

CONSTITUTION OF TWO COLORING MATTERS IN THE FLOWER PETALS
OF CARTHAMUS TINCTORIUS L.

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Summary. Structures of the safflower red pigment carthamin and the yellow pigment safflor yellow A are shown to be expressed by 3 and 4 respectively, mainly on the basis of spectroscopic evidence.

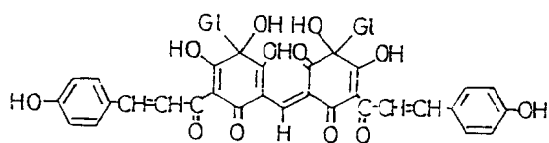
The structure of red¹⁻⁴⁾ and yellow^{3,5)} pigments in the flower petals of Carthamus tinctorius L. has been studied for many years by a number of workers, including one of us (M.W.). Recently, tentative formulas 1⁴⁾ and 2⁵⁾ have been proposed by other workers for the main red pigment, carthamin, and one of the yellow pigments, respectively. Stereochemistry at the glycosidic carbon atom and the ring-size of the glucose moiety in 1 and 2 were not explicitly described, however. Moreover evidence for the structure of the polyol side chain moiety in 2 is not clearly stated. We wish to report herein our own findings, which are best explained by assigning an equatorially C-1-alkylated glucopyranoside formula 3⁶⁾ to carthamin and by giving a structure 4⁶⁾ for the yellow pigment, for which we suggest the name safflor yellow A.⁷⁾

The ¹H nmr spectrum of carthamin at 400 MHz (py-d₅/CD₃OD=95/5) indicated a symmetrical structure for the pigment and exhibited well resolved signals due to the protons attached to oxygen-bearing carbon atoms (Fig 1). Clearly, all the substituents of the hexose moiety are equatorially oriented on a six-membered ring, judging from the coupling constants J_{1,2}=J_{2,3}=J_{3,4}=J_{4,5}=8.5 Hz. Configuration at C* on the quinonoid ring is, however, still obscure.

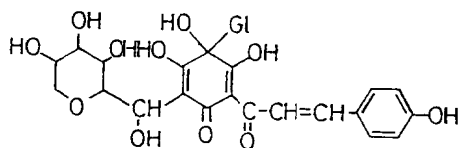
The FD mass spectrum of safflor yellow A (4) exhibited the M+1 peak at m/z 595 and the spectrum of its nonacetate (M=972), prepared with acetic anhydride-pyridine, presented FD peaks at m/z 972, 973 and 996 (M+23+1). Comparison of ¹H

nmr spectra of 4 and the nonacetate indicated that 4 contained thirty protons. On the other hand the noise decoupled ^{13}C spectrum of 4 showed that it is a C_{27} compound. Therefore 4 has a molecular formula $\text{C}_{27}\text{H}_{30}\text{O}_{15}$ ($M=594$, nitrogen was not detected) and hence is supposed to be formally constituted by two hexoses ($\text{C}_{12}\text{H}_{24}\text{O}_{12}$) and one pentahydroxychalcone ($\text{C}_{15}\text{H}_{12}\text{O}_6$), under elimination of three moles of water. Supporting evidence for this hypothesis was obtained from further study of the ^{13}C and ^1H nmr spectra of the pigment. In the former spectrum ($\text{DMSO}-d_6$), the twelve peaks ascribed to the hexose moieties appeared at δ 61.2(t), 61.7(t), 69.0(d), 70.0(d) x 2, 71.0(d), 74.1(d), 78.0(d), 79.0(d), 80.0(d), 80.7(d) and 85.8(d). Since in the spectrum of carthamin 3 essentially same δ values with those underlined were found and in 4 as well as in 3, a singlet at δ 85.8 (C^* , overlapped on the doublet) was observed, the presence of a carthamin-like C-glucopyranoside moiety in 4 was suggested. The signal anticipated for an acetal carbon (ca 100 ppm, d) was not observed. Therefore in 4 the presence of a second C-hexoside moiety was inferred. Other peaks representative of the quinochalconoid moiety of 4 are recognized at δ 158.6(s), 115.6(d) x 2, 130.0(d) x 2, 127.8(s) (phenyl); 136.8(d), 123.6(d) ($-\text{CH}=\text{CH}=\text{C}=\text{O}$); 99.4(s), 106.0(s) ($\text{RO}-\text{C}=\text{C}=\text{O}$); 170.0(s), 183.2(s), 189.4(s), 194.4(s) ($\text{C}=\text{O}$, $\text{RO}-\text{C}=\text{C}=\text{O}$).⁸ The ^1H nmr spectrum (200 MHz, $\text{DMSO}-d_6$) of 4 exhibited two peaks allocated to acidic protons at δ 18.25(1H, s, chelated OH), and 10.08(1H, s, phenolic OH) as well as peaks due to a *p*-hydroxycinnamoyl moiety at δ 6.88, 7.55 (each 2H, d, $J=9$ Hz), 7.36 and 7.68 (each 1H, d, $J=15$ Hz). Further, in addition to the complex resonance lines arising from twelve protons attached to oxygen-bearing carbon atoms between δ 3.0 and δ 3.7, characteristic peaks at δ 4.32(1H, d, $J=10$ Hz) and 3.98(1H, t, $J=10$ Hz) were observed. The last two peaks are coupled each other and must be due to the C-1 and C-2 protons of the second hexose, respectively, since no corresponding signals are observed at such a low field region in the spectrum of carthamin in the same solvent. In D_2O solution at 400 MHz, the proton signals attributed to the two hexoses of 4 were well separated and the 2D J-resolved spectrum⁹) as well as decoupling experiments¹⁰) allowed us to assign the signals as shown in Fig 2.¹⁰) J values (~ 9 Hz for doublets and triplets) thus obtained for the two hexoses indicated that their substituents also are all equatorially oriented on a six-membered ring, viz. the yellow pigment contains two C-glucopyranoside moieties. Taking into consideration all the above results, in particular the large δ_{H} values for C-1 and C-2 protons of the second hexose, MW, and the number of acidic protons, we conclude that the chemical constitution of safflor yellow A is expressed by formula 4. The stereochemistry at C^* is, however, not yet clear.

Treatment of 4 with 12% phosphoric acid in ether gave glucose and a chalconoid 5 mp 260-262 °C, m/z 432(M^+), ^1H nmr (200 MHz, $\text{py}-d_5$) δ 8.46(1H, d, $J=15$ Hz), 8.16(1H, d, $J=15$ Hz), 7.72(2H, d, $J=6$ Hz), 7.26(2H, d, $J=6$ Hz), 4.69,



1



2

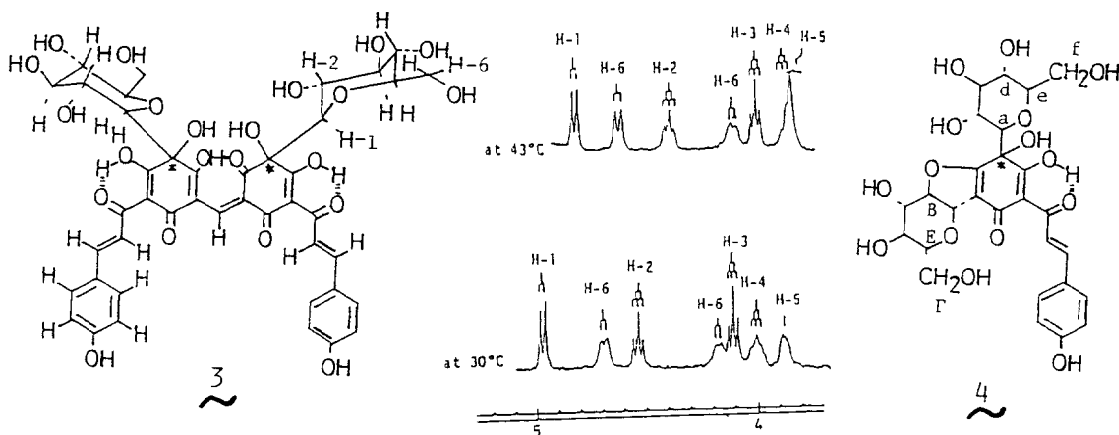
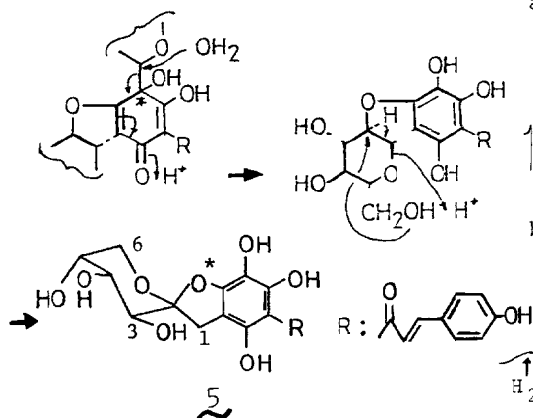


Fig 1

Scheme 1



5

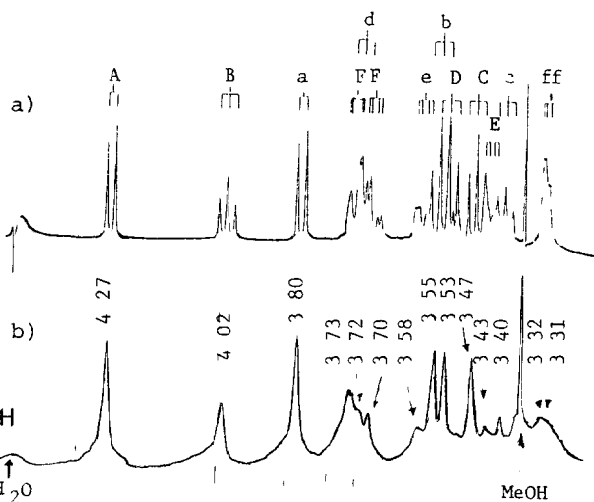


Fig 2 a) Normal spectrum b) 45° Projection of the 2D J spectrum

4.30 (each 1H, ABq, $J=18$ Hz, 1-H₂), 4.91 (1H, d, $J=9.5$ Hz, 3-H), 4.99 (1H, dd, $J=9.5, 4$ Hz, 4-H), 4.69 (1H, br.d, $J=4$ Hz, 5-H), 4.66, 4.32 (each 1H, br, ABq, $J=12.5$ Hz, 6-H₂, $J_{5,6a}=J_{5,6b}=1$ Hz). In DMSO-d₆, the chalconoid exhibited peaks due to acidic protons at δ 13.8 (2H, s) and 10.2 (2H, s). Acetylation of 5 with acetic anhydride-conc sulfuric acid gave an exhaustively acetylated heptaacetate, mp 119-120 °C, m/z 726 (M⁺), FeCl₃(-), ¹H nmr (200 MHz, CDCl₃) δ 3.22, 3.12 (each 1H, ABq, $J=16$ Hz, 1-H₂), 5.60 (1H, d, $J=10$ Hz, 3-H), 7.35 (2H, m, 4-H, 5-H), 4.10, 3.77 (each 1H, br, ABq, $J=12.5$ Hz, 6-H₂), 2.35, 2.30, 2.28, 2.27, 2.17, 2.07 and 2.06 (each 3H, s, CH₃CO₂). On the basis of these findings, the chalconoid was formulated as shown by 5.¹¹⁾ Formation of 5 from 4 is reasonably explained as depicted in Scheme 1 and this, together with the J values of the protons on the modified hexose of 5, supports the validity of formula 4.

References and Notes

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- 5) J. Onodera, H. Obara, M. Osone, Y. Maruyama, and S. Sato, Chem. Lett., 433 (1981).
- 6) This formula was presented before the 23rd Symposium on the Chemistry of Natural Products (Nagoya, 1980). Symposium Papers p.538.
- 7) Designated tentatively SP₂ in ref 6. In ref 5, the name safflomin A is proposed for the same compound. However, since it is now clear that the structure of safflomin A is erroneous and the name safflor yellow has been used for a long time for the yellow coloring matters, we would like to give the name safflor yellow A for the first pure yellow pigment.
- 8) For δ_C values of a similar system, see for example M. Tanabe and G. Detre, J. Am. Chem. Soc., 88, 4515 (1966).
- 9) For a review see R. Freeman, Proc. Roy. Soc. London, A 373, 149 (1980).
- 10) Our thanks are due to Drs. Y. Naya and A. Naoki, Suntory Research Institute for Bioorganic Science, for measuring decoupled nmr spectra at 360 MHz.
- 11) The configuration at C-2 is most probably expressed by 5, since in this structure the anomeric oxygen atom (O*) and the C-1 methylene group take thermodynamically stable axial and equatorial orientations respectively.

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