Tetrahedron Vol. 49, No. 40, pp. 9067-9078, 1993
Printed in Great Britain

0040-4020/93 $6.00+.00

Neo-clerodane Diterpenoids from Aerial Parts of *Linaria Saxatilis* var. *Glutinosa*

Arturo San Feliciano, Marina Gordaliza*, José M. Miguel del Corral, María L. de la Puente

Departamento de Química Orgánica. Facultad de Farmacia. Universidad de Salamanca. Campo Charro s/n. E-37007. Salamanca. Spain.

Santiago García-Granda and Miguel A. Salvado

Departamento de Química Física y Analítica. Facultad de Química. Universidad de Oviedo. Julián Clavería s/n. E-33006-Oviedo. Spain

(*Received in UK* 14 *June* 1993; *accepted* 23 *July* 1993)

Abstract: From the less polar fractions of the aerial parts of *L. saxatilis* var.*glutinosa*, one new *neo*-clerodane diterpenoid, highly oxygenated at the side chain, was isolated in addition to several known compounds. The structure of the new compound was established by a combination of ^{1}H and ^{13}C NMR spectral data analysis, while the assignment of the absolute stereochemistry of the side chain was performed by X-ray crystallographic analysis. Varied spectroscopic studies and several chemical correlations were used to confirm the structures of the remaining compounds.

INTRODUCTION

The study of components of plants belonging to the genus *Linaria* (family: Schrophulariaceae) is of particular interest from the medicinal point of view, due to the variety of biological activities and therapeutical uses reported for them. As examples we can cite that *L. vulgaris* has been used in the treatment of several vascular diseases[1], *L. cymbalaria* possess diuretic, tonic and antiscorbutic properties[2] and *L. japonica* has been traditionally used in Japan as diuretic and laxative[3].

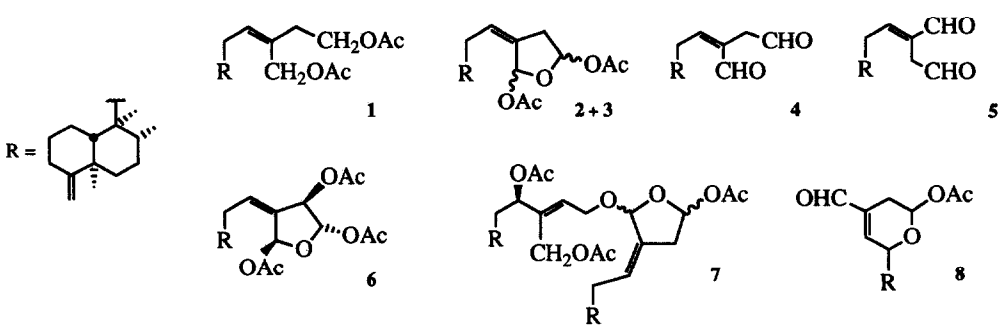
In previous studies on the variety *saxatilis* of *L. saxatilis* we reported the existence of *neo*-clerodane diterpenoids[4,6]. Following our systematic study of *Linaria* spp. we recently described the constituents isolated from root extracts of *L. saxatilis* var.*glutinosa* [7], a herbaceous plant endemic to the Western of the Iberian Peninsula, whose chemical composition had never been investigated. This report deals with the characterization of *neo*-clerodane diterpenoids isolated from the less polar fractions of the *n*-hexane extract of its aerial parts.

RESULTS AND DISCUSSION

Extraction of the air-dried aerial parts of *L.saxatilis* var.*glutinosa* with *n*-hexane, followed by dewaxing with acetone and chromatography afforded, in the elution order, the already known *neo*-clerodane diterpenoids **1** to **5**, **7** and **8**, together with the new natural product **6**, which was obtained as crystals, m.p. 141-143°C (*n*-hexane). Its mass spectrum showed the peak at *m/z* 191, assigned to a [$C_{14}H_{23}^+$] fragment, characteristic for a bicyclic monounsaturated diterpenoid with no oxygen functions on the ring system, together with three other peaks at *m/z* 402 [M^+ - HOAc], 342 [M^+ - 2HOAc] and 284 [M^+ - 3HOAc], which suggested the molecular formula $C_{26}H_{38}O_7$ for **6** and, correspondingly, the IR spectrum showed absorptions for acetates (1750, 1240 cm^{-1}) and unsaturations (3090, 1640, 890 cm^{-1}). Its ^1H and ^{13}C NMR spectra showed signals for three methyl and one vinyllidene groups whose chemical shifts and multiplicities correctly matched for a bicyclic *neo*-clerod-4(18)-ene moiety.

In relation with ^1H NMR signals associated to the side chain, the spectrum contained those characteristic for three protons geminal to acetoxyl groups, two of them of acetalic nature (δ 6.3, s and 6.8, *s*, ppm), and the third allylic to the Δ^{12} double bond (*s*, 5.4 ppm). These functions were also confirmed by the ^{13}C NMR spectrum, which showed methine signals at δ 94.2, 98.9 and 76.4 ppm. All these data were very close to those of the other compound previously isolated from the roots and for which we proposed the structure of 14,15,16-triacetoxy-15,16-epoxy-*ent*-cleroda-3,12*Z*-diene but, without establishing the absolute stereochemistry of the side chain[7]. Indeed, the only difference between both compounds seemed to be the Δ^3 endocyclic or $\Delta^{4(18)}$ exocyclic nature of the double bond of the bicyclic system.

Information about the stereochemistry of the chain was obtained from the results of some NOE difference measurements. Irradiation at δ 6.2 ppm (H-12) cause NOE in the H-14 (*s*, 5.4 ppm) signal, whereas on irradiation of H-14, NOE enhancements in the signals of H-12 and H-16 were observed. Consequently, the acetoxyl groups on those positions must be *cis* to each other. Furthermore, the absence of NOE between H-15 and H-16 and between H-15 and H-14, as well as the absence of coupling between H-14 and H-15, allowed us to propose the *Z* configuration for the Δ^{12} double bond and the relative *trans* configuration between the acetate at C-15 and those at C-14 and C-16. However, attempts to determine the absolute configuration of the side chain through spectral or conformational analysis were unsuccessful and the complete structure of **6** was finally established by X-ray diffraction. Fig. 1 shows a stereoscopic view of the molecule. The molecular structure was solved by Direct Methods and Fourier Synthesis (see experimental). Distances and angles (Table 1) are normal.



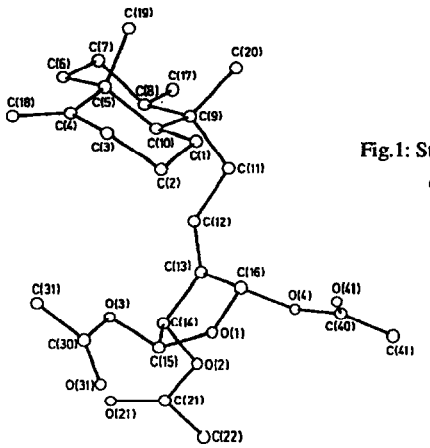


Fig.1: Stereoscopic view of compound **6**

Table 1: Bond Lengths(Å) and Bond Angles(°)(with esd's).

O1	-C15	1.416(4)	O1	-C16	1.419(4)	O2	-C14	1.456(4)
O2	-C21	1.344(4)	O3	-C15	1.424(4)	O3	-C30	1.366(4)
O4	-C16	1.445(4)	O4	-C40	1.362(4)	O2	-C21	1.200(5)
O31	-C30	1.191(5)	O41	-C4	1.185(6)	C1	-C2	1.514(5)
C1	-C10	1.533(4)	C2	-C3	1.520(6)	C3	-C4	1.483(6)
C4	-C5	1.527(5)	C4	-C18	1.322(6)	C5	-C6	1.543(4)
C5	-C10	1.573(4)	C5	-C19	1.552(5)	C6	-C7	1.531(5)
C7	-C8	1.525(5)	C8	-C9	1.545(4)	C8	-C17	1.533(5)
C9	-C10	1.557(4)	C9	-C11	1.560(4)	C9	-C20	1.564(4)
C1	-C12	1.514(4)	C12	-C13	1.338(4)	C13	-C14	1.498(4)
C13	-C16	1.498(4)	C14	-C15	1.522(5)	C21	-C22	1.491(5)
C30	-C31	1.479(6)	C40	-C41	1.486(6)			

C16	-O1	-C15	110.7(2)	C21	-O2	-C14	115.7(3)
C30	-O3	-C15	117.2(3)	C40	-O4	-C16	116.7(3)
C10	-C1	-C2	110.5(3)	C3	-C2	-C1	110.8(3)
C4	-C3	-C2	114.2(3)	C5	-C4	-C3	116.4(3)
C18	-C4	-C3	119.7(4)	C18	-C4	-C5	123.9(4)
C6	-C5	-C4	110.5(3)	C10	-C5	-C4	106.7(2)
C10	-C5	-C6	108.8(2)	C19	-C5	-C4	108.1(3)
C19	-C5	-C6	108.3(3)	C19	-C5	-C10	114.5(3)
C7	-C6	-C5	111.5(3)	C8	-C7	-C6	111.5(3)
C9	-C8	-C7	112.5(3)	C17	-C8	-C7	110.1(3)
C17	-C8	-C9	114.5(3)	C10	-C9	-C8	109.1(2)
C11	-C9	-C8	109.9(2)	C11	-C9	-C10	108.9(2)
C20	-C9	-C8	112.0(3)	C20	-C9	-C10	112.4(3)
C20	-C9	-C11	104.4(2)	C5	-C10	-C1	109.9(2)
C9	-C10	-C1	114.4(2)	C9	-C10	-C5	116.5(2)
C12	-C11	-C9	115.1(2)	C13	-C12	-C11	126.0(3)
C14	-C13	-C12	124.9(3)	C16	-C13	-C12	128.8(3)
C16	-C13	-C14	105.8(2)	C13	-C14	-O2	107.9(2)
C15	-C14	-O2	109.0(2)	C15	-C14	-C13	101.0(2)
O3	-C15	-O1	109.5(3)	C14	-C15	-O1	106.1(3)
C14	-C15	-O3	104.7(3)	O4	-C16	-O1	108.2(2)
C13	-C16	-O1	105.7(3)	C13	-C16	-O4	107.1(3)
O21	-C21	-O2	122.6(3)	C22	-C21	-O2	111.0(3)
C22	-C21	-O21	126.4(4)	O31	-C30	-O3	122.5(4)
C31	-C30	-O3	111.0(4)	C31	-C30	-O31	126.5(4)
O41	-C4	-O4	121.9(4)	C41	-C40	-O4	109.7(4)
C41	-C40	-O41	128.4(4)				

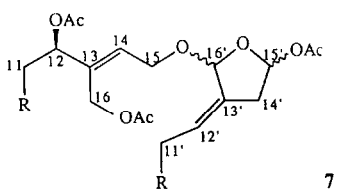
Table 2: ^{1}H NMR spectral data of compounds **1**, **2**, **3**, **4**, **5**, **6**, **8**, **9**, **10**, **11**, **12** and **15**.(200MHz, $CDCl_3$, TMS).

H	1	2	3	4	5	6	8	9	10	11	12	15
Me-5	1.04*s*	1.04*s*	1.04*s*	1.06*s*	1.05*s*	1.04*s*	1.03*s*	1.01*s*	1.03*s*	1.04*s*	1.07*s*	1.09*s*
Me-8	0.81*d* 6.1	0.79*d* 5.2	0.78*d* 6.1	0.87*d* 5.6	0.84*d* 5.8	0.83*d* 5.8	0.97*br.d*	0.82*d* 5.5	0.82*d* 5.8	0.81*d* 6.2	0.75*br.d*	0.76*d* 5.8
Me-9	0.75*s*	0.75*s*	0.73*s*	0.85*s*	0.83*s*	0.77*s*	0.98*s*	0.73*s*	0.75*s*	0.76*s*	0.91*s*	0.72*s*
H-11	2.38*d* 7.5	2.11*d* 7.4		2.60*d* 7.5	2.28*d* 7.5		5.08*dd* 3.8-2.2	2.03*t* 7.2	2.10*m*	2.27*d* 7.5	5.52*d* 16.2	2.12*d* 6.8
				2.70*d* 7.5	2.34*d* 7.5							
H-12	5.44*t* 7.6	5.61*t* 7.1	5.60*t* 7.2	6.45*t* 8.4	6.80*t* 7.5	6.23*dd* 5.3-10.3	6.71*m*	5.29*t* 7.5	5.48*t* 7.0	5.57*t* 7.2	6.05*d* 16.2	5.72*t* 7.3
H-14	2.42*d* 7.6	2.50*br.d*	2.50*br.d*	3.28*s*	3.35*br.s*	5.39*s*	2.85*cc* 9.3-5.0	2.31*t* 5.8	2.38*t* 5.8	2.43*t* 7.3	6.48*d* 2.0	2.50*br.d*
		3.00*m*	2.97*m*									3.02*m*
H-15	4.12*d.t* 6.8	6.40*d* 5.4	6.37*dd* 2.1-6.1	9.62*br.s*	9.39*br.s*	6.31*s*	6.34*dd* 5.5-9.3	3.64*t* 5.7	3.65*t* 5.7	4.09*t* 7.1	7.33*d* 2.0	6.34*d* 5.4
H-16	4.61*s*	6.74*s*	6.74*s*	10.12*s*	9.53*s*	6.83*s*	9.46*s*	4.08*s*	4.00*s*	4.50*s*	7.32*s*	6.78*s*
H-18	4.49*br.s*	4.50*br.s*	4.51*br.s*	4.50*br.s*	4.49*br.s*	4.51*br.s*	4.50*br.s*	4.46*br.s*	4.50*br.s*	4.50*br.s*	4.50*br.s*	2.33*d* 4.5
												2.77*d* 4.5
OAc	2.02	1.98	2.05			2.02	2.14			2.03		2.03
	2.05	2.04	2.08			2.11				2.05		2.06
						2.11						

Table 3: ^{13}C NMR spectral data of compounds **1, 2, 3, 4, 5, 6, 8, 9, 10, 11, 12** and **15**. (50 MHz, $CDCl_3$, TMS)

C	1	2	3	4	5	6	8	9	10	11	12	15
1	22.0	22.4	21.7	22.3	22.3	22.5	23.6	22.1	22.2	22.3	23.3	21.7
2	28.5	28.4	29.0	28.4	28.4	28.4	28.2	28.7	28.6	28.6*	28.7	24.1
3	33.1	33.1	33.0	33.0	32.9	33.0	32.8	33.1	33.1	33.2	33.3	30.4
4	160.2	160.1	160.3	159.6	159.6	159.8	159.1	160.4	160.4	160.3	160.3	65.2
5	40.1	40.2	40.1	40.2	40.2	40.2	40.4	40.1	40.2	40.2	39.7	37.6
6	37.2	37.5	37.1	37.1	37.0	37.9	36.5	37.2	37.2	37.3	37.2	30.8
7	27.5	27.5	27.5	27.5	27.4	27.4	28.0*	27.6	27.6	27.7	26.9	26.6
8	37.2	37.1	37.1	37.4	38.0	36.2	36.7	37.1	37.2	37.3	41.8	36.4
9	40.4	40.5	40.4	41.4	41.4	40.7	46.4	40.5	40.4	40.5	44.1	39.9
10	49.3	49.8	49.3	49.8	50.4	50.3	49.5	49.4	49.3	49.5	53.2	46.5
11	36.1	36.6	37.6	35.3	38.2	37.1	84.4	36.0	36.0	36.1	117.4	36.8
12	129.6	125.3	124.9	149.6	154.6	135.6	142.9	127.1	127.1	129.7	142.6	125.2
13	130.7	133.3	134.1	133.9	136.4	132.7	135.1	137.5	137.4	131.3	124.7	133.3
14	34.8	37.2	37.6	45.5	39.4	76.4	27.1*	39.8	32.7	28.2*	107.8	37.3
15	61.9	97.0	97.7	189.3	193.5	98.9	96.0	60.3	61.7	62.8	143.2	97.0
16	63.1	93.9	94.8	198.2	197.0	94.2	189.7	63.0	68.8	69.2	139.3	94.0
17	16.3	16.1	16.4	16.2	16.3	16.1	13.2	16.3	16.5	17.0	12.3	16.0
18	102.9	102.9	102.8	103.3	103.2	103.1	103.6	102.7	102.8	102.9	102.7	49.8
19	20.7	20.6	20.6	20.7	20.6	20.6	20.7	20.7	20.8	20.7	20.7	19.9
20	17.7	17.4	17.5	17.6	17.5	17.4	17.4	17.7	17.8	17.7	16.9	17.3
CH_3COO	20.9	21.0	21.1			20.6	20.7			21.0		21.1
	21.1					20.7						
CH_3COO	170.0	169.6	169.8			169.8	167.4			170.9		169.8
						169.9						
						170.1						

*Assignments may be reversed in the same column.



7

Table 4: ^{1}H NMR spectral data of compound 7

H	7
Me 5 - 5'	1.04 *s*
Me 8 - 8'	0.76 *brd*
Me 9 - 9'	0.71 *s*
12 12'	5.22*d* 9.7 5.77*t* 6.0
14	5.49 *s*
15 15'	4.23 *m* 6.34 *d* 5.3
16 16'	4.59-4.68
18 18'	4.50
CH_3COO-	1.99 1.99 2.05

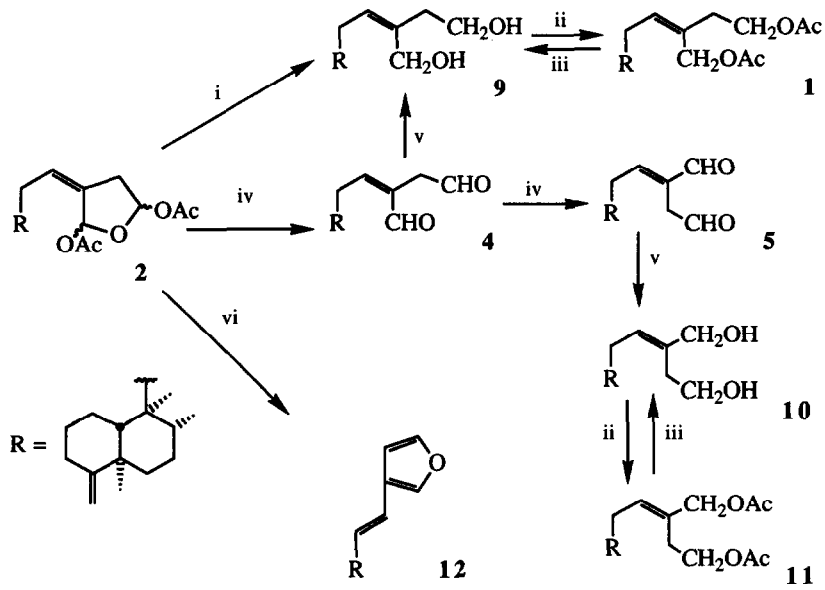
Table 5: ^{13}C NMR spectral data of compound 7

C		7		
1	1'	22.2		
2	2'	28.3		
3	3'	33.1		
4	4'	159.7*	160.3*	
5	5'	40.2		
6	6'	37.3		
7	7'	27.6		
8	8'	37.1*	38.1*	
9	9'	40.2		
10	10'	49.2	49.8	
11	11'	41.9	37.3	
12	12'	72.5	124.6	
13	13'	138.0	134.5	
14	14'	128.6	37.3	
15	15'	62.9	101.4	
16	16'	59.8	97.0	
17	17'	16.3		
18	18'	102.8*	103.1*	
19	19'	20.9	20.7	
20	20'	17.6	17.8	
CH_3COO-		20.9	20.9	21.2
CH_3COO-		169.8	170.0	170.5

* Values can be interchangeable within the same line.

In order to ascertain the structure of the remaining compounds, several chemical transformations and comparison with authentic samples were performed (Scheme 1). Thus, compound **1** was correlated with compound **2** through LAH reduction of the latter to give diol **9** which on acetylation produced **1** which, in turn reverted to **9** by saponification.

Compounds **2** and **3** were epimers at C-15 and were identified by comparison with similar samples isolated from *L.saxatilis* var.*saxatilis* [4]. They decompose on standing and during chromatographic separations on silica gel to the corresponding dialdehydes **4** and **5**. **2** in AcOH/ether evolved to **4**, which on prolonged treatment isomerised to the more stable dialdehyde **5**. Compounds **4** and **5** were easily separated by $AgNO_3/SiO_2$ column chromatography and reduced to the corresponding diols **9** and **10**, which were transformed into their respective diacetates **1** and **11**. In addition, pyrolisis of **2** at 200°C under N_2 gave **12**, whose ^{13}C NMR data are included in Table 3.

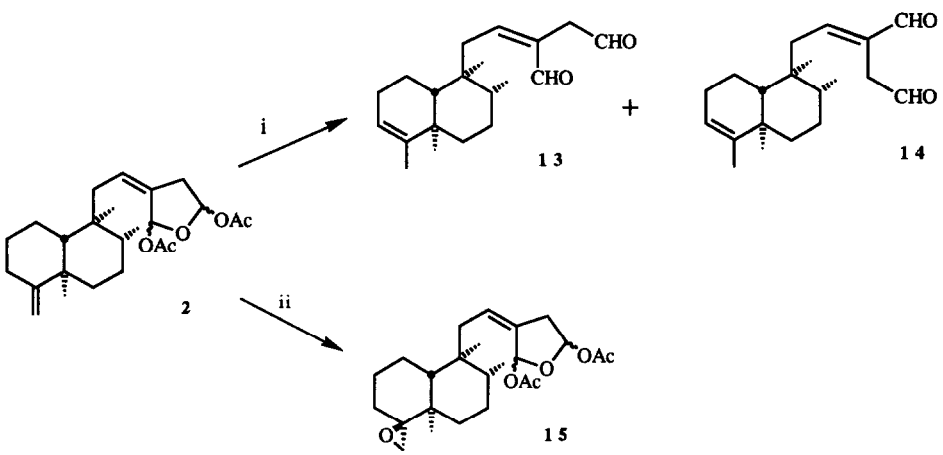


i/ $AlLiH_4$-Et_2O; **ii/** Ac_2O/py; **iii/** NaOH-MeOH; **iv/** AcOH/Et_2O 3h; **v/** $AlLiH_4$/ Et_2O; **vi/** direct pyrolysis 200°C 10min.

Scheme 1

Therefore, the main difference between *neo*-clerodanes isolated from aerial parts or from roots of the plant seemed to lie on to the exocyclic or endocyclic nature of the double bond on ring A. With the aim of correlating both series of compounds, the representative compound **2** was transformed into the mixture of the endocyclic dialdehydes **13+14** by treatment with H_2SO_4/AcOH/H_2O, the mixture being identified through spectroscopic and TLC comparison with authentic samples[7] (Scheme 2).

The antifeedant activity of several representatives of those series of compounds has been tested with promising results, but the necessity of functionalizing the ring-A part of the molecule for increasing potency and duration of the effects, emerged from comparison with literature reports[8]. At this respect, the presence of the Δ^3 or $\Delta^{4(18)}$ unsaturations could easily permit the functionalization of positions 2, 3, 4 and 18. In fact, treatment of compound **2** with *m*-CPBA promoted the regio and stereoselective epoxidation of the $\Delta^{4(18)}$ double bond affording **15** in quantitative yield (Scheme 2).



i/ H_2SO_4/AcOH/H_2O, 2.5h; ii/ *m*-CPB/$NaHCO_3$, 5 min.

Scheme 2

EXPERIMENTAL

General. Mps: uncorr. Optical rotations: $CHCl_3$. UV: EtOH, λ_{max} nm (ε). IR in NaCl films unless stated otherwise, ν_{max} values are expressed in cm^{-1}. ^1H NMR (200.13 MHz) and ^{13}C NMR (50.3 MHz) spectra were measured in $CDCl_3$. TMS was used as an int. standard and chemical shifts are given as δ-values (ppm), coupling constants in Hz. EIMS were recorded by direct inlet at 70 eV and the relative intensities of

peaks are reported with reference to the most intense peak, *m/z* = 100. Flash chromatography was run on silica gel (Merck No. 9385).

Plant material. Linaria saxatilis var.*glutinosa* was collected in June 1990 at Bermellar de Camazas (Salamanca, Spain) and identified by Dr. C. del Valle. A voucher specimen is deposited at the herbarium of the Botany Department, Faculty of Pharmacy, Salamanca. (Register No SALAF-21386).

Extraction and isolation. The air-dried aerial parts (1080 g) were finely ground and extracted by Soxhlet procedure with *n*-hexane and the resulting extract (51.3 g, 4.7 % over dried material) cooled overnight at -20°C. The soluble fraction (40.6 g) was defatted with acetone yielding 38.0 g (3.5 %) of a viscous residue which was chromatographed on a silica gel column and eluted with *n*-hexane-EtOAc mixtures of increasing polarity. Frs were purified by repeated silica gel CC, silica gel impregnated with $AgNO_3$ (20%) CC and by prep. TLC with a suitable solvent system, yielding **1** (169 mg), **2** (3.1 g), **3** (25 mg), **4** (970 mg), **5** (248 mg), **6** (615 mg), **7** (60 mg) and **8** (109 mg).

Z-isolinaridiol diacetate (**1**)

Eluted with *n*-hexane-EtOAc (95/5). Oil. $[\alpha]_D$ = -1.8° (c 0.5%). IR (CH_2Cl_2): 2920, 1735, 1675, 1635, 1230, 1025, 970, 890. EIMS: 390 (0.1), 330 (0.1), 315 (0.2), 270 (0.5), 191 (39.9), 175 (12.9), 140 (16), 121 (17.8), 109 (20.4), 95 (100), 81 (18.2), 55 (12.2). ^1H and ^{13}C NMR : Tables 2 and 3. A solution of 92.0 mg of **9** and 1 ml of acetic anhydride (Ac_2O) in 1 ml of pyridine was kept at room temperature for 12 h. After the usual work-up the resulting product was identical to diacetate **1**. A soln of **1** (55 mg) in Et_2O (7 ml) was added to a suspension of $LiAlH_4$ (100 mg) in dry Et_2O (7 ml). The reaction mixture was stirred at room temp. for 4h 30 min. Usual work-up afforded **9** (34 mg).

15,16-diacetoxy-15,16-epoxy-ent-*cleroda-4(18),12Z-diene* (**2**)

Eluted with *n*-hexane-EtOAc (9/1). Oil. $[\alpha]_D$ = -61.1° (c 0.90%). IR: 2940, 1750, 1640, 1450, 1380, 1230, 975. EIMS: 284 (78.3), 202 (50.4), 177 (39.4), 161 (48.2), 148 (73.8), 133 (85.0), 121 (100), 105 (67.6), 91 (87.6), 81 (59.0). ^1H and ^{13}C NMR : Tables 2 and 3. A mixture of acetic acid AcOH (7.5 ml), H_2SO_4 (0.5 ml) and water (2 ml) was added dropwise to a solution of **2** (130 mg) and stirred at room temperature for 2 h 30 min, washed with saturated aq. $NaHCO_3$ and water until neutral, dried (Na_2SO_4) and evaporated under reduced pressure. This yielded 13 mg of **13** and 4 mg of **14**.

15,16-diacetoxy-15,16-epoxy-ent-*cleroda-4(18),12Z-diene* (**3**)

Eluted with *n*-hexane-EtOAc (9/1). Oil. $[\alpha]_D$ = -2.3° (c 0.75%). IR: 2940, 1750, 1640, 1450, 1380, 1230, 975. EIMS: 284 (78.3), 202 (50.4), 177 (39.4), 161 (48.2), 148 (73.8), 133 (85.0), 121 (100), 105 (67.6), 91 (87.6), 81 (59.0) .^1H and ^{13}C NMR : Tables 2 and 3. Reduction of **3** (40.0 mg) with $LiAlH_4$ (50.1 mg) in dry Et_2O for 5 h, yielded 30.0 mg of **9**.

Z-isolinaridial (**4**)

Eluted with *n*-hexane-EtOAc (8/2). Oil.$[\alpha]_D$ = +10.0° (c 1.0%). IR: 2730, 1725, 1675, 1640, 890. UV: 240 nm (ε 9060), 201nm (ε 12760). EIMS: 302 (0.6), 216 (0.7), 203 (0.7), 191 (36.6), 175 (5.8), 149 (3.6), 135 (21.4), 121 (22.4), 95 (100), 81 (17.1), 55 (13.3). ^{1}H and ^{13}C NMR : Tables 2 and 3. A soln of **4** (590 mg) in Et_2O (50 ml) was added to a suspension of $LiAlH_4$ (2.5 g) in dry Et_2O (20 ml) and the mixture was stirred at room temp. for 5 h. 30 min. After the usual work-up, **10** (580 mg) was isolated.

E-*isolinaridial* (**5**)

Eluted with *n*-hexane-EtOAc 8/2. Oil. $[\alpha]_D$ = -2.1° (c 0.98%). IR: 2760, 1735, 1690, 1645, 1390, 900. UV: 234 nm (ε 11670), 204nm (ε 10300). EIMS: 302 (0.7), 216 (1.0), 191 (37.8), 175 (5.6), 149 (3.7), 135 (21.0), 121 (21.5), 95 (100), 79 (16.9), 55 (13.2). ^{1}H and ^{13}C NMR: Tables 2 and 3. 4 ml of HOAc were added to a solution of **2** (130 mg) in Et_2O (7 ml) and the mixture was refluxed for 2 h 45 min. After usual work-up, 118 mg of *E*-isolinaridial (**5**) was isolated. A solution of **5** (15.0 mg) in MeOH (11 ml) at room temp. was treated with an aq NaOH (5%, 3 ml), the mixture stirred for 30 min. After the usual work-up, **9** (11 mg) was isolated.

14,15,16-triacetoxy-15,16-epoxy-ent-*cleroda-4(18),12*E-*diene* (**6**)

Eluted with *n*-hexane-EtOAc (8/2). M.p. 143°C (*n*-hexane). $[\alpha]_D$ = -76.4° (c 1.0%). IR (CH_2Cl_2): 3060, 2920, 1750, 1640, 1375, 1220, 1030, 1000, 950, 890. EIMS: 402 (0.4), 342 (0.3 [M^+-HOAc]), 284 (0.2, [M^+-2HOAc]), 212 (23.5), 191 (38.0), 175 (13.0), 152 (19.0), 135 (16.5), 121 (19.0), 109 (22.1), 95 (100), 81 (17.9), 67 (11.7), 55 (15.5).^{1}H and ^{13}C NMR : Tables 2 and 3. Anal. calcd for $C_{26}H_{38}O_7$: C, 67.51; H, 8.28. Found: C, 67.65; H, 8.15.

16-[(12,16-diacetoxy-ent-*cleroda-4(18),13*E-*dien-15-il)-oxy] -15-acetoxy-15,16-diepoxy*-ent-*cleroda-4(18), 12Z-diene* (**7**)

Eluted with *n*-hexane-EtOAc (75/25). Oil. $[\alpha]_D$ = -9.7°(c 1.2%). IR (CH_2Cl_2): 2920, 1740, 1645, 1450, 1375, 1230, 1170, 980, 890. ^{1}H and ^{13}C NMR : Tables 4 and 5.

15-acetoxy-11,15-epoxy-ent-*cleroda-4(18),12-dien-16-al* (**8**)

Eluted with *n*-hexane-EtOAc (7/3). Oil.$[\alpha]_D$ = -14.7° (c 0.25%). IR: 2700, 1750, 1690, 1640, 1220, 970, 895. EIMS: 300 (1.1), 284 (1.1), 271 (1.6), 257 (1.7), 233 (1.6), 210 (1.6), 202 (1.6), 191 (18.8), 136 (15.0), 96 (65.0), 60 (76.2), 32 (100). ^{1}H and ^{13}C NMR : Tables 2 and 3.

Z-isolinaridiol (**9**)

Eluted with *n*-hexane-EtOAc (7/3). M.p: 87-88°C ($CHCl_3$). $[\alpha]_D$ = +19.8° (c 1.2%). IR ($CHCl_3$): 3640, 3425, 1090, 2940, 1730, 1635, 1390, 1210, 1050, 1010, 890. EIMS: 360 (0.2), 288 (1.1), 250 (1.8), 235 (2.8), 191 (11.1), 175 (11.2), 109 (23.1), 95 (100), 79 (40.0). ^{1}H and ^{13}C RMN : Tables 2 and 3.

E-*isolinaridiol* (**10**)

Eluted with *n*-hexane-EtOAc (7/3). M.p: 87.5°C ($CHCl_3$). $[\alpha]_D$ = +13.3° (c 1.0%). IR (CH_2Cl_2): 3320, 3090, 2920, 1735, 1635, 1390, 1255, 1130, 1050, 890. ^1H and ^{13}C NMR: Tables 2 and 3. Anal. Calcd for $C_{20}H_{34}O_2$: C, 78.38; H, 11.18. Found: C, 78.46; H, 11.15. **10** (580.0 mg) was treated with Ac_2O (2 ml) and pyridine (2 ml) for 12 h to afford **11** (727 mg).

E-*isolinaridiol diacetate* (**11**)

Oil. $[\alpha]_D$ = +7.0° (c 1.0%). IR (CH_2Cl_2): 2920, 1735, 1640, 1375, 1230, 1130, 1030, 960, 890. EIMS: 330 (0.2), 315 (0.1), 270 (0.7), 191 (53.7), 175 (19.0), 140 (27.1), 121 (21.4), 109 (23.2), 95 (100) 81 (17.1), 55 (9.8). ^1H and ^{13}C NMR: Tables 2 and 3. A soln of **11** (65.0 mg) in 5% aq NaOH (13 ml) was stirred for 30 min. at room temp. Usual work-up gave 49 mg of **10**.

15,16-epoxy-ent-*cleroda-4(18),13(16),14-tetraene* (**12**)

Oil. $[\alpha]_D$ = -17.2° (c 1.0%). UV : 230 nm (ε 9285), 225 nm (ε 9140), 212 nm (ε 9570), 205 nm (ε 11285), 201 nm (ε 10930). IR : 1640, 1510, 1455, 1380, 1160, 1070, 1030, 970, 890. EIMS: 330 (0.2), 315 (0.1), 270 (0.7), 191 (53.7), 175 (19.0), 140 (27.1), 121 (21.4), 109 (23.2), 95 (100), 81 (17.1), 55 (9.8). ^1H and ^{13}C NMR: Tables 2 and 3. 220 mg of **2** were kept at 200°C under N_2 atmosphere for 10 min. After purification through Si gel 151 mg of **12** was isolated.

15,16-diacetoxy-15,16-4,18-diepoxy-ent-*cleroda-12Z-diene* (**15**)

M.p: 117°C (*n*-hexane). $[\alpha]_D$ = -94.8° (c 0.5%). IR ($CHCl_3$): 2940, 2860, 2420, 1745, 1450, 1380, 1235, 1170, 1030, 970, 890. EIMS: 300 (100), 285 (3.1), 269 (10.0), 201 (5.1), 175 (90.1), 161 (35.2), 148 (42.9), 133 (36.4), 121 (60.6), 108 (78.7), 91 (51.6), 81 (48.7), 55 (44.1). ^1H and ^{13}C NMR : Tables 2 and 3. Anal. calcd for $C_{24}H_{36}O_6$: C, 68.55; H, 8.63. Found: C, 68.71; H, 8.55. To a stirred suspension of 202 mg of **2** and 0.45 g of $NaHCO_3$ in anhydrous CH_2Cl_2 (25 ml), *m*-CPBA (0.42 g) in the same solvent, was added dropwise. After 15 min, the reaction mixture was poured into water, washed with 10% aq. $Na_2S_2O_3$, saturated aq $NaHCO_3$ and water until neutral. Evaporation of solvent under reduced pressure followed by chromatography afforded 194 mg of **15**.

X-Ray Crystallographic Data.

Crystal data for compound **6** : $C_{26}H_{38}O_7$, M_r = 462.58, monoclinic, space group $P2_1$, a = 8.592(3), b = 13.778(6), c = 10.898(4) Å , b = 93.87(3), V = 1287(1) $Å^3$, Z = 2, D_x = 1.194 g/cm^3. MoK_α radiation (graphite crystal monochromator, λ = 0.71073 Å), $\mu(MoK_\alpha)$ = 0.80 cm^{-1}, F(000) = 500, T = 293°K. Colorless crystal, 0.46 x 0.30 x 0.23 mm size.

Data collection, Analysis and Refinement. MoK_α radiation used with a graphite monochromator on a Nonius CAD4 single crystal diffractometer (λ = 0.71073 Å). Unit cell dimensions determined from the angular settings of 25 reflections with $15° < \Theta < 19°$. Space group $P2_1$ from the systematic absences and the structure determination.

5038 reflections measured, hkl range (-10, -16,- 12) to (10, 16, 12), theta limits ($0° < \Theta < 25°$); ω-2Θ scan technique with a variable scan rate and a maximum scan time of 60 s per reflection. Intensity checked throughout data collection by monitoring three standard reflections every 60 minutes. Final drift corrections factors between 0.97 and 1.04 . On all reflections profile analysis performed[8,9]; empirical absorption correction was applied, using psi-scans[10], $\mu(MoK_\alpha)$ = 0.80 cm^{-1} (correction factors range 0.96 to 1.00). Some double measured reflections averaged, $R_{int} = \Sigma(|I|-<I>)/\Sigma I = 0.024$, 4494 unique reflections and 3370 'observed' with $I>3s(I)$. Lorentz and polarization corrections applied and the data reduced to $|F_o|$-values.

Structure solved by Direct Methods, using the program SHELXS86[11]. Isotropic least-squares refinement, using a locally modified version of the program SHELX76[12] , converged to R = 0.010. At this stage additional empirical absorption correction was applied[13]. Maximum and minimum absorption correction factors were respectively 1.06 and 0.70 .

Anisotropic least-squares refinements followed by a difference Fourier synthesis allowed the location of some hydrogen atoms, the rest of H-atoms were geometrically placed. During the final stages of the refinement the positional parameters and the anisotropic thermal parameters of the non-hydrogen atoms were refined. All the hydrogen atoms were refined from his geometrically idealized positions riding in their parent atoms with a refined common isotropic thermal parameter. The final conventional agreement factors were R = 0.049 and R_w = 0.053 for the 3370 'observed' reflections and 298 variables.

The function minimized was $\Sigma w(F_o-F_c)^2$, with $w = 1/(\sigma^2(F) + 0.0003\ F^2)$. The maximum shift over error ratio in the last full matrix least-squares cycle was less than 0.010. The final Difference Fourier map showed no peaks higher than 0.25 e/Å^3 nor deeper than - 0.34 e/Å^3. Atomic scattering factors were taken from the International Tables for X-ray Crystallography[14]. The absolute configuration of the compound were confirm using the statistical analysis of the measured pairs of Friedel using a new version of the program BIJVOET [15]. Bijvoet coefficient, B = 0.09(7) for the 100 strongest Friedel pairs.

The plot was made with PLUTO program[16]. Geometrical calculations were made with PARST [17]. Final positional and thermal parameters are given in Table I. Molecular geometry data are collected in Table II. Figure I shows the atomic numbering scheme. A selection of angles between least-squares planes and main torsion angles have been deposited. All calculations made on a MicroVax-3400 at the Scientific Computer Center, University of Oviedo. Lists of structure amplitudes, anisotropic thermal parameters, H-atom parameters, distances, angles, least-squares planes and lines data and principal torsion angles have been deposited.

Acknowledgements:: Financial support came from Spanish DGICYT (Grant No. PB 89/394).

REFERENCES

1. Pahlow, M. " *El gran libro de las plantas medicinales*" , 4th ed, Everest S.A.: Madrid. 1979; 273.
2. Dobrescu, D.; Cristea, A.; Susanu, M. *Farmacia* **1985**, *33*, 215 .
3. Kitagawa, I.; Tani, T.; Akita, K.; Yosioka, I. *Chem. Pharm. Bull.* **1973**, *21*, 1978.
4. San Feliciano, A.; Barrero, A. F.; Miguel del Corral, J. M.; Gordaliza, M.; Medarde, M. *Tetrahedron* **1985**, *41*, 671.
5. Pascual teresa, J. de; San Feliciano, A.; Barrero, A. F. ; Gordaliza, M.; Miguel del Corral, J. M.; Medarde, M. *An. Quim.*, **1982**, *78*, 425.
6. San Feliciano, A.; Barrero, A. F.; Miguel del Corral, J. M.; Gordaliza, M.; Medarde, M. *An. Quim.* **1985**, *81C*, 244.
7. San Feliciano, A.; Miguel del Corral, J. M.; Gordaliza, M.; de la Puente, M.L. *Phytochemistry* **1993**, *32*, 0000.
8. Lehman, M.S.; Larsen, F. K. *Acta Cryst.* **1974**, *A30*, 580-584.
9. Grant, D. F.; Gabe, E. J. J. *Appl. Cryst.* **1978**, *11*, 114-120.
10. North, A. C. T.; Philips, D. C.; Mathews, F. S. *Acta Cryst.* **1968**, *A24*, 351-359.
11. Sheldrick, G. M. SHELX86, " Crystallographic Computing 3 ", 175-189. Sheldrick, G. M.; Kruger, C. Goddard, R., Eds., Clarendon Press, Oxford, 1985.
12. Sheldrick, G. M. SHELX, A program for crystal structure determination. University Chemical Laboratory Cambridge, England, 1976.
13. Walker, N.; Stuart, D. *Acta Cryst.* **1983**, *A39*, 158-166.
14. " International Tables for X-ray Crystallography " , Vol. IV, Birmingham : Kynoch Press, (Present distributor Kluwer Acad. Publ., Dordrecht), 1974.
15. Beurskens, G.; Noordik, J. H.; Beurskens, P. T. *Cryst. Struct. Commun.* **1980**, *9*, 23-28.
16. Motherwell, W. D. S.; Clegg, W. PLUTO, A Program for plotting molecular and crystal structures. University Chemical Laboratory, Cambridge, England.1978.
17. Nardelli, M. *Comput. Chem.* **1983**, *7*, 95-98.