Novel C_{19} Homolignans, Taiwanschirin A, B, and Cytotoxic Taiwanschirin C, and a New C_{18} Lignan, Schizanrin A, from *Schizandra arisanensis*

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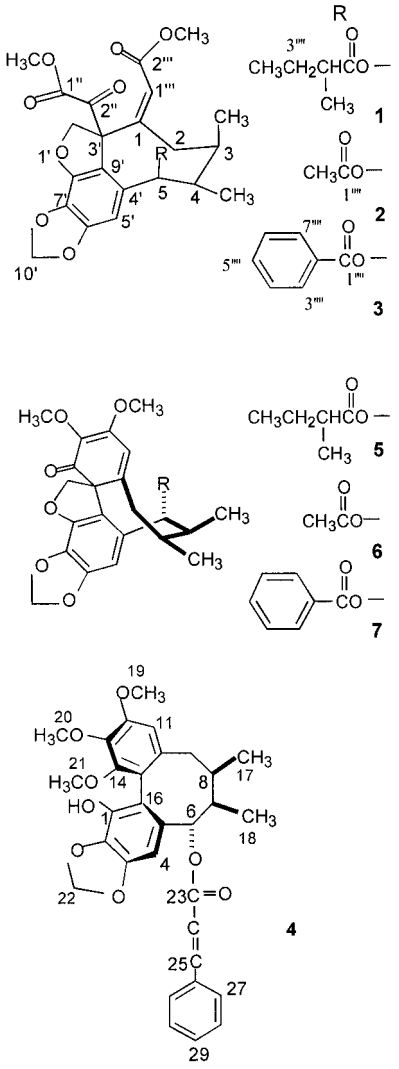
Three novel C_{19} homolignans with a 3,4-pentano-2,3-dihydrobenzo[*b*]furan skeleton, taiwanschirin A (**1**), taiwanschirin B (**2**), and taiwanschirin C (**3**), and a new C_{18} dibenzocyclooctadiene lignan, schizanrin A (**4**) were isolated from *Schizandra arisanensis*. Taiwanschirin C (**3**) and schizarin A (**4**) exhibited in vitro cytotoxicity against hepatoma (Hepa-3B, $ED_{50} = 2.2$ μg/mL for **3**, and $ED_{50} = 4.2$ μg/mL for **4**). Compound **4** also showed marginal activity against colon carcinoma (COLO-205, $ED_{50} = 2.9$ μg/mL). Their structural elucidation by spectral and single-crystal X-ray analyses, and structure−activity relationships are discussed.

Introduction

In the course of our searching on the development of naturally occurring antitumor agents,[1,2] we have recently reported the isolation of four novel C_{19} homolignans with 5,4-butano-2,4-cyclohexadienone-6-spiro-3-(2,3-dihydrobenzo[*b*]furan) skeleton, schiarisanrin A (**5**), B (**6**), C (**7**), and D (**8**), from *Schizandra arisanensis*.[3] The isolated C_{19} homolignans have a substituted cyclohexadienone moiety with an oxygenated methylene group, instead of the substituted benzene moiety found before in C_{18} dibenzocyclooctadiene lignans from *Schizandra* spp.[4−9] Several of these C_{18} type lignans showed some pharmacological effects such as antioxidant, antihepatitis, antihepatotoxic, and antilipid peroxidative effects.[4−9] It is note that schiarisanrin C, one of C_{19} homolignans from *S. arisanssesis*, has the cytotoxicity against human epidermoid carcinoma of nasopharynx (KB), colon carcinoma (COLO-205), hepatoma (Hepa-3B), and cervix (Hela) tumor cells.[3] Thus, further investigation of the titled plant led to the isolation and characterization of three novel C_{19} homolignans, taiwanschirin A (**1**), taiwanschirin B (**2**), and taiwanschirin C (**3**), as well as a new C_{18} dibenzocyclooctadiene lignan, schizanrin A (**4**). These new C_{19} homolignans possessing a 3,4-{1-[(*Z*)-2-methoxy-2-oxoethylidene]}-pentano(2,3-dihydrobenzo[*b*]furan)-3 (2-oxoacetate) skeleton are different from reported C_{19} homolignans. For the compounds **1**−**4**, their structures were unambiguously established by employing 2D NMR techniques including



$^1H-^1H$ COSY, $^1H-^{13}C$ heteronuclear COSY, long-range $^1H-^{13}C$ COSY, and NOESY spectra (Tables 1 and 2) as well as X-ray data (for **1**). Moreover, biological evaluation of these new compounds exhibited that compound **3** had

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Table 1. ^{1}H NMR (300 MHz, $CDCl_3$) Data[a] for Compounds 1−3

proton	1	2	3
2	2.45 (brs)	2.24 (m)	2.50 (m)
3	2.06 (m)	2.04 (m)	2.18 (m)
4	1.93 (m)	1.94 (m)	2.05 (m)
5	5.77 (s)	5.82 (s)	5.81 (s)
2′	4.60, 5.90 (Abq, 10.3)	4.68, 5.92 (Abq, 10.2)	4.64, 5.82 (Abq, 10.2)
5′	6.35 (s)	6.30 (s)	6.49 (s)
10′	5.96 (d, 0.9)	5.98 (s)	5.98 (d, 0.9)
3-CH_3	1.07 (d, 8.7)	1.02 (d, 7.5)	1.24 (d, 7.5)
4-CH_3	1.01 (d, 7.3)	1.02 (d, 7.5)	1.14 (d, 7.5)
1‴	6.07 (s)	6.09 (s)	6.14 (s)
1″-OCH_3	3.59 (s)	3.57 (s)	3.31 (s)
2‴-OCH_3	3.67 (s)	3.68 (s)	3.64 (s)
2⁗	2.52 (q, 6.8)	2.00 (s)	7.92 (d, 7.6)
3⁗	1.45, 1.62 (m)		7.42 (t, 7.6)
4⁗	0.87 (t, 7.2)		7.54 (t, 7.6)
5⁗	0.95 (d, 10.7)		7.42 (t, 7.6)
6⁗			7.92 (d, 7.6)

[a] All assignments are based on 1D and 2D NMR experiments including COSY90, HETCOR, HMBC, and NOESY spectra.

Table 2. ^{13}C NMR (75.5 MHz, $CDCl_3$) Data[a] for Compounds 1−3

carbon	1	2	3	C-H
1	155.70 (s)	154.60 (s)	156.08 (s)	H-2,2′
2	41.62 (t)	41.59 (t)	41.56 (t)	H-1‴, H-2
3	35.88 (d)	35.86 (d)	35.69 (d)	H-5, 3-CH_3
4	43.95 (d)	43.65 (d)	43.37 (d)	H-2,3,5
5	81.14 (d)	80.71 (d)	83.80 (d)	H-5′, 4-CH_3
CH_3-3	20.09 (q)	20.86 (q)	19.90 (q)	H-5,3
CH_3-4	14.01 (q)	13.58 (q)	14.56 (q)	H-3,5
2′	84.90 (t)	85.50 (t)	85.27 (t)	
3′	65.06 (d)	64.88 (d)	65.05 (d)	H-1‴
4′	133.02 (s)	132.70 (s)	132.99 (s)	H-5
5′	102.28 (d)	102.10 (d)	102.31 (d)	H-5
6′	130.52 (s)	130.45 (s)	130.09 (s)	H-5′
7′	150.68 (s)	150.78 (s)	150.74 (s)	H-10′
8′	145.46 (s)	145.55 (d)	145.45 (s)	H-2′
9′	118.09 (s)	118.50 (s)	118.50 (s)	H-5′, 5
10′	102.90 (t)	102.29 (t)	103.20 (t)	
1″	165.96 (s)	166.13 (s)	165.68 (s)	1″-OCH_3
2″	184.39 (s)	184.75 (s)	183.16 (s)	H-2′[a,b]
OCH_3-1″	51.46 (q)	51.42 (q)	51.41 (q)	
1‴	123.74 (d)	124.41(d)	123.87 (d)	H-2
2‴	161.51 (s)	161.67 (s)	160.64 (s)	2‴-OCH_3
OCH_3-2‴	52.65 (q)	52.50 (q)	52.13 (q)	
1⁗	177.06 (s)	170.92	167.56 (s)	*c*
2⁗	39.50 (d)	19.91 (q)	130.08 (s)	*c*
3⁗	26.99 (t)		128.03 (d)	*c*
4⁗	10.96 (q)		129.68 (d)	*c*
5⁗	14.81 (q)		132.44 (d)	*c*
6⁗			129.68 (d)	*c*
7⁗			128.02 (d)	*c*

[a] Multiplicity was determined from DEPT spectra. [b] ^{1}H−^{13}C long-range correlation (HMBC) corresponded to two- or three-bond bonds. [c] The assignments were explained in the text.

the inhibitory activity in vitro against human hepatoma (Hepa-3B); compound **4** demonstrated the inhibition of colon carcinoma (COLO-205) and hepatoma (Hepa-3B) tumor cells. The preliminary structure−activity relationship studies would conclude that the cytotoxicity is affected by the functional group substitution at the C-5 position in the 3,4-{1-[(*Z*)-2-methoxy-2-oxoethylidene]}-pentano(2,3-dihydrobenzo[*b*]furan)-3 (2-oxoacetate) skeleton, and at C-10 position in the 5,4-butano-2,4-cyclohexadienone-6-spiro-3-(2,3-dihydrobenzo[*b*]furan) skeleton.

Results and Discussion

An EtOH extract of the dried stems of *S. arisanensis* was extracted successively with *n*-hexane, EtOAc, and BuOH. Repeated column chromatography and/or HPLC of the EtOAc extract yielded taiwanschirin A (**1**), taiwanschirin B (**2**), taiwanschirin C (**3**), and schizanrin A (**4**).

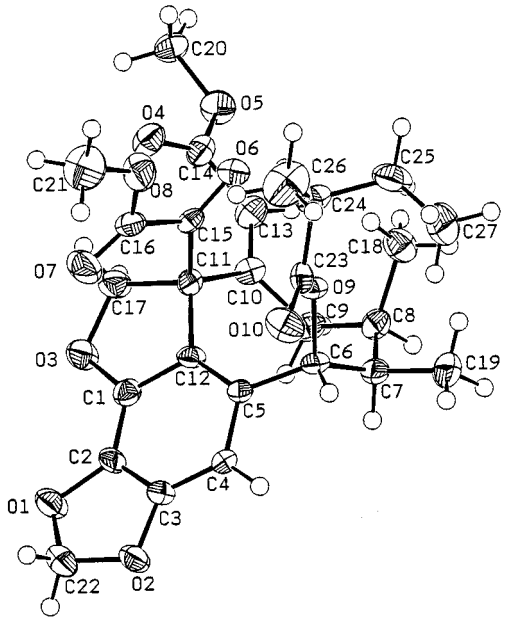

Figure 1. Computer-generated perspective drawing of taiwanschirin A (1). (The tentative numberings are different from the text described.)

Taiwanschirin A (**1**) was obtained as acolorless, cubic crystals (Figure 1). Its IR spectrum showed absorption bands at 1745, 1735 (C═O), 1718 (conjugated ester) 1655 (C═C−CO), and 1620 (aromatic) cm^{-1}. The ^{13}C NMR of **1** exhibited the signals for an aromatic group at δ_C 133.0, 102.3, 130.5, 150.7, 145.5 and 118.1 ppm (C-4′, -5′, -6′, -7′, -8′, and -9′, respectively). In the heteronuclear long-range correlation (HMBC) spectrum, the cross-peaks of oxygenated methylene protons (H-2′) to C-8′ and -9′ and a methylenedioxy protons (H-10′) to C-6′ and -7′ indicated the presence of an oxygenated benzene moiety. The correlations between H-2′ and a quaternary carbon (C-3′) and C-9′ indicated the connectivity of a dihydrofuran linkage an oxygenated benzene, which suggested a 2,3-dihydrobenzo[*b*]furan with a methylenedioxy moiety. Other evidence for structural elucidation comes from the ^{1}H−^{1}H COSY studies, the spectrum showing the cross-peaks of H-3, -2, -4, and H-4, -3, -5, as well as two methyl groups correlated with H-3 and -4, respectively, suggested a C_2−C_3−C_4−C_5 butano moiety. Moreover, the couplings between H-2′ and C-1, between H-2 and C-1, and between H-4′ and C-5 occurring in the HMBC spectrum revealed that the dihydrobenzo[*b*]furan was connected to a pentano moiety at 3′-4′ positions. The presence of signals for one methoxy and two carbonyl groups constructed an oxoacetate, which was assigned at C-3′ position, due to the long-range correlation between H-2′ and C-2″ (C═O). In addition, inspection of HMBC spectrum of **1**, the cross-peaks of the remaining methoxy and a carbonyl (C═O, 2‴), and the correlation between olefinic proton (H-1‴) and a carbonyl carbon (C-2‴) and a quaternary carbons (C-1) deduced a 2-methoxy-2-oxoethylidene group at C-1 position. All of these results suggested that **1** possesses a substituted dihydrobenzo-[*b*]furan, a substituted cyclooctene, a substituted oxoacetate, and a substituted methoxy oxoethylidene moieties. The mass spectrum of **1** exhibited a molecular ion at *m*/*z*

516 and an intense peak at *m*/*z* 327 [$M^+ - CH_3OCOCO - C_4H_9COO$] corresponding to the fragmentation of an oxoacetate and suggested the 1,2-elimination of a pentanoic acid via McLafferty rearrangement of ester. To clarify the nature of the ester group, the heteronuclear long-range correlation was observed. The cross signals between Me-2‴, H-5, and an ester carbonyl carbon at δ_C 177.06 ppm (C-1‴), consequently indicated a 2-methylbutanoic acid ester at C-5. On the basis of above corroboration, the whole structure of taiwainschrin A (**1**) was concluded to have a 3,4-{1-[(*Z*)-2-methoxy-2-oxoethylidene]}pentano(2,3-dihydrobenzo[*b*]furan)-3 (2-oxoacetate) skeleton with a 2-methylbutyroxyl group at C-5.

The complete structure and stereochemistry of **1** can be unequivocally confirmed as shown by single-crystal X-ray analysis on a Nonius (CAD4) diffractometer. Crystal data: $C_{27}H_{32}O_{10}$, $M_w = 518.55$, orthorhombic, space group *P*212121, $a = 9.699(3)$ Å, $b = 13.967(5)$ Å, $c = 18.780(14)$ Å, $V = 2544.0$ Å^3, $Z = 4$, $D_{calc} = 1.354$ g cm^{-3}, $\lambda = 0.717$, $F(000) = 1105$, $m\mu = 0.886$ cm^{-1}.

Taiwanschirin B (**2**) showed a molecular ion at *m*/*z* 474, which is consistent with the molecular formula of $C_{24}H_{26}O_{10}$. The IR and NMR spectra revealing **2** also has a 3,4-{1-[(*Z*)-2-methoxy-2-oxoethylidene]}pentano(2,3-dihydrobenzo[*b*]furan)-3 (2-oxoacetate) skeleton with an oxygenated methylene group, which is similar to those of **1** except for the missing of the signal of 2-methylbutyroxyl group and the presence of an acetate at δ_H 2.00, δ_C 170.9 (C-1‴), δ_C 19.9 (C-2‴). Proposed intense fragment ion at *m*/*z* 327 [$M^+ - CH_3OCOCO - CH_3COOH$] and a molecular ion at *m*/*z* 474 were also observed in the EIMS, revealing that **2** is corresponding to the replacement of a 2-methylbutyroxyl group with an acetoxyl group. Also, as compound **1**, the molecular ion of **2** undergoes the elimination of an acetic acid via McLafferty rearrangement involving the acetoxyl group. Extensive spectroscopic investigation by using the heteronuclear C−H COSY technique allowed us to assign the signal of secondary methyl group at δ_H 1.02 (d, 7.5, 6H). Thus, it was correlated with δ_C 20.86 and 13.58, respectively, which is attributable to C-3 and C-4 connecting a methyl group, respectively. A HMBC experiment of **2** displayed cross signals between the carbonyl carbon at δ_C 170.9 (C-1‴) and protons at δ_H 2.0 (H-2‴) and δ_H 5.82 (H-5), revealing that the acetoxyl group is located at C-5, undoubtedly verifying the structure of **2** as shown.

Taiwanschirin C (**3**) gave *m*/*z* 536.1678 [M^+], for $C_{29}H_{28}O_{10}$ by HREIMS. The ^{1}H and ^{13}C spectra of **3** were similar to those of **1** and indicated the characteristic signals for a substituted dihydrobenzo[*b*]furan, a substituted cyclooctene, a substituted oxoacetate, and a substituted methoxy oxoethylidene moieties. However, no signals for 2-methylbutyroxyl group were observed in the NMR spectra, but the proton signals (δ_H 7.42, 7.54, and 7.92) as well as the carbon signals (δ_C 128.0, 129.7, 130.0, 132.4, and 167.6), revealing that compound **3** possesses a benzoate group ($C_7H_5O_2$) instead of a 2-methylbutyroxyl group. Moreover, the correlation between the carbonyl carbon (C-1‴) and H-5 in HMBC spectrum, and a corresponding fragment ion at *m*/*z* 373 [$M^+ - CH_3OCOCO$ − benzoic acid] in the mass spectrum, which are in good agreement with the benzoic acid ester at C-5. In addition to the above evidence, the complete assignments for the chemical shifts of ^{1}H and ^{13}C NMR of **3** were performed by 2D ^{1}H−^{1}H COSY and ^{1}H−^{13}C COSY spectra and therefore confirmed the structure of **3** as 3,4-{1-[(*Z*)-2-methoxy-2-oxoethylidene]}pentano(2,3-dihydrobenzo[*b*]furan)-3 (2-oxoacetate) skeleton with a benzoic acid ester at C-5.

Schizarin A (**4**) had a molecular weight of 532 (HRMS) corresponding to the molecular formula $C_{31}H_{32}O_8$. The nature of the structure was deduced from ^{13}C and ^{1}H NMR spectra, together with the IR spectrum with bands at 3400 (OH), 1715 (ester), 1610, and 1590 (aromatic) cm^{-1}, suggesting that **4** could possess dibenzocyclooctadiene lignan with a hydroxyl and an ester groups. The ^{13}C NMR spectrum of **4** clearly indicates the presence of 12 carbon atoms (δ_C 146.8, 148.9, 136.1, 102.8, 119.3, and 135.6 for C-1, -2, -3, -4, -5, and -16, respectively; δ_C 133.4, 107.0, 133.4, 150.2, 141.2, and 117.2 for C-10, -11, -12, -13, -14, and -15, respectively), revealing the possibility of a biphenyl moiety. As compounds **1**−**3**, a butano moiety was predicted due to the prominent cross-peaks of H-6 (δ_H 5.70, s), H-7 (δ_H 2.12, m), H-8 (δ_H 2.12, m), and H-9 (δ_H 2.67, m) in the COSY90 spectrum. Moreover, long-range correlation (HMBC) spectrum showed the couplings between H-9 and C-15 and C-11, and between H-6 and C-16 and C-4 of respective aromatic rings, implying that **4** possesses substituted 10−5 butano moiety and that the two substituted aromatic moieties possess a C-15 and -16 linkage. From the above data, the skeleton of **4** was deduced as a substituted dibenzocyclooctadiene lignan isolated previously from Schizandraceae plants.[8,9] The other signals of functional groups including three methoxy groups, an oxygenated methylene group, and two secondary methyl groups appearing in the ^{13}C and ^{1}H NMR spectra, were assigned as C-19, -20, -21, and C-22, and C-17, -18, respectively, on the basis of the HMBC and NOESY studies. The presence of a cinnamic acid ester was shown by carbon signals at δ = 117.9, 144.1 (C-23, -24), 128.0 (C-27, -31), 128.7 (C-28, -30), 130.0 (C-29), as well as quaternary at δ_C 134.4 (C-26), and a carbonyl carbon at δ_C 166.0 (C-23) in the ^{13}C NMR spectrum. Also, the proton signals at δ_H 5.85 and 7.04 (d, $J = 16.2$, H-24, -25) corresponding to the trans double bond were found in the ^{1}H NMR spectrum. The mass spectrum of **4** exhibited a molecular ion at *m*/*z* 532 and a characteristic peak at *m*/*z* 384 [$M^+ - C_6H_5C_2H_2COO$], which are also corresponding to the presence of a cinnamic acid ester. Detailed examination of the HMBC spectrum of **4** revealed that the olefinic proton (H-24) was correlated with both carbonyl carbon (C-23) and benzyl carbon (C-26); the correlation between H-6 and C-23 revealed a *trans*-cinnamic acid ester at C-6. On the basis of NOESY spectrum, three methoxy groups correlated each other and the methoxy group at C-12 correlated H-11, suggesting that three methoxy groups are adjacent at the same aromatic ring. Consequently, the remaining quaternary carbon at C-1 (δ_C 146.8) would have a phenolic hydroxyl group for fitting the molecular weight of **4**, and then its structure was established unambiguously.

Comparing the structures of **1**−**3** and the other C_{19} homolignans (**5**−**8**) with 5,4-butano-2,4-cyclohexadienone-6-spiro-3-(2,3-dihydrobenzo[*b*]furan) isolated previously from the title plant, they contain a similar C_{19} hydrobenzo[*b*]furan-substituted cyclooctene skeleton, and hence compounds **1**−**3** could be named as homolignan. However, a substituted oxoacetate moiety and a substituted methoxy oxoethylidene moiety found in **1**−**3** instead of a

cyclohexadienone moiety in **5**−**8** would propose a biogenetic pathway by the oxidation in cyclohexadienone. Moreover, from the plant taxonomy viewpoint, the evidence that the specimen of *S. arisanensis* possesses C_{19} lignans with hydrobenzo[*b*]furan-substituted cyclooctene skeleton more than those of C_{18} lignans with dibenzocyclooctadiene skeleton could further assist the identification of morphological diversities of plants.

To study the stereochemistry of compounds **1**−**6** from *S. arisanensis*, their respective absorption values of circular dichroism (CD) spectra were examined. The dihydrobenzo[*b*]furan derivatives **1**, **2**, and **3**, showed a negative Cotton effect at 290, 290, and 255 nm, respectively, and a positive Cotton effect at 340, 340, and 330 nm, respectively. Schiarisanrin A (**5**) and schiarisanrin B (**6**), homolignans with dihydrobenzo[*b*]furan cyclohexadienone, exhibit a negative Cotton effect at 319, 330 nm, respectively, and two positive Cotton effects at 247, 247 nm, respectively, and at 369, 365 nm, respectively. These obtained data indicates the esters of compounds **1**−**3** have different orientations from those of and **5** and **6**. The figures of X-ray diffraction revealing that the cyclooctene of **1**−**3** have a chairlike shape, whereas schiarisanrins (**5**, **6**) have a boatlike shape, which is also consistent with the CD evidence. Furthermore, the correlation between H-6 and H-4, CH_3-18, and between CH_3-17, and H-19 and H-11 in the NOESY spectrum of **4**, together with the circular dichroism showing a negative Cotton effect at 260 nm, suggested that compound **4** possessed an (*S*)-biphenyl configuration as Gomisin B.[10,11]

Furthermore, those novel homolignans, **1**−**4**, were assayed for cytotoxicity in human KB, COLO-205, Hepa-3B, and Hela cell lines. Taiwanschirin C (**3**) and schizarin A (**4**) exhibited cytotoxicity against hepatoma (Hepa-3B, ED_{50} = 2.2 μg/mL for **3**, and ED_{50} = 4.2 μg/mL for **4**). Conversely, the ED_{50} values for compounds **1** and **2** exceeded 10 μg/mL. Compound **4** also showed cytotoxicity against colon carcinoma (COLO-205, ED_{50} = 2.9 μg/mL). It is first reported that C_{18} lignans with dibenzooctadiene such as compound **4** have cytotoxic activity. Notably, C_{19} homolignans, such as schiarisanrin C (**7**) and compound **3** with a benzoate ester at C-10 and C-5, respectively, demonstrated the cytotoxicity. It seems that dihydrobenzo[*b*]furan-substituted cyclooctene moiety as well as the substituent at C-5 (or different carbon numbering but same position) could be significant for cytotoxicity in C_{19} homolignans. The detailed structure−activity relationships of the dihydrobenzo[*b*]furan-substituted cyclooctene type of homolignans are under investigation.

Experimental Section

General Experimental Procedures. NMR spectra were measured at 300 MHz for ^{1}H and 75 MHz for ^{13}C. Heteronuclear long-range correlation (HMBC) spectra were performed by using coupling constants of 8 Hz. Samples for IR spectral measurements were prepared as KBr disks. EIMS were performed in the electron impact mode (70 eV). HPLC was employed by using the semipreparative $5C_{18}$ column.

Plant Material. The stems of *S. arisanensis* were collected in July 1993 in Taipei County, Taiwan. A voucher specimen was deposited at National Research Institute of Chinese Medicine, Taipei, Taiwan, R.O.C.

Extraction and Isolation. The crude $CHCl_3$ extract (67 g) of *S. arisanensis* was yielded as the reference reported.[5] After chromatography on silica gel with hexanes−EtOAc by gradually increasing EtOAc as the eluent, 10 fractions were furnished. Fraction 4 was further rechromatographyed over Si gel with hexanes−EtOAc (10:1, 7:1, 5:1, 3:1, 1:1) as the eluent to acquire nine fractions, 4−1 to 4−9. Compound **1** (20 mg) was obtained and recrystallized from fractions 4−5. Fraction 4−3 was further separated repeatedly by HPLC ($5C_{18}$, 250 × 10 mm) with MeOH−H_2O (2.7:1) as the eluent to afford compound **2** (5.7 mg), **3** (4.2 mg), and **4** (4.6 mg).

Table 3. ^{1}H and ^{13}C NMR Data ($CDCl_3$) for Compound 4[a]

position	carbon	proton	C-H connectivities[b]
1	146.80 (s)		*c*
2	148.86 (s)		H-22, 4
3	136.08 (s)		H-22, 4
4	102.75 (d)	6.54 (s)	H-6, 4
5	119.29 (s)		H-6, 4
6	82.74 (d)	5.70 (s)	H-4
7	41.76 (d)	2.12 (m)	H-6
8	34.83 (d)	2.12 (m)	H-6,9,17,18
9	38.67 (t)	2.67 (m)	H-11,17
10	133.43 (s)		H-9,11
11	107.00 (d)	6.43 (s)	H-9
12	133.43 (s)		H-11,19
13	150.25 (s)		H-11,20
14	141.28 (s)		H-21
15	117.16 (s)		H-11,9
16	135.57 (s)		H-6,4
17	15.12 (q)	0.99 (d, 7.6)	H-9
18	19.64 (q)	1.10 (d, 7.6)	H-6
19	59.74 (q)	3.09 (s)	*c*
20	60.53 (q)	3.52 (s)	*c*
21	55.72 (q)	3.81 (s)	
22	101.18 (t)	5.96, 5.92 (d, 1.2)	*c*
23	165.98 (s)		H-6
24	117.88 (d)	5.85 (d, 16.2)	*c*
25	144.13 (d)	7.04 (d, 16.2)	Bz
26	134.42 (s)		H-24,25,Bz
27	128.00 (d)	7.28 (m)	H-25
28	128.66 (d)	7.28 (m)	Bz
29	130.01 (d)	7.28 (m)	Bz
30	128.66 (d)	7.28 (m)	Bz
31	128.00 (d)	7.28 (m)	H-25

[a] Multiplicity was determined from DEPT spectra. [b] ^{1}H−^{13}C long-range correlation (HMBC) corresponded to two- or three-bond bonds. [c] The assignments were explained in the text.

Taiwanschirin A (**1**): colorless prism; mp 147−150 °C; IR (KBr) 1745, 1735, 1718, 1655, 1620 cm^{-1}; $[\alpha]_D$ +415° ($CHCl_3$, *c* 0.1); EIMS *m/z* (rel intensity) 516 (M^+, 8), 429 (11), 327(100), 295 (30), 271 (27), 267(25); HREIMS *m/z* 516.1990 (M^+, calcd $C_{27}H_{32}O_{10}$, 0.5 mmu); ^{1}H and ^{13}C NMR, see Tables 1 and 2, respectively.

Taiwanschirin B (**2**): colorless prism; mp 190−193 °C; IR (KBr) 1740, 1735,1715, 1650, 1610 cm^{-1}; $[\alpha]_D$ +344° ($CHCl_3$, *c* 0.1); EIMS *m/z* (rel intensity) 474 (M^+, 9), 384 (15), 327(100), 295 (41), 271 (28), 267(30); HREIMS *m/z* + 474.1523 (M^+, calcd $C_{24}H_{26}O_{10}$, 0.3 mmu); ^{1}H and ^{13}C NMR, see Tables 1 and 2, respectively.

Taiwanschirin C (**3**): colorless prism; mp 86−89 °C; IR (KBr) 1740, 1735, 1715, 1655, 1615 cm^{-1}; $[\alpha]_D$ +128° ($CHCl_3$, *c* 0.1); EIMS *m/z* (rel intensity) 536 (M^+, 7), 384 (7), 328 (34), 327(100), 295 (51), 271 (38), 267 (40), 105 (44); HREIMS *m/z* 536.1678 (M^+, calcd $C_{29}H_{28}O_{10}$, 0.4 mmu); ^{1}H and ^{13}C NMR, see Tables 1 and 2, respectively.

Schizarin A (**4**): colorless prism; mp 173−175 °C; IR (KBr) 3400, 1715, 1610, 1590 cm^{-1}; $[\alpha]_D$ +48° ($CHCl_3$, *c* 0.1); EIMS *m/z* (rel intensity) 532 (M^+, 36), 385 (55), 384 (100), 369 (21), 353 (33), 131 (52); HREIMS *m/z* 532.2092 (M^+, calcd $C_{31}H_{32}O_{10}$, 0.6 mmu); ^{1}H and ^{13}C NMR, see Table 3.

Cytotoxicity Assay. The in vitro cytotoxicity assay against KB (nasal pharnegeal carcinoma), Hepa-3B (hepatoma), Hela (cervix carcinoma), and COLO-205 (colon carcinoma) tumor cells by the methylene blue dying method was based on reported procedures.[12−15] The cells for bioassay were cultured

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in RPMI-1640 medium supplemented with a 5% CO_2 incubator at 37 °C. In summary, the assay depends on binding the methylene blue to the fixed monolayer at pH 8.5 and, after washing the monolayer, releasing dye by lowering the pH. Entries and control standard agents were prepared at concentrations of 1, 10, 40, and 100 μg/mL. The detailed procedures of this experiment are in previous report.[3] Finally, the 96-well tray was dipped into a 0.01 M borated-buffer solution four times for the removing the dye. Then, 100 μL/well ethanol−0.1 M HCl (1/1 v/v) was measured on a microtiter plate reader (Dynatech, MR 7000) at wavelength of 650 nm. The ED_{50} value was defined during a comparison with the untreated cells at the concentration of test sample resulting in 50% reduction of absorbance. Mitomycin C was employed as standard compound, which exhibited ED_{50} value of 0.05 μg/mL under above conditions.

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