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Chalcanol glucosides from seeds of *Trifolium alexandrinum*

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Abstract

Three new chalcanol glucosides have been isolated from the seeds of *Trifolium alexandrinum*, of which the first two are α′-chalcanol-α,β-epoxides and the third one is an α,β-dihydroxy-α′-chalcanol. The structures of the isolated compounds were verified by means of MS and 2D NMR spectral analyses. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Trifolium alexandrinum*; Leguminosae; Chalcanol glucosides

1. Introduction

In Egyptian folk-medicine, the seeds of *Trifolium alexandrinum* L. were used as an antidiabetic agent (Salah & El-Awady, 1961; Helmi, El-Mahdy, Ali & Khayyal, 1969). Earlier work on the seeds of this plant has resulted in the isolation of triterpenoidal saponins (Mohamed, Ohtani, Kasai & Yamasaki, 1995), flavonoids (Maatooq, 1997) and megastigmane glycosides (Mohamed, Mohamed, Ohtani, Kasai & Yamasaki, 1999). In continuation of our phytochemical investigation of the seeds of the same plant, the isolation and structure elucidation of α′-chalcanol-4-*O*-glucosides (**1**–**3**) are reported herein. Compounds **1** and **2** are α′-chalcanol-α,β-epoxides, while **3** is an α,β-dihydroxy-α′-chalcanol derivative (Agrawal, 1989).

2. Results and discussion

The molecular formula of compound **1** was deduced to be $C_{22}H_{26}O_{10}$ from negative mode of HR FAB–MS (see Section 3) and NMR spectral data (Tables 1 and 2). The ^{1}H-NMR spectrum displayed an anomeric proton signal of one sugar unit having β-configuration at δ_H 4.84 (1H, *d*, J = 7.3 Hz). Furthermore, the ^{13}C-NMR signals at δ_C 104.0, 74.9, 77.9, 71.3, 78.3 and 62.4 were characteristic for β-D-glucopyranosyl moiety. The remaining signals in ^{13}C- and ^{1}H-NMR together with DEPT mode measurement assigned by HSQC and H–H COSY indicated the presence of α′-chalcanol moiety with an α,β-epoxide functionality as deduced from signals at δ_C 62.8, 72.2 and 77.8 with corresponding δ_H 4.89 (1H, *d*, J = 3.7 Hz, H-β), 3.81 (1H, *dd*, J = 3.7 and 10.2 Hz, H-α) and 4.94 (1H, *d*, J = 10.2 Hz, H-α′). The signals at δ_C 140.3 (C-1′), 129.2 (C-2′, C-4′, C-6′) and 129.1 (C-3′, C-5′) were characteristic for the monosubstituted aromatic ring A with δ_H 7.45–7.47 (3H, *m*) and 7.32–7.39 (2H, *m*). The signals at δ_C 94.5 and 97.7 of C-3 and C-5, respectively, with δ_H 6.38 and 6.23 (each 1H, *d*, J = 2.2 Hz) were attributed to the *meta*-coupled H-3 and H-5 of ring B, respectively. The presence of a methoxyl group was indicated by the signal at δ_C 56.2 with δ_H 3.84 (3H, *s*). The signals at δ_C 114.6, 161.1, 161.2 and 156.9 were assigned to C-1, the methoxylated carbon C-2, the glycosylated carbon C-4 and the hydroxylated carbon C-6, respectively.

The above mentioned assignments were confirmed by measurement of HMBC and ROE spectra (Fig. 1). In the latter, on irradiation of H-α, a ROE was

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Table 1
^{13}C-NMR data of compounds **1**–**3**, (100 MHz, CD_3OD)

C	1	2	3
1	114.6	113.4	111.4
2	161.1	148.3	148.1
3	94.5	135.5	135.0
4	161.2	148.9	149.1
5	97.7	101.6	101.3
6	156.9	148.5	148.3
1′	140.3	140.4	140.6
2′	129.2	129.2	129.2
3′	129.1	129.1	129.1
4′	129.2	129.2	129.2
5′	129.1	129.1	129.1
6′	129.2	129.2	129.2
OMe	56.2	61.7	59.7 (at C-3) 61.1 (at C-2)
β	62.8	63.5	72.8
α	72.2	72.3	73.3
α′	77.8	77.6	78.1
Glc			
1	104.0	104.0	103.8
2	74.9	74.9	74.8
3	77.5	77.5	77.5
4	71.3	71.3	71.2
5	78.3	78.3	78.3
6	62.4	62.4	62.3

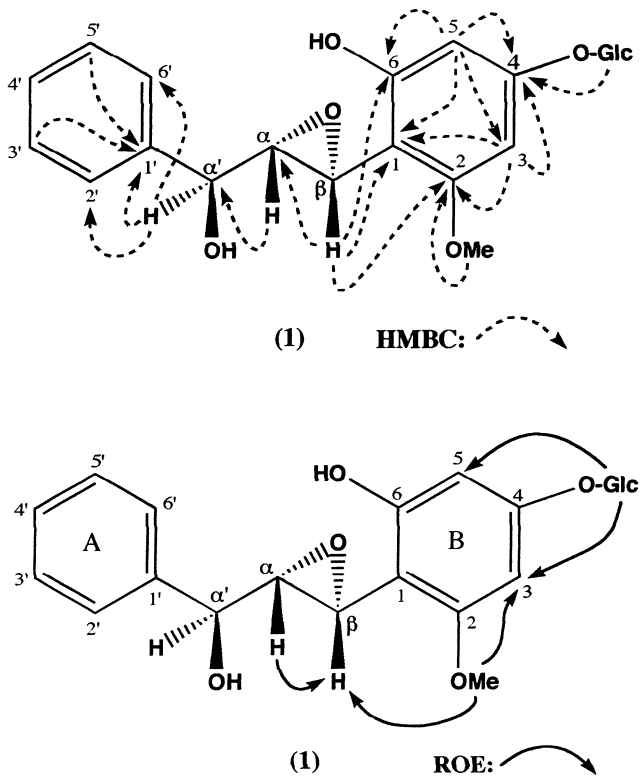


Fig. 1. Important HMBC correlations and results of ROE of (**1**).

observed with H-β and on irradiation of the methoxyl protons, ROEs were detected with both H-β and H-3 which established the location of the methoxyl group at C-2. Furthermore, on irradiation of the anomeric proton of glucose, ROEs were observed with both H-3 and H-5.

The relative configuration of the three chiral carbons C-β, C-α and C-α′ was determined from the ^{1}H-NMR mutual *J* values of their respective protons and by the help of molecular models. The magnitude of the *J* value of 3.7 Hz between H-β and H-α reflected *cis* orientation of the oxirane ring (Khan, Agrawal & Kapil, 1996). The mutual relationship between the *sec*-OH and the epoxy oxygen was deduced to be *trans* from the large *J* value between H-α and H-α′ (10.2 Hz) and by comparison of the ^{1}H-NMR spectral data

Table 2
^{1}H-NMR data of compounds **1**–**3**, (400 MHz, CD_3OD)[a]

H	1	2	3
3	6.38, 1H, *d* (2.2)		
5	6.23, 1H, *d* (2.2)	6.53, 1H, *s*	6.56, 1H, *s*
2′, 6′	7.45–7.47, 2H, *m*	7.44–7.47, 2H, *m*	7.48–7.50, 2H, *m*
3′, 4′, 5′	7.32–7.39, 3H, *m*	7.31–7.38, 3H, *m*	7.37–7.44, 3H, *m*
–OMe	3.84, 3H, *s*	3.96, 3H, *s*	3.66 (at C-3), 3.98 (at C-2), each 3H, *s*
β	4.89, 1H, *d* (3.7)	4.89, 1H, *d* (3.7)	4.58, 1H, *d* (3.4)
α	3.81, 1H, *dd* (3.7, 10.2)	3.82, 1H, *dd* (3.7, 10.3)	3.96, 1H, *dd* (3.4, 10.4)
α′	4.94, 1H, *d* (10.2)	4.91, 1H, *d* (10.3)	4.98, 1H, *d* (10.4)
Glc			
1″	4.84, 1H, *d* (7.3)	4.75, 1H, *d* (7.6)	4.82, 1H, *d* (7.6)
2″	3.50, 1H, *m*	3.52, 1H, *m*	3.54, 1H, *m*
3″	3.45, 1H, *m*	3.46, 1H, *m*	3.46, 1H, *m*
4″	3.39, 1H, *m*	3.39, 1H, *m*	4.0, 1H, *m*
5″	3.38, 1H, *m*	3.37, 1H, *m*	3.38, 1H, *m*
6″a	3.63, 1H, *dd* (5.2, 11.5)	3.64, 1H, *dd* (5.1, 11.7)	3.65, 1H, *dd* (5.1, 11.4)
6″b	3.84, 1H, *dd* (3.1, 11.5)	3.85, 1H, *dd* (3.0, 11.7)	3.85, 1H, *dd* (3.0, 11.4)

[a] Chemical shifts in ppm, *J* values in parentheses are recorded in Hz.

of **1** with related naturally occurring compounds having epoxy alcohol as a structural fragment (Wakabayashi, Spencer & Waters, 1991; Alfatafta, Gloer, Scott & Malloch, 1994; Sakagami, Sano, Hara, Mikawa & Marumo, 1995; Khan et al., 1996).

From the aforementioned results, the structure of compound **1** could be formulated as 2-methoxy-4,6-dihydroxy-α′-chalcanol-α,β-epoxide-4-*O*-β-D-glucopyranoside and named trifochalcanoloside **I**.

The molecular formula of compound **2** was determined to be $C_{22}H_{26}O_{11}$ from the negative HR FAB–MS (see Section 3) and NMR spectral data (Tables 1 and 2). The NMR spectral data of compound **2** showed a close similarity to **1** having the presence of an additional hydroxyl group attached to C-3 suggested from the ^{13}C-NMR data where a downfield shift of C-3 (41 ppm) and upfield shifts of C-2 and C-4 (12.8 and 12.3 ppm, respectively) were observed when compared with **1**. In addition, ^{1}H-NMR further confirmed the previous conclusion by displaying a singlet at δ_H 6.53 (1H, *s*) assigned for H-5. The downfield shift of the methoxyl group attached to C-2 to δ_C 61.7 is a further confirmation of its presence flanked by two substituents when compared with the corresponding shift of **1** (Harborne, 1988; Agrawal, 1989). The measurement of HMBC and ROE spectra for compound **2** supported the assignments mentioned above. In HMBC, correlation peaks were displayed between H-5 and both C-4 and C-6 while in ROE experiments, irradiation of H-1 of glucose gave a ROE to H-5. The absence of any ROE between the methoxyl group at C-2 and any aromatic proton in ring B was additional proof for the presence of the hydroxyl group at C-3. Consequently, the new compound **2** was identified as 2-methoxy-3,4,6-trihydroxy-α′-chalcanol-α,β-epoxide-4-*O*-β-D-glucopyranoside and was named trifochalcanoloside **II**.

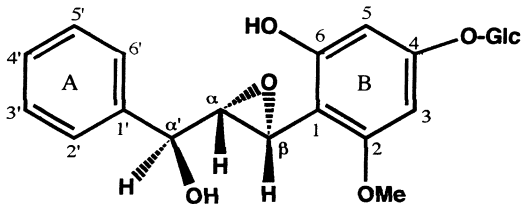
The molecular formula of compound **3** was determined to be $C_{23}H_{30}O_{12}$ from the negative HR FAB–MS (see Section 3) and NMR spectral data (Tables 1 and 2). The NMR spectral data of **3** were similar to those of **2** and showed the presence of an α′-chalcanol skeleton with hydroxylated carbons at δ_C 78.1, 73.3 and 72.8 corresponding to C-α′, C-α and C-β, respectively, with corresponding geminal methine protons at δ_H 4.98 (1H, *d*, J = 10.4 Hz, H-α′), 3.96 (1H, *dd*, J = 3.4 and 10.4 Hz, H-α) and 4.58 (1H, *d*, J = 3.4 Hz, H-β). The NMR shifts at δ_C 59.7 and 61.1 with δ_H 3.66 and 3.98 (each 3H, *s*) were attributed to the downfield shifted methoxyl groups located at C-2 and C-3, where each is flanked by two substituents (Harborne, 1988; Agrawal, 1989). The results of HMBC and ROE spectra for compound **3** confirmed these findings.

Therefore, compound **3** was characterized as 2,3-dimethoxy-4,6,α,β-tetrahydroxy-α′-chalcanol-4-*O*-β-D-glucopyranoside and named trifochalcanoloside **III**.

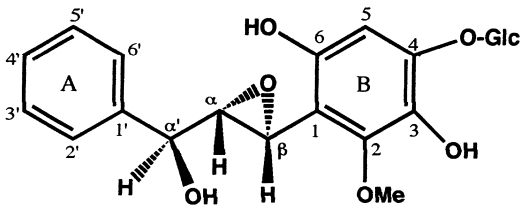
It is pertinent to emphasize that the relative configuration of the three chiral carbons C-β, C-α and C-α′ is the same for compounds **1**–**3** according to closely similar mutual J values of their relevant protons, (see Table 2).

Wong reported that chalcone epoxides are intermediates in the biosynthesis of aurones from chalcones (Wong, 1967). Hypothetically, α′-chalcanol-α,β-epoxides could originate by enzyme-activated reduction of the carbonyl group of chalcone epoxide precursors. Action of hydrolases on α′-chalcanol-α,β-epoxides may afford α,β-dihydroxy-α′-chalcanols.

Chalcone derivatives with the oxygenation pattern of **1**–**3** in ring B are rarely encountered as natural products (Panichpol & Waterman, 1978; El-Feraly & Hufford, 1982; Harborne & Mabry, 1982; Harborne, 1988; Ichino, Tanaka, Ito, Tanaka & Mizuno, 1988; Agrawal, 1989).



(1)



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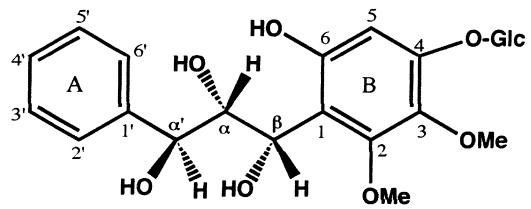

(3)

3. Experimental

For general experimental procedures, plant material and extraction, (see Mohamed et al., 1999).

3.1. Isolation of compounds (*1*–*3*)

The total methanolic extract of 1 kg powdered seeds of *Trifolium alexandrinum* L. was partitioned with EtOAc. The aq. fr. was applied to a column of Diaion HP 20 and the 50% MeOH eluate was chromatographed by silica gel CC using EtOAc–MeOH–H_2O (8:2:1 and 6:2:1, successively) to give 6 frs. from frs. 1 and 3 megastigmane glycosides were isolated (Mohamed et al., 1999). Fr. 4 (180 mg) was chromatographed on a column of RP-18 using 40% MeOH followed by prep. ODS HPLC using 35% MeOH to give **1** (10.7 mg), **2** (14.5 mg) and **3** (7.5 mg).

3.2. Compound (*1*)

2-methoxy-4,6-dihydroxy-α′-chalcanol-α,β-epoxide-4-*O*-β-D-glucopyranoside, amorphous powder, HR FAB–MS (negative) *m*/*z*: 449.1482 $[M-H]^-$ $C_{22}H_{25}O_{10}$ (req. 449.1448). ^{13}C- and ^1H-NMR (CD_3OD, Tables 1 and 2).

3.3. Compound (*2*)

2-methoxy-3,4,6-trihydroxy-α′-chalcanol-α,β-epoxide-4-*O*-β-D-glucopyranoside, amorphous powder, HR FAB–MS (negative) *m*/*z*: 465.1409 $[M-H]^-$ $C_{22}H_{25}O_{11}$ (req. 465.1397). ^{13}C- and ^1H-NMR (CD_3OD, Tables 1 and 2).

3.4. Compound (*3*)

2,3-dimethoxy-4,6,α,β-tetrahydroxy-α′-chalcanol-4-*O*-β-D-glucopyranoside, amorphous powder, HR FAB–MS (negative) *m*/*z*: 479.1589 $[M-H-H_2O]^-$ $C_{23}H_{27}O_{11}$ (req. 479.1553). ^{13}C- and ^1H-NMR (CD_3OD, Tables 1 and 2).

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