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Precursor of Carthamin, A Constituent of Safflower

Toshihiro KUMAZAWA, Shingo SATO, Daisuke KANENARI, Akira KUNIMATSU, Ryoji HIROSE, Shigeru MATSUBA, Heitaro OBARA, Masanobu SUZUKI, † Masaya SATO, † and Jun-ichi ONODERA*

Department of Materials Science and Engineering, Faculty of Engineering, Yamagata University, Jonan, Yonezawa 992

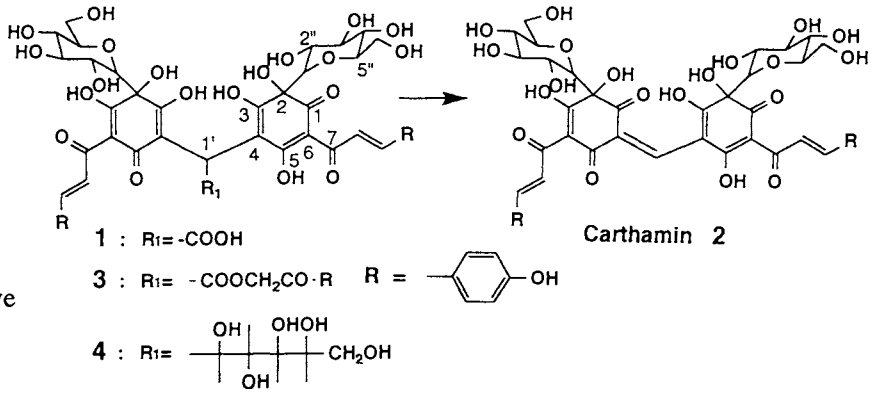
† Research Laboratories Pharmaceutical Div., Nippon Kayaku Co. Ltd., Shimo, Tokyo 115

Precursor of Carthamin was isolated from the flowers of *Carthamus tinctorius* L. (Safflower) and its structure was characterized as **1** on the basis of spectroscopic analysis and by comparing its properties with those of synthetic model compound.

Carthamin (**2**)[1] is a glycosidic red coloring matter found in Safflower. Safflower which is yellow after just having opened changes to red within few days. The homoginate of fresh yellow petals also immediately turns red, forming red carthamin. From these behaviors, it has been thought that red carthamin arises from an unstable yellow precursor(PRE),[2] however, the structure of PRE has not been elucidated because of its instability. In this paper, we wish to report on the isolation and structure elucidation of the precursor of carthamin.

Fresh Safflower petals(10 kg) were extracted with MeOH at room temp. for 7 days and concentrated in vacuo. Repeated column chromatography on polyamide, silica gel, Sephadex LH-20 and Toyopearl gel of water soluble parts of the extract gave PRE as micro needles(100 mg).

PRE, mp >300 ℃, gradually changed to red when exposured to air and rapidly changed to red carthamin when treated with peroxidase(horse radish)-H_2O_2 solution. In analogy with other coloring matters of Safflower,[3] PRE afforded glucose and *p*-hydroxybenzaldehyde by acid hydrolysis and alkali-degradation, respectively. Treatment with diazomethane in methanol-ethyl acetate, acetic anhydride in pyridine, resulted in no definite product. Whereas, treatment with *p*-bromophenacyl bromide and NaH in DMSO gave the corresponding *p*-hydroxyphenacyl ester (**3**) in good yield. These results suggested the existence of carboxyl moiety in its molecule.



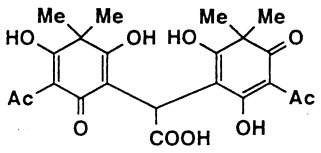
1 : R_1=-COOH

3 : R_1= -$COOCH_2CO$-R R =

4 : R_1=

The electronic spectrum of PRE exhibited two absorption maxima at 423(ε =36900) and 343(ε =17600) nm and was very similar to those of other yellow coloring matters, especially Safflower yellow B(**4**).[3] The negative FAB-MS (glycerol-thioglycerol, Xe) and negative SIMS (glycerol, Xe) exhibited a quasi-molecular ion peak at m/z 955 $[M-H]^-$. The positive FAB-MS (glycerol- thioglycerol, Xe)

exhibited peaks due to $[M+Na]^+$ and $[M+K]^+$ at m/z 979 and 995, respectively. This result suggests that the molecular weight of PRE is 956 and that it exists as a stable salt of K and Na. Although this compound is analytically pure, the signals of both ^1H- and ^{13}C -NMR spectra in various solvents were complex, acompanying several minor signals. These features suggested the presence of keto-enol tautomerism in PRE.



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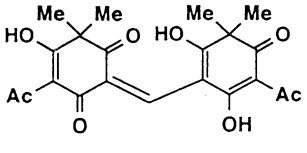

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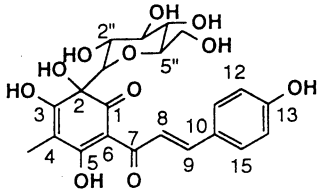

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The 400 MHz ^1H-NMR spectrum (DMSO-d6) of PRE exhibited signals of two *p*-hydroxycinnamoyl moieties at δ 6.85, 7.51 (each 4H, d, J=7.8 Hz), 7.40, 7.53 (each 2H, d, J=16.1 Hz) and signals corresponding to two glucosyl moieties between 2.8-4.0 (ca. 14H, m) ppm. Moreover, the characteristic chelated enol proton for these type of compounds[1] was observed at 18.84 (2H, br.s) ppm. The comparison of 100 MHz ^{13}C-NMR spectrum[4] of PRE with those of other yellow coloring matters showed the existence of skeleton **5** in its molecule. Except for the above skeletal signals, a signal of quaternary carbon, corresponding to carboxyl carbon, was observed at 173.4(br. s) ppm and one of methine carbon at 40.6 (d) ppm. The ^1H-^1H-cosy spectrum of PRE exhibited a methine proton which has no correlation with other protons at 5.42 (1H, m) ppm. These results suggested the existence of >CH-COOH moiety in the PRE molecule. Because it was difficult to get further information from NMR spectrum because of the keto-enol tautomerism of PRE, we synthesized the model compound **6**.[5] In analogy with PRE, the NMR spectrum of **6** was complex and **6** was rapidly converted to carthamin type compound **7** when sprayed with peroxidase-H_2O_2 solution.

Based on all of the foregoing findings, the structure of PRE was determined to be **1** and **1** may be converted to cartamin by oxidative decarboxylation. The mechanism of this conversion is now under investigation.

References

1) H. Obara and J. Onodera, *Chem. Lett.*, **1979**, 201; J. Onodera, T. Saito, and H. Obara, *ibid.*, **1979**,1327.

2) M. Wada, *Proc. Japan Acad.*, **29**, 218, 351 (1953); M. Shimokoriyama and S. Hattori, *Arch. Biochem. Biophy.*, **54**, 93 (1954).

3)The main coloring matters of Safflower which has been reported are carthamin,[1] Safflomin-A,[a] Safflower yellow B,[b] Safflomin-C.[c] a) J. Onodera, *Chem. Lett.*, **1981**, 887; Y. Takahashi,*Tetrahedron Lett.*, **23**, 5163 (1983) ; M. R. Meselhy, *J. Nat. Prod.*, **56**, 39 (1993). b) Y. Takahashi, *Tetrahedron Lett.*, **25**, 2471 (1984). c) J. Onodera, *Chem. Lett.*, **1989**, 1571; H. Obara, *Bull. Chem. Soc. Jpn.*,**64**, 309 (1991).

4) ^{13}C-NMR of PRE (100MHz, CD_3OD-D_2O 4:1) : δ (ppm) 193.8, 193.7(C-1, s), 82.6, 82.2(C-2, s), 178.1, 178.0(C-3,7, s), 188.0(C-5, s), 107.7,107.0,106.2,106.0(C-4,6, s), 120.2, 120.0(C-8,d), 139.4, 138.7(C-9, d), 126.5, 126.4(C-10, s), 130.0(C-11,15, d), 115.6(C-12,14, d), 161.1(C-13, s), 173.6(-COOH, s), 40.6(C-1'), 86.2, 85.3(C-1", d), 69.5, 69.2(C-2", d), 68.4, 68.7(C-4", d), 78.1(C-3", d), 79.5(C-5", d), 59.8, 60.4(C-6", d).

5) Compound **6** was synthesized by the reaction of 3-acetylfilicinic acid with glyoxilic acid. Mp 103-105 °C, FAB-MS m/z 449 $[M+H]^+$. The details will be published elsewhere.

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