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THREE NEW MELAMPOLIDE SESQUITERPENES, POLYMATIN A, B AND C, FROM *POLYMNIA MACULATA* CAV. VAR. *MACULATA*

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Key Word Index—*Polymnia maculata* Cav. var. *maculata*; Melampodiinae; Heliantheae; Compositae; new melampolide type sesquiterpene lactones.

Abstract—Three new melampolide type sesquiterpene lactones were isolated from *Polymnia maculata* Cav. var. *maculata*. Their structures were determined by spectroscopic and chemical means. Based on the presently available chemical data, the chemotaxonomic position of *Polymnia* within the subtribe is discussed.

INTRODUCTION

In continuation of our search for sesquiterpene lactones with potential biological activity in Compositae, we have investigated one species of the genus *Polymnia*. Several members of this genus (tribe Heliantheae, subtribe Melampodiinae) have been investigated. Besides pentaynen [1], a number of germacranolides of the melampolide type [2, 3] and the eudesmanolide ivalin [2] were found. Also, the flavones naringenin, sakuranetin [4] and artemetin [2], the diterpenes of the kaurene type [4] and some esters of cinnamic acid [4] have been found as *Polymnia* constituents.

We now report the isolation and structure elucidation of three new highly oxygenated melampolides, which we have named polymatin A (**3**), B (**4**) and C (**6**), from *Polymnia maculata* Cav. var. *maculata*. Structures and stereochemistry were established mainly by extensive use of ^{1}H NMR spectroscopy involving double resonance experiments and Eu(fod)$_3$ shift reagents as well as chemical transformations.

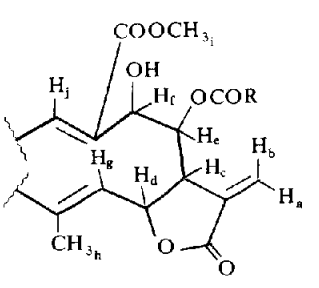
RESULTS AND DISCUSSION

The aerial parts of *P. maculata* var. *maculata* afforded in high yield the known kaurenic acids **1** and **2** [5, 6] together with four sesquiterpene lactones, two of them representing a crystalline mixture which could not be separated.

Polymatin A (**3**)

The most polar lactonic constituent with the molecular formula $C_{21}H_{26}O_7$ (high resolution MS) represented an alcohol (IR band at 3560 cm^{-1}) and contained an $\alpha\beta$-unsaturated lactone of the partial structure **A** (strong UV end absorption, IR bands at 1780 and 1630 cm^{-1}, narrowly split ^{1}H NMR doublets of H_a and H_b at δ 6.24 (J = 3.5 Hz)) and 5.67 (J = 3 Hz). The location of H_c as a multiplet at 2.80 was established by double irradiation at the frequencies of H_a and H_b. Irradiation at the frequency of H_c caused collapse of H_a and H_b into singlets and also converted a doublet of doublets at 5.09 (J_1 = 10, J_2 = 9 Hz) into a doublet (J = 10 Hz), and a narrowly split doublet of doublets at 6.33 (J_1 = 8, J_2 = 2 Hz) into a sharp doublet (J = 8 Hz). The absorptions at δ 5.09 and 6.33 were assigned H_d and H_e respectively or vice versa. The chemical shift of the lower field proton suggested that it was under an ester rather than under the lactone oxygen, especially since the IR spectrum indicated the presence of additional carbonyl functions at 1720 and 1725 cm^{-1} associated with unsaturated esters. One of these must be due to the carbomethoxy group indicated also by a ^{1}H NMR singlet at δ 3.81 (3H). The nature of the other ester side chain was deduced as follows. The ^{1}H NMR spectrum exhibited one methyl quartet of quartets at δ 1.89 ($J_1 = J_2$ = 1 Hz), one methyl doublet of quartets at 1.98 (J_1 = 7, J_2 = 1 Hz) each coupled to a one proton quartet of quartets at 6.10 (J_1 = 7, J_2 = 1 Hz), thus indicating the presence of an angeloyl side chain. This was confirmed by the appearance of a M-100 peak at *m/e* 290 due to the loss of angelic acid from the parent ion at *m/e* 390. Strong peaks at *m/e* 83 (acylium ion of angelic acid) and *m/e* 55 ($C_4H_7^+$) represented further typical markers for this side chain.

Irradiation at the frequency of H_e caused collapse of one doublet of doublets at δ 4.00 (H_f, J_1 = 9, J_2 = 8 Hz) into one doublet (J = 9 Hz). From chemical shift considerations, H_f must be under an oxygen function, most likely an OH group. This was confirmed by an ^{1}H



A

* Dedicated to my major professor, Professor F. Bohlmann.

NMR spectrum of a D_2O-exchanged sample. The frequency of H_f simplified to a doublet ($J = 8$ Hz). Also, acetylation of **3** with acetic anhydride in pyridine gave **4**. The doublet of doublets at δ 4.00 of the alcohol **3** moved downfield to 5.40 ($J = 8.5$ Hz) in acetate **4**.

Irradiation at the frequency of H_d converted H_c into a broad singlet and changed a broadened doublet at δ 4.94 (H_g, $J = 10$ Hz) into a broadened singlet. The broadening was caused by allylic coupling with a vinylic methyl (H_h) which appeared as a broadened singlet at 1.89.

The ^{1}H NMR spectrum exhibited also a one-proton doublet of doublets at δ 6.86 (H_i, $J_1 = 10$, $J_2 = 7$ Hz) typical of a β-proton at an α,β-unsaturated methyl ester (H_i); this olefinic proton coupled with two protons at 2.30. To complete the partial structure **A** only two methylenes had to be added permitting extension of **A** to **3**, exclusive of stereochemistry.

^{1}H NMR and CD correlation of **3** with melampodin A [7], whose structure and absolute configuration were determined by X-ray diffraction by Neidle and Rogers [8] and by neutron diffraction by Watkins *et al.* [9], permitted determination of the relative stereochemistry of polymatin A as **3**. The medium ring must be a *cis*-1(10)-*trans*-4,5-cyclodecadiene (melampolide) with anti-arrangement of C-14 and C-15 [8–10] and the lactone ring was *trans*-fused ($J_{5,6} = 10$, $J_{6,7} = 9$ Hz). The stereochemistry at C-8 and C-9 was deduced by comparison of ^{1}H NMR parameters with those of other melampolides and stereomodel considerations. The small coupling constant between H-7 and H-8 ($J = 2$ Hz) required H-8 to adopt an α-orientation if H-7 was α as in all other lactones. A large coupling between H-8 and H-9 ($J = 8$ Hz) indicated that H-9 was *trans* to H-8, that is, β. The paramagnetic shift of H-8 (6.33 ppm) must be caused by the orientation of the carbomethoxy carbonyl group, H-8 being placed in the plane of the carbonyl, and therefore strongly deshielded as previously observed for melampolides [11].

Polymatin B (**4**)

Compound **4**, $C_{23}H_{28}O_8$ (high resolution MS) which was less polar than **3** exhibited an IR spectrum, which indicated the absence of an OH group. An acetate absorption at 1.94 (3H) in the ^{1}H NMR spectrum and the empirical formula suggested the presence of an acetoxy function at C-9 in **4** instead of an OH group as in **3**. Acetylation of polymatin A (**3**) with acetic anhydride in pyridine gave an acetate which was identical with the natural polymatin B (**4**).

Polymatin C (**6**)

The major lactonic fractions contained a crystalline mixture of two closely related compounds, mp 183°, which were indistinguishable on TLC, but the 100 MHz ^{1}H NMR spectrum with distinct double patterns indicated a 5:2 mixture of two lactones. The major constituent in the mixture was shown to be identical with enhydrin (**5**) by comparison with ^{1}H NMR spectra in $CDCl_3$ and C_6D_6 [12].

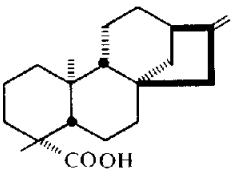
The ^{1}H NMR spectrum of the minor lactone, which we named polymatin C (**6**), differed only minimally from that of enhydrin (**5**). In addition, the ^{1}H NMR parameters did not match those of maculatin, an isomer of enhydrin [3]. The identity of the coupling constants and near identity of chemical shifts of **5** and **6** (see Table 1) requires that the two lactones possess the same configuration and conformation around the medium ring. Both contain two ester moieties, one being an acetate group and the other epoxyangelate. Epoxytiglate was excluded on the basis of ^{1}H NMR parameters, especially the H-3′ chemical shift.

Table 1. ^{1}H NMR data* of sesquiterpene lactones **3–6**

	5	+ Eu(fod)$_3$	**6**	+ Eu(fod)$_3$	**4**	**3**
1-H	7.15 *dd* (10, 7)	7.5–7.7 *m*	7.15 *dd* (10, 7)	7.5–7.7 *m*	7.00 *dd* (10, 7)	6.84 *dd* (10, 7)
2-H	2.3–2.5 *m*†	—	2.3–2.5 *m*†	—	2.48 *m*†	2.30 *m*†
5-H	2.67 *d* (9.5)	4.44 *d*	2.67 *d* (9.5)	4.59 *d*	4.94 *dbr* (9.5)	4.94 *ddr* (10)
6-H	4.27 *dd* (9.5, 9.5)	5.90 *dd*	4.27 *dd* (9.5, 9.5)	5.84 *dd*	5.11 *dd* (10, 9.5)	5.09 *dd* (10, 9)
7-H	3.00 *m*	—	3.00 *m*	—	2.79 *m*	2.80 *m*
8-H	6.71 *dd* (8.5, 1)	7.5–7.7 *m*	6.71 *dd* (8.5, 1)	7.5–7.7 *m*	6.67 *dd* (8.5, 1.5)	6.33 *dd* (8, 2)
9-H	5.87 *d* (8.5)	6.74 *d*	5.87 *d* (8.5)	6.84 *d*	5.40 *d* (8.5)	4.00‡ *dd* (9, 8)
13-H	5.81 *d* (3)	6.41 *d*	5.90 *d* (3)	6.23 *d*	5.78 *d* (3)	5.67 *d* (3)
13-H	6.30 *d* (3.5)	7.09 *d*	6.34 *d* (3.5)	6.80 *d*	6.25 *d* (3)	6.24 *d* (3.5)
—COOCH$_3$	3.80§ *s*	4.06 *s*	3.80§ *s*	4.02 *s*	3.80§ *s*	3.81§ *s*
15-H	1.70§ *s*	2.55 *s*	1.70§ *s*	2.60 *s*	1.98§ *sbr*	1.89§ *sbr*
OAc	2.03§ *s*	2.28 *s*	2.00§ *s*	2.35 *s*	1.94§ *s*	—
OEpAng‖	3.00 *m*	3.49 *q*	3.00 *m*	3.90 *m*	—	—
	1.43§ *s*	1.90 *s*	1.43§ *s*	2.05 *s*		
	1.16§ *d* (5)	1.65 *d*	1.20§ *d* (5)	2.10 *d*		
OAng‖	—	—	—	—	6.05 *qq* (7, 1)	6.10 *qq* (7, 1)
					1.95§ *dq* (7, 1)	1.98§ *dq* (7, 1)
					1.82§ *qq* (1, 1)	1.89§ *qq* (1, 1)

* Spectra were run in $CDCl_3$, at 100 MHz and Me_4Si was used as internal standard. Chemical shifts are in parts per million relative to TMS. Signals are designated as follows: *s*, singlet; *d*, doublet; *t*, triplet; *q*, quartet; *m*, multiplet whose center is given; *br*, broad. Figures in parentheses are coupling constants in hertz. Signals correspond to one proton unless otherwise given.

† Intensity two protons. ‡ $J_{9,OH} = 9$ Hz. § Intensity three protons. ‖ OEpAng = epoxyangelate; OAng = angelate.



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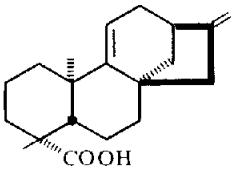

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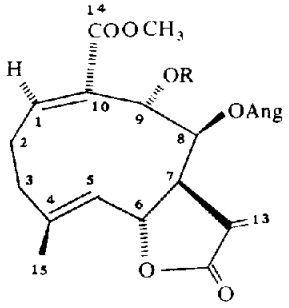
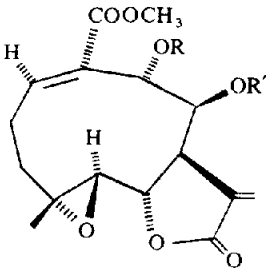


3 R = H
4 R = Ac



5 R = Ac; R' = epoxang*
6 R = epoxang; R' = Ac

The difference in the ^1H NMR spectra between polymatin C (**6**) and enhydrin (**5**) could either be due to a reverse attachment of the two ester groups at C-8 and C-9, or they could be stereoisomeric in the five carbon ester side chain. To decide between these two possibilities we have carried out extensive ^1H NMR studies of the 5:2 mixture involving $Eu(fod)_3$ shift reagent. While H-6 and H-13a and b of polymatin C shifted to δ 6.23, 6.80 and 5.84, respectively, enhydrin gave respective shifts at 6.41, 7.09 and 5.90. $Eu(fod)_3$ prefers to interact with the epoxide center of the expoxyangelic acid ester than the acetate group [13].

The observed differences in the downfield shifting of polymatin C and enhydrin in the presence of $Eu(fod)_3$, in particular H-13a, H-13b, H-6 and H-9 (Table 1) lead us to conclude that most probably it must be a reversal of the two ester groups in polymatin C and enhydrin, that is, the acetate group being attached to C-8 and the epoxyangelate at C-9 in polymatin C (**6**). In comparison to enhydrin, this would explain the lesser deshieldings of H-13a, H-13b and H-6 in polymatin C and a greater downfield shift of H-9 after $Eu(fod)_3$ addition. Upon $Eu(fod)_3$ complexation, the stronger downfield chemical shift of the epoxyangelate group in polymatin C, compared with those of enhydrin is in good agreement with structure **6**. This can be interpreted as an additional complexing effect of the carbomethoxy group.

The MS of the mixture showed only a small molecular ion at *m/e* 464.168 ($C_{23}H_{28}O_{10}$) and provided evidence for the presence of one acetate group and one epoxyangelic ester group in each compound by showing a strong peak at *m/e* 405 ($M^+ - {}^{\cdot}OAc$) and 348 ($M^+ -$ $HOOCC(CH_3)CHCH_3$). A small peak at *m/e* 433 must

be due to the loss of the methoxy radical (31 mu) from the parent ion, and a strong peak at *m/e* 256 to the loss of epoxyangelic acid, acetic acid and methanol (208 mu) from the molecular ion.

The chemical findings within this genus are relatively diverse. In two species, *P. uvedalia* and *P. maculata*, melampolides have been found [2, 3]. *P. laerigata* contains the eudesmanolide ivalin [2], and *P. canadensis* the flavone artemetin [2], whereas in two other species, *P. fructicosa* and *P. pyramidalis*, kaurene derivatives seem to be characteristic [4]. For the first time, melampolides and kaurenic acids were found together in one species, *Polymnia maculata* Cav. var. *maculata*. It appears that the genus *Polymnia* is an intermediate to the first group of the subtribe [4, 14]. While the first group (*Acanthospermum* and *Melampodium*) typically produces melampolides [11, 15] diterpenes seem to be more characteristic for the second group which includes *Espeletia* [5, 16–24] and *Siegesbeckia* [25, 26]. However, more chemical and botanical investigations are necessary to shed light on the relationships within the subtribe.

EXPERIMENTAL

IR spectra were run in CCl_4 or $CHCl_3$. ^1H NMR spectra were run at 100 MHz (interpretations were verified by extensive double resonance experiments); MS were 70 eV with high resolution; CD were run in MeOH; mps were determined in capillaries and are uncorr.

Polymnia maculata *Cav. var.* maculata. The air-dried plant material collected on 1 November 1976, in Costa Rica; Alajuda: 5 10 mile W. of Narakjo on route CA-1 (Stuessey and Gardner, No. 4453, Voucher deposited at O.H.), was extracted with Et_2O–petrol at room temp. overnight and the resulting extracts were separated first by CC (Si gel, 30–70 mesh) and further by repeated TLC (pre-coated TLC plates Si G-100 UV_{254} of Brinkman Instruments, Inc.) using $CHCl_3$, Et_2O–petrol or C_6H_6–Et_2O mixtures. Known compounds were identified by comparison of their ^1H NMR and IR spectra with authentic samples.

730 g aerial parts of *P. maculata* Cav. var. *maculata* afforded 2.8 g of a mixture of **1** and **2** (ratio ~ 3:1). The Et_2O–MeOH

* Ang = Angelic acid; epoxang = epoxyangelic acid.

fractions gave 300 mg of a mixture of **5** and **6** (ratio 5:2), which was recrystallized from *iso*-PrOH and then run 3× on PLC in $CHCl_3$–Et_2O (2:1). In addition, 20 mg **4** (6 runs in $CHCl_3$) and 70 mg **3** [6 runs in $CHCl_3$ Et_2O, 2 runs in C_6H_6 Et_2O (5:1)] were obtained.

Polymatin C (**6**). Contaminated with **5**; colourless crystalline mixture, mp 183° (*iso*-PrOH). IR ($CHCl_3$): γ-lactone 1780; C=CCOOCH$_3$ 1725; saturated COOR 1740, 1250 cm^{-1}. MS *m/e* (rel. int.): 464.168 (M^+, 2%) (calc. for $C_{23}H_{28}O_{10}$ 464.168); 433 (M − $^{\cdot}OCH_3$ 4); 405 (M − $^{\cdot}$Ac, 15); 348 (M − 
HOOCC(CH$_3$)CHCH$_3$, 62); 256 (348 − HOAc–CH$_3$OH, 62); 289 (348 − $^{\cdot}COOCH_3$, 12); 229 (289 − HOAc, 52); 201 (229 − CO, 30); 43 (CH_3CO^+, 100).

Polymatin B (**4**). Colourless gum, IR (CCl_4): γ-lactone 1780; C=C—COOR 1730, 1660; OAc 1745, 1250 cm^{-1}. MS *m/e* (rel. int.): 432.178 (M^+, 0.8%) (calc. for $C_{23}H_{28}O_8$ 432.178); 401 (M − $^{\cdot}OCH_3$, 0.1); 372 (M − HOAc, 0.7); 272 (372 − HOOC—C(CH$_3$)=CH—CH$_3$, 9.4); 240 (272 − CH$_3$OH, 3); 213 (272 − $^{\cdot}COOCH_3$, 8); 83 (CH_3—CH=C(CH$_3$)CO$^+$, 100); 55 ($C_4H_7^+$, 62); 43 (CH_3CO^+, 87). CD: (*c*, 1.24 × 10^{-4}, MeOH) $[\theta]^{27}_{265}$ 1393; $[\theta]^{27}_{247}$ − 72464.

Polymatin A (**3**). Colourless gum, IR (CCl_4): OH 3560; γ-lactone 1780; C=C—COOR 1725, 1710 cm^{-1}. MS *m/e* (rel. int.): 390.168 (M^+, 0.3%) (calc. for $C_{21}H_{26}O_7$ 390.168); 359 (M − $^{\cdot}OCH_3$, 0.6); 290 (M − HOOCC(CH$_3$)=CH—CH$_3$, 3); 272 (290 − H$_2$O, 2.1); 213 (272 − $^{\cdot}COOCH_3$, 6.4); 258 (290 − CH$_3$OH, 7.7); 83 (CH_3—CH=C(CH$_3$)CO$^+$, 100); 55 ($C_4H_7^+$, 62.8). CD: (*c*, 1.74 × 10^{-4}, MeOH) $[\theta]^{27}_{276}$ − 852; $[\theta]^{27}_{247}$ + 2555; $[\theta]^{27}_{214}$ − 27681.

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