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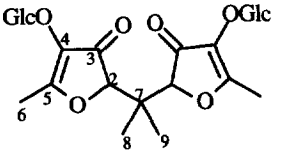
Phœniceroside, the First Natural Bis-Furanone Propane Derivative from *Juniperus phœnicea* L.

Gilles Comte, Daovy P. Allais and Albert J. Chulia*
Laboratoire de Pharmacognosie et de Phytochimie, Faculté de Pharmacie, 2, rue du Dr Marcland, 87025 Limoges, France,
Joseph Vercauteren
Laboratoire de Pharmacognosie, Faculté de Pharmacie, 146, rue Léo Saignat, 33076 Bordeaux, France,
Claude Bosso
CERMAV-CNRS, Domaine Universitaire de Grenoble, BP53X, 38041 Grenoble, France.

Abstract: Phœniceroside **1** or 2,2-bis-(5-methyl-4-(*O*-β-D-glucopyranosyl)-3(2H)-furanone)-propane have been isolated from *Juniperus phœnicea* L. (Cupressaceae). The structural elucidation of this new natural product was achieved by UV, MS, ^{1}H, ^{13}C NMR spectroscopy. The structure was confirmed by chemical way. Copyright © 1996 Elsevier Science Ltd

Recently, a German team had isolated from a plant and identified psydrin, a furanone glucoside derivative[1]. A few weeks later, we caracterized the same compound from *Juniperus phœnicea* along with phœnicein, another new natural furanone derivative[2]. These compounds are members of the furaneol family described to have mutagenic properties[3,4]. In this paper, we report the isolation of a new dimer related to these molecules from the same *Juniperus* and its caracterization by spectroscopy and chemical correlation.



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The M_r 592 and the molecular formula $C_{25}H_{36}O_{16}$ for **1** were deduced from its MS spectrum. Full assignments of ^{1}H and ^{13}C peaks, the connectivities between protons and carbons, as well as linkages remaining between glucosyl and aglycone units were established by 2D ^{1}H-^{1}H homonuclear correlation spectroscopy, *J* modulated ^{13}C, HMQC and HMBC experiments (Table 1). The positive and negative FAB mass spectra[5] showed respectively quasi-molecular ions at *m/z* 593 $[M+H]^+$ and 591 $[M-H]^-$. This was confirmed by the DCI and electrospray mass spectra[5]. The glucosyl moiety in the natural product **1** was revealed evidently in the ^{1}H NMR spectrum by the anomeric proton signal at δ 4.80 (J = 8Hz)[6], by the pattern of the carbon signals[7] and by the loss of 162 atomic mass units noted in the FAB mass spectra[5]. The glucosyl unit was also identified after acid hydrolysis of compound **1**. The linkage between the sugar residue and the aglycone part was given by the HMBC ^{3}J correlation observed between the anomeric osidic proton and the ethylenic carbon at δ 136.3 (C-4) (Table 1).

	Phœniceroside			Psydrin		Phœnicein	
	^1H	^{13}C	HMBC	^1H	^{13}C	^1H	^{13}C
2	4.85 *s*	86.5*	16.9; 43.0; 136.3; 184.2; 198.1	4.30 *s*(2H)	74.8		144.4
3		198.1			198.0		182.2
4		136.3			135.7		137.3
5		184.2			186.0		171.4
6	2.36 *s*	13.9	136.3; 184.2; 198.1	2.31 *s*(3H)	14.2	2.34 *s*(3H)	12.7
7		43.0*					137.0
8/9	1.10* *s*	16.9* 17.1*	16.9; 17.1; 43.0; 86.5			2.30 *s*(3H) 2.06 *s*(3H)	17.0/19.3
1'	4.80* *d*(8)	104.5*	78.4; 136.3	4.75 *d*(7.8)	104.5	4.74 *d*(7.9)	105.3
2'	3.27 *m*	74.8	77.8; 104.5	*ca* 3.32 *m*	74.8	*ca* 3.28 *m*	74.8
3'	3.39 *m*	77.8	71.3; 74.8; 78.4	*ca* 3.32 *m*	77.8	3.41 *t*(8.8)	76.8
4'	3.35 *m*	71.3	62.5; 77.8; 78.4	*ca* 3.32 *m*	71.3	3.35 *t*(9.5)	71.1
5'	3.25 *m*	78.4	62.5; 71.3; 77.8	*ca* 3.32 *m*	78.4	*ca* 3.30 *m*	78.5
6'	3.72 *m* 3.86 *m*	62.5	71.3; 78.4	3.67 *dd*(12; 5.3) 3.83 *dd*(12; 2.1)	62.6	3.69 *dd*(11.9; 5.3) 3.83 *dd*(11.9; 2.3)	62.6

Table 1 : ^1H and ^{13}C NMR data of psydrin, phœnicein[2] and phœniceroside **1** (CD_3OD, 500 MHz) (* multiple signals).

Both the ^1H and ^{13}C NMR spectra of **1** showed some great similarities with those of psydrin and phœnicein we had already described (Table 1)[2]. The most important difference was the appearance of a quaternary aliphatic carbon signal at δ 43.0 (C-7) instead of a quaternary ethylenic one in the phœnicein. The deshielding of H-2 (δ 4.85) and C-2 (δ 86.5) relative to psydrin was in agreement with the higher substitution of C-2. The good matching of the chemical shifts of C-3, C-4, C-5 and C-6 with the data for psydrin indicated the identity of structure for this part of the molecule. The molecular weight obtained from all MS data[5] indicated that compound **1** was constituted by two furanone derivative units and the simplicity of the NMR spectra showed a relative symetry in the structure. Final structural elucidation was given by the HMBC experiment. Actually, a ^2J cross peak observed between H-2 and C-7 indicated the link between the quaternary carbon and the C-2. Moreover, the ^3J cross peaks between H-2 and C-8/C-9, between H-8/H-9 and C-7 demonstrated the propane chain. Finally, a ^3J correlation observed between the H-2 of a furanone unit and the C-2 of the other one gave proof of the pseudo-dimerization. Moreover, the chromatographic behaviour of disaccharide type comparatively to those of psydrin and phœnicein[2] was in agreement with a diglucoside structure. However, the multiplicity of ^1H and ^{13}C signals for the positions 2, 8, 9, 7 and 1' indicated a mixture of isomers.

Finally, the acid hydrolyzis of **1** (Figure 1) led to two purified derivatives **1a** and **1b** identified mass spectrometry and NMR spectroscopy[8,9]. Actually, in DCI$^+$MS showed in both cases quasi-molecular ions at *m/z* 286 $[M+NH_4]^+$, 269 $[M+H]^+$ and 268 $[M+NH_4-H_2O]^+$ indicated the molecular weight of hydrolyzed compounds at MM 268 in agreement with the structural hypothesis for **1**. Furthermore, the NMR spectra of these compounds showed the expected signals. The difference between the two hydrolyzed compounds was the multiplicity of C-8/H-8 and C-9/H-9. Compound **1a** showed methyls 8 and 9 as singlet at δ 17.1 corresponding to the relative *meso* form. Compound **1b** showed for the same methyls two signals at δ16.5 and δ17.8 corresponding to the *threo* form.

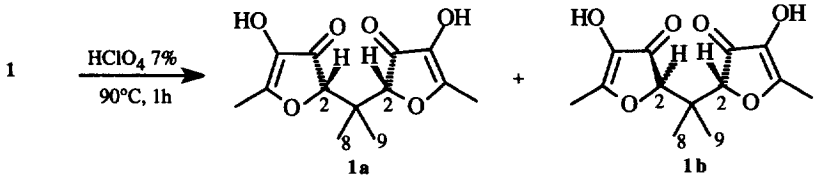

Figure 1 : Acid hydrolyzis of phœniceroside **1**.

The structure of **1** was confirmed by the hemisynthetic compound obtained from the condensation of stœchiometric amounts of phœnicein and psydrin (Figure 2). The synthetic product had completely identical NMR data (^1H and ^{13}C) and chromatographic behaviour (TLC and HPLC) as the natural one.

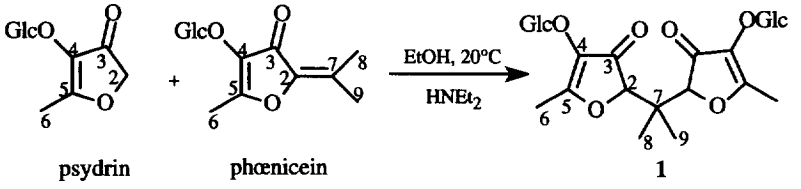

Figure 2 : Condensation of psydrin and phœnicein leading to compound **1**.

These experiments showed us that natural compound **1** was a mixture of diastereomers. The relative lability of the H-2 seemed to be at the origin of the isomerization. During the last 30 years, studies on volatile flavour compounds from a large spectra of fruit-producing plants led to the discovery of the very important family of the Furaneol™ and related compounds. The 2,5-dimethyl-4-hydroxy-3(2H)-furanone (furaneol) was first isolated from pineapple[10]. Some authors have reported the 4-methoxy-furaneol (mesifurane)[11], and recently the furaneol glucoside occuring as a mixture of diastereomers[12]. We previously described in *Juniperus phœnicea* two other glucoside derivatives of this family, psydrin and phœnicein[2]. The new phœniceroside seems to result of the condensation of the both compounds, but the question is asked to know if this mix is due to the plant or to our working methods using aqueous solutions during the purification. However, the very low efficiency (7%) of the hemisynthesis in connection with the relative amounts obtained for the three furanone derivatives during the purification indicated a biogenetic pathway for **1**. Consequently, it would be interesting to study the mutagenic potentialities of this new compound comparatively to those described for the furaneol[3,4] even if antiproliferative tests on human tumoral and leucemic cell lines we have made on psydrin and phœnicein have not given significative results.

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5. phœniceroside **1**. $[\alpha]_D^{20°}$ = -80° (*c* = 0,15; MeOH). UV λ_{max}^{MeOH} nm (ε): 279 (36700). Chromatographic mobilities were recorded in four systems : system 1 (Si gel F-254, EtOAc:HCOOH:HOAc:H_2O = 20:1:1:2) R*f* 0.26, system 2 [cellulose F-254, *n*-BuOH:HOAc:H_2O = 4:1:5 (upper phase)] R*f* 0.14, system 3 (cellulose F-254, HOAc:H_2O = 3:17) R*f* 0.87, system 4 [LichroCART 250-4 Lichrospher 100 DIOL 5 µm, *n*-Hexane:MeOH:*i*-PrOH = 63:30:7, 1 ml.min^{-1}] R_t 12 min. ^1H and ^{13}C NMR (see Table 1). DCI$^+$MS (NH_3 + isobutane) : *m/z* 610 $[M+NH_4]^+$, 593 $[M+H]^+$, 317 [M-psydrin+H]$^+$, 155 [M-psydrin-Glc+H]$^+$, 115 [M-hydrophœnicein-Glc+H]$^+$. Electrospray MS : *m/z* 615 $[M+Na]^+$. EIMS : *m/z* 276 [M-hydrophœnicein]$^-$, 154 [M-psydrin-Glc]$^-$, 114 [M-hydrophœnicein-Glc]$^-$. FAB$^+$MS (glycerol + KCl) : *m/z* 632 $[M+K]^+$, 593 $[M+H]^+$, 431 [M-Glc+H]$^+$, 317 [M-psydrin+H]$^+$, 277 [M-hydrophœnicein+H]$^+$, 155 [M-psydrin-Glc+H]$^+$. FAB$^-$MS (glycerol + KCl) : *m/z* 627 $[M+Cl]^-$, 591 $[M-H]^-$, 429 [M-Glc-H]$^-$, 315 [M-psydrin-H]$^-$, 275 [M-hydrophœnicein-H]$^-$, 113 [M-hydrophœnicein-Glc-H]$^-$.

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8. Compound **1a**. UV λ_{max}^{MeOH} nm : 289 . Chromatographic mobilities were recorded in two systems : system 5 (Si gel F-254, $CHCl_3$:MeOH = 9:1) R_f 0.45, system 6 [LichroCART 250-4 Lichrospher 100 DIOL 5 µm, *n*-Hexane:MeOH:*i*-PrOH = 18:5:2, 1 ml.min^{-1}] R_t 8.5 min.^1H NMR (500 MHz in MeOH) : δ 1.05 (H-8/9), 2.19 (H-6), 4.63 (H-2). ^{13}C NMR (125 MHz in MeOH) : 12.9 (C-6), 17.1 (C-8/9), 43.0 (C-7), 85.7 (C-2), 136.7 (C-4), 174.7 (C-5), 198.3 (C-3). DCI$^+$MS (NH_3 + isobutane) (rel. int.) : *m/z* 286 (13) $[M+NH_4]^+$, 269 (100) $[M+H]^+$, 268 (22) $[M+NH_4-H_2O]^+$, 155 (85) [M-$C_5H_5O_3$+H]$^+$, 114 (6) [M-$C_8H_{11}O_3$+H]$^+$.

9. Compound **1b**. UV λ_{max}^{MeOH} nm : 289 . Chromatographic mobilities were recorded in two systems : system 5 (Si gel F-254, $CHCl_3$:MeOH = 9:1) R_f 0.45, system 6 [LichroCART 250-4 Lichrospher 100 DIOL 5 µm, *n*-Hexane:MeOH:*i*-PrOH = 18:5:2, 1 ml.min^{-1}] R_t 8.5 min.^1H NMR (500 MHz in MeOH) : δ 0.98 and 1.08 (H-8/9), 2.21 (H-6), 4.64 (H-2). ^{13}C NMR (125 MHz in MeOH) : 12.9 (C-6), 16.5 and 17.8 (C-8/9), 42.8 (C-7), 85.2 (C-2), 136.7 (C-4), 174.9 (C-5), 198.4 (C-3). DCI$^+$MS (NH_3 + isobutane) (rel. int.) : *m/z* 286 (13) $[M+NH_4]^+$, 269 (100) $[M+H]^+$, 268 (22) $[M+NH_4-H_2O]^+$, 155 (85) [M-$C_5H_5O_3$+H]$^+$, 114 (6) [M-$C_8H_{11}O_3$+H]$^+$.

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