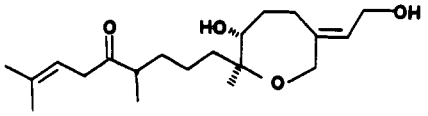
Journal of Natural Products
Vol. 49, No. 2, pp. 313-317, Mar-Apr 1986

STUDIES ON ZOAPATLE, II. LEUCANTHANOLIDE, A NOVEL SESQUITERPENE LACTONE FROM *MONTANOA LEUCANTHA* SSP. *LEUCANTHA*[1]

YOSHITERU OSHIMA,[2] SIU-MING WONG, CHOHACHI KONNO, GEOFFREY A. CORDELL,* DONALD P. WALLER, D. DOEL SOEJARTO, and HARRY H.S. FONG

Program for Collaborative Research in the Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612

The tea prepared from the leaves of *Montanoa tomentosa* Cerv. (Compositae), the zoapatle plant, reportedly has been used in Mexico for more than 400 years to induce menses and labor, and to terminate early unwanted pregnancy (2-4). A number of reports have appeared in the literature concerning the isolation, structure elucidation, biological effects, and synthesis of zoapatanol (**1**), an oxepane diterpene claimed to be responsible for the fertility regulating effect of the tea (2,3,5-9). The potential development of zoapatanol for use in reproductive medicine has also been reviewed (3,10,11).



1 Zoapatanol

In an earlier study of seven species or subspecies of *Montanoa* cultivated at the University of Illinois Pharmacognosy Field Station, we have shown that *Montanoa leucantha* (Lag.) Blake ssp. *leucantha* (Compositae) contained the highest concentration of zoapatanol (ca. 0.04%) by hplc analysis (1,11). An attempt was made, therefore, to isolate zoapatanol from this source for biological studies. Due to the complexity of the zoapatanol-containing fraction, however, the isolation of the desired compound proved rather difficult. During the course of this investigation, a novel sesquiterpene lactone was isolated and given the trivial name leucanthanolide. We describe here the structure elucidation and biological evaluation of leucanthanolide (**2**).

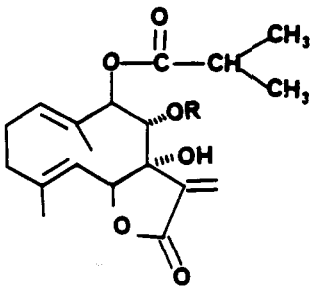
The mass spectrum established **2** to have a molecular ion at *m/z* 350 for a molecular formula $C_{19}H_{26}O_6$, and its ir spectrum showed strong bands at 3456, 1761, and 1713 cm^{-1} associated with hydroxyl, five-membered lactone and ester group absorbances, respectively. In the ^{1}H-nmr spectrum of **2**, two broad singlets at δ 1.65 and 1.73, and two singlets at 6.29 and 6.47 revealed the presence of two olefinic methyl groups and a terminal methylene moiety. Moreover, ^{1}H-nmr signals assignable to olefinic and methine protons were observed at δ 5.23 (br t, J=8.1 Hz), 4.64 (br d, J=10.4 Hz), 4.98 (d, J=10.4 Hz), 4.07 (br d, J=9.0 Hz) and 5.04 (br t, J=9.0 Hz), corresponding well with those of **3** isolated from *Montanoa atriplicifolia* (12). These spectral data suggested that **2** had the same germacradienolide skeleton as in **3**.

The ^{1}H-nmr signals due to the ester side chain were observed at δ 2.56 (septet J=6.8 Hz) and 1.15 (d, J=6.8 Hz), which, together with the fragment ions at *m/z* 280 $(M-C_4H_6O)^+$, 262 $(M-C_4H_8O_2)^+$, 71 $(C_4H_7O)^+$, and 43 $(C_3H_7)^+$, indicated the presence of an isobutyryloxy group. Leucanthanolide was therefore tentatively assigned the structure **2**, in which it remained to unambiguously demonstrate the various regio- and stereo-chemical attributions.

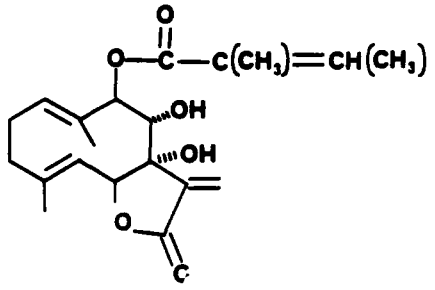
The isolate exhibited a strong nega-

[1]For the previous paper in this series, see Marcelle *et al.* (1).

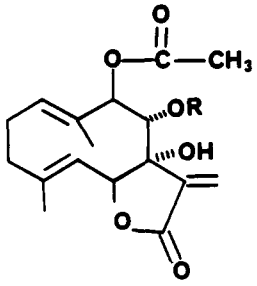
[2]Present address: Pharmaceutical Institute, Tohoku University, Aobayama 980, Sendai, Japan.



2 Leucanthanolide, R=H
3 Leucanthanolide Acetate, R=OAc



4



5 R=Epoxang

tive Cotton effect around 220 nm due to the interaction of the two macrocyclic double bonds and a positive Cotton effect near 260 nm associated with the n-π^* transition of the α,β-unsaturated lactone. These Cotton effects, together with the fact that the resonance signals in the ^{13}C-nmr spectrum of the monoacetate **4** were very close to those of the germacranolide lactone monoacetate **5** isolated from *Montanoa hibiscifolia* (13), demonstrated that **2** adopted the same conformation and had the same configuration as the germacradienolides **3** and **5** mentioned above.

A more detailed analysis of the ^{1}H-nmr spectrum could be obtained from the homonuclear two-dimensional *J*-correlation spectrum, and the results of this analysis are presented in Figure 1 and Table 1. In this way, the placement of the ester at C-9 rather than C-8 was made as follows.

Allylic coupling was observed between the C-15 methyl protons and the doublet at δ 4.64 attributed to H-5. Coupling between the doublet at δ 4.64 and the doublet at δ 4.98 showed that the latter signal belonged to H-6. Dreiding models indicated that long range coupling through a **W** relationship may be possible between H-6α and H-8β. Inasmuch as the 2-D COSY experiment indicated a broad doublet at δ 4.07 was, indeed, weakly coupled to H-6, this should be the 8β-proton. This resonance at δ 4.07 also exhibited coupling (J=8.3 Hz) to a proton at δ 5.04 which can be assigned to H-9α.

All 19 carbon signals were clearly displayed, and the APT spectrum permitted a preliminary assignment of the protonated and quaternary carbons. A distinction between C-10 and C-11 was made on the basis that, in the coupled carbon spectrum, the signal at δ 139.8 (C-10) was substantially broader than the signal at δ 140.44 (C-11). Irradiation of the H-2 signal caused a sharpening of the signal at δ 139.8, while irradiation of the H-6 signal did not result in any change. Completion of the assignment of the ^{13}C-nmr spectrum was made through the acquisition of a heteronuclear correlation spectrum, and these data are summarized in Table 1. Of particular

TABLE 1. Nuclear Magnetic Resonance Assignments of Leucanthanolide (**2**)

^{1}H-nmr[a]			^{13}C-nmr[a]	
Signal[b]	Assignment	Multiplicity; Coupling Constant	Signal[b]	Assignment
5.23	H-1	br, t; 8.1	129.57	C-1
2.24	H_2-2	m	25.37	C-2
1.96	H-3e	dd; 8.6, 19.4	39.01	C-3
2.31	H-3a	m		
			136.78	C-4
4.64	H-5	bd d; 10.5	122.13	C-5
4.98	H-6	bd d; 10.5	83.46	C-6
			81.61	C-7
4.07	H-8	d; 8.3	74.66	C-8
5.04	H-9	d; 8.3	79.21	C-9
			139.86	C-10
			140.44	C-11
			169.14	C-12
6.29	H-13	s	128.62	C-13
6.47	H-13	s		
1.65	H_3-14	s	18.02	C-14
1.73	H_3-15	s	17.17	C-15
			176.43	C-1′
2.56	H-2′	septet; 6.8	33.85	C-2′
1.15	H_3-3′	d; 6.8	18.57	C-3′
1.15	H_3-4′	d; 6.8	18.71	C-4′

[a]Obtained in $CDCl_3$, TMS as internal standard.
[b]δ values, TMS=0 ppm.

interest was the facile distinction between the four methyl carbon resonances in the region δ 17.17-18.71. These spectroscopic data firmly establish the structure and stereochemistry of leucanthanolide to be that shown in **2**.

Evaluation of the isolate in a pregnant guinea pig assay showed the compound to be devoid of activity. Both the isolate and extract were cytotoxic in the in vitro KB and P-388 test systems.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting point was determined by means of a Kofler hotplate and is uncorrected. The uv spectrum was obtained with a Beckman model DB-G grating spectrometer. The ir spectrum was determined on a Nicolet MX-1 interferometer. ^{1}H-nmr spectra were recorded on a Nicolet NMC 360 (360 MHz) with a Nicolet Fourier-Transform attachment using $CDCl_3$ as solvent and TMS as an internal standard. The ^{13}C-nmr spectrum was obtained on the Nicolet NMC 360 instrument operating at 90.8 MHz. The mass spectra were obtained with a Varian MAT 112S double focusing spectrometer operating at 70 eV. Optical rotations were measured with Perkin-Elmer, Model 241 polarimeter. Silica gel for chromatography was purchased from E. Merck, Darmstadt, W. Germany, and preparative tlc plates were from Analtech, Newark, NJ.

PLANT MATERIAL.—The plant material used in this investigation was originally obtained in 1979 from Dr. V.A. Funk of the Ohio State University. It was cultivated at our Pharmacognosy Field Station, Lisle, IL, in the summer of 1982. A herbarium specimen has been deposited at the Field Museum of Natural History, Chicago, IL.

EXTRACTION, FRACTIONATION AND ISOLATION.—The leaves of *M. leucantha* ssp. *leucantha* (1.5 kg) were exhaustively extracted with EtOAc at room temperature and the solvent removed in vacuo to afford an extract (138 g). Chromatography of this extract on silica gel (500 g) eluting successively, through gradient elution, with C_6H_6, C_6H_6-EtOAc (1:1), EtOAc, and MeOH afforded seven fractions. Fractions 4 and 5 (15.9 g) from the column, eluted with C_6H_6-EtOAc (1:1) were rechromatographed over silica gel (200 g) using $CHCl_3$/iPrOH mixtures. A fraction (2.7 g) from this separation was submitted to further silica gel column chromatography using hexane/EtOAc mixtures as a solvent to afford **2** (80 mg) as a colorless solid, mp 85-87°; $[\alpha]_D$ −153.3° (c

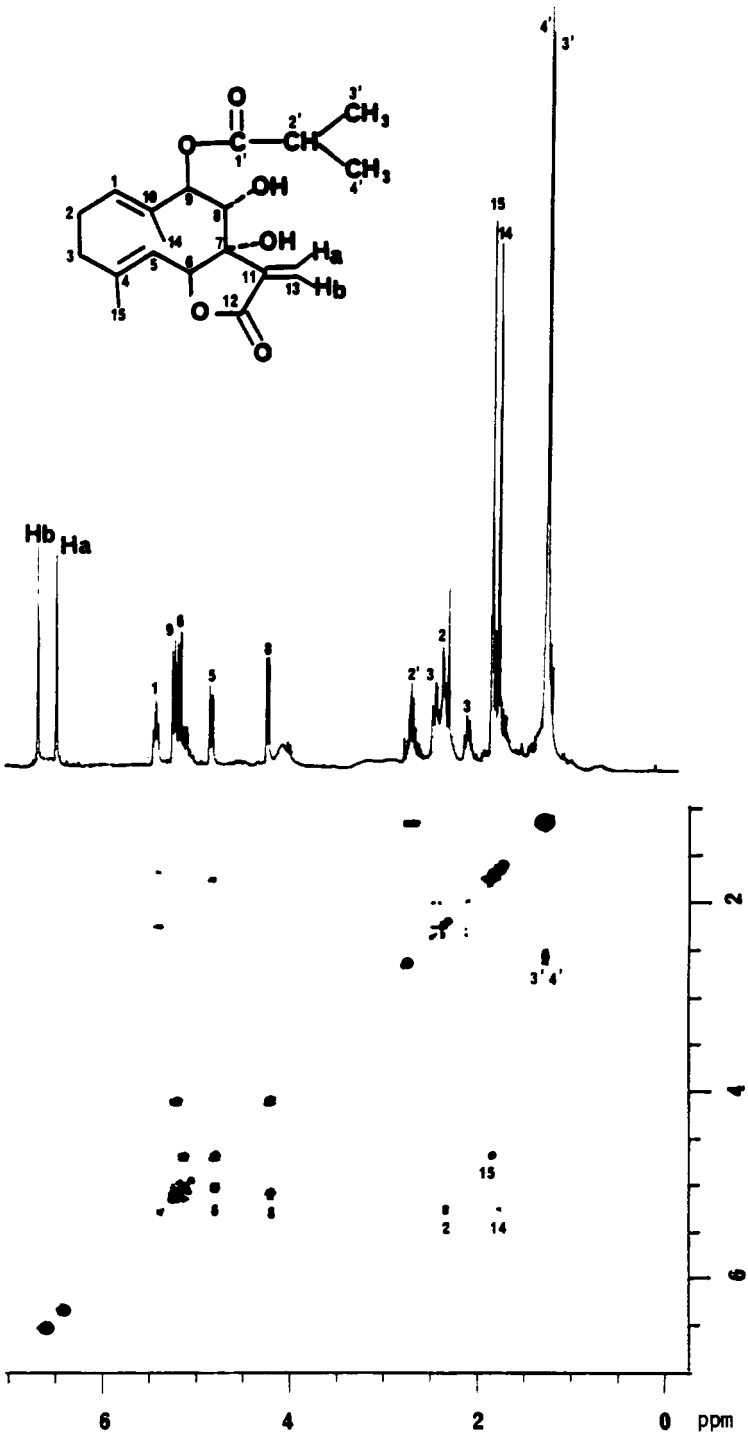


FIGURE 1. Homonuclear 2-D Nmr of Leucanthanolide.

1.18, $CHCl_3$); uv λ max (MeOH) (ε) 213 nm (10,200); ir ν max (neat) 3456, 1761, and 1713 cm^{-1}; cd (MeOH) 263 [θ] +1,860 and 219 [θ] −113,000; ^1H nmr (360 MHz, $CDCl_3$) see Table 1; ^{13}C nmr (90.80 MHz, $CDCl_3$) see Table 1; ms *m/z* (rel. int.) 350 (M^+, 1); 280 (2), 262 (2), 244 (6), 71 (70), and 43 (100).

ACETYLATION OF **2**.—A sample of **2** (10 mg) was treated with Ac_2O-pyridine (1:2, 0.3 ml) at

room temperature overnight. Workup in the usual way afforded a monoacetate derivative **3** (10 mg) as a colorless solid having the following spectroscopic properties; ^{1}H nmr (360 MHz, $CDCl_3$) δ 1.075 (6H, d, J=6.8 Hz), 1.690 (3H, br s), 1.702 (3H, br s), 1.918 (3H, s), 2.10-2.35 (4H, m), 2.437 (1H, septet, J=6.8 Hz), 3.296 (1H, br s, OH), 4.920 (2H, m), 5.250 (2H, s), 6.316 (1H, m), 5.932 (1H, s), and 6.412 (1H, s); ^{13}C nmr (90 MHz, $CDCl_3$) δ 175.97 (C-1′), 169.70 (C-12), 167.68 ($COCH_3$), 142.20 (C-11), 140.04 (C-10), 135.68 (C-4), 131.63 (C-1), 127.19 (C-13), 122.51 (C-5), 83.64 (C-6), 82.61 (C-7), 76.45 (C-8), 74.48 (C-9), 39.10 (C-3), 34.13 (C-2′), 25.26 (C-2), 21.11 ($COCH_3$), 18.96 (C-3′), 18.70 (C-4′), 18.04 (C-14), and 17.04 (C-15); ms *m/z* (rel. int.) 392 (M^+, 2), 350 (1), 32 (1), 71 (43), and 43 (100).

BIOLOGICAL EVALUATIONS.—*Fertility regulating.*—Leucanthanolide (**2**) was evaluated for uteroevacuant activity in a pregnant-guinea pig assay modified after the method of Hahn *et al.* (9). The compound was solubilized as its polyvinylpyrrolidone (PVP) co-precipitate (1:4) (12), administered as a single ip injection at a dose of 20 mg/kg to six guinea pigs on day 22 of gestation. All animals were observed daily for any signs of pregnancy interruption. On day 27 of gestation, all animals were autopsied. One of the treated animals was nonpregnant and was deleted from the assay (no CLP, no implantation sites). Two of the pregnancies were totally normal. Three of the pregnancies had abnormal fetuses, but no clearly identifiable activity was observed.

Cytotoxic activity.—The EtOAc extract was active (14) in the KB and P-388 test systems in cell culture, showing ED_{50} 1.35 μg/ml and 5.2 μg/ml, respectively. Leucanthanolide (**2**) also displayed activity in these in vitro systems showing ED_{50} 0.57 μg/ml (KB) and 0.93 μg/ml (P-388).

ACKNOWLEDGMENTS

This work was supported, in part, by the Special Programme of Research, Development and Research Training in Human Reproduction, World Health Organization (HRP Project 77918C).

The authors gratefully acknowledge the Nuclear Magnetic Resonance Laboratory of the Research Resources Center, University of Illinois at Chicago, which provided facilities and assistance necessary in order to conduct the spectroscopic aspects of this study.

LITERATURE CITED

1. G.B. Marcelle, N. Bunyapraphatsara, G.A. Cordell, and H.H.S. Fong, *J. Nat. Prod.*, (in press).
2. S.D. Levine, R.E. Adams, R. Chen, M.L. Cotter, A.F. Hirsch, V.V. Kane, R.M. Kanojia, C. Shaw, M.P. Wachter, E. Chin, E. Huettemann, P. Ostrowski, J.L. Mateos, L. Noriega, A. Guzman, A. Maijarez, and L. Tovar, *J. Am. Chem. Soc.*, **101**, 3404 (1979).
3. S.D. Levine, D.W. Hahn, M.L. Cotter, F.C. Greenslade, R.M. Kanojia, S.A. Pasquale, M. Wachter, and J.L. McGuire, *J. Reprod. Med.*, **26**, 524 (1981).
4. A.J. Gallegos, *Contraception*, **27**, 211 (1983).
5. R.M. Kanojia, M.P. Wachter, S.D. Levine, R.E. Adams, R. Chen, E. Chin, M.L. Cotter, A.F. Hirsch, R. Huettemann, V.V. Kane, P. Ostrowski, and C.J. Shaw, *J. Org. Chem.*, **47**, 1310 (1982).
6. R. Chen and D.A. Rowand, *J. Am. Chem. Soc.*, **102**, 6609 (1980).
7. K.C. Nicolaou, D.A. Claremon, and W.E. Barnette, *J. Am. Chem. Soc.*, **102**, 6611 (1980).
8. V.V. Kane and D. Doyle, *Tetrahedron Lett.*, **22**, 3031 (1981).
9. D.W. Hahn, E.W. Ericson, M.T. Lai, and A. Probst, *Contraception*, **23**, 133 (1981).
10. N.R. Farnsworth, H.H.S. Fong, and E. Diczfalusy, in: "Proc. Internat. Symposium on Research for the Regulation of Human Fertility, Stockholm, Sweden, Feb. 1983." Ed. by E. Diczfalusy and A. Diczfalusy, Scriptor, Copenhagen, 1983, p. 776.
11. H.H.S. Fong, in: "Natural Products and Drug Development, Alfred Benzon Symposium 20." Ed. by P. Krosgaard-Larsen, S. Brøgger Christensen, and H. Kofod, Munksgaard, Copenhagen, 1984, p. 355.
12. F. Bohlmann, V. Castro, and J. Jakupovic, *Phytochemistry*, **22**, 1233 (1983).
13. W. Herz, S.V. Govindan, and J.F. Blount, *J. Org. Chem.*, **45**, 1113 (1980).
14. R.I. Geran, N.H. Greenberg, M.M. MacDonald, A.M. Schumacher, and B.J. Abbott, *Cancer Chemother. Rep.*, **3**(2), 1 (1972).

Received 28 May 1985