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New Antifeedant *Neo*–Clerodane Triol. Semisynthesis and Antifeedant Activity of *Neo*–Clerodane Diterpenoids.

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Abstract: Methyl 2α,3β,4β–trihydroxy–*neo*–clerodan–15–oate was isolated from the acid fraction of *Cistus populifolius* and its structure determined by semisynthesis and confirmed by X–ray diffraction. Three other triols (**2**, **3** and **4**) and an intermediate diol (**5**) were also synthesized as potential antifeedants. The functional groups and stereochemistry for antifeedancy are discussed.

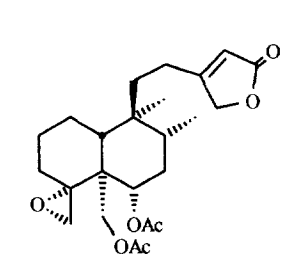
INTRODUCTION

Previous studies on *Cistus populifolius*[1] have shown that it is a rich source of *neo*–clerodane diterpenoids[2], a class of compounds that has received much attention due to the wide spectrum of biological activities of several members of this class, especially as antifeedants.[3]

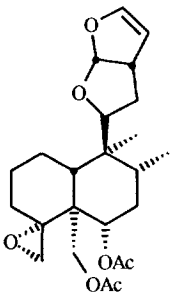
We report now on the isolation and structure elucidation of a new *neo*–clerodane triol **1**, whose structure has been confirmed by X–ray diffraction and its absolute configuration was established by chemical correlation, through **7**, with (5*R*,8*R*,9*S*,10*R*)–2–oxo–3–*neo*–cleroden–15–oic acid of which there is a D.C. study[1b] (populifolic acid : $\Delta\varepsilon_{341} = -0.19$, $\Delta\varepsilon_{230} = -0.97$, and $\Delta\varepsilon_{204} = -1.56$).

In the scheme below, it is shown, ajugarin **I**,[4] and clerodin **II**,[5] both compounds showed antifeedant activity.[6] In ajugarin **I** the butenolide part play an important part in the extension of the antifeedant activity of the decalin fragment. In clerodin the furofurane group and the epoxy diacetate playing a synergistic effect.[7] In this respect Kato[6b], showed that the difference of activity between clerodin **II** and clerodendrin **III** was due to the

substituents on ring A, with compound **IV** not active as an antifeedant.



I



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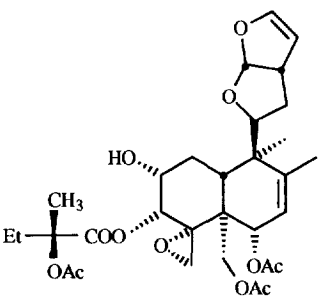
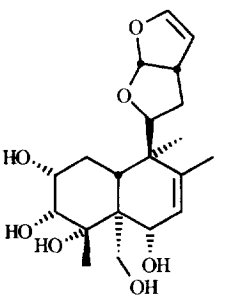
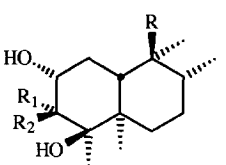


III

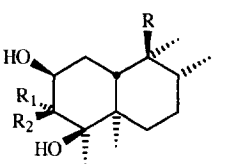


IV

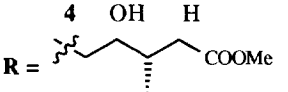
Taking advantage of the availability of triol **1** and diol **6**, also isolated from the same plant,[8] we have designed a synthetic strategy to prepare triols **2**, **3** and **4** and diol **5**, for structure–activity relationship studies, in particular the influence of ring–A hydroxyl group stereochemistries on the antifeedancy.

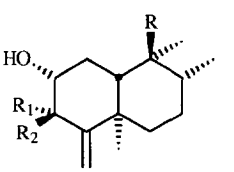


	R_1	R_2
1	H	OH
2	OH	H



	R_1	R_2
3	H	OH
4	OH	H





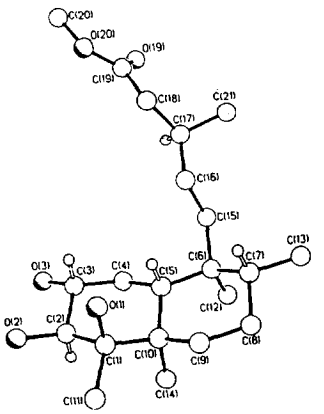
	R_1	R_2
5	OH	H
6	H	OH

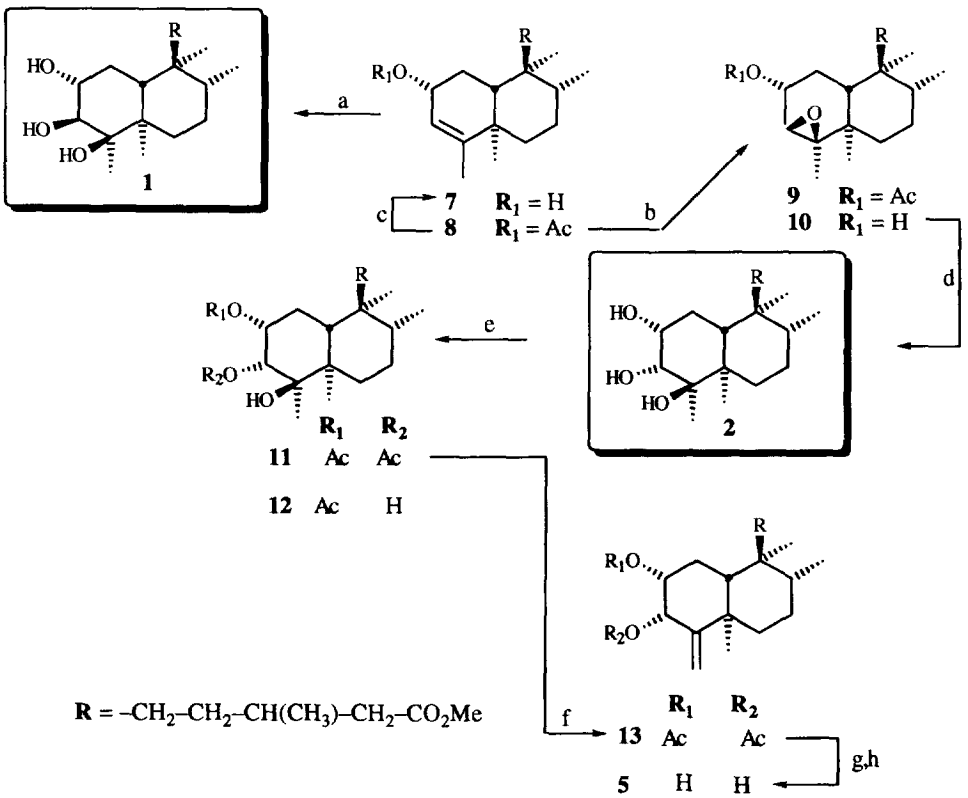
RESULTS AND DISCUSSION

The most polar fractions of the hexane extract of *C. populifolius* afforded compound **1**, isolated as its methyl ester. The EIMS (see experimental) showed a molecular ion peak at m/z 370 corresponding to a molecular formula $C_{21}H_{38}O_5$. The IR spectrum indicated the presence of a hydroxy ester and the ^1H NMR spectrum showed the characteristic methyl signals of *neo*–clerodane skeleton and the peaks corresponding to the *gem*–hydrogens to a hydroxyl group at δ 3.64 (1H, m) and δ 3.36 (1H, d, J = 6.5 Hz). Acetylation of **1** afforded a hydroxy diacetate. Based on the fact that one hydroxyl group was not acetylated with acetic anhydride and pyridine overnight, a tertiary hydroxyl group must be located at C–4 or C-8 since a deshielded methyl group at δ 1.15 (3H, s) was observed in the ^1H NMR spectrum. The COSY spectrum indicates that the two secondary hydroxyl groups are located on contiguous carbons. The ^{13}C NMR spectrum showed signals for twenty one carbon atoms: four of them are quaternaries (one directly bonded to an oxygen function at δ 78.0) and the protonated ones are sorted by DEPT subspectra as six methyl groups, six methylenes and five methines, two of them bearing an oxygenated function at δ 77.7 and δ 73.2. The signals corresponding to ring B and to the side chain carbon atoms are very similar to those observed for other *neo*–clerodane diterpenoids, suggesting that the hydroxyl groups must be on ring A. The elucidation of the configuration of both secondary hydroxyl groups at C–2 and C–3, respectively, has been done on the basis of the coupling constant values for the *gem*–hydrogens. The tertiary hydroxyl group at C–4 should be β, because **1** was prepared by *cis*–hydroxylation of **7**, 2α–oxy–populifolic acid methyl ester, with OsO_4, in which the reagent could access the substrate only by the less hindered β–face. The structure of **1** was confirmed by X–ray diffraction studies as methyl 2α,3β,4β–trihydroxy–*neo*–clerodan–15–oate (Figure 1).

Triols **2**, **3** and **4** were prepared from **8**, 2α–acetoxy–populifolic acid methyl ester, the major component of the plant extract.

Treatment of **8** with MCPBA afforded β–epoxide **9**,[9] being the β–face more accessible than the α–face for steric reasons. Hydrolysis of the acetoxyl group led to the epoxy alcohol **10**, that after treatment with $HClO_4$[9] afforded triol **2** (Scheme 1).



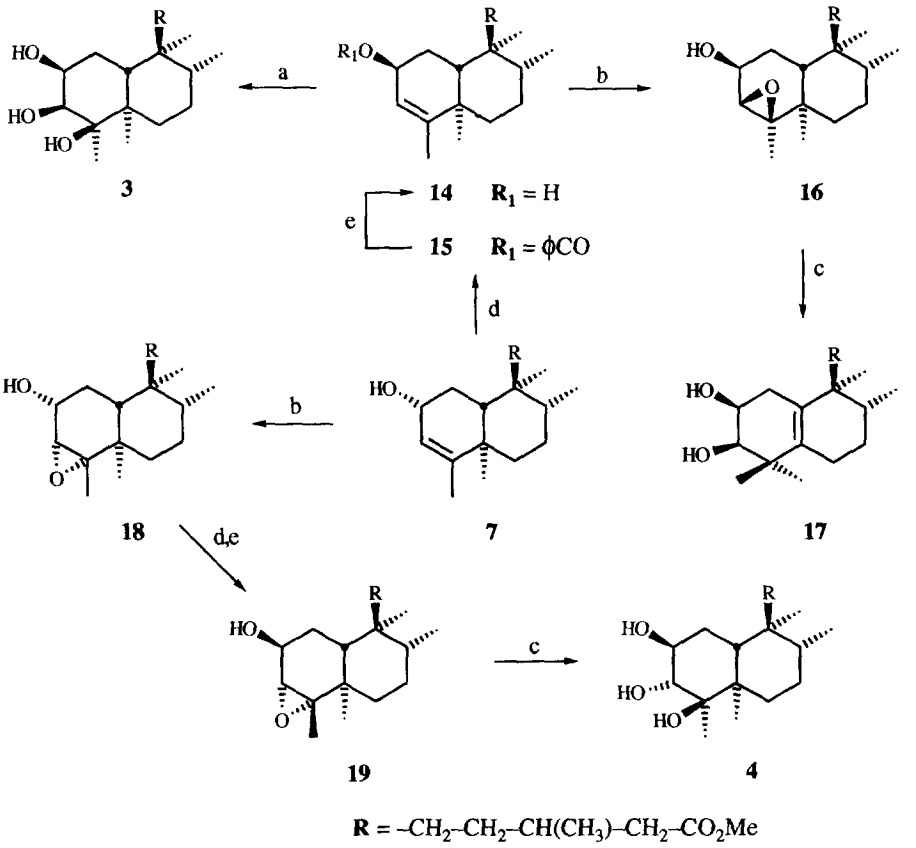


Scheme 1. a) OsO_4; b) MCPBA; c) K_2CO_3/MeOH; d) $HClO_4$; e) Ac_2O/Py; f) $POCl_3$; g) KOH/MeOH; h) CH_2N_2

The syntheses of triols **3** and **4** were designed originally from natural alcohol **14** because of the 2β–hydroxyl group, but **14** was isolated in very low quantity from *C. populifolius*, thus **7** was used as the starting material and Mitsunobu reaction conditions[10] were used to invert the configuration at C–2.

Treatment of **14** with OsO_4 under catalytic conditions[11] afforded **3** (Scheme 2). Epoxidation of **14** with MCPBA led to the β–epoxide **16**, because the β–hydroxyl group at C–2 favoured the entrance of the reactants from the β–face. Treatment of **16** with $HClO_4$ led to the rearranged diol **17** instead of the desired triol **4**. Instead of a *trans*–diaxial opening of the epoxide, which did not proceed because of the hydrogen bonding between the proton of the hydroxyl group at C–2 and the oxiranic oxygen, a carbocationic epoxide ring opening occurs with Me–19 migration.

Thus, approach to **4** has been done by means of the hydroxy epoxide **18** prepared from **7** by epoxidation.[4] Inversion of C–2 configuration afforded epoxy alcohol **19**, in which epoxide ring opening with $HClO_4$ afforded **4** (Scheme 2).



Scheme 2. a) OsO_4; b) MCPBA; c) $HClO_4$; d) $\phi COOH/(Ph)_3P/DEAE$; e) $K_2CO_3/MeOH$

Acetylation of **2** (Scheme 1) at room temperature with Ac_2O/Py led to a mixture of diacetate **11** and monoacetate **12**, separable by CC. Treatment of **11** with $POCl_3$ gave diacetate **13** and hydrolysis and esterification of the latter afforded diol **5**.

Finally, compounds 1-4 were tested for antifeedant activity against larvae of *Spodoptera littoralis*. The results of these bioassays are shown in Table 1, along with the activity of ajugarin (**I**) for comparative purposes[12].

Table 1. Effect of some Natural *Neo*-Clerodanes and their Derivatives on the Feeding Behaviour of Larvae of *Spodoptera littoralis*.

Compound	Antifeedant Index[a] Conc. 10^{-4} M	Conc. 10^{-5} M
I	25-29[13]	
1	52.0 ± 6.8	10 ±3.6
2	-62 ±16	-54 ±1.3
3	36 ± 9.7	1 ±14
4	33 ± 7.6	-12 ±28

a. Antifeedant Index: [(C-T)/(+T)] x 100; C= weight of control disc eaten, T= weight of treatment disc eaten. This index identifies both phagostimulants (-ve values) and antifeedants (+ ve values)

Thus, compounds **1**, **3** and **4** have more potent antifeedant activity against *Spodoptera littoralis* than ajugarin. These four compounds illustrate the effect changing the isomer/configuration of the hydroxy group at C2, C3 or C4 can have on the biological activity of the compounds. Very little difference between the activity of **3** and **4**; suggesting than the configuration of the hydroxy on C3 is not too important as long as the two adjacent hydroxy groups are in the β configuration. **1** is more active than either **3** or **4** which suggests that the configuration of the hydroxy groups at C2 and C3 is again shown by the activity of **2**. This compound has no antifeedant activity, in fact it stimulates feeding. Then the activity on neo-clerodanes with α β-hydroxyl group on C4 needs another β-hydroxyl on C2 or C3, increasing with the no formation of hydrogen bonds that activity, this corroborate in part the no antifeedant activity of **IV**. These results suggest that the receptors on the taste neurones of the insect are very responsive to small changes on this part of the molecule. We do not know from these studies whether the changes in the molecule influence directly the receptors on the so-called "deterrent taste neurones" or whether changes in the compounds influence the ability of the insect to perceive the sucrose on the bioassay discs that stimulates feeding. We are now changing the stereochemistry of C4 and the transformation of the side change to corroborate the theories of Kato[6b] of the synergistic effect and to approve our theory of the β effect in this type of clerodanes.

EXPERIMENTAL PART

Unless otherwise 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.

ISOLATION OF *Methyl 2α,3β,4β-trihydroxy–neo–clerodan–15–oate*, **1**.

The plant extraction and treatment of the hexane extract have been done as described in reference 4. Triol **1** was isolated in very low yield (40 mg) by CC of the esterified Na_2CO_3 soluble fraction of the extract . Triol **1** was separated from the most polar fractions of the original chromatography, eluted with ethyl acetate. $[\alpha]_D$ = +3.0 ($CHCl_3$, 0.8). IR (film) ν_{max} cm^{-1}: 3420, 2900, 1740, 1650, 1460, 1390 1060, 960, 860, 820, 740. MS: 370 ([M+], 90), 352 (70), 335 (95), 317 (40), 109 (25), 52 (100). Anal. Calc. for $C_{21}H_{38}O_5$ m/z 388.3300 ([M–H_2O+ 2NH_4^+], Δmmu = 0.3, HRCIMS).^1H δ: 3.64 (1H, s, H-2), 3.64 (3H, s, COO**Me**), 3.36 (1H, d, J = 6.5, H-3), 1.15 (3H, s, Me-18), 0.92 (3H, s, Me-19), 0.90 (3H, d, J = 6.0, Me-16), 0.74 (3H, d, J = 6.0, Me-17), 0.69 (3H, s, Me-20). ^{13}C δ: see Table 2.

Crystal data. $C_{21}H_{38}O_5$, M = 370.5, monoclinic, a = 10.548(6), b = 6.725(3), c = 15.336(12) Å, β = 104.52(2)°, V = 1053 $Å^3$, space group $P2_1$, Z = 2, D_c = 1.17 g cm^{-3}, μ(Cu–K_α) = 6.5 cm^{-1}, F(000) = 408. Data were measured on a Siemens P4/R4 diffractometer (graphite monochromator) using ω-scans. 1813 reflections

Table 2. ^{13}C NMR data.

C	**1**	**2**	**3**	**4***	**5**	**11**	**12**	**13**	**15**	**16**	**17**	**19**
1	29.3	29.9	27.9	25.0	25.7	22.9	22.2	22.7	25.2	27.8	30.8	25.1
2	73.2	69.7	70.8	73.1	72.9	70.7	73.7	73.1	69.5	64.2	67.2	67.3
3	77.7	79.8	71.8	76.7	77.2	76.4	77.1	75.5	118.4	62.9	78.8	63.7
4	78.0	77.1	80.4	77.5	157.9	76.0	76.4	152.7	152.0	68.2	40.9	67.7
5	41.7	41.5	41.9	41.7	41.7	41.3	41.0	41.5	38.4	38.0	134.2	38.5
6	31.6	33.0	31.3	32.1	37.8	32.0	32.2	37.4	36.2	34.7	25.9	37.1
7	26.8	27.2	26.8	26.7	27.0	26.7	26.6	27.0	27.5	27.0	27.3	28.2
8	35.9	36.7	35.9	36.4	36.2	36.0	36.1	36.1	36.2	36.0	30.8	36.2
9	38.4	39.0	38.1	38.2	38.7	38.7	38.7	39.1	38.6	36.9	41.8	36.9
10	37.8	39.3	33.2	34.0	45.1	38.9	38.9	45.5	40.7	35.9	130.7	40.3
11	35.7	36.4	36.0	36.4	35.5	35.8	35.7	35.4	34.5	34.5	33.3	36.2
12	28.4	27.4	28.4	28.4	29.7	29.5	29.5	29.3	29.1	28.9	29.7	28.2
13	31.1	31.4	31.2	31.4	31.0	31.1	31.0	30.9	30.8	31.1	31.4	31.1
14	41.5	41.6	41.1	41.1	41.4	41.5	41.5	41.4	41.7	41.5	41.2	41.0
15	173.8	173.5	174.4	174.8	173.7	173.8	173.7	173.7	173.5	173.7	173.8	174.7
16	19.9	20.1	20.1	20.2	19.9	20.0	20.0	19.9	19.7	20.0	19.7	20.2
17	15.8	16.3	15.8	15.9	15.9	16.0	16.0	15.9	15.8	15.8	16.0	15.8
18	19.6	22.3	18.4	20.6	112.1	21.4	21.3	114.8	18.0	17.2	23.6	18.9
19	17.2	17.9	17.1	17.7	22.5	17.0	17.3	21.9	18.7	18.0	27.5	17.1
20	18.5	19.0	18.4	18.6	18.4	18.5	18.6	18.4	18.3	18.2	21.1	18.9
COO<u>Me</u>	51.4	51.2	51.6	51.6	51.4	51.3	51.4	51.4	51.1	51.3	51.3	51.6
O<u>C</u>OMe						170.1	173.7	170.0				
OCO<u>Me</u>						21.1	21.9	21.1				
O<u>C</u>OMe						170.1		170.0				
OCO<u>Me</u>						21.1		21.3				
1'									131.0			
2'									129.7			
3'									128.3			
4'									132.8			
5'									128.3			
6'									129.7			
O<u>C</u>OPh									168.0			

* Assignment has been done by 2D heteronuclear experiment (HCCORR).

were measure ($2\theta \leq 120°$) of which 1724 were independent, $R_{int} = 0.045$, and of these 1164 had $|F_o| > 4\sigma(|F_o|)$ and were considered to be observed. The data were corrected for Lorentz and polarization factors; no absorption correction was applied. The structure was solved by direct methods and the non–hydrogen atoms were refined anisotropically. The positions of the hydrogen atoms idealized (C–H 0.96), assigned isotropic thermal parameters, $U(H) = 1.2U_{eq}(C)$, and allowed to ride on their parent carbon atoms. Two of the hydroxyl protons were located from ΔF maps, assigned isotropic thermal parameters, $U(H) = 1.2U_{eq}(O)$, and refined subject to a distance constraint (O–H 0.90 Å). The third hydroxyl proton, however, could not be located. Refinement was by full–matrix least squares to give $R = 0.057$, $R_w = 0.053$ [$w^{-1} = \sigma^2(F) + 0.0005F^2$]. The maximum and minimum residual electron densities in the final ΔF map were 0.20 and –0.21 eÅ^{-3} respectively. The mean and maximum shift/error ratios in the final refinement cycle were 0.001 and 0.004 respectively. Computations were carried out on an EuroBell 50 MHz 486 PC computer using the SHELXTLPC program system (version 4.1, Siemens Analytical X–Ray Instruments, Madison, WI, 1990).

TREATMENT OF **7** WITH OsO_4: **1**.

N–Methyl–morpholine–N–oxide (NMO, 48 mg, 0.35 mmol) and a solution of *t*–BuOH/THF/H_2O (10:3:1, 5.6 ml) were added to **7** (119 mg, 0.35 mmol) under Ar atmosphere. Then, a 2.5% solution of OsO_4 in *t*–BuOH (0.08 ml) was added and the mixture stirred at room temperature for 6 days. The reaction mixture was cooled at 0 °C and a 10% solution of sodium sulfite (4 ml) added. The mixture was warmed to room temperature and stirred for 3.5 h. The reaction mixture was extracted with CH_2Cl_2 and the organic layer washed with water, dried with anhydrous Na_2SO_4 and concentrated to give 95 mg of crude reaction product. Column chromatography (CC) on flash silica gel eluting with hexane/ethyl acetate (9:1) gave **7** (24 mg) and with hexane/EtOAc (1:1) **1** (30 mg, 23 %). The spectroscopic properties of the latter are identical to those of the natural compound.

SYNTHESIS OF *Methyl 2α–hydroxy–3,4β–epoxy–neo–clerodan–15–oate*, **10**.

Compound **10** was prepared as described in reference 4 by epoxidation and hydrolysis of acetate **8**.

ACID RING–OPENING OF **10**: *Methyl 2α,3α,4β–trihydroxy-neo-clerodan-15-oate* , **2**.

To a solution of **10** (49 mg, 0.14 mmol) in 1,2–dimethoxyethane (DME, 1 ml) was slowly added 6% $HClO_4$ (0.4 ml) while cooling at 0 °C in an ice–bath. The resulting mixture was stirred at 4 °C for 4 h, water was added and the mixture extracted with ether. The organic layer was washed with 10% $NaHCO_3$ and water, dried with Na_2SO_4 and concentrated. By crystallization from benzene/hexane **2** was separated (40 mg, 77%). mp: 150-152 °C. $[\alpha]_D = +32.5$ ($CHCl_3$, 0.08). MS: 352 ([M$^+$]–H_2O, 63), 308 (6), 266 (11), 241 (18), 223 (16), 205 (15), 167 (21), 137 (64), 123 (100), 95 (36), 69 (29), 55 (52). Anal. Calc. for $C_{21}H_{38}O_5$ m/z 388.3060 ([M+ NH_4^+], Δmmu = 0.3, HRCIMS). ^{1}H (Py-d_6) δ: 4.68 (1H, m, H-2), 4.24 (1H, bs, H-3), 3.63 (3H, s, COOMe), 1.66 (3H, s, Me-18), 1.46 (3H, s, Me-19), 0.85 (3H, d, J = 6.5, Me-16), 0.82 (3H, s, Me-20), 0.77 (3H, d, J = 5.5, Me-17).^{13}C (Py-d_6) δ: see Table 2.

ACETYLATION OF **2**: *Methyl 2α,3α–diacetoxy-4β–hydroxy-neo-clerodan-15-oate*, **11** and *2α–acetoxy-3α,4β–dihydroxy–neo-clerodan-15-oate*, **12**

Compound **2** (40 mg) was dissolved in pyridine (0.3 ml) and acetic anhydride (0.6 ml) was added. The mixture was kept at room temperature for 12 h. Ice was added and the mixture extracted with ether, the organic

layer was separated and washed with 2N HCl, 5 % $NaHCO_3$ and water, dried with Na_2SO_4 and evaporated to afford a mixture of diacetate **11** and monoacetate **12**. Both compounds were separated by CC, **11** (25 mg) was separated eluting with hexane/EtOAc (4:1) and **12** (14 mg) with hexane/EtOAc (1:1).

Compound **11**: IR (film) ν_{max} cm^{-1}: 3520, 2900, 1740, 1490, 1460, 1380, 1240, 1260, 1050. ^1H δ: 5.22 (1H, m, H-2), 5.18 (1H, m, H-3), 3.85 (3H, s, COOMe), 2.11(3H, s, OCOMe), 1.97 (3H, s, OCOMe), 1.12(3H, s, Me-18), 1.07 (3H, s, Me-19), 0.94 (3H, d, J = 6.5, Me-16), 0.78 (3H, d, J = 6.5, Me-17), 0.74 (3H, s, Me-20). ^{13}C δ: see Table 2.

Compound **12**: MS: 412 ([M+], 2), 394 (18), 352 (100), 205(32), 187 (43), 123 (95), 95 (18), 81 (22), 69(24). IR (film) ν_{max} cm^{-1}: 3460, 2890, 1740, 1720, 1490, 1360, 1260, 1110. ^1H δ: 5.30 (1H, m, H-2), 3.60 (1H, m, H-3), 3.66 (3H, s, COOMe), 2.08 (3H, s, OCOMe), 1.28 (3H, s, Me-18), 1.10 (3H, s, Me-19), 0.95 (3H, d, J = 6.5, Me-16), 0.76 (3H, d, J = 6.5, Me-17), 0.72 (3H, s, Me-20). ^{13}C δ: see Table 1.

Compound **11** (14 mg) was acetylated with Ac_2O/Py (0.2 ml: 0.1 ml) by heating at 70 °C during 12 h. After usual work–up **12** (15 mg) was obtained.

DEHYDRATION OF **11**: SYNTHESIS OF *Methyl 2α,3α–diacetoxy4(18)-neo-cleroden-15-oate*, **13**.

$POCl_3$ (0.15 ml) was added to a solution of **11** (35 mg, 0.08 mmol) dissolved in pyridine cooled in an ice–bath.. The reaction mixture was warmed to room temperature and monitored by TLC, then heated for 24 h at 50 °C. Ice was added and the reaction mixture extracted with ether. The organic layer was washed with 2N HCl, 5% $NaHCO_3$ and water, dried with anhydrous Na_2SO_4 and concentrated to give **13** (30 mg, 86%).

IR (film) ν_{max} cm^{-1}: 2900, 1740, 1440, 1420, 1360, 1240, 1040. ^1H δ: 5.70 (1H, d, J = 3.1, H–3), 5.08 (1H, s, H_a-18), 4.96 (1H, s, H_b-18), 4.69 (1H, dt, J_1 = 4.3 and J_2= 11.6, H-2), 3.63 (3H, s, COOMe), 2.03 (3H, s, OCOMe), 1.99 (3H, s, OCOMe), 1.10 (3H, s, Me-19), 0.91 (3H, d, J = 6.7, Me-16), 0.79 (3H, d, J = 6.1, Me-17), 0.68 (3H, s, Me-20). ^{13}C δ: see Table 2.

HYDROLYSIS OF **13** AND ESTERIFICATION : SYNTHESIS OF *Methyl 2α,3α-dihydroxy-4(18)-neo-cleroden-15-oate*, **5**.

2N KOH in MeOH (1 ml) was added to **13** (30 mg, 0.07 mmol) and stirred for 7 h at room temperature. The solvent was evaporated under reduced pressure and water was added. The reaction mixture was acidified with 2N HCl and extracted with ether. The organic layer was washed with water until neutrality, dried with Na_2SO_4 and evaporated to give the crude product (27 mg) which was esterified with diazomethane to give **5** (27 mg). IR (film) ν_{max} cm^{-1}: 3440, 2850, 1740, 1590, 1560, 1390. ^1H δ: 4.92 (1H, s, H_a-18), 4.23 (1H, s, H_b-18), 4.23 (1H, d, J = 4.2, H-3), 3.64 (3H, s, COOMe), 3.41 (1H, m, H-2), 1.15 (3H, s, Me-19), 0.90 (3H, d, J = 6.5, Me-16), 0.78 (3H, d, J = 6.5, Me-17), 0.73 (3H, s, Me-20). ^{13}C δ: see Table 2.

MITSUNOBU REACTION OF **7**: SYNTHESIS OF *Methyl 2β–benzoyloxy-4-neo-cleroden-15-oate*, **15** AND *Methyl 2β–hydroxy-4-neo-cleroden-15-oate*, **14**.

Benzoic acid (44 mg, 0.36 mmol) and triphenylphosphine (94 mg, 0.36 mmol) were added to a stirred solution of **7** (60 mg, 0.18 mmol) in dry ether (0.7 ml) under Ar atmosphere. The solution was cooled in an ice–bath and diethylazodicarboxylate (0.05 ml) was added and stirring was continued for 24 h until the reaction was judge complete. The mixture was extracted with ether and the organic layer washed with 10% $NaHCO_3$ and water, dried with Na_2SO_4 and evaporated to give 232 mg of a crude reaction product. The residue was

chromatographed on silica gel eluting with hexane/EtOAc (9:1) to afford **15** (68 mg, 87%).

IR (film) ν_{max} cm^{-1}: 2850, 1740, 1710, 1600, 1460,1440, 1280, 1180, 1160, 1110, 710. ^1H δ: 8.05 (2H, m), 7.44 (3H, m), 5.46 (1H, m, H-2), 5.42 (1H, m, H-3), 3.55 (3H, s, COOMe), 1.68 (3H, d, Me-18), 0.99 (3H, s, Me-19), 0.80 (3H, d, J = 6.8, Me-16),0.72 (3H, s, Me-20), 0.69(3H, d, J = 5.3, Me-17). ^{13}C δ: see Table 2.

Anhydrous K_2CO_3 (1.44 g, 11.2 mmol) was added to a solution of **15** (68 mg, 0.15 mmol) in MeOH (1.6 ml) and stirred for 5 days at room temperature and then heated to reflux for 24 h. The reaction mixture was filtered and the solvent evaporated under reduced pressure, water was added and the mixture extracted with ether. The organic layer was washed with water, dried with Na_2SO_4, filtered, evaporated and chromatographed (hexane/EtOAc, 9:1) to give **14** (19 mg, 38%). Spectroscopic data for compound **14** are given in references 1 and 4.

TREATMENT OF **14** WITH MCPBA: SYNTHESIS OF*Methyl–2β–hydroxy-3,4β–epoxy-neo-clerodan-15-oate*, **16**.

MCPBA (39 mg, 0.23 mmol) in dry CH_2Cl_2 (1.2 ml) was added to a cooled solution of **14** (69 mg, 0.21 mmol) dissolved in CH_2Cl_2 (1 ml). The reaction mixture was stirred at room temperature for 6 h. The solvent was removed and the residue extracted with ether, washed with 10% Na_2SO_3, 10% $NaHCO_3$ and water, dried with Na_2SO_4, filtered, evaporated and chromatographed (hexane/EtOAc, 7:3) to afford **16** (49 mg, 67% yield). $[\alpha]_D$ = -4.4 ($CHCl_3$, 0.70). IR (film) ν_{max} cm^{-1}: 3420, 2900, 1740, 1460, 1390, 1240, 1050. MS: 370 (100), 335 (45), 285 (28), 205 (10). Anal. Calc. for $C_{21}H_{36}O_4$ m/z 370.2957 ([M+ NH_4^+], Δmmu = 0.0, HRCIMS).^1H δ: 4.05 (1H, m, H-2), 3.63 (3H, s, COOMe), 3.09 (1H, d, J = 4.4, H-3), 1.21 (3H, s, Me-18), 0.97 (3H, s, Me-19), 0.89 (3H, d, J = 6.8, Me-16), 0.77(3H, d, J = 6.0, Me-17), 0.63 (3H, s, Me-20). ^{13}C δ: see Table 2.

TREATMENT OF **16** with 6% $HClO_4$, **17**

To a solution of **16** (39 mg, 0.11 mmol) in 1,2–dimethoxyethane (DME, 0.8 ml) was carefully added 6% $HClO_4$ (0.2 ml) while cooling at 0 °C in an ice–bath. The resulting mixture was stirred at room temperature for 6 h, water was added and the mixture extracted with ether. The organic layer was washed with 10% $NaHCO_3$ and water, dried with Na_2SO_4 and concentrated. After chromatography on silica gel (hexane/EtOAc, 4:1) **17** (22 mg, 57%) was separated.

$[\alpha]_D$ = +56.0 ($CHCl_3$, 0.9). IR (film) ν_{max} cm^{-1}: 3420, 2850, 1740, 1720, 1460,1430, 1380, 1210, 1150, 1080, 1050. ^1H δ: 3.98 (1H, m, H-2), 3.64 (3H, s, COOMe), 3.44 (1H, bs, H-3), 1.08 (3H, s, Me-18), 1.02 (3H, s, Me-19), 0.91 (3H, d, J = 6.8, Me-16), 0.82(3H, d, J = 6.0, Me-17), 0.82 (3H, s, Me-20). ^{13}C δ: see Table 2.

TREATMENT OF **14** WITH OsO_4: *Methyl 2β,3β,4β–trihydroxy–neo–clerodan-15-oate*, **3**.

N–Methyl–morpholine–N–oxide (NMO, 24.4 mg) and a solution of *t*–BuOH/THF/H_2O (10:3:1, 3.3 ml) were added to **14** (69 mg, 0.2 mmol) under Ar atmosphere. Then, a 2.5% solution of OsO_4 in *t*–BuOH (0.05 ml) was added and the mixture stirred at room temperature for 17 h, and then for 7 days at reflux (60 °C). The reaction mixture was cooled at 0 °C and a 10% solution of sodium sulfite (2.5 ml) added. The mixture was warmed to room temperature and stirred for 6 h. The reaction mixture was extracted with CH_2Cl_2 and the organic

layer washed with water, dried with anhydrous Na_2SO_4 and concentrated to give 44 mg of crude reaction product. Column chromatography (CC) on flash silica gel eluting with hexane/ethyl acetate (1:1) gave **3** (20 mg, 20%). $[\alpha]_D$= +4.9 ($CHCl_3$, 1.44). IR (film) ν_{max} cm^{-1}: 3410, 2890, 1740,1650, 1440, 1380,1240, 1110. ^1H δ: 4.16(1H, m, H-2), 3.66 (3H, s, COO**Me**), 3.45 (1H, bs, H-3), 1.15 (3H, s, Me-18), 0.93 (3H, s, Me-19), 0.95 (3H, d, J = 6.5, Me-16), 0.79 (3H, d, J = 5.5, Me-17), 0.73 (3H, s, Me-20). ^{13}C δ: see Table 2.

MITSUNOBU REACTION OF **18**: SYNTHESIS OF *Methyl 2β–hydroxy-3,4α–epoxy-neo-clerodan-15-oate*, **19**.

The epoxyalcohol **18** was prepared by epoxidation of **7** as described in reference 9. Benzoic acid (137 mg, 0.56 mmol) and triphenylphosphine (295 mg, 1.12 mmol) were added to a stirred solution of **18** (197 mg, 0.56 mmol) in dry ether (4.9 ml) under Ar atmosphere. The solution was cooled in an ice–bath and diethylazodicarboxylate (0.17 ml) was added and stirring was continued for 24 h until the reaction was judge complete. The mixture was extracted with ether and the organic layer washed with 10% $NaHCO_3$ and water, dried with Na_2SO_4 and evaporated to give 675 mg of a crude reaction product. The residue was chromatographed on silica gel eluting with hexane/EtOAc (9:1) to afford Methyl 2β–benzoyloxy-3α,4α–epoxy-neo-clerodan-15-oate (147 mg). Anhydrous K_2CO_3 (3.24 g, 23.4 mmol) was added to a solution of the latter in MeOH (25.4 ml) and stirred for 16 h at room temperature. Usual work–up afforded **19** (83 mg, 74%).

$[\alpha]_D$ = –29.5 ($CHCl_3$, 0.22). IR (film) ν_{max} cm^{-1}: 3490, 2850, 1740, 1450, 1390, 1280, 1090, 1010, 890, 740. MS: 370 (48), 335 (100), 317 (50), 285 (25), 205 (10). Anal. Calc. for $C_{21}H_{36}O_4$ m/z 370.2957 ([M+ NH_4^+], Δmmu = 0.0, HRCIMS). ^1H δ: 4.39 (1H, m, H-2), 3.64 (3H, s, COO**Me**), 2.90 (1H, bs, H-3), 1.16 (3H, s, Me-18), 0.98 (3H, s, Me-19), 0.88 (3H, d, J = 6.7, Me-16), 0.73 (3H, d, J = 5.0, Me-17), 0.61 (3H, s, Me-20). ^{13}C δ: see Table 2.

ACID RING–OPENING OF **19**: *Methyl 2β,3α,4β–trihydroxy-neo-clerodan-15-oate*, **4**.

3% $HClO_4$ (38 ml) was carefully added to a solution of **19** (392 mg, 1.11 mmol) in DME (6.9 ml) at room temperature. The mixture was stirred for 2.5 h, water was added and the reaction mixture was extracted with ether, washed with 5% $NaHCO_3$ and water, dried with Na_2SO_4. Removal of the solvent afforded **4** (406 mg, 99%). $[\alpha]_D$ = -0.69 ($CHCl_3$, 2.69). IR (film) ν_{max} cm^{-1}: 3390, 2880, 1740, 1460, 1450, 1390, 1380, 1280, 1180, 1120, 1050. ^1H δ: 4.06 (1H, bs, H-2), 3.64 (3H, s, COO**Me**), 3.57 (1H, bs, H-3), 1.18 (3H, s, Me-18), 1.06 (3H, s, Me-19), 0.94 (3H, d, J = 6.4, Me-16), 0.76 (3H, d, J = 5.9, Me-17), 0.73 (3H, s, Me-20). ^{13}C δ: see Table 2.

ANTIFEEDANCY BIOASSAYS.

A two-choice bioassy was used to assess the antifeedant activity of the compounds against larvae of *Spodoptera littoralis* (Boisduval). The compounds were presented to final stadium larvae on glass-fibre discs (Wathman GF/A 2.1 cm diam.) made palatable by the addition of 100µl of 50mM sucrose solution and subsequently air-dried. Discs receiving sucrose only were used as controls. Treatment discs were made by adding a further 100µl of a solution containing one of the test compounds at one of two concentrations (10^{-4} and 10^{-5} M). These discs were the redried and all the discs were weighed. Larvae 24-36h into the final stadium that had been deprived of food for 4 hours were placed singly in Petri dishes along with a control disc and treatment disc. The larvae remained in the Petri dishes for 18h or until approximately 50% of either disc had been

consumed. The discs were re-weighed and the Antifeedant Index [(C-T)/(C+T)] x 100 calculated, where C and T represent the amount of control and treatment disc eaten, respectively. Each concentration of the test compounds was tested against 10 different larvae.

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