Antimycobacterial Metabolites from *Plectranthus:* Royleanone Derivatives against *Mycobacterium tuberculosis* Strains

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The antimycobacterial activities of eight diterpenes, **1**–**8**, isolated previously from *Plectranthus* and eleven esters, **9**–**19**, of 7α-acetoxy-6β,12-dihydroxyabieta-8,12-diene-11,14-dione (**5**) were evaluated against the MTB strains H_{37}Rv and MDR. Only diterpenoids with a quinone framework revealed anti-MTB activity. Abietane **5** and its 6,12-dibenzoyl, 12-methoxybenzoyl, 12-chlorobenzoyl, and 12-nitrobenzoyl esters, **9**, **11**, **12**, and **13**, respectively, showed potent activities against the MDR strain with *MIC* values between 3.12 and 0.39 μg/ml. Cytotoxic activities towards 3T3 and *Vero* cells were also evaluated. Compound **11**, with the best selectivity index, may be a suitable lead for further chemical modifications. The complete structural elucidation of the new esters, **9**–**14**, **16**, **18**, and **19**, as well as the NMR data of known derivatives **15** and **17** are reported.

Introduction. – Tuberculosis (TB) is an old human infectious disease that still remains a leading cause of death worldwide, both in developing and developed countries. *Mycobacterium tuberculosis* (MTB) is the most important mycobacterium causing human TB. Nowadays, with the emergence of drug- and multidrug-resistant *M. tuberculosis* strains (MDR TB, XDR TB) treatment of this disease is of great concern. Though most people with normal immune systems resist to be infected, those with weakened immune systems, *e.g.*, caused by other diseases (such as HIV), by malnutrition, or by inadequate treatment, are seriously infected [1]. A large number of newly infected people emerge every year, mostly in Asia and in sub-Saharan Africa with TB being the fifth cause of mortality in South Africa [2].

References to natural products with anti-TB activity are increasingly reported, and thus it is expected that these compounds will enlarge the diversity of novel scaffolds for the antimycobacterial drug development [1][3]. *Plectranthus* species (Labiatae) are largely distributed in tropical and subtropical Africa, and are traditionally used for the treatment of respiratory complaints [2][4]. *P. barbatus* and *P. bojeri* are indicated for the treatment of pneumonia, and *P. amboinicus* revealed anti-MTB activity [4]. Moreover *P. aegypticus*, *P. ambiguus*, *P. caninus*, *P. edulis*, *P. elegans*, *P. glandulosus*, *P.*

hadiensis, *P. lanceolatus*, *P. laxiflorus*, *P. madagascarensis*, *P. mollis*, *P. montanus*, and *P. stolzii* are used to relieve several respiratory diseases [4].

The constituents of *Plectranthus* plants are mainly diterpenoids which belong to abietane, kaurane, and labdane classes, royleanone abietanes being a well-represented subclass [5]. In a few royleanone abietanes, antimycobacterial activities were found, *e.g.*, in derivatives of carnosic acid, isolated from *Salvia africana-lutea* [2], and in horminone, obtained from *S. reptans* [6] and *S. multicaulis* [7]. These last abietanes resemble royleanones isolated from *P. grandidentatus* and *P. hereroensis*, which revealed anti-methicillin-resistant *Staphylococcus aureus* (MRSA) properties [8].

Royleanones are hydroquinonic abietanes with a 12-hydroxy-11,14-dioxo-quinone moiety in *C* ring. Several natural quinone metabolites showed antimycobacterial activity [3], namely naphthoquinones and benzoquinones such as 7-methyljuglone [9] and primin [10], respectively.

Pursuing our research efforts to find antimicrobial metabolites from *Plectranthus* spp., we report here for the first time the antimycobacterial study of eight natural diterpenes, **1**–**8**, obtained previously from *Plectranthus* spp., and a set of eleven hemisynthetic royleanone derivatives, **9**–**19**, against two MTB strains. Cytotoxicity assays against eukaryotic murine (3T3) and mammalian (*Vero*) cell lines are also reported.

Results and Discussion. – 1. *Natural Diterpenoids.* Natural diterpenes were previously isolated from *P. fruticosus* (three *ent*-kaurenes: *ent*-kaur-15-en-19-oic acid (**1**), *ent*-12β-acetoxykaur-16-en-19-oic acid (**2**), and *ent*-7β-hydroxy-15β,16β-epoxy-kauran-19-oic acid (**3**)) [11], from *P. ornatus* (one labdane: rhinocerotinoic acid (**4**)) [12] and from *P. grandidentatus* (four royleanone abietanes: 7α-acetoxy-6β-hydroxy-royleanone (**5**), 6β,7α-dihydroxyroyleanone (**6**), horminone (**7**), 6,7-dehydroroyleanone (**8**)) [8][12][13] (*Fig. 1*). Preliminary results highlighted that only royleanone

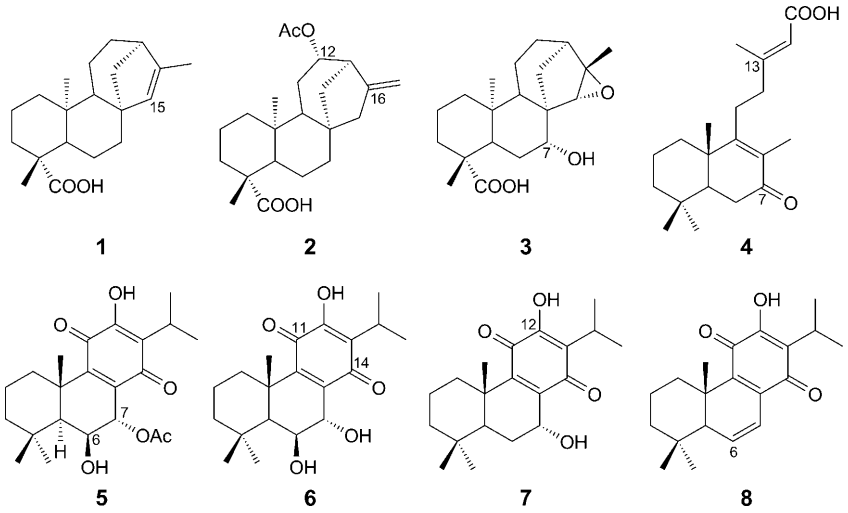
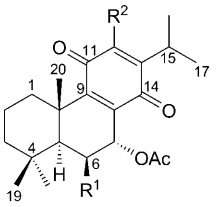


Fig. 1. *Diterpenes Isolated from* Plectranthus *spp.*

abietanes were active against the assayed MTB strains 7α-acetoxy-6β-hydroxyroyleanone (**5**), isolated from *P. grandidentatus* being the most potent of them, suggesting that it could be a lead for future drug development.

2. *Hemisynthetic Diterpenoids.* Assuming to keep the chromophoric system of the lead royleanone **5** (tautomeric 6-*O*-hydroxy-7-*O*-acetoxy-12-*O*-hydroxy-11,14-dioxoquinone motif), eleven royleanone derivatives, **9**–**19**, were prepared (*Fig. 2*). Regarding the complex lipophilic cell envelope of mycobacterium, modulation of lipophilicity was achieved by esterification at C(6) and C(12).



	R^1	R^2
9	BzO	BzO
10	4-Cl–C_6H_4COO	4-Cl–C_6H_4COO
11	OH	4-MeO–C_6H_4COO
12	OH	4-Cl–C_6H_4COO
13	OH	4-NO_2–C_6H_4COO
14	4-NO_2–C_6H_4COO	OH
15	AcO	AcO
16	$MeCH_2COO$	$MeCH_2COO$
17	AcO	OH
18	$MeCH_2COO$	OH
19	$Me(CH_2)_2COO$	OH

Fig. 2. *Ester Derivatives* **9**–**19** *of 7α-acetoxy-6β-hydroxyroyleanone* (**5**)

Aromatic and alkylic ester derivatives of **5** were synthesized according to a known methodology [6]. First, by reaction with the corresponding aromatic acyl chloride, two highly lipophilic diesters, **9** and **10**, [14] (*Table*) were prepared (*Fig. 2*), followed by the hemisynthesis of the aromatic monoesters **11**–**14** (*Fig. 2*; with medium log $P \approx 5.00$ [14]; *Table*). To study the effect of the alkylic esterification on the activity of **5**, a set of further five derivatives **15**–**19** (*Fig. 2*) was prepared [6].

3. *Structure Elucidation.* The structures of diterpenoids **9**–**14**, **16**, **18**, and **19** were elucidated on the basis of their spectroscopic data (see *Exper. Part*), mainly the ^{1}H- and ^{13}C-NMR spectra, and COSY, HSQC, and HMBC experiments, in addition to comparison with literature data [12][13][15–17]. The attachment of the acyl groups at C(6) and/or C(7) was firmly established by the correlations observed in the HMBC spectra, whereas the esterification of the OH group at C(12) was evident by the absence of the signal of the H-atom of 12-OH moiety in the ^{1}H-NMR spectra of **9**–**13** and **15** and **16**.

Though compounds **15** [15] and **17** [17] were already known, the ^{1}H-NMR data of **15** are now complete and its ^{13}C-NMR spectrum is reported here for the first time. The ^{1}H- and ^{13}C-NMR data of **17** were in good agreement with those reported in literature [17], except for the assignments of C(20) and the Me C-atom of the 6β-AcO group which must be reversed. Moreover, several J(H,H) values for **17**, not determined previously, are now reported.

4. *Antimycobacterial Activity.* Antimycobacterial assays [18] carried on *ent*-kaurene and labdane diterpenes, **1**–**3** and **4**, respectively, showed that they have no activity against MDR and H_{37}Rv strains (*MIC* values >25 μg/ml) (*Table*). In contrast, the royleanones, which bear a *p*-benzoquinone *C* ring, *i.e.*, 6β,7α-dihydroxyroyleanone (**6**),

Table. In vitro *Antimycobacterial and Cytotoxic Activities of Royleanone Diterpenoids*

Compounds	*Mycobacteria* (*MIC* [μg/ml][a]))		Eukaryotic cell lines (GI_{50} [μg/ml][b]))		*SI*[c])		log *P* [14]
	H_{37}Rv	MDR	3T3	*Vero*	3T3	*Vero*	
5	25	3.12	12.96	12.80	4.15	4.10	3.16 ± 0.62
9	> 25	1.56	1.44	2.52	0.92	1.62	7.02 ± 1.05
10	> 25	> 25	nd[d])	nd	nd	nd	8.07 ± 1.29
11	3.12	0.39	1.58	2.58	4.05	6.62	4.92 ± 0.83
12	25	0.78	2.42	4.42	3.10	5.67	5.52 ± 0.77
13	> 25	3.12	4.90	2.70	1.57	0.87	4.88 ± 0.67
14	25	25	2.34	2.86	0.09	0.11	5.13 ± 0.75
15	25	12.50	4.30	3.20	0.34	0.26	4.01 ± 0.69
16	25	6.25	4.00	2.70	0.64	0.43	4.87 ± 0.91
17	> 25	12.50	10.30	10.54	0.82	0.84	3.60 ± 0.56
18	> 25	6.25	4.90	2.90	0.78	0.46	4.18 ± 0.64
19	25	12.50	9.92	5.94	0.79	0.48	4.59 ± 0.73
6	> 25	12.50	nd	nd	nd	nd	2.44 ± 0.52
7	25	12.50	18.58	16.66	1.49	1.33	3.52 ± 0.52
8	> 25	≤ 12.50	nd	nd	nd	nd	4.52 ± 0.58
Isoniazid[e])	0.125	4	nd	nd	nd	nd	nd
Rifampicin[e])	0.063	16	nd	nd	nd	nd	nd

[a]) *MIC* Value is the minimum inhibitory concentration of the tested compounds effecting 100% of inhibition. [b]) GI_{50} Value is the concentration of the tested samples resulting in 50% inhibition of cell growth. [c]) $SI = GI_{50}$ values (3T3 or *Vero* cells)/*MIC* value (MDR strain). [d]) nd = Not determined. [e]) Positive controls.

horminone (**7**), and 6,7-dehydroroyleanone (**8**), showed mild activities against the MDR strain (*MIC* values ≤ 12.5 μg/ml), and, distinctively, 7α-acetoxy-6β-hydroxyroyleanone (**5**) exhibited the more potent antimycobacterial activity against the MDR strain with a *MIC* value of 3.12 μg/ml, and against H_{37}Rv strain with a *MIC* value of 25 μg/ml (*Table*). These results may suggest that the presence of the 7α-AcO group at *B* ring is essential to increase the MDR-MTB activity. As already emphasized, these results were not unexpected due to the *p*-benzoquinone framework, present on *C* ring of royleanones, responsible for the antimycobacterial activities of several quinonic compounds [10]. Moreover, it was observed that both acetone extract of *P. grandidentatus* and the chromatographic fraction containing these active four royleanones were inactive against both MTB strains (*MIC* 12.50–25 μg/ml), indicating that activity is only exerted by pure metabolites.

Since abietane **5** exhibited the best *MIC* value, and aiming at achieving compounds with a improved anti-TB activities, novel abietane derivatives **9**–**19** were tested.

Of the more lipophilic aromatic derivatives **9** and **10** only the dibenzoyl ester **9** showed slightly higher potency than **5** against the MDR strain (*MIC* 1.56 μg/ml; *Table*). The less lipophilic aromatic 12-esters **11**–**13** were particularly active against the MDR strain, but only compound **11** (with a methoxybenzoyl substituent) showed a promising antimycobacterial activity against both MDR and H_{37}Rv MTB strains with *MIC* values of 0.39 and of 3.12 μg/ml, respectively. It is also interesting to note that chlorobenzoyl

derivative **12** was fourfold more active, against MDR (*MIC* 0.78 μg/ml) than **13**, which has a nitrobenzoyl substituent (*MIC* 3.12 μg/ml against MDR). In contrast, the only assayed 6-aromatic ester (**14**, an isomer of **13**) was inactive. Lead royleanone **5**, and its esters **9**, and **11**–**13** are more active than the first-line antituberculous drugs isoniazid (*MIC* 4 μg/ml) and rifampicin (*MIC* 16 μg/ml) against the MDR-MTB strain. In these tested derivatives, **9**–**14**, there is no correlation between lipophilicity and activity; however, the presence of a *p*-MeO group in the benzoyl moiety (*i.e.*, **11**) afforded a more potent anti-MTB derivative (*Table*).

Apolar alkyl esters of **5** are less active than the parent diterpene **5**, the aromatic esters **9**, **11**–**13**, and isoniazid, the positive control (*Table*). Neither diesters of **5**, *i.e.*, 6,12-*O*-diacetyl and 6,12-*O*-dipropanoyl derivatives, **15** and **16**, respectively, nor 6-*O*-acetyl, 6-*O*-propanoyl, and 6-*O*-butanoyl derivatives, **17**, **18**, and **19**, respectively, showed significant antimycobacterial-activity enhancement, even though the 6-*O*-derivatives of **5** were slightly active against the MDR strain (*MIC* values ranging from 6.25 to 12.5 μg/ml). These results show that the insertion of an acyloxy chain at C(6) did not provide active compounds (*Table*), nevertheless the propanoyl group seems to increase the activity against MDR strain (*MIC* 6.25 μg/ml).

Surprisingly, all the royleanones are much more potent against MDR than against sensitive H_{37}Rv strains, with the exception of the diterpenoid **11**.

5. In vitro *Cytotoxicity.* In accord with the purpose of searching for potential antimycobacterial lead diterpenes, the cytotoxic bioactivity against the two eukaryotic 3T3 and *Vero* cell lines were also investigated, according to the recommendations of the *Tuberculosis Drug Screening Program* [18]. In this work, we found similar toxicities for all compounds against both cell lines.

Abietanes **5**, **9**, **10**–**19**, and horminone (**7**) did not show good *in vitro* cytotoxic profiles either in 3T3 cells (GI_{50} range 1.44 to 18.58 μg/ml) or against *Vero* cells (GI_{50} range 2.58 to 16.6 μg/ml), thus revealing high-to-moderate cytotoxicity [19]. It can be suggested that the chromophore present in the rings *B* and *C* of these royleanones contributes both to anti-MDR activity and cytotoxicity, once the more potent anti-MTB abietanoids exhibited also the higher cytotoxicities.

Finally, the anti-TB selectivity (therapeutic) indexes for both MTB assayed strains were determined towards both 3T3 and *Vero* cell lines. The majority of the diterpenoids were non-selective ($SI < 1$), and only **5** and its derivatives **11** and **12** exhibited selectivity (*SI* 3.2 and 6.62, resp.) for both the tested MDR strain *vs.* both cell lines (*Table*).

Even though the mode of action of the quinonic diterpenoids mentioned above was not studied, it should be quite complex, targeting different biological systems of both prokaryotic and eukaryotic cells [10]. In the literature [20] it is mentioned that the biological activity of some similarly substituted quinones, mostly 1,4-naphthoquinones, is related with the ability to accept electrons to forming radical anions and further to generate reactive oxygen species (ROSs) causing cell damage. Cellular damage has also been attributed to alkylation, whenever quinones are activated inside the cell, by binding to cellular nucleophiles (proteins, DNA) forming *Michael* adducts [10][20]. Other mechanisms related with extrusion pumps or cell-penetration abilities have also been described [20], as well as the interaction with the potential *M. tuberculosis* target Mtr (the NADPH-dependent enzyme mycothiol disulfide reductase) [9].

Conclusions. – Screening antimycobacterial activities of 7α-acetoxy-6β-hydroxyroyleanone (**5**) and its derivatives led to the conclusion, considering potency and selectivity observed, that 12-*O*-(4-methoxybenzoyl) derivative **11** may become a valid lead for further modifications to obtain compounds with better potency, lower cytotoxicity, and better selectivity. Similarly, 12-*O*-(4-chlorobenzoyl) derivative **12** and surely metabolite **5** may be suitable to prepare more derivatives with a valid therapeutic profile. Certainly, further chemical transformations are required to obtain, at least, other derivatives with adequate *SI* values (>10) [18], seeking to determine their possible inhibition of MTB in intracellular macrophage infection [18]. Besides this, there is a need to extend antimycobacterial and cytotoxicity studies, namely testing other resistant *Mycobacterium* strains, evaluating toxicity on prokaryotic Mycobacteriae, and analyzing selectivity differences of MDR- *vs.* H_{37}Rv-sensitive strains. The final goal will be to propose derivatives of these natural compounds, *i.e.*, royleanones from *Plectranthus* spp., as a new class of antimycobacterial agents.

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Experimental Part

General. M.p.: *Kofler* block; uncorrected. Optical rotations: in $CHCl_3$ soln., *Perkin-Elmer 241 MC* polarimeter. IR: in KBr, *Perkin-Elmer Spectrum One* spectrophotometer. ^{1}H- and ^{13}C-NMR spectra: at r.t.; *Varian INOVA-400* spectrometer, equipped with a 5-mm gradient inverse detection probe, operating at 399.90 and 100.55 MHz, resp.; chemical shifts with respect to the residual $CHCl_3$ signal (δ 7.25) and to the solvent signals (δ 77.00), resp.; all the ^{1}H- and ^{13}C-NMR assignments were in agreement with COSY, HSQC, and HMBC experiments. MS: positive EI mode, 70 eV, *Hewlett-Packard 5973* spectrometer.

Plant Material. Natural diterpenes extracted from *Plectranthus fruticosus* L'HÉRIT: ent-*kaur-15-en-19-oic acid* (**1**), ent-*12β-acetoxykaur-16-en-19-oic acid* (**2**), and ent-*7β-hydroxy-15β,16β-epoxykauran-19-oic acid* (**3**) [11]; from *P. ornatus* CODD.: *rhinocerotinoic acid* (**4**; *(13*E*)-7-oxolabda-8,13-dien-15-oic acid*) [12]; and from *P. grandidentatus* GÜRKE: *7α-acetoxy-6β-hydroxyroyleanone* (**5**), *6β,7α-dihydroxyroyleanone* (**6**), *horminone* (**7**), and *6,7-dehydroroyleanone* (**8**) [8][12][13]. Compound **5**, used as starting material for the esterification reactions, was re-isolated from an acetone extract of *P. grandidentatus*, which was kept in the Faculty of Pharmacy Hortum.

Hemisynthesis of Royleanone Derivatives **9**–**19**. A soln. of **5** (*=(6β,7α)-6,12-dihydroxy-11,14-dioxoabieta-8,12-dien-7-yl acetate*) in pyridine (Py) and eventually CH_2Cl_2, and the suitable benzoyl chloride or appropriate alkyl anhydride was allowed to stand under adequate conditions. The mixture was then diluted with CH_2Cl_2 and successively washed with H_2O and NaOH soln. (5%), and the org. layer was dried (anh. Na_2SO_4). The residue was purified by prep. TLC (silica gel; CH_2Cl_2/MeCOMe 98:2) to afford **9**–**19** [6]. The conditions to obtain each one of the derivatives were as follows. 30.0 mg of **5**, BzOCl (2 ml), Py (0.5 ml), r.t., 3 d, gave **9** (22.4% yield); 20.0 mg of **5**, 4-chlorobenzoyl chloride (32 µl), Py (2 ml), CH_2Cl_2 (2 ml), r.t., 7 d, gave **10** (66.4%, less polar compound) and **12** (27.9%); 32.2 mg of **5**, 4-methoxybenzoyl chloride (27 µl), Py (2 ml), CH_2Cl_2 (2 ml), r.t, 24 h, yielded 96.6% of **11**; 30.3 mg of **5**, 4-nitrobenzoyl chloride (141 mg), Py (2 ml), CH_2Cl_2 (2 ml), r.t., 40 min, gave **13** (16.8%, most polar compound) and 11.9% of **14**; 30.6 mg of **5**, Ac_2O (2 ml), Py (2 ml), CH_2Cl_2 (1 ml), r.t., 24 h, yielded **15** (65.6%) and 8.5% of **17** (most polar compound); 40.5 mg of **5**, propanoic anhydride (1 ml), Py (1 ml), 0°, 7 d, gave 73.5% of **16** and **18** (8.6%, most polar compound); 15.0 mg of **5**, butanoic anhydride (1 ml), Py (1 ml), 0°, 24 h afforded 35.2% of **19**.

*7α-Acetoxy-6β-benzoyloxy-12-*O*-benzoylroyleanone* (*=(6β,7α)-7-(Acetyloxy)-11,14-dioxoabieta-8,12-diene-6,12-diyl Dibenzoate*; **9**). Yellow quadrangular plates (AcOEt/pentane). M.p. 238–241°.

$[\alpha]_D^{18} = -29.2$ ($c = 0.24$, $CHCl_3$). IR (KBr): 3062, 2962, 2933, 2867, 1755, 1738, 1726, 1666, 1612, 1601, 1452, 1371, 1315, 1269, 1220, 1177, 1138, 1093, 1061, 1016, 932, 887, 808, 754, 712. ^{1}H-NMR (400 MHz, $CDCl_3$): 8.15 (*dt*, *J*(2′,3′) = 7.6, *J*(2′,4′) = 2.0, H−C(2′), H−C(6′) of BzO−C(6)); 7.99 (*dt*, *J*(2′,3′) = 7.2, *J*(2′,4′) = 1.2, H−C(2′), H−C(6′) of BzO−C(12)); 7.69–7.40 (*m*, H−C(3′), H−C(4′), H−C(5′) of BzO−C(6), BzO−C(12)); 5.90 (*dd*, *J*(7*β*,6*α*) = 2.0, *J*(7*β*,5*α*) = 0.9, H_β−C(7)); 5.77 (*dd*, *J*(6*α*,7*β*) = 2.0, *J*(6*α*,5*α*) = 1.6, H_α−C(6)); 3.17 (*sept.*, *J*(15,16(17)) = 7.1, H−C(15)); *ca.* 2.58 (overlapped, H_β−C(1)), 2.10 (*s*, MeCOO); *ca.* 1.78 (overlapped, H_β−C(2)); 1.77 (*s*, Me(20)); 1.69 (br. *d*, *J*(5*α*,6*α*) = 1.6, H_α−C(5)); 1.58 (overlapped, H_α−C(2)); 1.49 (*ddd*, *J*(3*β*,3*α*) = 13.2, *J*(3*β*,2*α*) = 3.6, *J*(3*β*,2*β*) = 2.8, H_β−C(3)); 1.29 (*td*, *J*(1*α*,1*β*) = *J*(1*α*,2*β*) = 13.2, *J*(1*α*,2*α*) = 3.8, H_α−C(1)); *ca.* 1.38 (overlapped, H_α−C(3)); 1.21 (*d*, *J*(17,15) = 7.1, Me(17)); 1.19 (*d*, *J*(16,15) = 7.1, Me(16)); 1.06 (*s*, Me(18)); 0.99 (*s*, Me(19)). ^{13}C-NMR (100 MHz, $CDCl_3$): 185.3 (C(14)); 180.0 (C(11)); 168.1 (Me*C*OO); 165.3 (C_6H_5COO−C(6)); 164.0 (C(7′) of C_6H_5COO−C(12)); 152.5 (C(9)); 150.0 (C(12)); 140.0 (C(13)); 136.2 (C(8)); 134.3 (H−C(4′) of BzO−C(12)); 133.1 (C(4′) of BzO−C(6)); 130.5 (C(3′), C(5′) of BzO−C(12)); 129.8 (C(3′), C(5′) of BzO−C(6)); 129.7 (C(1′) of BzO−C(6)); 128.8 (C(2′), C(6′) of BzO−C(12)); 128.5 (C(2′), C(6′) of BzO−C(6)); 127.9 (C(1′) of BzO−C(12)); 68.4 (C(6)), 65.2 (C(7)); 49.3 (C(5)); 42.5 (CH_2(3)); 38.8 (C(10)); 38.4 (CH_2(1)); 33.8 (C(4)), 33.3 (Me(18)); 25.2 (C(15)), 23.2 (Me(19)); 22.2 (Me(20)), 20.9 (*Me*COO); 20.4 (Me(16)), 20.0 (Me(17)), 18.8 (CH_2(2)). EI-MS: 539 (1, $[M - AcO]^+$), 510 (1), 451 (2), 434 (3, $[M - AcO - C_6H_5CO]^+$), 406 (20), 391 (5), 313 (7), 284 (6), 269 (12), 105 (100, $[C_6H_5CO]^+$), 77 (15).

*7α-Acetoxy-6α-[(4-chlorobenzoyl)oxy]-12-*O*-(4-chlorobenzoyl)royleanone* (=*(6β,7α)-7-(Acetyloxy)-11,14-dioxoabieta-8,12-diene-6,12-diyl Bis(4-chlorobenzoate)*; **10**). Yellow amorphous powder. $[\alpha]_D^{22} = -40.8$ ($c = 0.262$, $CHCl_3$). IR (KBr): 3074, 3097, 2961, 2932, 2867, 1751, 1726, 1669, 1594, 1488, 1402, 1371, 1268, 1216, 1173, 1137, 1092, 1064, 1012, 931, 887, 847, 757, 732. ^{1}H-NMR (400 MHz, $CDCl_3$): 8.08 (*d*, $J_o = 8.6$, H−C(2′), H−C(6′) of 4-Cl−C_6H_4COO−C(12)); 7.92 (*d*, $J_o = 8.6$, H−C(2′), H−C(6′) of 4-Cl−C_6H_4COO−C(6)); 7.51 (*d*, $J_o = 8.6$, H−C(3′), H−C(5′) of 4-Cl−C_6H_4COO−C(12)); 7.39 (*d*, $J_o = 8.6$, H−C(3′), H−C(5′) of 4-Cl−C_6H_4COO−C(6)); 5.88 (*d*, *J*(7*β*,6*α*) = 1.6, H_β−C(7)); 5.76 (*t*, *J*(6*α*,7*β*) = *J*(6*α*,5*α*) = 1.6, H_α−C(6)); 3.17 (*sept.*, *J*(15,16(17)) = 7.0, H−C(15)); 2.58 (overlapped, H_β−C(1)); 2.10 (*s*, MeCOO); 1.79 (*qt*, *J*(2*β*,1*α*) = *J*(2*β*,2*α*) = *J*(2*β*,3*α*) = 14.0, *J*(2*β*,1*β*) = *J*(2*β*,3*β*) = 3.7, H_β−C(2)); 1.74 (*s*, Me(20)); 1.70 (*d*, *J*(5*α*,6*α*) = 1.6, H_α−C(5)); 1.60 (*dquint.*, *J*(2*α*,2*β*) = 14.0, *J*(2*α*,1*α*) = *J*(2*α*,1*β*) = *J*(2*α*,3*α*) = *J*(2*α*,3*β*) = 3.6, H_α−C(2)); 1.48 (*dtd*, *J*(3*β*,3*α*) = 13.4, *J*(3*β*, 2*β*) = 3.7, *J*(3*β*,2*α*) = 3.6, *J*(3*β*,1*β*) = 1.0, H_β−C(3)); 1.30 (overlapped, H_α−C(1)); 1.28 (overlapped, H_α−C(3)); 1.21 (*d*, *J*(16,15) = 7.0, Me(16)); 1.19 (*d*, *J*(17,15) = 7.0, Me(17)); 1.06 (*s*, Me(18)); 0.97 (*s*, Me(19)). ^{13}C-NMR (100 MHz, $CDCl_3$): 185.2 (C(14)); 179.7 (C(11)); 168.1 (Me*C*OO); 164.5 (4-Cl−C_6H_4COO−C(6)), 163.2 (4-Cl−C_6H_4COO−C(12)); 152.4 (C(9)); 149.5 (C(12)); 141.0 (C(4′) of 4-Cl−C_6H_4COO−C(12)); 139.8 (C(13)); 139.4 (C(4′) of 4-Cl−C_6H_4COO−C(6)); 135.7 (C(8)); 131.8 (C(2′), C(6′) of 4-Cl−C_6H_4COO−C(12)); 131.2 (C(2′), C(6′) of 4-Cl−C_6H_4COO−C(6)); 129.2 (C(3′), C(5′) of 4-Cl−C_6H_4COO−C(12)); 128.9 (C(3′), C(5′) of 4-Cl−C_6H_4COO−C(6)); 128.1 (C(1′) of 4-Cl−C_6H_4COO−C(6)); 126.4 (C(1′) of 4-Cl−C_6H_4COO−C(12)); 68.7 (C(6)); 65.2 (C(7)); 49.3 (C(5)); 42.5 (C(3)); 38.8 (C(10)); 38.4 (C(1)); 33.8 (C(4)); 33.3 (Me(18)); 25.2 (C(15)); 23.2 (Me(19)); 22.2 (Me(20)); 20.4 (Me(16)); 20.2 (Me(17)); 18.8 (CH_2(2)). EI-MS: 607 (3, $[M - AcO]^+$), 468 (3, $[M - AcO - OC - C_6H_5Cl]^+$), 440 (9), 313 (6), 269 (9), 139 (100, [4-Cl−C_6H_4−CO]$^+$), 111 (16).

*7α-Acetoxy-6β-hydroxy-12-*O*-(4-methoxybenzoyl)royleanone* (=*(6β,7α)-7-(Acetyloxy)-6-hydroxy-11,14-dioxoabieta-8,12-dien-12-yl 4-Methoxybenzoate*; **11**). Yellow fine needles (AcOEt/pentane). M.p. 215–218°. $[\alpha]_D^{18} = +43.9$ ($c = 0.353$, $CHCl_3$). IR (KBr): 3474, 2962, 2931, 2867, 1745, 1724, 1668, 1607, 1581, 1512, 1463, 1371, 1256, 1221, 1168, 1141, 1100, 1063, 1019, 1002, 963, 936, 898, 843, 816, 759, 746. ^{1}H-NMR (400 MHz, $CDCl_3$): 8.09 (*d*, *J*(2′,3′(6′,5′)) = 8.9, H−C(2′), H−C(6′)); 6.98 (*d*, *J*(3′,2′(5′,6′)) = 8.9, H−C(3′), H−C(5′)); 5.67 (*d*, *J*(7*β*,6*α*) = 1.7, H−C(7*β*)); 4.32 (*dd*, *J*(6*α*,5*α*) = 1.9, *J*(6*α*,7*β*) = 1.7, H_α−C(6)); 3.89 (*s*, MeO); 3.17 (*sept.*, *J*(15,16(17)) = 7.0, H−C(15)); 2.49 (br. *m*, H_β−C(1)); 2.06 (*s*, MeCOO); 1.79 (*qt*, *J*(2*β*,1*α*) = *J*(2*β*,2*α*) = *J*(2*β*,3*α*) = 13.7, *J*(2*β*,1*β*) = *J*(2*β*,3*β*) = 3.4, H_β−C(2)); 1.62 (*s*, Me(20)); 1.53 (*dquint.*, *J*(2*α*,2*β*) = 13.7, *J*(2*α*,1*α*) = *J*(2*α*,1*β*) = *J*(2*α*,3*α*) = *J*(2*α*,3*β*) = 3.7, H_α−C(2)); 1.45 (*ddd*, *J*(3*β*,3*α*) = 13.2, *J*(3*β*,2*α*) = 3.7, *J*(3*β*,2*β*) = 3.4, H_β−C(3)); 1.36 (*d*, *J*(5*α*,6*α*) = 1.9, H_α−C(5)); 1.23 (overlapped, H_α−C(1)); 1.22 (*d*, *J*(17,15) = 7.1, Me(17)); 1.21 (*s*, Me(19)); 1.19 (overlapped, H_α−C(3)); 1.19 (*d*, *J*(16,15) = 7.0, Me(16)); 0.94 (*s*, Me(18)). ^{13}C-NMR (100 MHz, $CDCl_3$): 185.9 (C(14)); 179.9

(C(11)); 169.7 (Me*C*OO); 164.4 (C_6H_4*C*O); 163.7 (C(4′)); 153.0 (C(9)); 149.9 (C(12)); 139.5 (C(13)); 135.5 (C(8)); 132.7 (H–C(2′), H–C(6′)); 120.2 (C(1′)); 114.1 (H–C(3′)), H–C(5′)); 68.9 (C(7)); 67.3 (C(6)); 55.6 (MeO); 49.8 (C(5)); 42.3 (C(3)); 38.9 (C(10)); 38.3 (C(1)); 33.7 (Me(18)); 33.5 (C(4)); 25.1 (C(15)); 23.8 (Me(19)); 21.7 (Me(20)); 20.9 (*Me*COO); 20.4 (Me(16)); 20.2 (Me(17)); 18.9 (C(2)). EI-MS: 524 (0.5, M^+), 496 (1, $[M-CO]^+$), 464 (0.5, $[M-AcOH]^+$), 436 (3, $[M-AcOH-CO]^+$), 137 (2), 136 (17), 135 (100, $[MeOC_6H_4CO]^+$), 93 (1), 92 (3), 91 (1), 79 (1), 78 (1), 77 (6), 55 (1).

*7α-Acetoxy-6β-hydroxy-12-*O*-(4-chlorobenzoyl)royleanone* (*=(6β,7α)-7-(Acetyloxy)-6-hydroxy-11,14-dioxoabieta-8,12-dien-12-yl 4-Chlorobenzoate*; **12**). Yellow fine needles (AcOEt/pentane). M.p. 221–223°. $[\alpha]_D^{18} = +44.8$ ($c = 0.355$, $CHCl_3$). IR (KBr): 3485, 2962, 2929, 2867, 1749, 1732, 1667, 1596, 1461, 1371, 1252, 1220, 1141, 1094, 1066, 1008, 896, 844, 749. ^{1}H-NMR (400 MHz, $CDCl_3$): 8.07 (*d*, $J_o = 8.5$, H–C(2′), H–C(6′)); 7.49 (*d*, $J_o = 8.5$, H–C(3′), H–C(5′)); 5.68 (*dd*, $J(7\beta,6\alpha) = 2.0$, $J(7\beta,5\alpha) = 0.6$, H_β–C(7)); 4.33 (*dd*, $J(6\alpha,7\beta) = 2.0$, $J(6\alpha,5\alpha) = 1.6$, H_α–C(6)); 3.17 (*sept.*, $J(15,16(17)) = 7.1$, H–C(15)); 2.49 (*m*, H_β–C(1)); 2.06 (*s*, (MeCOO); 1.92 (br., OH); 1.80 (*qt*, $J(2\beta,1\alpha) = J(2\beta,2\alpha) = J(2\beta,3\alpha) = 13.6$, $J(2\beta,1\beta) = J(2\beta,3\beta) = 3.6$, H_β–C(2)); 1.62 (*s*, Me(20)); 1.54 (*dquint.*, $J(2\alpha,2\beta) = 13.6$, $J(2\alpha,1\alpha) = J(2\alpha,1\beta) = J(2\alpha,3\alpha) = J(2\alpha,3\beta) = 3.7$, H_α–C(2)); 1.45 (*dtd*, $J(3\beta,3\alpha) = 13.3$, $J(3\beta,2\alpha) = 3.7$, $J(3\beta,2\beta) = 3.6$, $J(3\beta,1\beta) = 0.9$, H_β–C(3)); 1.36 (*dd*, $J(5\alpha,6\alpha) = 1.6$, $J(5\alpha,7\beta) = 0.6$, H_α–C(5)); 1.24 (*s*, Me(19)); 1.24 (overlapped, H_α–C(1)); 1.21 (*d*, $J(16(17),15) = 7.1$, Me(16), Me(17)); 1.20 (overlapped, H_α–C(3)); 0.94 (*s*, Me(18)). ^{13}C-NMR (100 MHz, $CDCl_3$): 185.7 (C(14)); 179.6 (C(11)); 169.7 (Me*C*OO); 163.2 (C_6H_4*C*O); 152.5 (C(9)); 149.8 (C(12)); 140.9 (C(4′)); 139.5 (C(13)); 135.7 (C(8)), 131.8 (C(2′), C(6′)); 129.2 (C(3′), C(5′)); 126.5 (C(1′)); 68.9 (C(7)); 67.2 (C(6)); 49.8 (C(5)); 42.3 (C(3)); 38.9 (C(10)); 38.3 (C(1)); 33.7 (C(4)); 33.5 (Me(18)); 25.2 (H–C(15)); 23.8 (Me(19)); 21.7 (Me(20)); 20.9 (*Me*COO); 20.4 (Me(16)); 20.2 (Me(17)); 18.9 (C(2)). EI-MS: 486 (1, $[M-CH_2{=}C{=}O]^+$), 470 (3, $[M-AcOH]^+$), 468 (7, $[M-AcOH]^+$), 329 (9), 302 (8), 301 (8), 283 (6), 269 (7), 141 (43), 140 (10), 139 (100), 111 (14), 91 (2), 77 (1).

*7α-Acetoxy-6β-hydroxy-12-*O*-(4-nitrobenzoyl)royleanone* (*=(6β,7α)-7-(Acetyloxy)-6-hydroxy-11,14-dioxoabieta-8,12-dien-12-yl 4-Nitrobenzoate*; **13**). Yellow rectangular plates (AcOEt/pentane). M.p. 217–219°. $[\alpha]_D^{18} = +36.2$ ($c = 0.174$, $CHCl_3$). IR (KBr): 3452, 3109, 3056, 2956, 2933, 2867, 1752, 1733, 1668, 1608, 1530, 1462, 1369, 1347, 1320, 1252, 1218, 1140, 1101, 1070, 1011, 966, 932, 897, 869, 854, 712. ^{1}H-NMR (400 MHz, $CDCl_3$): 8.37 (*d*, $J(2',3'(6',5')) = 9.0$, H–C(2′), H–C(6′)); 8.32 (*d*, $J(3',2'(5',6')) = 9.0$, H–C(3′), H–C(5′)); 5.68 (*d*, $J(7\beta,6\alpha) = 1.7$, H_β–C(7)); 4.34 (*dd*, $J(6\alpha,5\alpha) = 1.9$, $J(6\alpha,7\beta) = 1.7$, H_α–C(6)); 3.18 (*sept.*, $J(15,16(17)) = 7.1$, H–C(15)); 2.49 (*m*, H_β–C(1)); 2.07 (*s*, MeCOO); 1.81 (*qt*, $J(2\beta,1\alpha) = J(2\beta,2\alpha) = J(2\beta,3\alpha) = 13.6$, $J(2\beta,3\beta) = 3.4$, $J(2\beta,1\beta) = 3.2$, H_β–C(2)); 1.62 (*s*, Me(20)); 1.55 (*dquint.*, $J(2\alpha,2\beta) = 13.6$, $J(2\alpha,3\beta) = 3.8$, $J(2\alpha,1\alpha) = J(2\alpha,1\beta) = J(2\alpha,3\alpha) = 3.7$, H_α–C(2)); 1.46 (*ddd*, $J(3\beta,3\alpha) = 13.4$, $J(3\beta,2\alpha) = 3.8$, $J(3\beta,2\beta) = 3.4$, H_β–C(3)); 1.37 (*d*, $J(5\alpha,6\alpha) = 1.9$, H_α–C(5)); *ca.* 1.23 (overlapped, H_α–C(1)); *ca.* 1.22 (overlapped, Me(16), Me(17)); 1.22 (*s*, Me(19)); *ca.* 1.20 (overlapped, H_α–C(3)); 0.95 (*s*, Me(18)). ^{13}C-NMR (100 MHz, $CDCl_3$): 185.5 (C(14)); 179.2 (C(11)); 169.6 (Me*C*OO); 164.6 (C_6H_4*C*O); 152.9 (C(9)); 151.2 (C(4′)); 149.7 (C(12)); 139.8 (C(13)); 135.9 (C(8)); 133.4 (C(1′)); 131.6 (C(2′), C(6′)); 123.9 (C(3′), C(5′)); 68.8 (C(7)); 67.2 (C(6)); 49.8 (C(5)); 42.2 (C(3)); 39.0 (C(10)); 38.4 (C(1)); 33.7 (Me(18)); 33.5 (C(4)); 25.3 (C(15)); 23.8 (Me(19)); 21.7 (Me(20)); 20.9 (*Me*COO); 20.4 (Me(16), Me(17)); 18.9 (C(2)). EI-MS: 539 (1, M^+), 521 (6, $[M-H_2O]^+$), 506 (2, $[M-H_2O-Me]^+$), 497 (14), 479 (15, $[M-AcOH]^+$), 450 (10), 329 (18), 283 (15), 150 (100, $[O_2N-C_6H_4CO]^+$), 120 (63), 92 (13), 76 (8), 55 (7).

7α-Acetoxy-6β-(4-nitrobenzoyl)oxyroyleanone (*=(6β,7α)-7-(Acetyloxy)-12-hydroxy-11,14-dioxo-abieta-8,12-dien-6-yl 4-Nitrobenzoate*; **14**). Red amorphous solid. $[\alpha]_D^{18} = -42.5$ ($c = 0.113$, $CHCl_3$). IR (KBr): 3383, 3109, 3080, 3056, 2962, 2931, 2867, 1753, 1732, 1643, 1609, 1531, 1462, 1374, 1347, 1270, 1216, 1168, 1146, 1098, 1016, 981, 873, 756, 719. ^{1}H-NMR (400 MHz, $CDCl_3$): 8.27 (*d*, $J(2',3'(6',5')) = 9.0$, H–C(2′), H–C(6′)); 8.14 (*d*, $J(3',2'(5',6')) = 9.0$, H–C(3′), H–C(5′)); 7.19 (*s*, OH); 5.87 (*d*, $J(7\beta,6\alpha) = 1.6$, H_β–C(7)); 5.78 (*t*, $J(6\alpha,5\alpha) = J(6\alpha,7\beta) = 1.6$, H_α–C(6)); 3.15 (*sept.*, $J(15,16(17)) = 7.0$, H–C(15)), 2.75 (br. *d*, $J(1\beta,1\alpha) = 12.9$, H_β–C(1)); 2.09 (*s*, MeCOO); 1.84 (*qt*, $J(2\beta,1\alpha) = J(2\beta,2\alpha) = J(2\beta,3\alpha) = 14.0$, $J(2\beta,1\beta) = J(2\beta,3\beta) = 3.5$, H_β–C(2)); 1.73 (*s*, Me(20)); 1.70 (*d*, $J(5\alpha,6\alpha) = 1.6$, H_α–C(5)); 1.64 (*dquint.*, $J(2\alpha,2\beta) = 14.0$, $J(2\alpha,1\alpha) = J(2\alpha,1\beta) = J(2\alpha,3\alpha) = J(2\alpha,3\beta) = 3.4$, H_α–C(2)); 1.52 (br. *d*, $J(3\beta,3\alpha) = 13.7$, H_β–C(3)); 1.28 (overlapped, H_α–C(3)); 1.25 (overlapped, H_α–C(1)); 1.21 (*d*, $J(16,15) = 7.0$, Me(16)); 1.17 (*d*, $J(17,15) = 7.0$, Me(17)); 1.05 (*s*, Me(19)); 0.98 (*s*, Me(18)). ^{13}C-NMR (100 MHz, $CDCl_3$): 185.2

(C(14)); 183.1 (C(11)); 168.1 (Me*C*OO); 163.6 (C_6H_4CO); 150.9 (C(4′)); 150.6 (C(12)); 149.2 (C(9)); 136.7 (C(8)); 135.0 (C(1′)); 130.9 (C(2′), C(6′)); 125.0 (C(13)); 123.7 (C(3′), C(5′)); 69.4 (C(6)); 64.9 (C(7)); 49.2 (C(5)); 42.4 (C(3)); 38.5 (C(1)); 38.4 (C(10)); 33.7 (Me(18)); 33.3 (C(4)); 24.2 (C(15)); 23.3 (Me(19)); 22.0 (Me(20)); 20.8 (*Me*COO); 19.8 (Me(16)); 19.6 (Me(17)); 18.9 (C(2)). EI-MS: 539 (0.5, M^+), 497 (1, $[M-CH_2CO]^+$), 479 (7, $[M-AcOH]^+$), 372 (1, $[M-HOOC-Ph-NO_2]^+$), 330 (43, $[M-CH_2CO-HOOC-Ph-NO_2]^+$), 314 (100), 298 (46), 283 (20), 271 (23), 245 (68), 232 (84), 213 (25), 201 (18), 187 (24), 167 (54).

*6β,7α-Diacetoxy-12-*O*-acetylroyleanone* (*=(6β,7α)-11,14-Dioxoabieta-8,12-diene-6,7,12-triyl Triacetate*; **15**). M.p., $[\alpha]_D$, IR, and MS data identical to those reported in [15]. ^{1}H-NMR (400 MHz, $CDCl_3$): 5.68 (*d*, *J*(7*β*,6*α*) = 2.0, H_β–C(7)); 5.48 (*dd*, *J*(6*α*,7*β*) = 2.0, *J*(6*α*,5*α*) = 1.6, H_α–C(6)); 3.09 (*sept.*, *J*(15,16(17)) = 7.0, H–C(15)); 2.52 (br. *d*, *J*(1*β*,1*α*) = 12.5, H_β–C(1)); 2.34 (*s*, MeCOO–C(12)); 2.04 (*s*, MeCOO–C(6)); 2.03 (*s*, MeCOO–C(7)); 1.78 (*qt*, *J*(2*β*,1*α*) = *J*(2*β*,2*α*) = *J*(2*β*,3*α*) = 14.0, *J*(2*β*,1*β*) = *J*(2*β*,3*β*) = 3.7, H_β–C(2)); 1.59 (*s*, Me(20)); 1.56 (overlapped, H_α–C(2)); 1.52 (*d*, *J*(5*α*,6*α*) = 1.6, H_α–C(5)); 1.45 (*dddd*, *J*(3*β*,3*α*) = 13.4, *J*(3*β*,2*β*) = 3.7, *J*(3*β*,2*α*) = 3.3, *J*(3*β*,1*β*) = 1.4, H_β–C(3)); 1.24 (overlapped, H_α–C(1)); 1.23 (overlapped, H_α–C(3)); 1.18 (*d*, *J*(16,15) = 7.0, Me(16)); 1.17 (*d*, *J*(17,15) = 7.0, Me(17)), 0.98 (*s*, Me(18)); 0.97 (*s*, Me(19)). ^{13}C-NMR (100 MHz, $CDCl_3$): 185.4 (C(14)); 179.7 (C(11)); 169.0 (Me*C*OO–C(6)); 168.3 (Me*C*OO–C(12)); 168.2 (Me*C*OO–C(7)); 152.2 (C(9)); 149.3 (C(12)); 139.4 (C(13)); 135.7 (C(8)); 67.2 (C(6)); 65.2 (C(7)); 49.0 (C(5)); 42.4 (C(3)); 38.9 (C(10)); 38.3 (C(1)); 33.6 (C(4)); 33.2 (Me(18)); 25.2 (C(15)); 22.9 (Me(19)); 21.4 (Me(20)); 21.3 (*Me*COO–C(7)); 20.8 (*Me*COO–C(6)); 20.4 (*Me*COO–C(7)); 20.2 (Me(16)); 20.2 (Me(17)); 18.8 (C(2)).

*7α-Acetoxy-6β-(propanoyloxy)-12-*O*-propanoylroyleanone* (*=(6β,7α)-7-(Acetyloxy)-11,14-dioxoabieta-8,12-diene-6,12-diyl Dipropanoate*; **16**). Yellow rectangular plates (AcOEt/pentane). M.p. 137–139°. $[\alpha]_D^{18} = +29.9$ (*c* = 0.257, $CHCl_3$). IR (KBr): 2962, 2940, 2873, 1776, 1764, 1744, 1668, 1612, 1462, 1375, 1275, 1210, 1172, 1136, 1113, 1079, 1027, 931, 893, 804, 750. ^{1}H-NMR (400 MHz, $CDCl_3$): 5.68 (*dd*, *J*(7*β*,6*α*) = 2.0, *J*(7*β*,5*α*) = 0.5, H_β–C(7)); 5.49 (br. *dd*, *J*(6*α*,7*β*) = 2.0, *J*(6*α*,5*α*) = 1.6, H_α–C(6)); 3.09 (*sept.*, *J*(15,16(17)) = 7.1, H–C(15)); 2.66 (*dq*, *J*(2′A,2′B) = 17.2, *J*(2′A,3′) = 7.6, $MeCH_AH_BCOO$–C(12)); 2.61 (*dq*, *J*(2′B,2′A) = 17.2, *J*(2′B,3′) = 7.6, $MeCH_AH_BCOO$–C(12)); 2.51 (br. *d*, *J*(1*α*,1*β*) = 12.4, H_β–C(1)); 2.32 (*dq*, *J*(2′A,2′B) = 16.8, *J*(2′A,3′) = 7.6, $MeCH_AH_BCOO$–C(6)); 2.25 (*q*, *J*(2′B,2′A) = 16.8, *J*(2′B,3′) = 7.6, $MeCH_AH_BCOO$–C(6)); 2.04 (*s*, MeCOO); 1.77 (*dddt*, *J*(2*β*,2*α*) = 14.4, *J*(2*β*,1*α*) = *J*(2*β*,3*α*) = 13.8, *J*(2*β*,1*β*) = *J*(2*β*,3*β*) = 3.6, H_β–C(2)); 1.59 (*s*, Me(20)); 1.55 (*dquint.*, *J*(2*α*,2*β*) = 14.4, *J*(2*α*,1*α*) = *J*(2*α*,1*β*) = *J*(2*α*,3*α*) = *J*(2*α*,3*β*) = 3.6, H_α–C(2)); 1.53 (*dd*, *J*(5*α*,6*α*) = 1.6, *J*(5*α*,7*β*) = 0.5, H_α–C(5)); 1.44 (*dtd*, *J*(3*β*,3*α*) = 13.8, *J*(3*β*,2*α*) = *J*(3*β*,2*β*) = 3.6, *J*(1*β*,3*β*) = 1.6, H_β–C(3)); 1.27 (*t*, *J*(3″,2″A) = *J*(3″,2″B) = 7.6, *Me*CH_2COO–C(12)); 1.24 (overlapped, H_α–C(1)); 1.23 (overlapped, H_α–C(3)); 1.17 (*d*, *J*(16,15) = 7.1, Me(16)); 1.16 (*d*, *J*(17,15) = 7.1, Me(17)); 1.11 (*t*, *J*(3′,2′A) = *J*(3′,2′B) = 7.6, *Me*CH_2COO–C(6)); 0.98 (*s*, Me(18)); 0.96 (*s*, Me(19)). ^{13}C-NMR (100 MHz, $CDCl_3$): 185.4 (C(14)); 179.7 (C(11)); 172.5 (MeCH_2*C*OO–C(6)); 171.7 (MeCH_2*C*OO–C(12)); 168.1 (Me*C*OO); 152.3 (C(9)); 149.4 (C(12)); 139.3 (C(13)); 135.6 (C(8)); 67.2 (C(6)); 65.3 (C(7)); 49.0 (C(5)); 42.4 (C(3)); 38.8 (C(10)); 38.3 (C(1)); 33.6 (C(4)); 33.2 (Me(18)); 27.8 (Me*C*H_2COO–C(6)); 27.2 (Me*C*H_2COO–C(12)); 25.1 (H–C(15)); 23.0 (Me(19)); 21.5 (Me(20)); 20.7 (*Me*COO); 20.2 (Me(16)); 20.1 (Me(17)); 18.8 (C(2)), 8.9 (*Me*CH_2COO–C(12)); 8.8 (*Me*CH_2COO–C(6)). EI-MS: 502 (0.3, M^+), 460 (1, $[M-CH_2{=}C{=}O]^+$), 443 (2, $[M-CMeOO]^+$), 428 (0.5, $[M-CMeCH_2OOH]^+$), 386 (34, $[M-CH_2CO-CMeH_2CO_2H]^+$), 330 (100), 312 (28), 302 (27), 269 (26), 57 (48).

6β,7α-Diacetoxyroyleanone (*=(6β,7α)-12-Hydroxy-11,14-dioxoabieta-8,12-diene-6,7-diyl Diacetate*; **17**). Yellow amorphous solid. $[\alpha]_D^{18} = -10.7$ (*c* = 0.056, $CHCl_3$). IR and MS data identical to those described in [17], whereas the ^{13}C-NMR spectrum is also identical to that described in [17], but the signals at *δ*(C) 21.3 (Me) and 20.8 (Me) must be assigned to Me(20) and *Me*COO–C(6), resp. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.17 (br. *s*, OH); 5.70 (*d*, *J*(7*β*,6*α*) = 1.7, H_β–C(7)); 5.50 (*dd*, *J*(6*α*,7*β*) = 1.7, *J*(6*α*,5*α*) = 1.5, H_α–C(6)); 3.16 (*sept.*, *J*(15,16(17)) = 7.1, H–C(15)); 2.67 (*dddd*, *J*(1*β*,1*α*) = 12.9, *J*(1*β*,2*β*) = 3.1, *J*(1*β*,2*α*) = 2.7, *J*(1*β*,3*β*) = 1.2, H_β–C(1)); 2.04 (*s*, MeCOO–C(6)); 2.03 (*s*, MeCOO–C(7)); 1.82 (*qt*, *J*(2*β*,1*α*) = *J*(2*β*,2*α*) = *J*(2*β*,3*α*) = 14.0, *J*(2*β*,1*β*) = *J*(2*β*,3*β*) = 3.1, H_β–C(2)); *ca.* 1.60 (overlapped, H_α–C(2)); 1.59 (*s*, Me(20)); 1.56 (*d*, *J*(5*α*,6*α*) = 1.5, H_α–C(5)); 1.49 (*dt*, *J*(3*β*,3*α*) = 13.4, *J*(3*β*,2*β*) = *J*(3*β*,2*α*) = 3.1, H_β–C(3)); 1.24 (overlapped, H_α–C(1)); 1.24 (overlapped, H_α–C(3)); 1.22 (*d*, *J*(16,15) = 7.1, Me(16)); 1.19 (*d*, *J*(17,15) = 7.1, Me(17)); 0.99 (*s*, Me(18)); 0.98 (*s*, Me(19)).

7α-Acetoxy-6β-propanoyloxyroyleanone (*=(6β,7α)-7-(Acetyloxy)-12-hydroxy-11,14-dioxoabieta-8,12-dien-6-yl Propanoate*; **18**). Yellow amorphous solid. $[\alpha]_{546}^{18} = -25$ ($c = 0.044$, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$): 7.17 (br., HO–C(12)); 5.70 (br. *d*, $J(7\beta,6\alpha) = 2.0$, $J(7\beta,5\alpha) < 0.4$, H_β–C(7)); 5.51 (*dd*, $J(6\alpha,7\beta) = 2.0$, $J(6\alpha,5\alpha) = 1.6$, H_α–C(6)); 3.16 (*sept.*, $J(15,16(17)) = 7.1$, H–C(15)); 2.66 (*dtd*, $J(1\beta,1\alpha) = 12.9$, $J(1\beta,2\beta) = 3.6$, $J(1\beta,2\alpha) = 3.4$, $J(1\beta,3\beta) = 0.8$, H_β–C(1)); 2.29 (*m*, CH_2(2′)), 2.04 (*s*, MeCOO); 1.82 (*qt*, $J(2\beta,1\alpha) = J(2\beta,2\alpha) = J(2\beta,3\alpha) = 14.0$, $J(2\beta,1\beta) = J(2\beta,3\beta) = 3.6$, H_β–C(2)); 1.59 (*s*, Me(20)); 1.58 (overlapped *m*, H_α–C(2)), 1.54 (br. *d*, $J(5\alpha,6\alpha) = 1.6$, $J(5\alpha,7\beta) < 0.4$, H_α–C(5)); 1.48 (*dtd*, $J(3\beta,3\alpha) = 13.6$, $J(3\beta,2\alpha) = J(3\beta,2\beta) = 3.6$, $J(3\beta,1\beta) = 0.8$, H_β–C(3)); 1.22 (overlapped, H_α–C(1), H_α–C(3)); 1.22 (*d*, $J(16,15) = 7.1$, Me(16)); 1.19 (*d*, $J(17,15) = 7.1$, Me(17)); 1.12 (*t*, $J(3',2'A) = J(3',2'B) = 7.6$, *Me*$CH_2$-COO); 0.99 (*s*, Me(18)); 0.97 (*s*, Me(19)). ^{13}C-NMR (100 MHz, $CDCl_3$): 185.4 (C(14)); 183.2 (C(11); 172.5 (MeCH_2*C*OO); 168.0 (Me*C*OO); 150.8 (C(l2)); 149.3 (C(9)); 137.1 (C(8)); 124.8 (C(13)); 67.1 (C(6)); 65.2 (C(7)); 49.0 (C(5)); 42.4 (C(3)); 38.7 (C(10)); 38.4 (C(1)); 33.6 (C(4)); 33.2 (Me(18)); 27.8 (Me*C*H_2COO); 24.2 (C(15)); 23.0 (Me(19)); 21.4 (Me(20)); 20.8 (*Me*COO); 19.8 (Me(16)); 19.7 (Me(17)); 18.9 (C(2)); 8.9 (*Me*CH_2COO). EI-MS: 446 (0.4, M^+), 404 (1, $[M - CH_2CO]^+$), 386 (4, $[M - AcOH]^+$), 372 (2, $[M - MeCH_2CO_2H]^+$), 348 (4), 330 (100, $[M - MeCH_2CO_2H - CH_2CO]^+$), 315 (20), 298 (12), 287 (10), 261 (21), 260 (15), 248 (20), 245 (13), 232 (12), 57 (18, $[C_2H_5C{=}O]^+$).

7α-Acetoxy-6β-butanoyloxyroyleanone (*=(6β,7α)-7-(Acetyloxy)-12-hydroxy-11,14-dioxoabieta-8,12-dien-6-yl Butanoate*; **19**). Amorphous solid. $[\alpha]_D^{18} = +12.1$ ($c = 0.19$, $CHCl_3$). IR (KBr): 3378, 2962, 2931, 2873, 1755, 1659, 1638, 1612, 1462, 1374, 1282, 1220, 1168, 1144, 1103, 1030, 959, 900, 756. ^{1}H-NMR (400 MHz, $CDCl_3$): 7.18 (br. *s*, OH); 5.69 (*dd*, $J(7\beta,6\alpha) = 2.1$, $J(7\beta,5\alpha) = 0.6$, H_β–C(7)); 5.50 (*t*, $J(6\alpha,7\beta) = J(6\alpha,5\alpha) = 2.1$, H_α–C(6)); 3.16 (*sept.*, $J(15,16(17)) = 7.1$, H–C(15)); 2.66 (*ddd*, $J(1\beta,1\alpha) = 13.0$, $J(1\beta,2\alpha) = 3.3$, $J(1\beta,2\beta) = 2.9$, H_β–C(1)); 2.28 (*dt*, $J(2'A,2'B) = 15.9$, $J(2'A,3') = 7.1$, $MeCH_2CH_AH_B$-COO); 2.20 (*dt*, $J(2'B,2'A) = 15.9$, $J(2'B,3') = 8.0$, $MeCH_2CH_AH_BCOO$); 2.04 (*s*, MeCOO); 1.82 (*qdd*, $J(2\beta,1\alpha) = J(2\beta,2\alpha) = J(2\beta,3\alpha) = 13.8$, $J(2\beta,1\beta) = J(2\beta,3\beta) = 2.9$, H_β–C(2)); 1.63 (overlapped, $MeCH_2CH_2COO$); 1.60 (overlapped, H_α–C(5)); 1.59 (*s*, Me(20)); 1.58 (overlapped, H_α–C(2)); 1.47 (*ddd*, $J(3\beta,3\alpha) = 13.0$, $J(3\beta,2\alpha) = 3.6$, $J(3\beta,2\beta) = 2.9$, H_β–C(3)); 1.23 (overlapped, H_α–C(1), H_α–C(3)); 1.22 (*d*, $J(16,15) = 7.1$, Me(16)); 1.19 (*d*, $J(17,15) = 7.1$, Me(17)); 0.99 (*s*, Me(18)); 0.98 (*s*, Me(19)); 0.91 (*t*, $J(4',3') = 7.4$, *Me*CH_2CH_2COO). ^{13}C-NMR (100 MHz, $CDCl_3$): 185.3 (C(14)); 183.2 (C(11)); 171.7 (MeCH_2CH_2*C*OO); 168.1 (Me*C*OO); 150.8 (C(12); 149.3 (C(9)); 137.1 (C(8)); 124.8 (C(13)); 67.0 (C(6)); 65.2 (C(7)); 49.0 (C(5)); 42.4 (C(3)); 38.7 (C(10)), 38.4 (C(1)), 36.4 ($MeCH_2CH_2$*C*OO), 33.6 (C(4)); 33.2 (Me(18)); 24.1 (C(15)); 23.0 (Me(19)); 21.3 (Me(20)); 20.8 (*Me*COO); 19.8 (Me(16)); 19.7 (Me(17)); 18.9 (C(2)); 18.1 (Me*C*H_2CH_2COO); 13.7 (*Me*CH_2CH_2COO). EI-MS: 460 (0.3, M^+) 418 (1, $[M - CH_2{=}C{=}O]^+$), 401 (5, $[M - AcO]^+$), 348 (5), 330 (100, $[M - AcO - MeCH_2CH_2CO]^+$), 315 (24, $[M - AcO - MeCH_2CH_2CO - Me]^+$), 297 (11), 287 (12), 261 (26), 248 (24), 232 (14), 217 (7), 201 (8), 187 (6), 83 (8), 71 (13), 55 (5).

Antimycobacterial Activity in vitro. Bioassays were conducted on sensitive H_{37}Rv ATCC 27294 (American Type Culture Collection) and multidrug-resistant (clinical isolate, strain 02TBDM039EP097) *Mycobacterium tuberculosis* strains. For the preparation of the inoculums, a suspension of MTB was prepared by mixing growth from slants (20–30-d-old) with 100 µl of *Tween 80* into 0.2% (BSA; *Sigma Chemical Co.*, St. Louis, MO). Turbidity of the suspension was then adjusted to a *McFarland* standard No. 1 (3×10^7 CFU/ml) by adding *Tween 80* and BSA. The bacterial suspension (300 µl) was further transferred to 7.2 ml of 7 H9GC broth (4.7 g of *Middlebrook 7 H9* broth base (*Difco*, Detroit, MI), 20 ml of 10% glycerol, 1 g of *Bacto Casitone* (*Difco*), 880 ml of distilled H_2O, 100 ml of OADC (oleic acid, albumin, dextrose, catalase; *Remel*, Lenexa, KS)). For the bioassay, the compounds were resuspended in DMSO at a concentration of 1 mg/ml (stock soln.). These stock solns. were further diluted with appropriate volumes of 7 H9GC broth to yield final concentrations of 0.1 to 50 µg/ml. Final drug concentration ranges of standard antibiotics used as positive controls were 0.125 to 32 µg/ml for isoniazid and 0.063 to 16 µg/ml for rifampicin (*Sigma Chemical Co.*, St. Louis, MO). The standard drugs or compounds (100 µl) were mixed in the wells with 100 µl of bacterial inoculum, resulting in a final bacterial concentration of *ca.* 1.2×10^6 CFU/ml. Solvent (DMSO) was included in every experiment as a negative control. The plates were sealed in plastic bags and then incubated at 37° for 5 d. On day 5, 50 µl of the MTT/*Tween 80* mixture (1.5 ml of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide; *Aldrich Chemical Co.*, Milwaukee, WI) at a dilution of 1 mg/ml in absolute EtOH and 1.5 ml of

10% *Tween 80*) was added to the wells, and the plate was incubated at 37° for 24 h. After this incubation period, the growth of the microorganism was visualized by the change in color of the dye from yellow to purple. The tests were carried out in triplicate. *MIC* Value is defined as the lowest drug concentration that prevents the aforementioned change in color.

Cell Growth-Inhibition Assay. The percent inhibition of cell growth relative to the negative control (solvent) was evaluated colorimetrically using a sulforhodamine B dye, according to a published procedure [19], by comparison to the control. The GI_{50} value was defined as the concentration of test sample resulting in a 50% reduction of absorbance as compared with untreated controls that received a serial dilution of the solvent in which the test samples were dissolved, and was determined by linear regression analysis.

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