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THE STRUCTURE AND CHEMISTRY OF SESQUITERPENE LACTONES FROM *POLYMNIA MACULATA*

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ABSTRACT.—The isolation and structure determination of two sesquiterpene lactones, the *cis,cis*-germacranolide 9-desacetoxymelcanthin F [**1**] and the leucantholide melampodin D [**2**] from *Polymnia maculata* var. *maculata* are reported. Treatment of **1** with sodium methoxide produced the 8-desacyl-13-methoxyderivative [**4**] and the 13-methoxydilactone [**5**]. Compounds **4** and **5** were also obtained in the reaction of **2** with $NaBH_4$ followed by sodium methoxide treatment. Formation of **4** from **2** represents the first transformation of a leucantholide into a *cis,cis*-germacranolide.

In continuation of our chemical study of members of the tribe Heliantheae (Asteraceae) we have investigated *Polymnia maculata* Cav. var. *maculata* of the subtribe Melampodiinae (1) from San Luis Potosi, Mexico. We had previously isolated three melampolides, polymatin A-C, from a Costa Rican population of this species (2). We now describe the isolation and structure determination of a new *cis,cis*-germacranolide, 9-desacetoxymelcanthin F [**1**], as well as the leucantholide melampodin D [**2**], which had previously been detected in *Melampodium argophyllum* by gc/ms analysis (3,4). Because the ^{1}H-nmr spectrum fro melampodin D [**2**] has not been reported before, the data are included in Table 1.

The finding of a *cis,cis*-germacranolide and a leucantholide, which are typical constituents of the genus *Melampodium*, represents the first case of occurrence of these skeletal types in the genus *Polymnia*. This further supports previous biochemical systematic and morphological considerations which indicated a close phylogenetic relationship of the two genera (1).

Structural information on the new compounds was obtained from nmr and mass spectral data and by performing chemical transformations, which include the first example for the conversion of a leucantholide into a *cis,cis*-germacranolide skeleton.

RESULTS AND DISCUSSION

9-Desacetoxymelcanthin F [**1**], $C_{21}H_{28}O_7$, was a gum that exhibited in the 200 MHz ^{1}H-nmr spectrum ($CDCl_3$) two one-proton doublets at δ 6.33 (H-13a) and 5.68 (H-13b) and a multiplet at δ 2.79 (H-7) that are characteristic of an α-methylene-γ-lactone. An ir band at 1765 cm^{-1} corroborated the presence of a γ-lactone moiety. Strong mass spectral peaks at *m/z* 85 (**A′**) and 57 (**A″**) along with diagnostic ^{1}H-nmr signals, a triplet at δ 0.88 (C-18-Me), a doublet at δ 1.07 (C-17-Me), and a multiplet at 1.52 (2H-18), indicated the presence of a 2-methylbutanoate group in **1**. Further assignments of the basic skeleton of 9-desacetoxymelcanthin F were deduced from extensive double irradiation experiments and ^{13}C-nmr data, the results being summarized in Tables 1 and 2. The great similarity of the nmr spectral data of lactone **1** with *cis,cis*-germacranolides from *Melampodium* species (5,6) suggested a similar skeleton for compound **1**. A three-proton singlet at δ 3.79, typical of a carbomethoxy methyl, together with the downfield absorption of H-1 at δ 6.81 indicated a *cis*-1(10)-double bond (6). The two-proton absorption at δ 4.01 (H-15) suggested an allylic alcohol which upon oxidation gave an aldehyde [**3**]; the chemical shift of which (H-15, δ 9.38) supported the presence of a 4-*cis*-double bond in **1** (6). The new lactone differed from all previously isolated *cis,cis*-germacranolides in that it lacked an acyloxy function at C-9. This was clearly shown by the presence of two C-9 protons.

TABLE 1. ^{1}H-nmr Spectral Data[a] of 9-Desacetoxymelcanthin F [**1**], Melampodin D [**2**] and Derivatives

Assignments	Compounds					
	1 (acetone-d_6)	**2** ($CDCl_3$)	**4** ($CDCl_3$)	**5** ($CDCl_3$)	**6** ($CDCl_3$)	**7** (C_6D_6)
H-1	6.84 [6.81] dt (7.8,7.8,1.3)	5.65 m	6.81 [6.67] dd (7.5)	6.78 br m	6.75 [6.35] brm	6.38 brm
H-2a/b	[2.56] m	2.0-2.5	2.30-2.60 [1.86-2.14]	—	2.18-2.65 [1.46-1.76]	—
H-3a/b	3.39 m	2.0-2.5	2.30-2.60 [1.86-2.14]	2.20-2.80	2.18-2.65 [1.46-1.76]	1.75-2.22
H-5	5.51 [5.51] dd (10,1.0)	5.57 d (10.0)	6.63 [5.70] d (9.8)	5.58 d (10.0)	5.65 [5.16] d (10.0)	5.36 d (10.0)
H-6	5.60 [5.51] dd (10,4.4)	4.66 dd (10.0,10.0)	5.37 [5.48] dd (9.8,5.0)	5.07 dd (10.0,10.0)	5.17 [4.77] dd (10.0,9.0)	4.55 dd (10.0,10.0)
H-7	2.89 [2.79] dddd (4.4,3.0,2.5,2.4)	3.25 ddd (10.0,3.5, 3.0)	2.18 [2.27] ddd (6.0,5.0,2.0)	2.66 ddd 12.0,10.0,3.9)	3.11 [2.15] dddd (9.0,3.5,3.5,3.0)	1.72 ddd (13.0,10.0,4.4)
H-8	5.81 [5.81] ddd (9.5,7.1,2.5)	5.62 m	4.46 [4.47] ddd (9.8,7.2,2.0)	4.89 dd (9.0,3.9)	5.15 [4.11] dd (9.0,3.5)	3.80 dd (9.0,4.4)
H-9a	2.93 [2.95] ddd (14,7.1,1.3)	7.19 br	2.70 dd (14.0,7.0)	3.21 d (14.8)	3.21 [2.35] d (15.0)	3.39 d (14.5)
H-9b	2.78 [2.60] dd (14,9.5)	—	[2.51] dd (14.0,10.0)	2.85 ddd 14.8,9.0,3.0)	2.93 [1.96] ddd (15.0,9.0,3.0)	2.33 ddd 14.5,9.0,3.0)
H-11	—	—	2.91 [2.97] ddd (6.0,5.0,3.8)	3.07 ddd (12.0,4.7,3.2)	—	2.57 dq (13.0,7.0)
H-13a	6.20 [6.33] d (3.0)	6.42 d (3.5)	3.68 [3.60] dd (9.5,5.0)	3.79 dd (10.0,4.07)	6.42 [6.32] d (3.5)	1.5 d (7.0)
H-13b	5.69 [5.68] d (2.4)	5.83 d (3.0)	3.53 [3.34] dd (9.5,3.8)	3.69 dd (10.0,4.7)	5.73 [5.21] d (3.0)	
H-15	3.97 [4.01] br s	4.14 br s	4.02 [3.68]	4.15	4.16 [3.55]	3.51
H-17	2.30 [2.32] m (7.0)	2.38 m (7.0)	—	—	—	—
H-18a/b	1.50 [1.52] m (7.0)	1.58 m (7.0)	—	—	—	—
H-20	1.05 [1.07] d (7.0)	1.15 d (7.0)	—	—	—	—
H-19	0.86 [0.88] t (7.0)	0.89 t (7.0)	—	—	—	—
CO_2CH_3	3.75 [3.97]		3.73 [3.37]	—	—	—
O-CH_3	—		3.34 [3.00]	3.40	—	—

[a]Spectra were obtained at 200 MHz in the solvents indicated. Data in brackets for compound **1** are chemical shifts in $CDCl_3$ and for compounds **4** and **6** are chemical shifts in C_6D_6. Chemical shifts are recorded in ppm relative to TMS. Singlets are unmarked and multiplets are designated by usual symbols. Figures in parentheses are coupling constants in hertz.

TABLE 2. ^{13}C-nmr Spectral Data[a] of 9-Desacetoxymelcanthin F [1]

Carbon	δ, multiplicity	Carbon	δ, multiplicity
1	142.8 d	11	135.7 s
2	25.0 t[b]	12	175.5 s[d]
3	25.8 t[c]	13	124.0 t
4	130.3 s	14	169.6 s[d]
5	125.7 d	15	66.2 t
6	73.6 d[c]	16	167.3 s[d]
7	47.1 d	17	41.1 d
8	74.0 d[c]	18	26.4 t
9	30.1 t	19	16.7 q
10	139.4 s	20	11.5 d
		-O-CH_3	52.0 q

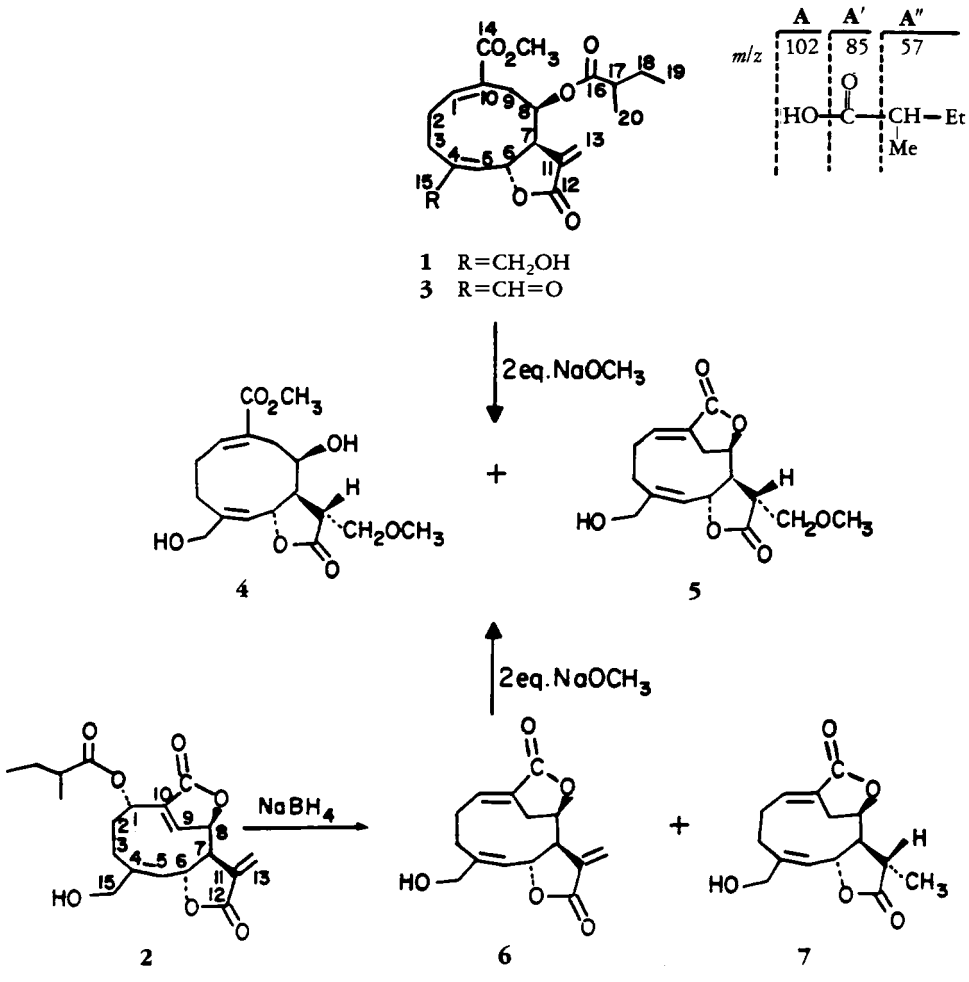
[a]The spectrum was obtained in $CDCl_3$ atambient temperature at 50.32 MHz. Chemical shifts are given in ppm relative to TMS as determined by proton noise decoupling. Peak multiplicity was obtained by off-resonance decoupling (2.5, ppm above TMS).

[b,c,d]Assignments interchangeable.

The attachment of the 2-methylbutanoate group to C-8 was in agreement with the chemical shift observed for H-8 (δ 5.81), which is generally more deshielded than the lactonic proton-signal at C-6 (δ 5.51). This was confirmed by the reaction of **1** with 2.5 molar equivalents of sodium methoxide in MeOH, which produced two major compounds [**4** and **5**]. The upfield shift of H-8 from δ 5.81 in **1** to 4.66 ppm in the transesterification product **4** (Table 1) further supported the attachment of the 2-methylbutanoate to C-8 in **1**.

The transformation of 9-desacetoxymelcanthin F [**1**] into **4** and **5** must involve conjugate addition of the methoxide to the 11,13-double bond combined with a base-mediated transesterification of the 2-methylbutanoate moiety at C-8 producing compound **4**. Subsequent 14,8-lactonization by intramolecular transesterification would give rise to the dilactone **5**. The stereochemistry of H-11 in **5** was tentatively assigned on the basis of its large coupling constant ($J_{7,11}$ = 12 Hz). This was in agreement with an antiperiplanar arrangement of H-7 and H-11 suggesting a H-11β in **5** assuming an H-7α as in all sesquiterpene lactones from higher plants (7). The $J_{7,11}$ value observed for compound **4** was 6.0 Hz which did not allow a clear assignment of the stereochemistry of C-11. When compound **4** was stirred with 20% aqueous HCl in MeOH for 2 h, the dilactone **5** was obtained as the major product. Although epimerization at C-11 could have taken place in the latter lactonization, the difference between the $J_{7,11}$ values observed for compounds **4** and **5** is more likely due to the change in conformation of the medium ring upon lactonization. Formation of the 14,8-lactone imposes additional ring strain on the medium ring, which also changes the flexibility of the lactone ring, resulting in a dihedral angle (~180°) between H-7 and H-11, which agrees with the observed $J_{7,11}$ value. The large coupling between H-5, H-6, and H-7 ($J_{5,6}$=$J_{6,7}$ = 10.0 Hz) in compound **5** is indicative of a near antiperiplanar orientation of the three protons. Based on the biogenetic assumption that all sesquiterpene lactones from higher plants have an H-7α configuration, H-6 must be β-oriented, that is, a *trans*-lactone must be present in **1**.

The configurations of C-6, C-7, and C-8 in compound **1** were further assigned on the basis of chemical correlation with melampodin D [**2**], which involved conversion of melampodin D into compounds **4** and **5** via the dilactone **6**. Reaction of melampodin D with $NaBH_4$ provided the dilactones **6** and **7**; the formation of which can be formulated as an S_N1-type reaction or may proceed via a C-10 carbanion followed by the loss of



the C-1 acyloxy group. The identities of lactones **6** and **7** were established by detailed spectral correlations (^{1}H nmr, ir, ms) the data being summarized in Table 1 and the Experimental section. Transformation of dilactone **6** into compounds **4** and **5** involves a base-mediated conjugate addition to the methylene lactone moiety combined with a partial transesterification leading to the *cis,cis*-germacranolide [**4**]. The transformation of melampodin D [**2**] into compound **4** represents the first example of the conversion of a leucantholide into a *cis,cis*-germacranolide. It is the reverse reaction of a previously described interconversion of the two biogenetically related skeletal types (8). Assuming that the conversion of melampodin D [**2**] into compounds **4** and **5** does not involve inversion of stereochemistry at C-8; the configurations in **1** must be H-6β, H-7α, and H-8α as in **2**.

Detailed ^{13}C-nmr studies involving proton noise decoupling (PND) along with single-frequency off-center decoupling (SFOCD) permitted partial assignment of the carbons in compound **1**. The use of selective single-frequency heteronuclear decoupled ^{13}C nmr (9) led to more conclusive assignments as summarized in Table 2.

This completed the structural arrangement of the carbon skeleton of 9-desacetoxymelcanthin F [**1**] except for the overall conformation of the medium ring. Since compound **1** is a *cis,cis*-germacranolide, its medium ring conformation in the solid state should be similar to [$^1D^{14}$,$_{15}D_5$]-longicornin A, a compound with known molecular structure (6).

EXPERIMENTAL

P. maculata var. *maculata* was collected on October 6, 1976, 10 miles west of Rayon, San Luis Potosi, Mexico (Stuessy-Gardner No. 4045; voucher at the Herbarium of Ohio State University). Dried leaves (62.0 g) were extracted and worked up as previously described (10) to yield 587 mg of crude syrup. Column chromatography on 60 g of silica gel using Et_2O-petroleum ether followed by mixtures of Et_2O/Me_2CO with increasing polarity gave 36 fractions of 50 ml each which were monitored by tlc. Fractions 8-16 (63.0 mg) afforded 9-desacetoxymelcanthin F [**1**]. Fractions 17-18 gave a mixture of terpenoid compounds that could not be further investigated due to limited amounts of material. Fraction 20 yielded 87 mg of melampodin D [**2**].

9-Desacetoxymelcanthin F [**1**]. $C_{21}H_{28}O_7$, gum; cd (MeOH) $[\theta]_{227}=+2.1\times10^4$; ir ν max ($CHCl_3$) 3600 (OH), 1765 (α,β-unsatd-γ-lactone), 1740, 1715 (esters), 1665, 1645 (double bonds); ms 70 eV *m/z* (rel. int.) 360 (0.5, M-MeOH), 335 (0.3, M-**A**″), 317 (0.3, M-**A**″-H_2O), 307 (0.3, M-**A**′), 290 (11.3, M-**A**), 289 (1.4, M-**A**′-H_2O), 272 (11.3, M-**A**-H_2O), 85 (84.1, **A**′), 57 (100, **A**″).

Anal. calcd for $C_{16}H_{18}O_5$ (M-**A** 290.1154. Found (ms) 290.1122. Ci (isobutane) *m/z* 393 (M+1).

REACTION OF 9-DESACETOXYMELCANTHIN F [**1**] WITH SODIUM METHOXIDE.—To a solution of **1** (32 mg, 0.082 mmoles) in 5 ml of absolute MeOH at 0° was added 0.25 mmoles of MeONa in 6 ml of MeOH. The mixture was allowed to warm up to ambient temperature, and after 3 h the reaction was quenched by the addition of aqueous 5% HCl. The reaction mixture was evaporated to dryness, and the residue partitioned between CH_2Cl_2 and H_2O. The organic layer was dried over Na_2SO_4 and evaporated. The oily residue was separated by preparative tlc (EtOAc-petroleum ether, 3:1). The band with Rf=0.26 gave 5.1 mg of **5** and the band at Rf=0.34 provided 6.0 mg of **4**.

Dilactone **5**, $C_{16}H_{20}O_6$, gum; ir ν max ($CHCl_3$) 3520 (OH), 1763 (γ-lactone), 1685 (double bonds); ms 70 eV *m/z* (rel. int.) 308 (1.4, M^+), 290 (0.8, M-H_2O), 276 (0.9, M-MeOH), 258 (0.9, M-MeOH -H_2O), 231 (100, $C_{14}H_{15}O_3$).

Anal. calcd for $C_{16}H_{20}O_6$: 308.1258. Found (ms): 308.1262.

Compound **4**. $C_{17}H_{24}O_7$, gum; ir ν max ($CHCl_3$) 3540 (OH), 1765 (γ-lactone), 1700 (α,β-unsatd ester), 1650 (double bonds); ms 70 eV *m/z* (rel. int.) 308 (2.7, M-MeOH), 290 (6.7, M-MeOH -H_2O), 272 (2.7, M-MeOH-2H_2O); ci (isobutane) *m/z* 341 (M+1).

OXIDATION OF 9-DESACETOXYMELCANTHIN F [**1**] WITH 2,2-BIPYRIDINIUMCHLOROCHROMATE.—To a stirred solution of 30 mg (0.0765 mmoles) of **1** in 5 ml of dry CH_2Cl_2 at room temperature was added 81 mg of reagent. The mixture was reacted for 3 h, filtered, and the filtrate washed with H_2O and dried over Na_2SO_4. The oily residue was separated by preparative tlc (EtOAc-petroleum ether, 1:1). The band at Rf=0.5 afforded 10 mg of aldehyde **3** $C_{21}H_{26}O_7$, gum; ir ν max ($CHCl_3$) 1779 (γ-lactone) 1739 (ester), 1724 (aldehyde); ms 70 eV *m/z* (rel. int.) 390 (0.3, M^+), 333 (0.6, M-**A**″), 305 (0.6, M-**A**′), 288 (3.6, M-**A**), 256 (7.0, M-**A**-MeOH).

Anal. calcd for $C_{21}H_{26}O_7$: 390.1676. Found (ms): 390.1671.

Melampodin D [**2**]. $C_{20}H_{24}O_7$; ms 70 eV *m/z* (rel. int.) 319 (0.6, M-**A**″), 274 (11.3, M-**A**), 256 (17.5, M-**A**-H_2O).

ACID-CATALYZED LACTONIZATION OF **4**.—Compound **4** (6 mg) was added to 5 ml of a solution of 20% aqueous HCl in MeOH and stirred a room temperature for 2 h. The solution was adjusted to pH 6 with 10% aqueous NaOH. The solvent was evaporated, CH_2Cl_2 was added, washed twice with H_2O, dried over Na_2SO_4, and the solvent removed. The residual oil showed one spot on tlc and the ^{1}H-nmr, ir, and ms data were identical with those of compound **5**.

REDUCTION OF MELAMPODIN D [**2**] WITH $NaBH_4$.—To a stirred solution of 86.0 mg (0.23 mmoles) of melampodin D [**2**] in 10 ml of absolute MeOH at 0° was added 22.0 mg (0.57 mmoles) of $NaBH_4$ and reacted for 25 min, after which it was adjusted to pH 5 with 5% aqueous HCl. The solution was evaporated to dryness, H_2O was added and extracted with CH_2Cl_2 and dried over Na_2SO_4. The oily residue was separated by preparative tlc (EtOAc-cyclohexane, 3:1). The band with Rf=0.38 gave 8.6 mg of compound **7**, and the band at Rf=0.30 provided 18.6 mg of compound **6**.

Compound **7**. $C_{15}H_{18}O_5$, gum; ir ν max ($CHCl_3$) 3500 (OH), 1770 (γ-lactone), 1690 (double bonds); ms 70 eV *m/z* (rel. int.) 278 (4.3, M^+), 260 (6.0, M-H_2O), 232 (25.5, M-H_2O-CO), 216 (5.0, M-H_2O-CO_2).

Anal. calcd for $C_{15}H_{18}O_5$: 278.1153. Found (ms): 278.1093.

Compound **6**. $C_{15}H_{16}O_5$, gum; ir ν max ($CHCl_3$) 3490 (OH)), 1765 (γ-lactone), 1685 (double bonds); ms 70 eV *m/z* (rel. int.) 276 (0.9, M^+), 258 (3.7, M-H_2O), 230 (12.9, M-H_2O-CO), 214 (7.0, M-H_2O-CO_2).

Anal. calcd for $C_{15}H_{14}O_4$ (M-H_2O): 258.0892. Found (ms): 258.0892.

REACTION OF COMPOUND **6** WITH MeONa.—At room temperature, compound **6** (18.0 mg, 0.065 mmoles) in 5 ml of absolute MeOH and 1.6 ml of a solution containing 0.13 mmoles of MeONa were stirred. After 2 h 5% aqueous HCl was added. Standard work-up gave an oil which was separated by preparative tlc (EtOAc-cyclohexane, 3:1). The less polar band afforded 6 mg of compound **4**, and the more polar band gave 3 mg of compound **5**.

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