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# CHLOROHYSSOPIFOLIN C, D, E AND VAHLENIN, FOUR NEW SESQUITERPENE LACTONES FROM *CENTAUREA HYSSOPIFOLIA**

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**Key Word Index**—*Centaurea hyssopifolia*; Compositae; sesquiterpene lactones; chlorohyssopifolin C, D and E; vahlenin.

**Abstract**—On the basis of chemical and spectroscopic evidence, structures are assigned to four new sesquiterpene lactones: chlorohyssopifolin C, D and E (guaianolides) and vahlenin (eudesmanolide), isolated from *Centaurea hyssopifolia* Vahl.

## INTRODUCTION

FROM *Centaurea hyssopifolia* Vahl. we previously isolated chlorohyssopifolin A (**1a**) and B (**1b**).[1] Simultaneously, Harley-Mason *et al.* determined by X-ray analysis the structure of a new sesquiterpene lactone called centaurepensin.[2] Direct comparison of this compound with our **1a** by physical methods (m.m.p., $[\alpha]_D$, TLC, IR) showed them to be identical. The differences between the two structures proposed lie in that the aforementioned authors situate an OH group at $C_3$ whereas we, based on the formation of an $\alpha,\beta$-unsaturated ketone,[1] locate it at $C_2$, and in the position of the Cl atom in the esterifying function, which will be discussed below. The present work reports the isolation from the same plant of the four new sesquiterpene lactones chlorohyssopifolin C (**2a**), D (**3a**), E (**3b**) and vahl. (**4a**). The first three are related with **1a**, hence their structures are based on that given by us for chlorohyssopifolin A.

## RESULTS AND DISCUSSION

Chlorohyssopifolin C (**2a**), $C_{19}H_{23}O_7Cl$, has spectral properties analogous to those of **1a**.[1] The IR spectrum indicates the presence of OH, $\alpha$-methylene-$\gamma$-lactone and ester functions, double bonds and halogen. The NMR spectrum displays the characteristic signals of an exocyclic $-CH_2$ group conjugated with a lactone CO ($\delta$ 6·05 and 5·71, each 1H, *d*, *J* 3·5 Hz), an isolated $-CH_2$ group ($\delta$ 5·21 and 5·03, each 1H, *d*, *J* 2 Hz), an Me function

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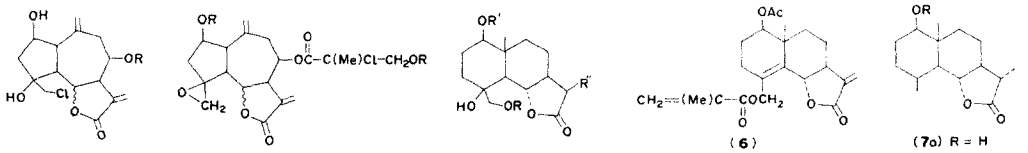
on a completely substituted C atom ($\delta$ 1·55, 3H, *s*), an AB quartet of a —$CH_2$OH group ($\delta$ 3·90, 3·76, 2H, $J$ 11 Hz) and a double doublet attributed to the proton geminal to the lactonic O atom ($\delta$ 4·80, 1H, $J$ 9 and 10·5 Hz). Under mild conditions **2**a forms the diacetate **2**b which in the IR has no OH absorptions. So far, the nature of six O atoms is explained; the remaining one must be present as an ether function. In fact, the NMR spectrum of **2**a shows two doublets in the region of the epoxide protons ($\delta$ 3·17 and 3·06, 2H, $J$ 5 Hz). The esterifying group must be the same as in **1**a because both MS show a fragment corresponding to $M^+-C_4H_7O_3Cl$ and the base peak at *m/e* 93 ($C_3H_6OCl$) and in their NMR spectra the same AB quartet of a —$CH_2$OH function is observed. We formulate the esterifying group as $\alpha$-chloro-$\beta$-hydroxyisobutyric acid, and not as the corresponding $\alpha$-hydroxy-$\beta$-chloro isomer, because **1**a gives a triacetate in whose NMR spectrum the AB quartet of the $CH_2$OAc function appears paramagnetically shifted by approximately 0·5 ppm with respect to the corresponding signal of the alcohol.[1] Furthermore, mild oxidation of tetrahydrochlorohyssopifolin A with Jones reagent yielded an acid (IR: 3400–3500, 1780, 1740, 1710 $cm^{-1}$) which was characterized by preparing its methyl ester. Under identical conditions **1**a gave also an acid which was converted into the pyrazoline methyl ester. Structure **2**a was confirmed by treating the compound with HCl gas, which gave **1**a.

Chlorohyssopifolin D (**3**a), $C_{21}H_{29}O_8Cl$, has the same lactone moiety as **1**a, from the similarity of their IR and NMR spectra. The MS shows a fragment at *m/e* 296 ($M^+-C_6H_{12}O_4$) and the base peak at *m/e* 103 ($C_5H_{11}O_2$). These data together with the NMR signals at 3·63, 3·49 (2H, each *d*, $J$ 7 Hz) and 1·16 (3H, *t*, $J$ 7 Hz) attributed to an $A_2X_3$ system, suggest that the esterifying function has an EtO group. This must be situated in $\alpha$ position to the CO since oxidation of **3**a with Jones reagent afforded an acid. By treatment with $CH_2N_2$ the corresponding pyrazoline methyl ester was obtained, whose NMR spectrum shows a three-protons singlet of a MeO group at $\delta$ 3·65, the signals of the exocyclic —$CH_3$ group having disappeared.

Chlorohyssopifolin E (**3**b), $C_{19}H_{25}O_8Cl$, shows the same spectral behaviour as **3**a except that it has no EtO group. As in the former cases, the nature of the esterifying function was inferred from the MS which presents a fragment at *m/e* 296 ($M^+-C_4H_8O_4$) and the base peak at *m/e* 75 ($C_3H_7O_2$). Hence, **3**b must be an $\alpha,\beta$-dihydroxyisobutyric acid ester.

Chlorohyssopifolin A, D and E show very similar MS fragmentations, which suggests that they have a common carbon skeleton.




(1a) R = O=C–C(Me)Cl–$CH_2$OH
(1b) R = H

(2a) R = H
(2b) R = Ac

(4a) R = O=C–C(Me)=$CH_2$; R'= H; R''=$CH_2$
(4b) R = O=C–C(Me)=$CH_2$; R'= Ac; R''=$CH_2$
(5) R = O=C–CH(Me)$_2$; R'= H; R''= Me

(6)

(7a) R = H
(7b) R = Ac

(3a) R = O=C–C(Me)(OEt)–$CH_2$OH
(3b) R = O=C–C(Me)(OH)–$CH_2$OH

Vahlenin (**4**a), $C_{19}H_{26}O_6$, has IR bands indicative of OH, $\alpha$-methylene-$\gamma$-lactone and ester functions and double bonds. Its NMR spectrum displays two broad singlets of a terminal —$CH_2$ group ($\delta$ 6·09, 5·59, 1H each), the typical doublets of an exocyclic —$CH_2$ group conjugated with a lactone CO ($\delta$ 5·94, 5·44, 1H each, $J$ 3·5 Hz), two doublets of a

—$CH_2OR$ group (4·36, 4·17, 2H, *J* 11 Hz), a triplet corresponding to a proton geminal to a lactone oxygen (4·28, *J* 10 Hz) and two three-protons singlets of an angular and a vinylic Me group (1·24 and 1·94 respectively). From the presence of the fragments at *m/e* 281 ($M^+-C_4H_5O$), 264 ($M^+-C_4H_6O_2$) and the base peak at *m/e* 69 ($C_4H_5O$) in the MS we deduce that **4a** must be a methacrylic ester, which is also supported by the NMR data.

Hydrogenation of **4a** with $PtO_2$ gave **5**, in the NMR spectrum of which appears a nine-protons doublet ($\delta$ 1·20, *J* 7 Hz) attributed to three >CHMe groups. Aromatization of **4a** with Se gave no azulenes so that it must have an eudesmane skeleton. Structure **4a** was confirmed by the following reactions: mild acetylation of vahlenin gave a monoacetate (**4b**) with OH absorptions in the IR. Subsequent dehydration with $SOCl_2$ afforded a compound formulated as **6** because its NMR spectrum shows no new vinyl protons, the singlet of the angular Me group is found at the same position as in **4a**, whereas the signal of the H–$C_6$ now appears as a doublet centred at $\delta$ 4·95 and that of the —$CH_2OR$ has suffered a paramagnetic shift of approximately 0·5 ppm. These results agree with the presence in **4a** of a tertiary OH and $C_4$ and the angular Me group at $C_{10}$. The esterifying function must be located at $C_{15}$ since **4a** has no vicinal diol (no reaction with $NaIO_4$) and its MS shows a fragment at *m/e* 251 ($M^+-C_6H_8O_2$). The position of the remaining OH group was proved by relating **4a** with tetrahydrosantamarin (**7a**) as follows: hydrogenolysis of **6** over $PtO_2$ in acid medium gave **7b**, whose IR and NMR spectra are superimposable with those of acetyltetrahydrosantamarin. Saponification yielded **7a** which was shown to be identical with an authentic sample of tetrahydrosantamarin.[3]

The possibility that the Cl atom in **2a**, **3a** and **3b** was introduced during the extraction process can be discarded because no solvents or reagents containing Cl were employed. The EtO group in chlorohyssopifolin D (**3a**) could, however, be an artefact produced during the Soxhlet extraction with EtOH. Several halogenated sesquiterpene lactones have been isolated from Compositae.[4,5] From the biogenetic viewpoint the co-occurrence of oxyranes and their related chlorohydrins in the same plant is of great interest.[4,6] The simultaneous formation of the chlorohyssopifolins (guaianolides) and vahlenin (eudesmanolide) may arise from a common germacranolide precursor[7] and follow a pathway similar to that proposed by Lee *et al.* for sesquiterpene lactones of *Artemisia* species.[8] To our knowledge, this is the first time that an eudesmanolide has been isolated from a plant of the tribe Cynareae, a fact which may be of taxonomic interest.[9]

## EXPERIMENTAL

M.p's, determined on a Kofler block, are uncorrected. If not otherwise stated, optical rotations and IR spectra were measured in $CHCl_3$ and UV spectra in EtOH. The NMR spectra of the natural compounds were taken at 100 MHz and the remaining ones at 60 MHz using TMS as internal reference. Column and dry column chro-

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matography was realized on silica gel 0·2–0·5 and 0·063–0·20 mm respectively. Acetates were prepared with $Ac_2O$ in pyridine at 25° overnight. Unless otherwise indicated, compounds were recrystallized from EtOAc–petrol.

*Extraction and separation.* The dry, chopped plant (16·2 kg), collected near Valdemoro (Spain) in June 1970, was exhaustively extracted with EtOH in a Soxhlet. The extract was filtered, concentrated to 1 l. and after adding $Pb(OAc)_2$ (100 g) in hot $H_2O$ (2 l.) left for 24 hr. Then it was filtered, concentrated and extracted with EtOAc, the extract dried over $Na_2SO_4$ and the solvent evaporated *in vacuo*. The residue (394 g), of strong bitter taste, was chromatographed on a column. $C_6H_6$–$Me_2CO$ (92:8) eluted first chlorohyssopifolin A (**1a**), followed by a mixture of **1a** and chlorohyssopifolin D (**3a**), pure **3a** and finally a mixture (5·5 g) of several compounds from which chlorohyssopifolin C (**2a**) and vahlenin (**4a**) were isolated by dry column chromatography ($C_6H_6$–EtOAc 1:1). Elution with $C_6H_6$–$Me_2CO$ (9:1) gave chlorohyssopifolin B (**1b**) and with $C_6H_6$–$Me_2CO$ (86:14) an oil (5 g) from which on repeated dry column chromatography ($C_6H_6$–EtOAc 1:1: $CHCl_3$–$Me_2CO$ 7:3) chlorohyssopifolin E (**3b**) was obtained.

*Chlorohyssopifolin C* **2a** (0·74 g), needles, m.p. 197–199°, $[\alpha]_D$ 100° (*c*, 1·29; MeOH). (Found: C, 57·10; H, 6·03; Cl, 8·38. $C_{19}H_{23}O_7Cl$ requires: C, 57·28; H, 5·77; Cl, 8·79%.) IR (KBr): 3450 (OH), 1740 (γ-lactone, ester), 1660 and 920 (C=C), 720 $cm^{-1}$ (C–Cl). NMR ($d_6$-acetone/$d_6$-DMSO): see text. MS: *m/e* (%) no $M^+$ peak, 278 (1), 260 (22), 243 (13), 230 (20), 173 (30), 148 (33), 129 (26), 93 (100), 91 (73). *Diacetate* **2b**, obtained as an oil which would not crystallize but was found homogeneous by TLC; $[\alpha]_D$ 73° (*c*, 1·93); IR: 1750 (γ-lactone, ester, OAc), 1660 (C=C), 1230 $cm^{-1}$ (OAc). **2a** and **2b** gave a positive Beilstein test for halogen.

*Chlorohyssopifolin A* (**1a**) *from* **2a**. A soln **2a** (80 mg) in MeOH (5 ml) was treated with HCl gas for 5 min. The MeOH was removed *in vacuo* and the residue washed several times with $H_2O$ till neutral. Recrystallization gave **1a** (60 mg), m.p. 218–219°, identical with an authentic sample (m.m.p., TLC, IR spectra superimposable).[1]

*Chlorohyssopifolin D* **3a** (1·5 g), needles, m.p. 186–188°, $[\alpha]_D$ 89° (*c*, 1·26; MeOH). (Found: C, 57·01; H, 6·57; Cl, 7·97. $C_{21}H_{29}O_8Cl$ requires: C, 56·75; H, 6·53; Cl, 7·88%.) IR (KBr): 3500 (OH), 1740 (γ-lactone, ester), 1660, 1640 and 830 (C=C), 740 $cm^{-1}$ (C–Cl). NMR ($d_6$-acetone): δ 6·03, 5·73 (1H each, *dd*, *J* 3·5 Hz, $CH_2$–$C_{11}$), 5·11, 4·90 (1H each, *dd*, *J* 2 Hz, $CH_2$–$C_{10}$), 4·93 (1H, *dd*, *J* 10 and 9 Hz, H–$C_6$), 4·53 (1H, *d*, *J* 4 Hz, OH), 4·28, 3·85 (2H, AB quartet, *J* 11 Hz, –$CH_2Cl$), 4·24 (1H, *s*, OH), 4 (1H, *s*, OH), 3·76, 3·44 (2H, AB quartet, *J* 10 Hz, –$CH_2OH$), 3·63, 3·49 (2H, *dd*, *J* 7 Hz, –$OCH_2Me$), 1·40 [3H, *s*, >C(OEt)Me], 1·16, (3H, *t*, *J* 7 Hz, –$OCH_2Me$). MS: *m/e* (%) 444 ($M^+$, 1), 414 (1), 398 (0·5), 368 (3), 343 (1), 337 (2), 298 (7), 279 (10), 261 (11), 243 (13), 229 (12), 201 (23), 189 (16), 175 (20), 103 (100), 75 (96). Gave a positive Beilstein test for halogen.

*Pyrazoline methyl ester of* **3a**. A soln of **3a** (190 mg) in $Me_2CO$ (7 ml) was treated with Jones reagent at 5° till the orange colour persisted. The soln was diluted with EtOAc, washed with $H_2O$, dried and concentrated. The residue (160 mg) gave positive Beilstein and Zimmermann tests; IR: 3500 (OH), 1780 (γ-lactone), 1740 (ester, cyclopentanone), 1720 (acid), 1640 $cm^{-1}$ (C=C). It was dissolved in $CHCl_3$ (10 ml), allowed to stand with $CH_2N_2$ in ether (50 ml) at 4° overnight and concentrated *in vacuo*. The oily residue (180 mg) would not crystallize; UV: 328 nm (ε 302); IR: 3530 (OH), 1780 (γ-lactone), 1730 (ester, cyclopentanone), 1640 $cm^{-1}$ (C=C).

*Chlorohyssopifolin E* **3b** (0·4 g), m.p. 118–119° (from $C_6H_6$–EtOAc–petrol.), $[\alpha]_D$ 91° (*c*, 1·20; MeOH). (Found: C, 50·44; H, 6·13; Cl, 8·26. $C_{19}H_{25}O_8Cl.2H_2O$ requires: C, 50·44; H, 6·41; Cl, 7·74%.) IR (KBr): 3410 (OH), 1760 (γ-lactone), 1720 (ester), 1660, 1640 and 820 (C=C), 740 $cm^{-1}$ (C–Cl). NMR ($d_6$-acetone): δ 6·03, 5·70 (1H each, *dd*, *J* 3·5 Hz, $CH_2$–$C_{11}$), 5·09, 5·01 (1H each, *dd*, *J* 2 Hz, $CH_2$–$C_{10}$), 4·93 (1H, *dd*, *J* 10 and 9 Hz, H–$C_6$), 4·51 (1H, *d*, *J* 4 Hz, OH), 4·28, 3·84 (2H, AB quartet, *J* 11 Hz, –$CH_2Cl$), 4·20 (1H, *s*, OH), 3·99 (1H, *s*, OH), 3·86, 3·56 (2H, AB quartet, *J* 10 Hz, –$CH_2OH$), 1·39 (3H, *s*, >C(OH)Me). MS: *m/e* (%) no $M^+$ peak, 296 (29), 278 (34), 260 (29), 243 (45), 229 (58), 189 (58), 175 (58), 91 (47), 75 (100). Gave a positive Beilstein test for halogen.

*Vahlenin* **4a** (0·7 g), sinters at 200° but fails to melt at higher temp., $[\alpha]_D$ 17° (*c*, 1·68; MeOH). (Found: C, 65·31; H, 7·68. $C_{19}H_{26}O_6$ requires: C, 65·13; H, 7·48%.) IR (KBr): 3470 (OH), 1750 (γ-lactone), 1710 (ester), 1640 and 825 $cm^{-1}$ (C=C). NMR ($d_6$-acetone): see text. MS: *m/e* (%) no $M^+$ peak, 332 (1), 319 (0·5), 281 (1), 264 (4), 251 (89), 233 (45), 215 (35), 187 (37), 169 (18), 69 (100).

*Tetrahydrovahlenin* **5**. A soln of **4a** (200 mg) in HOAc–EtOAc (1:1) (20 ml) was hydrogenated over $PtO_2$ (30 mg) at 25° and atm. pressure until $H_2$ uptake ceased. Dry column chromatography ($C_6H_6$–EtOAc 4:6) of the residue gave **5** (100 mg), m.p. 226–229°, $[\alpha]_D$ 19° (*c*, 1·62). (Found: C, 64·51; H, 8·54. $C_{19}H_{30}O_6$ requires: C, 64·39; H, 8·53%.) IR: 3600, 3470 (OH), 1770 (γ-lactone), 1730 $cm^{-1}$ (ester).

*Anhydroacetylvahlenin* **6**. Acetylation of **4a** (280 mg) gave **4b** as an oil (320 mg) which would not crystallize; IR: 3590, 3460 (OH), 1770 (γ-lactone), 1730 (ester), 1640 (C=C), 1230 $cm^{-1}$ (OAc). It was dissolved in pyridine (2 ml) and treated with $SOCl_2$ at 5° for 5 min. The soln was poured into ice-$H_2O$ and extracted with $Et_2O$. The organic layer was washed with dil. $H_2SO_4$ and $H_2O$, dried and evaporated *in vacuo*, giving **6** (220 mg) which was purified by dry column chromatography ($C_6H_6$–EtOAc 7:3), m.p. 98–99°, $[\alpha]_D$ 14° (*c*, 2·06). (Found: C, 67·32; H, 7. $C_{21}H_{26}O_6$ requires: C, 67·36; H, 7·00%.) IR: 1770 (γ-lactone), 1720 (ester), 1640 $cm^{-1}$ (C=C).

*Hydrogenolysis of* **6**. A soln of **6** (200 mg) in HOAc (15 ml) was hydrogenated over $PtO_2$ (25 mg) till $H_2$ uptake ceased. Dry column chromatography ($C_6H_6$–EtOAc 9:1) of the oily residue (150 mg) gave **7b** (50 mg), m.p. 141–145° (from $C_6H_6$–petrol.), identical with tetrahydrosantamarin acetate (IR and NMR spectra superimposable).[3] Saponification of **7b** (50 mg) in MeOH (2 ml) with 5% aq. $K_2CO_3$ (5 ml) at 25° for 14 hr followed by reflux on a steam-bath for 1 hr and usual work-up gave a residue (40 mg) which on dry column chromatography ($C_6H_6$–EtOAc 6:4) yielded **7a** (30 mg), m.p. 166–170° (from *i*-propyl ether), identical with tetrahydrosantamarin (m.m.p., TLC, IR spectra superimposable).

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