Flavonol glycosides in the flowers of the Himalayan *Meconopsis paniculata* and *Meconopsis integrifolia* as yellow pigments

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**ABSTRACT**

Two flavonol glycosides were isolated from the yellow flowers of Bhutan Himalayan *Meconopsis paniculata*, together with other two minor flavonol glycosides. They were identified as 6-hydroxykaempferol 3-O-glucoside and 6-hydroxykaempferol 3-O-sophoroside by UV, LC-MS, acid hydrolysis and \(^1\)H and \(^13\)C NMR. Two minor flavonol glycosides were identified as kaempferol 3-O-glucoside and kaempferol 3-O-sophoroside. Although herbacetin (8-hydroxykaempferol) 3-O-glucoside has been reported from *M. paniculata* as a yellow pigment by Harborne (1969), 6-hydroxykaempferol 3-O-glucoside and 3-O-sophoroside were found in this survey. The latter flavonoid was reported in nature for the first time. On the other hand, four flavonol glycosides were isolated from the yellow flowers of *M. integrifolia* and characterized as quer cetin 3-O-gentiobioside and three 3-O-(acetylgentiobiosides). Thus, it was proved that the yellow flower colour of two *Meconopsis* species, *M. paniculata* and *M. integrifolia*, are due to flavonol glycosides.

1. Subject and source

The genus *Meconopsis* (Papaveraceae) consists of ca. 150 species and is growing in 2000–5000 m alt. of the Himalayan Mountains and adjacent Tibet Highland (Grey-Wilson, 2014). A few species are cultivated as ornamentals. Flowers of *Meconopsis paniculata* (D. Don) Prain were collected in near Mt. Jomolhari, Bhutan, ca. 3600 m alt. and 4050 m alt., 30 June and 2 July 2013, and Jangchulakha, Lake Jimi, near Palo, Bhutan, 3550 m alt., July 2015. Voucher specimens were deposited in the Herbarium of National Biodiversity Centre (THIM), Thimpu, 873, Bhutan. *Meconopsis integrifolia* (Maxim.) Franch. were cultivated in the Hakuba Goryu Alpine Botanical Garden, Nagano Pref., Japan.

2. Previous work

Flower colours of *Meconopsis* species are variegated from white, yellow, red, purple to blue. Of their flower colours, three purple and blue species, *Meconopsis horridula* Hook. f. & Thomson, *M. grandis* Prain and *M. betonicifolia* Franch. have been surveyed for pigments. Their *Meconopsis* species had the same anthocyanin and flavonol composition, i.e. cyanidin 3-O-(xylosyl-(1→2)-(6‴-malonylglycoside))-7-O-glucoside and cyanidin 3-O-sambubioside-7-O-glucoside as anthocyanins, and kaempferol 3-O-gentiobioside and kaempferol 3-O-glucosyl(1→6)-galactoside as flavonols (Takeda et al., 1996; Tanaka et al., 2001; Yoshida et al., 2006). Moreover, the presence of two metals, Fe and Mg, were also shown with their anthocyanins and flavonols in blue *M. grandis* flowers (Yoshida et al., 2006). Recently, cyanidin 3-O-glucoside, 3-O-sambubioside and 3-O-(succinylsambubioside) were reported from the red flowers of *Meconopsis wallchii* Hook in Bhutan without the presence of other flavonoids (Iwashina et al., 2018). On the other hand, a flavonol glycoside, herbacetin 3-O-glucoside, was reported from the flowers of *M. paniculata* as a yellow pigment (Harborne, 1969). Quercetin 3-O-gentiobioside, 3-O-{[2‴-acetylglucosyl]-(1→6)-glucoside}, 3-O-{[3‴-acetylglucosyl]-(1→6)-glucoside} and 3-O-{[6‴-acetylglucosyl]-(1→6)-glucoside} were isolated from the yellow flowers of *M. integrifolia* as medicinal resources (Huang et al., 2015). However, contribution to the flower colour was not referred. Harborne (1969) suggested that the yellow pigments of this species are carotenoids without chemical data.

3. Present study

Dry petals (ca. 30 g) and fresh petals (1.47 g) of *Meconopsis paniculata* and *M. integrifolia* were extracted with MeOH, respectively. The
concentrated extracts were applied to preparative paper chromatography using solvent systems, BAW (n-ButOH/HOAc/H2O = 4:1:5, upper phase) and then 15% HOAc. The isolated flavonoids were purified by Sephadex LH-20 column chromatography using solvent system, 70% MeOH. Flavonoids were obtained as pale yellow powders or pure MeOH solutions. Relative amounts (%) of the pigments in the flowers were calculated by peak area (420 nm) of HPLC chromatograms. Retention times (min) (L-column2 ODS column, Chemicals Evaluation and research Institute, Tokyo, flowrate of 1.0 min min−1, and eluent was MeCN/H2O/H3PO4 = 15:85:0.2) of the pigments are as follows. * M. paniculata: 1, 5.27, 2, 11.27, 3, 1555, and 4, 3829. M. integifolia: 5, 11.54, 6, 13.87, 7, 17.74 and 8, 20.94.

Yellow water-soluble pigment 1 from *M. paniculata* flowers had absorption maxima *λ*max 278, 306, 329sh and 371sh nm which are different with that of common flavonols such as quercetin and kaempferol (*Mabry et al., 1970*). However, colour (dark purple and dark yellow) under UV light (365 nm) before and after exposure to fuming ammonia were those of typical flavones or 3-substituted flavonols (*Mabry et al., 1970*). Since molecular ion peaks, *m/z* 465 [M + H]+ and 463 [M-H]-, and fragment ion peak, *m/z* 303 [M-162 + H]+ appeared by LC-MS survey of 1, it was shown that the original compound is pentahydroxyflavone monohexoside such as quercetin monoglucoside. An aglycone and a sugar were liberated by acid hydrolysis. Although a sugar was identified as glucose by PC comparison with authentic sample, retention time of aglycone on HPLC did not agree with that of authentic quercetin. Final estimation of 1 was performed by NMR survey, and the proton and carbon signals were assigned by 1H-1H COSY, 1H-1H NOESY, HSQC and HMBC. 1H NMR data of 1 showed the presence of five aromatic protons, i.e. H-2′, 6′ at δ 8.59 (dd), H-3′,5′ at δ 7.18 (dd) and H-6 or H-8 (s) at δ 6.90, and a glucosyl anomeric proton at δ 6.25 (d, *J* = 7.4 Hz) (Table 1). Since the aglycone was identified as 6-hydroxykaempferol but not herbacitin by HPLC comparison with authentic sample (ALB Technology Limited, Hong Kong), a signal at δ 6.90 was shown to be that of H-8. This was also proved by the HMBC correlation between H-8 proton and C-6, C-9 and C-10 carbon signals of 6-hydroxykaempferol at δ 126.9, 154.5 and 105.2 (Fig. 1). The attachment of a glucose to 3-position of 6-hydroxykaempferol was determined by the HMBC correlation between a glucosyl anomeric proton at δ 6.25 and C-3 carbon signal of 6-hydroxykaempferol at δ 134.9. Thus, 1 was identified as 3′-O-glucosides of 6-hydroxykaempferol (Fig. 1).

UV spectral properties of 2 were essentially the same with those of 1, presumed that this flavonoid was also 6-hydroxykaempferol 3-O-glucoside. Practically, an aglycone was liberated by acid hydrolysis of 2 together with glucose and identified as 6-hydroxykaempferol by HPLC comparison with authentic sample. However, since molecular ion peaks, *m/z* 627 [M + H]+ and 625 [M-H]-, and fragment ion peak, *m/z* 303 [M-162 + H]+ appeared on LC-MS of 2, it was shown that the original glucoside is 6-hydroxykaempferol diglucoside. The attachment of one glucose to 3-position of 6-hydroxykaempferol was determined by HMBC correlation between glucosyl anomeric proton at δ 6.25 and C-3 carbon signal of 6-hydroxykaempferol at δ 134.9. Thus, 1 was identified as 6-hydroxykaempferol 3-O-β-glucopyranoside (Fig. 1).

**Table 1**

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UV spectral properties of 3 were essentially the same with those of 2, presumed that this flavonoid was also 6-hydroxykaempferol 3-O-glucoside. Practically, an aglycone was liberated by acid hydrolysis of 2 together with glucose and identified as 6-hydroxykaempferol by HPLC comparison with authentic sample. However, since molecular ion peaks, *m/z* 627 [M + H]+ and 625 [M-H]-, and fragment ion peak, *m/z* 303 [M-162 + H]+ appeared on LC-MS of 2, it was shown that the original glucoside is 6-hydroxykaempferol diglucoside. The attachment of one glucose to 3-position of 6-hydroxykaempferol was determined by HMBC correlation between glucosyl anomeric proton at δ 6.25 and C-3 carbon signal of 6-hydroxykaempferol at δ 134.9. Thus, 1 was identified as 3′-O-glucosides of 6-hydroxykaempferol (Fig. 1). Observed important HMBC correlations are indicated by arrows (1H→13C).

Fig. 1. 6-Hydroxykaempferol 3-O-glucoside (1). Glycosides from *M. paniculata* flowers were identified as kaempferol 3-O-glucoside (3) and kaempferol 3-O-sophoroside (4) by UV, LC-MS, acid hydrolysis, and TLC and HPLC comparisons with authentic samples.

Four flavonoids (5–8) were isolated from the yellow flowers of *M. integifolia*. Of their compounds, 5 was identified as quercetin 3-O-gentiobioside by UV, LC-MS, acid hydrolysis, and TLC and HPLC comparison with authentic sample. Other three flavonols were characterized as quercetin 3-O-gentiobiosides which attached 1 mol acetate acid by UV spectral properties, appearance of the molecular ion peaks, *m/z* 669 [M + H]+ and 667 [M-H]-, and fragment ion peaks, *m/z* 625 [M-42-H]+, *m/z* 465 [M-204 + H]+ and *m/z* 303 [M-366 + H]+ by LC-MS, and acid hydrolysis.
4. Contribution to flower colour

Two major flavonol glycosides were isolated from the yellow flowers of Meconopsis paniculata, together with two minor flavonols, kaempferol 3-O-glucoside (3) and kaempferol 3-O-sophoroside (4). They were identified as 6-hydroxykaempferol 3-O-glucoside (1) and 6-hydroxykaempferol 3-O-sophoroside (2). Of their flavonoids, the latter compound was found in nature for the first time. Thus, it was shown that the yellow pigments of M. paniculata are 6-hydroxykaempferol 3-O-glucoside (88.7% of total pigments) and 3-O-sophoroside (11.3%), together with minor kaempferol 3-O-glucoside (trace) and 3-O-sophoroside (trace). Herbacetin (8-hydroxykaempferol) 3-O-glucoside has been isolated and identified by UV spectral survey, acid hydrolysis and H₂O₂ oxidation as a yellow pigment (Harborne, 1969). However, this glycoside was not detected in this survey. Instead, 6-hydroxykaempferol 3-O-glucoside was isolated from the yellow flowers of the same species in this survey, together with 6-hydroxykaempferol 3-O-sophoroside, and kaempferol 3-O-glucoside and 3-O-sophoroside (trace). Since the chemical properties of 6-hydroxykaempferol and 8-hydroxykaempferol are very similar to each other, either previous report (Harborne, 1969) might have been mistaken or chemical intraspecific variation occurred. Although Bhutanese individuals were used as plant materials in this survey, the collection site was not shown in the previous report (Harborne, 1969).

On the other hand, four flavonol glycosides, quercetin 3-O-gentiobioside (5) and three quercetin 3-O-(acytetylgentiobiosides) (6-8) were found in yellow M. integrifolia flowers. From this species, quercetin 3-O-gentiobioside, 3-O-[2"-acetylglucosyl](1→6)-glucoside, 3-O-[3"-acetylglucosyl](1→6)-glucoside and 3-O-[6"-acetylglucosyl](1→6)-glucoside have been isolated from the flowers as medicinal resources (Huang et al., 2015). However, contribution to flower colour was not referred. Although we isolated three quercetin 3-O-(acetylgentiobiosides), together with quercetin 3-O-gentiobioside, they may be the same flavonoids with those which were found by Huang et al. (2015). Harborne (1969) has described yellow pigments of M. integrifolia as carotenoids without any data. However, we could not prove the presence of carotenoids by intact visible absorption spectra of the flowers (data not shown). Thus, it was shown in this survey that yellow flower pigments of M. integrifolia were also flavonol glycosides, quercetin 3-O-gentiobioside (48.3% of total pigments) and its three acetates (6, 20.3%, 7, 5.7% and 8, 21.0%, respectively).

In yellow flowers of Papaveraceous species, some indole alkaloids such as nudicaulins I and II have been reported from Papaver nudicaule L. as yellow pigments (Schlieman et al., 2006; Tatsis and Schneider, 2008; Tatsis et al., 2013a, 2013b). In addition, it was recently proved that the yellow pigments from the yellow flowers of the Himalayan Cathcartia villosa Hook. f. ex Hook. are carotenoids. In this survey, it was shown that major yellow pigments of Meconopsis species were some flavonol glycosides.

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References