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TWO FURANONE GLUCOSIDE DERIVATIVES FROM *JUNIPERUS PHŒNICEA*

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Key Word Index — *Juniperus phœnicea*; Cupressaceae; leaves; furanone glucoside derivatives; psydrin; phœnicein; 5-methyl-4-*O*-(β-D-glucopyranosyl)-3(2H)-furanone; 5-methyl-4-*O*-(β-D-glucopyranosyl)-3(2-*iso*propylidenyl)-furanone.

Abstract—The rare compound, psydrin (5-methyl-4-*O*-(β-D-glucopyranosyl)-3(2H)-furanone) and the new 5-methyl-4-*O*-(β-D-glucopyranosyl)-3(2-*iso*propylidenyl)-furanone have been isolated from an acetone extract of the aerial parts of *Juniperus phœnicea*. Structural elucidation of the new product was achieved mainly by spectroscopic methods.

INTRODUCTION

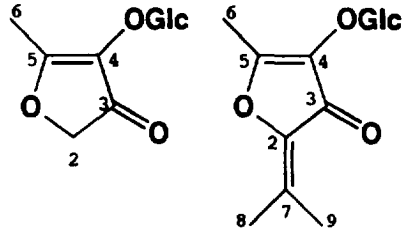
Juniperus species are well known for containing lignans which are considered to be antitumorals and antivirals of considerable interest [1–7]. *J. phœnicea*, has been the subject of previous phytochemical investigations. The essential oil is composed of large amounts of α-pinene [8], and the plant was reported to accumulate sesquiterpenes and diterpenes in particular. These diterpenoids had labdane, abietane and primarane skeletons [9–16], the most important ones being sandaracopimaric acid and eperuene diol [13]. A few phenolic compounds have been reported in this species. Lignan compounds of the aryltetrahydronaphthalen-type [5, 11, 12], and biflavonoids based on apigenin, such as amentoflavone and cupressuflavone [17, 18], are cited. During the course of our studies on structure–activity relationships between *J. phœnicea* compounds, we have described the crystallographic data of the sandaracopimaric acid, a cytotoxic and antilipoxygenasic constituent [19].

In the present paper, we report the presence of psydrin (**1**), a rare furanone, and the new 5-methyl-4-*O*-(β-D-glucosyl)-3(2-*iso*propylidenyl)-furanone (**2**), named phœnicein, isolated from an acetone extract of the leaves of *J. phœnicea*. Identification of these compounds was achieved by analysis of their UV, IR, ^{1}H and ^{13}C NMR, EI- and FAB-mass spectral data.

RESULTS AND DISCUSSION

Psydrin (**1**) and compound **2** were isolated from an acetone extract of dried leaves of *J. phœnicea*. This extract was submitted first to liquid–liquid extraction and then to chromatographic analysis (reverse-phase HPLC and diol MPLC). Final purification was performed by reverse-phase HPLC for psydrin (**1**) and by diol MPLC for **2**. All the work was monitored by analytical TLC and HPLC. Compounds **1** and **2** were obtained as amorphous powders.

The NMR data recorded for **1** agreed well with those in the literature [20]. Furthermore, additional spectral data from FAB- and EI-mass spectra were also in agreement with this structure; the furanone ring was confirmed by IR data (see Experimental) [21]. Unlike NMR data previously described for psydrin (**1**) [20], an HMBC



1 **2**

Fig. 1.

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Table 1. ^1H and ^{13}C connectivities of phenicein (**2**) and their ^2J, ^3J and ^4J interactions obtained from HMBC, HMQC and J mod ^{13}C NMR experiments (δ values)

Atom	^1H	^{13}C	^2J	^3J	^4J
Aglycone					
2		144.4			
3		182.2			
4		137.3			
5		171.4			
6	2.34 *s*	12.7	171.4 (C5)	137.3 (C4)	182.2 (C3)
7		137.0			
8	2.30 *br s*	17.0	136.0 (C7)	144.4 (C2); 19.3 (C9)	182.2 (C3)
9	2.06 *br s*	19.3	136.0 (C7)	144.4 (C2); 17.0 (C8)	182.2 (C3)
Glucosyl moiety					
1′	4.74 *d* (7.9)	105.3		137.3 (C4)	
2′	*ca* 3.28 *m*	74.8	76.8 (C3′)		
3′	3.41 *t* (8.8)	76.8	74.8 (C2′); 71.1 (C4′)		
4′	3.35 *t* (9.5)	71.1	78.5 (C5′); 76.8 (C3′)		
5′	*ca* 3.30 *m*	78.5	71.1 (C4′)		
6′A	3.69 *dd* (11.9; 5.3)	62.6	78.5 (C5′)		
6′B	3.84 *dd* (11.9; 2.3)			71.1 (C4′)	

NMR experiment showed a supplementary cross-peak (^4J correlation) between H-6 and C-3.

The molecular formula $C_{14}H_{20}O_8$ for compound **2** was deduced from EI- and FAB-mass spectrometry, and ^1H and ^{13}C NMR spectra. Both ^1H and ^{13}C NMR data revealed the presence of a glucosyl moiety in the pyranose form (see Table 1 and Experimental) [22, 23]. The FAB-mass spectrum of **2** showed quasi-molecular ion peaks at m/z 315 and 317, and the loss of a glucosyl unit was confirmed by the respective ions at m/z 153 $[M - Glc - H]^-$ and 155 $[M - Glc + H]^+$, m/z 135 $[M - OGlc - H]^-$ and 137 $[M - OGlc + H]^+$. This was corroborated in the EI mass spectrum by the ions at m/z 154 $[M - Glc]^-$ and m/z 136 $[M - OGlc]^-$. The glucosyl moiety was also identified after acid hydrolysis of compound **2**. The linkage between the sugar residue and the aglycone part was deduced from an HMBC experiment. A ^3J correlation was observed between the anomeric osidic proton and the ethylenic carbon at δ137.3 (C-4) (Table 1). The 500 MHz ^1H NMR spectrum (Table 1) of **2**, showed the corresponding glucosyl signals and only three other methyl signals appearing as singlets at δ2.30, δ2.06 and δ2.34 for the aglycone part ($C_8H_9O_3$). The chemical shifts of these methyl groups in the ^1H NMR spectrum and the corresponding chemical shifts at δ17.0, δ19.3 and δ12.7 in the ^{13}C NMR spectrum, indicated that they were linked to a carbon with a sp_2 configuration [23]. This was confirmed by an HMBC experiment which indicated a cross-peak between the methyl protons at δ2.30 and 2.06 and the C-7 at δ136.0, and between the methyl protons at δ2.34 and the olefinic carbon at δ171.4 (C-5). The methyl signal at δ2.34 also showed correlations with the C-4 at δ137.3 (^3J) and with the carbonyl group at δ182.2 (^4J) in agreement with the presence of a $CH_3 - C(R) = C(OGlc) - CO(R')$ unit. Furthermore, symmetry was observed in the correlation pattern between the methyl protons (δ2.30 and δ2.06) and C-7 (δ 136.0), C-2 (δ144.4), and C-3 (δ182.2) (Table 1). Moreover, reciprocal ^3J correlations between the methyl protons CH_3-8 (δ2.30) and CH_3-9 (δ2.06) and the methyl carbons C-9 (δ19.3) and C-8 (δ17.0) were observed. These data clearly indicated the presence of an *iso*propylidenyl group [23] and defined the following unit: $(CH_3)_2C=C(R)-CO(R')$. Finally, the deshielding of both the two olefinic carbons at δ144.4 (C-2) and δ171.4 (C-5) indicated their link to an oxygen atom. These data clearly defined the aglycone part as a furanone ring substituted by an *iso*propylidenyl group, a glucosyl moiety and a methyl group. Moreover, the UV data (261 and 322 nm) were in accordance with the above-defined ring. Consequently, compound **2** was identified as 5-methyl-4-*O*-(β-D-glucopyranosyl)-3(2-*iso*propylidenyl)-furanone, a new natural compound named phœnicein.

Psydrin (**1**) and phenicein (**2**) are of phytochemical interest having structures similar to some volatile compounds. During the last 30 years, studies on volatile flavour compounds from a large number of fruit-producing plants led to the discovery of the very important family of furaneol™, 2,5-dimethyl-4-hydroxy-3(2H)-furanone and related compounds. Furaneol™ was first isolated from pineapples [24, 25], then in many other species, e.g. strawberries, cape gooseberries, tomatoes and mangoes [26–29], and has been identified in various cooked, roasted and fermented foods [30]. 4-Methoxy-furaneol (mesifurane) has also been described [25] and more recently furaneol glucoside has been isolated from strawberries as a mixture of diastereoisomeres [31]. Furthermore, an attempt to identify 5-methyl-4-hydroxy-3(2H)-furanone (norfuraneol) was previously reported [28]. 5-Methyl-4-*O*-(β-D-glucopyranosyl)-3(2H)-furanone has been recently isolated from *Psydrax livida* [22].

EXPERIMENTAL

Plant material. Juniperus phœnicea was collected near Roquemaure in Vaucluse, France. A voucher specimen is deposited at our Laboratory (n°103).

General. TLC was carried out on precoated silica gel 60F-254 aluminium sheets (Merck). Analytical HPLC used a variable wavelength UV detector and a radial Nova-pak C18 cartridge (4 μm, 8 × 100 mm). Batch was achieved on Macherey Nagel polyamide SC-6. Semi-prep. HPLC was carried out on Lichroprep DIOL (25–40 μm), Lichroprep RP18 (15–25 μm) and μBondapak C_{18} (10 μm) columns. Chromatographic mobilities were recorded in five systems: system 1 (Silica gel F-254, EtOAc–HCO_2H–HOAc–H_2O, 20:1:1:2), system 2 [cellulose F-254, *n*-BuOH–HOAc–H_2O, 4:1:5 (upper phase)], system 3 (polyamide, toluene–MeOH, 9:1), systems 4 and 5 [radial Nova-pak C18 (4 μm 8 × 100 mm), MeOH–H_2O, (1:20), 1 ml min^{-1} and MeOH–H_2O (3:7), 1 ml min^{-1}, respectively]. IR were recorded as KBr pellets. NMR were measured at 500 MHz for ^1H and 125 MHz for ^{13}C; the solvent signal was used as ref. (CD_3OD, δ3.32 and 49.0). Complete proton and carbon assignments were based on 1D (^1H standard and ^{13}C *J* mod) 2D ^1H–^1H COSY, ^1H–^{13}C HMQC and ^1H–^{13}C HMBC NMR expts. EIMS were obtained at 70 eV. Acid hydrolysis was with 2 N HCl under reflux. Glucose was identified by TLC on silica gel (EtOAc–H_2O–MeOH– HOAc, 13:3:3:4) using *p*-anisidine phthalate spray reagent [32].

Extraction and isolation. Air-dried leaves (637 g) were successively extracted at room temp. with different solvents of increasing polarity: petroleum (53.5 g), $CHCl_3$ (45 g), EtOAc (11 g), Me_2CO (52 g) and MeOH (81.6 g). The Me_2CO extract was solubilized in 300 ml of H_2O and then dil. 3-fold with 100 ml each of EtOAc and *n*-BuOH. After concn, residues were 30 g of the *n*-BuOH part and 10 g in the EtOAc part. The aq. residual phase (12 g) was added to 90 g of polyamide MN SC-6 in batches. Then, extraction × 5 with 400 ml of MeOH was carried out. After analysis of the five frs by TLC (silica gel, EtOAc–H_2O–HOAc–HCO_2H, 20:2:1:1) and HPLC (Nova-pak C18 4 μm, 8 × 100 mm, MeOH–H_2O, 5–80% MeOH in 50 min), the residues of the first three extracts were combined (11 g). This was then submitted to prep. HPLC fractionation on a Merck 200 × 40 mm Prep Septech column using a Lichroprep 100 RP18 15–25 μm as stationary phase with an aq. MeOH stepped gradient. Ten frs were obtained: A, B and C (MeOH 5%), D and E (MeOH 10%), F and G (MeOH 20%), H and I (MeOH 40%) and J (MeOH 100%). Chromatographic stages were monitored by UV detection at 254 nm. Frs B (375 mg) and I (275 mg) were kept for further investigations. Compound **1** was purified from fr. B by semi-prep. HPLC (C18 μBondapak 25 × 100 mm, aq. MeOH 2%); 230 mg of pure psydrin were obtained. Compound **2** was purified from frs H + I by MPLC on Merck Lichroprep DIOL (15–25 μm, 15 × 460 mm, hexane–MeOH–*iso*-PrOH, 18:5:2) yielding 28 mg of pure compound.

Psydrin (**1**). IR (cm^{-1}): intense at 3350, medium at 2900, 1680, 1600, 1410, 1070, weak at 1320, 1200, 925, 695. EIMS *m/z* (rel. int.): 245 (15) $[M - 31]^+$, 162 (32) $[M - 114]^+$, 114 (100) $[M - 162]^+$, 96 (17) $[M - 180]^+$, 84 (27) $[M - 162 - 30]^+$, 72 (42) $[M - 162 - 42]^+$, 58 (72) $[M - 162 - 42 - 14]^+$. FAB (pos.) MS *m/z*: 277 $[M + H]^+$, 115 $[M - Glc + H]^+$. FAB (neg.) MS *m/z*: 275 $[M - H]^-$, 113 $[M - Glc - H]^-$. Chromatographic behaviour: R_f 0.28 (system 1), R_f 0.18 (system 2), R_f 0.13 (system 3) and R_t 8 min (system 4).

5-Methyl-4-O-(β-D-glucopyranosyl)-3(2-isopropylidenyl)-furanone (**2**). UV: λ_{max}^{MeOH} (nm) = 261, 322. EI-MS *m/z* (rel. int.): 154 (100) $[M - 162]^+$, 136 (26) $[M - 180]^+$, 108 (34) $[M - 180 - 28]^+$. FAB (pos.) MS *m/z*: 317 $[M + H]^+$, 155 $[M - Glc + H]^+$, 137 $[M - OGlc + H]^+$. FAB (neg.) MS *m/z*: 315 $[M - H]^-$, 153 $[M - Glc - H]^-$. Chromatographic behaviour: R_f 0.39 (system 1), R_f 0.62 (system 2), R_f 0.24 (system 3) and R_t 16 min (system 5).

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