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ARTICLE

Anti-Tobacco Mosaic Virus (TMV) Quassinoids from *Brucea javanica* (L.) Merr.

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Two new quassinoids, javanicolide E (**1**) and javanicolide F (**2**), along with fifteen known C-20 quassinoids were isolated from the seeds of *Brucea javanica* (L.) Merr. The antitobacco mosaic virus (TMV) activity of these quassinoids was screened by the conventional half-leaf and leaf-disk method along with Western blot analysis. All of the seventeen quassinoids showed potent anti-TMV activity. Among them, eight compounds, brusatol (**3**), bruceine B (**4**), bruceoside B (**5**), yadanzioside I (**6**), yadanzioside L (**7**), bruceine D (**8**), yadanziolide A (**9**), and aglycone of yadanziolide D (**17**), showed strong antiviral activities, with IC_{50} values in the range of 3.42−5.66 μM, and were much more effective than the positive control, ningnanmycin (IC_{50} = 117.3 μM). The antiviral structure−activity relationships of quassinoids against TMV were also discussed.

KEYWORDS: Tobacco mosaic virus (TMV); *Brucea javanica* (L.) Merr.; quassinoids; structure−activity relationship (SAR).

INTRODUCTION

Plant viruses cause dramatic losses in agriculture and horticulture all over the world (*1*). Tobacco mosaic virus (TMV), one of the most well-studied plant viruses, infects more than 400 plant species belonging to 36 families, such as tobacco, tomato, potato, and cucumber (*2*−*4*). However, there are no chemical treatments that can absolutely inhibit TMV once it does infect plants. In finding an effective way to protect plants from TMV infection, natural products from plants have been proved to be a rich resource, as plants have already evolved multiple mechanisms to selectively suppress pathogens by production of secondary metabolites with antimicrobial activities. Guided by such a principle, our previous research has identified a series of natural products from plants with anti-TMV background (*5*−*8*). The antiviral mechanism of some compounds was clarified (*5*), and a synergistic effect between naturally occurring anti-TMV compounds was also reported (*9*).

Brucea javanica (L.) Merr. (Simaroubaceae) is a shrub widely distributed from Southeast Asia to Northern Australia (*10*). The seeds of *B. javanica* (L.) Merr. are known as "Ya dan zi" in Chinese folk medicine and contain predominantly quassinoids. Previously, Shen et al. found that the ethanol extract of the seeds of *B. javanica* showed dramatic anti-TMV activity (*11*), and only one quassinoid, bruceine D, was isolated and was proposed to be responsible for the anti-TMV activity (*12*). Although quassinoids have exhibited many biological activities (*13*−*16*), it is the only report concerning the anti-TMV activity (*12*). Therefore, we systematically investigate the quassinoids from *B. javanica* in an attempt to find more active quassinoids and clarify the structure−activity relationship on TMV inhibiton activities of these compounds.

In the present work, according to bioassay-guided isolation of crude extract, two new quassinoids, along with fifteen known quassinoids, were isolated from the seeds of *B. javanica*. Herein we report the structural elucidation of new compounds and the anti-TMV activity of all isolated quassinoids.

MATERIALS AND METHODS

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were acquired on Bruker DRX-500 and AM-400 spectrometers (Massachusetts) with TMS as an internal standard. MS spectra were measured on a Waters HPLC-Thermo Finnigan LCQ Advantage ion trap mass spectrometer (Milford, PA). Optical rotation was determined on a Horiba SEPA-300 polarimeter (Horiba,Tokyo, Japan). Column chromatography (CC) (Qingdao Haiyang Chemical Co., Qingdao, China) was carried out on silica gel G (100−200 mesh, 200−300 mesh), silica gel H (10−40 μm), and Sephadex LH-20 (40−70 μm) (Amersham Pharmacia Biotech AB, Uppsala, Sweden). Thin-layer chromatography was conducted on silica gel plates GF254 (Qingdao Haiyang Chemical Co., Qingdao, China). Spots on chromatograms were detected by spraying with 10% H_2SO_4−EtOH. Leaf disks were kept in a RXZ280B culture chamber (Ningbo, Zhejiang, China). SDS-PAGE and Western blotting were carried out using a Bio-Rad electrotransfer system (Bio-Rad, Hercules, CA).

Plant Material. The seeds of *B. javanica* (L.) Merr. were bought from the Kunming Juhuacun herb medicine market. The specimen was identified by Dr. Xujia Hu of the Kunming Institute of Food and Drug Control. A voucher specimen (No. 200708312) has been deposited in the State Key

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Extraction and Isolation. Dried powder of seeds of *B. javanica* (21 kg) was extracted with MeOH (three times under reflux for 4, 3, and 3 h). The solvent was removed under reduced pressure to give a residue (1772 g), which was suspended with water and then extracted with petroleum ether, chloroform, and *n*-BuOH, successively. The extracts were evaporated under vacuum to afford corresponding extracts of petroleum ether (310 g), chloroform (75 g), and *n*-BuOH (340 g). The chloroform extract was separated with a 1.5 kg silica gel G (100−200 mesh) column eluted with petroleum ether and EtOAc to give five fractions. Fraction 2 (petroleum ether/EtOAc, 7:3) gave compounds **3** (7.3 g), and fraction 3 (petroleum ether/EtOAc, 6:4) was extensively chromatographed over columns of silica gel and Sephadex LH-20 to afford **1** (11.5 mg), **2** (15 mg), **4** (4.1 g), **16** (35 mg), and **17** (20 mg). By Diaion SP-700 resin column chromatography (H_2O/MeOH, 1:0, 4:1, 3:2, 2:3, and 0:1), the *n*-BuOH extract of *B. javanica* gave five fractions. The H_2O/MeOH (3:2) and H_2O/MeOH (2:3) eluates were subjected to a reversed-phased silica gel column and Sephadex LH-20 to give compounds **5** (1.32 g), **6** (23 mg), **7** (124 mg), **8** (3.7 g), **9** (418 mg), **10** (4.5 g), **11** (32.5 g), **12** (27 mg), **13** (4.6 g), **14** (0.8 g), and **15** (643 mg).

Javanicolide E (***1***). Amorphous powder, $C_{26}H_{34}O_{11}$, $[\alpha]_D^{24} = +55.7$ (*c* 0.4, MeOH). UV λ_{max} (MeOH): 219.2 nm. IR (KBr) ν_{max} (cm^{-1}): 3451, 1738, 1644, 1378, 1264, 1139. ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data were presented in **Table 1**. HRESIMS: *m*/*z* 545.1985, $[M + Na]^+$ calculated for 545.1998.

Javanicolide F (***2***). Amorphous powder, $C_{30}H_{40}O_{13}$, $[\alpha]_D^{24} = +52.6$ (*c* 0.4, MeOH). UV λ_{max} (MeOH): 376.6, 220.8, 198.8 nm. IR (KBr) ν_{max} (cm^{-1}): 3441, 1738, 1726, 1640, 1253, 1113. ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data were presented in **Table 1**. HRESIMS: *m*/*z* 631.2364, $[M + Na]^+$ calculated for 631.2366.

Anti-TMV Assays. *Preparation of Screening Materials*. TMV (U1 strain) was obtained from the Yunnan Key Laboratory of Agricultural Biotechnology, Yunnan Academy of Agricultural Sciences, P. R. China. The virus was multiplied in *Nicotiana tabacum* cv.K326 and purified as described by Gooding and Hebert (*17*). The concentration of TMV was determined as 16 mg/mL with an ultraviolet spectrophotometer [virus concentration = $(A_{260} \times$ dilution ratio$)/E_{1cm}^{0.1\%,260nm}$]. The purified virus was kept at −20 °C and was diluted to 32 μg/mL with 0.01 M PBS before use.

Nicotiana glutinosa and *N. tabacum* cv.K326 plants were cultivated in an insect-free greenhouse. *N. glutinosa* was used as a local lesion host, and *N. tabacum* cv.K326. was used to determine systemic TMV infection. The experiments could be conducted when the plants grow to the 5−6-leaf stage.

The tested compounds were dissolved in DMSO and diluted with distilled H_2O to the required concentrations. The solution of equal concentration of DMSO was used as negative control. The commercial antiviral agent ningnanmycin was used as a positive control.

Half-Leaf Method (18). The virus was inhibited by mixing with the solution of compound. 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.

Leaf Disk Method (8,9). Growing leaves of *N. tabacum* cv. K326 were mechanically inoculated with equal volumes of TMV (32 μg/mL). After 6 h, 10 or 15 leaf disks that were smooth and thin and without major veins were cut from the leaf surface with an inside diameter of 1 cm. The leaf disks were floated on solutions of compounds or ningnanmycin and solutions of DMSO as negative control. Disks of healthy leaves were floated on DMSO solution as mock. All leaf disks were kept in a culture chamber at 25 °C for 48 h. Then the coat protein of TMV was analyzed by Western blot analysis.

SDS-PAGE and Western Blot Analysis of TMV Coat Protein (CP). SDS-PAGE was performed as described by Sambrook et al. (*19*). Briefly, leaf disks from the leaf-disk method were ground in protein loading buffer (40 g/L SDS, 10 mL/L β-ME, 200 mL/L glycerin, 2 g/L bromophenol blue, 0.1 mol/L Tris-HCl, pH 6.8) and then 5 μL of sample and 3 μL of marker were loaded on a polyacrylamide gel (5% stacking gel, 12.5% separating gel). Samples were run in duplicate. After SDS-PAGE, TMV protein bands were transferred at 200 mA for 45 min onto a nitrocellulose membrane (0.2 μm) using an electrotransfer system (Bio-Rad, Hercules, CA). The membrane was washed in TBST (1 mol/L Tris-HCl, pH 7.5; 1 mol/L NaCl; 0.05% Tween-20) and blocked with 5% nonfat milk powder in TBST for 1 h at 37 °C. The membrane was washed three times for 15 min with TBST and reacted with a mixture of 1:5000 alkaline phosphatase-conjugated antirabbit IgG (Sigma, St. Louis, MO) and 1:8000 polyclonal antibodies of TMV for 1 h at 37 °C. After it was washed three times for 15 min with TBST, the membrane was incubated in substrate buffer (1 mol/L Tris-HCl, pH 9.5; 1 mol/L NaCl; 1 mol/L MgCl) with 330 μL/mL NBT and 165 μL/mL BCIP for 3−5 min in the dark until the bands of the CP were clear.

Table 1. ^1H NMR and ^{13}C NMR Spectral Data of **1** and **2** in $CDCl_3$

	javanicolide E (**1**)		javanicolide F (**2**)	
position	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1 α	2.37 (1H, d, *J* = 12.5)	50.2 t	2.36 (1H, d, *J* = 13.0)	50.2 t
1 β	2.89 (1H, d, *J* = 12.5)		2.90 (1H, d, *J* = 13.0)	
2		209.2 s		209.2 s
3	3.72 (1H, d, *J* = 10.5)	79.8 d	3.72 (1H, d, *J* = 10.5)	79.8 d
4	1.66 (1H, m)	40.8 d	1.66 (1H, m)	40.8 d
5	1.87 (1H, d, *J* = 12.5)	43.2 d	1.89 (1H, t, *J* = 11.0)	43.2 d
6 α	2.23 (1H, d, *J* = 14.5)	29.1 t	2.22 (1H, d, *J* = 15.0)	29.1 t
6 β	1.53 (1H, t, *J* = 13.0)		1.54 (1H, d, *J* = 14.0)	
7	4.70 (1H, brs)	82.2 d	4.72 (1H, brs)	82.2 d
8		45.7 s		45.7 s
9	2.05 (1H, brs)	42.2 d	2.03[a]	42.1 d
10		42.3 s		42.3 s
11	4.22 (1H, d, *J* = 4.0)	71.2 d	4.21 (1H, d, *J* = 4.5)	71.3 d
12	4.18 (1H, s)	75.8 d	4.19 (1H, s)	75.7 d
13		81.2 s		81.2 s
14	3.10 (1H, brs)	51.2 d	3.14 (1H, brs)	51.2 t
15	b	65.7 d	b	66.2 d
16		167.1 s		167.0 s
18	1.17 (3H, d, *J* = 6.0)	16.19 q	1.18 (3H, d, *J* = 6.5)	16.18 q
19	1.26 (3H, s)	16.14 q	1.26 (3H, s)	16.15 q
20 a	3.76[a]	73.6 t	3.75 (1H, d, *J* = 8.0)	73.6 d
20 b	4.60 (1H, d, *J* = 7.5)		4.60 (1H, d, *J* = 8.0)	
21		171.9 s		171.6 s
OCH_3	3.77 (3H, s)	52.9	3.79 (3H, s)	53.2
1′		164.4 s		164.6 s
2′	5.61 (1H, s)	114.0 d	5.75 (1H, s)	111.8 d
3′		160.9 s		165.2 s
4′	1.92 (3H, s)	27.6 q		82.3 s
5′	2.18 (3H, s)	20.5 q	2.12 (3H, s)	14.5 q
6′			1.51 (3H, s)	26.1 q
7′			1.51 (3H, s)	26.1 q
8′				169.6 s
9′			2.01 (3H, s)	21.6 q

[a] Signals overlap each other. [b] Not detectable.

RESULTS AND DISCUSSION

Bioassay Guided Isolation. We tested inhibition activities against TMV using the crude extracts of *B. javanica* by the half-leaf method. The methanol extracts at a concentration of 1 mg/mL showed the inhibition rate of 73.9%. Then the methanol extracts were suspended in water and extracted with petroleum ether, chloroform, and *n*-BuOH, successively. At the concentration of 1 mg/mL, the chloroform and the *n*-BuOH extracts showed a high inhibition rate against TMV with the inhibition rates of 100% and 76.2%, respectively. Further separation of the two extracts afforded two new compounds, javanicolide E (**1**) and javanicolide F (**2**), as shown in **Figure 1**. In addition, 15 known

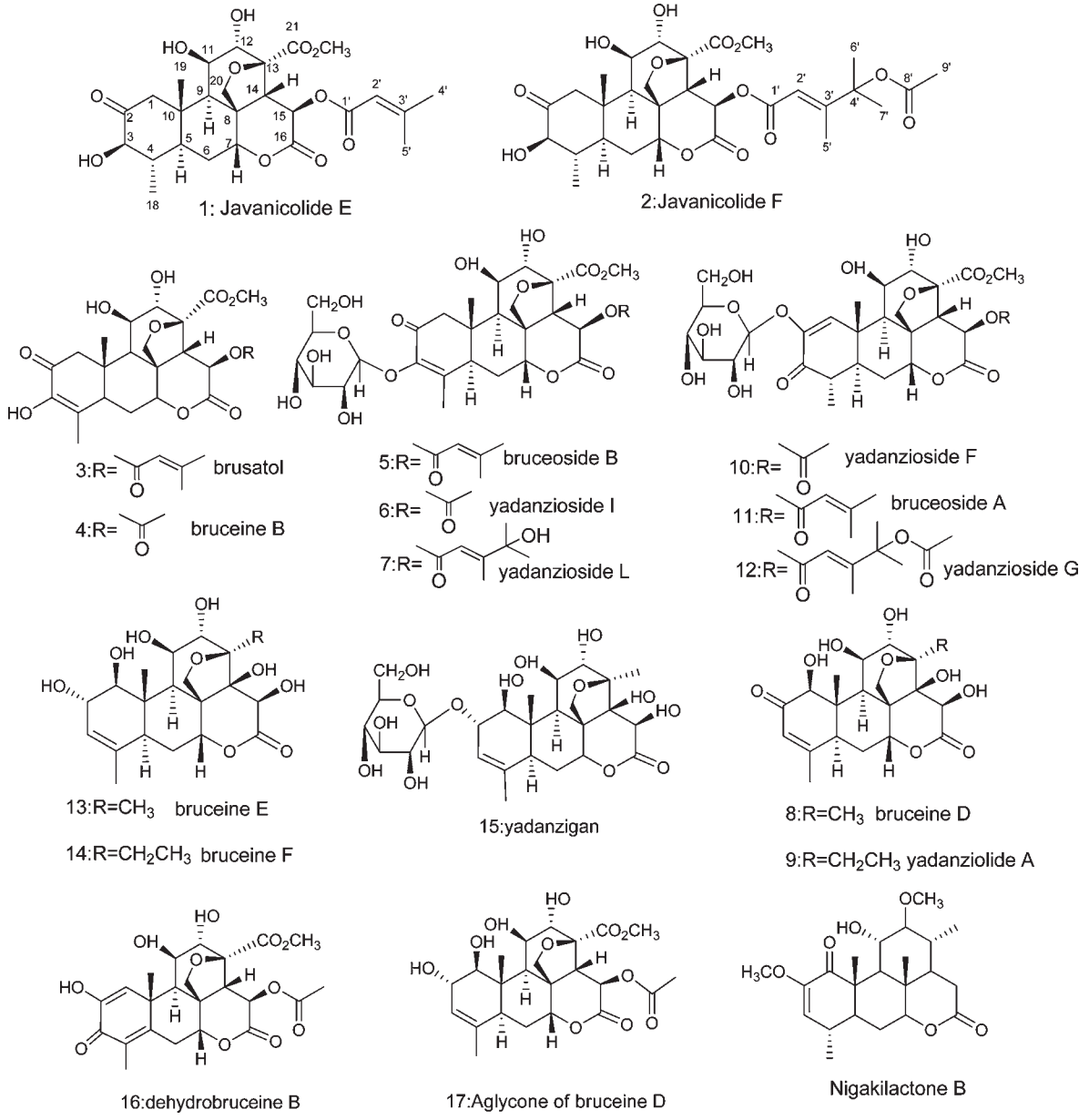

Figure 1. Structures of compounds **1**−**17** (from *Brucea javanica*) and nigakilactone B (from *Picrasma quassioides*).

compounds were also obtained and identified as brusatol (**3**) (*20*), bruceine B (**4**) (*21*), bruceoside B (**5**) (*22*), yadanzioside I (**6**) (*23*), yadanziosides L (**7**) (*23*), bruceine D (**8**) (*22*), yadanziolide A (**9**) (*24*), yadanziosides F (**10**) (*23*), bruceoside A (**11**) (*22*), yadanzioside G (**12**) (*25*), bruceine E (**13**) (*26*), bruceine F (**14**) (*26*), yadanzigan (**15**) (*27*), dedydrobruceine B (**16**) (*28*), and aglycone of yadanzioside D (**17**) (*29*), respectively.

Structure Elucidation of New Compounds. Javanicolide E (**1**) was obtained as an amorphous powder. Its molecular formula was determined to be $C_{26}H_{34}O_{11}$, indicating 10 degrees of unsaturation. The IR spectrum showed the bands of hydroxyl (3450 cm^{-1}) as well as carbonyl (1738 cm^{-1}) groups. Its ^1H NMR spectrum showed resonances of one secondary methyl (δ 1.17), one tertiary methyl (δ 1.26), two olefinic methyls (δ 1.92 and 2.18), one carbomethoxy group (δ 3.77), and one olefinic proton (δ 5.61) (**Table 1**), which indicated that **1** was a quassinoid with a picrasane skeleton. The ^1H and ^{13}C NMR data of **1** exhibited close similarity to those of bruceine D (**8**) (*21*), indicating the structures of the two compounds were very similar to each other except for ring A. The AB system of two protons of C-1 as well as HMBC correlations of H_2-1, H-3, 3-OH, and H_3-18 to a carbonyl (δ 209.2) suggested it is located at C-2. Unlike the case of javanicolide C (**18**) (*17*), a large coupling constant of H-3 with H-4

Table 2. TMV Infection Inhibition Activities of Compounds on *N. glutinosa in Vivo*[a]

compd	inhibition rate (%)	compd	inhibition rate (%)
javanicolide E (**1**)	52 ± 7.5	yandanzioside F (**10**)	31.1 ± 8.9
javanicolide F (**2**)	52.1 ± 6.8	bruceoside A (**11**)	24.1 ± 7.9
brusatol (**3**)	94.0 ± 5.3	yandanzioside G (**12**)	33.7 ± 5.3
bruceine B (**4**)	94.6 ± 7.3	bruceine E (**13**)	28.7 ± 6.9
bruceoside B (**5**)	78.4 ± 11.1	bruceine F (**14**)	31.2 ± 5.3
yadanzioside I (**6**)	81.5 ± 5.4	yadanzigan (**15**)	30.6 ± 2.4
yandanzioside L (**7**)	73.8 ± 4.3	dehydrobruceine B (**16**)	59.1 ± 5.1
bruceine D (**8**)	84.7 ± 6.5	aglycone of yadanziolide D (**17**)	70.8 ± 3.6
yadanziolide A (**9**)	83.4 ± 4.9	ningnamycin	25.3 ± 2.4

[a] The concentrations of compounds were 20 μM. All results are expressed as mean ± SD; n = 3 for all groups.

Table 3. IC_{50} Values of Selected Compounds against TMV on *N. glutinosa*

	inhibition rate					
compd	1.25 μM	2.5 μM	5 μM	10 μM	20 μM	IC_{50} (μM)
brusatol	20.0%	48.8%	64.7%	71.5%	88.9%	3.42
bruceine B	20.5%	40.6%	65.2%	81.5%	88.9%	3.47
bruceoside B	21.4%	31.7%	53.8%	66.7%	78.9%	4.64
yadanzioside I	26.1%	35.4%	54.9%	67.9%	83.7%	4.22
yadanzioside L	20.0%	35.0%	53.3%	64.3%	80.6%	4.86
bruceine D	21.4%	29.1%	47.9%	66.7%	78.6%	5.29
yadanziolide A	22.4%	39.2%	46.1%	58.7%	75.0%	5.50
aglycone of yadanziolide D	21.4%	38.5%	48.4%	59.2%	71.7%	5.66
	12.5 μg/mL	25 μg/mL	50 μg/mL	100 μg/mL	200 μg/mL	52.1 μg/mL
ningnamycin	28.0%	37.9%	46.6%	67.5%	86.2%	117.3 μM

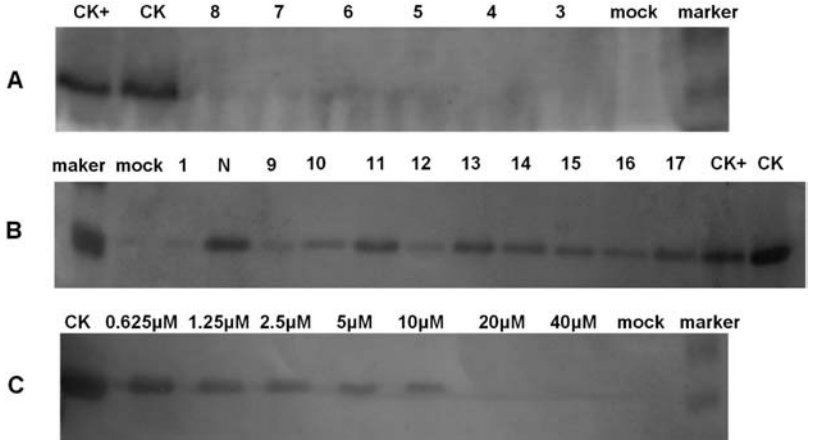

Figure 2. Western blot analysis. (**A**) Inhibition activities of compounds **3**−**8** (20 μM); CK^+, ningnanmycin; CK, negative control. (**B**) Inhibition activities of compounds **1** and **9**−**17**; N, nigakilactone B. (**C**) Dose-dependent inhibition of TMV replication (compound **3**).

(d, J = 10.5 Hz) indicated both H-3 and H-4 take up the axial position in the chair conformation of ring A. Due to the observed ROESY correlations of H-3 with H-5 and of H-4 with H-19, H-3 was therefore designated to the α-orientation while H-4 was β-oriented. Thus, the structure of javanicolide E (**1**) was elucidated as shown in **Figure 1**.

Javanicolide F (**2**) was obtained as an amorphous powder. Its molecular formula was determined to be $C_{30}H_{40}O_{13}$. The ^{1}H NMR and ^{13}C NMR spectra (**Table 1**) of **2** showed high similarity with those of **1**, except for the side chain at C-15. The HMBC spectrum revealed that **2** bears an (*E*)-4-hydroxy-3,4-dimethyl-2-pentenoyloxy group at C-15, just like yadanzioside G (**12**) (*24*) isolated from the same plants. Thus, the structure of javanicolide F (**2**) was elucidated as shown in **Figure 1**.

To the best of our knowledge, javanicolide E (**1**) and F (**2**) are among the very few examples of picrasane-type quassinoid with saturated ring A from *B. javanica*.

TMV Inhibition Activities of Compounds. The inhibitory activities of compounds **1**−**17** against TMV replication were tested using two approaches. First, the half-leaf method was used to test the antiviral activity in the local lesion host *N. glutinosa in vivo*. Then, the leaf-disk method was used to evaluate the antiviral activity of each compound in the systemic infection host *N. tabacum* cv. K326. Ningnanmycin, a commercial product for plant disease in China, was used as a positive control.

The antiviral inhibition rates of compounds **1**−**17** at the concentration of 20 μM tested by the half-leaf method were listed in **Table 2**. The results showed that all the compounds exhibited inhibition activities against TMV replication with inhibition rates ranging from 24.1% to 94.6%. Except compound **11** (bruceoside A, 24.1%), all the other compounds showed higher inhibition rates than that of the positive control, ningnanmycin (25.3%). Among the 17 quassinoids, brusatol (**3**) and bruceine B (**4**), sharing a diosphenol (3-hydroxy-3-en-2-one) unit in ring A, exhibited the best activity, with the inhibition rates 94% and 94.6%, respectively. Replacement with β-glucose at 3-OH (**5**−**7**) or a hydrogenation at 3-OH (**8**−**9**) gave a slight decrease of activity (**Table 2**). The saturation of the 3,4-double bond resulted in great loss of the antiviral activity from 94% (**3**) to 52% (**1**). The lost of the carbonyl at C-2 can also led to great lost in antiviral activity; the inhibition rate decreased from 84.7% (**8**) to 31.1% (**13**) and 30.6% (**15**) and from 83.4% (**9**) to 24.1% (**14**) with the carbonyl at C-2 in **8**−**9** substituted by the hydrogen (**13**−**14**) or β-glucose (**15**), which indicated the C-2 carbonyl was essential for the antiviral activity. Compared with compounds **1**−**17**, nigakilactone B (a quassinoid from *Picrasma quassioides*), without an epoxymethano bridge from C-8 to C-13, showed no anti-TMV activity (*9*), indicating the epoxymethano bridge moiety might be essential for anti-TMV activity.

Table 4. Protective Effects of Selective Compounds on TMV Infection[a]

compd	conc (μM)	inhibition rates (%)
brusatol	20	77.5 ± 2.5
bruceine B	20	86.3 ± 5.2
bruceine D	20	72.2 ± 4.4
bruceoside B	20	90.2 ± 1.5
yadanzioside L	20	54.4 ± 2.5

[a] All results are expressed as mean ± SD; n = 3 for all groups.

The inhibition rates of eight compounds, brusatol (**3**), bruceine B (**4**), bruceine D (**8**), yadanziolide A (**9**), yadanzioside I (**6**), yadanzioside F (**10**), dedydrobruceine B (**16**), and bruceoside B

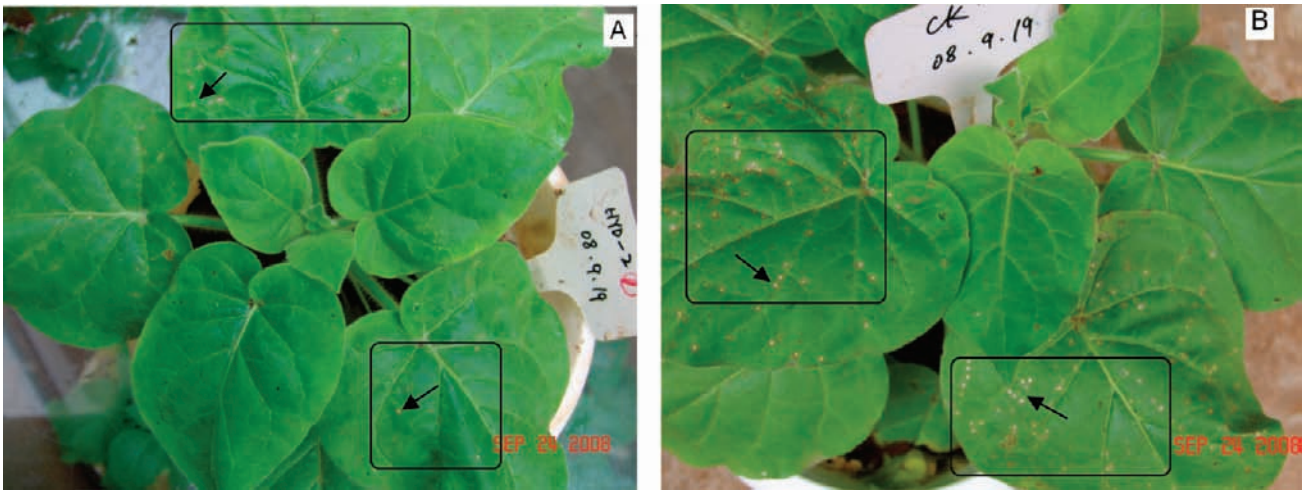

Figure 3. Protective effects of bruceine B on TMV infection. (**A**) *N. glutinosa* plants treated with 20 μM bruceine B 6 h before inoculation with TMV; (**B**) *N. glutinosa* plants treated with DMSO solution 6 h before inoculation with TMV. After inoculation with TMV, the plants were cultivated in an insect-free greenhouse for 3−4 days until the local lesions were clearly visible.

(**5**), were more than 70% .The IC_{50} value of the eight compounds were tested and listed in **Table 3**, with ningnanmycin as the positive control. Among them, brusatol (**3**) exhibited the best activity, with the IC_{50} value of 3.42 μM; the efficiency was 34-fold that of ningnamycin (117.3 μM).

To assess whether these quassinoids inhibit TMV replication in systemic infection host *N. tabacum* cv. K326, the leaf-disk method along with Western blot analysis of TMV coat protein (CP) in the presence of 20 μM compound was carried out (**Figure 2**). The bands of CP were not detected, when treated with compounds **3**−**9** (**Figure 2A** and **B**); while treated with compounds with moderate activity (**16**, **17**) or low activity (**10**−**15**), weak or strong bands were detected (**Figure 2B**). This result was in accordance with their inhibition rates in **Table 2**. However, nigakilactone B (*9*), a quassinoid from *Picrasma quassioides* Benn., showed no activity with a strong CP band as that of negative control (**Figure 2B**). Western-blot analysis further confirmed quassinoids from *Brucea javanica* could inhibit the accumulation of TMV CP *in vitro*. The quantity of TMV CP decreased with increasing concentrations of compounds in a dose-dependent manner (e.g., **Figure 2C**, compound **3**).

Protective Effects of Compounds on TMV *in Vivo*. *N. glutinosa* were pretreated with solutions of compounds or a solution of DMSO for 6 h before inoculation with TMV (*6*). The protective effects of five compounds were evaluated (**Table 4**). The results showed that, at the concentration of 20 μM, all the five tested compounds showed potent protective effects to the host plants, with the inhibition rates ranging from 54.4% to 90.2%. The results indicated that pretreatment with these quassinoids could increase the resistance of the host plant to TMV infection. **Figure 3** showed the protective effect of bruceine B (**4**); pretreated with **4** (**Figure 3A**), the local lesion number was much less than that of the control (**Figure 3B**), with the inhibition rate of 86.3 ± 5.2%.

In summary, a series of C-20 quassinoids with TMV inhibition activities were isolated from *B. javanica*. The *in vivo* tests showed that most quassinoids were more effective than the positive control ningnanmycin. Further study suggested that some quassinoids could not only inhibit the accumulation of TMV CP but also enhance the host plant's resistance to TMV infection. Quassinoids are the character components of the Simaroubeacea family, and recently, over 150 quassinoids have been isolated (*13*). Since there has been no effective antiviral product against TMV, perhaps quassinoids would be considered as lead structures for anti-TMV agents.

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Supporting Information Available: Supporting figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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