Biotransformation of Safflower Yellow B to Carthamin, A Coloring Matter of Safflower

Jun-ichi Onodera,* Ken-ichi Kawamoto, Shigeru Matsuba, Shingo Sato, Hironori Kojima, Yuichi Kaneya, and Heitaro Obara
Department of Materials Science and Engineering, Faculty of Engineering, Yamagata University, Jonan, Yonezawa, Yamagata 992

(Received June 21, 1995)

Safflower yellow B, one of the yellow coloring components of safflower (*Carthamus tinctorius* L.), was transformed to red carthamin and safflomin-A in cultured safflower cells. This result suggests that carthamin and other yellow coloring matters are biogenetically derived from safflower yellow B in the florets of safflower.

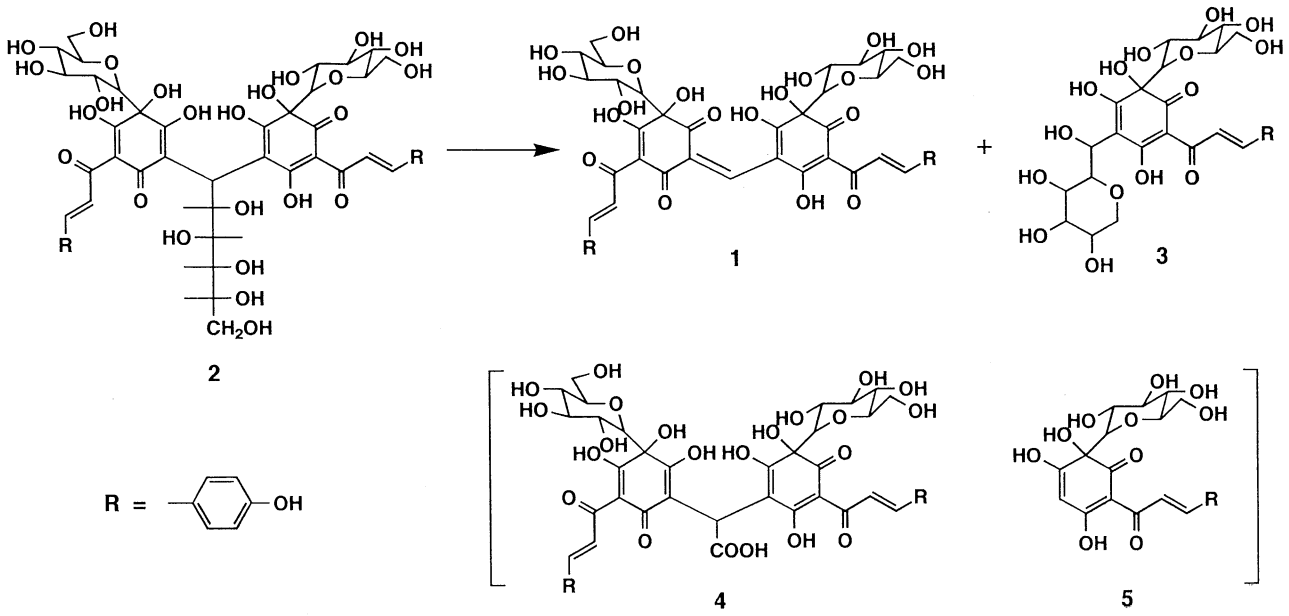
In our previous paper,[1,2] we elucidated the structure of the unstable yellow precursor of carthamin (**4**) on the basis of its spectral data and a comparison of its spectral and chemical properties with those of synthetic model compounds. The elucidated planner structure of this precursor suggested that it was biogenetically derived from safflower yellow B (**2**)[3] in the florets of safflower. In this paper, we wish to report on the biotransformation of **2** with cultured safflower cells to prove the above hypothesis.

Safflower yellow B (**2**) was extracted from fresh safflower florets and purified by successive chromatography with polyamide, silica gel, Toyopearl gel and Sephadex LH-20 columns. The HPLC analysis of the resulting safflower yellow B showed its purity of over 99.8 %.[4]

The callus of safflower was induced from seeds of cultivar Mogami on a Murashige-Skoog (MS) medium[5] containing 30 g/l sucrose, 2 g/l Gellangum, 1 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.5 mg/l benzyladenine (BA) and cultured for 1 month at 25 °C. Proliferating callus cultures were then routinely transferred to a fresh MS medium (pH 5.7) containing 0.2 mg/l 2,4-D and 1 mg/l BA every 4 weeks under a 16 h-light/8 h-dark cycle. Biotransformation of **2** under various conditions using the callus cultured on the above eutrophic medium gave no definite pigments. It is well-known that the transformation of the callus to an oligotrophic medium sometimes activates the enzymes concerned in the secondary metabolism. Therefore, the above callus was transferred to the MS medium which lacks NH_4NO_3 and KH_2PO_4 and contains 1.5 mg/l indole acetic acid and 2.4 g/l Gellangum.[6] After 3 days, 390 mg of powdered **2** was sprinkled on the surface of this callus (ca. 50g fr. wt). The colorless callus turned yellow, absorbed **2** and then gradually changed to red. After 5 days this callus was extracted with MeOH. Chromatography of the extracts with Sephadex LH-20 column showed a large amount of unreacted **2** and a small amount of safflomin-A (**3**) and carthamin (**1**).[7] Identification of these products were carried out by IR, electoronic, mass spectra[8] and retention times on HPLC. The transformation of **2** to carthamin may proceed via **4**, but its formation was not detected. Since **4** was quantitatively converted to carthamin by a cell-free homogenate of safflower callus,[2] the **4** formed is thought to be rapidly converted to **1** in the present culture system. Further, the treatment of a mixture of yellow coloring components of safflower, other than **2**, gave no carthamin under the conditions mentioned above.

This experiment confirmed the formation of **1** and **3**[9] from **2**, but the low yields of both compounds suggested the possibility that the stimulus of administrated **2** caused the callus to form **1** and **3**. Therefore, the transformation of **2** by the crude enzyme of safflower callus was then investigated.

Cell-free enzymes were extracted from freeze-dried safflower callus (1.5 g) cultured on an oligotrophic medium with phosphate buffer (pH 6.8). This extract was centrifuged at 15000 g for 15



Scheme 1.

min and the supernatant was fractionated with solid ammonium sulfate. The fraction precipitated between 40 and 90 % ammonium sulfate was collected and this crude enzyme was then desalted using Molecut L-LCC (size 5000, Millipore Co Ltd.). Components smaller than molecular weight around 5000 were thereby removed. Thirty mg of **2** was added to the solution of this crude enzyme in 6 ml phosphate buffer (pH 6.8), and the solution was allowed to stand at room temperature for 3 days.

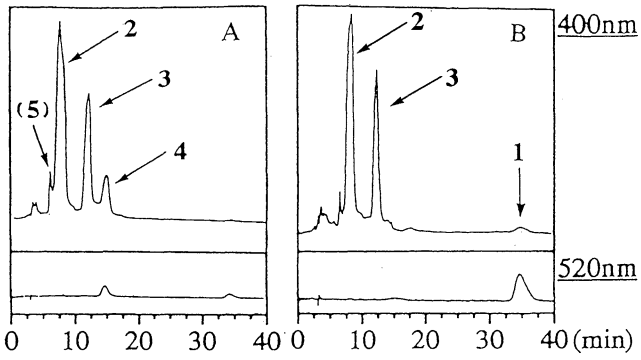
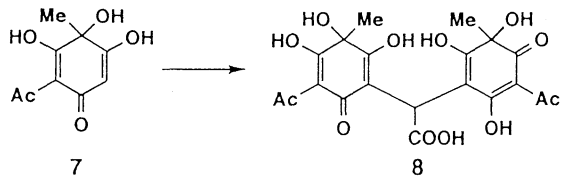


Figure 1. HPLC chromatograms of the reaction mixture of **2** with crude enzyme. A: After addition of glyoxylic acid. B: After addition of a cell-free homogenate to A. Column: ODS (4.6×250 mm). Eluent: MeOH-H2O-AcOH (70-30-0.5) 1 ml/min.

The HPLC analysis of this reaction mixture showed the existence of about 9 mg of **3**, a very small amount of **1**, 10 mg of unreacted **2** and a small amount of an unknown yellow product, however, the existence of **4** was not detected by both chemical and HPLC analyses. The unknown yellow product could not be purified because of its instability. Recently, Meselhy *et al.* reported the formation of compound **5** by the metabolism of **2** by human intestinal bacteria.[11] The retention time, electronic spectrum and the instability of this yellow product suggested that it is identical with compound **5**. Further, it was confirmed that the treatment of compound **7**,[2] a model compound of **5**, with glyoxylic acid in the above crude enzyme system afforded precursor-type compound **8** in a good yield. From these results it was thought that the reaction of compound **5** with glyoxylic acid affords **4** and the compound **4** was not formed in the mixuture of the above crude enzyme reaction because of the lacking of glyoxylic acid. Therefore, 5 mg of glyoxylic acid was added to the above reaction mixture. After 10 min the peak of the unstable yellow product decreased and a new peak appeared at the retention time of 15 min (Figure 1). The retention time on HPLC and the electronic and IR spectra of this product were identical with those of authentic **4**. As has been described above, **4** was immediately transformed to **1** by the addition of a cell-free homogenate of safflower callus which contains various low-molecular weight components such as co-enzymes (Figure 1). The electronic, IR and mass spectra of the resulting **1** were identical with those of authentic **1**. The yields of **4** and **1** estimated by HPLC analysis were about 2.2 and 2.0 mg, respectively. The structure of the unstable yellow product formed by the crude enzyme reaction has not yet been confirmed, however, the above results supported the assumption that this product is identical with compound **5**. Further, the above results also suggested that the transformation of **2** to **1** in the safflower cell may proceed by way of compound **5**.

From all of the above results, it was confirmed that **1** and **3**



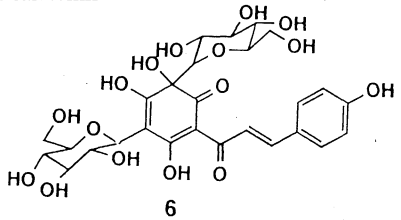
7 8

were biogenetically derived from **2** in the safflower cell. The mechanism of this transformation is now under investigation.

We are grateful to Mr. Yasuo Goto for his helpful supply of safflower.

References and Notes

1 T. Kumazawa, S. Sato, D. Kanenari, A. Kunimatsu, R. Hirose, H. Obara, M. Suzuki, M. Sato, and J. Onodera, *Chem. Lett.*, **1994**, 2343.
2 T. Kumazawa, Y. Amano, T. Haga, S. Matsuba, S. Sato, K. Kawamoto, and J. Onodera, *Chem. Lett.*, **1995**, in press.
3 Y. Takahashi, K. Saito, M. Yanagiya, M. Ikura, K. Hikichi, T. Matsumoto, and M. Wada, *Tetrahedron Lett.*, **25**, 2471 (1984).
4 Neither carthamin (**1**) nor safflomin-A (**3**) was detected in **2** thus obtained.
5 T. Murashige and F. Skoog, *Physiol. Plant.*, **15**, 473 (1962).
6 The callus cultured on this oligotrophic medium without **2** gave neither **1** nor yellow coloring components of safflower.
7 The yields of carthamin and safflomin-A were 0.6 and 2.0 mg, respectively.
8 FAB-MS (*m*-NBA): carthamin positive 933 (M+Na), negative 909 (M-H); safflomin-A positive 613 (M+H), negative 611 (M-H).
9 J. Onodera, H. Obara, M. Osone, Y. Maruyama, and S. Sato, *Chem. Lett.*, **1981**, 433. Recently, Goda[10] proposed the structure **6** for safflomin-A.



6

10 Y. Goda, J. Suzuki, T. Kometani, and T. Yamada, 114th Annual Meeting of Pharmaceutical Society of Japan, Tokyo, **1994**,Abstr.,197.
11 M. R. Meselhy, S. Kadota, M. Hattori, and T. Namba, *J. Natural Products*, **56**, 39 (1993).