New Acetylenic Glucosides from *Carthamus tinctorius*

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Two new acetylenic glucosides, 4′,6′-acetonide-8*Z*-decaene-4,6-diyne-1-*O*-β-D-glucopyranoside named carthamoside A_1 (1) and 4,6-decadiyne-1-*O*-β-D-glucopyranoside named carthamoside A_2 (2), along with one known acetylenic glucoside, 8*Z*-decaene-4,6-diyne-1-*O*-β-D-glucopyranoside (3), have been isolated from the air-dried flower of *Carthamus tinctorius*, these structures have been identified on the basis of spectroscopic methods.

Key words *Carthamus tinctorius*; acetylenic compound; carthamoside A_1; carthamoside A_2

The dried flower of safflower, *Carthamus tinctorius* L., is a widely used traditional Chinese plant medicine having the function of promoting blood circulation by removing blood stasis.[1)] During the course of our investigation, two new acetylenic glucosides were isolated from *C. tinctorius*. This paper describes the isolation and characterization of carthamoside A_1 and A_2.

Compound **1**, a brown syrup, was determined to be $C_{19}H_{26}O_6$ by HR (high-resolution)-FAB-MS (*m/z* 351.1840, $[M+H]^+$). In the IR spectra, absorption bands attributable to hydroxyl (3416 cm^{-1}), acetylene (2232, 2135 cm^{-1}) and ethylene (1675 cm^{-1}) groups were observed. The UV spectra of **1** were typical for an ene-diyne chromophore (λ_{max}=226, 238, 251, 265, 281 nm).[2)] The ^{1}H-NMR spectra and the heteronuclear multiple quantum coherence (HMQC) spectra indicated the presence of a methyl proton at δ 1.81 (3H, dd, *J*=7.0, 1.7 Hz, Me-10) coupling with two *Z*-configured olefinic protons at 6.21 (1H, dq, *J*=10.8, 7.0 Hz, H-9) and δ 5.59 (1H, br d, *J*=10.8 Hz, H-8) indicating a methyally moiety, a methylene at δ 2.45 (2H, t, *J*=7.0 Hz, H-3), a methylene at δ 1.70 (2H, m), and nonequivalent methylene protons at δ 3.49 (1H, dt, *J*=10.0, 6.2 Hz, H-1a) and 3.72 (1H, dt, *J*=10.0, 6.2 Hz, H-1b). C-4, C-5, C-6 and C-7 were confirmed to be ethynyl carbons in a ^{13}C-DEPT experiment showing four quaternary carbon signals at δ_C 65.0, 72.2, 78.5, and 85.5. The ^{1}H- and ^{13}C-NMR spectra showed the presence of a glucopyranosyl unit [δ_H 4.23 (anomeric proton), δ_C 103.5, 74.5, 73.2, 73.3, 66.8, 61.6]. The coupling constant (*J*=7.8 Hz) of the anomeric proton indicated the β-configuration of the glucose. Long-range correlations were observed in the heteronuclear multiple-bond connectivity (HMBC) spectra between the following: Me-10/C-9 (δ 143.0), C-8 (δ 108.8) and C-7 (δ 72.2); H-9/C-10 (δ 16.4), C-8 (δ 108.8) and C-7 (δ 72.2); H-3/C-1 (δ 67.5), C-2 (δ 28.1), C-4 (δ 85.5), C-5 (δ 65.0), C-6 (δ 78.5) and C-7 (δ 72.2); H-2/C-1 (δ 67.5), C-3 (δ 15.6) and C-4 (δ 85.5); and H-1a, H-1b/C-1′ (δ 103.5), C-2 (δ 28.1) and C-3 (δ 15.6). In the HMBC spectra of **1**, the glucose anomeric proton (δ_H 4.23) showed a correlation with a methylene carbon at δ 67.5, indicating that the glucose must be linked at position of C-1 of the aglycone. The presence and position of the acetonide group was provided by: (a) two characteristic additional methyl signals at δ 1.28 (3H, s) and 1.39 (3H, s); (b) a set of acetonide carbon signals at δ 98.8, 19.2 and 29.2 accompanied by downfield shift of C-4′ and C-6′ and significant upfield shifts of C-3′ and C-5′ in comparison with **3**; (c) In the HMBC spectra, Long-range correlations CH_3 (δ 1.28)/C (δ 98.8); CH_3 (δ 1.39)/C (δ 98.8); and H-6′a (δ 3.73)/C (δ 98.8) was observed indicating that the acetonide group must be linked at the position C-4′ and C-6′ of the glucose.

Acid hydrolysis of **1** produced sugar component, which was identified as glucose by PC comparison with an authentic sample. Thus, compound **1** was established to be 4′,6′-acetonide-8*Z*-decaene-4,6-diyne-1-*O*-β-D-glucopyranoside.

Compound **2** was obtained as a colorless powder. The HR-FAB-MS established that the molecular formula was $C_{16}H_{24}O_6$ (*m/z* 313.1669, $[M+H]^+$). The IR spectra of **2** exhibited the presence of a hydroxyl at 3400 cm^{-1}, and a acetylene at 2254 and 2160 cm^{-1}. By the comparison of ^{1}H- and ^{13}C-NMR spectral data with those of **1**, it was suggested that **2** also has two acetylenic bonds, but no ethylene group. Extensive analysis of the ^{1}H-NMR spectra indicated the presence of two separate spin systems. The first was a

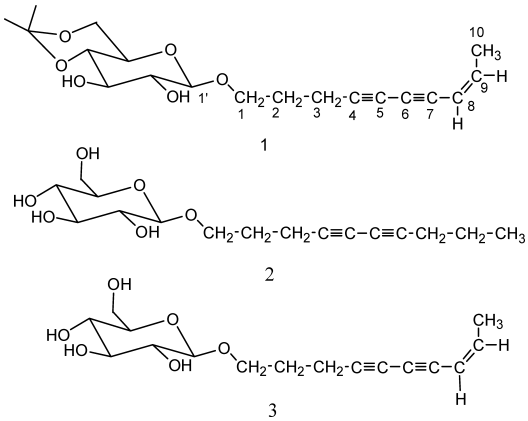

Fig. 1. Structures of **1**, **2** and **3**

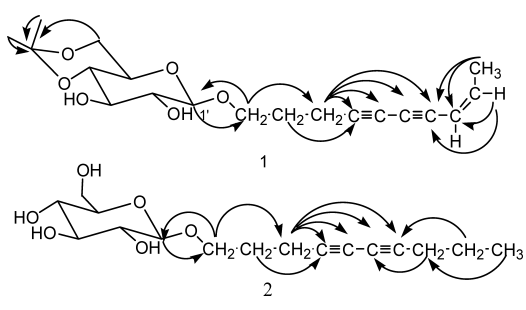

Fig. 2. Key HMBC Correlations of **1** and **2**

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Table 1. ^{1}H- and ^{13}C-NMR Spectral Data of Compounds **1**, **2** and **3** (600, 150 MHz)[a)]

Position	**1**		**2**		**3**
	H (*J* Hz)	C	H (*J* Hz)	C	C
1	3.49 dt (10.0, 6.2)	67.5	3.47 dt (9.9, 6.3)	67.6	67.2
	3.72 dt (10.0, 6.2)		3.78 dt (9.9, 6.3)		
2	1.70 m	28.1	1.70 m	28.7	28.2
3	2.45 t (7.0)	15.6	2.26 t (6.7)	15.6	15.6
4		85.5		78.2	85.7
5		65.0		65.9	65.0
6		78.5		65.8	78.5
7		72.2		78.4	72.2
8	5.59 br d (10.8)	108.8	2.38 t (7.2)	20.7	108.8
9	6.21 dq (10.8, 7.0)	143.0	1.47 m	21.7	143.3
10	1.81 dd (7.0, 1.7)	16.4	0.92 t (7.2)	13.7	16.4
1′	4.23 d (7.8)	103.5	4.20 d (7.8)	103.3	103.0
2′		74.5		73.9	73.5
3′		73.2		77.0	76.9
4′		73.3		70.5	70.1
5′		66.8		77.3	76.7
6′		61.6		61.5	61.1
$-C(Me)_2-$		98.8			
	1.28 s	29.2			
	1.39 s	19.2			

a) ppm from TMS, in DMSO-d_6, room temperature.

$CH_3CH_2CH_2-$ unit related to seven protons at δ 0.92 (3H, t, J=7.2 Hz), 1.47 (2H, m,) and 2.38 (2H, t, J=7.2 Hz), respectively. The second was a $-CH_2CH_2CH_2O-$ unit, which corresponded with six protons at δ 2.26 (2H, t, J=6.7 Hz), 1.70 (2H, m,), 3.47 and 3.78 (each 1H, dt, J= 9.9, 6.3 Hz), the chemical shifts of the last two protons showing that this methylene must be bounded to an oxygen atom. The two units were linked through two acetylenic bonds, therefore, the aglycone was determined to be $CH_3CH_2CH_2-C\equiv C-C\equiv C-CH_2CH_2CH_2O-$. The ^{1}H- and ^{13}C-NMR spectra showed the presence of a glucopyranosyl unit [δ_H 4.20 (anomeric proton), δ_C 103.3, 73.9, 77.0, 70.5, 77.3, 61.5]. The coupling constant (J=7.8 Hz) of the anomeric proton indicated the β-configuration of the glucose. The ^{13}C-NMR and DEPT spectra revealed the presence of four –C≡C– bonded quaternary carbons at δ 65.8, 65.9, 78.2 and 78.4. Taken together, this conclusion was confirmed by 2D NMR experiments, including HMQC and HMBC, which demonstrated the various chemical shifts assignments and their connectivities. Furthermore, by the comparison of chemical shifts with similar compounds,[2,3)] compound **2** was established to be 4,6-decadiyne-1-*O*-β-D-glucopyranoside.

Compound **3** was characterized by comparison of its ^{1}H- and ^{13}C-NMR spectra with reported values.[4)]

Experimental

General Experimental Procedures Optical rotations on a P-E 241 MC polarimeter using methanol as solvent. IR spectra were taken on a Bruker IFS-55 infrared spectrophotometer. UV spectra was obtained on a Hitachi 200-10 spectrophotometer. The NMR data were recorded on Bruker AV-600 (600 MHz for ^{1}H and 150 MHz for ^{13}C) in DMSO with TMS as internal standard. The HR-FAB-MS data were obtained on the Micross Mass Autospec-UltimaE TOF mass spectrophotometer. Chromatography was performed on silica gel (200—300 mesh), Sephadex LH-20, reversed-phase HPLC.

Plant Materials Dried petals (5 kg) of *C. tinctorius*, cultivated in Xinjiang province of China, were bought from the Cooperation of Traditional Chinese Medicine of Shenyang, China. A voucher specimen was identified by Prof. Qi-shi Sun and deposited in the School of Traditional Chinese Medicine of Shenyang Pharmaceutical University, China.

Extraction and Isolation Dried petals (5 kg) of *C. tinctorius* were extracted three times with hot 95% EtOH for 2 h, and the combined solution was concentrated *in vacuo* to a syrup (1200 g), followed by suspension in water. The suspension was extracted with Petroleum ether, ethyl acetate and then *n*-butanol. The ethyl acetate fraction (67.5 g) was further fractionated by silica gel column chromatography (eluted with $CHCl_3$ and MeOH in increasing polarity) to obtain nine fractions (I—IX). Fraction V was then purified by sephadex LH-20 column chromatography eluted with MeOH, and further separated by preparative HPLC eluted with 58% aqueous MeOH to give compounds **1** (20 mg), **2** (15 mg) and **3** (50 mg).

4′,6′-Acetonide-8*Z*-decaene-4,6-diyne-1-*O*-β-D-glucopyranoside (**1**): A brown syrup, $[\alpha]_D^{25}$ −15° (c=0.01, MeOH), HR-FAB-MS *m*/*z*: 351.1840 $[M+H]^+$ (Calcd for $C_{19}H_{27}O_6$: 351.1808). IR (KBr) cm^{-1}: 3416 (OH), 2232 (–C≡C–), 2135 (–C≡C–), 1675 (–C=C–), 1379, 1266, 1083. UV λ_{nm} (ε): 226 (7546), 238 (8820), 251 (12209), 265 (16235), 281 (13291). ^{1}H- and ^{13}C-NMR spectra are shown in Table 1.

4,6-Decadiyne-1-*O*-β-D-glucopyranoside (**2**): A colorless powder, $[\alpha]_D^{25}$ −18° (c=0.01, MeOH), HR-FAB-MS *m*/*z*: 313.1669 $[M+H]^+$ (Calcd for $C_{16}H_{25}O_6$: 313.1651). IR (KBr) cm^{-1}: 3400 (OH), 2254 (–C≡C–), 2160 (–C≡C–), 1029. ^{1}H- and ^{13}C-NMR spectra are shown in Table 1.

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References

1) Jiangsu New Medical College, "A Dictionary of the Traditional Chinese Medicines," Shanghai People's Publishing House, Shanghai, 1986, pp. 992—994.
2) Wang N. L., Yao X. S., Ishii R., Kitanaka S., *Chem. Pharm. Bull.*, **49**, 938—942 (2001).
3) Wang C. Z., Yu D. Q., *Phytochemistry*, **48**, 711—717 (1998).
4) Li S., Kuang H. X., Okada Y., Okuyama T., *Chem. Pharm. Bull.*, **52**, 439—440 (2004).