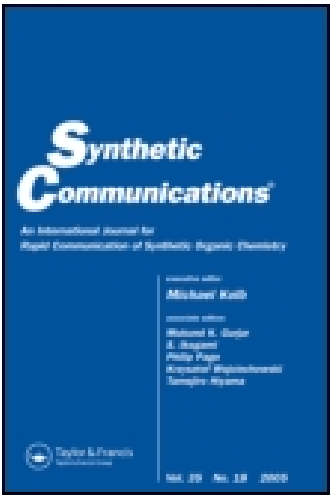

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Practical and Efficient 1α-Hydroxylation of 4,4-Dimethyl-2-Ene Derivatives in Terpenic Series

Gérard Aranda [a], Mireille Bertranne-Delahaye [a], Robert Azerad [b], Michèle Maurs [b], Manuel Cortés [c], Hector Ramirez [c], Gonzalo Vernal [c] & Thierry Prangé [d]

[a] Laboratoire de Synthèse Organique, Associé au CNRS, Ecole Polytechnique, Palaiseau Cedex, 91128, France

[b] Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, Université René Descartes-Paris V, 45 Rue des Saints Pères, Paris, 75006, France

[c] Pontificia Universidad Católica de Chile, Facultad de Química, Casilla 306, Correo 22, Santiago, Chile

[d] Chimie Structurale Biomoléculaire, URA 1430, Rue M. Cachin, 93017, Bobigny Cedex, France

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PRACTICAL AND EFFICIENT 1α-HYDROXYLATION OF 4,4-DIMETHYL-2-ENE DERIVATIVES IN TERPENIC SERIES.

Gérard Aranda*, Mireille Bertranne-Delahaye

Laboratoire de Synthèse Organique, Associé au CNRS,
Ecole Polytechnique, 91128-Palaiseau Cedex, France

Robert Azerad, Michèle Maurs

Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques,
Université René Descartes-Paris V, 45 Rue des Saints Pères, 75006-Paris, France

Manuel Cortés, Hector Ramirez, Gonzalo Vernal

Pontificia Universidad Católica de Chile, Facultad de Química,
Casilla 306, Correo 22 - Santiago, Chile

and Thierry Prangé

Chimie Structurale Biomoléculaire, URA 1430, Rue M. Cachin,
93017 - Bobigny Cedex, France

ABSTRACT: The third part of a triptycal synthesis providing 1α-hydroxy compounds, through microbial hydroxylation in position-3 of terpenoid substrates, followed by dehydration to 4,4-dimethyl-2-ene compounds and subsequent allylic hydroxylation by SeO_2/pyridine N-oxide, is described.

Introduction

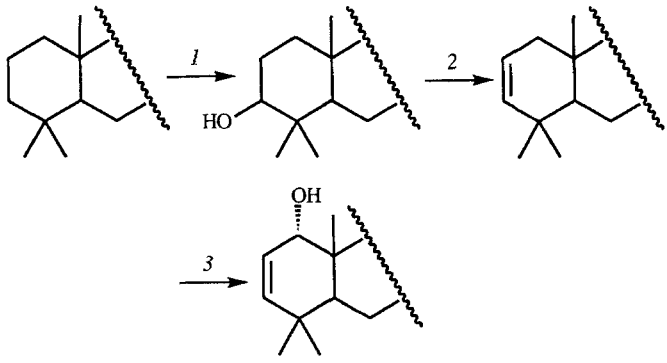
The significance of some peculiar asymmetric synthons as key structural units as well as initial building blocks in the synthesis of bioactive terpenoids such as forskolin, taxol, strigol, erigerol, etc... has promoted a general interest for an easier and cheaper synthesis of such precursors [1].

* to whom correspondence should be addressed

Our current efforts are directed toward the elaboration of new synthons involving the 1(α)-hydroxy-4,4-dimethyl-cyclohexane pattern. Although possible in the monocyclic series [2-8], a direct microorganism-mediated 1-hydroxylation has been mostly ruled out in the case of the drimane and the labdane series, and more generally for diterpenoid compounds [9], whatever the microorganism used.

We have previously described the direct 3β-biohydroxylation of various cyclic terpenoid substances by incubation with *Mucor plumbeus* ATCC 4740 [10,11] or *Rhizopus arrhizus* [12]. Such a high yield 3β-hydroxylation (or 3-ketonisation) of sesqui- and diterpenic bi- or tricyclic substances has also been repeatedly described with various microorganisms, including bacteria such as *Bacillus* species [13,14], or fungi such as *Aspergillus* [15-17], *Cunninghamella* [15,18], *Mortierella* [19], *Neurospora* [20], *Septomyxa* [21], *Syncephalostrum* [22], *Cephalosporium* [23], or *Mucor* [24] strains. The same microbial hydroxylation reaction has been similarly described in the natural *ent*-series, where 3α(*equat.*)-hydroxy derivatives were currently obtained [25,26].

In a second step, starting from 3β-hydroxy natural compounds or 3β-hydroxy microbially-functionalized derivatives, we have recently described a dehydration procedure affording in high yield the corresponding Δ-2,3-derivatives [27].



Scheme 1

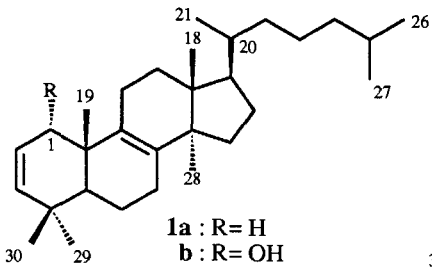
We want now to report herein the subsequent stereoselective introduction of the 1-hydroxy group, which constitutes the third part of our triptycal synthesis (Scheme 1), involving the (stereoselective) transfer of a C-3 hydroxy group to C-1 [22], and illustrating the use of combined microbial and chemical methods for the elaboration of such specifically functionalized synthons.

Results and discussion

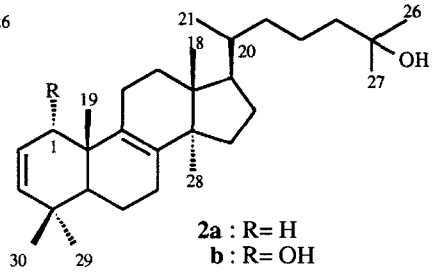
The attempted allylic microbial hydroxylation of 4,4-dimethyl-2-ene terpenoid compounds, such as **4**, originating from the dehydration of the corresponding 3β-hydroxy precursor [27], obtained from sclareol [10,11], was rather deceptive, despite several examples based on similar microbial allylic hydroxylations in other positions [12,28-30].

As a continuation of our ongoing studies, we decided to investigate the allylic hydroxylation of 4,4-dimethyl-2-ene compounds by chemical methods. Selenium dioxide (SeO_2) appears as the simplest effective oxidative reagent, although a combined chromic anhydride/3,5-dimethylpyrazole (DMP) reagent has been successfully employed at low temperatures [31,32]. The direct hydroxylation by SeO_2 in dioxane has to be secured in the presence of N-methylmorpholine N-oxide (NMO), available as a monohydrate [33], or preferably, with pyridine N-oxide (PNO) [34], commercially available as an anhydrous reagent. Several variously functionalized 2,3-dehydro molecules **1-8** were thus selected to investigate the scope and limitations of this reaction.

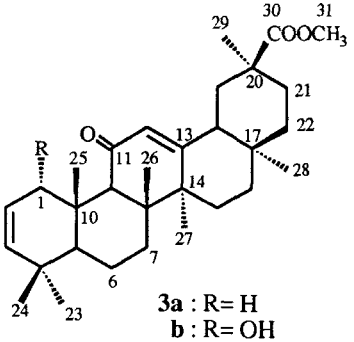
Although the yields reported in the Table were not optimized in all cases, we were able, by modifying some of the reaction conditions, such as temperature and reagent concentrations, to transform completely the starting Δ-2 compounds. Excess PNO (5-8 equivalents) in anhydrous dioxane was systematically utilized [35].



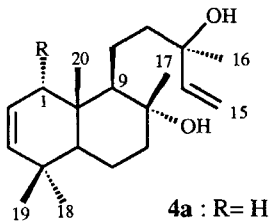
1a : R= H
b : R= OH



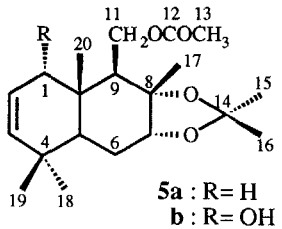
2a : R= H
b : R= OH



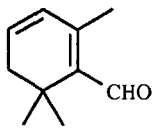
3a : R= H
b : R= OH



4a : R= H
b : R= OH



5a : R= H
b : R= OH



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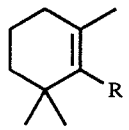

7 : R = CH_2CHO
8 : R = CH_2CH_2OH

The final worked-up mixtures were clean and unprovided of the usual bad smelling inherent to selenium by-products.

In the lanostadiene series (**1a**, **2a**), the major desired 1α-hydroxylation product (**1b** or **2b**, respectively) (50-70%) was associated with a minor 1α-hydroxylated Δ-7,9(11)-dehydrogenation product (**1c** or **2c**, 15-20%). Starting from the glycyrrhetinate derivative **3a**, the 1α-hydroxylation yield was higher (**3b**, about 90%). The highly functionalized dehydrosclareol **4a** afforded a mixture from which about 40% of a 1α-hydroxy derivative **4b** could be isolated. The yield was not improved by using a selenium monoxyde derivative $(CH_3OC_6H_4)_2SeO$ [36,37].

Table: 1α-Hydroxylation of terpenoid substances

	Reagent	Time (h)	Temperature (°C)	Conversion (%)	1-hydroxy derivatives (% yield [a])
1a	SeO_2 + PNO	72	90	69	2,8-lanostadiene-1α-ol **1b** (50) 2,7,9(11)-lanostatriene-1α-ol **1c** (12)
	SeO_2 + PNO	24	95	84	2,8-lanostadiene-1α-ol **1b** (53) 2,7,9(11)-lanostatriene-1α-ol **1c** (15)
	SeO_2 + PNO	22	100	95	2,8-lanostadiene-1α-ol **1b** (53) 2,7,9(11)-lanostatriene-1α-ol **1c** (20)
2a	SeO_2 + PNO	30	95	89	2,8-lanostadiene-1α-25-diol **2b** (70) 2,7,9(11)-lanostatriene-1α,25-diol **2c** (19)
3a	SeO_2 + PNO	24	100	98	1α-ol derivative **3b** (88)
	$CH_3OC_6H_4SeO$ +SeO_2	3	reflux	100	1α-ol derivative **3b** (40)
4a	SeO_2 + PNO	30	100	80	1α-ol derivative **4b** (40) [b]
	$CH_3OC_6H_4SeO$ +SeO_2	8	reflux	86	1α-ol derivative **4b** (40) [b]
5a	SeO_2 + PNO	14	95	67	1α-ol,11-OAc derivative **5b** (54) 1α-OAc,11-ol derivative **5c** (11)
6	SeO_2 + PNO	4	80	100	(untractable mixture)
7	SeO_2 + PNO	4	80	100	1-ol derivative (64) 1-keto derivative (15)
8	SeO_2 + PNO	6	85	75	1-ol derivative (60) 1-keto derivative (10)

[a] after chromatographic purification.

[b] Partial epimerization at C-13. The epi-sclareol derivative was removed after crystallization.

Although other methods [38] have been reported for the allylic hydroxylation of monocyclic unsaturated terpenoids, such as **7** or **8**, the use of SeO_2/PNO led to comparable conversion yields, the 1-hydroxylation products being partially oxidized to 1-keto-derivatives. Oxidation of safranal **6**, a well-known polymerizable molecule, in the hope to obtain an hydroxy- or a ketodiene product was misleading, as only an untractable mixture was obtained, even at moderate

temperature; the yield was not improved by using the selenium monoxyde derivative $(CH_3OC_6H_4)_2SeO$.

Nearly quantitative results were obtained with the protected trihydroxy-drimenyl derivative **5a**, which was the most probing example illustrating our synthetic scheme. The microbial hydroxylation of 7α,8α-isopropylidenedioxy-11-acetoxydrimane by *Aspergillus niger* ATCC 9142 provided 85-90 % overall yield of compounds functionalized at C-3 (80 % of 3β-hydroxy-, 1-2 % of 3-keto- and 5-7 % of the 3β-hydroxy-7,8-deprotected compound) [39]. The Δ-2 derivative **5a** was then prepared from the purified 3β-hydroxy derivative following our dehydration protocol [27] and subsequently submitted to SeO_2/PNO oxidation. Surprisingly, two isomeric alcohols were obtained, both presenting the characteristic ^{1}H-NMR features of a 1α-hydroxy derivative. Individual doublets at 3.71 ppm (J = 5.9 Hz) and 4.79 ppm (J = 5.5 Hz) were respectively observed for the major and the minor alcohol. The major compound was characterized by infrared absorption maxima at 3614, 3573, 3493 cm^{-1} and a carbonyl function at 1752 and 1724 cm^{-1}. The minor alcohol showed hydroxyl absorption at 3620 cm^{-1} and a sharp carbonyl absorption at 1738 cm^{-1}; furthermore, its ^{1}H-NMR spectrum showed undoubtely a strong interaction due to hydrogen bonding between the two functions in C-1 and C-11, which could be removed by D_2O exchange. All spectroscopic data of the major alcohol were in agreement with the proposed structure of a 1α-hydroxy derivative **5b**, whilst the molecular structure of the minor alcohol was deduced from X-ray crystallographic analysis (Figure 1) and corresponded to a 1α-acetoxy-11-hydroxy derivative **5c** resulting from an acetyl group migration between the primary C-11 acetate and the new secondary C-1 alcohol function, occurring during the reaction. Such an easy acyl migration during microbial incubation has been already observed for similar spatial configurations in

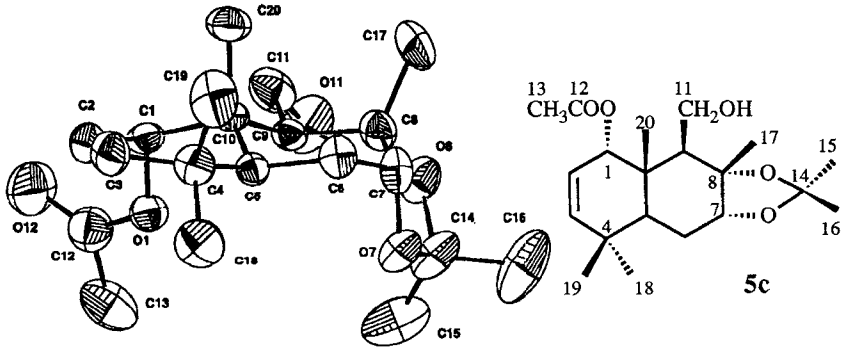

FIG.1
Ortep drawing of **5c**. Hydrogen atoms have been omitted for clarity. The ellipsoids are drawn at the 50% probability level.

the terpenoid series [26]. This explains the high correlation of ^{1}H and ^{13}C-NMR spectroscopic data of these two isomeric compounds.

Stereochemical assignments

The isolated 1α-hydroxy-2-ene compounds were typically characterized by a 1β-H, 2-H coupling constant in the 5.5-5.7 Hz range. After computer modelization of **5b** and other compounds, the measured dihedral angle value between those hydrogen atoms (about 34° *vs.* 77° for a 1α-H, 2-H dihedral angle) could be correctly correlated with the observed coupling constant. In addition, earlier described results in the same series, corresponding to isomeric allylic 1α– and 1β–alcohols [22], indicated respectively 5.5 and 1.5 Hz *J* values, corroborating our modeling results. Stereochemical assignments may be additionally checked after hydrogenation of the Δ-2 insaturation: hydrogenation of the 1α-hydroxy-Δ-2-glycyrrhetinate derivative **3b** afforded a new compound where the H-1 proton was characterized as a triplet at 4.66 ppm ($J_{1\beta H\text{-}2\alpha H} = J_{1\beta H\text{-}2\beta H} = 3.0$ Hz), in agreement with earlier described results concerning the same ring A functionalized structure [22].

The X-ray crystallographic analysis of the minor alcohol **5c**, resulting from an acetyl migration in the major 1-hydroxylated product **5b**, entirely confirms the 1α-hydroxy configuration assigned to all SeO_2/PNO oxidation products.

Conclusion

SeO_2 associated with PNO is a very efficient hydroxylating reagent, in spite of a relatively unreactive hindered C-1 position [19,20,40]. An advantage of this feature is the resulting stereospecific functionalization. The triptycal reaction scheme presented provides allylic 1α-hydroxylated derivatives in three steps, comparatively to a recent example where the same goal was reached through nine steps, also starting with an initial microbial 3β-hydroxylation [22]. Furthermore, while the reduction of 1-keto-2-ene-4,4-dimethyl derivatives always results in a mixture of 1α- and 1β- alcohols [22,40,41] whatever the reducing reagent used, our protocol ensures a stereospecific 1α-hydroxylation.

Experimental

General. Experimental conditions have been detailed in the preceeding publication [27]. NMR spectra were routinely realized in deuterated chloroform except otherwise indicated. Chemical shifts were assigned by comparison with previous results [22,41-44], determination of hydrogen-multiplicity by DEPT experiments and were ascertained in most cases by 2D-^{1}H-^{13}C chemical shift correlations. Mass spectra measurements (EI and CI) were obtained with a Fisons ZAB HSQ spectrometer and agreed with proposed structures.

Starting materials. Methyl glycyrrhetinate and drimenyl acetate have been obtained from chilian source. Sclareol, safranal **6** and 25-hydroxylanosterol (m.p. 182-185°C) were respectively gifts from Synarom (Paris, France), Robertet (Grasse, France), and P. Beuchet and Y. Letourneux (Pôle Sciences et Techniques,

La Rochelle, France). The synthesis of the drimenol derivative **5a** will be described elsewhere [39].

Typical general procedure. Pyridine N-oxide (0.30-0.40 g) was carefully dried under high vacuum at room temperature in the presence of P_2O_5 during 4 h. Over it, was added, under nitrogen, the Δ-2,3 derivative **1a** (0.230 g) in anhydrous dioxane (8 ml) distilled from sodium/benzophenone, and SeO_2 (63 mg). This solution was vigorously stirred at 100°C (bath temperature) during 22 h. After cooling, diethyl ether (200 ml) was added and the solution washed with a 10 % sodium hydroxide solution (10 ml), a 10 % hydrochloric acid solution (10 ml) and brine (3 x 10 ml). After drying over Na_2SO_4, removal of the solvent, the chromatography of the yellow residual oil over silica gel provides 10-12 mg of the starting material, 127 mg of 1α-hydroxylanosta-2,8-diene **1b** (53 % yield) and 47 mg of 1α-hydroxylanosta-2,7,9(11)-triene (20 % yield).

*1α-Hydroxy-lanosta-2,8-diene **1b***. M.p.123-124.5°C (from methanol). IR (CCl_4) cm^{-1}: 3614, 2956, 2870, 1468, 1372, 1026, 997. ^{1}H-NMR (δ ppm): 0.87 (6H, d, J = 6.4 Hz, 26- and 27-CH_3); 0.94 (3H, d, J = 4.1 Hz, 21-CH_3); 0.73, 0.93, 0.97, 1.02 (15H, 4s, 18-, 19-, 28-, 29- and 30-CH_3); 3.89 (1H, d, J = 5.7 Hz, 1β-H) ; 5.60 - 5.76 (2H, AB part of an ABX system with 1β-H, J_{AB} = 9.9 Hz, J_{AX} = 0, J_{BX} = 5.7 Hz, H-2 and H-3). ^{13}C-NMR (δ ppm): 69.9, C-1; 123.6, C-2; 142.1, C-3; 36.6, C-4; 41.6, C-5; 20.9, C-6; 28.2, C-7; 139.8, C-8; 129.7, C-9; 35.3, C-10; 19.0, C-11; 26.6, C-12; 44.5, C-13; 42.1, C-14; 31.0, C-15; 31.0, C-16; 50.6, C-17; 16.0, C-18; 18.9, C-19; 36.6, C-20; 22.3, C-21; 36.5, C-22; 24.2, C-23; 39.6, C-24; 31.6, C-25; 22.6, C-26; 22.9, C-27; 25.1, C-28; 31.6, C-29; 18.9, C-30. $[\alpha]_D^{22}$ +172; $[\alpha]_{578}^{22}$ +180; $[\alpha]_{546}^{22}$ +205; $[\alpha]_{436}^{22}$ +356; $[\alpha]_{365}^{22}$ +571 (c 0.735, $CHCl_3$). HR-MS for $C_{30}H_{50}O$, calc. 426.3861, found 426.3859.

*1α-Hydroxy-lanosta-2,7,9(11)-triene **1c***. M.p.120-121°C (from methanol). IR (CCl_4) cm^{-1}: 3560, 3018, 2959, 2931, 2870, 1467, 1375, 1247, 1217, 1073,

1001. ^{1}H-NMR (δ ppm): 0.88 (6H, d, J = 6.4 Hz, 26- and 27-CH_3); 0.91 (3H, d, J = 5.4 Hz, 21-CH_3); 0.58, 0.92, 0.98, 1.01 and 1.06 (15H, 5s, 18-, 19-, 28-, 29- and 30-CH_3); 4.21 (1H, d, J = 5.9 Hz, 1β-H); 5.52 - 5.62 (2H, m., H-7 and H-11); 5.65 - 5.84 (2H, AB part of an ABX system with 1β-H, J_{AB} = 10.0 Hz, J_{AX} = 0, J_{BX} = 5.9 Hz, H-2 and H-3). ^{13}C-NMR (δ ppm) : 69.8, C-1; 122.1, C-2; 142.3, C-3; 35.2, C-4; 40.3, C-5; 27.9, C-6; 119.0, C-7; 143.0, C-8; 140.7, C-9; 50.4, C-10; 121.4, C-11; 38.3, C-12; 43.9, C-13; 43.2, C-14; 31.5, C-15; 31.5, C-16; 51.2, C-17; 15.9, C-18; 18.6, C-19; 36.4, C-20; 23.2, C-21; 36.6, C-22; 24.3, C-23; 39.6, C-24; 31.9, C-25; 22.7, C-26; 22.9, C-27; 24.8, C-28; 31.9, C-29; 23.4, C-30. $[\alpha]_D^{22}$ +258; $[\alpha]_{578}^{22}$ +269; $[\alpha]_{546}^{22}$ +308; $[\alpha]_{436}^{22}$ +555; $[\alpha]_{365}^{22}$ +940 (c 0.772, $CHCl_3$). HR-MS for $C_{30}H_{48}O$: calc. 424.3705, found 424.3703.

1α,25-Dihydroxy-lanosta-2,8-diene ***2b***. M.p.158-160°C (from methanol). IR (CCl_4) cm^{-1}: 3615, 3013, 2960, 2875, 1469, 1371, 1027, 996. ^{1}H-NMR (δ ppm): 0.72, 0.92, 0.94, 0.96, 1.00 (18H, 5s, 18-, 19-, 21-, 28-, 29- and 30-CH_3); 1.20 (6H, s, 26- and 27-CH_3); 3.88 (1H, d, J = 5.8 Hz, 1β-H); 5.58 - 5.74 (2H, AB part of an ABX system with 1β-H, J_{AB} = 9.9 Hz, J_{AX} = 0, J_{BX} = 5.8 Hz, H-2 and H-3). ^{13}C-NMR (δ ppm): 69.6, C-1; 123.6, C-2; 142.1, C-3; 36.6, C-4; 41.6, C-5; 20.9, C-6; 28.2, C-7; 139.7, C-8; 129.8, C-9; 35.3, C-10; 19.0, C-11; 26.6, C-12; 44.8, C-13; 43.1, C-14; 31.1, C-15; 31.1, C-16; 50.6, C-17; 16.0, C-18; 18.8, C-19; 36.6, C-20; 22.3, C-21; 36.9, C-22; 21.3, C-23; 44.6, C-24; 71.1, C-25; 29.4, C-26; 29.3, C-27; 25.1, C-28; 31.6, C-29; 18.9, C-30. $[\alpha]_D^{22}$ +174.9; $[\alpha]_{578}^{22}$ +182; $[\alpha]_{546}^{22}$ +207; $[\alpha]_{436}^{22}$ +359; $[\alpha]_{365}^{22}$ +583 (c 1.2, $CHCl_3$). HR-MS for $C_{30}H_{49}O$ (M+1-H_2O = 443-18 = 425), calc. 425.3783, found 425.3785.

1α,25-Dihydroxy-lanosta-2,7,9(11)-triene ***2c***. M.p.163-166°C (from methanol). IR (CCl_4) cm^{-1}: 3615, 3561, 3017, 2961, 2928, 1542, 1467, 1375, 1246, 1218, 1029, 976, 908. ^{1}H-NMR (δ ppm): 0.58, 0.94, 0.98, 1.01, 1.06 (15H, 5s, 18-,

19-, 28-, 29- and 30-CH_3); 0.93 (3H, d, J = 6.1 Hz, 21-CH_3); 1.23 (6H, s, 26- and 27-CH_3); 4.21 (1H, d, J = 5.9 Hz, 1β-H); 5.52 - 5.62 (2H, br.d, J = 5.8 Hz, H-7 and H-11); 5.65 - 5.82 (AB part of an ABX system with 1β-H, J_{AB} = 9.9 Hz, J_{AX} = 0, J_{BX} = 5.9 Hz, H-2 and H-3). ^{13}C-NMR (δ ppm): 69.7, C-1; 121.9, C-2; 142.3, C-3; 35.2, C-4; 40.2, C-5; 27.9, C-6; 118.9, C-7; 140.6, C-8; 142.9, C-9; 50.3, C-10; 121.5, C-11; 38.2, C-12; 43.9, C-13; 43.1, C-14; 31.4, C-15; 31.4, C-16; 51.1, C-17; 15.8, C-18; 18.6, C-19; 36.4, C-20; 23.2, C-21; 36.7, C-22; 21.2, C-23; 44.5, C-24; 71.2, C-25; 29.5, C-26; 29.4, C-27; 24.7, C-28; 31.8, C-29; 23.4, C-30. $[\alpha]_D^{22}$ +145; $[\alpha]_{578}^{22}$ +151; $[\alpha]_{546}^{22}$ +173; $[\alpha]_{436}^{22}$ +311; $[\alpha]_{365}^{22}$ +526 (c 1.335, $CHCl_3$). HR-MS for $C_{30}H_{47}O$ (M+1–H_2O = 441–18 = 423) calc. 423.3627, found 423.3629.

Methyl 1α-hydroxy,11-oxo,18β,20β-olean-2,12-dien-30-oate ***3b***. M.p. 236-238°C (from methanol or ethanol). IR (CCl_4) cm^{-1}: 3550, 3000, 2950, 2920, 2850, 1720, 1640, 1610, 1225, 1170. ^{1}H-NMR (δ ppm): 0.82 (3H, s, 29-CH_3); 0.89 (3H, s, 25-CH_3); 0.99 (3H, s, 23-CH_3); 1.07, 1.13, 1.18 (9H, 3s, 24-, 26- and 28-CH_3); 1.38 (3H, s, 27-CH_3); 3.31 (1H, s, 9α-H); 3.68 (3H, s, $COOCH_3$); 4.67 (1H, d, J = 5.6 Hz, 1β-H), 5.53 (1H, d, J = 10.0 Hz, H-3); 5.67 (1H, dd, J = 5.6 and 10 Hz, H-2); 5.71 (1H, s, H-12). ^{13}C-NMR (δ ppm): 69.8, C-1; 123.6, C-2; 140.5, C-3; 34.6, C-4; 44.6, C-5; 18.6, C-6; 31.3, C-7; 43.6, C-8; 52.4, C-9; 40.8, C-10; 201.5, C-11; 128.7, C-12; 170.2, C-13; 44.6, C-14; 26.6, C-15; 26.5, C-16; 31.8, C-17; 48.3, C-18; 41.3, C-19; 44.0, C-20; 31.1, C-21; 37.8, C-22; 31.6, C-23; 23.5, C-24; 17.0, C-25; 18.6, C-26; 22.7, C-27; 28.3, C-28; 28.6, C-29; 176.9, C-30; 51.7, C-31. $[\alpha]_D^{22}$ +263 (c 1.6, $CHCl_3$). HR-MS for $C_{31}H_{46}O_4$, calc. 482.3396, found 482.3399.

Methyl 1α-hydroxy,11-oxo,18β,20β-olean-12-en-30-oate ***3c***. 25 mg of **3b** in methanol (5 ml) were hydrogenated in the presence of palladium oxide under hydrogen pressure (30-50 psi). After total conversion and usual work-up, 20 mg of

a colorless compound were isolated by preparative TLC. M.p. 237-239°C (from hexane-acetone mixture). IR (CCl_4) cm^{-1}: 3510, 2960, 2930, 1710, 1650, 1620, 1220, 1170. ^{1}H-NMR (δ ppm): 0.82, 0.85, 0.91, 1.14, 1.15, 1.39 (21H, 6s, 23-, 24-, 25-, 26-, 27-, 28- and 29-CH_3); 3.30 (1H, s, 9α-H); 3.69 (3H, s, $COOCH_3$); 4.66 (1H, t, J = 3.0 Hz, 1β-H); 5.68 (1H, s, H-12). ^{13}C-NMR (δ ppm): 71.4, C-1; 25.2, C-2; 34.5, C-3; 33.2, C-4; 47.6, C-5; 17.6, C-6; 32.1, C-7; 43.6, C-8; 53.7, C-9; 41.8, C-10; 201.9, C-11; 128.6, C-12; 170.0, C-13; 45.2, C-14; 26.5, C-15; 26.5, C-16; 31.8, C-17; 48.3, C-18; 41.2, C-19; 44.1, C-20; 31.2, C-21; 37.8, C-22; 33.2, C-23; 23.6, C-24; 17.8, C-25; 19.0, C-26; 21.5, C-27; 28.3, C-28; 28.5, C-29; 177.0, C-30; 51.8, C-31. $[\alpha]_D^{22}$ +146 (c 0.45, $CHCl_3$). Anal. for $C_{31}H_{48}O_4$, calc. C 76.81, H 9.98; found C 76.60, H 10.12

1α,8α,13(R)-Trihydroxylabd-2,14-diene ***4b***. M.p.132-134°C (from pentane-diethyl ether). IR (CCl_4) cm^{-1}: 3610, 3495 (shoulder), 3373, 2961, 2930, 2869, 1454, 1384, 1036, 1002, 923. ^{1}H-NMR (δ ppm): 0.75 (3H, s, 20-CH_3); 0.87 (3H, s, 19-CH_3); 1.00 (3H, s, 18-CH_3); 1.17 (3H, s, 17-CH_3); 1.23 (3H, s, 16-CH_3); 5.13 - 5.31 (2H, 2dd, H-15 and H-15', $J_{15,14}$ = 17.4 Hz, $J_{15',14}$ = 10.8 Hz and $J_{15,15'}$ = 1.3 Hz); 5.47 - 5.61 (2H, AB part of an ABX system with 1β-H, J_{AB} = 9.9 Hz, J_{AX} = 0, J_{BX} = 5.6 Hz, H-2 and H-3); 5.82 (1H, dd, H-14, $J_{14,15}$ = 17.3 Hz, $J_{14,15'}$ = 10.8 Hz). ^{13}C-NMR (δ ppm) (CD_3OD): 70.1, C-1; 124.7, C-2; 141.7, C-3; 36.2, C-4; 45.7, C-5; 22.3, C-6; 44.4, C-7; 75.7, C-8; 52.1, C-9; 43.5, C-10; 20.1, C-11; 44.0, C-12; 74.8, C-13; 146.7, C-14; 111.7, C-15; 28.6, C-16; 23.5, C-17; 31.6 ,C-18; 23.3, C-19; 16.2, C-20. $[\alpha]_D^{22}$ +90; $[\alpha]_{578}^{22}$ +94.5; $[\alpha]_{546}^{22}$ +107.5; $[\alpha]_{436}^{22}$ +186; $[\alpha]_{365}^{22}$ +295.5 (c 1.63, CH_3OH). MS (EI), m/z: 304 (M^+-H_2O), 286 (M^+-$2H_2O$), 271 (286-CH_3). HR-MS for $C_{19}H_{27}O$, calc. 271.2062, found 271.2064.

1α-hydroxy-7α,8α-isopropylidenedioxy-11-acetoxydrim-2-ene ***5b***. Colorless oil. IR (CCl_4) cm^{-1}: 3614, 3573, 3492, 3019, 2984, 2935, 2895, 2868, 1752, 1724, 1454, 1380, 1368, 1256, 1248, 1219, 1208, 1095, 1040, 998, 911, 847. ^{1}H-NMR (δ ppm) after D_2O addition: 0.76 and 0.86 (6H, 2s, 19- and 20-CH_3); 1.02 (3H, s, 18-CH_3), 1.27 (3H, s, 17-CH_3), 1.31 and 1.43 (6H, 2s, 15 and 16-CH_3); 2.05 (3H, s, 13-CH_3); 2.50 (1H, br.t, J = 6.0 Hz, 9α-H); 3.12 (1H, br.s, OH), 3.71 (1H, d, J = 5.9 Hz, 1β-H); 3.93 (1H, br.t, J = 2.7 Hz, 7β-H); 4.20 - 4.38 (2H, AB part of an ABX system with 9α-H, J_{AB} = 11.6 Hz, J_{AX} = J_{BX} = 6 Hz, H_2-11); 5.54 - 5.68 (2H, AB part of an ABX system with 1β-H, J_{AB} = 10.0 Hz, J_{AX} = 0, J_{BX} = 5.9 Hz, H-2 and H-3). ^{13}C-NMR (δ ppm): 69.3, C-1; 122.6, C-2; 141.2, C-3; 34.5, C-4; 36.2, C-5; 23.4, C-6; 79.8, C-7; 80.7, C-8; 44.3, C-9; 40.6, C-10; 62.1, C-11; 171.2, C-12; 21.2, C-13; 107.3, C-14; 28.4 and 27.3, C-15 and C-16; 21.6, C-17; 31.2, C-18; 23.0, C-19; 14.5, C-20. $[\alpha]_D^{22}$ +53; $[\alpha]_{578}^{22}$ +55; $[\alpha]_{546}^{22}$ +63; $[\alpha]_{436}^{22}$ +110; $[\alpha]_{365}^{22}$ +175 (c 1.98, CH_3OH). MS (EI), m/z: 352 (M^+).

1α-acetoxy-7α,8α-isopropylidenedioxy-11-hydroxydrim-2-ene ***5c***. M.p.140-143°C (with sublimation) from methanol. IR (CCl_4) cm^{-1}: 3520, 2986, 2960, 2931, 2869, 1738, 1454, 1380, 1370, 1248, 1238, 1219, 1208, 1081, 1034, 1012, 1002, 994, 976, 905, 845. ^{1}H-NMR (δ ppm) (after D_2O addition): 0.78 and 0.89 (6H, 2s, 19- and 20-CH_3); 1.05 (3H, s, 18-CH_3), 1.57 (3H, s, 17-CH_3), 1.38 and 1.47 (6H, 2s, 15 and 16-CH_3); 2.07 (3H, s, 13-CH_3); 2.50 (1H, dd, J = 10.5 Hz and J = 3.6 Hz, 9α-H); 3.58 - 3.86 (2H, AB part of an ABX system with 9α-H, J_{AB} = 10.6 Hz, JAX = 3.6 Hz, J_{BX} = 10.6 Hz, H_2-11); 4.02 (1H, br.t, J = 3.0 Hz, 7β-H); 4.79 (1H, d, J = 5.5 Hz, 1β-H); 5.64 - 5.72 (2H, AB part of an ABX system with 1β-H, J_{AB} = 9.95 Hz, J_{AX} = 0, J_{BX} = 5.5 Hz, H-2 and H-3). ^{13}C NMR (δ ppm) : 71.2, C-1; 119.5, C-2; 143.1, C-3; 34.6, C-4; 37.6, C-5;

23.3, C-6; 79.7, C-7; 83.0, C-8; 46.1, C-9; 38.9, C-10; 59.9, C-11; 170.4, C-12; 21.1, C-13; 108.0, C-14, 28.4 and 27.4, C-15 and C-16; 20.9, C-17; 31.1, C-18; 22.9, C-19; 15.0, C-20. $[\alpha]_D^{22}$ +114°; $[\alpha]_{578}^{22}$ +119; $[\alpha]_{546}^{22}$ +136.5; $[\alpha]_{436}^{22}$ +243; $[\alpha]_{365}^{22}$ +402 (c 1.19, CH_3OH). HR-MS for $C_{20}H_{32}O_5$, calc. 352.2249, found 352.2250.

X-ray structure analysis of ***5c*** [45]. A crystal was obtained from methanol by slow evaporation. X-ray diffraction data were recorded on a Philips PW1100 diffractometer. Orthorhombic, space group Pbca (Z = 8) with a = 27.937(6); b = 14.774(3); c = 9.613(2). The structure was solved by direct methods and refined by anisotropic thermal factors for the non-hydrogen atoms to a R value = 6.5% on F and 17.5% on intensities (SHELX93 program), for 3438 observed amplitudes with $I \geq 3\ \sigma(I)$.

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