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MELAMPOLIDES FROM *LECOCARPUS PINNATIFIDUS*

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Key Word Index—*Lecocarpus pinnatifidus*; Asteraceae; Heliantheae; Melampodiinae; sesquiterpene lactones; melampolides; flavonol; penduletin.

Abstract—Chemical analysis of *Lecocarpus pinnatifidus* afforded 11 melampolide-type sesquiterpene lactones and the known flavonoid penduletin. Eight lactones, which were named lecocarpinolides A–H, are new. Their structures were elucidated by spectroscopic methods and chemical transformations. The biochemical systematic implications of these findings are discussed briefly.

INTRODUCTION

Lecocarpus of the tribe Heliantheae, subtribe Melampodiinae (Asteraceae) is a small genus of shrubs endemic to the Galápagos Islands. Based on morphological grounds, it is closely related to the genera *Acanthospermum* and *Melampodium*, which are common on the American continent [1]. *Lecocarpus pinnatifidus* was established by Decaisne (1846) as a monotypic genus [2]. Cronquist and Stuessy [3] and Eliasson [4] recognised three species: *L. pinnatifidus* from Isla Santa Maria (Charles Island), *L. lecocarpoides* from Isla Española (Hood Island) and Gardner (Gardner near Hood) and *L. leptolobus* from Isla San Cristobal (Chatham Island). Based on new collections and extensive field work, Adsersen more recently revised this genus [5] recognising *L. pinnatifidus* Decne, an endemic of Isla Santa Maria, *L. lecocarpoides* from Isla Española and adjacent Gardner and *L. darwinii* sp. nov. from Isla San Cristobal. For the plant material used for our chemical studies the nomenclature by Adsersen [5] is used.

In continuation of our biochemical systematic study within the subtribe Melampodiinae [6] we have analysed *L. pinnatifidus* for its sesquiterpene lactones. We wished to learn whether the close morphological relationships between the genera *Melampodium*, *Acanthospermum* and *Lecocarpus* are supported by the chemical data using sesquiterpene lactones as systematic markers. In spite of the limited amount of plant material (< 50 g) of this rare species, 11 melampolide-type sesquiterpene lactones were identified by spectroscopic and chemical methods. Eight of the lactones were new; they were named lecocarpinolide A–H. Their structural elucidcations are described herein.

RESULTS AND DISCUSSION

Extraction of the aerial parts of *L. pinnatifidus* with CH_2Cl_2 afforded, after chromatography, 11 melampolide-type sesquiterpene lactones of increasing polarity, the known 15-hydroxy-8β-(2-methylbutyryloxy)-14-oxo-acanthospermolide (**1**) [7], lecocarpinolide A (**2**) acanthospermal B (**3**) [8], lecocarpinolides B–E (**4–7**) 9α,15-dihydroxy-8β-(2-methylbutyryloxy)-14-oxo-acanthospermolide (**8**) [7], lecocarpinolides F–H (**9–11**) and the flavonoid penduletin (**12**) [9]. Since the ^{13}C NMR spectral data of compounds **1** and **8** had not been previously reported, they are included in Table 2. Also, the ^{1}H NMR spectral data for lactones **1** and **8** are given in Table 1 since they include revisions of previous assignments [7].

Lecocarpinolide A (2) is a gum with a $[M]^+$ at *m/z* 360 which together with the ^{1}H and ^{13}C NMR data (Tables 1 and 2) was in agreement with the molecular formula $C_{20}H_{24}O_6$. Additional mass spectral peaks at *m/z* 260 $[M-A]^+$, 83 $[A^1]^+$ and 55 $[A^2]^+$ as well as ^{1}H NMR resonances at δ1.84 *dq*, 1.98 *dq*, 6.11 *qq* and ^{13}C NMR signals at δ15.82 *q* and 20.53 *q* were diagnostic of an angelate moiety [10]. The ^{1}H NMR data of **2** (Table 1) were similar to those of structurally related melampolides [7]. The presence of a 1,(10)-*cis* double bond with an aldehyde group at C-10 followed from characteristic chemical shifts of the aldehydic H-14 (δ9.47 *d*), and H-1 (δ6.64 *dd*). The 4,5-double bond was assigned a *trans*-configuration on the basis of typical chemical shift of H-5 (5.18 *d*), H-6 (5.24 *dd*) and large couplings ($J_{6,7}$ = 10.5 Hz) [7]. The ^{1}H NMR spectrum also suggested a hydroxyl group at C-15, as indicated by doublets of doublets at δ4.52 and 4.31 which were assigned to the geminal C-15 protons. The angelate moiety must be at C-8 since H-8 appeared at δ6.46 (*ddd*). The small coupling value $J_{7,8\alpha}$ (1.4 Hz) required an α-orientation of H-8, the strongly deshielded H-8 being in close proximity of the aldehyde carbonyl at C-10 [11]. Signals typical for an α-methylene-γ-lactone moiety appeared as two one-proton doublets at δ6.26 (H-13b) and 5.62 (H-13a), both being coupled to a one-proton multiplet at 2.48 (H-7). Strong IR absorptions at 3462, 1765, 1718 and 1680 cm^{-1} corroborated the presence of hydroxyls, a γ-lactone moiety, an unsaturated ester and an α,β-unsaturated aldehyde group, respectively. The ^{1}H NMR 2D COSY spectrum of **2** showed two series of protons couplings, H-1 (*dd*, 6.64) with H-2α (*dddd*, 2.39) and H-2β (*dddd*, 2.56), the two C-2 protons with H-3α (*ddd*, 2.04) and H-3β (*ddd*, 2.83), H-6 (*dd*, 5.24)

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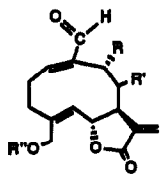
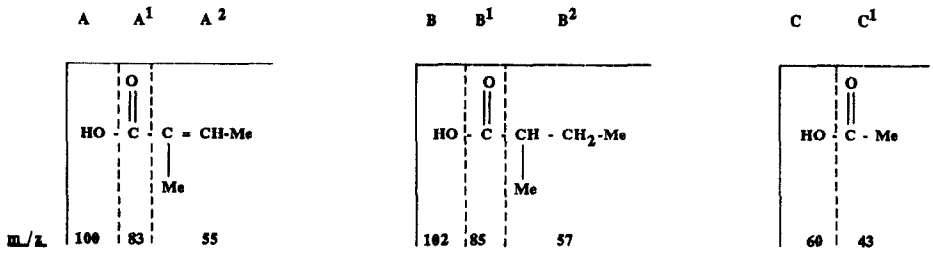
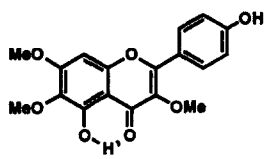


LECOCARPINOLIDES A-H

		R	R'	R''
	1	H	β-OMeBu	H
	1a	H	β-OMeBu	Ac
L-A	2	H	β-OAng	H
	2a	H	β-OAng	Ac
	3	OAc	β-OMeBu	H
	3a	OAc	β-OMeBu	Ac
L-B	4	OAc	β-OAng	H
	4a	OAc	β-OAng	Ac
L-C	5	H	α-OMeBu	H
L-D	6	H	α-OAng	H
L-E	7	OAc	β-OH	H
	7a	OAc	β-OAc	Ac
	8	OH	β-OMeBu	H
L-F	9	OH	β-OAng	H
L-G	10	OMeBu	β-OH	H
	10a	OH	β-OH	H
L-H	11	H	β-OH	H





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with H-5 (*d*, 5.18) and H-7 (*dddd*, 2.48), as well as H-8α (*ddd*, 6.37) with H-7, H-9α (*dd*, 2.66) and H-9β (*ddd*, 2.01). These findings required that the hydroxyl group had to be at C-15 as found in related melampolides [12]. The stereochemistry at C-6 was derived from the couplings between H-5, H-6 and H-7 ($J_{5,6}=J_{6,7}=10.5$ Hz) which were in agreement with a *trans*-diaxial orientation of these three protons. Assuming that H-7 is α as in all lactones from higher plants [13] the hydrogens at C-5 and C-6 had to be α and β, respectively. This was further substantiated by NOE difference experiments which showed effects between H-7 and H-8 and between H-5

Table 1. ^{1}H NMR spectral data of compounds **2**, **4–7**, **9–11**, **2a**, **4a**, **7a** and **10a** (400 MHz, $CDCl_3$, TMS as int. std)

H	**2**	**2a**	**4**	**4a**	**5**	**6**	**7**	**7a**	**9**	**10**	**10a**	**11**
1	6.64 *dd*	6.63 *dd*	6.85 *dd*	6.79 *dd*	6.62 *dd*	6.63 *dd*	6.75 *dd*	6.72 *dd*	6.67 *dd*	6.61 *dd*	6.60 *dd*	6.61 *dd*
2α	2.39 *dddd*	2.42–2.31 *m**	2.66 *dddd*	2.68 *dddd*	2.71 *dddd*	2.71 *dddd*	2.66 *dddd*	2.68 *dddd*	2.73–2.62 *m**	2.50 *dddd*	2.50 *dddd*	2.51 *dddd*
2β	2.56 *dddd*	2.57 *dddd*	2.81 *dddd*	2.94 *dddd*	2.84 *dddd*	2.86 *dddd*	2.95 *dddd*	2.96 *dddd*	2.57 *dddd*	2.79 *dddd*	2.78 *dddd*	2.79 *dddd*
3α	2.04 *ddd*	2.01 *ddd*	1.98 *ddd*	2.08 *ddd*	2.46 *ddd*	2.47 *ddd*	2.01 *ddd*	2.04 *ddd*	2.01 *ddd*	2.04 *ddd*	2.04 *ddd*	2.04 *ddd*
3β	2.83 *ddd*	2.68 *ddd*	2.91 *ddd*	2.76 *ddd*	3.09–2.94 *m**	3.09–2.94 *m**	2.81 *ddd*	2.68 *ddd*	2.84 *ddd*	2.72 *ddd*	2.68 *ddd*	2.72 *ddd*
5	5.18 *d*	5.29 *d*	5.03 *d*	5.12 *d*	5.54 *d*	5.56 *d*	5.01 *d*	5.09 *d*	5.02 *d*	5.17 *d*	5.17 *d*	5.16 *d*
6β	5.24 *dd*	5.15 *dd*	5.28 *dd*	5.18 *dd*	5.46 *dd*	5.48 *dd*	5.41 *dd*	5.32 *dd*	5.23 *dd*	5.31 *dd*	5.32 *dd*	5.31 *dd*
7α	2.48 *dddd*	2.50 *dddd*	2.63 *dddd*	2.64 *dddd*	2.65 *dddd*	2.65 *dddd*	2.45 *dddd*	2.46 *dddd*	2.73–2.62 *m**	2.49 *dddd*	2.47 *dddd*	2.32 *dddd*
8α	6.46 *ddd*	6.47 *ddd*	6.78 *dd*	6.75 *dd*	—	—	5.24 *br d*	6.68 *dd*	6.38 *dd*	5.46 *dd*	5.44 *dd*	5.20 *ddd*
8β	—	—	—	—	5.91 *ddd*	5.93 *ddd*	—	—	—	—	—	—
9α	2.71 *br dd*	2.88 *dd*	—	—	2.94 *dd*	3.00 *dd*	—	—	—	—	—	2.70 *dd*
9β	2.11 *ddd*	2.11 *m**	5.33 *dd*	5.35 *dd*	2.54 *ddd*	2.55 *ddd*	5.29 *dd*	5.30 *dd*	4.11 *dd*	5.56 *br d*	4.11 *dd*	2.21 *ddd*
13a	5.62 *d*	5.63 *d*	5.81 *d*	5.87 *d*	5.69 *d*	5.70 *d*	5.64 *d*	5.80 *d*	5.67 *d*	5.68 *d*	5.68 *d*	5.59 *d*
13b	6.26 *d*	6.26 *d*	5.24 *d*	6.31 *d*	6.32 *d*	6.34 *d*	6.35 *d*	6.34 *d*	6.27 *d*	6.42 *d*	6.41 *d*	6.34 *d*
14	9.47 *d*	9.47 *d*	9.48 *d*	9.50 *d*	9.40 *d*	9.41 *d*	9.44 *d*	9.45 *d*	9.50 *d*	9.40 *d*	9.41 *d*	9.45 *d*
15	4.52 *dd*	4.83 *d*	4.53 *d*	4.93 *d*	4.07 *br s*	4.07 *br s*	4.46 *br s*	4.90 *d*	4.48 *dd*	4.06 *br s*	4.04 *br s*	4.46 *d*
15′	4.31 *dd*	4.80 *dd*	3.97 *d*	4.80 *d*				4.81 *d*	4.31 *br d*			4.39 *d*
8-OAc	—	—	—	—	—	—	—	2.10 *s*	—	—	—	—
9-OAc	—	—	1.94 *s*	1.94 *s*	—	—	2.06 *s*	2.06 *s*	—	—	—	—
15-OAc	—	2.10 *s*	—	2.10 *s*	—	—	—	2.12 *s*	—	—	—	—
2′	—	—	—	—	2.30 *tq*	—	—	—	2.42 *tq*	2.41 *tq*	—	—
$3'_1$	6.11 *qq*	6.11 *qq*	6.10 *qq*	6.09 *qq*	1.61 *ddq*	6.07 *qq*	—	—	1.65 *ddq*	1.60 *ddq*	—	—
$3'_2$					1.41 *ddq*		—	—	1.49 *ddq*	1.42 *ddq*	—	—
4′	1.98 *dq*	1.97 *dq*	1.95 *dq*	1.93 *dq*	0.86 *t*	1.95 *dq*	—	—	0.91 *t*	0.85 *t*	—	—
5′	1.84 *dq*	1.84 *dq*	1.82 *dq*	1.83 *dq*	1.06 *d*	1.81 *dq*	—	—	1.13 *d*	1.07 *d*	—	—

J (Hz): **2, 4–7, 9–11, 1a–4a, 7a, 10a**: 1,2α = 10.0, 1,2β = 7.6; 2α,2β = 12.2; 2α,3α = 3.0; 2α,3β = 2.3; 2β,3α = 10.0; 2β,3β = 4.5; 3α,3β = 11.6; 5,6 = 6,7 = 10.5. **2, 4, 7, 9–11, 1a–4a, 7a, 10a:** 8α,9β = 9.0. **2, 4, 7, 9, 11, 1a–4a, 7a:** 7.13b = 3.4; 7.13a = 3.1. **2, 4, 9, 11, 1a–4a:** 7,8α = 1.4; 15.15′ = 12.7. **4, 7, 9, 10, 3a, 4a, 7a, 10a:** 9β,14 = 1.8. **2, 5, 6,11, 1a, 2a:** 9α,9β = 13.6; 9β,14 = 1.2. **2, 11, 1a, 2a:** 8α,9α = 7.8. **5, 6, 10, 10a:** 7,13b = 2.6; 7,13a = 2.3. **2, 1a, 1a:** 15, OH = 15′, OH = 3.8. **5, 6:** 7,8β = 10.0; 8β,9α = 2.5; 8β,9β = 5.3. **10,10a:** 7,8α = 3.5. **9**: 15,OH = 3.0; 15′, OH = 5.0; 9α,OH = 5.5. *Ang*: 3′,4′ = 7.2; 3′,5′ = 4′,5′ = 1.5. *MeBu*: 2′,5′ = 2′,$3'_1$ = 2′,$3'_2$ = 7.0; $3'_1$,4′ = $3'_2$,4′ = 7.4; $3'_1$,$3'_2$ = 13.5.

*Obscured by other signals.

and H-7. Acetylation of the hydroxyl group corroborated the above findings. The ^{1}H NMR spectrum of the acetate derivative (**2a**) showed a significant paramagnetic acylation shift of the two doublet of doublets at 4.52 (H-15) and 4.31 (H-15′) in **2** to doublets at 4.83 ($\Delta\delta=0.31$) and 4.80 ($\Delta\delta=0.49$) in **2a**, which were consistent with a geminal position of H-15 and H-15′ and the presence of a hydroxyl group at C-15 in **2**. All other proton signals of **2** and **2a** were assigned on the basis of 2D COSY and NOE difference experiments (Table 1). The ^{13}C NMR spectrum of **2** (Table 2) was assigned with the aid of heteronuclear multipulse DEPT experiments, 2D COSY and ^{1}H–^{13}C correlations.

Leocarpinolide B (**4**), $C_{22}H_{26}O_8$, showed in its IR spectrum strong absorptions at 3478 (OH), 1759 (γ-lactone), 1740, 1238 (acetate), 1720 (unsaturated ester) and 1690 cm^{-1} (unsaturated aldehyde). The presence of an acetate group was indicated by a three-proton singlet at δ1.94 and a mass spectral peak at m/z 43 $[C^1]^+$. On the basis of the great similarities between the ^{1}H NMR spectral data of compounds **2** and **4**, the stereochemical structure of the melampolide skeleton of compound **4** must be the same as that in lecocarpinolide A (**2**) with the presence of a new chiral centre at C-9. The ^{1}H NMR spectrum of compound **4** (Table 1) differed mainly from that of lactone **2** by the presence of a doublet of doublets at 5.33 (H-9β), which is due to an acetoxy substituent at C-9. The H-9β orientation was deduced from the typical W-coupling between H-9 and H-14 ($J_{9,14}=1.2$ Hz) [7]. This was further corroborated from the large H-8α, H-9β-coupling ($J_{8\alpha,9\beta}=9.0$ Hz) which was in agreement with a *trans*-diaxial orientation of these two protons.

As found in compound **2**, an angelate ester side chain was present at C-8, in lactone **4**. This was derived from the 2D COSY studies and from inspection of its ^{1}H NMR spectrum which displayed a one-proton quartet of quartets at δ6.10 (H-3′), a three-proton doublet of quartets at 1.95 (H-4′) and a three-proton doublet of quartets at 1.82 (H-5′). Strong mass spectral peaks at m/z $[M-A]^+$, 83 $[A^1]^+$ and 55 $[A^2]^+$ as well as ^{13}C NMR methyl resonances at 15.62 q and 20.31 q [10] were also diagnostic of an angelate moiety. The ^{13}C NMR spectrum of compound **4** (Table 2) supported the presence of four carbonyl groups, eight olefinic carbons and three methyl groups. The ^{13}C NMR signals were assigned on the basis of 2D COSY, ^{1}H–^{13}C correlation and DEPT experiments. The multiplicity of the signals in the ^{13}C spectrum indicated that compound **4** contains oxygen substituents at C-8 and C-9 and a hydroxyl group at C-15. The site of attachment of the acetyl group and the angelate ester in lecocarpinolide B (**4**) was confirmed by hydrolysis to the 8β-hydroxy derivative **7** which was identical with the co-occurring lecocarpinolide E (**7**) (Scheme 1). Acetylation of **4** provided the acetate **4a**, the ^{1}H NMR spectrum of which (Table 1) clearly showed that one acetate group was introduced at C-15 as indicated by the deshielded H-15 doublets at 4.93 ($\Delta\delta=0.40$) and 4.80 ($\Delta\delta=0.83$). The acetyl derivative (**4a**) was also obtained by acetylation of lecocarpinolide F (**9**) (Scheme 1). Consequently, lecocarpinolides B (**4**), E (**7**) and F (**9**) were correlated with the structures of the known compounds **3** and **8**, thus clearly establishing their stereostructures and ester attachments.

Leocarpinolides C and D (**5**, **6**), $C_{20}H_{26}O_6$ and $C_{20}H_{24}O_6$, respectively, were isolated as a gummy mixture (*ca* 1:1, by ^{1}H NMR). The ^{1}H NMR spectrum showed two series of proton signals and the ^{13}C NMR signals exhibited sufficient chemical shift differences to carry out detailed spectroscopic studies. A strong IR

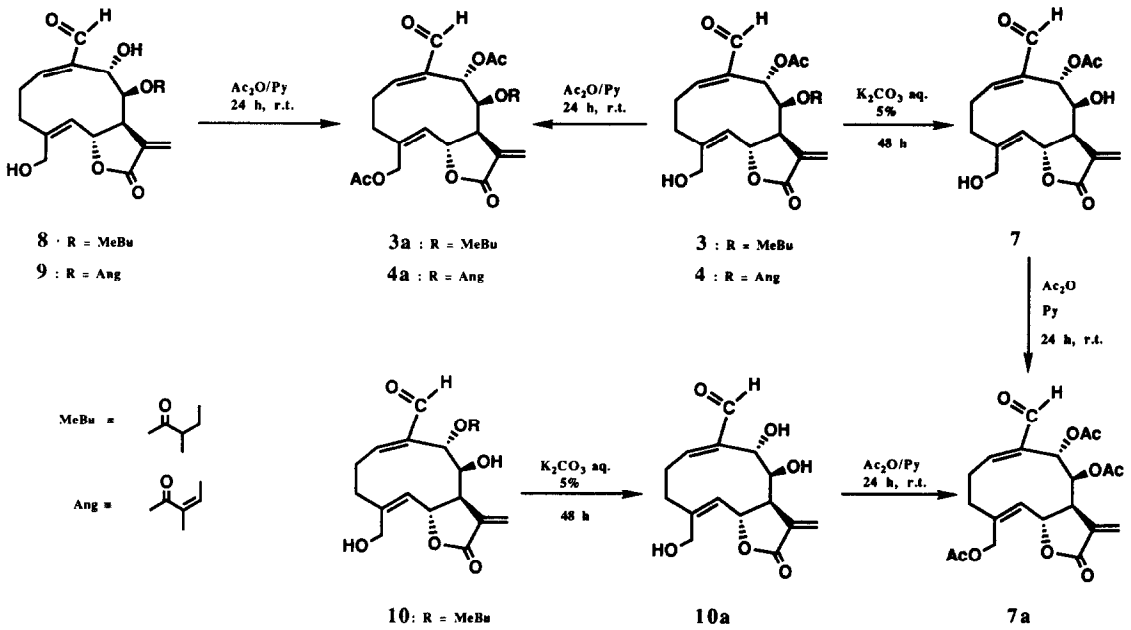
Table 2. ^{13}C NMR data of compounds **1**, **2**, **4**–**11** (100.62 MHz, $CDCl_3$, TMS as int. std)*

C	1†	2†	4†	5	6	7	8	9	10	11
1	153.63 *d*‡	153.63	158.70	153.76	153.84	158.79	155.70	155.46	155.04	155.12
2	27.02 *t*	27.12	26.41	26 48	26.48	27.78	27.39	26.79	27.39	27.29
3	32.64 *t*	32.64	32.11	28.59	28.72	32.80	32.06	32.50	32.49	32.40
4	142.79 *s*	142 79	140.67	141.89	142.01	140.21	140.00	140.22	140.30	140.52
5	128.38 *d*	127.14	128.17	126.75	126.75	129.32	129 00	129.31	128.99	128.60
6	73.82 *d*	74.03	73.61	73.62	73.75	72.83	73.75	73 57	73.75	73 57
7	49.54 *d*	49.65	50.86	46.97	47.00	51.83	51.52	51.44	51.51	51.43
8	65.54 *d*	65.59	70.04	72.08	72.59	68.30	71.25	71.12	70.38	60 82
9	28.73 *t*	28.90	67.77 *d*	25.91 *t*	25.26	72.83	70.44	70.38	71.24	26.77
10	140.42 *s*	140.32	141.39	138.99	139.42	139.98	139.97	139.22	139.51	140.42
11	134.95 *s*	134.85	133.89	135.25	135.45	134.31	134.28	134.28	133.99	134.05
12	169.27 *s*	169.27	169.08	169.50	169.50	169.31	169.30	169.10	168.95	168.80
13	120.94 *t*	121.13	122.10	124.49	124.49	120.55	121.86	121.56	123.10	122.90
14	195.41 *d*	195.4†	194.00	194.92	194.92	194.32	195.39	195.39	195.37	195.37
15	60.59 t	60.66	60.10	66.18	66.18	61.18	60 73	60.73	60.72	60.82
1′	175.19 *s*	166.38	166.02	175.36	167 32	—	176.01	165.66	176.03	—
2′	41.16 *d*	127.14 *s*	126.72	41.15 *d*	126.75 *s*	—	41.24 *d*	126.56 *s*	41.24 *t*	—
3′	26.74 *t*	139.19 *d*	141.54	26.48 *t*	141.89 *d*	—	26.79 *t*	139.97 *d*	26.79 *t*	—
4′	11.50 *q*	15.82	15 62	11.57	15.76	—	11.38	15.91	11.48	—
5′	16.73 *q*	20.53	20.31	16.79	20.42	—	16.78	20 53	16.78	—
OAc	—	—	170.21 *s*	—	—	170.25 *s*	—	—	—	—
	—	—	20.53 *q*	—	—	21.01 *q*	—	—	—	—

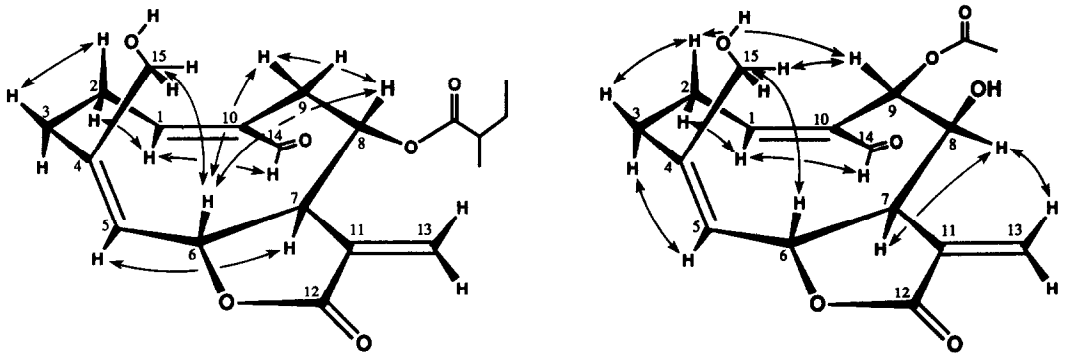
*Peak multiplicity was obtained by heteronuclear multipulse programmes.

†Assignments for **1**, **2** and **4** were confirmed by ^{13}C–^{1}H correlation.

‡Multiplicities are not repeated if identical with those in preceding column.



Scheme 1. Chemical transformations used in the structural elucidation of melampolides from *Leucocarpus pinnatifidus*.



L-C **L-E**

Fig. 1. NOE difference experiments on lecocarpinolides C (**5**) and E (**7**).

absorption at 1763 cm^{-1} corroborated the presence of a γ-lactone, and bands at 3466 and 1682 suggested the presence of hydroxyl(s) and an unsaturated aldehyde, respectively. Unsaturated and saturated ester side chains were indicated by respective absorptions at 1719 and 1736 cm^{-1}. Comparison of the ^1H NMR spectra of compounds **5** and **6** with the previously described lactones **1** and **2** showed three significant differences. First, the large coupling $J_{7,8} = 10.0$ Hz) required β-orientation of H-8. Second, the chemical shift of H-8 (**5**, δ5.91 *ddd*; **6**, 5.93 *ddd*) showed no deshielding effect by the carbonyl group C-10 commonly observed in 8β-acyl-substituted melampolides [8]. Third, the multiplicity and chemical shift of the two C-15 protons, which appear as a broad singlet at 4.07, suggests that free rotation of the $-CH_2OH$ at C-4 occurs, which is uncommon in 8β-acylated melampolides. This stereochemistry was further substantiated by NOE difference experiments (Fig. 1), which showed effects between H-5 and H-7, between H-6 and H-15 as well as H-6 and H-8. Comparison of the ^1H NMR signals of lecocarpinolide C (**5**) and D (**6**) suggested that the only structural difference resides in the C-8 ester side chain. The ^1H NMR spectral absorptions for **5** and **6** were very similar except for the signals due to the ester moieties, which in **5** was identified as 2-methylbutyrate on the basis of diagnostic ^1H and ^{13}C NMR signals together with the characteristic mass spectral peaks *m/z* 85 $[B^1]^+$ and 57 $[B^2]^+$ as well as the ^1H NMR 2D COSY spectrum. Compound **5** displayed a one-proton triplet of quartet at δ2.36 (H-2'), two one-proton multiplets (doublet of a doublet of a quartet) at 1.61 (H-3'$_1$) and 1.41 (H-3'$_2$), a three-proton triplet at 0.86 (H-4') and a three-proton doublet at 1.06 (H-5'). These ^1H NMR resonances together with the ^{13}C NMR peaks at 175.36 *s*, 41.15 *d*,

26.48 *t*, 11.57 *q* and 16.79 *q* are typical for the 2-methylbutyrate moiety [10]. The ester side chain at C-8 in **6** was identified as angelate on the basis of diagnostic ^{1}H and ^{13}C NMR signals, as described previously for lecocarpinolides A (**2**) and B (**4**) together with the characteristic mass spectral peaks at *m/z* 83 $[A^1]^+$ and 55 $[A^2]^+$.

All proton signals of **5** and **6** (Table 1) were assigned on the basis of 2D COSY and the stereochemistry was supported by NOE difference experiments (Fig. 1). The ^{13}C NMR spectrum of **5** and **6** (Table 2) was assigned with the aid of heteronuclear multipulse DEPT experiments and by comparison with data described above for compounds **1** and **2**.

Lecocarpinolide E (**7**), $C_{17}H_{20}O_7$, was a gum with an IR band at 1760 cm^{-1} indicating a γ-lactone. This was confirmed by diagnostic ^{1}H NMR signals: two one-proton doublets at δ5.64 (H-13a) and 6.35 (H-13b) which were coupled to the doublet of a doublet of a triplet at 2.45 (H-7). Additional IR bands appeared at 3434, 1740, 1242 and 1686 cm^{-1}, suggesting the presence of hydroxyl(s), acetate ester and a conjugated aldehyde, respectively. Comparison of the ^{1}H NMR spectrum of compound **7** with the previously described lactones **3** and **4** showed clearly that it represents the 8β-desacyl derivative of **3** and **4** which followed from the chemical shift of H-8 (δ5.24 *br d*) and the simplification of the spectrum due to the lack of ester side chain signals. A free rotation of the $-CH_2OH$ at C-4 was suggested by a broad two-proton singlet at 4.46 (H-15, H-15′). An α-orientation of H-8 is consistent with the large coupling $J_{8\alpha,9\beta}$ = 9.0 Hz [8]. This was further substantiated by NOE difference experiments (Fig. 1) with effects between H-7 and H-8. Further NOEs in **7** are summarised in Fig. 1. Acetylation of lecocarpinolide E (**7**) corroborated the above findings. The ^{1}H NMR spectrum of the acetate (**7a**) showed a significant paramagnetic acylation shift of the broad two-proton singlet at 4.46 (H-15, H-15′) in **7** to two doublets at 4.90 (H-15) and 4.81 (H-15′) in **7a**. In addition, the broad one-proton doublet at 5.24 (H-8) in **7** was changed to a doublet of doublets at 6.68 in **7a**, which is in agreement with the presence of hydroxyl groups at C-15 and at C-8 in **7**.

All proton signals of lactones **7** and **7a** were assigned on the basis of 2D COSY and NOE difference experiments, which are summarised in Table 1 and Fig. 1, respectively. The ^{13}C NMR spectrum of **7** (Table 2) was assigned using DEPT experiments and by comparison with compounds **3** and **4**. As described above, the relationships between compounds **3**, **4**, and **7** were confirmed chemically by saponification of **3** and **4** to give **7**. As outlined in Scheme 1, the preparation of **7a** from lecocarpinolide G (**10**) was accomplished by hydrolysis of the ester side chain of **10** to obtain **10a** which upon acetylation gave **7a**. These transformations correlated the chemistry of lecocarpinolides E (**7**) and G (**10**).

Lecocarpinolide F (**9**), $C_{20}H_{24}O_7$, was isolated as a gummy mixture with the known lactone **8** [7] (*ca* 2:1, by ^{1}H NMR). The ^{1}H NMR spectrum of the new melampolide **9** (Table 1) clearly indicated the presence of the same medium ring skeleton as **4**, differing only in the presence of a hydroxy group at C-9 instead of a C-9 acetate. This was supported by acetylation of **9** to obtain the diacetate derivative **4a** (Scheme 1). The ^{1}H NMR spectrum of **4a** (Table 1) differed mainly from that of lactone **9** by the signals due to the introduction of two acetate groups at C-9 and C-15. Typical paramagnetic acylation shifts of the doublet of doublets at 4.18 (H-9β) to a doublet at 5.35 and the two doublet of doublets at 4.47 (H-15) and 4.31 (H-15′) to the respective doublets at 4.93 and 4.80 were observed. All proton signals of **9** were assigned on the basis of 2D COSY experiments (Table 1). The ^{13}C NMR spectral assignments (Table 2) involved DEPT experiments and spectral comparison with compounds **3**, **4** and **7**.

Lecocarpinolide G (**10**), $C_{20}H_{26}O_7$, was a gummy mixture with lecocarpinolide H (**11**) (*ca* 1:2, by ^{1}H NMR). Saponification of this mixture provided pure **11** and the corresponding 9α-desacyl derivative **10a**. Acetylation of the desacyl derivative **10a** provided **7a**, which was also obtained by the previously outlined acetylation of **7** (Scheme 1). Comparison of the ^{1}H NMR spectrum of compound **10** (Table 1) with the data described for lecocarpinolide E (**7**) showed clearly that the only difference is due to the ester side chain at C-9. 2D COSY studies revealed that **7** is a 9α acetate and **10** represents the 9α-(2-methylbutanoate). Since the ^{1}H NMR spectra signals for the medium ring portion were essentially superimposable, the two lactones must possess the same medium ring stereochemistry. The ^{13}C NMR spectral signals (Table 2) were assigned on the basis of DEPT experiments and by comparison with lactones **3**, **4** and **7**.

Lecocarpinolide H (**11**), $C_{15}H_{18}O_5$, could only be separated from **10** after saponification of the mixture of **10** and **11** followed by separation from **10a**. On the basis of the great similarities of the ^{1}H NMR spectral data of compounds **1**, **2** and **11**, the stereochemical structure of the melampolide skeleton of compound **11** must be the same as that in lecocarpinolide A (**2**) and in **1**. Comparison of the ^{1}H NMR spectrum of compound **11** with lactones **1** and **2** showed clearly that lecocarpinolide H (**11**) is the 8β-desacyl derivative of **1** and **2**. This followed from the chemical shift of H-8 (δ5.20 *ddd*) and the absence of ester side chain signals. This was further substantiated by the saponification of a mixture of **1** and **2** which provided lecocarpinolide H (**11**).

The close taxonomic association of *L. pinnatifidus* with *Acanthospermum* was first proposed by Baillon [14]. Today, the genus is recognised as a member of the subtribe Melampodiinae in close association with *Acanthospermum* and *Melampodium* [1, 4]. Our chemical data strongly support the close relationship of *L. pinnatifidus* with the above two genera of the Melampodiinae. Among the 11 melampolides found in this species, three had been previously isolated from *Acanthospermum* species [7, 8, 12, 15]; lactones **1** and **8** in *A. australe* [7, 12] and **3** from *A. hispidum* [8]. 12,6βH-Lactonised melampolides with oxygen functions at C-8 and C-9 and C-10 aldehydic functions have also been reported from *Milleria guingelfora* [16], a monotypic genus in the subtribe Milleriinae within the tribe Heliantheae [17]. Other aldehyde-bearing melampolides structurally related to the *Lecocarpus* lactones have been isolated from *Ichthyothere ulei* [18] and *Smallanthus fruticosus* (= *Polymnia fruticosa*) [19] of the subtribe Melampodiinae [20]. The South American genus *Ichthyothere* was placed by Hoffmann in the subtribe Melampodiinae [21] but was later transferred to the subtribe Millerinae by Stuessy [1]. However, Robinson [note in ref. 18] suggested that it should be retained in the Melampodiinae.

One unique difference on the chemical pattern of *L. pinnatifidus*, when compared with other melampolide-producing members of the subtribe Melampodiinae, is

the occurrence of two melampolides [leocarpinolide C (**5**) and D (**6**)] with 8α-ester functions instead of the common 8β-oriented ester substituents. The change in stereochemistry at C-8 in compounds **5** and **6** clearly represents a difference in the genetic expression of this species and separates it from the other taxa of the subtribe, most significantly from *Acanthospermum*, with which it is otherwise most closely associated taxonomically and chemically. Our future chemical studies of the two other *Lecocarpus* species, *L. lecocarpoides and L. darwinii* will hopefully shed further light on this question.

EXPERIMENTAL

L. pinnatifidus Decne. was collected on 17 April 1986, in the Cerro Ventana area, elevation 100 m, on Isla Santa María (Isla Floreana; Charles Island), Galapagos Islands, Ecuador (F. Cruz and J. E. Lawesson; No. 2950; the voucher is deposited at the Missouri Botanical Garden Herbarium No. 3381370).

Air-dried flowers and leaves (47 g) were ground and extd at room temp. for 24 hr successively with *n*-hexane and CH_2Cl_2, providing 2.245 g of crude ext. (CH_2Cl_2). The ext. obtained was sepd by CC on silica gel using *n*-hexane–EtOAc mixts of increasing polarity, 300 frs of 30 ml each being collected. Upon further CC and prep. TLC of the various frs, 11 sesquiterpene lactones, 15-hydroxy-8β-(2-methylbutyryloxy)-14-oxo-acanthospermolide (**1**), [7], lecocarpinolide A (**2**), acanthospermal B (**3**), [8], lecocarpinolides B–E (**4–7**), 9α,15-dihydroxy-8β-(2-methylbutyryloxy)-14-oxo-acanthospermolide (**8**), [7] lecocarpinolides F–H (**9–11**), and the flavonoid penduletin (**12**), [9] were isolated. Frs 114–116 provided 40 mg of **12** as a yellow crystalline compound. Frs 164–200 (150 mg), after CC in *n*-hexane–EtOAc (7:3) and mixts of increasing polarity, further prep. TLC of frs 62′–78′ (CH_2Cl_2–MeOH, 97:3, ×3) gave 31 mg of **1**, 20 mg of **2** and 40 mg of **3** and **4** (*ca* 1:1, by ^{1}H NMR) sepd by prep. TLC (CH_2Cl_2–MeOH, 99:1, ×5) providing 16 mg of **3** and 18 mg of **4**. Frs 201–262 (190 mg), after CC in *n*-hexane–EtOAc (1:1), and mixs of increasing polarity, and further prep. TLC of frs 66′–93′ (CH_2Cl_2–MeOH, 9:1, ×3) afforded 22 mg of **5** and **6** (*ca* 1:1, by ^{1}H NMR) and 10 mg of **7**. Frs 94′–128′, after prep. TLC (CH_2Cl_2–MeOH, 17:3, ×4) provided 15 mg of a mixt. of **8** and **9** (*ca* 2:1, by ^{1}H NMR) and 8 mg of a mixt. of **10** and **11** (*ca* 1:2, by ^{1}H NMR). Known compounds were identified by comparison of physical data (mp, IR, MS, ^{1}H and ^{13}C NMR) with that in the lit.

Lecocarpinolide A (**2**). $C_{20}H_{24}O_6$, colourless gum. IR $\nu_{max}^{neat,KBr}$ cm^{-1}: 3462 (OH), 1765 (γ-lactone), 1718 (C=CCO_2R), 1680 (C=CCHO), 1626 (double bonds). MS (70 eV) *m/z* (rel. int.): 360 [M]$^+$ (0.4), 260 [M−A]$^+$ (5), 242 [M−A−H_2O]$^+$ (23), 214 [M−A−H_2O−CO]$^+$ (11), 213 [M−A−H_2O−CHO]$^+$ (9), 83 [A^1]$^+$ (99), 55 [A^2]$^+$ (75). ^{1}H NMR see Table 1; ^{13}C NMR see Table 2.

Preparation of **2a**. Acetylation of **2** (7 mg) with 1 ml of Ac_2O–pyridine (3:1) for 24 hr at room temp. followed by usual work-up and prep. TLC (*n*-hexane–EtOAc, 4:1, ×2) gave 5 mg of **2a** as a colourless gum. IR $\nu_{max}^{neat,KBr}$ cm^{-1}: 1769 (γ-lactone), 1740, 1231 (OAc), 1720sh (C=CCO_2R), 1680 (C=CCHO), 1628 (double bonds). MS (70 eV) *m/z* (rel. int.): 402 [M]$^+$ (0.1), 342 [M−C]$^+$ (6), 319 [M−A^1]$^+$ (8), 259 [M−C−A^1]$^+$ (15), 242 [M−C−A^1]$^+$ (80), 213 [M−C−A^1−CHO]$^+$ (60), 185 [M−C−A−CHO−CO]$^+$ (30), 83 [A^1]$^+$ (57), 55 [A^2]$^+$ (70), 43 [C^1]$^+$ (52). ^{1}H NMR see Table 1.

Lecocarpinolide B (**4**). $C_{22}H_{26}O_8$, colourless gum. IR $\nu_{max}^{neat,KBr}$ cm^{-1}: 3478 (OH), 1759 (γ-lactone), 1740, 1238 (OAc), 1720sh (C=CCO_2R), 1688 (C=CCHO), 1628 (double bonds). MS (70 eV) *m/z* (rel. int.): 418 [M]$^+$ (0.4), 318 [M−A]$^+$ (2), 258 [M−A−C]$^+$ (15), 240 [M−A−C−H_2O]$^+$ (21), 212 [M−A−C−H_2O−CO]$^+$ (15), 211 [M−A−C−H_2O−CHO]$^+$ (10), 83 [A^1]$^+$ (100), 55 [A^2]$^+$ (36), 43 [C^1]$^+$ (34). ^{1}H NMR see Table 1; ^{13}C NMR see Table 2.

Preparation of **4a**. Acetylation of **4** (6 mg), as described for **2a**, gave 4 mg of **4a** as a colourless gum. IR $\nu_{max}^{neat,KBr}$ cm^{-1}: 1775 (γ-lactone) 1742, 1234 (OAc), 1720sh (C=CCO_2R), 1690 (C=CCHO), 1628 (double bonds). MS (70 eV) *m/z* (rel. int.): 460 [M]$^+$ (0.3), 400 [M−C]$^+$ (11), 377 [M−A^1]$^+$ (12), 317 [M−C−A^1]$^+$ (14), 240 [M−C−C−A]$^+$ (100), 211 [M−C−C−A−CHO]$^+$ (70), 183 [M−2C−A−CHO−CO]$^+$ (58), 83 [A^1]$^+$ (59), 55 [A^2]$^+$ (83), 43 [C^1]$^+$ (56). ^{1}H NMR see Table 1. Compound **4a** was also prepd by acetylation of **9**.

Lecocarpinolides C and D (**5**, **6**). $C_{20}H_{26}O_6$ and $C_{22}H_{24}O_8$, respectively, colourless gums. IR $\nu_{max}^{neat,KBr}$ cm^{-1}: 3466 (OH), 1763 (γ-lactone), 1736 (CO_2R), 1719sh (C=CCO_2R), 1682 (C=CCHO), 1640 (double bonds). MS (70 eV) *m/z* (rel. int.): 362 [M]$^+$ (0.2), 360 [M_1]$^+$ (0.3), 344 [M−H_2O]$^+$ (0.5), 342 [M_1−H_2O]$^+$ (0.2), 260 [M−B]$^+$ and [M_1−A]$^+$ (5), 242 [M−B−H_2O]$^+$ and [M_1−A−H_2O]$^+$ (26), 214 [M−B−H_2O−CO]$^+$ and [M_1−A−H_2O−CO]$^+$ (14), 213 [M−B−H_2O−CHO]$^+$ and [M_1−A−H_2O−CHO]$^+$ (13), 85 [B^1]$^+$ (32), 57 [B^2]$^+$ (100), 83 [A^1]$^+$ (92), 55 [A^2]$^+$ (73). ^{1}H NMR see Table 1; ^{13}C NMR see Table 2.

Lecocarpinolide E (**7**). $C_{17}H_{20}O_7$, colourless gum. IR $\nu_{max}^{neat,KBr}$ cm^{-1}: 3434 (OH), 1760 (γ-lactone), 1740, 1242 (OAc), 1686 (C=CCHO), 1626 (double bonds). MS (70 eV) *m/z* (rel. int.): 336 [M]$^+$ (0.7), 276 [M−C]$^+$ (15), 258 [M−C−H_2O] (24), 240 [M−C−2H_2O]$^+$ (18), 212 [M−C−2H_2O−CO]$^+$ (16), 211 [M−C−2H_2O−CHO]$^+$ (10), 43 [C^1]$^+$ (90). ^{1}H NMR see Table 1; ^{13}C NMR see Table 2.

Preparation of **7a**. Acetylation of **7** (4 mg), as described previously, gave 2 mg of **7a** as a colourless gum. IR $\nu_{max}^{neat,KBr}$ cm^{-1}: 1762 (γ-lactone), 1742, 1239 (OAc), 1686 (C=CCHO), 1625 (double bonds). MS (70 eV) *m/z* (rel. int.): 420 [M]$^+$ (0.2), 360 [M−C]$^+$ (19), 300 [M−2C]$^+$ (70), 240 [M−3C]$^+$ (85), 212 [M−3C−CO]$^+$ (60), 211 [M−3C−CHO]$^+$ (52), 43 [C^1]$^+$ (87). ^{1}H NMR see Table 1. Compound **7a** was also prepd by acetylation of **10a**.

Preparation of **7**. A mixt. of **3** and **4** (15 mg) was mixed with aq. 5% K_2CO_3 (4 ml) and stirred for 48 hr. After acidification with dil. aq. HCl the mixt. was extd with EtOAc, the combined organic exts washed with H_2O, dried and evapd to dryness. Purification by prep. TLC (*n*-hexane–EtOAc, 7:3, ×2) gave 7 mg of gummy **7**.

Lecocarpinolide F (**9**). $C_{20}H_{24}O_7$, isolated as a gummy mixture with **8**. IR $\nu_{max}^{neat,KBr}$ cm^{-1}: 3455 (OH), 1765 (γ-lactone), 1750 (CO_2R), 1725 (C=CCO_2R), 1684 (C=CCHO), 1628 (double bonds). MS (70 eV) *m/z* (rel. int.): 378 [M]$^+$ (0.1), 376 [M_1]$^+$ (0.2), 276 [M−B]$^+$ and [M_1−A]$^+$ (5), 258 [M−B−H_2O]$^+$ and [M_1−A−H_2O]$^+$ (12), 240 [M−B−2H_2O]$^+$ and [M_1−A−2H_2O]$^+$ (20), 212 [M−B−2H_2O−CO]$^+$ and [M_1−A−2H_2O−CO]$^+$ (11), 211 [M−B−2H_2O−CHO]$^+$ and [M_1−A−2H_2O−CHO]$^+$ (9), 85 [B^1]$^+$ (70), 57 [B^2]$^+$ (100), 83 [A^1]$^+$ (95), 55 [A^2]$^+$ (73). ^{1}H NMR see Table 1; ^{13}C NMR Table 2.

Lecocarpinolides G (**10**) *and H* (**11**). $C_{20}H_{26}O_7$ and $C_{15}H_{18}O_5$, respectively, gummy mixt. IR $\nu_{max}^{neat,KBr}$ cm^{-1}: 3414 (OH), 1760 (γ-lactone), 1746 (CO_2R), 1680 (C=CCHO), 1632 (double bonds). MS (70 eV) *m/z* (rel. int.): 378 [M]$^+$ (0.2), 360 [M−H_2O]$^+$ (0.5), 278 [M_1]$^+$ (17), 276 [M−B]$^+$ (15), 260 [M_1−2H_2O]$^+$ (12), 258 [M−B−H_2O]$^+$ (26), 242 [M_1−2H_2O]$^+$ (10), 240 [M−B−2H_2O]$^+$ (18), 214 [M−B−2H_2O−CO]$^+$ and [M_1−2H_2O−CHO]$^+$ (15), 213 [M−B−2H_2O−CHO]$^+$ and [M_1−2H_2O−CHO]$^+$ (10), 85 [B^1]$^+$ (45), 57 [B^2]$^+$ (100). ^{1}H NMR see Table 1; ^{13}C NMR see Table 2.

Preparation of **10a**. Saponification of a mixt. of **10** and **11** (6 mg), as described above gave 2 mg of pure **11** and 2 mg of **10a** as colourless gums. IR $\nu_{max}^{neat,KBr}$ cm^{-1}: 3414 (OH), 1760 (γ-lactone), 1680 (C=CCHO), 1635 (double bonds). MS (70 eV) *m/z* (rel. int.): 294 $[M]^+$ (0.5), 276 $[M-H_2O]^+$ (19), 258 $[M-2H_2O]^+$ (8), 240 $[M-3H_2O]^+$ (70), 212 $[M-3H_2O-CO]^+$ (68), 211 $[M-3H_2O-CHO]^+$ (32). ^1H NMR see Table 1.

Preparation of **11**. Saponification of a mixt. **1** and **2** (8 mg) as described above gave 5 mg of gummy **11**.

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