at t_R (min) 15.90 and 16.92 for D- and L-glucose, 8.33 and 9.29 for L- and D-arabinose, 8.40 and 9.12 for D- and L-xylose, 10.27 for L-rhamnose, and 10.69 and 11.95 for D- and L-fucose, respectively.[16]

Acid Hydrolysis of 8. A solution of **8** (8 mg) in 1 M HCl (dioxane−H_2O, 1:1; 2 mL) was heated at 100 °C for 2 h. After cooling, H_2O was added and the solution was extracted with EtOAc three times. The EtOAc solutions were combined, dried, and subjected to normal-phase PTLC ($CHCl_3$−MeOH−H_2O, 14:6:0.5) to give ruscogenin[9] (1 mg). The aqueous layer was neutralized with Amberlite IRA67 (OH^- form), concentrated, and subjected to sugar analysis by GC.

Antiproliferative Assay. Human HT-1080 and murine B-16 BL6 melanoma cells were maintained in Eagle's minimum essential medium, whereas murine colon 26-L5 carcinoma cells were in RPMI medium (both Nissui Pharmaceutical Co., Ltd., Tokyo, Japan). These media were supplemented with 10% fetal calf serum (Gibco BRL Products, Gaithersburg, MD), 0.1% sodium bicarbonate, and 2 mM glutamine (Wako Pure Chemical Industries, Ltd., Kyoto, Japan). Cellular viability in the presence and absence of test samples was determined using the standard 3-(4,5-dimethylthiazol-2-yl)-2,5-dimethyltetrazolium bromide (MTT, Sigma, St. Louis, MO) assay[17] as described previously with minor modification.[18] In brief, exponentially growing cells were harvested, and a 100 μL suspension containing 2500 cells was plated in 96-well microtiter plates (Falcon, Becton Dickinson, NJ). After 24 h of incubation at 37 °C under 5% CO_2 to allow cell attachment, the cells were treated with varying concentrations of test samples in their respective medium (100 μL) and incubated for another 72 h under the same conditions. After adding a solution of MTT for 2 h, the amount of formazan formed was measured spectrophotometrically at 540 nm using Immuno Mini NJ-2300 plate reader.

Test samples were dissolved in DMSO and then diluted with medium. DMSO less than 0.1% in test solution had no effect. Doxorubicin HCl (Kyowa Hakko Co., Ltd., Tokyo, Japan) and 5-fluorouracil (Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan) were used as possitive controls, and IC_{50} values were calculated from the mean values from four wells.

Acknowledgment. This work was supported in part by a Grant-in-Aid for International Scientific Research (No. 09041177) from the Ministry of Education, Science, Sports and Culture, Japan.

References and Notes

(1) Tanaka, O.; Kasai, R. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Grisebach, H., Eds.; Springer-Verlag: Vienna, 1984; Vol. 46, pp 1−76.

(2) Vo, V. C. *Dictionary of Vietnamese Medicinal Plants*; Medicine Publisher: Hochiminh City, 1996; p 128.

(3) (a) Banskota, A. H.; Tezuka, Y.; Tran, Q. K.; Tanaka, K.; Saiki, I.; Kadota, S. *Chem. Pharm. Bull.* **2000**, *48*, 496−504. (b) Adnyana, I K.; Tezuka, Y.; Banskota, A. H.; Xiong, Q.; Tran, Q. K.; Kadota, S. *J. Nat. Prod.* **2000**, *63*, 496−500. See also references therein.

(4) Tran, Q. L.; Adnyana, I K.; Tezuka, Y.; Nagaoka, T.; Tran, Q. K.; Kadota, S. *J. Nat. Prod.* **2001**, *64*, 456−461.

(5) Miyakoshi, M.; Tamua, Y.; Masuda, H.; Mizutani, K.; Tanaka, O.; Ikeda, T. *J. Nat. Prod.* **2000**, *63*, 332−338.

(6) Nakanishi, H.; Kaneda, N. *Yakugaku Zasshi* **1987**, *197*, 780−784.

(7) (a) Hoyer, G.-A.; Sucrow, W.; Winkler, D. *Phytochemistry* **1975**, *14*, 539−542. (b) Jaffer, J. A.; Crabb, T. A.; Turner, C. H.; Blunden, G. *Org. Magn. Reson.* **1983**, *21*, 576−579.

(8) Hara, S.; Okabe, H.; Mihashi, K. *Chem. Pharm. Bull.* **1987**, *35*, 501−506.

(9) Agrawal, P. K.; Jain, D. C.; Gupta, R. K.; Thakur, R. S. *Phytochemistry* **1985**, *24*, 2479−2496.

(10) Mimaki, Y.; Inoue, T.; Kuroda, M.; Sashida, Y. *Phytochemistry* **1996**, *43*, 1325−1331.

(11) Mimaki, Y.; Kuroda, M.; Ide, A.; Kameyama, A.; Yokosuka, A.; Sashida, Y. *Phytochemistry* **1999**, *50*, 805−813.

(12) Mimaki, Y.; Takaashi, Y.; Kuroda, M.; Sashida, Y. *Phytochemistry* **1996**, *42*, 1609−1615.

(13) Pourrat, H.; Lamaison, J. L.; Gramain, J. C.; Remuson, R. *Ann. Pharm. Fr.* **1982**, *40*, 451−458.

(14) Mimaki, Y.; Inoue, T.; Kuroda, M.; Sashida, Y. *Phytochemistry* **1997**, *44*, 107−111.

(15) (a) Ikeda, T.; Tsumagari, H.; Nohara, T. *Chem. Pharm. Bull.* **2000**, *48*, 362−365. (b) Yokosuka, A.; Mimaki, Y.; Sashida, Y. *J. Nat. Prod.* **2000**, *63*, 1239−1243.

(16) Although we could not obtain D-rhamnose commercially, it was reported that derivatives of L- and D-rhamnose were well separated from each other as those of other sugars (see ref 15).

(17) (a) Rubinstein, L. V.; Shoemaker, R. H.; Paull, K. D.; Simon, R. M.; Tosini, S.; Skehan, P.; Scudiero, D. A.; Monsk, A.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1113−1118. (b) Goren, N.; Woerdenbag, H. J.; Bozok-Johansson, C. *Planta Med.* **1996**, *62*, 419−422.

(18) Banskota, A. H.; Tezuka, Y.; Prasain, J. K.; Matsushige, K.; Saiki, I.; Kadota, S. *J. Nat. Prod.* **1998**, *61*, 896−900.

NP0100385