Bioactive Constituents from *Asparagus cochinchinensis*[⊥]

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Bioassay-directed fractionation of the dried roots of *Asparagus cochinchinensis* led to the isolation of a new spirostanol saponin, asparacoside (**1**), two new C-27 spirosteroids, asparacosins A (**2**) and B (**3**), a new acetylenic derivative, 3″-methoxyasparenydiol (**4**), and a new polyphenol, 3′-hydroxy-4′-methoxy-4′-dehydroxynyasol (**6**), as well as five known phenolic compounds, asparenydiol (**5**), nyasol (**7**), 3″-methoxynyasol (**8**), 1,3-bis-di-*p*-hydroxyphenyl-4-penten-1-one (**9**), and *trans*-coniferyl alcohol (**10**). Compounds **1**, **6**, and **8** demonstrated moderate cytotoxicities in a panel comprised of KB, Col-2, LNCaP, Lu-1, and HUVEC cells, with IC_{50} values ranging from 4 to 12 μg/mL. The structures were determined by spectroscopic and chemical methods.

The dried roots of *Asparagus cochinchinensis* (Lourerio) Merrill (Asparagaceae) are used in Laos to treat chronic fever [Lao name of plant: Kheua Ya Nang Xang; voucher specimen K.Sydara037]. The plant also has a long history of use for treating fever, cough, kidney diseases, and benign breast tumors in China.[1] Phytochemically, they have been reported to contain monosaccharides, oligosaccharides,[2] polysaccharides,[3] furostanol oligosides,[4] and phenolic compounds.[5] As part of an International Cooperative Biodiversity Group (ICBG) involving the collaboration of institutions in Vietnam, Laos, and the United States,[6] a MeOH extract prepared from the roots of *A. cochinchinensis* collected in Laos was shown initially to inhibit HIV-1 replication by 78% at 20 μg/mL, while being devoid of cytoxicity in the HOG.R5 cell line. Dried roots (5 kg) of this plant were, therefore, re-collected for bioassay-directed fractionation studies aimed at identifying novel anti-HIV constituents. However, as the anti-HIV bioassay-directed fractionation proceeded, cytotoxic fractions emerged. With each level of separation, the cytotoxicity of concentrated fractions increased, which led us to redirect our efforts toward the isolation of potential antitumor compounds. As a result, six cytotoxic compounds were isolated from the roots of *A. cochinchinensis*. The current paper describes the isolation, structure elucidation, and biological evaluation of the compounds isolated from this plant.

Results and Discussion

Separation of the $CHCl_3$-soluble fraction of the MeOH extract of the dried roots of *A. cochinchinensis* utilizing parallel HIV-infectivity and toxicity assays in the HOG.R5 reporter cell line[7] led to the isolation of a new spirostanol saponin, asparacoside (**1**), two new C-27 spirosteroids, asparacosins A (**2**) and B (**3**), a new acetylenic derivative, 3″-methoxyasparenydiol (**4**), and a new polyphenol, 3′-hydroxy-4′-methoxy-4′-dehydroxynyasol (**6**). In addition, the known compounds asparenydiol (**5**),[8] nyasol (**7**),[9] 3″-methoxynyasol (**8**),[10] 1,3-bis-di-*p*-hydroxyphenyl-4-penten-1-one (**9**),[11] and *trans*-coniferyl alcohol (**10**) were also obtained.[12]

Asparacoside (**1**) was obtained as a white powder with a molecular formula of $C_{49}H_{80}O_{21}$ based on HRTOFMS and NMR (Tables 1−4) studies. Anomeric signals of four sugar units were observed in the ^1H and ^{13}C NMR spectra of **1** [δ_H 5.38 (d, J = 7.7 Hz), 5.30 (d, J = 7.7 Hz), 5.01 (d, J = 7.4 Hz), 4.74 (d, J = 7.7 Hz) and δ_C 105.7 (d), 105.3 (d), 105.2 (d), 101.4 (d)] (Tables 3 and 4). The aglycone of **1** was determined to be a spirostanol by comparison of its NMR data (Tables 1 and 2) with those of known spirostane-type steroids[13] and was identified as sarsasapogenin due to its NMR data being identical to those reported in the literature.[14,15] A partial acid hydrolysis of **1** afforded a mixture containing sarsasapogenin glycosides **1a**−**d**, which were separated by preparative HPLC chromatography. Compound **1a** contains a disaccharide group [δ_H 5.40 (d, J = 7.7 Hz), 4.96 (d, J = 7.6 Hz) and δ_C 106.0 (d), 102.0 (d)], which was determined to be a [β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl unit according to 1D and 2D NMR spectral data (Tables 3 and 4) including HMBC. The disaccharide unit attached to the C-3 of the sarsasapogenin aglycone was determined by the presence of the HMBC correlation between the anomeric proton signal at δ_H 4.96 and the signal at δ_C 75.2 (d). Compound **1a** was identified as 25(*S*)-5β-spirostan-3β-ol 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside, a component of a mixture of spirostanol saponins, known as 25*S*-schidigerasaponin D5, which was originally reported as a 25*S*/25*R* mixture from the stems of *Yucca schdigera*.[15] Compounds **1b**−**d** were elucidated as sarsasaponenin trisaccharides due to their characteristic sugar anomeric signals observed in the ^1H and ^{13}C NMR spectra (Tables 3 and 4). In addition to the glucopyranosyl-(1→2)]-β-D-glucopyranosyl unit, an additional sugar unit was revealed in the NMR spectra for both **1b** and **1d**. The additional sugar unit in both compounds was identified as α-L-arabinopyranosyl through analysis of the NMR spectral data. The α-L-arabinopyranosyl unit of **1b** was connected to C-4′ of the inner β-D-glucopyranosyl unit based on the presence of a HMBC correlation between the α-L-arabinopyranosyl anomeric proton signal at δ_H 4.98 and the C-4′ NMR signal at δ_C 81.4 (d), which resulted in a

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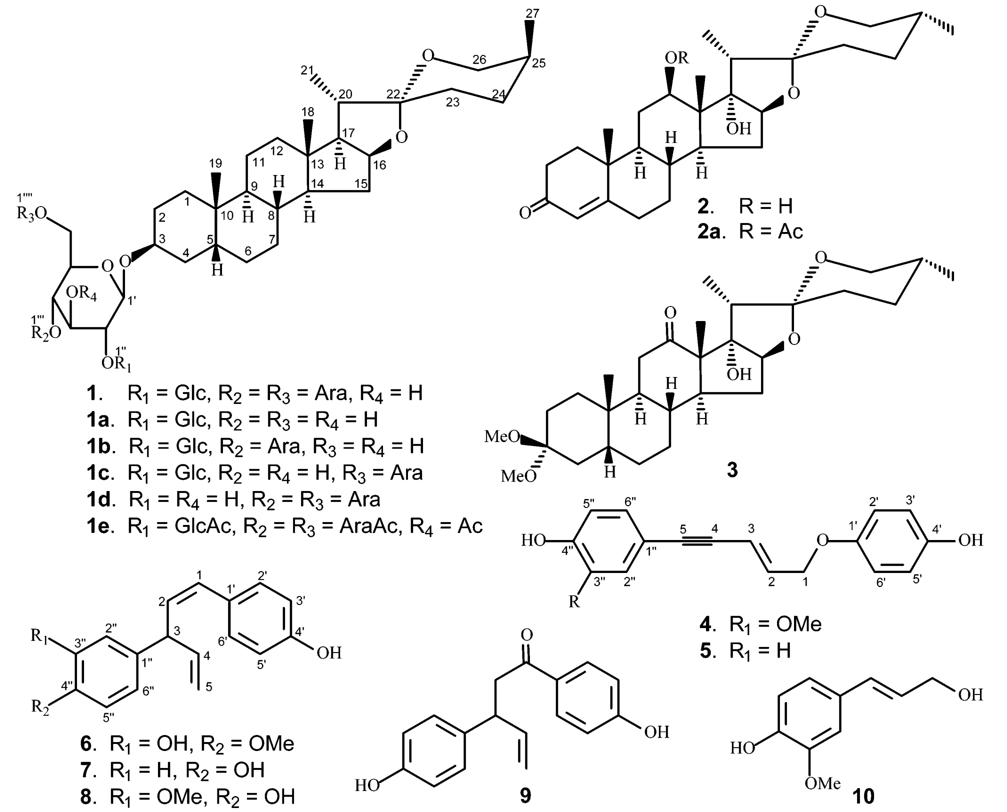
Chart 1



significant downfield shift of the ^{13}C signal of C-4′ in **1b** when compared to **1a**. The α-l-arabinopyranosyl unit of **1c** was deduced to be connected to the C-6′ of the inner β-D-glucopyranosyl unit due to the presence of the HMBC correlation between the α-l-arabinopyranosyl anomeric proton signal at δ_H 4.94 (d, J = 6.7 Hz) and the C-6′ NMR signal at δ_C 69.5 (t), which also resulted in a dramatic downfield shift of the ^{13}C signal of C-6′ in **1c** from that in **1a**. Interestingly, all nine proton signals of the sugar hydroxy group in **1c** were clearly observed in the ^{1}H NMR spectra, and the ^{1}H−^{1}H COSY correlations between these hydroxyl proton signals and the proton signals of their corresponding carbons strongly supported the presence of the two sugar units in **1c** connected to the C-2 and C-6, respectively, of a third sugar unit. Thereby, **1b** and **1c** were determined to be 25(*S*)-5β-spirostan-3β-ol 3-*O*-α-L-arabinopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranoside and 25(*S*)-5β-spirostan-3β-ol 3-*O*-α-L-arabinopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranoside, respectively. Differing from **1b** and **1c**, compound **1d** contains one hexapyranosyl unit and two pentapyranosyl units. The hexapyranosyl was identified as β-D-glucopyranosyl, and the two pentapyranosyls were elucidated to be α-L-arabinopyranosyls according to the NMR spectral data (Tables 3 and 4). The hexapyranosyl anomeric proton signal at δ_H 4.81 (d, J = 7.8 Hz) correlated to the ^{13}C signal at δ_C 74.6 (d) in the HMBC spectrum suggested that the β-D-glucopyranosyl was attached to the C-3 of the aglycone of **1d**. One of the α-L-arabinopyranosyl units in **1d** was positioned at C-4′ of the β-D-glucopyranosyl unit due to the presence of a HMBC correlation between the α-L-arabinopyranosyl anomeric proton signal at δ_H 5.38 (d, J = 7.8 Hz) and the ^{13}C signal at δ_C 80.0 (d). A second α-L-arabinopyranosyl unit in **1d** was found to be positioned at C-6′ of the β-D-glucopyranosyl unit due to the presence of the HMBC correlation between the α-L-arabinopyranosyl anomeric proton signal at δ_H 5.07 (d, J = 7.4 Hz) and the ^{13}C signal at δ_C 68.2 (t). The attachment of α-L-arabinopyranosyl units to β-D-glucopyranosyl in **1d** resulted in very dramatic downfield shifts of the ^{13}C signals of C-4′ and C-6′ in comparison to **1a**. Accordingly, **1d** was determined to be 25(*S*)-5β-spirostan-3β-ol 3-*O*-α-L-arabinopyranosyl-(1→6)-[α-L-arabinopyranosyl-(1→4)]-β-D-glucopyranoside. Compound **1b** had been reported as an isolate from *Asparagus curillus*,[16] while compounds **1c** and **1d** have not been reported from nature. Since no spectral data of **1b** are found in the literature, these data are presented in Tables 1−4 of the current report. For reference purposes, the ^{13}C NMR data of compounds **1a** are also included in Tables 1−4. The structure of **1** was thus determined to be (25*S*)-5β-spirostan-3β-ol 3-*O*-α-L-arabinopyranosyl-(1→6)-[α-L-arabinopyranosyl-(1→4)]-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranoside through the combination of the structural information of **1** and its acid-hydrolyzed products **1a**,**b**. The linkage of each sugar unit in **1** was further confirmed by 2D NMR spectral data including ^{1}H−^{1}H COSY, HMQC, HMBC, ROESY, and TOCSY techniques. For further supportive evidence in the structural determination, a total acetylation experiment of **1** was also performed. Redistribution of the ^{1}H NMR signals of the sugar units of the acetate derivative resolved the congested area in the middle range of the ^{1}H NMR spectra (δ_H 3.8−4.8), which, in turn, facilitated the application of the HMBC and TOCSY analyses. The full assignments of the ^{1}H and ^{13}C NMR data of compounds **1**, **1a**−**d**, and those of the acetate derivative (**1e**) were performed by analysis of their 2D NMR spectral data (Tables 1−4).

Asparacosin A (**2**) was shown to have molecular formula $C_{27}H_{40}O_5$ (HRTOFMS), which was consistent with the results of ^{13}C NMR and DEPT experiments. The similarity of the NMR data (Tables 1 and 2) relative to those of the aglycone of **1** suggested that **2** was also a spirostanol. Compound **2** aglycone differs from that of **1** by having an α,β-conjugated keto group [δ_H 5.71 (d, J = 1.2 Hz) and δ_C

Table 1. ^{1}H NMR Spectral Data (δ) of Compounds **2** and **3** and Aglycones of Compounds **1** and **1a**−**e** (500 MHz, pyridine-d_5, J in Hz)

position	1	1a	1b	1c	1d	1e[a]	2[b]	2a[b,c]	3[b,d]
H-1a	1.85 ddd (11.2. 8.4, 3.4)	1.85 m	1.85 m	1.89 m	1.85 m	1.79 m	2.00 ddd (13.4, 4.9, 3.1)	1.93 m	1.45 brd (12.5)
H-1b	1.45 brd (11.1)	1.45 m	1.45 m	1.53 m	1.48 m	1.52 m	1.69 tt (13.8, 4.7)	1.69 m	1.18 brtd (12.1, 3.0)
H-2a	1.83 m	1.83 m	1.83 m	1.92 m	1.88 m	1.82 m	2.39 ddd (17.0, 12.9, 5.0)	2.38 m	2.39 ddd (17.0, 12.9, 5.0)
H-2b	1.49 m	1.49 m	1.49 m	1.46 m	1.44 m	1.66 m	2.32 ddd (17.0, 4.9, 3.5)	2.32 m	1.28 m
H-3	4.22 m	4.34 m	4.22 m	4.32 m	4.31 m	4.24 m			
H-4a	1.75 m	1.75 m	1.77 m	1.78 m	1.75 m	1.82 m			1.67 m
H-4b	1.75 m	1.75 m	1.77 m	1.78 m	1.75 m	1.82 m			1.57 m
H-4							5.71 d (1.2)	5.71 brs	
H-5	2.21 m	2.19 m	2.20 m	2.20 m	2.04 m	2.17 m			1.57 m
H-6a	1.77 m	1.78 m	1.78 m	1.78 m	1.72 m	1.79 m	2.37 m	2.35 m	1.73 m
H-6b	1.11 brd (12.5)	1.13 m	1.13 m	1.15 m	1.08 m	1.32 m	2.26 ddd (14.8, 4.1, 2.3)	2.26 brd (15.0)	1.22 m
H-7a	1.19 m	1.20 m	1.20 m	1.21 m	1.25 m	1.28 m	1.82 ddt (12.7, 5.4, 2.7)	1.82 m	1.55 m
H-7b	0.90 brqd (12.7, 3.5)	0.90 m	0.91 m	0.91 m	0.93 m	0.95 m	0.99 dddd (14.1,13.2, 4.1, 2.2)	1.01 brqd (13.3, 4.5)	1.15 m
H-8	1.46 m	1.48 m	1.48 m	1.48 m	1.48 m	1.50 m	1.65 m	1.64 m	1.87 m
H-9	1.24 m	1.24 m	1.24 m	1.24 m	1.26 m	1.26 m	1.06 ddd (12.8, 10.5, 4.5)	1.10 brtd (10.8, 3.9)	1.86 m
H-11a	1.30 m	1.32 m	1.30 m	1.30 m	1.32 m	1.32 m	1.76 dt (12.8, 4.7)	1.82 brdt (12.6, 4.0)	2.27 dd (14.5, 13.1)
H-11b	1.23 m	1.21 m	1.21 m	1.21 m	1.23 m	1.21 m	1.45 brq (12.7)	1.43 brq (12.4)	2.07 dd (14.5, 4.0)
H-12a	1.65 brdt (12.5, 3.2)	1.67 brd (12.3)	1.67 brd (12.3)	1.64 brd (12.3)	1.66 brd (12.3)	1.69 m			
H-12b	1.03 m	1.08 m	1.07 m	1.07 m	1.07 m	1.09 m			
H-12							3.99 overlap	5.06 dd (11.4, 4.8)	
H-14	1.01 m	1.03 m	1.03 m	1.03 m	1.05 m	1.07 m	1.58 ddd (13.5, 11.1, 4.8)	1.75 ddd (13.5, 11.5, 5.6)	2.32 m
H-15a	2.00 ddd (12.0, 7.5, 5.9)	2.00 ddd (12.4, 7.5, 5.0)	2.00 m	2.02 m	2.00 m	2.04 overlap	2.09 ddd (11.9, 7.3, 4.8)	2.11 ddd (12.6, 7.5, 5.9)	2.19 ddd (12.3, 7.7, 5.9)
H-15b	1.41 m	1.40 m	1.40 m	1.41 m	1.41 m	1.43 m	1.36 ddd (13.5, 11.7, 8.3)	1.38 brtd (12.9, 7.1)	1.45 m
H-16	4.57 brq (7.5)	4.57 brq (7.8)	4.57 overlap	4.59 brq (7.4)	4.57 brq (7.7)		3.98t (7.9)	3.97t (7.4)	4.03 dd (7.7, 6.2)
H-17	1.81 dd (8.5, 6.5)	1.81 m	1.81 m	1.82 dd (8.1, 6.6)	1.81 m	1.85 m			
Me-18	0.80 s	0.81 s	0.81 s	0.80 s	0.80 s	0.85 s	0.83 s	0.89 s	1.02 s
Me-19	0.96 s	0.98 s	0.97 s	0.98 s	0.84 s	1.08 s	1.18 s	1.17 s	0.98 s
H-20	1.92 m	1.92 m	1.92 m	1.92 m	1.92 m	1.92 m	1.95 q (7.1)	1.93 q (7.4)	1.93 q (7.2)
Me −21	1.14 d (7.0)	1.14 d (6.9)	1.14 d (6.9)	1.15 d (6.9)	1.14 d (6.9)	1.15 d (6.9)	0.92 d (7.1)	0.78 d (7.1)	0.99 d (7.3)
H-23a	1.89 brdd (9.6, 6.5)	1.89 m	1.89 m	1.90 m	1.89 m	1.91 m	1.69 m	1.63 m	1.69 m
H-23b	1.42 m	1.42 m	1.42 m	1.42 m	1.42 m	1.42 m	1.60 m	1.58 m	1.51 m
H-24a	2.13 tt (13.2, 4.6)	2.13 tt (13.1, 4.7)	2.13 m	2.13 m	2.12 m	2.13 m	1.60 m	1.60 m	1.60 m
H-24b	1.33 m	1.33 m	1.33 m	1.33 m	1.33 m	1.33 m	1.44 m	1.41 m	1.41m
H-25	1.57 m	1.57 m	1.57 m	1.57 m	1.57 m	1.57 m	1.61 m	1.60 m	1.65 m
H-26a	4.06 overlap	4.06 dd (10.9, 2.6)	4.06 dd (11.1, 2.8)	4.06 dd (10.6, 2.2)	4.06 brd (9.6)	4.06 overlap	3.48 ddd (10.9, 4.2, 2.0)	3.45 brd (9.2)	3.53 ddd (11.0, 4.2, 2.2)
H-26b	3.35 d (11.3)	3.35 d (11.1)	3.35 d (11.1)	3.36 d (10.8)	3.36 d (10.7)	3.36 d (11.0)	3.36 t (10.9)	3.31 t (10.9)	3.31 t (11.1)
Me-27	1.06 d (7.1)	1.06 d (7.0)	1.06 d (7.1)	1.06 d (7.1)	1.06 d (7.0)	1.06 d (7.0)	0.76 d (6.2)	0.76 d (6.3)	0.76 d (6.4)

[a] Ac: 2.23 s, 2.225 s, 2.220 s, 2.19 s, 2.12 s, 2.06 s (×2), 2.05 s, 2.03 s, 2.01 s, 1.95 s. [b] Data measured in $CDCl_3$. [c] Ac: 2.01 s. [d] OMe: 3.15 s, 3.10 s.

199.4 (s), 170.3 (s), 124.1 (d)], an additional oxymethine group [δ_H 3.99 (overlap) and δ_C 71.3 (d)], and an oxyquaternary carbon [δ_C 90.4 (s)]. The α,β-conjugated keto group was assigned to ring A at C-3, -4, and -5 with the carbonyl carbon at C-3 because of the presence of HMBC correlations of H_2-1 signals [δ_H 2.00 (ddd, J = 13.4, 4.9, 3.1 Hz), 1.69 (tt, J = 13.8, 4.7 Hz)] to the ^{13}C signal of the carbonyl carbon at δ_C 199.4 (s), H_3-19 signals at δ_H 1.18 (s) to the ^{13}C signal of the olefinic quaternary carbon at δ_C 170.3 (s), and the olefinic proton signal at δ_H 5.71 (d, J = 1.2 Hz) to the ^{13}C signals at δ_C 199.4 (s), 170.3 (s), 33.8 (t, C-2), 32.6 (t, C-6), and 38.5 (s, C-10). On acetylation, the overlapped signal of an oxymethine group at δ_H 3.99 was shifted downfield to δ_H 5.06 (dd, J = 11.4, 4.8 Hz). The HMBC correlation between the proton signal at δ_H 5.06 (dd, J = 11.4, 4.8 Hz) and the ^{13}C signal of C-18 at δ_C 11.8

Table 2. ^{13}C NMR Spectral Data (δ) of Compounds **2** and **3** and Aglycones of Compounds **1** and **1a**−**e** (125 MHz, pyridine-d_5)

position	1	1a	1b	1c	1d	1e[a]	2[b]	2a[b,c]	3[b,d]
C-1	30.8 t	31.0 t	30.9 t	31.0 t	31.0 t	30.7 t	35.5 t	35.5 t	32.8 t
C-2	26.8 t	26.8 t	26.8 t	26.8 t	26.8 t	26.8 t	33.8 t	33.7 t	26.4 t
C-3	75.1 d	75.2 d	75.3 d	75.3 d	74.6 d	75.1 d	199.4 s	199.1 s	100.5 s
C-4	30.6 t	30.8 t	30.7 t	30.9 t	30.5 t	30.6 t	124.1 d	124.3 d	33.2 t
C-5	36.5 d	36.9 d	36.7 d	36.9 d	37.0 d	36.5 d	170.3 s	169.7 s	39.1 d
C-6	26.8 t	26.9 t	26.9 t	26.9 t	27.0 t	26.8 t	32.6 t	32.6 t	27.2 t
C-7	26.9 t	27.0 t	27.0 t	27.0 t	27.0 t	26.9 t	31.4 t	31.2 t	25.7 t
C-8	35.5 d	35.6 d	35.6 d	35.6 d	35.6 d	35.6 d	33.9 d	34.4 d	34.8 d
C-9	40.2 d	40.26 d	40.25 d	40.26 d	40.28 d	40.3 d	52.3 d	51.7 d	41.0 d
C-10	35.3 s	35.3 s	35.3 s	35.3 s	35.3 s	35.4 s	38.5 s	38.4 s	35.2 s
C-11	21.2 t	21.2 t	21.2 t	21.2 t	21.2 t	21.2 t	28.4 t	26.4 t	38.3 t
C-12	40.3 t	40.3 t	40.3 t	40.3 t	40.3 t	40.4 t	71.3 d	73.7 d	216.4 s
C-13	40.9 s	40.9 s	40.9 s	40.9 s	40.9 s	40.9 s	49.0 s	48.3 s	60.0 s
C-14	56.5 d	56.5 d	56.5 d	56.5 d	56.5 d	56.5 d	50.5 d	50.7 d	52.2 d
C-15	32.2 t	32.2 t	32.2 t	32.2 t	32.2 t	32.2 t	31.1 t	31.1 t	29.86 t
C-16	81.4 d	81.4 d	81.5 d	81.4 d	81.4 d	81.4 d	90.6 d	89.5 d	86.2 d
C-17	63.0 d	63.0 d	63.0 d	63.0 d	63.0 d	63.0 d	90.4 s	89.4 s	89.3 s
C-18	16.6 q	16.6 q	16.6 q	16.6 q	16.6 q	16.7 q	11.2 q	11.8 q	16.6 q
C-19	24.0 q	24.0 q	24.0 q	24.1 q	23.9 q	24.0 q	17.0 q	17.2 q	22.5 q
C-20	42.5 d	42.5 d	42.5 d	42.5 d	42.5 d	42.5 d	45.1 d	44.8 d	44.6 d
C-21	15.0 q	14.9 q	14.9 q	14.9 q	14.9 q	15.0 q	7.2 q	7.4 q	7.81 q
C-22	109.7 s	109.7 s	109.7 s	109.7 s	109.7 s	109.8 s	110.3 s	109.9 s	109.6 s
C-23	26.4 t	26.4 t	26.4 t	26.4 t	26.4 t	26.4 t	30.6 t	30.6 t	31.9 t
C-24	26.2 t	26.2 t	26.2 t	26.2 t	26.2 t	26.2 t	27.9 t	28.1 t	28.4 t
C-25	27.6 d	27.6 d	27.6 d	27.6 d	27.6 d	27.6 d	29.9 d	30.0 d	29.9 d
C-26	65.1 t	65.1 t	65.1 t	65.1 t	65.1 t	65.1 t	66.9 t	66.8 t	66.8 t
C-27	16.3 q	16.3 q	16.3 q	16.3 q	16.3 q	16.3 q	17.1 q	17.0 q	17.1 q

[a] Ac-Me: 21.3 q, 20.9 q (×2), 20.7 q (×5), 20.6 q, 20.5 q (×2); Ac-CO: 170.7 s, 170.4 s (×4), 170.3 s, 170.2 s, 170.1 s, 170.0 s, 169.96 s, 169.8 s. [b] Data measured in $CDCl_3$. [c] Ac-Me: 21.5 q; Ac-CO: 170.7 s. [d] OMe: 47.5 q, 47.4 q.

(q) for the acetate of **2a** determined the additional oxymethine carbon as C-12. Further analysis of the HMBC spectrum of **2a** assigned the oxy-quaternary carbon to C-17 due to the long-range correlations of its signal at δ_C 89.4 (s) to the proton signals at δ_H 5.06, 0.78, and 3.97. The configuration of H-12 of **2a** was established as α-oriented by its coupling pattern (J = 11.4, 4.8 Hz)[16] and by its ROE correlation to H-16α. The α-configuration of H-16 was confirmed by the ROE correlations of its proton signal to H_2-26 [δ_H 3.45 (brd, J = 9.2 Hz), 3.31 (t, J = 10.9 Hz)]. In comparison with compounds not having a hydroxyl group at C-17,[17] the ^{13}C signal of C-12 in **2** was dramatically shifted upfield (up ~8 ppm) due to a γ-gauche shielding effect from the hydroxyl group of C-17, which in turn established 17-OH as α-oriented. The methyl group at C-25 was assigned an α-orientation by ^{13}C NMR chemical shifts of C-23, -24, -25, and -27 identical to those reported for the (25*R*)-spirostanol epimers.[15] This assignment was confirmed by the presence of ROE correlations between H_3-27 [δ_H 0.76 (d, J = 6.2 Hz)] and H_2-26 [δ_H 3.48 (ddd, J = 10.9, 4.2, 2.0 Hz), 3.36 (t, J = 10.9 Hz)]. The structure of **2** was thus elucidated to be (25*R*)-12β,17α-dihydroxyspirost-4-en-3-one and was given the trivial name of asparacosin A.

Asparacosin B (**3**), $C_{29}H_{46}O_6$ (HRTOFMS), was shown to be a homologue of **2** by comparison of the NMR data of these two compounds (Tables 1 and 2). Analysis of the NMR data revealed that **3** is a second (25*R*)-spirostanol with a 17α-hydroxyl group isolated in this study. In contrast to **2**, compound **3** contains no carbon−carbon double bond according to the NMR spectra. However, a nonconjugated carbonyl carbon at δ_C 216.4 (s) and an additional oxy-quaternary carbon at δ_C 100.5 (s) were observed in its ^{13}C NMR spectrum. The carbonyl carbon in **3** was determined to be C-12 on the basis of the observed HMBC correlation between the ^{13}C signal of δ_C 216.4 (s) and the proton signals of Me-18 [δ_H 1.02 (s)] as opposed to C-3 in **2**. The second oxy-quaternary carbon in **3** was found to be an acetal carbon with two methoxy groups attached, according to the HMBC correlations of the proton signals at δ_H 3.15 (s) and 3.10 (s) to the ^{13}C signal at δ_C 100.5 (s). The acetal carbon was further determined to be C-3 on the basis of analysis of HMBC and ROESY spectral data. Accordingly, the structure of **3** was elucidated as (25*R*)-3,3-dimethoxy-17α-hydroxyspirostan-3-al-12-one and was given the trivial name asparacosin B.

3″-Methoxyasparenydiol (**4**) showed $[M + H]^+$ at m/z 297, corresponding to a molecular formula of $C_{18}H_{16}O_4$ in the ESIMS, which was consistent with ^{13}C NMR and DEPT experiments. The NMR spectra disclosed the presence of a 4-hydroxyphenyl group, a 3,4-dioxyphenyl group, a CH═CH double bond, a C≡C triple bond, and an oxymethylene group. On the basis of the long-range correlations observed in an HMBC experiment (Figure 1), the triple bond was conjugated to the double bond, which was coupled by the oxymethylene group to form an acetylenyl-allyloxyl group. The HMBC spectral data further connected the 3,4-dioxyphenyl group to the terminal acetylenyl carbon of the acetylenyl-allyloxyl group, and the 4-hydroxyphenyl group to the oxygen of the acetylenyl-allyloxyl group. The 3,4-dioxyphenyl group was identified as 3-methoxy-4-hydroxyphenyl since no long-range correlation between the H-6″ signal at δ_H 7.19 (dd, J = 8.1, 2.0 Hz) and the C-3″ at δ_C 148.3 (s) was observed in the HMBC spectrum. An *E*-configuration was assigned to the double bond due to the existence of a large coupling constant between its two protons (J = 15.8 Hz). The structure of **4** was thus determined to be 1-[4-hydroxyphenoxy]-5-[3-methoxy-4-hydroxyphenyl]pent-2-en-3-yne. This structural assignment was confirmed when the NMR data were compared with those of the known compound, asparenydiol (**5**), previously reported from the same plant by others.[8]

3′-Hydroxy-4′-methoxy-4′-dehydroxynyasol (**6**), $C_{18}H_{18}O_3$ (HRTOFMS), was shown to possess a 4-hydroxylphenyl group, a 3,4-dioxyphenyl group, a CH═CH double bond, a CH═CH_2 double bond, and a methine group by ^{1}H, ^{13}C, and DEPT NMR data. Analysis of the ^{1}H−^{1}H COSY and HMQC spectral data linked both the CH═CH double bond

Table 3. ^{1}H NMR Spectral Data (δ) of the Sugar Moieties of Compounds **1** and **1a**−**e** (500 MHz, pyridine-d_5, *J* in Hz)

position	1	1a	1b	1c	1d	1e
Glc-1′						
H-1′	4.74 d (7.7)	4.96 d (7.6)	4.82 brd (7.1)	4.88 d (7.6)	4.81 d (7.8)	4.72 d (7.9)
H-2′	4.09 t (8.8)	4.26 t (9.0)	4.26 overlap	4.18 t (8.9)	3.89 brt (8.1)	3.99 brt (7.9)
H-3′	4.15 t (9.1)	4.33 t (9.0)	4.26 overlap	4.25 overlap	4.17 t (9.1)	5.64 t (9.4)
H-4′	4.36 t (9.3)	4.19 t (9.2)	4.26 overlap	4.07 overlap	4.45 brt (9.4)	4.01 t (9.6)
H-5′	3.72 brdt (9.9, 2.7)	3.88 ddd (9.6, 5.2, 2.4)	3.72 m	3.99 m	3.86 brd (10.2)	3.75 dd (9.3, 3.5)
H-6′a	4.69 ABd (9.5)	4.52 dd (11.7, 2.4)	4.50 overlap	4.75 brdd (11.2, 1.7)	4.77 ABd (10.6)	4.36 brd (11.2)
H-6′b	4.60 overlap	4.34 dd (11.6, 5.1)	4.41 dd (12.2, 2.2)	4.25 overlap	4.77 brd (9.2)	4.06 overlap
Glc-1″						
H-1″	5.38 d (7.7)	5.40 d (7.7)	5.45 d (7.7)	5.35 d (7.7)		5.23 d (8.0)
H-2″	4.02 dd (9.0, 7.8)	4.10 t (8.0)	4.07 t (8.3)	4.07 overlap		5.40 dd (9.4, 8.1)
H-3″	4.28 overlap	4.26 t (9.0)	4.28 t (8.8)	4.25 overlap		5.81 t (9.5)
H-4″	4.28 overlap	4.34 t (9.2)	4.32 t (9.1)	4.32 overlap		5.52 t (9.5)
H-5″	3.99 m	3.97 brdt (9.2, 3.9)	3.99 ddd (8.9, 3.6, 2.8)	3.96 m		4.24 m
H-6″a	4.60 dd (11.6, 3.0)	4.56 dd (11.4, 2.7)	4.57 brd (9.2)	4.54 m		4.72 dd (12.1, 4.8)
H-6″b	4.48 dd (11.3, 5.1)	4.50 dd (11.5, 4.4)	4.48 dd (11.6, 4.5)	4.49 m		4.45 dd (12.2, 2.6)
Ara-1‴						
H-1‴	5.30 d (7.7)		4.98 d (7.6)		5.38 d (7.8)	4.94 d (5.7)
H-2‴	4.43 brt (8.2)		4.45 dd (8.8, 8.0)		4.47 overlap	5.57 overlap
H-3‴	4.26 overlap		4.09 dd (9.2, 3.3)		4.25 overlap	5.57 overlap
H-4‴	4.25 overlap		4.22 overlap		4.24 overlap	5.57 overlap
H-5‴a	4.22 brd (12.6)		4.26 overlap		4.22 brd (12.7)	4.17 dd (12.5, 4.2)
H-5‴b	3.99 d (11.4)		3.73 d (11.4)		3.98 d (12.1)	3.92 d (11.0)
Ara-1⁗						
H-1⁗	5.01 d (7.4)			4.94 d (6.7)	5.07 d (7.4)	5.01 d (6.6)
H-2⁗	4.45 dd (8.9, 7.2)			4.46 m	4.44 overlap	5.71 dd (9.0, 6.6)
H-3⁗	4.06 overlap			4.18 overlap	4.06 overlap	5.55 overlap
H-4⁗	4.25 overlap			4.32 overlap	4.24 overlap	5.62 overlap
H-5⁗a	4.25 overlap			4.30 overlap	4.25 brd (13.0)	4.22 overlap
H-5⁗b	3.71 d (10.9)			3.76 d (11.0)	3.72 d (11.7)	3.86 d (11.2)
2′-OH					7.13 brs	
3′-OH				7.86 brd (3.5) or 7.24 brd (3.2)	5.54 brs	
4′-OH				6.24 brd (3.5)		
2″-OH				7.30brd (2.8)		
3″-OH				7.24brd (3.2) or 7.86 brd (3.5)		
4″-OH				7.36 brd (4.9)		
6″-OH				6.13 brt (5.7)		
2‴-OH					7.41 brs	
3‴-OH					6.80 brs	
4‴-OH					6.41 brs	
2⁗-OH				7.13 brd (3.7)	7.41 brs	
3⁗-OH				6.52 brd (5.2)	6.59 brs	
4⁗-OH				7.17 brd (4.5)	6.29 brs	

Table 4. ^{13}C NMR Spectral Data (δ) of the Sugar Moieties of Compounds **1** and **1a**−**e** (125 MHz, pyridine-d_5)

position	1	1a	1b	1c	1d	1e
Glc-1′	101.4 d	102.0 d	101.7 d	101.9 d	103.0 d	99.6 d
2′	80.8 d	83.2 d	81.4 d	83.1 d	74.8 d	77.8 d
3′	76.1 d	78.2 d	76.3 d	77.95 d	76.5 d	75.2 d
4′	79.3 d	71.6 d	80.4 d	71.8 d	80.0 d	76.9 d
5′	74.7 d	78.2 d	76.4 d	76.8 d	75.0 d	74.7 d
6′	68.0 t	62.7 t	61.7 t	69.5 d	68.2 t	67.7 t
Glc-1″	105.3 d	106.0 d	105.4 d	106.1 d		101.1 d
2″	77.0 d	77.1 d	77.1 d	77.1 d		72.4 d
3″	78.0 d	78.0 d	78.0 d	78.04 d		73.8 d
4″	72.2 d	71.8 d	72.0 d	71.8 d		70.1 d
5″	78.6 d	78.6 d	78.6 d	78.6 d		72.2 d
6″	63.2 t	62.9 t	63.1 t	62.9 t		63.1 t
Ara-1‴	105.2 d		105.6 d		105.3 d	100.7 d
2‴	72.6 d		72.6 d		72.6 d	70.1 d
3‴	74.8 d		74.6 d		74.8 d	70.4 d
4‴	69.9 d		69.6 d		70.0 d	68.0 d
5‴	67.8 t		67.7 t		67.8 t	62.5 t
Ara-1⁗	105.7 d			105.3 d	105.7 d	101.0 d
2⁗	72.6 d			72.4 d	72.6 d	69.5 d
3⁗	74.68 d			74.4 d	74.7 d	70.8 d
4⁗	69.8 d			69.1 d	69.8 d	68.4 d
5⁗	67.4 t			66.4 t	67.3 t	63.1 t

and the CH=CH$_2$ double bond to a methine carbon to form a penta-1,4-dienyl group, which was in turn attached to a 4-hydroxylphenyl group at C-1 and a 3,4-dioxyphenyl group at C-3, through analysis of HMBC spectral data. The 3,4-

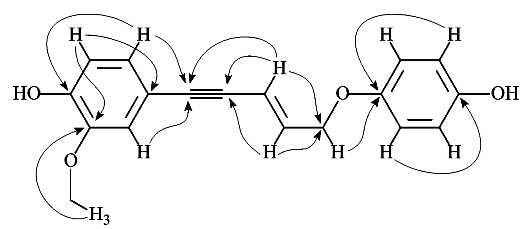


Figure 1. Selected HMBC correlations for compound **4** (pyridine-d_5).

dioxyphenyl group was determined to be a 3-hydroxy-4-methoxyphenyl, as the HMBC correlation was clearly observed between the proton signal [δ_H 4.84 (brs)] of the 3″-phenolic hydroxyl group and C-2″ [δ_C 114.0 (d)]. The CH=CH double bond was assigned a *Z*-configuration on the basis of the coupling constant (J = 11.4 Hz) between its two protons. The structure of **6** was thus determined to be 1-[4-hydroxyphenoxy]-3-[3-hydroxy-4-methoxyphenyl]-penta-1,4-diene.

Compounds **7**−**9** were shown to have structures similar to **6**. They were identified as the known compounds nyasol (**7**),[9] 3″-methoxynyasol (**8**),[10] and 1,3-bis-di-*p*-hydroxyphenyl-4-penten-1-one (**9**),[11] by comparison of their NMR data to those reported in the literature. It should be noted that the NMR assignments of **8** were incomplete, especially with regard to the observation of the long-range correlation of

Table 5. Cytotoxic Activity of Compounds **1**−**10** in Cell Culture[a]

compound	KB	Col2	LNCaP	Lu1	HUVEC	HOG.R5
1	4.8 (4.8)	5.4 (5.4)	10.1 (10.1)	4.2 (4.2)	4.1 (4.1)	<10 (<10)
2	10.7 (24.1)	>20	>20	>20	>20	
3	>20	>20	>20	>20	>20	
4	12.0 (40.5)	>20	>20	19.7 (66.5)	>20	<5 (<17)
5	2.4 (8.5)	>20	>20	19.8 (70.1)	>20	<5 (<18)
6	9.0 (31.9)	11.7 (41.4)	11.6 (41.1)	7.2 (25.5)	16.4 (58.1)	3.4 (12.0)
7	>20	>20	>20	>20	>20	15.6 (58.1)
8	9.0 (31.9)	6.3 (22.3)	6.6 (23.4)	4.5 (15.9)	6.7 (23.7)	6.8 (24.1)
9	>20	>20	>20	>20	>20	20.6 (76.8)
10	>20	>20	>20	>20	>20	
ellipticine	0.04 (0.16)	0.3 (1.22)	0.8 (3.25)	0.02 (0.08)	0.09 (0.37)	0.02 (0.08)

[a] Results are expressed as IC_{50} values [concentration required to inhibit cell growth by 50%] in μg/mL (μM). Ellipticine was used as a positive control.

the proton signal of the phenolic hydroxy group at δ_H 4.79 (brs) to the ^{13}C signal at δ_C 114.4 (d, C-5″).

Except for major compound **2**, which was obtained by direct crystallization from a first pass silica gel column chromatographic fraction, all other compounds (**1**, **3**−**10**) were isolated by bioassay-directed fractionation. Although compounds **7** and **9** exhibited moderate anti-HIV activities with IC_{50} values of 11.7 μg/mL (46.4 μM) and 20.0 μg/mL (74.6 μM), respectively, they were cytotoxic to HOG.R5 cells at similar concentrations [CC_{50} values of 15.6 μg/mL (58.1 μM) and 20.6 μg/mL (76.8 μM), respectively]. The toxicity and poor antiviral selectivity of compounds **1**, **4**−**6**, and **8** precluded further evaluation of their anti-HIV activity. Prompted by their toxicity to the HOG.R5 cell line that is based on parental HOS (human osteosarcoma) cells, we decided to evaluate these compounds in a broader panel of human cancer cell lines[18] for potential antitumor activity. The results of these assays showed **1**, **6**, and **8** to exhibit moderate cytotoxicity against the Lu1 (human lung cancer), LNCaP (hormone-dependent human prostate cancer), Col2 (human colon carcinoma), HUVEC (human umbilical vein endothelial carcinoma), KB (human oral epidermoid carcinoma), and HOS (human osteosarcoma) cell lines, while compounds **2** and **4** demonstrated moderate cytotoxicity toward KB cells only (Table 5). In addition, the greater cytotoxic response mediated by compound **5** appears to be selective for the KB and HOG.R5 cell lines.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer model 241 polarimeter. IR spectra were run on a Jasco FT/IR-410 spectrometer, equipped with a Specac Silver Gate ATR system by applying a film on a germanium plate. 1D and 2D NMR spectra were recorded on a Bruker DRX-500 MHz spectrometer. Chemical shifts (δ) were expressed in ppm with reference to the solvent signals. All NMR data were obtained by using standard pulse sequences supplied by the vendor. Column chromatography was carried out on silica gel (200−400 mesh, Natland International Corp.). Reversed-phase flash chromatography was accomplished with RP-18 silica gel (40−63 μm, EM Science), and reversed-phase HPLC was carried out on a Waters 600E delivery system pump, equipped with a Waters 996 photodiode detector and a Watrex GROM-Saphir 110 C18 column (120 Å,12 μm, 300 × 40 mm), which also resulted in extracted UV spectral data of each purified compound. Thin-layer chromatography was performed on Whatman glass-backed plates coated with 0.25 mm layers of silica gel 60. HRTOFMS and MS/MS spectra were recorded on a Micromass QTOF-2 spectrometer, a VG 7070-HF spectrometer, or a Finnigan LCQ.

Plant Material. The initial collection of root sample (SL-7037) of *Asparagus cochinchinensis* (Lourerio) Merrill (Asparagaceae) was made at Ban Kaxa, Saravanh District, Saravanh Province in Laos, and was documented by voucher specimens #037. A larger amount of the roots of the plant sample (SLA-7037, 5.0 kg) was subsequently re-collected at Ban Kaxa, Saravanh District, Saravanh Province in Laos, for current isolation work. Duplicate voucher specimens of the initial collection have been deposited at the Herbarium of the Traditional Medicine Research Center in Vientiane (Laos) and the John G. Searle Herbarium of the Field Museum of Natural History (Chicago, IL).

Anti-HIV Assay. Anti-HIV and toxicity assays were performed in parallel utilizing the green fluorescent protein (GFP)-based HOG.R5 reporter cell line that was constructed and developed specifically for quantitating HIV-1 infectivity. The system was validated and adapted as a moderately high-throughput procedure for screening natural products for anti-HIV activity in our laboratory.[7] Briefly, cultures in microtiter wells were infected with HIV-1_{IIIB} (2.5 ng/mL p24) in the presence of plant extracts, after which the fluorescence output was measured at the end of 4 days. Virus was omitted from parallel cultures treated with identical concentrations of plant extracts in order to monitor changes in cellular viability by a combination of microscopic and fluorometric measurements.

Cytotoxicity Assay. Compounds (**1**−**10**) were evaluated for cytotoxicity against a panel comprised of the following human cells in culture: Lu1, Col2, LNCaP, HUVEC, and KB. Assays involving Lu1, Col-2, LNCaP, and KB cell lines utilized established protocols,[18] while HUVEC were propagated and assayed in more specialized medium. HUVEC were purchased and grown in media and components supplied in the EGM-2 BulletKit (Cambrex Bio Science Walkersville, Inc., MD) with 2% fetal bovine serum (FBS). The HUVEC line constitutes a test system to identify samples with potential antiangiogenic activity.

Extraction and Isolation. The dried, milled plant material (5.0 kg) was extracted with MeOH and concentrated. The resulting syrup (350 g) was subsequently defatted with petroleum ether and partitioned with $CHCl_3$. The $CHCl_3$-soluble fraction (31.6 g) was chromatographed over a silica gel column (1 kg), which was developed by gradient elution with $CHCl_3$ and increasing concentrations of Me_2CO and MeOH to afford 31 fractions [$CHCl_3$ (eluates F1−F6, each 1.0 L); $CHCl_3$−Me_2CO, 9:1 (eluates F7−F11, each 1.0 L), 8:2 (eluates F12−F18, each 1.0 L), 7:3 (eluates F19−F22, each 1.0 L); $CHCl_3$−MeOH, 95:5 (eluates F23−F26, each 1.0 L), 90:10 (eluates F27−F30, each 1.0 L), 80:20 (eluate F31, 3.0 L), respectively]. Bioassay localized the anti-HIV activity in fractions F5 (65 mg), F6 (793 mg), F8 (454 mg), F16 (452 mg), F17 (643 mg), F27 (1331 mg), F28 (2423 mg), and F29 (812 mg). Asparacoside (**1**, 771 mg) was obtained as a precipitate from the MeOH solution of fraction F28, and asparacosin A (**2**, 372 mg) was obtained from fraction F7 (1805 mg) by direct crystallization from MeOH. Fraction F6 was subjected to a C-18 reversed-phase flash column (130 g, gradient elution with MeOH and H_2O) to yield eight fractions [MeOH−H_2O, 4:6 (eluates F32 and F33, each 300 mL), 5:5 (eluate F34, 500 mL), 6:4 (eluate F35, 500 mL), 7:3 (eluate F36, 500 mL), 8:2 (eluate F37, 500 mL), 9:1 (eluate F38, 500 mL); MeOH (eluate F39, 1.0 mL), respectively]. Asparacosin B (**3**, 18.2 mg) was obtained from fraction F38 by crystallization from MeOH. Fractions F35 and F36 were pooled and subjected to preparative HPLC separation on a GROM-Saphir 110 C18 column (solvent system: MeCN−H_2O, 45:55) to afford 3″-methoxyasparenydiol (**4**, 9.6 mg), 3′-hydroxy-4′-methoxy-4′-dehydroxynyasol (**6**, 2.5 mg), nyasol [**7**, 5.6 mg, $[\alpha]^{20}_D$ +154.0° (*c* 0.43, MeOH)], and 3″-methoxynyasol [**8**, 5.7 mg, $[\alpha]^{20}_D$ +146.2° (*c* 0.05, MeOH)]. Fraction F8 was subjected to a C-18 reversed-phase flash column (130 g, gradient elution with MeOH and H_2O) to yield seven fractions [MeOH−H_2O, 4:6 (eluate F41, 500 mL), 5:5 (eluate F42, 500 mL), 6:4 (eluate F43, 500 mL), 7:3 (eluate F44, 500 mL), 8:2 (eluate F45, 500 mL), 9:1 (eluate F46, 500 mL); MeOH (eluate F47, 1.0 mL), respectively]. Fractions F43 and F44 were pooled and subjected to preparative HPLC separation on a GROM-Saphir 110 C18 column (solvent system: MeCN−H_2O, 45:55) to afford asparenydiol (**5**, 2.0 mg), 1,3-bis-di-*p*-hydroxyphenyl-4-penten-1-one [**9**, 2.3 mg, $[\alpha]^{20}_D$ −9.8° (*c* 0.07, MeOH)], and *trans*-coniferyl alcohol (**10**, 3.5 mg).

Asparacoside (1): white powder, $[\alpha]^{20}_D$ −35.2° (*c* 0.57, MeOH−$CHCl_3$, 1:1); IR (film) ν_{max} 3378.7 (br), 2929.3, 1452.6, 1368.7, 1338.4, 1254.5, 1231.8, 1163.4, 1125.3, 1071.8, 1041.4, 996.1, 988.3, 912.2, 843.7, 782.5 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1−4; ESIMS *m/z* (%) 1243 $[M + K]^+$ (34), 1027 $[M + Na]^+$ (100), 977 (8), 737 (8), 645 (12), 513 (15); HRTOFMS *m/z* 1027.5100 $[M + Na]^+$ (calcd for $C_{49}H_{80}O_{21}Na$, 1027.5090).

Partial Acid Hydrolysis of Asparacoside (1). To a solution of **1** (85.66 mg) in MeOH−$CHCl_3$ (1:1, 8.0 mL) was added 3 mL of 1 N HCl. The solution was allowed to react at 50 °C for 2 h and was then evaporated to dryness to yield 83.2 mg of a mixture, which was separated by preparative HPLC (GROM-Saphir 110 C18 column; solvent system: MeCN−H_2O, 55:45) to yield **1a** [3.26 mg, $[\alpha]^{20}_D$ −70.7° (*c* 0.10, MeOH−$CHCl_3$, 1:1)], **1b** [2.60 mg, $[\alpha]^{20}_D$ −19.7° (*c* 0.16, MeOH)], **1c** [4.74 mg, $[\alpha]^{20}_D$ −45.2° (*c* 0.30, MeOH)], and **1d** [2.07 mg, $[\alpha]^{20}_D$ −23.2° (*c* 0.13, MeOH)]. The NMR data of **1a**−**d** are presented in Tables 1−4.

Acetylation of Asparacoside (1). A sample of **1** (10.07 mg) in a mixture of 2.5 mL each pyridine and Ac_2O was allowed to react for 36 h at room temperature. The reaction product was evaporated to dryness to yield acetylated asparacoside (**1e**, 14.21 mg). The NMR data of the acetate (**1e**) are presented in Tables 1−4.

Asparacosin A (2): colorless flake, $[\alpha]^{20}_D$ −13.0° (*c* 0.53, MeOH); IR (film) ν_{max} 3462.1 (br), 2944.8, 2929.3, 2876.3, 1673.4, 1656.6, 1612.7, 1460.3, 1376.4, 1338.4, 1238.5, 1178.8, 1117.6, 1079.5, 1049.1, 980.6, 896.7, 866.4 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* (%) 467 $[M + Na]^+$ (44), 445 $[M + H]^+$ (100), 427 (27), 408 (32), 381 (38), 359 (10), 353 (15), 313 (22); HRTOFMS *m/z* 467.2782 $[M + Na]^+$ (calcd for $C_{27}H_{40}O_5Na$, 467.2773).

Acetylation of Asparacosin A (2). A sample of **2** (17.53 mg) was acetylated in 5.0 mL of pyridine−Ac_2O (3:2) at room temperature for 26 h to afford **2a** (19.22 mg). The NMR data of **2a** are presented in Tables 1 and 2.

Asparacosin B (3): colorless flake, $[\alpha]^{20}_D$ −21.7° (*c* 0.73, MeOH); IR (film) ν_{max} 2952.5, 2929.3, 2860.9, 1681.1, 1452.6, 1430.0, 1385.1, 1362.5, 1339.8, 1324.4, 1294.5, 1241.5, 1155.6, 1098.3, 1082.0, 985.0, 972.9, 942.5, 902.0 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* (%) 513 $[M + Na]^+$ (100), 491 $[M + H]^+$ (7), 459 (25), 408 (5), 381 (8); HRTOFMS *m/z* 513.3179 $[M + Na]^+$ (calcd for $C_{29}H_{46}O_6Na$, 513.3192).

3″-Methoxyasparenydiol (4): yellowish powder, UV λ_{max} [AU (absorbance units)] 202.4 (3.94), 220.3 (2.30), 283.9 (2.23), 299.8 (1.81) nm; IR (film) ν_{max} 3371.0 (br), 2192.7, 1648.8, 1611.2, 1573.6, 1513.4, 1452.6, 1376.4, 1294.5, 1247.2, 1224.1, 1133.0, 1018.7, 950.3, 874.1, 820.6, 805.6, 759.8 cm^{-1}; ^1H NMR (pyridine-d_5) δ 11.50 (1H, brs, OH), 11.10 (1H, brs, OH), 7.47 (1H, d, J = 2.0 Hz, H-2″), 7.19 (1H, dd, J = 8.1, 2.0 Hz, H-6″), 7.15 (2H, ABd, J = 8.9 Hz, H-3′ and H-5′), 7.03 (2H, ABd, J = 8.9 Hz, H-2′ and H-6′), 6.91 (1H, d, J = 8.4 Hz, H-5″), 6.46 (1H, dt, J = 15.8, 5.2 Hz, H-2), 6.27 (1H, dt, J = 15.9, 1.8 Hz, H-3), 4.59 (2H, dd, J = 5.3, 1.8 Hz, H_2-1), 3.70 (3H, s, OMe); ^{13}C NMR (pyridine-d_5) δ 68.7 (t, C-1), 138.5 (d, C-2), 112.6 (d, C-3), 86.5 (s, C-4), 91.9 (s, C-5), 152.0 (s, C-1′), 116.6 (d, C-2′), 117.0 (d, C-3′), 153.3 (s, C-4′), 117.0 (d, C-5′), 116.6 (d, C-6′), 116.3 (s, C-1″), 119.5 (d, C-2″), 148.3 (s, C-3″), 149.7 (s, C-4″), 112.4 (d, C-5″), 123.8 (d, C-6″), 55.8 (q, OMe); ESIMS *m/z* (%) 297 $[M + H]^+$ (21), 272 (100), 203 (22), 188 (56).

3′-Hydroxy-4′-methoxy-4′-dehydroxynyasol (6): white powder, $[\alpha]^{20}_D$ +85.8° (*c* 0.09, MeOH); UV λ_{max} (AU) 197.7 (3.67), 255.5 (0.89) nm; IR (film) ν_{max} 3386.4 (br), 1643.1, 1605.0, 1505.7, 1444.9, 1353.8, 1262.2, 1233.7, 1218.8, 1171.1, 1125.3, 1026.4, 927.1, 752.1 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.15 (2H, ABd, J = 8.5 Hz, H-2′ and H-6′), 6.82 (1H, d, J = 2.1 Hz, H-2″), 6.77 (2H, ABd, J = 8.5 Hz, H-3′ and H-5′), 6.77 (1H, d, J = 8.3 Hz, H-5″), 6.69 (1H, dd, J = 8.3, 2.1 Hz, H-6″), 6.50 (1H, d, J = 11.4 Hz, H-1), 5.98 (1H, ddd, J = 17.1, 10.2, 6.1, H-4), 5.66 (1H, dd, J = 11.4, 10.1, H-2), 5.56 (1H, brs, 4′-OH), 5.14 (1H, dt, J = 17.2, 1.6, H-5a), 5.12 (1H, dt, J = 10.1, 1.5, H-5b), 4.84 (1H, brs, 3′-OH), 4.44 (1H, dd, J = 9.9, 6.1, H-3), 3.85 (3H, s, OMe); ^{13}C NMR ($CDCl_3$) δ 128.6 (d, C-1), 131.6 (d, C-2), 47.0 (d, C-3), 140.6 (d, C-4), 115.05 (t, C-5), 129.9 (s, C-1′), 130.0 (d, C-2′), 115.1 (d, C-3′), 154.5 (s, C-4′), 115.1 (d, C-5′), 130.0 (d, C-6′), 136.7 (s, C-1″), 114.0 (d, C-2″), 145.1 (s, C-3″), 145.6 (s, C-4″), 110.7 (d, C-5″), 119.0 (d, C-6″), 56.0 (q, OMe); ESIMS *m/z* (%) 281 $[M - H]^+$ (100), 266 (10), 121 (7); HRTOFMS *m/z* 281.1171 $[M - H]^+$ (calcd for $C_{18}H_{17}O_3$, 281.1178).

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References and Notes

(1) Jiangsu Medical College. *Zhongyaodacidian* (A Dictionary of Traditional Chinese Medicines); Shanghai Science and Technology Publishers: Shanghai, 1985; p 318.
(2) Tomoda, M.; Satoh, N. *Chem. Pharm. Bull.* **1974**, *22*, 2306−2310.
(3) Du, X. H.; Guo, Y. Z. *Shenyang Yaoxueyuan Xuebao* **1990**, *7*, 197−201.
(4) Konishi, T.; Shoji, J. *Chem. Pharm. Bull.* **1979**, *27*, 3086−94.
(5) Tsui, W. Y.; Brown, G. D. *Phytochemistry* **1996**, *43*, 1413−1415.
(6) Soejarto, D. D.; Gyllenhaal, C.; Regalado, J. C.; Pezzuto, J. M.; Fong, H. H. S.; Tan, G. T.; Hiep, N. T.; Xuan, L. T.; Binh, D. Q.; Hung, N. V.; Bich, T. Q.; Thin, N. N.; Loc, P. K.; Vu, B. M.; Southavong, B. H.; Sydara, K.; Bouamanivong, S.; O'Neill, M. J.; Lewis, J.; Xie, X.; Dietzman, G. *Pharm. Biol.* **1999**, *37* (Suppl.), 100−113.
(7) Hoang, V. D.; Tan, G. T.; Zhang, H. J.; Tamez, P. A.; Hung, N. V.; Cuong, N. M.; Soejarto, D. D.; Fong, H. H. S.; Pezzuto, J. M. *Phytochemistry* **2002**, *59*, 325−329.
(8) Terada, K.; Honda, C.; Suwa, K.; Takeyama, S.; Oku, H.; Kamisako, W. *Chem. Pharm. Bull.* **1995**, *43*, 564−566.
(9) Marini-Bettolo, G. B.; Nicoletti, M.; Messana, I.; Galeffi, C.; Msonthi, J. D.; Chapya, W. A. *Tetrahedron* **1985**, *41*, 665−670.
(10) Fujita, N.; Yoshimoto, T.; Samejima, M. *Mokuzai Gakkaishi* **1984**, *30*, 264−268.
(11) Jeong, S. J.; Ahn, N. H.; Kim, Y. C.; Inagaki, M.; Miyamoto, T.; Higuchi, R. *Planta Med.* **1999**, *65*, 367−368.
(12) Gagnaire, D.; Robert, D.; Vignon, M.; Vottero, P. *Eur. Polym. J.* **1971**, *7*, 965−975.
(13) Agrawal, P. K.; Jain, D. C.; Gupta, R. K.; Thakur, R. S. *Phytochemistry* **1985**, *24*, 2479−2496.
(14) Agrawal, P. K.; Bunsawansong, P.; Morris, G. A. *Magn. Reson. Chem.* **1997**, *35*, 441−446.
(15) Miyakoshi, M.; Tamura, Y.; Masuda, H.; Mizutani, K.; Tanaka, O.; Ikeda, T.; Ohtani, K.; Kasai, R.; Yamasaki, K. *J. Nat. Prod.* **2000**, *63*, 332−338.
(16) Sharma, S. C.; Sati, O. P.; Chand, R. *Phytochemistry* **1982**, *21*, 1711−1714.
(17) Nakano, K.; Hara, Y.; Murakami, K.; Takaishi, Y.; Tomimatsu, T. *Phytochemistry* **1991**, *30*, 1993−1995.
(18) Likhitwitayawuid, K.; Angerhofer, C. K.; Cordell, G. A.; Pezzuto, J. M.; Ruangrungsi, N. *J. Nat. Prod.* **1993**, *56*, 30−38.

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