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STRUCTURE OF CARTHAMIN

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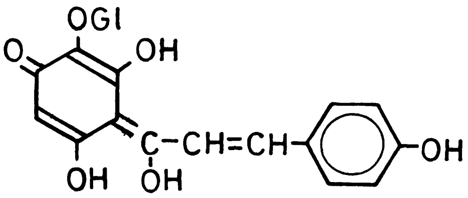
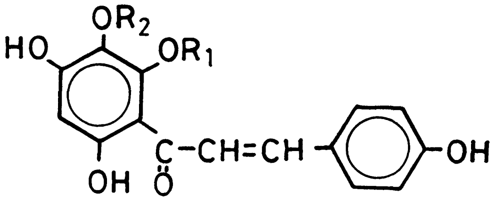
The structure 6, 1-(2,6-diketo-3-glucosyl-3,4-dihydroxy-5-p-hydroxycinnamoyl)-cyclohex-4-enylidene-1'-(2',3',4'-trihydroxy-3'-glucosyl-5'-p-hydroxycinnamoyl-6'-keto)-cyclohexa-1',4'-dienylmethane, was assigned for carthamin on the basis of its spectroscopic evidence and hydrolytic behavior.

In a previous paper,[1)] we have reported that the old structure (1, 2, or 3)[2,3)] of carthamin, the red coloring matter of the flowers of Safflower (Carthamus tinctorius L.), should be subjected to reexamination from the comparison of its properties with those of two synthetic analogs (4 and 5). In this communication, we wish to propose the structure 6 for carthamin from the comparison of its PMR and ^{13}C-NMR spectra with those of the synthetic analogs and hydrolytic behavior.



(1)



(2) R_1=H, R_2=Gl (3) R_1=Gl, R_2=H

(4) R_1=H, R_2=Me (5) R_1=Me, R_2=H

Because of the satisfactory PMR spectrum of the unstable carthamin[4)] was not obtained, the stable calcium salt of carthamin was used in the measurement of PMR spectrum. The chemical shifts and coupling constants of carthamin, 4, and 5 are listed in Table 1. In the PMR spectrum of carthamin, the signals of p-substituted phenyl protons (J=8.5Hz) and trans-vinylene protons (J=16.0Hz) due to the p-hydroxycinnamoyl group were observed in the aromatic region. However, in spite of the signals of 5'-proton in 4 or 5 were observed at 6.00 and 6.15 ppm, respectively, no signal could be found in the same region in carthamin. On the other hand, a signal corresponding to one proton against twelve protons of two p-substituted cinnamoyl groups was found at 8.42 ppm in carthamin. This signal did not disappear after the D_2O addition. In cooperation with the peak area of this signal and the lacking of the signal of 5'-position in carthamin, it is assumed that this signal must be the proton of one methine group linking the two polyhydroxychalcone groups. Further, a characteristic enol proton signal was observed in the very low field at 19.0 ppm in carthamin. Such low values have been found in ceroptene,5) usnic acid,6) and humulone,7) which have a enolized β-triketone group in their molecules. Since such a low field proton signal cannot be regarded in the phenolic aromatic chelated compounds as 4 or 5, it is thought that the two groups which are linked with one methine group are not necessarily the polyhydroxychalcone groups. From these results, it is assumed that carthamin has two enolized β-triketone-, two p-hydroxycinnamoyl-, one methine-, and glucosyl-group as the partial structures.

Table 1. Chemical Shifts (δ) and Coupling Constants (Hz) of Carthamin (Ca salt), 3'-Methoxy-2',4,4',6'- and 2'-Methoxy-3',4,4',6'-tetrahydroxychalcone (4 and 5).a)

Carthamin (Ca salt)b)	4c)	5b)
2.80-4.80 (m, glucosyl)	3.78 (3H, s, -OMe)	3.76 (3H, s, -OMe)
——	6.00 (1H, s, $C_{5'}$-H)	6.15 (1H, s, $C_{5'}$-H)
6.87 (4H, d, J=8.5) 7.60 (4H, d, J=8.5)	6.92 (2H,d,J=8.5,$C_{3,5}$-H) 7.57 (2H,d,J=8.5,$C_{2,6}$-H)	6.86 (2H,d,J=8.5,$C_{3,5}$-H) 7.57 (2H,d,J=8.5,$C_{2,6}$-H)
7.46 (2H, d, J=16.0) 7.60 (2H, d, J=16.0)	7.76 (1H,d,J=16.0,C_{α}-H) 8.13 (1H,d,J=16.0,C_{β}-H)	7.62 (2H, s, $C_{\alpha,\beta}$-H)
8.42 (1H, s)	——	——
10.00 and 19.00 (2H, s, OH×2)	8.93, 9.42, 11.10 and 12.18 (4H, s, OH×4)	8.23, 10.00, 10.40, and 12.80 (4H, s, OH×4)

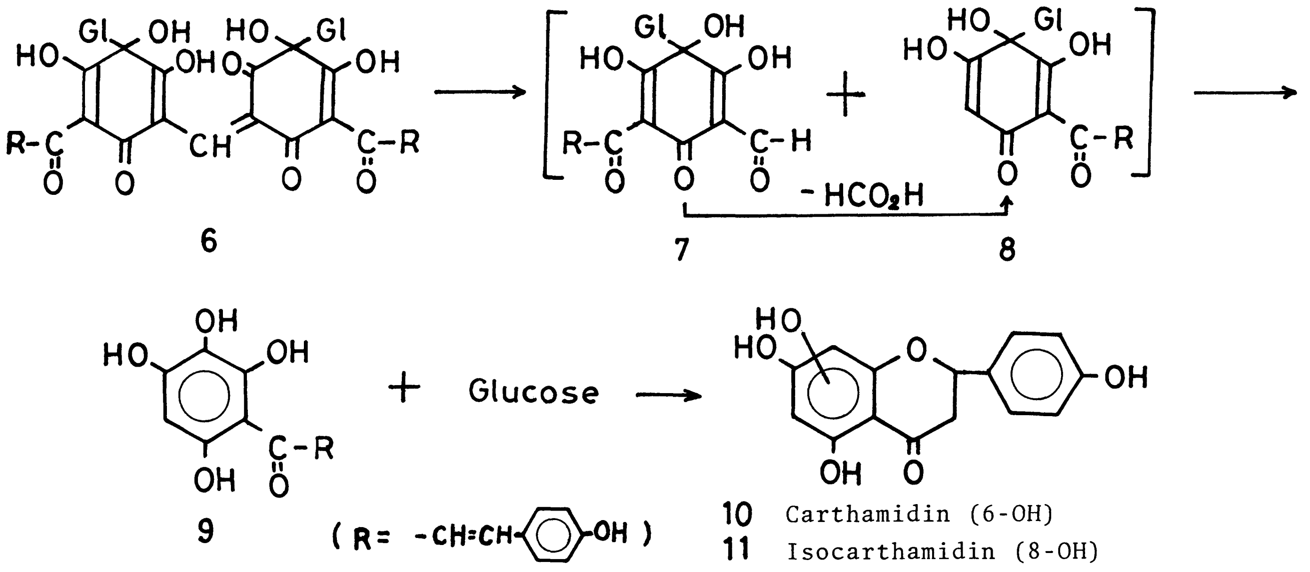
a) Tetramethylsilane was used as an internal standard. b) in DMSO-d_6 c) in Acetone-d_6

Carthamin is hydrolyzed with 10% phosphoric acid to give two flavanones, carthamidin (10) and isocarthamidin (11), and glucose.2) However, a large amount of resinous substances was formed and the yields of these flavanones extremely decreased in the hydrolysis with dilute mineral acid. Recently, we have first identified the formation of formic acid in the hydrolysis of carthamin besides the above hydrolysis products. Although carthamin is hydrolyzed by acid as mentioned above, the enzymatic hydrolysis by emulsin has not occurred. From such behavior of the hydrolysis of carthamin, a paticular glycoside structure in carthamin may be expected.

On the basis of the studies of the PMR spectra and hydrolytic behavior described above, a presumable structure 6 was given for carthamin. It is presumed that the hydrolysis of carthamin proceeds via the intermediates, 7, 8, and 9, as showed in scheme 1.

It seems reasonable to assume that the elimination of glucose occurs in the quinol structure such as carthamin, though the general C-glycosides don't hydrolyze by dilute acid.

Scheme 1



The skeletal structure of 6 was also supported from the electronic spectrum of the synthetic analog, dehydro-3,3'-diacetyl-5,5'-methylenedifilicinic acid (12).8) (Fig. 2). This compound 12, light yellow crystals, mp 173-174°C, UV (EtOH) max: 300 nm ($\log\varepsilon$ = 4.17) and 490 nm ($\log\varepsilon$ =4.85), showed a red color on dissolving in ethanol. Such behavior is very similar to that of carthamin.4)

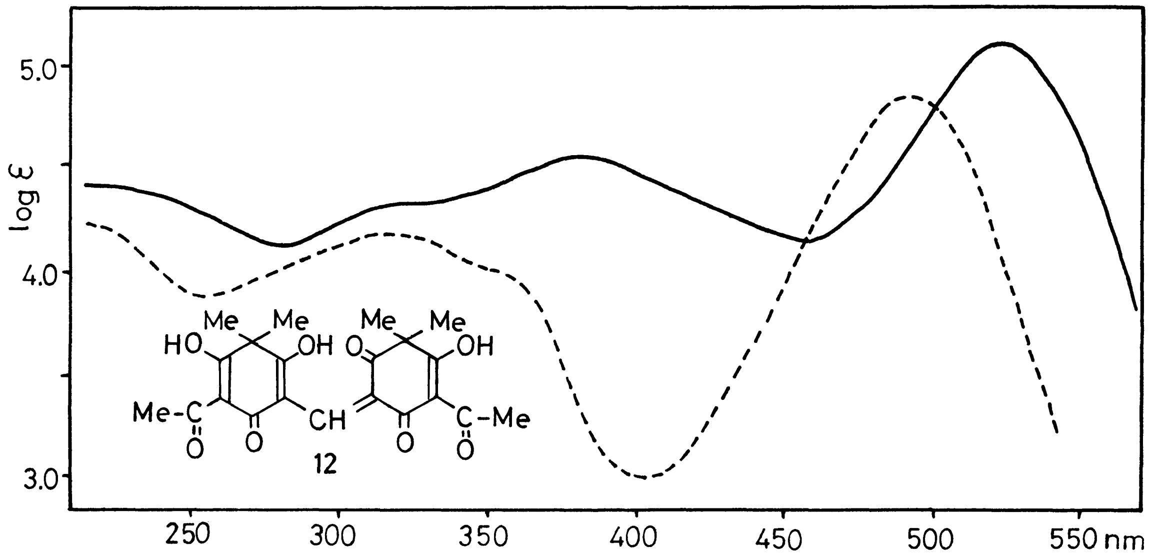


Fig. 2. The electronic spectra of 12 (-----) and carthamin (———) in ethanol.

Unfortunately, carthamin does not give a molecular ion peak in its EI-MS spectrum, but, a maximal fragment ion peak was observed at m/e 568 in its FD-MS spectrum. This peak corresponds to a value which was lost one molecular water from the aglycon of 6.

Finally, the structure of 6 was supported by the comparison of its ^{13}C-NMR spectrum with that of 3-(p-hydroxycinnamoyl)-5-methylfilicinic acid (13)[8] as shown in Fig. 2. The potassium salt of carthamin was used in this measurement for the similar reason of the PMR spectrum measurement described above. From this ^{13}C-NMR spectrum of carthamin, it is assumed that the potassium salt of carthamin exists in a symmetrical structure through its keto-enol tautomerism in solution.

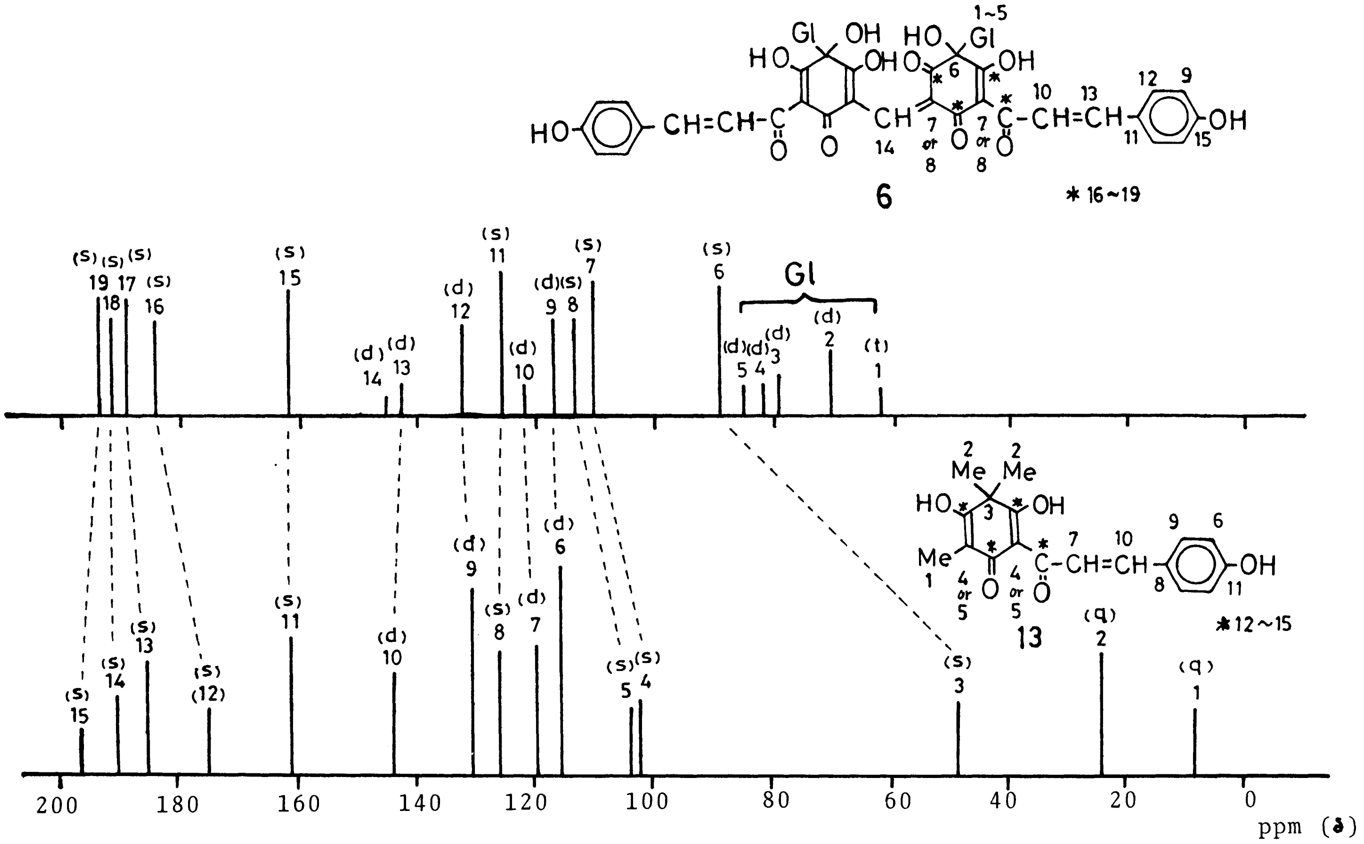


Fig. 2. The ^{13}C-NMR spectra of carthamin (K salt) and 13 in DMSO-d_6. The letter q, t, d, and s show the quartet, triplet, doublet, and singlet in the offresonance ^{1}H-decoupling spectra, respectively.

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References and Notes

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8) The synthetic method of this compound will be reported elsewhere.

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