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Flavonoids, including an unusual flavonoid-Mg^{2+} salt, from roots of *Cudrania cochinchinensis*

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Abstract

Four flavonoids with 2′,4′-di-oxygenated B-rings, cochinchinol A (**1**), cochinchinol **B** (**2**), (2R,3R)-4′,7-dihydroxy-2′,5-dimethoxydihydroflavonol (**3**), 4′,7-dihydroxy-2′,5-dimethoxyflavonol (**4**), along with 11 known compounds, were isolated from an ethanolic extract of the roots of *Cudrania cochinchinensis*. Their structures were elucidated by chemical and spectroscopic methods. Cochinchinol A (**1**) and cochinchinol B (**2**) have two hitherto unprecedented flavonol salt structures in natural product chemistry. Cytotoxic activities were evaluated against several different cell lines.

Keywords: *Cudrania cochinchinensis*; Moraceae; Flavonoids; Flavonol salt; Cochinchinol A; Cochinchinol B; (2R,3R)-4′,7-dihydroxy-2′,5-dimethoxydihydroflavonol; 4′,7-dihydroxy-2′,5-dimethoxy flavonol

1. Introduction

Cudrania cochinchinensis (Moraceae) is distributed throughout the southern part of China, Korea and Japan. Its roots are used for the treatment of gonorrhea, rheumatism, jaundice, boils, scabies, bruising, and dysmenorrhea in Chinese folk medicine "Chuan-Po-Shi", as are the roots of *Cudrania tricuspidata*. Previous investigations of this plant revealed the presence of prenylated benzophenones, prenylated xanthones (Hou et al., 2001; Chang et al., 1989), and flavonoids (Sun et al., 1988) with cytotoxic activities. Recently, we evaluated the cytotoxic potential of flavonoids (Zhang et al., 2001). However, as a part of our systematic search on flavonoids of Moraceae plants, further investigations of the roots of *C. cochinchinensis* were carried out and resulted in the isolation of four new flavonoids with 2′,4′-di-oxygenated B-rings, namely cochinchinol A (**1**), cochinchinol B (**2**), (2R,3R)-4′,7-dihydroxy-2′, 5-dimethoxydihydroflavonol (**3**), 4′,7-dihydroxy-2′,5-dimethoxyflavonol (**4**), along with 11 known compounds, 4′5,7-trihydroxydihydroflavonol (**5**), 3′,4′,5,7-tetrahydroxyisoflavone (**6**), 4′,5,7-trihydroxydihydroflavonol-7-O-glucoside (**7**), 4′,5,7-trihydroxyflavonol-7-O-glucoside (**8**), 3′,4′,5,7-tetrahydroxydihydroflavonol (**9**), 2′,4′5,7,-tetrahydroxydihydroflavonol (**10**), 4′,5,7-trihydroxyisoflavone (**11**), l,3,6-trihydroxy-5-methoxyxanthone (**12**), 1,3,5,6-tetrahydroxyxanthone (**13**), 4′,5,7-trihydroxyflavonol (**14**), and 4′,5,7-trihydroxyflavonol-3,7-di-*O*-glucoside (**15**). Cochinchinol A (**1**) and cochinchinol B (**2**) were unprecedented flavonol natural product salts. Cytotoxic activities of compounds **1**–**9** were evaluated against several different cell lines.

2. Results and discussion

Compound **1** was obtained as a bright yellow powder. The IR spectrum indicated the presence of hydroxyl, carbonyl and aromatic groups. The ^{1}H NMR spectrum of **1** (Table 1) indicated the existence of two *meta*-coupled

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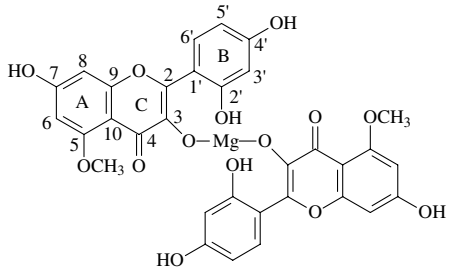
Table 1
^{1}H NMR spectroscopic data of **1**, **1a**, **2**, **3** and **4** (DMSO-d_6)

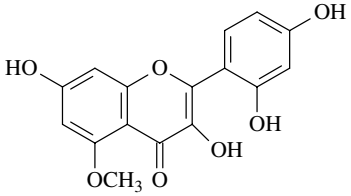
C	1	1a	2	3	4
2					
				5.25 *d*(11.1)	
3				4.36 *d*(11.1)	8.00 (OH)
4					
5			12.50 (OH)		
6	6.34 *d*(2.1)	6.33 *d*(2.1)	6.15 *d*(2.1)	6.06 *d*(2.1)	6.33 *d*(2.1)
7	10.67 (OH)		10.67 (OH)	10.55 (OH)	10.60 (OH)
8	6.46 *d*(2.1)	6.33 *d*(2.1)	6.37 *d*(2.1)	5.88 *d*(2.1)	6.30 *d*(2.1)
2′	13.73 (OH)		14.70 (OH)		
3′	6.09 *d*(2.1)	6.33 *d*(2.1)	6.17 *d*(2.1)	6.42 *d*(2.1)	6.51 *d*(2.1)
4′	9.47 (OH)		9.40 (OH)	9.62 (OH)	9.90 (OH)
5′	6.29 *dd*	6.35 *dd*	6.30 *dd*	6.38 *dd*	6.44 *dd*
	(9.0, 2.1)	(8.4, 2.1)	(9.0, 2.1)	(8.1, 2.1)	(8.1, 2.1)
6′	7.47 *d*(9.0)	7.19 (8.4)	7.48 (9.0)	7.21 *d*(8.1)	7.21 *d*(8.1)
5-OCH_3	3.81	3.81		3.74	3.81
2′-OCH_3				3.71	3.71

aromatic protons at δ 6.34 (1H, *d*, $J = 2.1$ Hz), and 6.46 (1H, *d*, $J = 2.1$ Hz), a methoxyl group at δ 3.81, and three phenolic hydroxyl protons at δ 13.73, 10.67, and 9.47, as well as resonances for an ABX aromatic spin system at δ 6.09 (1H, *d*, $J = 2.1$, H-3′), 6.29 (1H, *dd*, $J = 9.0$, 2.1 Hz, H-5′), and 7.47 (1H, *d*, $J = 9.0$ Hz, H-6′). The ^{13}C NMR spectroscopic data (Table 2) were consistent with a flavonol in which the B ring was hydroxylated at C-2′ and C-4′.

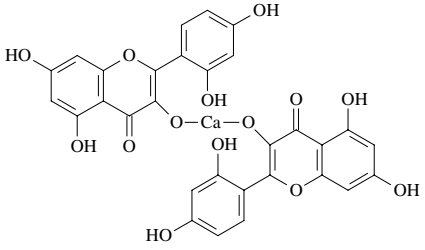
The two *meta*-coupled doublets at δ 6.34 and 6.46 were assigned to H-6 and H-8, respectively, on the basis of HMBC analysis (Fig. 1). The HMBC experiment showed ^{1}H–^{13}C long-range correlations between H-8 at δ 6.46



1



1a



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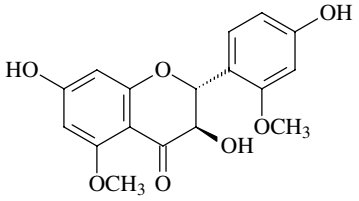

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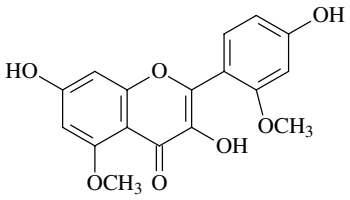

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Table 2
^{13} **C NMR** spectroscopic data of **1**, **1a**, **2**, **3** and **4** (DMSO-d_6)

C	1	1a	2	3	4
2	144.2	143.7	148.0	76.7	143.1
3	142.6	137.7	142.4	71.2	138.2
4	176.3	171.1	178.9	190.2	171.1
5	160.2	160.7	160.4	162.1	160.7
6	95.6	95.7	97.5	93.1	95.6
7	162.2	162.1	162.9	164.4	162.0
8	94.6	94.7	93.1	95.2	94.6
9	158.4	158.7	156.5	164.0	158.5
10	104.9	105.9	103.0	102.5	105.8
1′	112.5	109.5	113.0	115.3	110.7
2′	159.5	156.6	160.0	158.8	158.6
3′	104.6	102.9	105.1	98.9	99.3
4′	160.1	160.0	161.3	158.9	160.3
5′	106.8	106.7	106.5	106.8	106.9
6′	128.0	131.7	128.2	129.5	131.9
5-OCH_3	55.9	56.1		55.7	56.0
2′-OCH_3				55.4	55.5

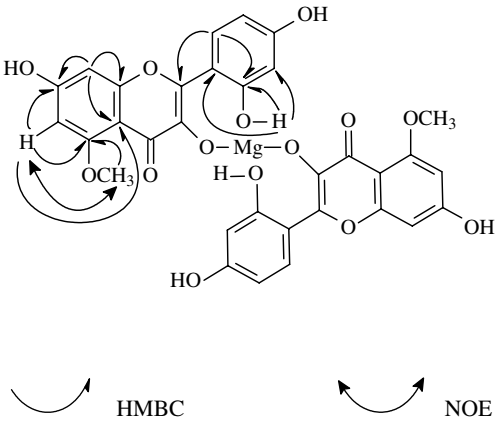

Fig. 1. HMBC and NOE correlations for **1**.

and C-9, C-10, C-7, C-6, and between H-6 at δ 6.34 and C-5, C-7, C-8, C-10. The *O*-methyl group at δ 3.81 should be located at C-5 based on the observed NOE enhancement of H-6 at δ 6.34 upon irradiation of the *O*-methyl signal at δ 3.81 (Fig. 1) and the ^{1}H–^{13}C long-range correlation (Fig. 1) of the carbon signal at δ 160.2 with the *O*-methyl proton at δ 3.81 and H-6 at δ 6.34. Comparing the ^{13}C NMR spectroscopic signals of **1** with those of a 5-methoxyflavonol, the signals of C-3 and C-4 in **1** displayed a ca. 5 ppm downfield shift (Yu and Yang, 1999). The UV spectrum of **1** showed an absorption maxima at 267 (Band II) and 399 nm (Band I) in MeOH, and no significant bathochromic shift was observed upon addition of $AlCl_3$, indicating the absence of a free hydroxyl at either C-3 or C-5. However, addition of HCl caused a hypsochromic shift of about 48 nm in band I. Compound **1** was subjected to 001 H^+ cation exchange resin column chromatography eluted with H_2O-EtOH (1:1), yielding compound **1a**, which was elucidated as 7,2′,4′-trihydroxy-5-methoxyflavonol by ^{1}H NMR (Table 1), ^{13}C NMR (Table 2) and ESIMS analyses. From these facts, it was deduced that the C-3 hydroxyl of **1** might be in the form of a salt. This was confirmed by semi-quantitative analysis of cation on ICP-MS which revealed that compound **1** contained a magnesium ion. The positive ESIMS data [*m*/*z* 339, 655 $[M + H]^+$, 677 $[M + Na]^+$] as well as the negative ESIMS data [*m*/*z* 315, 653 $[M - H]^-$ and 675 $[M + Na - 2H]^-$] for **1** were in agreement with this result. In the ^{1}H NMR spectrum of **1**, the sharp singlet at δ 13.73 was assigned to the C-2′ hydroxyl group, as it showed ^{1}H–^{13}C long-range correlations with C-1′,2′ and 3′ at δ 112.5, 159.5, and 104.6. This significant downfield chemical shift was obviously caused by a hydrogen bond with either a carbonyl or O^- at C-3. Thus, compound **1** was elucidated as depicted and named cochinchinol A.

Compound **2** was obtained as a bright yellow powder. The IR spectrum also indicated hydroxyl, carbonyl and aromatic groups. The ^{1}H NMR spectrum of **2** indicated the existence of two *meta*-coupled protons at δ 6.15 (1H, *d*, $J = 2.1$ Hz, H-6) and 6.37 (1H, *d*, $J = 2.1$ Hz, H-8) in ring A, the resonances for an ABX system at δ 6.17 (1H, *d*, $J = 2.1$, H-3′), 6.30 (1H, *dd*, $J = 9.0$, 2.1 Hz, H-5′), and 7.48 (1H, *d*, $J = 9.0$ Hz, H-6′) in ring B, and four phenolic hydroxyl proton signals at δ 14.70, 12.50, 10.67 and 9.40, suggesting that the structure of **2** was similar to that of **1** except for a 5-OH group.

The ^{13}C NMR spectroscopic data (Table 2) showed 15 signals, suggesting a flavonoid nucleus. The carbon signals of ring B were similar to those of the corresponding data in **1**. The chemical shift values of C-2, 3, 4 in **2** were δ 148.0, 142.4, and 178.9, therefore suggesting a substitution pattern of C-3 in **2** was no free hydroxy. In the ^{1}H NMR spectrum of **2**, the proton at δ 12.50 was assigned to 5-OH, while the chemical shift value of the hydroxy proton at C-2′ shifted downfield to δ 14.70 owing to the formation of a hydrogen bond. The UV spectrum of **2** showed an absorption maxima at 268 and 418 nm in MeOH, and similar shifts to **1** were observed upon addition of $AlCl_3$ and HCl. Compound **2** was subjected to 001 H^+ cation exchange resin column chromatography eluted with H_2O–EtOH (1:1) to yield compound **2a**, which was elucidated as morin by ^{1}H NMR and ^{13}C NMR spectroscopic analyses. From these facts, it was deduced that the C-3 hydroxyl of **2** might be in the form of a salt. This was also confirmed by semi-quantitative analysis of the cations on ICP-MS which revealed that compound **2** contained a calcium ion. The negative ESIMS data [*m*/*z* 641, 301, 943] and the positive ESIMS data [*m*/*z* 643, 341, 303] were in agreement with this result. Thus, compound **2** was elucidated as depicted and named cochinchinol B.

Compounds **1** and **2** are two unprecedented flavonols in the form of salts. In order to further confirm the structural characteristics of the flavonol salts, the flavonol salt **2b** was prepared by using morin and K^+-cation exchange resin. The ^{1}H NMR spectrum of **2b** indicated the existence of two *meta*-coupled aromatic protons at δ 5.96 (1H, *d*, $J = 2.1$ Hz), and 6.00 (1H, *d*, $J = 2.1$ Hz), two phenolic hydroxyl protons at δ 15.60 and 13.54, and an ABX aromatic spin system at δ 6.16 (1H, *d*, $J = 2.1$, H-3′), 6.12 (1H, *dd*, $J = 9.0$, 2.1 Hz, H-5′), and 7.35 (1H, *d*, $J = 9.0$ Hz, H-6′)

but no phenolic hydroxyl protons at C-7 and C-4′. The ^{13}C NMR spectroscopic data of **2b** were analogous to those of the corresponding data in **2**, but the signals for C-7, C-3, and C-4′ at δ 164.1, 140.8 and 163.7 were shifted downfield by $\Delta\delta$ 1.2, 1.6, and 1.4, respectively. Combined with the change of the ^1H NMR spectrum of **2b**, **2b** was assigned as tri-potassium salts of morin at C-7, C-3 and C-4′. Comparison of the ^{13}C NMR spectroscopic data of flavonol salts **1**, **2** and **2b** with the corresponding flavonols **1a** and **2a**, it is obvious that the signals of C-3 and C-4 in ring C, and C-1′, C-2′, and C-3′ in ring B, are downfield shifted whereas the signal of C-6′ appears a typical upfield shift when the C-3 hydroxyl is in the form of a salt. Additionally, in the ^1H NMR spectrum of **2b**, the hydroxyl proton at C-2′ also had a significant downfield shift such as **1** and **2**. Thus, it is more reasonable that such a downfield shift might be caused by a hydrogen bond with O^- at C-3 instead of a carbonyl in the flavonol salts. At the same time, the existence of the hydroxyl proton at C-2′ may favor a flavonol salt. Many flavonols with 2′-hydroxy and 2′,4′-dihydroxy groups in ring B were isolated from members of the family Moraceae plants, but a flavonol salt was never found thus far. So, flavonol salts should be a new type of flavone in chemotaxonomy.

Compound **3** was obtained as a yellow powder, $[\alpha]_D^{25}$ +82.7 (*c* 0.12, MeOH), and gave a positive ferric chloride test. The IR spectrum indicated the presence of hydroxyl, carbonyl and aromatic groups. The molecular formula was determined as $C_{17}H_{16}O_7$ by negative HRFABMS (found: 331.0816; calcd: 331.0818). The UV spectrum of **3** showed an absorption maxima at 205 and 285 nm in MeOH. The ^1H NMR spectrum of **3** (Table 1) exhibited two characteristic proton signals for a *trans*-dihydroflavonol at δ 5.25 (1H, *d*, $J = 11.1$ Hz, H-2) and 4.36 (1H, *d*, $J = 11.1$ Hz, H-3). It also revealed resonances for signals of two phenolic hydroxyl protons at δ 10.55 and 9.62, two *meta*-coupled aromatic protons at δ 6.06 (1H, *d*, $J = 2.1$ Hz, H-6) and 5.88 (1H, *d*, $J = 2.1$ Hz, H-8), and two methoxyl groups at δ 3.74 and 3.71. In addition, the signals for an ABX system at δ 6.42 (1H, *d*, $J = 2.1$, H-3′), 6.38 (1H, *dd*, $J = 8.1$, 2.1 Hz, H-5′), and 7.21 (1H, *d*, $J = 8.1$ Hz, H-6′) suggested that the B-ring was di-oxygenated at C-2′ and C-4′.

The ^{13}C NMR spectroscopic data (Table 2) of **3** were consistent with a dihydroflavonol having two methoxyl groups and a C-2′, C-4′ di-oxygenated B-ring. The HMBC experiment (Fig. 2) showed ^1H-^{13}C long-rang correlations between the proton at δ 5.88 and C-9, C-7, C-6, C-10, and between the proton at δ 6.06 and C-5, C-7, C-8, C-10, suggesting that the two *meta*-coupled doublets at δ 6.06 and 5.88 were assigned to H-6 and H-8, respectively. The positions of the two methoxyl groups were determined by detailed NMR spectroscopic analysis. In the ^{13}C NMR spectrum, carbonyl carbons generally appear at δ 195–199 in the case of dihydroflavonols having a hydroxyl group at C-5, but appear at δ189–192 in the case of dihydroflavonols having an OMe group at C-5 (Yu and Yang, 1999). The carbonyl carbon of **3** appeared at δ190.2, so an OMe group should be located at C-5. This was further supported by HMBC and NOE experiments (Fig. 2). On irradiation of the OMe signal at δ 3.74 an enhancement of the proton signal at δ 6.06 was observed. The location of the other methoxyl group was assigned to be at C-2′ from the fact that an enhancement of the proton signal at δ 6.42 (H-3′) upon irra-

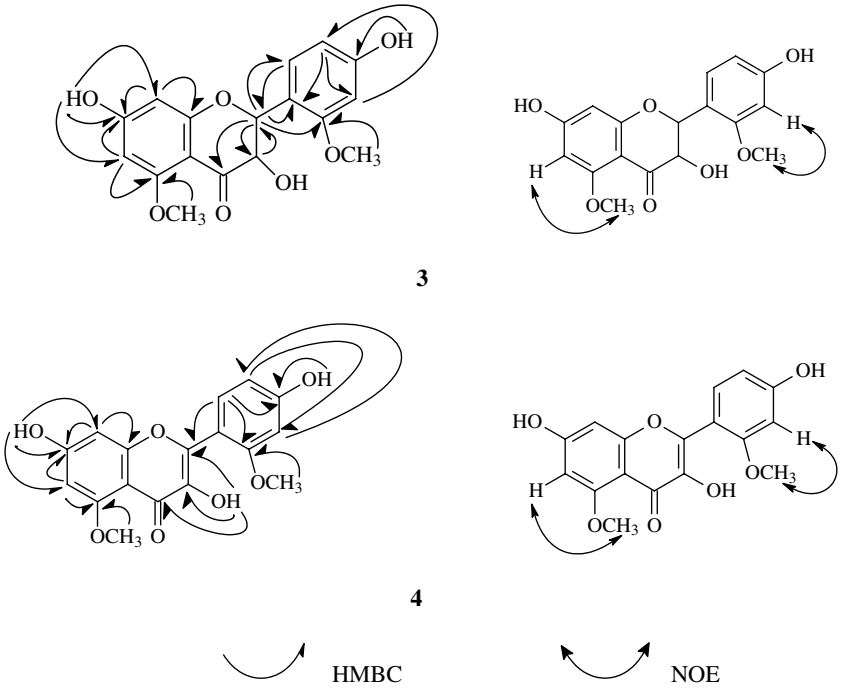

Fig. 2. HMBC and NOE correlations for **3** and **4**.

diation of the OMe signal at δ 3.71, while this resonance also showed a ^{1}H–^{13}C long-range correlation with C-2′ at δ 158.8 in the HMBC. Finally, the 2R,3R configuration was deduced by comparing the optical rotation of **3** with that of dihydromorin (Shimizu et al., 1998). Thus, **3** was assigned as (2R,3R)-4′,7-dihydroxy-2′,5-dimethoxydihydroflavonol.

Compound **4** was obtained as a yellow powder and gave a positive ferric chloride and magnesium hydrochloric acid test. The IR spectrum indicated the presence of hydroxyl, carbonyl, and aromatic groups. The molecular formula was determined as $C_{17}H_{14}O_7$ by the positive HREIMS (found: 330.0729; calcd: 330.0739). The UV spectrum of **4** showed an absorption maxima at 248 (Band II) and 347 (Band I) nm in MeOH, and bathochromic shifts of 50 nm with $AlCl_3$ and $AlCl_3 + HC1$ in Band I, indicating that **4** was a flavonol with no *ortho*-dihydroxyl groups.

The ^{13}C NMR spectroscopic data (Table 2) of **4** were consistent with a flavonol having two methoxyl groups. The chemical shift values of ring B in **4** were similar to those of the corresponding data of 3,5,7,4′-tetrahydroxy-2′-methoxyflavone (Gonda et al., 2000). The ^{1}H NMR spectrum (Table 1) of **4** showed signals for three phenolic hydroxyl protons at δ 10.60, 9.90, and 8.00, two *meta*-coupled aromatic protons at δ 6.33 (1H, *d*, $J = 2.1$ Hz) and 6.30 (1H, *d*, $J = 2.1$ Hz) in ring A, two methoxyl groups at δ 3.71 and 3.81, and resonances for an ABX system at δ 6.51 (1H, *d*, $J = 2.1$, H-3′), 6.44 (1H, *dd*, $J = 8.1$, 2.1 Hz, H-5′) and 7.21 (1H, *d*, $J = 8.1$ Hz, H-6′) in ring B. One methoxyl group was located at C-5 on the basis of the observed NOE enhancement of H-6 at δ 6.33 upon irradiation of the *O*-methyl signal at δ 3.81 (Fig. 2), and the ^{1}H–^{13}C long-range correlation (Fig. 2) of the carbon signal at δ 160.7 (C-5) with the *O*-methyl proton at δ 3.81 and H-6 at δ 6.33. The HMBC of **4** also showed the ^{1}H–^{13}C long-range correlations of the proton at δ 6.33 with C-7 and C-5 bearing an *O*-methyl functionality, and the proton at δ 6.30 with C-9 and C-7. Thus, the two *meta*-coupled protons at δ 6.33 (1H, *d*, $J = 2.1$ Hz) and 6.30 (1H, *d*, $J = 2.1$ Hz) in ring A should be assigned to H-6 and H-8, respectively. The other methoxyl group was located at C-2′ by detailed analysis of the HMBC and NOE spectra (Fig. 2). Thus, the structure of **4** was established as 4′,7-dihydroxy-2′,5-dimethoxyflavonol.

In addition, the **11** known compounds were elucidated as 4′5,7-trihydroxydihydroflavonol (**5**) (ElSohly et al., 1999), 3′,4′,5,7-tetrahydroxyisoflavone (**6**) (Nunes et al., 1989), 4′,5,7-trihydroxydihydroflavonol-7-*O*-glucoside (**7**), 4′,5,7-trihydroxyflavonol-7-*O*-glucoside (**8**) (Ternai and Markham, 1976), 3′,4′,5,7-tetrahydroxydihydroflavonol (**9**), 2′,4′5,7-tetrahydroxydihydroflavonol (**10**) (Su et al., 2002), 4′,5,7-trihydroxyisoflavone (**11**) (Wagner and Sonnenbichler, 1976), 1,3,6-trihydroxy-5-methoxyxanthone (**12**) (Gonda et al., 2000), 1,3,5,6-tetrahydroxyxanthone (**13**) (Yu and Yang, 1999), 4′,5,7-trihydroxyflavonol (**14**) and 4′,5,7-trihydroxyfiavonol-3,7-di-*O*-glucoside (**15**) (Kamiya et al., 1997), by comparison with authentic samples on TLC and spectroscopic analysis.

Table 3
Cytotoxicity (ED_{50} μg/mL) of compounds **1**–**9** against some human tumor cell Lines by the MTT method

Compound	Bel-7402	BGC823	HCT-8	A549	MCF-7
1	>10	>10	>10	>10	>10
2	>10	>10	>10	>10	>10
3	>10	>10	>10	>10	>10
4	>10	>10	>10	>10	>10
5	1.47	2.62	1.34	>10	>10
6	2.16	>10	>10	>10	>10
7	>10	>10	>10	>10	>10
8	6.21	>10	5.33	>10	>10
9	>10	>10	>10	>10	>10
Camptothecin (control)	0.06	0.09	0.14	0.09	0.01

Cytotoxic activities of compounds **1**–**9** were evaluated against several different cell lines. Bioassay experiments using the MTT method revealed that compound **5** was weakly cytotoxic against Bel-7402, BGC823, and HCT-8 human tumor cell lines, compound **6** was weakly cytotoxic against Bel-7402 human tumor cells, and compound **8** was weakly cytotoxic against Bel-7402 and HCT-8 human tumor cells. The other compounds exhibited no cytotoxicity at 10 μg/mL (Table 3).

Moraceae comprises a large family of sixty genera and about 1400 species, which are found in temperate, subtropical, and tropical regions of the world (Nomura and Hano, 1994). Moraceous plants are rich sources of the phenolic compounds, including flavonoids, xanthones, benzophenones and stibenes. The phenolic compounds from the genus *Morus* and related plants were reviewed in the literature (Nomura, 1988). Flavonoids of Moraceous plants have structural diversity, such as the flavonoids we isolated from *C. cochinchinensis*. But to our knowledge, flavonoids with 2′-hydroxy and 2′,4′-dihydroxy groups in ring B were common in the members of the family Moraceae plants. For instance, many isoprenoid substituted flavonoids and their Diels–Alder-type adducts with 2′-hydroxy and 2′,4′-dihydroxy groups in ring B were isolated from the genus *Morus*, *Ficus*, and *Artocarpus* (Tahara and Ibrahim, 1995). Morin and its derivative are typical flavonoids and have been isolated from many Moraceae plants including *C. cochinchinensis*. But the flavonol salts such as compound **1** and **2** were hitherto unprecedented. From a biosynthetic pathway perspective, it is possible that the flavonol salts were formed by a flavonol and insertion of Mg^{2+} or Ca^{2+} in plant under special condition. On the other hand, the existence of the hydroxyl proton at C-2′ favors a flavonol salt. Thus, flavonol salts are a new type of flavone in chemotaxonomy.

3. Experimental

All melting points were determined on a Reichert Nr-229 micromelting point apparatus and are uncorrected. The optical rotations were measured on a Perkin–Elmer 241 digital polarimeter in CH_3OH. UV spectra were

determined with a Hitachi UV-240 spectrophotometer. IR spectra were recorded on an IMPACT 400 (KBr) spectrometer. ^{1}H NMR (500 MHz, 300 MHz), ^{13}C NMR (125 MHz, 75 MHz), NOE, and HMQC, HMBC spectra were run on an INOVA-500 and Mercury 300 spectrometers with TMS as internal standard and values were given in ppm (δ). HR-mass spectra were performed on VG-Autospec-300 mass spectrometer. Quantitative analysis of cation was determined on Agilent 7500a ICP-MS. Silica gel (160–200, 200–300 mesh) (Qingdao) was used for CC and silica gel 60 F-254 (Qingdao) for TLC and preparative TLC.

3.1. Plant material

The roots of *Cudrania cochinchinensis* were collected from Hainan province of People's Republic of China in July 2002. The plant material was identified by Professor Shi-Man Huang. A voucher specimen has been deposited in the Herbarium of the Department of Medicinal plants, Institute of Materia Media, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, P.R. China.

3.2. Extraction and isolation

The dried roots of *C. cochinchinensis* (10 kg) were exhaustively extracted with EtOH–H_2O (95:5) under reflux for 3 h. The EtOH extract was concentrated under reduced pressure to give a residue (980 g), which was suspended in H_2O with the suspension sequentially extracted with petroleum ether, EtOAc, and nBuOH. The EtOAc extract was evaporated in vacuo to give a residue (233 g), which was subjected to silica gel CC eluting with a $CHCl_3$–CH_3OH gradient to afford 22 fractions (Fr 1-22). The first fraction (non-polar compounds) and the MeOH fraction were not investigated further. Fr. 2 (1 g), Fr. 3 (1 g), Fr. 4 (1 g), Fr. 5 (1 g), and Fr. 6 (1 g) were crystallized in acetone to give compound **12** (10 mg), **11** (20 mg), **14** (60 mg), **6** (300 mg), and **5** (500 mg), respectively. Fr. 7 (4 g) was applied to a polyamide column eluted with H_2O–EtOH to yield **9** (90 mg) (H_2O–EtOH = 4:1), **10** (70 mg) (H_2O–EtOH = 4:1), and **13** (20 mg), whereas Fr. 10 (2 g) was subjected to polyamide CC eluted with $CHCl_3$–MeOH to yield **3** (12 mg) ($CHCl_3$–MeOH = 10:1) and **4** (90 mg) ($CHCl_2$–MeOH = 9:1). Fr. 13 (10 g) was further purified by polyamide CC eluted with H_2O–EtOH to yield **7** (1 g) (H_2O–EtOH = 4:1) and **8** (5 g), whereas Fr. 15 (4.2 g), following polyamide CC eluted with H_2O–EtOH, yield **15** (10 mg) (H_2O–EtOH = 5:1) and **1** (100 mg) (H_2O–EtOH = 4:1). Fr. 16 (2.5 g) was subjected to polyamide column chromatography with $CHCl_3$–MeOH to yield **2** (400 mg) ($CHCl_3$–MeOH = 3:1).

3.3. Cochinchinol A (*1*)

Bright yellow powder; UV (MeOH) λ_{max} ($\log\varepsilon$) 263 (4.47), 399 (4.31) nm, λ_{max} ($AlCl_3$) 263, 414 nm, λ_{max} ($AlCl_3$ + HCl) 263, 411 nm, λ_{max} (HCl) 250, 260 (sh), 351 nm; IR (KBr) ν_{max} 3415, 1624, 1597, 1512, 1448, 1201, 1099, 984, 827 cm^{-1}; for ^{1}H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 2; ESIMS(+) *m/z* 655, 677, 339, 317; ESIMS(−) *m/z* 653, 315, 675. **1a**: for ^{1}H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 2; ESIMS(−) *m/z* 315 $[M-1]^-$, 631 $[2M-1]^-$.

3.4. Cochinchinol B (*2*)

Bright yellow powder; UV (MeOH) λ_{max} ($\log\varepsilon$) 268 (4.58), 418 (4.37) nm, λ_{max} ($AlCl_3$) 265, 420 nm, λ_{max} ($AlCl_3$ + HCl) 266, 417 nm, λ_{max} (HCl) 255, 263 (sh), 358 nm; IR(KBr) ν_{max} 3467, 3338, 1651, 1610, 1564, 1500, 1446, 1371, 1255, 974, 837 cm^{-1}; for ^{1}H NMR (DMSO-d_6, 300 MHz) and ^{13}C NMR spectroscopic data, see Tables 1 and 2; ESIMS(+) *m/z* 643, 341, 303; ESIMS(−) *m/z* 641, 301, 943. **2a**: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 6.28 (1H, *d*, $J = 1.8$ Hz), 6.16 (1H, *d*, $J = 1.8$), 6.33 (1H, *dd*, $J = 8.4$, 2.4 Hz), 6.36 (1H, *d*, $J = 2.4$ Hz), 7.22 (1H, $J = 8.4$ Hz), 12.68 (1H, s), 10.70 (1H, s), 9.75 (1H, s); ^{13}C NMR (DMSO-d_6, 300 MHz) δ 149.0, 136.2, 176.2, 160.9, 98.0, 163.6, 93.3, 156.7, 103.5, 109.1, 156.7, 102.9, 160.4, 106.8, 131.7. **2b**: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 5.96 (1H, *d*, $J =$ 2.1 Hz), 6.00 (1H, *d*, $J = 2.1$ Hz), 15.60 (1H, s), 13.54 (1H, s), 6.16 (1H, *d*, $J = 2.1$), 6.12 (1H, *dd*, $J = 9.0$, 2.1 Hz), 7.35 (1H, *d*, $J = 9.0$ Hz); ^{13}C NMR (DMSO-d_6, 300 MHz) δ 148.8, 140.8, 178.3, 160.0, 97.4, 164.1, 93.0, 156.6, 103.1, 114.1, 160.9, 104.8, 163.7, 105.2, 127.9.

3.5. (2R,3R)-4′,7-Dihydroxy-2′,5-dimethoxydihydroflavonol (*3*)

White powder; $[\alpha]_D^{25} + 82.7$ (*c* 0.12, MeOH); UV (MeOH) λ_{max} ($\log\varepsilon$) 205 (4.55), 286 (4.58) nm; IR (KBr) ν_{max} 3352, 3035, 1660, 1608, 1593, 1514, 1479, 1111, 997, 831 cm^{-1}; for ^{1}H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 2; FABMS *m/z* 331 $[M-1]^-$, 283, 255, 177, 139; HRFABMS *m/z* 331.0816 $[M-1]^-$.

3.6. 4′,7-dihydroxy-2′,5-dimethoxyflavonol (*4*)

Yellow powder; UV (MeOH) λ_{max} ($\log\varepsilon$) 205 (4.55), 248 (4.58), 347 nm; λ_{max} ($AlCl_3$) 210, 259, 400 nm, λ_{max} ($AlCl_3$ + HCl) 204, 259, 397 nm, λ_{max} (HCl) 203, 247, 343 nm; IR (KBr) ν_{max} 3408, 3240, 3018, 2584, 1666, 1622, 1601, 1568, 1509, 1456, 1173, 958, 831 cm^{-1}; for ^{1}H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 2; EIMS *m/z* 330 $[M]^+$, 314, 299, 284, 167, 162, 134; HREIMS *m/z* 330.0729 $[M]^+$.

3.7. Cytotoxicity experiments

Cytotoxicity against human tumor cell was measured in a 5-day MTT test for HCT-8, A549, Bel-7402, BGC823 and MCF-7. Briefly, 1×10^3 cells/100 μL were seeded in 96-well

microplates and preincubated for 24 h to allow cell attachment. This medium was then aspirated and 100 μL of fresh medium containing various concentrations of test drug were added to the cultures. The cells were incubated with each drug for 5 days. Cell survival was evaluated by adding 50 μL of MTT reagent (5 mg MTT/ml in RPMI 1640 medium) to each well. After 4 h reincubation at 37 °C, 100 μL DMSO was added to dissolve the precipitate of reduced MTT. Microplates were agitated on a rotation platform at room temperature for 15 min, and the absorbance of the reaction mixtures was determined at 570 nm with a multiwell scanning spectrophotometer.

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