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RESEARCH ARTICLE

A new flavonoid glycoside from the aerial parts of *Diplotaxis erucoides* (L.) DC. growing in Algeria

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Abstract

The phytochemical study of the 70% ethanol extract of the aerial parts of *Diplotaxis erucoides* afforded one new flavonoid glycoside, namely kaempferol-3-*O*-[*α*-L-rhamnopyranosyl-(1→2)-*β*-D-xylopyranoside]-7-*O*-*α*-L-rhamnopyranoside (**1**), named diploerucoside A and seven known compounds including one flavonoid (**2**), one phenolic glycoside (**3**), one monoterpene (**4**), one triterpene (**5**), one sitosterol (**6**) and two monoglycerolipids (**7**, **8**). Their structures were established by extensive spectroscopic analysis including 1D- and 2D-NMR (^{1}H, ^{13}C, ^{1}H-^{1}H COSY, HSQC and HMBC), mass spectrometry (HR-ESI-MS) and by comparison with the data reported in the literature.

Keywords: Brassicaceae, *Diplotaxis erucoides*, Kaempferol-3-*O*-[*α*-L-rhamnopyranosyl-(1→2)-*β*-D-xylopyranoside]-7-*O*-*α*-L-rhamnopyranoside, flavonoids, NMR

1. Introduction

The genus *Diplotaxis* (wall-rocket), belonging to the Brassicaceae (Cruciferae) family, is represented by more than 32 species (Warwicket al. 2006) which are mainly distributed in the Mediterranean region, Southwest Asia and Europe (Pignone and Martinez-Laborde, 2010). Plants of this genus have been used in folk medicine as aphrodisiac and anti-diarrheic (De Feoet al., 1992). They have also mucolytic properties (Caudron, 2005).

Diplotaxis erucoides (L.) D.C. (white wall-rocket) is an herbaceous plant up to 30–50 cm tall. Its flowers are white veined with purple (Quezel and Santa, 1963). It is a wild vegetable with high content in ascorbic acid, glucosinolates, flavonoids, and it is also a NO_3^- bioaccumulator. (Bennett et al., 2006, Guijarro-Real et al., 2020). Previous phytochemical investigations have reported that *n*-hexane and *n*-butanol fractions of the aerial parts of *Diplotaxis erucoides* contained fatty acid and flavonoids (Ben Salah et al., 2015).

In the present study, we describe the isolation and structural elucidation of one new flavonoid glycoside (**1**) named diploerucoside A with seven known compounds (**2-8**) from the aerial parts of *D. erucoides.* All compounds were identified based on spectroscopic data (1D- and 2D-NMR, HR-ESI-MS) and comparison with data from the literature.

2. Results and discussion

Compound **1** was isolated as yellow crystals. Its molecular formula was assigned as $C_{32}H_{38}O_{18}$ on the basis of the high-resolution ESI-MS experiment (negative-ion mode) by observation of pseudo-molecular ion peak at *m/z* = 709.1985 $[M–H]^-$ (calcd $[C_{32}H_{37}O_{18}]^-$, 709.1985) which is consistent with the presence of 32 signals on the ^{13}C-NMR spectrum. The analysis of the ^{1}H- and ^{13}C-NMR spectra of **1** showed characteristic signals of a glycosylated flavonoid. The^{1}H NMR and ^{1}H-^{1}H COSY spectra displayed two *meta* coupled signals at δ_H 6.46 (1H, d, *J* = 1.5 Hz, H-6) and 6.75 (1H, d, *J* = 1.5 Hz, H-8), characteristic of the 5,7-

distributed A ring of flavonoid. These protons were resonating over 6.4 ppm indicating that glycosylation site is position 7 (Vassallo et al., 2006). The ^{1}H NMR spectrum also showed two *ortho*-coupled doublet signals at δ_H 8.06 (2H, d, *J*= 8.5 Hz) and 6.91 (2H, d, *J*= 8.5 Hz) of a *para*-substituted aromatic nucleus. According to the ^{1}H-^{1}H COSY spectrum, these protons belong to the same spins system, attributable to the H-2'/H-6' and H-3'/H-5' protons. Their carbons were detected by HSQC spectrum at δ_C 116.2 and 132.2, respectively. The assignments of all carbons of the flavonol moiety were accomplished on the basis of $^{3}J_{H\text{-}C}$ HMBC correlations depicted between H-2′/H-6′ and two quaternary carbons C-2 (δ_C 159.1) and C-4' (δ_C 161.7); H-3'/H-5' and the quaternary carbon at δ_C 122.7 (C-1'); H-8 and four carbons, among them three are quaternary at δ_C 100.5 (C-6), 107.5 (C-10), 158 (C-9) and 163.5 (C-7); H-6 and C-5 (δ_C 163), C-8 (δ_C 95.5). Thus, the aglycone part of **1** was identified as kaempferol (Aisyah et al., 2017). Furthermore, three anomeric proton signals were clearly observed at δ_H 5.63 (d, *J* = 7 Hz), 5.22 (brs) and 5.56 (brs) correlating with carbon signals at δ_C 101.2, 99.9 and 102.7 in the HSQC spectrum, respectively. The analysis of ^{1}H-^{1}H COSY and HSQC experiments, and acid hydrolysis led to the identification of *β*-D-xylose and two *α*-L-rhamnose units. In fact, the two *α*-L-rhamnopyranosyl units with anomeric proton signals at δ_H 5.56 (brs, H-1"") and 5.22 (brs, H-1‴) were characterized by the presence of eight proton spins system with their methyl groups at δ_H1.33 (d, *J* = 6.2 Hz, H_3-6"") and 1.12 (d, *J* = 6 Hz, H_3-6‴), respectively. Additionally, the *β*-D-xylopyranosyl unit was assigned on the basis of six proton spins system starting from the third anomeric proton H-1" (δ_H 5.63). This latter (H-1") correlated with H-2" (δ_H 3.66, dd, *J* = 9.5, 7 Hz) in the ^{1}H-^{1}H COSY spectrum, which coupled with H-3" (δ_H 3.51, t, *J* = 9.5 Hz). The proton H-4" resonating as a triplet at δ_H 3.49 (*J* = 9.5 Hz) correlated with the protons H_2-5" at δ_H 3.74 (dd, *J* = 11.5, 5 Hz) and 3.1 (dd, *J* = 11.5, 7.8 Hz) (Serge et al., 2017). The values of the coupling constants are all greater than 7 Hz indicating a *trans*-diaxial position of these protons. The correlation observed in the HMBC

spectrum (Figure 1) from anomeric proton H-1"" (δ_H 5.56) of rhamnose to carbon C-7 (δ_H 163.5) revealed the point of attachment of one of the *α*-L-rhamnopyranosyl units to the aglycone part at C-7 position. On the other hand, the long-range correlations (Figure 1) observed between the anomeric proton H-1‴ (δ_H 5.22) of the second rhamnose with C-2″ (δ_C 79.5) of xylose indicated the interglycosidic linkage between these glycosyl units identified as *α*-L-rhamnopyranosyl-(1→2)-*β*-D-xylopyranoside. Moreover, the glycosylation position of this diglycoside (Figure 1) was deduced into C-3 based on the chemical shift of this carbon at δ_C 134.7 and C-2 at δ_C 159.1 (Chabani et al. 2013; Okoye et al. 2015). Thus, **1** was determined to be kaempferol-3-*O*-[*α*-L-rhamnopyranosyl-(1→2)-*β*-D-xylopyranoside]-7-*O*-*α*-L-rhamnopyranoside, a new flavonoid glycoside named diploerucoside A.

Insert figure 1 here

Compared the physical and spectral data (1D- and 2D-NMR, HR-ESI-MS) with the literature data, 7 known compounds (**2**–**7**) were identified as one flavonoid: astragalin (**2**) (Fawzi et al., 2019; Ghavam-Haghi et al., 2017); one phenolic glucoside: 1-*O*-*p*-coumaroyl-*β*-D-glucose (**3**) (Strack et al. 1989; Baderschneider et al., 2001); one monoterpene: loliolide (**4**) (Hodges and Porte, 1964); one triterpene: lupeol (**5**) (Saratha et al., 2011); *β*-sitosterol (**6**) (Nyigo et al., 2012), and two monoglycerolipids: (10*E*,12*Z*)-2,3-dihydroxypropyl octadeca-10,12-dienoate (**7**) (Eleni et al., 2017), and (9*Z*,11*E*)-2,3-dihydroxypropyl octadeca-9,11-dienoate (**8**) (Chin et al.,1992; Eleni et al., 2017) (Figure 2). Compounds **3**, **4**, **7** and **8** were isolated from *Diplotaxis* genus for the first time.

Insert figure 2 here

This study allowed particularly the isolation and identification of two flavonoids named diploerucoside A (**1**) and astragalin (**2**). This type of secondary metabolites is used as a chemotaxonomic marker in genus *Diplotaxis* including *D. erucoides, D. acris, D. harra* and *D. muralis* (Hussein et al., 2017). The new flavonol glycoside diploerucoside A (**1**) is a

kaempferol derivative having two sugar moieties in positions C-3 (*α*-L-rhamnopyranosyl-(1→2)-*β*-D-xylopyranoside) and C-7 (*α*-L-rhamnopyranoside), whereas the known flavonol (**2**) possesses a kaempferol skeleton with *β*-D-glucose in position C-3. Furthermore, previous studies revealed that *D. erucoides* contained flavonol glycosides with kaempferol, quercetin and rhamnetin skeletons and that glycosylation linkage occurred mainly at positions C-3 and C-7 of the aglycone (Ben Salah et al., 2015; Sanchez-Yelamo, 1994). This constatation is notably in agreement with our results helping to further clarify the involvement of flavonoids in the chemotaxonomic profile of *Diplotaxis* species.

3. The Experimental

3.1. General experimental procedures

The 1D- (^{1}H and ^{13}C) and 2D- (^{1}H-^{1}H COSY, HSQC, HMBC) NMR spectra were recorded on a Brüker type device at 500 MHz for ^{1}H and 125 MHz for ^{13}C. Samples were dissolved in CD_3OD or $CDCl_3$ deuterated solvents depending on the solubility of the compounds. The chemical shifts *(δ*) are expressed in ppm relative to tetramethylsilane (TMS); the coupling constants are defined in Hz. HR-ESI-MS experiments were performed on a Waters SYNAPT G2-Si High Resolution Q-TOF Mass Spectrometer equipped with electrospray ionization (ESI) source. The optical rotation $[\alpha]_D$ of the isolated compounds was determined using a Perkin-Elmer 241 polarimeter type at 20 °C. The fractionation was carried out on vacuum liquid chromatography (VLC) using SiO_2 (Kieselgel Merck 70-230 mesh). The purification of isolated compounds was performed over column chromatography (CC) on SiO_2 (Kieselgel Merck 70-230 mesh), polyamide SC6 and Sephadex LH-20 (25–100 μM). Analytical and preparative TLCs were conducted on Silica gel [Kiselgel 60 F_{254}, Merck 250 μM (20 × 20 cm)] plates, detection at 254 and 366 nm and by spraying with sulfuric acid reagent (50%) followed by heating.

3.2. Plant material

The aerial parts of *Diplotaxis erucoides* (L.) DC. were harvested in November 2018 in El Madher from region of Batna (North eastern of Algeria). The plant was identified by Prof. Bachir Oudjehih from the Agronomy Department of the Institute of Veterinary and Agronomic Sciences at University of Batna-1 (Algeria). A voucher specimen was listed under the number 977/LCCE.

3.3. Extraction and isolation

Powdered air-dried aerial parts (1.4 kg) of *Diplotaxis erucoides* were extracted with 70% aqueous ethanol for 48 h at room temperature. The extraction was repeated twice with the renewal of the solvent. After filtration and concentration under vacuum, the concentrated aqueous residue (400 mL) was submitted to liquid – liquid partition, with petroleum ether (PE), ethyl acetate (EtOAc), and *n*-butanol (*n*-BuOH), successively (each solvent, 250 mL × 3). The evaporation of the organic phases gave 15.59 g of PE, 14.17 g of EtOAc and 18.48 g of *n*-BuOH fractions.

A part of the EtOAc fraction (7 g) was separated by VLC performed over a silica gel (5 × 5 cm; fractions of 100 mL). The elution was carried out with PE/EtOAc (10:0 to 0:10), then EtOAc/MeOH (10:0 to 0:10) to yield fractions (F1– F9). Fraction F2 (28 mg) was fractionated over a silica gel CC, eluted with PE/EtOAc (10:0 to 0:10) to give seventeen sub-fractions. The sub-fractions F_2-5 (6.1 mg) and F_2-7 (5.7 mg) were subjected successively to Sephadex LH-20 column, eluted with $CHCl_3$ to provide compounds **5** (3 mg) and **6** (3 mg). Fraction F5 (88.2 mg) was purified by silica gel CC eluted with a gradient of PE/EtOAc (10:0 to 0:10) to give four sub-fractions (F_5-1 – F_5-4). Compounds **4** (2.8 mg), **7** (2.5 mg) and **8** (2 mg) were obtained from sub-fraction F_5-3 (42.6 mg) using Sephadex LH-20 CC, eluted with

CH_2Cl_2 followed by silica gel preparative thin layer chromatography (prep. TLC) eluted with CH_2Cl_2/MeOH (9:1).

The *n*-BuOH extract (7 g) was subjected to a silica gel VLC, eluted with $CHCl_3$/MeOH/H_2O solvent system to give eight fractions. Fraction F4 (1.292 g) was subjected to a polyamide SC-6 CC eluted with the H_2O/MeOH system (10:0 to 0:10). Ten sub-fractions were obtained after fractionation and pooling (A_1 to A_{10}). The A_4 sub-fraction (106.2 mg) was separated on Sephadex LH-20 CC, eluted with MeOH, followed by silica gel CC, eluted with $CHCl_3$/MeOH (10:0 to 5:5), to afford eight sub-fractions (A_4-1 to A_4-8). Compound **3** (1.5 mg) was obtained by precipitation of the sub-fraction A_4-4 (11.4 mg) in MeOH. The sub-fraction A_4-7 (6.7 mg) was precipitated in diethyl ether followed by filtration through a Sephadex LH-20 CC using MeOH as eluent to provide compound **1** (6.4 mg). Sub-fraction A_4-8 (62.9 mg) was chromatographed on Sephadex LH-20 CC, eluted with $CHCl_3$/MeOH to yield 9 sub-fractions (A_4-8a to A_4-8H). The sub-fraction A_4-8f (11 mg) was purified by silica gel prep. TLC in ($CHCl_3$/MeOH/H_2O; 7:3:0.3) to furnish compound **2** (3.2 mg).

3.4. Acid hydrolysis

An acid hydrolysis was carried out on compound **1** to establish the configuration of monosaccharide residues. Indeed, 5 mg of **1** was refluxed with 5 mL of 2N HCl for 2h, then it was neutralized with 0.5 M KOH. After extraction with CH_2Cl_2 (3 × 5 mL), the aqueous solution was evaporated and then purified by prep. TLC using the solvents system MeCOEt–isoPrOH–Me_2CO–H_2O (10:5:3.5) to afford two monosaccharides identified as rhamnose and xylose by comparison with authentic simples on TLC in the same solvents system. The optical rotation of each purified monosaccharide was measured [rhamnose $[\alpha]_D^{20}$ +2.4 (c 0.96, H_2O) and xylose $[\alpha]_D^{20}$ +15.3 (c 0.96, H_2O)] and the absolute configurations were

determined as L for rhamnose and D for xylose by comparison with $[\alpha]^{20}_{D}$ values of authentic samples of D-xylose and L-rhamnose (Sigma–Aldrich).

3.5. Spectral data

Kaempferol-3-*O*-[*α*-L-rhamnopyranosyl-(1→2)-*β*-D-xylopyranoside]-7-*O*-*α*-L-rhamnopyranoside **(1):** yellow crystals; mp 252-253 °C; $[\alpha]_D^{20}$ – 21.6 (*c* 0.15, MeOH); HR-ESI-MS *m/z* = 709.1985 $[M - H]^-$ (calcd $[C_{32}H_{37}O_{18}]^-$, 709.1985); ^{1}H-NMR (500 MHz, CD_3OD) δ_H 8.06 (2H, d, *J* = 8.5 Hz, H-2′/H-6′), 6.91 (2H, d, *J* = 8.5 Hz, H-3′/H-5′), 6.75 (1H, d, *J* = 1.5 Hz, H-8), 6.46 (1H, d, *J* = 1.5 Hz, H-6), 5.63 (1H, d, *J* = 7 Hz, H-1″), 3.66 (1H, dd, *J* =9.5, 7 Hz, H-2″), 3.51 (1H, t, *J* = 9.5 Hz, H-3″), 3.49 (1H, t, *J* = 9.5 Hz, H-4″), 3.74 (1H, dd, *J* = 11.5, 5 Hz, H-5″a), 3.1 (1H,dd, *J* = 11.5, 7.8 Hz, H-5″b), 5.22 (1H, brs, H-1‴), 4.01 (1H, dd, *J* = 3.5, 1.2 Hz, H-2‴), 3.77 (1H, dd, *J* = 9.5, 3.5 Hz, H-3‴),3.36 (1H, t, *J* = 9.5 Hz, H-4‴), 4.06 (1H, dd, *J* = 9.8, 6 Hz, H-5‴), 1.26 (3H, d, *J* = 6 Hz, H_3-6‴),5.56 (1 H, brs, H-1⁗), 4.02 (1H, dd, *J* = 3.4, 1.1 Hz, H-2⁗), 3.83 (1H, dd, *J* = 9.5, 3.4 Hz, H-3⁗), 3.48 (1H, t, *J* = 9.5 Hz, H-4⁗), 3.6 (1H, dd, *J* = 10.2, 6.4 Hz, H-5⁗), 1.04 (3H, d, *J* =6.5 Hz, H_3-6⁗).^{13}C-NMR (125 MHz, CD_3OD) δ_C 179.0 (C-4), 163.5 (C-7), 163.0 (C-5), 161.7 (C-4′), 159.1 (C-2), 159.0 (C-9), 134.7 (C-3), 132.2 (C-2′/C-6′), 122.7 (C-1′), 116.2 (C-3′/C-5′), 107.5 (C-10), 100.5 (C-6), 95.5 (C-8), 101.2 (C1″), 79.5 (C-2″), 77.5 (C-3″),71.4 (C-4″), 67.2 (C-5″), 102.7 (C-1‴), 72.4(C-2‴), 72.3(C-3‴), 74.0 (C-4‴), 70.0 (C-5‴), 17.7 (C-6‴), 99.9 (C-1⁗), 71.7 (C-2⁗), 72.1 (C-3⁗),73.6 (C-4⁗), 71.3 (C-5⁗), 18.1 (C-6⁗).

4. Conclusion

A new flavonol glycoside (**1**) named diploerucoside A along with seven known compounds including one flavonoid, one phenolic glucoside, one monoterpene, two monoglycerolipids, one sitosterol and one triterpene have been isolated from the EtOAc and *n*-BuOH fractions of

the 70% ethanol extract of the aerial parts of *Diplotaxis erucoides* belonging to the Brassicaceae family.This study reports for the first time the occurrence of compounds **3**, **4**, **7** and **8** in the *Diplotaxis* genus.

Supplementary material

HR-ESI-MS, ^{1}H and ^{13}C NMR, ^{1}H-^{1}H-COSY, HSQC and HMBC spectra of all compoundsare available online.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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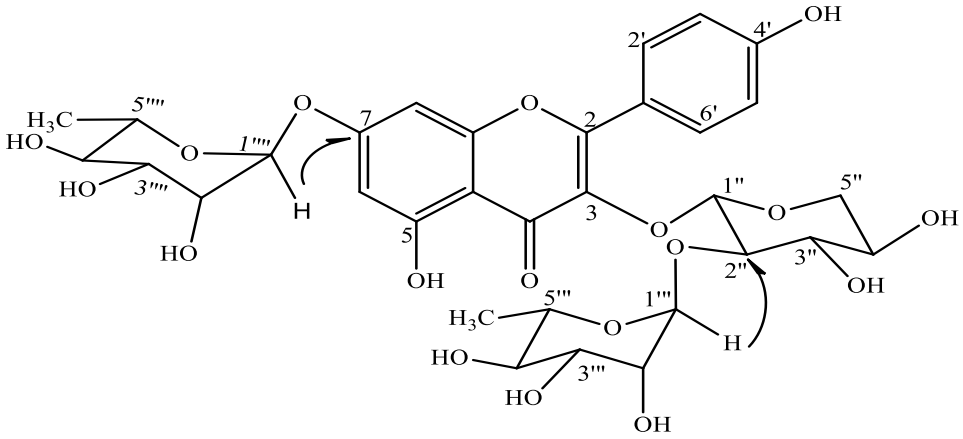

Figure 1. HMBC (H-C) correlations for compound **1**

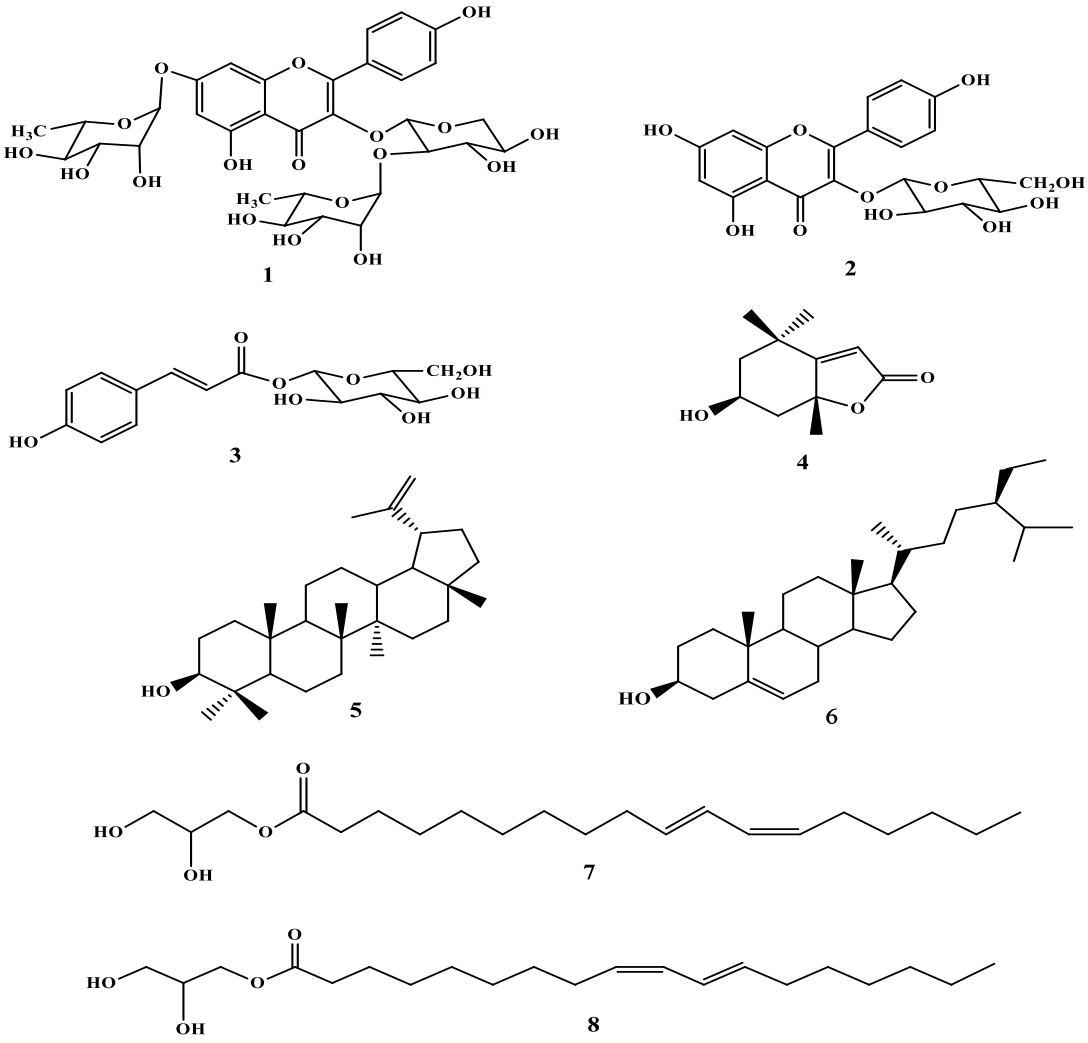

Figure 2. Structures of compounds **1–8** isolated from *D. erucoides*