Sesquiterpene lactones from *Centaurea linifolia* Vahl¹

A. G. GONZÁLEZ, J. BERMEJO, J. M. AMARO, G. M. MASSANET,
A. GALINDO, AND I. CABRERA

Department of Organic Chemistry and Biochemistry, University of La Laguna,
Instituto de Productos Naturales Organicos del CSIC, Tenerife, Spain

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Three new sesquiterpene lactones, linichlorins A, B, and C were isolated from the aerial parts of *Centaurea linifolia* Vahl and their structures were determined by physical and chemical methods. The sesquiterpene lactones chlorohyssopifolins A, B, C, D, E, aguerin B, and vahlenin were also isolated from the same plant.

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Trois nouvelles lactones sesquiterpéniques ont été isolées à partir de *Centaurea linifolia* Vahl; ses structures ont été déterminées par des méthodes physiques et chimiques. On a isolé aussi les lactones sesquiterpéniques, chlorohyssopifolins A, B, C, D, E, aguerin B et vahlenin.

As part of our studies of the chemical constituents of the genus *Centaurea* (Compositae) we examined *Centaurea linifolia* Vahl, endemic to the Iberian peninsula. The plant was gathered in July at Los Monegros (Zaragoza) and extracted as usual (see Experimental). By column chromatography 10 sesquiterpene lactones were separated and 7 were identified, by comparison with authentic samples, as centaurepensin (chlorohyssopifolin A) (**1b**), acroptilin (chlorohyssopifolin C) (**2b**), chlorohyssopifolins B (**1a**), D (**1c**), and E (**1d**), vahlenin (**4e**), and aguerin B (**3e**), isolated from other species of *Centaureae* (1-6). The other three lactones were chlorinated guaianolides found for the first time and were named linichlorin A (**1e**), B (**3b**), and C (**5b**).

Linichlorin A (**1e**): $C_{19}H_{23}O_6Cl$, mol. wt. (ms) 382, mp 153-155°C, $[\alpha]_D + 89^\circ$. The ir spectrum showed bands at 3450 (OH), 1740 (α,β' -unsaturated γ -lactone), 1710 (ester), and 1640 cm^{-1} (double bonds). Its nmr (Table 1) and mass spectra clearly show that it is a methacrylic acid ester (doublets at δ 6.20 and 5.65 ($J = 2$ Hz), singlet of a vinyl methyl at δ 2.0, and prominent peaks at m/e 296 ($M^+ - C_4H_6O_2$) and 69 (C_4H_5O). A pair of doublets at δ 4.35 and 3.95 ($J = 11$ Hz) is attributable to a $-C-(OH)-CH_2Cl$ group at C-4, a pair of lowfield doublets at δ 6.20 and 5.60 ($J = 3$ Hz) confirms the presence of an exocyclic methylene in **1e** while two signals at δ 5.10 and 4.80 represent a methylene at C-10.

From these data, linichlorin A was assigned the formula **1e**. $NaBH_4$ reduction led to the corresponding 11,13-dihydroderivative (**6e**), with a new doublet at δ 1.25 ($J = 9$ Hz) typical of a secondary methyl.

This compound proved to be the same as 8 α -metaacryloyl-11,13 α -dihydrochlorohyssopifolin B, obtained in this laboratory (7) by treating centaurepensin with a zinc-copper couple.

Linichlorin B (**3b**): $C_{19}H_{23}O_6Cl$, mol. wt. (ms) 382, mp 144-146°C, $[\alpha]_D + 95^\circ$ has an ir spectrum similar to that of linichlorin A. Its nmr shows signals in the vinyl region which correspond to three methylenes, at C-4, C-10, and C-11 (Table 1). A β -chloro- α -hydroxyisobutyric group is indicated by nmr (pair of doublets at δ 3.90 and 3.65 ($J = 11$ Hz), singlet at 1.50 ($-C-(OH)Me-CH_2Cl$) and by ms (m/e 244 ($M^+ - C_4H_7O_3Cl$)). These data suggest the formula **3b** for linichlorin B. By $NaBH_4$ treatment of **3b**, the 11,13 α -dihydroderivative (**7b**) was obtained (8 α,β -chloro- α -hydroxyisobutyrate of 11,13 α -dihydrocynaropicrin) plus its saponification product **7a** (11,13 α -dihydrodeacylcynaropicrin). Both of these had been obtained in this laboratory from centaurepensin, the structure and stereochemistry of which, in its turn, had previously been established by González *et al.* (7) by dehalogenation and saponification, thus giving the deacyldihydrocynaropicrin derivative of cynaropicrin for which the asymmetrical centres C-1 [α -H], C-5 (α -H), C-6 (β -H), and C-7 (α -H) have been rigorously established. By reductive elimination of the spiroepoxide at C-4 with $MgBr_2-(H_2)Mg$ (8), **3b** can be obtained from acroptilin (**2b**), further confirming the structure of this lactone as **3b**.

Linichlorin C (**5b**): mp 160-162°C, $[\alpha]_D + 83^\circ$, $C_{21}H_{27}O_9Cl$ from its elemental analysis and ms (fragment at m/e 427 ($M^+ - CH_2OH$)), shows bands of hydroxyl groups, of an α,β' -unsaturated γ -lactone, esters and double bonds in its ir. Its nmr and ms reveal an acetyl group (singlet at 2.20 and peak at m/e 43 (C_2H_3O)) and a β -chloro- α -hydroxy-

¹Part 35 of the series "Chemical constituents of the Compositae." For Part 34 see ref. 6.

isobutyric grouping. A prominent peak in its ms spectrum at m/e 31 (CH_2OH) suggests the existence of a primary hydroxyl group in the molecule. By mild acetylation **8b** was formed with a paramagnetic shift of 0.5 ppm from the CH_2OH signals recorded in the nmr of **5b**. Protracted treatment produced **9f**. Alkaline hydrolysis of **5b** gave **10** as major product and **11** in lower yield. Compound **11** proved to be identical (nmr and mp) to the compound obtained by Rybalko *et al.* (9) upon saponifying acroptilin. Compound **8b** showed no reaction on treatment with

Jones' reagent, leading to the assumption that the acetate group in **5b** esterified the hydroxyl at C-3. This was confirmed when **5b** was obtained from acroptilin. In fact the acetylation at 0°C of **2b** yielded a mixture of **12b** and **12f** and treatment of **12b** with HClO_4 at room temperature (10) caused the opening of the oxiran ring to give a mixture of diols (**5b**) epimeric at C-4 and identical to **5b** and **13b**. A comparison of the nmr spectra of both diols showed a downfield chemical shift of the H-6 in **5b** relative to **13b** ($\Delta\delta = +0.30$ ppm probably due to the 1-3

TABLE 1. Proton chemical shifts of linichlorins and related compounds*

Compound	H-3	H-6(q)	H-8(c)	H-13(d)	H-14	H-15(d)	3'H-2†	2'Me†	OAc
1e	4.15	4.75	5.05	6.20 (3) 5.60 (3)	5.10 4.80	4.35 (11) 3.95 (11)	6.20 (2) 5.65 (2)	2.0 s	
3b	4.55	4.30	5.25	6.25 (3) 5.60	5.20 5.10	5.45 (6)	3.90 (11) 3.65 (11)	1.50 s	
5b	4.90	4.85	5.20	6.20 (3) 5.60 (3)	5.15 5.02	3.90 (11) 3.65 (11)	3.90 (11) 3.65 (11)	1.55 s	2.20 s
6e	4.15	4.65	5.05	1.25 (7)	5.10 4.90	4.25 (11) 3.85 (11)	6.15 (2) 5.60 (2)	1.95 d	
7a		4.50		1.32 (7)	5.05 4.95	5.25			
7b	4.55	4.20	5.20	1.25 (7)	5.15 5.10	5.43 5.35	3.86 (11) 3.59 (11)	1.50 s	
8b	4.45		5.25	6.25 (3) 5.60 (3)	5.20 5.10	4.65 (11) 4.30 (11)	3.90 (11) 3.65 (11)	1.55 s	2.10 s
9b	4.45		5.40	6.25 (3) 5.78 (3)	5.13 5.03	4.95 (11) 4.70 (11)	4.05 (11) 3.75 (11)	1.70 s	2.10 c
10	4.05	4.55	4.10	6.15 c	5.10 4.95	4.05 (11) 3.80 (11)			
11	3.60	4.20	3.80	6.30 c	5.05	3.95 s			
12b	4.90	4.40	5.25	6.20 (3) 5.55 (3)	5.20 5.10	3.25 (6) 3.05 (6)	3.85 (11) 3.55 (11)	1.50 s	2.0 s
13b	5.05	4.55	5.20	6.25 (3) 5.65 (3)	5.20 5.10	4.35 (11) 4.15 (11)	3.85 (11) 3.65 (11)	1.55 s	2.10 s

*All values are in δ relative to internal TMS; s, singlet; d, doublet; q, quartet; c, complex; J values (Hz) in parentheses.
†Acyl group C(1')—C(2')Me—C(3').

diaxial interaction between the β H-6 and the C-4 OH. This suggests a β orientation for the C-4 OH in 5b.

Experimental

Melting points were taken on a Kofler block and are uncorrected. Optical activities were measured in CHCl_3 , ir spectra in CHCl_3 on a Perkin-Elmer spectrophotometer, and nmr spectra in CDCl_3 with TMS as internal standard on a 90 MHz Perkin-Elmer instrument.

Extraction and Separation

The dried plant (12 kg) collected at Los Monegros (Zaragoza) in July was chopped and exhaustively extracted with EtOH in a Soxhlet. The extract was filtered, concentrated to 500 ml and, after adding $\text{Pb}(\text{OAc})_2$ (15 g) in hot H_2O (500 ml), was left for 24 h. It was then filtered, concentrated until free of EtOH and the residue (630 g) was extracted with CHCl_3 and chromatographed on a column of silica gel. Elution with benzene—ethyl acetate yielded, in order of increasing polarity, linichlorin A, aguerin B, chlorohyssopifolins A, D, and C, linichlorin B, chlorohyssopifolins B and E, vahlenin, and linichlorin C.

Linichlorin A (1e) was purified by preparative tlc with petrol—ethyl acetate (7:3) (six elutions) and crystallized from chloroform—iisopropyl ether: mp 153–155°C, $[\alpha]_D + 89^\circ$ (c 0.58); ir (KBr) ν_{max} : 3450, 1740, 1710, 1630, and 740 cm^{-1} ; ms m/e : 382 (M^+), 296, 278, 260, 69. *Anal.* calcd. for $\text{C}_{19}\text{H}_{23}\text{O}_6\text{Cl}$: C 59.60, H 6.01, Cl 9.28; found: C 59.81, H 6.24, Cl 9.32.

Linichlorin B (3b): mp 144–146°C (EtOAc—petrol), $[\alpha]_D + 95^\circ$ (c 0.45); ir ν_{max} : 3560, 3540, 1760, 1740, and 1640 cm^{-1} ; ms m/e : 382 (M^+), 296, 278, 260, 69. *Anal.* calcd. for $\text{C}_{19}\text{H}_{23}\text{O}_6\text{Cl}$: C 59.60, H 6.01, Cl 9.28; found: C 59.59, H 6.13, Cl 8.98.

Linichlorin C (5b): mp 160–162°C (EtOAc—petrol), $[\alpha]_D + 83^\circ$ (c 0.36); ir (KBr) ν_{max} : 3550, 3360, 1760, 1720, and 1660 cm^{-1} ; ms m/e : 427 ($\text{M}^+ - 31$), 391, 368, 320, 302, 260, 242, 93, 57, 43. *Anal.* calcd. for $\text{C}_{21}\text{H}_{27}\text{O}_9\text{Cl}$: C 55.02, H 5.89, Cl 7.75; found: C 54.79, H 6.11, Cl 7.77.

NaBH₄ Reduction of 1e

Sodium borohydride (600 mg) was added to compound (1e) (300 mg) dissolved in methanol (50 ml) and the solution was stirred for 10 min at 0°C. The solution was evaporated and the residue acidified with 5% hydrochloric acid and extracted with ethyl acetate. The resulting solid was chromatographed on silica gel, yielding the product (6e): mp 164–167°C, $[\alpha]_D + 33.4^\circ$ (c 0.25); ir ν_{max} : 3540, 1760, and 1640 cm^{-1} ; ms m/e : 298 ($\text{M}^+ - 86$), 280, 262, 91, and 69.

NaBH₄ Reduction of 3b

A solution of compound (3b) (500 mg) in methanol (30 ml) was treated with sodium borohydride (100 mg). Under the same conditions as above, a mixture of two products was obtained which was separated by preparative tlc with petrol—ethyl acetate (4:6). Derivative 7b, oil: $[\alpha]_D + 88^\circ$ (c 0.24); ir ν_{max} : 3460, 1770, 1730, and 1640 cm^{-1} ; ms m/e : 384 (M^+), 264, 246, 93. Derivative 7a: mp 134–136°C (EtOAc—petrol), $[\alpha]_D + 70^\circ$ (c 0.27 in MeOH); ir (KBr) ν_{max} : 3330, 1730, and 1635 cm^{-1} ; ms m/e : 264 (M^+), 246, 218, and 200.

Treatment of 2b with MgBr₂—Mg(Hg)

A solution of 2b (500 mg) in THF (20 ml) was added to magnesium amalgam (Hg 3.53 g, Mg 0.072 g) under argon atmosphere. A solution of MgBr₂ (180 mg) in THF was then introduced and the mixture was continually stirred for 1 night under argon atmosphere. It was filtered, the solvent being eliminated, poured into water, and extracted with ethyl acetate. By chromatography on silica gel (petrol—ethyl

acetate 6:4), two products were separated, one proving to be linichlorin B (3b).

Diacetate (8b)

A solution of 5b (300 mg) in pyridine (0.2 ml) and Ac_2O (1 ml) was left standing for 2 h at room temperature. By silica gel chromatography (petrol-EtOAc 7:3) monoacetyl linichlorin C (8b) was obtained, oil: $[\alpha]_D + 82^\circ$ (c 0.43); ν_{max} 3530, 1770, 1740, and 1640 cm^{-1} ; ms *m/e*: 464 ($M^+ - 36$), 427, 462, 302, 242, 93, and 43.

Tetraacetate (9f)

Treatment of 5b (150 mg) with Ac_2O (3 ml) in pyridine (0.2 ml) at room temperature during 48 h yielded a liquid tetraacetate (9f) which was purified by silica gel chromatography (benzene-ethyl acetate 1:1), $[\alpha]_D + 56^\circ$ (c 0.36); ν_{max} : 1770, 1740, and 1640 cm^{-1} ; ms *m/e* 524 ($M^+ - 60$), 481, 421, 404, 284, 241, 224, 93, 43.

Alkaline Hydrolysis of 5b

A solution of compound (5b) (500 mg) in methanol (10 ml) was treated with aqueous 5% potassium carbonate (50 ml) during 48 h at room temperature. The soln was acidified with 5% hydrochloric acid until pH 6, saturated with NaCl and continuously extracted with EtOAc. The crude product (350 mg) was eluted on a column of silica gel (benzene-EtOAc 1:4). Crystallization from EtOAc-petrol gave a solid (11): mp $164-166^\circ\text{C}$, $[\alpha]_D + 61^\circ$ (c 0.2); ir (KBr) ν_{max} : 3460, 1760, and 1640 cm^{-1} ; ms *m/e* 278 (M^+), 260, 247, 231. The other product was an amorphous solid (10): $[\alpha]_D + 32^\circ$ (c 0.64 in MeOH); ir (film) ν_{max} : 3500-3300, 1740, and 1640 cm^{-1} ; ms *m/e* 296 (M^+), 278, 265, 248, 229. Anal. calcd. for $\text{C}_{15}\text{H}_{20}\text{O}_6$: C 60.80, H 6.80; found: C 60.88, H 6.82.

Treatment of Diacetate with Jones' Reagent

Diacetate 8b (250 mg) was dissolved in acetone (20 ml) and Jones' reagent (8 ml) was added in drops. The solution was stirred for 1 h at 0°C and then brought to room temperature and kept overnight. Excess reagent was destroyed with MeOH and the solution was filtered and evaporated at reduced pressure. The residue was extracted with EtOAc, dried on Na_2SO_4 , and concentrated, recovering the starting material.

Acetylation of 2b

Treatment of 2b (500 mg) with Ac_2O -Py at 0°C produced a mixture of acetates (480 mg) separated by silica gel chroma-

tography (petrol-EtOAc 6:4). Monoacetate (12b): mp $173-176^\circ\text{C}$ (CH_2Cl_2 -isopropyl ether), $[\alpha]_D + 79^\circ$ (c 0.48); ir ν_{max} : 3540, 1765, 1740, and 1650 cm^{-1} ; ms *m/e* 440 (M^+), 380, 370, 302, 93, 43. Diacetate (12f): mp $111-112^\circ\text{C}$, $[\alpha]_D + 72^\circ$ (c 1.75); ir ν_{max} : 1765, 1735, and 1640 cm^{-1} ; ms *m/e* 302 ($M^+ - 180$), 259, 242, 91, 43.

Treatment of 12b with HClO_4

One millilitre of 3 N HClO_4 was added to a solution of 12b (300 mg) in THF (15 ml) and the mixture was left at room temperature. The reaction was followed by tlc until the starting material was virtually eliminated (1 h). The products were extracted with CHCl_3 , dried, distilled, and the residue was separated by preparative tlc with CHCl_3 - Me_2CO (8:2) (three elutions). Diol 5b: mp 160°C , $[\alpha]_D + 83^\circ$ (identical with natural linichlorin C (mixture mp, ir, nmr, and chromatography results)). Diol 13b: $[\alpha]_D + 41^\circ$ (c 0.53); ir ν_{max} : 3520, 1760-1710, and 1640 cm^{-1} ; ms *m/e* 427 ($M^+ - 31$), 368, 321, 302, 260, 242, 229, and 93.

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