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The Absolute Stereochemistry at C-12 in 12-Hydroxylated *Neo*-Clerodane Diterpenoids

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Key Words: Neo-clerodane diterpenes; epimeric C-12 alcohols, acetates and benzoates; absolute configuration at C-12; differences in ^{1}H and ^{13}C NMR spectroscopy and molecular rotations; application of the CD exciton chirality method; X-ray crystallographic analysis.

Abstract: *A study of the ^{1}H and ^{13}C NMR spectra, variation of the molecular rotations and behaviour in the CD exciton chirality method of several C-12 epimers of 12-hydroxy-, 12-acetoxy- and 12-benzoyloxy-*neo*-clerodane derivatives has been achieved, providing useful alternative methods for establishing the C-12 absolute configuration of this kind of compounds.*

A large number of diterpenoids belonging to the *neo*-clerodane class have been isolated from *Teucrium* species (Labiatae) over the last few years[1-4]. These compounds have attracted a great interest on account of their useful insect antifeedant activity[5,6] and fascinating and challenging structures[4,7,8].

An almost general structural feature of the *neo*-clerodanes found in *Teucrium* species is the existence of an alcohol function at the C-12 position. In the different diterpenes, this hydroxyl function appears as a free alcohol or as its acetyl derivative, as well as forming a C-20,C-12 lactone or hemiacetal or, less frequently, involved in a C-20,C-19,C-12 acetal grouping. The vast majority of the C-12 hydroxylated *neo*-clerodanes isolated from *Teucrium* possesses a 12*S* absolute stereochemistry, but there are some substances with an opposite absolute configuration (12*R*)[1-11].

For compounds in which the C-12 hydroxyl group is involved in a lactone, hemiacetal or acetal function, their C-12 configuration is easily solved by NOE experiments[9-11], whereas in the case of the diterpenoids having a C-12 hydroxyl or acetoxyl group, the absolute configuration of the C-12 asymmetric centre has been determined by X-ray diffraction analyses[12,13] or by Horeau's method[14,15]. However, these two methods can not be applied in all the cases and there are some natural C-12 hydroxyl or acetoxyl *neo*-clerodane derivatives isolated from *Teucrium* whose C-12 configuration has not been established[13, 16-19].

Since we were interested in finding alternative methods for establishing the C-12 absolute configuration in *neo*-clerodane derivatives possessing a C-12 alcohol function, we have undertaken a comparative study of the C-12 epimers **3** and **4** and some of their derivatives (**5-10**) in order to establish some criteria for defining the C-12 configuration of this kind of compounds, even when only one epimer is available. These criteria are based on ^{1}H and ^{13}C NMR spectroscopic data, variations of the molecular rotations and different behaviour of each epimer in CD and the CD exciton chirality method[20].

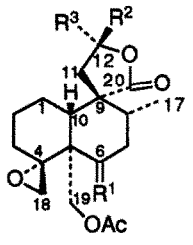
RESULTS AND DISCUSSION

Compounds **3** and **4** , epimers at the C-12 position, have previously been obtained by us starting from the natural diterpenoids 19-acetylgnaphalin (**1**) and montanin C (**2**), respectively[21]. Acetic anhydride-pyridine treatment of the diterpenoids **3** and **4** yielded the corresponding 6,12-diacetyl derivatives **5** and **6**, respectively. On the other hand, reaction of compound **3** with an equimolecular amount of benzoyl chloride in pyridine solution for 3 hours at room temperature gave the chlorohydrin **7**, whereas treatment of compound **4** with an excess of benzoyl chloride in pyridine solution (36 hours at room temperature) not only caused the opening of its oxirane to the corresponding chlorohydrin but also a selective esterification of the C-12 hydroxyl group yielding the derivative **9**. The C-12 epimeric benzoate **8** was obtained by treating compound **7** with an excess of benzoyl chloride at room temperature for 24 hours. Finally, the 12-keto derivative **10** has previously been synthesized by selective oxidation of both C-12 epimeric alcohols (**3** and **4**)[21].

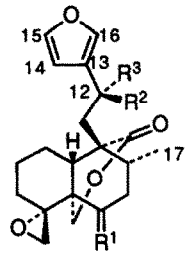
The ^{13}C NMR spectroscopic data of compounds **3-9** collected in Table 1 reveal that each pair of the C-12 epimers (**3** and **4**, **5** and **6**, and **8** and **9**) shows almost identical ^{13}C NMR spectra and there are not significant differences which allow an unambiguous assignment of the C-12 stereochemistry. On the contrary, the ^{1}H NMR spectra of compounds **3-6**, **8** and **9** (Table 2), although they are similar for each pair of epimers, provide some criteria which can be useful for distinguishing the configuration at the C-12 asymmetric centre, even when only one epimer is available. In fact, all the compounds with a 12*R* stereochemistry (**4**, **6** and **9**) showed the C-17 methyl protons resonance at slightly lower field than the compounds belonging to the 12*S* epimeric series (**3**, **5** and **8**), as has been pointed out previously[9,22] for some 12*R*- and 12*S*-*neo*-clerodan-20,12-olide derivatives. Moreover, in compounds of the 12*R* series (**4** and **6**), the acetylation causes a noticeable upfield shift of the signal of the H-8β proton ($\Delta\delta$ -0.61 ppm) and a very slight diamagnetic effect on the H-10β proton ($\Delta\delta$ -0.03 ppm), whereas in compounds with a 12*S* stereochemistry (**3** and **5**) the esterification produces a clear upfield shift of the H-10β proton ($\Delta\delta$ -0.47 ppm) and a little variation in the H-8β proton ($\Delta\delta$ -0.06 ppm). The ^{1}H NMR behaviour of the C-11 methylene protons [$J_{11A,12}$ always of minor magnitude in compounds **4**, **6** and **9** (12*R*) than in their 12*S* epimers (**3**, **5** and **8**); $J_{11B,12}$ value of the 12*R* derivatives larger than in compounds of the 12*S* series, see

Table 2] does not provide an adequate criterion for establishing the C-12 configuration, because it can occasionally be due to the substitution pattern in the rest of the diterpenic skeleton[12,23].

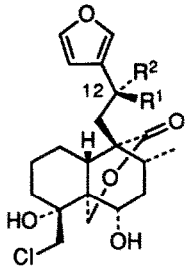
It is of interest to note that the ^1H and ^{13}C NMR spectra of compounds **3** and **4** recorded in deuterochloroform, pyridine-d_5 and mixtures of these solvents, and also at different temperatures, showed striking similarities and the observed variations were not suitable for establishing additional criteria in order to define their C-12 stereochemistries.



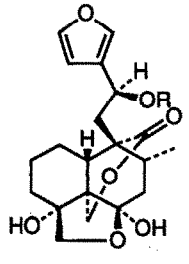
	R^1	R^2	R^3
1	O	β-furyl	H
2	αOAc,βH	H	β-furyl



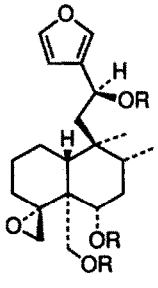
	R^1	R^2	R^3
3	αOH,βH	OH	H
4	αOH,βH	H	OH
5	αOAc,βH	OAc	H
6	αOAc,βH	H	OAc
10	αOH,βH	O	
19	αH,βOH	OH	H
20	αH,βOAc	OAc	H



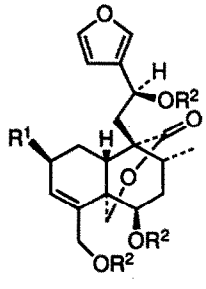
	R^1	R^2
7	OH	H
8	OBz	H
9	H	OBz



11 R=H
12 R=Ac



13 R=H
14 R=Ac



	R^1	R^2
15	OH	H
16	OAc	Ac
17	H	H
18	H	Ac

Apart from the above criteria based on ^1H NMR spectroscopy, there is an easy method for establishing the C-12 configuration of 12-hydroxy-*neo*-clerodane diterpenoids, namely comparison between the molecular rotation values of the C-12 alcohols and those of their corresponding 12-acetyl or 12-benzoyl or 12-keto derivatives. In the 12-hydroxy derivative having a 12*S* absolute configuration (**3**), acetylation of both C-6 and C-12 hydroxyl groups (compound **5**) produces a weak negative increment of the molecular rotation value, whereas

Table 1. ^{13}C NMR Chemical Shifts of Compounds 3-9*

C	3†	4†	5	6	7	8	9
1	23.8 *t*	23.8 *t*	24.0 *t*	23.6 *t*	25.5 *t*#	24.7 *t*#	24.8 *t*#
2	24.7 *t*	24.4 *t*	24.5 *t*	24.5 *t*	21.8 *t*#	20.9 *t*#	21.2 *t*#
3	31.6 *t*	31.4 *t*	32.3 *t*	32.1 *t*	30.4 *t*	30.2 *t*	30.2 *t*
4	65.3 *s*	65.0 *s*	61.8 *s*	61.6 *s*	76.9 *s*	76.7 *s*	76.7 *s*
5	40.1 *s*	40.0 *s*	39.9 *s*	39.9 *s*	44.1 *s*	43.3 *s*	43.5 *s*
6	72.8 *d*	72.7 *d*	72.1 *d*	72.1 *d*	73.0 *d*	73.0 *d*	72.9 *d*
7	36.2 *t*	36.3 *t*	35.4 *t*	35.7 *t*	37.4 *t*	37.8 *t*	38.0 *t*
8	34.4 *d*	34.8 *d*	34.6 *d*	35.2 *d*	34.7 *d*	34.3 *d*	34.9 *d*
9	50.0 *s*	49.3 *s*	49.4 *s*	49.1 *s*	50.2 *s*	49.4 *s*	49.2 *s*
10	44.4 *d*	43.0 *d*	44.5 *d*	43.2 *d*	42.1 *d*	41.0 *d*	40.7 *d*
11	37.0 *t*	37.1 *t*	33.5 *t*	33.7 *t*	39.0 *t*	33.4 *t*	33.9 *t*
12	63.6 *d*	62.7 *d*	64.2 *d*	63.3 *d*	62.8 *d*	65.1 *d*	64.3 *d*
13	130.2 *s*	130.0 *s*	125.4 *s*	125.4 *s*	132.6 *s*	125.4 *s*	125.5 *s*
14	108.3 *d*	108.4 *d*	108.5 *d*	108.6 *d*	109.6 *d*	108.6 *d*	108.6 *d*
15	143.7 *d*	143.7 *d*	143.5 *d*	143.5 *d*	143.8 *d*	143.6 *d*	143.7 *d*
16	138.5 *d*	138.5 *d*	140.0 *d*	140.1 *d*	139.1 *d*	139.8 *d*	140.1 *d*
17	16.5 *q*	17.0 *q*	16.2 *q*	16.4 *q*	17.0 *q*	16.4 *q*	16.8 *q*
18	50.9 *t*	50.7 *t*	50.1 *t*	50.2 *t*	51.0 *t*	48.7 *t*	49.0 *t*
19	67.1 *t*	66.9 *t*	68.1 *t*	68.0 *t*	69.4 *t*	68.4 *t*	68.5 *t*
20	172.1 *s*	172.1 *s*	171.1 *s*	171.3 *s*	172.6 *s*	171.6 *s*	171.9 *s*
OAc			170.1 *s*	170.2 *s*			
			169.8 *s*	170.1 *s*			
			21.2 *q*	21.2 *q*			
			21.2 *q*	21.2 *q*			
OBz (CO)						165.9 *s*	165.8 *s*
1'						129.5 *s*	129.7 *s*
2',6'						129.4 *d*	129.6 *d*
3',5'						128.8 *d*	128.6 *d*
4'						133.9 *d*	133.5 *d*

*At 50.3 MHz, all in $CDCl_3$ solution except for 7, which was recorded in pyridine-d_5 solution; TMS as internal standard. Multiplicities were established by the DEPT pulse sequence.
†Taken from ref (21).
#These assignments may be reversed, but those given here are considered to be the most likely.

in the case of the 12*R* epimer (compound **4**) this transformation causes a strong positive increment of the molecular rotation of the derivative **6** (see Table 3). Although these comparisons could be taken cautiously due to the additional esterification of the C-6 hydroxyl group in compounds **5** and **6**, the acetylation at C-12 seems to be the greatest contributor to the change of the molecular rotation, taking into account that the furan is the most polarizable group. This is also supported by the behaviour of several 12*S*-hydroxy-*neo*-clerodane diterpenoids and their peracetyl derivatives previously described, and whose 12*S* absolute stereochemistry is firmly established, as in the cases of teugnaphalodin[15] (**11**) and its 12-monoacetate (**12**), in which the ΔM_D=[M]$_D$(OAc)-[M]$_D$(OH) is -81, teumassilin[14] (**13**) and its 6,12,19-triacetate (**14**) (ΔM_D -181), teusalvin E[24] (**15**) and its 2,6,12,19-tetraacetate (**16**) (ΔM_D -304), teubotrin[25] (**17**, also named teulamifin B[26]) and its 6,12,18-triacetyl derivative (**18**) (ΔM_D -132) and teucroxylepin[27] (**19**) and its 6,12-diacetate (**20**) (ΔM_D -193).

The method of the variation of molecular rotations can also be used comparing the M_D value of the C-12 alcohol with that of its corresponding 12-keto derivative, which is easily available by selective oxidation of the C-12 hydroxyl group [21] In this case, there is a strong positive increment (+183) of the molecular rotation of the keto derivative when the starting alcohol possesses a 12*S* configuration and a weak positive increment (+32) for the 12*R* epimer (see Table 3, compounds **3**, **4** and **10**). Furthermore, when a selective benzoylation at C-12 is achieved, the magnitude of the rotation difference between the benzoate and the carbinol follows the Brewster's

Table 2. ^{1}H NMR Spectroscopic Data of Compounds 3-9 (TMS as int. standard)*

H	3†	4†	5	6	7	8	9
1α	1.21 *qd*	1.22 *qd*	1.27 *qd*	1.21 *qd*	#	#	#
1β	2.42#	1.75#	2.42#	#	#	#	#
2α	2.00#	1.99 *ddddd*	2.00#	#	#	#	#
2β	1.50 *qt*	1.45 *qt*	1.42 *qt*	1.46 *qt*	#	#	#
3α	2.25 *tdd*	2.24 *tdd*	2.18 *tdd*	2.13 *tdd*	#	#	#
3β	1.14 *ddd*	1.14 *ddd*	1.09 *ddd*	1.06#	#	#	#
6β	3.77 *ddd*	3.76 *ddd*	4.87 *dd*	4.87 *dd*	4.25#	3.84 *ddd*	3.90 *ddd*
7α	1.41 *ddd*	1.35 *ddd*	1.45 *ddd*	1.42 *ddd*	#	#	#
7β	1.92 *ddd*	1.91 *ddd*	1.92 *ddd*	1.92#	#	#	#
8β	2.11 *ddq*	2.45 *ddq*	2.05#	1.84#	#	#	#
10β	2.24 *dd*	1.75#	1.77 *dd*	1.72#	#	#	#
11A	2.13 *dd*	1.86 *dd*	2.36 *dd*	1.96 *dd*	2.49 *dd*	2.47 *dd*	2.05 *dd*
11B	2.45 *dd*	2.68 *dd*	2.65 *dd*	2.98 *dd*	2.84 *dd*	2.73 *dd*	3.08 *dd*
12	4.85 *ddd*§	4.66 *ddd*§	5.94 *dd*	5.79 *dd*	5.20 *ddd*§	6.32 *dd*	6.07 *dd*
14	6.43 *dd*	6.46 *dd*	6.42 *dd*	6.44 *dd*	6.72 *dd*	6.46 *dd*	6.48 *dd*
15	7.40 *t*	7.41#	7.38 *t*	7.39 *t*	7.60 *t*	7.39 *t*	7.38 *t*
16	7.41 *m*	7.41#	7.46 *m*	7.46 *m*	7.75 *m*	7.49 *m*	7.51 *m*
Me-17	0.92 *d*	1.05 *d*	0.86 *d*	1.05 *d*	1.01 *d*	0.97 *d*	1.07 *d*
18A	2.64 *d*π	2.64 *d*π	2.36 *d*π	2.36 *d*π	4.23 *d*	3.23 *d*	3.97 *s*
18B	3.32 *dd*¶	3.33 *dd*¶	2.95 *dd*¶	2.95 *dd*¶	4.34 *d*	3.68 *d*	3.97 *s*
19A	4.74 *dd*	4.70 *dd*	4.81 *d*	4.81 *s*	5.00 *dd*	4.62 *dd*	4.66 *dd*
19B	4.83 *d*	4.83 *d*	4.86 *d*	4.81 *s*	5.14 *d*	4.73 *d*	4.76 *d*
OAc			2.05 *s* 2.03 *s*	2.05 *s* 2.04 *s*			
OBz						8.05-8.00 (2H, *m*) 7.60-7.45 (3H, *m*)	8.05-7.99 (2H, *m*) 7.60-7.37 (3H, *m*)
OH(C-4)**					6.59 *br s*††	3.26 *s*††	3.45 *br s*††
OH(C-6)**	3.83 *s*	3.79 *s*			5.79 *br s*††	3.17 *s*††	3.33 *br s*††
OH(C-12)**	1.79 *d*	1.75 *d*			4.90 *br*††		
J(Hz)							
1α,1β	13.4	13.4	13.0	13.0	#	#	#
1α,2α	3.7	4.2	3.0	3.8	#	#	#
1α,2β	13.4	13.4	13.2	13.2	#	#	#
1α,10β	13.4	13.4	12.4	13.0	#	#	#
1β,2α	3.9	3.4	#	#	#	#	#
1β,2β	3.8	3.7	3.9	3.8	#	#	#
1β,10β	3.8	#	3.7	#	#	#	#
2α,2β	13.4	13.4	13.2	13.2	#	#	#
2α,3α	4.2	4.2	4.3	4.3	#	#	#
2α,3β	3.1	3.0	3.2	#	#	#	#
2β,3α	13.4	13.4	13.7	13.6	#	#	#
2β,3β	3.8	3.7	3.9	3.8	#	#	#
3α,3β	13.4	13.4	13.7	13.6	#	#	#
6β,7α	11.3	11.4	11.5	11.5	#	11.5	11.4
6β,7β	5.4	5.4	5.1	5.6	#	5.1	5.1
7α,7β	13.7	13.6	13.3	13.1	#	#	#
7α,8β	11.5	11.4	11.3	11.5	#	#	#
7β,8β	4.7	4.1	4.5	#	#	#	#
8β,17	6.7	6.6	6.6	6.5	6.7	6.7	6.5
11A,11B	15.8	16.1	16.4	16.4	15.7	16.5	16.5
11A,12	10.5	2.0	9.7	1.9	10.5	10.5	2.6
11B,12	1.3	10.2	2.9	9.6	2.6	2.4	9.7
12,12(OH)	3.4	4.9			#		
14,15	1.7	1.5	1.7	1.8	1.8	1.9	1.8
14,16	0.9	1.2	0.8	0.8	0.8	0.8	0.8
15,16	1.7	#	1.7	1.8	1.8	1.9	1.8
18A,18B	3.3	3.3	3.9	3.9	11.6	11.6	0
18B,3α	2.6	2.4	2.3	2.4	0	0	0
19A,19B	12.5	12.5	12.9	0	13.2	13.5	13.5
19A,6β	1.5	1.5	0	0	1.5	1.0	1.4

*Spectral parameters were obtained by first order approximation. All these assignments have been confirmed by double resonance experiments. All the spectra were recorded at 300 MHz, in $CDCl_3$ solution except for 7 (pyridine-d_5). †Taken from ref (21). #Overlapped signal. §Collapsed into *dd* after addition of D_2O. π*Exo* hydrogen with respect to ring B. ¶*Endo* hydrogen with respect to ring B. **Disappeared after addition of D_2O. ††Interchangeable assignments.

rule[28,29], considering that the furan is the most polarizable substituent [see Table 3 and compare the M_D values of the 12*S* compounds **3**, **7** and **8** and those of the 12*R* derivatives **4** and **9**].

Table 3. Specific and Molecular Rotations of Compounds **3-10** (values in degrees)*

Compound	Temperature(°)	*c*	$[\alpha]_D$	$[M]_D$
3†	22	0.413	-52.8	-191
4†	19	0.988	-10.9	-39
5	24	0.407	-46.4	-207
6	20	0.568	+25.0	+111
7	24	0.740	-20.3	-81
8	22	0.972	-66.0	-332
9	24	0.427	+78.7	+395
10†	20	0.217	-2.3	-8

*All in $CHCl_3$ solution except for **7**, which was measured in $CHCl_3$:MeOH 9:1 solution.
†Taken from ref (21).

The CD spectral data of the furan-alcohols **3**, **4** and **7**, are also useful for the determination of the absolute configuration at C-12. The UV spectra of these compounds exhibit a $\pi \rightarrow \pi^*$ absorption band around 210 nm (ε 5,400). In the corresponding region, the CD spectra of **3** and **7**, with the 12*S* absolute configuration, show negative Cotton effects, while that of **4**, with 12*R* absolute configuration, exhibits a positive Cotton effect (Table 4). The sign of the CD Cotton effects around 205-214 nm thus reflects the absolute configuration of 3-furan-alcohols, i.e., that at C-12. All the furan-alcohols **3**, **4** and **7** give positive weak CD Cotton effects around 234 nm, which may be due to the chirality of the decalin part.

Table 4. UV and CD Spectra of 3-Furylcarbinols and Benzoate Derivatives in Ethanol

Compound	UV, λ_{max} nm (ε)	CD, λ_{ext} nm (Δε)	
3 (12*S*)	210.0 (5,200)	233 (+0.3)	207 (-1.9)
4 (12*R*)	210.2 (5,600)	236 (+0.6) sh	214 (+2.7)
7 (12*S*)	209.0 (5,500)	233 (+0.6)	205 (-1.8)
8 (12*S*)	230.6 (13,400)	277 (+0.5)	
		244 (-4.0)	224 (+6.0)
9 (12*R*)	230.6 (14,700)	265 (-0.5)	
		240 (+1.4)	223 (-0.7)

Recently, to determine the absolute configuration of 2-furylcarbinols, the CD exciton chirality method has been applied to the interaction between furan and benzoate chromophores[30]. The exciton method was similarly applied to the 3-furylcarbinol benzoates **8** and **9**. The UV spectra of **8** and **9** show the $\pi \rightarrow \pi^*$ absorption band due to the intrachromophoric charge transfer transition at 230 nm (ε 14,000). The CD spectrum of **8** exhibits negative first and positive second Cotton effects at 244 nm and 224 nm, respectively. These Cotton effects may be attributable to the exciton coupling between furan and benzoate transitions. The $\pi \rightarrow \pi^*$ band of furan chromophore around 210 nm has been assigned to the $^1B_2$ transition, which is polarized parallel to the long axis of the diene part[30,31].

Therefore, the sign of bisignate Cotton effects depends on the exciton chirality between the long axes of diene and benzoate chromophores. Since the furan ring part can rotate around the bond of C-12,C-13, it is difficult to determine the stable conformation of the rotation. Therefore, we assumed that the stable conformation in solution state was similar to that in crystalline state. As depicted in the ORTEP drawing of the benzoate **8**, the furan and benzoate chromophores constitute a negative exciton chirality which agree with the negative sign of the observed first CD Cotton effect (Figure 1). The bisignate Cotton effects of **8** was thus explained by the exciton coupling mechanism. The CD spectrum of **9**, with 12*R* absolute configuration, exhibits positive first and negative second Cotton effects at 240 nm and 223 nm, respectively. These Cotton effects are opposite in sign to those of **8** with 12*S* absolute configuration. Although the amplitude is small, the Cotton effects reflect the absolute configuration at C-12.

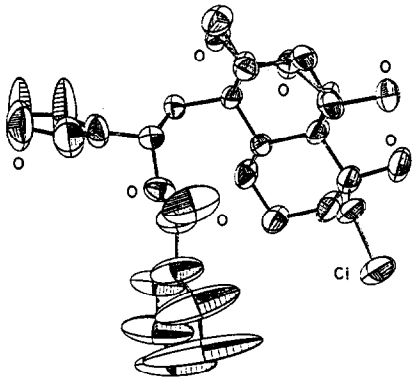


Figure 1. ORTEP drawing of the absolute configuration and crystalline conformation of (4*R*,5*R*,6*S*,8*R*,9*R*,10*S*,12*S*)-(-)-**8**.

The absolute configuration of some 12-hydroxylated *neo*-clerodanes isolated from *Teucrium* has previously been determined by X-ray crystallographic method[12,13]. However, the determination of the absolute configuration was based on the heavy atom effect of oxygen and carbon atoms. To determine the absolute configuration by use of the anomalous dispersion effect of heavier atom, we performed the X-ray crystallographic analysis of the chlorohydrin derivative (-)-**8**. The heavy atom effect of chlorine atom led to the unambiguous determination of the absolute configuration (4*R*,5*R*,6*S*,8*R*,9*R*,10*S*,12*S*) as illustrated in Figure 1.

In summary, we believe we have suggested some useful alternative methods for establishing the C-12 stereochemistry of 12-hydroxy-*neo*-clerodane derivatives, which are interesting on account of their biological activities.

EXPERIMENTAL

Mps are uncorrected. Starting materials (compounds **3** and **4**) were available from a previous work [21].

Diacetate **5** *from compound* **3**. Treatment of **3** (60 mg) with Ac_2O-pyridine (1:1, 10 ml) at room temperature for 48 h yielded **5** (56 mg after crystallization from EtOAc - *n*-hexane): mp 207-209°; $[\alpha]_D$: see Table 3. IR (KBr)

ν_{max} cm^{-1}: 3145, 3120, 1505, 875 (furan); 3070 (epoxide); 1730 br (δ-lactone and OAc); 1250, 1230 (OAc). ^1H NMR: see Table 2. ^{13}C NMR: see Table 1. EIMS (70 eV, direct inlet) *m/z* (rel. int.): 446 $[M]^+$(2), 404 (60), 387 (74), 386 (83), 272 (43), 187 (48), 95 (23), 94 (11), 91 (16), 81 (16), 43 (100). (Found: C, 64.41; H, 6.91. $C_{24}H_{30}O_8$ requires: C, 64.56; H, 6.77%.)

Diacetate **6** *from compound* **4**. Ac_2O-pyridine treatment of **4** (80 mg) as above gave the diacetyl derivative **6** (80 mg) as an amorphous solid: mp 57-68°; $[\alpha]_D$: see Table 3. IR (KBr) ν_{max} cm^{-1}: 3140, 1505, 875 (furan); 3060 (epoxide); 1730 br (δ-lactone and OAc); 1250 (OAc). ^1H NMR: see Table 2. ^{13}C NMR: see Table 1. EIMS (70 eV, direct inlet) *m/z* (rel. int.): 446 $[M]^+$ (1), 404 (56), 387 (57), 386 (100), 272 (82), 187 (44), 95 (34), 94 (16), 91 (26), 81 (32), 43 (89). (Found: C, 64.89; H, 6.79. $C_{24}H_{30}O_8$ requires: C, 64.56; H, 6.77%.)

Chlorohydrin **7** *from compound* **3**. Benzoyl chloride (70 mg, 0.5 mmol) was added to a solution of **3** (160 mg, 0.44 mmol) in pyridine (5 ml) and the solution was left at room temperature for 3 h. Work-up in the usual manner gave a residue (170 mg) which was subjected to column chromatography (silica gel, *n*-hexane - EtOAc 2:1 as eluent) yielding 150 mg of the derivative **7**. Mp 196-198° (EtOAc - *n*-hexane); $[\alpha]_D$: see Table 3. IR (KBr) ν_{max} cm^{-1}: 3460, 3350 (OH); 3140, 3120, 1502, 875 (furan); 1705 (δ-lactone). ^1H NMR: see Table 2. ^{13}C NMR: see Table 1. EIMS (70 eV, direct inlet) *m/z* (rel. int.): 400 (14) and 398 (44) $[M]^+$, 382 (3), 380 (13), 362 (30), 245 (27), 111 (87), 105 (60), 97 (89), 95 (100), 91 (97), 79 (82), 77 (72), 41 (30). (Found: C, 60.49; H, 6.63; Cl, 8.69. $C_{20}H_{27}O_6Cl$ requires: C, 60.22; H, 6.82; Cl, 8.88%.)

Benzoate **8** *from compound* **7**. Treatment of **7** (50 mg, 0.125 mmol) with an excess of benzoyl chloride (100 mg, 0.71 mmol) in pyridine solution (10 ml) at room temperature for 24 h. yielded compound **8** (58 mg, after crystallization from EtOAc - *n*-hexane), mp 192-194°; $[\alpha]_D$: see Table 3. IR (KBr) ν_{max} cm^{-1}: 3490, 3420 (OH); 3150, 3125, 1507, 875 (furan); 3090, 3070, 1710, 1600, 1585 (OBz); 1700 (δ-lactone). ^1H NMR: see Table 2. ^{13}C NMR: see Table 1. EIMS (70 eV, direct inlet) *m/z* (rel. int.): $[M]^+$ absent, 382 (11) and 380 (25)[M-BzOH]$^+$, 344 [M-BzOH-HCl]$^+$ (28), 259 (11), 159 (10, 148 (12), 145 (10), 105 (100), 95 (21), 91 (32), 77 (40). (Found: C, 64.53; H, 6.39; Cl, 6.98. $C_{27}H_{31}O_7Cl$ requires: C, 64.47; H, 6.21; Cl, 7.05%.)

X-ray crystallography of the benzoate **8**. Colourless single crystals of compound **8** were obtained by crystallization from EtOAc - *n*-hexane. A crystal (dimensions 0.30x0.18x0.09 mm) was selected for data collection and mounted on a Rigaku AFC-6B automated four-circle diffractometer. The crystal was found to be monoclinic, and unit cell parameters and the orientation matrix were obtained. Data collection was carried out by using 2θ-θ: formula, $C_{27}H_{31}O_7Cl$; M_r = 502.99; space group, $P2_1$; a=10.056(1) Å, b=13.843(1) Å, c=9.057(1) Å, β=94.393(11)°; V=1257.1(3) $Å^3$; Z=2; D_o=1.350 g/cm^3, D_c=1.329 g/cm^3; radiation, CuK_α (1.54178 Å); monochrometer, graphite crystal; linear absorption coefficient, 17.139 cm^{-1}; temperature 20 °C; scan speed, 2.0 °/min; scan range, 1.2 °+0.5° tan θ; 2θ scan limits, 2.0 -130.0°; standard reflections, 3 per 50 reflections; indices, (1,-2,-1), (-2,-2,-1), (0,0,-2); crystal stability, no indication of standard reflection decay during data collection; total reflections scanned, 2012; unique data F_o›3.0σ(F_o), 1732.

The positions of the most non-hydrogen atoms were found by the direct method, and then those of the remaining atoms were found by successive Fourier synthesis. Absorption correction was made by using the data of face indices and the size of the crystal. All hydrogen atoms were placed in idealized positions. Block diagonal

least-squares refinement of positional parameters, anisotropic thermal parameters for non-hydrogen atoms and isotropic parameters for hydrogen atoms, including anomalous scattering factors of chlorine, oxygen and carbon atoms, led to the final convergence with R=0.0678 (final no. of variables, 441) for the (4*R*,5*R*,6*S*,8*R*,9*R*,10*S*,12*S*) absolute configuration, while a similar calculation for the mirror image structure gave R=0.0696. So, the absolute stereochemistry of compound **8** was determined as illustrated in Figure 1 and formula **8**. Lists of atomic parameters of compound **8** are deposited at the Cambridge Crystallographic Data Centre.

Benzoate **9** *from compound* **4**. A pyridine solution of **4** (100 mg in 10 ml) was treated with benzoyl chloride (200 mg) at room temperature for 36 h. Work-up in the usual way yielded the derivative **9** (123 mg, after crystallization from EtOAc - *n*-hexane), mp 198-200°; $[\alpha]_D$: see Table 3. IR (KBr) ν_{max} cm^{-1}: 3500, 3450 (OH); 3140, 1508, 878 (furan); 3080, 3070, 1725, 1600, 1590 (OBz); 1710 (δ-lactone). ^1H NMR: see Table 2. ^{13}C NMR: see Table 1. EIMS (70 eV, direct inlet) *m/z* (rel. int.): $[M]^+$ absent, 382 (5) and 380 (15) $[M\text{-}BzOH]^+$, 344 $[M\text{-}BzOH\text{-}HCl]^+$ (21), 259 (4), 159 (6), 105 (100), 95 (13), 91 (12), 81 (12), 77 (20). (Found: C, 64.38; H, 6.43; Cl, 7.18. $C_{27}H_{31}O_7Cl$ requires: C, 64.47; H, 6.21; Cl, 7.05%.)

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