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TWO NEW PYRANOFLAVANONES FROM THE STEMS OF *DERRIS RETICULATA*

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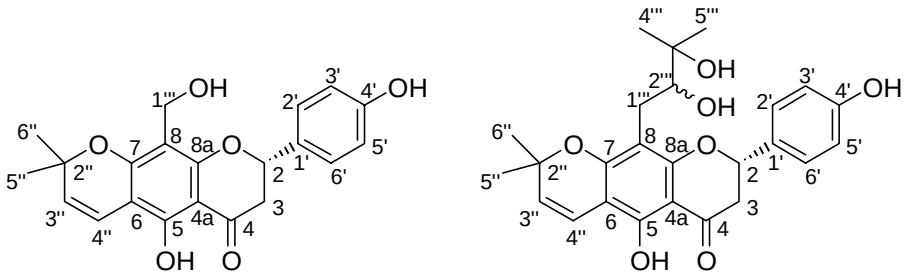
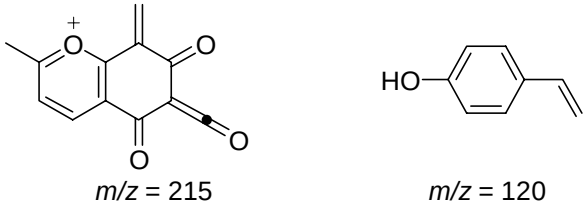
Abstract- Two new flavonoids, 4′,5-dihydroxy-8-hydroxymethyl-6″,6″-dimethylpyrano[2″,3″:7,6]flavanone (**1**), and 2″,3″-dihydroxylupinifolin (**2**) were isolated from the stems of *Derris reticulata*. Both compounds showed cytotoxic activity in the P-388 cell line. The structures were established by spectroscopic analysis and chemical transformations. The biosynthetic origin and the mechanism of formation of the hydroxymethyl group in compound (**1**) are proposed.

The Leguminosae is known to be a rich source of flavonoids and most of the prenyl derivatives have been found in this family.[1,2] The flavonoids exhibit diverse biological activities and recent interest has been focussed on their medicinal and nutritional values.[3] Recently, some biologically active prenylated flavonoids have been reported[4-6] and, significantly, it was found that the prenyl groups on the flavonoid skeleton play an important role in anti-HIV activity.[7] *Derris reticulata* (Leguminosae) is a well known Thai herbal medicine used for the relief of thirst and as an expectorant. Previously, we have reported the isolation and structural characterization of four prenylated flavanones, lupinifolin, 2‴,3‴-epoxylupinifolin, dereticulatin,[8] and 1‴-hydroxy-2‴,3‴-epoxylupinifolin from the stems *of Derris reticulata*.[9] Further investigation of this herb has led to the isolation of two new cytotoxic flavonoids, 4′,5-dihydroxy-8-hydroxymethyl-6″,6″-dimethylpyrano[2″,3″:7,6]flavanone (**1**), and 2″,3″-dihydroxy-lupinifolin (**2**). In this report we present the isolation, structure determination, and biological evaluation of these two new compounds.

Compound (**1**) was obtained as a yellow solid, mp 141-142 °C, $[\alpha]_D$ –18.2°(*c* 0.12, $CHCl_3$). The UV

spectrum (λ_{max} 226, 273, 298 nm) suggested the presence of a pyranoflavanone chromophore.[10] HREIMS of **1** gave a molecular ion at *m/z* 368.1260, with the calculated value for $C_{21}H_{20}O_6$ being 368.1260. Its IR spectrum (EXPERIMENTAL) showed absorptions typical of hydroxyl, substituted aromatic ring, and carbonyl groups. The ^{1}H NMR spectrum of **1** showed the typical pattern of protons at C-2 and C-3 in a flavanone skeleton as three one-proton doublet of doublets at δ 5.34 (*J* = 12.9, 3.0 Hz), 3.07 (*J* = 17.2, 12.9 Hz), and 2.77 (*J* = 17.2, 3.0 Hz). In the aromatic region of the ^{1}H NMR spectrum of **1**, two doublets appearing at δ 7.27 and 6.83 (each 2H, *J* = 8.6 Hz) were assigned to the protons of *para*-substituted ring B. The signal at δ 12.32 (1H, s) was ascribed to a phenolic group hydrogen bonded to an acyl group and the signals at δ 4.54 and 4.59 (each 1H, d, ^2J = 11.7 Hz) were assigned to methylene protons of the benzylic alcohol. The signals that could be assigned to the 2,2-dimethylpyran group were at δ 1.43 and 1.45 (each 3H, s, 2 × Me), δ 5.51 (d, *J* = 10.0 Hz), and 6.58 (d, *J* = 10.0 Hz). No more protons in the aromatic region was observed in the NMR spectrum, suggesting the absence of aromatic protons on ring A. Therefore, the three groups mentioned above were linked to ring A. The ^{13}C NMR spectrum of **1** showed signals of four oxygenated aromatic carbons (160.1, 160.2, 157.2, 157.4) and a carbonyl carbon (196.6). The ^{1}H and ^{13}C NMR (Table 1) spectra of **1** showed that the phenolic groups were located at C-5, and C-4′. HMBC (Figure 1) experiments showed long-range C-H correlation in which two methylene protons of the benzylic alcohol (δ 3.07 and 2.77) correlated with C-7, C-8, and C-8a. Furthermore, the structure of compound (**1**) was confirmed by the EIMS spectrum which showed ions at *m/z* 215, and 120, resulting from the retro Diels-Alder cleavage of the ion at *m/z* 353 [M^+-CH_3], 335 [M^+-CH_3 -H_2O] (Scheme 1). The presence of an ion at *m/z* 120 confirmed that one phenolic group was located in ring B. Based on the above spectroscopic evidence, the structure of **1** is proposed to be 4′,5-dihydroxy-8-hydroxymethyl-6″,6″-dimethylpyrano[2″,3″:7,6]flavanone.[11]





Scheme 1. Mass fragmentation of compound (**1**).

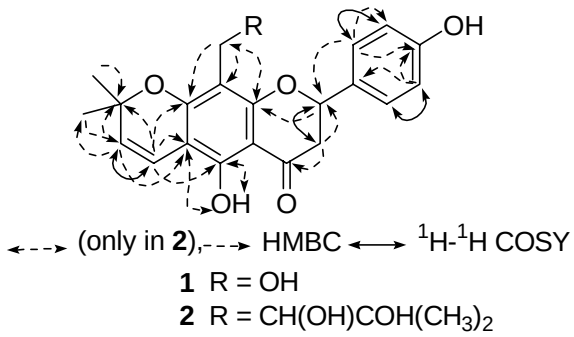
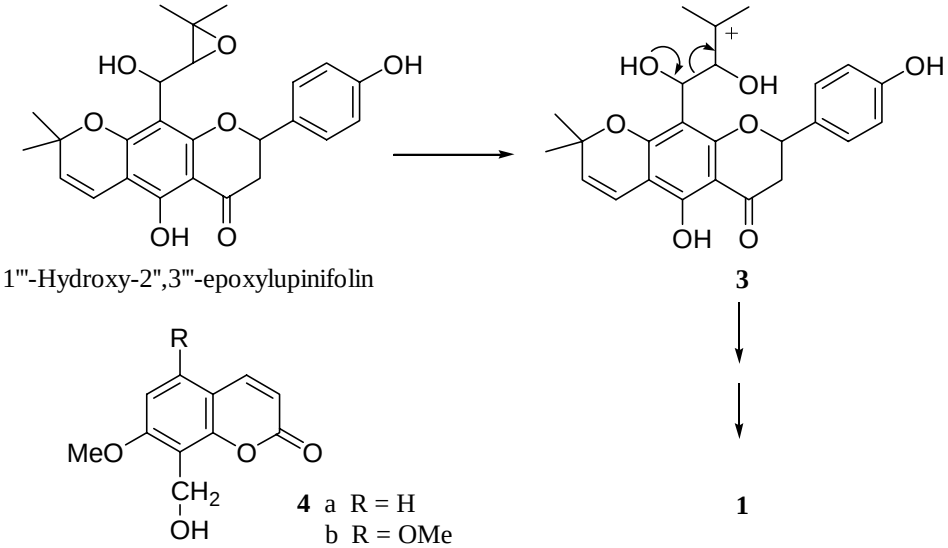


Figure 1. Summary of important connectivities observed in **1** and **2** by HMBC and COSY.

Compound (**2**), $[\alpha]_D$ –26.89°(*c* 0.10, $CHCl_3$) and the UV absorptions at 225, 266, 274, 300, 312, 363 nm were indicative of a pyranoflavanone chromophore.[5] HRFABMS (positive mode) exhibited $[M^+ +1]$ *m/z* 441.1913 corresponding to a molecular formula of $C_{25}H_{28}O_7$, The ^1H and ^{13}C NMR spectral data of **2** (Table 1) exhibited two sets of signals with partial overlapping suggesting the presence of two forms of the vicinal diol. The ^1H and ^{13}C NMR spectra of flavanone (**2**) were nearly identical with those of 2‴,3‴-epoxylupinifolin previously isolated from this plant.[8] Comparison of the molecular formula of **2** and 2‴,3‴-epoxylupinifolin suggested an additional H_2O molecule for **2**. The structure was also confirmed by reaction of epoxylupinifolin with 1% sulfuric acid in THF at room temperature for 24 h. From this reaction compound (**2**) was isolated as yellow solid. The structure of this compound was further confirmed by various spectroscopic methods including the correlations of the ^{13}C-^1H and ^1H-^1H in HMBC and COSY respectively as shown in Figure 1. Thus, from the spectroscopic studies and from semisynthesis, **2** was deduced to be 2‴,3‴-dihydroxylupinifolin. The stereochemistry at C-2 in compounds (**1**) and (**2**) was assigned to be *S* configuration by correlation with other related derivatives[8,10] isolated from this plant.



Scheme 2. The proposed biosynthesis of compound (**1**).

The co-occurrence of compound (**1**) with 1'''-hydroxy-2''',3'''-epoxylupinifolin and compound (**2**) lends support to the proposal in Scheme 2 that the hydroxymethyl group in compound (**1**) could be derived

from this compound by acid catalyzed opening of the epoxide ring or protonation of the hydroxyl group in compound (**2**) to give the corresponding stable carbonium ion followed by carbon-carbon bond cleavage as shown in **3** to give the aldehyde, an immediate precursor to compound (**1**). This biosynthetic route apparently could be applied to rationalize the biosynthesis of the extremely rare hydroxymethyl coumarins, murrayacarpin-A and –B (**4a** and **4b**) which also co-occur with the prenyl derivatives.[12]

In vitro bioassay evaluation of compounds (**1**) and (**2**) showed cytoxic activity in the P-388 cell line with IC_{50} values of 6.4 and 1.3 μg/mL respectively, but were inactive against the KB cell line.

Table 1: ^{1}H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR Spectral Data for **1** and **2**.

position	**1**		**2**	
	δH (mult., *J* in Hz)	δC (mult.)	δH (mult., *J* in Hz)	δC (mult.)
2	5.34 (dd, *J* = 12.9, 3.0)	78.8 (d)	5.27, 5.33 (dd, *J* = 13.1, 3.0)	78.9, 79.0[a] (d)
3	3.07 (dd, *J* = 17.2, 12.9)	42.5 (t)	2.99, 2.97 (dd, *J* = 17.1, 13.1)	43.0, 43.1 (t)
	2.77 (dd, *J* = 17.2, 3.0)		2.73, 2.71 (dd, *J* = 17.1, 3.0)	
4		196.6 (s)		196.4 (s)
4a		102.4 (s)		102.7, 102.8 (s)
5	OH 12.32 (s)	157.2[a] (s)	OH 12.18 (s)	157.0, 157.1[b] (s)
6		102.2 (s)		102.9 (s)
7		160.2[b] (s)		159.8, 159.7[c] (s)
8		107.4 (s)		105.6 (s)
8a		160.1[b] (s)		159.6, 159.5[c] (s)
1′		128.8 (s)		129.9, 129.8 (s)
2′, 6′	7.27 (d, *J* = 8.5)	127.4 (d)	7.19 (d, *J* = 8.3)	127.6, 127.7 (d)
3′, 5′	6.83 (d, *J* = 8.5)	115.1 (d)	6.76, 6.75 (d, *J* = 8.3)	115.8, 115.7 (d)
4′		157.4[a] (s)		156.7, 156.6[b] (s)
2″		78.4 (s)		78.8 (s)
3″	5.51 (d, *J* = 10)	126.0 (d)	5.44 (d, *J* = 10.0)	125.9, 125.8 (d)
4″	6.58 (d, *J* = 10)	114.9 (d)	6.57 (d, *J* = 10.0)	115.6, 115.5 (d)
5″	1.43 (s)	27.8 (q)	1.40, 1.38[a] (all s)	28.6, 28.5[d] (all s)
6″	1.45 (s)	27.6 (q)	1.37, 1.36[a] (all s)	28.4, 28.3[d] (all s)
1‴	4.59 (d, *J* = 11.7)	52.3 (t)	2.76, 2.75 (dd, *J* = 14.0, 1.9)	25.3, 25.2 (t)
	4.54 (d, *J* = 11.7)		2.53, 2.51 (dd, *J* = 14.0, 10.4)	
2‴			3.45, 3.42 (dd, *J* = 10.4, 1.9)	79.2, 79.1[a] (d)
3‴				73.2 (s)
4‴, 5‴	-		1.14, 1.13, 1.23, 1.12 (all s)	25.9, 23.4 (all s)

[a-d] Assignments may be interchangeable

EXPERIMENTAL

General: Melting points were determined on a Buchi 535 and are uncorrected. Optical rotations were measured with a JASCO P-1020 Digital Polarimeter. UV (MeOH) spectra were measured on a Shimadzu UV-2100S spectrophotometer. IR (KBr) spectra were recorded on a Perkin-Elmer system 2000 FT-IR spectrophotometer. The NMR spectra were recorded on Bruker AM 400 (400 MHz for ^{1}H and 100 MHz for ^{13}C) spectrometer and chemical shifts were recorded in δ (ppm) using TMS as internal standard. MS spectra were determined on Finnigan Mat 90 and Finnigan Polaris instruments. HPLC was performed on the Thermo Separation Products, San Jose, CA, U. S. A. (pump, P4000; detector, UV6000LP for analysis, UV2000 for preparative isolation).

Plant material: Dry stems of *Derris reticulata* were purchased from a local traditional drug store in Bangkok, Thailand (June 1998). Botanical identification was achieved through comparison by Prof. Nijsiri Ruangrungsi with the authentic specimen in the Bangkok Herbarium (BK 36776), Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok, Thailand.

Extraction and Isolation: The dichloromethane extract (20 g) of dry powdered stems of *Derris reticulata* was separated by VLC (Vacuum Liquid Chromatography) using hexane and increasing amounts of ethyl acetate to give 8 fractions. Fraction 5 (200 mg) was resolved by preparative HPLC using Luna C_8 and UV detection at 274 nm. Elution with MeOH: H_2O (77:23), flow rate 8 mL/min, afforded compound (**1**) (3 mg); and 1‴-hydroxy-2‴,3‴-epoxylupinifolin (15 mg). Fraction 6 (429 mg) was further purified by repeated preparative TLC developed twice with a mixture of methanol:acetone:hexane (1:16:33) as eluent to yield 2 bands. The final purification of band 1 was achieved by preparative HPLC using Luna C_8 and UV detection at 280 nm, eluted with MeOH:H_2O (62.5:37.5) to furnish compound (**2**) (10 mg).

4′,5-Dihydroxy-8-hydroxymethyl-6″,6″-dimethylpyrano[2″,3″:7,6]flavanone (1): yellow solid (3 mg); mp 141-142 °C; $[\alpha]^{26}_{D}$ -18.2° (*c* 0.12, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 226 (4.16), 267 (4.41), 273 (4.47), 298 (3.94), 312 (3.92), 360 (3.29) nm; IR (KBr) ν_{max} 3300 (OH), 2925, 1647 (C=O), 1600, 1520, 1460, 1380, 1133, 830 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$), see Table 1; EIMS *m/z* 368 $[M]^+$ (73), 353 $[M-CH_3]^+$ (37), 350 $[M-H_2O]^+$ (36), 335 $[M-CH_3, -H_2O]^+$ (87), 215 (100), 120 (12); HREIMS *m/z* 368.1260 (calcd for $C_{21}H_{20}O_6$, 368.1260).

2″,3″-Dihydroxylupinifolin (2): as yellow solid, $[\alpha]_D$ –26.89° (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 225 (4.14), 266 (4.48), 274 (4.56), 300 (3.91), 312 (3.93), 363 (3.36) nm; IR (KBr) ν_{max} 3411, 3244, 2974, 1620, 1521, 1451, 1382, 832 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$), see Table 1; EIMS *m/z* 440 $[M]^+$ (50), 425

$[M\text{-}CH_3]^+$ (70), 407 (12), 381 (60), 351 (78), 335 (20), 305 (5), 287 (23), 261 (83), 231 (100), 215 (48), 120 (13); HRFABMS (positive ion) *m/z* 441.1913 (calcd for $C_{25}H_{29}O_7$, 441.1913).

Preparation of 2‴,3‴-dihydroxylupinifolin from 2‴,3‴-epoxylupinifolin: A solution of 2‴,3‴-epoxylupinifolin (100 mg) in 1% H_2SO_4 in THF (5 mL) was stirred at rt for 24 h. The reaction mixture was poured into water (25 mL) and extracted with CH_2Cl_2 (3×20 mL). The organic layer was evaporated to give crude product which was separated by silica gel preparative TLC using 1% MeOH in CH_2Cl_2 to give 2‴,3‴-dihydroxylupinifolin (53%).

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