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SESQUITERPENE LACTONES OF *ARTEMISIA* CONSTITUENTS OF *A. LUDOVICIANA* SSP. *MEXICANA**

K. H. LEE and T. A. GEISSMAN

Department of Chemistry, University of California, Los Angeles, California, 90024

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Abstract—Five sesquiterpenoid lactones have been isolated from *Artemisia ludoviciana* Nutt. ssp. *mexicana* (Willd.) Keck. These include three new santanolides, ludovicin-A (III), -B (VI) and -C (VIII), and the known douglanine (I). The co-occurrence of these four closely related compounds suggests a realistic sequence of biosynthetic transformations.

INTRODUCTION

Artemisia ludoviciana Nutt. ssp. *mexicana* (Willd.) Keck[1] (Compositae, tribe *Anthemideae*, section *Abrotanum*) is one of a number of closely related species of the *A. vulgaris* complex, the examination of which has been undertaken as a part of a study of chemical taxonomy and phylogeny in the genus. Five sesquiterpene lactones have been isolated from a specimen obtained from Arizona,[2] one of which is the known compound douglanine (I), and three of which are the new ludovicins-A, -B and -C (III, VI and VIII). The fourth (ludovicin-D) is a minor component which is still under investigation. The structures of douglanine and the three ludovicins form a closely related group, in the structures of which can be discerned what appears to be a rational sequence of biosynthetic transformations.

RESULTS AND DISCUSSION

Chromatographic separation of the components of the extract of the plant led to the isolation in the less-polar fraction of douglanine (I),[3] m.p. 116–118°.

It showed a molecular ion peak at m/e 248 in the mass spectrum and analyzed for $C_{15}H_{20}O_3$. The u.v. and i.r. spectra showed the presence of a hydroxyl group and an α-methylene-γ-lactone, both of which were confirmed by the NMR spectrum (Table 1). The latter also showed a vinylic methyl group (C-4), a quaternary methyl group (C-10), and a quartet for the lactonic proton at C-6 which indicated the *trans*-disposition of the protons at C-5, C-6 and C-7. These data are all consistent with the structure (I), previously established for douglanine.[3, 4]

Acetylation of I led to the formation of the acetate (II), the identity of which with douglanine acetate was established by comparison with an authentic sample (mixed m.p., i.r., TLC). Ludovicin-A (III), $C_{15}H_{20}O_4$, m.p. 215°, $[\alpha]_D^{25}$ 128°, showed prominent mass spectral

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[1] D. D. KECK, *Proc. Calif. Acad. Sci.* **25**, 421 (1946).
[2] Collected and identified by Mr. R. J. Barr, Tucson, Arizona, voucher No. 67343 (RJB).
[3] S. MATSUEDA and T. A. GEISSMAN, *Tetrahedron Letters* 2159 (1967).
[4] A direct comparison was not possible, for douglanine is unstable, and specimens isolated earlier had undergone deterioration.

peaks at *m/e* 264 (M^+), 249 (M-15), 246 (M-18) and 231 (M-15-18), and its u.v. i.r. and NMR spectra disclosed the presence of a hydroxyl group, an α-methylene-γ-lactone, and a *trans*-disposed C-6/C-7 lactone. Two 3-proton singlets at δ 0·90 and 1·50 indicated that ludovicin-A contained, in addition to the methyl group at C-10, a methyl group attached to carbon bearing an oxygen substituent. That this substituent was a C-3/C-4 epoxide ring was suggested

Table 1. NMR data for ludovicins and derivatives

	C-1	H-2	C-3	C-4	H-5	H-6	C-10 (CH_3)	C-11 (=CH_2)
I*	H: 3·50 (1H) m	—	H: 5·30 (1H)† d, 3	CH_3: 1·95 (3H) d, 1·5	—	3·95 (1H) q, 11, 10·5	0·81 (3H) s	6·08 (1H) d, 3 5·30 (1H) d, 3
III	H: 3·23 (1H) m‡ OH: 2·46 (1H) s§	—	H: 3·01 (1H) t, 1·5	CH_3: 1·50 (3H) s	2·44 (1H) d, 12	3·97 (1H) q, 12, 10.5	0·90 (3H) s	6·05 (1H) d, 3 5·43 (1H) d, 3
IV	H: 4·51 (1H) t, 3 OAC: 2·03 (3H) s	—	H: 2·88 (1H) t, 2	CH_3: 1·49 (3H) s	2·46 (1H) d, 12	3·88 (1H) q, 12, 10·5	0·97 (3H) s	6·03 (1H) d, 3 5·38 (1H) d, 3
V	—	5·87 (1H) d, 10·5	H: 6·60 (1H) d, 10·5	CH_3: 1·57 (3H) s OH: 2·85 (1H) s	2·53 (1H) d, 11	4·13 (1H) t, 11	1·19 (3H) s	6·12 (1H) d, 3 5·48 (1H) d, 3
VI	H: 3·39 (1H) m‖	—	H: 4·38 (1H) t, 3	CH_2: 5·15 (1H) br. s 5·02 (1H) br. s	3·10 (1H) d, 11 t, 1·5	4·10 (1H) t, 11	0·78 (3H) s	6·05 (1H) d, 3 5·42 (1H) d, 3
VII	H: 4·62 (1H) t, 3 OAC: 2·00 (3H) s	—	H: 5·38 (1H)† t, 3 OAC: 2·08 (3H) s	CH_2: 5·33 (1H) br. s 5·17 (1H) d, 2	3·11 (1H) d, 11, t, 1·5	3·98 (1H) t, 11	0·90 (3H) s	6·08 (1H) d, 3 5·40 (1H) d, 3
VIII	H: 3·75 (1H) q, 4, 3 OH: 3·01§	—	—	CH_3: 2·00 (3H) d, 1·5	—	4·67 (1H) d, 11·5, q, 1·5	1·32 (3H) s	6·18 (1H) d, 3 5·53 (1H) d, 3

* In pyridine-d_5.
† Overlapping signals.
‡ Became t (4) after addn. of D_2O.
§ Disappeared after addn. of D_2O.
‖ Became t (3) after addn. of D_2O.

by the elementary composition of the compound and confirmed by the appearance of a 1-proton triplet (δ 3·01, $J = 1·5$ Hz) for the proton at C-3. These data are all accommodated by structure III. The acetate (IV) of ludovicin-A contained one acetyl residue and no hydroxyl group (by i.r.), and its NMR spectrum showed the proton at C-1 at δ 4·51 (t, $J = 3$ Hz), shifted from δ 3·23 in III.

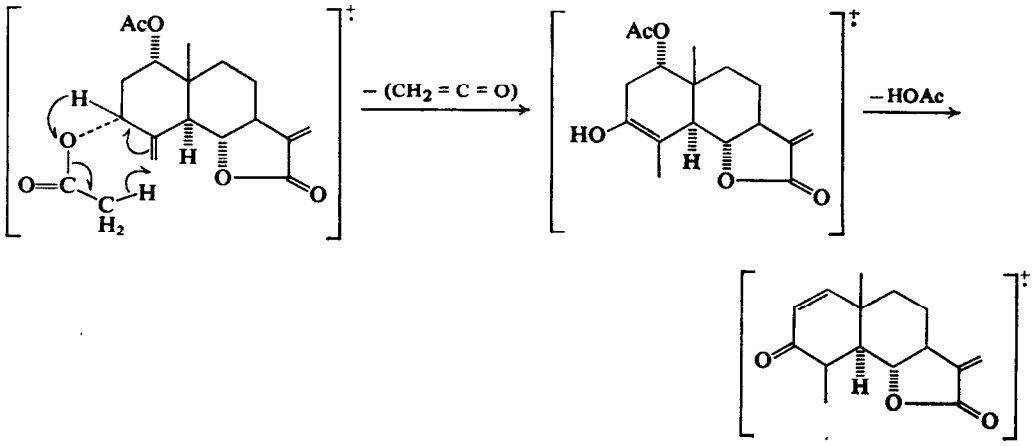
Oxidation of III with chromium trioxide provided the α,β-unsaturated hydroxy ketone (V), which proved to be identical with arglanine, previously found as a constituent of *A.*

douglasiana Bess., where it occurs along with douglanine.[5] Finally, ludovicin-A was prepared by epoxidation of douglanine; the synthetic and natural materials were identical.

The stereochemistry of the hydroxyl group at C-1 in I, and thus in III and V, is clearly revealed by the signal for the proton at C-1; in IV, for example, this proton is seen as a 1-proton signal with the small coupling constant of 3 Hz (δ 4·51), showing the absence of axial–axial coupling between the protons at C-1 and C-2. This conclusion is in accord with the demonstration, by X-ray crystallography, of the structure of douglanine.[6]

Ludovicin-B (VI), m.p. 152°, $[\alpha]_D^{25}$ 138°, has the composition $C_{15}H_{20}O_4$, and showed the molecular ion at *m/e* 264. It is an α-methylene-γ-lactone which contains two hydroxyl groups, both of which could be readily acetylated with acetic anhydride–pyridine. The NMR spectrum of ludovicin-B showed, in addition to the familiar signals for the exocyclic methylene protons of the lactone grouping, a well-defined triplet (δ 4·01, $J = 11$ Hz) for the proton at C-6 (showing the *trans*-disposition of the lactone fusion). A doublet at δ 3·10 ($J = 11$ Hz), further split ($J = 1{\cdot}5$ Hz) by allylic coupling to the exocyclic methylene protons (of the $=CH_2$ group at C-4), could be attributed to the proton at C-5. The exocyclic methylene group at C-4 was seen as two broad singlets at δ 5·15 (1 H) and 5·02 (1 H). A multiplet at δ 3·39 (1 H) which was reduced to a triplet ($J = 3$ Hz) after addition of D_2O, was ascribed to the proton at C-1, where one of the two hydroxyl groups is found. The triplet at δ 4·38 (1 H, $J = 3$ Hz) corresponds to the proton at the other hydroxyl-bearing position, C-3. These observations are in accord with the structure VI assigned to ludovicin-B. The stereochemistry at positions C-1 and C-3 is assigned as shown in VI from the nearly identical coupling constants of the C-1 and C-3 protons ($J = 3$ Hz), which show the absence of axial–axial interaction and establish the α-disposition of the hydroxyl groups at these positions.

It is of interest to note that ludovicin-B diacetate (VII) showed prominent peaks in the mass spectrum at *m/e* 306 (M-42), 288 (M-60), 246 (M-42-60) and 228 (M-60-60), the latter being the base peak. The loss of the fragments of 42 and (42 + 60) mass units is suggestive of a fragmentation of the following kind:

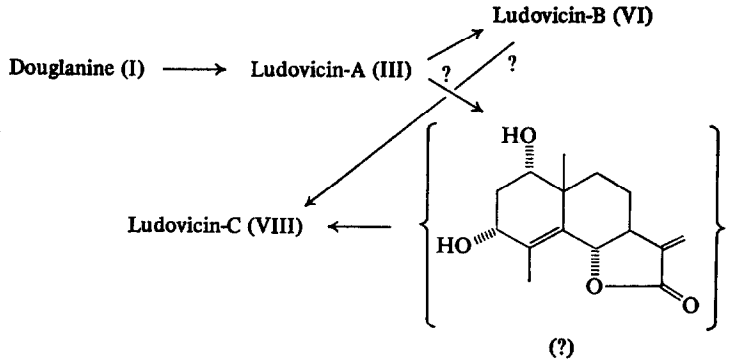


Ludovicin-C (VIII), m.p. 193–195°, $[\alpha]_D^{25}$ 95°, has the composition $C_{15}H_{18}O_4$ and showed the molecular ion at *m/e* 262. The spectral properties (i.r., u.v.) of the compound showed that it

[5] S. MATSUEDA and T. A. GEISSMAN, *Tetrahedron Letters* 2013 (1967).
[6] M. U. HAGUE, C. N. CANGHLAN, M. T. EMERSON, S. MATSUEDA and T. A. GEISSMAN, *J. Chem. Soc.*, in press.

possesses the α-methylene-γ-lactone grouping, a hydroxyl group, and is an α,β-unsaturated ketone. The NMR spectrum confirmed these assignments, showing further the *trans*-disposition of the C-6/C-7 lactone (H at C-6 at δ 4·61, t, $J = 11$ Hz). The appearance of a 3-proton singlet at δ 2·00 for the C-4 methyl group, and the absence of vinylic protons (other than those of the methylene group of the lactone) indicated that ludovicin-C was a 4-ene-3-one, and the position and character of the proton of the carbinolic carbon atom at C-1 (δ 3·75, $J = 3,4$ Hz) led to the complete formulation of the compound as VIII. Confirmation of this structure was accomplished by dehydration of the compound with thionyl chloride–pyridine, with the formation of 11,13-dehydrosantonin (IX). The dienone system present in IX showed i.r. absorption substantially identical in the 1600–1800 cm^{-1} region with those of santonin (X) and yomogin (XI).[7] The u.v. spectrum of IX showed a maximum at 238 nm, identical with those reported for X and XI. Further, comparison of the NMR spectrum of IX with those of X and XI showed the AB system (C-1/C-2) of the dienone as a pair of doublets at δ 6·75 and 6·30, $J = 10$ Hz (for IX), compared with 6·72 and 6·18, $J = 10$ Hz (for X), and 6·65 and 6·18, $J = 10$ Hz (for XI).

The co-occurrence of I, III, VI and VIII in the single plant is suggestive of their biosynthetic interrelationship. The transformations shown in the following scheme are clearly mechanistically rational and provide a picture of a sequence of changes which appears highly probable:



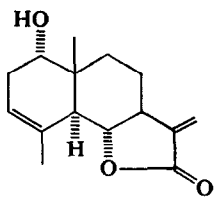
EXPERIMENTAL

M.ps were taken in capillaries and are corrected. Spectral measurements were made in the usual way; NMR data are gathered together in Table 1, where chemical shifts are in ppm (δ) and J values in Hz (cps), and s, d, t, m and br refer, respectively, to singlet, doublet, triplet, multiplet and broad. Mass spectra were measured on an AEI-MS 9 instrument at 70 eV using direct insertion. TLC was carried out with silica gel G Merck, with benzene–EtOAc (1 : 1) as the solvent. Optical rotations were measured on solutions in $CHCl_3$.

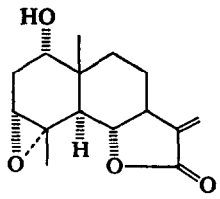
Extraction of Plant Material

Artemisia ludoviciana Nutt. ssp. *mexicana* (Willd.) Keck, was collected in September 1967 along Arizona Highway 73 near Eagar, at 7000 ft elevation in the White Mountains, Arizona.[2] The dried, milled plant (5·85 kg) was exhaustively extracted with $CHCl_3$ at room temperature, yielding, after removal of the solvent, 212 g of crude extractives. This was shaken with a mixture of methanol (2·4 l.), hexane (10 l.) and water (800 ml) and the aqueous layer separated and washed with hexane. The combined aqueous extracts were concentrated somewhat *in vacuo* and extracted with $CHCl_3$. The $CHCl_3$ extract, upon evaporation, yielded 60·4 g of a brown syrup.

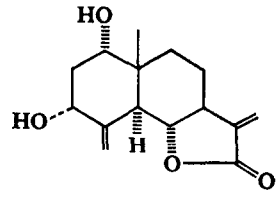
[7] T. A. GEISSMAN, *J. Org. Chem.* **31**, 2523 (1966).



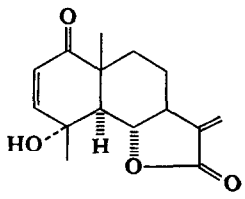
(I) Douglanine
(II) (OAc for OH)



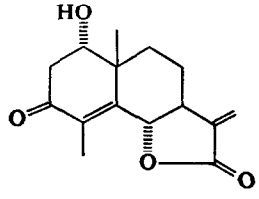
(III) Ludovicin-A
(IV) (OAc for OH)



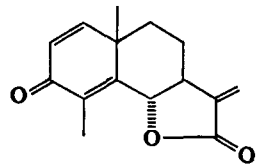
(VI) Ludovicin-B
(VII) (OAc for OH)



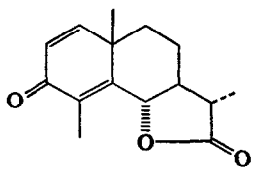
(V) Arglanine



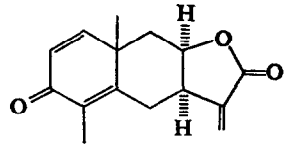
(VIII) Ludovicin-C



(IX) 11,13-Dehydrosantonin



(X) α-Santonin



(XI) Yomogin

Isolation of Douglanine and Ludovicins-A, -B, -C and -D

The crude extract (60·4 g) was chromatographed on silica gel (800 g, 8 × 50 cm), with elution with $CHCl_3$, $CHCl_3$–EtOAc, EtOAc with increasing amounts of acetone, and acetone. Thirty-three fractions of about 800 ml each were collected and examined by TLC. Fractions 1–7 (5·6 l.) contained two components (TLC) and fractions 8–11 (3·2 l.) one, which corresponded to the lower R_f spot of fractions 1–7. Fractions 12–14 ($CHCl_3$–EtOAc, 9:1) gave a yellow-green spot, fractions 15–18 a single component, 19–21 an orange-yellow spot, and later fractions (EtOAc–acetone, acetone) only oily, polar substances from which no satisfactory compounds could be obtained.

Douglanine (I). The brownish syrup (15 g) obtained from fractions 1–7 yielded crystals (4·26 g) when triturated with ether–CH_2Cl_2 (1:1). Rechromatography of 1·82 g of this crystalline material yielded a single, pure compound (0·82 g), m.p. 110°, which upon recrystallization from light petroleum had m.p. 116–118°. Its spectral characteristics were: i.r., 3500 (OH), 1750 (lactone), 1665 cm^{-1} (α-methylene C=C); mass spectrum, *m/e* 248 (M^+), 230 (M-18), 215 (M-18-15). The NMR spectrum has been described. The m.p. reported for douglanine is 115–117°.[3,4] (Found: C, 72·79; H, 7·92. Calc. for $C_{15}H_{20}O_3$: C, 72·55; H, 8·12%.)

Ludovicin-A (III). The residual syrup from fractions 8–11 was rubbed with ether and the crystalline material that formed was rechromatographed on silica gel. CH_2Cl_2 eluted a material that crystallized from ether–CH_2Cl_2, yielding 0·8 g of colorless needles, m.p. 215°, $[\alpha]_D^{25}$ 128°. Spectral data have been discussed above. (Found: C, 67·99; H, 7·72. Calc. for $C_{15}H_{20}O_4$; C, 68·16; H, 7·63%.)

Ludovicin-B (VI). The residual syrup from fractions 15–18 crystallized when ether was added. Recrystallized from CH_2Cl_2–EtOAc, the product formed colorless needles, m.p. 152°, $[\alpha]_D^{25}$ 138°. Its u.v. spectrum showed end absorption at 208 nm (ε 12,800), and i.r. bands were observed at 3300 (broad, strong; OH), 1765 and 1660 cm^{-1} (α-methylene-γ-lactone). (Found: C, 68·13; H, 7·65. Calc. for $C_{15}H_{20}O_4$; C, 68·16; H, 7·63%.)

Ludovicin-C (VIII). The EtOAc eluate (fractions 19–21) upon evaporation and trituration with ether afforded a crystalline compound which, after recrystallization from ether–CH_2Cl_2, formed colorless needles, m.p. 193–195°, $[\alpha]_D^{25}$ 95°. It had u.v. maximum at 243 nm (ε 15,200) and end absorption at 208 nm (ε 13,700). The i.r. spectrum showed bands at 3525, 1760, 1670 and 1630 cm^{-1}. (Found: C, 68·70; H, 6·83. Calc. for $C_{15}H_{18}O_4$; C, 68·70; H, 6·87%.)

Ludovicin-D. The residual syrup from the $CHCl_3$–EtOAc (9:1) eluate (fractions 12–14) was rubbed with ether, yielding 60 mg of crystalline material. Recrystallized from CH_2Cl_2, it formed colorless needles, m.p. 230–232°. Spectral data: u.v., 243 nm max. (ϵ 15,400), end absorption 208 nm (ϵ 15,200); i.r., 3370, 1765, 1665, 1615 cm^{-1}; mass spectrum, *m/e* 262 (M^+), 244 (M-18). (Found: C, 68·43; H, 7·17. Calc. for $C_{15}H_{18}O_4$; C, 68·70; H, 6·87%.)

Douglanine acetate (II). Acetylation of douglanine (I), described above, yielded the acetate, m.p. 143–145°. The compound was identical with the material prepared in the earlier study[3] as shown mixed m.p. and spectral comparison.

Ludovicin-A acetate (IV). Treatment of ludovicin-A with acetic anhydride–pyridine in the usual way afforded the acetate, colorless needles from ether–CH_2Cl_2, m.p. 164–165°. The i.r. spectrum showed absorption at 1775, 1725, 1660 and 1250 cm^{-1}, and the mass spectrum showed principal peaks at *m/e* 306 (M^+), 291 (M-15), 228 (M-18), 264 (M-42) and 246 (M-60). (Found: C, 66·46; H, 7·41. Calc. for $C_{17}H_{22}O_5$: C, 66·65; H, 7·24%.)

Oxidation of Ludovicin-A; Arglanine (V)

A solution of 100 mg of ludovicin-A in 2 ml of pyridine was oxidized with 100 mg CrO_3 at room temperature. The solution was diluted and extracted with $CHCl_3$, and the oily material obtained by removal of the solvent chromatographed on silica gel. Elution with $CHCl_3$ yielded a crystalline product (65 mg) which crystallized from ether–CH_2Cl_2 as colorless needles, m.p. 197°. The mass spectrum showed the molecular ion at *m/e* 262 and a peak at 247 (M-15). The identity of the compound with arglanine[5] was established by direct comparison (mixed m.p., TLC, spectra). (Found: C, 68·87; H, 7·08. Calc. for $C_{15}H_{18}O_4$: C, 68·70; H, 6·87%.)

Epoxidation of douglanine; ludovicin-A. To a solution of 100 mg of douglanine (I) in 3 ml $CHCl_3$ was added a solution of 100 mg of *m*-chloroperbenzoic acid in 3 ml $CHCl_3$. The mixture was kept overnight, then washed with aqueous $NaHCO_3$ and with water, dried and evaporated. The residual material crystallized, and after recrystallization from ether–CH_2Cl_2 formed colorless needles, m.p. 215°. The compound was identical with ludovicin-A (III) in all respects.

Ludovicin-B acetate (VII). Acetylation of ludovicin-B in the usual way afforded the diacetate, m.p. 209°. It showed strong i.r. bands at 1770, 1735, 1650, 1375 and 1250 cm^{-1}, and no absorption in the hydroxyl region. The mass spectrum has been described above. (Found: C, 65·63; H, 6·92. Calc. for $C_{19}H_{24}O_6$: C, 65·52; H, 6·89%.)

11,13-*Dehydrosantonin (IX).* A solution of 120 mg ludovicin-C (VIII) in 0·6 ml of dry pyridine was treated with 0·4 ml $SOCl_2$ for 1 hr at −5°. The solution was diluted with water and extracted with EtOAc. The crude product, a brown syrup, was chromatographed over silica gel to afford a substance which did not crystallize but gave a single spot on TLC and had an u.v. maximum at 238 nm. The i.r. spectrum of IX was nearly identical with those of α-santonin (X) and yomogin (XI) in the 1600–1800 cm^{-1} region. The relevant peaks were as follows: IX: 1775, 1665, 1638, 1618; X: 1785, 1667, 1638, 1615; XI: 1768, 1665, 1533, 1615 cm^{-1}; all were of the same relative intensity. The NMR spectra have been described above.

Acknowledgements—This study is part of an investigation of sesquiterpene lactones supported by a research grant GM–14240–03 from the U.S. Public Health Service. Analyses are by Miss Heather King, UCLA. The NMR and mass spectrometers were purchased with the aid of financial support from the National Science Foundation and E.I. du Pont de Nemours and Co.