Iridoid and flavonoid glycosides from the aerial part of *Prasium majus* growing in Tunisia

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ABSTRACT: The aerial part of *Prasium majus* afforded Melittoside **1** and a flavone glycoside Chrysoeriol 7-(2"-O-β-D-allopyranosyl)-β-D-glycopyranoside **2**. Their structures were established via their uncompletely acetylated derivatives on the basis of spectroscopic measurements, mainly 2D NMR.

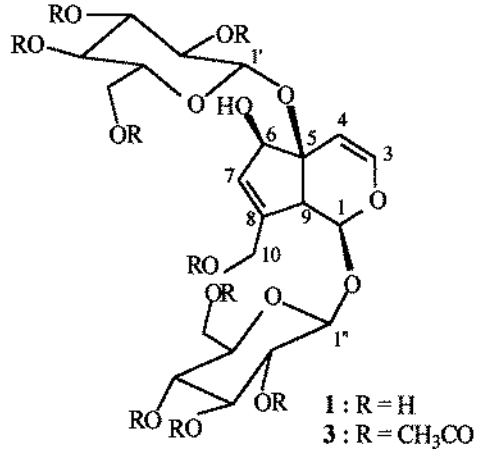
Keywords: *Prasium majus*, Lamiaciatae, iridoid glycoside, flavonoid glycoside, 2D NMR

RESUME: Le Melittoside **1** et un hétéroside à génine flavonoïque le 7-(2"-O-β-D-allopyranosyl)-β-D-glycopyranosyl Chrysoeriol **2** ont été isolés de la partie aérienne de la plante *Prasium majus*. Leurs structures ont été élucidées à l'aide de leurs dérivés acétylés en utilisant quelques techniques spectroscopiques en RMN 2D.

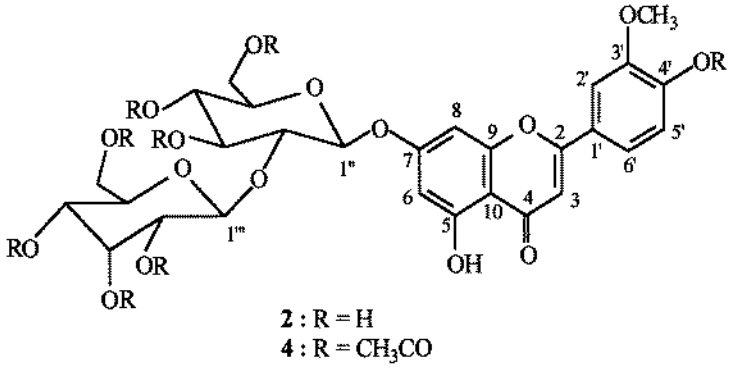
Mots clés : *Prasium majus*, lamiacées, iridoïde, flavonoïde, hétéroside, RMN 2D.

INTRODUCTION

In continuation of our work on some medicinal plants growing in Tunisia [1-10] and in the course of a search for the bioactive principles of *Prasium majus* (Lamiaciatae), we now report the identification of an iridoid glycoside, Melittoside **1** and a flavone glycoside, Chrysoeriol 7-(2"-O-β-D-allopyranosyl)-β-D-glycopyranoside **2** in the aerial part methanolic extract of the indicated plant. The structures of these two compounds were established via their uncompletely acetylated derivatives **3** and **4** respectively on the basis of 2D-NMR spectroscopic data and by comparison with those of closely related compounds. This plant has been used traditionaly in Greece as a tranquillizer [11] and the only study that was devoted to it only indicated the presence of **1** as a major component. The authors did not describe its structure elucidation and reported only its optical rotation and Rf [11].



1 : R = H
3 : R = CH_3CO



2 : R = H
4 : R = CH_3CO

* corresponding author

RESULTS AND DISCUSSION

Compound 3 The ^{13}C NMR Spectrum exhibited 21 carbon signals, twelve of them were assigned to both β-D-glycopyranosidic moieties and nine to the iridoid skeleton.

The caracteristic chemical shifts of some protons revealed from the ^{1}H NMR spectrum such as $H_{1'}$ (δ4.75; d; J=8.1Hz; β-anomer), $H_{1''}$ (δ4.88; d; J=8.3Hz; β-anomer), H_3 (δ6.38; d; J=6.3Hz) and H_4 (δ5.28; d; J=6.3Hz) as well as the spectral data deduced from the ^{13}C NMR spectrum were compatible with the molecular formular $C_{21}H_{32}O_{14}$ of the non-acetylated compound **1**.

Table I : ^{13}C (125 MHz, $CDCl_3$) and ^{1}H (500 MHz, $CDCl_3$) spectral data of compound 3

Atom	δ ^{13}C	δ ^{1}H	mult.	J (Hz)	HMBC	ROESY
1	93.9	5.29	d	2.2	H_1--H_3	H_9
3	142.5	6.38	d	6.3	H_1-H_4	
4	109.8	5.28	d	6.3	H_3	
5	67.7				H_1-$H_{1''}$-H_3-H_4-H_6-H_7	
6	54.8	3.83	bd		H_4-H_3-H_7	H_1
7	127.0	5.78	m		H_6-H_{10}	
8	140.9				H_1-H_7-H_9-H_{10}	
9	82.7	5.38	t	1.8	H_1-H_4-H_7-H_{10}	
10	60.9	4.71	s		H_7	
1'	95.7	4.75	d	8.1	$H_{2'}$	$H_{3'}$-$H_{5'}$
2'	71.2	4.89	dd	8.1; 9.8	$H_{1'}$--$H_{3'}$--$H_{4'}$	$H_{4'}$
3'	72.6	5.52	t	9.6	$H_{2'}$-$H_{4'}$	$H_{1'}$-$H_{5'}$
4'	68.2	5.08	t	9.6	$H_{3'}$-$H_{5'}$	$H_{2'}$
5'	71.0	3.95	ddd	2.3; 5.1; 10.0	$H_{3'}$-$H_{4'}$--$H_{6'a-b}$	$H_{1'}$
6'	61.9	4.13 (a)	dd	2.2; 12.1	$H_{4'}$-$H_{5'}$	
		4.27 (b)	dd	5.1; 12.1		
1''	96.8	4.88	d	8.3	H_1-$H_{2''}$--$H_{3''}$	$H_{3''}$
2''	70.3	4.99	dd	8.5; 9.5	$H_{1''}$--$H_{3''}$--$H_{4''}$	
3''	72.4	5.26	t	9.6	$H_{1''}$--$H_{4''}$	$H_{1''}$--$H_{5''}$
4''	67.9	5.13	t	9.6	$H_{2''}$--$H_{3''}$--$H_{5''}$--$H_{6''}$	
5''	72.3	3.76	ddd	2.2; 4.3; 10.0	$H_{1''}$--$H_{3''}$--$H_{4''}$--$H_{6''}$	$H_{1''}$--$H_{3''}$
6''	61.4	4.15 (a)	dd	2.0; 12.4	$H_{4''}$	
		4.31 (b)	dd	4.5; 12.4		
CO-O10	170.2				H_{10}	
CO-O2'	169.5				$H_{2'}$	
CO-O3'	170.2				$H_{3'}$	
CO-O4'	169.7				$H_{4'}$	
CO-O6'	170.6				$H_{6'a-b}$	
CO-O2''	169.5				$H_{2''}$	
CO-O3''	170.1				$H_{3''}$	
CO-O4''	169.5				$H_{4''}$	
CO-O6''	170.5				$H_{6''a-b}$	
CH_3-CO-O6	20.5-20.7	2.06	s		H_6 (^4J)	
CH_3-CO-O10	20.5-20.7	2.09	s		H_{10} (^4J)	
CH_3-CO-O2'	20.5-20.7	2.08	s		$H_{2'}$ (^4J)	
CH_3-CO-O3'	20.5-20.7	2.00	s		$H_{3'}$ (^4J)	
CH_3-CO-O4'	20.5-20.7	2.10	s		$H_{4'}$ (^4J)	
CH_3-CO-O6'	20.5-20.7	2.10	s		$H_{6'a-b}$ (^4J)	
CH_3-CO-O2''	20.5-20.7	2.14	s		$H_{2''}$ (^4J)	
CH_3-CO-O3''	20.5-20.7	2.06	s		$H_{3''}$ (^4J)	
CH_3-CO-O4''	20.5-20.7	2.06	s		$H_{4''}$ (^4J)	
CH_3-CO-O6''	20.5-20.7	2.09	s		$H_{6''a-b}$ (^4J)	

The presence of two acetylated β-D-glycopyranosidic units was suggested by the resonances between 60 and 75 ppm in the ^{13}C NMR spectrum as well as the resonances at 95.7 and 96.8 ppm relative to the anomeric carbon atoms $C_{1'}$ and $C_{1''}$, respectively (Table I).The HSQC spectrum was in agreement with this attribution and permitted the assignement of the protons attached to the glycopyranosidic carbons (Table I) [12].

The complete interpretation of the remaining NMR data was undertaken and established on the result of conclusive COSY, TOCSY and HMBC experiments.

The observation of the doublet at 5.29 ppm (H_1) coupled in tbe HSQC spectrum with the corresponding carbon C_1 (δ93.9 ppm) and the long range couplings H_1-C_3, H_1-C_5 and H_1-C_9 deduced from the HMBC spectrum confirmed the iridoid six membered ring structure. Furthermore, HMBC correlations H_6-C_5; H_6-C_7; H_7-C_5 and H_7-C_9 confirmed the structure of the iridoid five membered ring. The substitution of C_8 by the CH_2OAc system in **3** was proved by the long range correlations H_{10}-C_8; H_7-C_8 and H_{10}-C_{OAc}.

The homonuclear TOCSY experiment (fig 1) proved the structure of the two glycopyranosidic moieties, thus it was possible to trace correlations of $H_{1'}$ to $H_{2'}$, $H_{1''}$ to $H_{2''}$ and sequentially to $H_{3'}$, $H_{4'}$, $H_{5'}$, $H_{6'a,b}$ (for the first sugar) and to $H_{3''}$, $H_{4''}$, $H_{5''}$ and $H_{6''a,b}$ (for the second one).Their nature was, on the other hand, reinforced by the calculation of the coupling constants between the corresponding protons (Table I). The branching point of these two sugars at positions C_1 and C_5 of the aglycone was deduced from the HMBC spectrum showing clearly correlations between C_5 and the anomeric proton $H_{1'}$ and also between C_1 and the second anomeric proton $H_{1''}$ (Table I).

The relative stereochemistry of the aglycone was proved by the ROESY spectrum (fig 2) assuming a β-orientation of H_9 and of glycopyranose at C_5, tbe strong dipolar coupling of H_1 with H_9 and the lack of one between H_6 and H_9 confirm the common α-orientation of H_1 and H_6. The absolute configuration was proposed on biogenetic reason.

Compound 4 The identification of the aglycone skeleton as chrysoeriol was deduced from a detailed analysis of 1D and 2D NMR (HSQC, COSY, HMBC and TOCSY) spectra and was confirmed by comparison with literature data [13-14].

Analysis of the ^1H NMR spectum of 4 revealed caracteristic resonances of aromatic and glycosidic protons and one methoxyl group. Comparison of the chemical shift values and coupling constant data of tbe aromatic protons at δ6.60 (1H, d, J=2Hz), δ6.50 (1H, d, J=2.0Hz); δ7.48 (1H, d, J=1.7Hz); δ7.53 (1H, dd, J=1.8, 8.3Hz); δ3.94 (1H, m) and δ6.68 (1H, S) to those of some flavonoïds suggested that the aglycone was a flavone moiety methoxylated at $C_{3'}$.

In addition, the ^1H NMR spectrum showed signals from seven aliphatic acetoxy groups (1.9-2.2 ppm) and only one phenolic acetoxy group (2.07 ppm). Thus the carbohydrate must be the peracetate of a disaccharide (Table II).

This result was confirmed by the ^{13}C NMR spectrum showing in the region 60-78 ppm ten signals attribuables to the sugar carbons. On the other hand, the observation in the ^1H NMR spectrum of two doudlets at 5.17 ppm ($H_{1''}$) and 5.05 ppm ($H_{1'''}$) which correlated in the HSQC spectrum with carbons at 98.2 ($C_{1''}$) and 99.1 ppm ($C_{1'''}$), respectively, reinforced the above conclusion.

A weak intensity ^4J $C_{4''}$-H(Ac) HMBC correlation indicated that the phenoxy group at position C_5 was left unchanged, as supported by a chemical shift comparison with the non-acetylated material [13]. The additional methoxyl group at δ3.95 was assigned to $C_{3'}$ in B-ring due to $H_{2''}$-$C_{2'}$; $H_{2''}$-$C_{1'}$; $H_{2''}$-$C_{3'}$ and H(OMe)-$C_{3'}$ HMBC correlations. On the other hand, C_7 glycosidation was confirmed in the HMBC map showing a conclusive ^3J correlation $H_{1'''}$- C_7.

As for compound **3**, the exploration of COSY and TOCSY spectra permitted us to determine the nature of the carbohydrate as (2''-O-β-D-allopyranosyl)-β-D-glycopyranoside by the observation of the homonuclear correlations $H_{1''}$-$H_{2''}$ and sequentially $H_{3''}$, $H_{4''}$, $H_{5''}$ and $H_{6''a,b}$ (for the glycopy-

ranosyl moiety attached at C_7) and $H_{1'''}$-$H_{2'''}$, $H_{2'''}$-$H_{3'''}$, $H_{3'''}$-$H_{4'''}$, $H_{4'''}$-$H_{5'''}$ and $H_{5'''}$-$H_{6'''a,b}$ (for the allopyranosyl system).

Table II : ^{13}C (125 MHz, $CDCl_3$) and ^{1}H (500 MHz, $CDCl_3$) spectral data of compound 4

Atom	δ ^{13}C	δ ^{1}H	mult.	J (Hz)	HMBC
2	163.7				H_3-$H_{2'}$-$H_{6'}$
3	106.2	6.68	s		
4	182.4				H_3-H_8
5	162.2				H_6
6	99.9	6.50	d	2.0	H_8
7	162.4				H_6-H_8-$H_{1''}$
8	95.2	6.60	d	2.0	H_6
9	157.3				H_8
10	106.8				H_3-H_6-H_8
1'	129.9				$H_{2'}$-H_3-$H_{5'}$
2'	110.2	7.48	d	1.7	$H_{6'}$
3'	151.7				$H_{2'}$-$H_{5'}$-$H_{(OMe)}$
4'	142.8				$H_{2'}$-$H_{5'}$-H_6-$H_{(OAC)}$
5'	123.5	7.21	d	8.3	
6'	119.3	7.53	dd	1.8; 8.3	$H_{2'}$
1''	98.2	5.17	d	7.5	$H_{2''}$-$H_{3''}$
2''	77.8	4.03	dd	7.6; 9.2	$H_{1''}$-$H_{3''}$-$H_{4''}$
3''	74.2	5.31	t	9.4	$H_{1''}$-$H_{2''}$-$H_{4''}$
4''	68.2	5.07	t	9.7	$H_{3''}$-$H_{5''}$-$H_{6''a-b}$
5''	72.0	3.94	m		$H_{3''}$-$H_{4''}$-$H_{6''a-b}$
6''	61.8	4.20 (a)	t	12.1	$H_{4''}$
		4.34 (b)	dd	5.8; 12.3	
1'''	99.1	5.05	d	8.2	$H_{2'''}$-$H_{3'''}$
2'''	68.7	4.88	dd	3.0; 8.3	$H_{2'''}$-$H_{1'''}$-$H_{3'''}$
3'''	68.6	5.64	t	2.8	$H_{2'''}$-$H_{4'''}$
4'''	65.9	5.00	dd	2.8; 10.3	$H_{3'''}$-$H_{5'''}$-$H_{6'''a-b}$
5'''	70.2	4.10	m		$H_{3'''}$-$H_{4'''}$-$H_{6'''a-b}$
6'''	61.9	4.20 (a)	t	12.1	$H_{4'''}$
		4.31 (b)	dd	4.2; 12.6	
CO-O4'	168.5				
CO-O3''	169.5				$H_{3''}$
CO-O4''	169.7				$H_{4''}$
CO-O6''	170.5				$H_{6''}$
CO-O2'''	169.0				$H_{2'''}$
CO-O3'''	169.4				$H_{3'''}$
CO-O4'''	169.0				$H_{4'''}$
CO-O6'''	170.8				$H_{6'''}$
CH_3-CO-O4'	56.2	2.38	s		$H_{4'}$ (^4J)
CH_3-CO-O3''	20.5	2.13	s		$H_{3''}$ (^4J)
CH_3-CO-O4''	20.6	2.07	s		$H_{4''}$ (^4J)
CH_3-CO-O6''	20.6	2.11	s		$H_{6''}$ (^4J)
CH_3-CO-O2'''	20.3	2.04	s		$H_{2'''}$ (^4J)
CH_3-CO-O3'''	20.6	2.17	s		$H_{3'''}$ (^4J)
CH_3-CO-O4'''	20.5	2.02	s		$H_{4'''}$ (^4J)
CH_3-CO-O6'''	20.6	2.11	s		$H_{6'''}$ (^4J)
CH_3-O3'	56.2	3.95	s		$H_{3'}$

fig 1. TOCSY Spectrum of compound 3.

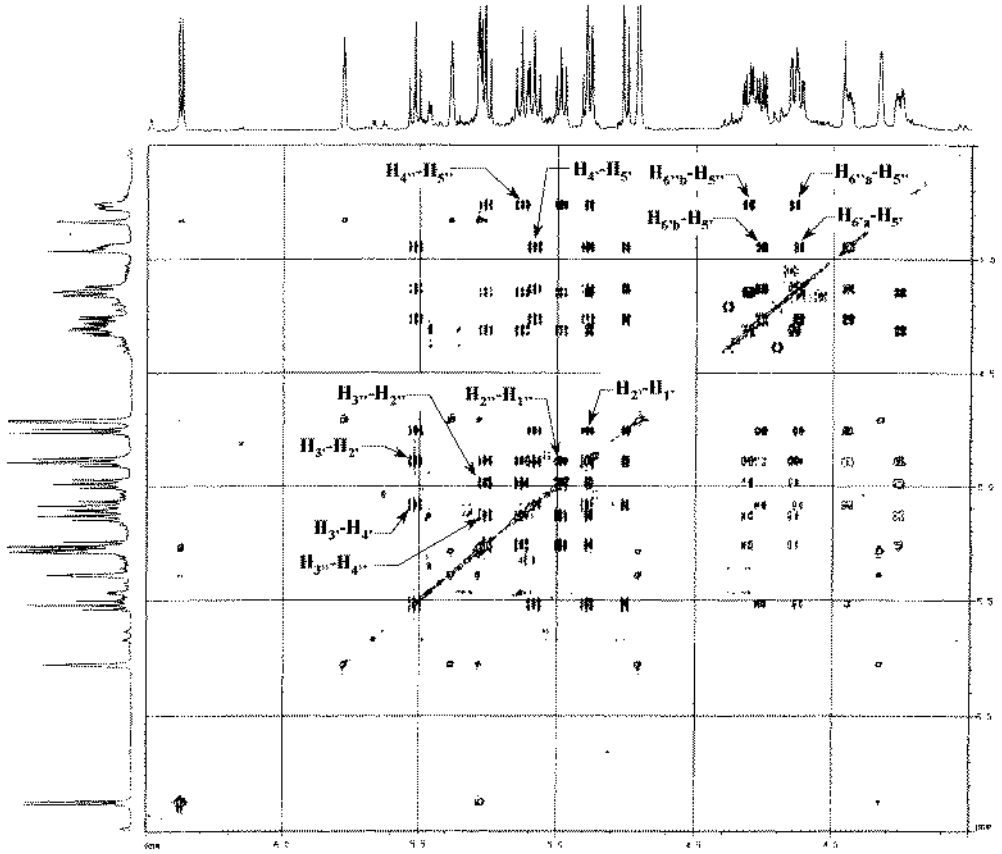
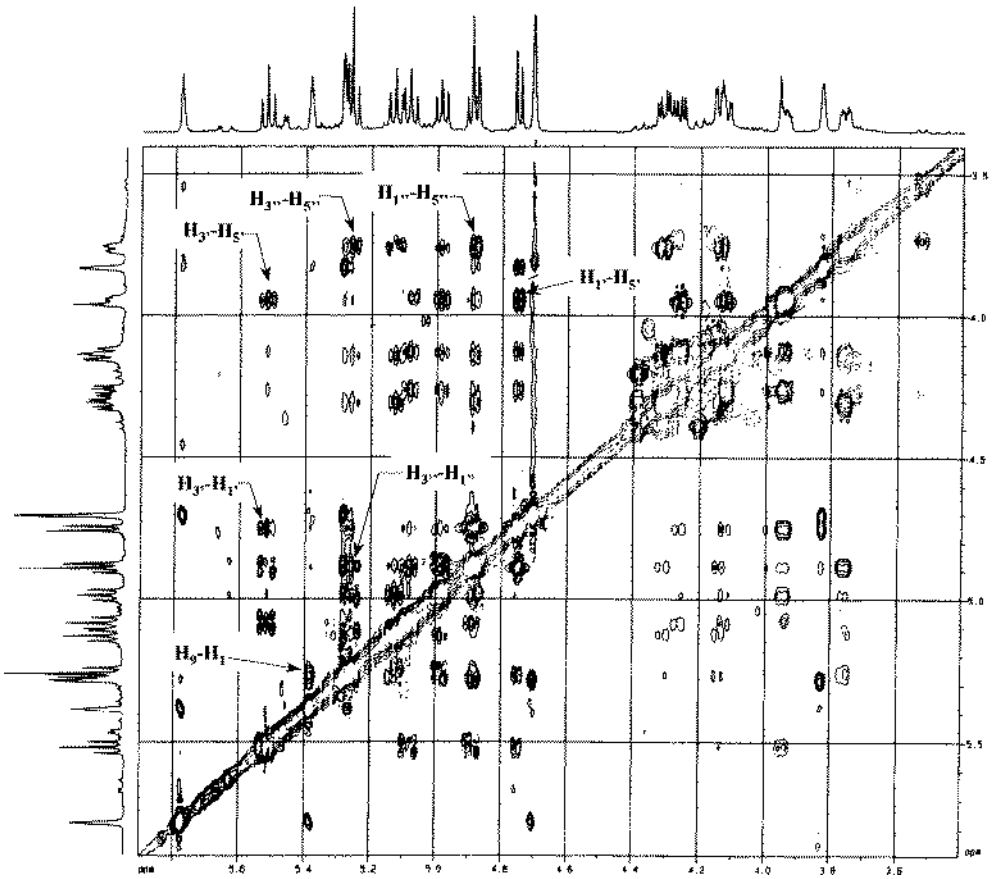


fig 2. ROESY Spectrum of compound 3.



This structure and more precisely the branching point ($C_{2''}$) of the two sugars was inferred from the HMBC spectrum showing $H_{2'''}$-$C_{1'''}$ and $H_{1'''}$-$C_{2''}$ long range connectivities.

The identification of the stereochemistry of these sugar residues was carried out by measuring ^1H-^1H coupling constants: in allose, both coupling constants of $H_{3'''}$ are small (2.8 Hz) proving thus its axial anomeric configuration followed from the large coupling constant value (J=8.2Hz) of $H_{1'''}$.

EXPERIMENTAL

Plant material. Was collected in June 1999 in Sousse (Tunisia) and voucher specimen is deposited in the herbarium of the Ecole Supérieure d'Horticulture de Chott Meriem, Université du Centre, Sousse, Tunisia.

Extraction and isolation. Dried and finely powdered *Prasium majus* aerial part (1300 g) was extracted with petroleum ether (40-60°C), methylene chloride, acetone, methanol and water successively using a soxhlet apparatus. Methanol extract was filtered then evaporated to dryness. The residue (130 g) was extracted with chloroform giving two fractions. The polar one was evaporated yielding a residue (120 g) which was acetylated with Ac_2O and pyridine in order to decrease its polarity then fractionated in a silica gel column. This fractionation allowed us to obtain 17 crude fractions. Fraction 10 was subjected to cc witb petroleum ether/EtOAc 3:7 to give 16 mg of an oily substance (compound **3**). Fraction 13 was previously separated on preparative TLC to afford 400 mg of an oily fraction less complex which was purified over repeated cc silica gel using petroleum ether/$CHCl_3$/acetone 1.5:7:1.5 as a mobile phase, yielded compound **4** in a pure form (29 mg).

Compound 3. White granular compound; $[\alpha]_D^{22}$ -32 (C = 0.58; MeOH), Rf = 0.65 (petroleum ether/EtOAc 3:7); IR (KBr) ν_{max} 3484 cm^{-1} (br OH), 1757 ($C{=}O_{ac}$), 1248 ($C\text{-}O\text{-}C_{ac}$); ^1H and ^{13}C NMR (see Table1).

Compound 4. Yellow granular compound; $[\alpha]_D^{22}$ -38 (C = 1; MeOH), m.p = 134 °C; Rf = 0.6 (petroleum ether/ $CHCl_3$/acetone 1.5:7:1.5); IR (KBr) ν_{max} 3468 cm^{-1} (br OH), 1752 ($C{=}O_{ac}$), 1657 (C=O, α,β-insaturated system), 1372 (CH_{3ac}), 1228 ($C\text{-}O\text{-}C_{ac}$); ^1H and ^{13}C NMR (see Table 2).

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