NEW THIAZINEDIONES AND OTHER COMPONENTS FROM *Xanthium strumarium*

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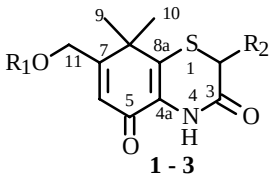
Two new thiazinediones along with five known compounds were isolated from the fruits of Xanthium strumarium *L. The structures of the two new compounds were determined to be 7-hydroxymethyl-8,8-dimethyl-4,8-dihydrobenzol[1,4]thiazine-3,5-dione-11*-O-β-*D-glucopyranoside (**1**) and 2-hydroxy-7-hydroxymethyl-8,8-dimethyl-4,8-dihydrobenzol[1,4]thiazine-3,5-dione-11*-O-β-*D-glucopyranoside (**2**). The five known compounds were identified as xanthiazone (**3**), chlorogenic acid (**4**), ferulic acid (**5**), formononetin (**6**), and ononin (**7**), respectively.*

Key words: *Xanthium strumarium* L., Compositae, two new thiazinediones, five known compounds.

The genus *Xanthium* (Compositae) is represented by 25 species in the world and 3 species and 1 variety in China [1]. *Xanthium* species have been used as traditional herb medicines for a long time in oriental countries. *Xanthium strumarium* L. is the principal species found abundantly throughout China, and its fruits, Fructus Xanthii (Chinese name Cang-Er-Zi), are used in China for the treatment of nasal sinusitis, headache caused by wind-cold, urticaria, and arthritis [2]. The chemical composition of ent-kaurane diterpenoids, sesquiterpene lactones, caffeoylquinic acids, and a thiazinedione from this plant (leaves or fruits) have been reported [3–5]. The composition of the seed oil of *Xanthium strumarium* L. was reported in 1965 [6] and 1976 [7].



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1, 2: R_1= *β*-D-Glc*p*; **3:** R_1 = H
1, 3: R_2 = H; **2:** R_2 = OH

In continuation of our research on biologically active compounds, we conducted a study of *Xanthium strumarium* L. By using a combination of silica gel and sephadex LH-20 in various solvent systems, we obtained two new compounds **1** and **2**, along with five known compounds: 7-hydroxymethyl-8,8-dimethyl-4,8-dihydrobenzol[1,4]- thiazine-3,5-dione (xanthiazone, **3**), chlorogenic acid (**4**), ferulic acid (**5**) from the *n*-butanol fraction, formononetin (**6**), and ononin (**7**) from the ethyl acetate fraction. In this paper, we report the isolation and structure elucidation of these compounds.

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TABLE 1. ^{1}H and ^{13}C NMR Data of Compounds **1**, **2** and **3**, J/Hz

C atom	Compound							
	3		**1**			**2**		
	δ_H	δ_C	δ_H	δ_C	HMBC	δ_H	δ_C	HMBC
2	3.46 s	28.5 d	3.32 s	30.3 s		5.30 s	71.2 d	
3		162.8 s		165.2 s	H-2		162.9 s	H-2
4	9.36							
4a		130.0 s		131.5 s	H-6		130.8 s	H-6
5		175.2 s		177.6 s	H-6		177.4 s	H-6
6	6.37 s	120.2 d	6.51 s	123.6 d	H-11	6.55 s	123.2 d	H-11
7		167.8 s		167.8 s	H-6, H-9, H-10, H-11		170.2 s	H-6, H-9, H-10, H-11
8		42.1 s		43.9 s	H-6, H-9, H-10		43.2 s	H-6, H-9, H-10
8a		141.1 s		143.9 s	H-2, H-9, H-10		144.1 s	H-2, H-9, H-10
9	1.35 s	26.9 q	1.29 s	27.7 q	H-10	1.31 s	27.1 q	H-10
10	1.35 s	26.8 q	1.31 s	28.1 q	H-9	1.30 s	28.3 q	H-9
11	4.30 s	59.4 t	4.36 d (J = 16)	68.1 t	H-6, H-1′	4.39 d (J = 16)	67.8 t	H-6, H-1′
			4.66 d (J = 16)			4.68 d (J = 16)		
1′			4.20 d (J = 7.5)	104.3 d	H-11, H-3′, H-5′	4.31 d (J = 7.7)	104.8 d	H-11, H-3′, H-5′
2′			3.12*	75.6 d	H-4′	3.13*	77.2 d	H-4′
3′			3.15*	78.7 d	H-1′, H-5′	3.15*	78.9 d	H-1′, H-5′
4′			3.15*	72.4 d	H-2′, H-6′	3.15*	74.2 d	H-2′, H-6′
5′			3.20 m	78.5 d	H-1′, H-3′	3.20 m	78.3 d	H-1′, H-3′
6′			3.48 m	63.3 t		3.49 m	65.1 t	
			3.71 d (J = 11)			3.75 d (J = 11)		

*Glucosyl proton signals unclear due to overlapping.

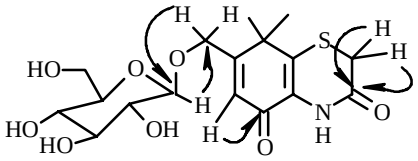

Fig. 1. Selected HMBC correlation of compound **1**.

Compound **1** was obtained as a white amorphous powder, mp. 180~182°C, UV λ_{max} (MeOH): 280 nm; the ESI-MS afforded the positive ion at *m/z* 402 $[M+H]^+$, implying a molecular formula of $C_{17}H_{23}N_2SO_8$, which was confirmed by HR-EI-MS ($[M]^+$ found 401.1155, calcd. 401.1144). The IR spectrum of **1** indicated the presence of the hydroxyl group (3369 cm^{-1}) and two carbonyl groups (1680 cm^{-1}, 1655 cm^{-1}). The signals at δH 4.20 (d, 1H, J = 7.7 Hz) and δC 104.3 in the ^{1}H, ^{13}C, and DEPT NMR spectra of compound **1** indicated that **1** possessed a monoglycosidic structure with an α-sugar unit. The ^{1}H and ^{13}C NMR data of the skeleton of compound **1** were quite similar to those of compound **3** [5]. The glycosylation of **1** occurred at C-11 on the basis of the carbon signals at δ 68.1 (C-11) in the ^{13}C NMR spectrum, and this was confirmed by the HMBC experiments (Fig. 1). Consequently, the structure of **1** was assigned as 7-hydroxymethyl-8,8-dimethyl-4,8-dihydrobenzol[1,4]thiazine-3,5-dione-11-*O*-β-D-glucopyranoside.

Compound **2** was also obtained as a white amorphous powder, mp 190~192°C, UV λ_{max} (MeOH): 280 nm; the ESI-MS afforded the positive ion at *m/z* 418 $[M+H]^+$, implying a molecular formula of $C_{17}H_{23}N_2SO_9$, which was confirmed by the HR-EI-MS ($[M]^+$ found 417.0566, calcd. 417.0562). The ^{13}C NMR (DEPT) spectrum of compound **2** was found to differ from that of compound **1** by the presence of a hydroxyl methine group signal (δ 71.2) and the absence of one methylene group signal (C-2, δ 30.3). Therefore, compound **2** was suggested to be a C-2 hydroxy substituent of compound **1**. The structure of **2** was thus established as 2-hydroxy-7-hydroxymethyl-8,8-dimethyl-4,8-dihydrobenzol[1,4]thiazine-3,5-dione-11-*O*-β-D-glucopyranoside.

EXPERIMENTAL

General Experimental Procedures. NMR spectra were operated on a Bruker DRX-500 spectrometer at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR expressed in δ values with reference to TMS as internal standard, and coupling constants J/Hz; EI-MS were recorded on a Varian MAT-212 mass spectrometer and HRESI on a Q-TOF micro mass spectrometer; melting points were measured on a RY-2 melting point apparatus and are uncorrected; IR were recorded on a Bruker Vector 22 spectrometer with KBr pellet; column chromatography was performed on silica gel (200–300 mesh, Yantai, P. R. China), silica gel H (10–40 μm, Yantai, P. R. China), and Sephadex LH-20 (Pharmacia); TLC analysis was run on HSGF254 precoated silica gel plates (10–40 μm, Yantai, P. R. China).

Plant Material. The ripe fruits of *Xanthium strumarium* L., Xanthii Fructus, were collected from a local research farm in Sunqiao town, Shanghai, P. R. China in November 2003 and authenticated by Prof. Hanchen Zheng, Second Military Medical University. A voucher specimen of the plant was deposited at the Herbarium of the Department of Pharmacognosy, School of Pharmacy, Second Military Medical University, Shanghai, P. R. China under the acquisition number of CE20031107.

Extraction and Isolation. Dried fruits of *Xanthium strumarium* L. (20 kg) were ground and extracted with 75% aqueous ethanol by infiltration. The solvent was evaporated under vacuum to afford 1100 g crude extract (yield, 5.5%). Then the extract was suspended in water and partitioned with petroleum ether, chloroform, ethyl acetate, and aqua-saturated *n*-butanol successively. Each fraction was evaporated under vacuum to yield residues of petroleum ether 55 g (5.0%), chloroform 60 g (5.4%), ethyl acetate 60.5 g (5.5%), *n*-butanol 200 g (18.2%), and aqueous 725 g (65.9%), respectively. The *n*-butanol fraction (170 g) was subjected to silica gel column chromatography (2000 g) and eluted with ethyl acetate–methanol (20:1~1:5). Combination of similar fractions on the basis of TLC analysis afforded 5 fractions (1 to 5). Fraction 1 was separated on a silica gel column (chloroform/methanol 5:1, 1000 mL each fraction). The second fraction afforded compound **5** (30 mg) after being concentrated to a small volume and recrystallized with methanol. Fraction 2 was purified by Sephadex LH-20 and eluted with methanol to get compound **4** (100 mg). Chromatography of fraction 3 on Sephadex LH-20 (60% aqueous methanol) gave two fractions (3-I, 3-II). Fraction 3-I was further chromatographed on Sephadex LH-20 (50% aqueous methanol) to give compound **1** (20 mg). Purification of fraction 3-II on Sephadex LH-20 with methanol gave compound **2** (22 mg) and compound **3** (30 mg). The ethyl acetate extraction (40 g) was separated on a silica gel column (600 g, chloroform–methanol 50:1~1:1) to give six fractions. The third fraction afforded a white powder after concentration, which was purified by recrystallization in ethanol to give compound **6** (20 mg). Compound **7** (50 mg) was obtained from the fourth part of the ethyl acetate extraction by further chromatography on silica gel column.

7-Hydroxymethyl-8,8-dimethyl-4,8-dihydro-benzol[1,4]thiazine-3,5-dione-11-*O*-*β*-D-glucopyranoside (1): white powder, mp 180–182°C; IR (KBr, cm^{-1}): 3369 (OH), 1680 (C=O), 1655 (C=O), 1624 (Ar); EI-MS *m/z*: 401.2 [M$^+$], 239.2 [M-Glu]$^+$; HR-EI-MS *m/z*: 401.1155 [M]$^+$ (calcd. for $C_{17}H_{23}NSO_8$ 401.1144); ^{1}H and ^{13}C NMR data were listed in Table 1.

2-Hydroxy-7-hydroxymethyl-8,8-dimethyl-4,8-dihydrobenzol[1,4]thiazine-3,5-dione-11-*O*-*β*-D-glucopyranoside (2): white powder, mp 190–192°C; IR (KBr, cm^{-1}): 3512 (OH), 1685 (C=O), 1657 (C=O), 1630, 1605 (Ar); EI-MS *m/z*: 417.3 [M$^+$], 255.3 [M-Glu]$^+$; HR-EI-MS *m/z*: 417.0566 [M]$^+$ (calcd. for $C_{17}H_{23}NSO_9$ 417.0562); ^{1}H and ^{13}C NMR data were listed in Table 1.

Xanthiazone (3): colorless cubic crystal (MeOH), mp 159–161°C; IR (KBr, cm^{-1}): 3512 (OH), 1682 (C=O), 1657 (C=O), 1630, 1605 (Ar); EI-MS *m/z*: 240.1[M+H]$^+$; ^{1}H and ^{13}C NMR data were listed in Table 1 [5].

Chlorogenic acid (4): colorless needles (MeOH), mp 204–206°C; IR (KBr, cm^{-1}): 3362 (OH), 1726 (C-O), 1687, 1640; EI-MS *m/z* (%): 354.2 [M]$^+$, 180 (92), 163 (100), 136 (54). ^{1}H NMR (DMSO-d_6, δ, ppm, J/Hz): 7.42 (1H, d, J = 16, H-7′), 7.03 (1H, d, J = 2, H-2′), 6.98 (1H, dd, J = 2 and 8, H-6′), 6.77 (1H, d, J = 8, H-5′), 6.15 (1H, d, J = 16, H-8′), 5.08 (1H, br.d, J = 5, H-5), 3.94 (1H, br.s, H-3), 3.55 (1H, br.d, J = 4, H-4), 1.7~2.1 (2H, m, H-6), 1.98 (2H, br.d, J = 5, H-2); ^{13}C NMR (DMSO-d_6): δ 174.9 (C-7), 165.7 (C-9′), 148.3 (C-4′), 145.5 (C-3′), 144.8 (C-7′), 125.6 (C-1′), 121.2 (C-6′), 115.7 (C-5′), 114.7 (C-2′), 114.3 (C-8′), 73.6 (C-1), 70.9 (C-3), 70.6 (C-4), 68.3 (C-5), 37.2 (C-6), 36.5 (C-2). ^{1}H and ^{13}C NMR data of the compound were identical to the authentic 5-caffeoylquinic acid (chlorogenic acid) [8].

Ferulic acid (5): colorless needles (MeOH), mp 170–172°C; EI-MS *m/z* (%): 194 (M$^+$, 100), 179 (24), 177 (7), 151 (8), 145 (10), 133 (39), 107 (10), 105 (12), 89 (12), 77 (25). ^{1}H NMR ($CDCl_3$, δ, ppm, J/Hz): 7.71 (1H, d, J = 15.5, H-7), 7.11 (1H, dd, J = 1.5, 8, H-6), 7.06 (1H, d, J = 1.5, H-2), 6.94 (1H, d, J = 8, H-5), 6.30 (1H, d, J = 15.5, H-8), 3.94 (3H, s, OCH_3) ^{13}C NMR ($CDCl_3$): δ 168.8 (C-9), 148.3 (C-3), 147.0 (C-4), 146.8 (C-7), 126.6 (C-1), 123.5 (C-6), 114.7 (C-2), 114.3 (C-5), 109.4 (C-8), 55.9 (OCH_3) [9].

Formononetin (6): white powder, mp 250–252°C; IR (KBr, cm^{-1}): 3148 (OH), 1640 (C=O), 1610, 1570, l515 (Ar), 2980, 2835; EI-MS *m/z* (%): 269.1 $[M+H]^+$ (100), 268.1 (M^+, 27), 253.0 (3), 225 (2), 185.1 (13), 136.0 (10), 132.0 (3), 89.0 (20); ^1H NMR (DMSO-d_6, δ, ppm, J/Hz): 10.77 (1H, s, Ar-OH), 8.32 (1H, s, H-2), 7.96 (1H, d, J = 8.7, H-5), 7.48~7.51 (2H, dd, J = 1.9, 6.8, H-2′, H-6′), 6.96~6.99 (2H, dd, J = 1.9, 6.8, H-3′, H-5′), 6.95 (1H, dd, J = 2.2, 8.7, H-6), 6.85 (1H, d, J = 2.2, H-8), 3.78 (3H, s, OCH_3); ^{13}C NMR (DMSO-d_6): δ 174.9 (C-4), 162.8 (C-7), 159.3 (C-4′), 157.8 (C-9), 153.4 (C-2), 130.4 (C-2′, 6′), 127.6 (C-5), 124.6 (C-1′), 123.5 (C-3), 117.0 (C-10), 115.5 (C-6), 113.9 (C-3′, 5′), 102.4 (C-8), 55.4 (4′-OCH_3) [10].

Ononin (7): white needles (MeOH), mp 220–223°C; ^1H NMR (DMSO-d_6, δ, ppm, J/Hz): 8.43 (1H, s, H-2), 8.06 (1H, d, J = 9.0, H-5), 7.54 (2H, d, J = 9.0, H-2′, 6′), 7.24 (1H, d, J = 2.1, H-8), 7.16 (1H, dd, J = 2.4, 9.0, H-6), 7.0l (2H, d, J = 8.7, H-3′, 5′), 5.05 (1H, d, J = 4.8, Glu H-l″), 3.78 (3H, s, OCH_3); ^{13}C NMR (DMSO-d_6): δ 174.7 (C-4), 161.5 (C-7), 159.0 (C-4′), 157.1 (C-9), 153.6 (C-2), 130.1 (C-2′, 6′), 127.0 (C-5), 124.0 (C-3), 123.4 (C-1′), 118.5 (C-10), 115.6 (C-6), 113.6 (C-3′, 5′), 103.4 (C-8), 100.0 (C-1″), 77.2 (C-3″), 76.5 (C-5″), 73.13 (C-2″), 69.6 (C-4″), 60.6 (C-6″), 55.2 (OCH_3) [11].

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