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LONGIPILIN, A NEW MELAMPOLIDE FROM *MELAMPODIUM LONGIPILUM*

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Key Word Index—*Melampodium longipilum*; Heliantheae; Compositae; melampolide; sesquiterpene lactone.

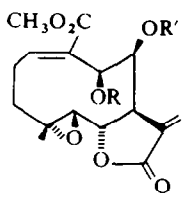
INTRODUCTION

The sesquiterpene lactone analysis of *Melampodium longipilum* yielded two constituents, the known melampolide enhydrin (**1a**) and a new lactone which we named longipilin. Enhydrin was first isolated from *Enhydra fluctuans* [1] and subsequently found in *Melampodium perfoliatum* [2] and *Polymnia uvedalia* [3]. We report here the isolation and structural elucidation of longipilin, which has also been found more recently in *Tetragonatheca repanda* [4].

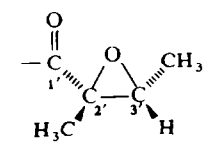
RESULTS AND DISCUSSION

The structure of longipilin (**1b**) $C_{21}H_{26}O_8$ (high resolution MS) was deduced by comparison of NMR and MS data with the enhydrin spectral parameters. The low resolution MS of longipilin displayed, besides a parent peak at *m/e* 406, a peak at *m/e* 388 (M − H_2O), indicating the presence of a hydroxyl. Together with the diagnostic NMR signals, the base peak at *m/e* 83 (C_5H_7O) and peaks at *m/e* 323 (M − C_5H_7O), *m/e* 55 (C_4H_7), and *m/e* 306 (M − $C_5H_8O_2$) suggested an angeloyl side chain in **1b**.

The 100 MHz NMR spectrum of longipilin (Table 1) displayed two single proton doublets at 6.33 (J = 3.5 Hz) and 5.80 (J = 3.5 Hz) and a broad multiplet at 2.90 ppm that are characteristic of α,β-unsaturated γ-lactones. The spectrum closely resembled that of enhydrin except for (1) the appearance of peaks diagnostic for an angeloyl side chain in longipilin instead of an 8-epoxyangeloyl moiety in **1a** and (2) the different chemical shifts associated with the replacement of the C-9 acetate by a hydroxyl group. Accordingly, the H-8 and H-9 signals, located at 6.85 ppm and 5.86 ppm, respectively, in enhydrin (**1a**) were shifted upfield to 6.39 ppm and 4.45 ppm, respectively



1a R = Ac, R′ =



1b R = H, R′ = $-C(=O)-C(CH_3)=CH-CH_3$

1c R = Ac, R′ = $-C(=O)-C(CH_3)=CH-CH_3$

Table 1. ^{1}H-NMR parameters* of longipilin (**1b**) and longipilin acetate (**1c**)

	1b	**1c**
H-1	7.01 *dd* (9.07; 7.5)	7.01 *dd* (9.0; 7.5)
H-2α	3.31 *m*	3.31 *m*
H-2β	*ca* 2.52 *m*	*ca* 2.52 *m*
H-3α	1.22 *m*	1.22 *m*
H-3β	2.58 *m*	2.58 *m*
H-5	2.69 *d* (9.5)	2.69 *d* (9.5)
H-6	4.27 *dd* (9.5; 10.0)	4.29 *dd* (9.5; 10.0)
H-7	2.90 *m*	2.95 *m*
H-8	6.39 *dd* (1.5; 9.0)	6.76 *dd* (1.5; 9.0)
H-9	4.45 d	5.87 *d* (8.5)
C-9-O<u>H</u>	2.98 *br*	
H-13a	5.80 (3.5)	5.92 *d* (3.5)
H-13b	6.33 *d* (3.5)	6.36 *d* (3.5)
C-4-C<u>H</u>$_3$	1.58 *s*	1.69 *s*
H-3′	6.15 *qq* (1.5; 7.0)	6.11 *qq* (1.5; 7.0)
C-2′-CH$_3$	1.85 *m*	1.85 *m*
C-3′-CH$_3$	1.97 *dq*	1.97 *dq*
—COOC<u>H</u>$_3$	3.84 *s*	3.84 *s*

* Run at 270 MHz in $CDCl_3$ with TMS as internal standard. Chemical shifts are given in δ-values in ppm. Figures in parentheses are coupling constants in Hz or line separations.

in longipilin. In addition, there was a corresponding appearance of a hydroxyl proton signal at 2.98 ppm due to C-9-O<u>H</u>. As expected, the acetate (**1c**) produced NMR chemical shifts for H-8 (6.76 ppm) and H-9 (5.87 ppm) very similar to those of enhydrin.

Double irradiation experiments were used in determining the proton resonance assignments of longipilin. Irradiation of the multiplet at 2.90 ppm (H-7) reduced the paired doublets at 5.80 ppm (H-13a) and 6.33 ppm (H-13b) to singlets, collapsed at triplet at 4.27 ppm (H-6) to a doublet ($J_{5,6}$ = 9 Hz), and changed the doublet of doublets at 6.39 ppm to a doublet ($J_{8,9}$ = 8.0 Hz). When the H-6 triplet at 4.27 ppm was irradiated the H-7 multiplet at 2.90 ppm sharpened and the H-5 doublet at 2.69 ppm collapsed to a broadened singlet. Irradiation at 6.39 ppm (H-8) reduced the H-7 multiplet to a doublet of a doublet ($J_{7,13}$ = 3.5 Hz, $J_{6,7}$ = 9.5 Hz) and collapsed the H-9 doublet at 4.45 ppm to a singlet. The H-8 signal at 6.39 ppm simplified to a doublet ($J_{7,8}$ = 1.5) when the H-9 doublet at 4.45 ppm was irradiated.

Irradiation at 1.85 ppm (C-2′ methyl) changed the 3′-H multiplet to a quartet ($J_{3',3'\text{-}CH_3}$ = 4.0 Hz). When the region containing both the C-2′ and C-3′ methyl signals was irradiated the 3′-H multiplet collapsed to a singlet.

^{13}C-NMR data (see Experimental) obtained under proton noise decoupling and single-frequency off-center decoupled conditions and the ^{13}C chemical shifts are in agreement with the above NMR and MS results.

The NMR spectra assignments, the MS fragmentation patterns and CD-data are in full agreement with a melampolide skeleton as shown for **1b**. On the basis of the similarity of the NMR parameters, longipilin (**1b**) should exhibit the same configurational and conformational relationships as enhydrin (**1a**), the structure of which has been established by X-ray diffraction [5].

EXPERIMENTAL

Melampodium longipilum (Hartman-Funk No. 4151; Mexico: Puebla: roadside 1 mile S of Ascuncion de Chila) leaves and stems (800 g) were extracted in 3 l. $CHCl_3$. After the standard workup [6], 8.9 g crude syrup were obtained which yielded upon titration with iso-PrOH 2.0 g enhydrin. The remaining syrup was chromatographed over 250 g Si gel, using $CHCl_3$–*n*-propyl acetate (6:4) as eluant. Longipilin (40 mg) crystallized readily from fractions 35–50 when treated with *iso*-PrOH–petrol mp 170–173°; λ_{max}^{MeOH} 213 nm (ε, 2.1 × 10^4); CD (c 4.93 × 10^{-5}, MeOH): $[\theta]_{216} = -5.38 \times 10^4$, $[\theta]_{247} = 7.1 \times 10^3$; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (*br*.OH), 1770 (γ-lactone), 1710 (ester). The low resolution MS exhibited significant peaks at *m/e* (rel. int): 406 (2.4, M^+), 388 (1.7, M − H_2O), 375 (0.5, M − OCH_3), 323 (2.5, M − C_5H_7O), 306 (5.1, M − $C_5H_8O_2$), 291 (6.8, M − $C_5H_8O_2$ − CH_3), 83 (100, C_5H_7O). ^{13}C NMR ($CDCl_3$) 168.32, 167.32, 166.54 (>C=O); 145.31, 140.21 (—CH=); 133.92, 133.33 (>C=); 122.50 (=CH_2); 76.28, 72.24, 70.95, 62.80 (HCO); 59.18 (>CO); 52.40 (OCH_3); 46.07 (>CH); 35.44, 24.71 (>CH_2); 20.51, 17.72, 15.92 (—CH_3). (Calc. for $C_{21}H_{26}O_8$: MW, 406.1628. Found: MW (MS), 406.1628).

Longipilin acetate (**1c**) (30 mg) was prepared from 40 mg **1b** in 1 ml Py and 1 ml Ac_2O under standard conditions: oil; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1770 (γ-lactone) 1735 and 1245 (acetate) and 1710 (ester).

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13-HYDROXYBALLONIGRINOLIDE, A NEW DITERPENOID FROM *BALLOTA LANATA*

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Key Word Index—*Ballota lanata*, Labiatae; diterpenoid, ballonigrin.

We have described the isolation of a number of new bicyclic diterpenoids from *Ballota* (Labiatae) species including *B. nigra* [1, 2], *B. rupestris* [3] and *B. acetobulosa* [4]. In continuation of this work we have examined *B. lanata* which is used as a herbal medicine, Wolfstrappkraut. Chromatography of the acetone extract afforded ballonigrin (**1**) previously obtained from *B. nigra* [1, 2] and *B. rupestris* [3], together with a new diterpenoid, $C_{20}H_{26}O_6$. The IR and UV spectra showed that the oxygen functions were disposed as a hydroxyl, two γ-lactones and an αβ-unsaturated ketone implying a similarity to ballonigrin. However the spectra lacked absorption associated with the furan ring. The ^1H and ^{13}C NMR spectra (Table 1) led to the structure **2** for the diterpenoid, 13-hydroxyballonigrinolide. The ^1H NMR spectrum contained two tertiary C Me resonances and one olefinic C-Me resonance whilst spin decoupling of two ^1H doublets (δ 4.95 and 2.18, J = 8 Hz) established their vicinal relationship as H(6) and H(5), respectively. The spectrum also contained an AB quartet, δ 4.36 and 4.57, associated with a —C(|)—CH_2—O—C=O grouping together with a two proton singlet, δ 2.94, which was assigned to a —C(|)—CH_2—C=O grouping. The ^{13}C NMR spectrum showed marked similarities to that of ballonigrin [1, 2] and rupestralic acid (**3**) [5]. In particular the SFORD showed that the additional hydroxyl group was tertiary in character and was associated with the side chain. The ^{13}C NMR spectrum may be interpreted in terms of structure **2**.

The MS of 13-hydroxyballongrinolide showed substantial ions at *m/e* 237 and 219 associated with the fragment **4** and its dehydration product. Two other major ions at *m/e* 233 (**5**) and 109 (**6**) are associated with the bicyclic ring systen and provide strong evidence for the location of the functional groups.

The isolation of this compound with a $\Delta^{8,9}$-double