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MELROSINS A, B AND C, THREE *cis*-1 (10)-*cis*-4-GERMACRADIENOLIDES FROM *MELAMPODIUM ROSEI**

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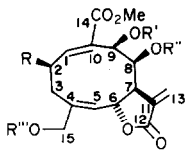
Key Word Index—*Melampodium rosei*; Asteraceae; Heliantheae; sesquiterpene lactones; *cis,cis*-germacradienolides; melrosins A, B and C.

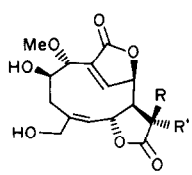
Abstract—The isolation and structure elucidation of the three new *cis*-1(10)-*cis*-4-germacradienolides, melrosins A, B and C, from *Melapodium rosei* are reported. The structure determination involved chemical and spectral methods including ^{1}H NMR correlations with melrosin A the structure of which had been determined by single crystal X-ray diffraction.

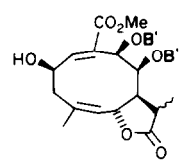
INTRODUCTION

In our biochemical systematic study of the genus *Melampodium* [1] we have analysed the sesquiterpene lactones of the Mexican species *M. rosei* which is the first member of the series Cupulata Stuessy in the section Melampodium [1] to be studied chemically. Major structural representatives of sesquiterpene lactones in other *Melampodium* species are melampolides and leucantholides [2]. More recently, members of the less common group of *cis,cis*-germacradienolides [3] were found in *M. leucanthum* [4] and *M. cinereum* [5]. Therefore, it was of particular interest to learn about the structural types of lactones in *M. rosei*. Structural data for the three new compounds, which we named melrosins A, B and C, were obtained by physical methods, chemical transformations and ^{1}H NMR correlations with melrosin A (**3a**), the structure of which was recently determined by X-ray diffraction [6]. The new findings suggested configurational revisions of the related melcanthins A–G [6].



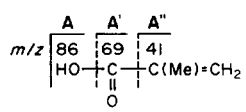
	R	R'	R''	R'''
1	OH	Ac	Ang	H
2	OH	B'	B'	H
3a	OH	A'	A'	H
3b	OAc	A'	A'	Ac
4	OH	A'	B'	H
5	H	A'	A'	H

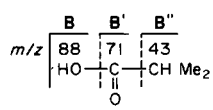


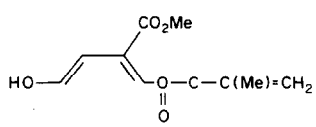


6 R=H; R'=$-CH_2-OMe$
7 R=R'=$=CH_2$

8







m/z 212
C

RESULTS AND DISCUSSION

Melrosin A (**3a**), $C_{24}H_{28}O_{20}$, mp 159–161°, exhibited strong UV end absorption and IR bands indicating hydroxyl(s) (3500 cm^{-1}), α,β-unsaturated-γ-lactone (1768 cm^{-1}) and α,β-unsaturated ester (1730 cm^{-1}) functions. The ^{1}H NMR spectrum (Table 1) of **3a** showed doublets typical for the lactonic exocyclic methylene protons at δ 6.42 (J = 3.3 Hz) and 5.91 (J = 2.6 Hz) as well as a three-proton singlet at 3.63 attributed to the methyl protons of a carbomethoxy group. A one-proton doublet centered at δ 7.22 (J = 7.3 Hz) was assigned to H-1 of an α,β-unsaturated carbomethoxy system typical of *cis,cis*-germacranolides and melampolides with a substituent at the C-2 position [4]. The presence of two methacrylate side chains was deduced from two sets of signals typical of vinylic methyl groups at δ 1.94 and 1.87 together with pairs of broadened vinylic proton absorptions at 6.21 and 5.99 and narrow quartets (J = 1.5 Hz) at 5.65 and 5.57. The mass spectrum of **3a** showed diagnostic peaks at *m/z* 69 (A') and *m/z* 41 (A'') which also supported the presence of methacrylate side chains.

^{1}H NMR decoupling experiments were employed to define the substitution pattern of the medium ring. Irradiation of a multiplet at δ 3.26 (H-7) caused the collapse of the two doublets assigned to H-13a and H-13b

*The X-ray structure of melrosin A was published in ref. [6].
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Table 1. ^{1}H NMR data for compounds **3a**, **3b** and **4–8** (200 MHz, TMS as internal standard, 27°)*

Signal	**3a** ($CDCl_3$)†	**3b** ($CDCl_3$)	**4** ($CDCl_3$)	**5** ($CDCl_3$)	**6** (Me_2CO-d_6)	**7** (Me_2CO-d_6)	**8** ($CDCl_3$)†
H-1	7.22 *d* (7.3)	7.05 *d* (7.4)	7.23 *d* (7.0)	7.24 *br dd*	3.95 *d* (8.3)	3.94 *d* (8.7)	7.06 *d* (8.4)
H-2, H-2′	4.80 *ddd* (7.3, 5.1, 2.1)	5.58‡	4.80 *br s*	2.3–2.9‡	3.82‡ *m*	2.78 *m*	4.58 *ddd* (8.4, 4.9, 2.0)
H-3	2.83 *dd* (15.2, 5.1)	2.86 *dd* (15.5, 1.5)	2.83 *dd* (15.0, 5.0)	2.3–2.9‡	2.26 *dd* (14.7, 6.5)	2.30 *dd* (15.0, 6.5)	2.87‡ *m*
H-3′	2.54 *dd* (15.2, 2.1)	2.52 *dd* (15.5, 1.5)	2.53 *br d* (15.0)	2.3–2.9‡	2.04 *br d* (14.7)	2.01 *br d* (15.0)	2.29 *dd* (15.2, 2.0)
H-5	5.75 *br d* (9.7)	5.76–5.93‡	5.76 *br d* (10.0)	5.7 *d* (8.0)	5.47 *br d* (10.0)	5.62 *br d* (10.0)	5.47 *br d* (10.0)
H-6	5.23 *dd* (9.7, 6.6)	5.20 *dd* (9.9, 6.5)	5.22 *dd* (10.0, 6.5)	5.42 *br d* (8.0)	4.43 *dd* (10.0, 10.0)	4.42 *dd* (10.0, 9.1)	5.19 *br d* (10.0)
H-7	3.26 *m*	3.27 *m*	3.22 *m*	3.26 *m*	2.90 *ddd* (11.9, 10.0, 1.9)	3.51 *dddd* (9.1, 3.5, 3.0, 1.6)	2.58‡ *m*
H-8	5.94 *dd* (3.8, 2.0)	5.76–5.93‡	5.91 *d* (4.0, 2.0)	5.87‡	5.58 *dd* (1.9, 1.9)	5.94 *dd* (1.6, 1.6)	5.51 *br dd* (3.2, 0.9)
H-9	5.81 *d* (3.8)	5.76–5.93‡	5.70 *d* (4.0)	6.06 *d* (4.0)	7.57 *dd* (1.9, 0.5)	7.63 *d* (1.6)	5.64 *br s*
H-13	6.42 *d* (3.3)	6.42 *d* (3.0)	6.41 *d* (3.0)	6.4 *d* (3.0)	3.79	6.63 *d* (3.5)	1.23 *d* (7.3)
H-13′	5.91*d* (2.6)	5.76–5.93‡	5.89 (2.9)	5.87 *d* (3.0)	3.79	6.0 *d* (3.0)	— —
H-15, H-15′	4.36 *br s*	4.88/4.65 *br d* (14.0)	4.38 *br s*	4.17 *br s*	4.12	4.14	—
Me-4	—	—	—	—	—	—	1.94
CO_2Me	3.63	3.62	3.74	3.66	—	—	3.72
Me-2′	1.94/1.87 *br s*	1.94/1.85 *br s*	1.93 *br s*	1.86/1.94 *br s*	—	—	—
C-3′H/H	6.21/5.99 *br s*	6.19/5.98 *br s*	6.21/5.67 *br s*	6.19/6.0 *br s*	—	—	—
C-3′H′/H′	5.65 *q*/5.57 *q* (1.5)	—	—	5.66/5.46 *br s*	—	—	—

*Figures in parentheses are coupling constants or line separations in Hz.

†Run at 55°. Miscellaneous signals: **3b**, OAc = 2.10/2.10; **4**, Me-2″ = 1.07 *d*/1.03 *d* (7.0), H-2″ = 2.41 *h* (7.0); **6**, OMe = 3.37/3.31, H-11 = 3.14 *ddd* (11.9, 5.5, 4.1); **7**, OMe = 3.31; **8**, Me-2″ = 1.0–1.20, H-2″ = 2.58‡ *m*/2.44 *h* (7.0), H-11 = 2.87 (*dq* (9.9, 7.3).

‡Obscured by other signals.

and simplified the doublet of doublets at 5.23 (J = 9.7, 6.6 Hz), which was assigned to H-6. Irradiation of the H-6 signal effected the collapse of H-5, a broad doublet at δ 5.75 (J = 9.7 Hz). The doublet at δ 5.81 (J = 3.8 Hz) was assigned to H-9; the signal was partially obscured by H-8. Saturation of the multiplet centered at δ 4.80 (J = 7.3, 5.1, 2.1 Hz) caused the collapse of the doublet at 7.22 (H-1) and was, therefore, assigned to H-2. Finally, a broad two-proton singlet centered at δ 4.36 was assigned to the C-15 methylene protons. Based on the chemical shifts of the H-2 and H-15 absorptions, it was deduced that positions C-2 and C-15 must contain hydroxyl groups. This was confirmed by the acetylation of **3a** which produced a diacetate (**3b**) whose ^{1}H NMR spectrum exhibited the expected downfield shifts of the H-2 and H-15 signals.

The above spectral features suggested a strong similarity between the structure of melrosin A (**3a**) and that of melcanthin B, a *cis*-germacradienolide previously isolated from *Melampodium leucanthum* [4]. Their structural relationship was established unambiguously by the reactions of the two compounds with sodium methoxide to give the leucantholide, **6**. Reaction of melcanthin B with one equivalent of sodium methoxide at 0° provided a mixture of **6** and **7**. Melrosin A (**3a**), when reacted at ambient temperature with 2 mols of sodium methoxide gave **6**. This addition–rearrangement reaction [7, 8] established that all chiral centers of the two medium ring compounds are the same with a possible exception at C-9.

Single crystal X-ray diffraction data of melrosin A solved the remaining structural ambiguities and established its configurations at all chiral centers as shown in structure **3a** [6]. The X-ray data obtained for melrosin A (**3a**), together with the above chemical correlations, necessitated configurational revision from H-2β to H-2α of melcanthin B. On the basis of very similar ^{1}H NMR parameters of melrosin A as well as longicornin A (**2**), the X-ray structures of which have been established [6], configurational changes from H-2α in melcanthin C [4] and from H-9β to H-9α in melcanthins A–G [4, 5] are also suggested. The revised configurational representation for melcanthin B is shown in structure **1**.

Catalytic hydrogenation of melrosin A (**3a**) provided the reduction-hydrogenolysis product, **8**. This reaction involved, besides the expected saturation of three double bonds, an uncommon hydrogenolysis of the hydroxyl group at C-15.

The structures of melrosin B, $C_{24}H_{30}O_{10}$, and melrosin C, $C_{24}H_{28}O_9$, were determined by correlations of their ^{1}H NMR and mass spectral data with those of known *cis,cis*-germacradienolides. On the basis of the extreme similarities of the ^{1}H NMR parameters for the medium ring protons of melrosins A and B their structural difference must only be in the ester side chains. Indeed, melrosin B showed ^{1}H NMR and mass spectral signals typical of the isobutyrate and methacrylate moieties which, on the basis of ^{1}H NMR chemical shift considerations, must be attached to C-8 and C-9, or vice versa. Evidence for the attachment of the methacrylate ester C-9 was provided by a strong diagnostic mass spectral peak at m/z 212 ($C_{10}H_{12}O_5$) which was assigned the radical ion **C** [4]. In contrast, the peak at m/z 214, which would have indicated the presence of the isobutyrate moiety at C-9, was of low intensity suggesting that melrosin B must be represented by structure **4**.

Melrosin C exihibited ^{1}H NMR and mass spectral signals typical of the melcanthin skeleton which, in contrast to melcanthin B (**1**), does not possess a hydroxyl group at C-2 [4]. The ^{1}H NMR spectrum indicated two methacrylate side chains (Table 1) which, on the basis of chemical shift arguments, must be attached to C-8 and C-9. Diagnostic peaks at m/z 69 (**A′**) and 41 (**A″**) corroborated the ^{1}H NMR data. Therefore, melrosin C is represented by structure **5**.

EXPERIMENTAL

Melampodium rosei Robins was collected on 10 September 1976 on Highway 40 *ca* 3 miles east of the junction with Highway 15, Sinaloa, Mexico; (Hartman & Funk, No. 4310, voucher deposited at O.S., U.S.A.). Aerial parts (100 g) were extracted with 100 ml CH_2Cl_2. Standard work-up [9] yielded 734 mg of terpenoid material which was chromatographed over 60 g Si gel, starting with a mixture of Et_2O–petrol (1:1) and followed by Et_2O–petrol (75:25), Et_2O, Me_2CO–petrol (1:1) and Me_2CO; 42 fractions of 50 ml were taken and all fractions were monitored by TLC.

Fractions 22–24 afforded 8 mg **5**. Fraction 31 (147.7 mg) yielded a mixture of **3a** and **4** which was separated by reverse phase HPLC. Six injections of 20 mg/200 μl each on a C-18 semiprep. column were made: flow 2.3 ml; solvent MeOH–H_2O (1:1) with 3% HOAc; UV detector at 254 nm. After work-up, 13.9 mg **4** was obtained along with 37.4 mg **3a**. Fraction 33 (108 mg) afforded 89.2 mg **1** after rechromatography by prep. TLC (Et_2O; four elutions).

Another collection of plant material was investigated (Hartman & Funk, No. 4288). This was collected on 8 September 1976 in Nayarit, Mexico on Highway 15, 2 miles north north-west of Esquinapa. The distribution and concn of terpenoids in this collection were very similar to collection No. 4310.

Melrosin A (**3a**). $C_{24}H_{28}O_{10}$, mp 159–161°; UV, end absorption; CD (MeOH): $[\theta]_{258} - 8.7 \times 10^3$, $[\theta]_{238} + 6.5 \times 10^4$, $[\theta]_{219} - 3.2 \times 10^4$; IR ν_{max}^{film} cm^{-1}: 3475 (OH), 1768 (α,β-unsatd-γ-lactone), 1730 (α',β-unsatd ester), 1640 (double bonds), 1143, 1043 and 995 (C–O; MS m/z (rel. int.): 476 $[M]^+$ (not present), 304 $[M-2A]^+$ (2.3), 286 $[M-2A-H_2O]^+$ (2.2), 272 $[M-2A-MeOH]^+$ (3.8), 254 $[M-2A-MeOH-H_2O]^+$ (2.8), 212 $[C]^+$ (16.7), 69 $[A']^+$ (100), 41 $[A'']^+$ (41.2). (Calcd for $C_{24}H_{28}O_{10}$: C, 60.47; H, 5.94; MW 476. Found: C, 60.63; H, 5.94%. Calcd for $C_{16}H_{16}O_6$ $[M-2A]^+$: 304.0947. Found: MS 304.0928.)

Melrosin B (**4**). $C_{24}H_{30}O_{10}$, gum; UV, end absorption; IR ν_{max}^{film} cm^{-1}: 3465 (OH), 1750 (lactone), 1715 (ester), 1625 (double bonds), 1130, 1030 and 990 (C–O); MS m/z (rel. int.): 478 $[M]^+$ (not present), 446 $[M-MeOH]^+$ (0.3), 304 $[M-A-B]^+$ (1.7), 286 $[M-A, B-H_2O]^+$ (2.0), 272 $[M-A-B-MeOH]^+$ (2.7), 254 $[M-A-B-MeOH-H_2O]^+$ (3.4), 212 $[C]^+$ (19.0), 71 $[B']^+$ (44.8), 69 $[A']^+$(100.0), 43 $[B'']^+$(27.8), 41 $[A'']^+$ (26.1). (Calcd for $C_{16}H_{16}O_6$ $[M-A-B]^+$: 304.0946. Found: MS 304.0925.)

Melrosin C (**5**). $C_{24}H_{28}O_9$, gum; UV, end absorption; IR ν_{max}^{film} cm^{-1}: 3490 (OH), 1745 (lactone), 1730 (ester), 1185, 1120 (C–O); MS m/z (rel. int.): 460 $[M]^+$ (not present), 374 $[M-A]^+$ (3.6), 356 $[M-A-H_2O]^+$ (0.9), 288 $[M-2A]^+$ (2.1), 270 $[M-2A-H_2O]^+$ (2.9), 256 $[M-2A-MeOH]^+$ (6.0), 238 $[M-2A-MeOH-H_2O]^+$ (2.9), 69 $[A']^+$ (100), 41 $[A'']^+$ (18.4).

Melrosin A diacetate (**3b**). Acetylation (Ac_2O–pyridine) of 25 mg **3a** afforded after usual work-up 18.2 mg (60%) of diacetate **3b**, $C_{28}H_{32}O_{12}$, gum; strong UV end absorption; CD (MeOH): $[\theta]_{271} - 3.8 \times 10^2$, $[\theta]_{234} + 8.1 \times 10^3$; IR ν_{max}^{film} cm^{-1}: 1770 (lactone), 1735 (esters), 1640 (double bonds), 1240, 1140, 1033 and 1000 (C–O); MS m/z (rel. int.): 560 $[M]^+$ (not present), 458 $[M-OCCH_2-HOAc]^+$ (0.5), 346 $[M-2A-OCCH_2]^+$

(1.4), 328 $[M-2A-HOAc]^+$ (1.5), 286 $[M-2A-HOAc-OCCH_2]^+$ (8.1), 268 $[M-2A-2HOAc]^+$ (9.0), 254 $[C_{12}H_{14}O_6]^+$ (9.1), 69 [A'] (100.0), 41 $[A'']^+$ (29.7).

Reaction of melrosin A (**3a**) *with sodium methoxide.* Compound **6** (50 mg, 0.11 mmol) in 10 ml MeOH was treated with 0.22 mmol NaOMe for 1.5 hr at 25°. Usual work-up provided 16.2 mg **6**, $C_{17}H_{22}O_8$, mp 95–100°; UV, end absorption; CD (MeOH): $[\theta]_{237}+3.1\times10^4$, $[\theta]_{210}+5.0\times10^4$; IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1775 (lactone), 1753 (lactone), 1070 and 997 (C–O); MS *m/z* (rel. int.): 354 $[M]^+$ (1.2), 336 $[M-H_2O]^+$ (0.7), 325 $[M-HC\equiv O]^+$ (3.0), 307 $[M-H_2O-HC\equiv O]^+$ (4.8), 212 $[C]^+$ (25.3), 198 $[C_{10}H_{12}O_4]^+$ (62.2), 166 $[C_9H_{10}O_3]^+$ (27.3), 126 $[C_6H_6O_3]^+$ (100.0), 75 (91.7), 45 $[CH_2=O-Me]^+$ (65.9).

Reaction of melcanthin B (**1**) *with* NaOMe. Compound **1** (60 mg, 0.13 mmol) in 5 ml MeOH was treated with 0.26 mmol NaOMe for 15 min at 0°. Prep. TLC gave 10.6 mg **7** and 15.9 mg **6**. Compound **7**, $C_{16}H_{18}O_7$, mp 223–225°; UV, end absorption; CD (MeOH): $[\theta]_{243}+2.1\times10^4$, $[\theta]_{219}-4.9\times10^4$; IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1775 (γ-lactone), 1753 (γ-lactone), 1638 ($C=CH_2$), 1070 and 985 (C–O); MS *m/z* (rel. int.): 322 $[M]^+$ (not present), 304 $[M-H_2O]^+$ (0.5), 293 $[M-HC\equiv O\cdot]^+$ (3.2), 275 $[M-H_2O-HC\equiv O\cdot]^+$ (2.4), 194 $[C_{10}H_{10}O_4]^+$ (23.2), 176 $[C_{10}H_8O_3]^+$ (100.0), 166 $[C_9H_{10}O_3]^+$ (19.6), 126 $[C_6H_6O_3]^+$ (69.3), 91 $[C_7H_7]^+$ (21.2), 77 $[C_6H_5]^+$ (29.9), 69 $[C_4H_5O]^+$ (40.8).

Catalytic reduction of melrosin A (**3a**). Melrosin A (50 mg, 0.11 mmol) was reduced catalytically at atm. pres. using 25 ml dry MeOH as solvent and 40 mg pre-reduced 5% Pd/C as catalyst. The consumption of 7.4 ml (0.33 mmol) of H_2 was allowed over a period of 30 min. TLC work-up gave 4.7 mg **8**, $C_{24}H_{34}O_9$ gum; UV, end absorption; CD (MeOH): $[\theta]_{220}-3.9\times10^4$; IR ν_{max}^{film} cm^{-1}: 3455 (OH), 1755 (γ-lactone), 1740 (ester), 1728 (α,β-unsatd ester), 1198, 1145 and 1050 (C–O); MS 70 eV *m/z* (rel. int.): 466 $[M]^+$ (not present), 378 $[M-B]^+$ (0.7), 364 $[M-MeOH\ O{=}C{=}CMe_2]^+$ (2.0), 308 $[M-B-O{=}C{=}CMe_2]^+$ (2.2), 290 $[M-2B]^+$ (2.6), 258 $[M-2B-MeOH]^+$ (7.2), 230 $[M-2B-MeOH-CO]^+$ (7.2), 214 $[C_{10}H_{14}O_5]^+$ (32.9), 71 $[B']^+$ (100.0), 43 $[B'']^+$ (96.3).

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REFERENCES

1. Stuessy, T. F. (1972) *Rhodora* **74**, 1.
2. Seaman, F. C., Fischer, N. H. and Stuessy, T. F. (1980) *Biochem. Syst. Ecol.* **19**, 1485.
3. Fischer, N. H., Olivier, E. J. and Fischer, H. D. (1979) in *Progress in the Chemistry of Organic Natural Products* (Herz, W., Grisebach, H. and Kirby, G. W., eds.) Vol. 38, p. 48. Springer, Wien.
4. Fischer, N. H., Seaman, F. C., Wiley, R. A. and Haegele, K. D. (1978) *J. Org. Chem.* **43**, 4984.
5. Klimash, J. W. and Fischer, N. H. (1982) *Phytochemistry* **20**, 840.
6. Fischer, N. H., Malcolm, A. J., Olivier, E. J., Fronczek, F. R., Delord, T. J. and Watkins, S. F. (1982) *J. Chem. Soc. Chem. Commun.* 1243.
7. Olivier, E. J. (1980) Ph.D. dissertation, Louisiana State University, Baton Rouge.
8. Olivier, E. J., Perry, D. L. and Fischer, N. H. (1980) *J. Org. Chem.* **45**, 4028.
9. Fischer, N. H., Wiley, R. A., Lin, H. N., Karimian, K. and Politz, S. M. (1975) *Phytochemistry* **14**, 2241.