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Three Phenolic and a Sterol Glycosides Identified for the First Time in *Matthiola longipetala* Growing in Tunisia

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Three phenolic glycosides: 4-*O*-β-D-glycopyranosyl zingerone **1**, 4-*O*-β-D-glycopyranosylhomovanillyl alcohol **2** and eugenol glycoside **3,** together with 3-*O*-β-D-glycopyranosyl sitosterol **4,** were isolated and identified for the first time from the flowers of *Matthiola longipetala* (Crucifers) growing in Tunisia. The structures of **1**, **2** and **3** were identified *via* their acetylated derivatives on the basis of the 1 and 2D NMR data analysis, mass spectrometry and IR spectroscopy.

INTRODUCTION

The present research is one of our contributions to the biological and chemical studies of Tunisian plants.[1,2] *Matthiola longipetala (ssp livida)* is a plant living in a narrow geographical area, in particular from Egypt to Morocco. G. Pottier Alapetite reports that, along with *longipetala*, four other species from the *Matthiola* genus were found in Tunisia: *fruticulosa*, *lunata*, *parviflora* and *tricuspidata.*[3] To our knowledge, there are no reports on the use of these species in folk medicine in Tunisia. We report here on the results of an investigation of the methanolic extract of fresh flowers of *Matthiola longipetala* and on the structural elucidation of three phenolic glycosides: 4-*O*-β-D-glycopyranosyl zingerone **1**, 4-*O*-β-D-glycopyranosylhomovanillyl alcohol **2** and eugenol glycoside **3,** together with the heteroside: 3-*O*-β-D-glycopyranosyl sitosterol **4,** isolated for the first time from this plant**.**

EXPERIMENTAL

General

Mass spectra were obtained with a micromass spectrometer (Q-Tof micro) linked to an ESI source. UV spectra were recorded on a JASCO V-530 UV-Vis spectrophotometer. ^{1}H (400 MHz), ^{13}C (100 MHz) and 2D-NMR spectra of **1a** and **4** were recorded in $CDCl_3$ and in a mixture of $CD_3OD + CDCl_3$, respectively, with a Bruker Avance AM-400 spectrometer. The ^{1}H (500 MHz), ^{13}C (125 MHz) and 2D-NMR spectra of **2a** were obtained in $CDCl_3$ with a Bruker Avance AM-500 spectrometer. The ^{1}H (300 MHz), ^{13}C (75 MHz) and 2D-NMR spectra of **3a** were recorded in $CDCl_3$ using a Bruker NMR-300 spectrometer. Chemical shifts (δ) are given in ppm and coupling constants (J) in Hz. Residual solvent resonances were used as internal standards.

Plant Material

Matthiola longipetala was harvested in March 2003 at Gabes (Southeastern Tunisia). A voucher specimen was deposited

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TABLE I. ^{1}H and ^{13}C NMR spectral data for compounds **1a**, **2a** and **3a**

Compound	**1a**		**2a**		**3a**	
Position	^{13}C	^{1}H (J / Hz)	^{13}C	^{1}H (J / Hz)	^{13}C	^{1}H (J / Hz)
1	137.5		134.9		135.3	
2	112.5	6.72; d (1.7)	113.5	6.75; d (1.6)	121.0	6.71; m
3	150.0		150.1		118.8	6.69; dd (8.1; 2.4)
4	145.0		145.0		144.7	
5	119.9	7.01; d (8.1)	121.5	7.04; d (8.1)	150.9	7.04; d (8.1)
6	120.0	6.65; dd (8.1; 1.7)	122.5	6.71; dd (8.1; 1.6)	113.7	6.71; d (8.1)
7	30.0	2.85; t (7.1)	37.0	2.87; t (7.0)	40.3	3.31; m
8	45.1	2.75; t (7.1)	66.8	4.27; m	137.6	5.93; m
9	208.0		172.0		116.3	5.09; m
10	30.1	2.13; s				
1'	101.0	4.90; d (7.4)	102.5	4.93; d (7.4)	101.4	4.91; d (7.5)
2'	72.1	5.26; m	73.0	5.27; m	71.6	5.27; m
3'	72.1	5.26; m	73.0	5.27; m	73.0	5.27; m
4'	68.5	5.16; t (4)	69.5	5.16; t (6.3)	68.8	5.15; t (4.5)
5'	72.0	3.75; m	73.0	3.75; m	72.3	3.71; m
6'a	61.0	4.15; dd (12.2; 2.4)	62.9	4.19; dd (12.2; 2.2)	62.3	4.16; dd (12.2; 2.4)
6'b	61.0	4.29; dd (12.2; 5)	62.9	4.27; m	62.3	4.27; dd (12.2; 5)
OCH_3	56.0	3.79; s	58.0	3.81; s	56.3	3.81; s

in the herbarium of the Ecole Supérieure d'Horticulture et d'Elevage de Chott Mériem, Université du Centre, Sousse, Tunisia.

Extraction and Isolation

*Compound **1a**:* Fresh flowers of *Matthiola longipetala* (1kg) were extracted with pure methanol to yield 50 g of a dry residue. Methanolic extract was simplified over a silica gel column (sds, 60 AC. C 70–200 μm, petroleum ether, AcOEt, MeOH gradients). 105×400 mL fractions were obtained. Similar fractions were pooled together based on the TLC analysis. Fractions 77–85 (2.5 g) were rechromatographed on a silica gel column eluted with methylene chloride, increasing the amounts of methanol and collecting 10 mL fractions. The eluted fractions examined by TLC and similar fractions were pooled to give 5 main fractions. Fraction 3 (250 mg) was further chromatographed on a silica gel column eluted with methylene chloride. Four main subfractions were obtained. The more polar one (190 mg) was chromatographed over a silica gel column eluted with a mixture of 9:1 methylene chloride/methanol into 74×5 mL fractions. Acetylation at room temperature with acetic anhydride in pyridine of 5 mg of the mixture 48–64 (30 mg) containing an impure major compound difficult to be purified by classic chromatographic methods (cc or prep TLC) followed by purification of the acetylated residue over a silica gel column eluted with petroleum ether/acetone 8:2 yielded compound **1a** as a white solid (3 mg), $[\alpha]_D^{22}$ + 22 (c = 0.06; $CHCl_3$): IR (KBr) ν_{max} / cm^{-1}: 1750; UV (MeOH) λ_{max} / nm (A): 234 (2,878), 267 (2,717). ^{1}H and ^{13}C NMR – see Table I. The ^{1}H NMR spectrum of fractions 48–64 before acetylation showed no presence of acetoxy signal.

*Compound **2a**:* Fractions 86–95 (2.5 g) from the first liquid chromatography column were separated over a silica gel column with a 9:1 mixture of methylene chloride/methanol as eluent. 140×10 mL fractions were collected and combined according to the TLC analysis. Fractions 76–120 (320 mg) were acetylated as indicated above to give an acetylated residue (459.2 mg), which was in turn chromatographed over a silica gel column eluted with a 9.5:0.5 mixture of petroleum ether/acetone, increasing the amounts of acetone. 225×10 mL fractions were collected and combined according to the TLC analysis. Fractions 111–122 (11.5 mg) were purified on preparative TLC to furnish compound **2a** as a white solid (2.8 mg), $[\alpha]_D^{22}$ +13.75 (c = 0.02; $CHCl_3$); IR (KBr) ν_{max} / cm^{-1}: 1750. ^{1}H and ^{13}C NMR – see Table I.

*Compound **3**:* Fractions 33–56 (850 mg) obtained from the first liquid chromatography column were subjected to silica gel column chromatography eluted with methylene chloride to give 5 crude fractions. The fourth one was further purified on a silica gel column eluted with a 9:1 mixture of chloroform/methanol to afford compound **3** as a white solid (7 mg).

Acetylation of compound **3:** eugenol glycoside tetraacetate and 3-*O*-β-D-glycopyranosyl sitosterol were prepared by acetylation (pyridine-Ac_2O, 2:1; 5 h at room temperature).

*Compound **3a**:* white solid (11 mg), UV (MeOH) λ_{max} / nm (A): 232 (2,625), 278 (1,755). ESMS *m/z* 1011 ($[2M+Na]^+$) 517 ($[M+Na]^+$), 331 (35), 169 (8), 109 (10) (calculated for $C_{24}H_{30}O_{11}$), ^{1}H and ^{13}C NMR – see Table I.

RESULTS AND DISCUSSION

*Compound **1a**:* The ESMS of **1a** exhibiting a pseudomolecular ion peak $[M+Na]^+$ at *m/z* 547 revealed the molecular formula of $C_{25}H_{32}O_{12}$. The ^{1}H NMR spectrum of **1a** displayed signals at: δ 7.01 ppm (d, J = 8.1 Hz, H_5), δ 6.72 ppm (bd, J = 1.7 Hz, H_2) and δ 6.65 ppm (dd, J_1 = 1.7 Hz, J_2 = 8.1 Hz, H_6) showing the presence of 1,3,4-

trisubstituted benzene ring. A singlet at 3.79 ppm (s, 3H) indicates that one of the substituents is a methoxy group. The signal at δ 4.90 ppm (d, J = 7.4 Hz, $H_{1'}$) assigned to a β-coupled anomeric proton was in agreement with the presence of a β-D-glycopyranoside moiety. Signals centered at 2.85 (t, J = 7.1 Hz, H_7) and 2.75 (t, J = 7.1 Hz, H_8), in addition to the significant acetoxy signal observed at 2.13 ppm, suggested linkage of a CH_2-CH_2-CO-CH_3 moiety to the aromatic ring. This hypothesis was confirmed by the ^{1}H-^{1}H correlation spectroscopy (COSY) spectrum revealing the connectivity of H_7 to H_8. The C-H long-range HMBC spectrum (Figure 1) allowed us to connect H_7 (δ 2.85 ppm) to C_1 (δ 137.5 ppm) of the aromatic ring on the basis of the correlations H_7-C_1, H_2-C_7 and H_6-C_7. The location of the methoxy group at C_3 was proved by the long range coupling between H_{OMe} (δ 3.79 ppm) and C_3 (δ 150.0 ppm) deduced from the same HMBC spectrum (Figure 1). The analysis of COSY datasets showing correlations $H_{1'}$-$H_{2'}$; $H_{2'}$-$H_{3'}$; $H_{4'}$-$H_{5'}$; $H_{5'}$-$H_{6'a}$; $H_{5'}$-$H_{6'b}$ and $H_{6'a}$-$H_{6'b}$ as well as the observation on the HMQC spectrum of only one anomeric carbon $C_{1'}$ (δ 101.0 ppm) made it possible to establish the skeleton of the monoglycopyranoside. The linkage of the monosaccharide moiety at C_4 of the aglycone was deduced from the HMBC spectrum showing the characteristic correlation between the anomeric proton $H_{1'}$ (δ 4.90 ppm) and carbon C_4 (δ 145.0 ppm). Comparison of these spectral data to those previously reported in literature[4] confirmed that **1a** is the 4-*O*-β-D-glycopyranosyl zingerone tetraacetate.

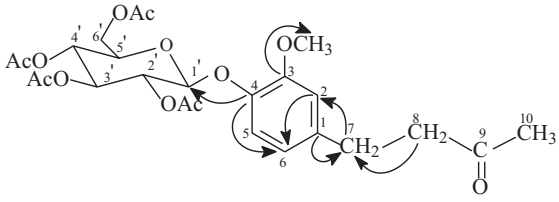


Figure 1. Heteronuclear multiple-bond correlations (HMBC) for **1a.** Arrows point from carbon to proton.

*Compound **2a**:* Its molecular formula $C_{25}H_{32}O_{13}$ was deduced from the ESMS showing a pseudomolecular ion peak $[M+Na]^+$ at *m/z* 563. The spectral data of compound **2a** were similar to those of compound **1a,** with the exception that H_8 was shifted at δ 4.26 ppm, which suggested the homovanillyl alcohol structure for the aglycone. This hypothesis was reinforced by the HMQC spectrum showing a direct correlation between H_8 (δ 4.26 ppm) and C_8 (δ 67.0 ppm) as well as by the HMBC spectrum, in which we picked up the significant long range correlation H_8-CO_{OAc}. The difference of 16 a.m.u (oxygen atom) between the two molecular weights of **1a** and **2a** reinforced the aglycone structure. Comparison of these spectral data to those previously reported in literature[5] confirmed that **2a** is 4-*O*-β-D-glycopyranosylhomovanillyl alcohol pentaacetate.

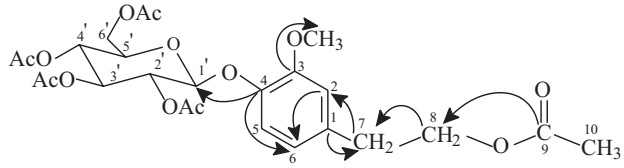
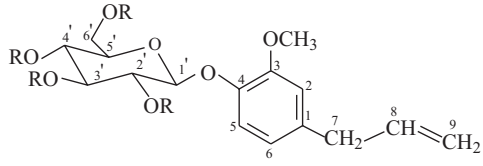


Figure 2. Heteronuclear multiple-bond correlations (HMBC) for **2a**. Arrows point from carbon to proton.

*Compounds **3** and **3a**:* The molecular formula of **3a** was determined to be $C_{24}H_{30}O_{11}$ by ESMS showing a pseudomolecular ion peak $[M+Na]^+$ at *m/z* 517. The spectral data of **3** and those of **3a** deduced from ^{1}H, ^{13}C and COSY NMR spectra were very similar to those of eugenol glycoside.[6,7]



3 R = H
3a R = Ac

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SAŽETAK

Tri fenolna i sterolni glikozidi identificirani prvi put u *Matthiola longipetala* koja raste u Tunisu

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Tri fenolna glikozida: 4-*O*-β-D-glukopiranozilzingeron **1**, 4-*O*-β-D-glukopiranozilhomovanilil-alkohol **2** i eugenil-glikozid **3** i 3-*O*-β-D-glukopiranozilsitosterol **4** izolirani su i identificirani prvi put iz cvijetova *Matthiola longipetala* (Crucuferae), koja raste u Tunisu. Strukture **1**, **2** i **3** su identificirane preko njihovih acetiliranih derivata iz 1- i 2-D NMR podataka, masenom spekrometrijom i IR-spektroskopijom.