CONTRIBUTION TO THE PHYTOCHEMICAL INVESTIGATION OF THE PLANT *Eryngium dichotomum* Desf. (APIACEAE) FROM TUNISIA

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Abstract : The butanolic extract of *Eryngium dichotomum* Desf. aerial parts afforded a Cyclohexenone glycoside **1**, flavone glycoside : Naringenine 7-*O*-α-L-Rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranoside **2**, Sugar : β-D-fructofuranose **3,** 2-*O*-methyl-α-D-fructofuranose **4,** Sucrose **5** and D-mannitol **6**. Their structures were principally elucidated by spectroscopic procedures and chemical data. These natural substances are signalled for the first time in *Eryngium dichotomum* Desf.

Keywords: Cyclohexenone glycoside, Flavone glycoside, Sugar, *Eryngium dichotomum*, NMR.

Résumé : L'étude chromatographique de l'extrait butanolique de la partie aérienne de la plante *Eryngium dichotomum* Desf. Nous a permis d'isoler : une Cyclohexenone glycoside **1**, une flavone glycoside : Naringenine 7-*O*-α-L-Rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranoside **2**, Sucres : β-D-fructofuranose **3,** 2-*O*-méthyl-α-D-fructofuranose **4,** Sucrose **5** et le D-mannitol **6**. Les structures de ces produits naturels ont été établies à l'aide de techniques spectroscopiques. Ces substances naturelles sont signalées pour la première fois dans cette plante.

Mots clés: Cyclohexenone glycoside, Flavone glycoside, Sucres, *Eryngium dichotomum*, RMN.

1. Introduction

Eryngium dichotomum Desf. (Apiaceae) is one of the Tunisian medicinal plant is in our Laboratory the subject of phytochemical and biological studies in order to characterize its secondary metabolites which could be biologically and therapeutically active. This Mediterranean plant is widely distributed in Tunisia [1]. The same reference indicates the presence of seven other species: *E. barrelieri*, *E. triquetrum*, *E. glomeratum*, *E. campestre*, *E. tricuspidatum*, *E. maritimum* and *E. Ilicifolium*.

Our earlier chemical investigations on the *E. dichotomum* have led to the isolation of stigmasterol and stigmasterol-3-*O*-β-D-glucoside [2]. The present study describes the isolation and the structure determination of a cyclohexenone-*O*-glycoside **1,** Naringenine 7-*O*-α-L-Rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranoside **2**, β-D-fructofuranose **3,** 2-*O*-methyl-α-D-fructofuranose **4,** Sucrose **5** and D-mannitol **6**, from its aerial parts. Structural characterisation of the isolated compounds achieved by extensive one and two dimensional NMR experiments and by comparison of some spectral data with those of the literature. Our bibliographic research showed that the isolated compounds were previously identified in other natural sources and indicated for the first time in *E. dichotomum*.

2. Results and Discussion

Compound **1:** Was isolated as an oil from the butanolic extract of the aerial parts of *Eryngium dichotomum*. Its molecular formula $C_{19}H_{30}O_9$ was deduced from the ESMS $[M+Na]^+$ at *m/z* 425. The IR spectrum displayed absorption bands attributable to alcohol groups (3368 cm^{-1}) and an α,β

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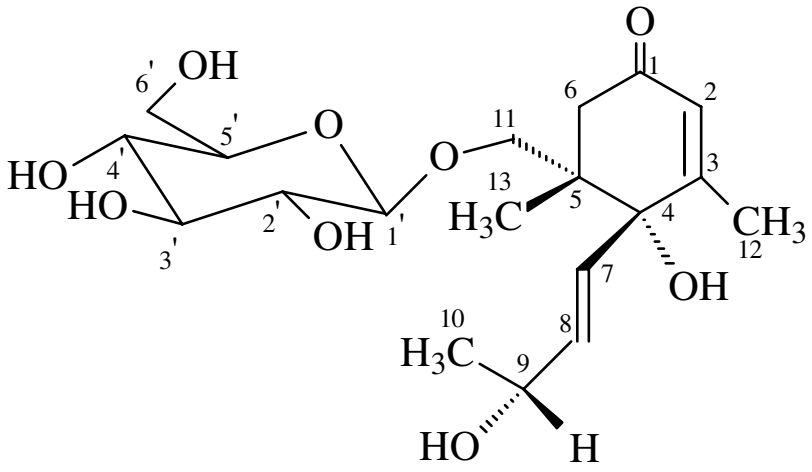
unsaturated ketone function (1652 cm^{-1}). NMR spectral data are presented in Table 1. The ^{1}H-NMR spectrum showed two singlets of methyl groups attributed to H_{12} (3H, δ 1.93, s) and H_{13} (3H, δ 1.07, s). a doublet at 1.25 ppm was also observed and assigned to the third methyl group H_{10} (3H, *J*=7.3 Hz). An AB system centred at 2.51 ppm in the ^{1}H-NMR spectrum was assigned to the methylene group $H_{6a,b}$ (Table 1) [3-7]. The same spectrum revealed three olefinic proton signals between 5.7 and 5.95 ppm attributable to H_2 (1H, δ 5.91, s), H_7 (1H, δ 5.75, d, *J*=15.6 Hz) and H_8 (1H, δ 5.84, dd, $J_{8\text{-}7}$=15.6 Hz and $J_{8\text{-}9}$=5.1 Hz) [3,5-7].

Analysis of the ^{13}C-NMR spectrum confirmed the above spectral data and revealed, in particular, a signal at 201.1 ppm corresponding to the α,β unsaturated ketone function (C_1) in agreement with the absorption at 1652 cm^{-1} showen in the IR spectrum as indicated above. Four signals at 127.8, 167.3, 129.7 and 137.2 ppm were also observed and attributed to C_2, C_3, C_7 and C_8, respectively [3, 5, 6]. The existence of a sugar moiety in the molecule was ascertained by the observation of the anomeric carbon signal at 104.6 ppm. The correlation $H_{1'}/C_{1'}$ observed in the HMQC spectrum reinforced this result and permitted to know in an other hand the anomeric proton $H_{1'}$ resonating at 4.14 ppm which was used as starting point for the interpretation of COSY datasets allowing the identification of the sugar as β-D-glucopyranose. Sugar system spectral data (Table 1) were in agreement with those of the literature [4,6,7] and the proposed stereochemistry was supported by the trans di-axial couplings between $H_{1'}/H_{2'}$ (*J*=7.8 Hz) and $H_{2'}/H_{3'}$ (*J*=8.1 Hz) in agreement with nOe correlations $H_{1'}/H_{3'}$ and $H_{1'}/H_{5'}$ deduced from the NOESY experiment.



Compound **1**

The HMBC spectrum showed that the carbonyl group C_1 (δ 201.1 ppm) correlated with the AB system $H_{6a,b}$ which in turn correlated with C_4, C_5 and C_{11}. Furthermore, long range correlations between the methyl group at δ 1.93 (3H, H_{12}) with C_2, C_3 and C_4 supported the structure of the cyclohexenone moiety. The position of the methyl group H_{12} was reinforced by the significant ^4J peak correlating signal H_{12}/H_2 observed in the COSY spectrum. On an other hand, the same COSY spectrum revealed correlations between the olefinic proton H_7 (δ 5.75 ppm) and the second olefinic proton H_8 (δ 5.84 ppm) which in turn had a cross peak with H_9 (δ 4.34 ppm) confirming the structure of the side chain at C_4. The HMBC spectrum allowed us to reinforce this result and pointed to the attachment of this side chain at C_4 by the observation of the significant correlations H_7/C_4 and H_8/C_4.

The location of the sugar system at C_{11} was inferred from the HMBC spectrum showing a ^3J correlation between the anomeric proton $H_{1'}$ (δ 4.14 ppm) and the secondary carbon C_{11} (δ 74.6 ppm) via oxygen atom.

Elucidation of the relative stereochemistry of compound **1** was based on the results deduced from the NOESY spectrum. However, dipolar couplings of H_{13} to H_7 and to H_8 confirmed the proposal

stereochemistry of the molecule. The close similarity of the ^{13}C chemical shift of C_9 with that of the literature permitted to maintain the same configuration (*R*) [3,5]. The (*E*) configuration of the Δ^7 double bond as depicted in formula **1** was confirmed by the coupling constant value ($J_{7\text{-}8}$=15.6 Hz).

Table 1. ^1H- and ^{13}C-NMR spectral data of Compound **1**.

Position	^{13}C	^1H	COSY	HMBC (H to C)
1	201.1			
2	127.8	5.91; s	12	4; 12
3	167.3			
4	79.4			
5	46.3			
6a	45.5	2.38; d; 13	6b	1; 5; 11
6b		2.64; d; 13	6a	1; 4; 5
7	129.7	5.75; d; 15.6	8	4; 9
8	137.2	5.84; dd; 15.6; 5.1	7; 9	4; 9
9	68.6	4.34; m	8; 10	
10	23.8	1.25; d; 7.3	9	8; 9
11a	74.6	3.59; d; 10	11b	13
11b		3.96; d; 10	11a	6
12	19.6	1.93; s	2	2; 3; 4
13	20.1	1.07; s		4; 6; 11
1’	104.6	4.14; d; 7.8	2’	11
2’	75.0	3.14; t; 8.1	1’; 3’	
3’	77.9	3.2-3.4; m	2’; 4’	
4’	71.5	3.2-3.4; m	3’; 5’	3’; 5’
5’	77.9	3.2-3.4; m	4’; 6’a; 6’b	
6’a	62.7	3.66; dd; 12; 5.4	6’b; 5’	
6’b		3.84; dd; 12; 2.1	6’a; 5’	

Compound **2:** Was isolated as an oil from the butanolic extract of the aerial parts of *Eryngium dichotomum*. Its molecular formula $C_{27}H_{32}O_{14}$ was deduced from the ESMS $[M+Na]^+$ at *m/z* 603.

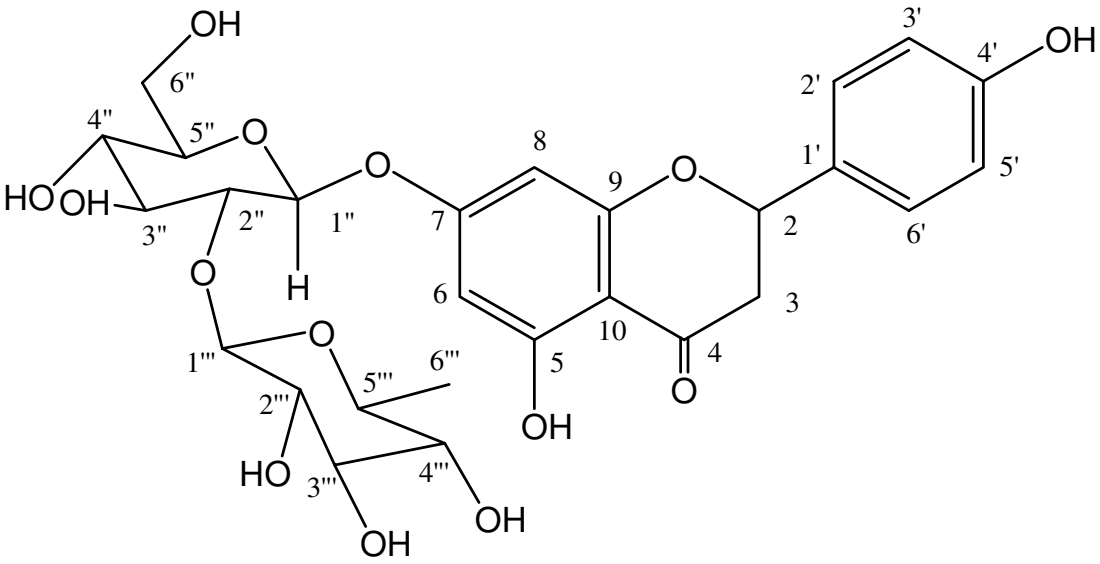
The identification of the aglycone skeleton as Naringenine was deduced from a detailed analysis of 1D and 2D NMR (HMQC, ^1H-^1H COSY and NOESY) spectral data presented in Table 2, and was confirmed by comparison with literature data [8-10].

Analysis of the ^1H-NMR spectrum of compound **2** revealed caracteristic resonances of aromatic and glycosidic protons. Comparison of the aromatic protons at δ 6.15 (1H, d, *J*=2.1 Hz); δ 6.17 (1H, d, *J*=2.1 Hz); δ 6.82 (2H, d, *J*=8.4 Hz) and δ 7.32 (2H, d, *J*=8.7 Hz) to those of some flavonoids suggested that the aglycone was the Naringenine [10,11] . In addition, the ^1H-NMR spectrum showed signals of ten protons (3.3-4 ppm) indicating that the carbohydrate must be a disaccharide. This result was confirmed by the ^{13}C-NMR spectrum showing in the region 60-78 ppm nine signals attributable to the sugar carbons. On the other hand, the observation in the ^1H-NMR spectrum of two signals at δ 5.08 (1H, d, *J*=7.5 Hz) and δ 5.24 (1H, brs) which correlated in the HMQC spectrum with carbons at 96.1 (C''_1) and 101.2 (C'''_1), respectively, reinforced the above conclusion.

The multiplicities and the weak coupling constants of H_6 and H_8 were in agreement with the existence of the etherified hydroxyl group at C_7 (δ 165.1). The branching point of the glucopyranosyl moiety (C_7) was inferred from the NOESY spectrum showing a *nOe* between the anomeric proton $H_{1''}$ and H_6 in A-ring. This result was confirmed by the UV spectra, which were indicative of the presence of phenolic functionality in positions 4’ and 5. The absence of any

bathochromic shifs upon addition of NaOAc to compound **2** dissolved in MeOH showed that the hydroxyl group at C_7 is not free.

The sequence of the disaccharide moiety was evidenced by the observation in the NOESY spectrum of the *nOe* $H_{1'''}/H_{2''}$. Moreover, the dipolar correlation of the rhamnopyranoside methylic protons $H_{6'''}$ with the aromatic proton H_6 observed in the NOESY spectrum supported the attachment of this sugar at $C_{2''}$ of the glucopyranoside. Measurement of through–space interatomic distance based on molecular model reinforces the proposed position.



Compound **2**

Table 2**.** ^{1}H- and ^{13}C-NMR spectral data of Compound **2**.

Position	^{13}C	^{1}H	COSY
2	79.3	5.35; dd; 12; 3	3α; 3β
3α	42.8	3.15; dd; 18; 12	2; 3β
3β	42.8	2.75; dd; 18; 3	2; 3α
4	197.2		
5	163.2		
6	96.4	6.15; d; 2.1	
7	165.1		
8	95.3	6.17; d; 2.1	
9	157,7		
10	103.5		
1’	129.4		
2’	127.8	7.32; d; 8.7	3’
3’	114.9	6.82; d; 8.4	2’
4’	163.5		
5’	114.9	6.82; d; 8.4	6’
6’	127.8	7.32; d; 8.7	5’
Glu			
1 ”	96.1	5.08; d; 7.5	2’’
2”	70. 8	3.54-3.72	1’’
3”	77. 6	3.54-3.72	4’’

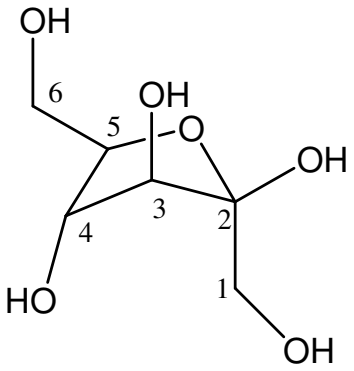
4”	69, 8	3.28-3.5	5’’
5”	77.6	3.54-3.72	4’’
6”a	60.8	3.54-3.72	6’’b
6”b	60.8	3.82-3.96	6’’a
Rha			
1 ”’	101.2	5.24; brs	2’’’
2”’	70. 8	3.82-3.96	1’’’
3”’	76,8	3.28-3.5	2’’’;4’’’
4”’	72.5	3.28-3.5	3’’’;5’’’
5”’	68.6	3.82-3.96	4’’’;6’’’
6”’	16.9	1.28; d; 6	5’’’

Compounds **3** and **4:** Were isolated as white solids from the butanolic extract of the aerial parts of *Eryngium dichotomum*. The molecular formula of compounds **3** ($C_6H_{12}O_6$) and **4** ($C_7H_{14}O_6$) were deduced from the ESMS $[M+K]^+$ at *m/z* 219, from the ESMS $[M+Na]^+$ at *m/z* 217, respectively.

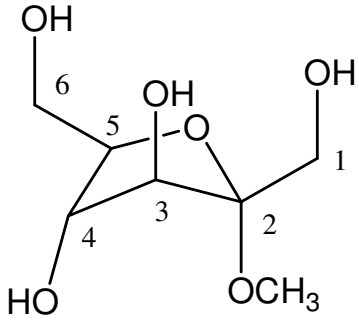
The ^{13}C-NMR spectra of compounds **3** and **4**, displayed two CH_2, three CH and one tetrasubstitued carbon linked to oxygen, whereas the corresponding ^{1}H-NMR spectra lack the anomeric proton, which suggested cyclic form of ketoses [11]

Compound **4** had in addition, a methoxyl group δ 3.11 (s, 3H, OCH_3) that correlated in the CHCorr spectrum with C_{OCH3} (δ 48.6) and placed at the tetrasubstituted carbon C_2. The analysis of proton-proton couplings in ^{1}H-^{1}H COSY spectra of **3** and **4** displayed correlations between H_3 and H_4, H_4 and H_5, H_5 and $H_{6a,b}$ and H_{6a}-H_{6b} .

From the above-mentioned data and comparison with literature data [12] compound **3** was identified as β-D-fructofuranose and compound **4** as 2-*O*-methyl-α-D-fructofuranose.



Compound **3**



Compound **4**

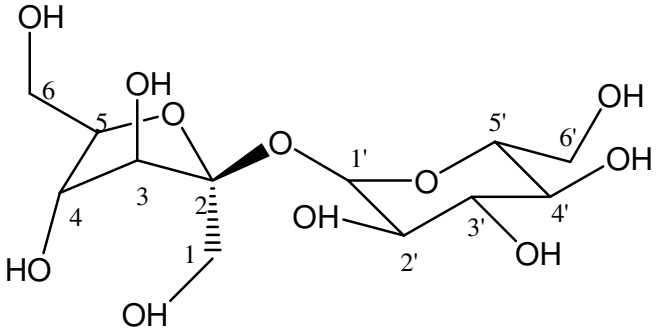
Table 3**.** ^{1}H- and ^{13}C-NMR spectral data of Compound **3**.

Position	^{13}C	^{1}H	COSY
1a	61.9	3.67; d; 11.7	1b
1b		3.55; d; 11.7	1a
2	105.6		
3	79.1	4.13; d; 7.8	4
4	77.6	3.95; t; 7.5	3;5
5	83.9	3.72-3.8; m	4;6b
6a	65.1	3.72-3.8; m	6b
6b		3.6; dd; 1.2; 7.2	5;6a

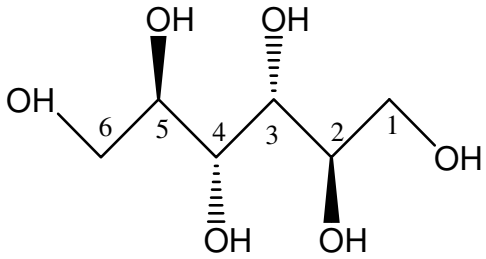
Table 4.^{1}H- and ^{13}C-NMR spectral data of Compound **4**.

Positic	^{13}C	^{1}H	COSY
1a	60.9	3.43; d; 12	1b
1b		3.52; d; 12	1a
2	109.6		
3	82.9	3.83; d; 3.9	4
4	79.3	3.7; dd; 6.6; 3.9	3;5
5	85.0	3.59; ddd; 7.5; 4.8;	4;6b
6a	63.2	3.55; dd; 12; 3	6b
6b		3.43; dd; 12; 4.2	5;6a
OCH	48.6	3.11	

Compounds **5** and **6:** These compounds exhibited spectral data (^{1}H, ^{13}C NMR) comparable to published values [13-17].



Compound **5**



Compound **6**

3. Experimental

3.1. General

^{1}H, ^{13}C 1 and 2D NMR spectra of compound **1**, were recorded in CD_3OD with a Bruker 400 spectrometer operating at 400 MHz (^{1}H) and at 100 MHz (^{13}C). The residual solvent resonance is used as internal reference. Chemical shifts (δ) are given in ppm and coupling constants (*J*) in Hz.The fourier transform infrared absorption spectrum was recorded on a salt plate using a Perkin-Elmer FT-IR spectrometer 1000. The mass spectrum was obtained with a micromass spectrometer (Q-Tofmicro) linked to an ESI source. The optical rotation was measured at room temperature with a Perkin-Elmer 141 MC polarimeter and was referenced to the D-line of sodium.

^{1}H, ^{13}C 1 and 2D NMR spectra of compounds **2**, **3, 4, 5** and **6** were recorded in CD_3OD with a Bruker spectrometer AM operating at 300 MHz (^{1}H) and at 75 MHz (^{13}C). The residual solvent resonance is used as internal reference. Chemical shifts (δ) are given in ppm and coupling constants (*J*) in Hz.

3.2. Plant material

Eryngium dichotomum was harvested at Zaghouan (Tunisia) in May 2001. Voucher specimen (n° ED.111) of the plant was deposited in the Herbarium of the Ecole Supérieure d'Horticulture et d'Elevage de Chott mériem, Université de Sousse, Sousse, Tunisia.

3.3. Extraction and Isolation

Air dried and powdered aerial parts of *E. dichotomum* (2kg) were extracted in a Soxhlet apparatus with methanol. 170g of the crude residue (250g) obtained after filtration and evaporation of the solvent under reduced pressure, were dissolved in water then extracted successively with $CHCl_3$, AcOEt and BuOH yielding 15g, 5g and 30g subextracts, respectively. 28.5g of the butanolic extract were simplified by silica gel column chromatography (sds 60 AC.C 70-200µm, petroleum ether, AcOEt, acetone gradients). in 300x200mL fractions ((a_1-a_{10} (100mg); a_{10}-a_{20} (72mg); a_{21}-a_{24} (75mg); a_{25}-a_{29} (90mg); a_{30}-a_{37} (185mg); a_{38}-a_{45} (775mg); a_{46}-a_{54} (900mg); a_{55}-a_{62} (640mg); a_{63}-a_{75} (900mg); a_{76}-a_{85} (850mg); a_{86}-a_{105} (1.4g); a_{106}-a_{151} (450mg) and a_{152}-a_{300} (1.2g)).
The mixture of fractions a_{106}-a_{151} (450mg) was rechromatographed on silica gel (sds 60 AC.C 70-200µm, $CHCl_3$/MeOH 8:2) in 80x3mL fractions ((b_1-b_{20} (90mg); b_{21}-b_{43} (100mg); b_{44}-b_{70} (40mg); b_{71}-b_{80} (80mg)). The mixture of fractions b_{44}-b_{70} (40mg) was separated by silica gel column (sds 60 AC.C 70-200µm, $CHCl_3$/MeOH 8:2) to furnish compound **1** as an oily substance (12mg); $[\alpha]_D^{20}$ +76 (C = 1.2; MeOH), Rf = 0.45 ($CHCl_3$/MeOH 8:2); IR (NaCl) ν_{max} cm^{-1} 3368 (br OH), 1652 (α,β unsaturated CO); ESMS *m/z* (%) 245 $[M+Na]^+$ (100), 409 (33), 301 (30), 270 (31), 149 (20), 79 (19); for ^{1}H and ^{13}C-NMR data see Table 1.
The mixture of fractions a_{38}-a_{45} (755mg) was rechromatographed on silica gel (sds 60 AC.C 70-200µm, $CHCl_3$/MeOH 8.5:1.5) in 70x5mL fractions ((c_1-c_{18} (130mg); c_{19}-c_{50} (400mg); c_{21}-c_{70} (150mg)). The mixture of fractions c_{19}-c_{50} (400mg) was separated by silica gel column (sds 60 AC.C 70-200µm, $CHCl_3$/MeOH 9:1) to furnish compound **2** as an oily substance (100mg); for ^{1}H and ^{13}C-NMR data see Table 2.
The mixture of fractions a_{86}-a_{105} (1,4g) was rechromatographed on silica gel (sds 60 AC.C 70-200µm, $CHCl_3$/MeOH 8:2; $CHCl_3$/MeOH 7:3 then MeOH) in 140x20mL fractions ((d_1-d_{40} (180mg); d_{41}-d_{60} (200mg); d_{61}-d_{79} (190mg); d_{80}-d_{100} (300mg); d_{101}-d_{140} (390mg)) . The mixture of fractions d_{80}-d_{100} (300mg) was separated by silica gel column (sds 60 AC.C 70-200µm, $CHCl_3$/MeOH 8:2) to furnish compounds **3** (11mg) and **4** (7mg) as white solids; for ^{1}H and ^{13}C-NMR data see Tables 3 and 4. The mixture of fractions d_{101}-d_{140} (390mg) was separated by silica gel column (sds 60 AC.C 70-200µm, $CHCl_3$/MeOH 9:1) to furnish compounds **5** (12mg) and compound **6** (5mg) as white solids.

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