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QUINOLINE ALKALOIDS FROM THE LEAVES OF CIGAR TOBACCO AND THEIR ANTI-TOBACCO MOSAIC VIRUS ACTIVITY

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Abstract – Three new (**1-3**), together with three known (**4-6**) quinoline alkaloids were isolated from the leaves of cigar tobacco (a variety of *Nicotiana tabacum*). Their structures were elucidated by spectroscopic methods, including extensive ^{1}H, ^{13}C, and 2D-NMR techniques. Compounds **1**-**6** were tested for their anti-tobacco mosaic virus (anti-TMV) activity. The results showed that compound **3** exhibited high anti-TMV activity with inhibition rate of 42.2% at the concentration of 20 μM. This rate in higher than that of positive control (with inhibition rate of 32.5% at the concentration of 20 μM). Compounds **1**, **2**, **4**~**6** also showed potential anti-TMV activity with inhibition rates of 28.2, 31.0, 22.8, 26.6, and 29.5% at the concentration of 20 μM, respectively. These rates are slightly lower than that of positive control.

Nicotiana tabacum is the principal source of nicotine. The stems and leaves of the plant are used for commercial purposes in many countries to be processed into the production of cigars, cigarettes, chewing tobacco, and nicotine replacement products.[1-3] The leaves of *N. tabacum* are used to treat arthritis, bronchitis, digestive system problems, sinusitis, and skin problems,[2,4] and also used for general gynecological disorders, narcotic, toothache remedy, general skin diseases, and the treatment of ear problems.[5] In previous phytochemical studies, some bioactive metabolites, such as sesquiterpenoids,[6,7] flavonoids,[8,9] alkaloids,[10,11] furans,[12,13] coumarins,[14,15] had been isolated from this plant.

Cigar (a variety of *N. tabacum*), originally from South America, has a main use of rolling cigar wrapper that is different from flue-cured tobacco. Cigar tobacco had been introduced into Yunnan Province, and

started large-scale cultivation in 2019. However, the secondary metabolites of cigar grown in Yunnan have not been studied. In continuing our efforts to utilize *N. tabacum* and identify bioactive natural products from this plant, the phytochemistry investigation of the leaves of Yunxue-8 (a cigar variety cultivated in Yunnan) led to the isolation of three new (**1-3**) and three known (**4-6**) quinoline alkaloids. Their structures were determined by means of HRESIMS and extensive 1D- and 2D- NMR spectroscopic studies. Compounds **1-6** were also tested for their anti-tobacco mosaic virus (anti-TMV) activity. The structure elucidation of these compounds and a preliminary evaluation of their anti-TMV are reported in this manuscript.

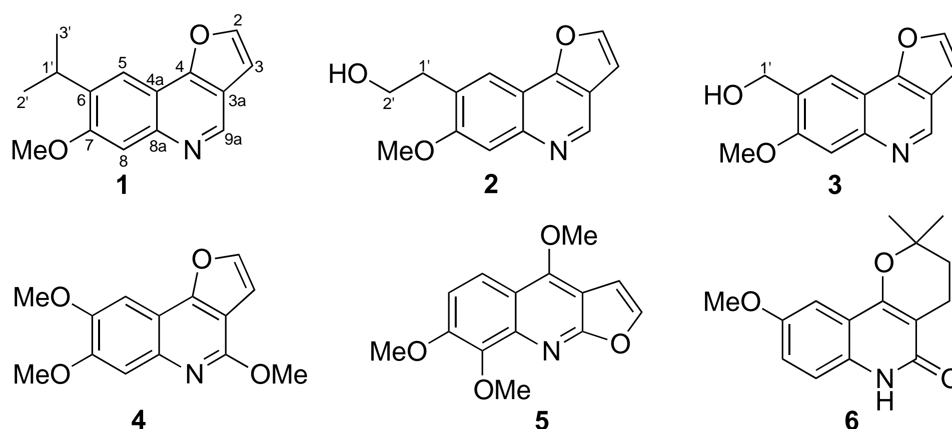

Figure 1. Quinoline alkaloids from the leaves of *N. tabacum*

A 95% aq. ethanol extract prepared from leaves of *N. tabacum* 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 alkaloid materials were subjected repeatedly to column chromatography and preparative HPLC to afford three new (**1-3**), together with three known quinoline alkaloids (**4-6**). Their structures of the compounds **1-6** were as shown in Figure 1, and the ^{1}H and ^{13}C NMR data of compounds **1-3** were listed in Table 1. The known compounds, compared with literatures, were identified as kokusaginine B (**4**),[16] skimmianine (**5**),[17] and dihydrohaplamine (**6**).[18]

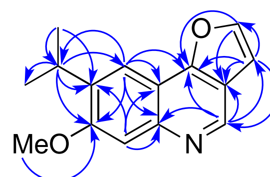

Figure 2. Key HMBC (↷) correlations of 1

Compound **1** was obtained as a pale-yellow gum. Its molecular formula $C_{15}H_{15}NO_2$ was deduced from its HRESIMS which showed the pseudo-molecular ion $[M+Na]^+$ at *m/z* 264.1008 (calc. 264.1001 for $C_{15}H_{15}NNaO_2$), with 9 degree of unsaturations. Its infrared spectrum exhibited bands due to aromatic protons (=C-H at 3096 cm^{-1}), imines (-C=N- at 1825 cm^{-1}), and aromatic carbons (-C=C- at 1612, 1470, and 1368 cm^{-1}). Its ^{1}H, ^{13}C, and DEPT NMR data displayed resonances for 15 carbons and 15 hydrogen atoms, which were ascribed to a tetrasubstituted

benzene ring (C-5~C-8, C-4a and C-8a, H-5 and H-8), a furan moiety (C-2 and C-3, H-2 and H-3), a isopropyl moiety (-CH(CH_3)$_2$, C-1'~C-3', H-1', H_6-2',3'),[19] a methoxy group (-MeO-7, δ_H 3.80 s and δ_C 56.5 q), and a –C=C-CH=N- moiety (C-4, C-3a, C-9a, and H-9a). The NMR data of **1** (Table 1) showed similarity to those of known compound (**4**).[20] The main difference was that two methoxy groups in **4** was replaced by an isopropyl moiety in **1**, which suggested that **1** should be a furo[3,2-*c*]quinoline analogue.[20] In addition, on the basis of the carbon chemical shifts, benzene ring, furan ring and –C=C-CH=N- moiety in compound **1** were accounted for 8 of the 9 degrees of unsaturation. The benzene and –C=C-CH=N- moiety should be fused to a quinoline moiety to meet 9 degrees of unsaturation, and these deductions were also supported by the and the HMBC correlations (Figure 2) from H-5 to C-4, C-4a, C-8a, from H-8 to C-6, C-4a, C-8a, from H-9a to C-3, C-4, C-3a, C-8a, from H-2 to C-4, C-3a, and from H-3 to C-4, C-3a, C-9a. Based on above information, the skeleton of **1** can be definitely confirmed as furo[3,2-*c*]quinoline.

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

No.	**Compound 1**		**Compound 2**		**Compound 3**	
	δ_C	δ_H (mult, *J*, Hz)	δ_C	δ_H (mult, *J*, Hz)	δ_C	δ_H (mult, *J*, Hz)
2	143.2 d	7.94 d (2.8)	143.2 d	7.92 d (2.8)	143.4 d	7.93 d (2.8)
3	105.0 d	7.57 d (2.8)	105.6 d	7.55 d (2.8)	105.7 d	7.54 d (2.8)
4	156.6 s		156.3 s		156.8 s	
5	126.8 d	7.70 s	128.3 d	7.75 s	127.4 d	7.73 s
6	134.4 s		132.0 s		132.0 s	
7	161.3 s		162.9 s		162.1 s	
8	105.9 d	7.39 s	106.8 d	7.41 s	106.9 d	7.43 s
3a	115.9 s		115.4 s		115.6 s	
4a	113.3 s		112.3 s		112.9 s	
8a	136.9 s		138.3 s		138.5 s	
9a	142.6 s	8.77 s	142.5 s	8.79 s	142.5 s	8.75 s
1'	28.7 d	2.85 m	32.9 t	2.55 t (7.2)	62.8 t	4.54 s
2',3'	22.6 q	1.33 d (6.8)	63.5 t	3.62 t (7.2)		
-OMe	56.5 q	3.80 s	56.3 q	3.79 s	56.3 q	3.78 s

Since the furo[3,2-*c*]quinoline skeleton was determined, the positions of substituents (isopropyl and methoxy groups) can also be determined by further analysis of its HMBC data. The isopropyl group located at C-6 was supported by the HMBC correlation from H-1' to C-5, C-6, and C-7. The HMBC correlation from the methoxy proton (δ_H 3.80 s) to C-7 confirmed that the methoxy group was located at

C-7. Thus, the structure of **1** was established, and gave the systematic name of 6-isopropyl-7-methoxy-furo[3,2-*c*]quinoline.

Compound **2** was also obtained as pale-yellow gum with a molecular formula as $C_{14}H_{13}NO_3$, according to the ion peak of *m/z* 266.0787 ($[M+Na]^+$) in the HRESIMS. The UV, IR spectra and NMR signal pattern of **2** were highly similar to those of **1**. The chemical shift differences resulted from the disappearance of an isopropyl group and appearance of the ^1H and ^{13}C NMR chemical shift value of a hydroxyethyl group (C-1' and C-2', H_2-1' and H_2-2') in **2**. These changes indicated that the isopropyl group at C-6 in **1** was converted into a hydroxyethyl group in **2**. The HMBC correlation from H_2-1' to C-5, C-6, C-7, and from H-5 to C-1' also supported the hydroxyethyl group located at C-6. In addition, the positions of the methoxy group can also be determination by further analysis of its HMBC correlations. The structure of 2-(7-methoxyfuro[3,2-*c*]quinolin-6-yl)ethanol (**2**) was therefore defined.

Compound **3** was obtained as a pale-yellow gum and showed a quasi-molecular ion at *m/z* 252.0645 $[M+Na]^+$ in the HRESIMS (calcd *m/z* 252.0637), corresponding to the molecular formula $C_{13}H_{11}NO_3$. The ^1H and ^{13}C NMR spectra of **3** were highly similar to those of **1**. These indicated that compounds **1** and **3** have very similar structures. The marked differences between them were due to the disappearance of an isopropyl group signals, and appearance of a hydroxymethyl signal (the ^1H and ^{13}C NMR chemical shift value of C-1′ and H_2-1′). This indicated that the isopropyl group in **1** was substituted by a hydroxymethyl group in **3**. The 2D HMBC data of two or three-bond correlations from H_2-1′ to C-5, C-6, C-7, and from H-5 to C-1′ revealed that the hydroxymethyl group located at C-6. Therefore, the structure of (7-methoxyfuro[3,2-*c*]quinolin-6-yl)methanol (**3**) was established as shown.

Since certain of the alkaloids from *N. tabacum* exhibit potential anti-TMV activity,[10,11,21] compounds **1-6** were tested for their anti-TMV activity. The anti-TMV activity were tested by half-leaf method, using ningnanmycin ($C_{16}H_{25}N_7O_8$, CAS#: 156410-09-2, a commercial new cytosine nucleoside peptide antibiotics for plant viral diseases in China) as a positive control.[22,23] The results showed that compound **3** exhibited high anti-TMV activity with inhibition rate of 42.2% at the concentration of 20 μM, respectively. This rate in higher than that of positive control (with inhibition rate of 32.5% at the concentration of 20 μM). Compounds **1**, **2**, **4~6** also showed potential anti-TMV activity with inhibition rates of 28.2, 31.0, 22.8, 26.6, and 29.5%, respectively, at the concentration of 20 μM. These rates are slightly lower than that of positive control.

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. ^1H, ^{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 C_{18} (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), 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 ethanol and heating.

Plant Material. The leaves of cigar tobacco (Yunxue-8, a cigar variety cultivated in Yunnan), which was planted in Lincang prefecture, Yunnan Province. The tobacco leaves were picked at the mature stage, and voucher specimen (Ynni-19-10-049) 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 leaves of cigar tobacco (5.0 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 9.5 with saturated Na_2CO_3 aq., extracted with EtOAc, and removed the pigments with MCI gel. The purified EtOAc-soluble alkaloidal materials (82.3 g) were applied to silica gel 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, 6.22 g) by silica gel column chromatography, eluted with $CHCl_3$/Me_2CO (9:1-2:1), yielded mixtures B1–B7. Sub-fraction B1 (9:1, 1.85 g) was subjected to silica gel column chromatography using petroleum ether/acetone, and then semi-preparative HPLC (62% MeOH/H_2O, flow rate 20 mL/min) to give the crude compounds. The crude compounds was applied to Sephadex LH-20 column eluting with MeOH to give **1** (12.2 mg), and **6** (13.8 mg). Sub-fraction B2 (8:2, 5.67 g) was subjected to silica gel column chromatography using petroleum ether/acetone, and then semi-preparative HPLC (55% MeOH/H_2O, flow rate 20 mL/min) to give the crude compounds. The crude compounds was applied to Sephadex LH-20 column eluting with MeOH to give **2** (10.5 mg), **3** (13.4 mg), **4** (15.8 mg), and **5** (14.4 mg).

Anti-TMV Assays. The anti-TMV activities were tested using the half-leaf method,[22,23] 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 *N. 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.

6-Isopropyl-7-methoxyfuro[3,2-*c*]quinoline (**1**), $C_{15}H_{15}NO_2$, obtained as yellow powder; UV (MeOH), λ_{max} (log ε) 210 (4.68), 245 (4.38), 336 (3.97) nm; IR (KBr) ν_{max} 3096, 2328, 1825, 1612, 1470, 1368, 1174, 1049, 918 cm^{-1}; ^1H and ^{13}C NMR data ($CDCl_3$, 500 and 125 MHz), Table 1; ESI-MS (positive ion mode) *m/z* 264 $[M+Na]^+$; HR-ESI-MS (positive ion mode) *m/z* 264.1008 $[M+Na]^+$ (calcd 264.1001 for $C_{15}H_{15}NNaO_2$).

2-(7-Methoxyfuro[3,2-*c*]quinolin-6-yl)ethanol (**2**), $C_{14}H_{13}NO_3$, obtained as yellow powder; UV (MeOH), λ_{max} (log ε) 210 (4.65), 246 (4.34), 338 (3.95) nm; IR (KBr) ν_{max} 3392, 3090, 2325, 1822, 1612, 1464, 1371, 1168, 1053, 927 cm^{-1}; ^1H and ^{13}C NMR data ($CDCl_3$, 500 and 125 MHz), Table 1; ESI-MS (positive ion mode) *m/z* 266 $[M+Na]^+$; HR-ESI-MS (positive ion mode) *m/z* 266.0787 $[M+Na]^+$ (calcd 266.0793 for $C_{14}H_{13}NNaO_3$).

(7-Methoxyfuro[3,2-*c*]quinolin-6-yl)methanol (**3**), $C_{13}H_{11}NO_3$, obtained as yellow powder; UV (MeOH), λ_{max} (log ε) 210 (4.58), 242 (4.32), 334 (3.90) nm; IR (KBr) ν_{max} 3389, 3084, 2322, 1819, 1614, 1469, 1374, 1162, 1058, 936 cm^{-1}; ^1H and ^{13}C NMR data ($CDCl_3$, 500 and 125 MHz), Table 1; ESI-MS (positive ion mode) *m/z* 252 $[M+Na]^+$; HR-ESI-MS (positive ion mode) *m/z* 252.0645 $[M+Na]^+$ (calcd 252.0637 for $C_{13}H_{11}NNaO_3$).

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