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PHENYLPROPANOIDS FROM LEAVES OF *JUNIPERUS PHOENICEA*

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Key Word Index—*Juniperus phoenicea*; Cupressaceae; leaves; phenylpropane glucoside derivatives; guaiacylglycerol; junipetriolosides A and B.

Abstract—Two new compounds, junipetriolosides A (3-methoxy-4-hydroxy-phenylpropane-7,8-(2′,1′-*O*-β-D-glucopyranosyl)-7,8,9-triol) and B (3-methoxy-4-*O*-β-D-glucopyranosyl-phenylpropane-7,8,9-triol) have been isolated from a methanolic extract of the aerial parts of *Juniperus phoenicea*, along with the rare compound, guaiacylglycerol (3-methoxy-4-hydroxy-phenylpropane-7,8,9,-triol). Structural elucidation of these natural products was achieved mainly by spectroscopic methods.

INTRODUCTION

Previous phytochemical studies on phenolics from *Juniperus phoenicea* showed that this species accumulates only a few phenolic derivatives: bisflavones [1, 2] and lignans [3, 4]. We have reported the presence of three phenylpropane glycosides (juniperoside, rosarin and skimmin) [5] and two furanone glucoside derivatives (psydrin and phoenicein) [6] in this species. More recently, we have demonstrated the presence of phoeniceroside, a pseudo-dimer of the two previously reported furanones [7] and the occurrence of phenylisopropane derivatives in the same species [8]. In this paper, we report on the isolation and structural elucidation of two novel glucosylated phenylpropanoids and their rare aglucone described here for the first time in *Juniperus*. These compounds were isolated from a methanolic extract of leaves of *J. phoenicea* and identified by spectroscopic methods and by acid hydrolysis in order to confirm the sugar moiety.

RESULTS AND DISCUSSION

The molecular formulae $C_{10}H_{14}O_5$, $C_{16}H_{22}O_9$ and $C_{16}H_{24}O_{10}$ for **1**–**3**, respectively, were deduced from DCI mass spectrometry, ^1H and ^{13}C NMR.

NMR data of compound **1** revealed the presence of an aromatic ring and a propane chain. The DCI mass spectrum of **1** showed quasimolecular ion peaks at m/z 232 $[M+NH_4]^+$ and m/z 214 $[M+NH_4-H_2O]^+$ in accordance with the presence of a phenylpropane aglycone. In the aromatic part of the ^1H NMR, the signals at δ 6.98 (*d*, J = 1.8 Hz: H-2), δ 6.75 (*d*, J = 8.2 Hz: H-5) and δ 6.79 (*dd*, J = 8.2 and 1.8 Hz; H-6) defined a 1,3,4 tri-substituted aromatic ring. This was confirmed in the ^{13}C NMR spectrum by three methine peaks at δ 111.6 (C-2), δ 115.9 (C-5) and δ 120.7 (C-6), and three quaternary aromatic signals at δ 134.8 (C-1), δ 148.9 (C-3) and δ 147.1 (C-4) (see Experimental). The propane chain was defined in the ^1H NMR spectrum by a signal at δ 4.51 (*d*, J = 6.3 Hz; H-7), a multiplet at δ 3.66 (*ddd*, J = 6.3, 6.3 and 3.9 Hz: H-8) and two double doublets at δ 3.34 (*dd*, J = 11.4 and 6.3 Hz; H-9$_A$) and δ 3.47 (*dd*, J = 11.4 and 3.9 Hz; H-9$_B$). This aliphatic part was confirmed in the ^{13}C NMR spectrum by two methine carbon signals at δ 75.5 (C-7) and δ 77.6 (C-8), and a methylene peak at δ 64.3 (C-9). Moreover, ^3J cross-peaks noted during the HMBC experiment confirmed the propane chain. Whereas the chemical shift of C-9 was in accordance with a free methyleneoxy group, those of C-7 and C-8 indicated clearly the methineoxy configuration of these carbons. The HMBC cross-peaks noted for H-7 and/or C-7 and the other surrounding positions showed the link between the propane chain and the aromatic ring. The singlet at δ 3.85 (H-10) in the ^1H NMR spectrum and δ 56.4 (C-10) in the ^{13}C NMR spectrum, indicated the presence of a methoxyl group which was confirmed by an HMBC cross-peak between the H-10 protons and the quaternary aromatic carbon at δ 148.9 (C-3). At the end, the chemical shift of the quaternary carbon at δ 147.1 (C-4) indicated an hydroxyl group. Moreover, the relative pos-

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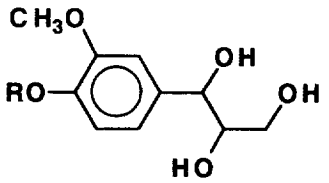
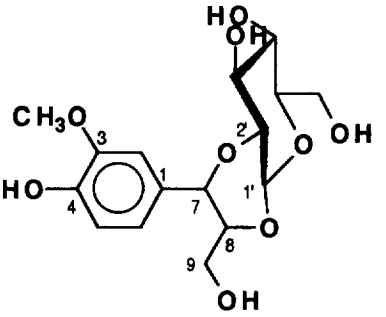


guaiacylglycerol [1] : R = H
junipetrioloside B [3] : R = Glc



junipetrioloside A [2]

itions of the methoxyl and the hydroxyl were confirmed by a 1D NOE difference experiment which showed a correlation between the H-10 and the H-2 protons. Based on the spectral data, compound **1** was thus identified as 3-methoxy-4-hydroxy-phenylpropane-7,8,9-triol or guaiacylglycerol.

Compounds **2** and **3** were analogous to **1** (similar NMR and UV data) but their more polar chromatographic characters indicated glycosylated compounds. The DCI mass spectra showed quasimolecular ion peaks at *m/z* 376 $[M+NH_4]^+$ and *m/z* 358 $[M+NH_4-H_2O]^+$ for compound **2**, and *m/z* 394 $[M+NH_4]^+$ and *m/z* 376 $[M+NH_4-H_2O]^+$ for compound **3**. In both cases, the loss of a glucosyl unit was confirmed by the same ion at *m/z* 214 $[M-OGlc+H]^+$. Moreover, ^1H and ^{13}C NMR data for both compounds showed the presence of a glucosyl moiety in the β-pyranose form (Tables 1 and 2) [9, 10]. Furthermore, the glucosyl units were also identified after acid hydrolysis of **2** and **3**. The linkage between the sugar residue and the aglycone part was in both cases deduced from HMBC experiments.

The 500 MHz ^1H NMR spectra for **2** and **3** (Tables 1 and 2) showed a 1,3,4-trisubstituted aromatic ring and an aliphatic trioxygenated propane chain, as found in **1** (see Experimental). This skeleton was confirmed in the respective ^{13}C NMR spectra. As for **1**, a methoxyl and a hydroxyl group were attached to the aromatic ring in **2** and **3**. The linkages between the propane chains and their respective aromatic rings were established unambiguously by an HMBC experiment. The HMBC correlation patterns observed in both cases for H-7 (Tables 1 and 2) confirmed the link between C-1 and C-7. However, the deshielded chemical shifts for the carbon signals of C-7 and C-8 of compound **2** (Table 1), as compared to those for **1** and **3** (see Experimental and Table 2) indicated etherification of these positions in **2**. ^3J correlations were observed between the anomeric proton at δ 4.58 (H-1′) and the aliphatic carbon at δ 82.7 (C-8) on

Table 1. NMR data for junipetrioloside A (**2**) at 125 MHz (^{13}C *J* mod) and 500 MHz (^1H) in CD_3OD

	^1H	^{13}C	HMBC
	Phenylpropanoid part		
1		130.1	
2	6.98 *d* (1.8)	112.5	80.2; 121.9; 130.1; 148.0; 149.0
3		149.0	
4		148.0	
5	6.77 *d* (8.2)	116.1	121.9; 130.1; 148.0; 149.0
6	6.85 *dd* (8.2; 1.8)	121.9	80.2; 112.5; 148.0
7	4.43 *d* (9.6)	80.2	62.1; 80.8; 82.7; 112.5; 121.9; 130.1
8	3.79 *ddd* (12; 9.6; 2.4)	82.7	62.1; 80.2; 99.8
9A	3.38 *dd* (12; 5.2)	62.1	80.2; 82.7
9B	3.45 *dd* (12; 2.4)		
10	3.85 *s*	56.5	149.0
	Glucosyl part		
1′	4.58 *d* (7.7)	99.8	71.9; 79.8; 80.8; 82.7
2′	3.14 *dd* (9.6; 7.7)	80.8	75.1; 80.2; 99.8
3′	3.58 *dd* (9.6; 8.6)	75.1	71.9; 80.8; 99.8
4′	3.39 *m*	71.9	62.6; 75.1; 79.8
5′	3.48 *m*	79.8	62.6; 71.9
6′A	3.72 *dd* (12; 5.6)	62.6	71.9; 79.8
6′B	3.89 *dd* (12; 2.3)		

Table 2. NMR data for junipetrioloside B (**3**) at 125 MHz (^{13}C *J* mod) and 500 MHz (^{1}H) in CD_3OD

	^{1}H	^{13}C	HMBC
	Phenylpropanoid part		
1		138.5	
2	7.07 *d* (1.8)	112.5	75.1; 120.6; 138.5; 147.4; 150.7
3		150.7	
4		147.4	
5	7.13 *d* (8.3)	117.8	112.5; 120.6; 138.5; 147.4; 150.7
6	6.90 *dd* (8.3; 1.8)	120.6	75.1; 112.5; 117.8; 147.4; 150.7
7	4.58 *d* (5.8)	75.1	64.2; 77.4; 112.5; 120.6; 138.5
8	3.65 *m*	77.4	64.2; 75.1; 138.5
9A	3.37 *dd* (11.4; 6.3)	64.2	75.1; 77.4
9B	3.50 *dd* (11.4; 4.3)		
10	3.86 *s*	56.7	150.7
	Glucosyl part		
1′	4.86 *d* (7.3)	103.0	77.8; 78.2
2′	3.48 *m*	74.9	71.4; 78.2; 103.0
3′	3.40 *m*	78.2	71.4; 74.9
4′	3.38 *m*	71.4	77.8; 78.2
5′	3.45 *m*	77.8	62.5; 71.4
6′A	3.68 *dd* (9.8; 4.5)	62.5	71.4; 77.8
6′B	3.85 *m*		

one side; a ^{3}J cross peak was also noted between the glucoside proton at δ 3.14 (H-2′) and the carbon signal at δ 80.2 (C-7) on the other side. Moreover, in the DCI mass spectrum of **2**, the loss of a glucosyl unit was confirmed by a peak at *m/z* 144 corresponding with a glucosyl unit which has lost both oxygen atoms at positions 1′ and 2′. Chemical shifts of the glucosidic carbons C-4′ (δ 71.9), C-5′ (δ 79.8) and C-6′ (δ 62.6) were in accordance with a β-glucosyl moiety. Those noted for C-1′ (δ 99.8), C-2′ (δ 80.8) and C-3′ (δ 75.1) could easily be explained by the β-effects due to etherification of positions 1′ and 2′. The down-shifting of the C-1′ and C-3′ signals compared with usual values was the consequence of the 2′ substitution of the sugar. Moreover, the glucosyl unit was also identified after acid hydrolysis of **2**. All these data established unambiguously the nature of the glycoside moiety and defined the quite unusual glucosylation pattern of compound **2**. In the NMR spectra of **3** (Table 2), the changes in the chemical shifts of aromatic carbons, as compared with those of **1**, indicated that glucosylation was on the aromatic ring. ^{3}J correlations observed between the anomeric proton at δ 4.86 (H-1′) and the aromatic carbon at δ 147.4 (C-4) confirmed the link between the sugar residue and the phenyl moiety.

Thus, **2** was identified as 3-methoxy-4-hydroxyphenylpropane-7,8-(2′,1′-*O*-β-D-glucopyranosyl)-7,8,9-triol and **3** as 3-methoxy-4-*O*-β-D-glucopyranosylphenylpropane-7,8,9-triol. The compounds were named junipetriolosides A and B, respectively. In these natural products, the relative coupling constants between the aliphatic protons H-7 and H-8 (J = 9.6 Hz and J = 5.8 Hz) indicated a *trans*-configuration of both H-atoms.

Guaiacylglycerol [**1**] was first isolated from *Ginkgo biloba*, another coniferophyte [11]. It is here described only for the second time in the plant kingdom. Junipetriolosides A (**2**) and B (**3**) are the first reported natural glucosides of guaiacylglycerol. The unusual trioxidation of the propane chain in these compounds has yet been encountered in neolignans in the free and 4-glucosylated forms [12, 13]. However, the particular glucosylation pattern of junipetrioloside A, to the best of our knowledge, is the first one described for natural phenylpropanoids.

EXPERIMENTAL

Plant material. Juniperus phoenicea L. was collected near Roquemaure in Vaucluse (France). A voucher specimen is deposited at our Laboratory (no 103).

General. TLC was carried out on pre-coated silica gel 60F-254 aluminium sheets (Merck). Analytical HPLC used a variable wavelength UV detector and a radial Novapak C18 cartridge (4 μm, 8 × 100 mm). Batch was achieved on Macherey Nagel polyamide SC-6. Semi-prep HPLC was carried out with Lichroprep DIOL (25–40 μm) and Lichroprep RP18 (15–25 and 25–40 μm) columns. Chromatographic mobilities were recorded in four systems: 1 (silica gel F-254, EtOAc–Me_2CO–HCO_2H–HOAc–H_2O, 10:6:1:1:2), 2 [cellulose F-254, *n*-BuOH–HOAc–H_2O, 4:1:5 (upper phase)], 3 (polyamide, toluene–MeOH, 4:1) and 4 (Waters radial Novapak C18 (4 μm, 8 × 100 mm), H_2O 1 ml min^{-1}). NMR were mea-

sured at 500 MHz for ^1H and 125 MHz for ^{13}C. The solvent signal was used as ref. (CD_3OD, δ 3.32 for ^1H and δ 49.0 for ^{13}C). 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. Acid hydrolysis was made in 2N HCl under reflux for 1 hr. Glucose was identified by TLC on silica gel (EtOAc–H_2O–MeOH–HOAc, 13:3:3:4) with *p*-anisidine phthalate reagent [14].

Extraction and isolation. Air dried leaves (637 g) were successively extracted at room temp. with petrol (4 l), $CHCl_3$ (6 l), EtOAc (6 l), Me_2CO (6 l) and MeOH (6 l) and gave residues of 53 g, 45 g, 11 g, 52 g and 130 g, respectively. The whole methanolic residue was solubilized in 300 ml of H_2O and then extracted $\times 3$ with 100 ml of EtOAc and *n*-BuOH. After concn, the EtOAc and *n*-BuOH phases gave 10 and 50 g, respectively. The aq. residual phase (70 g) was then submitted to a prep. fractionation on a 900×90 mm column using Macherey–Nagel polyamide MN SC-6 as stationary phase with an aq. MeOH stepped gradient. Six frs were obtained: A (H_2O), B (MeOH 10%), C (MeOH 20%), D (MeOH 40%), E (MeOH 60%) and F (MeOH). The cc was monitored by TLC (silica gel, EtOAc–H_2O–HOAc–HCO_2H, 20:2:1:1). Fr. A (50 g) was kept for further investigations. Fr. A was then submitted to a 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. Six frs were obtained A1 and A2 (MeOH), A3 (MeOH 10%), A4 (MeOH 20%), A5 (MeOH 50%) and A6 (MeOH 80%). The sepn was followed by UV detection at 280 nm. Fr. A3 (1.5 g) was submitted to fractionation on a 570×25 mm column using Sephadex LH-20 eluted by MeOH. The resulting most important fr. (900 mg) was submitted to MPLC on a Merck Lichroprep DIOL column (15–25 μm, 15×460 mm, hexane–MeOH–*i*-PrOH, 18:5:2 to 27:20:3). Compound **1** was present in the fr. eluted with hexane–MeOH–*i*-PrOH (18:5:2) and purified by MPLC 230×15 mm on Merck Lichroprep 100 RP18 (25–40 μm), H_2O affording 22 mg of pure product. Compounds **2** and **3** were present in the fr. eluted with hexane–MeOH–*i*-PrOH (27:20:3) and purified by two successive MPLC 230×15 mm on Merck Lichroprep 100 RP18 (25–40 μm), H_2O affording 30 and 52 mg, respectively, of the pure compounds.

Guaiacylglycerol (**1**). UV λ_{max}^{MeOH} (nm) 229, 277. DCIMS (NH_3 + isobutane): *m/z* 232 $[M+NH_4]^+$ (10), 214 $[M+NH_4-H_2O]^+$ (80). ^1H NMR: δ 6.98 (*d*, $J = 1.8$ Hz, H-2), 6.75 (*d*, $J = 8.2$ Hz, H-5), 6.79 (*dd*, $J = 8.2$, 1.8 Hz, H-6), 4.51 (*d*, $J = 6.3$ Hz, H-7), 3.66 (*ddd*, $J = 6.3$, 6.3, 3.9 Hz, H-8), 3.34 (*dd*, $J = 11.4$, 6.3 Hz, H-9A), 3.47 (*dd*, $J = 11.4$, 3.9 Hz, H-9B), 3.85 (*s*, 3H-10). ^{13}C NMR: δ 134.8 (C-1), 111.6 (C-2), 148.9 (C-3), 147.1 (C-4), 115.9 (C-5), 120.7 (C-6), 75.5 (C-7), 77.6 (C-8), 64.3 (C-9), 56.4 (C-10). Chromatographic behaviour: R_f 0.77 (system 1), R_f 0.40 (system 2), R_f 0.23 (system 3), R_t 12 min. (system 4).

Junipetrioloside A (**2**). UV: λ_{max}^{MeOH} (nm) 229, 277. DCIMS (NH_3 + isobutane): *m/z* 376 $[M+NH_4]^-$ (32), 358 $[M+NH_4-H_2O]^+$ (4), 214 $[M-Glc+H]^+$ (50), 144 $[Glc+H]^+$ (40). ^1H and ^{13}C NMR: Table 1. Chromatographic behaviour: R_f 0.62 (system 1), R_f 0.22 (system 2), R_f 0.20 (system 3), R_t 8 min. (system 4).

Junipetrioloside B (**3**). UV: λ_{max}^{MeOH} (nm) 227, 274. DCIMS (NH_3 + isobutane): *m/z* 394 $[M+NH_4]^+$ (89), 214 $[M-Glc+H]^+$ (49), 197 $[M-OGlc+H]^+$ (100). ^1H and ^{13}C NMR: Table 2. Chromatographic behaviour: R_f 0.20 (system 1), R_f 0.04 (system 2), R_f 0.11 (system 3), R_t 9 min. (system 4).

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