Studies on the Constituents of Seeds of *Pachyrrhizus erosus* and Their Anti Herpes Simplex Virus (HSV) Activities

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Studies on the chemical constituents of the seeds of *Pachyrrhizus erosus* (Leguminosae) resulted in the isolation of nine known components: five rotenoids [dolineone (3), pachyrrhizone (5), 12a-hydroxydolineone (7), 12a-hydroxypachyrrhizone (9), and 12a-hydroxyrotenone (2)], two isoflavonoids [neotenone (4) and dehydroneotenone (8)], one phenylfuranocoumarin [pachyrrhizine (6)], and a monosaccharide (dulcitol). The full ^{1}H- and ^{13}C-NMR assignments for the isolated products except a sugar, including revision of previous assignments in the literature, are reported. Moderate anti herpes simplex virus (HSV) activity was observed in 12a-hydroxydolineone (7) and 12a-hydroxypachyrrhizone (9) among the isolated products.

Key words *Pachyrrhizus erosus*; anti HSV activity; rotenoid; isoflavonoid; phenylfuranocoumarin; assignment revision

Pachyrrhizus erosus (L.) URBAN (Leguminosae) is cultivated in Southeast Asia including India and the pulverized seeds have been used as piscicides[1] and pesticides.[1,2] The isolation of rotenoids, isoflavonoids, and phenylfuranocoumarin derivatives as chemical components of this plant was reported.[3] It was also shown that rotenone (**1**) and 12a-hydroxyrotenone (**2**) among the isolates exhibited cytotoxic activities against various tumor cell lines including human cancer cells.[4] In a preliminary examination using plaque reduction assay we observed that the chloroform ($CHCl_3$) extract of the seeds of *P. erosus* showed potent activity against herpes simplex virus (HSV) types 1 and 2. In this paper we describe the isolation of nine known components: five rotenoids [dolineone[3a—e] (**3**), pachyrrhizone[3a—c,e,f] (**5**), 12a-hydroxydolineone[3c—e,4] (**7**), 12a-hydroxypachyrrhizone[3a—e,4] (**9**), and 12a-hydroxyrotenone[3d,e,g,4] (**2**)], two isoflavonoids [neotenone[3a—e] (**4**) and dehydroneotenone[3a—e] (**8**)], one phenylfuranocoumarin [pachyrrhizine[3a—e] (**6**)], and a monosaccharide (dulcitol[5]), their full ^{1}H- and ^{13}C-NMR assignments except a sugar including revision of previous assignments in the literature, and two possible rotenoids [12a-hydroxydolineone (**7**) and 12a-hydroxypachyrrhizone (**9**)] as active components against HSV.

The $CHCl_3$ and ethanol (EtOH) extracts were obtained as a viscous oil and a semi-solid, respectively, from the dried and powdered plant material by successive extraction followed by evaporation of the solvents after removal of less polar substances dissolved in cyclohexane. Purification of the $CHCl_3$ extract by repeated column chromatography afforded five known components: three rotenoids [**3**,[3a—e] **5**,[3a—c,e,f] and **7**[3c—e,4]], an isoflavonoid [**4**[3a—e]], and a phenylfuranocoumarin [**6**[3a—e]]. A monosaccharide, dulcitol,[5] was directly obtained by recrystallization of the EtOH extract from methanol (MeOH). Purification of the mother liquor by repeated column chromatography afforded three other known components: two rotenoids [**9**[3c,e,4] and **2**[3d,e,g,4]] and an isoflavonoid [**8**[3a—e]]. Thus, a total of nine compounds were isolated and were spectroscopically identified with the expected products, including the sign of optical rotation.

The full ^{1}H- and ^{13}C-NMR assignments of five isolated rotenoids are given in Table 1. Thus, in the ^{13}C-NMR spectrum of dolineone[3d] (**3**) the signals at δ 159.8 and 158.6 ppm had been assigned to the carbons 7a and 9, respectively; however, these should be reversed. Similarly, two relations between carbons 1 and 3′ and between carbons 7a and 9 in 12a-hydroxydolineone[3d] (**7**) and one relation between carbons 1 and 12b in 12a-hydroxyrotenone[3d] (**2**) should also be exchanged. The signals of pachyrrhizone (**5**) and 12a-hydroxypachyrrhizone (**9**) could be safely deduced as shown in Table 1.

The full ^{1}H- and ^{13}C-NMR assignments of two isolated isoflavonoids and one phenylfuranocoumarin are given in Table 2. Thus, in the ^{13}C-NMR spectrum of neotenone[3d] (**4**) the signals at δ 115.6 and 122.6 ppm had been assigned to carbon 6 and 1″, respectively, but these should be revised. Furthermore, the signal at δ 116.1 was newly assigned to be carbon 1″ in pachyrrhizine[3d] (**6**). The signals of dehydroneotenone (**8**) could be reasonably deduced as shown in Table 2.

All assignments of ^{1}H- and ^{13}C-NMR spectra were con-

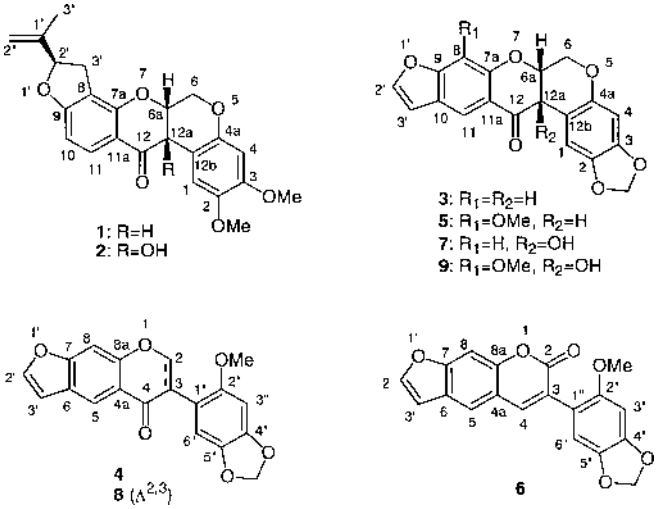

Fig. 1. The Rotenoids, Isoflavonoids and a Phenylfuranocoumarin Isolated from the Seeds of *P. erosus*

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Table 1. ^{1}H- and ^{13}C-NMR Data[a)] of the Isolated Rotenoids

C#	3	7	5	9	2
1	6.72 (s) [106.9]	6.52 (s) [105.8]	6.71 (s) [106.9]	6.49 (s) [105.6]	6.55 (s) [109.3]
2	— [142.3]	— [142.3]	— [142.2]	— [142.8]	— [143.9]
3	— [147.9]	— [149.5]	— [147.3]	— [149.4]	— [151.1]
4	6.45 (s) [98.6]	6.47 (s) [99.3]	6.42 (s) [98.9]	6.46 (s) [99.2]	6.48 (s) [101.0]
4a	— [148.4]	— [149.6]	— [148.6]	— [149.7]	— [148.3]
6	4.19 (d, *J*=12) [66.3]	4.50 (dd, *J*=15.8, 4) [64.0]	4.20 (d, *J*=11.9) [66.2]	4.50 (dd, *J*=12.1, 1) [63.8]	4.50 (m) [63.8]
	4.64 (dd, *J*=12, 3)	4.63 (dd, *J*=15.8, 2.6)	4.69 (dd, *J*=11.9, 3.5)	4.71 (dd, *J*=12.1, 2.4)	4.62 (m)
6a	4.96 (dd, *J*=4, 3) [72.0]	4.62 (dd, *J*=4, 2.6) [75.9]	4.97 (dd, *J*=3.8, 3.5) [72.3]	4.61 (dd, *J*=2.4, 1) [75.9]	4.58 (m) [76.0]
7a	— [158.6]	— [158.3]	— [149.7]	— [149.3]	— [157.7]
8	7.06 (d, *J*=1) [99.8]	7.02 (d, *J*=1) [100.1]	— [133.5]	— [133.5]	— [113.2]
9	— [159.8]	— [160.3]	— [150.9]	— [151.4]	— [168.0]
10	— [123.0]	— [123.4]	— [123.9]	— [124.3]	6.53 (d, *J*=8.5) [105.3]
11	8.21 (s) [120.9]	8.19 (s) [121.1]	7.91 (s) [114.0]	7.88 (s) [113.9]	7.82 (d, *J*=8.5) [130.1]
11a	— [116.0]	— [114.6]	— [117.1]	— [115.7]	— [111.7]
12	— [190.5]	— [193.0]	— [190.7]	— [193.1]	— [191.1]
12a	3.89 (d, *J*=4) [45.2]	— [68.4]	3.88 (d, *J*=3.8) [45.2]	— [68.2]	— [67.5]
12b	— [105.3]	— [109.3]	— [105.2]	— [109.0]	— [108.7]
2′	7.54 (d, *J*=2.5) [146.2]	7.55 (d, *J*=2.3) [146.5]	7.55 (d, *J*=2.2) [146.1]	7.55 (d, *J*=2.2) [146.3]	5.24 (t, *J*=9) [87.9]
3′	6.73 (dd, *J*=2.5, 1) [106.8]	6.74 (dd, *J*=2.3, 1) [106.9]	6.73 (d, *J*=2.2) [107.3]	6.73 (d, *J*=2.2) [107.2]	2.94 (dd, *J*=16, 9) [31.1]
					3.29 (dd, *J*=16, 9)
1″	— [—]	— [—]	— [—]	— [—]	— [142.8]
2″	— [—]	— [—]	— [—]	— [—]	4.94 (s) [112.7]
					5.07 (s)
3″	— [—]	— [—]	— [—]	— [—]	1.76 (s) [17.1]
OMe	— [—]	— [—]	4.13 (s) [61.1]	4.10 (s) [61.1]	3.72 (s) [56.3]
OMe	— [—]	— [—]	— [—]	— [—]	3.82 (s) [55.8]
OCH_2O	5.81 (d, *J*=1.3) [101.1]	5.80 (d, *J*=1.4) [101.4]	5.80 (d, *J*=1.1) [101.2]	5.80 (d, *J*=1.5) [101.3]	— [—]
	5.87 (d, *J*=1.3)	5.84 (d, *J*=1.4)	5.85 (d, *J*=1.1)	5.84 (d, *J*=1.5)	

a) ^{1}H-NMR (400 MHz in $CDCl_3$) are reported downfield from internal TMS at 0.00 ppm and peak multiplicities are quoted in Hz. ^{13}C-NMR assignments are related to internal $CDCl_3$ at 77.00 ppm and the data are given in square brackets. ^{1}H- and ^{13}C-NMR assignments are based on decoupling, HMQC, and HMBC experiments.

Table 2. ^{1}H- and ^{13}C-NMR Data[a)] of the Isolated Isoflavonoids and Coumarin

C#	4	8	6
2	4.50 (dd, *J*=10.6, 5.5) [71.3]	7.98 (s) [154.7]	— [160.7]
	4.56 (dd, *J*=11.3, 10.6)		
3	4.31 (dd, *J*=11.3, 5.5) [48.3]	— [121.1]	— [123.9]
4	— [192.8]	— [176.6]	7.80 (s) [142.4]
4a	— [118.8]	— [121.1]	— [116.1]
5	8.25 (s) [120.9]	8.54 (s) [119.0]	7.68 (s) [119.6]
6	— [122.6]	— [126.0]	— [124.8]
7	— [159.3]	— [157.2]	— [156.1]
8	7.08 (s) [99.7]	7.57 (s) [99.8]	7.49 (s) [99.4]
8a	— [159.9]	— [154.2]	— [151.6]
2′	7.57 (d, *J*=2.3) [146.0]	7.72 (d, *J*=2.2) [147.4]	7.69 (d, *J*=2.2) [146.7]
3′	6.76 (d, *J*=2.3) [107.0]	6.91 (d, *J*=2.2) [107.0]	6.82 (d, *J*=2.2) [106.4]
1″	— [115.5]	— [112.8]	— [116.1]
2″	— [152.7]	— [153.0]	— [152.9]
3″	6.57 (s) [95.4]	6.63 (s) [95.5]	6.63 (s) [95.4]
4″	— [147.8]	— [148.4]	— [148.7]
5″	— [141.3]	— [141.2]	— [141.2]
6″	6.61 (s) [109.8]	6.85 (s) [111.3]	6.89 (s) [110.3]
OMe	3.72 (s) [56.5]	3.73 (s) [56.9]	3.77 (s) [56.8]
OCH_2O	5.91 (s) [101.3]	5.96 (s) [101.4]	5.96 (s) [101.5]

a) ^{1}H-NMR (400 MHz in $CDCl_3$) are reported downfield from internal TMS at 0.00 ppm and peak multiplicities are quoted in Hz. ^{13}C-NMR assignments are related to internal $CDCl_3$ at 77.00 ppm and the data are given in square brackets. ^{1}H- and ^{13}C-NMR assignments are based on decoupling, HMQC, and HMBC experiments.

firmed by application of heteronuclear multiple-quantum coherence (HMQC) and heteronuclear multiple-bond connectivitiy (HMBC) experiments. Selected HMBC correlations of **2**—**4**, **6** and **7** are shown in Fig. 2.

Among nine isolated products four rotenoids [dolineone (**3**), pachyrrhizone (**5**), 12a-hydroxydolineone (**7**), and 12a-hydroxypachyrrhizone (**9**)], an isoflavonoid [neotenone (**4**)], and a phenylfuranocoumarin [pachyrrhizine (**6**)] were subjected to antiviral tests using plaque reduction assay method[6)] (Table 3). Either no or weak activity was observed in isoflavonoid (run 2) and phenylfuranocoumarin derivatives (run 4). On the other hand, in rotenoid series products with-

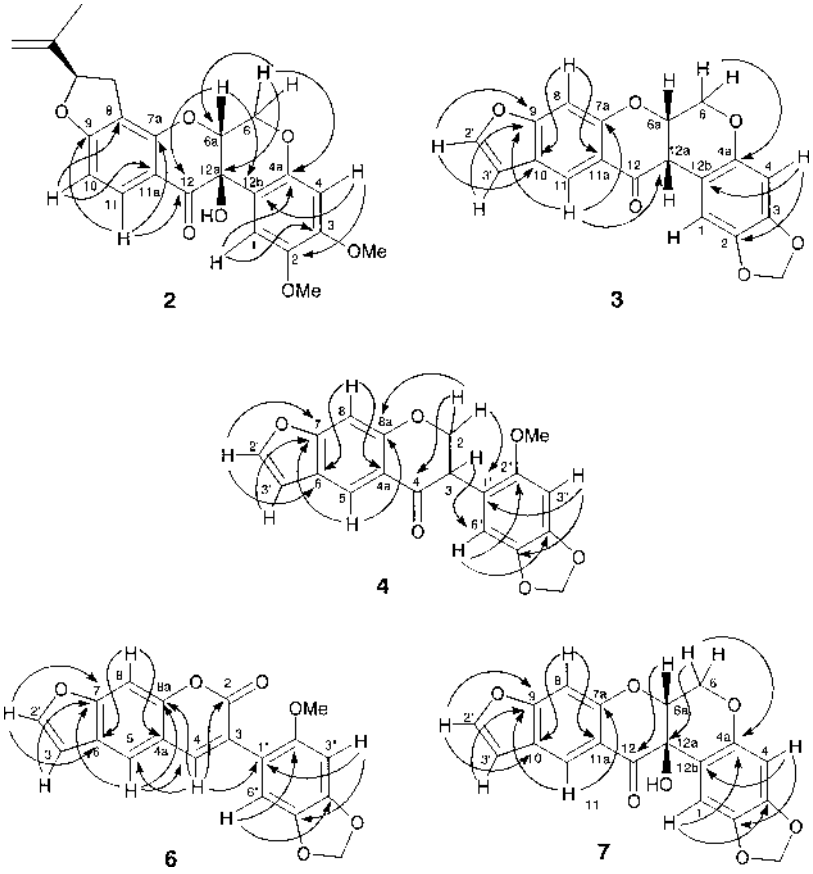

Fig. 2. Selected HMBC Correlations of **2**—**4**, **6** and **7**

Table 3. Inhibition (%) of Isolated Products against HSV-1 and 2 Viruses[a)]

Run	Products (μg/ml)	HSV-1	HSV-2
1	**3** (50)	15.4	24.4
2	**4** (50)	—[b)]	—[b)]
3	**5** (50)	—[b)]	15.5
4	**6** (50)	26.1	23.7
5	**7** (50)	82.7[c)]	42.5
6	**9** (20)	56.6[d)]	56.3[e)]

a) Cell toxicity dose to Vero cell: >50 μg/ml for **3**, **4**, **5**, **6**, and **7**; 50 μg/ml for **9**. *b*) —: negative. *c*) IC_{50}: 25.5 μg/ml. *d*) IC_{50}: 18.0 μg/ml. *e*) IC_{50}: 18.5 μg/ml.

out a hydroxy group such as **3** and **5** (runs 1, 3) showed only weak activity, whereas 12a-hydroxy derivatives **7** and **9** showed good to moderate activity (runs 5, 6). Interestingly the former **7** was found to be more effective against HSV-1 than HSV-2. These facts may suggest that the presence of a hydroxy group at the 12 position in rotenoids may be crucial for the antiviral activity.

In conclusion, we fully assigned the ^{1}H- and ^{13}C-NMR signals of the isolated compounds from the seeds of *P. erosus*. In addition, it was found that two rotenoids, 12a-hydroxydolineone (**7**) and 12a-hydroxypachyrrhizone (**9**), showed moderate activity against HSV types 1 and 2.

Experimental

General Procedures ^{1}H- (400, 600 MHz) and ^{13}C-NMR (100, 150 MHz) spectra were measured on a JEOL JNM ECP 400 and 600 (TMS as an internal standard). Electron impact (EI)-MS was obtained by a JEOL JMS-HX110 spectrometer. Optical rotation, $[\alpha]_D$, was measured with a Perkin Elmer 341 polarimeter. IR and UV spectra were measured on a Perkin Elmer FT-IR 2000 spectrometer and a Shimadzu UV-160A spectrophotometer. TLC was performed using Merck precoated TLC plates (Kieselgel 60 F_{254}). Column chromatography was conducted with Kieselgel (70—230 and 230—400 mesh, Merck).

Plant Material and Preparation of Crude Extracts The seeds of *P. erosus* (L.) URBAN (Leguminosae) were collected in the Nakornsawan province of central Thailand in April 1996. Voucher herbarium specimens (No. AP 9601) of the plant were identified and deposited at the Forest Herbarium (BKF), Royal Forest Department, Ministry of Agriculture and Cooperatives, Bangken, Bangkok, Thailand. The dried and powdered plant material (2.0 kg) was successively macerated for 45 d with cyclohexane (12 l), $CHCl_3$ (12 l), and then 95% EtOH (10 l) and evaporation of the solvents afforded the corresponding cyclohexane (0.48 l), $CHCl_3$ (43 g), and EtOH extracts (127 g), respectively.

Isolation of the $CHCl_3$ Extract Repeated column chromatography of the $CHCl_3$ extract using hexane, $CHCl_3$, and MeOH gave five isoflavonoids [**3**[3a—e)] (28 mg, 1.4×10^{-3}%), **4**[3a—e)] (82 mg, 4.1×10^{-3}%), **5**[3a—c,e,f)] (234 mg, 11.7×10^{-3}%), **6**[3a—e)] (107 mg, 5.4×10^{-3}%), and **7**[3c—e,4)] (62 mg, 3.1×10^{-3}%)] in order of polarity.

Isolation of the EtOH Extract Recrystallization of the EtOH extract from MeOH gave dulcitol[5)] (190.2 mg, 9.5×10^{-3}%). Repeated column chromatography of the mother liquor using hexane, $CHCl_3$, and MeOH afforded three further isoflavonoids [**8**[3a—e)] (15 mg, 0.8×10^{-3}%), **9**[3c,e,4)] (35 mg, 1.8×10^{-3}%), and **2**[3d,e,g,4)] (17 mg, 0.9×10^{-3}%)] in order of polarity.

Bioassay Method Plaque reduction assay was performed according to the reported method.[6)] HSV-1 (KOS) or HSV-2 (186) [30 plaque forming unit (PFU)/25 μl] was mixed with 25 μl of complete medium containing various concentrations of a test compound and incubated at 37 °C for 1 h. After incubation, the mixtures were added into Vero cells (6×10^5 cells/ml, 50 μl/well) in 96-well microtiter plates and incubated at 37 °C for 2 h. The overlay medium containing various concentrations of a test compound (100 μl/well) was added to the Vero cells and incubated at 37 °C in a humidified CO_2 incubator for 2 d. The plaques were counted under an inverted mi-

croscope. The cells were stained with 1% crystal violet in 10% formalin for 1 h, and the percent plaque inhibition was determined.

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