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CHEMICAL CONSTITUENTS OF *PTEROCAULON REDOLENS*

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Abstract - Studies on the chemical constituents of the aerial parts of *Pterocaulon redolens* (Forst.f) F. Vill.(Asteraceae) resulted in the isolation of ten components: seven coumarins [5-methoxy-6,7-methylenedioxycoumarin (**1**), ayapin (**2**), puberulin (**3**), 5-methoxyscopoletin (**4**), 2',3'-dihydroxypuberulin (**5**), isofraxidin (**6**), and 5-(2',3'-dihydroxy-3'-methylbutyloxy)-6, 7-methylenedioxycoumarin (**7**)] and three flavonoids [luteolin (**8**), tomentin (**9**), and chrysosplenol C (**10**)], among which **5** was firstly isolated as a natural product. The full ^{1}H- and ^{13}C-NMR spectral assignments for the isolated products, including revision of previous assignment in the literature are reported. Six coumarins (**1-4, 6**, and **7**) and one flavonoid (**8**) displayed mild activity against *Mycobacterium tuberculosis* H_{37}Ra. In addition, flavonoid (**10**) was firstly found to possess moderate cytotoxicity against breast cancer (BC) and human small cell lung cancer (NCI-H187) cell lines.

INTRODUCTION

The genus *Pterocaulon* (Asteraceae) is widely distributed in tropical America, Australia, and Asia.[1] Various species are used in folk medicine as insecticides and agents against snake bites.[2] The isolation of coumarins, flavonoids, and caffeic acid derivatives as chemical components of the aerial parts of this particular genus have been reported.[3] *P. redolens* (Forst. f) F. Vill, locally known in Thailand as "Jon Jan", is a plant indigenous to central Thailand and is used for the same purpose described above. No previous phytochemical analysis of this plant has been reported. The present paper deals with the isolation of seven coumarins (**1-7**) and three flavonoids (**8-10**) from the aerial parts of this plant and their biological activities against *Mycobacterium tuberculosis* H_{37}Ra and human cancer cell lines.

RESULTS AND DISCUSSION

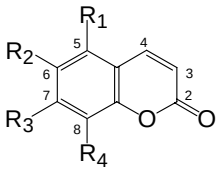
The EtOH extract from aerial parts of *P. redolens* was chromatographically separated by silica gel and a gel filtration technique as described in experimental section. Structure determination of the isolates were performed by spectroscopic means, mainly ^{1}H- and ^{13}C- NMR spectra. Full assignment of signals in the NMR spectra of coumarins (Table 1) was confirmed by application of heteronuclear multiple-quantum coherence (HMQC) and heteronuclear multiple-bond connectivity (HMBC) experiments. Comparison of signals assigned by us with those in the literatures resulted in the revision of assignments of some signals in the NMR spectra of six compounds except 2',3'-dihydroxypuberulin (**5**).[4]

Signals at δ 151.49 and 152.63 previously assigned to carbons 7 and 8a in 5-methoxy-6,7-methylenedioxycoumarin (**1**),[5] those at δ 3.94 and 3.99 to methoxy protons at 5 and 6 in 5-methoxyscopoletin (**4**),[6] and those at δ 70.57 and 76.05 to carbon

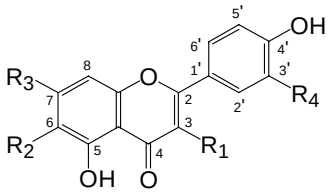
Table 1. ^{1}H- and ^{13}C-NMR Data [a] of the Isolated Coumarins

C[#]	1	2	3	4	5	6	7
	($CDCl_3$)	($CDCl_3$)	($CDCl_3$)	($CDCl_3$)	($CDCl_3$)	($CDCl_3$)	(DMSO-d_6)
2	- [161.33]	- [161.18]	- [160.55]	- [161.43]	- [160.10]	- [160.58]	- [160.26]
3	6.20(d, *J*= 9.5) [111.68]	6.28(d, *J*= 9.7) [113.37]	6.34(d, *J*= 9.5) [115.09]	6.23(d, *J*= 9.5) [112.44]	6.37(d, *J*= 9.6) [115.49]	6.28(d, *J*= 9.5) [113.55]	6.27(d, *J*= 9.8) [111.22]
4	7.94(d, *J*= 9.5) [138.84]	7.58(d, *J*= 9.7) [143.44]	7.61(d , *J*= 9.5) [143.44]	7.91(d, *J*= 10) [138.56]	7.62(d, *J*= 9.2) [143.34]	7.60(d, *J*= 9.5) [143.79]	8.15(d, *J*= 9.8) [139.60]
4a	- [106.56]	- [112.66]	- [114.38]	- [107.22]	- [114.77]	- [111.23]	- [106.59]
5	- [137.99]	6.828(s)[b] [105.00]	- [103.55]	- [148.38]	6.70(s) [103.81]	6.66(s) [103.20]	- [137.09]
6	- [131.69]	- [144.89] [b]	6.66(s) [150.63]	- [136.25]	- [149.65]	- [144.58]	- [132.24]
7	- [152.63]	- [151.24] [b]	- [144.90]	- [153.29]	- [144.64]	- [142.42]	- [152.39]
8	6.53(s) [92.32]	6.833(s)[b] [98.37]	- [141.74]	6.70(s) [98.82]	- [140.99]	- [134.49]	6.80(s) [92.16]
8a	- [151.49]	- [151.25] [b]	- [142.99]	- [151.55]	- [142.41]	- [143.05]	- [150.87]
1'	-	-	4.64(d, *J*= 7.0) [70.24]	-	4.00(dd, *J*= 10.4, 7.8) [76.26]	-	4.11(dd, *J*= 10.1, 8.6) [74.37]
					4.54(dd, *J*= 10.4, 2.6)		4.60(dd, *J*= 10.1, 2.5)
2'	-	-	5.56(t like, *J*= 7.0) [119.09]	-	3.67(ddd, *J*= 7.8, 3.6, 2.6) [75.68]	-	3.53(ddd, *J*= 8.6, 5.8, 2.5) [76.05]
3'	-	-	- [139.25]	-	- [71.29]	-	- [70.57]
Me	-	-	1.71(s) [17.90]	-	1.23(s) [25.06]	-	1.02(s) [24.25]
			1.77(s) [25.78]		1.28(s) [26.65]		1.22(s) [27.56]
OMe	4.14(s) [59.92]	-	3.89(s) [56.27]	3.94(s) [61.22]	3.92(s) [56.31]	3.95(s) [56.50]	-
			4.09(s) [61.69]	3.99(s) [61.48]	4.07(s) [61.96]	4.10(s) [61.63]	
OH	-	-	-	6.43(br s)	2.71(s)	6.13(br s)	4.40(s)
					3.83(d, *J*= 3.6)		5.13(d, *J*= 5.8)
OCH_2O	6.01(s) [101.79]	6.07(s) [102.32]	-	-	-	-	6.11(s) [102.25]

[a] ^{1}H-NMR (500 MHz in $CDCl_3$ and DMSO-d_6) are reported downfield from internal standard TMS at 0.00 ppm and peak multiplicities are quoted in Hz. ^{13}C-NMR assingnments are related to internal $CDCl_3$ at 77.0 ppm, DMSO-d_6 at 39.5 ppm and the data are given in square brackets. ^{1}H- and ^{13}C-NMR spectral assignments are based on decoupling, NOEs, HMQC and HMBC experiments. [b] Interchangeable



1: R_1= OCH_3 R_2+R_3= OCH_2O R_4= H
2: R_1=R_4= H R_2+R_3= OCH_2O
3: R_1= H R_2=R_4= OCH_3 R_3= $OCH_2CH=C(CH_3)_2$
4: R_1=R_2= OCH_3 R_3= OH R_4= H
5: R_1= H R_2=R_4= OCH_3 R_3= $OCH_2CH(OH)C(OH)(CH_3)_2$
6: R_1= H R_2=R_4= OCH_3 R_3= OH
7: R_1= $OCH_2CH(OH)C(OH)(CH_3)_2$ R_2+R_3= OCH_2O R_4= H



8: R_1=R_2= H R_3=R_4= OH
9: R_1=R_3= OCH_3 R_2=R_4= OH
10: R_1=R_3=R_4= OCH_3 R_2= OH

2' and 3' in 5-(2',3'-dihydroxy-3'-methylbutyloxy)-6,7-methylenedioxycoumarin (**7**)[7] should be revised, respectively. In the case of puberulin (**3**)[8] signals at δ 144.90, 150.63, 142.99, and 141.74 previously assigned to carbons 6, 7, 8, and 8a should be revised to 7, 6, 8a, and 8, and in the case of isofraxidin (**6**)[9] those at δ 103.20, 113.55, 134.49, 142.42, and 143.05 to carbons 3, 5, 7, 8a, and 8 to 5, 3, 8, 7, and 8a, respectively. In ayapin (**2**), literature[10] noted that three signals were observed at δ 143.4, 144.9, and 151.3 in the lower field than δ 140 and assigned them to be carbons 4 and 6, 8a, and 7. Precise examination of the ^{13}C-NMR spectrum of our sample showed that the corresponding signals were separatedly observed at δ 143.44, 144.89, 151.24, and 151.25. These four signals could be assigned to carbons 4, 6, 7, and 8a.

Coumarins (**5** and **7**) with a 2,3-dihydroxy-3-methylbutyloxy substituent were isolated as optically active forms, $[\alpha]_D^{23}$+25.0° (*c* 0.9, $CHCl_3$) in **5** and $[\alpha]_D^{24}$ +30.9° (*c* 0.65, MeOH) in **7**; however, these may not be perfectly optically pure because of lower value in **5** compared with the reported $[\alpha]_D^{25}$ +80.6° (*c* 0.9, $CHCl_3$) in the coumarin semi-synthesized from 2',3'-epoxypuberulin.[4] It is noted here that 2',3'-dihydroxypuberulin (**5**) is firstly isolated from natural sources.

Similar full assignment of signals in the NMR spectra of three flavonoids, luteolin (**8**),[11] tomentin (**9**),[12] and chrysosplenol C (**10**),[13] were also performed (Table 2). The ^{13}C-NMR data of **9** was firstly given here.

The EtOH extract of this plant was preliminarily subjected to biological tests for antimalarial, antifungal, anti herpes simplex virus, antituberculosis, and cytotoxic activities, among which positive results were obtained in the last two tests. Thus, isolated products were further examined antituberculosis activity using *M. tuberculosis* H_{37}Ra and cytotoxic activity using breast cancer (BC) and human small lung cancer (NCI-H187) cell lines. In the former case mild activity was observed in six coumarins (**1-4**, **6**, and **7**) and one flavonoid (**8**) with MICs 100, 50, 100, 100, 100, 200 and 100 μg/mL, respectively. On the other hand, chrysosplenol C (**10**) showed moderate cytotoxic activity to the both cell lines with IC_{50} 5.52 and 9.25 μg/mL, respectively.

In conclusion, seven coumarins and three flavonoids were isolated as the chemical constituents of the aerial parts of *P. redolens*. Detailed examination of NMR spectral data led to revision of some previous assignments in the literature. Mild antituberculosis activity was observed in the compounds (**1-4**, **6-7**, and **8**) and moderate cytotoxic activity was in **10**. Although antipoliovirus activity[13] had been reported on **10**, this is the first example of cytotoxicity.

EXPERIMENTAL

Material : The aerial parts of *P. redolens* were collected at Kanchanaburi province, Thailand, in August 2000. Authentication was achieved by comparison with the herbarium specimen (BKF No. 1482) at the Royal Forest Department, Ministry of Agriculture and Cooperatives, Bangkok, Thailand. A voucher specimen has been deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

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

C#	8	9	10
	(DMSO-d_6)	(DMSO-d_6)	(DMSO-d_6)
2	- [163.86]	- [155.69]	- [155.53]
3	6.66(s) [102.84]	- [137.52]	- [137.61]
4	- [181.62]	- [178.08]	- [178.10]
4a	- [103.66]	- [105.48]	- [105.52]
5	- [161.45]	- [145.67]	- [145.64]
6	6.18(d, *J*= 2) [98.79]	- [129.57]	- [129.62]
7	- [164.11]	- [154.50]	- [154.53]
8	6.43(d, *J*= 2) [93.80]	6.83(s) [90.78]	6.89(s) [90.99]
8a	- [157.26]	- [148.77]	- [148.81]
1'	- [121.46]	- [120.96]	- [120.95]
2'	7.38(d, *J*=2.5) [113.33]	7.58(d, *J*= 2) [115.48]	7.66(d, *J*= 2.5) [111.99]
3'	- [145.70]	- [145.22]	- [147.47]
4'	- [149.67]	- [148.62]	- [149.73]
5'	6.88(d, *J*= 8.5) [115.98]	6.90(d, *J*= 8.5) [115.66]	6.95(d, *J*= 8.5) [115.61]
6'	7.41(dd, *J*= 2.5, 8.5) [118.96]	7.47(dd, *J*= 2, 8.5) [120.49]	7.61(dd, *J*= 2.5, 8.5) [122.21]
OH	9.69(br s)	8.70(s)	8.71(s)
	10.68(br s)	9.35(br s)	9.91(s)
	12.97(s)	9.77(br s)	12.35(s)
		12.36(s)	
OMe	-	3.78(s) [59.61]	3.80(s) [59.66]
		3.90(s) [56.28]	3.86(s) [55.77]
			3.90(s) [56.33]

See the footnote *a* in Table 1.

Extraction and isolation : The pulverized dried aerial parts of *P. redolens* (1.5 kg) were macerated 4 times at rt with 95% EtOH (*ca*. 10 L for each) for 3 days. The EtOH was removed under reduced pressure and the alcoholic extract (141 g) was partitioned with hexane, $CHCl_3$ and MeOH, respectively. The concentrated $CHCl_3$ extract (30.8 g) was chromatographed on silica gel and eluted successively with a mixed solvent of hexane-$CHCl_3$ and then $CHCl_3$-MeOH according to polarity. The crude fractions obtained were repeatedly purified using silica gel column chromatography (with hexane-$CHCl_3$ and hexane-EtOAc) to give seven pure coumarins [**1** (60 mg), **2** (30.8 mg), **3** (18 mg), **4** (20 mg), **5** (40.1 mg), **6** (10.1 mg), and **7** (20.3 mg)] in the order of polarities of the isolates. On the other hand, the MeOH extract was further partitioned with BuOH and then the concentrated BuOH extract (4.5g) was purified by column chromatography on Sephadex LH20 (with hexane-EtOAc-MeOH, $CHCl_3$-MeOH, acetone-MeOH and acetone) to give three pure flavonoids [**8** (20.9 mg)**, 9** (12 mg), and **10** (25 mg)]. These purified isolates were characterized by comparison with the reported data.

***In Vitro* antituberculosis activity test**[14] **:** Antituberculosis activity was performed by a microplate alamar blue assay. *M. tuberculosis* $H_{37}Ra$ was used as a tested microorganism. The MICs of the tested compounds were measured in µg/mL. ***In Vitro* cytotoxic activity test**[15] **:** Cytotoxicity was assessed using the sulforhodamine B (SRB)-assay using human tumor cell lines of BC and NCI-H187. The cells were incubated at 37 ºC for 72 h, at which time the SRB was added. The results are expressed as an IC_{50}.

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