Prenylisoflavone Derivatives from the Roots of *Hedysarum scoparium*

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Four new prenylisoflavone derivatives, namely, 5-hydroxy-4′-methoxy-8-prenyl-2″-hydroxyisopropyldihydrofurano[4,5:6,7]-isoflavone (1), 5-hydroxy-4′-methoxy-6-prenyl-2‴-hydroxyisopropyldihydrofurano[4,5:8,7]-isoflavone (2), 5-hydroxy-4′-methoxy-8-prenyl-1″,2″-peroxyl-3″,3″-dimethyldihydropyrano[5,6:6,7]-isoflavone (3), and 5-hydroxy-4′-methoxy-6-prenyl-1‴,2‴-peroxyl-3‴,3‴-dimethyldihydropyrano[5,6:8,7]-isoflavone (4), together with three known ones 5—7, were isolated from the roots of *Hedysarum scoparium*. Their structures were established by means of detailed spectroscopic analysis (IR, EI- or HR-ESI-MS as well as 1D- and 2D-NMR), and by comparison of their spectroscopic data with those reported for structurally related compounds.

Key words *Hedysarum scoparium*; Leguminosae; isoflavone; prenylisoflavone derivative

The genus *Hedysarum* belongs to the Leguminosae family. Many of the *Hedysarum* plants have been used as folk remedies in China, especially as detoxifying and diuretic agents, and also with some efficacy against diabetes and chronic nephritis as recorded in some Chinese herbal books.[1—3)]

Extracts of some species of *Hedysarum* have been reported to have the potential to treat dementia[4)] and Alzheimer's disease (AD) from the results of their improving learning ability and memory of mice *in vivo* and showing remarkable acctylcholinesterase (AChE) inhibitory activity *in vitro*.[5)] Furthermore, these extracts might show promise against myocarditis[6)] and to stop low-level replication of HBV, combination with anti-hepatitis B vaccine (EV therapy),[7)] as well as to have antioxidant effect, cell-regenerating effect, and anti-aging effect.[8,9)]

The chemical components of 40 species were studied from 285 genus *Hedysarum* species. The compounds found in the plants include isoprenoids, alkaloids, amino acids, phenol compounds (flavoids, coumarins, xanthones, phenolic acids), mono-, oligo-, and polysaccharides, aliph compounds, macro- and microelements, and vitamins, many of them being of interest for traditional and modern medicines.[10)] We studied the dried rhizomes of *Hedysarum scoparium* found in desert areas of northwestern China and obtained four new prenylisoflavone derivatives **1**—**4**, and three known ones **5**—**7** from this species. Herein, we report their isolation and structural determination.

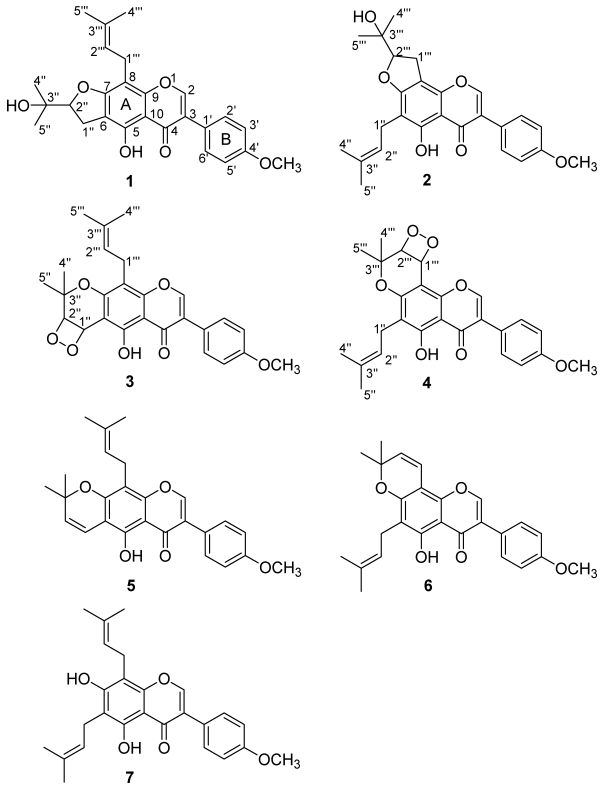

Fig. 1. Structures of Compounds **1**—**7**

Results and Discusion

The known compounds were identified by comparing their physical (mp), spectroscopic (IR, NMR), and mass-spectrometric (MS) data with those reported in the literature, as warangalone 4′-methyl ether (**5**),[11,12)] osajin 4′-methyl ether (**6**),[11,12)] and 5,7-dihydroxy-4′-methoxy-6,8-diprenylisoflavone (**7**).[13,14)] The data of ^{13}C-NMR of **6** were reported for the first time, and its structure was supported by the HMBC correlations.

The new compound **1**, $[\alpha]_D^{20}=+5°$ ($c=0.1$, $CHCl_3$), was obtained as a yellow oil. Its EI-MS gave the $[M]^+$ signal at *m*/*z* 436 and HR-ESI-MS showed the $[M+H]^+$ peak at *m*/*z* 437.1962 (Calcd for $C_{26}H_{28}O_6+H$ 437.1959), corresponding to the molecular formula $C_{26}H_{28}O_6$. The IR spectrum (ν_{max} 3347 cm^{-1} for OH, 1660 cm^{-1} for conjugated C=O) and the UV spectrum (λ_{max} 269 nm) indicated the presence of an

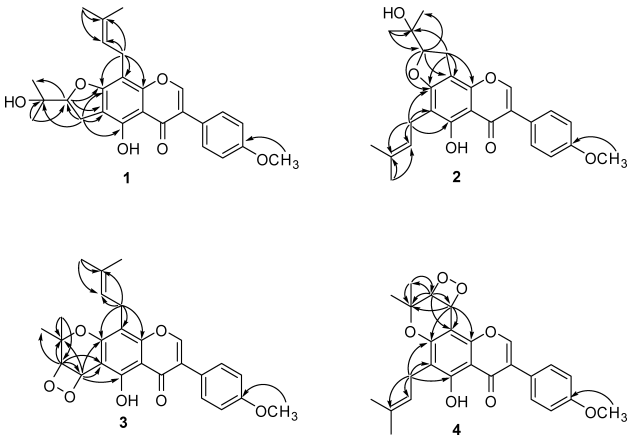

Fig. 2. Important HMBC Correlations of **1**—**4**

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isoflavone nucleus.[15)]

The ^{1}H-NMR spectrum of **1** showed the characteristic signal of H-2 of an isoflavone at δ 7.92 (1H, s) and AA′BB′-type signals at δ 7.46 (2H, d, J=8.7 Hz) and δ 6.98 (2H, d, J=8.7 Hz) assignable to H-2′, 6′ and H-3′, 5′ of the B-ring of the isoflavone, together with typical signals due to a HO-5 of a flavonoid at δ 13.02 (1H, s)[16)] and one set of 3,3-dimethylallyl group (or prenyl group) (Table 1). The ^{13}C-NMR spectrum showed 13 signals from δ 102.1 to δ 181.3 due to the isoflavone skeleton including two intense signals at δ 114.1 (CH) and 130.1 (CH) assigned respectively to two equivalent aromatic carbons (H-3′ and 5′ and H-2′ and 6′) of the symmetrical B-ring and also gave 5 signals due to the prenyl unit (Table 2). The remaining proton and carbon signals, apart from δ_H 3.84 and δ_C 55.4 considered as a methoxyl group, were deduced as a partial structure, –CH_2CH(O–)C(CH_3)$_2$(OH) from the ^{1}H–^{1}H COSY and HMBC correlations. Furthermore, the position of the partial structure was determined by the following HMBC correlations: from H_2-1″ to C-5, C-6 and C-7, from H- 2″ to C-6 and C-7, which suggested that it was connected at C-6 and C-7 of the isoflavone skeleton through the CH_2 and the oxygen atom, respectively, resulting in the formation of a dihydrobenzofuran ring composed of A-ring. This deduction was in accordance with the thirteen unsaturated degrees of the molecular formula of **1**. Also, the positions of the prenyl and OMe were determined as at C-8 and C-4′ for the HMBC correlations of H_2-1‴ to C-7, C-8, and C-9 and OMe to C-4′. Therefore the structure of **1** was concluded as a prenylisoflavone and named as 5-hydroxy-4′-methoxy-8-prenyl-2″-hydroxyisopropyldihydrofurano[4,5:6,7]-isoflavone.

Further structural confirmation came from comparing the observed ^{1}H and ^{13}C shifts with those of similar known compounds that have been reported in the literature.[17,18)] Unfortunately, the absolute configuration at C-2″ was unable to be determined for the scarcity of a very effective method.

Compound **2**, $[\alpha]_D^{20}$=+9° (c=0.1, $CHCl_3$), was obtained as a yellow oil and its molecular formula was determined to be $C_{26}H_{28}O_6$ from the HR-ESI-MS for the $[M+H]^+$ signal at m/z 437.1952 (Calcd for $C_{26}H_{28}O_6$+H 437.1959) and from the EI-MS for the $[M]^+$ peak at m/z 436, indicating thirteen degrees of unsaturation as that of **1**. The IR (3357, 1662 cm^{-1}) and UV (λ_{max} 269 nm) spectra of **2** were closely similar to those of **1**, which suggested that **2** had the same chromophores and functionalities as **1**. The NMR spectral data of **2** and **1** were also much the same except for the ^{13}C-NMR data of the A-ring (Table 2) and the ^{1}H-NMR shift of HO-5 (Table 1), which showed that an evident difference between **2** and **1** was the locations of the dihydrobenzofuran ring and the prenyl group in the A-ring. In the HMBC experiment, the cross peaks of H_2-1‴ to C-7, C-8 and C-9, H-2‴ to C-7 and C-8 suggested that the dihydrobenzofuran ring was composed of A-ring at C-7 and C-8 of the isoflavone skeleton, whereas, the prenyl group was located at C-6 due to the following HMBC correlations: from H_2-1″ to C-5, C-6, and C-7. Similarly, the uncertainty of the absolute configuration of C-2‴ of **2** was also as a result of the absence of a very effective method. Thus the structure of **2** was concluded to be 5-hydroxy-4′-methoxy-6-prenyl-2‴-hydroxyisopropyldihydrofurano[4,5:8,7]-isoflavone.

Compound **3** was obtained as a white amorphous solid showing $[\alpha]_D^{20}$=+6° (c=0.1, $CHCl_3$). Its HR-ESI-MS showed the $[M+H]^+$ signal at m/z 451.1759 (Calcd for $C_{26}H_{26}O_7$+H 451.1751), and the EI-MS gave the $[M]^+$ peak at m/z 450, corresponding to the molecular formula $C_{26}H_{26}O_7$, in accordance with fourteen degrees of unsaturation. **3** was also an isoflavone derivative as deduced from the IR spectrum (3413 cm^{-1} for OH, 1656 cm^{-1} for conjugated C=O) and the UV spectrum (λ_{max} 268 nm). The ^{13}C-NMR spectrum also showed 13 signals (δ from 102.1 to 181.5) due to the isoflavone skeleton including two intense signals at δ 114.1 (C-3′ and C-5′) and δ 130.1 (C-2′ and C-6′) of the symmetrical B-ring and 5 signals arising from one prenyl group (Table 2). The signal of δ 55.3 was considered as a methoxyl group from its chemical shift and DEPT spectrum.

These data suggested that the structure of **3** was similar to that of **1**. However an evident difference was the presence of a 1″,2″-peroxybenzopyran ring composed of A-ring in **3** instead of the dihydrobenzofuran in **1**, which was deduced by

Table 1. ^{1}H-NMR Data for **1**—**4** (in $CDCl_3$, 300 MHz, δ ppm, J Hz).

Position	1	2	3	4
2	7.92 s	7.80 s	7.92 s	7.86 s
5-OH	13.02 s	13.23 s	13.31 s	13.43 s
2′, 6′	7.46 d (8.7)	7.44 d (9)	7.46 d (8.4)	7.44 d (8.7)
3′, 5′	6.98 d (8.7)	6.97 d (9)	6.98 d (8.4)	6.98 d (8.7)
1″	3.18 m	3.34 d (7.5)	6.13 d (6.6)	3.34 d (7.8)
2″	4.78 t (8.6)	5.28 br t (7.5)	5.30 d (6.6)	5.25 br t (7.8)
4″	1.34 s	1.69 br s	1.43 s	1.67 br s
5″	1.22 s	1.78 br s	1.39 s	1.77 br s
1‴	3.39 d (7.5)	3.23 m	3.39 d (7.5)	6.18 d (5.7)
2‴	5.21 br t (7.5)	4.79 t (8.0)	5.20 br t (7.5)	5.33 d (5.7)
4‴	1.69 br s	1.36 s	1.68 br s	1.44 s
5‴	1.79 br s	1.25 s	1.78 br s	1.40 s
MeO-4′	3.84 s	3.84 s	3.84 s	3.84 s

Table 2. ^{13}C-NMR Data for **1**—**4**, and **6** (in $CDCl_3$, 75 MHz, δ ppm)

Position	1	2	3	4	6
2	152.4 (d)	152.1 (d)	152.6 (d)	152.0 (d)	152.1 (d)
3	123.1 (s)	123.7 (s)	123.3 (s)	125.1 (s)	123.4 (s)
4	181.3 (s)	181.1 (s)	181.5 (s)	180.8 (s)	180.9 (s)
5	155.1 (s)	160.5 (s)	157.8 (s)	162.5 (s)	159.3 (s)
6	108.6 (s)	107.8 (s)	106.4 (s)	107.5 (s)	105.6 (s)
7	164.0 (s)	164.6 (s)	164.7 (s)	165.5 (s)	157.2 (s)
8	102.1 (s)	102.8 (s)	102.1 (s)	100.8 (s)	100.7 (s)
9	155.3 (s)	151.2 (s)	156.9 (s)	152.4 (s)	150.5 (s)
10	106.7 (s)	106.2 (s)	106.9 (s)	106.6 (s)	105.6 (s)
1′	123.1 (s)	123.5 (s)	122.8 (s)	122.9 (s)	123.2 (s)
2′	130.1 (d)	130.5 (d)	130.1 (d)	130.2 (d)	130.1 (d)
3′	114.1 (d)	114.3 (d)	114.1 (d)	114.1 (d)	114.1 (d)
4′	159.7 (s)	160.0 (s)	159.8 (s)	159.9 (s)	159.7 (s)
5′	114.1 (d)	114.3 (d)	114.1 (d)	114.1 (d)	114.1 (d)
6′	130.1 (d)	130.5 (d)	130.1 (d)	130.2 (d)	130.1 (d)
1″	27.1 (t)	22.2 (t)	82.6 (d)	21.6 (t)	21.3 (t)
2″	91.3 (d)	121.8 (d)	98.2 (d)	121.1 (d)	121.9 (d)
3″	72.0 (s)	132.4 (s)	85.6 (s)	132.4 (s)	131.6 (s)
4″	25.6 (q)	26.0 (q)	23.8 (q)	25.7 (q)	25.8 (q)
5″	23.8 (q)	18.1 (q)	18.1 (q)	17.8 (q)	17.9 (q)
1‴	21.9 (t)	27.5 (t)	21.2 (t)	83.0 (d)	115.0 (d)
2‴	121.4 (d)	91.3 (d)	121.1 (d)	98.2 (d)	127.1 (d)
3‴	132.3 (s)	72.2 (s)	132.5 (s)	85.8 (s)	77.8 (s)
4‴	25.7 (q)	26.1 (q)	25.7 (q)	23.9 (q)	28.1 (q)
5‴	17.8 (q)	24.4 (q)	17.8 (q)	18.1 (q)	28.1 (q)
MeO-4′	55.4 (q)	55.6 (q)	55.3 (q)	55.4 (q)	55.3 (q)

the following proton and carbon shifts: δ_H 6.13 (1H, d, J=6.6 Hz, H-1‴) and 5.30 (1H, d, J=6.6 Hz, H-2‴); δ_C 82.6 (C-1‴), 98.2 (C-2‴), 85.6 (C-3‴), 23.8 (Me-4‴) and 18.1 (Me-5‴), and by the HMBC correlations: from H-1″ to C-5, C-6, C-7, C-2″, and C-3″ and from H-2″ to C-1″, C-3″, and C-6. This conclusion was supported by coupling with the molecular formula and the degree of unsaturation. The relative configuration of H-1″ and H-2″ in **3** should be *cis*-orientated due to the coupling constant between H-1″ and H-2″ (J (1″,2″)=6.6 Hz),[19] which was confirmed by the NOESY spectrum: the signal of H-4″ correlated to those of H-1″ and H-2″, and the NOE difference spectrum: irradiation of H-1″ enhanced the signals of H-2″ (7.22%). Hence the structure of compound **3** was finally determined as 5-hydroxy-4′-methoxy-8-prenyl-1″,2″-peroxyl-3″,3″-dimethyldihydropyrano[5,6:6,7]-isoflavone.

Compound **4** was obtained as a white amorphous solid showing $[\alpha]_D^{20}=-5°$ (c=0.1, $CHCl_3$). It was found that **4** has the same molecular formula as compound **3**, $C_{26}H_{26}O_7$, from HR-ESI-MS (m/z 451.1756 $[M+H]^+$, Calcd 451.1751) and very similar UV (λ_{max} 269 nm) and IR (3406, 1656, 1579, 1217 cm^{-1}) spectra to those of **3**, which indicated that both compounds had an identical carbon skeleton and were isomers to each other. The close resemblance of ^{13}C and ^1H chemical shifts of **4** with those of **3** (Tables 1, 2) also strongly indicated that **4** and **3** were another pair of positional isomers of substituents in the A-ring. The validity of this deduction was demonstrated by careful investigation of the HMBC spectrum. The HMBC spectrum of **4** showed cross peaks: H-1‴ of the benzopyran ring with C-7, C-8 and C-9; H-1″ of the prenyl unit with C-5, C-6 and C-7. Based on these spectroscopic data, **4** was identified as 5-hydroxy-4′-methoxy-6-prenyl-1‴,2‴-peroxyl-3‴,3‴-dimethyldihydropyrano[5,6:8,7]-isoflavone. The relative configuration of H-1‴ and H-2‴ in **4** should also be *cis*-orientated due to the coupling constant between H-1‴ and H-2‴ (J=5.7 Hz),[19] which was confirmed by the NOE difference spectrum: irradiation of H-1‴ enhanced the signals of H-2‴ (3.48%).

Experimental

General Procedure Optical rotations were measured on a Perkin-Elmer-341 polarimeter. IR spectra were taken on a Nicolet FT-IR-360 spectrometer. UV spectrum was measured by Shimadzu UV-260 spectrophotometer. ^1H- and ^{13}C-NMR spectra were recorded on: Varian Mercury-300BB (300/75 MHz) spectrometer, in $CDCl_3$; δ in ppm rel. to Me_4Si, J in Hz. EI-MS data were obtained on a VG ZAB-HS instrument, at 70 eV; in m/z. HR-ESI-MS data were measured on a Bruker APEX-II instrument with glycerol as matrix. Silica gel (200—300 mesh) used for column chromatography (CC) and silica gel GF_{254} (10—40 μ) used for TLC were supplied by the Qingdao Marine Chemical Factory, Qingdao, P. R. China. Spots were detected on TLC under UV light, then by heating after spraying with 5% H_2SO_4 in C_2H_5OH (v/v) or with 5% $FeCl_3$ solution in C_2H_5OH (m/v).

Plant Material *Hedysarum scoparium* plants were collected from the Minqin County, Gansu Province, P. R. China, in 2004, and identified by Prof. Guo-liang Zhang, School of Life Science, Lanzhou University, P. R. China.

Extraction and Isolation The air-dried whole plant material was separated into two parts: 1) roots and 2) stems—leaves. The dried roots was reduced to a fine powder (400 g) and extracted at r.t. with MeOH (4×1.5 h) by Ultrasound Extraction. The combined extract was evaporated to give a brown gum (50.5 g). Fractionating by CC (SiO_2; petroleum ether (PE)–AcOEt step gradient 100 : 0, 50 : 1, 20 : 1, 10 : 1, 5 : 1, 2 : 1, 1 : 1, and 0 : 1, and finally MeOH), 300 ml each, gave nine fractions (Fr. 1—9). From Fr. 3, the crude crystal **7** was obtained and then recrystallized from PE–acetone 20 : 1 to afford pure **7** (181 mg). The residue (1.5 g) of Fr. 3 was separated into two parts, Fr. 3.1 and Fr. 3.2. Further purification of Fr. 3.1 (0.4 g) by CC (SiO_2; PE–AcOEt from 10 : 1 to 2 : 1 yielded four subfractions, Fr. 3.1a, Fr. 3.1b, Fr. 3.1c, and Fr. 3.1d from TLC analysis. Fraction Fr. 3.1b was purified by prep. TLC (SiO_2; PE–acetone 8 : 1) to afford **6** (6 mg). Fraction Fr. 3.2 (1.1 g) was purified in a similar procedure (SiO_2; PE–$CHCl_3$ from 3 : 2 to 1 : 1) to give 86 fractions of 25 ml each, combined into seven subfractions, Fr. 3.2a, Fr. 3.2b, Fr. 3.2c, Fr. 3.2d, Fr. 3.2e, Fr. 3.2f, and Fr. 3.2g from TLC analysis. Fraction Fr. 3.2a was purified by prep. TLC (SiO_2; PE–acetone 8 : 1) to provide **5** (2 mg). Fraction Fr. 3.2e afforded a mixture of **3** and **4**, which was purified by prep. TLC (SiO_2; PE–AcOEt 5 : 1) to give pure **3** (6 mg) and **4** (5 mg). Fraction Fr. 3.2f was further purified by CC (SiO_2; PE–AcOEt 8 : 1) to provide Fr. 3.2f.1 and Fr. 3.2f.2. Fraction Fr. 3.2f.2 was purified by prep. TLC (SiO_2; first PE–acetone 4 : 1, then PE–AcOEt 5 : 1) to give pure **1** (4 mg) and **2** (5 mg).

5-Hydroxy-4′-methoxy-8-prenyl-2″-hydroxyisopropyldihydrofurano-[4,5:6,7]-isoflavone (**1**): Yellow oil. $[\alpha]_D^{20}=+5°$ (c=0.1, $CHCl_3$). UV λ_{max}^{MeOH} nm: 269, 221. IR (KBr) cm^{-1}: 3347, 2945, 2832, 1660, 1451, 1115, 1029, 673. ^1H- and ^{13}C-NMR: Tables 1 and 2. EI-MS (70 eV) m/z (%): 436 (44, $[M]^+$), 421 (11), 403 (11), 377 (14), 363 (20), 349 (12), 335 (12), 321 (10), 309 (23), 135 (10), 105 (6), 91 (10), 89 (13), 77 (14), 69 (15), 59 (100), 43 (87), 41(44). HR-ESI-MS m/z: 437.1962 $[M+H]^+$, (Calcd for $C_{26}H_{28}O_6$+H 437.1959).

5-Hydroxy-4′-methoxy-6-prenyl-2‴-hydroxyisopropyldihydrofurano-[4,5:8,7]-isoflavone (**2**): Yellow oil. $[\alpha]_D^{20}=+9°$ (c=0.1, $CHCl_3$). UV λ_{max}^{MeOH} nm: 269, 221. IR (KBr) cm^{-1}: 3357, 2946, 2833, 1662, 1451, 1416, 1115, 1029, 662. ^1H- and ^{13}C-NMR: Tables 1 and 2. EI-MS (70 eV) m/z (%): 436 (26, $[M]^+$), 421 (4), 403 (4), 393 (21), 381 (22), 363 (9), 349 (7), 335 (7), 321 (24), 309 (24), 135 (10), 117 (7), 105 (5), 91 (11), 89 (15), 77 (14), 69 (14), 59 (88), 43 (100), 41 (50). HR-ESI-MS m/z: 437.1952 $[M+H]^+$ (Calcd for $C_{26}H_{28}O_6$+H 437.1959).

5-Hydroxy-4′-methoxy-8-prenyl-1″,2″-peroxyl-3″,3″-dimethyldihydropyrano[5,6:6,7]-isoflavone (**3**): White amorphous solid. $[\alpha]_D^{20}=+6°$ (c=0.1, $CHCl_3$). UV $\lambda_{max}^{CHCl_3}$ nm: 267. IR (KBr) cm^{-1}: 3413, 2923, 1656, 1579, 1514, 1469, 1431, 1379, 1219, 1158, 1087, 1065, 1026, 772. ^1H- and ^{13}C-NMR: Tables 1 and 2. EI-MS (70 eV) m/z (%): 450 (1, $[M]^+$), 403 (6), 392 (25), 377 (42), 349 (4), 337 (6), 324 (15), 132 (18), 117 (14), 89 (27), 77 (17), 58 (14), 43 (100). HR-ESI-MS m/z: 451.1751 $[M+H]^+$ (Calcd for $C_{26}H_{26}O_7$+H 451.1759).

5-Hydroxy-4′-methoxy-6-prenyl-1‴,2‴-peroxyl-3‴,3‴-dimethyldihydropyrano[5,6:8,7]-isoflavone (**4**): White amorphous solid. $[\alpha]_D^{20}=-5°$ (c=0.1, $CHCl_3$). UV $\lambda_{max}^{CHCl_3}$ nm: 269. IR (KBr) cm^{-1}: 3406, 2923, 1656, 1629, 1579, 1514, 1428, 1376, 1217, 1154, 1066, 1032, 838, 772. ^1H- and ^{13}C-NMR: Tables 1 and 2. EI-MS (70 eV) m/z (%): 450 (5, $[M]^+$), 403 (3), 392 (21), 377 (13), 349 (38), 337 (11), 132 (10), 117 (9), 89 (20), 77 (14), 58 (16), 43 (100). HR-ESI-MS m/z: 451.1756 $[M+H]^+$ (Calcd for $C_{26}H_{26}O_7$+H 451.1759).

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