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^{1}H-NMR Spectroscopy as a Tool for Establishing the C-12 Stereochemistry and the Conformation of the Side Chain in 12-Hydroxylated *Neo*-clerodanes Isolated from *Teucrium* Species.

Jesús Jiménez-Barbero

Instituto de Química Orgánica General (C. S. I. C.), Juan de la Cierva 3, 28006 Madrid (Spain)

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Abstract: The analysis of the DQF-COSY, TOCSY, and NOESY ^{1}H-NMR spectra, assisted by molecular mechanics calculations, of several *neo*-clerodane diterpenoids possessing hydroxyl or acetoxyl substituents at the C-12 position, provided an useful method for establishing the C-12 stereochemistry. The preferred rotamer of the C-9 side-chain of these compounds, as well as the identification of their diastereotopic protons at C-11 was also achieved. Thus, the method allowed the assignment of a 12*S* stereochemistry for teupyrin B (**7**), teugracilin C (**10**), montanin H (**13**), and the diacetyl derivative of teulepicephin (**15**), all of them previously isolated from *Teucrium* species, and whose configuration at C-12 remained unknown.

The determination of the absolute configuration of stereogenic centers in natural products as well as their conformational preferences is a topic of major importance. X-ray crystallography provides the most secure method to solve structural problems in the solid state, but it requires single crystals, and, on the other hand, the conformation in solution can be different from that in the crystal.

The naturally occurring *neo*-clerodane diterpenoids have attracted interest because of their biological activity as insect antifeedants and as antifungal, antitumour, and antimicrobial agents. Although a large number of these compounds have been isolated from different plants in the last few years, the genus *Teucrium* (family Labiatae) is far and away the most abundant natural source of these substances[1,2].

Recently, a study of the ^{1}H and ^{13}C-NMR chemical shifts, the variation of the molecular rotations, and the behaviour in the circular dichroism exciton chirality method of several pairs of epimers at C-12 of 12-hydroxy, 12-acetoxy, and 12-benzoyloxy-*neo*-clerodane derivatives has been performed, providing alternative methods for establishing the C-12 absolute configuration of these compounds[3].

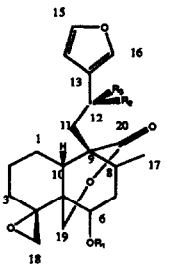
Nevertheless, the method of choice to determine the configuration and/or conformation of molecular entities in solution is obviously NMR, as has been demonstrated in the last years in the field of biopolymers[4,5]. NMR experiments providing chemical shifts, coupling constants and NOE data are an extraordinary tools to analyze the different structural features of molecules in solution[6]. Nevertheless, the analysis of these features for small molecules can be extremely complicated because of the occurrence of conformational flexibility.

However, provided that a single conformation is uniquely present or strongly preponderant in solution, nuclear Overhauser enhancements may be estimated for its corresponding geometry and may be compared with the experimental results to assess its presence and, therefore discriminate among several alternatives. We have used this approach to establish the absolute stereochemistry at C-12 of some 12-hydroxylated *neo*-clerodane diterpenoids, according to the following protocol: First, vicinal coupling constants analysis allowed to ascertain the presence of a major conformation in solution for the region of interest. This conformation was characterized with the help of molecular mechanics calculations. Second, the diastereotopic protons at C-11 were identified from unique contacts observed in NOE experiments, which permitted to confirm the expectations from MM calculations. Finally, NOE contacts were observed that were only compatible with a given configuration for the C12-stereogenic centre.

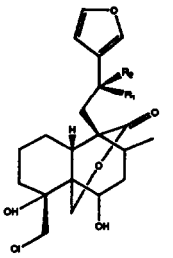
RESULTS AND DISCUSSION

The complete ^{1}H-NMR spectra of compounds **1-15** were assigned through homonuclear 2D-COSY and 2D-TOCSY experiments. The whole structures of compounds **1-6, 8, 9, 11,** and **12,** including their absolute configuration of *neo*-clerodane[7] and the stereochemistry at the C-12 centre are known[3,8-10], whereas compounds **7** (teupyrin B)[10], **10** (teugracilin C)[11], **13** (montanin H)[12,13], **14** (synthetic diacetate of **13**)[13], and **15** (*3, 12*-diacetylteulepicephin)[14] are substances isolated from *Teucrium* species whose stereochemistry at C-12 and the absolute configuration of the decalin moiety have not been ascertained. The chemical shift values are gathered in Table 1, along with the coupling constant values between H-12 and the methylene protons at C-11. There is always a value higher than 8.5 Hz, while the other one is smaller than 3.0 Hz, indicating the occurrence of a major rotamer around the C-11-C-12 bond in solution, independently of the configuration at C-12. It is noteworthy that only compounds **8-10** had the larger coupling smaller than 9 Hz, probably due to the presence of a certain degree of flexibility around C-11-C-12, induced by the lack of the lactone or hemicetal moiety involving the C-20 carbon atom, which in these compounds (**8-10**) is a methyl group. The favored rotamers around this C-11-C-12 bond as well as the C-9-C11 one were estimated by using molecular mechanics MM2 calculations. The three possible staggered rotamers around each of the bonds were used as starting geometries for the calculations. The geometry of compound **5**, whose structure is well known from an X-ray analysis[3], was used for the rest of the molecular skeleton. The calculations were also performed for the *R*-stereochemistry at the C-12 stereogenic center. Table 2 shows the steric energy differences found in the calculations for the different conformers, along with their relevant torsion angles. It can be observed that conformer A is the lowest energy minimum for both the 12-*R* and the 12-*S* configurations. A view of this conformer for compounds **1** and **3** is given in Figure 1.

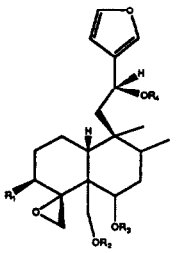
Although steric energy values for these systems may be only approximated, conformer A appears to be populated more than ca. 90% in both cases. Since the experimental *J*-values for **1-15** also indicate the presence of one major conformer in solution, it seems reasonable to assume that this is the corresponding A-type conformer. The relevant interproton distances for the A-type conformer for both 12-*R* and 12-*S* configurations are given in Table 3. It can be observed that protons 11-pro*R* and 11-pro*S* may be differentiated in base of their different proximities to the 17-methyl group and to protons 1α and 1ß. The values in Table 3 also show that, in the case of the C-12-*S* configuration there are close proximities between protons H-12 and H-17, and



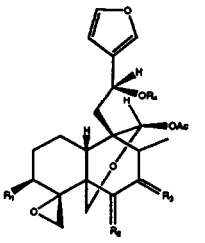
	R1	R2	R3
1	H	OH	H
2	Ac	OAc	H
3	H	H	OH
4	Ac	H	OAc



	R1	R2
5	OBz	O
6	H	OBz



	R1	R2	R3	R4
7	OH	H	Ac	H
8	H	Ac	Ac	H
9	H	Ac	Ac	Ac
10	OH	Ac	H	Ac



	R1	R2	R3	R4
11	OAc	O	H2	OH
12	OAc	O	H2	OAc
13	H	αOH,βH	O	OH
14	H	αOAc,βH	O	OAc

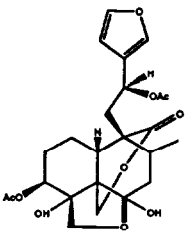


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TABLE 1. Chemical shifts (δ, ppm) and coupling constants (*J*, Hz) for compounds **1-15** in $CDCl_3$ at 30°C.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
1α	1.21	1.27	1.22	1.21	1.19	1.19	1.55	1.52	1.56	1.49	2.20	2.22		1.42	1.38
1β	2.42	2.42	1.75	1.71	2.29	1.72	2.10	2.02	2.04	1.67	2.23	2.14	2.30	2.26	2.26
2α	2.00	2.00	1.99	1.85	2.02	1.77	2.16	1.90	1.92	2.23	2.25	2.30		2.22	2.26
2β	1.50	1.42	1.45	1.46	1.52	1.30	1.36	1.46	1.38	1.22	1.60	1.67			1.36
3α	2.25	2.18	2.24	2.13	1.68	1.56	4.22	1.02	1.01	4.03	4.50	4.50			5.00
3β	1.14	1.09	1.14	1.06	0.81	2.18	-	2.11	2.08	-	-	-			-
6	3.77	4.87	3.76	4.87	3.84	3.90	4.91	4.75	4.75	3.60	-	-	3.81	5.06	-
7α	1.41	1.45	1.35	1.42	1.49	1.43	1.61	1.62	1.47	1.45	2.75	2.74	-	-	2.08
7β	1.92	1.92	1.91	1.92	1.92	1.73	1.60	1.48	1.58	1.52	2.55	2.55	-	-	1.58
8	2.11	2.05	2.45	1.84	2.09	1.82	1.77	1.76	1.66	1.65	2.16	2.22	2.58	2.60	2.04
10	2.24	1.77	1.50	1.72	1.65	1.75	1.98	1.98	1.98	1.98	2.31	2.36		2.06	1.78
11_S	2.45	2.65	2.68	2.98	2.73	3.08	1.54	1.57	1.68	1.67	1.65	1.59	1.62	1.55	2.71
11_R	2.13	2.36	1.86	1.96	2.47	2.05	1.92	1.93	2.10	2.09	2.63	2.30	2.80	2.37	2.24
12	4.84	5.94	4.66	5.79	6.32	6.07	4.76	4.74	4.69	3.60	6.01	6.02	6.00	5.98	5.92
14	6.43	6.42	6.46	6.44	6.46	6.48	6.35	6.36	6.34	6.32	6.43	6.39	6.46	6.37	
15	7.40	7.38	7.41	7.39	7.39	7.38	7.36	7.37	7.33	7.36	7.36	7.38	7.39	7.38	
16	7.41	7.46	7.41	7.46	7.49	7.51	7.38	7.37	7.39	7.34	7.44	7.40	7.47	7.42	
17	0.92	0.86	1.05	1.05	0.97	1.07	0.77	0.77	0.74	0.76	0.98	1.06	1.08	1.11	0.85
18a	2.64	2.36	2.64	2.36	3.23	3.97	2.82	2.99	2.95	3.04	3.14	3.16	2.53	2.40	4.30
18b	3.32	2.95	3.33	2.95	3.68	3.97	2.79	2.20	2.18	2.98	2.76	2.74	3.28	2.90	4.00
19a	4.74	4.81	4.70	4.81	4.62	4.66	4.29	4.84	4.79	4.55	4.41	4.20	3.79	3.91	4.70
19b	4.83	4.86	4.83	4.81	4.73	4.76	3.95	4.38	4.35	4.35	2.95	3.00	4.44	4.26	4.50
20	-	-	-	-	-	-	0.61	0.69	0.69	0.65	5.00	5.98	4.81	5.76	-
$J_{11S,12}$	1.3	2.9	10.2	9.6	2.4	9.7	2.5	2.7	3.1	2.8	2.5	2.6	2.5	2.5	2.7
$J_{11R,12}$	10.5	9.7	2.0	1.9	10.5	2.6	9.2	8.6	8.5	8.5	11.2	11.1	10.2	9.8	9.7

H-12 and H-8, while for C-12-*R* isomers, there are short distances between protons H-12 and H-10, and H-12 and H-1β. Since the local geometry around these centers in this *neo*-clerodane skeleton is fairly similar, it may be assumed that these or similar distances are kept in the different compounds, so the corresponding NOEs can be used as key data to ascertain the configuration at C-12. The methodology was first tested with compounds **1-6**, whose stereochemistry at C-12 is known from other methods. The steady-state 1D-NOE experiments were of limited use due to the frequent overlap among key protons. On the other hand, the integration of 2D-NOESY matrices may lead to important errors. Thus, the observed NOEs were classified as strong (s), medium(m) or weak(w). The results are shown in Table 4. It can be observed that the experimental NOEs agree with the expectations from MM calculations, and thus, strong H-12/H-17 and H-12/H-8 NOEs are observed for *S*-type compounds, while clear H-12/H-10 and H-12/H-1β contacts are present for the *R*-type analogues. A closer inspection of Table 4 indicates that a weak NOE between H-12 and H-10 also appears in some cases for *S*-configurations, although its intensity is much weaker than that of H-12/H-8. On the other hand, a weak H-12/H-8 NOE can be occasionally measured for C-12-*R* configurations. Also here, the corresponding H-12/H-10 NOE is much stronger. A view of the NOESY spectra recorded for **5** and **6** is given in Figure 2. Once the validity of the method was checked, the configuration of compounds **7-15**, which in some cases was unknown (*i.e.*, **7**, **10**, **13-15**), was studied. The results are also gathered in Table 4, and the analysis indicates that their resulting stereochemistry is *S* for all of them.

Consequently, the previously unknown stereochemistry at the C-12 centre of teupyrin B[10] (**7**), teugracilin C[11] (**10**), montanin H[12,13] (**13**), its synthetic diacetate (**14**)[13], and *3,12*-diacetylteulepicephin[14] (**15**) must be *S*, *if the absolute configuration of their decalin moiety belongs to the* neo-*clerodane series.*[1,2,7] This assumption is highly likely, since this is the case for all the clerodane diterpenoids isolated from *Teucrium* species, whose structures have been rigorously established so far.

Table 2. Relevant torsion angles and corresponding relative steric energy values for the low energy rotamers of compounds 1 and 3

Torsion (º)	Conformer					
	S-AA	*S*-AB	*S*-AC	*R*-AA	*R*-AB	*R*-AC
C9-O12	-68	-171	66	67	174	-66
C9-C13	171	67	-62	-173	-65	62
C9-H12	52	-55	-179	-56	56	176
C10-C12	64	51	66	52	69	60
C10-H11$_R$	-62	-71	-61	-70	-58	-65
C10-H11$_S$	-174	177	-172	178	-170	-176
H12-H11$_R$	179	67	-52	67	-176	-57
H12-H11$_S$	-71	178	57	177	-67	51
ΔE (Kcal/mol)	0.00	0.94	1.80	0.00	0.76	2.55

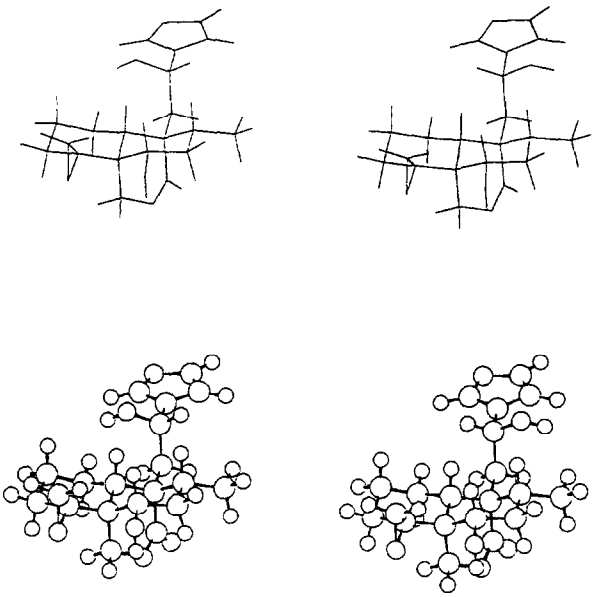

Figure 1.- Low energy conformers for compounds **1** (12-*S*, left) and **3** (12-*R*, right)

Table 3. Relevant interproton distances for the global minima of compounds 1 and 3, according to MM2 calculations.

Proton pair	CONFORMER			
	S-AA	*S*-AB	*R*-AA	*R*-AB
$H11_S$-H12	2.5	3.1	3.1	2.5
$H11_R$-H12	3.1	2.5	2.5	3.1
H12-$H17_{ABC}$	2.6/>3.5/>3.5	>3.5	>3.5	2.6/>3.5/>3.5
H12-H8	2.2	3.3	3.3	2.2
H12-H10	3.1	2.3	2.3	3 3
H12-$H1_b$	>3.5	2.2	2.3	>3.5
$H11_S$-$H17_{ABC}$	2.2/2.9/>3.5	2.1/3.0/>3.5	2.2/2.9/>3.5	2.2/2.9/>3.5
$H11_S$-H8	3.2	3.1	3.2	3.2
$H11_S$-H10	>3.5	>3.5	>3.5	>3.5
$H11_S$-$H1_\alpha$	>3.5	>3.5	>3.5	>3.5
$H11_S$-$H1_\beta$	>3.5	>3.5	>3.5	>3.5
$H11_R$-$H17_{ABC}$	>3.5	>3.5	>3.5	>3.5
$H11_R$-H8	>3.5	>3.5	>3.5	>3.5
$H11_R$-H10	3.3	3.3	3.3	3.2
$H11_R$-$H1_\alpha$	2.8	2.9	2.8	2.9
$H11_R$-$H1_\beta$	2.1	2.2	2.2	2.1

Table 4. Nuclear Overhauser effects classified as strong (s), medium (m), or weak (w) for compounds **1-15**.

Proton Pair	Compound														
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
11_S-12	s	s	w	w	s	w	s	s	s	s	s	s	s	s	s
11_R-12	w	w	s	s	w	s	w	w	w	w	w	w	w	w	w
12-17	s	s	nd	w	m	nd	w	w	m	m	m	s	s	s	s
12-8	s	s	w	nd	s	w	s	s	m	m	s	s	s	s	s
12-10	w	w	s	s	w	s	nd	nd	nd	nd	nd	w	w	w	w
12-1β	nd	nd	m	m	nd	m	nd	nd	nd	nd	nd	nd	nd	nd	nd
11_S-17	s	s	s	s	s	s	s	s	s	s	s	s	s	s	s
11_S-8	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
11_S-10	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
11_S-1β	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
11_R-17	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
11_R-8	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
11_R-10	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
11_R-1α	w-m	over	over	w-m	w-m	w-m	w-m	over	over	w-m	w-m	over	over	w-m	over
11_S-1β	over	over	m	m	s	s	m	m	over	nd	m	m	m	nd	m

nd, not determined. over, overlapped signal.

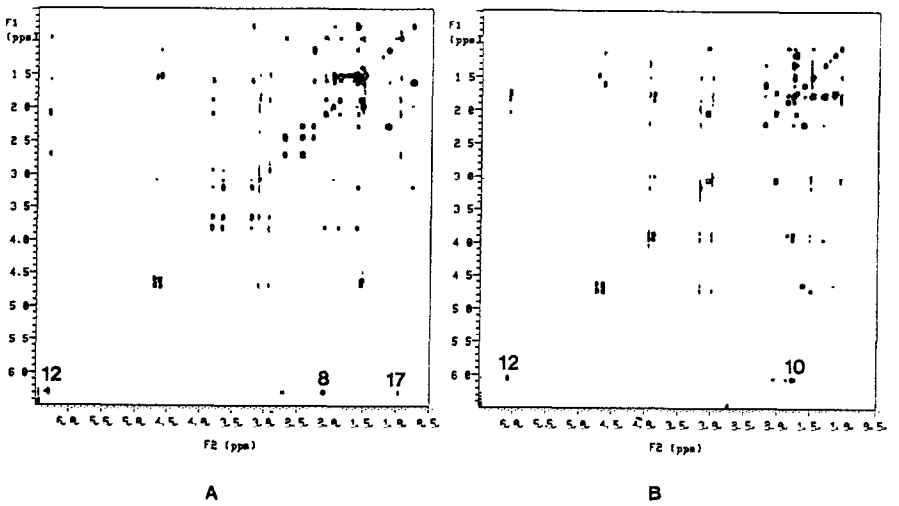

Figure 2. NOESY spectra for (A) compounds **5** (12-*S*) and (B) **6** (12-*R*). Characteristic cross peaks are indicated.

In conclusion, we have shown that the absolute configuration at C-12, and the assignment of the diastereotopic protons at C-11, of some *neo*-clerodane diterpenoids may be unambiguously determined by a careful analysis of NMR data, assisted by molecular mechanics calculations, provided that a major conformation exists for the region of interest. The presence of flexibility would imply the use of NOE-restrained molecular dynamics simulations[15], as currently used in the conformational analysis of biopolymers[16].

EXPERIMENTAL

Materials.- Compounds **1**[8], **2**[8], **3**-**6**[3], **7**[10], **8** and **9**[9], **10**[11], **11** and **12**[10], **13** and **14**[12,13], and **15**[14] were available from previous studies.

NMR experiments.- NMR spectra were recorded at 30º C in $CDCl_3$ on a Varian Unity 500 spectrometer.

The double quantum filtered DQF-COSY experiment were performed in the phase sensitive mode using the standard Varian sequence. A data matrix of 256 * 2K points was used to digitize a spectral width of 4000 Hz. 16 scans were used per increment with a relaxation delay of 2 s. Prior to Fourier transformation, zero filling was used in F_1 to expand the data to 2K * 2K.

The 2D-TOCSY experiments[17] were carried out in the phase sensitive mode using MLEV-17 for isotropic mixing. The mixing time was set to 80 ms. A data matrix of 256 * 2K points was used to digitize a

spectral width of 4000 Hz. 16 scans were used per increment with a relaxation delay of 2 s. Squared cosine bell functions were applied in both dimensions and zero filling was used to expand the data to 2K * 2K.

The 2D NOESY experiments were recorded[6] in the phase sensitive mode. The mixing time was set to 500 ms. A data matrix of 256 * 2K points was used to resolve a spectral width of 4000 Hz. 32 scans were used per increment with a relaxation delay of 2 s. Prior to Fourier transformation, squared sine bell functions shifted by $\pi/3$ were applied in both dimensions and zero filling was used in F_1 to expand the data.

The steady state NOE experiments were performed through the interleaved differential technique using a saturation delay of 7s. Between 256 and 512 free induction decays were accumulated for each irradiation site.

Molecular mechanics calculations.- MM2 calculations[18] were performed using a dielectric constant ε=1.5D, and using as starting geometry the crystallographic coordinates of compound **5**, and modified when necessary. The opposite configuration at C-12 was generated also from these coordinates and submitted to further minimization. Interproton distances were calculated from the final geometries.

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