## **Supporting Information**

## Rec. Nat. Prod. 16:6 (2023) 1090-1094

# **5,6-Dihydroxypyranoflavone, a New Flavonoid with an Oxidized Prenyl Group from Dietary plant** *Citrus hystrix*

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Figure S1: The HR-ESI-MS spectrum of 1

Sample Name	Xn27	Position InjPosition	P1-F9	Instrument Name SampleType	Instrument 1 Sample	User Name IRM Calibration Status Acquired Time	Success 4/14/2023 10:
Inj voi Data Filename	Xn27.d	ACQ Method	s.m	Comment		All shares and shares a	
to 5 FES	Scan (0.14-0.1	7 min, 3 Scans) Frag=	135.0V Xn27.d	Subtract (2)			
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0.05- 1	24 188,	205 31	3 39	422 448 491	1 1 1 1 1 1 1 1 1 1 1 1	710 705 705 775 800	925 950 875

Figure S2: The ESI-MS spectrum of 1



Figure S4: The <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>) spectrum of 1



Figure S6: The <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) spectrum of 1



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Figure S9: The <sup>13</sup>C NMR (100 MHz, DMSO-*d*6) spectrum of 1 (From  $\delta_{\rm C}$  120 to 150 ppm)



Figure S10: The HSQC spectrum of 1

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**Figure S11**: The HSQC spectrum of **1** (From  $\delta_{\rm C}$  100 to140 ppm)



Figure S12: The <sup>1</sup>H-<sup>1</sup>H COSY spectrum of 1



Figure S13: The <sup>1</sup>H-<sup>1</sup>H COSY of H-1"/H-2", H-2'(6')/H-3'(5'), and H-2'(6')/H-4' of 1



Figure S14: The HMBC spectrum of 1

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Figure S15: The HMBC correlations of 5-OH to C-5, C-6 and C-10 of 1



Figure S16: The HMBC correlations of  $CH_3-4"/5"$  to C-3" and C-2" of 1



Figure S17: The HMBC correlations of CH-1" to C-7, C-8 and C-9 of 1



Figure S18: The HMBC correlations of CH-3 to C-2, C-4, C-10 and C-1' of 1





Figure S22: The <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD) spectrum of 3



Figure S24: The <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD) spectrum of 4



Figure S25: The image of the Citrus hystrix

The herbarium number of *Citrus hystrix* registered at https://sweetgum.nybg.org/science/ih/ was 3787305.

Structure Match	1 Result			
As Drawn (0)	□ 1 •••			
Substructure (1)	2166018-83-1 <b>د</b>			
Similarity (85K)	the contract			
Analyze Structure Precision	HO HO O			
Chemscape Analysis	C <sub>20</sub> H <sub>16</sub> O <sub>6</sub>			
Visually explore structure similarity with a powerful new tool. Learn more about Chemscape.	5,6-Dihydroxy-2-(4-hydroxyphenyl)-8,8- dimethyl-4 <i>H</i> ,8 <i>H</i> -benzo[1,2- <i>b</i> :3,4- <i>b</i> '] dipyran			
Create Chemscape Analysis	2 References Reactions Suppliers			

Figure S26: The exact search report from scifinder of 1

Structure Match	Filtering: Similarity	: 95-98 × Nu	mber of Compo	nents: 1 🗙				Clear All Filters
As Drawn (0)	3 Results					Sort: R	elevance 👻	View: Partial 🗸
Substructure (1)	□ 1	97 •••	2		97 •••	3		95 •••
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Chemscape Analysis Visually explore structure similarity with a powerful new tool. Learn more about Chemscape.		Ф	HO		$\bigcirc$	t		он
Create Chemscape Analysis	C <sub>20</sub> H <sub>16</sub> O <sub>6</sub> 5,6-Dihydroxy-2-(4-hyd dimethyl-4 <i>H</i> ,8 <i>H</i> -benzo[	roxyphenyl)-8,8- 1,2- <i>b</i> :3,4- <i>b</i> ']	C <sub>20</sub> H <sub>16</sub> O <sub>4</sub> 6-Hydroxy-8 benzo[1,2- <i>b</i> :	,8-dimethyl-2- 3,4- <i>b</i> ']dipyran	phenyl-4 <i>H</i> ,8 <i>H-</i> -4-one	C <sub>20</sub> H <sub>16</sub> O <sub>5</sub> Atalantoflavo	one	
Filter Behavior	dipyran							
Filter by Exclude	2 References Reaction	s Suppliers	2 References	▲ 0 Reactions	2 Suppliers	40 References	▲ 3 Reactions	19 Suppliers
✓ Search Within Results								

Figure S27: The 95%-98% similarity search report from scifinder of 1



Figure S28: The 94%-95% similarity search report from scifinder of 1

Table S1: 1H and 13C NMR Data of Compound 1 in DMSO-d6 and 5,6,4'-<br/>trihydroxypyranoflavone in CDCl3





5,6-dihydroxypyranoflavone (1)

5,6,4'-trihydroxypyranoflavone

No		1	5,6,4'-trihydroxypyranoflavone		
INO.	$\delta_{ m C}$	$\delta_{ m H}$	$\delta_{ m C}$	$\delta_{ m H}$	
2	162.9 s	-	159.4 s	-	
3	104.6 d	6.93 s	99.4 d	6.21 s	
4	182.4 s	-	175.4 s	-	
5	147.3 s	-	160.1 s	-	
6	129.8 s	-	135.4 s	-	
7	147.5 s	-	150.9 s	-	
8	101.2 s	-	101.3 s	-	
9	144.2 s	-	146.0 s	-	
10	104.7 s	-	103.7 s	-	
1'	130.8 s	-	122.4 s	-	
2'/6'	127.3 d	8.03 overlapped	129.4 d	8.04 d (8.8)	
3'/5'	129.2 d	7.53 overlapped	115.6 d	6.92 d (8.8)	
4'	132.0 d	7.53 overlapped	158.8 d	-	
1"	114.7 d	6.86 d (8.9)	114.7 d	6.75 d (9.6)	
2"	128.4 d	5.76 d (8.9)	127.2 d	5.56 d (10.0)	
3"	78.0 s	-	78.1 s	-	
4"/5"	27.6 q	1.41 s	28.0 q	1.41 s	
5-OH	-	12.77 s		-	

#### S1: Synthesis of 5,6-dihydroxypyranoflavone (1)

Baicalein (1 equiv, 135 mg, 0.5 mmol) and 3-methyl-2-butenal (2 equiv, 84 mg, 1.0 mmol) were dissolved in anhydrous pyridine (2 mL), and the reaction was performed by stirring the mixture under nitrogen at 110 °C for 10 hours. Then, the solution was reduced under a vacuum. The resulting mixture was directly subjected to silica gel column eluted with petroleum ether/ethyl acetate (ratio 8:2) to afford compound 1 as a yellow solid (59 mg, 0.175 mmol, 35%).

### S2: DPPH Radical Scavenging Assay

The DPPH assay was carried out as previously described [1-4]. L-Ascorbic acid was used as positive controls, and reaction mixtures containing 100  $\mu$ L of 200  $\mu$ M DPPH solution and 100  $\mu$ L of 2-fold serial dilutions of the sample with concentrations in the range of 160, 80, 40, 20, 10, 5, and 2.5  $\mu$ M were placed in a 96-well microplate and incubated at 37 °C for 30 min. After incubation, the absorbance was read at 517 nm by an Emax precision microplate reader, and the mean of three readings was obtained. Scavenging activity was calculated by the following equation:

Level of inhibition (%) =  $[1 - (A_{control} - A_{sample})/A_{control}] \times 100\%$ 

The IC<sub>50</sub> value was obtained through extrapolation from linear regression analysis and denoted the concentration of sample required to scavenge 50% of DPPH radicals.

#### **S3: ABTS Radical Scavenging Assay**

The ABTS assay was carried out as previously described [1-4] The ABTS<sup>+</sup> radical was obtained by the reaction of a 6 mM ABTS solution in water with potassium persulfate (2.45 mM) without light at 25 °C for 16 h before use. The absorbance of the ABTS<sup>+</sup> dilution was regulated with ethanol to  $0.70 \pm 0.02$  at 734 nm at 25 °C. L-Ascorbic acid was used as positive controls, and reaction mixtures containing 100  $\mu$ L of ABTS solution and 2-fold serial dilutions of the sample with concentrations in the range of 30, 15, 7. 5, 3. 75, 1.875, and 0.9375  $\mu$ M were placed in a 96-well microplate and incubated at 25 °C for 30 min. After incubation, the absorbance was read at 734 nm by an Emax precision microplate reader, and the mean of three readings was obtained. Scavenging activity was calculated by the following equation:

Level of inhibition (%) =  $[1 - (A_{control} - A_{sample})/A_{control}] \times 100\%$ 

The  $IC_{50}$  value was obtained through extrapolation from linear regression analysis and denoted the concentration of sample required to scavenge 50% of ABTS radicals.

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