Synthesis of Model Compounds of the Precursor of Carthamin, a Colouring Matter of Safflower, and Their Conversion into Carthamin-Type Compounds

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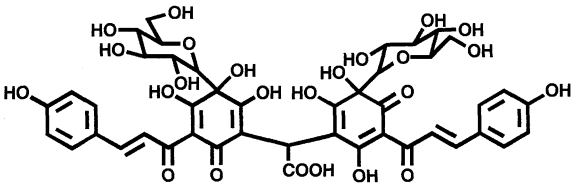
Model compounds of the precursor of carthamin **3**, **4**, and **5** were prepared. And in analogy with the precursor of carthamin (PRE) **1**, they were converted into carthamin-type compounds **13**, **14**, and **15**, respectively, when sprayed with peroxidase-H_2O_2 solution. It was also found that these conversions proceeded quantitatively by treatment with phosphate buffer extract of a cultured safflower cell and this conversion reaction proceeded by oxidative decarboxylation.

It has been thought that carthamin **2**[1], a red colouring matter Safflower flowers (*Carthamus tinctorius* L.) may be formed via an unstable yellow precursor (PRE) in the florets of the safflower.[2] In our previous communication, we extracted this unstable precursor and proposed the structure **1** for it, mainly on the basis of its spectral data.[3] But the NMR spectral data of PRE were insufficient to elucidate its structure, because of the existence of keto-enol tautomerism in its molecule. This structure **1** was supported by comparison of its spectral and chemical evidences with those of the model compound **4**.

In this communication, we wish to report the synthesis and properties of **4** and two more analogous compounds **3** and **5** of PRE to comfirm the structure **1**.



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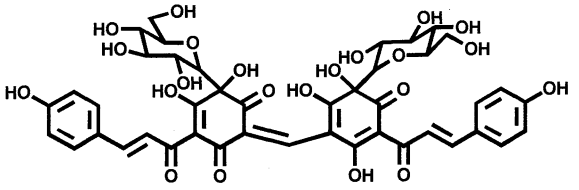

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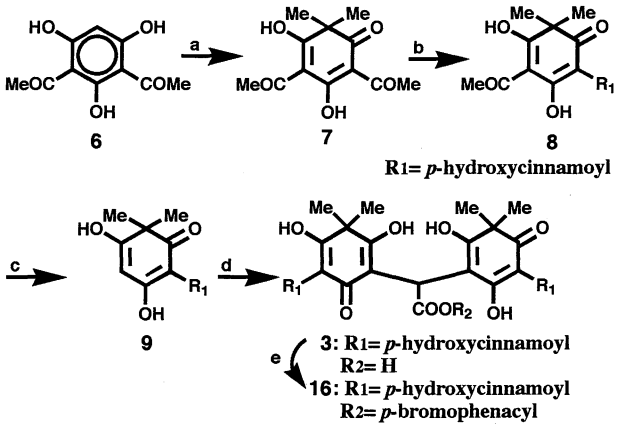
Figure 1.

Model compound **3** was synthesized by the following method. Methylation of **6**[4] with methyl iodide in the presence of sodium methoxide in methanol at room temperature afforded **7**[4] in 78% yield. Aldol condensation of **7** with *p*-hydroxybenzaldehyde in piperidine gave **8** in 60% yield, and deacetylation of **8** with 10% Na_2CO_3aq. afforded **9**[5] in 36% yield. The reaction of **9** with glyoxylic acid monohydrate in 0.5% NaOHaq. gave **3**[6] in 61% yield (Scheme 1).

As shown in Scheme 2, deacetylation of **7** with 80% H_2SO_4 afforded **10**[4] in 72% yield. The reaction of **10** with glyoxylic acid under the conditions described above gave **4**[7] in 53% yield (Scheme 2).

Finally, model compound **5** was synthesized by the following method. Methylation of **11**[8] with methyl iodide in the presence of sodium hydride in DMSO under N_2 afforded **12**[9] in 52% yield. In a similar manner described above, a reaction of **12** with glyoxylic acid monohydrate gave **5**[10] in 52% yield (Scheme 3).

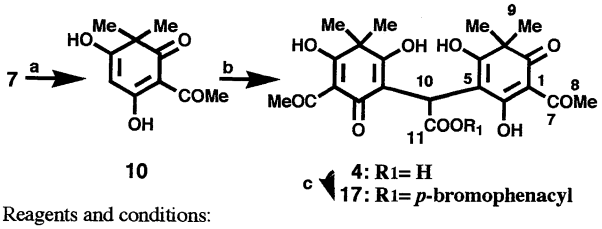
The structures of the above synthetic model compounds were determined by IR, MS and NMR spectral analysis. In analogy with PRE, the NMR spectra of these model compounds were very complex, accompanying many signals of their tautomers. However this complexity was not improved by measurements in various solvents. Treatment of the aqueous solution of PRE with peroxidase (horseradish)-H_2O_2 solution gave red carthamin **2**. Similarly, three model compounds **3**, **4**, and **5** which adhered to filter paper also gave carthamin-type compounds **13**[5], **14**, and **15**[9], respectively, when sprayed with peroxidase-H_2O_2 solution. However, the conversion ratio of this reaction was too low to elucidate the conversion mechanism. Among several oxidizing



R1= *p*-hydroxycinnamoyl

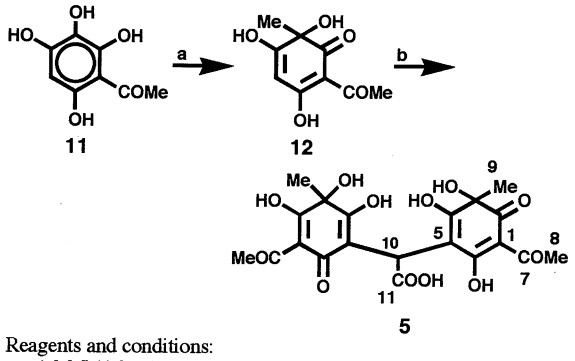
Reagents and conditions:
a) MeI (6.0eq.), NaOMe (5.0eq.), MeOH, 2d, 78%;
b) *p*-Hydroxybenzaldehyde (1.1eq.), piperidine, 50 °C, 3h, 60%;
c) 10%NaOHaq., 90 °C, 4h, 36%;
d) OHCCO2H-H2O (1.6eq.), 0.5% NaOHaq., 0 °C, 15min, 61%.
e) *p*-Bromophenacyl bromide (2.0eq.), NaH (4.0eq.), DMSO, 27%

Scheme 1.



Reagents and conditions:
a) 80%H2SO4, 80 °C, 15min, 72%;
b) OHCCO2H-H2O (1.6eq.), 0.5% NaOHaq., 0 °C, 15min, 53%.
c) *p*-Bromophenacyl bromide (1.3eq.), NaH (1.6eq.), DMSO, 72%

Scheme 2.



Reagents and conditions:
a) MeI (4.0eq.), NaH (2.0eq.), DMSO, N2, 15min, 72%;
b) OHCCO2H-H2O (5.0eq.), 0.5% NaOHaq., 0 °C , 5min, 52%.

Scheme 3.

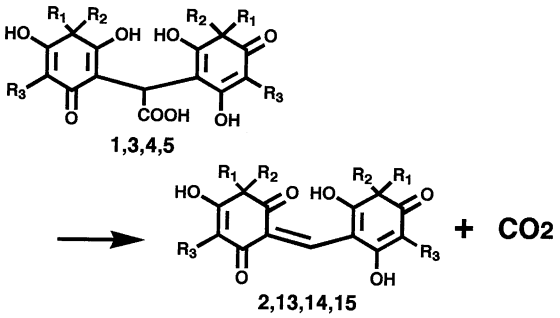


Figure 2.

reagents and enzymes, the phosphate buffer extract of a cultured safflower cell gave an excellent result. The safflower cultivar used in this experiment was "Mogami". Safflower callus was induced from its seed on MS medium containing 30g/l sucrose, 2g/l Gellungum, 1mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.5mg/l benzyladenine (BA) and cultured for 30 days at 25 °C. Proliferating callus cultures were then routinely transferred to fresh MS medium containing 0.2mg/l 2,4-D and 1mg/l BA every 4 weeks under a 16h-light/8h-dark cycle. Treatment of PRE with the homogenate of Safflower callus in disodium hydrogenphosphate buffer (pH8.2) gave red carthamin quantitatively. This reaction proceeded immediately with the evolution of gas and the three model compounds **3**, **4**, and **5** were also converted quantitatively to carthamin-type compounds, respectively. The gas evolved in this reaction was characterized as CO_2 by the formation of calcium carbonate when introduced to aqueous calcium hydroxide solution. On the other hand, formic acid was not detected in this reaction mixture by HPLC analysis. Further, treatment of formic acid, *p*-bromophenacyl esters of **3** and **4** (**16**[11] and **17**[11]) with the above phosphate buffer extract gave no evolution of CO_2.

The NMR spectral patterns of the above synthetic model compounds and their chemical and enzymatical conversion to carthamin-type compounds were very analogous to those of PRE. These results strongly support the proposed structure **1** for PRE. Furthermore, it has become apparent that the conversion of PRE to carthamin proceeds by oxidative decarboxylation with oxidative decarboxylase in the florets of safflower. Purification and identification of its enzyme is now in progress.

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References and Notes

1 H. Obara and J. Onodera, *Chem. Lett.*, **1979**, 201; J. Onodera, T. Saito, and H. Obara, *Chem. Lett.*, **1979**, 1327.
2 M. Wada, *Proc.Japan Acad.*, **29**, 218, 351 (1953); M.Shimokoriyama and S.Hattori, *Arch. Biochem. Biophy.*, **54**, 93 (1954).
3 36th Symposium on the Chemistry of Natural Products, Hiroshima, 1994, Abstr., 665; T. Kumazawa, S. Sato, D. Kanenari, A. Kunimatsu, R. Hirose, S. Matsuba, H.Obara M. Suzuki, M. Sato, and J. Onodera, *Chem. Lett.*, **1994**, 2343; 36th Symposium on the Chemistry of Natural Products, Hiroshima, 1994, Abstr., 619.
4 T. Meikle and R. Stevens, *J. Chem. Soc., Perkin Trans. 1*, **1978**, 1303.
5 H. Obara, J. Onodera, and F. Shirasaki, *Chem.Lett.*, **1980**, 1095: compound **9** was previously synthesized by a route different from that employed in the present case.
6 **3**: mp 195 °C (dec.); FAB-MS m/z 657 (M+H)+; ^{1}H-NMR (DMSO-d6) δ = 1.25 and 1.31 (12H, each s, -Me×4), 4.41 (1H, s, =CH-), 6.94 and 7.54 (each 4H, d, J = 9.0 Hz, -Ar × 2), 7.99 (4H, s, trans -CH=CH-×2), 19.01 (br., chelated -OH)
7 **4**: mp 102-105 °C (dec.); FAB-MS m/z 449 (M+H)+; ^{1}H-NMR (CDCl3) δ = 1.41 and 1.47 (12H, each s , -Me ×4), 2.55, 2.60, 2.70, and 2.75 (6H, each s, -COMe ×2), 5.13 (1H, s, =CH-), 18.44 (2H, m, chelated-OH).; ^{13}C-NMR (CDCl3) δ = 24.3, 25.1 (C-9a, 9b), 29.0 (C-8), 35.5, 36.3, 36.8 (C-10), 44.5, 45.1 (C-3), 106.7, 108.3, 109.2, 110.1 (C-1, 5), 174.6, 175.1 (C-11), 186.8 (C-4, 6), 198.9 (C-2), 202.9, 203.1 (C-7).
8 H. Obara, J. Onodera, Y. Kurihara, and F. Yamamoto, *Bull. Chem. Soc. Jpn.*, **51**, 3627 (1978).
9 Presented at the 66th Annual Meetings of the Chemical Society of Japan, Nishinomiya, 1993, Abstr., 51.
10 **5**: Amorphous pale yellow powder; FAB-MS m/z 453 (M+H)+; ^{13}C-NMR (CDCl3) δ = 25.86, 25.95 (C-9), 26.86, 26.99 (C-8), 28. 2, 28.6 (C-10), 74.4, 77.1, 79.1 (C-3), 99.6, 100.5, 102.3, 102.4, 102.5 (C-5), 105.4, 105.6, 109.4, 109.8 (C-1), 165.0, 165.2 (C-11), 187.5, 187.66, 187.75 (C-4), 193.1, 194.1, 194.3, 194.4 (C-6), 198.5, 198.6, 198.8, 199.2 (C-2), 201.56, 201.69, 201.72, 201.81, 201.87, 201.96 (C-7).
11 **16** and **17** were prepared from the corresponding **3** and **4** in the presence of sodium hydride with *p*-bromophenacyl bromide in DMSO as shown in schemes, respectively.