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CHEMICAL CONSTITUTION OF SAFFLOR YELLOW B, A QUINOCHALCONE C-GLYCOSIDE FROM THE FLOWER PETALS OF CARTHAMUS TINCTORIUS L.

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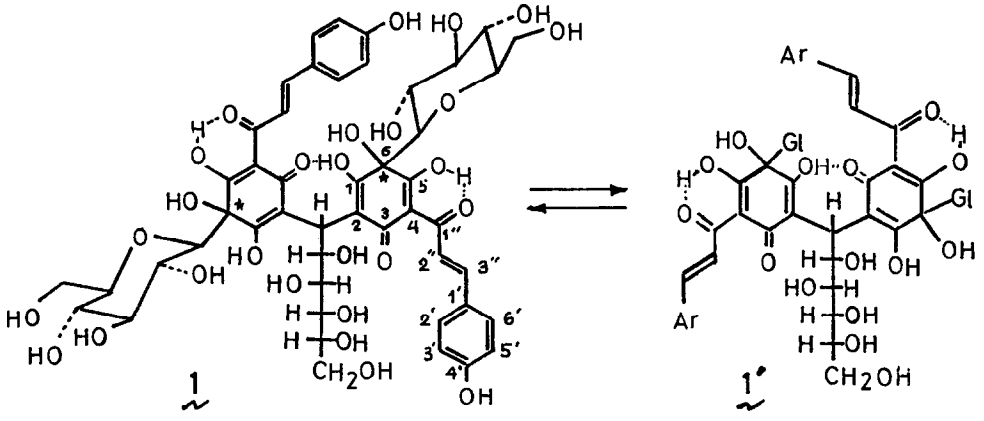
Summary: The chemical constitution of safflor yellow B, a quinochalcone glycoside from the flower petals of Carthamus tinctorius L, has been characterized as 1, mainly on the basis of spectral means.

We have recently reported the structures of carthamin (red pigment) and safflor yellow A (yellow pegment) isolated from the flower petals of C. tinctorius.[1] We now wish to report the isolation and structural determination of the second yellow quinochalcone C-glycoside, safflor yellow B (1),[2] contained in the same flower petals.



1 ⇌ 1'

Crude yellow pigment juice, squeezed from reddened flower petals[2] of C. tinctorius, was treated with active carbon, which in turn extracted with pyridine to give yellow powder. Successive column chromatography of the pigment on cellulose with different solvent systems (e.g. BuOH/AcOH/H_2O=4:1:2) gave a yellow substance exhibiting a single spot on a TLC plate. Further chromatography of the pigment on Sephadex LH-20 (H_2O) and then on Toyo Pearl

Fig. 1

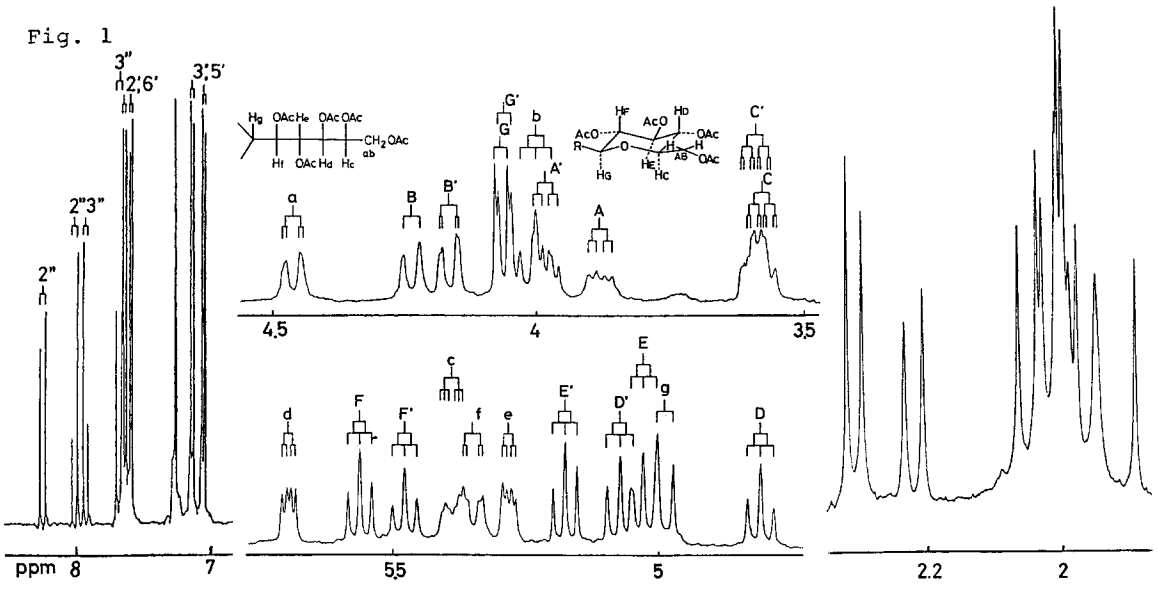
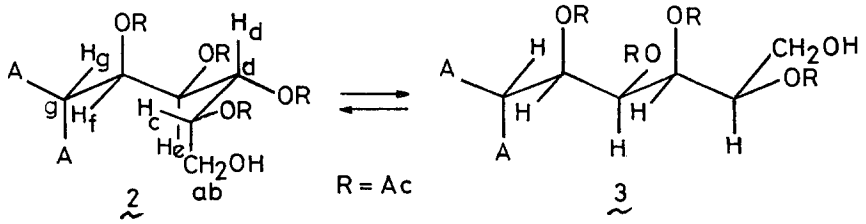


Table 1. ^{1}H Chemical shift values (δ)

A 3.88, B 4.23, C 3.58, D 4.83, E 5.03, F 5.55, G 4.06, A' 3.99, B' 4.16, C' 3.59, D' 5.07, E' 5.17, F' 5.49, G' 4.05, a 4.43, b 4.00, c 5.39, d 5.69, e 5.28, f 5.35, g 4.98, 2' 7.64, 3' 7.14, 5' 7.06, 6' 7.59, $2''_a$ 8.01, $2''_b$ 8.23, $3''_a$ 7.93, $3''_b$ 7.60

Table 2. Coupling constants (Hz)

AB 12.6, AC 7.2, BC 1.8, CD 9.4, DE 9.4, EF 9.4, FG 9.4, A' B' 12.6, A' C' 8.5, B' C' 1.8, C' D' 9.4, D' E' 9.4, E' F' 9.4, F' G' 9.4, ab 12.6, ac 3.6, bc 12.6, cd 3.6, de 7.2, ef 3.6, fg 11.4, 2' 3' 9.0, 5' 6' 9.0, $2''_a 3''_a$ 16.4, $2''_b 3''_b$ 16.4



HW-40F (65% MeOH) gave the yellow pigment safflor yellow B (0.0044% yield from fresh flower petals), which showed R_f 0.59 on cellulose TLC using n-BuOH/AcOH/H_2O (=4:1:2).

The 400 MHz ^1H nmr spectrum (D_2O)[3] of the pigment exhibited peaks due to two p-hydroxycinnamoyl moieties at δ6.80 and 7.43 (each 4H, AB, J=9.0 Hz), δ 7.27 and 7.39 (each 2H, AB, J=15.0 Hz) and signals corresponding to three hexose moieties between δ2.6-5.0 ppm. An AMX system, confirmed by decoupling experiments, was observed at δ 4.92 (t, J=7.5 Hz), 4.42 (d, J=7.5 Hz), 3.89 (d, J=7.5 Hz). Moreover, an unusually shielded broad doublet (1H, J=8.2 Hz), with a characteristic splitting pattern for H_5 of glucopyranoside, appeared at δ 2.72. However, since the ^1H spectrum exhibited a complex overlapped pattern except for the above peaks, safflor yellow B heptadecaacetate[4] was prepared and its ^1H spectrum ($CDCl_3$, 500 MHz) was studies (see Fig. 1, Table 1 and 2). The acetate displayed four phenolic and enolic acetyl groups and thirteen alcoholic acetyl groups, at 2.32-2.40 and 1.87-2.06 respectively. Other signals were correlated with each other by extensive decoupling experiments. The presence of two non-equivalent glucopyranoside moieties with all of the J_{CD} , J_{DE} , J_{EF} , J_{FG} of 9.4 Hz were clearly demonstrated. The third sugar moiety turned out to be an open-chain hexose, by assigning H_{a-g} protons of the acetyl compound as shown in Fig. 1. The coupling constants in Table 2 can be explained satisfactorily by allotting glucitol structure to this moiety. Since C_g of this moiety bears bulky groups, C_g is expected to be anti to C_d, and the attractive gauche interaction[5] between the C-O bonds of the glucitol moietity predicts the presence of almost equal amount of two main conformations 2 and 3 in equilibrium, in conformity with the observed coupling constants.[6,7] The FAB mass spectrum (DMSO-glycerol, Xe) of 1 displayed a peak at m/z 1067, corresponding to $C_{48}H_{58}O_{27}$+1.[8] In the UV-VIS spectrum (MeOH), absorption maxima at 408 (ε 22,600), 330 (ε 14,600) and 240 nm (ε 10,900) were observed. Comparison of these data with those of safflor yellow A [400 (10,200), 335 (6,200), 226 (7,800)] coupled with the above gross molecular weight and ^1H nmr data, suggested that safflor yellow B was composed of two unequivalent chalcoquinoids similar to that of safflor yellow A, two unequivalent glycopyranose and one glucitol. The ^{13}C nmr data[9] supported this conclusion and moreover, showed that these components are connected together through C-glycosidic linkages, like safflor yellow A.

Taking into consideration all of the above results, together with biogenetic relationship, the constitution of safflor yellow B is best expressed by formula 1. The configurations at C*s are not yet clear at present, however. If they are not identical, non-equivalency of the two chalcoquinoids and two glucopyranose moieties in the nmr is readily understood. However, even if both of chiralities are identical, and moreover rotation around the C_g-quinoid bonds is not hindered as shown by formula 1', they can be magnetically unequivalent, since they correspond to A part of $\begin{matrix} A \\ A \end{matrix}\!>\!CH\text{-}\overset{H}{\overset{|}{C^{**}}}\text{-}OR$ moiety, where C** is asymmetric.

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References and Notes

1) Y. Takahashi, T. Miyasaka, S. Tasaka, I. Miura, S. Urano, M. Ikura, K. Hikichi, T. Matsumoto, and M. Wada, Tetrahedron Lett., 23, 5163 (1982).
2) M. Wada, Japan patent No. 8943 (1955); M. Wada, Japan patent No. 2383 (1960).
3) The ^{1}H nmr spectrum of 1 is susceptible to be affected probably by the presence of acidic or basis impurities. Under certain circumstances the two chalcoquinoid moieties exhibit unequivalent peaks at 400 MHz.
4) Safflor yellow B acetate, yellow needles, mp 200 °C (dec.) was prepared by treating 1 with Ac_2O/Py at 85 °C. All hydroxyl groups except for the tertiary groups were acetylted.
5) H. S. Zefirov, L. G. Gurvich, A. S. Shashkov, M. Z. Krimer, E. A. Vorobeva, Tetrahedron, 32, 1211 (1976) and references cited there. This concept seems useful for similar case. For example relative configurations of the segment 15-20, and other vic-glycolic segments of palytoxin can be inferred correctly as determined by Kishi, Hirata et al. [H. Fujioka, W. J. Christ, J. K. Cha, J. Leder, Y. Kishi, D. Uemura and Y. Hirata, J. Am. Chem. Soc., 104, 8367 (1982)], from the reported J values of its acetate [R. E. Moore, G. Bartolihi, J. Barchi, A. A. Bothner-By, J. Dadok and J. Ford, J. Am. Chem. Soc., 104, 3776 (1982)], if this effect is taken into account.
6) For reference $J_{2,3}$'s of meso and dl forms of 2,3-butanediol diacetate were measured by the ^{13}C satellite method [H. A. Bent, Chem. Rev., 61, 275 (1961)]: meso form, 3.66 Hz; dl form, 6.35 Hz.
7) The coupling constant at H_{cd} of the glucitol moiety of free safflor yellow B was nearly 0 Hz. Clearly conformation of the glycitol moiety is considerably different from that of the acetate as a result of hydrogen bonding.
8) Reduction of a quinonoid to its dihydro compound is often observed in the FAB MS using glycerol (private communication from the Mass Spectrometry Laboratory, Jeol). Difference between the observed and calculated m/z for 1 ($C_{48}H_{54}O_{27}$, 1062) may be due to this effect.
9) ^{13}C NMR of safflor yellow B (100.7 MHz, D_2O): δ (ppm) 197.2 (s), 195.6 (s), 188.6 (s), 185.1 (s), 181.9 (s), 180.8 (s), 180.7 (s) [C=O and C(OH)=C, one carbon atom was not clearly observed]; 158.7 (s), 157.6 (s) (C_4'); 144.7 (d), 140.1 (d) (C_1); 131.1 (d), 130.3 (d, strong) (C_3' and C_5'); 128.0 (s) and 127.6 (s) (C_1'); 121.0 (d) and 117.6 (d) (C_2); 116.1 (d, stron g) (C_2' and C_6'); 113.2 (s), 108.2 (s), 106.1 (s), 102.1 (s), [C(OH)=C]; 85.3 (s) and 77.9 (s) (C_7); 93.0 (d), 85.9 (d), 83.5 (d), 80.0 (d), 78.9 (d), 77.8 (d), 77.7 (d), 71.8 (d), 71.6 (d), 71.3 (d), 70.1 (d), 69.8 (d), 69.4 (d), 69.2 (d), 67.9 (d) (CHOH and C-C-H); 63.2 (t), 61.1 (t), 59.4 (t) (CH_2O).

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