The Constituents of the Root and Stem of *Aristolochia cucurbitifolia* Hayata and Their Biological Activity

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Four new compounds, three phenanthrene derivatives, aristolochic acid-III methyl ester (1), cepharanone C (2), and sodium 7-hydroxyl-8-methoxyaristolate (3), and the benzoate derivative, sodium 3,4-dimethoxybenzoate (4), together with 53 known compounds were isolated and characterized from the fresh root and stem of *Aristolochia cucurbitifolia*. Their structures were elucidated by spectral analyses and chemical transformations. The cytotoxicity and antiplatelet activity of the isolated compounds are also discussed.

Key words *Aristolochia cucurbitifolia*; Aristolochiaceae; aristolochic acid derivatives; antiplatelet agent; cytotoxic agent

The genus *Aristolochia* grows over wide areas from the tropics to temperate zones and consists of about 400 species.[1)] The fruits and roots have been used in traditional Chinese medicine as anodynes, antiphlogistics, antitussives, expectorants, and antiasthmatic agents, and also for the treatment of snakebite and lung inflammation.[2)] Aristolochic acid derivatives, often isolated as major components from the plant of the genus *Aristolochia*, have been characterized as tumor inhibitors.[3,4)] Our chemotaxonomic and pharmacological interests led us to investigate the constituents of the stem and root of *A. cucurbitifolia*. We report here the isolation and structural elucidation of four new compounds, **1**—**4**, and 53 known compounds. In addition, the antiplatelet activity and cytotoxicity of the compounds isolated from the root and stem of *A. cucurbitifolia* are also discussed.

Results and Discussion

Aristolochic acid-III methyl ester (**1**) was obtained as yellowish needles. The high resolution electron ionization mass spectrum (HREIMS) of **1** showed a molecular ion peak at *m/z* 355.0695, indicating a molecular formula of $C_{18}H_{13}NO_7$. The UV absorption of **1** at 216, 256, 278, 300, 350, and 384 nm showed it was a phenanthrene derivative.[5)] The IR spectrum showed the presence of a nitro group at 1525 and 1346 cm^{-1}. In the aromatic region of the ^{1}H-NMR spectrum, ABX-type signals at δ 7.34 (1H, dd, J=8.8, 2.4 Hz), 7.90 (1H, d, J=8.8 Hz), and 8.60 (1H, d, J=2.4 Hz) were attributed to H-7, H-8, and H-5, respectively. The signal of H-5 appeared at lower field at δ 8.60, due to the deshielding effect of the A ring in the aristolochic acid derivative. Two singlet signals appearing at δ 7.76 and 8.31 (each 1H) were assigned to H-2 and H-9, respectively. The methoxyl groups appeared at δ 4.02 (3H, s) and 3.87 (3H, s). In addition, δ 6.39 (2H, s) was assigned to the methylenedioxy group which was further confirmed by peaks in the IR spectrum at 1045 and 941 cm^{-1}. Based on the above results, the structure of aristolochic acid-III methyl ester is represented as **1**, which is reported for the first time to isolate from nature.

Cepharanone-C (**2**) was isolated as a yellowish powder. Its molecular formula was determined to be $C_{17}H_{11}NO_5$ by HR-EIMS ($[M]^+$ *m/z* 309.0634). The UV absorption at 236 (sh), 241, 276 (sh), 288, 357, 373 and 395 nm was consistent with a typical aristolactam derivative,[5)] which was also supported by the positive reaction with Dragendroff reagent. The ^{1}H-NMR spectrum of **2** showed signals attributable to a methylenedioxy, a methoxyl, and a hydroxyl group at δ 6.42 (2H, s), 3.85 (3H, s) and 10.73 (1H, br s, D_2O exchange disap.), respectively. A set of *ortho* coupling aromatic protons appeared at δ 8.13 and 7.14 (each 1H, d, J=8.8 Hz). The lower field signal is the characteristic signal of H-5 in an aristolactam derivative. Two singlet signals at δ 7.55 (1H, s) and δ 7.18 (1H, s) could be assigned to H-2 and H-9, respectively. The relative substitution of methoxy and hydroxyl groups was deduced from the nuclear Overhauser effect spectroscopy (NOESY) experiment (Fig. 1), which showed the correlations of H-6 (δ 7.14) to H-5 and 7-OMe (δ 3.85). Thus, the structure of cepharanone-C was assigned as **2**.

Sodium 7-hydroxyl-8-methoxyaristolate (**3**) was isolated as a colorless powder. The UV absorption at 225, 265 (sh), 273, 296, 319, 352 and 371 nm, combined with the lack of a NO_2 band in the IR spectrum indicated **3** was a phenanthrene derivative.[5)] In the ^{1}H-NMR spectrum, a set of doublet signals at δ 8.27 and 7.78 (each 1H, d, J=9.5 Hz) were assigned to H-10 and H-9, respectively. Two *ortho*-coupling protons at δ 8.64 and 7.12 (each 1H, d, J=9.1 Hz) were assigned to H-5 and H-6. A singlet at δ 7.31 (1H, s) was attributed to H-2. The methoxyl and methylenedioxy groups appeared at δ 3.92 (3H, s) and 6.20 (2H, s). The positions of the methoxy and hydroxyl groups were determined by the observed NOESY correlation of 8-OMe (δ 3.92) to H-9 (δ 7.78). Based on these data, compound **3** was proposed to be 7-hydroxyl-8-methoxyaristolic acid. However, the carboxylic group appearing at 1558 cm^{-1} in the IR spectrum suggested compound **3** was in salt form.[6)] Treatment of **3** with 5% HCl, followed by purification of the solution on a Sephadex LH-20

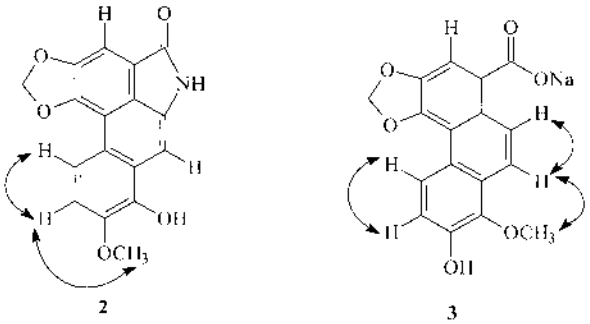

Fig. 1. The NOE Correlations of **2** and **3**

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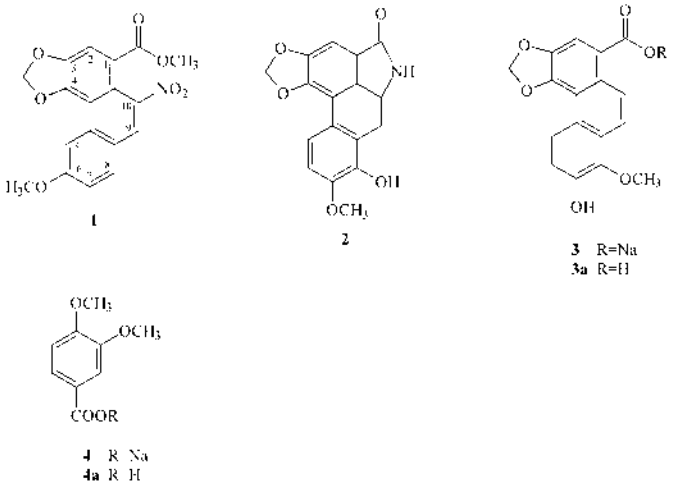
column, afforded sodium chloride which was determined by atomic absorption spectrometry. Thus, the structure of **3** was assigned as sodium 7-hydroxyl-8-methoxyaristolate.

Sodium 3,4-dimethoxybenzoate (**4**) was isolated as a colorless powder. The UV spectrum showed absorptions at 205, 252, 286 and 291 nm, which suggested a benzenoid. In the ^{1}H-NMR spectrum, there was a set of ABX-type signals at δ 7.57 (1H, d, J=1.9 Hz), 7.47 (1H, dd, J=8.0, 1.9 Hz) and 6.75 (1H, d, J=8.0 Hz), and two methoxyl groups at δ 3.89 and 3.61 (each 3H, s). These data are similar to those of 3,4-dimethoxy benzoic acid.[7] However, the IR spectrum of **4** displayed a carboxylic absorption at 1602 cm^{-1}, indicating a salt. Following acidification of **4** with 5% HCl, the solution was applied to a Sephadex LH-20 column and eluted with H_2O and then MeOH. The residue obtained from the H_2O fraction was confirmed to contain sodium ion by atomic absorption spectroscopy. The MeOH eluted fraction afforded **4a** which was identified as 3,4-dimethoxy benzoic acid. On the basis of the above data, compound **4** was assigned as sodium 3,4-dimethoxybenzoate.



Although aristolochic acid derivatives are usually isolated from the *Aristolochia* genus in free form, we had also isolated them in salt form.[6,8—11)]

The known compounds, sodium (2*R*)-3-(*p*-hydroxylphenyl) lactate (**5**)[8] methyl pheophorbide-a (**6**),[12] aristolochic acid-I methyl ester (**7**),[13] methyl-21-hydroxyl-(21*R*)-pheophorbide-a (**8**),[14] isoscopoletin (**9**),[15] aristolochic acid-II methyl ester (**10**),[16] (−)hinokinin (**11**),[17] aristolide-A (**12**),[18] aristolide-B (**13**),[18] aristolochic acid-IV methyl ester (**14**),[16] aristolactam-I (**15**),[16] -AII (**16**),[16] -BII (**17**),[19] -IIIa (**18**),[8] 9-methoxyaristolactam-I (**19**),[16] 9-methoxyaristolactam IV (**20**),[20] piperolactam-A (**21**),[21] cepharadione-A (**22**),[16] cepharanone-A (**23**),[16] *N-trans*-cinnamoyltyramine (**24**),[22] sodium aristolochate-I (**25**),[6] -II (**26**),[9] -III (**27**),[6] -IVa (**28**),[9] sodium 7-hydroxylaristolochate (**29**),[6] aristolic acid (**30**),[10] asimilobine (**31**),[23] 6-*O*-(*E*)-feruloyl-(α and β)-glucopyranoside (**32**),[24] adenine (**33**),[25] glucosyringic acid (**34**),[26] 4-hydroxylbenzoic acid (**35**),[8] salidroside (**36**),[27] 4-hydroxylcinnamic acid (**37**),[8] 4-hydroxyl-3-methoxycinnamic acid (**38**),[8] vanillic acid (**39**),[28] isorhamnetin-3-*O*-robinobioside (**40**),[16] 4,5-dioxodehydroasimilobine (**41**),[11] aristolochic acid-C (**42**),[16] -I (**43**),[16] *cis*- and *trans*-4-hydroxyl-3-methoxycinnamic acid methyl ester (**44**),[8] allantoin (**45**),[16] aristoloterpenate-III (**46**),[29] friedelin (**47**),[30] stigment-4-ene-3,6-dione (**48**),[9] β-sitosterol (**49**),[9] stigmasterol (**50**),[9] madolin-A (**51**),[31] -B (**52**),[31] -C (**53**),[31] -D (**54**),[31] -E (**55**),[31] aristolactone (**56**)[31] and manshurolide (**57**)[31] were also isolated and characterized by comparison of their spectroscopic data (UV, IR, NMR and MS) with literature values.

Table 1. The Effects of Compounds Isolated from the Stem and Root *Aristolochia cucurbitifolia* on the Aggregation of Washed Rabbit Platelets Induced by Collagen, Thrombin (Thr), Arachidonic Acid (AA) and Platelet Activation Factor (PAF)

Compounds	Inhibition (%)									
Ind. agent	Thr (0.1 μM/ml)		AA (100 μM)			Collagen (10 μg/ml)			PAF (2 ng/ml)	
Compds conc. (μg/ml)	100	50	100	50	20	100	50	20	100	50
Methyl pheophorbide-a (**6**)	A	A	A	A	0.9±1.1	N	N	0.1±4.1	N	N
Aristolochic acid-I methyl ester (**7**)	N	1.7±1.7	N	N	N	N	1.6±5.1	N	N	11.3±0.8‡
Aristolide-A (**12**)	−0.6±1.8	N	100.0±0.0‡	89.6±8.6‡	11.26±7.0	45.2±15.1*	15.5±6.4*	0.5±3.8	22.5±4.8‡	N
Aristolactone (**56**)	−1.5±0.8	N	91.7±6.8‡	11.2±7.3	N	13.5±4.5*	N	N	48.3±21.7*	N
Manshurolide (**57**)	−3.2±2.6	N	100.0±0.0‡	77.3±11.5‡	18.2±6.7*	21.9±5.9†	N	N	51.1±12.3‡	N
Aristolactam-I (**15**)	1.5±1.3	N	13.1±2.8	N	N	10.3±2.4	N	N	31.4±2.9	N
Aristolactam-AII (**16**)	0.8±3.9	N	100.0±0.0‡	100.0±0.0‡	95.3±3.7‡	46.8±10.2†	N	N	10.6±4.1†	N
Piperolactam-A (**21**)	4.0±3.4	N	100.0±0.0‡	100.0±0.0‡	10.3±1.6‡	100.0±0.0‡	60.6±21.1*	22.3±2.4	38.8±13.8†	N
Cepharanone-A (**23**)	4.0±1.7	N	4.7±1.6	N	N	8.3±1.9	N	N	25.6±1.5	N
Sodium aristolochate-I (**25**)	1.9±1.5	N	0.1±1.8	N	N	3.3±2.7	N	N	1.8±1.4	N
Sodium aristolochate-II (**26**)	−3.7±1.0	N	4.3±1.8	N	N	1.8±4.9	N	N	7.5±1.8†	N
Sodium aristolochate-III (**27**)	−4.1±3.8	N	2.6±1.2	N	N	1.7±2.6	N	N	5.8±0.5†	N
Sodium aristolochate-IVa (**28**)	4.6±2.3	N	10.8±2.9†	N	N	21.5±8.9*	N	N	1.7±0.4	N
Aristolactam-IIIa (**18**)	−0.1±1.7	N	−0.5±1.8	N	N	11.6±1.0	N	N	6.0±2.4	N
Sodium (2*R*)-3-(*p*-hydroxylphenyl) lactate (**5**)	2.9±1.3	N	0.8±0.2	N	N	2.4±2.0	N	N	0.7±1.0	N
Adenine (**33**)	3.8±2.3	N	0.0±0.4	N	N	2.6±1.9	N	N	1.7±0.3	N
Glucosyringic acid (**34**)	3.1±1.6	N	4.5±1.8*	N	N	2.8±2.0	N	N	2.1±0.8	N
Isorhamnetin-3-*O*-robinobioside (**40**)	−0.4±1.4	N	18.3±1.1	N	N	3.9±1.1	N	N	0.5±2.1	N
Aristolochic acid-C (**42**)	−0.1±1.7	N	−0.5±1.8	N	N	11.6±1.1	N	N	6.0±2.4	N
Aristolochic acid-I (**43**)	4.4±1.2‡	N	21.66±5.3†	N	N	22.4±7.9*	N	N	10.4±1.7*	N
Allantoin (**45**)	4.3±3.2	N	3.2±1.4	N	N	1.4±1.9	N	N	1.2±1.1	N

Platelets were preincubated with compounds or DMSO (0.5%, control) at 37 °C for 3 min; the inducer was added. Values are means±S.E.M. (n=3—4). N=not tested. A=platelet aggregation promoted. *p<0.05, †p<0.01, ‡p<0.001 were compared with the respective control.

Table 2. Cytotoxicity of Compounds Isolated from the Stem and Root *Aristolochia cucurbitifolia*

Compounds	ED_{50} conc. (μg/ml)				
Cell lines	KB	P-388	A-549	HT-29	HL-60
Aristolactam-I (**15**)	3.3	1.0	3.2	2.6	2.4
Aristolactam-AII (**16**)	>50	1.3	2.7	13.2	12.9
Aristolactam-IIIa (**18**)	>50	8.8	>20	17.1	40
Cepharanone-A (**23**)	4.1	2.3	1.7	3.3	3.9
Aristolochic acid-C (**42**)	>50	8.8	>20	17.1	40
Aristolochic acid-I (**43**)	4.0	0.7	5.0	8.3×10^{-4}	3.4

KB: human epidermoid carcinoma; P-388: mouse lymphocytic leukaemia; A-549: lung adenocarcinoma; HT-29: colon adenocarcinoma; HL-60: human leukaemia.

Compounds **5**, **7**, **12**, **15**, **16**, **18**, **21**, **23**, **25**—**28**, **33**, **34**, **40**, **42**, **43**, **45**, **56** and **57** were subjected to cytotoxicity testing and evaluation of their platelet aggregation effect. Among them, **12**, **16**, **21**, **56**, **57** at 100 μM showed 91—100% inhibition of rabbit platelet aggregation induced by arachidonic acid (100 μM) and **21** also produced 100% inhibition of aggregation induced by collagen (10 $\mu g\,ml^{-1}$) (Table 1). In addition, aristolochic acid-I (**43**) exhibited the most potent inhibition of the growth of HT-29 cells (Table 2).[32,33]

Experimental

General Procedures Melting points (Yanagimoto apparatus) are uncorrected. Optical rotations were recorded on a Jasco DIP-370 digital polarimeter. UV spectra in MeOH solution were obtained on a Hitachi UV-3210 spectrophotometer. IR spectra in KBr discs were recorded on a Shimadzu FT-IR DR-8011 spectrophotometer. Mass and high resolution mass spectra were measured on a VG-70-250S spectrometer with a direct inlet system. ^{1}H-NMR and ^{13}C-NMR spectra were determined on Bruker AMX-400 and Varian Unity plus 400 spectrometers. Chemical shifts are shown in δ values (ppm) with tetramethylsilane (TMS) as an internal standard.

Plant Material *Aristolochia cucurbitafolia* Hayata was collected in Yen Chao, Kaohsiung Hsien, Taiwan, in April, 1992, and verified by Prof. C.-S. Kuoh. A voucher specimen is deposited in the Herbarium of the National Cheng Kung University, Tainan, Taiwan, R.O.C.

Extraction and Isolation The stem and root (3.1 kg) were extracted with MeOH (×10) at room temperature, and concentrated to give a dark brown syrup. The MeOH extract was partitioned successively between H_2O and $CHCl_3$, and then *n*-BuOH. The $CHCl_3$ layer was filtered to obtain precipitate and filtrate. The filtrate was dried over Na_2SO_4 and then concentrated under reduced pressure to leave a brown syrup which was chromatographed directly on silica-gel and eluted with a gradient of $CHCl_3$ and MeOH to afford 3 fractions. Fraction 1 underwent column chromatography on silica gel and eluted with *n*-hexane–EtOAc (15 : 1) to obtain methyl pheophorbide-a (**6**) (24 mg), aristolochic acid-I methyl ester (**7**) (6 mg), methyl-21-hydroxyl-(21*R*)-pheophorbide-a (**8**) (23 mg), aristoloterpenate-III (**46**) (3 mg), isoscopoletin (**9**) (6 mg), aristolochic acid-II methyl ester (**10**) (4.5 mg), (−)hinokinin (**11**) (10 mg), aristolide-A (**12**) (6 mg), aristolide-B (**13**) (0.6 mg), aristolochic acid-IV methyl ester (**14**) (2.7 mg), aristolochic acid III methyl ester (**1**) (3 mg), madolin-A (**51**) (15 mg), -B (**52**) (5 mg), -C (**53**) (13 mg), -D (**54**) (3 mg), -E (**55**) (1 mg), aristolactone (**56**) (683 mg) and manshurolide (**57**) (23 mg), successively. Fraction 3 underwent column chromatography on silica-gel and eluted with iso-Pr_2O–Me_2CO (9 : 1) to give aristolactam-I (**15**) (2.1 mg), cepharanone-C (**2**) (1 mg), aristolactam-AII (**16**) (2.7 mg), piperolactam-A (**21**) (6 mg), cepharadione-A (**22**) (2 mg), 9-methoxyaristolactam-I (**19**) (2 mg), aristolactam-BII (**17**) (3 mg), cepharanone-A (**23**) (17 mg), *N-trans*-cinnamoyltyramine (**24**) (2 mg), and 9-methoxyaristolactam-IV (**20**) (1 mg). The precipitate was chromatographed on Sephadex LH-20 and eluted with a gradient of H_2O–MeOH to afford sodium aristolochate-I (**25**) (290 mg), sodium 7-hydroxyl-aristolochate (**29**) (2 mg), sodium aristolochate-II (**26**) (6 mg), sodium aristolochate-III (**27**) (6 mg), aristolic acid (**30**) (3 mg), sodium aristolochate-IVa (**28**) (24 mg), asimilobine (**31**) (5 mg), and aristololactam IIIa (**18**) (2 mg). The *n*-BuOH layer was chromatographed directly on Diaion HP-20 and eluted with a gradient of MeOH and H_2O to afford 22 fractions and allantoin (**45**) (7 g). Fraction 5 underwent column chromatography on Sephadex LH-20 and eluted with a gradient of MeOH and H_2O to give sodium (2*R*)-3-(*p*-hydroxylphenyl) lactate (**5**) (13 mg) and adenine (**33**) (10 mg). Fraction 9 underwent column chromatography on Sephadex LH-20 and eluted with a gradient of MeOH and H_2O to give glucosyringic acid (**34**) (13 mg), 4-hydroxylbenzoic acid (**35**) (4 mg), and salidroside (**36**) (11 mg). Fraction 12 underwent column chromatography on Sephadex LH-20 and eluted with a gradient of MeOH and H_2O to give sodium 3,4-dimethoxybenzoate (**4**) (13.5 mg), 4-hydroxylcinnamic acid (**37**) (2 mg), and 4-hydroxyl-3-methoxy-cinnamic acid (**38**) (2 mg). Fraction 13 underwent column chromatography on Sephadex LH-20 and eluted with a gradient of MeOH and H_2O to give 6-*O*-(*E*)-feruloyl-(α and β)-glucopyanoside (**32**) (10 mg), vanillic acid (**39**) (1 mg). Fraction 16 underwent column chromatography on silica-gel and eluted with $CHCl_3$ and MeOH (15 : 1) to give isorhamnetin 3-*O*-robinobioside (**40**) (1.09 g). Fraction 19 underwent column chromatography on silica-gel and eluted with $CHCl_3$ and MeOH (15 : 1) to afford 4,5-dioxodehydroasimilobine (**41**) (3 mg), aristolochic acid-C (**42**) (20 mg), *cis*- and *trans*-4-hydroxyl-3-methoxycinnamic acid methyl ester (**44**) (6 mg), sodium 7-hydroxyl-8-methoxyaristolate (**3**) (1 mg), and aristolochic acid-I (**43**) (280 mg), successively.

Aristolochic Acid III Methyl Ester (**1**): Yellowish needles. $C_{18}H_{13}NO_7$. mp 272—274 °C. UV λ_{max}^{MeOH} nm (log ε): 216 (4.06), 256 (4.33), 278 (sh, 3.91), 300 (3.81), 350 (3.63) and 384 (3.59). IR ν_{max}^{KBr} cm^{-1}: 1710 (C=O), 1612, 1525 (NO_2), 1346, 1228, 1045 and 941 (OCH_2O). HRMS: *Anal*. calcd for $C_{18}H_{13}NO_7$ (355.0692). Found: $C_{18}H_{13}NO_7$ (*m*/*z*, 355.0695). EI-MS *m*/*z* (rel. int.): 355 ($[M]^+$, 43), 310 (20), 309 (100), 294 (67), 278 (35), 266 (16). ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 3.87 (3H, s, OCH_3), 4.02 (3H, s, OCH_3), 6.39 (2H, s, OCH_2O), 7.34 (1H, dd, *J*=8.8, 2.4 Hz, H-7), 7.76 (1H, s, H-2), 7.90 (1H, d, *J*=8.8 Hz, H-8), 8.31 (1H, s, H-9), 8.60 (1H, d, *J*=2.4 Hz, H-5).

Cepharanone-C (**2**): Yellowish powder. $C_{17}H_{11}NO_5$. UV λ_{max}^{MeOH} nm (log ε): 236 (sh, 4.45), 241 (4.49), 276 (sh, 4.35), 288 (4.49), 357 (3.85), 373 (3.83) and 395 (3.74). IR ν_{max}^{KBr} cm^{-1}: 3453 (OH), 1651 (C=O), 1560 (C=C), 1417, 1282, 1097 and 931 (OCH_2O). HRMS: *Anal.* calcd $C_{17}H_{11}NO_5$ (309.0637), Found (*m*/*z*, 309.0634). EI-MS *m*/*z* (rel. int.): 309 ($[M]^+$, 3), 294 (17), 293 (100), 278 (68), 263 (31), 132 (36), 131 (33), 62 (33). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 3.85 (3H, s, OCH_3), 6.42 (2H, s, OCH_2O), 7.14 (1H, d, *J*=8.8 Hz, H-6), 7.18 (1H, s, H-9), 7.55 (1H, s, H-2), 7.65 (1H, br s, NH), 8.13 (1H, d, *J*=8.8 Hz, H-5), 10.73 (1H, s, OH).

Sodium 7-Hydroxyl-8-methoxyaristolate (**3**): Colorless powder. $C_{17}H_{11}O_6Na$. mp >300 °C. UV λ_{max}^{MeOH} nm (log ε): 225 (4.84), 265 (sh, 4.57), 273 (4.60), 296 (4.27), 319 (4.02), 352 (sh, 3.44) and 371 (3.15). IR ν_{max}^{KBr} cm^{-1}: 1558 (C=O), 1415, 1298, 1035 and 933 (OCH_2O). ^{1}H-NMR (CD_3OD, 400 MHz) δ: 3.92 (3H, s, OCH_3), 6.20 (2H, s, OCH_2O), 7.12 (1H, d, *J*=9.1 Hz, H-6), 7.31 (1H, s, H-2), 7.78 (1H, d, *J*=9.5 Hz, H-9), 8.27 (1H, d, *J*=9.5 Hz, H-10), 8.64 (1H, d, *J*=9.1 Hz, H-5).

Sodium 3,4-Dimethoxylbenzoate (**4**): Colorless powder. $C_9H_9O_4Na$. mp >300 °C. UV λ_{max}^{MeOH} nm: 205, 252, 286 and 291. IR ν_{max}^{KBr} cm^{-1}: 1602 (C=O), 1558, 1450, 1282 and 1224. ^{1}H-NMR (CD_3OD, 200 MHz) δ: 3.61 (3H, s, OCH_3), 3.89 (3H, s, OCH_3), 6.75 (1H, d, *J*=8.0 Hz, H-5), 7.47 (1H, dd, *J*=8.0, 1.9 Hz, H-6), 7.57 (1H, d, *J*=1.9 Hz, H-2).

Acidification of **3** and **4**: Isolated **3** (0.6 mg) and **4** (1 mg) were dissolved in 5% HCl (1 ml). The solution was eluted from a Sephadex LH-20 column with H_2O, then MeOH, to give NaCl and the acids **3a** (89%, 0.5 mg) and **4a** (85%, 0.76 mg), successively.

Antiplatelet Aggregation Assays: The antiplatelet aggregation assays were based on a method reported by Teng *et al.*[34]

Cytotoxicity Assays: The *in vitro* KB cytotoxicity assay was carried out according to procedures described by Geran *et al.* and Ferguson *et al.*[35,36] The assay against P-388, A-549, HT-29 and HL-60 tumor cells was based on a method reported by Lee *et al.*[37]

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