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Diterpenoid quinones from Rosemary (*Rosmarinus officinalis* L.)

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Abstract

Two new abietane-type diterpenoid *o*-quinones, 7β-methoxyabieta-8,13-diene-11,12-dione-(20,6β)-olide (rosmaquinone A) (**1**) and 7α-methoxyabieta-8,13-diene-11,12-dione-(20,6β)-olide (rosmaquinone B) (**2**), together with six known compounds were isolated from the aerial parts of *Rosmarinus officinalis* L. The structures of the new compounds were determined by extensive spectroscopic analysis, including IR, UV, HR-EIMS, 1D and 2D 400 MHz NMR data (^{1}H, ^{13}C NMR, DEPT, ^{1}H–^{1}H COSY, HMQC, HMBC and NOEs).

Keywords: *Rosmarinus officinalis* L.; Lamiaceae; Diterpenoid *o*-quinones; Rosmaquinone A and B

1. Introduction

Rosemary, *Rosmarinus officinalis* L. (Lamiaceae) is an aromatic evergreen shrubby herb highly distributed in the Mediterranean region. It is a well-known and greatly valued medicinal herb that is widely used in pharmaceutical products and folk medicine as a digestive, tonic, astringent, diuretic, diaphoretic, and useful for urinary ailments (Chang et al., 1977; Aqel, 1991; Leung and Foster, 1996; Haloui et al., 2000). Furthermore, several extracts, essential oils and chemical constituents isolated from this species demonstrated a number of interesting biological activities such as antioxidant (Inatani et al., 1983; Houlihan et al., 1985; Aruoma et al., 1992, 1996; Haraguchi et al., 1995; Cuvelier et al., 1996; Frankel et al., 1996; Richheimer et al., 1996; Sotelo-Felix et al., 2002; Dorman et al., 2003), anti HIV-1 (Paris et al., 1993), antiulcerogenic (Dias et al., 2000), anticarcinogenic (Offord et al., 1995), and antimicrobial (Takenaka et al., 1997). This biological importance prompted us to re-investigate the chemical constituents of the aerial parts of this species. Earlier work on the chemistry of this species showed that it contains mainly abietane-type diterpenoids including some diterpenoid quinones (Inatani et al., 1983; Nakatani and Inatani, 1983, 1984; Houlihan et al., 1985; Arisawa et al., 1987; Takenaka et al., 1997), however some triterpenoids (Ganeva et al., 1993) and flavonoids (Okamura et al., 1994) were also isolated. In this work, we reported the isolation and structure elucidation of two new abietane-type diterpenoid *o*-quinones, 7β-methoxyabieta-8,13-diene-11,12-dione-(20,6β)-olide (rosmaquinone A) (**1**) and 7α-methoxyabieta-8,13-diene-11,12-dione-(20,6β)-olide (rosmaquinone B) (**2**) together with the known compounds, royleanonic acid (**3**) (Gu and Weng, 2001), rosmanol (**4**) (Takenaka et al., 1997), betulin (**5**), betulinic acid (**6**), 23-hydroxybetulinic acid (**7**) (Macías et al., 1998) and rofficerone (**8**) (Ganeva et al., 1993). The structures of the new compounds **1** and **2** were determined by spectroscopic analysis, including IR, UV, HR-EIMS, 1D and 2D NMR data, whereas

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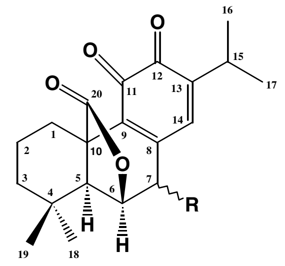
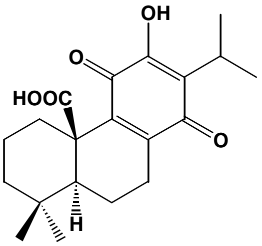
the known compounds (**3**–**8**) were identified by comparison of their IR, UV, ^1H and ^{13}C NMR analysis with those reported in the literature data. The diterpenoid *p*-quinone royleanonic acid (**3**) and the triterpene 23-hydroxybetulinic acid (**7**) were isolated from this species for the first time.



1: R = β-OCH$_3$

2: R = α-OCH$_3$



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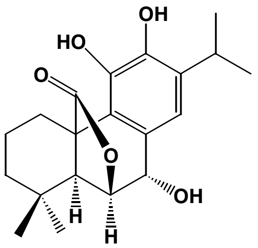

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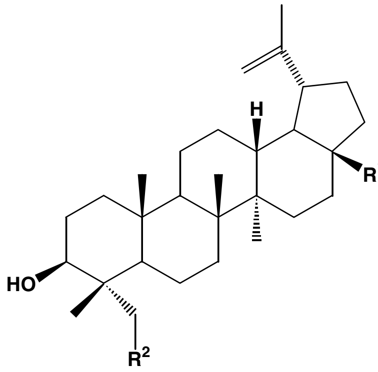
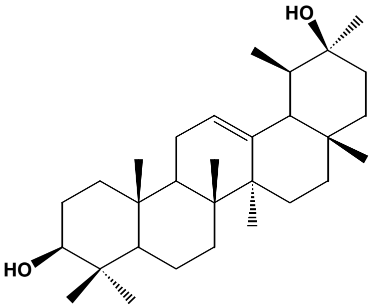


5: R^1 = CH$_2$OH, R^2 = H

6: R^1 = COOH, R^2 = H

7: R^1 = COOH, R^2 = OH



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2. Results and discussion

Rosmaqunione A **(1)** was isolated as a reddish yellow oil with $[\alpha]_D 25 + 3.2^\circ$ (*c* 0.08, MeOH). The molecular formula was determined to be $C_{21}H_{26}O_5$ by high-resolution EIMS (*m*/*z* 358.4362; calc. 358.4334), ^{13}C NMR and DEPT analysis. This formula indicated the existence of nine degrees of unsaturation. The oxygen-containing functionalities could be deduced from the IR, ^1H and ^{13}C NMR spectral data which showed the presence of a saturated γ-lactone function [C=O (IR: ν = 1780 cm^{-1}; ^{13}C NMR: δ = 175.0) and –CH–O– (^{13}C NMR: δ = 72.7], *o*-quinone carbonyl groups [2 × C=O (IR: ν =;1670 and 1660 cm^{-1}; ^{13}C NMR: δ = 179.5 and 180.2)], and a methoxyl group [^1H NMR: δ = 3.62 (3H, *s*); ^{13}C NMR: δ = 57.1 (CH$_3$)]. Furthermore, the maximum absorptions at 275 and 402 (sh) nm in the UV spectrum in addition to the presence of one tri- and one tetra-substituted double bonds [δ 149.7 (C); 132.7 (CH) and δ 146.6 (C); 137.9 (C)] as well as two carbonyl groups at δ 179.5 (C) and 180.2 (C), supported the presence of a tri-substituted *o*-bezoquinone moiety (González et al., 1989; Ulubelen et al., 1995; Pretsch et al., 2000). The ^{13}C NMR spectral data (Table 1) revealed 21 carbon atoms while their multiplicities were assigned by DEPT analysis. The carbons were assigned as 14 sp^3 (five CH$_3$, three CH$_2$, four CH and two C), 4 sp^2 (one tri- and one tetra-substituted double bonds) and three C=O (δ 175.0, 179.5 and 180.2). Taking into account the presence of three carbonyl groups, two double bonds, and one lactone ring, the remaining three degrees of unsaturation were therefore attributed to a tricyclic skeleton. The ^1H NMR spectrum showed characteristic signals of an isopropyl group attached to an aromatic ring, two aliphatic *ter*-methyl groups, one methoxyl group, an AB system (*J* = 2.60 Hz) of two oxymethine protons, and of one aromatic proton. Based on all these accumulated data together with the results of the 2D ^1H–^1H COSY, HMQC and HMBC analysis (Table 1), **1** was assumed to be a methoxy derivative of an abietane diterpenoid with an *o*-quinone group and a γ-lactone function similar to other abietanes pre-

Table 1
^{1}H- and ^{13}C NMR assignments for **1** and **2** ($CDCl_3$, 400 MHz, TMS)

Position	δ_C	DEPT	δ_H	HMBC
1[a,b]				
1α	25.1	CH_2	1.43m	C-20
1β			3.25 *br d*, $J = 12.0$ Hz	C-3, C-5, C-10, C-20
2α	18.2	CH_2	1.52 *m*	
2β			1.43 *m*	
3α	37.8	CH_2	1.12 *m*	
3β			1.43	
4	31.8	C		
5	55.3	CH	1.89 *s*	C-7, C-9, C-10, C-18, C-19, C-20
6	72.7	CH	4.88 *d*, $J = 2.60$ Hz	C-4, C-8, C-20
7	78.1	CH	4.09 *d*, $J = 2.60$ Hz	C-5, C-8, C-9, C-14, -OMe
8	146.6	C		
9	137.9	C		
10	46.6	C		
11	179.5			
12	180.2	C		
13	149.7	C		
14	132.7	CH	6.80 *d*, $J = 1.1$ Hz	C-7, C-9, C-12, C-15
15	27.5	CH	2.92 *d sept*, $J = 7.0$, 1.1 Hz	C-12, C-13, C-14, C-16, C-17
16	21.6	CH_3	1.12 *d*, $J = 7.0$ Hz	C-13, C-15, C-16
17	21.9	CH_3	1.11 *d*, $J = 7.0$ Hz	C-13, C-15, C-16
18	31.6	CH_3	1.00 *s*	C-3, C-4, C-5, C-19
19	21.3	CH_3	0.93 *s*	C-3, C-4, C-5, C-18
20	175.0	C		
OMe	57.1	CH_3	3.62 *s*	C-7
2[a,b]				
1α	25.1	CH_2	1.44 *m*	C-20
1β			3.21 *br d*, $J = 9.5$ Hz	C-3, C-5, C-10, C-20
2α	18.3	CH_2	1.59 *m*	
2β			1.44 *m*	
3α	38.0	CH_2	1.13 *m*	
3β			1.44 *m*	
4	31.1	C		
5	50.2	CH	2.00 *s*	C-4, C-9, C-10, C-18 C-19, C-20
6	72.4	CH	4.64 *d*, $J = 3.0$ Hz	C-4, C-8,C-10, C-20
7	77.6	CH	3.87 *d*, $J = 3.0$ Hz	C-5, C-6, C-8, C-9, C-14, -OMe
8	145.6	C		
9	138.2	C		
10	45.8	C		
11	179.5	C		
12	180.0	C		
13	150.1	C		
14	133.5	CH	6.62 *d*, $J = 1.2$ Hz	C-7, C-9, C-12, C-15
15	27.5	CH	2.92 *d sept*, $J = 7.0$, 1.2 Hz	C-12, C-14, C-16, C-17
16	21.3	CH_3	1.10 *d*, $J = 7.0$ Hz	C-13, C-15, C-17
17	21.2	CH_3	1.12 *d*, $J = 7.0$ Hz	C-13, C-15, C-16
18	31.4	CH_3	1.01 *s*	C-3, C-4, C-5, C-19
19	21.9	CH_3	0.90 *s*	C-3, C-4, C-5, C-18
20	175.5	C		
OMe	59.5	CH_3	3.68 *s*	C-7

[a] Assignments of all proton and carbon signals were deduced from ^{1}H–^{1}H COSY, HMQC and HMBC experiments.
[b] Multiplicities were deduced from DEPT experiments.

viously isolated (González et al., 1989; Takenaka et al., 1997). The ^{1}H NMR spectrum showed the presence of five methyl signals at δ 0.93, 1.00, 1.11, 1.12 and 3.62. The most downfield methyl singlet signal at δ 3.62 was due to a methoxyl group, which showed a correlation with a carbon signal at δ 57.1 (CH_3) in the HMQC spectrum. Attachment of this methoxyl group at C-7 of the abietane skeleton was established by the HMBC correlation between the $-OCH_3$ proton signal (δ 3.62) and the oxymethine carbon C-7 at δ 78.1. The two *sec*-methyl signals at δ 1.11 and 1.12 (each *d*, $J = 7.0$ Hz), which showed coupling with a septet proton at δ 2.92 (H-15) in the ^{1}H–^{1}H COSY spectrum, indicated the presence of an isopropyl group, while the down-field shift (δ

2.92) indicated the attachment with an aromatic ring. The HMBC spectrum, which showed correlations between this septet proton at δ 2.92 (H-15) with the carbonyl signal at δ 180.2 (C-12), two olefinic carbon signals at δ 149.7 (C-13) and 132.7 (C-14) as well as the two methyl carbon signals at δ 21.6 (CH_3-16) and δ 21.9 (CH_3-17), established the position of the isopropyl group at C-13 of the abietane skeleton. The remaining two *tert*-methyl groups at δ 1.00 and 0.93 suggested the presence of a *gem*-dimethyl group which was evident from the HMBC correlations of the methyl signals at δ 1.00 and 0.93 with their corresponding carbons [(δ 31.6 (C-18) and 21.3 (C-19)] in addition to a similar set of correlations with the neighboring carbons [(δ 37.8 (C-3), 31.8 (C-4) and 55.3 (C-5)]. The position of the *gem*-dimethyl group was assigned at C-4 from the HMBC correlations to CH_2-3 at δ 37.8, and the quaternary carbon at δ 31.8 (C-4) and the methine carbon at δ 21.3 (C-5). The ^1H and ^{13}C NMR spectra showed the absence of the methyl group at C-10 (CH_3-20) if compared with normal abietane skeletons previously isolated (Rodriguez et al., 1983; Ulubelen et al., 1995) and similar to rosmanol **4**. This C-20 was assigned as a carbonyl signal at δ 175.0 by the HMBC correlations to the proton signals at δ 1.43 (H-1α), 3.25 (H-1β) and 1.89 (H-5). These data clearly indicated that the carbon C-20 in **1** was oxidized and formed a part of a γ-lactone ring similar to rosmanol (**4**) and several abietanes previously isolated from the same species (Inatani et al., 1983; Nakatani and Inatani, 1983, 1984; Takenaka et al., 1997). On the other hand, the H-5 proton which was assigned from the 2D data at δ 1.89 as a singlet signal, indicating a single proton at C-6 forming a 90° angle with H-5, located the lactone ring closure at C-6 (Urones et al., 1998; Nakatani and Inatani, 1984). Confirmation of this was given by the HMBC correlations between the oxymethine proton H-6 at δ 4.88 with the carbonyl group at δ 175.0 (C-20). In the ^1H–^1H COSY spectrum, the signal at δ 4.88 (1H, d, $J = 2.60$ Hz) (H-6) showed an AB coupling with another proton at δ 4.09 (d, $J = 2.60$ Hz) which was assigned to a proton (H-7) attached to the methoxyl group. The ^1H–^1H COSY and HMQC spectra allowed the sequential assignments of hydrogens from H-1 to H-3 for ring A and from H-5 to H-7 for ring B. Finally, the presence of C-14 quinonoide proton and consequently the presence of an *o*-quinone group at C-11 and C-12 rather than a *p*-quinone group at C-11 and C-14 were followed from the aromatic proton signal which appeared at δ 6.80 (*d*, $J = 1.10$ Hz) and showed in the HMQC spectrum a correlation with the olefinic methine carbon signal at δ 132.7. This proton showed in the ^1H–^1H COSY spectrum an allylic coupling with H-15 at δ 2.92, while in the HMBC spectrum correlated with the oxymethine carbon C-7 (δ 78.1), and finally gave a clear NOE effect with the oxymethine proton H-7 (δ 4.09) (Fig. 1). The stereochemistry of **1** was followed from the coupling constants and NOE measurements with inspection of the Dreiding model (Fig. 1). The relative configuration and stereochemistry at C-5, C-6 and C-7 were derived from the coupling constants ($J_{5,6} = 0$ and $J_{6,7} = 2.60$ Hz) which were in a good agreement with an (axial-equatorial and equatorial-axial) pattern for these protons (Nakatani and Inatani, 1984; Urones et al., 1998). This 5α, 6α, 7α (H) configuration was further supported by the NOE effects, irradiation of H-5 signal enhanced the H-7 signal, which confirmed 1,3-diaxial relationship of these protons. Furthermore, irradiation of H-7 signal enhanced the H-5, H-6 and H-14 signals. Finally, the ^{13}C-chemical shifts of C-5, C-6 and C-7 were very similar to those reported of other compounds with the same configurations. (Urones et al., 1998; Miura et al., 2001). Therefore, compound **1** was identified as 7β-methoxyabieta-8,13-diene-11,12-dione-(20,6β)-olide and named rosmaquinone A.

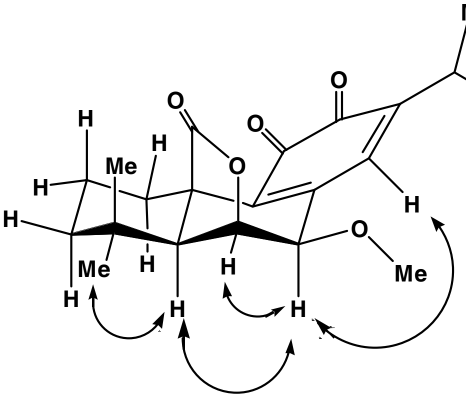

Fig. 1. Selected NOEs observed for **1**.

Rosmaqunione B **(2)** was isolated as a reddish yellow oil with $[\alpha]_D25 - 6.1°$ (*c* 0.05, MeOH). It had a molecular formula of $C_{21}H_{26}O_5$ by HR-EIMS, ^{13}C NMR and DEPT analysis. The IR and ^{13}C NMR spectral data showed the presence of a saturated γ-lactone function [C=O (IR: $\nu = 1780\ cm^{-1}$; ^{13}C NMR: $\delta = 175.5$) and –CH–O– [^{13}C NMR: $\delta = 72.4$] and *o*-quinone carbonyl groups (2 × C=O (IR: $\nu = 1670$ and $1662\ cm^{-1}$; ^{13}C NMR: $\delta = 179.5$, and 180.0)]. The UV spectrum which showed absorptions at 275 and 404 (sh) nm supported the presence of an *o*-quinone group. The remaining oxygen of the formula was deduced to be a methoxyl group from the ^1H and ^{13}C NMR spectra [$\delta_H = 3.62$ (3H, *s*) and δ_C=59.5 (*q*)]. The ^{13}C NMR spectral data (Table 1) revealed 21 carbon atoms while their multiplicities were assigned by DEPT analysis. The carbons were assigned as 14 sp^3 (five CH_3, three CH_2, four CH and two C), 4 sp^2 (one tri- and one tetra-substituted double bond) and three C=O (δ 175.5, 179.5 and 180.0). All these data which were very similar to those observed in rosmaquinone **1** in addition to the negative sign of the optical rotation ($[\alpha]^{25}D - 6.1°$), if compared with

+3.2° of **1**, suggested **2** to be a stereoisomer of **1**. The ^1H NMR spectrum of **2** was also similar to that of **1** except for chemical shift differences associated with the methine proton H-5 (δ 2.00 in **2**; δ 1.89 in **1**), the lactonic proton H-6 (δ 4.64 in **2**; δ 4.88 in **1**), and the oxymethine proton H-7 (δ 3.87 in **2**; δ 4.09 in **1**). These differences together with the similarity of the coupling constant between H-5 and H-6 ($J_{5,6} = 0$), with that obtained for **1**, suggested an inversion configuration at C-7 which was further supported by the NOEs analysis with inspection of the Dreiding model (Fig. 2). The absence of an NOE effect between H-5 and H-7 (in contrast to **1**) established the axial (α) configuration for the 7-OCH_3 in **2**. In agreement with this result the chemical shifts of these carbon signals of C-5, C-6 and C-7 at δ 50.2, 77.6 and 72.4, respectively, were very similar to those reported of other compounds with the same stereochemistry centers (Arisawa et al., 1987; Takenaka et al., 1997; Urones et al., 1998;). Therefore, compound **2** was identified as 7α-methoxyabieta-8,13-diene-11,12-dione-(20,6β)-olide and named rosmaquinone B.

The IR, UV, ^1H, and ^{13}C NMR spectral data of compounds (**3**–**8**) were identical with those previously reported for the same compounds.

3. Experimental

3.1. General

The ^1H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (100 MHz, $CDCl_3$), and 2D spectra were measured with a JEOL JNM-ECP 400 NMR spectrometer, with TMS as internal standard. The IR spectra (oily film, $CHCl_3$) were taken on a Shimadzu-8000 FT-IR spectrophotometer. The UV spectra were recorded on a Shimadzu UV-240 spectrometer. Mass spectra were recorded with a JEOL JMS 700 spectrometer. TLC: Precoated silica gel $60F_{254}$ plate (Merck); preparative TLC: silica gel PF_{254} (Merck, 200 × 200 × 0.25 mm); CC: silica gel type 60 (Merck). HPLC: column: RP-8; elution MeOH–H_2O, 3:1; flow = 6 mL/min; Knauer pump 64; detection: knauer differential refractometer. Optical rotation were determined with a Perkin–Elmer 341 polarimeter operating at sodium D line.

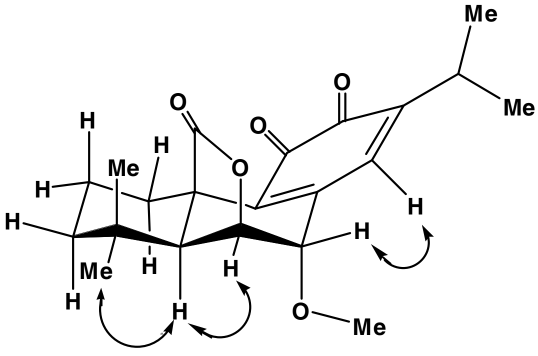

Fig. 2. Selected NOEs observed for **2**.

3.2. Plant material

Rosmarinus officinalis L. was collected in El-Minia, Egypt in May 2001. A voucher specimen (R-14-01) has been deposited in the Department of Botany, El-Minia University, El-Minia, Egypt.

3.3. Extraction and isolation

The air-dried aerial parts (1 kg) were powdered and extracted with CH_2Cl_2:MeOH (1:1) (10 L) at room temperature, and the extract was concentrated to obtain 80 g of residue. The extract was prefractionated by column chromatography (6 × 100 cm) on silica gel (900 g) eluting with *n*-hexane (2 L) followed by a gradient of *n*-hexane–Et_2O up to 100% Et_2O and Et_2O–MeOH (2 L of each solvent mixture) into five fractions: fraction 1 (*n*-hexane–Et_2O 3:1), fraction 2 (*n*-hexane–Et_2O 1:1), fraction 3 (*n*-hexane–Et_2O 1:3), fraction 4 (Et_2O 100%), fraction 5 (Et_2O–MeOH 9:1). Fractions 1 and 2 were collected and separated on silica gel column (600 g, 5 × 100 cm) eluted with *n*-hexane–CH_2Cl_2 (2:1) (ca. 500 mL × 2) to give fractions 1-A and 1-B. Fraction 1-A was further purified on a Sephadex LH-20 column (250 g, 4 × 90 cm) eluted with *n*-hexane–CH_2Cl_2–MeOH (4:7:0.5) to afford 8 (15 mg) and mixture from 1 and 2 which was further separated and purified by HPLC (MeOH–H_2O, 3:1, Rt = 1.4 min) to give 1 (9 mg) and 2 (11 mg). Fraction 3 was further purified on a sephadex LH-20 column (250 g, 4 × 80 cm) eluted with *n*-hexane–CH_2Cl_2–MeOH (4:7:0.5) and one of the subfraction was further purified by preparative TLC (silica gel PF_{254}) eluted with petroleum ether–Et_2O (1:2) gave 3 (20 mg) and 4 (18 mg). Fr. 4 was further purified on a sephadex LH-20 column (250 g, 4 × 90 cm) eluted with *n*-hexane–CH_2Cl_2–MeOH (4:7:1) to give 5 (22 mg), 6 (13 mg), and one of the subfraction was further purified by TLC (silica gel PF_{254}) eluted with petroleum ether–Et_2O–MeOH (1:3:0.1) to afford **7** (8 mg).

3.4. *7β-methoxyabieta-8,13-diene-11,12-dione-(20, 6β)-olide (**1**)*

Reddish yellow oil, $[\alpha]_D25 + 3.2°$ (c = 0.08, MeOH); IR (film) υ_{max} $CHCl_3$: 2927, 1780, 1670, 1660, 1508, 1459, 1395, 1254, 1172, 1091, 1034, 996 cm^{-1}; UV λ_{max} MeOH: 402, 275, 225 nm; HR-EIMS: 358.4362, (calc. for $C_{21}H_{26}O_5$, 358.4334); EI-MS: *m*/*z* (rel. int.) = 358 $[M]^+$ (23), 314 $[M - CO_2]^+$ (25), 299 $[M - CO_2 - Me]^+$ (15), 284 $[M - CO_2 - 2Me]^+$ (23), 269 $[M - CO_2 - 3Me]^+$ (70), 258 (100), 245 (69), 215 (20), 149 (30), 115

(15), 81 (25), 69 (5), 55 (40); ^{1}H NMR, ^{13}C NMR, DEPT, HMBC (Table 1); NOEs (Fig. 1).

3.5. *7α-methoxyabieta-8,13-diene-11,12-dione-(20,6β)-olide (**2**)*

Reddish yellow oil, $[\alpha]_D25 - 6.1°$ ($c = 0.05$, MeOH); IR (film) υ_{max} $CHCl_3$: 2927, 1785, 1670, 1662, 1508, 1459, 1395, 1255, 1172, 1091, 1034, 991 cm^{-1}; UV λ_{max} MeOH: 404, 275, 225 nm; HR-EIMS: 358.4300, (calc. for $C_{21}H_{26}O_5$, 358.4334); EI-MS: *m/z* (rel. int.) = 358 $[M]^+$ (15), 314 $[M - CO_2]^+$ (17), 299 $[M - CO_2 - Me]^+$ (18), 284 $[M - CO_2 - 2Me]^+$ (23), 269 $[M - CO_2 - 3Me]^+$ (70), 258 (35), 245 (38), 149 (100), 115, 81, 69 (100), 55 (90); ^{1}H NMR, ^{13}C NMR, DEPT, HMBC (Table 1); NOEs (Fig. 2).

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