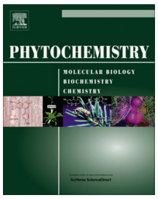
Phytochemistry 92 (2013) 105–112

Contents lists available at SciVerse ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem



Occurrence of nudicaulin structural variants in flowers of papaveraceous species

Evangelos C. Tatsis [a], Hartmut Böhm [b], Bernd Schneider [a,*]

[a] *Max Planck Institute for Chemical Ecology, Hans-Knöll-Straße 8, Beutenberg Campus, D-07745 Jena, Germany*
[b] *Friedrich-Wilhelm-Murnau-Straße 20, D-14480 Potsdam, Germany*

ARTICLE INFO

Article history:
Received 20 December 2012
Received in revised form 15 April 2013
Accepted 19 April 2013
Available online 14 May 2013

Keywords:
Meconopsis cambrica
Papaver alpinum
Papaver nudicaule
Anthocyanins
Flavonols
Flower pigments
Nudicaulins
HMG conjugates
Papaveraceae

ABSTRACT

The intense color of yellow *Papaver nudicaule* flowers is conferred by the presence of nudicaulins, a group of alkaloids with a unique pentacyclic skeleton composed of an indole ring and a polyphenolic moiety. Petals from eight different Papaveraceae species composed of different color varieties were probed for the presence of nudicaulins. In addition to their occurrence in yellow *P. nudicaule* flowers, nudicaulins **I**–**VIII** were detected and quantified in orange flowers of *P. nudicaule*, and in yellow and orange *Papaver alpinum* flowers. *Meconopsis cambrica* petals showed a divergent nudicaulin spectrum, with compounds having an attached 3-hydroxy-3-methyl-glutaryl group (HMG) instead of a malonyl unit at one of the glucose units. Flavonols and anthocyanins that accompany nudicaulins were identified. The taxonomical significance of the occurrence of nudicaulins is briefly discussed.

1. Introduction

Both the shape and the color of flowers, especially of petals, play a crucial role in how plants interact with their environment, specifically, in the chemical communication between plants and their pollinators. The color of petals usually is conferred by the presence of distinct chemical structures. Two major classes of flower pigments, anthocyanins and carotenoids, are responsible for the intense colors of most plants' flowers, while pigments such as betalains and aurones are restricted to a limited number of taxonomical units (Grotewold, 2006; Tanaka et al., 2008).

Nudicaulins, an elusive group of flower pigments, generate the color of the dark yellow petals of *Papaver nudicaule*. They were isolated for the first time by Price et al. (1939). Early trials to elucidate their structures led to the conclusion that nudicaulins did not belong to one of the known classes of flower pigments but seemed to be a new group of nitrogen-containing compounds (Harborne, 1965). Based on nuclear magnetic resonance (NMR) spectroscopic and mass spectrometric (MS) analyses, a pentacyclic indole alkaloid skeleton, decorated with a glucose and a sophorose unit, was proposed (Schliemann et al., 2006). Additional NMR experiments and chemical modifications resulted in a revised constitution of the nudicaulin aglycon, namely 12-(4-hydroxyphenyl)-3,11-dihydrobenzofuro[2′,3′:1]cyclopenta[1,2-*b*]indole-5,7,11-triol (Tatsis et al., 2013). Furthermore, the relative configuration of the two diastereomers, nudicaulin **I** and **II**, was substantiated by rotating-frame Overhauser effect spectroscopy (ROESY) and quantum-chemical calculations, and the absolute configuration was assigned by comparing the results of quantum-chemical circular-dichroism (CD) calculations and experimental CD spectra. From these data, nudicaulin **I** was assigned as the (3*S*,11*R*)-diastereomer and nudicaulin **II** as the (3*R*,11*S*)-diastereomer (Fig. 1), i.e. H-3 and glucose A are oriented to the same side of the aglycon skeleton (*cis* conformation) in each of the diastereomers. While the constitution of the aglycon and the carbohydrate units is not subject to variation in the nudicaulins known so far, acyl units attached to the sugars are the reason for some of the structural diversity of *P. nudicaule*'s typical flower pigments.

In the present study, the petals of four *P. nudicaule* color varieties, three *Papaver alpinum* color varieties, and six other Papaveraceae species were examined for the occurrence of nudicaulins; the structure of the nudicaulins discovered was elucidated.

2. Results

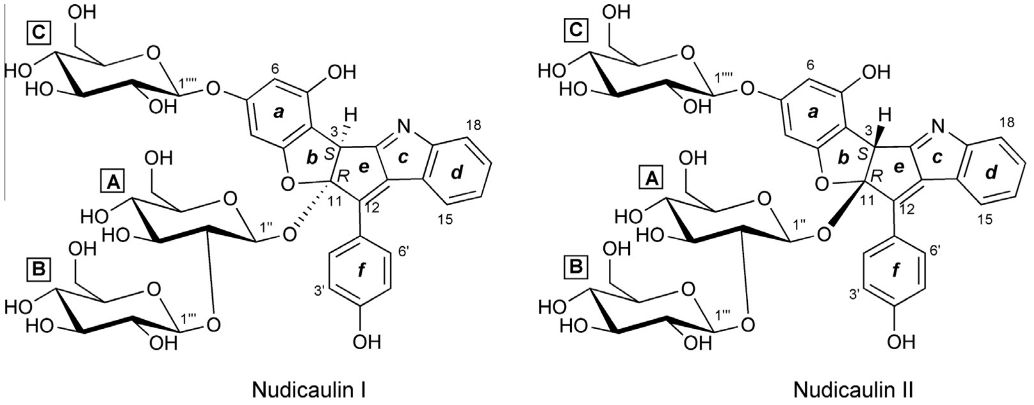
2.1. Identification of compounds

Papaveraceous plants were chosen according to taxonomical relationships, petal colors, and the availability of herbal material

---

* Corresponding author. Tel.: +49 3641 571600; fax: +49 3641 571601.
E-mail address: schneider@ice.mpg.de (B. Schneider).

0031-9422/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.phytochem.2013.04.011



Nudicaulin I

Nudicaulin II

Fig. 1. Structures of nudicaulins **I** and **II** (Tatsis et al., 2013).

or seeds. The analyzed plant species and varieties are listed in Table 1. The flavonoids and nudicaulins found in dark yellow petals of *P. nudicaule* were investigated in detail for their structures (Schliemann et al., 2006; Tatsis et al., 2013) and the present studies of respective plant parts follow the reported methods, briefly described in the experimental section. UV/VIS, NMR and MS data confirm a previous report, in which eight nudicaulins (nudicaulin **I**–**VIII**) and the eight flavonols (gossypitrin (**6**), kaempferol 3-*O*-β-sophoroside (**7**) and its isomer (**8**), kaempferol 3-*O*-β-sophoroside-7-*O*-β-glucoside (**1**), kaempferol 3-*O*-β-(sophoroside)-7-*O*-β-[(6-malonyl)glucoside] (**4**), kaempferol 3-*O*-β-[(6-malonyl)sophoroside]-7-*O*-β-glucoside (**2**) and kaempferol 3-*O*-β-[(6-malonyl)sophoroside]-7-*O*-β-[(6-malonyl)glucoside] (**5**) and its isomer (**3**)) were identified or partially characterized (Fig. 2; Schliemann et al., 2006). In the extract obtained from the orange petals of *P. nudicaule*, the anthocyanins pelargonidin 3-*O*-β-sophoroside-7-*O*-β-glucoside (**9**), pelargonidin 3-*O*-β-[(6-malonyl)-sophoroside]-7-*O*-β-glucoside (**10**) and pelargonidin 3-*O*-β-[(6-malonyl)-sophoroside]-7-*O*-β-[(6-malonyl)-glucoside] (**11**)] were found in addition to nudicaulins **I**–**VIII** and flavonols **1**–**8**. White petals from this species contain flavonols **1**–**8**, and red petals are colored by the pelargonidin derivatives, pelargonidin 3-*O*-β-sophoroside-7-*O*-β-glucoside (**9**), pelargonidin 3-*O*-β-sophoroside (**12**) and pelargonidin 3-*O*-β-[(6-malonyl)-sophoroside] (**13**) (Fig. 2) as reported earlier (Cornuz et al., 1981). Nudicaulins were not found in white and red petals. The spectroscopic data (UV/VIS, NMR, MS) of the isolated compounds are in full agreement with the published data.

P. alpinum was selected for the present study because this poppy is a close relative of *P. nudicaule* within the *Papaver* section *Meconella* Spach (Carolan et al., 2006). Like *P. nudicaule*, *P. alpinum* shows yellow and orange petals but no red pigmentation. Fabergé (1943) reported the occurrence of nudicaulin and pelargonidin glycosides for *P. alpinum* petals. Here, these plant parts were extracted, and their metabolites isolated and analyzed according to published protocols (Schliemann et al., 2006). The HPLC, UV, LC–MS and NMR analysis of extracts from yellow petals revealed the presence of nudicaulins **I**–**VIII** and flavonols **1**–**8**. In the extract obtained from orange petals, the anthocyanins pelargonidin 3-*O*-β-sophoroside-7-*O*-β-glucoside (**9**), pelargonidin 3-*O*-β-[(6-malonyl)-sophoroside]-7-*O*-β-glucoside (**10**) and pelargonidin 3-*O*-β-[(6-malonyl)-sophoroside]-7-*O*-β-[(6-malonyl)-glucoside] (**11**) were found, in addition to nudicaulins **I**–**VIII** and flavonols **1**–**8**. From the white petals of *P. alpinum* the flavonols **1**–**6** have been identified.

A number of other *Papaver* species, namely *P. pilosum*, *P. atlanticum*, *P. aculeatum*, *P. californicum* and *P. somniferum*, were selected for the present investigation in light of their different taxonomical relation to a member of the genus *Meconopsis*, *M. cambrica* (Carolan et al., 2006). However, nudicaulins were not found in the petals of all these *Papaver* species. Instead, the color of the petals seems to be dominated by anthocyanins (data not shown). After the lack of nudicaulins was proven unequivocally, the respective experiments were finished.

Price et al. (1939) reported the occurrence of nudicaulins in the yellow flowers of *Meconopsis cambrica*. This was confirmed by chromatographic comparison and UV/VIS spectroscopy with samples from *P. nudicaule* (Böhm, 1984, unpublished). However, the structural details of the nudicaulins in *M. cambrica* are still unknown. Investigation of flower extracts by HPLC analysis at 460 nm, a wavelength characteristic for nudicaulins, revealed the presence of five major peaks (Fig. 3). On the basis of their UV/VIS spectra, retention times, and mass spectra, nudicaulins **I**, **II**, **V** and **VI** have been identified. No HPLC peaks at R_t 19.1 and 19.3 min of molecular ion masses of *m*/*z* 958 were found, suggesting that the monomalonylated nudicaulins **III** and **IV** do not occur in *M. cambrica*. The same applies to dimalonylated nudicaulins **VII** (R_t 22.0 min, *m*/*z* 1044) and **VIII** (R_t 22.6 min, *m*/*z* 1044). Nudicaulins **III** and **IV**, where the malonyl unit is attached to 6-OH of glucose A, and nudicaulins **V** and **VI**, where the malonyl unit is attached to 6-OH of glucose C (Fig. 2), were not only distinguished by their retention times (Fig. 3) but also the MS/MS spectra of their molecular cations (Schliemann et al., 2006).

The absorption maxima at 214, 258, 335, and 458 nm in the UV/VIS spectra of the HPLC peak eluted at 21.2 min suggested the presence of nudicaulin in a *M. cambrica* flower extract (Fig. 3). This chromatographic peak showed a molecular ion mass of *m*/*z* 1016 and mass fragments at *m*/*z* 854, 692, and 386. The cation of *m*/*z* 386 corresponds to the mass of nudicaulin aglycon, while the cations with masses of *m*/*z* 854 and 692 indicate the loss of one and two glucose units from the molecular cation of *m*/*z* 1016, respectively. The MS data point to the presence of a nudicaulin derivative, probably one with the same glucosidic moiety as nudicaulins **I**–**VIII**. Given the difference of molecular mass ion *m*/*z* 1016 with the molecular ion of non-acylated nudicaulins **I** and **II** *m*/*z* 872, and taking into account the fact that water is eliminated during esterification, the conjugation of an acyl unit with the elemental composition $C_6H_9O_4$ was concluded to have taken place. The fraction eluted at R_t 21.2 was further separated by preparative HPLC and measured by NMR. The ^{1}H NMR spectrum shows two sets of analogous signals with minor chemical shift differences between them, suggesting the presence of two co-eluting compounds, namely **IX** and **X**, of the nudicaulin type. In the aglycon parts of the nudicaulin molecules, the only major chemical shift difference was observed for the signals of the protons (H-3) attached to the chiral center at C-3, appearing at δ 5.80 and 5.63. Similar chemical shift differences have been observed for diastereomeric pairs of nudicaulins (Schliemann et al., 2006; Tatsis et al., 2013). Therefore it was concluded that compounds **IX** and **X** are diastereomeric counterparts. Since the concentrations of the two co-eluted