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Seco-pregnane steroidal glycosides from the roots of *Cynanchum atratum* and their anti-TMV activity

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ABSTRACT

Fifteen new *seco*-pregnane steroidal glycosides cynanosides A–O (**1**–**15**) together with twenty-seven known ones were isolated from the roots of *Cynanchum atratum*. The structures of **1**–**15** were determined by extensive analysis of spectroscopic data. The anti-tobacco mosaic virus (TMV) activity of these steroidal glycosides was screened by the conventional half-leaf method, enzyme-linked immunosorbent assay, and Western blot methods, most of them showed potent anti-TMV activity. Among them, compounds **1**, **7**, **13**, **28** and **31** showed significantly anti-TMV activity with an IC_{50} value of 20.5, 18.6, 22.0, 19.2 and 22.2 μg/mL, respectively, and were much more effective than the positive control, ningnanmycin (IC_{50} = 49.6 μg/mL).

1. Introduction

The plant disease caused by tobacco mosaic virus (TMV) is found worldwide. TMV is known to infect members of 9 plant families and at least 125 individual species, including tobacco, pepper, tomato, cucumbers, and a number of ornamental flowers [1]. However, there are no chemical treatments that can absolutely inhibit TMV once it does infect plants. On the other hand, plants have evolved multiple mechanisms to selectively suppress pathogens by the production of secondary metabolites with antimicrobial and/or antivirus. Guided by such a principle, our previous research has identified a series of natural products from plants with anti-TMV background [2–6]. The antiviral mechanism of some compounds was clarified, and a synergistic effect between naturally occurring anti-TMV compounds was also reported [7].

Cynanchum atratum Bunge (Asclepiadaceae), is a vivacious herb broadly distributed in China [8], whose dried roots have been used as a Chinese folk medicine for the treatment of hectic fevers, acute urinary infection and abscesses, and named as "Pai-Wei" in traditional Chinese medicine [9]. *C. atratum* has been reported to be rich in C-21 steroidal glycosides [10,11], whose chemical structures are classified into polyhydroxypregnane-type [12,13] and *seco*-pregnane-type glycosides. The *seco*-pregnane glycosides are further classified into 14,15-*seco*-pregnane-type [14,15] and 13,14:14, 15-*diseco*-pregnane-type glycosides [16]. Pregnanes and their glycosides have shown antitumor, bone resorbing, anti-fungal activates [17]. The most interesting result is, however, glaucogenin C and its one monosugar- and three pentasugar-glycosides were identified as the new agent with potent antiviral activity but not toxic as found in our study [2]. These compounds are effective and are selective inhibitors to alpha virus-like positive-strand RNA viruses including plant-infecting tobacco mosaic virus (TMV) and animal-infecting Sindbis virus (SINV), eastern equine encephalitis virus (EEEV) and Getah virus. Our previous study also indicated that these compounds predominantly suppress the expression of viral

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subgenomic RNA without affecting the accumulation of viral genomic RNA [2]. This result attracted us to systematically investigate anti-TMV activity of the *seco*-pregnane steroids from *C. atratum* in an attempt to find more active anti-TMV agents (Fig. 1).

In the present work, according to bioassay-guided isolation of crude extract, fifteen new *seco*-pregnane steroids, along with twenty-seven known *seco*-pregnane steroids, were isolated from the roots of *C. atratum*. Herein we report the structural elucidation of new compounds and the anti-TMV activity of all isolated *seco*-pregnane steroids.

2. Experimental

2.1. General methods

Optical rotations were determined with a JASCO DIP-370 Digital Polarimeter. IR spectra were recorded on a Bio-Rad

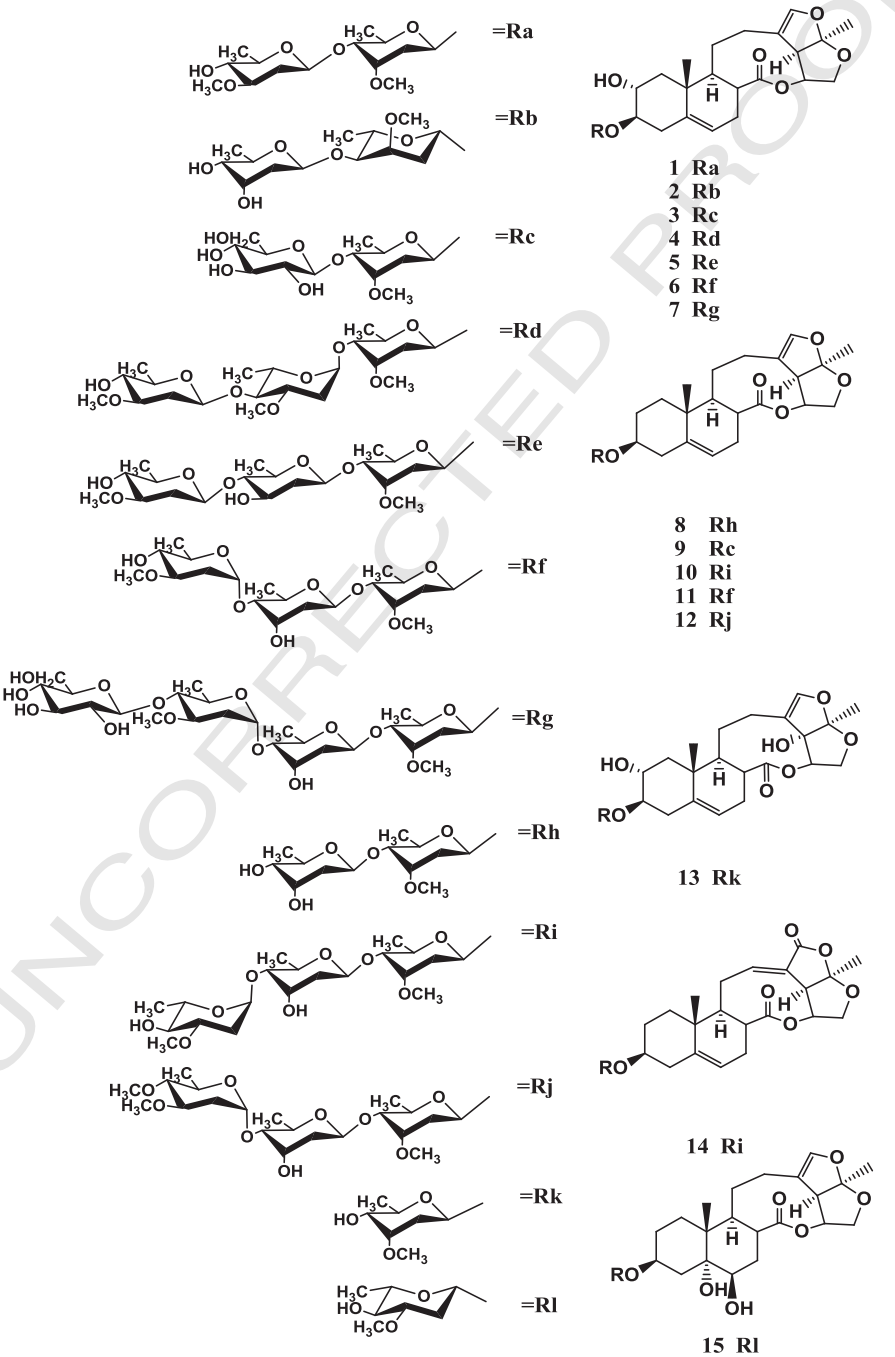

Fig. 1. Chemical structures of compounds **1**–**15**.

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