

STRUCTURE OF CHLOROREPDIOLIDE, A NEW SESQUITERPENE LACTONE FROM *CENTAUREA REPENS*

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ABSTRACT.—A new chlorinated sesquiterpene lactone, chlororepdiolide, has been isolated from *Centaurea repens*. Its structure and absolute configuration were determined by spectroscopic methods and single crystal X-ray analysis.

The noxious weed, *Centaurea repens* L. (Russian knapweed, Compositae) is a fast-growing perennial herb that is rapidly becoming a pest of major proportion in many parts of the United States. Not only is it suspected of being allelopathic (1), it is also very toxic to horses (2). The disease has been dubbed "chewing disease" or equine nigropallidal encephalomalacia (ENE), which is characterized by necrosis of specific brain tissue in the substantia nigra with subsequent loss of muscular control.

Previous work on *C. repens* has resulted in the isolation and characterization of several sesquiterpene lactones (1, 3-8) as well as a number of polyacetylenes (9). The present work describes the isolation and structural determination of a new chlorinated sesquiterpene lactone chlororepdiolide (**1**).

RESULTS AND DISCUSSION

Chlororepdiolide (**1**) was obtained as colorless needles, mp 207-208°, [α]²⁵_D +80.2 (*c* 0.109, CHCl₃), C₁₉H₂₃O₇Cl (*ci* with isobutane, [M+1]⁺399). Ir spectral analysis gave carbonyl stretching frequencies at 1720 and 1745 cm⁻¹ characteristic of an ester side chain and an α -methylene- γ -butyrolactone group, respectively.

The ¹³C-nmr spectrum of **1** exhibited resonances very similar to other guaianolide sesquiterpene lactones previously isolated from Russian knapweed and yellow starthistle (8, 10). For example, the methacrylate side chain was readily identified by resonances at δ 166.5 (C-16), 136.7 (C-17), 126.3 (C-18), and 18.3 (C-19), which are characteristic of such side chains (8, 10). In addition, the carbocyclic five-membered ring was suspected of having hydroxyl groups on carbons 2, 3, and 4 since resonances were found at δ 83.3 (C-2), 84.1 (C-3), and 83.9 (C-4) (8). Because the molecule contains chlorine and C-4 is hydroxylated, it was likely that **1** possessed a 4,15-epichlorohydrin. This was substantiated by a resonance at δ 50.9 (C-15), which is characteristic of a 4,15-epichlorohydrin in the guaianolide series of sesquiterpene lactones (8). Further confirmation of the structure was found by assignment of all ¹³C resonances. Assignments were aided by using a multiplicity separation pulse sequence (11).

Mass spectral analyses of **1** revealed the loss of three principal fragments, i.e., HCl (*m/z* 36), H₂O (*m/z* 18), and C₄H₆O₂ (*m/z* 86) from the MH⁺ ion. Table 1 gives the resultant fragments along with the relative abundance of each ion and the molecular composition as established by high resolution studies. The ion at *m/z* 363 could arise either

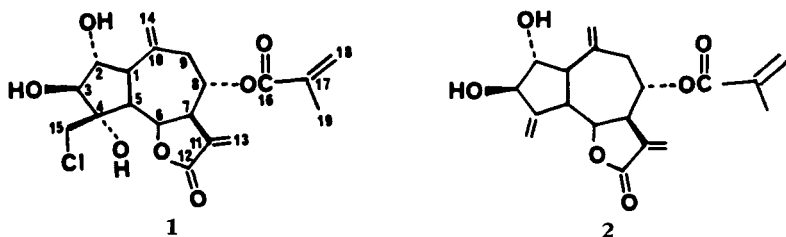


TABLE 1. Chemical Ionization Mass Spectral Analysis of **1**

Fragment <i>m/z</i>	Abundance (%)	Composition	Origin
399 ^a	40.4	C ₁₉ H ₂₄ O ₇ Cl	MH ⁺
381 ^a	5.1	C ₁₉ H ₂₂ O ₆ Cl	MH ⁺ -H ₂ O
363	33.1	C ₁₉ H ₂₃ O ₇	MH ⁺ -HCl
345	18.9	C ₁₉ H ₂₁ O ₆	MH ⁺ -HCl-H ₂ O
313 ^a	8.6	C ₁₅ H ₁₈ O ₅ Cl	MH ⁺ -C ₄ H ₆ O ₂
295 ^a	15.5	C ₁₅ H ₁₆ O ₄ Cl	MH ⁺ -C ₄ H ₆ O ₂ -H ₂ O
277	18.8	C ₁₈ H ₁₈ O ₅	MH ⁺ -C ₄ H ₆ O ₂ -HCl
259	38.8	C ₁₅ H ₁₆ O ₄	MH ⁺ -C ₄ H ₆ O ₂ -HCl-H ₂ O

^aMost abundant ion of chlorine isotopes.

by loss of HCl or two H₂O molecules. Because no chlorine was found in the ion, it was concluded that the fragment arose via loss of HCl. The loss of C₄H₆O₂ (methacrylate) without loss of chlorine substantiates the ¹³C-nmr data that the Cl atom is not located on the side chain.

The ¹H nmr assignments (Table 2) were made by extensive decoupling experiments in both pyridine-*d*₅ and CDCl₃. The *cis*-orientation of the guaianolide ring system was readily confirmed by the nOe found upon irradiation of H₆ (δ 4.66, CDCl₃), which increased the intensity of H₉ by about 15%. This is in accord with the results found with

TABLE 2. ¹H-nmr Spectrum of **1** in Pyridine-*d*₅ and CDCl₃

Atom	CDCl ₃	Pyridine- <i>d</i> ₅	<i>J</i> values
H-1	3.29 dd	4.10 dd	1,2 = 6.5
H-2	4.04 dd	4.68 dd	2,3 = 1
H-3	4.05 d	4.82 dd	1,5 = 9.5
H-5	2.59 dd	2.96 dd	5,6 = 9
H-6	4.66 dd	5.27 dd	6,7 = 11
H-7	3.14 ddt	3.29 ddt	7,8 = 9.5
H-8	5.08 ddd	5.39 ddd	7,13A = 3
H9A	2.66 ddt	2.97 ddt	7,13B = 3
H9B	2.42 ddt	2.66 ddt	8,9A = 5
H13A	5.64 dd	5.64 dd	8,9B = 3
H13B	6.21 dd	6.26 dd	9A,9B = 15
H-14A	4.99 dt	5.02 dt	9,14A = 1
H-14B	5.24 dt	5.35 dt	9A,14B = 1
H-15A	3.88 d	4.43 d	9B,14A = 1
H-15B	4.16 d	4.83 d	9B,14B = 1
H-18A	5.69 dq	5.68 dq	13A,13B = 3
H-18B	6.20 dq	6.30 dq	14A,14B = 2
H-19 (3H)	2.02 t	2.01 t	15A,15B = 11
			18A,18B < 1
			18A,19 = 1.5
			18B,19 = 1.5

repdiolide (**2**) and experiments conducted by Herz (12), namely, *cis*-guaianolides are "buckled" such that H₆ and H₉ are in close proximity. H₂ occurs at δ 4.04 (CDCl₃) and is coupled to both H₁ and H₃, with *J*_s = 6.5 and 1 Hz, respectively. Attempts to assign the relative configuration to these vicinal hydroxyl groups were unsuccessful due to the inaccuracies of applying the Karplus equation to five-membered rings (8). Repdiolide

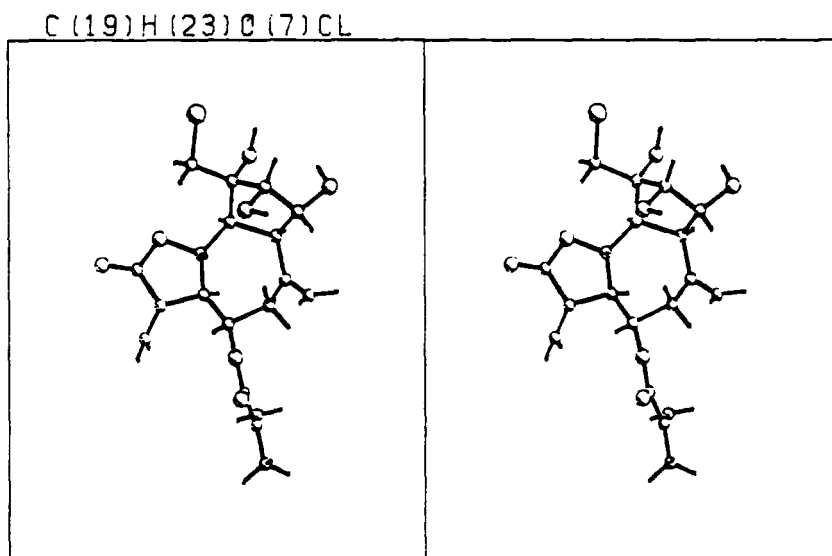


FIGURE 1. Stereoscopic view of chlororepdiolide (1)

(2) (8) had $J_{2,3} = 8.1$ Hz, which would seem to indicate that **1** and **2** have different configurations around carbons 2 and/or 3. Furthermore, the relative configuration of the 4,15-epichlorohydrin could not be assigned with certainty using spectral data, although one could argue on biogenetic precedence that the chloromethylene group was β and the C-4 hydroxyl group was α inasmuch as all of the sesquiterpene lactones isolated from Russian knapweed containing a C-4 oxygen function were found to be α -oriented. Thus, to alleviate any ambiguities about the relative configuration around C-2, C-3, and C-4, the absolute configuration of **1** was determined by single crystal X-ray crystallography.

Crystallization from Me_2CO /skellysolve-F gave crystals suitable for X-ray analysis. The molecular structure and absolute configuration of **1** (1*R*, 2*R*, 3*S*, 4*S*, 5*S*, 6*S*, 7*R*, 8*S*) is shown in Figure 1 with the numbering system shown in Figure 2, and the atomic coordinates are shown in Table 3. It is readily apparent that **1** has an α -OH at C-2 and a β -OH at C-3, i.e., they are *trans*-oriented, as in in repdiolide (**2**). Likewise, the C-4 hydroxyl group is α , and the chloromethylene group is β , again consistent with all previous guaianolides isolated from Russian knapweed containing a C-4 oxygen function. The bond distance between H_9 and H_6 is 2.3 Å thus confirming the relative close

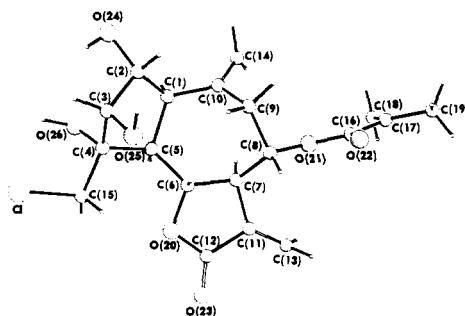


FIGURE 2. Perspective view of (1) with crystallographic numbering scheme. The open bonds represent double bonds

TABLE 3. Atomic Coordinates ($\times 10^4$) and Temperature Factors ($A_2 \times 10_3$)

Atom	x	y	z	U
C(1)	3095(8)	10966(4)	7085(2)	50(2)*
C(2)	3740(9)	11277(4)	7680(2)	57(2)*
C(3)	3370(8)	10279(4)	8039(2)	59(2)*
C(4)	1655(8)	9686(4)	7739(2)	51(2)*
C(5)	2191(8)	9773(4)	7125(2)	46(2)*
C(6)	3535(8)	8861(4)	6896(2)	48(2)*
C(7)	4302(7)	9052(4)	6312(2)	45(2)*
C(8)	6428(7)	9513(4)	6313(2)	49(2)*
C(9)	6584(8)	10483(4)	6699(2)	53(2)*
C(10)	4732(8)	11186(4)	6677(2)	51(2)*
C(11)	4067(8)	7945(4)	6045(2)	58(2)*
C(12)	2695(10)	7309(4)	6398(2)	65(2)*
C(13)	4703(13)	7563(6)	5587(3)	94(3)*
C(14)	4590(11)	12018(5)	6323(2)	79(2)*
C(15)	1227(9)	8556(4)	7946(2)	59(2)*
C(16)	8736(9)	9931(5)	5604(2)	69(2)*
C(17)	9077(10)	10419(7)	5058(3)	82(3)*
C(18)	7563(17)	10726(7)	4772(3)	150(5)*
C(19)	11153(12)	10483(8)	4906(3)	135(4)*
O(20)	2297(6)	7861(2)	6860(1)	58(1)*
O(21)	6806(5)	9856(3)	5754(1)	54(1)*
O(22)	10037(7)	9613(6)	5894(2)	117(3)*
O(23)	2039(8)	6398(3)	6318(2)	88(2)*
O(24)	2722(7)	12224(3)	7879(1)	74(1)*
O(25)	5055(5)	9565(3)	8042(2)	67(1)*
O(26)	-99(5)	10358(3)	7789(2)	65(1)*
CL	546(3)	8559(1)	8661(1)	81(1)*

*Equivalent isotropic U defined as one third of the trace of the orthogonalized Uij tensor.

proximity of these two atoms as determined by ^1H -nmr studies. As can be seen from Figure 2, the seven-membered ring is in a twist-chair conformation (13), while the five-membered carbocyclic ring is essentially planar.

EXPERIMENTAL

PLANT MATERIAL.—*C. repens* was collected during the summer of 1984 near Discovery Bay, California. A voucher sample is deposited with K.L. Stevens, USDA, Berkeley, California.

EXTRACTION.—Dried aerial parts of *C. repens* (65 lbs) were extracted sequentially with skellysolve-F, Me_2CO , and MeOH. The solvent from the Me_2CO extract was evaporated and the mixture extracted with EtOAc. The EtOAc extract was dried, filtered, and evaporated. One liter of EtOH and 1 liter of 4% aqueous lead acetate were added to precipitate the chlorophyll. After cooling about 0.5 h, the mixture was filtered, and the EtOH was removed under vacuum. The resulting aqueous mixture was extracted with CHCl_3 and dried with anhydrous MgSO_4 . Repeated chromatography on silica gel ($\text{CH}_2\text{Cl}_2/\text{MeOH}$), reverse phase C_{18} on silica gel ($\text{MeOH}/\text{H}_2\text{O}$), and silica gel ($\text{MeOH}/\text{CHCl}_3$) gave 243 mg of chlororepdiolide

$$(1), [\alpha]^{21}_D = \frac{589 \quad 578 \quad 546 \quad 436 \quad 365}{+80.2 + 84.8 + 95.9 + 165.0 + 257.1}$$

SPECTROSCOPIC DATA.— ^1H -nmr spectra were measured on a Nicolet superconducting 200 MHz spectrometer. ^{13}C -nmr spectra were measured on a JEOL PFT-100 operating at 22.05 MHz using a multiplicity pulse sequence (11) with a 90° flip angle at a 2 sec repetition rate. TMS was used as an internal standard. ^{13}C -nmr (pyridine- d_5) δ 58.4 (C-1); 83.3 (C-2); 84.1 (C-3); 83.9 (C-4); 60.1 (C-5); 77.6 (C-6); 47.2 (C-7); 74.4 (C-8); 36.7 (C-9); 142.8 (C-10); 138.8 (C-11); 169.0 (C-12); 120.9 (C-13); 118.0 (C-14); 50.9 (C-15); 166.5 (C-16); 136.7 (C-17); 126.3 (C-18); 18.3 (C-19).

CRYSTAL DATA.— $\text{C}_{19}\text{H}_{23}\text{O}_7\text{Cl}$, $M=398.9$, mp 207-208°, orthorhombic space group, $P2_12_12_1$,

$a=6.698(2)$, $b=12.228(5)$, $c=24.320(9)$ Å, $V=1991.9$ Å³, $Z=4$, $d_c=1.33$ g cm⁻³, $\mu(\text{Cu-K}\alpha)=20.3$ cm⁻¹ ($\lambda=1.5418$ Å).

X-RAY DATA¹.—Intensities were collected with a Nicolet R3 automatic diffractometer at room temperature using graphite monochromatized CuK α radiation and the θ - 2θ scan technique. A total of 1529 independent reflections were measured within the range of $3^\circ \leq 2\theta \leq 110^\circ$ while 1379 independent reflections were considered as observed with $|F_0| > 3\sigma |F_0|$. Intensity data were corrected for background and Lorentz-polarization but not for absorption or extinction. The crystal structure was solved by direct-methods using the SHELXTL (14) program and least-squares refined (244 parameters) to a final $R=0.059$ and $R(\text{weighted})=0.068$. The weighted scheme applied was $w=[\sigma^2(F_0)+0.001 F_0^2]^{-1}$. The absolute configuration was determined by comparing R_w -values for the two enantiomeric structures, yielding a ratio of R_w values of 1.153. The enantiomer with the lower R_w is considered correct at the 5% significance level according to Hamilton's (15) statistical test.

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¹Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW, UK.