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New Constituents and Antiplatelet Aggregation and Anti-HIV Principles of *Artemisia capillaris*

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Abstract—Five new constituents including a flavonoid, artemisidin A (**1**), and four coumarins, artemicapins A (**2**), B (**3**), C (**4**) and D (**5**), together with 70 known compounds (**6**–**75**), have been isolated and characterized from the aerial part of *Artemisia capillaris*. The structures of these compounds were determined from spectral analyses and/or chemical evidence. Among them, 15 compounds (**3**, **6**, **10**, **18**, **30**–**32**, **38**–**41**, **44**, **45**, **51**, and **55**) showed antiplatelet aggregation activity and three compounds (**10**, **17**, and **51**) demonstrated significant activity against HIV replication in H9 lymphocytic cells.

Introduction

Artemisia capillaris Thunb is a famous traditional Chinese medicine and used mainly as a choleretic, anti-inflammatory, and diuretic agent in the treatment of epidemic hepatitis.[1] In the course of our continuing search for novel biologically active compounds from natural sources, we screened extracts of this plant for thrombolytic and/or vasoactive activity. The methanol extract of the aerial part of *Artemisia capillaris* Thunb displayed antiplatelet aggregation activity. Fractionation and chemical profiling of this active extract led to the isolation and characterization of one new flavonoid, artemisidin A (**1**), and four new coumarins, artemicapins A (**2**), B (**3**), C (**4**), and D (**5**), together with 70 known compounds (**6**–**75**). We describe herein the isolation and structural determination of the new compounds. Based on the preliminary extract screening, all isolated compounds were screened for antiplatelet aggregation activity. In addition, because *A. capillaris* is a main constituent of a folkloric treatment for viral-induced liver inflammation and jaundice, we also screened the isolated compounds for anti-HIV activity. Fifteen compounds (**3**, **6**, **10**, **18**, **30**–**32**, **38**–**41**, **44**, **45**, **51**, and **55**) showed good antiplatelet aggregation activity and three compounds (**10**, **17**, and **51**) demonstrated significant anti-HIV activity.

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Results and Discussion

Artemisidin A (**1**) was isolated as yellow needles. The HRFABMS exhibited a *pseudo*-molecular ion at m/z 643.0843 for $[M^+ + H]$, consistent with the molecular formula $C_{27}H_{30}O_{18}$. The presence of UV bands at 218, 261, and 359 nm and a carbonyl IR band at 1645 cm^{-1} indicated that this compound is a flavonoid.[2] Band I (359 nm) in the UV spectrum was shifted bathochromically by 80 nm when $AlCl_3$ was added into the methanolic solution of **1**, and shifted hypsochromically by 69 nm when HCl was added subsequently. These shifts indicated the presence of two *ortho*-hydroxyl substituents on C-4′ and C-5′ in ring B. Bands I and II (261 nm) showed bathochromic shifts of 21 and 20 nm, respectively, after the addition of NaOAc, indicating the presence of hydroxyl group at C-4′ in ring B and C-7 in ring A.[2] In the ^1H NMR spectrum, four D_2O exchangeable phenolic protons were found at δ 8.48, 9.06, 9.97 and 12.82. The extreme downfield shift of the latter proton was attributed to intramolecular hydrogen bonding between C=O and OH; thus, a hydroxyl substituent should be located on C-5. In the aromatic region of the ^1H NMR spectrum, four singlets at δ 6.76, 6.88, 7.06, and 7.26 were assigned to H-3′, H-8, H-3, and H-6′, respectively. The absence of a substituent at C-8 was also supported by a signal at δ 93.7 in the ^{13}C NMR spectrum.[2] The remaining signals in the ^1H NMR spectrum indicated protons for two glucose moieties attached at C-2′ and C-6: eight D_2O exchangeable protons

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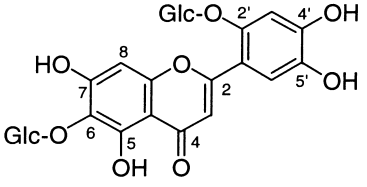
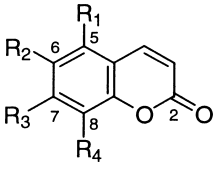
at δ 4.56 (1H, br s), 4.66 (1H, br s), 4.88 (1H, d, $J=7.2$ Hz), 5.0–5.1 (3H, br s), 5.26 (1H, d, $J=4.0$ Hz), and 5.41 (1H, br s) for the two glucose hydroxyls; 12 unresolvable protons at δ 3.1–38 for the two glucose aliphatic protons; and two downfield protons at δ 5.01 (d, $J=7.1$ Hz) and 5.05 (d, $J=8.2$ Hz) for the two glucose anomeric protons. Twelve ^{13}C NMR signals typical for glucose were present at δ 60.6, 60.7, 69.5, 69.6, 73.2, 73.4, 75.7, 75.8, 76.8, 77.2, 100.9, and 101.1. The coupling constant of the anomeric doublet (δ 5.01, 7.1 Hz; 5.05, 8.2 Hz) suggested a β-glucosidic linkage for the flavonoid. Accordingly, artemisidin A was assigned to the structure 4′,5′,5,7-tetrahydroxyflavon-2′,6-di-*O*-β-glucoside (**1**).

Artemicapin A (**2**) was obtained as yellow needles and had the molecular formula $C_{12}H_{10}O_6$. The UV absorptions at 261 and 326 nm and the IR band at 1728 (OC=O) cm^{-1} as well as two AB-type protons at δ 6.06 (d, $J=9.6$ Hz, H-3) and 7.77 (d, $J=9.6$ Hz, H-4) suggested that **2** is a coumarin derivative.[3] Other proton signals were characteristic of a methylenedioxy substituent (6.00, 2H, s) and two aromatic methoxy groups (δ 3.82 and 3.89) attached to the benzene ring. NOE correlation between the methylene (δ 6.00) and both methoxy groups indicated that the two methoxyls are located at C-5 and C-8 of the benzene ring, on either side of the methylenedioxy unit fused to C-6 and C-7. Thus, the structure of artemicapin A (**2**) was assigned as 5,8-dimethoxy-6,7-methylenedioxycoumarin. Additional data to support the structural assignment came from NOESY and HMBC spectral data. The former correlations are shown in Figure 1 and the latter in Figure 2.

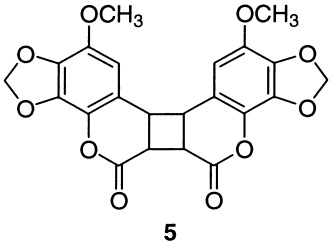
Artemicapin B (**3**), yellow needles, was determined to have the molecular formula $C_{11}H_8O_6$. It had similar spectral data to those of **2** except that one methoxy group in **2** was replaced by a phenolic hydroxyl at δ 8.60 in **3**. The sole methoxy group at δ 3.90 showed NOE correlation with the methylene at δ 6.16 and the olefinic



1



2: $R_1 = OCH_3$, R_2, $R_3 = -OCH_2O-$, $R_4 = OCH_3$
3: $R_1 = OCH_3$, R_2, $R_3 = -OCH_2O-$, $R_4 = OH$
4: $R_1 = H$, $R_2 = OH$, R_3, $R_4 = = -OCH_2O-$



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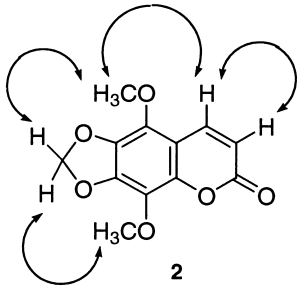

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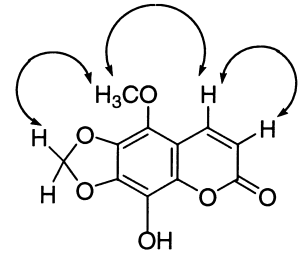

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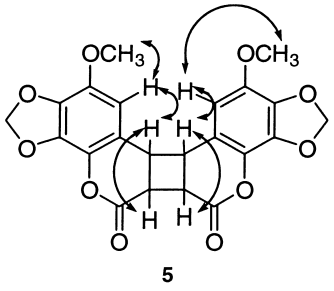
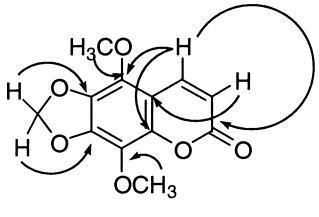


5

Figure 1. NOESY correlations of compounds **2** and **5**.



2

Figure 2. HMBC correlations of compound **2**.

proton H-4 at δ 7.98; these data further confirmed the structure of 8-hydroxy-5-methoxy-6,7-methylenedioxycoumarin (**3**) for artemicapin B.

Artemicapin C (**4**) was obtained as yellow needles and possessed the molecular formula $C_{10}H_6O_5$. The UV and IR spectra were very similar to those of **3**. In the ^1H NMR spectrum, an aromatic singlet at δ 6.78 was attributed to H-5, and two doublets at δ 6.21 and 7.80 (d, $J=9.6$ Hz) were assigned to H-3 and H-4, respectively. These assignments were further supported by the presence of NOE correlation between the signal at δ 6.78 (H-5) and H-4 (δ 7.80). A negative response to Gibbs indophenol test[4] indicated that a hydroxyl substituent (δ 8.19) is located at C-6, and a methylenedioxy (δ 6.19) is fused to C-7 and C-8. Therefore, the structure of 6-hydroxy-7,8-methylenedioxycoumarin (**4**) was assigned to artemicapin C.

Artemicapin D (**5**) was isolated as colorless needles. UV absorptions at 222 and 291 nm and an IR absorption at 1769 cm^{-1} suggested the presence of a benzo-δ-lactone nucleus. The molecular formula $C_{22}H_{16}O_{10}$ was obtained from the HREIMS, $[M]^+$ 440.0743. Two spectral results were indicative of a coumarin dimer: (1) the mass spectral base peak occurred at one-half of the molecular weight (m/z 220) and (2) only 11 carbon signals were present in the ^{13}C NMR spectrum. The ^1H NMR spectrum of **5** displayed a methoxy singlet at δ 3.68, an aromatic singlet at δ 5.95, a methylenedioxy singlet at δ 6.02, and two methine protons at δ 3.9–4.1. NOE correlations between the aromatic proton (δ 5.95), the methoxy group, and one of the methine protons (δ 4.0–4.1, H-4) confirmed one-half of a 6-methoxy-7,8-methylenedioxybenzo-δ-lactone structure. In addition, the presence of two different methines, instead of two vinyl protons, suggested that this dimer was a head-to-head [2+2] cycloadduct of two 6-methoxy-7,8-methylenedioxycoumarins (**43**). Hence, the structure of artemicapin D was established as the cycloadduct **5**.

In addition to those five new compounds described above, 70 known compounds were also isolated: four chromones [capillarisin (**6**),[5] 7-methylcapillarisin (**7**),[6] 6-demethoxycapillarisin (**8**),[6] and 6-demethoxy-4′-methylcapillarisin (**9**)];[6] 20 flavonoids [arcapillin (**10**),[7] cirsilineol (**11**),[6] cirsimaritin (**12**),[6] chrysoeriol (**13**),[8] velutin (**14**),[9] vicenin-II (**15**),[10] vitexin (**16**),[10] isorhamnetin (**17**),[11] quercetin (**18**),[12] kumatakenin (**19**),[13] isorhamnetin-3-*O*-β-D-galactoside (**20**),[14] quercetin-3-*O*-β-D-galactoside (**21**),[14] isorhamnetin-3-*O*-robinobioside (**22**),[15] quercetin-5-glucoside (**23**),[16] isorhamnetin-5-glucoside (**24**),[17] quercetin-3-robinobioside (**25**),[18] luteolin-3′,4′,7-trimethyl ether (**26**),[19] hesperidin (**27**),[20] liquiritin (**28**),[21] and lucenin (**29**)];[22] 11 phenylalkynes [capillaridins A (**30**),[23] B (**31**),[23] C (**32**),[23] D (**33**),[23] E (**34**),[23] F (**35**),[23] G (**36**),[23] and H (**37**),[23] capillene (**38**),[24] capillin (**39**),[25] and *O*-methoxycapillene (**40**)];[26] 13 coumarins [scoparone (**41**),[27] isosabaudin (**42**),[28] 6-methoxy-7,8-methylenedioxycoumarin (**43**),[29] isoscopoletin (**44**),[27] scopoletin (**45**),[33] 5,7,8-trimethoxycoumarin (**46**),[30] 5-hydroxy-6,7-dimethoxycoumarin (**47**),[31] arscotin (**48**),[32] leptodactylone (**49**),[30] scopolin (**50**),[31] aesculetin (**51**),[34] isoscopolin (**52**),[27] and fraxinol methyl ether (**53**)];[35] 15 benzenoids [alkyl-*p*-hydroxycinnamate (**54**),[36] caffeic acid (**55**),[37] ferulic acid (**56**),[38] methyl ferulate (**57**),[36] 3-hydroxy-4-methoxycinnamic acid (**58**),[38] methyl *p*-hydroxycinnamate (**59**),[36] *trans*-*p*-hydroxycinnamic acid (**60**),[39] methyl paraben (**61**),[40] vanillin (**62**),[41] 3,4-dimethoxybenzoic acid (**63**),[42] resacetophenone (**64**),[43] vanillic acid (**65**),[44] 2,6-dimethoxybenzoquinone (**66**),[45] quinic acid 4-*O*-coumarate (**67**),[46] and chlorogenic acid (**68**)];[46] four lignans [(+)-sesamin

Table 1. Effects of flavonoids and chromone isolated from *Artemisia capillaris* on the aggregation of washed rabbit platelets induced by arachidonic acid (AA), collagen (Col), PAF and thrombin (Thr)

Inducer	Control	Concn (μg/mL)	Inhibition (%)						
			Aspirin	**6**	**10**	**11**	**12**	**15**	**18**
AA	100.0±0.3	10.0	52.5±17.0		6.4±2.8				3.9±0.8[c]
(100 μM)		20.0	100.0±0.0		44.8±20.2				100.0±0.0[c]
		50.0			A[d]				
		100.0		20.2±9.9[b]	A	16.1±10.0[a]	29.2±16.1[a]	1.2±1.1	100.0±0.0[c]
Col (10 μg/mL)	100.0±1.0	10.0			7.3±5.0				
		20.0	4.6±4.2		27.1±17.4[a]				12.0±3.9[b]
		50.0			57.2±5.8				69.7±15.1[c]
		100.0		68.3±14.4[c]	13.8±3.0	44.2±7.7[c]	38.9±13.3[a]	1.7±1.5	100.0±0.0[c]
PAF (2 nM)	100.0±1.1	10.0							
		20.0			1.5±1.3				
		50.0							
		100.0		−0.7±0.9		0.2±1.2	0.4±0.7	2.8±0.8[b]	46.2±0.9[c]
Thr (0.1 μ/mL)	100.0±1.7	100.0		2.1±1.8	A	−1.3±0.9	3.2±2.9	−1.1±0.9	18.5±6.5[b]
		50.0			A				
		20.0			0.9±1.2				

Platelets were preincubated with flavonoids and chromone or DMSO (0.5%, control) at 37 °C for 3 min before the inducer was added. Values are means±s.e.m. ($n=3$–4).

[a] $P<0.05$.

[b] $P<0.01$.

[c] $P<0.001$ as compared with the respective control.

[d] 'A'—caused platelet aggregation.

Table 2. Effects of coumarins isolated from *Artemisia capillaris* on the aggregation of washed rabbit platelets induced by arachidonic acid (AA), collagen (Col), PAF and thrombin (Thr)

			Inhibition (%)											
Inducer	Control	Concn 4 (μg/mL)	Aspirin	**2**	**3**	**4**	**41**	**43**	**44**	**45**	**46**	**50**	**51**	**52**
AA (100 μM)	100.0±0.3	1.0					7.5±0.8[a]							
		2.0					8.6±0.8[b]							
		5.0	2.6±1.7				19.6±2.6[c]			16.2±6.2[a]			−1.1±1.7	
		10.0	52.5±17.0				27.6±1.1[c]			67.4±11.7[b]			10.8±4.1[a]	
		20.0	100.0±0.0				65.9±14.3[c]			79.9±19.5[c]			70.7±18.3[b]	
		50.0			3.6±1.1		100.0±0.0[c]		7.4±7.1	93.6±3.3[c]			100.0±0.0[c]	
		100.0		10.4±4.4[a]	100.0±0.0[c]	−0.1±2.3	100.0±0.0[c]	6.5±4.4	53.6±23.9[a]	100.0±0.0[c]	15.0±7.5	5.4±3.6	100.0±0.0[c]	0.8±1.5
Col (10 μg/mL)	100.0±1.0	1.0					7.3±1.9[a]							
		2.0					12.7±3.1[c]							
		5.0					26.1±9.3[b]							
		10.0					41.9±13.2[c]						2.0±2.2	
		20.0	4.6±4.2				68.3±12.0[c]						16.0±16.6	
		50.0			4.5±2.6		95.5±2.7[c]						54.7±16.6[b]	
		100.0		15.9±4.1[b]	53.1±17.5[b]	3.9±1.7	96.7±2.8[c]	10.0±4.8	49.2±14.1[b]	81.0±2.1[c]	8.7±3.0[b]	1.0±1.0	67.7±17.5[b]	3.2±2.4
PAF (2 nM)	100.0±1.1	100.0		11.1±3.6[a]	2.9±0.9[a]	2.5±1.4		1.8±2.6	1.2±1.6	3.9±1.5	9.9±0.9[a]	1.1±0.8	1.6±0.5	2.5±2.5
Thr (0.1 μ/mL)	100.0±1.7	100.0		6.6±3.3	1.6±0.9	1.6±1.3	16.3±3.6[b]	2.1±1.8	1.1±1.4	1.8±2.1	6.6±3.0	−2.0±1.4	1.6±1.8	1.6±1.3

Platelets were preincubated with coumarins or DMSO (0.5%, control) at 37 °C for 3 min before the inducer was added. Values are means±s.e.m. (n = 3-4).

[a] $P < 0.05$.

[b] $P < 0.01$.

[c] $P <$ 0.001 as compared with the respective control.

Table 3. Effects of phenylacetylenes and caffeic acid isolated from *Artemisia capillaris* on the aggregation of washed rabbit platelets induced by arachidonic acid (AA), collagen (Col), PAF and thrombin (Thr)

			Inhibition (%)								
Inducer	Control	Concn (μg/mL)	Aspirin	**30**	**31**	**32**	**35**	**38**	**39**	**40**	**55**
AA (100 μM)	100.0±0.3	2.0							9.2±0.9[c]		
		5.0	2.6±1.7			13.8±1.0[c]		4.4±1.5[a]	64.7±17.6[b]	19.7±7.1[a]	
		10.0	52.5±17.0		3.3±3.6	64.1±17.9[b]		58.5±20.7[a]	100.0±0.0[c]	82.2±12.7[c]	
		20.0	100.0±0.0		17.8±9.2	100.0±0.0[c]		100.0±0.0[c]		100.0±0.0[c]	2.1±2.3
		50.0		2.7±1.1[a]	100.0±0.0[c]						39.2±17.6[a]
		100.0		100.0±0.0[c]	100.0±0.0[c]	100.0±0.0[c]	4.2±3.4	100.0±0.0[c]	100.0±0.0[c]	100.0±0.0[c]	100.0±0.0c
Col (10 μg/mL)	100.0±1.0	2.0				0.9±1.2			10.1±2.4		
		5.0				57.2±21.5[a]	3.0±4.1	0.1±1.1	100.0±0.0[c]	5.8±3.0	
		10.0				88.6±9.9[c]		24.6±8.9[a]		43.7±17.1[a]	
		20.0	4.6±4.2		3.8±4.3	100.0±0.0[c]		83.4±10.1[c]		91.3±7.5[c]	
		50.0		−0.4±0.4	50.3±24.8[a]			100.0±0.0[c]		100.0±0.0[c]	
		100.0		71.6±19.1[c]	87.3±10.3[c]	100.0±0.0[c]	4.6±3.1	100.0±0.0[c]	100.0±0.0[c]	100.0±0.0[c]	13.3±5.2[a]
PAF (2 nM)	100.0±1.1	5.0				3.2±2.8			6.2±4.1		
		10.0				17.3±3.3[c]			25.1±1.8[c]	5.2±2.4[a]	
		20.0				76.6±10.8[c]		10.3±0.5[c]	65.8±2.8[c]	17.0±1.3	
		50.0				98.4±1.4[c]		59.8±2.3[c]	100.0±0.0[c]	85.2±2.8	
		100.0		8.3±2.0[c]	2.1±1.7	100.0±0.0[c]		98.4±1.4[c]	100.0±0.0[c]	100.0±0.0[c]	4.8±2.2[a]
Thr (0.1 μ/mL)	100.0±1.7	5.0				8.5±1.5[b]			12.1±1.9[c]	4.8±3.4	
		10.0				49.0±6.3[c]		4.3±2.0	46.3±7.4[c]	13.7±1.9[c]	
		20.0				94.8±4.5[c]		34.6±2.3[c]	96.6±2.9[c]	89.9±8.3[c]	
		100.0		−1.1±0.6	2.1±0.9	100.0±0.0[c]	−2.3±0.9	95.5±3.6	100.0±0.0[c]	100.0±0.0[c]	3.2±1.5

Platelets were preincubated with phenylacetylenes or DMSO (0.5%, control) at 37 °C for 3 min before the inducer was added. Values are means ±s.e.m. (n = 3-4).

[a] $P < 0.05$.

[b] $P < 0.01$.

[c] $P < 0.001$ as compared with the respective control.

(**69**),[47] 9-β-xylopyranosyl-(+)-isolariciresinol (**70**),[48] pluviatide (**71**),[49] and honokiol (**72**)];[50] one diterpene [phytal (**73**)];[51] and two chlorophylls [13^2-hydroxy(13^2-*R*)pheophytin b (**74**)[52] and 13^2-hydroxy(13^2-*S*)pheophytin a (**75**)].[52] The structures of these known compounds were characterized by spectroscopic analyses and/or by comparison with data reported in the literature.

All isolated compounds were evaluated for antiplatelet aggregation and anti-HIV activities. Among them, arcapillin (**10**), aesculetin (**51**) and isorhamnetin (**17**) inhibited HIV replication in H9 lymphocyte cells with ED_{50} values of 17.3, 2.51, and 1.78 μg/mL and therapeutic indexes of > 5.8, 11.2, and 15.9, respectively. On the other hand, capillarisin (**6**), quercetin (**18**), scopoletin (**45**), arcapillin (**10**), isoscopoletin (**44**), scoparone (**41**), aesculetin (**51**), artemicapin B (**3**), capillaridin A (**30**), capillaridin B (**31**), capillaridin C (**32**), capillene (**39**), capillin (**38**), *O*-methylcapillene (**40**) and caffeic acid (**55**) all showed significant antiplatelet aggregation activity (Tables 1–3). According to the different antiplatelet specificity with different inducers, these compounds can be classified into three groups. Group 1 compounds such as **3**, **6**, **18**, **30**, **31**, **44**, **45**, **51**, and **55** were more selective inhibitors of the platelet aggregation induced by arachidonic acid and collagen. Group 2 compounds such as **32** and **38**–**40** inhibited platelet aggregation induced by all four inducers (arachidonic acid, collagen, platelet-activating factor, and thrombin). A single group 3 compound (**10**) showed inhibition at low concentration, but induced spontaneous platelet aggregation at high concentrations, even in the absence of any inducer. The action of the first group is quite similar to that caused by aspirin. However, for groups 2 and 3, additional studies are needed to ascertain whether thromboxane formation is inhibited and to determine the detailed action mechanisms.

Experimental

General

Melting points were measured on a Yanagimoto MP-S_3 micromelting point apparatus. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AC-200, AMX-400, and Varian Unity Plus 400 spectrometers. Chemical shifts are shown in δ values (ppm) with tetramethylsilane (TMS) as internal reference. EIMS, FABMS, and HRMS were taken with a VG 70-250 S spectrometer by direct inlet system. UV spectra were recorded on a Hitachi UV-3210 double beam spectrophotometer. IR spectra were recorded on Jasco IR Report-100 and Shimadzu FTIR-8501 spectrophotometers. Specific optical rotations were recorded on a Jasco DIP-370 polarimeter. CD and ORD spectra were measured on a Jasco-J-720.

Plant material

Artemisia capillaris Thunb was collected from Chaiyi Hsien, Taiwan in September 1992 and verified by Prof.

C. S. Kuoh. A specimen of this plant is deposited in the herbarium of National Cheng Kung University, Tainan, Taiwan.

Extraction and isolation

The hot methanol extract of the aerial part of *A. capillaria* Thunb (12.5 kg) was concentrated under reduced pressure and the crude syrup was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ layer was subjected to chromatography on silica gel column eluted with $CHCl_3$–Me_2CO to give seven fractions. Rechromatography of each fraction gave the following pure compounds. Fraction 1 afforded **38** (1.98 g), **39** (0.15 g), **40** (43.3 mg), **30** (17.6 mg), **31** (0.35 g), **32** (50.9 mg), **33** (1.0 mg), **34** (4 mg), **35** (5.7 mg), **36** (2.5 mg), **37** (1.7 mg), **42** (0.28 g), **2** (0.11 g), **3** (7.4 mg), **46** (13.6 mg), **54** (3.2 mg), **69** (17.6 mg), **73** (1.0 mg), **74** (2.3 mg) and **75** (1.6 mg). Fraction 2 gave **43** (0.21 g) and **5** (7.6 mg). Fraction 3 yielded **7** (2.6 mg), **12** (0.56 g), **13** (4.4 mg), **48** (2.2 mg), **53** (1.8 mg), and **72** (1.6 mg). Fraction 4 afforded **9** (2.2 mg), **26** (1.0 mg), **44** (53.4 mg), **45** (80.4 mg), **47** (3.1 mg), **4** (3.4 mg), and **49** (2.5 mg). Fraction 5 provided **11** (2.1 mg). Fraction 6 furnished **6** (0.56 g) and **41** (12.00 g). Fraction 7 yielded **8** (4.3 mg), **13** (3.1 mg), **10** (36.6 mg), **61** (2.5 mg), **62** (1.0 mg), **66** (2.4 mg), and **71** (1.5 mg). The H_2O layer was repartitioned again between H_2O and *n*-BuOH. The *n*-BuOH layer was chromatographed on a dianion column and eluted with CH_3OH–H_2O to give 10 fractions. Each fraction was rechromatographed on a Sephadex LH-20 column. Fraction 2 gave **68** (0.20 g); fraction 3 gave **67** (30.5 mg); fraction 4 gave **1** (4.4 mg), **19** (6.2 mg), **51** (6.4 mg), **52** (1.82 g), **55** (15.8 mg), **56** (2.6 mg), **57** (2.0 mg), **58** (1.8 mg), **59** (2.9 mg), **63** (4.3 mg), **64** (2.5 mg) and **65** (5.7 mg); fraction 5 gave **50** (79.8 mg) and **60** (4.3 mg); fraction 7 gave **15** (1.4 mg), **21** (34.5 mg) and **29** (1.0 mg); fraction 8 gave **16** (5.5 mg), **17** (7.9 mg), **18** (19.0 mg), **20** (80.5 mg), **22** (0.14 g), **23** (97.3 mg), **24** (5.6 mg), **25** (70.6 mg), **27** (15.3 mg), **28** (45.1 mg) and **70** (1.2 mg).

Artemisidin A (1). Yellow needles (MeOH): mp $>300\,^{\circ}C$; HRFABMS *m/z* 643.0843 $[M+H]^+$ (calcd for $C_{27}H_{30}O_{18}$ 643.0845); UV λ_{max} (MeOH) nm 261, 280 (sh), 359; λ_{max} (MeOH + NaOAc) nm 281, 307 (sh), 380; λ_{max} (MeOH + $AlCl_3$) nm 267, 303 (sh), 439; λ_{max} (MeOH + $AlCl_3$ + HCl) nm 263 (sh), 298, 370; IR ν_{max} cm^{-1} 3450, 1645, 1562, 1546; FABMS *m/z* (rel. int.) 643 ($M^+ + H$, 100); ^1H NMR (DMSO-d_6) δ 3.1–3.8 (12H, m, sugar CH), 4.56 (1H, br s, OH), 4.66 (1H, br s, OH), 4.88 (1H, d, $J=7.2$ Hz, OH), 5.0–5.1 (3H, br s, 3OH), 5.01 (1H, d, $J=7.1$ Hz, H-1″), 5.05 (1H, d, $J=8.2$ Hz, H-1‴), 5.26 (1H, d, $J=4.0$ Hz, OH), 5.41 (1H, br s, OH), 6.76 (1H, s, H-3′), 6.88 (1H, s, H-8), 7.06 (1H, s, H-3), 7.26 (1H, s, H-6′), 8.48 (1H, br s, OH), 9.06 (1H, br s, OH), 9.97 (1H, br s, OH), 12.82 (1H, br s, OH); ^{13}C NMR (DMSO-d_6) δ 60.6 (C-6″), 60.7 (C-6‴), 69.5 (C-4″), 69.6 (C-4‴), 73.2 (C-2‴), 73.4 (C-2‴), 75.7 (C-5″), 75.8 (C-5‴), 76.8 (C-3‴), 77.2 (C-3″), 93.7 (C-8), 100.9 (C-1‴), 101.1 (C-1″), 104.0 (C-3′), 105.7 (C-10), 108.3 (C-1′), 110.5 (C-3), 114.5 (C-6′), 130.3 (C-6), 140.3 (C-2′), 146.6 (C-5), 149.2 (C-9), 150.1 (C-4′ and C-5′), 151.3 (C-7), 161.4 (C-2), 182.5 (C-4).

Artemicapin A (2). Yellow needles ($CHCl_3$): mp 120–121 °C; HREIMS *m/z* 250.0477 $[M]^+$ (calcd for $C_{12}H_{10}O_6$ 250.0477); UV λ_{max} (MeOH) nm 261, 316; λ_{max} cm^{-1} 1728, 1643, 1585; EIMS *m/z* (rel. int.) 250 (M^+, 100), 235 (83), 207 (6), 177 (8); ^1H NMR ($CDCl_3$) δ 3.82 (3H, s, 5-OMe), 3.89 (3H, s, 8-OMe), 6.00 (2H, s, OCH_2O), 6.06 (1H, d, $J=9.6$ Hz, H-3), 7.77 (1H, d, $J=9.6$ Hz, H-4); ^{13}C NMR ($CDCl_3$) δ 60.3 (8-OMe), 62.1 (5-OMe), 102.9 (OCH_2O), 108.1 (C-9), 112.6 (C-3), 130.2 (C-6), 132.6 (C-10), 133.8 (C-8), 138.8 (C-4), 142.3 (C-7), 143.2 (C-5), 159.2 (C-2).

Artemicapin B (3). Yellow needles (MeOH): mp 210–212 °C; HREIMS *m/z* 236.0320 $[M]^+$ (calcd for $C_{11}H_8O_6$ 236.0321); UV λ_{max} (MeOH) nm 210, 260, 334; IR ν_{max} cm^{-1} 3250, 1693, 1641, 1581; EIMS *m/z* (rel. int.) 236 (M^+, 100), 221 (91), 95 (16); ^1H NMR (acetone-d_6) δ 3.90 (3H, s, 5-OMe), 6.16 (2H, s, OCH_2O), 6.19 (1H, d, $J=9.8$ Hz, H-3), 7.98 (1H, d, $J=9.8$ Hz, H-4), 8.60 (1H, s, OH).

Artemicapin C (4). Yellow needles (MeOH): mp 225–226 °C; HREIMS *m/z* 206.0215 $[M]^+$ (calcd for $C_{10}H_6O_5$ 206.0216); UV λ_{max} (MeOH) nm 212, 254 (sh), 332; IR ν_{max} cm^{-1} 3220, 1679, 1626, 1585; EIMS *m/z* (rel. int.) 206 (M^+, 100), 178 (30), 120 (14), 108 (13), 92 (19), 79 (18); ^1H NMR (acetone-d_6) δ 6.19 (2H, s, OCH_2O), 6.21 (1H, d, $J=9.6$ Hz, H-3), 6.78 (1H, s, H-5), 7.80 (1H, d, $J=9.6$ Hz, H-4), 8.19 (1H, s, 6-OH).

Artemicapin D (5). Colorless needles ($CHCl_3$): mp 138–139 °C; HREIMS *m/z* 440.0743 $[M]^+$ (calcd for $C_{22}H_{16}O_{10}$ 440.0747); UV λ_{max} (MeOH) nm 222, 291 (sh); λ_{max} cm^{-1} 1769, 1514; EIMS *m/z* (rel. int.) 440 (M^+, 3), 220 (100), 205 (11), 192 (22), 91 (19), 79 (21); ^1H NMR ($CDCl_3$) δ 3.68 (6H, s, 2×6-OMe), 3.98 (2H, m, 2×H-3), 4.08 (2H, m, 2×H-4), 5.95 (2H, s, 2×H-5), 6.02 (4H, s, 2×OCH_2O); ^{13}C NMR ($CDCl_3$) δ 39.6 (C-3), 40.5 (C-4), 56.8 (6-OMe), 102.9 (OCH_2O), 106.7 (C-5), 111.2 (C-9), 131.6 (C-10), 135.8 (C-6), 137.1 (C-8), 140.4 (C-7), 163.1 (C-2).

Biology

Anti-HIV assay

The anti-HIV assay was carried out according to the procedure described in the literature.[53]

Antiplatelet aggregation assay

The antiplatelet aggregation assays were based on a method reported by Teng et al.[54]

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