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CHEMICAL CONSTITUENTS OF *MATTHIOLA LONGIPETALA* (ssp. *LIVIDA*) (Del.) MAIRE (BRASSICACEAE) GROWING IN TUNISIA

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ABSTRACT: Investigation of methanolic extract from *Matthiola longipetala (ssp. livida)* (Del.) Maire Brassicaceae of Tunisian origin, led to the isolation of methylfirulate-4'-*O*-β-D glycoside 1 together with 2-*O*-methyl α-D-fructofuranose tetraacetate 2 as well as α-D-glycopyranose pentaacetate 3 and β-D glycopyranose pentaacetate 4, all structures were established using 1 and 2D NMR experiments, carbohydrates were identified via their acetylated derivatives.

Key words: *Matthiola longipetala (ssp. livida)* (Del.) Maire, Brassicaceae, 4'-*O*-β-D-glycopyranosyl-methylfirulate 1,2-*O*-methyl α -D-fructofuranose tetraacetate 2, α and β-D-glycopyranoses pentaacetates 3 and 4.

RESUME: le 4'-*O*-β-D-glycosylfirulate de méthyle 1 a été identifié pour la première fois dans l'extrait méthanolique de l'espèce *Matthiola longipetala (ssp. livida)* (Del.) Maire (Brassicaceae) poussant en Tunisie, le 2-*O*-méthyl-α-D-fructofuranose, l'α et le β-D-glycopyranose, ont été identifiés dans le même extrait via leurs dérivés acétylés 2, 3 et 4.

Mots clés : *Matthiola longipetala (ssp. livida)* (Del.) Maire, Brassicaceae, 4'-*O*-β-D-glycosylfirulate de méthyle 1,2-*O*-méthyl-α-D-fructofuranose tétraacétate 2, l'α et le β-D-glycopyranoses petaacétylés 3 et 4.

INTRODUCTION

Naturally occuring plants and marine organisms posses the capacity to bio-synthesise a large number of organic compounds exhibiting a variety of biological activities and contributing to control the human health. Continuing our search for bioactive chemicals deriving from Tunisian flowering plants [1-6], this scientific activity has been focused on the chemical characterization of secondary metabolites from methanolic extract of *Matthiola longipetala (ssp. livida)* (Del.) Maire. This plant is widespread in a narrow geographical area in particular from Egypt to Marocco, four other species from *Matthiola* genus are found in TUNISIA: M. *fruticulosa* (L.) Maire, M. *lunata* (DC.), M. *parviflora* (R.Br.) and M. *tricuspidata* (L.) R.Br. [7]. Previous phytochemical study of *Matthiola longipetala* led to the isolation of 4-*O*-β-D-glycopyranosyl zingerone, 4-*O*-β-D-glycopyranosyl homovanillyl alcohol and eugenol glycoside [8]. In continuation to our application of solid/liquid chromatography for the isolation and of 1 and 2D (^1H and ^{13}C) NMR experiments for structure elucidation, led to the identification of methylfirulate-4'-*O*-β-D glycoside as well as the carbohydrates: 2-*O*-methyl α and β-D-fructofuranose tetraacetate, α and β-D-glycopyranose pentaacetates isolated for the first time from *Matthiola longipetala (ssp. livida)* (Del.) Maire specie.

RESULTS AND DISCUSSION

Compound 1: Compound 1 was isolated as a white solid from the methanolic extrac of Matthiola longipetala specie, its positive FABMS spectrum showed a pseudomolecular ion peak at m/z 371 $[M+H]^+$ suggesting a molecular fomula of $C_{17}H_{22}O_9$; ^1H NMR spectrum recorded in CD_3OD, suggested the presence of a trisubstituted aromatic ring by the observation of three protons appearing between 7.00ppm and 7.20ppm, in addition, two ethylenic protons at 6.46ppm

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(J=15.9*Hz*) and 7.64ppm (J=15.9*Hz*) were in agreement of a trans substituted double bond. Two methoxyl groups were also observed at δ_1 3.77 (s) and δ_2 3.90 (s). Following the analysis of ^{13}C NMR data, a glycopyranosic moiety was suggested since five oxygenated methine and one methylenic carbon were detected. Full assignement of ^{1}H and ^{13}C NMR data was established on the basis of CHCorr experiment. In the other hand, the Heteronuclear correlation of methoxy protons H_4 with quaternary carbon C_1 indicated the presence of a methoxycarbonyle group dirctly linked to the ethylenic carbon C_2. Additionally, the positions of aromatic methoxy group and of glycopyranose moiety fixed at C_3 and C_4 of the aglycone, were proved on the basis of the significat long range correlations $H_{2'}$-$C_{6'}$; $H_{2'}$-$C_{4'}$ and H_5-$C_{3'}$ observed in HMBC spectrum (Figure 1).

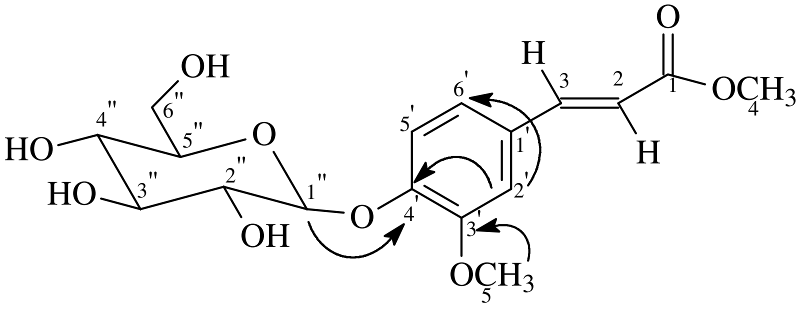


Figure 1: important HMBC correlations for compound 1

NOESY NMR experiment confirmed that the double bond was directly attached at $C_{1'}$ (observation of NOE correlations from H_3 to $H_{6'}$, from H_2 to $H_{2'}$ and from H_5 to $H_{2'}$) (Figure 2), thus, we proposed for compound 1, the structure of methylfirulate-4'-*O*-β-D glycoside indicated for the first time in *Matthiola* specie.

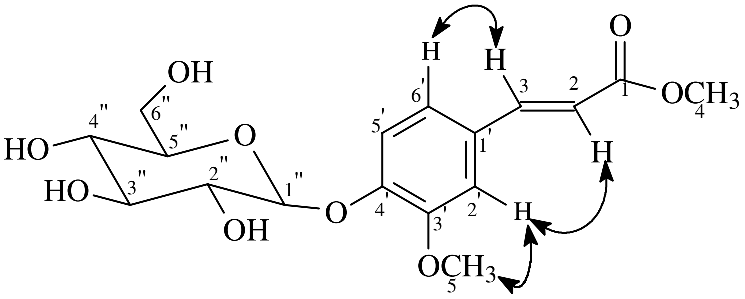
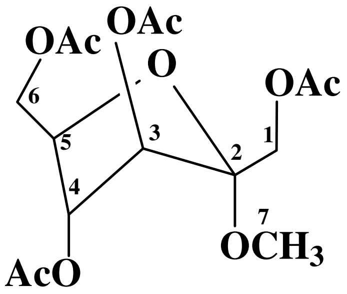


Figure 2: Significant NOES for compound 1

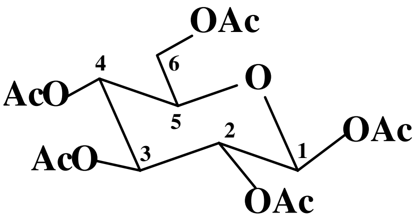
Compound 2: The acetylated derivative was isolated as a white solid from the acetylated methanolic extract of *Matthiola longipetala (ssp. livida)* (Del.) Maire, its ^{13}C and DEPT 135 NMR



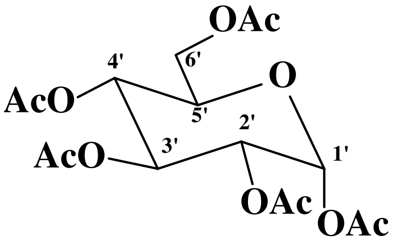
2-*O*-Methyl-α-D-fructofuranose tetraacetate (2)

spectra recorded in $CDCl_3$ at 75*MHz* indicated the presence of two oxygenated methylene, three oxygenated methine groups, a signal attribuable to a tetrasunstituted anomeric carbon C_2 (δ 107.9) and one methoxy group C_7 (δ 49.1). Further analysis of the same spectra, showed signals from four acetoxy groups (21.0-21.8ppm). These data compared with those of the litterature [9-11] were in agreement with that of the 2-*O*-methyl-α-D-fructofuranose tetraacetate. The exploration of COSY spectrum allowed us to confirm the nature of the carbohydrate by the observation of the homonuclear correlations of H_3 with H_4 and sequentially H_5, H_{6a} and H_{6b} observed in ^1H-^1H COSY spectrum. We remind that the identification of chemical shifts for all protons was determined on the basis of CHCorr spectrum.

Compounds 3 and 4: Compounds 3 and 4 were isolated in mixture as an oily substance. ^1H NMR spectrum of the indicated substance displayed many signals appearing between 3.70 and 5.60ppm characteristic of protons of acetylated carbohydrate. The observation of two doublets having significant coupling constants and chemical shifts at δ_1 5.69 (d, 1H, *J* 8.4*Hz*) and δ_2 6.30ppm (d, 1H, *J* 3.2*Hz*) suggested that the acetylated derivative is a mixture of α and β-Dglycopyranose. This proposition was reinforced by ^{13}C and DEPT 135 NMR spectra showing the presence of eight oxygenated methine groups ($C_{2/2'}$; $C_{3/3'}$; $C_{4/4'}$; $C_{5/5'}$), two methylenic carbons ($C_{6/6'}$) at δ 62.5 and two anomeric carbons at δ_1 92.2 and δ_2 92.9. Exploitation of 2D NMR spectra (^1H-^1H COSY, CHCorr and HMBC) reinforced by the litterature data [12-13] permitted us to precise the chemicals shifts of protons and carbons for both anomers.



Compound 3



Compound 4

In conclusion, the chemical investigation of the methanolic extract of *Mtthiola longipetala (ssp. livida)* (Del.) Maire led to the identification of 4'-*O*-β-D-glycosylfirulate de méthyle together with three carbohydrates: 2-*O*-methyl-α-D-fructofuranose, α-D-glycopyranose and β-D-glycopyranose, four compounds are signaled for the first time in the indicated plant.

EXPERIMENTAL

Plant material: Fresh flowers of *Matthiola longipetala* (*ssp. livida*) were collected from Gabes region in March 2003 and identified by Dr. Fethia Harzallah SKHIRI (Biothechnological Institute of Monastir). A voucher specieman was deposited in Natural Substances and Organic Chemistry Laboratory at the Faculty of Science of Monastir.

Extraction and isolation: *Matthiola longipetala (ssp. livida)* Fresh Flowers (1000g) were extracted extensively with methanol at room temperature, after evaporation of the solvent, the concentrated methanolic extract (50g) was further separated in eight main fractions (F1-F8) using silica gel Column Chromatography (70-200µm) and a gradient of PE/AcOEt, AcOEt/MeOH as eluent. Further purification of the most polar fraction by column chromatography on silica gel using EtOAc gradually increased by MeOH as eluent and TLC for analysis, afforded seven fractions having different chemical compositions. Acetylation of the sixth one with acetic anhydride in pyridine at room tempreture gave 1.64g of an impure fraction which constituents were separated by silica gel column chromatography and preparative plates eluted with dichloromethane gradually increased by methanol to afford 55mg and 5mg of a white solid (compound 2) and an oily substance (compounds 3 and 4 in mixture). The fifth fraction (F5) deriving from the first separation of the methanolic extract was subjected to a purification through two silica gel columns eluted with ethylacetate gradually increased with methanol yielding 4mg of a white solid (compound 1).

methylfirulate-4'-*O*-β-D glycoside 1

White solid, FABMS:*m/z* 371 $[M+H]^+$ ($C_{17}H_{22}O_9$); ^{1}H NMR (CD_3OD, 300*MHz*) δ:6.46 (d, 1H, $J_{2\text{-}3}$ 15.9*Hz*, H-2), 7.64 (d, 1H, $J_{3\text{-}2}$ 15.9*Hz*, H-3), 3.77 (s, 3H, H-4), 3.90 (s, 3H, H-5), 7.27 (s, 1H, H-2'), 7.12-7.17 (m, 2H, H-6', H-5'), 4.85, (d, 1H, $J_{1''\text{-}2''}$, 7.5Hz, H-1"), 3.44 (m, 1H, H-2"), 3.31 (m, 1H, H-3"), 3.23 (m, 1H, H-4"), 3.36 (m, 1H, 1H, H-5"), 3.61 (m, 1H, H-6"a), 3.81 (m, 1H, H-6"b); ^{13}C NMR (CD_3OD, 75*MHz*) δ: 170.0 (C-1), 115.6 (C-2), 144.7 (C-3), 50.7 (C-4), 55.3 (C-5), 130.0 (C-1'), 110.9 (C-2'), 150.5 (C-3'), 150.0 (C-4'), 115.9 (C-5'), 122.2 (C-6'), 100.8 (C-1"), 73.4 (C-2"), 76.9 (C-3"), 69.9 (C-4"), 76.5 (C-5"), 61.0 (C-6").

2-O-Methyl-α-D-fructofuranose tetraacetate 2

White solid, ^{1}H NMR ($CDCl_3$, 300*MHz*) δ: 4.16 (m, 1H, H-1a), 4.44 (m, 1H, H-1b), 5.29 (m, 1H, H-3), 4.91 (m, 1H, H-4), 4.16 (m, 2H, H-5, H-6a), 4.44 (m, 1H, H-6b), 3.31 (s, 3H, H-7), 2.00-2.10 (m, 12H, CH_3 (OAc). ^{13}C NMR ($CDCl_3$, 75*MHz*) δ: 58.5 (C-1), 107.3 (C-2), 79.9 (C-3), 78.5 (C-4), 80.7 (C-5), 63.6 (C-6), 49.1 (C-7), 169.3-171.1 (CO).

β-D-glycopyranose pentaacetate 3

White solid, ^{1}H NMR ($CDCl_3$, 300*MHz*) δ: 5.69 (d, 1H, $J_{1\text{-}2}$ 8.4Hz, H-1), 5.10 (m, 1H, H-2), 5.23 (t, 1H, $J_{4\text{-}3\text{-}2}$ 9.3, H-3), 5.10 (m, 1H, H-4), 5.82 (ddd, 1H, $J_{5\text{-}4}$ 9.9, J5-6^a 2.1, J5-6b 4.2, H-5), 4.08 (m, 1H, H-6^a), 4.24 (m, 1H, H-6b); ^{13}C NMR ($CDCl_3$, 75*MHz*) δ: 92.8 (C-1), 71.3 (C-2), 73.9 (C-3), 68.8 (C-4), 73.8 (C-5), 62.5 (C-6).

α-D-glycopyranose pentaacetate 4

White solid, ^{1}H NMR ($CDCl_3$, 300*MHz*) δ: 6.30 (d, 1H, J1'-2' 3.6Hz, H-1'), 5.10 (m, 1H, H-2'), 5.45 (t, 1H, $J_{4'\text{-}3'\text{-}2'}$ 9.9, H-3'), 5.10 (m, 1H, H-4'), 4.08 (m, 1H, H-5'), 4.08 (m, 1H, H-6'a), 4.24 (m, 1H, H-6'b); ^{13}C NMR ($CDCl_3$, 75*MHz*) δ: 92.2 (C-1'), 70.3 (C-2'), 70.9 (C-3'), 68.9 (C-4'), 70.9 (C-5'), 62.5 (C-6').

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