Four New Isoflavonoids from the Stem Bark of *Erythrina variegata*

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Bioassay-directed fractionation of the stem bark extract of *Erythrina variegata* L. has resulted in the isolation of three new isoflavones: 5,4′-dihydroxy-8-(3,3-dimethylallyl)-2″-methoxyisopropylfurano[4,5:6,7]isoflavone (1), 5,7,4′-trihydroxy-6-(3,3-dimethylallyloxiranylmethyl)isoflavone (2), 5,4′-dihydroxy-8-(3,3-dimethylallyl)-2″-hydroxymethyl-2″-methylpyrano[5,6:6,7]isoflavone (3) and a new isoflavanone, 5,4′-dihydroxy-2′-methoxy-8-(3,3-dimethylallyl)-2″,2″-dimethylpyrano[5,6:6,7]isoflavanone (4) together with seven known compounds, euchrenone b_{10} (5), isoerysenegalensein E (6), wighteone (7), laburnetin (8), lupiwighteone (9), erythrodiol (10), and oleanolic acid (11). The structures were determined on the basis of spectroscopic analyses and chemical evidence. The effect of these compounds on the proliferation of rat osteogenic sarcoma (UMR106) is also reported.

Key words *Erythrina variegata*; Leguminosae; isoflavone; UMR106 cell

Erythrina variegata L. (Leguminosae) is a folk medicine in China and has been mainly used as an antibacterial, anti-inflammatory, antipyretic, and antiseptic agent.[1)] Phytochemical investigation of the non-alkaloidal secondary metabolites of the genus *Eryhtrina* revealed the presence of one cinnamylphenol[2)] and several isoflavonoids,[3—7)] some of which have antimicrobial[2)] and anti-inflammatory activities.[3)] This study reports the isolation and structural elucidation of three new isoflavones (**1**—**3**), one new isoflavanone (**4**), and seven known compounds **5**—**11** from the stem bark of this plant *E. variegata* that was obtained from China.

Results and Discussion

Fresh stem bark of *Erythrina variegata* was refluxed with 65% EtOH and the extract was concentrated under vacuum to afford a viscous residue. The residue was dissolved in water and partitioned successively with EtOAc and *n*-BuOH in the same volume three times, obtaining three fractions: EtOAc-, *n*-BuOH- and H_2O-soluble fractions. The effects of these fractions on proliferation of Rat osteogenic sarcoma (UMR106), osteoblast-like (OB-like) cells, were evaluated. As a result, the EtOAc-soluble fraction promoted the cell proliferation by 47% at the concentration of 2×10^{-3} mg/ml after 48 h of co-culture with UMR106 cells, and the *n*-BuOH-soluble fraction promoted the cell proliferation by 33% at the concentration of 2×10^{-2} mg/ml. The H_2O-soluble fraction, however, showed no significant difference from control, indicating that the EtOAc-soluble fraction might contain active constituents stimulating proliferation of the osteoblast. We then elucidated its active principle(s). Extensive column chromatography on silica gel, Sephadex LH-20 and ODS of the fraction yielded pure compounds **1**—**4** together with seven known compounds, which were identified as euchrenone b_{10} (**5**),[6)] isoerysenegalensein E (**6**),[8)] wighteone (**7**),[9)] laburnetin (**8**),[10)] lupiwighteone (**9**),[11)] erythrodiol (**10**),[12)] and oleanolic acid (**11**)[13)] by comparison of their spectral data with literature values.

Compound **1** was determined to be $C_{26}H_{26}O_6$ using HR-EI-MS ($[M]^+$ *m*/*z* 434.1724). The IR spectrum exhibited vibration bands for free hydroxyl (3356), conjugated carbonyl (1655), olefine (1628, 1512), and ether (1107) cm^{-1} functionalities. The ^{1}H-NMR signal at δ 8.38 (H-2), ^{13}C-NMR signal at δ 155.2, and UV absorption band at λ_{max}^{MeOH} 268 nm ($\log\varepsilon$ 4.75) were all typical characteristics of an isoflavone. This skeleton was also supported by its color tests: positive to $FeCl_3$ (greenish-brown) and negative to Mg–HCl. Compound **1** has one γ,γ-dimethylally group which was confirmed in the ^{1}H-NMR spectrum by one 2H doublet at 3.72 (2H, J=7.5 Hz, H-1‴), one 1H triplet at 5.38 (1H, J=7.5, 1.3 Hz, H-2‴), two 3H singlets at 1.67 (Me, *trans*) and 1.89 (Me, *cis*). The presence of a methoxyisopropyl furan substituent was confirmed by the ^{1}H-NMR spectrum which showed a *gem*-dimethyl signal at 1.63 (6H, H-5″, H-6″), one 3H singlet at 3.11 (3H, 4″-OCH_3), and one 1H singlet at 6.91 (1H, H-3″). The isopropyl furan skeleton was also supported by the ^{13}C-NMR spectrum which showed peaks for C-2″, C-3″, and C-4″ at δ 161.6, 102.0, and 73.9, respectively. In the aromatic region, a typical AA′BB′ spin coupling system of four aromatic protons at δ 7.50 (2H, J=8.6 Hz, H-2′, H-6′) and 6.93 (2H, J=8.6 Hz, H-3′, H-5′) was also shown, which suggested the presence of a *para*-substituted ring B.

It remained for us to establish unambiguously the position of the free prenyl group on ring A of **1** and to see if the fusion of the methoxyisopropylfuran unit is linear or angular. In the HMBC experiment, the H-1‴ proton of the prenyl at δ 3.72 showed correlations to both C-7 and C-9 indicating its C-8 location of the prenyl group. Consequently, the structure of **1** was identified as 5,4′-dihydroxy-8-(3,3-dimethylally)-2″-methoxyisopropylfurano[4,5:6,7]isoflavone.

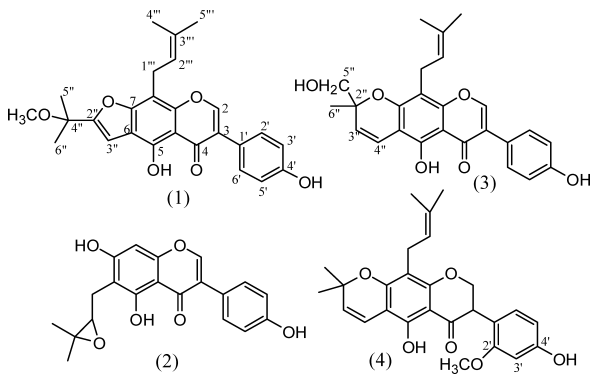

Fig. 1. Structures of Compounds **1**—**4**

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The molecular formula of **2** was determined as $C_{20}H_{18}O_6$ by HR-EI-MS ($[M]^+$ *m/z* 354.1109). The ^{1}H-NMR signal at δ 8.16 (H-2), ^{13}C-NMR signal at δ 154.4, and its UV spectrum revealed that it is also an isoflavone. This skeleton was also supported by its color tests: positive to $FeCl_3$ (greenish-brown) and negative to Mg–HCl. In the aromatic region, a typical AA′BB′ spin coupling system of four aromatic protons at δ 7.45 (2H, J=8.8 Hz, H-2′, H-6′) and 6.90 (2H, J=8.8 Hz, H-3′, H-5′) suggested the presence of a *para*-substituted ring B. Another aromatic proton at 6.36 indicated that either C-6 or C-8 of ring A was substituted. The presence of a 3,3-dimethyloxiranylmethyl substituent was confirmed by the ^{1}H-NMR spectrum which showed two 3H singlets at 1.33 (3H, 4″-CH_3) and 1.39 (3H, 5″-CH_3), one 1H multiplet at 3.88 (1H, H-2″), one 1H doublet of a doublet at 2.62 (1H, J=17.0, 7.2 Hz, H_a-1″) and one 1H multiplet at 2.94 (1H, H_b-1″). The skeleton was also supported by the ^{13}C-NMR spectrum which showed peaks for C-1″, C-2″, C-3″, C-4″ and C-5″ at δ 26.1, 68.8, 79.8, 21.2 and 25.8, respectively. And the attachment of the 3,3-dimethyloxiranylmethyl group was assigned to the C-6 from the HMBC spectrum that revealed correlations between the H_a-1″ proton of the unit and C-6, C-7, and between H_b-1″ proton and C-6 indicating its C-6 location. Consequently, **2** was deduced to be 5,7,4′-trihydroxy-6-(3,3-dimethyloxiranylmethyl)isoflavone.

The HR-EI-MS of **3** displayed a molecular ion $[M]^+$ at *m/z* 420.1576 in agreement with the formula $C_{25}H_{24}O_6$. The ^{1}H-NMR signal at δ 8.27 (H-2), ^{13}C-NMR signal at δ 154.4, and UV absorption band at λ_{max}^{MeOH} 289 nm ($\log \varepsilon$ 4.71) were all typical characteristics of an isoflavone. Its color tests: positive to $FeCl_3$ (greenish-brown) and negative to Mg–HCl also supported this skeleton. In the ^{1}H-NMR spectrum, the two doublets at δ 7.47 (2H, J=8.8 Hz, H-2′, H-6′) and 6.91 (2H, J=8.8 Hz, H-3′, H-5′) indicated the presence of a *para*-substituted ring B. On the other hand, the absence of other aromatic proton signals indicated that ring A was fully substituted. Moreover, the downfield chelated hydroxyl proton signal at δ 13.35 indicated that C-5 was attached by a hydroxyl group. The ^{1}H-NMR spectrum also revealed the presence of protons of a prenyl group (δ 1.82, 1.66, 3.43, 5.23), and protons of a 2-hydroxymethyl-2-methylpyran ring substituent, namely, one methyl signal at 1.44 (3H, H-6″), one 2H singlet at 3.67 (2H, H-5″), two 1H doublets at 6.78 (1H, J=10.1 Hz, H-4″), 5.76 (1H, J=10.1 Hz, H-3″). The skeleton was also supported by the ^{13}C-NMR spectrum which showed peaks for C-2″, C-3″, C-4″, C-5″ and C-6″ at δ 81.8, 126.3, 117.8, 68.7 and 23.6, respectively. An HMBC experiment was used to establish the position of the free prenyl group and to see if the fusion of the 2-hydroxymethyl-2-methylpyran unit is linear or angular. In the HMBC spectrum, the H-1‴ protons of the prenyl at δ 3.43 showed correlations to both C-7 and C-9, indicating its C-8 location of the prenyl group. H-3″ proton of the 2-hydroxymethyl-2-methylpyran moiety showed correlation to C-6, indicating that the 2-hydroxymethyl-2-methylpyran ring is linear.

Consequently, the structure of **3** was identified as 5,4′-dihydroxy-8-(3,3-dimethylally)-2″-hydroxymethyl-2″-methylpyrano[5,6:6,7]isoflavone.

The HR-EI-MS of **4** showed an $[M]^+$ at *m/z* 436.1878 consistent with the molecular formula $C_{26}H_{28}O_6$. All the carbons in the ^{13}C-NMR spectrum (Table 1) were sorted by DEPT experiment into four CH_3, one OCH_3, two CH_2, seven CH and 12 fully substituted carbons. The UV absorption bands at 275 nm, ^{1}H-NMR signals at 4.47 (2H, m, H-2), 4.28 (1H, dd, J=11.2, 5.6 Hz, H-3), and ^{13}C-NMR signals at δ 70.4 (C-2), 46.7 (C-3) were all diagnostic for an isoflavanone skeleton.[8] This skeleton was also supported by its color tests: positive to $FeCl_3$ (greenish-brown) and negative to Mg–HCl. ^{1}H-NMR spectrum showed ABX-type aromatic protons on the ring B, which was one 1H double doublet at 6.34 (IH, J=8.0, 2.0 Hz, H-5′), one 1H doublet at 6.92 (1H, J=8.0 Hz, H-6′), and a 1H broad singlet at 6.40 (1H, br s, H-3′). It also revealed the presence of protons of a prenyl group (δ 1.68, 1.76, 3.20, 5.16), and protons of a 2,2-dimethylpyran ring, a *gem*-dimethyl signal at 1.45 (6H, H-5″, H-6″), two 1H doublets at 5.49 (1H, J=10.0 Hz, H-3″), 6.64 (1H, J=10.0 Hz, H-4″). The relative positions of these groups were determined on the basis of the HMBC which showed that the 2H-1‴ protons of the prenyl at δ 3.20 were correlated to C-8, indicating its C-8 location of the prenyl group. Furthermore, the observed correlations between the H-4″ proton and C-5, C-7, and between the H-3″ proton and C-6 confirmed that the fusion of the 2,2-dimethylpyran ring is linear.

The location of a methoxyl group at C-2′ position was confirmed by the HMBC technique, which showed correlation between the methoxyl group and C-2′. Consequently, the structure of 4 was subsequently identified as 5,4′-dihydroxy-2′-methoxy-8-(3,3-dimethylally)-2″,2″-dimethylpyrano[5,6:6,7]isoflavanone.

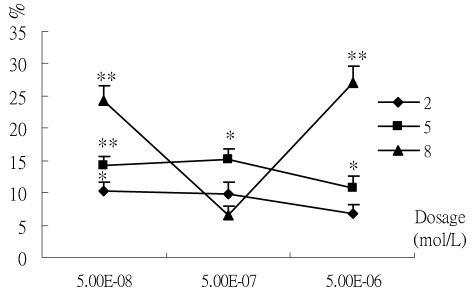

Fig. 2. Effects of Pure Compounds **2**, **5** and **8** on Proliferation of UMR106 Cells (n=4) at Dosage of 5×10^{-8}, 5×10^{-7} and 5×10^{-6}

$^*p<0.05$, $^{**}p<0.01$ as compared to control.

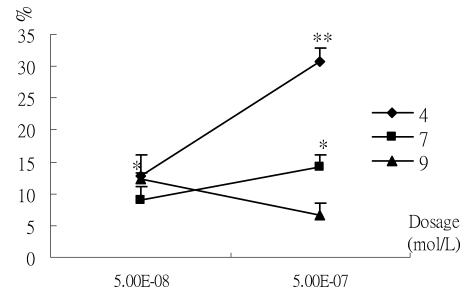

Fig. 3. Effects of Pure Compounds **4**, **7** and **9** on Proliferation of UMR106 Cells (n=4) at Dosage of 5×10^{-8} and 5×10^{-7}

$^*p<0.05$, $^{**}p<0.01$ as compared to control.

Experimental

General All melting points were determined on YANACO apparatus and are uncorrected. IR spectra were recorded on a SHIMADZU FTIR8900 spectrophotometer in a KBr disk and UV spectra were recorded on a SHIMADZU UV2401PC spectrophotometer. Optical rotations were taken on a

Table 1. Spectral Data for Compounds **1** and **2**[a)]

Position	**1**[b)]			**2**[b)]		
	^{13}C	^{1}H	HMBC	^{13}C	^{1}H	HMBC
2	155.2	8.38 (1H, s)	C-4, C-9, C-1′	154.4	8.16 (1H, s)	C-3, C-4, C-9
3	123.0			123.8		
4	183.8			181.8		
5	154.3			160.8		
6	113.8			105.1		
7	158.2			160.6		
8	104.6			95.1	6.36 (1H, s)	C-7, C-9, C-10
9	151.9			156.8		
10	107.4			105.7		
1′	123.1			123.1		
2′	131.3	7.50 (1H, d, J=8.6 Hz)	C-3, C-4′, C-6′	131.1	7.45 (1H, d, J=8.8 Hz)	C-3, C-4′, C-6′
3′	116.0	6.93 (1H, d, J=8.6 Hz)	C-1′, C-5′	116.0	6.90 (1H, d, J=8.8 Hz)	C-1′, C-4′, C-5′
4′	158.5			158.5		
5′	116.0	6.93 (1H, d, J=8.6 Hz)	C-1′, C-3′	116.0	6.90 (1H, d, J=8.8 Hz)	C-1′, C-3′, C-4′
6′	131.3	7.50 (1H, d, J=8.6 Hz)	C-3, C-2′, C-4′	131.1	7.45 (1H, d, J=8.8 Hz)	C-3, C-2′, C-4′
1″				26.1	2.94 (1H, m)	C-6
					2.62 (1H, dd, J=17.0, 7.2 Hz)	C-6, C-7, C-2″
2″	161.6	3.88 (1H, m)		68.8		
3″	102.0		C-6, C-7, C-2″	79.8	6.91 (1H, s)	
4″	73.9	1.33 (3H, s)		21.2		C-2″, C-3″, C-5″
5″	25.5	1.39 (3H, s)	C-2″, C-4″, C-6″	25.8	1.63 (3H, s)	C-2″, C-3″, C-4″
6″	25.5		C-2″, C-4″, C-5″		1.63 (3H, s)	
1‴	22.7		C-7, C-8, C-9, C-2‴, C-3‴		3.72 (2H, d, J=7.5 Hz)	
2‴	122.0				5.38 (1H, tt, J=7.5, 1.3 Hz)	
3‴	133.3					
4‴	17.9		C-2‴, C-3‴, C-5‴		1.89 (3H, s)	
5‴	25.9		C-2‴, C-3‴		1.67 (3H, s)	
OCH_3-4″	51.1	3.11 (3H, s)	C-4″			
OH-5		13.72 (1H, s)	C-5, C-6, C-10		13.36 (1H, s)	C-5, C-6, C-10
OH-4′					8.71 (1H, s)	C-3′, C-5′

a) Assignments made by combination of COSY, DEPT135 and HMQC experiments. *b*) Measured in acetone-d_6.

Table 2. Spectral Data for Compounds **3** and **4**[a)]

Position	**3**[b)]			**4**[c)]		
	^{13}C	^{1}H	HMBC	^{13}C	^{1}H	HMBC
2	154.4	8.27 (1H, s)	C-3, C-4, C-9	70.4	4.47 (2H, m)	C-4, C-9
3	123.8			46.7	4.28 (1H, dd, J=11.2, 5.6 Hz)	C-2, C-1′
4	182.2			198.1		
5	155.8			157.0		
6	107.9			102.8		
7	157.7			159.6		
8	106.0			108.4		
9	155.6			159.8		
10	106.5			103.0		
1′	123.1			115.1		
2′	131.2	7.47 (1H, d, J=8.8 Hz)	C-3, C-4′, C-6′	158.6		
3′	116.0	6.91 (1H, d, J=8.8 Hz)	C-1′, C-4′, C-5′	99.7	6.40 (1H, br s)	C-1′, C-2′, C-4′, C-5′
4′	158.5			156.8		
5′	116.0	6.91 (1H, d, J=8.8 Hz)	C-1′, C-3′, C-4′	107.5	6.34 (1H, dd, J=8.0, 2.0 Hz)	C-1′, C-3′
6′	131.2	7.47 (1H, d, J=8.8 Hz)	C-3, C-2′, C-4′	130.9	6.92 (1H, d, J=8.0 Hz)	C-3, C-2′, C-4′
2″	81.8			78.0		
3″	126.3	5.76 (1H, d, J=10.1 Hz)	C-6, C-2″	125.9	5.49 (1H, d, J=10.0 Hz)	C-6, C-2″
4″	117.8	6.78 (1H, d, J=10.1 Hz)	C-7, C-2″	115.8	6.64 (1H, d, J=10.0 Hz)	C-7, C-5, C-2″
5″	68.7	3.67 (2H, s)	C-3″, C-6″	28.4	1.45 (3H, s)	C-2″, C-3″, C-6″
6″	23.6	1.44 (3H, s)	C-2″, C-3″, C-5″	28.4	1.45 (3H, s)	C-2″, C-3″, C-5″
1‴	21.9	3.43 (2H, m)	C-7, C-8, C-9, C-2‴, C-3‴	21.3	3.20 (2H, d, J=7.2 Hz)	C-8, C-2‴, C-3‴
2‴	123.0	5.23 (1H, m)		122.6	5.16 (1H, t, J=7.2 Hz)	
3‴	132.1			131.1		
4‴	18.0	1.82 (3H, br s)	C-2‴, C-3‴, C-5‴	17.8	1.76 (3H, s)	C-2‴, C-3‴, C-5‴
5‴	25.9	1.66 (3H, br s)	C-2‴, C-3‴, C-4‴	25.8	1.68 (3H, s)	C-2‴, C-3‴, C-4‴
OCH_3-2′				55.5	3.75 (3H, s)	C-2′
OH-5		13.35 (1H, s)			12.45 (1H, s)	C-5, C-6, C-10

a) Assignments made by combination of COSY, DEPT135 and HMQC experiments. *b*) Measured in acetone-d_6. *c*) Measured in $CDCl_3$.

Table 3. Effects of Pure Compounds on Proliferation of UMR106 Cells (n=4)

Compounds	5×10^{-8} mol/l	5×10^{-7} mol/l	5×10^{-6} mol/l	5×10^{-5} mol/l
1	*a)*	2.67	*a)*	*a)*
2	10.37*	9.87	6.66	*a)*
3	14.83	4.57	5.67	*a)*
4	12.87	30.67**	*a)*	*a)*
5	14.23**	15.26*	10.81*	*a)*
6	*a)*	*a)*	20.87**	*a)*
7	9.04	14.21*	*a)*	*a)*
8	24.25**	6.59	27.00**	*a)*
9	12.31*	6.63	*a)*	*a)*
10	7.67	7.30	*a)*	*a)*
11	14.87	3.84	8.68	*a)*
IGF-1		21.16**		

a) Nonstimulating effect. $*p<0.05$, $**p<0.01$. Prol. Rat=$(OD_{570,treatment}/OD_{570,control}-1)\times100\%$.

JASCO P-1020 digital polarimeter. The ^{1}H-NMR spectra were obtained on a Bruker AVANCE 400 MHz spectrometer, while ^{13}C-NMR spectra were recorded at 100 MHz on the same instrument, with TMS as an internal standard. Mass spectra were determined on a Bruker Esquire 2000 spectrometer, and column chromatography was performed using silica gel (200—300 mesh), Sephadex^{TM} LH-20 and ODS.

Plant Material Stem bark of *E. variegata* was collected in May 2003 at Shenzhen (China). The identification of the plant was confirmed by one of the authors (Qi-shi Sun). A voucher specimen (No. Y0305EV) was deposited in the Shenzhen Research Center of traditional Chinese medicines & natural products.

Extraction and Isolation Fresh and sliced stem bark (6.0 kg) of *E. variegata* was refluxed with 65% EtOH and the extract was concentrated under vacuum to afford a viscous residue (320 g). The residue was dissolved in water and partitioned successively with EtOAc and *n*-BuOH in the same volume three times. Three fractions, EtOAc- (44 g), *n*-BuOH- (54 g) and H_2O-soluble (220 g) fractions were obtained. The effects of these fractions on proliferation of rat osteogenic sarcoma (UMR106), which were osteoblast-like (OB-like) cells, were evaluated. The EtOAc-soluble fraction promoted the cell proliferation by 47% at the concentration of 2×10^{-3} mg/ml after 48 h of co-culture with UMR106 cells; the *n*-BuOH-soluble fraction promoted the cell proliferation by 33% at the concentration of 2×10^{-2} mg/ml. But the H_2O-soluble fraction showed no significant difference from control. The results indicated that the EtOAc-soluble fraction might contain active constituents stimulating the proliferation of the osteoblast. We therefore elucidated its active principle(s). The EtOAc-soluble fraction was subjected to column chromatography over silica gel (200—300 mesh) and eluted with cyclohexane–EtOAc with increasing polarity to yield 78 fractions (each fraction; 400 ml, column A). Fractions A34—38 (4.1 g) were applied to a Sephadex LH-20 column chromatography using $CHCl_3$–MeOH (1 : 1) to yield 12 fractions (each fraction; 10 ml, column B). Fractions B6—11 (3.1 g) were separated by MPLC on ODS using MeOH–H_2O (70 : 30→85 : 15) to yield **4** (6 mg), isoerysenegalensein E (**6**) (30 mg), erythrodiol (**10**) (7 mg) and oleanolic acid (**11**) (175 mg). Fractions A39—46 (5.1 g) were subjected to Sephadex LH-20 chromatography using $CHCl_3$–MeOH (1 : 1) to yield 16 fractions (each fraction; 10 ml, column C). Fractions C8—10 (1.1 g) were purified by MPLC on ODS using MeOH–H_2O (63 : 27→77 : 23) to yield **1** (7 mg), **2** (5 mg), **3** (15 mg), euchrenone b_{10} (**5**) (50 mg), and laburnetin (**8**) (6 mg). Fractions C12—14 (1.5 g) were purified by MPLC on ODS using MeOH–H_2O (67 : 33) to yield wighteone (**7**) (140 mg) and lupiwighteone (**9**) (8 mg).

5,4′-Dihydroxy-8-(3,3-dimethylally)-2″-methoxyisopropylfurano-[4,5:6,7]isoflavone (1) Yellow amorphous powder, mp 101—102 °C; HR-EI-MS m/z: 434.1724 (Calcd for $C_{26}H_{26}O_6$: 434.1729); UV λ_{max} (MeOH) (log ε) nm: 268 (4.75), 353 (3.49); IR ν_{max} (KBr) cm^{-1}: 3356, 2982, 2932, 1655, 1628, 1562, 1512, 1423, 1365, 1335, 1223, 1107, 1069, 988, 837. ^{1}H-NMR; ^{13}C-NMR: Table 2.

5,7,4′-Trihydroxy-6-(3,3-dimethyloxiranylmethyl)isoflavone (2) Yellow amorphous powder, mp 252—254 °C; HR-EI-MS m/z: 354.1109 (Calcd for $C_{20}H_{18}O_6$: 354.1103); UV λ_{max} (MeOH) (log ε) nm: 212 (4.64), 265 (4.75), 300 (4.14), 339 (3.71); IR ν_{max} (KBr) cm^{-1}: 3441, 2932, 1655, 1616, 1578, 1516, 1462, 1369, 895, 837 and 818. $[\alpha]_D^{20}$ 10.8° (c=0.1, MeOH). ^{1}H-NMR; ^{13}C-NMR: Table 1.

5,4′-Dihydroxy-8-(3,3-dimethylally)-2″-hydroxymethyl-2″-methyl-pyrano[5,6:6,7]-isoflavone (3) Yellow amorphous powder, mp 205—207 °C; HR-EI-MS m/z: 420.1576 (Calcd for $C_{25}H_{24}O_6$: 420.1573); UV λ_{max} (MeOH) (log ε) nm: 289 (4.71), 226 (4.44); IR ν_{max} (KBr) cm^{-1}: 3456, 3283, 2909, 1659, 1616, 1570, 1516, 1435, 1230, 887, 837 and 818. $[\alpha]_D^{20}$ −7.8° (c=0.1, MeOH). ^{1}H-NMR; ^{13}C-NMR: Table 1.

5,4′-Dihydroxy-2′-methoxy-8-(3,3-dimethylally)-2″,2″-dimethyl-pyrano[5,6:6,7]-isoflavanone (4) Yellow oil, HR-EI-MS m/z: 436.1878 (Calcd for $C_{26}H_{28}O_6$: 436.1886); UV λ_{max} (MeOH) (log ε) nm 269 (4.48), 275 (4.49), 312 (3.90) and 360 (3.20). $[\alpha]_D^{20}$ 7.6° (c=0.1, MeOH). ^{1}H-NMR; ^{13}C-NMR: Table 2.

Proliferation Assay The effects of these fractions and compounds on proliferation of rat osteogenic sarcoma (UMR106), which was osteoblast-like (OB-like) cells, were evaluated. The cell line was purchased from the American Type Culture Collection. Each test material was added to cultivated UMR106 cells after 24 h and the cells were further cultivated for 48 h. The proliferation of UMR106 cells was measured using the MTT assay and the results of pure compounds are shown in Table 3.

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