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Biotransformation of oleanolic and maslinic acids by *Rhizomucor miehei*

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ABSTRACT

Microbial transformation of oleanolic acid by *Rhizomucor miehei* produced three metabolites. A known compound, a 30-hydroxyl derivative (queretaroic acid), and two 7β,30- and 1β,30-dihydroxylated metabolites, respectively. The action of the same fungus (*R. miehei*) on maslinic acid produced an olean-11-en-28,13β-olide derivative, a metabolite hydroxylated at C-30, an 11-oxo derivative, and two metabolites with an 11α,12α-epoxy group, hydroxylated or not at C-30. Their structures were elucidated by extensive analyses of their spectroscopic data, and also by chemical correlations.

1. Introduction

Triterpenoids are a large and structurally diverse group of natural products (Hill and Connolly, 2012) that display nearly 200 distinct skeletons. These compounds have been studied for their anti-neoplastic, anti-inflammatory, anti-ulcerogenic, antimicrobial, anti-plasmodial, antiviral (anti-HIV), hepato- and cardio-protective, analgesic, anti-mycotic, immunomodulatory, and tonic effects (Shanmugam et al., 2012; Cassels and Asencio, 2011; Rios, 2010; Yadav et al., 2010; Kuo et al., 2009; Petronelli et al., 2009; Dzubak et al., 2006; Akihisa and Yasukawa, 2006; Ovesna et al., 2004).

Oleanolic (3β-hydroxyolean-12-en-28-oic acid, **1**) and maslinic (2α,3β-dihydroxyolean-12-en-28-oic acid, **2**) acids are natural pentacyclic triterpenoid compounds (Pollier and Goossens, 2012; García-Granados et al., 1998a,b). These acids are present in high concentrations in olive-pomace oil, being the main components of the protective wax-like coating of the olive skin. Our group has reported a method to obtain large amounts of these compounds from olive-pressing residues (García-Granados, 1998c). Both triterpenic acids and some closely related compounds display remarkable pharmacological activities such as antitumor, antibacterial, anti-HIV, anti-inflammatory, antioxidant, and hepatoprotective (Pollier and Goossens, 2012; Salvador et al., 2010; Wolska et al., 2010; Wang et al., 2010; Reyes-Zurita et al., 2011; Huang et al., 2011; Parra et al., 2009a, 2011).

Microbial transformations of triterpenoids have been developed primarily in the last 10 or 15 years to produce new and useful compounds (Muffler et al., 2011; Parra et al., 2009b). Biotransformation methods can be used as an alternative to the traditional chemical synthesis in search of new production routes for fine chemical, pharmaceutical, and agrochemical compounds. Several biotransformations of oleanolic acid (**1**), using different microorganisms, have been published (Hikino et al., 1969, 1971, 1972a,b; Zhang et al., 2005; Wang et al., 2006; Choudhary et al., 2008; Sun et al., 2010; Capel et al., 2011; Liu et al., 2011; Zhu et al., 2011). Recently, the first biotransformation of maslinic acid by *Cunninghamella blakesleana* has been described (Feng et al., 2012). This paper concentrates on the production of new oleanolic and maslinic acid derivatives by biotransformation processes, using *Rhizomucor miehei*.

2. Results and discussion

2.1. Biotransformation of oleanolic acid (*1*)

Biotransformation of oleanolic acid (**1**) with *R. miehei* for 13 days yielded a mixture of metabolites. Chromatography on a silica-gel column of this mixture resulted in recovery of 64% of the substrate (**1**), and isolation of metabolites **3** (5%), **4** (6%), and **5** (5%) (Fig. 1).

Metabolite **3** had a molecular formula of $C_{30}H_{48}O_4$, indicating that the microorganism had inserted an oxygen atom onto the substrate (**1**). The main difference of the ^{1}H NMR spectra of compounds

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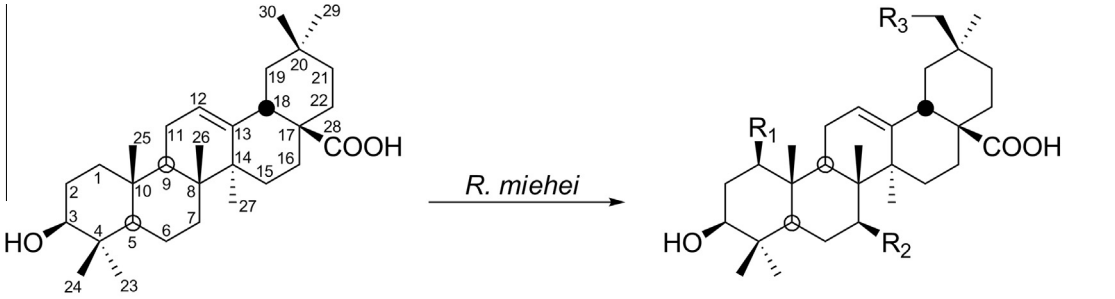

1 (oleanolic acid)

3 (queretaroic acid): $R_1 = R_2 = H$, $R_3 = OH$
4: $R_1 = H$, $R_2 = R_3 = OH$
5: $R_1 = R_3 = OH$, $R_2 = H$

Fig. 1. Metabolites isolated from the incubation of oleanolic acid (**1**) with *Rhizomucor miehei*.

1 and **3** was the presence in the latter of an AB system signal (δ_H 3.55 and 3.43), which was due to the coupling of two geminal protons to a hydroxyl group placed on a primary carbon. This signal suggested the hydroxylation, by the fungus, at one of the seven methyl groups of the substrate (**1**). The position of the microbial hydroxylation was deduced by comparison of the NMR data (^{13}C, HSQC, and HMBC) of metabolite **3** and substrate **1**. The analysis of the HMBC of metabolite **3** ruled out the possibility that the new hydroxyl group was located at C-23, C-24, C-25, C-26, or C-27. Therefore, this hydroxyl group may occur only at C-29 or C-30. The ^{13}C NMR chemical shifts of these carbon atoms, for the substrate **1**, were significantly different (δ_C 33.6 and 24.0, respectively), which made it easier to locate the position of the new hydroxyl group. A hydroxyl group at C-29 would result in a γ-effect on C-30 (δ_C 19.8) (Shao et al., 1989), whereas a hydroxyl group at C-30 a γ-effect on C-29 (δ_C 28.8) (Yang et al., 1998). These data confirmed that metabolite **3** had a structure of 3β,30-dihydroxyolean-12-en-28-oic acid, also called queretaroic acid, a natural product isolated from two Mexican cacti, *Lemaireocereus queretaroensis* and *Lemaireocereus beneckei* (Djerassi et al., 1956), and from *Machaerocereus eruca* (Yang et al., 1998). Queretaroic acid (**3**) has also been obtained from the hydroxylation of oleanolic acid (**1**) by *Escherichia coli* coexpressing a cytochrome P450 gene from *Nonomuraea recticatena* and the *Pseudomonas* redox partner *camAB* (Fujii et al., 2006). This compound (**3**) showed anti-tumour properties inhibiting ^{32}Pi-incorporation into phospholipids of HeLa cells enhanced by TPA (12-*O*-tetradecanoylphorbol-13-acetate) (Kinoshita et al., 1999).

Metabolite **4** had a molecular formula of $C_{30}H_{48}O_5$, implying that two oxygen atoms had been inserted onto the substrate (**1**) by the microorganism. In the ^1H NMR spectrum of metabolite **4** appeared an AB system signal (δ_H 3.56 and 3.44) similar to that of metabolite **3**, suggesting the presence of a 30-hydroxyl group in the molecule, being confirmed by the correlations of this signal with those of C-19, C-20, C-21, and C-29 in the HMBC spectrum of metabolite **4** (Fig. 2). In the ^1H NMR spectrum of this metabolite (**4**) a new signal also appeared at δ_H 3.86 (1H, dd, J = 11.2, 5.0 Hz), due to a geminal proton to an equatorial hydroxyl group. This second hydroxylation performed by the microorganism on a secondary carbon would be located on C-1, C-7, C-15, C-16, C-21, or C-22. The correlations observed in the HMBC spectrum of this metabolite (**4**) between this signal (δ_H 3.86) and C-6, C-8, C-14, and C-26, suggested that the new hydroxyl group was placed at C-7 (Fig. 2), being confirmed by the analysis of the ^{13}C NMR spectrum of metabolite **4** compared with that of metabolite **3**, where effects α (on C-7), β (on C-6 and C-8), and γ (on C-5 and C-26), were observed. These data are consistent with a structure of 3β,7β,30-trihydroxyolean-12-en-28-oic acid for metabolite **4**. A 7β-hydroxy derivative of oleanolic acid, called canthic acid, was tested for *in vitro* antimicrobial activity against six Gram-positive and twelve Gram-negative bacterial species as well as three yeasts of *Candida* species, being active against 95% of all of them (Kuete et al., 2007).

The molecular formula ($C_{30}H_{48}O_5$) of the last metabolite isolated (**5**) confirmed that the microorganism had inserted two oxygen atoms in the molecule of the substrate (**1**). The AB system signal (δ_H 3.55 and 3.42) that appeared in the ^1H NMR spectrum of metabolite **5** suggested the presence of a 30-hydroxyl group in the molecule, being confirmed again by the correlations existing in the HMBC spectrum of this metabolite (**5**), similar to those observed in metabolite **4** (Fig. 2). In the ^1H NMR spectrum of this metabolite (**5**), a new signal also appeared at δ_H 3.34 (1H, dd, J = 10.8, 4.9 Hz), due to a geminal proton to an equatorial hydroxyl group. The correlations observed in the HMBC spectrum of this metabolite (**5**) between this signal (δ_H 3.34) and C-2, C-9, C-10, and C-25, suggested that the new hydroxyl group was placed at C-1 (Fig. 2), being confirmed by the analysis of the ^{13}C NMR spectrum of metabolite **5** compared with that of metabolite **3**, where effects α (on C-1), β (on C-2 and C-10), and γ (on C-3, C-5, and C-25) were observed. These data confirmed the structure of 1β,3β,30-trihydroxyolean-12-en-28-oic acid for metabolite **5**.

2.2. Biotransformation of maslinic acid (**2**)

Biotransformation of maslinic acid (**2**) by *R. miehei* for 13 days produced a mixture of metabolites difficult to separate. Chromatography on a silica-gel column of this mixture resulted in recovery of 42% of the substrate (**2**), and isolation of metabolites **6** (0.5%) and **7** (11%). Acetylation of the rest of the fractions yielded the 2α,3β-diacetoxy derivatives **8**–**12** (Fig. 3).

The molecular formula of metabolite **6** ($C_{30}H_{48}O_4$) indicated that this metabolite had two hydrogen atoms less than the substrate (**2**). Its ^1H NMR spectrum showed two double doublets (δ_H 6.04 and 5.42) corresponding to the signals of two olefinic protons, which were correlated with the signal of H-9 (δ_H 1.94) in its COSY spectrum. In the ^{13}C NMR spectrum of this metabolite (**6**) appeared two signals of methyne double bond carbon (δ_C 135.5 and 127.3) that corresponded to a HC=CH system, and also a signal of a quaternary oxygenated carbon at 89.9 ppm. These spectroscopic data suggested that metabolite **6** had a structure of 28,13β-oleanolide with a double bond between C-11 and C-12. In its HMBC spectrum the correlations between H-11 and C-9, and between H-12 with C-9 and C-13, confirmed this structure (Fig. 4). Therefore, metabolite **6** was 2α,3β-dihydroxyolean-11-en-28,13β-olide. A general mechanism to rationalize the formation of this metabolite (**6**) could be the introduction of a hydroperoxyl group on the allylic position at C-11, by the microorganism (Fig. 5). Then there would be the loss of a hydrogen peroxide molecule, and the subsequent attack

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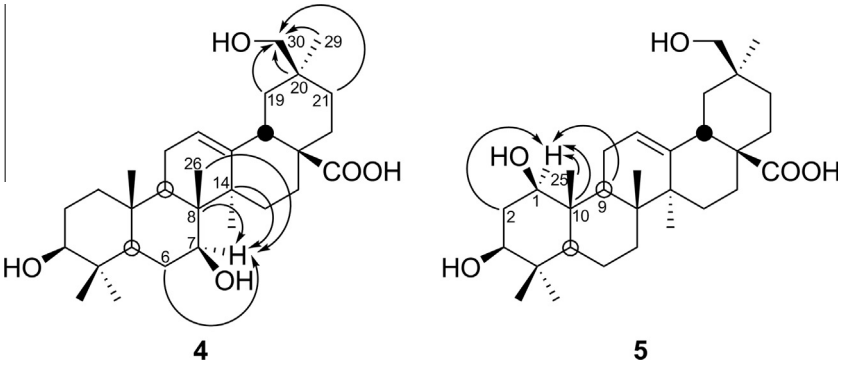

Fig. 2. Key HMBC (C→H) correlations in metabolites **4** and **5**.

of the carboxyl group of C-28 to the carbon atom of C-13 to form a 28,13β-olide group with a double bond at C-11. Moodley et al. (2011) reported that an 11-en-28,13β-olide derivative of oleanolic acid, with a structure similar to that of metabolite **6**, presented good antibacterial activity (MICs in the range 0.06–0.12 mg/mL) for all the bacterial strains studied, and declared that the lactone ring contributes significantly to the expression of antibacterial activity in these types of triterpenes.

Metabolite **7** had a molecular formula of $C_{30}H_{48}O_5$, with an extra oxygen atom with respect to that of the substrate (**2**). The AB system signal (δ_H 3.55 and 3.43) that appeared in the ^{1}H NMR spectrum, and the correlations of this signal with those of C-19, C-20, C-21, and C-29 observed in the HMBC spectrum of metabolite **7**, confirmed the presence of a 30-hydroxyl group in the molecule. Therefore, metabolite **7** had a structure of 2α,3β,30-trihydroxyolean-12-en-28-oic acid.

The rest of the fractions obtained from the chromatographic separation of the mixture of metabolites from the biotransformation with *R. miehei* of substrate **2** were combined and acetylated, yielding derivatives **8**–**12** (Fig. 3). The study of ^{1}H NMR, ^{13}C NMR, HSQC, and HMBC spectra of the acetoxy derivatives **8** and **9** confirmed the structures of 2α,3β-diacetoxyolean-11-en-28,13β-olide (**8**), and 2α,3β,30-triacetoxyolean-12-en-28-oic acid (**9**), respectively, for these derivatives. To confirm the structures of metabolite **6** and its acetoxy derivative **8**, both were chemically synthesised from maslinic acid (**2**). Bromination of maslinic acid (**2**) with Br_2/CCl_4 originated the monobrominated derivative **13** (Pereda-Miranda et al., 1992). This compound (**13**) had a molecular formula of $C_{30}H_{48}BrO_4$. The position and stereochemistry of the bromine atom was deduced from the NMR data for this compound (**13**): at δ_H 4.30 a signal due to the axial proton geminal to the bromine atom at C-12, and at δ_C 56.2 a signal of a methynic carbon atom assigned to C-12. Also, the presence of a signal of a totally substituted oxygenated carbon at δ_C 91.7, revealed the existence of a lactone group between C-28 and C-13. These data, together with those gained from the analysis of the HMBC spectrum, which showed a correlation between the signal of H-12 (δ_H 4.30) and C-9, C-11, C-13, and C-14 (Fig. 4), confirmed that compound **13** was 12α-bromine-2α,3β-dihydroxyolean-28,13β-olide. The proposed mechanism to explain the formation of this compound (**13**) could be the initial formation of a bromonium ion at C-12/C-13 by the α-face and the subsequent attack of the carboxyl group of C-28 to the carbon atom of C-13 to form the 28,13β-olide group (Fig. 6). Acetylation of the bromine derivative (**13**) originated the diacetoxy derivative **14**, which was treated with DBU/xylene to give a compound with a double bond at C-11, whose spectroscopic properties matched with those of the diacetoxy derivative **8**. The hydrolysis of compound **8** with KOH in methanol/water gave metabolite **6** (Fig. 6).

The third derivative (**10**) had a molecular formula of $C_{34}H_{50}O_7$. Its ^{1}H NMR spectrum showed two singlets (δ_H 5.63 and 2.37) corresponding to the signal of an olefinic proton at C-12, and to the signal of H-9, respectively. In the HSQC spectrum, these signals were correlated with those that appeared at δ_C 128.1 and 61.6, respectively. In the ^{13}C NMR spectrum of this metabolite (**10**) also appeared a signal of a carbonyl group at δ_C 199.7, and the other signal of the double bond (δ_C 168.8). The correlations observed in the HMBC spectrum of this compound (**10**) between the signal of H-9 (δ_H 2.37) and C-11, and the signal of H-12 (δ_H 5.63) and C-9, C-14, and C-18 (Fig. 4), suggested the presence of an α,β-unsaturated system on the C ring of the molecule, with a carbonyl group at C-11 and a double bond between C-12 and C-13. Therefore, compound **10** had a structure of 2α,3β-diacetoxy-11-oxoolean-12-en-28-oic acid. A mechanism to explain the formation of this compound (**10**) could be, as was the case for metabolite **6**, the introduction of a hydroperoxyl group on the allylic position at C-11, by the microorganism. Then, there would be the loss of a molecule of H_2O to form a carbonyl group at this position (Fig. 5). This α,β-unsaturated carbonyl group system on the C ring was previously achieved in the biotransformation of oleanolic acid (**1**) with *Cunninghamella blakesleeana* (Hikino et al., 1969), and this compound (**10**) was chemically synthesised through the modification of oleanolic acid (Zhao et al., 2010).

The fourth derivative isolated (**11**) had a molecular formula $C_{34}H_{50}O_7$, which indicated that the microorganism had inserted an oxygen atom onto the substrate. In the IR spectrum, there were no signals of hydroxyl groups. In the ^{1}H NMR spectrum, no signals of double bond protons appeared, but signals of two geminal protons to an oxygen atom (δ_H 3.02) were present, these being correlated, in the HSQC spectrum, with the signals of two oxygenated carbons (δ_C 57.1 and 52.4). Also, the presence of a signal of a totally substituted oxygenated carbon at δ_C 87.4, in the ^{13}C NMR spectrum, revealed the existence of a lactone group between C-28 and C-13. The correlations observed in the HMBC spectrum of this compound (**11**) between the signals of H-11 and H-12 (δ_H 3.02) with C-9, C10, C-13, and C-14 (Fig. 4), suggested the presence of an epoxy group at C-11 and C-12. Therefore, compound **11** had a structure of 2α,3β-diacetoxy-11α,12α-epoxyolean-28,13β-olide. Pereda-Miranda and Delgado (1990) described the chemical epoxidation of an olean-11-en-28,13β-olide compound that originated an 11α,12α-epoxiderivative. Thus, we achieved the epoxidation of the diacetoxy derivative **8** with peracetic acid resulting an 11α,12α-epoxy derivative, with identical spectroscopic properties to those of compound **11** (Fig. 6).

The last derivative isolated (**12**) had a molecular formula of $C_{36}H_{52}O_9$. Its ^{1}H NMR spectrum showed signals similar to those detected in compound **11**, due to an 11α,12α-epoxy group, along

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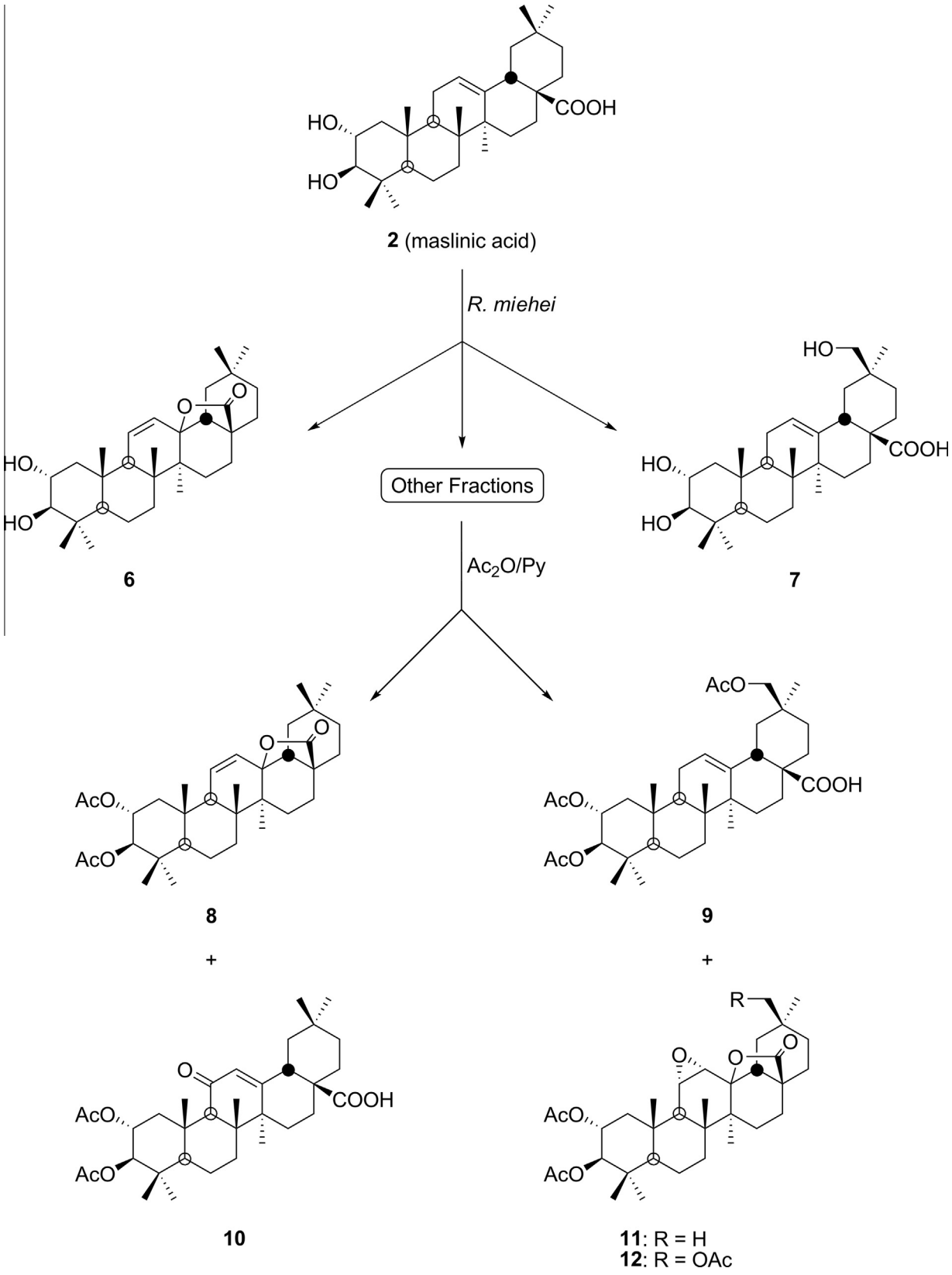

Fig. 3. Metabolites isolated and acetylated derivatives achieved from the incubation of maslinic acid (**2**) with *Rhizomucor miehei*.

with a new AB system signal with doublets centred at δ_H 4.20 and 3.78, as identified in compound **9** for an acetoxy group at C-30. These data indicated that the structure of compound **12** was 2α,3β,30-triacetoxy-11α,12α-epoxyolean-28,13β-olide.

3. Concluding remarks

The biotransformation processes carried out reveal that *R. miehei* can perform remarkable functionalizations on these

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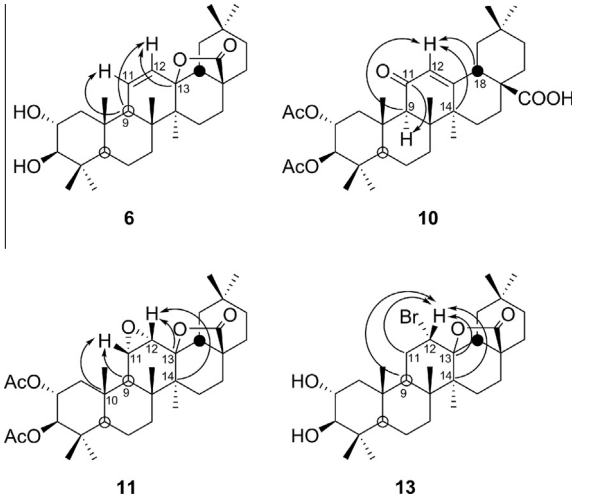

Fig. 4. Key HMBC (C→H) correlations in compounds **6**, **10**, **11**, and **13**.

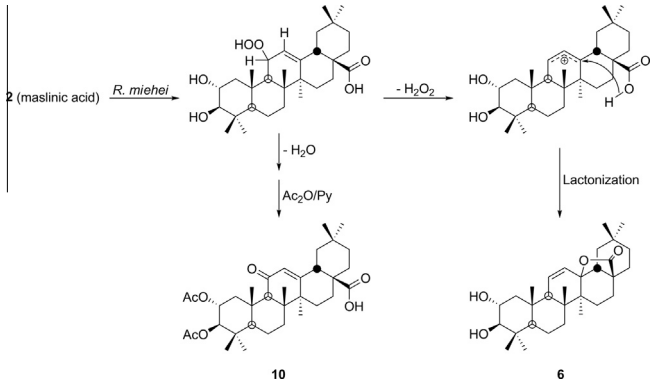

Fig. 5. The general mechanism proposed for the formation of the metabolite **6** and compound **10**.

triterpenoid compounds. This microorganism has achieved hydroxylation reactions at non-activated positions, oxidation reactions of a hydroxyl group and the double bond, and formation of a lactone group from the carboxyl group present in these triterpenic acids. Some of these metabolites isolated present remarkable biological properties, such as queretaroic acid, or have very similar structures of compounds that have noteworthy biological activities.

4. Experimental

4.1. General experimental procedures

Measurements of NMR spectra were made in a VARIAN Inova unity (300 MHz ^{1}H NMR), and a VARIAN direct drive (500 and 600 MHz ^{1}H NMR) spectrometers. Assignments of ^{13}C NMR chemical shifts (Table 1) were made with the aid of distortionless enhancement by polarization transfer (DEPT) using a flip angle of 135°. Several programs were used for COSY, HSQC, and HMBC experiments. IR spectra were recorded in a MATTSON SATELLITE FT-IR spectrometer. High-resolution mass spectra were made in a WATERS LCT Premier XE spectrometer or by LSIMS (FAB) ionization mode in a MICROMASS AUTOSPEC-Q spectrometer (EBE geometry). Melting points (mp) were determined using a Kofler (Reichert) apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 431 polarimeter at 25 °C. Incubations of substrates with the microorganism were shaken in CERTOMAT R B. Braun orbital shakers. Merck silica-gel 60 (0,040–0,063 mm, ref. 1.09385) was used for flash chromatography. CH_2Cl_2 (Fisher, ref. D/1852/17) or *n*-hexane (Merck, ref. 1.04374), with increasing amounts of Me_2CO (Fisher, ref. A/0600/17), MeOH (Fisher, ref. M/4000/17), or AcOEt (Fisher, ref. E/0900/17), were used as eluents (all the solvents had an analytical reagent grade purity). Merck TLC silica-gel 60 (ref. 1.16835) was rendered visible by spraying with H_2SO_4–HOAc–H_2O, followed by heating to 120 °C.

4.2. Isolation of starting materials

Oleanolic acid (3β-hydroxyolean-12-en-28-oic acid, **1**) and maslinic acid (2α,3β-dihydroxyolean-12-en-28-oic acid, **2**) were isolated from solid wastes of olive-oil production, extracting in a Soxhlet successively with hexane and EtOAc. The EtOAc extracts were concentrated under reduced pressure, and the solid powder obtained (10 g) was chromatographed on silica-gel by flash column chromatography to give 2 g of **1**, and 6 g of **2**.

4.2.1. 3β-Hydroxyolean-12-en-28-oic acid (oleanolic acid, *1*)

White solid; mp 306–8 °C; $[\alpha]_D$ +80 (*c* 1, $CHCl_3$); IR (KBr) ν_{max} 3438, 2930, 2869, 1690 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD) δ 5.25 (1H, dd, *J* = 3.6, 3.6 Hz, H-12), 3.16 (1H, dd, *J* = 11.2, 4.8 Hz, H-3), 2.84 (1H, dd, *J* = 13.6, 3.7 Hz, H-18), 1.99 (1H, ddd, *J* = 13.4, 13.4, 3.6 Hz, H-16), 1.15 (3H, s, 3H-27), 0.97 (3H, s, 3H-23), 0.93 (6H, s, 3H-25 and 3H-30), 0.90 (3H, s, 3H-29), 0.80 (3H, s, 3H-26), 0.77 (3H, s, 3H-24), 0.74 (1H, d, *J* = 11.5 Hz, H-5); for ^{13}C NMR (125 MHz, CD_3OD), see Table 1; HRLSIMS *m/z* 479.3504 [M+Na]$^+$ (calcd. for $C_{30}H_{48}O_3Na$, 479.3501).

4.2.2. 2α,3β-Dihydroxyolean-12-en-28-oic acid (maslinic acid, *2*)

White solid; mp 267-9 °C; $[\alpha]_D$ +54 (*c* 1, $CHCl_3$:MeOH, 2:1); IR (KBr) ν_{max} 3386, 2936, 2867, 1690 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ 5.26 (1H, dd, *J* = 3.5, 3.5 Hz, H-12), 3.62 (1H, ddd, *J* = 11.3, 9.6, 4.5 Hz, H-2), 2.91 (1H, d, *J* = 9.6 Hz, H-3), 2.86 (1H, dd, *J* = 14.1, 3.9 Hz, H-18), 2.02 (1H, ddd, *J* = 13.3, 13.3, 3.9 Hz, H-16), 1.70 (1H, dd, *J* = 14.0, 14.0 Hz, H-9), 1,17 (3H, s, 3H-27), 1.02 (3H, s, 3H-23), 1.01 (3H, s, 3H-25), 0.95 (3H, s, 3H-30), 0.91 (3H, s, 3H-29), 0.82 (3H, s, 3H-26), 0.81 (3H, s, 3H-24); for ^{13}C NMR (75 MHz, CD_3OD), see Table 1; HRLSIMS *m/z* 495.3458 [M+Na]$^+$ (calcd. for $C_{30}H_{48}O_4Na$, 495.3450).

4.3. Organism, media and culture conditions

R. miehei CECT 2749 was obtained from the Spanish Type Culture Collection (CECT), Departamento de Microbiología, Universidad de Valencia, Spain. Medium YEPGA containing 1% of yeast extract, 1% of peptone, 2% of glucose, and 2% of agar, at pH 5, was used to store the microorganisms. In all microbial transformation experiments, a medium of potato dextrose broth (Scharlau 02-483) was used for microorganism proliferation. Erlenmeyer flasks (250 ml) containing 90 ml of sterilized medium were inoculated with a dense microorganism suspension. Incubations were maintained at 28 °C with gyratory shaking (150 rpm) for 6 days after which the substrates (5–10%) in EtOH were added.

4.4. Oleanolic acid incubation with R. miehei

Oleanolic acid **1** (500 mg) was dissolved in EtOH (24 ml), distributed among 12 Erlenmeyer flask cultures (*R. miehei*) and incubated for 13 days. Then, the cultures were filtered and pooled, the cells were washed thoroughly with water, and the liquid was saturated with NaCl and extracted continuously with CH_2Cl_2 for four 6-h periods. Dry fungal cells were washed repeatedly with CH_2Cl_2. Both extracts were pooled, dried with dry Na_2SO_4, and evaporated under reduced pressure. The resulting mixture of compounds was

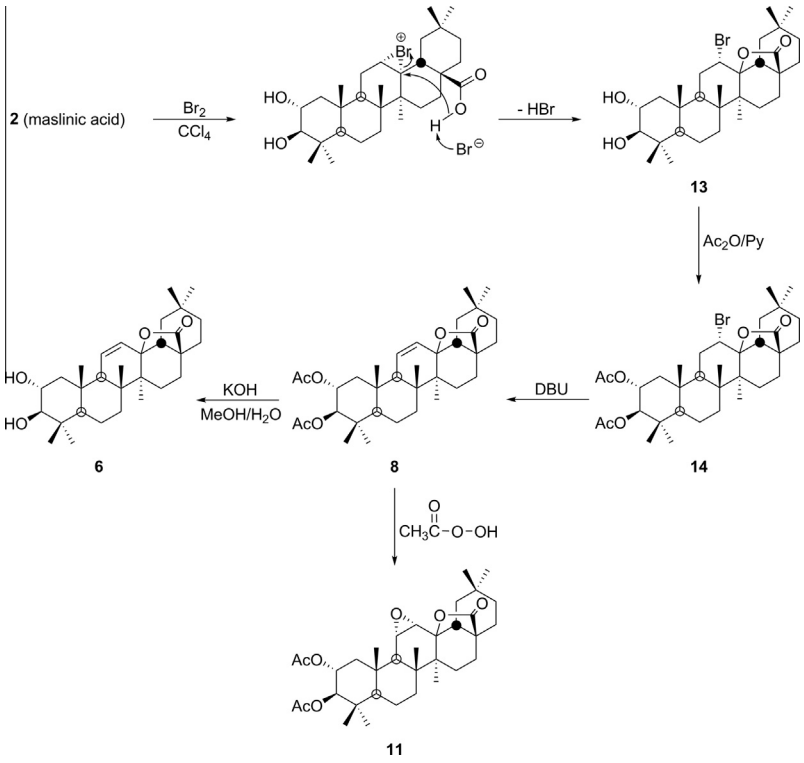

Fig. 6. The mechanism proposed for the formation of the compound **13**, and the chemical correlation of compounds **6**, **8**, and **11**.

chromatographed on a silica-gel column to obtain 320 mg of starting material (**1**, 64%), 25 mg of 3β,30-dihydroxyolean-12-en-28-oic acid (queretaroic acid, **3**, 5%), 32 mg of 3β,7β,30-trihydroxyolean-12-en-28-oic acid (**4**, 6%), and 28 mg of 1β,3β,30-trihydroxyolean-12-en-28-oic acid (**5**, 5%).

*4.4.1. 3β,30-Dihydroxyolean-12-en-28-oic acid (queretaroic acid, **3**)*

White solid; mp 159 °C; $[\alpha]_D$ +10 (*c* 1, $CHCl_3$); IR (KBr) ν_{max} 3386, 1689, 1460, 1034, 668 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ 5.27 (1H, dd, *J* = 3.5, 3.5 Hz, H-12), 3.55 and 3.43 (2H, AB system, *J* = 10.9 Hz, 2H-30), 3.15 (1H, dd, *J* = 10.9, 4.9 Hz, H-3), 2.82 (1H, dd, *J* = 13.8, 3.8 Hz, H-18), 1.18 (3H, s, 3H-27), 0.97 (3H, s, 3H-23), 0.94 (3H, s, 3H-25), 0.89 (3H, s, 3H-29), 0.82 (3H, s, 3H-26), 0.78 (3H, s, 3H-24); ^1H NMR (500 MHz, C_5D_5N) δ 5.53 (1H, dd, *J* = 3.5, 3.5 Hz, H-12), 4.03 and 3.95 (2H, AB system, *J* = 10.9 Hz, 2H-30), 3.48 (2H, m, H-3 and H-18), 1.36 (3H, s, 3H-27), 1.27 (3H, s, 3H-29), 1.25 (3H, s, 3H-23), 1.05 (6H, s, 3H-24 and 3H-26), 0.91 (3H, s, 3H-25); for ^{13}C NMR (150 MHz, CD_3OD, or 125 MHz, C_5D_5N), see Table 1; HRESIMS *m/z* 473.3627 $[M+H]^+$ (calcd. for $C_{30}H_{49}O_4$, 473.3631).

*4.4.2. 3β,7β,30-Trihydroxyolean-12-en-28-oic acid (**4**)*

White solid; mp 161 °C; $[\alpha]_D$ +41 (*c* 1, $CHCl_3$); IR (KBr) ν_{max} 3356, 1694, 1461, 1036, 996, 756, 668 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 5.34 (1H, dd, *J* = 3.6, 3.6 Hz, H-12), 3.86 (1H, dd, *J* = 11.2, 5.0 Hz, H-7), 3.56 and 3.44 (2H, AB system, *J* = 10.8 Hz, 2H-30), 3.18 (1H, dd, *J* = 11.3, 5.5 Hz, H-3), 2.83 (1H, dd, *J* = 14.0, 4.7 Hz, H-18), 1.27 (3H, s, 3H-27), 1.01 (3H, s, 3H-23), 0.96 (3H, s, 3H-25), 0.92 (3H, s, 3H-29), 0.85 (3H, s, 3H-26), 0.80 (3H, s, 3H-24); for ^{13}C NMR (125 MHz, CD_3OD), see Table 1; HRESIMS *m/z* 489.3586 $[M+H]^+$ (calcd. for $C_{30}H_{49}O_5$, 489.3580).

*4.4.3. 1β,3β,30-Trihydroxyolean-12-en-28-oic acid (**5**)*

White solid; mp 171 °C; $[\alpha]_D$ + 43 (*c* 1, $CHCl_3$); IR (KBr) ν_{max} 3375, 1693, 1460, 1040, 995, 756, 668 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ 5.27 (1H, m, *J* = 3.5, 3.5 Hz, H-12), 3.55 and 3.42 (2H, AB system, *J* = 10.5 Hz, 2H-30), 3.34 (1H, dd, *J* = 10.8, 4.9 Hz, H-1), 3.18 (1H, dd, *J* = 11.5, 4.9 Hz, H-3), 2.79 (1H, dd, *J* = 14.0, 4.7 Hz, H-18), 2.44 (1H, ddd, *J* = 19.3, 3.5, 3.5 Hz, H-11α), 2.09 (1H, ddd, *J* = 19.3, 11.0, 3.5 Hz, H-11β), 1.18 (3H, s, 3H-27), 0.97 (3H, s, 3H-25), 0.95 (3H, s, 3H-23), 0.89 (3H, s, 3H-29), 0.81 (3H, s, 3H-26), 0.76 (3H, s, 3H-24), 0.61 (1H, d, *J* = 10.8 Hz, H-5); for ^{13}C NMR (150 MHz, CD_3OD), see Table 1; HRESIMS *m/z* 489.3578 $[M+H]^+$ (calcd. for $C_{30}H_{49}O_5$, 489.3580).

4.5. Maslinic acid incubation with R. miehei

Maslinic acid (**2**, 2 g) was dissolved in EtOH (100 ml), distributed among 50 Erlenmeyer flask cultures (*R. miehei*) and incubated for 13 days. Then, the cultures were filtered and pooled, the cells were washed thoroughly with water, and the liquid was saturated with NaCl and extracted continuously with CH_2Cl_2 for four 6-h periods. Dry fungal cells were washed repeatedly with CH_2Cl_2. Both extracts were pooled, dried with dry Na_2SO_4, and evaporated under reduced pressure. The resulting mixture of compounds was chromatographed on a silica-gel column to obtain 845 mg of starting material (**2**, 42%), 10 mg of 2α,3β-dihydroxyolean-11-en-28,13β-olide (**6**, 0.5%), and 225 mg of 2α,3β,30-trihydroxyolean-12-en-28-oic acid (**7**, 11%). The rest of the fractions (200 mg) were dissolved in pyridine (4 ml) and Ac_2O (2 ml). After the reaction was maintained for 24-h at room temperature, it was diluted with cold H_2O (50 ml), extracted with CH_2Cl_2, washed with saturated aqueous $KHSO_4$, and dried over dry Na_2SO_4. Chromatography on silica-gel of the reaction mixture gave 8 mg of 2α,3β-diacetoxyolean-11-en-28,13β-olide (**8**, 0.3%), 30 mg of 2α,3β,30-triacetoxyolean-12-en-28-oic acid (**9**, 1.2%), 26 mg of 2α,3β-diacetoxy-11-oxoolean-12-en-28-oic acid (**10**, 1%), 6 mg of 2α,3β-diacetoxy-11α,12α-epoxyolean-28,13β-olide (**11**, 0.2%), and 23 mg of 2α,3β,30-triacetoxy-11α,12α-epoxyolean-28,13β-olide (**12**, 0.9%).

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Table 1
^{13}C NMR spectroscopic data for compounds **1**–**14**.

Position	1	2	3[a]	3[b]	4	5	6	7	8	9	10	11	12	13	14
1	39.9	48.1	39.8	39.3	39.7	80.5	46.2	48.1	43.8	44	44.6	43.7	43.8	46.4	43.8
2	27.9	69.5	27.9	28.5	27.9	38.2	68.8	69.5	69.9	70.1	69.9	69.5	69.5	69.1	70
3	79.7	84.4	79.8	78.5	79.5	76.8	83.9	84.4	80.6	80.8	80.8	80.4	80.4	83.9	80.5
4	39.9	40.5	39.8	40.2	39.6	39.8	39.4	40.6	39.6	39.5	39.5	39.5	39.6	39.4	39.5
5	56.8	56.7	56.8	56.2	54	54.5	55	56.7	54.6	55	54.8	54.4	54.5	55.5	55.1
6	19.5	19.6	19.5	19.2	30.4	19.2	17.8	19.6	17.7	18.3	17.5	17.6	17.6	17.9	17.7
7	33.8	33.8	34	33.7	73.9	34.1	31.2	33.9	31.1	32.6	32.8	31.1	31.1	34.7	34.5
8	40.6	40.6	40.6	39.8	46.2	41	41.8[c]	40.5	41.8[c]	39.5	45.2	41.5	41.5	42.7	42.7
9	49.1	49	49.1	48.5	49.9	49.7	53.2	49	53.1	47.7	61.6	50.7	50.8	45.7	45.6
10	38.2	39.3	38.2	37.8	38.2	44.3	37.7	39.2	37.5	38.3	38.3	37.6	37.6	38.1	37.9
11	24.5	24.6	24.5	24.2	24.6	28.1	127.3	24.3	127.6	23.6	199.7	52.4	52.4	30.7	30.7
12	123.7	123.5	123.9	123.1	124.1	125	135.5	123.7	135.1	123	128.1	57.1	57	56.2	56.1
13	145.2	145.3	145	145.2	144.5	143.6	89.9	145	89.6	143.2	168.8	87.4	87.3	91.7	91.5
14	42.9	42.9	42.9	42.6	44.3	42.7	41.5[c]	42.9	41.5[c]	41.7	43.6	40.7	40.7	43.6	43.6
15	28.9	28.8	28.9	28.8	32.3	28.9	25.5	28.8	25.5	27.7	27.9	26.8	26.8	29.3	29.3
16	24.1	24	24.4	24.5	24.8	24.3	21.4	24.6	21.4	23.2	22.7	21.4	21.4	21.5	21.4
17	47.6	47.6	47.4	47.1	47.5	47.4	44.2	47.5	44.1	46.4	46.1	43.9	43.7	45.7	45.6
18	42.8	42.7	42.2	42	42.8	41.9	50.7	42.1	50.6	40.6	41.5	49.7	48.9	52.5	52.4
19	47.3	47.2	42.6	42.4	42.7	42.5	37.4	42.5	37.5	41.5	44.3	37.8	32.9	40.1	40
20	31.6	31.6	36.1	36.3	36.2	36.1	31.6	36.1	31.6	34	30.8	31.6	34.9	32	32
21	34.9	34.9	29.9	30	29.9	29.8	34.5	29.8	34.5	29.4	31.8	34.4	30.5	34	34
22	34	33.9	33.4	33.4	33.2	33.3	27.3	33.3	27.3	32	33.8	27.1	26.8	27.7	27.7
23	28.8	29.3	28.8	28.8	28.7	28.7	28.4	29.3	28.2	28.6	28.6	28.2	28.2	28.6	28.4
24	16.3	17.1	16.3	16.9	16.4	16.1	16.2	17.5	17.1	17.8	17.7	17.3	17.3	16.7	17.5
25	15.9	17.5	15.9	15.9	15.9	11.9	19.3	17.1	19.1	16.6	17.5	18.3	18.3	18.3	18.2
26	17.7	17.7	17.8	17.9	10.7	18	19.2	17.7	19.1	17.2	19.4	20.1	20.1	19.3	19.2
27	26.4	26.5	26.4	26.6	26.4	26.5	18.4	26.5	18.4	26.1	23.7	18.9	19	21.3	21.3
28	180.8	181.8	180.5	180.7	181.7	181.7	180.2	181.8	179.9	183.2	182.5	179.3	178.7	178.9	178.8
29	33.6	33.6	28.1	29.2	28.1	28.2	33.4	28.1	33.4	27.9	33	33.3	28.4	33.4	33.4
30	24	24	66.3	66	66.3	66.3	23.7	66.3	23.7	67.8	23.5	23.7	66.9	23.7	23.7
MeCOO									170.9	171.4	171.1	170.8	171.2		170.9
MeCOO									170.6	171	170.3	170.6	170.9		170.6
MeCOO										170.7			170.6		
MeCOO									21.2	21.4	21.2	21.2	21.2		21.3
MeCOO									21	21	21	21	21		21
MeCOO										21			20.9		

[a] ^{13}C NMR data for compound **3** measured in CD_3OD.
[b] ^{13}C NMR data for compound **3** measured in C_5D_5N.
[c] Interchangeable within the same column.

*4.5.1. 2α,3β-Dihydroxyolean-11-en-28,13β-olide (**6**)*

White solid; mp 187 °C; $[\alpha]_D$ +26 (*c* 1, $CHCl_3$); IR (KBr) ν_{max} 3407, 1757, 1463, 1388, 1218, 1050, 755 cm^{-1}; ^{1}H NMR (300 Mz, CD_3OD) δ 6.04 (1H, dd, *J* = 10.3, 1.4 Hz, H-12), 5.42 (1H, dd, *J* = 10.3, 3.2 Hz, H-11), 3.74 (1H, ddd, *J* = 11.2, 9.5, 4.5, H-2), 2.99 (1H, d, *J* = 9.5 Hz, H-3), 2.18 (1H, dd, *J* = 12.3, 4.5 Hz, H-1), 1.94 (1H, bs, H-9), 1.79 (1H, dd, *J* = 13.5, 13.5 Hz, H-19), 1.04 (6H, s, 3H-26 and 3H-27), 1.01 (3H, s, 3H-23), 0.96 (6H, s, 3H-25 and 3H-29), 0.87 (3H, s, 3H-30), 0.81 (3H, s, 3H-24); for ^{13}C NMR (75 MHz, CD_3OD), see Table 1; HRESIMS *m/z* 471.3454 $[M+H]^+$ (calcd. for $C_{30}H_{47}O_4$, 471.3474).

*4.5.2. 2α,3β,30-Trihydroxyolean-12-en-28-oic acid (**7**)*

White solid; mp 183 °C; $[\alpha]_D$ +31 (*c* 1, MeOH); IR (KBr) ν_{max} 3355, 1685, 1455, 1388, 1047, 752 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.28 (1H, dd, *J* = 3.3, 3.3 Hz, H-12), 3.61 (1H, ddd, *J* = 10.1, 9.6, 4.4 Hz, H-2), 3.55 and 3.43 (2H, AB system, *J* = 10.9 Hz, 2H-30), 2.91 (1H, d, *J* = 9.6 Hz, H-3), 2.82 (1H, dd, *J* = 13.6, 4.1 Hz, H-18), 2.05 (1H, ddd, *J* = 13.4, 13.4, 3.8 Hz, H-16), 1.18 (3H, s, 3H-27), 1.01 (3H, s, 3H-23), 1.00 (3H, s, 3H-25), 0.89 (3H, s, 3H-29), 0.80 (6H, s, 3H-24 and 3H-26); for ^{13}C NMR (75 MHz, $CDCl_3$), see Table 1; HRESIMS *m/z* 489.3576 $[M+H]^+$ (calcd. for $C_{30}H_{49}O_5$, 489.3580).

*4.5.3. 2α,3β-Diacetoxyolean-11-en-28,13β-olide (**8**)*

White solid; mp 222 °C; $[\alpha]_D$ + 12 (*c* 1, $CHCl_3$); IR (KBr) ν_{max} 1765, 1743, 1368, 1253, 1030, 896, 755 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.00 (1H, dd, *J* = 10.3, 1.4 Hz, H-12), 5.43 (1H, dd, *J* = 10.3, 3.2 Hz, H-11), 5.14 (1H, ddd, *J* = 11.2, 9.5, 4.5, H-2), 4.74 (1H, d, *J* = 9.5 Hz, H-3), 2.24 (1H, dd, *J* = 12.3, 4.5 Hz, H-1), 2.05 and 1.98 (3H each, s, AcO groups), 1.79 (1H, dd, *J* = 13.5, 13.5 Hz, H-19), 1.05 (9H, s, 3H-25, 3H-26 and 3H-27), 0.97 (3H, s, 3H-29), 0.91 (3H, s, 3H-24), 0.89 (3H, s, 3H-23), 0.87 (3H, s, 3H-30); for ^{13}C NMR (75 MHz, $CDCl_3$), see Table 1; HRESIMS *m/z* 555.3683 $[M+H]^+$ (calcd. for $C_{34}H_{51}O_6$, 555.3686).

*4.5.4. 2α,3β,30-Triacetoxyolean-12-en-28-oic acid (**9**)*

White solid; mp 192 °C; $[\alpha]_D$ +28 (*c* 1, $CHCl_3$); IR (KBr) ν_{max} 1740, 1370, 1247, 1041, 756 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.26 (1H, *J* = 3.1, 3.1 Hz, H-12), 5.10 (1H, ddd, *J* = 11.6, 10.3, 4.2, H-2), 4.74 (1H, d, *J* = 10.3 Hz, H-3), 4.06 and 3.99 (2H, AB system, *J* = 11.0 Hz, 2H-30), 2.80 (1H, dd, *J* = 13.7, 4.2 Hz, H-18), 2.06, 2.05 and 1.97 (3H each, s, AcO groups), 1.13 (3H, s, 3H-27), 1.05 (3H, s, 3H-25), 0.92 (3H, s, 3H-29), 0.89 (6H, s, 3H-23 and 3H-24), 0.73 (3H, s, 3H-26); for ^{13}C NMR (75 MHz, $CDCl_3$), see Table 1; HRESIMS *m/z* 615.3893 $[M+H]^+$ (calcd. for $C_{36}H_{55}O_8$, 615.3897).

*4.5.5. 2α,3β-Diacetoxy-11-oxoolean-12-en-28-oic acid (**10**)*

White solid; mp 131 °C; $[\alpha]_D$ + 20 (*c* 1, $CHCl_3$); IR (KBr) ν_{max} 1740, 1658, 1370, 1246, 1042, 756 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.63 (1H, s, H-12), 5.22 (1H, ddd, *J* = 10.3, 10.3, 4.6 Hz, H-2), 4.71 (1H, d, *J* = 10.3 Hz, H-3), 3.20 (1H, dd, *J* = 12.9, 4.8 Hz, H-1), 2.97 (1H, dd, *J* = 13.8, 4.0 Hz, H-18), 2.37 (1H, s, H-9), 2.04 and 1.95 (3H each, s, AcO groups), 1.35 (3H, s, 3H-27), 1.23 (3H,

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s, 3H-25), 0.94 (6H, s, 3H-29 and 3H-30), 0.91 (6H, s, 3H-24 and 3H-26), 0.89 (3H, s, 3H-23); for ^{13}C NMR (75 MHz, $CDCl_3$), see Table 1; HRESIMS *m/z* 571.3634 $[M+H]^+$ (calcd. for $C_{34}H_{51}O_7$, 571.3635).

*4.5.6. 2α,3β-Diacetoxy-11α,12α-epoxyolean-28,13β-olide (**11**)*

White solid; mp 152 °C; $[\alpha]_D$ +19 (*c* 1, $CHCl_3$); IR (KBr) ν_{max} 1776, 1742, 1368, 1253, 1037, 931, 756 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 5.15 (1H, ddd, *J* = 10.3, 10.3, 4.5 Hz, H-2), 4.77 (1H, d, *J* = 10.3 Hz, H-3), 3.02 (2H, m, H-11 and H-12), 2.30 (2H, m, H-1 and H-18), 2.12 (1H, ddd, *J* = 13.1, 13.1, 5.6 Hz, H-16), 2.05 and 1.98 (3H each, s, AcO groups), 1.85 (1H, dd, *J* = 13.5, 13.5 Hz, H-19), 1.15 (3H, s, 3H-25), 1.08 (3H, s, 3H-27), 1.05 (3H, s, 3H-26), 0.99 (3H, s, 3H-29), 0.91 (6H, s, 3H-24 and 3H-30), 0.89 (3H, s, 3H-23); for ^{13}C NMR (75 MHz, CD_3OD), see Table 1; HRESIMS *m/z* 571.3641 $[M+H]^+$ (calcd. for $C_{34}H_{51}O_7$, 571.3635).

*4.5.7. 2α,3β,30-Triacetoxy-11α,12α-epoxyolean-28,13β-olide (**12**)*

Sirup; $[\alpha]_D$ +14 (*c* 1, $CHCl_3$); IR (film) ν_{max} 1775,1741, 1370, 1242, 1036, 932, 756 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 5.15 (1H, ddd, *J* = 10.3, 10.3, 4.5 Hz, H-2), 4.77 (1H, d, *J* = 10.3 Hz, H-3), 4.20 and 3.78 (2H, AB system, *J* = 10.5 Hz, 2H-30), 3.03 (2H, m, H-11 and H-12), 2.32 (2H, m, H-1 and H-18), 2.06 (6H, s, AcO groups), 1.98 (3H, s, AcO group), 1.82 (1H, dd, *J* = 13.7, 13.7 Hz, H-19), 1.16 (3H, s, 3H-25), 1.10 (3H, s, 3H-27), 1.06 (3H, s, 3H-26), 1.02 (3H, s, 3H-29), 0.92 (3H, s, 3H-24), 0.89 (3H, s, 3H-23); for ^{13}C NMR (75 MHz, CD_3OD), see Table 1; HRESIMS *m/z* 629.3693 $[M+H]^+$ (calcd. for $C_{36}H_{53}O_9$, 629.3690).

*4.6. Bromination of maslinic acid (**2**)*

After 500 mg of maslinic acid (**2**) were dissolved in 5 ml of CCl_4, 15 ml of a solution of bromine dissolved in CCl_4 (8.7×10^{-2} M) were added. The mixture was kept for 15 min at room temperature, and afterwards the solvent was evaporated and purified on column, yielding 12α-bromo-2α,3β-dihydroxyolean-28,13β-olide (**13**, 325 mg), as a white solid; mp 266 °C; $[\alpha]_D$ +49 (*c* 1, $CHCl_3$); IR (KBr) ν_{max} 3327, 1760, 1465, 1392, 1216, 1134, 1048, 927, 755 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.30 (1H, dd, *J* = 3.9, 2.3 Hz, H-12), 3.73 (1H, ddd, *J* = 11.3, 9.5, 4.6 Hz, H-2), 3.04 (1H, d, *J* = 9.5 Hz, H-3), 2.41 (1H, ddd, *J* = 15.0, 12.4, 3.9 Hz, H-11β), 2.17 (1H, ddd, *J* = 13.0, 13.0, 5.4 Hz, H-16α), 2.06 (1H, dd, *J* = 12.4, 4.6 Hz, H-1α), 1.45 (3H, s, 3H-27), 1.22 (3H, s, 3H-26), 1.04 (3H, s, 3H-24), 1.00 (3H, s, 3H-29), 0.96 (3H, s, 3H-25), 0.90 (3H, s, 3H-30), 0.82 (3H, s, 3H-23); for ^{13}C NMR (75 MHz, $CDCl_3$), see Table 1; HRESIMS *m/z* 551.2743 $[M+H]^+$ (calcd. for $C_{30}H_{48}BrO_4$, 551.2736).

*4.7. Acetylation of bromoderivative **13***

The bromine derivative (**13**, 300 mg) was acetylated with Ac_2O/Pyridine (10 ml/20 ml) for 12 h. Afterwards, cold water was added to the reaction mixture, and this was extracted repeatedly with CH_2Cl_2, washing the organic phase with a solution of $KHCO_3$. The organic phase was dried with dry Na_2SO_4 and the solvent was evaporated under reduced pressure. Finally, the reaction mixture was purified by column chromatography isolating 268 mg of 2α,3β-diacetoxy-12α-bromoolean-28,13β-olide (**14**, 325 mg) as a white solid; mp 257 °C; $[\alpha]_D$ +24 (*c* 1, $CHCl_3$); IR (KBr) ν_{max} 1773, 1741, 1368, 1253, 756 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.11 (1H, ddd, *J* = 11.4, 10.4, 4.7 Hz, H-2), 4.77 (1H, d, *J* = 10.4 Hz, H-3), 4.28 (1H, dd, *J* = 3.8, 2.2 Hz, H-12), 2.40 (1H, ddd, *J* = 15.4, 12.5, 3.8 Hz, H-11β), 2.16 (1H, ddd, *J* = 11.2, 11.2, 5.5 Hz, H-16α), 2.08 (1H, dd, *J* = 13.1, 4.7 Hz, H-1α), 2.05 and 1.99 (3H each, s, AcO groups), 1.44 (3H, s, 3H-27), 1.21 (3H, s, 3H-26), 1.03 (3H, s, 3H-25), 0.99 (3H, s, 3H-29), 0.90 (9H, s, 3H-23, 3H-24 and 3H-30); for ^{13}C NMR (75 MHz, $CDCl_3$), see Table 1; HRESIMS *m/z* 635.2941 $[M+H]^+$ (calcd. for $C_{34}H_{52}BrO_6$, 635.2947).

*4.8. Dehydrohalogenation of **14** with DBU*

After 200 mg of compound **14** were dissolved in xylene (10 ml), 2.5 ml of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) were added. The reaction was kept for 13 h at reflux. Next, ethyl acetate was added and the reaction mixture was extracted with aqueous HCl (5%). The organic phase was treated with an aqueous solution of Na_2CO_3 (5%), dried with dry Na_2SO_4, and evaporated under reduced pressure. Finally, after purification, 170 mg of 2α,3β-diacetoxyolean-11-en-28,13β-olide (**8**) were isolated.

*4.9. Saponification of **8***

First, 20 mg of compound **8** were dissolved in a mixture of methanol/water/KOH (100:70:5), and stirred for 12-h at room temperature. Next, the mixture was diluted with water and extracted repeatedly with CH_2Cl_2. From the column chromatography of the mixture, 15 mg of compound **6** were isolated.

*4.10. Epoxidation of **8***

After 70 mg of **8** were dissolved in CH_2Cl_2 (10 ml), 2.5 ml of peracetic acid with a few drops of ethanol were added until the turbidity disappeared. The reaction was kept under stirring for 24-h at room temperature. Afterwards, 20 ml of water were added to the reaction mixture, which was extracted repeatedly with CH_2Cl_2, and washed with a saturated solution of $NaHCO_3$. The organic phase was dried with dry Na_2SO_4, and evaporated under reduced pressure. After chromatographic purification of the mixture 40 mg of 2α,3β-diacetoxy-11α,12α-epoxyolean-28,13β-olide (**11**) were isolated.

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