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MELAMPOLIDES FROM *ENHYDRA FLUCTUANS* VAR. *FLUCTUANS**

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Key Word Index—*Enhydra fluctuans* var. *fluctuans*; Compositae; sesquiterpene lactones; melampolides.

Abstract—The investigation of the aerial parts of a variety of *Enhydra fluctuans* afforded in addition to 4-hydroxyfarnesyl acetate and fluctuadin five new melampolides elucidated by high field ^{1}H NMR spectroscopy. The chemotaxonomic situation is discussed briefly.

INTRODUCTION

The genus *Enhydra* (Compositae, tribe Heliantheae) was placed by Stuessy [1] in the subtribe Ecliptinae, while recently in a new grouping of the large tribe Heliantheae [2] a separate subtribe Enhydrinae has been proposed with *Enhydra* being the single genus. Previous chemical studies have shown that in addition to diterpenes, melampolides are present [3–6], which have been isolated previously from *Melampodium, Smallanthus, Tetragonotheca, Sigesbeckia, Schkuhria, Iva, Blainvillea, Grazielia* and *Acanthospermum* species. We have reinvestigated the aerial parts of a variety, *Enhydra fluctuans* Lour. var. *fluctuans* and again melampolides were isolated, five of them being new compounds.

RESULTS AND DISCUSSION

The aerial parts of *E. fluctuans* var. *fluctuans*, collected in north-eastern Brazil, afforded in addition to 4-hydroxyfarnesyl acetate (**1**) [7] and fluctuadin (**2**) [6] five further sesquiterpene lactones, the melampolides **3–6** and **8**. The ^{1}H NMR spectrum of **3** (Table 1), which could not be completely separated from **2**, showed by the typical signals that a tiglate was present (δ 6.76*qq*, 1.80*dq* and 1.78*dq*). The remaining signals were only slightly different from those of enhydrin (**7**). Accordingly, the same substitution pattern and stereochemistry could be assumed. Spin decoupling established the assignment of all signals. The extreme downfield shifts of H-8 clearly supported the proposed conformation of melampolides [8] with 4″β″-methyl and 10″α″-carbomethoxy groups, as in this conformation H-8 is deshielded by the carbonyl group. The *trans*-orientation of H-8 and H-9 followed from the coupling observed. Also the signals and the couplings of H-2 and H-3 could be assigned and they agreed nicely with the proposed conformation. The ^{1}H NMR spectrum of **4** (Table 1) clearly showed that the tiglate residue was replaced by hydroxymethacrylate, as could be deduced from the typical ^{1}H NMR signal (δ 6.15*br s*, 5.88 *dt* and 4.28 *br d*). Obviously, the stereochemistry again was the same as in **7**, as all couplings were the same and also the chemical shifts were nearly identical. In the ^{1}H NMR spectrum of **5** (Table 1) the characteristic signals of 4-hydroxytiglate were present. Again the substitution pattern and the stereochemistry obviously were the same, as the chemical shifts and the couplings were nearly the same as those of **7**. The ^{1}H NMR spectral data of **6** (Table 1) also differed from those of **2–5** only by the signals of the ester residue. The nature of this group followed from the corresponding signals (δ 2.93 *br d*, 2.73*d* and 1.50*s*), which clearly indicated the presence of an epoxy isobutyrate. Again the chemical shifts and couplings showed that identical stereochemistry had to be assumed. The ^{1}H NMR spectrum of **8** (Table 1) showed that the carbomethoxy group was replaced by an aldehyde group, while the ester groups were acetate and methacrylate, as clearly followed from the ^{1}H NMR spectral data. Again identical stereochemistry could be deduced from the observed couplings. The additional coupling of H-9 with the aldehyde proton was the typical *W*-coupling, which further supported the proposed stereochemistry and conformation of these lactones. Though the relative position of the acetate group in all lactones had not been established rigorously, the proposed one was very likely as the chemical shift of H-9 was nearly the same in all lactones and corresponded to that of enhydrin (**7**) with known relative position of the acetate group [9]. Surprisingly this variety did not contain enhydrin, the main constituent of *E. fluctuans*. However, as all lactones are closely related to **7**, this type of melampolide is probably characteristic for the genus. Therefore its separation from the subtribe Ecliptinae is strongly supported by the chemistry, as none of the genera of the Ecliptinae contains lactones of this type. These lactones have been isolated previously only from genera belonging to the subtribe Melampodiinae [8]. Similar lactones, where

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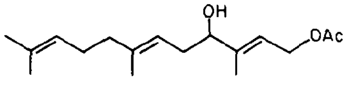
*Part 415 in the series "Naturally Occurring Terpene Derivatives". For Part 414 see Bohlmann, F., Singh, P., King, R. M. and Robinson, H. (1982) *Phytochemistry* **21**, 1171.

Table 1. ^1H NMR spectral data of compounds **3**–**6** and **8** (400 MHz, $CDCl_3$, TMS as int. standard)

	3	**4**	**4**(C_6D_6)	**5**	**5**(C_6D_6)	**6**	**6**(C_6D_6)	**8**	**8**(C_6D_6)
H-1	7.14 *dd*	7.16 *dd*	6.68 *dd*	7.15 *dd*	6.70 *dd*	7.15 *dd*	6.66 *dd*	6.95	5.73 *dd*
H-2β	2.98 *ddd br*	2.95 *ddd br*	2.69 *ddd br*	2.93 *m*	2.75 *ddd br*	2.94 *ddd br*	2.65 *m*	3.14 *ddd br*	2.71 *ddd br*
H-2α	2.45 *ddd*	2.46 *ddd*	1.65 *m*	2.46 *ddd*	1.65 *m*	2.45 *ddd*	1.7 *m*	2.62 *ddd*	1.65 *ddd*
H-3β	2.35 *ddd*	2.36 *ddd*		2.36 *ddd*		2.35 *ddd*		2.44 *ddd*	1.74 *ddd*
H-3α	1.24 *dd br*	1.23 *dd br*	0.68 *dd br*	1.24 *dd br*	0.67 *dd br*	1.21 *dd br*	0.67 *dd br*	1.28 *dd br*	0.64 *dd br*
H-5	2.69 *d*	2.69 *d*	1.97 *d*	2.69 *d*	1.99 *d*	2.66 *d*	1.98 *d*	2.62 *d*	1.89 *d*
H-6	4.30 *dd*	4.30 *dd*	4.23 *dd*	4.30 *dd*	4.28 *dd*	4.28 *dd*	4.25 *dd*	4.28 *dd*	4.15 *dd*
H-7	2.98 *d br*	2.99 *dddd*	2.66 *dddd*	2.99 *m*	2.70 *dddd*	2.96 *dddd*	2.65 *m*	2.83 *dddd*	2.34 *dddd*
H-8	6.69 *dd*	6.75 *dd*	6.98 *dd*	6.71 *dd*	6.98 *dd*	6.61 *dd*	6.87 *dd*	6.79 *d br*	7.02 *dd*
H-9	5.87 *d*	5.91 *d*	5.89 *d*	5.89 *d*	5.94 *d*	5.86 *d*	5.88 *d*	5.77 *dd*	5.67 *dd*
H-13	6.33 *d*	6.36 *d*	6.29 *d*	6.35 *d*	6.30 *d*	6.35 *d*	6.25 *d*	6.36 *d*	6.23 *d*
H-13′	5.93 *d*	5.93 *d*	5.84 *d*	5.92 *d*	5.89 *d*	5.85 *d*	5.73 *d*	5.96 *d*	5.84 *d*
H-14	—	—	—	—	—	—	—	9.53 *d*	8.95 *d*
H-15	1.74 *s*	1.75 *s*	1.49 *s*	1.75 *s*	1.54 *s*	1.70 *s*	1.49 *s*	1.74 *s*	1.45 *s*
OCOR	6.76 *qq*	6.15 *s br*	5.96 *s br*	6.72 *tq*	6.53 *tq*	2.93 *d br*	2.75 *s br*	6.01 *s br*	5.85 *s br*
	1.80 *dq*	5.88 *dt*	5.38 *dt*	4.35 *d br*	3.67 *d br*	2.73 *d*	2.05 *d*	5.60 *dq*	5.05 *dq*
	1.78 *dq*	4.28 *d br*	3.93 *d br* 3.86 *d br*	1.79 *td*	1.46 *td*	1.50 *s*	1.13 *s*	1.90 *s br*	1.61 *s br*
OAc	1.99 *s*	2.01 *s*	1.52 *s*	2.00 *s*	1.53 *s*	2.05 *s*	1.57 *s*	1.99 *s*	1.49 *s*
CO_2Me	3.83 *s*	3.84 *s*	3.34 *s*	3.84 *s*	3.36 *s*	3.83 *s*	3.35 *s*	—	—

J (Hz): 1, 2α = 10; 1, 2β = 7.5; 2α, 2β = 13; 2α, 3α = 2; 2α, 3β = 2; 2β, 3α = 7; 2β, 3β = 13; 3α, 3β = 13; 5, 6 = 9.5; 6, 7 = 10; 7, 8 ~ 1; 7, 13 = 3.5; 7, 13′ = 3; 8, 9 = 9; (compound **8**: 9, 14 = 2); Tigl: 3′, 4′ = 7; 3′, 5′ = 4′, 5′ = 1; $COC(CH_2OH) = CH_2$: 3′, 4′ ~ 1; 4′, 4′ = 14; 4′, OH = 5; $COC(Me)=CHCH_2OH$: 3′, 4′ = 5; 3′, 5′ = 1.5; 4′, OH = 5; epoxy Meacr: 3′, 3′ = 5.5.



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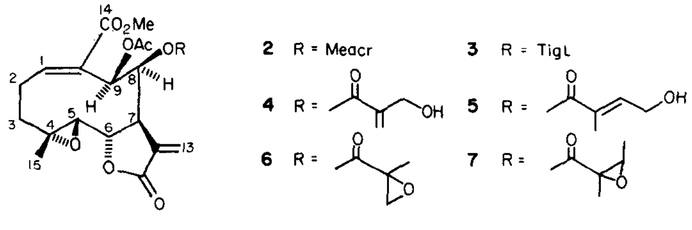

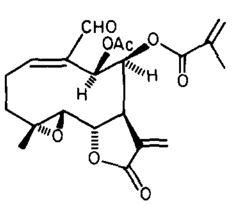

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the 4, 5-epoxide is replaced by a double bond, are present in *Sigesbeckia* [10], *Tetragonotheca* [11] and *Blainvillea* [12].

EXPERIMENTAL

The air-dried aerial parts (200 g), voucher RMK 8685, deposited in the U.S. National Herbarium, Washington, was extracted with Et_2O–petrol (1:2) and the resulting extract was separated by CC (Si gel). Known compounds were identified by comparing their ^1H NMR spectra with those of authentic material. In addition to 10 mg of **1** a complex mixture of sesquiterpene lactones were isolated from the column fractions with Et_2O and Et_2O–MeOH, which could be separated by TLC (Si gel) using Et_2O–petrol (9:1) and then CH_2Cl_2–C_6H_6–Et_2O (1:1:1) as solvents. Finally, 10 mg **2**, 10 mg **3** (not free from **2**), 3 mg **4**, 5 mg **5**, 10 mg **6** and 5 mg **8** were obtained. Due to the small amounts attempts at crystallization were not successful.

8-*Desacyl enhydrin tiglate* (**3**). Colourless crystals, not free from **2**, IR $\nu_{max}^{CCl_4}$, cm^{-1}: 1780 (γ-lactone), 1735, 1235 (OAc), 1720, 1640 (C=CCO_2R); MS *m/z* (rel. int.): 448 $[M]^+$ (0.1), .348.121 $[M-HOTigl]^+$ (16) ($C_{18}H_{20}O_7$), 288 $[348-HOAc]^+$ (2), 83 $[C_4H_7CO]^+$ (100), 55 $[83-CO]^+$ (61).

8-*Desacyl enhydrin*-[*4-hydroxymethacrylate*] (**4**). Colourless gum, IR $\nu_{max}^{CCl_4}$, cm^{-1}: 3600 (OH), 1780 (γ-lactone), 1740, 1235 (OAc), 1715 (C=CCO_2R); MS *m/z* (rel. int.): 348.121 $[M-HO_2CR]^+$ (32) ($C_{18}H_{20}O_7$), 288 $[348-HOAc]^+$ (4), 256 $[288-MeOH]^+$ (23), 85 $[C_3H_4(OH)CO]^+$ (100), 57 $[85-CO]^+$ (56).

8-*Desacyl enhydrin*-[*4-hydroxytiglate*] (**5**). Colourless gum, IR $\nu_{max}^{CCl_4}$, cm^{-1}: 3600 (OH), 1780 (γ-lactone), 1740 (OAc), 1720 (C=CCO_2R); MS *m/z* (rel. int.): 422 $[M-ketene]^+$ (12), 407 $[422-Me]^+$ (8), 348.121 $[M-HO_2CR]^+$ (18) ($C_{18}H_{20}O_7$), 288 $[348-HOAc]^+$ (3), 256 $[288-MeOH]^+$ (9), 99 $[RCO]^+$ (45), 71$[99-CO]^+$ (52), 55 (100).

8-*Desacyl enhydrin*-[*2, 3-epoxyisobutyrate*] (**6**). Colourless gum, IR $\nu_{max}^{CCl_4}$, cm^{-1} 1780 (γ-lactone), 1740 (OAc, CO_2R), 1640 (C=C); MS *m/z* (rel. int.): 450.153 $[M]^+$ (0.1) ($C_{22}H_{26}O_{10}$), 348 $[M-HO_2CR]^+$ (44), 288 $[348-HOAc]^+$ (3), 256 $[288-MeOH]^+$ (38), 85 $[RCO]^+$ (44), 288 $[348-HOAc]^+$ (3), 256 $[288-MeOH]^+$ (38), 85 $[RCO]^+$ (10), 57 $[85-CO]^+$ (100).

$$[\alpha]^{\lambda}_{24^\circ} = \frac{589 \quad 578 \quad 546 \quad 436\,\mathrm{nm}}{-36 \quad -39 \quad -46 \quad -94} (c = 0.58, CHCl_3).$$

8β-*Methacryloyloxy*-9α-*acetoxy*-14*oxo*-*acanthospermolide* (**8**). Colourless gum, IR $\nu_{max}^{CCl_4}$. cm^{-1}: 1780 (γ-lactone), 1740 (OAc, CO_2R), 2710, 1690, 1640 (C=CCHO); MS *m/z* (rel. int.): 404.147 $[M]^+$ (0.2) ($C_{21}H_{24}O_2$), 318 $[M-RCO_2H]^+$ (3), 290 $[318-CO]^+$ (12), 69 $[C_3H_5CO]^+$ (100).

$$[\alpha]^{\lambda}_{24^\circ} = \frac{589 \quad 578 \quad 546 \quad 436\,\mathrm{nm}}{-64 \quad -68 \quad -81 \quad -165} (c = 0.18, CHCl_3).$$

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