Phenolic Glucosides and a γ-Lactone Glucoside from the Sprouts of *Crocus sativus*

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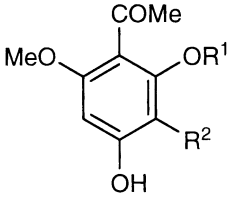
Abstract: Two new phenolic glucosides, a new γ-lactone glucoside, and adenosine were isolated from the sprouts of *Crocus sativus* L. The new compounds were characterized as 2,4-dihydroxy-6-methoxyacetophenone-2β-D-glucopyranoside (**1**), 2,3,4-trihydroxy-6-methoxyacetopenone-3-β-D-glucopyranoside (**2**) and 3-(*S*)-3-β-D-glucopyranosyloxybutanolide (**3**), respectively. All structures were elucidated on the basis of chemical and spectroscopic evidence.

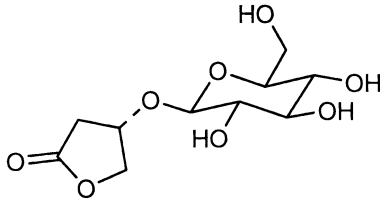
Key words: *Crocus sativus* L., Iridaceae, sprout, phenolic glucosides, γ-lactone glucoside.

Introduction

Crocus sativus L. is a rare medicinal herb. Its stigmas are used as medicine in Europe and Asia (1). Moreover, it is also used as a condiment because of its aroma and color. In pharmacological tests, it has been reported to possess many activities (2–4). Several investigations have been carried out on this plant (5–7). But because its stigmas are expensive, the use is limited. In order to expand the use of this medicinal herb, its sprouts, which can be collected more easily and cheaply, was studied. From the *n*-BuOH extract, three new compounds (**1**, **2** and **3**) accompanied by adenosine (**4**) were obtained. This paper deals with the isolation and characterization of these compounds.



1 R^1 = Glu, R^2 = H
2 R^1 = H, R^2 = OGlu



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Compound **1** was isolated as colorless needles with an m.p. 158–160 °C and a molecular formula of $C_{15}H_{20}O_9$. It gave a dark blue coloration with ethanolic $FeCl_3$ reagent which indicated that **1** was a phenolic compound. The IR spectrum of **1** showed the absorption bands of hydroxy, conjugated carbonyl, and aromatic groups. The EIMS and FABMS showed that its molecular weight was 344. Hydrolysis of **1** with HCl afforded glucose with a $[\alpha]_D$ value as 15.8° (H_2O) which showed it was D-glucose. Its ^{1}H-NMR indicated the presence of an acetyl at δ 2.65 (3H, s), a methoxy at δ 3.79 (3H, s) and aliphatic protons at δ 3.0 to 5.0 (7H) including an anomeric proton of a β-glucosyl moiety at δ 4.99 (1H, d, J = 7.2 Hz). Two signals at δ 6.28 (1H, d, J = 1.9 Hz) and δ 6.12 (1H, d, J = 1.9 Hz) were assigned to two aromatic protons located in the *meta*-position. A signal at δ 10.12 was attributed to a hydroxy proton on the aromatic ring and this datum showed that this hydroxy group located in the *meta*- or *para*-position of the acetyl group because no H-bonding was observed. Acetylation of **1** with Ac_2O and pyridine afforded a pentaacetate (**1-1**) whose ^{1}H-NMR showed four aliphatic and one aromatic acetoxy signals and an acetyl signal. The NOE difference spectrum of **1** showed that the proton at δ 6.28 correlated only with the methoxy at δ 3.79 and the proton at δ 6.12 only with the anomeric proton of the β-glucosyl moiety at δ 4.99. The results indicated that the two aromatic protons were located in the *ortho*-positions to the methoxy group and glucose, respectively, and both in the *meta*-positions to the acetyl group. Therefore, the structure of compound **1** was elucidated as 2,4-dihydroxy-6-methoxyacetophenone-2-β-D-glucopyranoside. This was further confirmed by the ^{13}C-NMR data of **1** (see Table **1**).

Compound **2**, colorless needles, showed an m.p. of 188–189 °C and a molecular formula of $C_{15}H_{20}O_{10}$. It also produced a positive reaction with ethanolic $FeCl_3$ reagent and its IR spectrum was almost the same as that of compound **1**. The EIMS and FABMS of **2** showed that its molecular weight was 360. Hydrolysis of **2** with HCl also afforded D-glucose ($[\alpha]_D$ = 14.7° in H_2O). In its ^{1}H-NMR spectrum there were an acetyl at δ 2.61 (3H, s), a methoxy at δ 3.93 (3H, s) and aliphatic protons at δ 3.0 to 5.0 (7H) including an anomeric proton of the β-glucosyl moiety at δ 4.48 (1H, d, J = 7.4 Hz), and an aromatic proton at δ 6.28 (1H, s). Two signals at δ 10.63 and 13.00 exhibited two hydroxy groups attached to the aromatic ring and one of them was H-bonded. Acetylation of **2** with Ac_2O and pyridine afforded a hexaacetate (**2-1**) whose ^{1}H-NMR contained four aliphatic and two aromatic acetoxy signals and an

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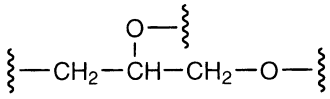
Table 1 ^{13}C-NMR data of **1**, **2** (in DMSO-d_6) and **3-1** (in $CDCl_3$).

Site		1	2	3-1
	C-1	105.88 s	104.38 s	174.28 s
	C-2	160.58 s	153.69 s	34.83 t
	C-3	94.99 d	126.44 s	74.15 d
	C-4	165.45 s*	161.98 s	74.04 t
	C-5	93.47 d	91.64 d	
	C-6	165.56 s*	159.89 s	
	-OCH_3	55.62 q	56.02 q	
	-$COCH_3$	203.39 s	203.07 s	
$OCOCH_3$	–			168.03, 169.16, 170.01, 170.35
	-$COCH_3$	33.12 q	32.45 q	
$OCOCH_3$	–			20.23 × 3, 20.53
Glucose	C-1′	100.70 d	105.71 d	99.35 d
	C-2′	73.11 d	73.84 d	70.74 d
	C-3′	76.68 d	76.05 d	72.59 d
	C-4′	69.45 d	69.65 d	68.00 d
	C-5′	77.27 d	77.16 d	72.33 d
	C-6′	60.63 t	60.80 t	61.62 t

* May be interchanged.

acetyl signal. The NOE difference spectrum of **2** showed the methoxy at δ 2.61 correlated with the proton at δ 6.28 only. With all the information obtained above and the HMBC spectrum of compound **2** (Fig. **1**), the structure of it was elucidated as 2,3,4-trihydroxy-6-methoxyacetophenone-3-β-D-glucopyranoside. The ^{13}C-NMR data of this compound are listed in Table **1**.

The fully acetylated derivative of **3** (compound **3-1**) are colorless needles, m.p. 108 – 110 °C, $C_{18}H_{24}O_{12}$. Its IR spectrum showed the existence of lactone, acetoxy group and glucosyl moiety. The EIMS of **3-1** indicated its molecular weight was 432. Mild hydrolysis of **3-1** with dilute NaOH solution afforded D-glucose ($[\alpha]_D$ = 15.4° in H_2O) in water layer and the aglycone of **3** (compound **3-2**) in ethyl acetate layer whose EIMS showed its molecular weight was 102. The ^{1}H-NMR of **3-1** showed four acetyls at δ 1.98 – 2.07 (3H × 4, s), four protons at δ 4.60 (1H, m), δ 4.40 (2H, d, J = 4.0 Hz), δ 2.70 (1H, dd, J = 18.0, 6.9 Hz), δ 2.54 (1H, dd, J = 18.0, 2.7 Hz), and the large proton coupling constant (J = 18.0 Hz) indicated the presence of γ-lactone moiety (9). In addition, the aliphatic protons at δ 3.5 – 5.4 (7H) and a proton at δ 4.55 (1H, d, J = 7.9 Hz) was assigned to the β-glucosyl moiety. The ^{1}H-^{1}H COSY of **3-1** indicated that it had the structural units as shown below:



From the above, compound **3-1** was characterized as 3-β-D-tetraacetylglucopyranosyloxybutanolide. So compound **3** should be 3-β-D-glucopyranosyloxybutanolide.

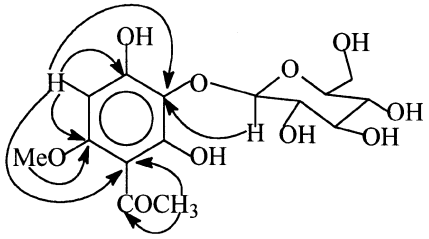


Fig. 1 HMBC of compound **2**.

In order to determine the absolute configuration of **3**, the aglycone of it (compound **3-2**), namely 3-hydroxybutanolide was obtained and its $[\alpha]_D^{28}$ was measured as – 71°. In reference (9), compound (*R*)-3-hydroxybutanolide was reported with an $[\alpha]_D^{28}$ value as + 78°. Therefore, compound **3-2** should be (*S*)-3-hydroxybutanolide. Thus compound **3** could be elucidated as 3-(*S*)-3-β-D-glucopyranosyloxybutanolide.

Materials and Methods

M.p.s. are uncorrected. IR spectra were measured on a Perkin-Elmer 599B infrared spectrometer. FABMS were recorded on a VG 2AB-HS mass spectrometer and EIMS on a MAT-95 mass spectrometer. ^{1}H-, ^{13}C-NMR, NOE difference spectrum were recorded on a Bruker AM-400 spectrometer. CC was carried out on Sephadex LH-20 and silica gel. Acetylation was carried out by the normal procedure. Sprouts of *C. sativus* L. were collected in autumn in Zhejiang province, China and identified by Dr. X. Q. Ma. A voucher specimen (No. 1688) has been deposited at the herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Extraction and isolation

Air-dried and pulverized sprouts (2 kg) were extracted with 95% ethanol at reflux (5 times, 4 h per time). After evaporation of the solvent under reduced pressure, the extract was dispersed with a small amount of distilled water and fractionated first with chloroform (5 × 100 ml), then with *n*-butanol (5 × 100 ml). The *n*-butanol part was concentrated under reduced pressure and the resulting extract (40 g) was applied to a Sephadex LH-20 column (100 g, 4 × 50 cm) with methanol and then chromatographed over silica gel column (3 × 50 cm) with chloroform-methanol to afford compounds **1** (15 mg, 6 : 1, 120 – 145 ml), **2** (80 mg, 5 : 1, 155 – 195 ml), **4** (20 mg, 5 : 1, 205 – 230 ml) and an oily mixture (200 mg, 3 : 1, 300 – 350 ml). The mixture was acetylated totally and chromatographed on silica gel column (2 × 50 cm) with chloroform-acetone. The acetylated compound **3-1** was then obtained (40 mg, 15 : 1, 85 – 105 ml).

Compound **1**: Colorless needles, m.p. 158 – 160 °C. $[\alpha]_D$ = 48.7° (*c* 0.05, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3350, 1630, 1610, 1110. EIMS *m/z* (%): 344 [M$^+$] (7), 182 [M – 162] (58), 167 [M – 162 – Me] (100), 149 [M – 162 – Me – H_2O] (65). FABMS *m/z*: 345 [M + 1]. ^{1}H-NMR (in DMSO-d_6): δ = 2.65 (3H, s, $COCH_3$), 3.79 (3H, s, OMe), 4.99 (1H, d, J = 7.2 Hz, glu-H-1), 6.12 (1H, d, J = 1.9 Hz, aromatic H-3), 6.28 (1H, d, J = 1.9 Hz, aromatic H-5), 10.12 (1H, br.s, OH-4, D_2O exchange). ^{13}C-NMR data see Table **1**.

Hydrolysis of **1**: Compound **1** (10 mg) was stirred with 2 N HCl (in methanol) at reflux for 10 h. The reaction mixture was concentrated under reduced pressure to dryness. The residue was dispersed with a small amount of water and extracted with ethyl acetate. The aqueous layer was neutralized with dilute NaOH solution and worked-up to obtain D-glucose by paper chromatography with *n*-BuOH-pyridine-H_2O (9 : 5 : 4) identified by $[\alpha]_D$ determination.

Acetylation of **1**: Compound **1** (5 mg) was acetylated with Ac_2O and pyridine by the usual manner to give the pentaacetate **1-1** (6 mg). ^{1}H-NMR (in $CDCl_3$): δ 2.01 (3H, s, OAc), 2.03 (3H, s, OAc), 2.08 (3H, s, OAc), 2.09 (3H, s, OAc), 2.39 (3H, s, OAc), 2.54 (3H, s, Ac), 3.82 (3H, s, OMe), 5.12 (1H, d, J = 7.3 Hz, Glu-H-1), 6.56 (1H, d, J = 2.1 Hz, aromatic H-3), 6.77 (1H, d, J = 2.1 Hz, aromatic H-5).

Identification of **2**: Colorless needles, m.p. 188 – 189 °C. $[\alpha]_D$ = 83.5° (*c* 0.08, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3380, 1629, 1611, 1305, 1117. EIMS *m/z* (%): 198 [M – 162] (72), 183 [M – 162 – Me] (100), 168 [M – 162 – 2Me] (17). FABMS *m/z*: 361 [M + 1]. ^{1}H-NMR (in DMSO-d_6): δ = 2.61 (3H, s, $COCH_3$), 3.93 (3H, s, OMe), 4.48 (1H, d, J = 7.4 Hz, glu-H-1), 6.28 (1H, s, aromatic H-5), 10.63 (1H, br.s, OH-4, D_2O exchange), 13.00 (1H, br.s, OH-2, D_2O exchange). ^{13}C-NMR data see Table **1** and the results of HMBC see Fig. **1**.

Hydrolysis of **2**: Compound **2** (10 mg) was hydrolyzed with the same method described in hydrolysis of **1**. Similarly, D-glucose was detected with the same approach as mentioned above.

Acetylation of **2**: Compound **2** (10 mg) was treated with Ac_2O and pyridine by the usual manner to give the hexaacetate **2-1** (15 mg). ^{1}H-NMR (in $CDCl_3$): δ = 2.00 (3H, s, OAc), 2.05 (3H, s, OAc), 2.06 (3H, s, OAc), 2.09 (3H, s, OAc), 2.31 (3H, s, OAc), 2.38 (3H, s, OAc), 2.53 (3H, s, Ac), 3.90 (3H, s, OMe), 5.01 (1H, d, J = 7.2 Hz, Glu-H-1), 6.84 (1H, s, aromatic H-5).

Identification of **3**: Acetylation of the oily mixture containing **3** in the usual manner led to the isolation of **3-1**. Compound **3-1**, is colorless needles, $C_{18}H_{24}O_{12}$, m.p. 108 – 110 °C, $[\alpha]_D$ = – 8.2° (*c* 0.08, $CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 1785.9, 1760.4, 1758.8, 1754.9, 1380.8, 1228.5, 1043.3; EIMS *m/z* (%): 432 [M$^+$] (5), 331 (61), 287 (14), 243 (28), 200 (47), 157 (100), 101 (52), 85 (57). ^{1}H-NMR (in $CDCl_3$): δ = 1.98 – 2.07 (CH_3 × 4, s), 2.54 (1H, dd, J = 18.0, 6.9 Hz, H-2a), 2.70 (1H, dd, J = 18.0, 2.7 Hz, H-2b), 3.68 (1H, m, glu-H-5), 4.12 (1H, dd, J = 12.0, 2.4 Hz, glu-H-6a), 4.19 (1H, dd, J = 12.0, 5.8 Hz, glu-H-6b), 4.40 (2H, d, J = 4.0 Hz, H-4), 4.55 (1H, d, J = 7.9 Hz, glu-H-1), 4.60 (1H, m, H-3), 4.94 (1H, t, J = 7.9 Hz, glu-H-2), 5.02 (1H, m, glu-H-4), 5.16 (1H, m, glu-H-3). ^{13}C-NMR see Table **1**.

Mild hydrolysis of **3-1**: Compound **3-1** (20 mg) was stirred with 1 % NaOH in methanol at room temperature for 8 h. Then the reaction mixture was evaporated to dryness under reduced pressure. The residue was dispersed with a little water and extracted with ethyl acetate. The aqueous layer was neutralized with dilute HCl solution, evaporated to near dryness, and D-glucose was detected by paper chromatography and $[\alpha]_D$ determination. The ethyl acetate layer was evaporated and compound **3-2** was obtained as a colorless oil (4 mg): $[\alpha]_D^{28}$: – 71°. IR ν_{max}^{KBr} cm^{-1}: 1780.5, 1324.5, 1222.4; EIMS *m/z* (%): 102 [M$^+$] (32], 84 [M$^+$ – 18] (44), 74 [M$^+$ – 28] (60), 58 [M$^+$ – 44] (100); ^{1}H-NMR (in $CDCl_3$): δ = 2.25 (1H, dd, J = 17.8, 6.4 Hz, H-2a), 2.79 (1H, dd, J = 17.8, 3.5 Hz, H-2b), 4.32 (2H, d, J = 4.2 Hz, H-4), 4.41 (1H, m, H-3).

Identification of **4**: Colorless powder, $C_{10}H_{13}N_5O_4$, m.p. 233 – 234 °C. IR ν_{max}^{KBr} cm^{-1}: 3500 – 3000, 1680, 1600, 1580. EIMS *m/z* (%): 267 (3), 178 (30), 164 (79), 136 (75), 135 (100). ^{1}H-NMR (in CD_3OD): δ = 8.15 (1H, s, H-8), 8.01 (1H, s, H-2), 5.80 (1H, d, J = 6.5 Hz, H-1′), 4.85 (1H, t, J_1 = 8.0 Hz, J_2 = 5.7 Hz, H-2′), 4.00 (1H, br.s, H-4′), 3.72 (1H, d, J = 12.3 Hz, H-5′α), 3.58 (1H, d, J = 12.3 Hz, H-5′β). ^{13}C-NMR (in C_5D_5N): δ = 157.32 (s, C-4), 152.96 (d, C-6), 140.25 (d, C-2), 135.49 (s, C-7a), 121.11 (s, C-3a), 90.52 (d, C-1′), 87.48 (d, C-4′), 75.23 (d, C-3′), 72.08 (d, C-2′), 62.72 (t, C-5′). Comparison of spectral and physical data of **4** with that reported for adenosine indicated them to be identical (10), (11).

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