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The chemical investigation of the chloroformic extract of *Ononis angustissima* Lam. Var. species

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ABSTRACT

Four flavonoids, 1-4, are isolated from the chloroform extract of the areal part of *Ononis angustissima* Lam. Var., belonging to Fabaceae family, by various chromatographic techniques. Their structures are elucidated on the basis of spectroscopic analysis, including HRESI-MS, UV and 2D NMR experiments (COSY, NOESY, HSQC and HMBC) and compared with literature data. Compounds 1 and 2 are new in the genus, thus they are isolated for the first time in this species.

Key words: Flavonoids, *Ononis angustissima* Lam. Var., Fabaceae

INTRODUCTION

Herbal medicine represents one of the most important fields of traditional medicine all over the world. Natural products have served as an important source of drugs since ancient times. In recent years, there is an increasing interest both in the industry and in scientific research, for finding antioxidant phytochemicals because they may inhibit the propagation of free radical reactions and protect the human body from diseases [1].

Several plants belonging to the *Ononis* genus are known to be used in the treatment of jaundice, urinary tract inflammations and kidney stones [2]. Besides, *Ononis* L. species have been used for healing of wounds, eczema and rheumatic complaints, against skin cancer and lesions and topically used as antiseptic and antimicrobial agent in folk medicine [3]. Also, the traditional use of this plant, for medicinal purposes, reports that a kind of this plant known as *Ononis natrix* is used to treat diarrhea and a urinary disorder and rheumatic [4].

Ononis is a large genus of perennial herbs and shrubs from the Fabaceae family [5]. *Ononis angustissima* is widely present in daya regions (Ouargla, Ghardaia, Bechar and Biskra of Algeria) from April to May.

The aim of this paper is to continue a previous research work on this species leading to isolation and identification of seven Flavonoids [6-7]. Our contribution here is to isolate and elucidate four others Compounds.

MATERIALS AND METHODS**General Procedures**

Silica gel 60F 254 (Merck). Column chromatography (CC): silica gel 60 (Merck 200-400 mesh). UV Spectra in (MeOH): Evolution 300 Thermo Electron corporation spectrometer. NMR Spectra: Bruker AMX-400 MHz, AMX-500 MHz and Advance DPX-250, 250 MHz spectrometers; chemical shifts (δ) are given in ppm using TMS as internal standard and coupling constants (J) are given in Hz. Mass spectra: Q-TOF micro (waters) spectrometer

The experimental work

The areal part of *Ononis angustissima* Lam. Var species (980 g) is air dried and extracted with MeOH-H₂O (70:30, v/v) three times during 24 hours. The solution is concentrated and extracted with increasing polarity of solvents, starting with CHCl₃, AcOEt, and n-BuOH.

12g of chloroformic extract is fractionated by column chromatography of silica gel eluted with (CHCl₃: AcOEt), (9:1), 26 fractions are obtained. The obtained products from the studied fractions are isolated and purified using TLC plats and eluted with the eluents systems: (Petroleum ether: AcOEt)/ (8:2) and (2:3), (Dichloromethane: Acetone)/ (2:0.5)/

The fraction 4, as a solid, is washed with hexane to give yellow crystals visible to the naked eye as P1.

A mass of 0.5 g of fraction F6 is chromatographed on a silica gel column eluted with P. Ether / AcOEt in a gradient polarity to obtain 7 fractions.

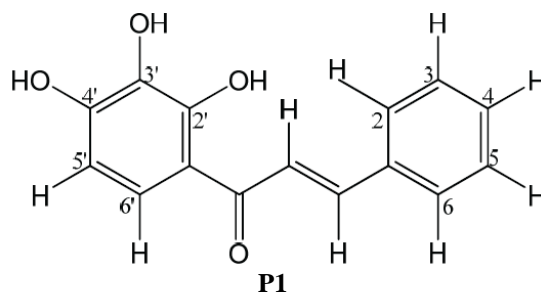
The sub-fraction 6-4 is washed with hexane and then washed with petroleum ether leading to the product P3 as yellow crystals visible to the naked eye.

The preparative thin layer is used for the separation of the fraction F11 eluted with P. Ether / AcOEt (2:3 / V: V) to obtain product P2.

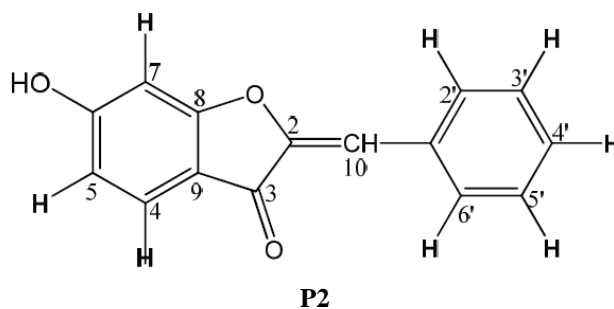
The last product is obtained by washing Fraction 16 with chloroform.

RESULTS AND DISCUSSION**Isolated and identified compounds**

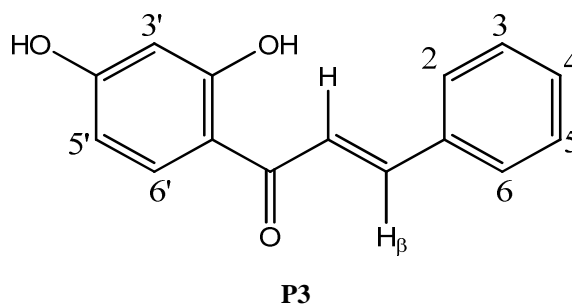
Product P1: RMN-¹H (400MHz, CDCl₃, δ ppm, J/Hz), 6.61 (1H, d, J =8.4, H-5'), 7.43-7.45 (3H, m, H-3, 4, 5), 7.58 (1H, d, J =15.2 Hz, H- α), 7.65-7.68 (2H, m, H-2, 6), 7.80 (1H, d, J =8.4, H-6')7.94 (1H, d, J =15.2 Hz, H- β); RMN-¹³C (CDCl₃, δ ppm, 100.61MHz): 192.15(C=O), 163.25 (C-4'), 162.60(C-2'), 145.33(C- β), 134.65(C-1), 130.95(C-4), 130.03 (C-6'), 129.07 (C-3,5), 128.70(C-2,6), 119.69(C- α), 114.38 (C-1'), 110.33(C-5'); UV (MeOH, λ_{max} , nm): 223, 316-349, +NaOH: 278, 390, + AlCl₃ 3: 263, 407, + AlCl₃+HCl: 263, 407, + NaOAc: 277, 388, + NaOAc+H₃BO₃: 223, 349. This compound is identified as: 2, 3, 4-Trihydroxyphenyl)-3-phenylprop-2-en-1-one or 2', 3', 4'-Trihydroxychalcone [8-9].



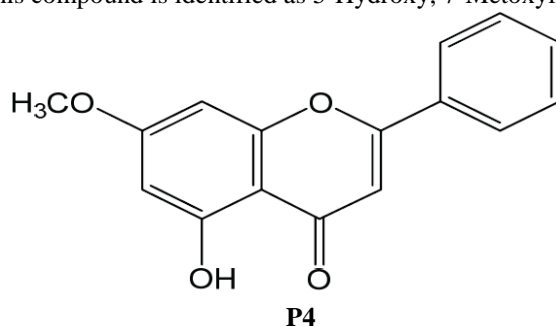
Product P2: RMN-¹H (400MHz, CD₃OD, δ ppm, J/Hz) : 6.78 (1H, s, H-10), 6.91 (1H, dd, J =9.6, 2.8 Hz, H-5), 6.93 (1H, dd, J =2.8, 0.5, H-7), 7.52-7.60 (3H, m, H-3', 4', 5'), 7.96 (1H, d, J =9.6, H-4), 7.98-8.01 (2H, m, H-2', 6'), 6.78 (1H, s, H- α ethelinic); RMN-¹³C (CDCl₃, δ ppm, 100.61MHz): 180.10 (C-3), 167.59(C-6), 165.23(C-8), 160.03(C-2), 133.12(C-1'), 116.13(C-9), 132.64(C-4'), 130.29(C-3', 5'), 127.63(C-4), 127.45(C-2',6'), 117.77(C-5), 107.15(C-10), 103.61(C-7); UV (MeOH, λ_{max} , nm): 252, 320, +NaOH: 252, 256, + AlCl₃ 3: 252, 310, + AlCl₃+HCl: 256, 260, + NaOAc: 256, 310, Sh 270, + NaOAc+H₃BO₃: 255, 312, Sh 280. This compound is identified as 6-Hydroxyaurone [10-11].



Product P3: The ESI mass spectrum in positive resolution of this compound gives an almost molecular peak of exact mass $m/z = 263.0666$ corresponding to the molecular formula $C_{15}H_{12}O_3Na$ (calculated 263.06841). This result leads to a molecular formula $C_{15}H_{12}O_3$; R MN- 1H (400MHz, $(CD_3)_2CO$, δ ppm, J/Hz): 6.39 (1H, d, $J=8.7$ Hz, H-3'), 6.50 (1H, dd, $J=8.7, 2.4$ Hz, H-5'), 7.45-7.49 (3H, m, H-3, 4, 5), 7.85-7.88 (2H, m, H-2, 6), 7.90 (1H, d, $J=15.6$ Hz, H- α), 7.97 (1H, d, $J=15.6$ Hz, H- β), 8.18 (1H, d, $J=2.4$ Hz, H-6'); RMN- ^{13}C (Acetone- d_6 , δ ppm, 75MHz): 192.81(C=O), 167.70(C-4'), 165.88(C-2'), 144.81(C- α), 135.94(C-1), 133.62(C-6'), 131.47(C-4), 129.84(C-3,5), 129.69(C-2,6), 121.70(C- β), 114.50(C-1'), 103.78(C-3'), 108.92(C-5'); UV (MeOH, λ_{max} , nm): 223, 316,349, +NaOH: 278, 390, + $AlCl_3$: 263, 407, + $AlCl_3+HCl$: 263, 407, + NaOAc: 277, 388, + NaOAc+ H_3BO_3 : 223, 349; This compound is identified as P2 (2', 4'-Dihydroxychalcone) [12-14].



Product 4: RMN- 1H (300MHz, CD_3OD , δ ppm, J/Hz) 3.80 (3H, s, OCH_3 -7), 6.39(1H, d, $J = 2.0$ Hz, H-6), 6.65 (1H, d, $J = 2.0$ Hz, H-8), 7.55 (3H, m, H-3', 4', 5'), 8.00 (2H, m, H-2', 6'), 10.65 (1H, s, OH-5); UV (MeOH, λ_{max} , nm): 266, 335, +NaOH: 273, 356, + $AlCl_3$: 264, 318, + $AlCl_3+HCl$: 264, 318, + NaOAc: 274, 351, + NaOAc+ H_3BO_3 : 264, 322. This compound is identified as 5-Hydroxy, 7-Methoxyflavone (tecto-chrysin)[15-16].



CONCLUSION

Four compounds namely: 2', 3', 4'-Trihydroxychalcone **P1**, 6-Hydroxyaurone **P2**, 2', 4'-Dihydroxychalcone **P3** and 5-Hydroxy, 7-Methoxyflavone (tecto-chrysin) **P4** have been isolated from the exudate extract of *Ononis angustissima* Lam. Var. The compounds P1 and P2 are described for the first time in the *Ononis* genus.

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