TINCTORMINE, A NOVEL Ca^{2+} ANTAGONIST *N*-CONTAINING QUINOCHALCONE *C*-GLYCOSIDE FROM *CARTHAMUS TINCTORIUS* L.

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Tinctormine (**1a**), *N*-containing quinochalcone glycoside, has been isolated from safflower and its structure has been determined by means of 2-D NMR spectroscopy including HMBC; **1a** was proved to be a potent Ca^{2+} antagonist.

KEYWORDS Tinctormine; Ca^{2+} antagonist; *Carthamus tinctorius* L.; Compositae

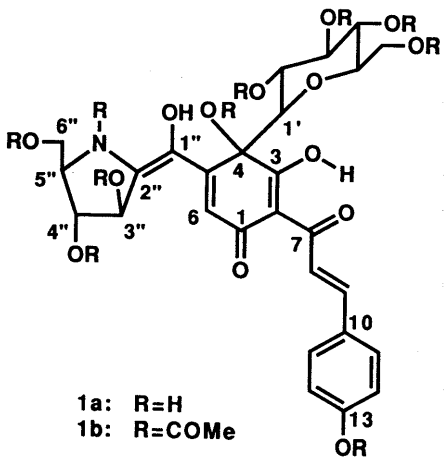
Safflower (*Carthamus tinctorius* L., Compositae), a common remedy in the Chinese Materia Medica, has been used for gynechological diseases and heart diseases[1] and as a sedative and anti-inflammatory.[2,3] Recently, the demand for Ca^{2+}antagonists[4] in the treatment of cardiovascular diseases as anti-anginal and anti-arrythmic, has been directed to the naturally occurring active compounds. During our research for a Ca^{2+} antagonist from safflower, we have isolated a new quinochalcone *C*-glucosyl yellow pigment, tinctormine (**1a**), from the acetone extract of the dried flower petals together with the known compounds carthamin[5] and safflor yellow B.[6] Among these, **1a** was found to be a potent Ca^{2+}antagonist. In this paper, we report the structure determination and the electrophysiological screening of tinctormine (**1a**).

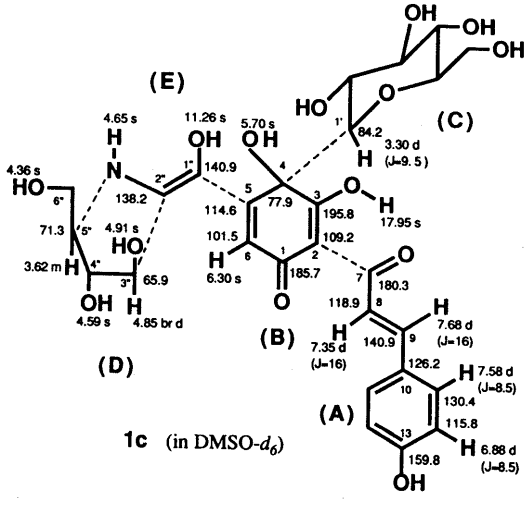
The dried flower petals of *Carthamus tinctorius* L. (3 kg) were extracted with 60% acetone at room temperature and concentrated *in vacuo*. Repeated column chromatography on Sephadex LH-20 and polyamide and subsequent preparative TLC gave tinctormine (**1a**, 0.001%).

Tinctormine (**1a**) was obtained as yellow amorphous powder, $[\alpha]_D$ - 206° (*c*=0.1, MeOH), and the IR spectrum suggested the presence of hydroxyl (3400 cm^{-1}) and 1,3-diketone (1620, 1600 cm^{-1}) moiety. The negative ion FAB-MS of **1a** exhibited a quasi-molecular ion peak at *m/z* 592 $[M-H]^-$, and its molecular formula was determined to be {$[(C_{27}H_{31}O_{14}N)-H]^-$; Found, 592.1652; Calcd for 592.1667} by high-resolution FAB-MS. In addition, a fragment ion peak at *m/z* 430 $[M-H-Glc]^-$ indicates the loss of glucose unit, and a fragment ion at *m/z* 147 is attributable to a cinnamoyl residue.

Acetylation of **1a** with acetic anhydride and pyridine (over night) afforded a deca-*O*,*N*-acetylated compound (**1b**), and the negative ion FAB-MS exhibited an $[M-H]^-$ peak at *m/z* 1013 {$[(C_{47}H_{52}O_{24}N)-H]^-$; Found, 1013.2781; Calcd for 1013.2801}. It showed IR ($CHCl_3$) absorption at ν_{max} 3330 (OH), 1760 (CO) and 1658 (N-CO) cm^{-1}. The ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of **1b** showed signals for deca acetyl methyls at δ_H 1.85, 1.98, 2.01 (Ac x 2), 2.05 (Ac x 2), 2.13, 2.18, 2.23, 2.32 and signals at δ_H 3.60 (1H, dd, *J*= 9, 2 Hz, H-5'), 3.70 (1H, dd, *J*= 12.5, 9 Hz, H-6'), 4.02 (1H, d, *J*= 9 Hz, H-1'), 4.03 (1H, dd, *J*= 12.5, 2 Hz, H-6'), 4.22 (2H, m, H-6"), 4.98 (1H, t, *J*= 9 Hz, H-4'), 5.24 (1H, dd, *J*= 7.5, 4 Hz, H-5"), 5.28 (1H, t, *J*= 9 Hz, H-3'), 5.30 (1H, t, *J*=9 Hz, H-2'), 5.55 (1H, dd, *J*= 7.5, 4 Hz, H-4"), 6.21 (1H, d, *J*= 4 Hz, 3"-H), 6.61 (1H, s, H-6), 7.14 (2H, d, *J*=8.5 Hz, H-12, H-14), 7.67 (2H, d, *J*=8.5 Hz, H-11, H-15), 7.88 (1H, d, *J*=16 Hz, H-8), 8.27 (1H, d, *J*= 16 Hz, H-9), 10.62 (1H, brs, 1"-OH) and 18.78 (1H, s, 3-OH).

The ^{1}H- and ^{13}C-NMR spectra of **1a** (in DMSO-d_6) indicate the presence of two carbonyl carbons (δ_C 185.7 and 180.3), four olefinic groups (δ_C 195.8, 140.9, 140.9,[7] 138.2, 118.9, 114.6, 109.2 and 101.5; δ_H 7.68, 7.35 and 6.30), an aromatic group (δ_C 159.8, 130.4, 126.2





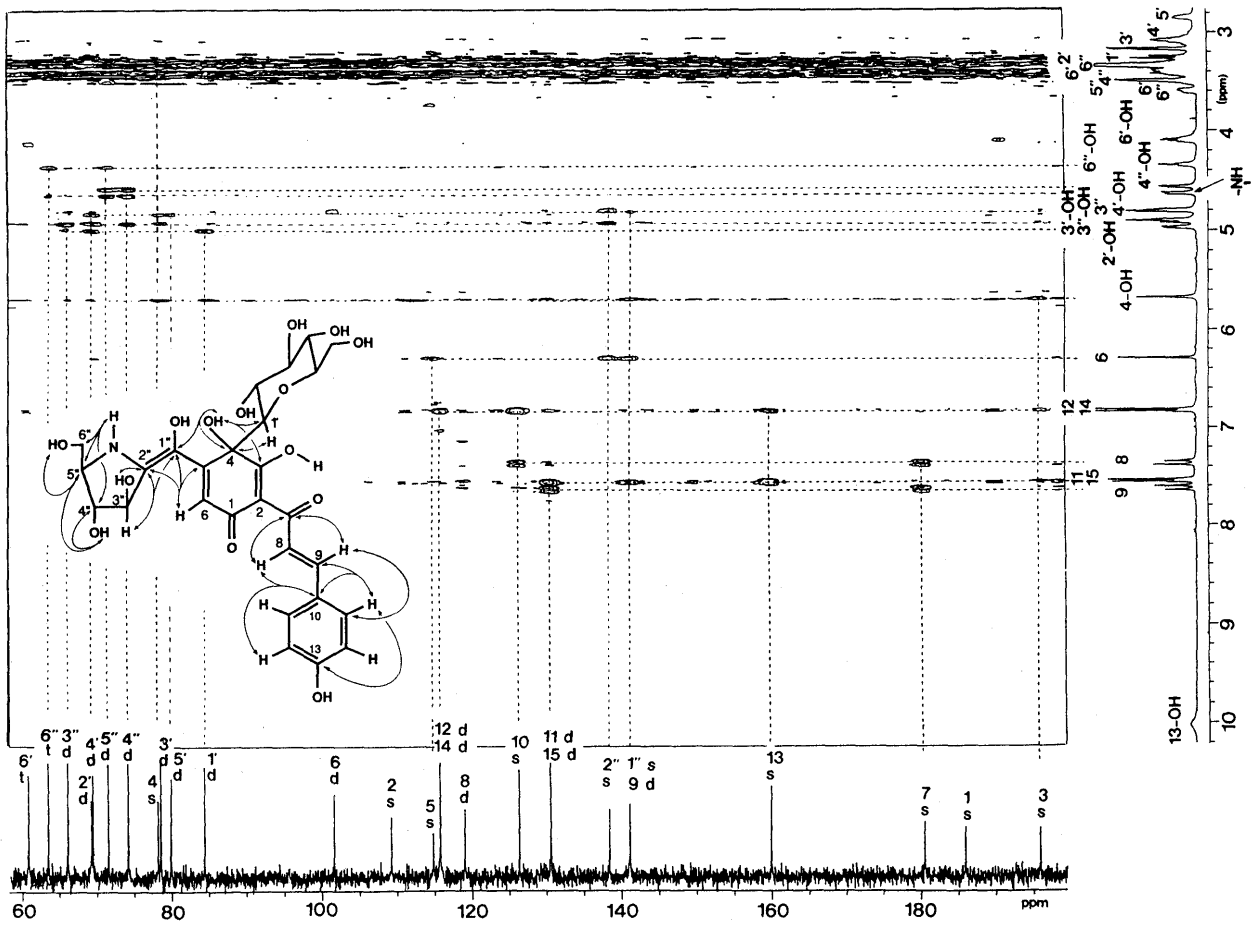


Fig. 1. HMBC Spectrum of Tinctormine (**1a**) in DMSO-*d6* (25 mg, 25°C, 14h run)

and 115.8; δ_H 6.88 and 7.58), a glucose unit (δ_C 84.2, 79.7, 78.3, 69.2, 69.0 and 60.7; δ_H 3.54, 3.50, 3.45, 3.30, 3.17, 3.15 and 2.95), three carbinols (δ_C 73.9, 65.9 and 63.3; δ_H 4.85, 3.67, 3.57 and 3.47), sp^3 methine carbon (δ_C 71.3; δ_H 3.62), an sp^3 quaternary carbon (δ_C 77.9) and two enolic hydroxyl (exchangeable with D_2O) at δ_H 17.95 and 11.26, respectively.[8] These data coupled with the detailed analysis of ^1H-^{13}C COSY spectrum suggested that **1a** has a pattern similar to that of safflor yellow A[5] or safflomin A,[9] and suggested five partial structures **A** – **E** (see Formula **1c**). The partial structure **A** was further detected by the loss of 147 mass unit in FAB-MS, indicating the cinnamoyl side chain with C-7 as an α,β-unsaturated carbonyl carbon. The partial structure **B** was confirmed with a carbonyl carbon C-1, an enolic carbon C-3, sp^2 methine carbon C-6 and sp^2 quaternary carbon (δ 114.6, C-5), suggesting a carbon substitution at C-5, not at C-6 which was assigned for an α-conjugated olefinic carbon.[10] The HMBC of **1a** showed a long-range correlation between the enolic carbon C-3 (δ_C 195.8) and the proton signal at δ_H 5.70 (4-OH), and no correlation was detected with the carbonyl carbon C-1 (Fig. 1). The carbon signal at δ_C 77.9 (C-4) is connected with the proton signals at δ_H 3.30 (1′-H) and 5.70 (4-OH) in terms of long-range correlation indicating the partial structure **C**. Although connectivities could not be detected between the quaternary carbons (C-2 and C-7), there must be a chemical bonding between them since the 3-OH group is internally hydrogen-bonded with the 7-ketone group. The ^1H-^1H COSY spectrum of **1a** suggested the partial structure **D**. The quaternary carbon signal at δ_C 140.9 (C-1") is correlated with the proton signal at δ_H 6.30 (6-H), 4-OH proton and the proton at δ_H 4.85 (3"-H), while the quaternary carbon signal at δ_C 138.2 (C-2") is correlated with 3"-H and the protons at δ_H 4.91 (3"-OH) and 6.30 (6-H). In turn, the carbon signal at δ_C 73.9 (C-4") was correlated with the proton signals at δ_H 4.59 (4"-OH) and 4.65 (NH-), and the signal at δ_C 71.3 (C-5") is correlated with proton signal at δ_H 4.36 (6"-OH), 4.65 (-NH-), and 4.59 (4"-OH). Some other significant long-range correlations are shown by arrows in the formula in Fig. 1. The ^1H-^1H COSY of **1a** (in DMSO-*d6*) detects a cross peak between 5"-H (δ_H 3.62) and -NH- (δ_H 4.65) indicates attachment of a nitrogen atom linking C-5" and C-2" rather than an oxygen to give ether. The above data suggest the partial structure **E**. Furthermore, C-1" is exist in the enol form rather than the keto form to establish the enamine system with -NH-group, and the ^{13}C-NMR spectrum of **1a** indicates only two carbonyls (C-1and C-7). The relative stereochemistry of **1a** was determined by the results obtained from NOE experiments and coupling constants. Irradiation of 6-H, a negative NOE,[11] was observed in 3"-H and irradiation of 3"-H, resulted in a negative NOE in 6-H: consequently, the geometry between C-1" and C-2" was established to be *Z* and all the sugar substitutions are equatorially oriented on a 6-membered ring; however, the stereochemistry around C-4 still not clear.

Based on the foregoing findings, the structure of tinctormine was determined to be **1a**.

The electrophysiological screening of tinctormine (**1a**) and safflor yellow B using a whole-cell voltage-clamp method[12] on single ventricular myocytes of dog revealed that tinctormine (**1a**, 10^{-5}M) selectively inhibited the slow inward Ca^{2+}currents

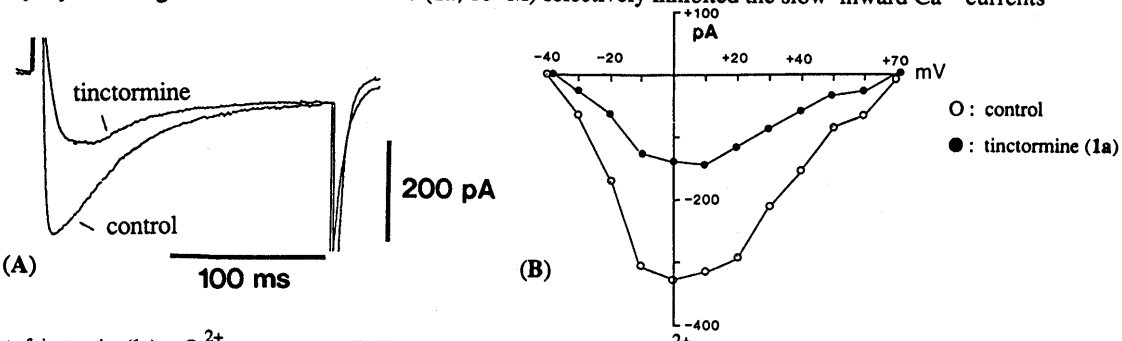


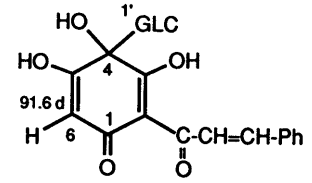
Fig. 2. Effect of tinctormine (**1a**) on Ca^{2+}currents were studied in single canine ventricle cells. (A). Ca^{2+}currents were measured by depolarized pulses (0.2 Hz, 200 msec duration) from holding potential of -40 mV to 0 mV. Tinctormine (**1a**, 10^{-5}M) was applied by perfusion. (B). Currents-voltage relation for Ca^{2+}currents is plotted against the peak amplitude taken before (O) and after (●) exposure to tinctormine (**1a**, 10^{-5}M).

(approx. 42% of control). These effects are dose-dependent and reversible. However, tinctormine (**1a**) did not affect the activation threshold (-40 mV) or the reversal potential (+70 mV) of the Ca^{2+}currents (Fig. 2). In addition, the inhibitory activity of **1a** on Ca^{2+} currents was close to that shown by diltiazem (IC_{50}: $5x10^{-6}$M) used in this preparation; thus, we conclude that tinctormine (**1a**) is a potant Ca^{2+} antagonist. On the other hand, safflor yellow B showed no significant effects on the Ca^{2+}currents.[13]

Our present results provided the first naturally occuring quinochalcone type as a Ca^{2+} antagonist from safflower; its pharmacological mechanism(s) will be a subject of particular interest to be discussed in the near future.

REFERENCES AND NOTES

1) "Dictionary of Chinese Materia Medica," Publishing House of Science and Technology of Shanghai, 1985, p. 992.
2) H. Kutsuna, S. Fujii, K. Kitamura, K. Komatsu and M. Nakano, *Yakugaku Zasshi*, **108**, 1101 (1988).
3) Y. Kasahara, K. Kumaki, T. Sato and S. Katagiri, *Shoyakugaku Zasshi*, **43**, 331(1989).
4) W. G. Nayler, " *Calcium Antagonists*," Academic Press, London, 1988.
5) Y. Takahashi, N. Miyasaka, S. Tasaka, I. Miura, S. Urano, M. Ikura, K. Hikichi, T. Matsumoto and M. Wada, *Tetrahedron Lett.*, **23**, 5163 (1982).
6) Y. Takahashi, K. Saito, M. Yanagiya, M. Ikura, K. Hikichi, T. Matsumoto and M. Wada, *Tetrahedron Lett.*, **25**, 2471 (1984).
7) The off-resonance spectrum was measured and it was clear that the carbon signal at δ 140.9 was assignable for two overlapping carbons, sp^2 methine carbon (C-9, d) and an oxygenated quaternary carbon (C-1", s).
8) **1a:** ^{1}H-NMR (400 MHz, DMSO-*d6*): δ_H 2.95 (1H, m, H-5'), 3.15 (1H, m, H-4'), 3.17 (1H, m, H-3'), 3.30 (1H, d, *J*= 9 Hz, H-1'), 3.45 (1H, m, H-2'), 3.47 (1H, m, H-6"), 3.50 (1H, m, H-6'), 3.54 (1H, m, H-6'), 3.57 (1H, m, H-4"), 3.62 (1H, m, H-5"), 3.67 (1H, m, H-6"), 4.11 (6'-OH), 4.36 (6"-OH), 4.59 (4"-OH), 4.65 (s, NH), 4.85 (1H, brd, H-3"), 4.91 (1H, 3"-OH), 4.94 (1H, 3'-OH), 4.98 (2'- OH), 5.70 (s, 4-OH), 6.30 (1H, s, H-6), 6.88 (2H, d, *J*= 8.5 Hz, H-12, H-14), 7.35 (1H, d, *J*=16 Hz, H-8), 7.58 (2H, d, *J*= 8.5 Hz, H-11, H-15), 7.68 (1H, d, *J*= 16 Hz, H-9), 11.26 (s, 1"-OH), 17.95 (s, 3-OH); ^{1}H-NMR (400 MHz, DMSO-*d6* + D_2O): δ_H 2.89 (1H, dd, *J*= 9.5, 2 Hz, H-5'),.3.10 (1H, t, *J*= 9.5 Hz, H-4'), 3.12 (1H, t, *J*= 9.5 Hz, H-3'), 3.26 (1H, d, *J*= 9.5 Hz, H-1'), 3.37 (1H, t, *J*= 9.5 Hz, H-2'), 3.38 (1H, dd, *J*= 11, 3.5 Hz, H-6"), 3.41 (1H, dd, *J*= 11, 2 Hz, H-6'), 3.46 (1H, dd, *J*= 7.5, 3.5 Hz, H-4"), 3.52 (1H, dd, *J*= 11, 9.5 Hz, H-6'), 3.57 (1H, br d, *J* = 7.5, 3.5 Hz, H-5"), 3.58 (1H, dd, *J*= 11, 7.5 Hz, H-6"), 4.79 (1H, d, *J*= 3.5 Hz, H-3"), 6.37 (1H, s, H-6), 6.81 (2H, d, *J*= 8.5 Hz, H-12, H-14), 7.28 (1H, d, *J*=16 Hz, H-8), 7.52 (2H, d, *J*= 8.5 Hz, H-11, H-15), 7.63 (1H, d, *J*= 16 Hz, H-9); ^{13}C-NMR (100 MHz, DMSO-*d6*): δ_C 185.7 (s, C1), 109.2 (s, C2), 195.8 (s, C3), 77.9 (s, C4), 114.6 (s, C5), 101.5 (d, C6), 180.3 (s, C7), 118.9 (d, C8), 140.9 (d, C9), 126.2 (s, C10), 130.4 (d, C11/15), 115.8 (d, C12/14), 159.8 (s, C13), 84.2 (d, C1'), 69.0 (d, C2'), 78.3 (d, C3'), 69.2 (d, C4'), 79.7 (d, C5'), 60.7 (t, C6'), 140.9 (s, C1"), 138.2 (s, C2"), 65.9 (d, C3"), 73.9 (d, C4"), 71.3 (d, C5"), 63.3 (t, C6").
9) J. Onodera, H. Obara, M. Osone, Y. Maruyama and S. Sato, *Chem. Lett.*, **1981**, 433.
10) Substitution at C-6 cannot be accepted in **1a**, and as such, C-5 cannot be detected around δ_C 101.5, d. A nearly similar chemical shift for C-6 was observed in safflor-metabolin (**2**), resulting from the anaerobic incubation of safflor yellow B with human intestinal bacteria, M. R. Meselhy, S. Kadota, M. Hattori and T. Namba, *J. Nat. Prod.*, (*in press*).
11) J. H. Noggle and R. E. Shirmer, *The Nuclear Overhauser Effect*, Academic Press, Londen, 1971, p. 17.
12) O. P. Hamill, A. Marty, E. Neher, B. Sakmann and F. J. Sigworth; *Pflugers Arch.*, **391**, 85 (1981).
13) Since carthamin was unstable and easily decomposed in aqueous solutions, we could not investigate its activity.



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(Received October 26, 1992)