CARBOHYDRATES FROM *MORICANDIA ARVENSIS* GROWING IN TUNISIA

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ABSTRACT: β-D-Glucopyranosyl-2-methylbutanoate, β-D-glucopyranosyl-2-methylpropanoate, 2-*O*-methyl-α-D-fructofuranose, 2-*O*-methyl-β-D-fructofuranose, and sucrose were isolated from the flowers of *Moricandia arvensis*, and characterized by spectroscopic methods.

Key words: *Moricandia arvensis*; β-D-Glucopyranosyl-2-methylbutanoate; β-Dglucopyranosyl-2-methylpropanoate; 2-*O*-methyl-α-D-fructofuranose; 2-*O*-methyl-β-D-fructofuranose

RESUME: Le β-D-Glucopyranosyl-2-methylbutanoate, β-D-glucopyranosyl-2-methylpropanoate, 2-*O*-méthyl-α-D-fructofuranose, 2-*O*-méthyl-β-D-fructofuranose et le sucrose ont été isolés pour la première fois des fleurs fraiches de la plante *Moricandia arvensis*. Leurs structures ont été confirmées à l'aide de méthodes spectroscopiques.

Mots clés: *Moricandia arvensis*; β-D-Glucopyranosyl-2-méthylbutanoate; β-Dglucopyranosyl-2-méthylpropanoate; 2-*O*-méthyl-α-D-fructofuranose; 2-*O*-méthyl-β-D-fructofuranose

INTRODUCTION

The genus *Moricandia* (Cruciferae) includes five species distributed in North Africa, South Europe, and Western Asia [1]. In previous phytochemical work reported for *Moricandia arvensis* (L.) DC, an indole derivative, glucosinolates, fatty acids, and phenolic glycosides have been characterized [2,3,4,5].

This note describes the isolation and characterization from flowers of *M. arvensis* growing in Tunisia [6] of β-D-glucopyranosyl-2-methylbutanoate (**1**), β-D-glucopyranosyl-2-methylpropanoate (**2**), 2-*O*-methyl-α D-fructofuranose (**3**), and 2-*O*-methyl-β-D-fructofuranose (**4**).

RESULTS AND DISCUSSION

The methanolic extract of flowers of *M. arvensis* was submitted to successive flash chromatography and LPLC on normal and reversed-phase silica, to yield compounds **1**-**4**, along with sucrose. The negative FABMS of **1** showed a pseudomolecular ion peak at *m/z* 263 $[M - H]^+$, whereas its ^1H and ^{13}C NMR, DEPT, and HMQC spectra displayed the characteristic signals of a pyranose ring, a carbonyl ester group at δ_C 177.0, a methyne group at δ_H 2.45/δ_C 42.2, two methylenic protons at 1.71 and 1.51 ppm linked to a carbon at δ 27.6, and two methyl groups at δ_H 1.16/δ_C 16.6 and δ_H 0.94/δ_C 11.8. The carbonyl group showed HMBC cross-peaks with the six

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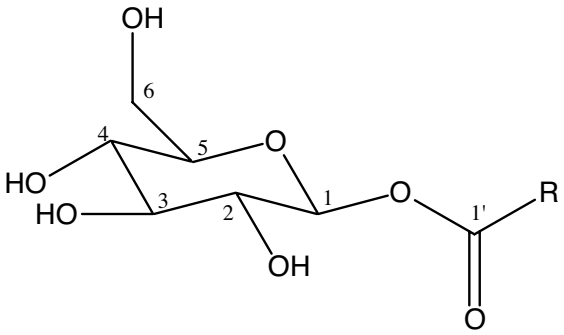
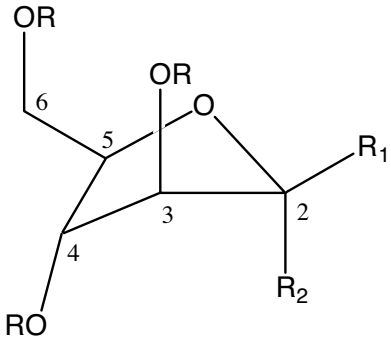
high-field 2´-CH, 4´-CH_2, and 3´-CH_3 protons, and with the pyranosyl anomeric proton at δ 5.45 (d, *J* 8.0 Hz). These data, in addition to the proton couplings observed in the COSY spectrum, accounted for the presence of a 2-methylbutanoate moiety attached to the anomeric carbon. The pyranose ring was identified as β-D-glucose from its carbon chemical shifts (see experimental section), which were identical to those reported for 1-*O*-acyl-β-D-glucoses [7], and by comparison of the hydrolysed product with a sugar standard. Compound **1** was thus identified as β-D-glucopyranosyl-2-methylbutanoate. The ^{1}H NMR spectrum of compound **2**, when compared to that of **1**, showed a different high-field pattern. No methylenic protons were observed, and the methyl group of **1** was here replaced by an isopropyl group appearing at 2.60 ppm (CH), and 1.18 ppm (2 × CH_3). The pseudomolecular ion peak of **2**, at *m/z* 249 $[M - H]^+$ confirmed its identity as β-D-glucopyranosyl-2-methylpropanoate. Compounds **1** and **2**, whose NMR data are reported herein for the first time, have been previously identified from mamee apple fruit pulp [8]. Due the lack of available sample, the configuration of C-2´ in **1** could not be determined. In a disaccharide glycoside previously isolated from *Acacia sieberana,* also bearing a 2-methylbutyrate aglycon, the configuration of this chiral carbon was assigned as *S* by comparison of the CD curve with that of 2*S*-methylbutyric acid [9].

Mono and bidimensional NMR spectra of compound **3** indicated the presence of two CH_2, three CH, an oxygenated tetrasubstituted carbon at δ 107.5, and a methoxyl group (δ_H 3.46/δ_C 40.2) placed on the tetrasubstituted carbon, as indicated by the corresponding $^3J_{C\text{-}H}$ correlation observed in the HMBC spectrum. The above data, and the absence of an anomeric proton, was in agreement with a 2-*O*-methylated cyclic form of ketose [10,11]. Extensive 2D NMR experiments on compound **3** and its acetylated derivative **3a**, and their ESIMS spectra confirmed the structure of 2-*O*-methyl-α-D-fructofuranose, whose configuration at C-2 was evidenced by the NOESY correlation of the methyl protons with H-3 (Fig. 1). The NMR spectra of compound **4** were similar to those of **3**, although high-field shifts of 2.8 and 4.1 ppm were observed for C-2 and C-3, respectively. This fact, in addition to the negative optical rotation of **4**, and the absence of NOESY correlation of the methoxy group with H-3, corroborated the structure of 2-*O*-methyl-β-D-fructofuranose, which was confirmed by the 2D NMR spectra of the acetate **4a**. The occurrence of the two anomers of 2-*O*-methyl-D-fructofuranose from plants, has been previously reported [11,12].



2' 3' 4' 5'
1 R = -CH(CH$_3$)CH$_2$CH$_3$
2 R = -CH(CH$_3$)$_2$



3 R = H, R_1 = CH_2OH, R_2 = OMe
3a R = Ac, R_1 = CH_2OAc, R_2 = OMe
4 R = H, R_1 = OMe, R_2 = CH_2OH
4a R = Ac, R_1 = OMe, R_2 = CH_2OAc

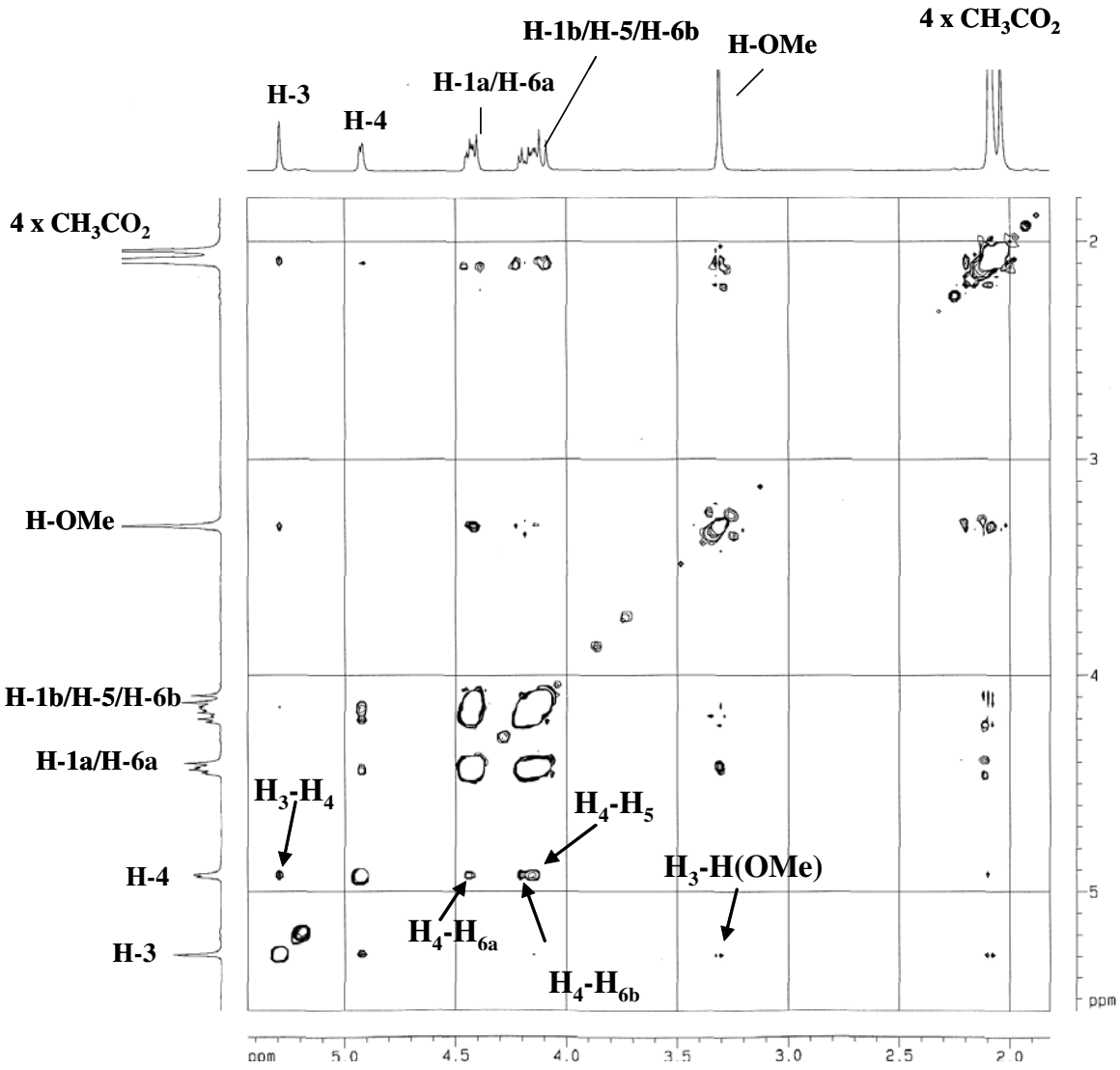


Fig.1. NOESY spectrum of compound 3a.

Materials and Methods

Plant material

The flowers of *Moricandia arvensis* were collected in Monastir, in March 2003, and identified by Dr. F. Harzallah-Skhiri, from Institut Supérieur de Biotechnologie de Monastir, Université de Monastir, Tunisia, where a voucher specimen (HCM-120) has been deposited.

General methods

Optical rotations were obtained using a Perkin-Elmer 241-MC polarimeter. UV spectra were recorded on a Milton Roy Spectronic 1201 spectrophotometer, and FTIR spectra on a Perkin-Elmer 157G infrared spectrophotometer. The NMR spectra were recorded on a Bruker ARX 400 NMR spectrometer (^{1}H at 400 MHz; ^{13}C at 100.61 MHz), using CD_3OD or $CDCl_3$ as solvent. ESIMS and FABMS experiments were performed on LCT and Micromass Autospec spectrometers, respectively. TLC was performed on plates of normal-phase silica, RP-18, and NH_2 phases (MN 818133, Merck 5559, and Merck 5533 respectively), using sulphuric acid, $CeSO_4$, and α-naphthol as spray reagents. Normal and reversed phase silica gel were used for flash chromatography and LPLC (Merck 13905 and 13900, respectively).

Extraction and isolation

Fresh flowers (1 kg) were macerated at room temperature in MeOH (3 × 5 L) for 48 hours. The combined methanolic extracts were concentrated to dryness, yielding a residue (37 g) which was further dissolved in methanol and defatted with petroleum ether. The resulting extract (30 g) was eluted on a silica gel column with CH_2Cl_2/MeOH (100:0 to 50:50), yielding twelve crude fractions (A_1-A_{12}). Fr A6 (240 mg; 96:4 to 94:6) was subjected to RP-18 flash CC (H_2O/MeOH 90:10 to 60:40) to yield **1** (8 mg) and **2** (2 mg). Fr A_{10} (500 mg; 74:26 to 66:34) was subjected to LPLC (CH_2Cl_2/MeOH 82:18), to yield seven sub-fractions (B_1-B_7). Fractions B_2 (124 mg; 82:18) and B_5 (185 mg; 82:18) were rechromatographed on flash CC (CH_2Cl_2/MeOH, 88:12), to afford **3** (25 mg) and **4** (22 mg). RP-18 flash CC of Fr A_{12} (8.4 g, 58:42 to 50:50) using a gradient elution of H_2O/MeOH (100:0 to 50:50) yielded 150 mg of sucrose, which was identified by comparison of its physical and spectral data with those of an authentic sample, and literature data [13,14].

Compounds **3** and **4** (10 mg) were acetylated at room temperature in a pyridine-acetic anhydride mixture (0.5:0.5 v/v). The reaction mixture was diluted with water, extracted three times with ethyl acetate, and the organic phase evaporated in vacuum. The acetylated compounds were further purified on flash CC (Hex/AcOEt, 70:30) to yield **3a** (12 mg) and **4a** (10 mg).

β-D-Glucopyranosyl-2-methylbutanoate (1)

Oil; $[\alpha]_D^{25}$ +2° (*c* 0.36, MeOH); UV (MeOH) λ_{max} 216, 264 nm; IR (NaCl) ν 3369 (alcohol), 1736 (ester) cm^{-1}; FABMS: *m/z* 263 $[M - H]^+$ ($C_{11}H_{19}O_7$); ^{1}H NMR (CD_3OD) δ: 5.45 (d, 1 H, *J* 8.0 Hz, H-1), 3.82 (dd, 1 H, $J_{6a\text{-}6b}$ 12.5 Hz, $J_{6a\text{-}5}$ 1.0 Hz, H-6a), 3.67 (dd, 1 H, $J_{6b\text{-}5}$ 4.3 Hz, H-6b), 3.45-3.25 (m, 4 H, H-2, H-3, H-4, H-5), 2.45 (m, 1 H, H-2'), 1.71 (m, 1 H, H-3a´), 1.51 (m, 1 H, H-3b´), 1.16 (d, 3 H, $J_{5'\text{-}2'}$ 7.3 Hz, H-5'), 0.94 (t, 3 H, $J_{4\text{-}3}$ 7.4 Hz, H-4'); ^{13}C NMR (CD_3OD) δ: 177.0 (C-1´), 95.6 (C-1), 78.8 (C-3), 78.1 (C-5),74.0 (C-2), 71.1 (C-4), 62.3 (C-6), 42.2 (C-2´), 27.6 (C-3´), 18.6 (C-4´), 11.8 (C-5´).

β-D-Glucopyranosyl-2-methylpropanoate (2)

Oil; FABMS: *m/z* 249 $[M - H]^+$ ($C_{10}H_{17}O_7$); ^{1}H NMR (CD_3OD) δ: 5.45 (d, 1 H, *J* 8.1 Hz, H-1); 3.83 (dd, 1 H, $J_{6a\text{-}6b}$ 12.5 Hz, $J_{6a\text{-}5}$ 1.0 Hz, H-6a); 3.67 (dd, 1 H, $J_{6b\text{-}5}$ 4.3 Hz, H-6b); 3.45-3.25 (m, 4 H, H-2, H-3, H-4, H-5), 2.60 (m, 1 H, H-2´), 1.18 (d, 6 H, $J_{3a'\text{-}2'}$, $J_{3b'\text{-}2'}$ 7.0 Hz, 3 × H-3a´, 3 × H-3b´).

2-*O*-Methyl-α-D-fructofuranose (3)

Oil; $[\alpha]_D^{25}$ +73° (*c* 0.30, MeOH); ESIMS: *m/z* 217 $[M + Na]^+$ ($C_7H_{14}O_6Na$); ^{1}H NMR (CD_3OD): δ 3.96 (d, 1 H, $J_{3\text{-}4}$ 5.0 Hz, H-3), 3.69 (dd, 1 H, $J_{4\text{-}3}$ 5.0, $J_{4\text{-}5}$ 7.0 Hz, H-4), 3.63 (ddd, 1 H, $J_{5\text{-}6a}$ 2.0, $J_{5\text{-}6b}$ 5.0, $J_{5\text{-}4}$ 7.0 Hz, H-5), 3.56 (dd, 1 H, $J_{6a\text{-}6b}$ 12.0 Hz, H-6a), 3.41 (dd, 1 H, H-6b), 3.50 (d, 1 H, $J_{1a\text{-}1b}$ 12.0 Hz, H-1a), 3.39 (d, 1 H, H-1b), 3.18 (s, 3H, OCH_3); ^{13}C NMR (CD_3OD): δ 107.5 (C-2), 82.8 (C-5), 81.0 (C-3), 77.0 (C-4), 61.4 (C-6), 59.7 (C-1), 48.2 (OCH_3).

2-*O*-Methyl-β-D-fructofuranose (4)

Oil; $[\alpha]_D^{25}$ −35° (*c* 0.33, MeOH); ESIMS: *m/z* 217 $[M + Na]^+$ ($C_7H_{14}O_6Na$); ^{1}H NMR (CD_3OD): δ 3.98 (brd, 1 H, H-3), 3.75 (m, 1 H, H-3), 3.52 (m, 1 H, H-4), 3.52 (m, 1 H, H-6a), 3.40 (m, 1 H, H-6b), 3.40 (m, 1 H, H-1a), 3.32 (m, 1 H, H-1b), 3.20 (s, 3 H, OCH_3); ^{13}C NMR (CD_3OD): δ 104.7 (C-2), 82.2 (C-5), 76.9 (C-3), 75.5 (C-4), 62.8 (C-6), 61.2 (C-1), 48.9 (OCH_3).

Methyl 1,3,4,6-tetra-*O*-acetyl-α-D-fructofuranose (3a)

Oil; $[\alpha]_D^{25}$ +78° (*c* 0.31, MeOH); ESIMS: *m/z* 385 $[M + Na]^+$ ($C_{15}H_{22}O_{10}Na$); IR (NaCl) ν 1747 and 1220 (ester) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.29 (d, 1 H, $J_{3\text{-}4}$ 1.2 Hz, H-3), 4.93 (dd, 1 H, $J_{4\text{-}5}$ 5.0 Hz, H-4), 4.16 (m, 1 H, H-5), 4.44 (dd, 1 H, $J_{6a\text{-}5}$ 3.0, $J_{6a\text{-}6b}$ 11.5 Hz, H-6a), 4.18 (dd, 1H, $J_{6b\text{-}5}$ 5.7, H-

6b), 4.42 (d, 1 H, J_{1a-1b} 12.2 Hz, H-1a), 4.06 (d, 1 H, H-1b), 3.31 (s, 3 H, OCH_3); ^{13}C NMR (CDCl3): δ 106.9 (C-2), 80.2 (C-5), 79.6 (C-3), 78.2 (C-4), 63.2 (C-6), 58.2 (C-1), 49.0 (OCH_3).

Methyl 1,3,4,6-tetra-*O*-acetyl-β-D-fructofuranose (4a)

Oil; $[\alpha]_D^{25}$ −20° (*c* 0.27, MeOH); ESIMS: *m/z* 385 $[M + Na]^+$ ($C_{15}H_{22}O_{10}Na$); IR (NaCl) ν 1746 and 1220 (ester) cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.50 (d, 1 H, J_{3-4} 7.2 Hz, H-3), 4.41 (dd, 1 H, J_{4-5} 5.8, H-3), 4.18 (m, 1 H, H-5), 4.40-4.10 (m, 4 H, H-6a, H-6b, H-1a, H-1b), 3.31 (s, 3 H, OCH_3); ^{13}C NMR ($CDCl_3$): δ 103.0 (C-2), 78.1 (C-5), 76.3 (C-3), 75.7 (C-4), 64.3 (C-6), 62.1 (C-1), 49.8 (OCH_3).

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