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ANTI-TMV ISOQUINOLINE ALKALOIDS FROM THE WHOLE PLANTS OF *THALICTRUM GLANDULOSISSIMUM*

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Abstract – Three new (**1-3**), together with three known (**4-6**) isoquinoline alkaloids were isolated from the whole plants of *Thalictrum glandulosissimum*. Their structures were elucidated by spectroscopic methods, including extensive ^{1}H, ^{13}C, and 2D-NMR techniques. Compounds **1-6** were evaluated for their anti-tobacco mosaic virus (Anti-TMV) activity. The results revealed that compounds **2**, **3** and **4** showed potential anti-TMV activities with inhibition rates of (35.6±3.5)%, (36.2%±3.8)%, (46.3%±3.2)%, at the concentration of 20 μM, respectively. These rates are higher than that of positive control.

Thalictrum is a genus of herbaceous perennial flowering plants in the Ranunculaceae (buttercup) family native mostly to temperate regions.[1] Various species from the genus Thalictrum have been used for centuries as herbal medicines especially in Russia, Japan and China.[2,3] This genus is well known for its diverse pharmacological activities, including anti-tumor, anti-virus, anti-microbial, anti-tuberculosis, anti-inflammatory, anti-malarial activities, and the like.[3,4]

T. glandulosissimum is a low-growing perennial herb plant belong to Thalictrum genus. It is of 60 - 85 cm in height, grown in meadow slopes, alpine meadows and river banks at cold plateau in Yunnan province, P. R. China.[1] The whole plants of *T. glandulosissimum* are used to treat some diseases, such as enteritis, dysentery, jaundice and throat by Bai nationality people in Yunnan Province of China.[5] Due to their versatile medicinal traditional uses, an increasing number of phytochemical studies have been carried out on this plant, and lot of bioactive natural products, especially some structural biodiversity alkaloids, have been reported.[6-9] Therefore, in the course of identifying bioactive compounds from local plants, we now

reinvestigated the chemical constituents of the whole plant of *T. glandulosissimum* collected in Lijiang Prefecture, Yunnan Province. As a results, three new (**1**-**3**), together with three known isoquinoline alkaloids (**4**-**6**) were isolated in this work. This paper describes the elucidation of the structures of these compounds and a preliminary evaluation of their anti-tobacco mosaic virus (anti-TMV) activity.

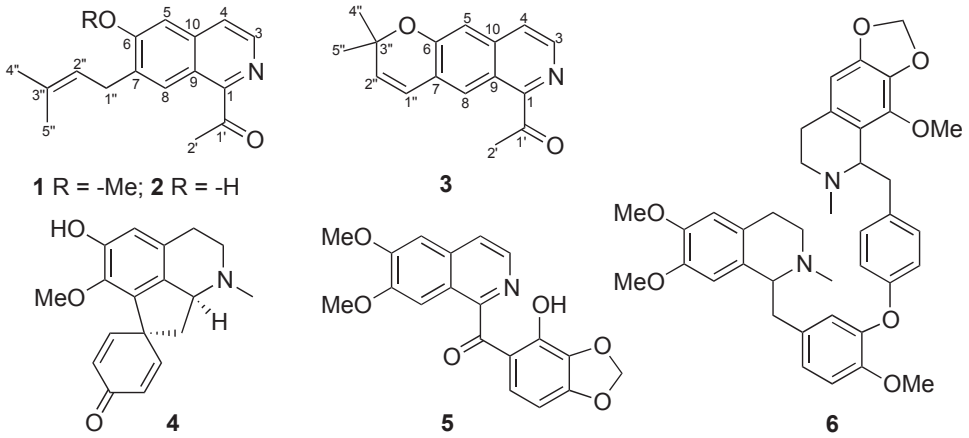

Figure 1. The isoquinoline alkaloids from the whole plants of *T. glandulosissimum*

A 95% aq. ethanol extract prepared from whole plants of *T. glandulosissimum* was partitioned between EtOAc and 3% tartaric acid. The aqueous layer was adjusted to pH 9.0 with saturated Na_2CO_3 aq. and extracted with EtOAc again. The EtOAc-soluble alkaloidal materials were subjected repeatedly to column chromatography on silica gel and preparative HPLC to afford three new isoquinoline alkaloids, 1-(6-methoxy-7-prenylisoquinolin-1-yl)ethanone (**1**), 1-(6-hydroxy-7-prenylisoquinolin-1-yl)ethanone (**2**) and 1-(2,2-dimethyl-2*H*-pyrano[2,3-*g*]isoquinolin-6-yl)ethanone (**3**), together with three known isoquinoline alkaloids (**4**-**6**). The structures of the compounds **1**-**6** were shown in Figure 1, and the ^1H and ^{13}C NMR data of **1**-**3** were listed in Table 1. The known compounds, compared with literature, were identified as linearisine (**4**),[10] rugosinone (**5**),[11] and thaliracebine (**6**).[12]

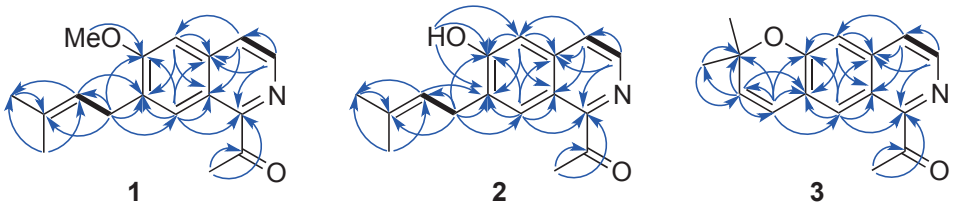

Figure 2. Key HMBC ( ) and ^1H-^1H COSY ( ) correlations of compounds **1**-**3**

Compound **1** was obtained as a yellow gum and assigned the molecular formula $C_{17}H_{19}NNaO_2$ from its HRESIMS at *m/z* 292.1318 $[M+Na]^+$ (calcd 292.1314). The IR absorption bands indicated the presence of carbonyl (1662 cm^{-1}), and aromatic ring (1614, 1573, and 1442 cm^{-1}) groups. UV absorptions at 222, 268, 302, and 341 nm suggested a conjugated aromatic ring system. Its ^1H, ^{13}C, and DEPT NMR data displayed resonances for 17 carbons and 19 hydrogen atoms, corresponding to one 1,6,7-trisubstituted

isoquinoline system[13,14] (C-1~C-10; H-3, H-4, H-5, and H-8), one acetyl group (-$COCH_3$, C-1′ and C-2′, H_3-2′),[15] one prenyl group (-CH_2CH=$C(CH_3)_2$, C-1″~C-5″, H_2-1″, H-2″, H_3-4″ and H_3-5″),[16] and one methoxy group (δ_C 56.3, δ_H 3.78). By analysis of its HMBC correlations (Figure 2), the existence of the isoquinoline system was supported by the HMBC correlations from H-3 to C-1, C-4, and C-10, from H-4 to C-5, C-9, and C-10, from H-5 to C-7, C-9, C-10, from H-8 to C-1, C-6, C-9, and C-10. The existence of prenyl group was supported by HMBC correlations from H_2-1″ to C-2″ and C-3″, from H-2″ to C-1″, C-3″, C-4″ and C-5″, from H_3-4″ to C-3″ and C-2″, and from H_3-5″ to C-3″ and C-2″, and the existence of acetyl group was also supported by the HMBC correlations from H_3-2′ to C-1′. Since the isoquinoline skeleton was determined, the positions of substituents (acetyl, prenyl and methoxy groups) also can determined by further analysis of its HMBC data (Figure 2). The acetyl group located at C-1 was supported by the HMBC correlation from the H_3-2′ (δ_H 2.33) to C-1 (δ_C 155.7). The HMBC correlations from the H_2-1″ (δ_H 3.36) to C-6 (δ_C 162.1), C-7 (δ_C 130.1), and C-8 (δ_C 126.6), from H-2″ (δ_H 5.22) to C-7 (δ_C 130.1), and from H-8 (δ_H 8.41) to C-1″ (δ_C 28.9) indicated that the prenyl group located at C-7. The HMBC correlations from the methoxy resonance (δ_H 3.78) to C-6 (δ_C 162.1) confirmed that the methoxy group was located at C-6. Thus, the structure of **1** was established, and gave the systematic name of 1-(6-hydroxy-7-prenylisoquinolin-1-yl)ethanone.

Table 1. ^{1}H NMR and ^{13}C NMR data (in $CDCl_3$) of compounds **1**-**3**

No.	Compound **1**		Compound **2**		Compound **3**	
	δ_C (m)	δ_H (m, *J*, Hz)	δ_C (m)	δ_H (m, *J*, Hz)	δ_C (m)	δ_H (m, *J*, Hz)
1	155.7 s		155.9 s		156.0 s	
3	140.4 d	8.06 (d) 6.4	140.2 d	7.99 (d) 6.4	140.9 d	8.09 (d) 6.4
4	122.5 d	7.47 (d) 6.4	122.1 d	7.43 (d) 6.4	123.6 d	7.52 (d) 6.4
5	108.5 d	7.11 s	110.2 d	7.10 s	106.9 d	7.13 s
6	162.1 s		158.8 s		157.2 s	
7	130.1 s		129.5 s		130.6 s	
8	126.6 d	8.41 s	127.7 d	8.38 s	126.6 d	8.63 s
9	121.4 s		120.6 s		121.1 s	
10	135.5 s		134.9 s		136.8 s	
1′	196.0 s		196.3 s		196.4 s	
2′	27.4 q	2.33 s	27.1 q	2.34 s	28.1 q	2.35 s
1″	28.9 t	3.36 (d) 6.8	28.9 t	3.38 (d) 6.8	116.7 d	6.67 (d) 9.8
2″	123.9 d	5.22 (t) 6.8	123.6 d	5.26 (t) 6.8	128.7 d	5.84 (d) 9.8
3″	134.1 s		133.6 s		78.9 s	
4″	18.2 q	1.53 s	18.0 q	1.52 s	26.3 q	1.64 s
5″	26.1 q	1.73 s	25.8 q	1.73 s	26.3 q	1.64 s
-OMe	56.3 q	3.78 s				
Ar-OH				10.80 s		

1-(6-Hydroxy-7-prenylisoquinolin-1-yl)ethanone (**2**) was obtained as a pale yellow gum and showed a quasi-molecular ion at *m/z* 278.1152 $[M+Na]^+$ in the HRESIMS (calcd *m/z* 278.1157), corresponding to the molecular formula $C_{16}H_{17}NNaO_2$. The ^1H and ^{13}C NMR spectra of **2** were similar to those of **1**. The chemical shift differences resulted from the highfield shift of C-6 from δ_C 162.1 ppm to δ_C 158.8 ppm, and the disappearance of a methoxy resonance and appearance of a phenolic hydroxy resonance (δ_H 10.08 s) in **2**. These changes indicated that a methoxy group at C-6 in **1** was converted into a phenolic hydroxy group in **2**. The HMBC correlation from the phenolic hydroxy proton (δ_H 10.08) to C-5 (δ_C 110.2), C-6 (δ_C 158.8) and C-7 (δ_C 129.5) indicated that the phenolic hydroxy group located at C-6. In addition, the positions of the prenyl group and acetyl group can also be determination by further analysis of its HMBC correlations (Figure 2). The structure of **2** was therefore defined.

Compound **3** was also obtained as a pale yellow gum, and its molecular formula was determined to be $C_{16}H_{15}NNaO_2$, by HREIMS experiment (*m/z* 276.1008 $[M+Na]^+$). The ^1H and ^{13}C spectral data of **3** also depict similar structure to compound **1**. The disappearance of a methoxy group and prenyl group resonance, and appearance of a 2,2-dimethyl-2*H*-pyran moiety (-CH=CH-C$(CH_3)_2$-O-, C-1″~C-5″, H-1″, H-2″, H_6-5″)[17] was obversed in its NMR spectra. This indicated that the methoxy group and prenyl group at C-6 and C-7 in **1** was converted into a 2,2-dimethyl-2*H*-pyran moiety group in **3**. The 2,2-dimethyl-2*H*-pyran moiety located at C-6 and C-7, and C-1″ linked to C-7, were supported by the HMBC correlations from H-1″ (δ_H 6.67) to C-6 (δ_C 157.2), C-7 (δ_C 130.6), and C-8 (δ_C 126.6), from H-2″ (δ_H 5.84) to C-7 (δ_C 130.6), and from H-8 (δ_H 8.63) to C-1″ (δ_C 116.7). In addition, the positions of the other substituents group can also be determination by further analysis of its HMBC correlations (Figure 2). Thus, the structure of 1-(2,2-dimethyl-2*H*-pyrano[2,3-*g*]isoquinolin-6-yl)ethanone (**3**) was determined.

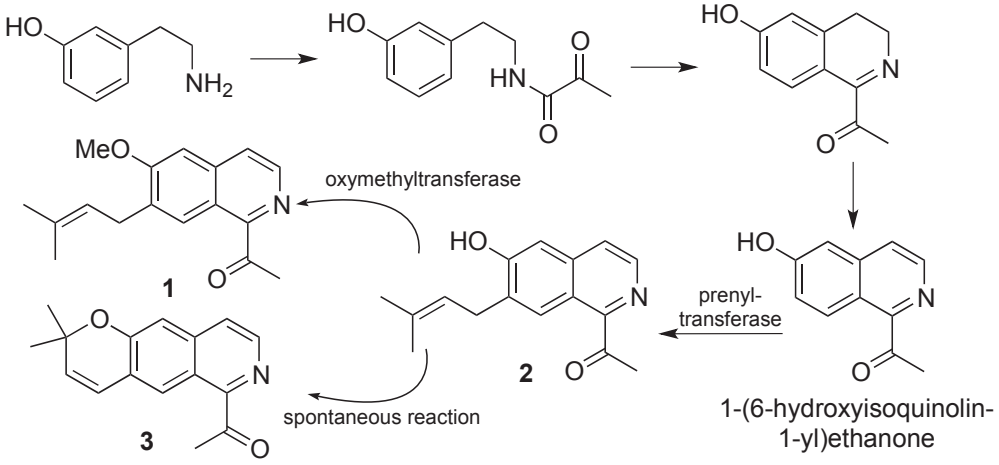

Figure 3. The possible biosynthetic pathway of compounds **1-3**

The possible biosynthetic pathway of compounds **1-3** are shown in Figure 3. Compound **2** might be produced by the precursor [1-(6-hydroxyisoquinolin-1-yl)ethanone] reacted with IPP (isopentenyl

pyrophosphate) by prenyltransferase and then resulted in the production of **1** and **3** under the action of oxymethyltransferase and a spontaneous reaction, respectively.

Since certain of the isoquinoline alkaloids exhibit potential anti-TMV activity,[15,18,19] compounds **1**-**6** were tested for their anti-TMV activity. The anti-TMV activity was tested using the half-leaf method. Ningnanmycin (a commercial product for plant disease in China) with inhibition rate of 32.5%, was used as a positive control.[20,21] The results revealed that compounds **2**, **3** and **4** showed potential anti-TMV activities with inhibition rates of (35.6±3.5)%, (36.2%±3.8)%, (46.3%±3.2)%, at the concentration of 20 μM, respectively. These rates are higher than that of positive control. Compounds **1**, **5** and **6** also showed inhibition rates of (22.3±3.2)%, (28.6±3.4)%, (18.4±3.5)%, at the concentration of 20 μM, respectively.

Since the compound **4** exhibited higher inhibition rate for TMV, its IC_{50} values was also tested with ningnanmycin as the positive control.[20,21] The results revealed that compound **4** exhibited the good activity with an IC_{50} value of 23.6 μM; the efficiency was higher than that of Ningnamycin (33.2 μM). In addition, the protective effects of compound **4** on TMV were also evaluated by pretreating the tobacco plant with 20 μM solutions of compounds or a solution of DMSO for 6 h before inoculation with TMV. The results showed that compound **4** showed protective effects to the host plants with the inhibition rates (48.6±3.8)%. This results indicated that pretreatment with compound **4** could increase the resistance of the host plant to TMV infection.

EXPERIMENTAL

General Experimental Procedures. UV spectra were obtained using a Shimadzu UV-1900 spectrophotometer. A Bio-Rad FTS185 spectrophotometer was used for scanning IR spectra. ^{1}H, ^{13}C, and 2D-NMR spectroscopic data were recorded on a DRX-500 NMR spectrometer with TMS as internal standard. ESIMS and HRESIMS analyses were measured on Agilent 1290 UPLC/6540 Q-TOF mass spectrometer. Chemical shifts (δ) are expressed in ppm with reference to the TMS signal. Semi-preparative HPLC was performed on an Agilent 1260 preparative liquid chromatograph with Zorbax PrepHT GF (2.12 mm × 25 cm) or Venusil MP C_{18} (2.0 mm × 25 cm) columns. Column chromatography was performed using silica gel (200-300 mesh, Qing-dao Marine Chemical, Inc., Qingdao, China), Lichroprep RP-18 gel (40 - 63 μm, Merck, Darmstadt, Germany), Sephadex LH-20 (Sigma-Aldrich, Inc, USA), or MCI gel (75 - 150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). Column fractions were monitored by TLC visualized by spraying with 5% H_2SO_4 in EtOH and heating.

Plant Material. The whole plants of *Thalictrum glandulosissimum* ((Finet & Gagnep.) W. T. Wang & S. H. Wang) were collected in Lijiang prefecture of Yunnan Province, People's Republic of China, in September 2017. The identification of plant material was verified by Prof. Ning Yuan. A voucher specimen (Ynni-17-09-93) has been deposited in Key Laboratory of Chemistry in Ethnic Medicinal

Resources, Yunnan Minzu University, P. R. China.

Extraction and Isolation. The air-dried and powdered whole plants of *T. glandulosissimum* (3.5 kg) were extracted with 95% aq. EtOH, and the extract was partitioned between EtOAc and 3% tartaric acid. The aqueous layer was adjusted to pH 9 with saturated Na_2CO_3 aq. and extracted with EtOAc. The EtOAc-soluble alkaloidal materials (34.5 g) were applied to silica gel (200–300 mesh) column chromatography, eluting with $CHCl_3$/MeOH gradient system (10:0, 9:1, 8:2, 7:3, 6:4, 5:5) to give six fractions A-F. Further separation of fraction B (9:1, 4.67 g) by silica gel column chromatography, eluted with $CHCl_3$/Me_2CO (9:1-2:1), yielded mixtures B1–B7. Subfraction B1 (9:1, 2.35 g) was subjected to silica gel column chromatography using petroleum ether/acetone, and then semi-preparative HPLC (78% MeOH/H_2O, flow rate 20 mL/min) to give **1** (25.5 mg). Subfraction B2 (8:2, 3.36 g) was loaded on another silica gel column using petroleum ether-ethyl acetate (EtOAc) elution, and then separated semi-preparative HPLC (65% MeOH-H_2O, flow rate 20 mL/min) to afford **2** (14.6 mg) and **3** (18.2 mg). Subfraction B3 (7:3, 3.04 g) was separated on the other silica gel column eluted by petroleum ether-EtOAc, followed by semi-preparative HPLC (58% MeOH-H_2O, flow rate 20 mL/min) to give **1** (12.6 mg), **4** (24.8 mg), and **5** (16.9 mg).

Anti-TMV Assays. The anti-TMV activities were tested using the half-leaf method,[20,21] and Ningnanmycin (2% water solution), a commercial product for plant disease in China, was used as a positive control. The virus was inhibited by mixing with the solution of tested compounds (20 μM in DMSO). After 30 min, the mixture was inoculated on the left side of the leaves of *Nicotiana. glutinosa*, whereas the right side of the leaves was inoculated with the mixture of DMSO solution and the virus as control. The local lesion numbers were recorded 3-4 days after inoculation. Three repetitions were conducted for each compound. The inhibition rates were calculated according to the formula:

$$\text{inhibition rate (\%)} = [\,(C-T) / C\,] \times 100\%$$

where C is the average number of local lesions of the control and T is the average number of local lesions of the treatment. Ningnanmycin (20 μM in DMSO), a commercial virucide for plant disease in China, was used as a positive control.

1-(6-Methoxy-7-prenylisoquinolin-1-yl)ethanone (1): Obtained as yellow gum; UV (MeOH) λ_{max} (log ε) 222 (4.15), 268 (3.64), 302 (3.35), 341 (3.48) nm; IR (KBr) ν_{max} 3048, 2947, 1662, 1614, 1573, 1442, 1356, 1246, 1163, 1058, 922, 789 cm^{-1}; ^1H NMR and ^{13}C NMR data (in $CDCl_3$, 500 and 125 MHz) see Table 1; positive ESIMS *m/z* 292 $[M+Na]^+$; HRESIMS *m/z* 292.1318 $[M+Na]^+$ (calcd for $C_{17}H_{19}NNaO_2$, 292.1314).

1-(6-Hydroxy-7-prenylisoquinolin-1-yl)ethanone (2): Obtained as yellow gum; UV (MeOH) λ_{max} (log ε) 220 (4.12), 264 (3.53), 299 (3.22), 338 (341) nm; IR (KBr) ν_{max} 3412, 3050, 2954, 1665, 1612, 1568, 1453, 1386, 1243, 1160, 1056, 936, 794 cm^{-1}; ^1H NMR and ^{13}C NMR data (in $CDCl_3$, 500 and 125 MHz) see

Table 1; positive ESIMS *m/z* 278 $[M+Na]^+$; HRESIMS *m/z* 278.1152 $[M+Na]^+$ (calcd for $C_{16}H_{17}NNaO_2$, 278.1157).

1-(2,2-Dimethyl-2*H*-pyrano[2,3-*g*]isoquinolin-6-yl)ethanone (3): Obtained as yellow gum; UV (MeOH) λ_{max} (log ε) 225 (4.22), 276 (3.62), 308 (3.39), 346 (348) nm; IR (KBr) ν_{max} 3074, 2962, 1644, 1610, 1538, 1469, 1357, 1266, 1164, 1047, 895, 762 cm^{-1}; ^1H NMR and ^{13}C NMR data (in $CDCl_3$, 500 and 125 MHz) see Table 1; positive ESIMS *m/z* 276 $[M+Na]^+$; HRESIMS *m/z* 276.1008 $[M+Na]^+$ (calcd for $C_{16}H_{15}NNaO_2$, 276.1001).

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