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Clerodane Diterpenoids and an Ursane Triterpenoid from *Salvia haenkei*. Computer-assisted Structural Elucidation

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Abstract: Five *ent*-clerodane diterpenoids, four new, and a new ursane triterpenoid have been isolated from *Salvia haenkei* collected in Bolivia. The structures of two *ent*-clerodanes *ent*-(4*S*,5*R*)-7α-acetoxy-15,16-epoxy-10-oxo-9,10-secocleroda-1,8,13(16),14-tetraene-17,12*S*;18,19-diolide **1** (salvianduline B) and *ent*-(5*R*,9*R*)-15,16-epoxy-10*S*-hydroxy-cleroda-3,7,13(16),14-tetraene-17,12*S*;18,19-diolide **2**, were elucidated from HMQC and HMBC data using an automatic structure elucidation computer program. The structures of the other clerodanes, *ent*-(5*R*,9*R*)-15,16-epoxy-10*S*-hydroxy-cleroda-3,13(16),14-triene-17,12*S*;18,19-diolide **6**, *ent*-(4*S*,5*R*,9*S*,10*R*)-15,16-epoxycleroda-1,13(16),14-triene-17,12*S*;18,19-diolide **7** and *ent*-(5*R*,9*R*,10*S*)-7*S*-acetoxy-15,16-epoxy-1*S*,2*S*,12ξ-trihydroxycleroda-3,13(16),14-trien-18,19-olide **8**, were derived in the usual manner from ^{1}H and ^{13}C NMR data. The stereochemistry of **2** was confirmed by an X-ray crystal structure analysis. The triterpenoid is 3-oxours-12-ene-1β,11α-diol **9**. © 1997 Elsevier Science Ltd.

INTRODUCTION

Ideally structural elucidation using modern NMR techniques should proceed without recourse to biogenetic thinking or the use of working structures. However many structures which appear in the literature are not derived in a rigorous manner and all possible solutions are not considered.[1] Structures are normally assigned on the basis of information from COSY, HMQC and HMBC experiments, despite the fact that the HMBC experiment itself does not distinguish between $^{2}J_{CH}$ and $^{3}J_{CH}$. Recently the 1,1-ADEQUATE experiment and varients have been used to make this distinction,[2] but unfortunately experiments of this type require long accumulation times. The DODO pulse, a modified BIRD pulse, has also been used successfully to identify two-bond correlations involving protonated carbons.[3] Another approach has been to identify and utilise correlations, such as from tertiary methyl protons to non-quaternary carbons, which necessarily arise from $^{3}J_{CH}$ interactions; in this way the structures of the rearranged limonoids entilins A and B were derived unambigously.[4] One of us (J.-M.N.) has developed a program for computer-assisted structural elucidation (**L**ogic for **S**tructure **D**etermination program) which does not depend on distinction between $^{2}J_{CH}$ and $^{3}J_{CH}$ correlations.[5,6] The **LSD** program considers all possible solutions and is not inhibited by considerations of biogenetic acceptability. The potential of this **LSD** program has recently been demonstrated with respect to the structure of the complex

limonoid azadirachtin.[6] We have now used the above computer approach for the structure elucidation of two clerodanes (**1** and **2**) from *Salvia haenkei*. Chromatography of the methylene chloride extract of *S. haenkei* afforded five clerodane diterpenoids and an ursane triterpenoid. All the clerodanes are assumed to belong to the *enantio*-series, the expected absolute configuration of *Salvia* diterpenoids.[7] The structures of the other compounds (**6** - **9**) have been determined conventionally, on the basis of their spectroscopic properties.

RESULTS AND DISCUSSION

Compound **1**, $C_{22}H_{22}O_8$, has spectroscopic properties (Tables 1 and 2) consistent with the presence of an αβ-unsaturated ketone, a β-substituted furan, a secondary acetate, a vinyl methyl and two lactones (δ_C 176.2 and 163.9), one of which is unsaturated. The compound is therefore monocarbocyclic and appeared to be a new structural type. The data set used for the **LSD** computer analysis of this compound did not include any information from the COSY spectrum but contained the multiplicity and hybridization state of all the carbons and oxygens, HMQC and HMBC data, and bonds deduced from the presence of a ketonic carbonyl and three

Table 1

Carbon Chemical shifts of the Clerodane Diterpenoids

	1	**2**	**6**	**7**	**8**†
1	129.4	25.6	26.1	126.9*	68.7
2	148.7	22.7	23.2	127.2*	68.7
3	23.4	135.8	135.7	21.2	129.4
4	42.9	131.6	132.1	46.3	141.3
5	51.5	45.0	47.3	41.8	43.8
6	39.0	39.7	31.3	18.1	38.6
7	68.0	132.8	16.5	17.0	73.9
8	125.1	135.2	43.3	41.1	41.0
9	153.0	40.8	42.1	36.4	38.3
10	197.6	72.0	73.8	51.8	43.8
11	37.5	38.4	34.6	37.0	45.6
12	71.0	72.8	71.3	70.6	62.3
13	123.1	123.0	126.8	127.2	131.0
14	108.3	108.1	108.5	108.6	108.5
15	143.7	143.2	144.1	144.3	143.0
16	140.8	139.4	138.5	138.6	138.4
17	163.9	168.2	174.0	172.7	11.4
18	176.2	170.0	169.7	175.8	170.2
19	74.2	74.0	71.2	76.3	74.6
20	20.1	29.0	27.3	31.6	20.0
Ac	169.4				170.2
Ac	20.6				21.2

* May be interchanged. † $CDCl_3$ + CD_3OD solution

$CDCl_3$ solutions. Multiplicities were derived from DEPT spectra.

Chemical shifts relative to $CDCl_3$ at δ 77.0 or CD_3OD at δ 49.0.

Table 2

Proton NMR Data of the Clerodane Diterpenoids

Proton	(1)	(2)	(6)	(7)	(8)*
1	6.08 (dq, 10.3, 1.1)	1.65 (2H, t, 6.5)	*ca* 1.7, 1.95	5.84 (s)	4.02 (br s)
2	6.92 (ddd, 10.3, 5.4, 2.3)	2.36 (m)	2.36 (m), 2.56 (m)	5.48 (s)	4.04 (dd, 7.0, 2.0)
3	2.97 (dd, 20.3, 5.3)	6.78 (t, 3.6)	6.90 (t, 3.6)	2.35 (m)	6.56 (d, 7.0)
	2.77 (ddd, 20.3 ,7.0, 2.7)			2.40 (m)	
4	3.07 (d, 6.7)				
6	2.59 (dd, 14.7, 11.0)	2.76 (dd, 20.8, 4.4)	*ca* 1.5, 1.7	1.17 (m)	1.53 (ddd, 14.6, 4.4, 2.2)
	2.01 (dd, 14.7, 2.2)	2.19 (ddd, 20.8, 1.6, 1.1)		1.80 (m)	2.19 (dd, 14.6, 1.2)
7	5.70 (dd, 11.0, 2.2)	6.42 (t, 3.7)	2.18 (m), *ca* 1.7	2.14 (m)	5.24(dt, 2.3, *ca* 3)
				1.65 (m)	
8			2.56 (br d, 4.4)	2.32 (m)	2.38 (dq, 4.4, 6.9)
10				2.23 (m)	2.37 (br s)
11	2.68 (dd, 18.0, 11.9)	2.59 (dd, 25.5, 2.2)	2.88 (dd, 15.9, 8.2)	2.72 (dd, 15.5, 8.4)	1.75 (br d, 15.6)
	2.38(dd, 18.0, 3.6)	1.63 (dd, 15.5, 12.3)	1.94 (dd, 15.9, 1.7)	2.06 (dd, 15.5, 1.5)	2.00 (dd, 15.6, 9.4)
12	5.25 (dd, 11.8, 3.6)	4.85 (dd, 12.3, 2.2)	5.65 (dt, 8.2, 1.7)	4.46 (dt, 8.4, 1.5)	4.81 (br d, 9.4)
14	6.37 (d, 2.0)	6.32 (d, 1.5)	6.39 (m)	6.38 (br s)	6.42 (dd, 1.8, 0.8)
15	7.37 (t, 1.5)	7.29 (br s)	7.38 (m)	7.35 (dt, 1.6, 1.0)	7.32 (t, 1.7)
16	7.42 (br s)	7.36 (t, 1.7)	7.45 (t, 1.7)	7.42 (t, 1.7)	7.38 (br s)
17					0.95 (d, 7.5)
19	4.78 (d, 9.0)	4.38 (dd, 8.3, 1.1)	4.35 (dd, 8.5, *ca* 1)	4.46 (d, 8.7)	4.85 (d, 7.5)
	3.97 (d, 9.0)	4.08 (d, 8.3)	4.22 (d, 8.5)	3.98 (dd, 8.7, 2.0)	4.52 (dd, 7.5, 2.2)
20	2.04	1.07	1.27	1.24	1.03
	1.97 (Ac)				

^{1}H NMR spectra of $CDCl_3$ solutions were run at 360MHz, 300MHz or 200MHz. Chemical shifts relative to $CHCl_3$ at δ 7.25.

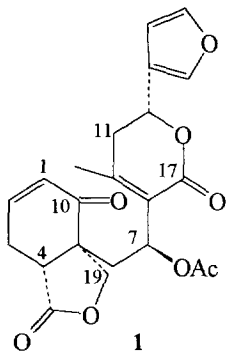
*$CDCl_3$ + CD_3OD. Chemical shifts relative to CHD_2OD at δ 3.30.

ester/lactone groups.[8] Carbons C-19, C-12 and C-7 must, on chemical shift grounds, be attached to oxygen atoms while C-20 and the acetate methyl must be connected to quaternary carbons. The analysis gave nine structures. Four of these contained a cyclobutadiene ring and no furan and could be discarded immediately. The 'correct' solution **1** was given twice but with different ^{13}C assignments. In the erroneously assigned solution and the three false structures **3**, **4** and **5** the carbon with an asterisk was assigned a chemical shift of δ_C 140.8, an unrealistic value for this kind of environment. During our work this interesting 9,10-secoclerodane structure was published for salviandulines A and B from *S. lavanduloides*.[9] The stereochemistry of salvianduline B as shown in **1** was derived from a crystal structure analysis.[9] The identity of our compound with salvianduline B rather than its 7-epimer salvianduline A is based on a comparison of ^1H and ^{13}C NMR data, especially of the vicinal couplings between H-7 and 2H-6. Thus compound **1** is *ent*-(4*S*,5*R*)-7α-acetoxy-15,16-epoxy-10-oxo-9,10-secocleroda-1,8,13(16),14-tetraene-17,12*S*;18,19-diolide.

Compound **2**, $C_{20}H_{20}O_6$, has one tertiary methyl, a β-substituted furan, a tertiary hydroxyl group and two trisubstituted double bonds [δ_H 6.78 (t, *J* 3.6 Hz, H-3) and 6.42 (t, *J* 3.7 Hz, H-7)], both conjugated with lactone carbonyls [δ_C 168.2 (C-17) and 170.0 (C-18)]. The molecule is therefore bicarbocyclic. The spin systems associated with H-12 and 2H-11, H-7 and 2H-6 and H-3, 2H-2 and 2H-1 are apparent in the COSY spectrum which also reveals long-range coupling between H-6β and H-19α (*J* 1.1 Hz). The assembly of these spin systems was achieved using the **LSD** computer program and structure **2** was obtained as the unique solution. The data set consisted of atom status and HMQC and HMBC as usual but also incorporated two COSY correlations which, in conjunction with HMQC data, led to the C-11/C-12 and C-1/C-2 bonds. The four C-O bonds of the two ester/lactone groups were also incorporated. Carbons C-15, C-16, C-19, C-12 and C-10 must be bound to oxygen atoms and C-5, C-9, C-6, C-11, C-20, C-1 and C-2 must have only carbon neighbours. As above, the methyl C-20 is bound to a quaternary carbon.

Several features of the stereochemistry of **2** were readily deduced from NOE experiments. Thus irradiation of Me-20 afforded significant NOEs at H-19*R* (7%), H-19*S* (6%) and H-11α (8%), revealing the 1,3-diaxial nature of C-19 and C-20. Because of the presence of a hydroxyl group at C-10 the nature of the AB ring junction was not immediately apparent. However, NOEs from 2H-1 to H-6β (6%) and to H-12 (15%, including a contribution from H-11β) are difficult to reconcile on the basis of an AB *trans* fusion. Inspection of molecular models suggested an AB *cis* ring junction. The NOE from 2H-1 to H-12 is also consistent with an α-configuration of the furan ring. H-12 shows only a small NOE to H-11β. Confirmation of the relative stereochemistry of **2** was obtained by an X-ray analysis, the details of which are given later. The crystal conformation of the molecule (Figure 1) readily accommodates the observed NOEs. Compound **2** is therefore *ent*-(5*R*,9*R*)-15,16-epoxy-10*S*-hydroxycleroda-3,7,13(16),14-tetraene-17,12*S*;18,19-diolide.

There is considerable advantage in using computer-based systems for structural elucidation, in particular when the structural class of the molecule under investigation is not known. The principal benefits are the exhaustiveness of candidate structure generation and the absence of any specialised chemical knowledge within the program, except that provided explicitly by the user. The latter point is particularly important if one is liable to be tempted to overinterpret chemical shift values. The **LSD** program is based on the analysis of carbon-proton chemical shift correlation experiments, which have presently reached considerable sensitivity and reliability. In the future, improvements of NMR and computer hardware and software will certainly encourage chemists to make routine use of automated structural elucidation and it is even possible to envisage its incorporation into automated spectrometer operation and data processing.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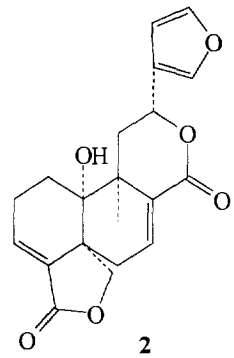
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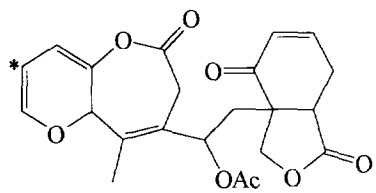
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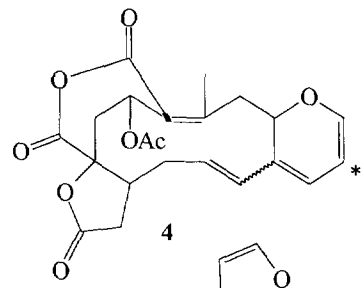
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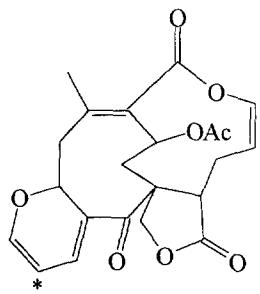
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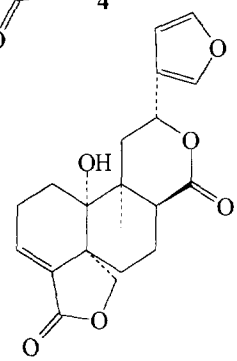
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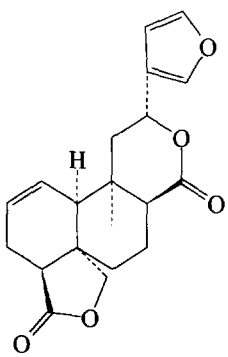
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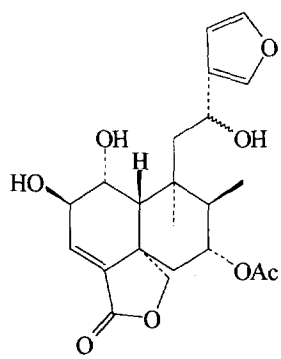
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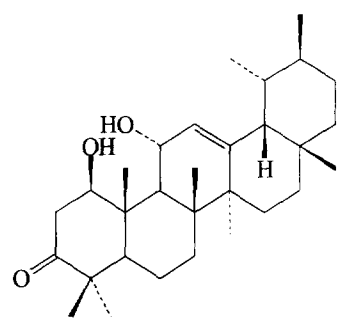
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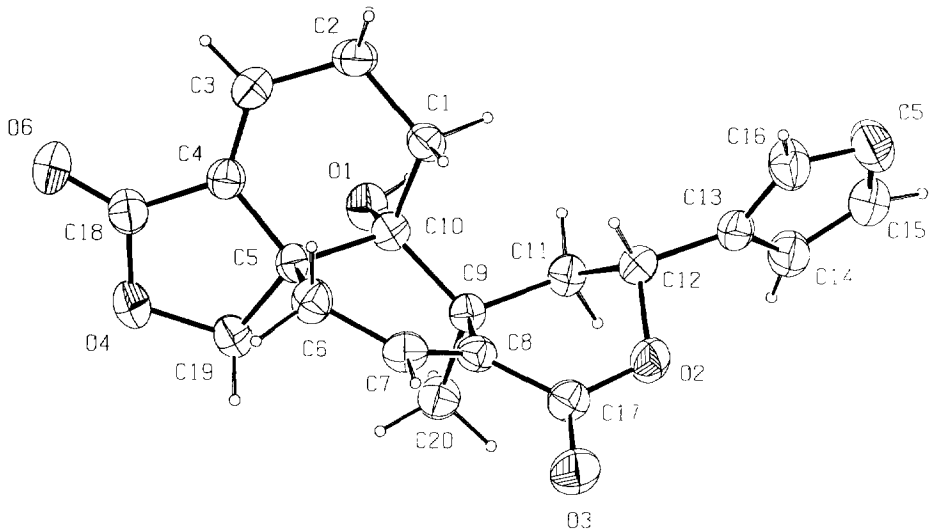

Figure 1. The molecular structure and atomic labelling scheme of **2**, $C_{20}H_{20}O_6$, with thermal ellipsoids shown at the 50% probability level.

The spectroscopic properties of the third compound, **6**, $C_{20}H_{22}O_6$, m/z 358 indicated that it is a dihydro-derivative of **2**. In particular it lacks the Δ^7 double bond and has a non-conjugated ring C lactone (δ_C 174.0) but retains the unsaturated γ-lactone [δ_H 6.90 (t, *J*=3.6 Hz), H-3], β-substituted furan and tertiary methyl group. The relative stereochemistry of most of the molecule was readily revealed. Irradiation of Me-20 afforded large NOEs at 2H-19 (each 5%), H-8 (7%), H-11α (5%) and the furan protons H-14 (1.5%) and H-16 (4%), thus showing that Me-20, 2H-19, H-8 and the furan ring all lie on the same face (α) of the molecule. However a decision on the nature of the AB ring fusion is less straightforward. With an AB-*trans* fusion a NOE from the tertiary methyl to H-1α would be expected but is not observed. However there is a NOE from the tertiary methyl to H-7α (3%). Moreover the chemical shift of the C-20 methyl group (δ_C 27.3) is similar to those of the other *cis*-clerodanes **2** and **7**. In general this methyl group is more shielded in *trans*-clerodanes.[10] Thus compound **6** is assigned the structure *ent*-(5*R*,9*R*)-15,16-epoxy-10*S*-hydroxycleroda-3,13(16),14-triene-17,12*S*;18,19-diolide. In 1973 Brieskorn and Stehle[11] isolated a diterpenoid from *Salvia rubescens* and assigned it structure **6** but without stereochemistry; it has not been possible to decide if the two compounds are identical because the proton NMR data were reported for a different solvent.

Compound **7**, $C_{20}H_{22}O_5$, m/z 342, has a disubstituted double bond whose protons are accidentally equivalent and whose carbon shifts are very similar. Its other features include two saturated lactone rings [δ_C 172.7 (C-17) and 175.8 (C-18)] and a β-substituted furan. It was possible to identify the various spin systems and to assign all the protons and carbons (Tables 1 and 2) by inspection of COSY, HMQC and HMBC spectra and to arrive at the planar structure (**7**). As usual, NOE experiments provided valuable information on the stereochemistry of the molecule. Irradiation of Me-20 gave NOEs at H-10 (5%) and 2H-19 (8 and 3%) indicating a *cis* AB ring junction, at H-8 (6%) indicating a *cis* BC ring junction and at the furan protons H-16 (4%) and H-14 (1.5%) indicating the *ent*-*S* configuration at C-12. The NOE from Me-20 to the H-11 signal at

δ_H 2.06 (7%) identified it as H-11α. Irradiation of the olefinic proton signal H-1 and H-2 afforded a large NOE at H-11β (8%), thus confirming the placement of the double bond at C-1, C-2. The remaining stereochemical centre is assigned as *ent-R* (4α-H) following the observation of NOEs from H-19 (δ_H 3.98) to H-4 (2%), H-10 (2%), and the other H-19 (δ_H 4.46, 8%). Thus compound (7) is *ent*-(4*R*,5*R*,9*S*,10*R*)-15,16-epoxycleroda-1,13(16),14-triene-17,12*S*;18,19-diolide. The conformation of the molecule suggested by these NOEs is similar to that found in the X-ray analysis of the corresponding 1α,2α-epoxide, isolated from *S. reptans*.[12] A small NOE from H-1 to H-12 supports this conclusion. A diterpenoid with the planar structure 7 has been isolated from *Salvia gensneraefolia*[13] but its spectroscopic properties are different and it is presumably a stereoisomer.

The fifth clerodane, **8**, $C_{20}H_{28}O_8$ (m/e 402, M^+-18), differs from the others in several respects. It has two methyl groups (one tertiary and one secondary), an unsaturated lactone [δ_C 170.2 (C-18), δ_H 4.56 (dd, *J*=7.5, 2.2 Hz) and 4.85 (d, *J*=7.5 Hz) (2H-19), 6.56 (d, *J*= 7.0 Hz, H-3)], a secondary acetate (δ_H 5.24 (dt, *J*= 2.3 and *ca* 3 Hz, H-7), three secondary hydroxyl groups [δ_H 4.02 (br s, H-1), 4.04 (dd, *J*=7.0, 2.0 Hz, H-2), 4.81 (br d, *J*=9.4 Hz, H-12)] and a typical β-substituted furan. Acetylation afforded a tetra-acetate. The spin systems associated with ring A, ring B and the side chain were established from the COSY spectrum and readily lead to the planar structure **8**.

As usual, elucidation of the relative stereochemistry rested largely on NOE experiments. Irradiation of Me-20 afforded NOEs at H-19*S* (5%) and H-19*R* (2%) indicating that they are on the same face (α) of the molecule. The H-6 proton at δ_H 2.19 gave a NOE at H-19*R* (1%) and is therefore H-6α. It also gave NOEs at H-6β and H-7. The AB ring fusion must be *trans* since irradiation of H-6β resulted in a NOE at H-10 (3%). Since $J_{1,10}$ is very small H-1 must be equatorial (β). The vicinal coupling of H-2 and the vinyl proton H-3 is *ca* 7 Hz and thus H-2 is equatorial (α), rather than axial (ß) and almost orthogonal to H-3. Proton H-7 of the acetate-bearing methine is a narrow doublet of triplets and is equatorial (α). Irradiation of Me-17 afforded NOEs at both H-8 and H-10 and must be axial (β). The configuration of the remaining chiral centre C-12 was not determined. It should be noted, however, that significant NOEs were observed from H-12 to H-1 and H-10. These results lead to structure **8**, *ent*-(5*R*,9*S*,10*S*)-7*S*-acetoxy-15,16-epoxy-1*S*,2*S*,12ξ-trihydroxycleroda-3,13(16),14-trien-18,19-olide.

The triterpenoid $C_{30}H_{48}O_3$, m/z 456, isolated from the methylene chloride extract, was assigned the structure 3-oxours-12-ene-1β,11α-diol **9**. It has resonances for two secondary alcohol methines [δ_H 3.85 (t, *J*=7.5 Hz, H-1), 4.33 (dd, *J*=9.0, 3.2 Hz, H-11)] of which one is coupled to an olefinic proton [δ_H 5.22 (d, *J*=3.2 Hz, H-12], a methylene group adjacent to a carbonyl group [δ_H 2.72 (dd, *J*=15.0, 7.1 Hz, H-2α), 2.58 (dd, *J*=15.0,7.8 Hz, H-2β)], eight methyls, of which two are secondary, and a ketonic carbonyl group [δ_C 215.5 (C-3)]. These data are consistent with an ursane-type pentacyclic triterpenoid with oxygen functions at 1, 3 and 11. Irradiation of H-1α gave strong NOEs at H-2α and H-9, which resonates as a doublet (*J*=9 Hz) at δ_H 1.73, while irradiation of H-11β gave a NOE at H-12. These results and the ^{13}C chemical shifts[14] are consistent with the proposed structure and stereochemistry for **9**.

Crystal structure analysis of compound 2. Details of the data collection procedures and structure refinement are given in Table **3**. Crystals of compound **2** were obtained as colourless prisms. A single crystal of suitable size was attached to a glass fibre using acrylic resin and mounted on a goniometer head in a general position. Data were collected on an Enraf-Nonius TurboCAD4 diffractometer, running under CAD4-Express software, and using graphite monochromated X-radiation (λ=0.71073 Å). Precise unit cell dimensions were

determined by refinement of the setting angles of 25 high-angle reflections which were flagged during data collection. Standard reflections were measured every 2 h during data collection; a small decay of *ca* 4% was noted and an interpolated correction was applied. Lorentz-polarisation corrections were also applied. The structure was solved by direct methods (SIR92).[15] All non-H atoms were allowed anisotropic thermal motion. In view of the relative paucity of data, the CH hydrogen atoms were included at calculated positions (although all appeared in difference Fourier maps), with C-H = 0.96 Å, but with freely refined isotropic thermal parameters. The OH hydrogen was found from difference Fourier maps and freely refined. An extinction correction was also applied. Refinement (SHELXL93)[16] was by full matrix least squares on F^2, using the weighting scheme $w = [\sigma^2(F_o^2) + (0.3660P)^2 + 0.3085P]^{-1}$ where $P = [F_o^2/3 + 2F_c^2/3]$. $\sigma(F_o^2)$ was estimated from counting statistics. Neutral atom scattering factors, coefficients of anomalous dispersion and absorption coefficients were obtained from reference 17. The thermal ellipsoid plot (Figure 1) was produced using PLATON.[18]

Table 3 : Experimental Details of the Crystallographic Analysis of 2

Molecular formula	$C_{20}H_{20}O_6$
M_r	356.36
space group	$P2_12_12_1$
crystal system	orthorhombic
a/Å	9.7271(4)
b/Å	12.2638(8)
c/Å	13.6592(8)
V/Å^{-3}	1629.4(1)
θ range for cell	17.8 to 18.3
Z	4
D_{calc}/g cm^{-3}	1.453
F (000)	752
μ(Mo-K$_\alpha$)/cm^{-1}	1.07
scan mode	ω/2θ
ω scan angle/deg	0.49 + 0.38tan(θ)
θ range/deg	2.2 to 25.0
crystal size/mm	0.6 x 0.5 x 0.4
no. of data collected	2242
no. of unique data	2066
hkl range	-11 → 1; -14 → 1; -2 → 16
R_{int}	0.0124
standard reflections	(-4 -2 2) (1 -3 6) (0 4 5)
no. of data in refinement	2066
no. of refined parameters	261
final *R* [$I > 2\sigma(I)$] (all data)	0.025 (0.0264)
R_w^2 [$I > 2\sigma(I)$] (all data)	0.065 (0.066)
Flack absolute structure parameter	1.5(10)
Extinction coefficient	0.024(2)
goodness of fit *S*	1.044
largest remaining feature in	0.18(max)
electron density map/ eÅ^{-3}	-0.13(min)
shift/esd in last cycle	0.0005(mean)
	0.002(max)

EXPERIMENTAL

Salvia haenkei Benth. was collected at the locality of Liriuni (Quillacollo, Bolivia), in May 1990, identified by Dr. S. Beck (Universidad Mayor de San Andres, La Paz) and at Huajchilla, La Paz, identified by Dr E. Garcia. Voucher specimens are kept at the National Herbarium in La Paz.

Fresh flowering tops (400 g) were immersed in CH_2Cl_2 (3L, rt, 1h) and the solvent removed under reduced pressure. Treatment of the extract (15 g) with petrol afforded a precipitate (2 g) that consisted mainly of ursolic acid, identified by comparison with an authentic sample (NMR, tlc). The petrol soluble fraction was subjected to vacuum liquid chromatography on silica gel eluting with increasing amounts of EtOAc in petrol and finally with MeOH. Repeated column chromatography of the intermediate fractions afforded 3-oxours-12-ene-1β,11α-diol **9** (240 mg, mp 222-223°C, IR (KBr) 3600-3100 (br),1710 cm^{-1}), δ_H 5.22 (d, *J*=3.2 Hz, H-12), 4.33 (dd, *J*=9.0, 3.2 Hz, H-11), 3.58 (t, *J*=7.5 Hz, H-1), 2.72 (dd, *J*=15.0, 7.1 Hz, H-2α), 2.58 (dd, *J*=15.0, 7.8 Hz, H-2β), 1.73 (d, *J*=9.0 Hz, H-9), 1.16, 1.11, 1.07, 1.06, 1.05, 0.79 (t-Me), 0.91 (br s) and 0.84 (d, *J*=5.9 Hz) (*sec*-Me), δ_C 78.2 (C-1), 42.9 (C-2), 215.5 (C-3), 47.4 (C-4), 51.1 (C-5), 18.8 (C-6), 32.6 (C-7), 43.7 (C-8), 55.2 (C-9), 43.4 (C-10), 67.6 (C-11), 126.7 (C-12), 144.0 (C-13), 42.1 (C-14), 27.8 (C-15), 26.8 (C-16), 33.6 (C-17), 58.0 (C-18), 39.3 (C-19), 39.3 (C-20), 31.0 (C-21), 41.1 (C-22), 26.6 (C-23), 20.9 (C-24), 12.6 (C-25), 17.9 (C-26), 22.8 (C-27), 28.6 (C-28), 17.6 (C-29), 21.3 (C-30), *ent*-(4*R*,5*R*,9*S*,10*R*)-15,16-epoxycleroda-1,13(16),14-triene-17,12*S*;18,19-diolide **7** (300 mg, mp 224-225°C, IR (KBr) 3570-3350, 3120(s), 1775(s), 1625, 1500, 1450, 870, 815 cm^{-1}), *ent*-(4*S*,5*R*)-7α-acetoxy-15,16-epoxy-10-oxo-9,10-secocleroda-1,8,13(16),14-tetraene-17,12*S*;18,19-diolide **1** (200 mg, gum), *ent*-(5*R*,9*R*)-15,16-epoxy-10*S*-hydroxycleroda-3,13(16),14-triene-17,12*S*;18,19-diolide **6** (120 mg, mp 274-275°C, IR (KBr) 3360-3350 (br), 3520 (s), 1720, 1675, 1650, 1500, 1450, 1420, 870, 815 cm^{-1}), *ent*-(5*R*,9*R*)-15,16-epoxy-10*S*-hydroxycleroda-3,7,13(16),14-tetraen-17,12*S*;18,19-diolide **2** (50 mg, mp 283-286°C, $[\alpha]_D$ -115 (c, 0.013, $CHCl_3$/MeOH) and *ent*-(5*R*,9*S*,10*S*)-7*S*-acetoxy-15,16-epoxy-1*S*,2*S*,12ξ-trihydroxycleroda-3,13(16),14-trien-18,19-olide **8** (26 mg, mp 183-186 °C).

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