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Ring A Functionalized *Neo*–Clerodane Diterpenoids from *Cistus populifolius*

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Abstract: The isolation and characterization of 11 *neo*–clerodane diterpenic acids, three of them described for the first time is reported. The absolute stereochemistry for some of them are determined unambiguously by hemisynthesis. This work is also an approach to the obtention of highly functionalized diterpenoids from readily available natural products, that could be a starting point for a future synthesis of new antifeedant agents.

INTRODUCTION

In previous papers on the chemical composition of *Cistus populifolius* L. a series of *neo*–clerodane diterpenoids were isolated and their structure established by means of spectroscopic techniques and thereafter confirmed by chemical transformations.[1,2] None of the *Cistus* species previously studied[3] contains this type of diterpenes, that has acquired a great interest due to the wide spectrum of biological activities of several members of this class.[4]

The use of readily available natural compounds as homochiral templates in the synthesis of other minor derivatives has attracted our attention and natural compounds as labdanolic and zamoranic acids[5,6] had been used in the synthesis of commercial compounds used in the perfumery industry[7] or antifeedant agents.[8] The requirement of the major components as homochiral templates for the synthesis of possibly bioactive *neo*–clerodanes has impelled a new study of the acid fraction.

This study described the isolation of eleven methyl esters of the *neo*–clerodane class: eight known components (**1–5, 8** and **10**) and three new compounds (**6, 9** and **11**) whose structure was determined by spectroscopic techniques and their absolute stereochemistry by unambiguous hemisynthesis. The ^{13}C NMR data are given for all of them for the first time and the assignment done for some of them by means of 2D heteronuclear correlation experiments (one bond and long range).

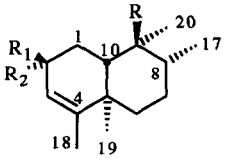
All the acids isolated posses the same side chain and their differences are restricted to the functionalization of ring A: a double bond (Δ^3) in **1**, two conjugated double bonds in **2** and **3**, a double bond (Δ^3) and a methoxyl, an acetoxyl or a hydroxyl group at C–2 in **4, 5, 6** and **9**, respectively; a double bond (Δ^3) and a carbonyl group in **8**, a hydroxyl group at C–4 in **7**, a carbonyl group at C–2 and an oxiranic ring (C–3, C–4) in **10** and two hydroxyl groups at C–3 and C–4 in **11**.

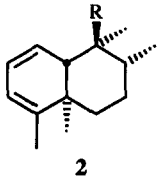
Compounds **5, 7, 8** and **10** have been tested as antifeedant agents agains *Spodoptera littoralis*. All of them

showed a low–medium level as antifeedants and that little changes in the functionality at C–2 modified the observed level of activity. These results are encouranging to proceed in this research modifying and increasing functionalization of A ring. The antifeedant activity as well as other biological activity results will be published elsewhere.

RESULTS AND DISCUSSION

The acid fraction soluble in Na_2CO_3 of the hexane extract of *Cistus populifolius*, L. was esterified with diazomethane solution and chromatographed over Silica gel (see details in experimental section). Compounds **1**–**11** (Figure 1) were separated and identified as: **1** Methyl 3–*neo*–cleroden–15–oate (populifolic acid methyl ester), **2** Methyl 1,3–*neo*–clerodadien–15–oate (dehydropopulifolic acid methyl ester), **3** Methyl 2,4(18)–*neo*–clerodadien–15–oate (isodehydropopulifolic acid methyl ester), **4** Methyl 2α–methoxy–3–*neo*–cleroden–15–oate (2α–methoxy–populifolic acid methyl ester), **5** Methyl 2α–acetoxy–3–*neo*–cleroden–15–oate, **6**, **7** Methyl 2β–hydroxy–3–*neo*–cleroden–15–oate (*epi*–oxypopulifolic acid methyl ester), **8** Methyl 2–oxo–3–*neo*–cleroden–15–oate (oxopopulifolic acid methyl ester), **9**, **10** Methyl 2α–hydroxy–3–*neo*–cleroden–15–oate (oxypopulifolic acid methyl ester) and **11**.





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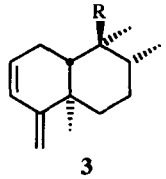
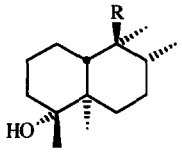


3

	R_1	R_2
1	H	H
4	H	OCH_3
5	H	OAc
7	OH	H
8	O	
10	H	OH

R = $(CH_2)_2$–CHMe–CH_2–CO_2Me (13, 16)



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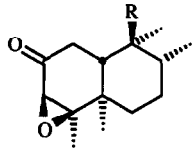

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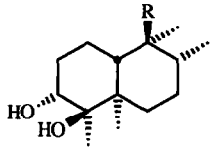


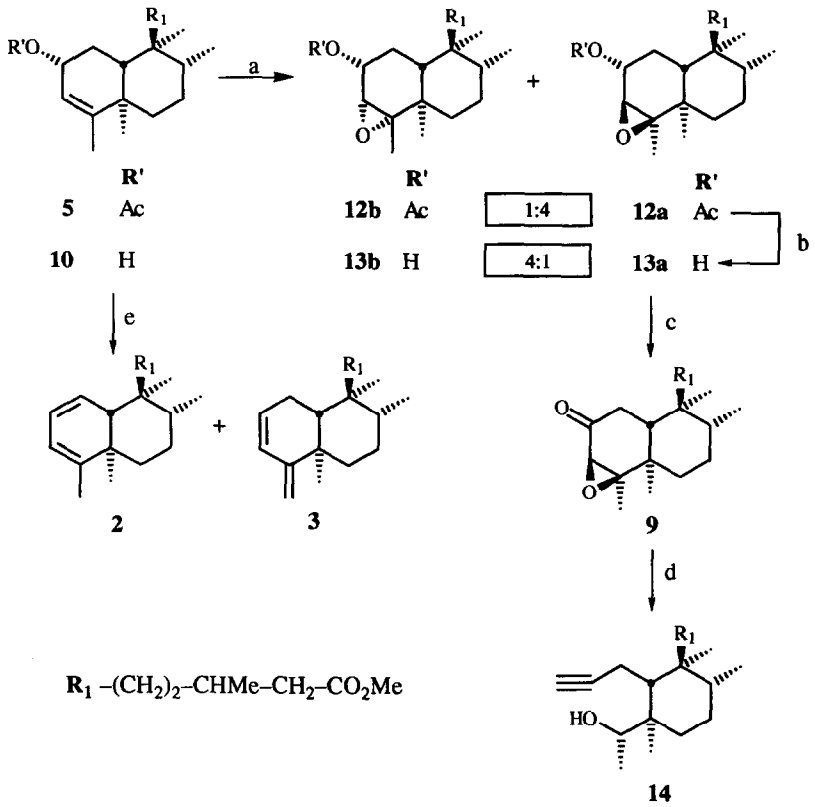
11

Figure 1. Natural *neo*–clerodane diterpenoids from *Cistus populifolius* L.

The hydroxy ester **6** (IR 3440 and 1740 cm^{-1}) shows a parent molecular ion at m/z 338 in its MS spectrum that corresponds to a molecular formula $C_{21}H_{38}O_3$ in agreement with a bicyclic diterpenoid with a carboxylic acid methyl ester and a hydroxyl group. Its ^1H NMR spectrum is characterized by six methyl groups: a methoxyl of the methyl ester, four corresponding to the bicyclic system of the *neo*–clerodane skeleton (two doublets, J = 6.4 Hz, and two singlets) and one deshielded (δ 1.27 ppm) corresponding to a –COH(CH_3) group. The ^{13}C NMR

spectrum shows signals corresponding to 21 carbon atoms: four of them are quaternary (a carboxyl and at δ 76.1 one directly bonded to oxygen), the protonated ones are sorted by DEPT as six CH_3, eight CH_2 and three CH. These data is in agreement with the structure Methyl 4–hydroxy–*neo*–clerodan–15–oate for compound **6**.

Compound **9** has in its MS spectrum a molecular parent ion at m/z 350 corresponding to a molecular formula $C_{21}H_{34}O_4$. In the IR spectrum there are two strong absorption bands at 1735 (ester) and 1720 cm^{-1} (carbonyl group). The two carbonyl functions are observed in the ^{13}C NMR spectrum at δ 207.9 and 173.4 ppm. The other signals are assigned as six methyl groups, six methylenes and four methines (one directly bonded to oxygen at δ 63.6 ppm) and three other quaternary carbon atoms, one of them supporting an oxygenated function at δ 69.0 ppm. The ^1H NMR spectrum shows in addition to the four methyl groups (two singlets and two doublets) of the *neo*–clerodane bicyclic system and the methyl ester, a singlet of one hydrogen at δ 3.08 ppm and a singlet of a deshielded methyl group at δ 1.33 ppm corresponding to a $CH_3-C(=O)-C-H$ group that allows to assign the structure as Methyl 2–oxo–3,4–epoxy–*neo*–clerodan–15–oate, that is confirmed by treatment of **8** with H_2O_2/OH^- and the stereochemistry of both C–3 and C–4 is determined and discussed later.



Scheme 1. a) *m*–CPBA; b) K_2CO_3/MeOH; c) CrO_3/Py; d) $TsNHNH_2/NaBH_4$; e) MsCl/Py

Table 1. ^{13}C NMR Data (50.3 MHz, $CDCl_3$)

C	1	2	3	4	5[a]	6	7	8[a]	9	10	11	12a	13a	13b
1	18.3	125.9	23.7	23.3	24.7	23.5	27.6	35.0	35.2	27.3	16.4	23.7	27.4	25.4
2	26.9	125.1	128.5	74.6	72.3	21.2	65.2	200.1	207.9	69.4	30.5	68.9	66.8	70.7
3	120.5	119.2	128.9	120.3	120.3	37.1	122.2	125.6	63.6	124.7	76.5	62.1	64.9	65.5
4	144.5	148.0	157.3	150.0	149.6	76.1	149.4	172.2	69.0	147.4	76.4	66.3	70.5	70.5
5	38.6	38.3	38.8	38.8	38.6	42.1	38.8	39.9	38.5	38.8	41.4	38.1	38.0	36.3
6	36.9	35.2	37.3	36.3	36.4	32.0	36.3	35.7	35.2	36.5	32.5	35.8	36.1	36.9
7	27.7	27.6	27.4	27.5	27.3	27.2	28.3	27.0	26.9	29.0	26.7	26.7	26.9	28.1
8	36.3	35.9	36.7	36.1	35.9	36.8	36.2	36.0	36.2	36.0	36.1	36.0	36.0	35.9
9	38.2	38.1	37.8	38.4	38.3	38.8	38.3	38.7	37.7	38.2	38.6	37.2	37.4	38.9
10	46.5	47.8	43.6	41.3	45.1	43.2	40.9	45.8	36.8	45.2	40.8	34.7	34.9	46.5
11	35.5	34.8	35.2	35.4	35.2	35.7	35.7	34.9	34.6	35.4	35.8	34.2	34.2	35.5
12	29.5	29.6	29.4	29.3	29.3	29.5	28.8	29.1	29.1	29.3	29.6	29.5	29.5	29.4
13	31.1	31.1	31.1	31.2	30.9	31.1	31.1	30.9	31.0	31.0	31.1	31.0	31.1	31.0
14	41.8	41.6	41.6	41.8	41.4	41.6	41.4	41.5	41.4	41.5	41.6	41.4	41.5	41.5
15	173.6	173.7	173.4	173.8	173.4	173.6	174.1	173.4	173.4	173.6	173.8	173.5	173.7	173.6
16	19.9	19.9	19.9	19.9	19.9	20.0	20.1	19.7	19.9	19.9	20.0	19.9	19.9	19.9
17	16.0	15.9	15.8	15.8	15.9	15.9	15.8	15.7	15.5	15.9	15.9	15.9	15.9	15.9
18	17.6	14.8	106.9	18.0	17.7	23.5	18.0	18.8	16.3	17.7	21.3	17.1	17.4	16.6
19	20.0	20.0	22.0	18.6	19.7	14.8	18.6	18.4	17.3	19.9	17.3	18.2	18.5	19.5
20	18.5	17.5	18.3	18.3	18.4	18.3	18.4	17.9	17.7	18.5	18.5	18.3	18.3	18.8
CO_2Me	51.3	51.3	51.3	51.2	51.3	51.3	51.4	51.4	51.3	51.3	51.3	51.3	51.5	51.4
$MeCO_2$					21.3							21.1		
$MeCO_2$					170.7							170.0		
OMe				56.3										

a. Assignment has been made by 2D heteronuclear (one bond and long range) experiments. All other assignments have been made by comparison.

Table 2. ^{13}C NMR Data (50.3 MHz, $CDCl_3$)

C	14	17	18	19	20	21
1	13.8	15.5	22.3	22.3	17.1	23.4
2	86.9	30.0	21.2	21.2	39.5	21.2
3	68.9	62.3	35.7	36.2	74.5	37.1
4	72.0	66.5	75.5	70.1	50.1	76.1
5	41.4	39.0	41.6	41.6	37.5	42.1
6	36.4	37.3	31.8	31.8	32.1	32.0
7	26.7	28.3	27.3	27.3	27.0	27.2
8	36.3	36.1	36.2	36.2	36.4	36.7
9	40.3	37.3	38.9	38.6	38.6	38.8
10	43.9	47.9	40.4	40.5	48.1	43.1
11	30.7	35.6	35.6	35.6	35.3	35.6
12	29.7	28.3	29.8	29.7	29.8	29.7
13	31.1	30.3	30.2	30.7	30.5	30.6
14	41.8	40.0	39.9	35.9	35.5	35.6
15	173.7	61.3	61.3	63.2	63.1	63.1
16	19.9	19.9	20.0	19.8	19.7	19.7
17	15.9	16.0	16.1	16.1	16.1	16.0
18	18.2	16.9	24.4	24.4	15.3	23.5
19	16.1	19.7	17.7	17.6	11.4	14.8
20	18.3	18.8	18.6	18.5	18.6	18.4
CO_2Me	51.4					
$MeCO_2$				21.0	21.5	21.0
$MeCO_2$				170.9	170.9	171.2
$MeCO_2$					21.1	
$MeCO_2$					171.3	

Compound **11** is also a hydroxy ester (IR 3440 and 1735 cm^{-1}) and its MS spectrum shows a parent molecular ion at m/z 354 corresponding to a molecular formula $C_{21}H_{38}O_4$. In its ^{13}C NMR spectrum peaks corresponding to 21 carbon atoms are observed. The protonated carbons are sorted and edited by DEPT as six methyl groups, seven methylenes and four methines (one directly bonded to a hydroxyl group at δ 76.5 ppm) and four quaternary carbons (a carboxyl group at δ 173.8 ppm and one directly bonded to oxygen at δ 76.4 ppm). The ^1H NMR spectrum is characterized by a –CHOH–C(OH)Me (δ 3.54 ppm, 1H, t, J = 2.9 Hz and 1.20 ppm, 3H, s). All these data allow the assignment of the structure of **9** as Methyl 3,4–dihydroxy–*neo*–clerodan–15–oate.

The absolute stereochemistry of C–3 and C–4 in compound **9**, C–4 in **6** and C–3 and C–4 in **11** has been assigned in a later stage by unambiguous hemisynthesis of all them using as the major components **5**, **8** and **10** starting materials, (Schemes **1** and **2**).

Treatment of the acetyl derivative **5** (Scheme **1**) with *m*–CPBA led to a mixture of epoxides **12a** and **12b** in a 4:1 ratio. The selective hydrolysis of **12a** with K_2CO_3 gave epoxide **13a**. However, when **10** is treated under the same reaction conditions with *m*–CPBA the mixture of epoxides **13a** and **13b** is obtained with a 1:4 ratio. This means that the peracid attack in compound **5** occurs from the opposite face of the acetoxy and methyl substituents, that is the less hindered face, leading to a *trans* relationship between the acetoxyl group and the oxiranic ring; while in the case of the free hydroxyl group, **10**, the formation of a hydrogen bond[9] between the

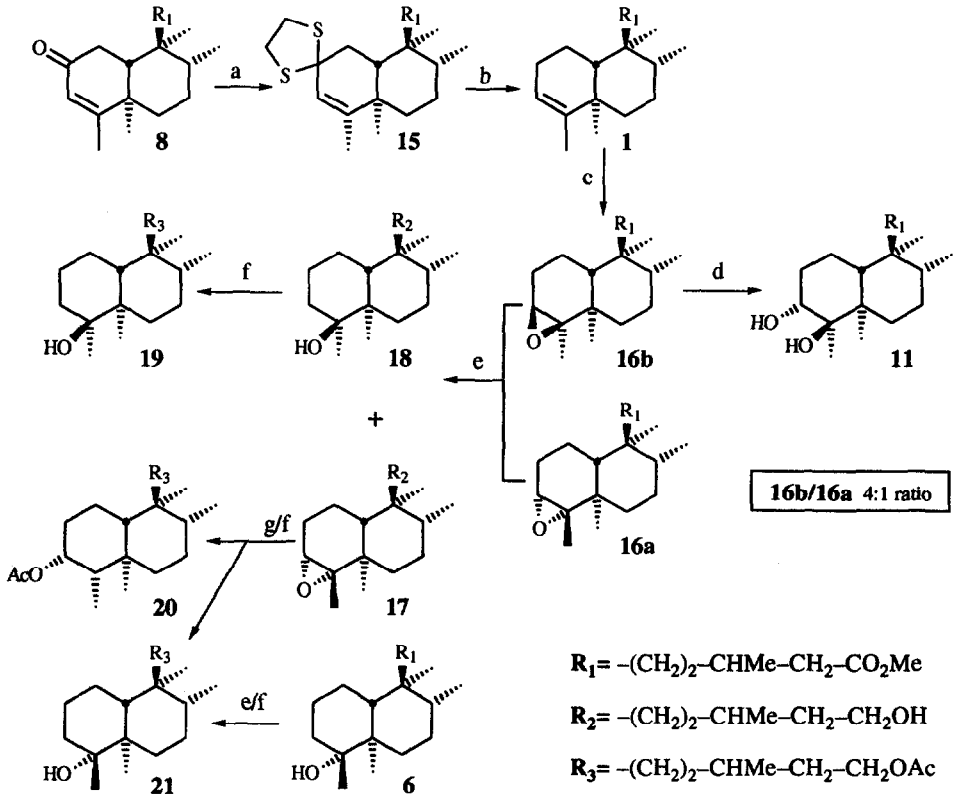
hydroxyl group and the peracid favoured the attack from the same face, the more hindered face, leading to the an inverse ratio, where the major product is epoxide **13b**.

Compound **13a** is oxidized with CrO_3/Py affording **9**, that could also be obtained from **8** with poor yield after treatment with H_2O_2/OH^-,[10] that confirmed unambiguously the stereochemistry of both C–3 and C–4 in compound **9** (Methyl 3β,4β–epoxy–2–oxo–*neo*–clerodan–15–oate).

Populifolic acid methyl ester **1** is isolated in very low yield from the extract and its hemisynthesis is necessary because it is the synthetic precursor for both **6** and **11** through an intermediate epoxide **17** (Scheme **2**).

When **5** is used as the starting material, the reduction of the mesyl derivative will afford 3–*neo*–cleroden–15–ol. However, when **5** is treated with MsCl two elimination products **2** and **3** were obtained.

The reduction with $NaBH_4$ of the tosylhydrazone of **9** will give **16b**,[11] however, when **9** is treated with tosyl hydrazine and $NaBH_4$, the acetylenic derivative **14**, the product of an Eschenmoser opening,[12] is obtained.



Scheme **2**. a) $HSCH_2CH_2SH$/HOAc/*p*–TsOH ; b) Raney Ni; c) *m*–CPBA; d) $HClO_4$; e) LAH/Et_2O; f) Ac_2O/Py; g) LAH/THF/Δ

Treatment of **8** with ethanedithiol in acidic medium gave the dithioacetal **15** that after hydrogenolysis with Raney Ni afforded **1**. Treatment of the latter with *m*–CPBA gave the mixture of epoxides **16a** and **16b**, that cannot be separated by chromatographic techniques. Reduction of the mixture with LAH afforded a separable

mixture of **17** and **18**. Compound **17** is an epoxide hard to reduce and **18** is the *trans* diaxial ring–opening product of the β-epoxide and reduction of the methoxycarbonyl group of the side chain. Compound **17** could be reduced treating it with LAH/THF under reflux. Subsequent acetylation afforded two separable compounds **20** and **21**. The latter is an epimer at C–4 of **19**, that is obtained after acetylation of **18**.

Reduction of **6** with LAH and acetylation afforded **21**, fixing the stereochemistry of the hydroxyl group at C–4 as α for compound **6**.

Finally, when epoxides **16a** and **16b** were treated with $HClO_4$, **11** was separated and the stereochemistry at C–3 and C–4 determined unequivocally.

EXPERIMENTAL

Unless otnerwise stated, all chemicals were purchased as the highest purity commercially available and were used without further purification. Melting points were determined with a Kofler hot stage melting point apparatus and are uncorrected. IR spectra were recorded on a BOMEM 100 FT IR spectrophotometer. ^1H and ^{13}C NMR spectra were performed in deuterochloroform and referenced to the residual peak of $CHCl_3$ at δ 7.26 ppm and δ 77.0 ppm, for ^1H and ^{13}C, respectively in a Bruker WP–200 SY. Chemical shifts are reported in δ, ppm and coupling constants (*J*) are given in Hz. MS spectra were performed in a VG–TS 250 spectrometer at 70 eV ionizing voltage. Mass Spectra are presented as m/z (% rel. int.) Optical Rotations were determined in a Perkin–Elmer 241 polarimeter in 1 dm cells. Diethyl ether, THF, benzene were distilled from Sodium and pyridine and dichloromethane were distilled from Calcium hydride under Ar atmosphere.

Plant Material.

Fresh plant material was coolected in June 1993 from Valle de las Batuecas (Salamanca, Spain). A voucher specimen is deposited in the herbarium of the Department of Botany, University of Salamanca.

Extraction and Initial Fractionation.

Air dried material (1.5 kg) was extracted continuously with hexane during 8 hours in a Soxhlet apparatus. The hexane extract was concentrated in vacuo to afford a green residue (62.5 g). Dewaxing with MeOH afforded 42.7 g of crude dewaxed extract.

Isolation of Compounds.

The dewaxed extract (42.7 g) was dissolved in ether and washed successively with 10 % Na_2CO_3 and 4 % NaOH aqueous solutions. After usual work–up three fractions were obtained: the neutral part (14.2 g), the Na_2CO_3 soluble fraction (26.7 g) and the NaOH fraction (600 mg).

The Na_2CO_3 soluble fraction after esterification with diazomethane was chromatographed over 750 g of SiO_2 eluting with increasing polarity mixtures of hexane/Ethyl acetate (9:1 → 1:1) affording thirteen fractions (**I** → **XIII**). Column chromatography over SiO_2 impregnated with 10 % $AgNO_3$ of fraction **I** gave **1** (Hexane/Benzene, 1:1, 60 mg), **2** (Benzene, 42 mg), **3** (Benzene, 210 mg). From fraction **II** were separated **4** (90 mg) and **5** (4.5 g) . Fraction **VI** afforded **6** (18 mg) after CC over SiO_2 impregnated with 10 % $AgNO_3$ and PTLC eluting with Benzene/Ether 4:1, three times. From fractions **VII–X** were purified **7** (260 mg) and **8** (1.5 g). From fraction **XI** after successive CC were isolated **9** (25 mg), **10** (600 mg) and **11** (40 mg). ^1H NMR data (60 MHz) for compounds **1**, **2**, **3**, **4**, **5**, **7**, **8** and **10** were reported in references 1 and 2. Now, we reported for these compounds ^{13}C NMR data, only.

Natural Products.

Methyl 4α-hydroxy-neo-clerodan-15-oate, **6**: $[\alpha]_D = -0.4°$ (c= 0.78, $CHCl_3$). IR: ν_{max} (film) cm^{-1}: 3440, 2940, 1740, 1465, 1440, 1390, 1270, 1175, 1010. ^1H NMR: 3.66 (3H, COOMe, s), 1.27 (3H, s, Me-18), 1.01 (3H, s, Me-19), 0.93 (3H, d, *J* = 6.4, Me-16), 0.78 (3H, d, *J* = 6.4, Me-17), 0.70 (3H, s, Me-20). ^{13}C NMR: see Table 1. Compound **6** was reduced with $LiAlH_4$ and after usual work–up acetylated affording an acetate identical to the semisynthetic compound **21**. Mass spectrum was obtained from semisynthetic acetyl derivative.

Methyl 2–oxo-3β,4β-epoxy-neo–clerodan-15-oate, **9**: $[\alpha]_D = +51.0°$ (c= 1.2, $CHCl_3$); MS: 350 (M^+, 28), 333 (40), 129 (46), 99 (100), 85 (56). Exact mass calculated for $C_{21}H_{34}O_4$: 350.4986 (Δ ± 0.3 mmu). IR: ν_{max} (film) cm^{-1}: 2940, 1735, 1720, 1460,1430,1380, 1165,1075, 1015. ^1H NMR:3.65 (3H, s, COOMe); 3.08 (1H, s, H-3); 1.32 (3H, s, Me-18); 0.94 (3H, s, Me-19); 0.92 (3H, d, *J* = 6.4, Me-16); 0.79 (3H, d, *J* = 6.4, Me-17); 0.70 (3H, s, Me-20).^{13}C NMR: see Table 1.

Methyl 3α,4β-dihydroxy-neo-clerodan-15-oate, **11**: $[\alpha]_D = -6.8°$ (c= 0.75, $CHCl_3$). MS: 354 (M^+, 100), 322 (60.9), 225 (72.5), 207 (61.2), 189 (46.3), 163 (36.5), 137 (80.7), 123 (48), 109 (50), 95 (57), 69 (50.5), 55 (51.2), 43 (60.5). Exact mass calculated for $C_{21}H_{38}O_4$: 354.5303 (Δ ± 0.4 mmu).IR: ν_{max} (film) cm^{-1}: 3490, 2950, 1735, 1490, 1360, 1110, 960. ^1H NMR: 3.66 (3H, COOMe), 3.58 (1H, t, *J* = 2.9, H-3),1.24 (3H, s, Me-18), 1.11 (3H, s, Me-19), 0.93 (3H, d, *J* = 6.8, Me-16), 0.77 (3H, d, *J* = 6.4, Me-17), 0.72 (3H, s, Me-20). ^{13}C NMR: see Table 1.

EPOXIDATION OF 5 WITH *m*–CPBA: **12a/12b**

To a solution of **5** (370 mg, 0.94 mmoles) in CH_2Cl_2 (2 ml) was added *m*–CPBA (300 mg, 1.74 mmoles) in 2 ml of CH_2Cl_2. The reaction was monitored by TLC. After 30 h the reaction mixture was extracted with ether. The organic phase washed with 10% Na_2SO_3 and 10% $NaHCO_3$, dried over Na_2SO_4, filtered and evaporated to afford 359 mg of crude reaction product. CC over SiO_2 eluting with *n*-hexane/EtOAc 9:1 afforded **5** (20 mg), **12a** (240 mg, 67%) and **12a/12b** (97 mg, 27%).

Methyl 2α–acetoxy-3β,4β-epoxy-neo-clerodan-15-oate, **12a**: IR: ν_{max} (film) cm^{-1}: 2990, 1740, 1440,1390, 1250, 1030. ^1H NMR: 4.93 (1H, dd, J_1 = 7.8, J_2 = 9.8, H-2), 3.66 (3H, s, COOMe), 2.79 (1H, s, H-3), 2.08 (3H, s, OCOMe), 1.21 (3H, s, Me-18), 1.10 (3H, s, Me-19), 0.92 (3H, d, *J* = 6.8, Me-16), 0.76 (3H, d, *J* = 5.9, Me-17), 0.67 (3H, s, Me-20). ^{13}C NMR: see Table 1.

HYDROLYSIS OF **12a** WITH K_2CO_3/MeOH: **13a**

To 146 mg (0.37 mmoles) of **12a** was added 2 ml of 3% K_2CO_3/MeOH stirring at room temperature. The reaction was monitored by TLC. After 1 h water was added and extracted with ether, the organic layer was washed with water until neutrality, dried over Na_2SO_4, filtered and evaporated to afford **13a** (117 mg, 0.33 mmoles, 90 %).

Methyl 2α–hydroxy–3β,4β –epoxy–neo-clerodan-15-oate , **13a**. MS: 352 (M^+, 2.5), 334 (10), 291 (11),

223 (84), 205 (93), 123 (78), 109 (60), 95 (58), 59 (85), 55 (50). IR: ν_{max} (film) cm^{-1}: 3520, 2900, 1740, 1460, 1390, 1150, 990. ^1H NMR: 3.93 (1H, dd, $J_1 = 7.3$, $J_2 = 9.8$, H-2), 3.63 (3H, s, COOMe), 2.82 (1H, s, H-3), 1.18 (3H, s, Me-18), 1.06 (3H, s, Me-19), 0.89 (3H, d, $J = 6.8$ Hz, Me-16), 0.73 (3H, d, $J = 5.9$ Hz, Me-17), 0.65 (3H, s, Me-20). ^{13}C NMR: see Table1.

TREATMENT OF **10** WITH *m*–CPBA: **13a/13b**

A solution of **10** (1.75 g, 5.21 mmol) in dry CH_2Cl_2 (20 ml) was chilled to 0° C and *m*–CPBA (988 mg, 5.73 mmol) in CH_2Cl_2 (20 ml) was added. The reaction was warmed to room temperature monitoring by TLC. After 3h was extracted with ether and the organic layer washed with 10% Na_2SO_3, 10% $NaHCO_3$ and water, dried over anhydrous Na_2SO_4, filtered and evaporated. After flash chromatography of the crude product (n-hexane/EtOAc, 7:3) 633 mg of a mixture of **13a/13b** (highly enriched in **13b**), and (n-hexane/EtOAc, 1:1) 934 mg (51 %) of **13b**.

Methyl 2α–hydroxy-3α,4α-epoxy-neo-clerodan-15-oate, **13b**. IR: ν_{max} (film) cm^{-1}: 3340, 1740, 1490, 1450, 1390, 1290, 1110, 890. ^1H NMR: 3.85 (1H, m, H-2), 3.66 (3H, s, COOMe), 3.05 (1H, s, H-3), 1.19 (3H, s, Me-18), 1.02 (3H, s, Me-19), 0.92 (3H, d, $J = 5.4$, Me-16), 0.74 (3H, d, $J = 6.5$, Me-17), 0.62 (3H, s, Me-20). ^{13}C NMR: see Table 1

OXIDATION OF **13a** WITH CrO_3-PYRIDINE: **9**

To 12 ml of dry CH_2Cl_2 were added 0.9 ml of pyridine and 450 mg of CrO_3. The mixture was stirred during 15 min at room temperature. Then **13a** (265 mg, 0.75 mmoles) in CH_2Cl_2 was added. The reaction was monitored by TLC and after 4 h at room temperature, ether was added and filtered. The organic phase was washed with 5% NaOH, 5% HCl, 5% $NaHCO_3$ and water. Dried over anhydrous Na_2SO_4, filtered and evaporated to afford **9** (240 mg, 88 %).

EPOXIDATION OF **8** WITH H_2O_2/OH^-: **9**

To 171 mg (0.5 mmoles) of oxopopulifolic acid **8**, were added 0.14 ml of H_2O_2 (30 %) and 1.5 ml of MeOH. The mixture chilled to 15° C and 0.08ml of 6N NaOH added and left to warm to room temperature. The mixture was stirred for 4 h at room temperature. The reaction was quenched with water and acified to pH 5-6. The reaction mixture was extracted with ether and the organic phase dried over anhydrous Na_2SO_4. The residue (149 mg) was esterified with ethereal diazomethane and further purified by PTLC (n-hexane/EtOAc, 8:2) affording **9** (14 %).

MESYLATION OF **10** AND ELIMINATION: **2** AND **3**

To a solution of **10** (814 mg, 242 mmol) in CH_2Cl_2 (10 ml) was added Et_3N (0.47 ml). The mixture under Ar atmosphere was cooled to –5° C and 0.12 ml of MsCl added. The reaction was maintained at that temperature during 8 h, then it was allowed to reached room temperature monitoring by TLC. Ice was added and the mixture was extracted with ether. The organic phase was washed successively with 2N HCl and H_2O until neutrality. The organic phase was dried over Na_2SO_4, filtered and evaporated in vacuo to afford 738 mg of crude reaction product. After CC over SiO_2 eluting with hexane/EtOAc 9:1 (700 mg, **2** and **3**), hexane/EtOAc 4:1 (34 mg, **10**) were separated. **2** and **3** were separated by CC over SiO_2/10 % $AgNO_3$ eluting with hexane/EtOAc 95:5.

Methyl 1,3-neo-clerodadien–15–oate, **2**. IR: ν_{max} (film) cm^{-1}: 2900, 1740, 1420, 1390, 1110. ^1H NMR:

5.9 (1H, m, H-1); 5.7 (1H, m, H-2); 5.6 (1H, m, H-3); 3.64 (3H, s, COOMe); 2.30 (1H, dd, H-14a); 2.10 (1H, dd, H-14b); 1.66 (3H, s, Me-18); 0.93 (3H, d, J = 6.4, Me-16); 0.83 (3H, s, Me-19); 0.80 (3H, d, J = 6.4, Me-17); 0.80 (3H, s, Me-20). ^{13}C NMR: see Table 1.

Methyl 2,4(18)–neo–clerodadien–15–oate, **3**. IR: ν_{max} (film) cm^{-1}: 2900, 1730, 1470, 1430, 1390, 1170, 1140, 1110, 1030, 880. ^1H NMR: 6.02 (1H, d, J = 12.0, H-3); 5.73 (1H, m, H-2); 4.78 (1H, s, H-18a); 4.62 (1H, s, H-18b); 3.64 (3H, s, COOMe); 0.99 (3H, s, Me-19); 0.93 (3H, d, J = 6.4, Me-16); 0.78 (3H, d, J = 6.4, Me-17); 0.79 (3H, s, Me-20). ^{13}C NMR: see Table 1.

TREATMENT OF **9** WITH TOSYLHYDRAZINE AND $NaBH_4$: **14**

To 159 mg of **9** (0.45 mmoles) in absolute EtOH (0.9 ml) a solution of tosylhydrazine (85 mg) in absolute EtOH was added. The reaction mixture was heated in a water bath for 15 min until a clear solution was obtained and stored in the refrigerator for 12 h (4°C). The reaction was monitored by TLC observing disappearance of **9**.

To the reaction mixture was added $NaBH_4$ (26 mg) and stirred for 3 h at room temperature. Then, water and a few drops of 2N HCl were added and extracted with ether. The organic phase was washed with water until neutrality, dried, filtered and evaporated giving 130 mg of crude reaction product. CC over SiO_2 (hexane/EtOAc 9:1) afforded **14**, 56 mg (37 %).

MS: 336 (M^+, 10), 291 (4), 252 (10), 163 (25), 123 (100), 107 (18), 95 (19), 81 (18), 69 (27). Exact mass calculated for $C_{21}H_{36}O_3$: 336.5150 ($\Delta \pm 0.2$ mmu). IR: ν_{max} (film) cm^{-1}: 3590, 3300, 2220, 1735, 1480, 1390, 1290, 1105, 1050, 1010, 910.^1H NMR: 3.77 (1H, q, H-4); 3.64 (3H, s, COOMe); 2.00 (1H, s, H–3), 1.08 (3H, d, J = 6.3, Me-18); 0.94 (3H, d, J = 6.8, Me-16); 0.79 (3H, s, Me-19); 0.77 (3H, d, J = 6.4, Me-17); 0.88 (3H, s, Me-20). ^{13}C NMR: see Table 2.

REACTION OF **8** WITH ETHANE DITHIOL: **15**

To a stirred solution of **8** (569 mg, 1.68 mmoles) in glacial HOAc (0.68 ml) were added ethanedithiol (0.14 ml) *p*-TsOH (147 mg) and glacial HOAc (1.57 ml). The reaction mixture was stirred for 6 h at room temperature. Water was added, stirring was continued for 30 min and more water was added, after usual work–up, **15** (696 mg) was obtained quantitatively.

^1H NMR: 5.38 (1H, bs, H-3); 3.66 (3H, s, COOMe); 1.60 (3H, s, Me-18); 0.99 (3H, s, Me-19); 0.95 (3H, d, J = 8.0, Me-16); 0.77 (3H, d, J = 8.0, Me-17); 0.72 (3H, s, Me-20).

REDUCTION WITH RANEY Ni OF **15**: **1**

To a solution of **15** (696 mg) in absolute EtOH (10 ml) were added three teaspoons of Raney Ni, adding also more absolute EtOH until a final volume of 50 ml. The reaction was refluxed for 21 h. The mixture was filtered through celite washing with absolute EtOH and percolating again the filtrate through a celite packed column. After evaporating the solvent under reduced pressure **1** (450 mg, 83% yield respect to ketone **8**) was obtained.

Methyl 3-neo-cleroden-15-oate, **1**. IR: ν_{max} (film) cm^{-1}: 2870, 1740, 1450, 1390, 1180, 1030. ^1H NMR: 5.18 (1H, bs, H-3); 3.64 (3H, s, COOMe); 1.55 (3H, s, Me-18); 0.97 (3H, s, Me-19); 0.92 (3H, d, J = 8.0, Me-16); 0.74 (3H, d, J = 8.0 , Me-17); 0.69 (3H, s, Me-20).

EPOXIDATION OF **1** AND REDUCTION WITH LAH: **17** AND **18**

To a solution of **1** (288 mg, 0.84 mmoles) in CH_2Cl_2 (2 ml) was added a solution of *m*–CPBA (157 mg) in CH_2Cl_2 (4 ml). The reaction mixtured was stirred at room temperature for 1 h. Filtered and washed

successively with 10 % Na_2SO_3, 5 % $NaHCO_3$ and water, dried over anhydrous Na_2SO_4 filtered and evaporated to afford 275 mg of crude reaction product that was flash chromatographed (hexane/EtOAc, 95:5) giving 220 mg of **16a + 16b** according to the ^1H NMR spectrum and that are not separated by CC. To a solution of the latter mixture of **16a/16b** (137 mg, 0.45 mmoles) in THF (3 ml) was added LAH (16 mg). The reaction mixture was stirred for 5 h at room temperature under Ar atmosphere. Wet ether was added and the mixture filtered. The organic phase was dried over Na_2SO_4 filtered and evaporated affording 130 mg that was chromatographed (hexane/EtOAc 4:1) affording **17** (90 mg) and **18** (30 mg).

Neo-clerodan-3β,15-diol, **17.** IR: ν_{max} (film) cm^{-1}: 3300, 2900, 1460, 1390, 1190, 1080, 940. ^1H NMR: 3.70 (2H, m, H-15); 1.06 (3H, s, Me-18); 0.95 (3H, s, Me-19); 0.90 (3H, d, J = 6.3, Me-16); 0.77 (3H, d, J = 6.3, Me-17); 0.70 (3H, s, Me-20). ^{13}C NMR: see Table 2.

3α,4α-epoxy-neo-clerodan-15-ol, **18.** IR: ν_{max} (film) cm^{-1}: 3410, 2900, 1450, 1390, 1220, 1110, 1090, 990, 890.^1H NMR: 3.69 (2H, m, H-15); 2.92 (1H, t, J = 1.9, H-3);1.17 (3H, s, Me-18); 1.04 (3H, s, Me-19); 0.91 (3H, d, J = 6.4, Me-16); 0.76 (3H, d, J = 5.9, Me-17); 0.64 (3H, s, Me-20). ^{13}C NMR: Table 2.

ACETYLATION OF **17**: **19**

To **17** (30 mg, 0.10 mmoles) pyridine (0.3 ml) and Ac_2O (0.6 ml) were added. The reaction was stirred at room temperature for 6 h, then crushed ice was added and the mixture extracted with ether. After usual work–up **19** (33 mg, 0.09 mmoles, 91%) was obtained.

15-acetoxy-neo-clerodan-3β-ol, **19.** IR: ν_{max} (film) cm^{-1}: 3400, 2900, 1740, 1460, 1390, 1370, 1250, 810. ^1H NMR: 4.18 (2H, m, H-15); 2.04 (3H, s, OCOMe); 1.08 (3H, s, Me-18); 0.95 (3H, s, Me-19); 0.87 (3H, d, J = 6.0, Me-16); 0.76 (3H, d, J = 6.0, Me-17); 0.70 (3H, s, Me-20). ^{13}C NMR: see Table 2.

REDUCTION AND ACETYLATION OF **17**: **20** AND **21**

To a solution of **17** (32 mg, 0.09 mmoles) in THF (3 ml) LAH (3 mg) was added. The reaction was refluxed for 4 h. After cooling, wet ether was added and dried over Na_2SO_4, filtered and evaporated to afford 31 mg of crude reaction product. Pyridine (0.4 ml) and Ac_2O (0.8 ml) were added. The reaction was stirred at room temperature for 8 h, then crushed ice was added and the mixture extracted with ether. PTLC of the crude acetylation product eluting with CH_2Cl_2 afforded **20** (17 mg) and **21** (14 mg).

3α,15-diacetoxy-neo-clerodane, **20.** IR: ν_{max} (film) cm^{-1}: 2900, 1740, 1735, 1480, 1390,1260, 1190, 810. ^1H NMR: 5.90 (1H, m, H-3); 4.16 (2H, m, H-15a, H-15b); 2.05 (6H, s, OCOMe); 0.93 (3H, s, Me-19); 0.90 (3H, d, J = 6.0, Me-18); 0.82 (3H, d, J = 6.0, Me-16); 0.79 (3H, d, J = 6.0, Me-17); 0.72 (3H, s, Me-20). ^{13}C NMR: see Table 2.

15 acetoxy-neo-clerodan-3α-ol, **21.** MS: 338 (M^+, 12), 325 (12), 321 (9), 305 (3), 293 (8), 265 (6), 251 (5), 209 (57), 191 (64), 177 (9),163 (8), 149 (10), 135 (19), 123 (42), 109 (8), 95 (48), 81 (47), 69 (60); 55 (86). Exact mass calculated for $C_{22}H_{40}O_3$: 352.2977 ($\Delta \pm 0.2$ mmu). IR: ν_{max} (film) cm^{-1}: 3590, 2900, 1740, 1460, 1390,1250, 1060, 940. ^1H NMR: 4.10 (2H, m, H-15a, H-15b); 2.05 (3H, s, COOMe); 1.29 (3H, s, Me-18); 1.03 (3H, s, Me-19); 0.92 (3H, d, J = 6.3, Me-16); 0.78 (3H, d, J = 6.3, Me-17); 0.72 (3H, s, Me-20). ^{13}C NMR: see Table 2.

REDUCTION AND ACETYLATION OF **6**: **21**

To a solution of **6** (15 mg, 0.04 mmoles) in THF (2 ml), LAH (1 mg) was added, the reaction was stirred at room temperature for 3 h. The crude product was acetylated adding pyridine (0.2 ml) and Ac_2O (0.4 ml) at room temperature for 5 h. Usual work–up afforded **21** (12 mg, 85.2%).

HYDROLYSIS OF **16b**: **11**

To the mixture of epoxides **16a/16b** (7:3, 56 mg) in 1 ml of dimethoxyethane chilled in an ice–bath was slowly added 6% $HClO_4$ (0.5 ml). The reaction was left at room temperature for 28 h. After that time, water was added and the mixture was extracted with ether. The organic phase was successively washed with 10 % $NaHCO_3$ and water. Dried over Na_2SO_4, filtered and evaporated to afford 54 mg of crude reaction product that was chromatographed over SiO_2 and eluting with hexane/EtOAc 4:1, **11** (25 mg) was separated.

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