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STRUCTURE, CHEMISTRY AND STEREOCHEMISTRY OF CLEMENTEINS, SESQUITERPENE LACTONES FROM CENTAUREA CLEMENTEI[1]

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Abstract: The stereochemistry of clementein (1) (the first oxetane-containing sesquiterpene lactone) is determined to be 16(S) on the basis of chemical and spectroscopic data. Clementeins B (2) and C (3), isolated from the same source (Centaurea clementei D.C.) were shown to be closely related guaianolides, with an 11,13-oxetane ring. A number of chemical transformations on the oxetane in 2 and 3 have been accomplished.

Previously, we have described the isolation and structural determination from the aerial parts of Centaurea clementei D.C., of clementein (1)[2]. The first described sesquiterpene lactone with a methyloxetane group, and four flavonoids: negletein, 7,4'-scutellarein dimethyl ether, isokaempferide and hispidulin[3]. In the present paper we confirm the given structure and stereochemistry of clementein (1), determine its configuration at C-16, and describe other two new lactones with the same type of functionalization: clementein B (2) and clementein C (3). In addition, several chemical transformations are carried out, to establish the chemical behaviour of these oxetanelactones.

RESULTS AND DISCUSSION

Clementein (1)

The structure of clementein (1), $C_{21}H_{26}O_7$ (mp 193-195° AcOEt:petrol), has been described[2], although the configuration of C-16 could not be determined solely on the basis of its ^1H NMR. Fortunately, one of the other two guaianolides that are here described, clementein B (2) proved to be an epimer at C-16 of clementein (1). Both compounds being available, the absolute configuration of the aforementioned carbon atom have been determined from the study of their spectroscopic data (IR, MS, ^1H NMR). The proposed configurations were confirmed by the study of the resulting derivatives.

Clementein B (2)

Clementein B (2), a crystalline compound, mp 189-191° (AcOEt:MeOH), $C_{21}H_{26}O_7$, obtained by MS m/z 390 (M^+, 0.3%) and by combustion analysis. Its IR spectrum revealed the presence of hydroxyl groups at 3440 and 3260 cm^{-1}, γ-lactone at 1767 cm^{-1}, α,β-unsaturated ester at 1690 cm^{-1} and double bonds at 1635 cm^{-1}. The presence of ions at m/z 208 $(M-102)^+$, 306 $(M-84)^+$ and 85 $(C_4H_5O_2)^+$ in MS and the signals at δ 6.25 d(br), at 5.96 m, and two doublets at δ 4.34 and 4.29 in the ^1H NMR spectrum (Table I) is characteristic of an α-hydroxymethacrylic ester.

TABLE I

^{1}H NMR spectral data of 2,3,5 (δ, $CDCl_3$-CD_3OD 1:1, 300 MHz), 1a,1b,2a,2b and 3a (δ, $CDCl_3$, 360 MHz)

	1a	1b	2	2a	2b	3	3a	5
H-1	2.96 ddd(br)	2.91 ddd(br)	2.93 ddd(br)	2.99 ddd(br)	2.97 ddd(br)	2.85 m	2.92 ddd(br)	2.92 ddd(br)
H-2	1.50-1.70*	1.50-1.70*	1.72 ddd	1.78 ddd	1.81 m	1.72 ddd	1.70 ddd	1.74 ddd
H-2´	2.30 dt	2.24 dt	2.18 dt	2.40 dt	2.40 dt	1.89 dt	1.82 dt	1.92 dt
H-3	5.53 dddd	5.47 dddd	4.49 dddd	5.54 dddd	5.55 dddd	3.66 dddd	4.69 dddd	3.71 dddd
H-4	--------	------	------	------	------	1.96 ddq	2.01-2.12*	2.07 ddq
H-5	2.67 dd(br)	2.60 dd(br)	2.82 dd(br)	2.73 dd(br)	2.73 dd(br)	2.05 ddd	2.29 ddd	2.06 ddd
H-6	4.45 dd	4.39 dd	4.36 dd	4.45 dd	4.28 dd	4.26 dd	4.40 dd	4.20 dd
H-7	2.75 dd(br)	2.69 dd(br)	2.61 dd	2.67 dd(br)	2.50 dd(br)	2.49 dd	2.64 dd(br)	3.19 dd
H-8	5.27 m	4.92 m	5.47 ddd	5.25 ddd	5.48 ddd	5.27 ddd	5.27 ddd	5.01 ddd
H-9	2.58 dd	2.51 dd	2.66 dd	2.62 dd	2.68 dd	2.85 dd	2.71 dd	2.87 dd
H-9´	2.28 dd	2.06 dd	2.38 dd	2.27 dd	2.36 dd	2.17 dd	2.42 dd	2.24 dd
H-13	2.23 dd	2.27 dd	2.10 dd	2.35 dd	2.35 dd	2.14 dd	2.18 dd	5.88 d
H-13´	2.13 dd	2.21 dd	1.65 dd	1.96 dd	1.81 dd	1.68 dd	1.96 dd	5.64 d
H-14	5.12 s(br)	5.06 s(br)	5.10 s(br)	5.16 s(br)	5.12 s(br)	5.08 s(br)	5.09 s(br)	5.06 s(br)
H-14´	4.95 s(br)	4.87 s(br)	4.94 s(br)	4.98 s(br)	5.04 s(br)	5.07 s(br)	5.07 s(br)	5.03 s(br)
H-15	5.53 s(br)	5.45 s(br)	5.43 s(br)	5.43 s(br)	5.42 s(br)	1.18 d	1.13 d	1.22 d
H-15´	5.34 s(br)	5.28 s(br)	5.35 s(br)	5.32 s(br)	5.35 s(br)	------	------	------
H-16	5.27 m	4.92 m	4.34 m	4.98 m	5.27 ddq	4.39 ddq	4.89 ddq	------
H-17	1.29 d	1.29 d	1.18 d	1.28 d	1.29 d	1.19 d	1.27 d	------
H-3$_1$´	4.87 dd	4.47 dd	4.34 dd	4.85 dd	4.86 dd	4.26 d(br)	4.88 dd	4.34 d(br)
H-3$_2$´	4.83 dd	4.43 dd	4.29 d(br)	4.78 dd	4.79 dd		4.86 dd	
H-4$_1$´	6.46 s(br)	6.29 s(br)	6.25 d(br)	6.36 s(br)	6.36 s(br)	6.22 d(br)	6.34 s(br)	6.37 d(br)
H-4$_2$´	5.98 s(br)	5.90 s(br)	5.96 ddd	5.95 s(br)	5.94 s(br)	5.96 m	5.95 s(br)	5.80 m
C_{11}-OCOC$\underline{H}_3$	2.11 s	2.03 s	------	2.09 s	------	------	2.06 s	------
C_3-OCOC$\underline{H}_3$	2.14 s	------	------	2.13 s	2.13 s	------	2.12 s	------
-OCOC$\underline{H}_3$	2.10 s	2.04 s	------	2.11 s	2.10 s	------	2.08 s	------
-OCOC$\underline{H}_3$	2.05 s	1.99 s	------	2.03 s	2.02 s	------	2.01 s	------

*Overlaped signals

J(Hz): 1a: 1,5=11; 8,9=4; 13,13=15; 3´,4=3; 4´$_1$,4´$_2$=2. 1b: 7,8=9; 13,16=7.5; 3´,4=2. 2: 2´,3=7.2; 8,9=5.6; 16,17=6.1. 2a: 2,2=13.5; 8,9=3.5; 8,9=6. 2b: 2,3=8; 7,8=10; 3´$_1$,3´$_2$=13.5. 3: 2´,3=8.5; 8,9=4.4; 8,9=9.7; 9,9=12.3; 13,13=14.5. 3a: 8,9=4.5; 8,9=11; 9,9=13; 13,16=10. 5: 1,2=6.2; 1,2=9.8; 2,2=9.6; 7,8=9.9; 8,9=4.6; 8,9=8; 9,9=13.2; 4,15=6.6; 7,13=3. 1a,1b: 2,2=12; 9,9=15; 13,16=4; 3´$_1$,3´$_2$=14.5. 1a,2a: 2,3=7. 1a,3: 1,2=7.5. 1b,2: 2,3=9. 1b,2b: 8,9=5. 2,2b: 1,5=9; 2,2=13; 1,2=9.4. 2,3: 7,8=9.7; 13,16=11. 2a,2b: 13,16=3; 3´,4=1.5. 2a,3a: 1,2=8. 3,3a: 1,2=12; 4,15=1,5=6; 2,2=1. 3,5: 3´$_1$,3´$_2$=15.4. 1a,1b,2a: 1,2=8. 1a,2a,2b: 2´,3=13,16=7. 1a,2a,3a: 7,8=9.5. 1b,2,2b: 1,2=7. 1b,2a,2b: 4´$_1$,4´$_2$=1.5. 1b,2a,5: 1,5=9.5. 1b,2b,3a: 2´,3=8. 1b,2b,5: 5,6=6,7=9.5. 2,2a,2b: 9,9=14. 2,2a,3a: 3´$_1$,3´$_2$=15. 2,3,3a: 13,16=2.7. 3,3a,5: 4,5=11; 2,3=5.8. 1a,1b,2,2b: 8,9=5. 2,3,3a,5: 3´,4=4´$_1$,4´$_2$=1.7. 1a,1b,2,2a,2b: 3,15=1.5. 1a,2,2a,3,3a: 5,6=6,7=10. 1b,2,2a,2b,3a: 13,13=14. 1a,1b,2a,2b,3,3a: 16,17=6.5.

Only small differences are observed when ^{1}H NMR (Table I) and MS data of clementein B (2) are compared to those of clementein (1). The conformation of the seven-membered ring must be the same in both compounds, as is deduced from the coupling constants $J_{8,9}$= 4.9 Hz, $J_{8,9'}$= 5.6 Hz, $J_{7,8}$ = 9.7 Hz · These values agreeing with a "twisted boat" preferential conformation [4]. The only differences in the ^{1}H NMR data correspond to the protons found in the area of the molecule close to the methyloxetane (H-7,H-16,H-13 and H-13′).

Spin-decoupling experiments allowed to the location of the H-16 (δ4.34) as a signal overlapped to H-6: Irradiating the methyl at the C-16, the multiplet signal at δ4.34 is simplified to two doublets with coupling constants $J_{16,13}$ = = 11 Hz and $J_{16,13}$= 2.7 Hz; irradiation of the signal at δ4.34, transformed the signal at δ2.10 (dd) and the signal at δ1.65 (dd), to two singlets, and the signal at δ1.18 (d), to a singlet, corresponding to the protons H-13, H-13′ and H--17 respectively.

The paramagnetic shift of the proton H-7 (0.28 ppm) in clementein (1) with respect to 2 could be explained by the shorter distance between the this proton and the methyl group at C-16 in 1; according to this, the configuration that should correspond to C-16 in clementein (1) is "S".

There are several references in the Literature [5, 6, 7] which describe the paramagnetic shift effect of an α 8-acyl group on the Ha of a C-6 closed α-methylene-γ-lactone (usually 0.5 ppm). When the chemical shift of H--16 in clementein B (2) is compared with that of its deacylderivative (2c), a similar effect, although of lesser extent (0.14 ppm), is observed. This could be attributed to the fact that H-16 in (2) has a similar orientation to Ha in the α,β-unsaturated-γ-lactones (Fig. 1). Consequently, the configuration of C-16 in clementein B (2) must be "R". The same relationship is observed when the chemical shift of H-13 and H-13′is compared in compounds (2 and 2c), the paramagnetic shift being 0.25 ppm in this case. In the H-13′proton of clementein (1), this effect is not observed probably by the fact that the methyl group at C-16, impedes the proximity of the oxetane ring and the C-8 acyl group.

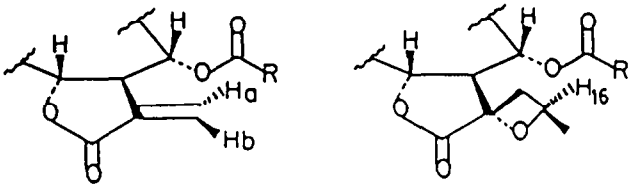


FIGURE 1

These above spectroscopic criteria, which have permitted the stereochemistry of the methyl group at the oxetane ring to be established, are applicable to other lactones having the same functionalization, e.g. the subexpinnatins B and C [8] should have configuration C-16 "R".

The data from the ^{13}C NMR spectrum of clementein B (Table II) clearly show a modification in carbons C-11 and C-13 with regards to the spectrum from cynaropicrin (4) [9], a lactone from which they may formally be considered derivatives; thus, while in 4, C-11 and C-13 appear at δ137.3 (s) and δ 121.6 (t),

revealing their olefinic nature, in clementein B (2) they appear at δ 75.5 (s) and 43.1 (t), and two new signals at 64.3 (d), assigned to C-16, and at 24.1 (q) to C-17.

TABLE II

^{13}C NMR spectral data of 2, 3 and 5 (δ, MeOD-d_4:$CDCl_3$ 1:1, 75 MHz).

	2	3	5
C-12	178.1 s	178.2 s	170.5 s
C-1′	165.5 s	165.5 s	165.5 s
C-4	153.5 s	47.1 d	46.7 d
C-10	143.0 s	142.8 s	142.2 s
C-2′	140.9 s	140.9 s	140.2 s
C-4′	125.0 t	125.0 t	126.0 t
C-14	117.1 t	115.9 t	116.9 t
C-15	112.5 t	18.6 q	18.4 q
C-6	79.0 d	81.4 d	81.9 d
C-11	75.5 s	75.9 d	137.8 s
C-3	73.3 d	78.2 d	78.1 d
C-8	70.3 d	71.9 d	75.2 d
C-16	64.3 d	64.4 d	-------
C-3′	61.1 t	61.1 t	61.1 t
C-5	51.7 d	54.6 d	51.7 d
C-7	51.0 d	51.2 d	50.4 d
C-1	44.9 d	42.9 d	43.4 d
C-13	43.1 t	43.2 t	123.0 t
C-2	39.1 t	41.3 t	40.7 t
C-9	30.0 t	38.5 t	--------
C-17	24.1 q	24.2 q	--------

A comparison of the IR spectra determined in solid state of clementein (1) and clementein B (2) reveals a surprising difference. While in 1 the carbonyl ester absorbs at 1720 cm^{-1}, in 2 it absorbs at 1690 cm^{-1}; when the spectra were determined in $CHCl_3$:MeOH (1:1), the same solvent used for their NMR spectra, this difference disappears and the absorption of both carbonyl esters are observed at 1720 cm^{-1}.

These data suggest that in the solid state clementein B acquires such a conformation which permits intramolecular interaction between the oxygen atom of the oxetane and the carbonyl carbon of the ester, lowering the frequency (ν C=O) [10].

Clementein C (3)

Clementein C (3) is a crystalline compound, mp 211-213° (AcOEt:petrol) $C_{21}H_{26}O_7$, obtained by MS m/z (M^+, 9.3%) and confirmed by combustion analysis.

In the IR spectrum, absortion of hydroxyl groups is observed at 3350 and 3200 cm^{-1}, of δ-lactone at 1775 cm^{-1}, α,β-unsaturated ester at 1720 cm^{-1} and double bonds at 1635 cm^{-1}. As in the above discussed compounds, the presence of a hydroxymethacrylic ester is revealed by ions at m/z 210 $(M-102)^+$, 308 $(M-84)^+$ and 85 $(C_4H_5O_2)^+$ in the MS, and the signals at δ 6.22 d(br), δ 5.96 m, and a wide doublet (2H) at 4.26 in the ^1H NMR spectrum (Table I).

The molecular ion at m/z 392 is two units greater than that of clementein (1) and of clementein B (2). This difference is mantained in the most significant fragments.

In its ^{1}H NMR spectrum, the signal corresponding to the C-4 exocyclic methylene does not appear, though a doublet is observed at δ 1.18 ($J_{4,15}$= 6 Hz) which may be attributed to a methyl group over the aforementioned carbon. The α-orientation of the methyl group is inferred from the coupling constant ($J_{4,5}$= 11 Hz); a β-orientation would give a smaller coupling constant.

The signals at δ 4.39 (m) corresponding to H-16, two doublets at δ 2.14 and 1.68 of the protons H-13 and H-13'respectively, and a doublet (3H) due to H-17 show that clementein C (3) have also a methyloxetane ring at C-11, C-13. These values, also confirmed by double resonance, are practically the same as for 2 (Table I), indicating the stereochemistry of C-16 must be the same in both compounds.

On the other hand, the comparative study of the ^{1}H NMR spectra of related lactones: muricatin (5) [11], clementein (1), clementein B (2), clementein C (3) and cynaropicrin (4) reveals differences which provide information about the preferential conformation of the cycloheptane ring in these products.

In Table III, diferences are observed between the chemical shifts of protons H-9 and H-9', these being much greater in the pair 5, 3 than in the lactones 1, 2 and 4; both groups also present different values of coupling constants for H-8, H-9 and H-9'. Taking into account these J values, the study of the Dreiding models shows a preferential conformation for the cycloheptane of "twisted chair" for clementein C (3) (Fig. 2), instead of a "twisted boat" as we proposed for clementein (1) and clementein B (2). Therefore we may conclude that clementein C (3) is a 4,15-dihydro derivative of clementein B (2) with configuration "R" in C-16.

TABLE III

	$\Delta\delta$(H-9,H-9')	$J_{7,8}$	$J_{8,9}$	$J_{8,9'}$
clementein (1)	0.25	9.5	4.5	5.0
clementein B (2)	0.28	9.7	4.9	5.6
clementein C (3)	0.68	9.7	9.7	4.4
muricatin (5)	0.63	9.9	8.0	4.6

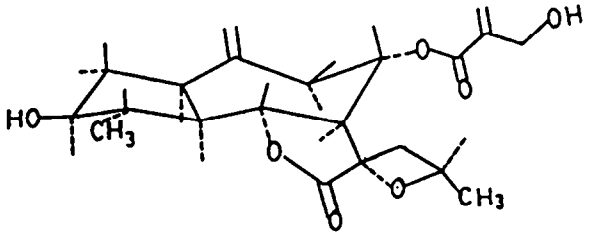


FIGURE 2

Finally,the ^{13}C NMR chemical shifts of C-11, C-13,C-16 and C-17 in clementein C (3) are practically the same as the corresponding of clementein B (2) (Table II), supporting the previous configurational assignation of C-16 ("R") in both lactones.

Chemical transformations

In order to ascertain the reactivity of the oxetane ring and to obtain further information concerning its proposed structure, clementeins were subjected to the chemical transformations shown in the Scheme 1.

Reaction with acetic anhydride-pyridine.- To our surprise, when clementeins were allowed to react with acetic anhydride-pyridine, ring opening of the four membered heterocyclic ring proceeded smoothly to give the corresponding 1,3-diacetylated derivatives. Generally, the oxetane ring undergoes this ring opening under more drastic conditions[12].

Reaction of 1 with Ac_2O/py at room temperature resulted in the almost exclusive formation of 1a along with small amounts of 1b. 1a is a tetraacetylated derivative as revealed by its ^1H NMR spectrum (Table V), with four (3H) singlets at 2.14 (C-3'OAc), 2.11 (C-11 OAc), 2.10 and 2.05 (C-3 OAc and C-16 OAc). The signal of H-16 (δ 4.98) undergoes a paramagnetic shift (δ 3.92 in 1) due to the presence of a geminal acyl group. Compound 1b is a triacetylated derivative, its ^1H NMR spectrum showing three (3H) singlets at δ 2.03 (C-11 OAc), 2.04 and 1.99 (C-3 OAc and C-16 OAc).

Treatment of 2 in the same conditions gave tetraacetate 2a (major compound) and triacetate 2b. The H-16 paramagnetic shift with respect to the same signal in 2 is in this case of 0.64 ppm, this difference being attributed to the different configuration of C-16 in both compounds. It can be deduced, from the conformations that the system (C-11)-(C-13)-(C-16) can adopt, that the paramagnetic shift of H-16 in 1a may be due to an 1,3-diaxial interaction with C-11-OAc. (fig. 3).

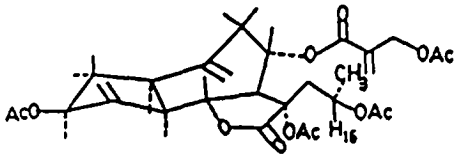


FIGURE 3

The position of the acetyl groups in triacetates 1b and 2b was inferred by the examination of their ^1H NMR spectra (Table I).

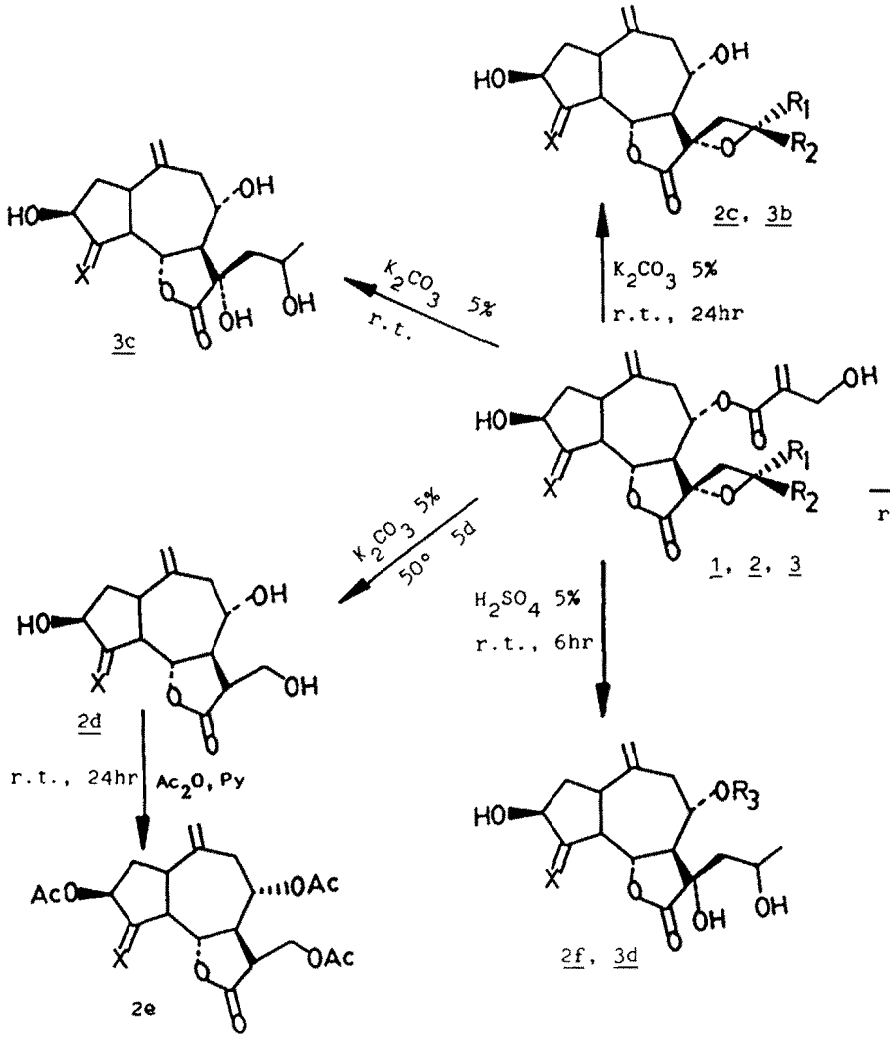
Finally, when clementein C (3) was subjected to the same acetylation conditions described for 1 and 2 afforded almost exclusively tetraacetate 3a.

Reaction with aqueous solution of potassium carbonate.-

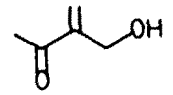
Treatment of the oxetanelactones 2 and 3 with aqueous solution of K_2CO_3 (5%) at room temperature for 24h, gives compounds 2c and 3b. Their spectroscopic study reveals the disappearance of the α-hydroxymethacrylic ester, while the methyloxetane ring remains unaltered. Lactone 2c gives mass spectrum peaks at m/z 306 (M^+), 288 ($M-H_2O)^+$, 278 ($M-CO)^+$, 270 ($M-2xH_2O)^+$ and 266 ($M-C_3H_4)^+$. The latter is also present in 1, 2 and 3 and seems to be a characteristic fragmentation of the methyloxetane ring of this particular class of oxetane lactones.

Saponification of 2 is evident from the absence in the ^1H NMR spectrum (Table IV) of the signals corresponding to the ester moeity. Furthermore, 2b displays its H-13, H-13'and H-16 at an almost identical chemical shifts as that of 2, indicating that the methyloxetane grouping is still present after the basic treatment.

Similar results are obtained in the basic treatment of clementein C (3). Thus, when 3 was treated under the same basic conditions lactone 3b was obtained. Its ^1H NMR spectrum (Table IV) reveals that it is a dihydro deriva-



	X	R_1	R_2	R_3	R_4
1	$=CH_2$	CH_3	H	--	--
1a	$=CH_2$	--	--	--	--
1b	$=CH_2$	--	--	Meacr	Ac
2	$=CH_2$	H	CH_3	--	--
2a	$=CH_2$	--	--	--	--
2b	$=CH_2$	--	--	MeAc	H
2c	$=CH_2$	H	CH_3	--	--
2d	$=CH_2$	--	--	--	--
2e	$=CH_2$	--	--	--	--
2f	$=CH_2$	--	--	--	--
3	$H\beta, CH_3\alpha$	H	CH_3	--	--
3a	$H\beta, CH_3\alpha$	--	--	--	--
3b	$H\beta, CH_3\alpha$	H	CH_3	--	--
3c	$H\beta, CH_3\alpha$	--	--	--	--
3d	$H\beta, CH_3\alpha$	--	--	--	Meacr

Meacr ≡ 

SCHEME 1

tive of 2c, in which the cycloheptane ring must adopt a "twisted chair" preferential configuration, while in 2c it should be "twisted boat", according to the coupling constant values of H-8, H-9, H-9'and H-7 for each compound.

TABLE IV

^{1}H NMR spectral data of 2c,2d,2e,2f,3b,3c and 3d (δ , MeOD-d_4:$CDCl_3$ 1:1. 360 MHz).

	2c	3b	2d	2e	3c	2f	3d
H-1	2.89 ddd(br)	2.80 ddd(br)	2.91ddd(br)	2.96 ddd(br)	2.82 ddd(br)	2.98 ddd(br)	2.85 ddd(br)
H-2	1.69 ddd	1.87 ddd	1.72 ddd	1.79 ddd	1.96 ddd	1.70 ddd	1.90 ddd
H-2'	2.25 dt	1.70 dt	2.29 dt	2.42 dt	1.71 dt	2.26 dt	1.71 dt
H-3	4.48 dddd	3.79 ddd(br)	4.51 dddd	5.52 dddd	3.67 ddd(br)	4.49 dddd	3.67 ddd
H-4	—	2.20-2.00*	—	—	2.20-2.00*	—	2.03 m
H-5	2.77 dd(br)	2.20-2.00*	2.82 dd(br)	2.93 dd(br)	2.20-2.00*	2.78 dd(br)	2.08 ddd
H-6	4.19 dd	4.24 dd	4.11 dd	4.08 dd	4.17 dd	4.25 dd	4.27 dd
H-7	2.23 dd(br)	2.32 dd(br)	2.10 m	2.58 dd(br)	2.20-2.00*	2.24 dd	2.50 dd
H-8	4.15 ddd	4.02 ddd	4.11 m	4.89 ddd	4.05 ddd	4.20 ddd	5.29 ddd
H-9	2.61 dd	2.76 dd	2.70 dd	2.76 dd	2.74 dd	2.62 dd	2.82 dd
H-9'	2.18 dd	2.20-2.00*	2.10 dd	2.17 dd	2.20-2.00*	2.20 dd	2.18 dd
H-11	—	—	2.70 m	2.72 m	—	—	—
H-13	2.21 dd	2.20-2.00*	3.71 m	4.40 dd	2.29 dd	2.10 dd	2.03 dd
H-13'	1.90 dd	1.72 dd	3.71 m	4.27 dd	1.91 dd	1.91 dd	1.68 dd
H-14	5.05 s(br)	5.00 s(br)	5.07 s(br)	5.07 s(br)	5.00 s(br)	5.05 s(br)	5.09 s(br)
H-14'	4.98 s(br)	5.00 s(br)	5.01 s(br)	5.05 s(br)	5.00 s(br)	4.98 s(br)	5.08 s(br)
H-15	5.38 s(br)	1.21 d	5.35 s(br)	5.49 s(br)	1.19 d	5.39 s(br)	1.19 d
H-15'	5.31 s(br)	—	5.30 s(br)	5.29 s(br)	—	5.32 s(br)	—
H-16	4.48 m	4.54 ddq	—	—	4.17 ddq	4.20 m	3.91 ddq
H-17	1.21 d	1.22 d	—	—	1.21 d	1.23 d	1.20 d
H-3'$_1$	—	—	—	—	—	—	4.35 d(br)
H-3'$_2$	—	—	—	—	—	—	4.30 d(br)
H-4'$_1$	—	—	—	—	—	—	6.23 d(br)
H-4'$_2$	—	—	—	—	—	—	5.97 d(br)
-OCOCH_3	—	—	—	2.08 s	—	—	—
2x-OCOCH_3	—	—	—	2.07 s	—	—	—

*Overlaped signals

J(Hz): 2c: 1,2=7; 1,5=10; 2,3=8.5; 3,15=15; 8,9=6; 9,9=14 13,16=2.5; 16,17=6. 2e: 11,13=3; 11,13=3; 13,13=11. 2c,2e: 2,3=7. 2d,2e: 2,3=7; 3,15=3. 2e,2f: 7,8=10. 2f,3c: 1,2=7.5; 2,3=8. 2f,3d: 16,17=6. 3b,3c: 16,17=6.5. 3b,3d: 1,2=11.5; 4,15=6.5. 2c,2d,2e: 1,2=9. 2c,2d,3b: 7,8=9.5; 8,9=4.5. 2d,2e.2f: 1,5=9. 2d,3b,3d: 2,3=7.5. 2f,3b,3c: 5,6=6,7=10. 3b,3c.3d: 1,5=2,2==6; 2,2=8,9=11. 2c,2d,2e,2f: 2,2=13. 2c,2d,2e,3d: 5,6=6,7=9.5. 2c,2f,3c,3d: 13,13=14. 2d,2e,3b,3c: 9,9=13. 2d,2e,3b,3d: 1,2=8. 2e,2f,3c,3d: 8,9=5.

Prolonged treatment of 3 under the same basic conditions resulted the saponification of the ester and opening of the oxetane ring, to give the 1,3-diol 3c, a crystalline solid mp 213-215° (AcOEt:MeOH). In the ^{1}H NMR spectrum the H-16 appears at δ 4.17 characteristic of proton geminal to an hydroxyl group, while H-13 and H-13'resonate at closer δ values (2.29 and 1.91 respectively). The rest of the spectrum of 3c is similar in appearance and chemical shifts to that of 3b, from which can be obtained under the same basic conditions described above.

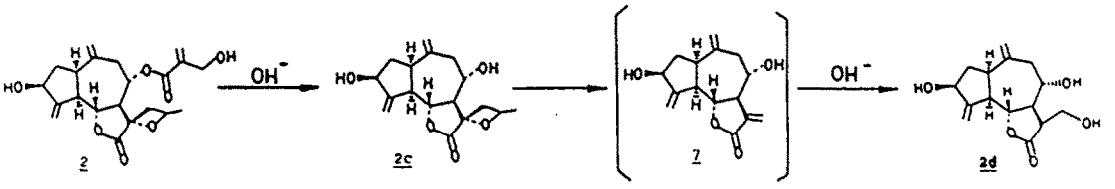
Treatment of clementein B (2) with aqueous solution of K_2CO_3 (5%) for 3 days at 50° gives the triol 2d. The mass spectrum shows peaks at m/z 280 (M^+), 262 ($M-H_2O)^+$, 244 ($M-2xH_2O)^+$, 226 ($M-3xH_2O)^+$ suggesting the presence of, at least, three hydroxyl groups. Degradation of the oxetane function is evidenced from the following facts: the signals corresponding to the secondary methyl at C-16 are absent and the H-13 and H-13'appear completely superimposed at δ 3.71.

Acetylation of 2d with acetic anhydride-pyridine yields 2e. Its ^{1}H NMR spectrum shows the incorporation of three acetyl groups, as well as the

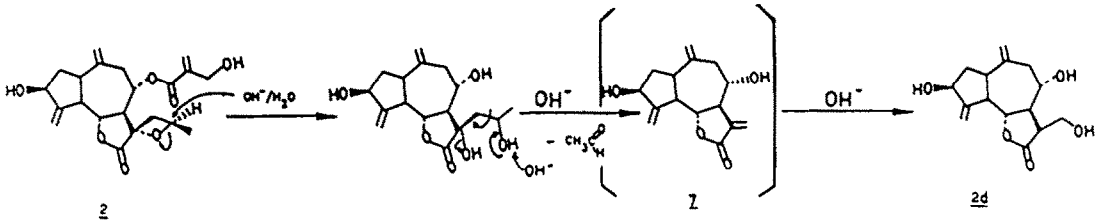
characteristic shifts of the protons at C-3 (δ 5.52), C-8 (δ 4.89) and C-13 (δ 4.27).

On the basis of the above MS and ^{1}H NMR data the structure of 2e is determined as 13-hydroxi-11,13-dihydrodeacylcynaropicrin.

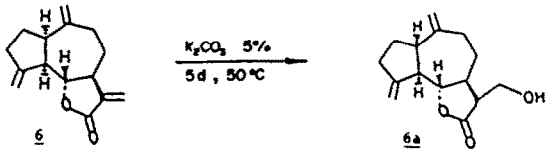
The formation of 2e could be explained through the previous fragmentation of the oxetane ring followed by a conjugated addition of the hydroxyl group (Schemes 2 and 3). We have proved that this reaction takes place when α, β-unsaturated-δ-lactones, such as dehydrocostuslactone (6) are submitted to treatment with an aqueous solution of K_2CO_3 (5%), at 50° for 5 days, thereby obtaining compound 6a (Scheme 4). However, we have been unable to detect product 7 in the aforementioned reaction conditions.



SCHEME 2



SCHEME 3



SCHEME 4

Reaction with sulfuric acid.- The reaction of the oxetanolactones 2 and 3 with H_2SO_4 (5%) at room temperature during 6h affords 2f and 3d. Lactone 3d is obtained as a crystalline product mp 205-207° (MeOH). Whose spectroscopic data show that the oxetane undergoes ring opening while the rest of the molecule remains unchanged. Thus, the ^{1}H NMR spectrum (Table IV) shows the signals correspondind to an 1,3-diol in positions C-11 and C-16 (H-16 at δ 3.91, H-13 and H-13'at δ 2.03 and 1.68 respectively). While the rest of the spectrum is similar to that of clementein C (3) (Table I). H-6 does not undergo any shift indicating the α orientation of the hydroxyl group at C-11.

The corresponding product of the acid treatment of 2 is a crystalline solid 2f mp 204-206° (MeOH). Its mass spectrum shows peaks at m/z 324 (M^+), 306 $(M-H_2O)^+$, 288 $(M-2xH_2O)^+$, 270 $(M-3xH_2O)^+$. As in 3d, the ^{1}H NMR spectrum (Table

IV) shows that the oxetane ring has been opened (the proton H-16 resonates at δ 4.20, and H-13 and H-13' appear at δ 2.10 and 1.91 respectively) whereas the absence of the characteristic signals of the hydroxymethacrylic ester indicates that the ester is hydrolyzed.

EXPERIMENTAL

Melting points were measured on a Kofler hot stage apparatus and are uncorrected. Infrared spectra were recorded on either a Pye-Unicam SP 3300 or a Digilab FTS-IMX spectrometers in KBr, nujol or solution using $CHCl_3$. ^1H NMR and ^{13}C NMR spectra were made on either a Bruker WB-360 or a Varfan FT-300 spectrometers using Me_3Si as an internal standard. Mass spectra were recorded on a VG-Micromass ZAB-2F spectrometer. Chromatographic separations were made on silica gel (Merck) and microanalyses were performed by the Departamento de Técnicas Instrumentales del Instituto de Química Orgánica General del C.S.I.C.

For general experimental details see reference [3]. The more polar fractions were chromatographed on a column of silica gel and after repeated preparatives TLC (silica gel) afforded: 1 [2], 2 (200 mg) and 3 (100 mg) ($CHCl_3$:n-BuOH, 75:15).

Tetraacetate 1a. Acetylation of 1 with 12 ml Ac_2O:pyridine (3:1) for 24hr, room temp., after purification by prep TLC (Be:AcOEt, 3:1) afforded 1a as a colourless gum. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1790,1740,1720,1640. MS m/z (rel. int.): 516 $[M-AcOH]^+$ (0.5), 372 $[M-AcOH-144]^+$ (1), 252 $[M-3xAcOH-144]^+$ (29), 144 $[C_6H_8O_4]^+$ (11), 85 $[C_4H_5O_2]^+$ (base peak). ^1H NMR: see Table I.

Triacetate 1b. Colourless gum. IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 3450,1780,1740,1720,1640. MS m/z (rel. int.): 354 $[M-3xAcOH]^+$ (1), 252 $[M-3xAcOH-102]^+$ (0.8), 85 $[C_4H_5O_2]^+$ (50). ^1H NMR: see Table I.

Clementein B (2). Mp 189-191°. IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 3440,3260,1765,1690,1635. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3436,1764,1685,1632,1599,1570. MS m/z (rel. int.): 390 $[M]^+$(0.3) ($C_{21}H_{26}O_7$), 362 $[M-CO]^+$ (0.3), 350 $[M-C_3H_4]^+$ (5), 306 $[M-84]^+$ (1), 288 $[M-102]^+$ (3), 270 $[M-102-H_2O]^+$ (2), 85 $[C_4H_5O_2]^+$ (39). ^1H NMR: see Table I. ^{13}C NMR: see Table II. (Found: C, 61.63; H, 7.10; Calc. for $C_{21}H_{26}O_7.H_2O$: C, 61.81; H, 6.91%).

Tetraacetate 2a. Acetylation of 2 with 12 ml Ac_2O:pyridine (3:1) for 24hr, room temp., after purification by prep. TLC (Be:AcOEt, 3:1) afforded 2a as a colourless gum. IR $\nu_{max}^{CHCl_3}$cm^{-1}: 1790,1740,1650,1610. MS m/z (rel. int.): 516 $[M-AcOH]^+$ (0.7), 372 $[M-AcOH-144]^+$ (1.5), 252 $[M-3xAcOH-144]^+$ (27). ^1H NMR: see Table I.

Triacetate 2b. Colourless gum. IR $\nu_{max}^{CHCl_3}$cm^{-1}: 3440,1777,1740, 1720,1600. MS m/z (rel. int.): 516 $[M-H_2O]^+$ (1), 354 $[M-3xAcOH]^+$ (6), 210 $[M-3xAcOH-144]^+$ (5), 144 $[C_6H_8O_4]^+$ (15). ^1H NMR: see Table I.

Deacylclementein B (2c). Clementein B (2) (12 mg) was mixed with aq. 5% K_2CO_3 (4 ml) and stirred for 24hr. After acidification with dil. HCl the mixture was extracted with AcOEt. The organic soln was washed with H_2O, dried and evaporated to dryness. After purification by repeated prep TLC in $CHCl_3$:n-BuOH (75:25), afforded 2c as a colourless gum (8 mg). MS m/z (rel. int.): 306 $[M]^+$ (0.8), 288 $[M-H_2O]^+$ (1.5), 278 $[M-CO]^+$ (3), 270 $[M-2xH_2O]^+$ (1), 266 $[M-C_3H_4]^+$ (12). ^1H NMR: see Table IV.

13-Hydroxydihydrodeacylcynaropicrin (2d). Clementein B (2) (15 mg) was mixed with aq. 5% K_2CO_3 (4.5 ml) and stirred for 5 days at 50°. After neutralization with dil. HCl the mixture was extracted with AcOEt. The organic soln was washed with H_2O, dried and evaporated to dryness. After purification by prep TLC twice in Be:AcOEt (1:1) afforded 2d as a colourless gum (7 mg). MS m/z (rel. int.): 280 $[M]^+$ (2) ($C_{15}H_{20}O_5$), 262 $[M-H_2O]^+$ (1.5), 244 $[M-2xH_2O]^+$ (8), 266 $[M-3xH_2O]^+$ (9). ^1H NMR: see Table IV.

Triacetate 2e. Acetylation of 2d (7 mg) as described for 1 and 2, after purification by prep TLC (twice in Be:AcOEt, 3:1) afforded 2e as a colourless gum (3 mg). IR $\nu_{max}^{CHCl_3}$cm^{-1}: 1765,1740,1650,1640. MS m/z (rel. int.): 406 $[M]^+$ (0.6) 346 $[M-AcOH]^+$ (1.5), 286 $[M-2xAcOH]^+$ (6), 226 $[M-3xAcOH]^+$ (6.5). ^1H NMR: see Table IV.

11α-Hydroxy-13-(1'-hydroxyethyl)-dihydrodeacylcynaropicrin (2f). 2f was prepared by mixing 10 mg 2 with aq. 5% H_2SO_4 (3ml) and stirring for 6hr. After neutralization with aq. 5% K_2CO_3 the mixture was extracted with AcOEt. The organic soln was dried and evaporated to dryness. After purification by TLC (twice in $CHCl_3$:n-BuOH, 75:25) afforded 2f as a crystalline compound mp 204-206° (7 mg). IR $\nu_{max}^{CHCl_3}$cm^{-1}: 3445,3270,1766,1653. MS m/z (rel. int.): 324 $[M]^+$ (1) ($C_{17}H_{24}O_6$), 306 $[M-H_2O]^+$ (0.8), 288 $[M-2xH_2O]^+$ (6), 270 $[M-3xH_2O]^+$ (12). ^1H NMR: see Table IV.

Clementein C (3). Mp 211-213°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3350,3200,1775,1720,1635. MS m/z (rel. int.): 392 $[M]^+$ (9.3) ($C_{21}H_{28}O_7$), 364 $[M-CO]^+$ (0.6), 352 $[M-C_3H_4]^+$ (48.4 308 $[M-84]^+$ (16), 290 $[M-102]^+$ (17.7), 272 $[M-102-H_2O]^+$ (10.3), 85 $[C_4H_5O_2]^+$ (base peak). ^{1}H NMR: see Table I. ^{13}C NMR: see Table II. (Found: C, 61.23; H, 7.50; Calc. for $C_{21}H_{28}O_7.H_2O$: C, 61.45; H, 7.37%).

Tetraacetate 3a. Acetylation of 3 (12 mg) as described for 1 and 2, after purification by prep TLC (Be:AcOEt, 3:1) afforded 3a as a colourless gum (9 mg). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1790,1740,1720,1610. MS m/z (rel. int.): 518 $[M-AcOH]^+$ (1), 374 $[M-AcOH-144]^+$ (2.8), 254 $[M-3xAcOH-144]^+$ (20). ^{1}H NMR: see Table I.

Deacylclementein C (3b). Clementein C (12mg) was mixed with aq. 5% K_2CO_3 (4ml) and stirred for 24hr. After acidification with dil. HCl the mixture was extracted with AcOEt. The organic soln was washed with H_2O, dried and evaporated to dryness. After purification by repeated prep TLC in $CHCl_3$:n-BuOH (75:25) afforded 3c as a colourless gum (2 mg). MS m/z (rel. int.): 308 $[M]^+$ (0.6), 300 $[M-H_2O]^+$ (1.3), 280 $[M-CO]^+$ (3), 272 $[M-2xH_2O]^+$ (1), 268 $[M-C_3H_4]^+$ (11). ^{1}H NMR see Table IV.

11α-Hydroxy-13-(1'-nydroxyethyl)-dihydrodeacylmuricatin (3c). Isolated as an oil in the saponification of 3 (6 mg). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3350,1775,1640. MS m/z (rel. int.): 326 $[M]^+$ (1) ($C_{17}H_{26}O_6$), 308 $[M-H_2O]^+$ (1.5), 290 $[M-2xH_2O]^+$ (4), 272 $[M-3xH_2O]^+$ (9). ^{1}H NMR: see Table IV.

11α-Hydroxy-13-(1'-hydroxyethyl)-dihydromuricatin (3d). Prepared as reported for 2f. After neutralization with aq. 5% K_2CO_3 the mixture was extracted with AcOEt. The organic soln was dried and evaporated to dryness. After purification by TLC (twice in $CHCl_3$:n-BuOH, 75:25) afforded 3d as a crystalline solid mp 205-207°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3350,3210,1770,1720,1635. MS m/z (rel. int.): 410 $[M]^+$ (1), 392 $[M-H_2O]^+$ (0.5), 382 $[M-CO]^+$ (2), 374 $[M-2xH_2O]^+$ (10), 338 $[M-4xH_2O]^+$ (4), 308 $[M-102]^+$ (11), 254 $[M-3xH_2O-102]^+$ (7). ^{1}H NMR: see Table IV.

13-Hydroxycostuslactone (6a). Dehidrocostuslactone (6) (35 mg) was mixed with aq. 5% K_2CO_3 (5 ml) and stirred for 5 days at 50°. After neutralization with dil. HCl the mixture was extracted with AcOEt. The organic soln was washed with H_2O, dried and evaporated to dryness. After purification by chromatography on silica gel and the fractions (25 ml) eluted with benceneyielded 6a a colourless gum. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450,1760,1660,1640. MS m/z (rel. int.): 248 $[M]^+$ (2) ($C_{15}H_{20}O_3$), 230 $[M-H_2O]^+$ (5), 202 $[M-H_2O-28]^+$ (3), 159 $[M-C_3H_5O_3]^+$ (23). ^{1}H NMR (200 MHz, $CDCl_3$) : 5.11 (d(br),H-15), 4.99 (d(br), H-15'), 4.82 (s(br), H-14), 4.72 (s(br), H-14'), 3.94 (dd, H-6), 3.92 (d(br), H-13), 3.68 (d(br), H-13'), 2.81 (m, H-1), 2.77 (dd(br), H-5), 2.52-2.29 (4H, m, H-3, H-3', H-9, H-9'), 2.27 (ddd, H-11), 2.14 -1.90 (4H, m, H-2, H-2', H-8, H-8'), 1.33 (m, H-7); J (Hz): 5,6=6,7=9; 11,13=11,13'=12; 11,7=3; 3,15=2; 5,15'=2.

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