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Flavonoids from the cocoon of *Rondotia menciana*

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ABSTRACT

Two flavonol glycosides along with four known flavonoids were isolated from the cocoon of the mulberry white caterpillar, *Rondotia menciana* (Lepidoptera: Bombycidae: Bombycinae), a closely related species of the domesticated silkworm *Bombyx mori*, both of which feed on leaves of mulberry (*Morus alba*). The two glycosides were characterized as quercetin 3-*O*-β-D-galactopyranosyl-(1 → 3)-β-D-galactopyranoside and kaempferol 3-*O*-β-D-galactopyranosyl-(1 → 3)-β-D-galactopyranoside, based on spectroscopic data and chemical evidence. The flavonol galactosides found in the cocoon were not present in the host plant, nor in the cocoon of the silkworm, *B. mori*. Notably, flavonol glucosides, which are the main constituents of cocoon flavonoids in *B. mori mori*, were not found in the *R. menciana* cocoon. The present result strongly suggests that *R. menciana* is quite unique in that they predominantly use an UDP-galactosyltransferase for conjugation of dietary flavonoids, whereas UDP-glucosyltransferases are generally used for conjugation of plant phenolics and xenobiotics in other insects.

1. Introduction

It has been known that cocoon shells of some strains of the domesticated silkworm, *Bombyx mori* (L.), contain flavonoids, which are derived from the leaves of their host plant, the mulberry tree (*Morus alba*) (Fujimoto and Hayashiya, 1972; Tamura et al., 2002; Kurioka and Yamazaki, 2002; Hirayama et al., 2006). Orally administrated quercetin (**5**) was preferably glucosylated at the 5-*O*-position in the midgut, which is the first step for the biosynthesis of cocoon flavonoids (Hirayama et al., 2008). It was also found that cocoon flavonoids increase the UV-shielding activity of cocoons, and protect individuals from harmful effects of sunlight during metamorphosis (Daimon et al., 2010).

Uptake and utilization of dietary flavonoids is relatively widespread in the Lepidoptera (Ferreres et al., 2007). Flavonoids sequestered by Lepidoptera larvae are subsequently metabolized, stored, and transferred into the target organs such as the wings (Geuder et al., 1997). However, as far as is known, there is no report on cocoon flavonoids of insects other than *B. mori*. The aim of this study was to increase the knowledge of cocoon flavonoids of insects. The mulberry white caterpillar, *Rondotia menciana* has a close relationship to *B. mori*, both belonging to the subfamily Bombycinae and feeding on mulberry leaves. It is considered herein that comparative studies on ecological aspects of *R. menciana* and *B. mori* (e.g. metabolism of plant secondary metabolites, such as flavonoids and alkaloids) would facilitate an understanding of molecular mechanisms underlying interactions of insects and plants.

In the present study, cocoon flavonoids of *R. menciana* were isolated and identified based on spectroscopic methods and chemical evidence. These results indicated that the main constituents of the insect cocoon flavonoids were flavonol galactosides. Flavonol glycosides, which have been found in the cocoon of *B. mori* and their host plant, were not detected in the cocoon of *R. menciana*. This is the first report suggesting that flavonoids are conjugated with galactose in animal species, highlighting the complex metabolism of flavonoids in insects and its great diversity. This study further suggests that insect metabolites are a source of potential bioactive compounds that are not found in plants.

2. Results and discussion

2.1. Structural determination of cocoon shell flavonoids of R. menciana

The HPLC-DAD profile of the aqueous methanolic extract of a *R. menciana* cocoon shows six peaks with UV spectra characteristic of flavonols (Fig. 1A, see also Table S1). These flavonols were isolated and identified as follows. Acid hydrolysis of peaks a and b gave quercetin (**5**) (Fig. 2) as the aglycone and D-galactose (**7**) as the sugar residue. The UV spectra of both peaks a and b in the

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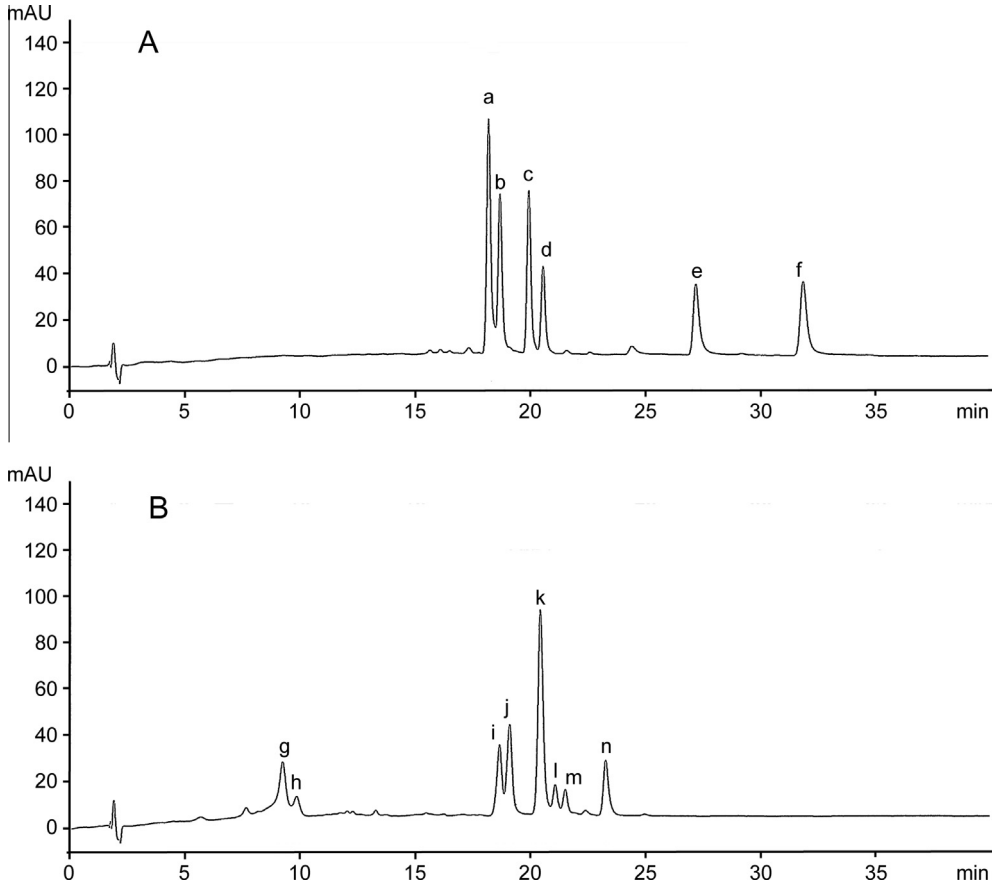

Fig. 1. Representative HPLC-DAD profiles of aqueous methanolic extracts of *R. menciana* cocoon (A) and *M. alba* leaves (B). Detection was performed at 365 nm. a, quercetin 3-*O*-galactosyl-galactoside (**1**); b, quercetin 3-*O*-galactoside (**3**); c, kaempferol 3-*O*-galactosyl-galactoside (**2**); d, kaempferol 3-*O*-galactoside (**4**); e, quercetin (**5**); f, kaempferol (**6**); g, 5-caffeoylquinic acid; h, caffeoylquinic acid; i, quercetin 3-*O*-rutinoside; j, quercetin 3-*O*-glucoside; k, quercetin 3-*O*-(6-malonylglucoside); l, quercetin derivative; m, kaempferol 3-*O*-glucoside; n, kaempferol derivative.

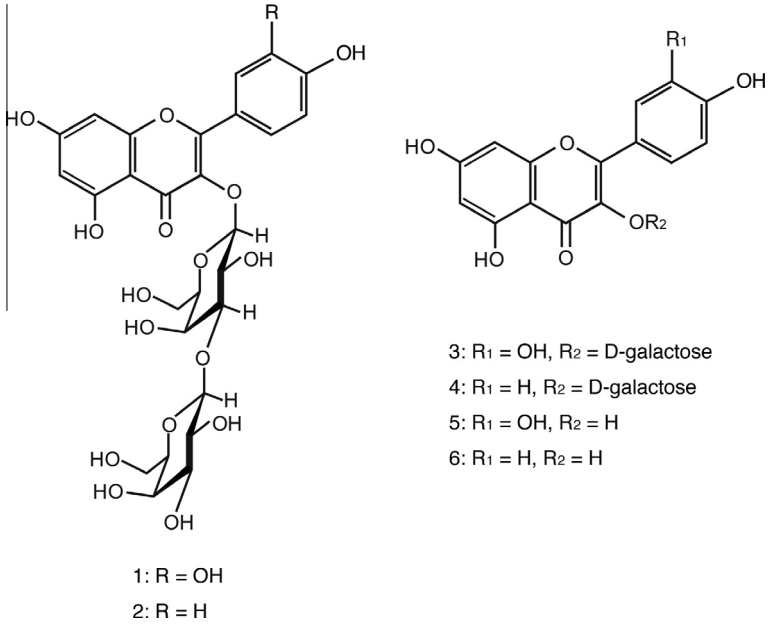

Fig. 2. Structures of flavonoids isolated from the cocoon of *Rondotia menciana*.

presence of shift reagents suggested free hydroxyl groups at C-5, C-7, C-3′ and C-4′, and substitution at the 3-*O* position of quercetin (**5**). LC–MS analysis of peaks a and b showed deprotonated ions $[M-H]^-$ with peaks at *m*/*z* 625, 463, respectively. Based on these data, peaks a and b were identified as quercetin 3-*O*-galactosyl-galactoside (**1**) and quercetin-3-*O*-galactoside (**3**), respectively.