

NOTE

GALLOCATECHIN AND TRANS SYRINGIN FROM *LIMONIASTRUM GUYONIANUM* BOIS GROWING IN TUNISIA

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ABSTRACT: Chemical investigation of butanol extract from *L. guyonianum* Bois roots led to the isolation of Gallocatechin **1** and Trans-Syringin **2**. Their structures were established by means of 1 and 2D NMR spectroscopy and ES Mass spectrometry.

Key words: *L. guyonianum* Bois, Plumbaginaceae, roots, Gallocatechin **1**, Trans Syringin **2**, 1 and 2D NMR.

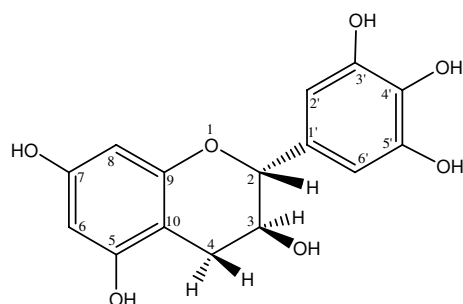
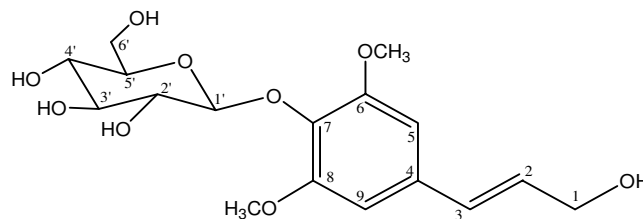
RESUME : des études chimiques de l'extrait butanolique des racines de l'espèce *L. guyonianum* Bois, nous ont permis d'isoler et d'identifier le Gallocatéchine **1** et le Trans-Syringine **2** signalés pour la première fois dans la plante. L'élucidation de leurs structures a été établie par le moyen de la RMN 1 et 2D confirmée par la spectrométrie de masse en mode Electro Spray.

Mots clés: *L. guyonianum*, Plumbaginaceae, racines, Gallocatéchine **1**, Trans Syringine **2**, RMN 1 et 2D.

INTRODUCTION

Limoniastrum guyonianum is a plant covered with calcareous concretions of 20 to 40 cm height, having erect branches, linear and semi-cylindrical leaves of 30 to 50mm, the sessiles are surrounding the stem [1]. The plant inflorescence is composed of Dinaric spikelet, 1-2 floras on an axis in a zig-zag. The decoction of its roots is used as depurative, galls are used for the tanning of leathers and also intervene for the tincture of hair in the Tunisian south [2], this specie is mainly distributed in the Sahara, it is known for its resistance to arid conditions. *Limoniastrum guyonianum* has not been reported for previous chemical investigation, therefore, bioguided fractionation of leaves extract from *Limoniastrum feei* endemic to Algeria led to the isolation of seven polyphenolic constituents: Gallic acid, Myriaphenone A, Myricetin-3-*O*- β -Galactopyranoside, Epigallocatechin gallate, Myrcetin-3-*O*- α -rhamnopyranoside, Quercetin and Myricetin [3]. In the course of searching new Natural products from Tunisian plants [4-8], we have examined the roots of *L. guyonianum* collected from Skanes, Monastir region, Tunisia and isolated for the first time from the indicated plant Gallocatechin **1** and Trans Syringin **2**. Their structure identification is described in this paper.

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**Galocatechin 1****Trans Syringin 2**

RESULTS AND DISCUSSION

Compound 1: The ElectroSpray Mass Spectrum of compound **1** gave pseudomolecular ions at 307 m/z $[M+H]^+$ and 329 m/z $[M+Na]^+$ in good agreement with the calculated mass for a molecular formula of $C_{15}H_{14}O_7$. 1H NMR spectrum of compound **1** displayed a doublet appearing at δ_H 4.44 (1H , $J=7.2Hz$, H_2) and two double doublets at δ_H 2.44 (1H , $J_1=16.8Hz$, $J_2=7.5Hz$, $H_{4\alpha}$) and δ_H 2.65 (1H , $J_1=16.8Hz$, $J_2=3.0Hz$, $H_{4\beta}$) which are characteristic signals of ring A from catechin nucleus (Table I). Two doublets at δ_H 5.81 and 5.85 ppm ($J=2.4Hz$) are assigned to H_6 and H_8 protons respectively. The observation of an additional singlet at δ_H 6.41 ppm (2H, $H_{2'}$, $H_{6'}$) suggested the presence of $C_{3'}$, $C_{4'}$ and $C_{5'}$ trihydroxy group substitutions in ring C. Comparison of 1H and ^{13}C NMR data with those of the literature [9], suggested that compound **1** has a catechin skeletal pattern. Furthermore, significant long range correlations H_2-C_3 ; H_2-C_4 ; H_4-C_{10} and H_4-C_9 confirming the presence of pyran ring having a C_3 hydroxyl group. 2D CHCorr and HMBC (300 MHz, CD_3OD) analysis showed that compound **1** was Gallocatechin. In fact, the identity of pyran ring having a C_3 hydroxyl group was confirmed by significant long range correlations H_2-C_3 ; H_2-C_4 ; H_4-C_9 and H_4-C_{10} observed in HMBC spectrum (Figure 1). The same spectrum allowed to suggest that C aromatic ring is trisubstituted by three hydroxyl groups in positions 3', 4' and 5' on the basis of HMBC correlations $H_2-C_{1'}$; $H_2-C_{2'}$; $H_2-C_{6'}$; $H_{6'}-C_{4'}$ and $H_{2'}-C_{4'}$. In conclusion, analysis of 1 and 2D NMR spectra compared with the literature values, allowed to propose for compound **1** the structure of Gallocatechin isolated for the first time from the butanol extract from *L. guyonianum* roots.

Table I. ^{13}C NMR and 1H NMR spectral data for compound **1****Compound 1 (CD_3OD , 300MHz)**

Position	^{13}C	1H
1	-	-
2	81.5	4.44 d ($J=7.2Hz$)
3	67.4	4.10 ; m
4 α	26.7	2.44; dd ($J_1=16.8Hz$; $J_2=7.5Hz$)
4 β	26.7	2.65; dd ($J_1=16.8Hz$; $J_2=3Hz$)
5	155.4	-
6	94.9	5.85; d; ($J=2.4Hz$)

7	156.6	-
8	98.7	5.81; d; ($J = 2.4 \text{ Hz}$)
9	156.2	-
10	99.3	-
1'	132.2	-
2'	105.6	6.41; s
3'	145.3	-
4'	132.6	-
5'	145.3	-
6'	105.6	6.41; s

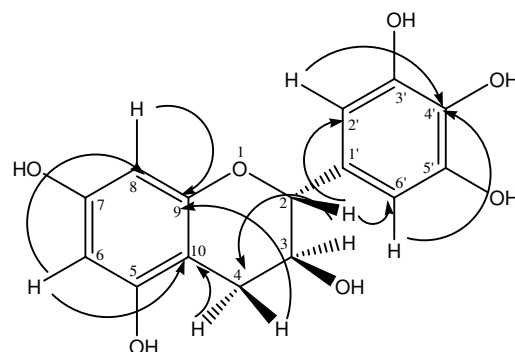


Figure 1: Heteronuclear multiple-bond correlations for compound **1**. Arrows point from proton to carbon

Compound 2: has been isolated from *L. guyonianum* butanol extract. Its ^1H NMR spectrum showed a typical singlet integrating two protons appearing at δ_{H} 6.75ppm (H_5 , H_9), indicating the presence of a symmetric tetra-substituted aromatic ring, another singlet at δ_{H} 3.86ppm (6H) attributable to two methoxyle groups. The observation of two characteristic doublet of triplets at δ_{H} 6.55ppm (1H, $J_1=15.9\text{Hz}$ and $J_2=2\text{Hz}$, H_3) and δ_{H} 6.32ppm (1H, $J_1=15.9\text{Hz}$ and $J_2=5.4\text{Hz}$, H_2) (Table II) suggested the presence of a Trans disubstituted double bond directly linked to a methylene group. The ^{13}C NMR spectrum obtained in methanol showed the famous peak of anomeric carbon $\text{C}_{1'}$ at δ_{C} 105.9ppm and five peaks appearing between δ_{C} 62.9ppm and δ_{C} 78.0ppm attributable to $\text{C}_{2'}$, $\text{C}_{3'}$, $\text{C}_{4'}$ and $\text{C}_{5'}$ of a glycopyranose moiety and indicating that compound **2** is an aromatic glycoside derivative. The structure of compound **2** as Trans-Syringin was established on the basis of HMBC spectrum which displayed significant ^1H - ^{13}C long range correlations: H_5 - C_6 ; H_5 - C_7 ; H_5 - C_3 ; H_9 - C_3 ; H_2 - C_1 ; H_9 - C_8 and H_1 - C_7 (Figure 2). In conclusion, comparison of 1 and 2D NMR data of compound **2** with those of the literature [10-12] allowed confirming its structure of Trans-Syringin isolated for the first time from *L. guyonianum* specie.

Table II. ^{13}C NMR and ^1H NMR spectral data for compound **2**
Compound **2** (CD_3OD , 300MHz)

Position	^{13}C	^1H
1	63.9	4.22 ; dd ($J_1=5.7\text{Hz}$, $J_2=1.2\text{Hz}$)
2	130.5	6.32 ; dt ($J_1=15.9\text{Hz}$, $J_2=5.4\text{Hz}$)
3	131.7	6.55 ; d ($J=15.9\text{Hz}$)
4	-	-
5,9	104.5	6.75; s
6,8	154.8	-

7	-	-
1'	105.8	m
2'	71.0-78.8	3.00-3.80; m
3'	71.0-78.8	3.00-3.80; m
4'	71.0-78.8	3.00-3.80; m
5'	71.0-78.8	3.00-3.80; m
6'a	62.9	3.64; dd ($J_1=11.7\text{Hz}$, $J_2=5.1\text{Hz}$)
6'b	62.9	3.77; dd ($J_1=12\text{Hz}$, $J_2=2.4\text{Hz}$)
10,11 (-OCH ₃)	57.5	3.86; s

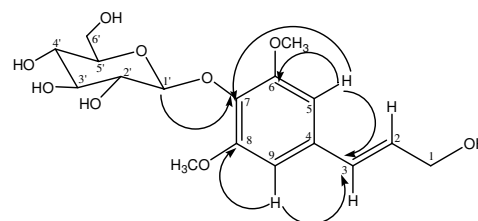


Figure 2: Heteronuclear multiple-bond correlations for compound **2**. Arrows point from proton to carbon

EXPERIMENTAL

Plant material: *Limoniastrum guyonianum* (Plumbaginaceae) was collected in Skanes region, Monastir, Tunisia in October 2008. It was identified by Dr. F. Harzallah-Skhiri (Institut Supérieur de Biothechnologie de Monastir) and a voucher specimens were deposited in Natural Substances and organic Synthesis Laboratory, Faculty of Sciences of Monastir, Tunisia. Roots were exhaustively extracted with methanol during 5 days. Evaporation of organic solvent under reduced pressure yielded 275g of methanolic crude extract which was concentrated on a rotator evaporator under reduced pressure.

Extraction and isolation of the chemical constituents:

Compound 1: The hydro-methanolic solution has been the subject of a liquid/liquid partition using PE, EtOAc and butanol as solvents having increasing polarity. The crude butanol extract (30g) thus obtained, was chromatographed on a first silica gel column using a mixture of PE/EtOAc (4:6) gradually increased with EtOAc and Methanol as eluent to collect 247x200 mL fractions regrouped on fifteen main groups having different chemical composition (F₁-F₁₅). The fifth one was further purified on a second silica gel column eluted with PE/EtOAc (8:2) mixture to afford 60mg of compound **1** as a white solid.

Compound 2: fraction F₁₁ deriving from butanol extract separation has been the subject of a solid-liquid chromatographic purifications successively over two silica gel columns eluted with PE/EtOAc (7:3) mixture gradually increased with EtOAc to afford 5mg of compound **2** as a white solid.

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