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Lignans and Other Constituents from *Helianthemum sessiliflorum* Pers.

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Abstract: One new lignan named 1-*O*-acetyl prinsepiol (**1**), in addition to nineteen known compounds including two lignans; 1*α*-hydroxypinoresinol (**2**) and (+)-cycloolivil (**3**), one fatty acid; (−)-pinellic acid (**4**), five phenolic acids; benzoic acid (**5**), *p*-hydroxybenzoic acid (**6**), protocatechuic acid (**7**), vanillic acid (**8**) and gallic acid (**9**), nine flavonoids; (−)-epicatechin (**10**), (−)-catechin (**11**), (−)-epigallocatechin (**12**), (−)-gallocatechin (**13**), astragalin (**14**), tiliroside (**15**), quercetrin (**16**), isoquercetrin (**17**) and myricitrin (**18**), and two phytosterols; *β*-sitosterol (**19**) and daucosterol (**20**) were isolated from the aerial parts of AcOEt extract of medicinal plant *Helianthemum sessiliflorum* Pers. of the family Cistaceae. The structures of all the isolated compounds **1**-**20** were determined by spectral methods including 1D (^{1}H and ^{13}C NMR) and 2D NMR (COSY, HSQC, HMBC and NOESY), HR-ESI-MS, values of optical rotation and chemical correlations with known compounds that have been described in the literature.

Keywords: *Helianthemum sessiliflorum*; Cistaceae; lignans; phenolics; NMR.

1. Introduction

Helianthemum is a genus of plants including around 110 species of evergreen and semi-evergreen shrubs and belongs to the Cistaceae family also known as rock rose, sun rose and rush rose. This genus can be found in America, Europe and Northern Africa. However, the Mediterranean region is considered its center of diversity [1]. *Helianthemum sessiliflorum* Pers. is a perennial plant usually grows in arid and semi-arid areas in the Mediterranean region [2,3]. A previous biological study revealed that *H. sessiliflorum* had interesting biological activities as analgesic and anti-inflammatory [4]. *H. sessiliflorum* held a place of importance from ancient times as many species of this genus due to their widely reported uses as folk medicinal plants [5] where the aerial parts of this plant have been frequently used for the treatment of cutaneous lesion [6]. *H. sessiliflorum* is known to possess an important ecological interest. It is related to hypogeous species commonly named desert truffles and establishes mycrrohizal symbiosis with them [7,8]. This plant and its associated fungi play a major role in the maintenance of Mediterranean shrublands and verophytic grasslands, in terms of preventing

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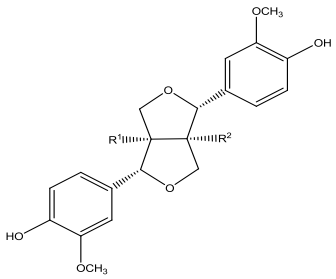
erosion and desertification [9,10]. The phytochemical investigation of the AcOEt extract of the aerial parts of *H. sessiliflorum* resulted in the identification of one new lignan; 1-*O*-acetyl prinsepiol (**1**), together with nineteen known compounds (**2**-**20**).



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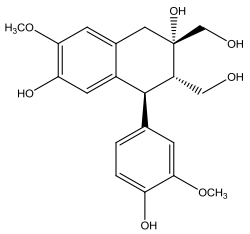

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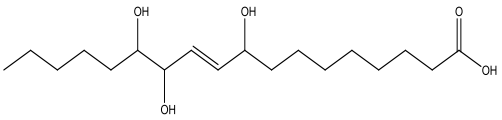

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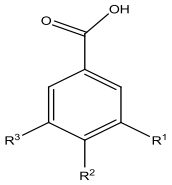
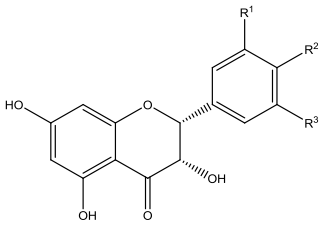


5 $R^1 = H$ $R^2 = H$ $R^3 = H$
6 $R^1 = H$ $R^2 = OH$ $R^3 = H$
7 $R^1 = OH$ $R^2 = OH$ $R^3 = H$
8 $R^1 = OCH_3$ $R^2 = OH$ $R^3 = H$
9 $R^1 = OH$ $R^2 = OH$ $R^3 = OH$



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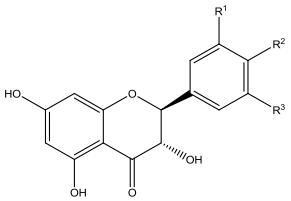
12 $R^1 = OH$ $R^2 = OH$ $R^3 = OH$



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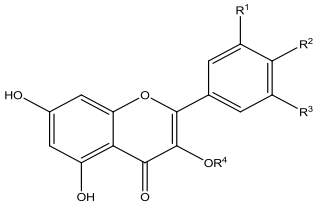
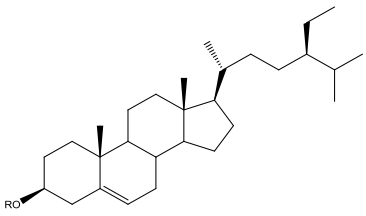


14 $R^1 = H$ $R^2 = OH$ $R^3 = H$ $R^4 = Glu$
15 $R^1 = H$ $R^2 = OH$ $R^3 = H$ $R^4 = E\text{-}p\text{-coum (1-6) Glu}$
16 $R^1 = OH$ $R^2 = OH$ $R^3 = H$ $R^4 = Rha$
17 $R^1 = OH$ $R^2 = OH$ $R^3 = H$ $R^4 = Glu$
18 $R^1 = OH$ $R^2 = OH$ $R^3 = OH$ $R^4 = Rha$



19 R = H
20 R = Glu

Figure 1. Structures of compounds **1**-**20** isolated from *Helianthemum sessiliflorum* Pers.

2. Materials and Methods

2.1. General procedures

Column chromatography (CC) was carried out using silica gel (SiO_2; 320 – 400 mesh; Merck), RP-18 reversed phase SiO_2 (40 – 63 μm, Merck), Polyamide (SC-6) and Sephadex LH-20 (25 – 100

μM). TLC and prep. TLC: Pre-coated silica gel (Kieselgel 60 F_{254}, Merck) and RP-18 reversed-phase SiO_2 (Kieselgel 60 F_{254S}, Merck) plates, detection at 254 and 366 nm and by spraying with sulfuric acid reagent (50%), followed by heating. HPLC was carried out on a *Dionex* (Luna 5μC 18 (2) 100 A column (250 mm × 10 mm × 5 μm); UVD detector). Optical rotations were measured on a Perkin-Elmer 241 polarimeter. UV Spectra were recorded on a Beckman DU-600 spectrophotometer; λ_{max} in nm. IR spectra were obtained on a Shimadzu IR-470 spectrometer; KBr pellets; $\bar{\upsilon}$ in cm^{-1}. NMR experiments were performed on a Bruker-Avance-600 Spectrometer; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. MS spectra were measured by a Bruker Esquire MSQ-Trap (ESI) and Bruker Micromass Q-TOF (HR-ESI) spectrometers; in *m/z*.

2.2. Plant material

Aerial parts of *H. sessiliflorum* Pers. were collected during the flowering period (May 2011) in southern Algeria (Biskra). A voucher specimen accession number (664/LCCE) is identified by Prof. Bachir Oudjehih of Agronomic Institute of Batna University.

2.3. Extraction and isolation

Dried aerial parts (1Kg) of the plant material were macerated with 70% EtOH (3 × 10 L) at room temperature. The EtOH extract was concentrated then diluted with H_2O and partitioned successively with cyclohexane (3 × 150 mL), AcOEt (3 × 150 mL) and *n*-buthanol (3 × 150 mL). The AcOEt extract (3.2 g) was fractionated on VLC (SiO_2) with the solvent system cyclohexane/AcOEt (100:0 to 0:100) then AcOEt/MeOH (100:0 to 0:100) to give four Fractions (1-4). Fraction 2 (450 mg) was subjected to reversed phase Silica gel column chromatography employing MeOH/H_2O (20:80 to 100:0) yielded twenty fractions (Fr. 2-1 to Fr. 2-20). Fr. 2-6 (30 mg) was submitted to semi-prep. HPLC using MeCN/H_2O (20:80) to provide **1** (3.5 mg) and **2** (4 mg). Further purification of Fr. 2-12 by CC (Sephadex LH-20, $CHCl_3$) gave **4** (16 mg). Fraction 1 (500 mg) was fractionated by CC (SiO_2, PE/AcOEt, 100:0 to 10:90) to get fifteen fractions (Fr. 1-1 to Fr. 1-15). Fr. 1-5 (100 mg) was subjected to prep. TLC (SiO_2) using $CHCl_3$/MeOH (9:1) to lead **7** (15 mg) and **8** (19 mg). Fr. 1-7 (55 mg) was separated by CC (Sephadex LH-20, $CHCl_3$) to give **9** (19.5 mg). Fr. 1-9 (90 mg) was subjected to Polyamide SC-6 CC eluting with toluene/MeOH (100:0 to 85:15), followed by prep. TLC (RP-18) using MeOH/H_2O (3:7) to obtain compounds **12** (18 mg) and **13** (10 mg). Purification of Fr. 1-14 (79 mg) over Polyamide SC-6 CC with the solvent system toluene/MeOH (100:0 to 80:20) allowed the isolation of **15** (20 mg). Fr. 1-2 (30 mg) was applied to CC (SiO_2, PE/AcOEt, 90:10 to 5:95) to yield **19** (18 mg). Fraction 3 (250 mg) of VLC was submitted to CC (SiO_2) with solvent system CH_2Cl_2/MeOH (100:0 to 50:50) and afforded ten fractions (Fr. 3-1 to Fr. 3-10). Fr. 3-5 (60 mg) gave, after purification on Sephadex LH-20 CC eluting with $CHCl_3$/MeOH (8:2) then on prep. TLC (RP-18) developed with MeOH/H_2O (1:3), two compounds **10** (10.5 mg) and **11** (11.8 mg). Fr. 3-6 (80 mg) was further subjected to Polyamide SC-6 CC using toluene/MeOH as eluent (95:5 to 85:15), then Sephadex LH-20 CC eluting with $CHCl_3$/MeOH (95:5 to 70:30) to provide compounds **3** (15 mg), **5** (9 mg) and **6** (10 mg). Fr. 3-7 (50 mg) was chromatographed over Polyamide SC-6 CC. Elution was performed by toluene/MeOH (95:5 to 60:40) to provide **14** (10 mg) and **16** (8 mg). Fr. 3-10 (45 mg) gave **17** (12 mg) and **18** (11 mg) after separation on Polyamide SC-6 CC eluting with toluene/MeOH (95:5 to 80:20), then on Sephadex LH-20 using $CHCl_3$/MeOH (100:0 to 85:15) and followed by prep. TLC (SiO_2) with $CHCl_3$/MeOH/H_2O (35:15:1). Fraction 4 of VLC (198 mg) was precipitated in MeOH to give 30 mg of compound **20**.

3. Results and Discussion

The 70% EtOH extract of the aerial parts of *H. sessiliflorum* was partitioned into fractions soluble in cyclohexane, AcOEt and *n*-butanol. Repeated column chromatography over silica gel (SiO_2), reversed-phase (RP-18), Polyamide SC-6 and Sephadex LH-20, and semi-prep. HPLC (RP-18) of the AcOEt extract afforded twenty compounds (Figure 1), the new furofuran lignan; 1-*O*-acetyl prinsepiol (**1**), along with nineteen known ones named 1*α*-hydroxypinoresinol (**2**) [11], (+)-cycloolivil (**3**) [12], (−)-pinellic acid (**4**) [13], benzoic acid (**5**) [14], *p*-hydroxybenzoic acid (**6**), protocatechuic acid (**7**),

vanillic acid (**8**) [15], gallic acid (**9**) [16], (–)-epicatechin (**10**) [17], (–)-catechin (**11**) [18], (–)-epigallocatechin (**12**) [16], (–)-gallocatechin (**13**) [19], astragalin (**14**) [20], tiliroside (**15**) [21], quercetrin (**16**), isoquercetrin (**17**) [22], myricitrin (**18**) [16], *β*-sitosterol (**19**) [23] and daucosterol (**20**) [24].

Compound **1** was obtained as colorless oil with the negative optical rotation $[\alpha]^{22}_{D} = -20.5$ (c = 0.275, AcOEt). Its ESI-MS$^+$ showed pseudomolecular ion peaks at *m/z* 455 $[M + Na]^+$ and 887 $[2M + Na]^+$. The formula as $C_{22}H_{24}O_9$ with 11 degrees of unsaturation was confirmed by HR-ESI-MS (*m/z* 455.1322 $[M + Na]^+$; calc. 455.1318). IR spectrum displayed absorption bands at 3475 cm^{-1} (hydroxyl), 1725 cm^{-1} (carbonyl) and 1605, 1580, 1515 cm^{-1} (aromatic ring). In the UV spectrum maxima of absorption were observed at 232 and 278 nm suggesting the presence of aromatic system. ^{1}H NMR and COSY spectra of **1** (Table 1) showed in the aromatic part signals of protons resonating at δ_H 7.00 (H-2′), 6.93 (H-2″), 6.87 (H-5′), 6.98 (H-5″), 6.95 (H-6′) and 6.91 (H-6″) due to the presence of two 1,3,4-trisubstituted aromatic rings A and B respectively. The ^{1}H NMR spectrum displayed also two large singlet signals at δ_H 5.64 and 5.68 that showed no correlations in the HSQC spectrum ascribable to two hydroxyl groups. Moreover, two singlet signals at δ_H 3.89 (3H) and 3.94 (3H) were observed in the ^{1}H NMR spectrum attributed to two methoxyl groups which were located at C-3′ and C-3″ positions according to HMBC cross-peaks from the methoxyl protons 3′-OMe/3″-OMe, H-2′/ H-2″, H-5′/H-5″ and 4′-OH (δ_H 5.64)/4″-OH (δ_H 5.68) to the quaternary aromatic carbons C-3′ (δ_C 145.9)/C-3″ (δ_C 146.6) respectively. So, both the aromatic moieties A and B were identified as 4-hydroxy-3-methoxyphenyl. The ^{1}H and ^{13}C NMR and HSQC spectra (Table 1) exhibited the characteristics of a pinoresinol-type lignan [25-28], with particularly the presence of two methylene groups bearing an oxygen function at δ_H 4.17 (H-4a); 4.12 (H-4b) and δ_C 74.9 (C-4), and 4.72 (H-8a); 4.28 (H-8b) and δ_C 75.9 (C-8), and two oxymethine protons at δ_H 5.33 (H-2) and δ_C 86.6 (C-2), and 4.92 (H-6), δ_C 87.1(C-6) of furofuran lignan. The only difference between **1** and 1*α*-hydroxy-pinoresinol **2** isolated also in this study (Table 1), was the lack in **1** of signals of methine group observed in the case of **2** at δ_H 3.15 (H-5) and δ_C 60.1 (C-5), and the appearance of resonances due to one acetoxyl group at δ_H 1.54 (CH_3-2‴) and δ_C 20.9 (C-2‴) and δ_C 168.8 (C-1‴), and one downfield oxygenated quaternary carbon resonating at 88.2 ppm of furofuran ring. The HMBC experiment (Figure 2) showed ^2J and ^3J correlations from protons H_2-4 and H_2-8 to carbons C-1 (δ_C 91.4), C-2 (δ_C 86.6), C-5 (δ_C 88.2) and C-6 (δ_C 87.1). The two oxymethine protons H-2 and H-6 correlated in the HMBC spectrum with oxymethylene carbons C-4 (δ_C 74.9) and C-8 (δ_C 75.9). These assignments confirmed the presence of furofuran ring substituted in C-1 and C-5 positions as observed in prinsepiol [25]. The ^2J cross-peak noted during the HMBC experiment between H-2 and the aromatic quaternary carbon C-1′ (δ_C 128.3) showed the link between C-2 and the first 1,3,4-trisubstituted aromatic ring A while the correlation depicted from H-6 to C-1″ (δ_C 127.5) providing evidence that C-6 was attached to ring B (Figure 2). These spectral data were in close agreement with those reported for prinsepiol except for the chemical shift observed for C-1 (δ_C 91.4) indicative of an acetoxyl group linked to C-1 [26,29]. The location of this group at C-1 was also confirmed on the basis of the comparison of chemical shift of the aromatic quaternary carbon C-1″ of ring B (δ_C 127.5) upfield (- 0.8 ppm) to the corresponding signal of C-1′ of ring A (δ_C 128.3). This upfield shift is due to the *γ* substituent effect of the hydroxyl group at the C-5 (δ_C 88.2) [29].

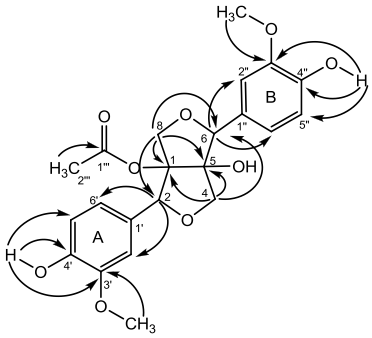


Figure 2. Pertinent HMBC (H → C) correlations of compound **1**

Table 1. ^{1}H NMR and ^{13}C NMR data of **1** and **2** in $CDCl_3$ (δ in ppm, *J* in Hz, recorded at 600 MHz and 150 MHz, respectively).

	1		**2**	
Position	δ_H, *m, J*	δ_C	δ_H, *m, J*	δ_C
1	-	91.4	-	91.6
2	5.33 (1H, *s*, H-2*β*)	86.6	4.87 (1H, *br. s*, H-2*β*)	87.8
4a	4.17 (1H, *d*, *J* = 9.8, H-4*α*)	74.9	4.56 (1H, *t*, *J* = 8.7, H-*4α*)	71.7
4b	4.12 (1H, *d*, *J* = 9.7, H-4*β*)		3.87 (1H, *dd*, *J* = 9.1, 6.3, H-4*β*)	
5	-	88.2	3.15 (1H, *td*, *J* = 7.9, 5.2, H-5*α*)	60.1
6	4.92 (1H, *s*, H-6*β*)	87.1	4.89 (1H, *d*, *J* = 4.8, H-6*β*)	85.8
8a	4.72 (1H, *d*, *J* = 11.2, H-8*β*)	75.9	4.08 (1H, *d*, *J* = 9.3, H-8*β*)	74.7
8b	4.28 (1H, *d*, *J* = 11.2, H-8*α*)		3.94 (1H, *d*, *J* = 9.2, H-8*α*)	
1′	-	128.3	-	127.0
1″	-	127.5	-	132.3
2′	7.00 (1H, *d*, *J* = 1.6)	111.9	7.01 (1H, *d*, *J* = 1.4)	109.3
2″	6.93 (1H, *d*, *J* = 1.5)	109.3	7.03 (1H, *br. s*)	109.0
3′	-	145.9	-	146.0
3″	-	146.6	-	145.4
4′	-	145.7	-	146.9
4″	-	145.8	-	146.7
5′	6.87 (1H, *d*, *J* = 8.2)	113.9	6.98 (1H, *d*, *J* = 8.1)	114.7
5″	6.98 (1H, *d*, *J* = 8.1)	114.5	6.93 (1H, *br. s*)	114.2
6′	6.95 (1H, *dd*, *J* = 8.2, 1.6)	122.5	6.90 (1H, *dd*, *J* = 8.1, 1.4)	119.7
6″	6.91 (1H, *dd*, *J* = 8.1, 1.5)	119.8	6.93 (1H, *br. s*)	119.6
3′-OMe	3.89 (3H, *s*)	55.9	3.95 (3H, *s*)	56.0
3″-OMe	3.94 (3H, *s*)	56.0	3.93 (3H, *s*)	55.9
4′-OH	5.64 (1H, *br. s*)	-		
4″-OH	5.68 (1H, *br. s*)	-		
1‴	-	168.8		
2‴	1.54 (3H, *s*)	20.9		

From the above data, **1** was an acetylated derivative of prinsepiol ($[\alpha]^{22}_D$ = −18.41) [26] and the same stereochemistry was expected for the both compounds. It was established previously that the junction between the two five-membered rings of pinoresinol-type lignans was always *cis* and both fusion rings adopt an envelope conformation, with the two oxygen atoms pointing away (Figure 3) [30-33]. This stereochemistry was confirmed by NOE correlations depicted in the NOESY spectrum between protons H-4b/H-2 and H-4b/H-6, as well as between H-8a/H-2 indicating that all these protons were on the same face of the molecule and deduced to be (*β*)-oriented. Thus, the aromatic rings A and B had an (*α*)-equatorial orientation (Figure 3). This (*α*)-arrangement was further confirmed by the upfield chemical shift of the acetyl protons at δ_H 1.54 (CH_3-2‴) due particularly to the shielding cone of the aryl group (ring A) [26], and by the downfield chemical shifts of oxymethine protons H-2*β* and H-6*β* at δ_H 5.33 and 4.92 respectively in comparison with the same protons for related structures having aryl moieties (*β*)-axial oriented [33-35]. The NOESY cross-peak observed between H-4b/H-2*β* and the absence of correlation from H-6*β* to H-8a supported a chair/boot conformation for furofuan ring (Figure 3) [25,30]. Thus, **1** was found to be a new compound identified as 1-acetoxy-5-hydroxy-2,6-di(4-hydroxy-3-methoxyphenyl)-3,7-dioxabicyclo[3.3.0]octane named 1-*O*-acetyl prinsepiol.

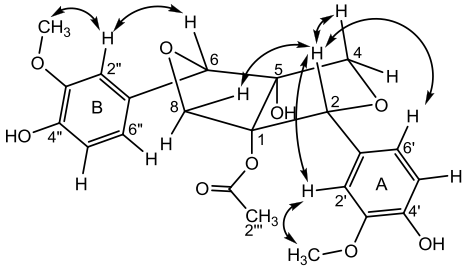


Figure 3. Key NOESY (H ↔ H) correlations of compound **1**

4. Conclusions

In this study we describe the isolation and identification of one new lignan; 1-*O*-acetyl prinsepiol (**1**), with nineteen known compounds (**2-20**), from Algerian medicinal plant *Helianthemum*

sessiliflorum. The phytochemistry of this species is dominated by both flavonoids and phenolic compounds. These results are in good agreement with other reports on the chemical composition of Cistaceae family, to which the genus *Helianthemum* belongs [36-38]. To the best of our knowledge, this is the first report on the occurrence of lignan compounds in plants of Cistaceae family. The major isolated compounds have been previously found to be effective in many biological tests [39-42] and showed a relationship between the chemical constituents of *H. sessiliflorum* and its reported biological activities [4].

Acknowledgments

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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