Two New Quinochalcone Yellow Pigments from *Carthamus tinctorius* and Ca^{2+} Antagonistic Activity of Tinctormine[1)]

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Two new quinochalcone *C*-glycosides, hydroxysafflor yellow A (1a) and tinctormine (2a), were isolated from *Carthamus tinctorius* L. (Compositae) together with carthamin, safflor yellow B and safflomin C. The structures of 1a and 2a have been determined by spectroscopic methods including heteronuclear multiple-bond multiple-quantum coherence and linked scan FAB-MS. The latter compound (2a) was demonstrated to have potent Ca^{2+} antagonistic action.

Keywords *Carthamus tinctorius* L.; Compositae; calcium ion antagonist; quinochalcone *C*-glycoside; hydroxysafflor yellow A; tinctormine

In our previous paper,[1)] we briefly reported the isolation and characterization of a new naturally occurring Ca^{2+} antagonist, tinctormine (**2a**), from the flower petals of safflower (*Carthamus tinctorius* L., Compositae). During the course of our phytochemical and pharmacological investigations of safflower, we isolated an additional new *C*-glucosyl quinochalcone named hydroxysafflor yellow A (**1a**), together with safflomin C.[2)] In the present paper, we report in more detail the isolation and structural determination of **1a** and **2a**, and the inhibition of the Ca^{2+}-currents on single ventricular myocytes of dog by **2a**, which was evaluated using electrophysiological techniques.

Results and Discussion

An acetone extract of the flower petals of *C. tinctorius* was subjected to further fractionation (Chart 2). Repeated column chromatography of an aqueous lyophilizate on Sephadex LH-20, polyamide and subsequent preparative thin-layer chromatography (prep. TLC) gave two compounds named hydroxysafflor yellow A (**1a**) and tinctormine (**2a**).

Hydroxysafflor yellow A (**1a**) was obtained as a yellow amorphous powder, $[\alpha]_D$ $-54.3°$ ($c=0.1$, MeOH) and showed infrared (IR) absorptions at 3400 cm^{-1} (OH) and 1640, 1610 cm^{-1} (1,3-diketone). The negative ion fast atom bombardment-mass (FAB-MS) spectrum of **1a** exhibited an $[M-H]^-$ ion peak at m/z 611 and the molecular formula was determined to be $C_{27}H_{32}O_{16}$ by high-resolution FAB-MS spectrometry.

Proton nuclear magnetic resonance (^1H-NMR) spectrum showed the presence of two *C*-glucosyl anomeric protons at δ_H 3.64 (1H, d, $J=9.5$ Hz, 1′-H) and 4.21 (1H, d, $J=9.5$ Hz, 1″-H), a pair of $A'_2B'_2$-type aromatic protons at δ_H 6.77 and 7.41 (each 2H, d, $J=9$ Hz, 12-, 14-H and 11-, 15-H, respectively) and *trans* (*E*)-olefinic protons at δ_H 7.31 and 7.42 (each 1H, d, $J=15.5$ Hz), a phenolic hydroxyl proton at δ_H 8.30 (C_{13}-OH), and two enolic hydroxyl protons at δ_H 9.75 (C_5-OH) and 18.61 (C_3-OH) (the last three were exchangeable with D_2O). Furthermore, on the basis of the proton–proton shift correlated spectroscopic (^1H–^1H COSY) experiment, **1a** was deduced to be a quinochalcone *C*-glycoside with a structure similar to that reported for safflor yellow A (**3**),[3)] or safflomin A.[4)] The FAB-MS gave a quasi-molecular ion peak at m/z 611 $[M-H]^-$, 18 mass units higher than that of **3** (m/z 593 $[M-H]^-$), indicating that an additional hydroxyl group is introduced to the molecule of **3**. The proton signal at δ_H 4.12 (2″-H) of **1a** showed good correlation with a signal at δ_H 4.01 (exchangeable with D_2O), assignable to a free hydroxyl group at C-2″. Moreover, the signals (6″-H_2) at δ_H 3.41 and 3.58 correlated with a hydroxyl proton at δ_H 4.45 (6″-OH). The ^1H–^{13}C COSY spectrum indicated the presence of two carbinols at δ_C 61.4 (t) and 68.7 (d) assignable to C-6″ and C-2″, respectively, as well as characteristic signals for two hexose units with chemical shifts similar to those of two *C*-glucosyl residues in **3**, except

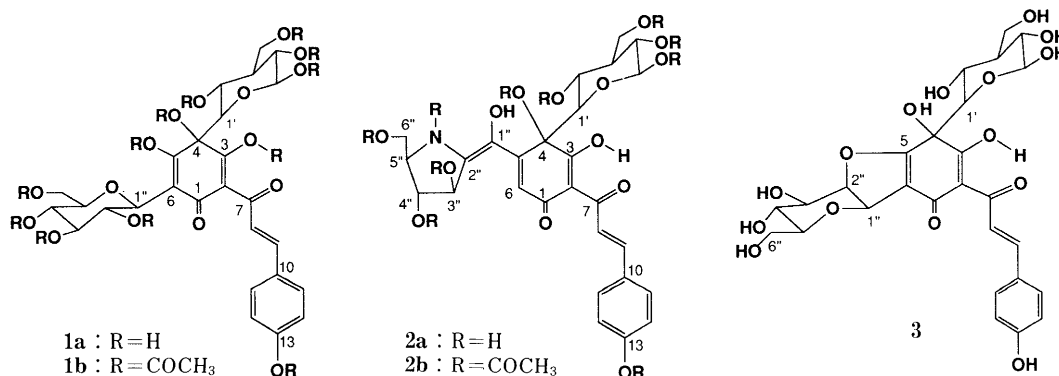

Chart 1

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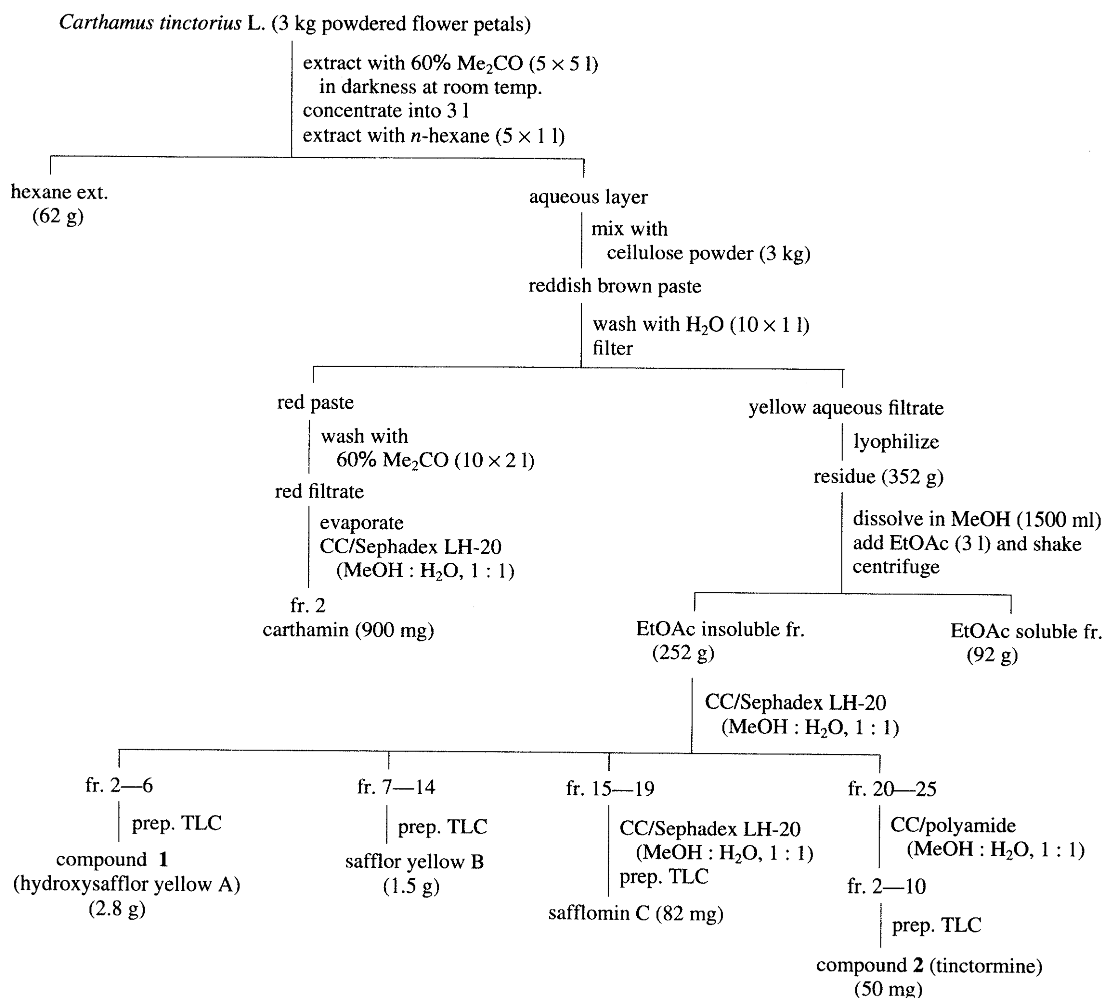

Chart 2. Flow Chart of the Isolation of Pigments from the Flower Petals of *C. tinctorius*

for C-2′ (Table I). The close similarity of the ^{1}H- and ^{13}C-NMR spectral data of **1a** with those of **3** led us to assume that **1a** might be safflor yellow A with free hydroxyl groups at C-5 and C-2″.

On acetylation with acetic anhydride–pyridine, **1a** gave a dodeca-*O*-acetyl derivative (**1b**), though the yield was poor (10%). The IR spectrum ($CHCl_3$) of **1b** showed a strong carbonyl absorption at 1750 cm^{-1} but no absorption due to a hydroxyl group, indicating the complete acetylation of **1a**. The positive ion FAB-MS spectrum showed a quasi-molecular ion peak at m/z 1117 $[M+H]^+$, indicating the presence of twelve acetoxyl groups in the molecule. The ^{1}H-NMR spectrum ($CDCl_3$) also exhibited signals for twelve acetoxyl groups at δ_H 1.84, 1.99, 2.01 (Ac × 2), 2.06, 2.08 (Ac × 3), 2.11, 2.32 and 2.35 (Ac × 2), but no signals were assignable to hydroxyl protons.

Next, we measured the long-range ^{1}H–^{13}C COSY spectrum of **1a** to confirm the proposed structure **1a**. The quaternary carbon signal at δ_C 85.2 (C-4, s) correlated with a proton signal at δ_H 3.64 (1′-H), and the sp^2 quaternary carbon signal at δ_C 99.3 (C-6, s) with a proton signal at δ_H 4.21 (1″-H), which unequivocally indicated the site of attachment of two *C*-glucosyl units at C-4 and C-6, respectively. The pertinent ^{1}H–^{13}C long-range correlations obtained here are illustrated in Chart 3. The ^{1}H-NMR spectrum of **1a** (in dimethyl sulfoxide-d_6 (DMSO-d_6)) showed well resolved signals for the *C*-glucosyl protons with

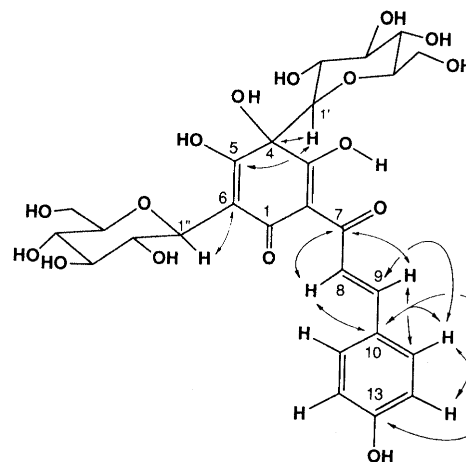

Chart 3. Significant Correlations Observed by Long-Range ^{1}H–^{13}C COSY of Hydroxysafflor Yellow A (**1a**)

coupling constants in the range of 9.5—10 Hz, indicating the equatorial orientations of all substituents.[5)]

From the above spectral data, the structure of hydroxysafflor yellow A was concluded to be **1a** as shown in Chart 1.

Tinctormine (**2a**) was obtained as a yellow amorphous powder, $[\alpha]_D$ $-260°$ ($c=0.1$, MeOH) and the IR spectrum indicated the presence of hydroxyl and 1,3-diketo groups

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as observed in **1a**. The negative ion FAB-MS spectrum of **2a** exhibited a quasi-molecular ion peak at *m/z* 592 $[M-H]^-$ and its molecular formula was determined to be $C_{27}H_{31}O_{14}N$ by high-resolution FAB-MS, indicating 13 degrees of unsaturation in the molecule.

Acetylation of **2a** with acetic anhydride in pyridine afforded a deca-*N,O*-acetate (**2b**), and the positive ion FAB-MS exhibited an $[M]^+$ peak at *m/z* 1013 $[C_{47}H_{51}O_{24}N]^+$. It showed IR ($CHCl_3$) absorptions at 3330 (OH), 1760 (C=O) and 1658 (N-CO) cm^{-1}. The ^{1}H-NMR ($CDCl_3$) spectrum of **2b** showed the presence of ten acetoxyl signals at δ_H 1.85, 1.98, 2.01 (Ac×2), 2.05 (Ac×2), 2.13, 2.18, 2.23, 2.32 and two hydroxyl signals at δ_H 10.62 (1H, br s, 1″-OH) and 18.78 (1H, s, 3-OH).

The ^{1}H- and ^{13}C-NMR spectra of **2a**, analyzed by ^{1}H–^{1}H COSY and ^{1}H–^{13}C COSY, were similar in part to those of hydroxysafflor yellow A (**1a**), but showed the presence of a sugar moiety with an anomeric carbon and proton signals at δ_C 84.2 and δ_H 3.30 (1H, d, J=9.5 Hz, 1′-H), respectively. The signal assignable to 3″-H shifted downfield at δ_H 4.85 (1H, br d), suggesting an α-vinyl group (a feature not found in **1a**). In addition, a characteristic singlet signal was observed at δ_H 6.30 (1H, s), attributable to a proton (6-H) of a conjugated double bond. Two carbonyl signals at δ_C 185.7 and 180.3 were assignable to C-1 and C-7, respectively, and three carbinols at δ_C 63.3 (t), 65.9 (d), 73.9 (d), an sp^3 methine carbon at δ_C 71.3 (d), two sp^2 quaternary carbons at δ_C 138.2 (s), 140.9 (s), an sp^2 methine carbon at δ_C 140.9 (d) and an enolic carbon at δ_C 195.8 (s) were observed (Table I).

Figure 1 shows partial structures for **2a** deduced by detailed analysis of ^{1}H–^{1}H COSY and ^{1}H–^{13}C COSY data. Partial structures A, C and most of B were reminiscent of those in hydroxysafflor yellow A (**1a**). The FAB-MS displayed a fragment ion peak at *m/z* 147 attributable to a cinnamoyl moiety, as indicated in the partial structure A.

The structure of tinctormine (**2a**) shown in Chart 4 was obtained by piecing together partial structures A—E on the basis of the ^{1}H-detected heteronuclear multiple-bond multiple-quantum coherence (HMBC) experiment. Although no connectivity was observed between the quaternary carbons at C-2 and C-7, the chemical bonding between them was deduced by the evidence that the 3-OH group (δ_H 17.95, s) is internally hydrogen-bonded with the 7-ketone group. The carbon signal at δ_C 77.9 (C-4) correlated with proton signals at δ_H 3.30 (1′-H) and 5.70 (4-OH) in terms of long-range correlation, indicating the connectivity of partial structure C with B. Moreover, a significant long-range correlation was observed between an enolic carbon at δ_C 195.8 (C-3, s) and proton signals at δ_H 3.30 (1′-H) and 5.70 (4-OH). By contrast, no correlation was detected between a carbonyl carbon at δ_C 185.7 (C-1) and the above mentioned protons (1′-H and 4-OH), which alternately supported partial structure B having a carbonyl carbon (C-1) and an enolic carbon (C-3). Since the signal of C-1″ was correlated with a 4-OH signal, C-1″ may be linked at C-5, not at C-6 (α-keto-olefinic carbon). Partial structure D was confirmed by the ^{1}H–^{1}H COSY and HMBC experiments. With the aid of the off-resonance experiment of **2a**, for example, a signal at δ_C 140.9 (d and s) could be assigned to two overlapping carbons, an sp^2 methine carbon (C-9, d) and an oxygenated quaternary carbon (C-1″, s). The C-9 signal correlated with a proton signal at δ_H 7.58 (11-H), while the C-1″ signal correlated with a proton signal at δ_H 6.30 (6-H). On the other hand, the C-1″ signal showed a long-range correlation with a proton signal at δ_H 4.85 (3″-H), and the quaternary carbon signal at δ_C 138.2 (C-2″) was correlated with proton signals at δ_H 4.85 (3″-H) and 6.30 (6-H). In turn, the carbon signal at δ_C 73.9 (C-4″) was correlated with proton signals at δ_H 4.59 (4″-OH) and 4.65 (–NH–), and the carbon signal at δ_C 71.3 (C-5″) showed correlations with proton signals at δ_H 4.36 (6″-OH), 4.65 (–NH–) and 4.59 (4″-OH). The significant long-range correlations observed are shown by arrows in the formula in Chart 4. In addition, the ^{1}H–^{1}H COSY experiment of **2a** showed a cross peak between a signal at δ_H 3.62 (5″-H) and signals at δ_H 4.36 (6″-OH), 4.65 (–NH–) and 4.59 (4″-OH), indicating a nitrogen atom linking C-5″ and C-2″, rather than an oxygen, to give an ether bond. On the other hand, the ^{13}C-NMR spectrum indicated only two carbonyls (C-1 and C-7), and suggested that the quaternary carbon C-1″ existed in an enol form rather than in a keto form, which in turn formed an enamine system with respect to an –NH– group. The above mentioned data indicated partial structure E.

Thus the planar structure of tinctormine was concluded to be that shown by formula **2a**.

The relative stereochemistry of **2a** was determined by

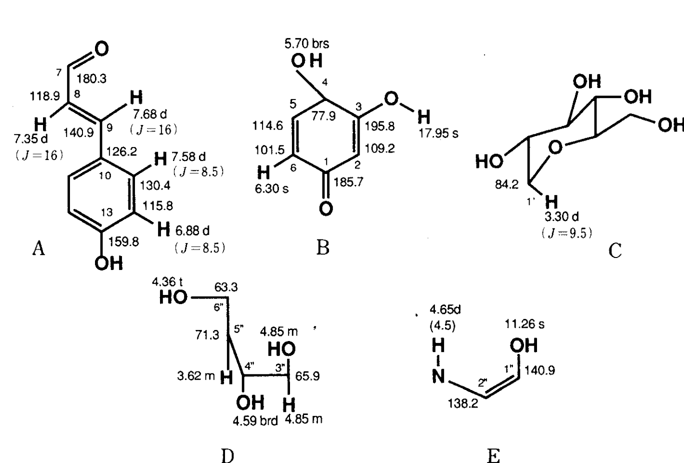

Fig. 1. Partial Structures (A—E) of Tinctormine (**2a**) Deduced from ^{1}H- and ^{13}C-NMR Data

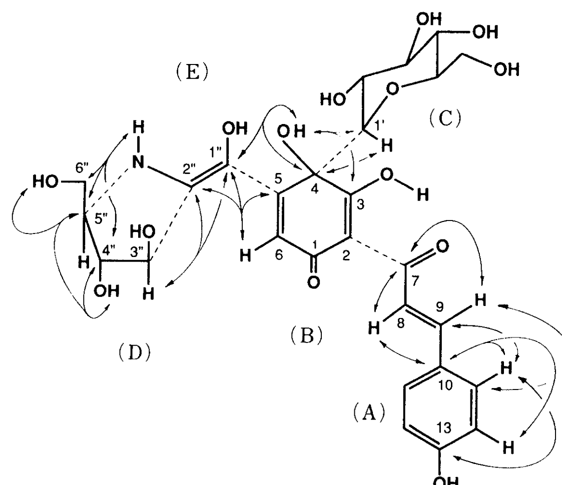

Chart 4. Connectivities of the Partial Structures Obtained from HMBC

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nuclear Overhauser effect (NOE) experiments and the coupling constants. Irradiation of the signal 6-H resulted in a negative NOE[6] on the signal 3″-H (or *vice versa*). This NOE provided experimental evidence that the geometry between C-1″ and C-2″ is *Z* (Fig. 2). From the examination of the dreiding model, these two protons (6-H and 3″-H) were found to be reasonably close to each other, and the 1″-OH and NH groups were arranged in the *Z* form. Moreover, the ^{1}H-NMR spectrum (DMSO-d_6+D_2O) showed characteristic signals assignable to an anomeric proton at δ_H 3.26 (1H, d, J=9.5 Hz), methylene protons at δ_H 3.41 (1H, dd, J=11, 2 Hz) and 3.52 (1H, dd, J=11, 9.5 Hz), and three hydroxy-bearing methine protons at δ_H 3.10 (1H, t, J=9.5 Hz), 3.12 (1H, t, J=9.5 Hz) and 3.37 (1H, t, J=9.5 Hz), suggesting the presence of a *C*-glucosyl residue in **2a**.

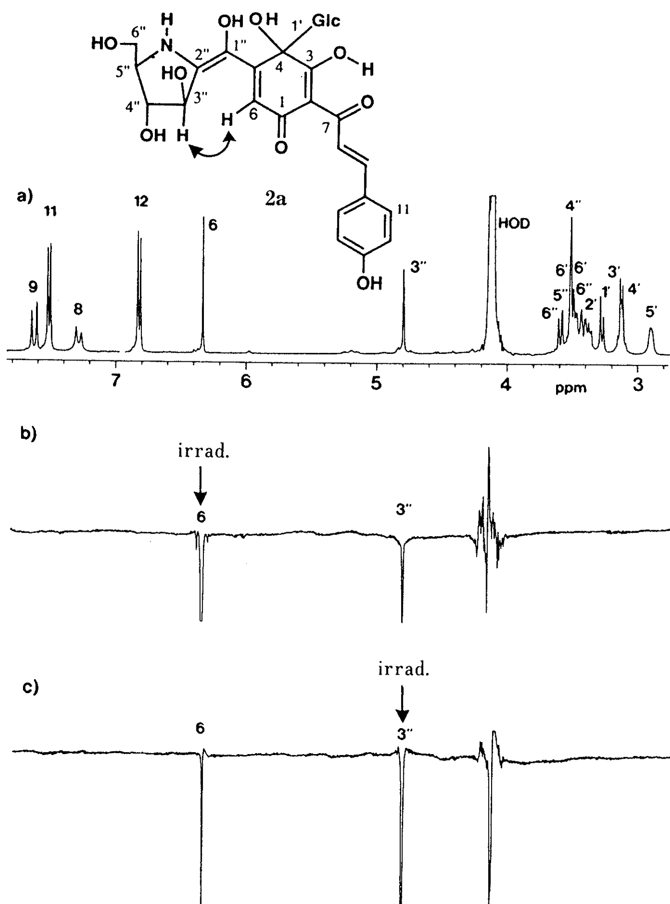

Fig. 2. ^{1}H-NMR (Normal and NOE) Spectra of Tinctormine (**2a**) (in DMSO-d_6+D_2O)

a) Normal spectrum. b, c) NOE difference spectra on irradiation at δ_H 6.37 and 4.79 ppm, respectively.

Based on the foregoing findings, the relative stereochemistry of **2a** was established.[5]

In accordance with this conclusion, the linked scan FAB-MS (positive ion mode) of **2a** displayed a quasi-molecular ion peak at *m/z* 594 $[M+H]^+$ and a characteristic fragment ion peak at *m/z* 432 $[(M+H)-Glc]^+$ which corresponds to the formula $C_{21}H_{22}O_9N$, generated by the loss of a glucose unit. In addition, the fragment ion peak at *m/z* 432 showed significant daughter ion peaks at *m/z* 289 and 142, which probably arose from the loss of an

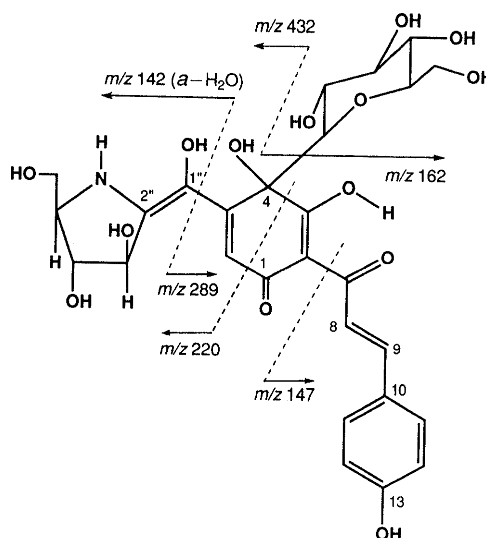

Chart 5

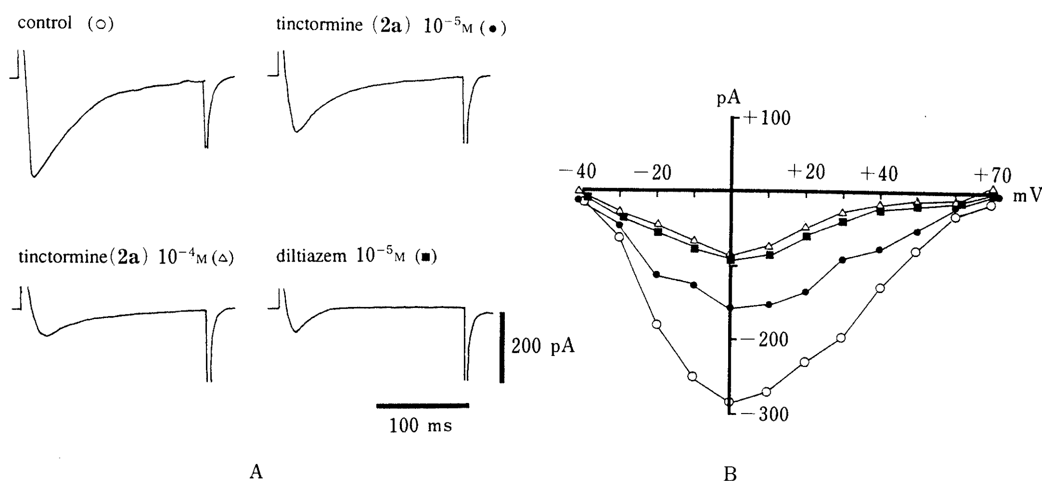

Fig. 3. Effect of Tinctormine (**2a**) and Diltiazem on Ca^{2+} Currents in Single Canine Ventricular Myocytes Using the Whole-Cell Voltage Clamp Method

A) Recording of Ca^{2+} currents generated depolarizing pulse from −40 mV to potential positive to 0 mV, 200 ms duration. Ca^{2+} currents were decreased by tinctormine (**2a**) in a dose-dependent manner. B) The peak current-voltage relationship for Ca^{2+} currents in: control (○), tinctormine (**2a**), 10^{-5} M (●), 10^{-4} M (△), and diltiazem (10^{-5} M) (■). Tinctormine (**2a**, 10^{-4} M and 10^{-5} M) significantly decreased the Ca^{2+} currents at all the voltages studied.

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Table I. ^{1}H- and ^{13}C-NMR Spectral Data of Hydroxysafflor Yellow A (**1a**) and Tinctormine (**2a**)

Position	Hydroxysafflor yellow A (**1a**)		Tinctormine (**2a**)			Safflor yellow A
	δ_H[b]	δ_C[b]	δ_H[b]	δ_H[c]	δ_C[b]	(**3**)[a] δ_C[b]
1	—	189.3 (s)	—	—	185.7 (s)	189.4 (s)
2	—	105.8 (s)	—	—	109.2 (s)	106.0 (s)
3	—	195.0 (s)	—	—	195.8 (s)	194.4 (s)
4	—	85.2 (s)	—	—	77.9 (s)	85.8 (s)
5	—	182.9 (s)	—	—	114.6 (s)	183.2 (s)
6	—	99.3 (s)	6.30 s	6.37 s	101.5 (d)	99.4 (s)
7	—	179.3 (s)	—	—	180.3 (s)	170.0 (s)
8	7.42 d (15.5)	123.1 (d)	7.35 d (16.0)	7.28 d (16.0)	118.9 (d)	123.6 (d)
9	7.31 d (15.5)	135.9 (d)	7.68 d (16.0)	7.63 d (16.0)	140.9 (d)	136.8 (d)
10	—	127.2 (s)	—	—	126.2 (s)	127.8 (s)
11	7.41 d (9.0)	129.2 (d)	7.58 d (8.5)	7.52 d (8.5)	130.4 (d)	130.0 (d)
12	6.77 d (9.0)	115.5 (d)	6.88 d (8.5)	6.81 d (8.5)	115.8 (d)	115.6 (d)
13	—	158.3 (s)	—	—	159.8 (s)	158.6 (s)
1′	3.64 d (9.5)	85.5 (d)	3.30 d (9.5)	3.26 d (9.5)	84.2 (d)	85.8 (d)
2′	3.35 dd (9.5, 4.5)	69.5 (d)	3.45 m	3.37 t (9.5)	69.0 (d)	69.0 (d)
3′	3.11 dd (9.5, 4.5)	78.2 (d)	3.17 m	3.12 t (9.5)	78.3 (d)	79.0 (d)
4′	2.89 td (9.5, 4.5)	69.7 (d)	3.15 m	3.10 t (9.5)	69.2 (d)	70.0 (d)
5′	2.96 td (9.5, 4.5)	80.0 (d)	2.95 m	2.98 dd (9.5, 2)	79.7 (d)	80.7 (d)
6′	3.37 t (10.0)	60.8 (t)	3.50 m	3.41 dd (11, 2)	60.7 (t)	61.2 (t)
	3.60 m		3.54 m	3.52 dd (11, 9.5)		
1″	4.21 d (9.5)	73.8 (d)	—	—	140.9 (s)	74.1 (d)
2″	4.12 td (9.5, 4.5)	68.7 (d)	—	—	138.2 (s)	71.0 (d)
3″	3.15 dd (10.0, 4.5)	79.1 (d)	4.85 m	4.79 d (3.5)	65.9 (d)	78.0 (d)
4″	3.10 dd (10.0, 4.5)	70.8 (d)	3.57 m	3.46 dd (7.5, 3.5)	73.9 (d)	70.0 (d)
5″	3.05 dd (10, 4.5)	80.5 (d)	3.62 m	3.57 br d (7.5, 3.5)	71.3 (d)	80.7 (d)
6″	3.41 m	61.4 (t)	3.47 m	3.38 dd (11, 3.5)	63.3 (t)	61.7 (t)
	3.58 ddd (12, 6.5, 4.5)		3.67 m	3.58 dd (11, 7.5)		
3-OH	18.61 s		17.95 s			
4-OH	4.53 d (4.5)		5.70 br s			
5-OH	9.75 br s		—			
13-OH	8.30 s		10.07 br s			
2′-OH	4.64 d (4.5)		4.98 d (5.5)			
3′-OH	4.78 d (4.5)		4.94 m			
4′-OH	4.76 d (4.5)		4.81 m			
6′-OH	4.80 t (4.5)		4.11 t (5.5)			
1″-OH	—		11.26 s			
2″-OH	4.01 d (4.5)		—			
3″-OH	4.12 m		4.85 m			
4″-OH	4.69 d (4.5)		4.59 br d			
6″-OH	4.45 t (4.5)		4.36 t (4.5)			
-NH-	—		4.65 d (4.5)			

δ Values in ppm. Values in parentheses are coupling constants (Hz). The ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY, long-range ^{1}H–^{13}C COSY and HMBC spectra were measured. Multiplicites of carbon signals were determined by the DEPT method and the off-resonance decoupling technique and are indicated as (s), (d) and (t). *a*) See ref. 3. *b*) Measured in DMSO-d_6. *c*) Measured in DMSO-d_6 + D_2O.

N-containing side chain (*a*). Furthermore, a fragment ion peak at *m*/*z* 147 was attributable to a cinnamoyl moiety (Chart 5).

The electrophysiological effects of the isolated pigments, hydroxysafflor yellow A (**1a**), tinctormine (**2a**), safflor yellow B, and safflomin C, were investigated on single ventricular myocytes of dog by the whole-cell voltage-clamp method.[7] Tinctormine (**2a**) apparently inhibited the slow inward Ca^{2+} currents (approx. 42% of the control) at a concentration of 10^{-5} M and the inhibitory activity was close to that of diltiazem (DIL, IC_{50}: 5×10^{-6} M). These effects were dose-dependent and reversible. However, tinctormine (**2a**) did not affect the activation threshold (-40 mV) or the reversal potential ($+70$ mV) of the Ca^{2+} currents (Fig. 3).

On the other hand, hydroxysafflor yellow A (**1a**), safflor yellow B and safflomin C showed no significant effect on the Ca^{2+} currents.[8]

Recently, a search for Ca^{2+} antagonists which are either more potent in action or more tissue-selective, have less side effects or longer-lasting action than the prototypes, DIL, VER and NIF, has been conducted using traditional medicines.[9,10] Tanshinone (diterpene) from *Salvia* sp.,[11] tetrandrine (bisbenzylisoquinoline alkaloid) from *Stephania* sp.[12] and liriodendrin (tetrahydrofuran lignan glucoside) from *Boerhaavia* sp.,[13] were reported to be selective Ca^{2+} antagonists. Furthermore, omega conotoxin (polypeptide) from a marine snail, *Concus geographus*,[14] and apamin (polypeptide) from bee venom[15] were isolated as Ca^{2+} antagonists which originated from animals. Tinctormine (**2a**) from *C. tinctorius* was demonstrated in this experiment to be a new quinochalcone type Ca^{2+} antagonist. Until more detailed electrophysiological studies are performed on the naturally occurring Ca^{2+} antagonists, it seems difficult to subclassify them. However, by using the data available at the moment and the modification of Fleckenstein's scheme,[16] (**2a**) was preliminarily classified under group B

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of Ca^{2+} antagonists. This subclassification may require some modifications as more information becomes available on a variety of Ca^{2+} antagonists from natural sources.

Experimental

Optical rotations were measured in MeOH solutions using a JASCO DIP-4 automatic polarimeter at 25 °C. IR spectra were taken using a JASCO IRA-2 spectrometer in KBr. UV spectra were taken on a Shimadzu UV-2200 UV-VIS spectrophotometer in MeOH solutions. ^{1}H- and ^{13}C-NMR spectra were taken on a JEOL-GX400 spectrometer in DMSO-d_6 (unless otherwise stated) with tetramethylsilane (TMS) as an internal standard, and chemical shifts are recorded in δ values. ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY, long-range ^{1}H–^{13}C COSY and HMBC were obtained using the usual pulse sequence, and data processing was performed with the standard JEOL software. FAB-MS and high-resolution FAB-MS were obtained with a JEOL JMS-SX 102A spectrometer (ionization voltage, 70 eV; accelerating voltage, 5.0 kV) using glycerol + *m*-nitrobenzyl alcohol or *m*-nitrobenzyl alcohol as a matrix. Sephadex LH-20 (Pharmacia) and polyamide (Wako Pure Chemicals, Osaka, Japan) were used for column chromatography (CC). Merck Kieselgel 60 F_{254} (layer thickness 0.25 mm, 0.5 mm) was used for TLC and prep. TLC, respectively, with solvent system A, *n*-BuOH–HOAc–H_2O (4 : 1 : 2), solvent system B, AcOEt–MeOH–H_2O (100 : 16 : 12) and spots were detected under a UV lamp or by heating after spraying with $Ce(SO_4)_2$–H_2SO_4.

Extraction and Isolation of the Flower Pigments of *Carthamus tinctorius* L. The dried powder of the flower petals (3 kg) of *C. tinctorius* purchased from Tochimoto Tenkaido Co. (Osaka, Japan) were extracted with 60% Me_2CO (5 × 5 l) in the dark at room temperature for 5 d. The combined Me_2CO solutions were concentrated to 3 l *in vacuo*, washed with *n*-hexane (5 × 1 l) and mixed with cellulose powder (3 kg) to give a reddish brown paste. The paste was washed with H_2O (10 × 1 l) and filtered *in vacuo* to give a red paste and a yellowish brown filtrate. The red paste was washed with 60% Me_2CO, and the washings (20 l) were evaporated at 30 °C *in vacuo* to give a reddish brown residue. The residue was applied to a column of Sephadex LH-20 (65 × 2.5 cm) using H_2O with an increasing proportion of MeOH as the eluent to give two fractions. Carthamin was obtained from fraction 2, after evaporation, as a reddish brown powder with a metallic luster (*Rf* 0.49 in solvent system A, 900 mg, 0.03%). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 2900, 1620 (conjugated C=O), 1600, 1500, 1400 (aromatic). UV λ_{max}^{MeOH} nm (log ε): 280 (4.80), 380 (6.83), 520 (4.40). FAB-MS (negative ion mode) *m/z*: 909 $[M-H]^-$. The ^{1}H- and ^{13}C-NMR spectra were in good agreement with those reported for carthamin.[3] The yellow brown filtrate (10 l) was concentrated *in vacuo* and lyophilized. The lyophilizate (352 g) was dissolved in MeOH (1500 ml) and filtered. AcOEt (3 l) was added to the filtrate with occasional shaking to give a yellow precipitate (252 g). The precipitates were chromatographed on a column of Sephadex LH-20 (70 × 6 cm i.d.) with 50% MeOH, and fractions (300 ml each) were collected. Fractions 2—6 were pooled, evaporated to dryness *in vacuo*, and subjected to further purification using prep. TLC with solvent system A to give hydroxysafflor yellow A (**1a**) (*Rf* 0.33, 2.8 g, 0.1% w/w dry powder) as an amorphous yellow powder. $[\alpha]_D$ −54.3° (*c* = 0.1, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1640, 1610 (C=O). UV λ_{max}^{MeOH} nm (log ε): 280 (4.6), 400 (4.4). High-resolution FAB-MS (negative ion mode) *m/z*: 611.1598 $[M-H]^-$, Calcd for $C_{27}H_{31}O_{16}$: 611.1612. ^{1}H-NMR (400 MHz, DMSO-d_6) and ^{13}C-NMR (100 MHz, DMSO-d_6) spectral data: see Table I. Safflor yellow B was obtained from fractions 7—14 by prep. TLC with solvent system A, as an amorphous yellow powder, (*Rf* 0.42, 1.5 g, 0.05%), $[\alpha]_D$ +208°C (*c* = 0.1, MeOH). The negative ion FAB-MS *m/z*: 1061 $[M-H]^-$ and the ^{1}H- and ^{13}C-NMR spectral data were superimposed over those reported for safflor yellow B.[17] Repeated column chromatography of fractions 15—19 on Sephadex LH-20 (35 × 1.5 cm i.d.) and prep. TLC using solvent system B afforded safflomin C as a yellow amorphous powder (*Rf* 0.2, 82 mg, 0.003%), $[\alpha]_D$ +50° (*c* = 0.3, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1700, 1650, 1600 (C=O), 1520, 1400, 1230. FAB-MS (negative ion mode) *m/z*: 613 $[M-H]^-$ and the ^{1}H- and ^{13}C-NMR spectral data obtained were consistent with those reported for safflomin C.[2] Fractions 20—25 were collected and evaporated to dryness *in vacuo*. The residue (1.3 g) was rechromatographed on a polyamide column (45 × 2.5 cm i.d.) using 50% MeOH as an eluent and was further purified by prep. TLC with solvent system B to afford tinctormine (**2a**) (*Rf* 0.15, 50 mg), as an amorphous yellow powder. $[\alpha]_D$ −206° (*c* = 0.1, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1620, 1600 (C=O). UV λ_{max}^{MeOH} (log ε): 405 (4.5), 275 (4.5). High-resolution FAB-MS (negative ion mode) *m/z*: 592.1652 $[M-H]^-$, Calcd for $C_{27}H_{30}NO_{14}$: 592.1667. Linked scan FAB-MS (positive ion mode) *m/z*: 594 $[M+H]^+$, 432 $[(M+H)-Glc]^+$, 289 $[(M+H)-Glc-160]^+$, 220, 147 [cinnamoyl residue]$^+$, 142 $[160-H_2O]^+$. ^{1}H-NMR (400 MHz, DMSO-d_6) and ^{13}C-NMR (100 MHz, DMSO-d_6) data are shown in Table I.

Acetylation of Hydroxysafflor Yellow A (1a) A mixture of **1a** (25 mg), acetic anhydride (0.5 ml) and pyridine (0.5 ml) was allowed to stand overnight at room temperature. The residue obtained by the usual work-up was purified by prep. TLC with AcOEt–benzene (1 : 1) to give a dodeca-*O*-acetyl derivative (**1b**). Other acetates produced were not further purified. Compound **1b** was obtained as a yellow amorphous powder (2.5 mg), $[\alpha]_D$ −67° (*c* = 0.22, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1750 (C=O), 1597, 1500, 1450. FAB-MS (positive ion mode) *m/z*: 1117 $[M+H]^+$, 1057 $[(M+H)-CH_3COOH]^+$, 727 $[(M+H)-Glc\ (Ac\times4)-CH_3COOH]^+$, 331 $[Glc\ (Ac\times4)]^+$; ^{1}H-NMR (270 MHz, $CDCl_3$): δ_H 1.84, 1.99, 2.01 (Ac × 2), 2.06, 2.08 (Ac × 3), 2.11, 2.32, 2.35 (Ac × 2), δ_H 3.4—5.5 (10H, C<u>H</u>−OAc × 10), 3.55 (1H, dd, *J* = 9.5, 4 Hz, 5′-H), 3.72 (1H, dd, *J* = 10, 4 Hz, 5″-H), 4.30 (1H, d, *J* = 9.5 Hz, 1′-H), 4.95 (1H, d, *J* = 10 Hz, 1″-H), 7.15 (2H, d, *J* = 8.5 Hz, 12-, 14-H), 7.63 (2H, d, *J* = 8.5 Hz, 11-, 15-H), 7.75 (1H, d, *J* = 15.5 Hz, 8-H), 8.05 (1H, d, *J* = 15.5 Hz, 9-H).

Acetylation of Tinctormine (2a) Tinctormine (**2a**) (10 mg) was treated with acetic anhydride (0.5 ml) and pyridine (0.5 ml) overnight at room temperature. The reaction mixture was worked up in the usual manner and purified as mentioned above to give a deca-*N*,*O*-acetyl derivative (**2b**, 3 mg) as a yellow amorphous powder, $[\alpha]_D$ −84° (*c* = 0.2, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3330 (–OH), 1760 (C=O), 1658 (N–CO) cm^{-1}. High resolution FAB-MS *m/z*: 1014.2854 $[M+H]^+$ (Calcd for $C_{47}H_{52}NO_{24}$: 1014.2879), 1013.2781 $[M]^+$ (Calcd for $C_{47}H_{51}NO_{24}$: 1013.2801), 954.2629 $[(M+H)-CH_3COOH]^+$ (Calcd for $C_{45}H_{48}NO_{22}$: 954.2668), 683.1830 $[(M+H)-Glc\ (Ac\times4)]^+$ (Calcd for $C_{33}H_{33}NO_{15}$: 683.1850), 641.1733 $[(M+H)-Glc\ (Ac\times4)-Ac]^+$ (Calcd for $C_{31}H_{31}NO_{14}$: 641.1744), 331.1038 $[Glc\ (Ac\times4)]^+$ (Calcd for $C_{14}H_{19}O_9$: 331.1029). ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H 1.85, 1.98, 2.01 (Ac × 2), 2.05 (Ac × 2), 2.13, 2.18, 2.23, 2.32, 3.60 (1H, dd, *J* = 9.5, 2 Hz, 5′-H), 3.70 (1H, dd, *J* = 11, 9.5 Hz, 6′-H), 4.02 (1H, d, *J* = 9.5 Hz, 1′-H), 4.03 (1H, dd, *J* = 11, 2 Hz, 6′-H), 4.22 (2H, m, 6″-H), 4.98 (1H, t, *J* = 9.5 Hz, 4′-H), 5.24 (1H, dd, *J* = 7.5, 3.5 Hz, 5″-H), 5.28 (1H, t, *J* = 9.5 Hz, 3′-H), 5.30 (1H, t, *J* = 9.5 Hz, 2′-H), 5.55 (1H, dd, *J* = 7.5, 3.5 Hz, 4″-H), 6.21 (1H, d, *J* = 3.5 Hz, 3″-H), 6.61 (1H, s, 6-H), 7.14 (2H, d, *J* = 8.5 Hz, 12-, 14-H), 7.67 (2H, d, *J* = 8.5 Hz, 11-, 15-H), 7.88 (1H, d, *J* = 16 Hz, 8-H), 8.27 (1H, d, *J* = 16 Hz, 9-H), 10.62 (1H, br s, 1″-OH), 18.78 (1H, s, 3-OH).

Assay of Ca^{2+} Current Inhibitory Activity Single verntricular myocytes of dogs were obtained by the enzymatic dissociation method.[18] Briefly, collagenase (0.04% w/v, Sigma, type 1) in nominally Ca^{2+}-free Tyrode solution was perfused through the coronary arteries with a Langendorff apparatus for 30 min at 37 °C. A small piece of the ventricular tissue was dissected and gently agitated in the recording chamber filled with Tyrode solution. Ca^{2+} currents were recorded using whole-cell voltage clamp techniques.[7] The tyrode solution consisted of 136.5 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.53 mM $MgCl_2$, 10.0 mM glucose and 5.5 mM HEPES, (pH 7.4 adjusted with NaOH). All experiments were performed at 35 °C.

References and Notes

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