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

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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-β-D-glycopyranosyl zingerone 1, 4-β-D-glycopyranosylhomovanillyl alcohol 2 and eugenol glycoside 3, together with 3-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.

EXPERIMENTAL

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. 1H (400 MHz), 13C (100 MHz) and 2D-NMR spectra of 1a and 4 were recorded in CDCl3 and in a mixture of CD3OD + CDCl3, respectively, with a Bruker Avance AM-400 spectrometer. The 1H (500 MHz), 13C (125 MHz) and 2D-NMR spectra of 2a were obtained in CDCl3, and in a mixture of CD3OD + CDCl3, respectively, with a Bruker Avance AM-400 spectrometer. The 1H (500 MHz), 13C (125 MHz) and 2D-NMR spectra of 3a were recorded in CDCl3 with a Bruker Avance AM-500 spectrometer. The 1H (300 MHz), 13C (75 MHz) and 2D-NMR spectra of 3a were recorded in CDCl3 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.
TABLE I. $^1$H and $^{13}$C NMR spectral data for compounds 1a, 2a, and 3a

<table>
<thead>
<tr>
<th>Compound</th>
<th>1a</th>
<th>2a</th>
<th>3a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Position</td>
<td>$^{13}$C</td>
<td>$^1$H (J / Hz)</td>
<td>$^{13}$C</td>
</tr>
<tr>
<td>1</td>
<td>137.5</td>
<td>134.9</td>
<td>135.3</td>
</tr>
<tr>
<td>2</td>
<td>112.5</td>
<td>6.72; d (1.7)</td>
<td>113.5</td>
</tr>
<tr>
<td>3</td>
<td>150.0</td>
<td>150.1</td>
<td>118.8</td>
</tr>
<tr>
<td>4</td>
<td>145.0</td>
<td>145.0</td>
<td>144.7</td>
</tr>
<tr>
<td>5</td>
<td>119.9</td>
<td>7.01; d (8.1)</td>
<td>121.5</td>
</tr>
<tr>
<td>6</td>
<td>120.0</td>
<td>6.65; dd (8.1; 1.7)</td>
<td>122.5</td>
</tr>
<tr>
<td>7</td>
<td>30.0</td>
<td>2.85; t (7.1)</td>
<td>37.0</td>
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<tr>
<td>8</td>
<td>45.1</td>
<td>2.75; t (7.1)</td>
<td>66.8</td>
</tr>
<tr>
<td>9</td>
<td>208.0</td>
<td>172.0</td>
<td>116.3</td>
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<tr>
<td>10</td>
<td>30.1</td>
<td>2.13; s</td>
<td>101.0</td>
</tr>
<tr>
<td>1′</td>
<td>72.1</td>
<td>5.26; m</td>
<td>73.0</td>
</tr>
<tr>
<td>2′</td>
<td>72.1</td>
<td>5.26; m</td>
<td>73.0</td>
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<tr>
<td>3′</td>
<td>68.5</td>
<td>5.16; t (4)</td>
<td>69.5</td>
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<tr>
<td>4′</td>
<td>72.0</td>
<td>3.75; m</td>
<td>73.0</td>
</tr>
<tr>
<td>5′</td>
<td>61.0</td>
<td>4.15; dd (12.2; 2.4)</td>
<td>62.9</td>
</tr>
<tr>
<td>6′</td>
<td>61.0</td>
<td>4.29; dd (12.2; 5)</td>
<td>62.9</td>
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<tr>
<td>OCH$_3$</td>
<td>56.0</td>
<td>3.79; s</td>
<td>58.0</td>
</tr>
</tbody>
</table>

in the herbarium of the Ecole Supérieure d’Horticulture et
de Elevage de Chott Mérem, 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.
Methodic 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 chromato-
graphed over 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 to give a mixture
of 9:1 methylene chloride/methanol and 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 acetyl-
ylated residue (459.2 mg), which was in turn chromatog-
graphed 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), [a]$_D^{22}$ +13.75 (c = 0.02; CHCl$_3$);
IR (KBr) $\nu_{max}$ / 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 tetra-
acetate and 3-O-β-D-glycopyranosyl sitosterol were prepared
by acetylation (pyridine-Ac$_2$O, 2:1; 5 h at room
temperature).

**Compound 3a:** white solid (11 mg), UV (MeOH) $\lambda_{max}$ / 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 pseudomo-
lar 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$_8$), 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, H3) 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, H7) and 2.75 (t, J = 7.1 Hz, H8), in addition to the significant acetoxyl signal observed at 2.13 ppm, suggested linkage of a CH2-CH2-CO-CH3 moiety to the aromatic ring. This hypothesis was confirmed by the 1H-1H correlation spectroscopy (COSY) spectrum revealing the connectivity of H7 to H8. The C-H long-range HMBC spectrum (Figure 1) allowed us to connect H7 (δ 2.85 ppm) to C1 (δ 137.5 ppm) of the aromatic ring on the basis of the correlations H7-C1, H2-C7 and H6-C7. The location of the methoxy group at C3 was proved by the long range coupling between HOMe (δ 3.79 ppm) and C3 (δ 150.0 ppm) deduced from the same HMBC spectrum (Figure 1). The analysis of COSY datasets showing correlations H1-H2; H2-H3; H3-H4; H5-H6a; H5-H6b and H6a-H6b, as well as the observation on the HMQC spectrum of only one anemic carbon C1 (δ 101.0 ppm) made it possible to establish the skeleton of the monoglycopyranoside. The linkage of the monosaccharide moiety at C4 of the aglycone was deduced from the HMBC spectrum showing the characteristic correlation between the anomeric proton H1 (δ 4.90 ppm) and carbon C4 (δ 145.0 ppm). Comparison of these spectral data to those previously reported in literature confirmed that 1a is the 4-O-β-D-glycopyranosylohomovanillyl alcohol pentaacetate.

**Compound 2a:** Its molecular formula C25H32O13 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 H8 was shifted at δ 4.26 ppm, which suggested the homovanillyl alcohol structure for the aglycone. This hypothesis was reinforced by the HMBC spectrum showing a direct correlation between H8 (δ 4.26 ppm) and C3 (δ 67.0 ppm) as well as by the HMBC spectrum, in which we picked up the significant long range correlation H8-COAc. 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 confirmed that 2a is 4-O-β-D-glycopyranosylhomovanillyl alcohol pentaacetate.

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

**Compounds 3 and 3a:** The molecular formula of 3a was determined to be C24H30O11 by ESMS showing a pseudomolecular ion peak [M+Na]+ at m/z 517. The spectral data of 3 and those of 3a deduced from 1H, 13C and COSY NMR spectra were very similar to those of eugenol glycoside.6,7

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

**References**

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

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Tri fenolna glikozida: 4-\(O\)-\(\beta\)-D-glukopiranozilzingeron 1, 4-\(O\)-\(\beta\)-D-glukopiranozilhomovanilil-alkohol 2 i eugenil-glikozid 3 i 3-\(O\)-\(\beta\)-D-glukopiranozilsitosterol 4 izolirani su i identificirani prvi put iz cvijetova *Matthiola longipetala* (Cruciferae), 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.