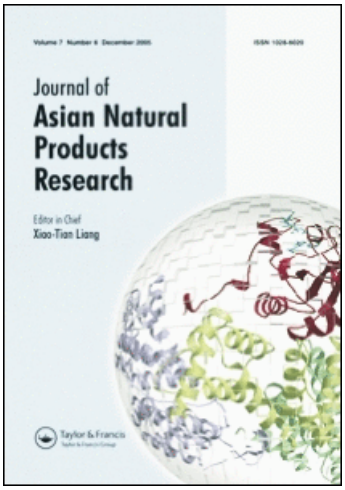


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# New spermidines from the florets of *Carthamus tinctorius*

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Two new spermidine compounds, namely safflospermidine A (**1**) and safflospermidine B (**2**), together with two known compounds, $N^1$,$N^5$,$N^{10}$-(*Z*)-tri-*p*-coumaroylspermidine (**3**) and $N^1$,$N^5$,$N^{10}$-(*E*)-tri-*p*-coumaroylspermidine (**4**), were isolated from the florets of *Carthamus tinctorius* L. Their structures were elucidated by spectroscopic means.

**Keywords:** *Carthamus tinctorius*; safflower; spermidine; tri-*p*-coumaroylspermidine; safflospermidine A; safflospermidine B



## 1. Introduction

The florets of *Carthamus tinctorius* (Compositae) have been used as Chinese folk medicine for over 2500 years. It showed therapeutic potential for coronary heart disease, stroke, gynecological disease, angina and hypertension.[1,2] Now, *C. tinctorius* L. is widely planted in Henan, Sichuan and the Xinjiang Uygur Autonomous Region of China. Up to now, many constituents such as flavonoids,[3] alkaloids,[4] lignans[2] and fatty acids[1] were isolated from safflower. In the course of search for active components from the fresh flowers of this plant, two new tri-*p*-coumaroylspermidines, known as safflospermidine A (**1**) and safflospermidine B (**2**), together with two known spermidines, $N^1$,$N^5$,$N^{10}$-(*Z*)-tri-*p*-coumaroylspermidine (**3**) and $N^1$,$N^5$,$N^{10}$-(*E*)-tri-*p*-coumaroylspermidine (**4**),[6] were isolated from the florets of safflower. Their structures were elucidated by spectroscopic means.

## 2. Results and discussion

Compound **1** was obtained as a white powder. The IR spectrum of **1** showed the presence of hydroxyl groups (3266 $cm^{-1}$), carbonyl groups (1650 $cm^{-1}$) and aromatic rings (1581 and 1513 $cm^{-1}$). It has the molecular formula $C_{34}H_{38}N_3O_6$, on the basis of a quasi-molecular ion peak $[M + H]^+$ at *m/z* 584.2767 in the positive ion HR-ESI-MS spectrum.

The $^1$H NMR spectrum showed a set of *cis* olefinic hydrogen signals at $\delta$ 6.47, 6.51 (1H, ABq, $J = 12.5$ Hz) and 5.86, 5.89 (1H, ABq, $J = 12.5$ Hz) and two sets of *trans* olefinic hydrogen signals at $\delta$ 7.37, 7.42 (1H, ABq, $J = 15.5$ Hz) and 6.34, 6.37 (1H, ABq, $J = 15.5$ Hz) and $\delta$ 7.37, 7.42 (1H, ABq, $J = 15.5$ Hz) and 6.29, 6.31 (1H, ABq, $J = 15.5$ Hz), which suggested the existence of three double bonds in the structure of **1**. Additionally, three sets of *p*-substituted phenyl hydrogen signals at $\delta$ 6.73, 7.33

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Table 1. $^{13}C$ NMR spectral data for compounds **1**, **2** and **3** (in $CD_3OD$).

| Position | 1 | 2 | 3 |
|---|---|---|---|
| 1 | | | |
| 2 | 38.2 | 38.3 | 38.0 |
| | 37.9 | 37.9 | 37.7 |
| 3 | 29.7 | 29.8 | 29.3 |
| | 28.3 | 28.3 | 27.9 |
| 4 | 46.0 | 46.0 | 46.0 |
| | 44.0 | 44.0 | 44.1 |
| 5 | | | |
| 6 | 49.7 | 49.7 | 49.7 |
| | 47.7 | 47.6 | 47.8 |
| 7 | 26.9 | 27.0 | 27.0 |
| | 25.7 | 25.8 | 25.8 |
| 8 | 28.0 | 27.7 | 27.7 |
| | 27.6 | 27.3 | 27.3 |
| 9 | 40.1 | 39.9 | 40.0 |
| | 39.9 | 39.7 | 39.8 |
| 10 | | | |
| 1′ | 127.7 | 127.7 | 128.1 |
| | 127.6 | 127.6 | 128.0 |
| 2′, 6′ | 130.6 | 130.6 | 131.2 |
| 3′, 5′ | 116.7 | 116.7 | 116.1 |
| | | | 116.0 |
| 4′ | 160.6 | 160.6 | 159.3 |
| | 160.5 | 160.5 | 159.2 |
| 7′ | 141.9 | 142.0 | 138.3 |
| | 141.8 | 141.9 | 138.0 |
| 8′ | 118.5 | 118.4 | 121.8 |
| | 118.4 | 118.2 | 121.7 |
| 9′ | 169.3 | 169.3 | 170.5 |
| | 169.2 | 169.2 | 170.4 |
| 1″ | 128.2 | 128.2 | 128.2 |
| | 128.1 | 128.1 | 128.1 |
| 2″, 6″ | 131.2 | 131.2 | 131.2 |
| | 131.1 | 131.1 | |
| 3″, 5″ | 116.4 | 116.4 | 116.4 |
| 4″ | 159.3 | 159.3 | 159.3 |
| | | 159.2 | 159.2 |
| 7″ | 134.9 | 134.9 | 134.9 |
| | 134.6 | 134.6 | 134.7 |
| 8″ | 121.0 | 121.0 | 121.0 |
| | 120.9 | | |
| 9″ | 172.0 | 172.0 | 172.0 |
| | 171.9 | 171.9 | 171.9 |
| 1‴ | 127.7 | 128.1 | 128.1 |
| | 127.6 | 128.0 | 128.0 |
| 2‴, 6‴ | 130.6 | 132.2 | 132.3 |
| | | 132.1 | 132.2 |
| 3‴, 5‴ | 116.7 | 116.0 | 116.1 |
| | | 115.9 | 116.0 |
| 4‴ | 160.6 | 159.3 | 159.3 |
| | 160.5 | 159.2 | 159.2 |
| 7‴ | 141.9 | 137.8 | 137.8 |
| | 141.8 | 137.7 | 137.7 |
| 8‴ | 118.5 | 121.8 | 121.8 |
| | 118.4 | 121.7 | 121.7 |
| 9‴ | 169.3 | 170.5 | 170.5 |
| | 169.2 | 170.4 | 170.4 |

(each 4H, AA′BB′, $J = 8$ Hz, Ar-H) and $\delta$ 6.66, 7.15 (each 2H, AA′BB′, $J = 8$ Hz, Ar-H), and seven methylene hydrogen signals were demonstrated. The $^{13}C$ NMR spectrum (Table 1) showed 28 sets of carbon signals (a majority of carbon signals were twin) with three sets of *p*-substituted phenyl carbon signals, seven sets of methylene carbon signals, three sets of olefinic carbon signals, and three sets carbonyl carbon signals. Based on the NMR data, it was concluded that there were a *cis*-*p*-coumaroyl group and two *trans*-*p*-coumaroyl groups in **1**. Furthermore, a spermidine $H_2NCH_2CH_2CH_2NHCH_2CH_2$-$CH_2CH_2NH_2$ moiety in **1** was deduced on the basis of seven methylene signals of NMR and the molecular formula ($C_{34}H_{38}N_3O_6$). Therefore, it was confirmed that compound **1** was a spermidine derivative with three *p*-coumaroyl moieties. Compared with those of $N^1$,$N^5$,$N^{10}$-(*E*)-tri-*p*-coumaroylspermidine,[6] the $^1H$ and $^{13}C$ NMR data of **1** were similar to those of $N^1$,$N^5$,$N^{10}$-(*E*)-tri-*p*-coumaroylspermidine, except for the NMR data of a carbonyl group and one double bond with *cis* configuration. The connectivity of the *cis*-*p*-coumaroyl group and spermidine was established on the basis of the key HMBC experiment. The HMBC correlations of H-4(H-6)/C-9″, H-2/C-9′ and H-9/C-9‴ suggested that a *cis*-*p*-coumaroyl group was linked to N-5 of the spermidine moiety, while two *trans*-*p*-coumaroyl moieties were located at N-1 and N-10, respectively. Furthermore, three carbonyl signals at $\delta$ 169.2/169.3, 172.0/172.9 and 169.2/169.3 could be assigned to C-9′, C-9″ and C-9‴, respectively, by means of the HMBC correlations of H-7′(H-8′)/C-9′, H-7″ (H-8″)/C-9″ and H-7‴(H-8‴)/C-9‴. All these results indicated that the structure of **1** was $N^1$, $N^{10}$-(*E*)-$N^5$-(*Z*)-tri-*p*-coumaroylspermidine, known as safflospermidine A (Figure 1).

Compound **2** was obtained as a white powder. The IR of **2** showed the presence of carbonyl groups (1649 $cm^{-1}$) and aromatic rings (1581 and 1514 $cm^{-1}$), and possessed the molecular formula of $C_{34}H_{38}N_3O_6$, based on HR-ESI-MS at *m/z* 584.2766 $[M + H]^+$.

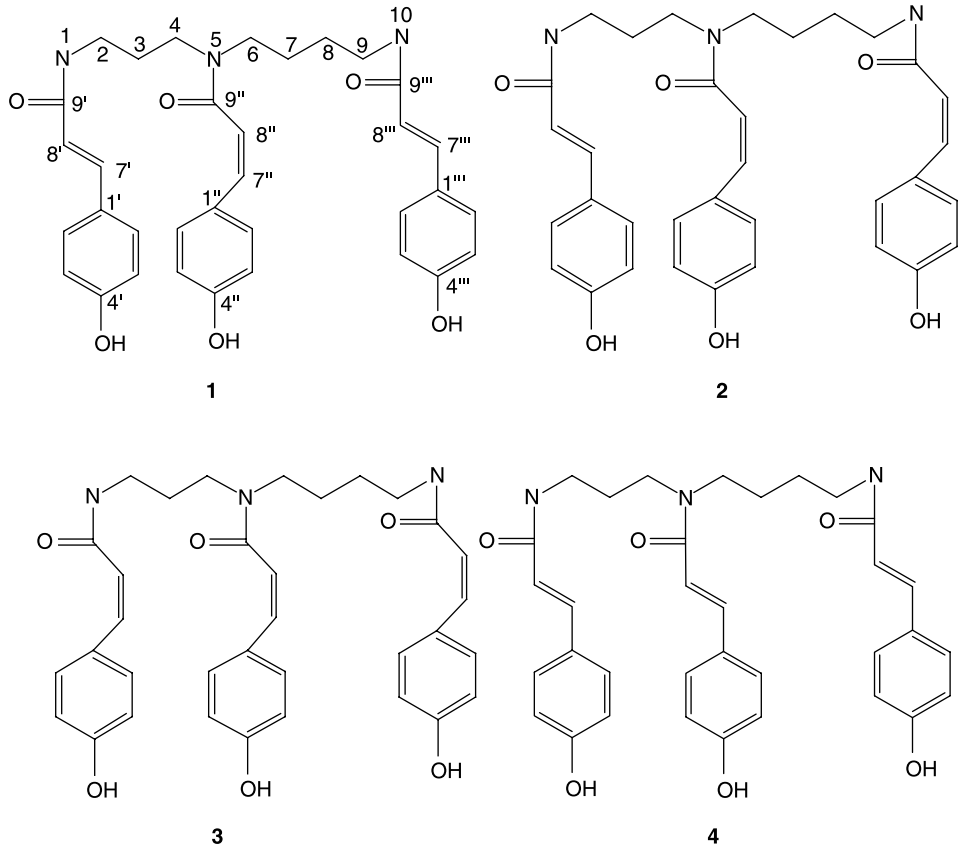


Figure 1. Structures of compounds **1**–**4**.

The $^{1}$H NMR spectrum showed two sets of *cis* olefinic hydrogen signals at $\delta$ 6.53, 6.56 (1H, ABq, $J = 12.5$ Hz) and 5.72, 5.78 (1H, ABq, $J = 12.5$ Hz) and $\delta$ 6.47, 6.50 (1H, ABq, $J = 12.5$ Hz) and 5.86, 5.88 (1H, ABq, $J = 12.5$ Hz), a set of *trans* olefinic hydrogen signals at $\delta$ 7.37, 7.39 (1H, ABq, $J = 15.5$ Hz) and 6.30, 6.36 (1H, ABq, $J = 15.5$ Hz), three sets of *p*-substituted phenyl signals at $\delta$ 6.74, 7.34 (2H, AA′BB′, $J = 8$ Hz, Ar-H), $\delta$ 6.67, 7.31 (each 2H, AA′BB′, $J = 8$ Hz, Ar-H), and $\delta$ 6.67, 7.16 (each 2H, AA′BB′, $J = 8$ Hz, Ar-H), and seven methylene proton signals. The $^{13}$C NMR spectrum also showed 28 sets of carbon signals and was similar to the corresponding carbon signals in **1**. These observations suggested that compound **2** was still a spermidine derivative with three *p*-coumaroyl moieties. But careful analysis revealed that a *cis*-*p*-coumaroyl moiety appeared in **2**, instead of one *trans*-*p*-coumaroyl moiety existed in **1**. These were further confirmed by the HMBC spectrum. In the HMBC spectrum, the correlations of H-9/C-9‴, H-4(H-6)/C-9″ and H-2/C-9′ suggested that two *cis*- and one *trans*-*p*-coumaroyl groups were linked to N-10, N-5 and N-1 of the spermidine moiety, respectively. Therefore, compound **2** was elucidated as $N^{1}$-(*E*)-$N^{5}$,$N^{10}$-(*Z*)-tri-*p*-coumaroylspermidine, known as safflospermidine B (Figure 1).

In addition, two known compounds $N^{1}$,$N^{5}$,$N^{10}$-(*Z*)-tri-*p*-coumaroylspermidine (**3**) and $N^{1}$,$N^{5}$,$N^{10}$-(*E*)-tri-*p*-coumaroylspermidine (**4**) were identified by the MS, $^{1}$H NMR, and $^{13}$C NMR spectra (Figure 1).

The spermidine derivatives such as mono-, di- and tri-*p*-coumaroylspermidines[5–8] have been reported. The cinnamonyl chromophores for the mono- or di-*p*-coumaroylspermidines undergo (*E*) → (*Z*) isomerization under UV light irradiation or normal daylight conditions,[7,8] thus implying that the geometric isomers [(*E*,*E*,*E*)-, (*Z*,*Z*,*Z*)-, (*E*,*Z*,*E*)- and (*E*,*Z*,*Z*)-] of tri-*p*-coumaroylspermidine, e.g. compounds **1**–**4** from *C. tinctorius*, could exist in the natural plants. Additionally, the NMR spectra of compounds **1**–**4** showed complex signals. The same phenomenon existed in the other spermidines. Those were owing to the restricted rotation around the N–C ($sp^2$) bond in the *p*-coumaroylspermidine.[6,9]

## 3. Experimental

### 3.1 General experimental procedures

The IR spectra were recorded on an IMPACT 400 (KBr) spectrometer. The $^1$H NMR and $^{13}$C NMR spectra were run on INOVA-500 spectrometers with TMS as an internal standard and the HR-MS on Finning LTQ FT mass spectrometer. Preparative HPLC was performed with LC-6AD pump (Shimadzu, Japan) equipped with SPD-20A detector (Shimadzu) using an ODS column (YMC, 20 × 250 mm, 5 μm). Silica gel (100–200, 200–300 mesh) and silica gel GF-254 (Branch of Qingdao Haiyang Chemical Plant, China) were used for CC and TLC, respectively.

### 3.2 Plant material

The florets of *C. tinctorius* L. were collected from Changji Hui tribe autonomous prefecture of the Xinjiang Uygur Autonomous Region of China in July 2005. The plant was identified by Professor Lin Ma (Institute of Materia Medica, Peking Union Medical College, and Chinese Academy of Medical Science, China). A voucher specimen has been deposited in the Herbarium of the Department of Medicinal Plants, Institute of Materia Media, Chinese Academy of Medical Sciences, and Peking Union Medical College, Beijing, China.

### 3.3 Extraction and isolation

The florets of *C. tinctorius* L. (5 kg) were exhaustively extracted with 95% EtOH under refluxed conditions. The EtOH extracts were then concentrated under reduced pressure to give a residue (650 g), which was suspended in $H_2O$, and the suspension was then extracted with petroleum ether and EtOAc, successively. The EtOAc extracts were evaporated in vacuum to give a residue (128 g), which was chromatographed over silica gel column eluting with $CHCl_3$–$CH_3OH$ (from 50:1 to 5:1). A total of 100 fractions (200 ml each) were collected and combined in 10 fractions (Fr. 1–10). Fr. 3 (20:1, 2 g) was chromatographed over Sephadex LH-20 column, and eluted with $CH_3OH$–$H_2O$ (45:55) to give three fractions and then Fr. 3–2 was further purified by reversed-phase preparative HPLC using $CH_3OH$–$H_2O$ (55:45, 3 ml/min) as mobile phase to yield compound **3** (90 mg). Fr. 5 (15:1, 3.5 g) was chromatographed over Sephadex LH-20 column and eluted with $CH_3OH$–$H_2O$ (45:55) to give two fractions and then Fr. 5–1 was further purified by reversed-phase preparative HPLC using $CH_3OH$–$H_2O$ (55:45, 3 ml/min) as mobile phase to yield compound **1** (90 mg). Fr. 5–2 was further purified by reversed-phase preparative HPLC using (55:45, 3 ml/min) as mobile phase to yield compound **2** (65 mg). Fr. 6 (10:1, 3 g) was chromatographed over Sephadex LH-20 column and eluted with $CH_3OH$–$H_2O$ (45:55) to give two fractions and then Fr. 6–1 was further purified by reversed-phase preparative HPLC using $CH_3OH$–$H_2O$ (55:45, 3 ml/min) as mobile phase to yield compound **4** (100 mg).

Safflospermidine A (**1**): White powder; IR (KBr) ($cm^{-1}$): 3266, 2942, 2813, 1650, 1581, 1513, 1440, 1221, 1170, 978, 829; $^1$H NMR ($CD_3OD$, 500 MHz) $\delta$: 1.38/1.52 (2H, m, H-8), 1.44/1.62 (2H, m, H-7), 1.64/1.81 (2H, m, H-3), 3.13/3.25 (2H, m, H-2), 3.14/3.28 (2H, m, H-9), 3.25/3.28 (2H, m, H-6), 3.38/3.42 (2H, m, H-4), 5.86/5.89 (ABq, $J$ = 12.5 Hz) (H-8″), 6.29/6.31 (ABq, $J$ = 15.5 Hz) (H-8‴),

6.34/6.37 (ABq, $J = 15.5$ Hz) (H-8′), 6.47/6.51 (ABq, $J = 12.5$ Hz) (H-7″), 6.66 (AA′BB′, $J = 8$ Hz) (H-3″, 5″), 6.73 (AA′BB′, $J = 8$ Hz) (H-3′, 5′), 6.73 (AA′BB′, $J = 8$ Hz) (3‴, 5‴), 7.15 (AA′BB′, $J = 8$ Hz) (H-2″, 6″), 7.33 (AA′BB′, $J = 8$ Hz) (H-2′, 6′), 7.33 (AA′BB′, $J = 8$ Hz) (2‴, 6″) and 7.37/7.42 (ABq, $J = 15.5$ Hz) (H-7′, 7‴); $^{13}$C NMR ($CD_3OD$, 125 MHz) (Table 1); ESI-MS *m/z*: 584 $[M + H]^+$; HR-ESI-MS *m/z*: 584.2767 $[M + H]^+$ (calcd for $C_{34}H_{38}N_3O_6$: 584.2761).

Safflospermidine B (**2**): White powder; IR (KBr) ($cm^{-1}$): 3268, 2940, 2815, 1649, 1581, 1514, 1439, 1220, 1172, 977, 830; $^1$H NMR ($CD_3OD$, 500 MHz) δ: 1.32/1.46 (2H, m, H-8), 1.36/1.54 (2H, m, H-7), 1.63/1.79 (2H, m, H-3), 3.06/3.20 (2H, m, H-9), 3.13/3.30 (2H, m, H-2), 3.27/3.30 (2H, m, H-6), 3.35/3.39 (2H, m, H-4), 5.86/5.88 (ABq, $J = 12.5$ Hz) (H-8″), 5.72/5.78 (ABq, $J = 12.5$ Hz) (H-8‴), 6.30/6.36 (ABq, $J = 15.5$ Hz) (H-8′), 6.47/6.50 (ABq, $J = 12.5$ Hz) (H-7″), 6.53/6.56 (ABq, $J = 12.5$ Hz) (H-7‴), 6.67 (AA′BB′, $J = 8$ Hz) (H-3″, 5″), 6.67 (AA′BB′, $J = 8$ Hz) (3‴, 5‴), 6.74 (AA′BB′, $J = 8$ Hz) (H-3′, 5′), 7.16 (AA′BB′, $J = 8$ Hz) (H-2″, 6″), 7.31 (AA′BB′, $J = 8$ Hz) (H-2‴, 6‴), 7.34 (AA′BB′, $J = 8$ Hz) (H-2′, 6′), and 7.37/7.39 (ABq, $J = 15.5$ Hz) (H-7′); $^{13}$C NMR ($CD_3OD$, 125 MHz) (Table 1); ESI-MS *m/z*: 584 $[M + H]^+$; HR-ESI-MS *m/z*: 584.2766 $[M + H]^+$ (calcd for $C_{34}H_{38}N_3O_6$: 584.2761).

$N^1$,$N^5$,$N^{10}$-(*Z*)-tri-*p*-coumaroylspermidine (**3**): White powder; ESI-MS *m/z*: 584 $[M + H]^+$; $^1$H NMR ($CD_3OD$, 500 MHz) δ: 1.30/1.45 (2H, m, H-8), 1.30/1.51 (2H, m, H-7), 1.55/1.72 (2H, m, H-3), 3.04/3.20 (4H, m, H-2, 9), 3.27/3.29 (2H, m, H-6), 3.30/3.31 (2H, m, H-4), 5.71/5.74 (ABq, $J = 12.5$ Hz) (H-8‴), 5.77/5.80 (ABq, $J = 12.5$ Hz) (H-8′), 5.83/5.86 (ABq, $J = 12.5$ Hz) (H-8″), 6.47/6.50 (ABq, $J = 12.5$ Hz) (H-7″), 6.53/6.56 (ABq, $J = 12.5$ Hz) (H-7′), 6.57/6.59 (ABq, $J = 12.5$ Hz) (H-7‴), 6.66 (AA′BB′, $J = 8$ Hz) (H-3′, 5′), 6.66 (AA′BB′, $J = 8$ Hz) (H-3″, 5″), 6.66 (AA′BB′, $J = 8$ Hz) (H-3‴, 5‴), 7.15 (AA′BB′, $J = 8$ Hz) (H-2″, 6″), 7.35 (AA′BB′, $J = 8$ Hz) (H-2′, 6′), and 7.35 (AA′BB′, $J = 8$ Hz) (H-2‴, 6‴); $^{13}$C NMR ($CD_3OD$, 125 MHz) (Table 1); ESI-MS *m/z*: 584 $[M + H]^+$.

$N^1$,$N^5$,$N^{10}$-(*E*)-tri-*p*-coumaroylspermidine (**4**): White powder; $^1$H NMR ($CD_3OD$, 500 MHz) and $^{13}$C NMR ($CD_3OD$, 125 MHz) were identical to those previously reported;[6] ESI-MS *m/z*: 584 $[M + H]^+$.

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